Kingdom of Saudi Arabia
Ministry of Education
Qassim University
College of Agriculture and Food
Department of Food Science and Human Nutrition

Protective Effect and Bioactivity of Polyphenolics Extract from Ajwa Dates Against the Drug Prednisolone-induced Antioxidant Disorders and Lipid Peroxidation in Male Rats.

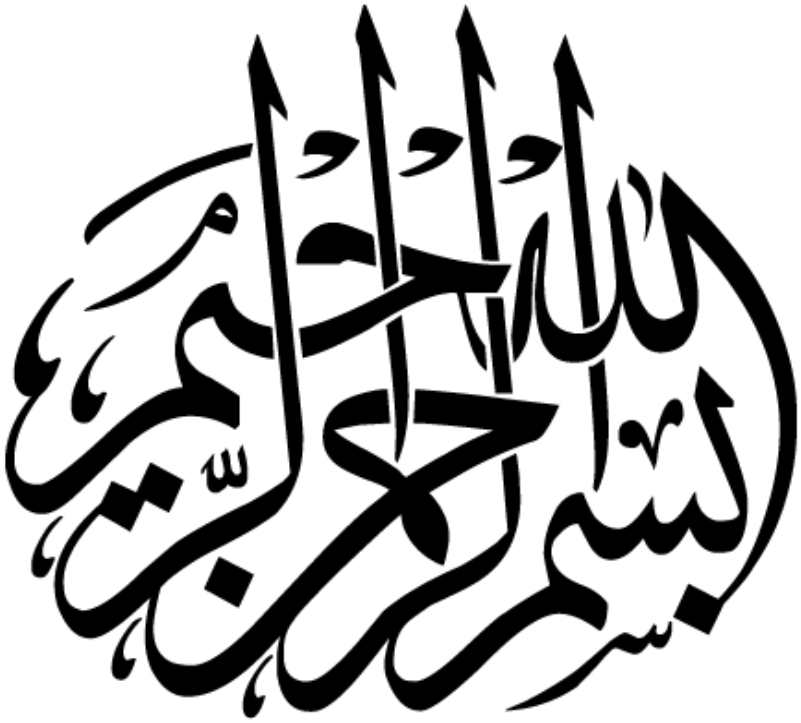
A Thesis submitted in Partial Fulfillment of the Requirement for the (Master) Degree in Human Nutrition

Submitted by:
Samiyah Saifi Eisa Al-Shelaly.
Student ID: 441212013

Supervisor:
Dr. Waheeba Elfaki Ahmed Mohammed Ahmed
Associate Professor at the Department of Food science and Human Nutrition – Faculty of Agriculture and Food – Qassim University

Co- Supervisor:
Prof. Dr. Hassan Mirghani Mousa
Professor at the Department of Food Science and Human Nutrition – Faculty of Agriculture and Food – Qassim University

(2025 Ad- 1447 H)


بسم الله الرحمن الرحيم

Kingdom of Saudi Arabia
Ministry of Education
Qassim University
College of Agriculture and Food
Department of Food Science and Human Nutrition

Protective Effect and Bioactivity of Polyphenolics Extract from Ajwa Dates Against the Drug Prednisolone-induced Antioxidant Disorders and Lipid Peroxidation in Male Rats.

By:

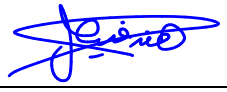
Samiyah Saifi Eisa Al-Shelaly.

Student ID: 441212013

Recommendation of The Committee:

The committee has approved this dissertation as a Patrial Completion of the requirement for the master's Degree in Human Nutrition.

Examination and Decision Making Committee

Committee Members	Name	Academic Degree	Specialies Action	Signature
Main Supervisor	Waheeba Elfaki Ahmed	Associate Professor	Human Nutrition	
Co-Supervisor	Hassan Mergani Mussa	Professor	Biochemistry	
Enternal Examiner	Hashim Suliman Ibrahim	Associate Professor	Nutrition and Dietetics	
Internal Examiner	Hind Faisal Al-Harbi	Associate Professor	Human Nutrition	
Internal Examiner	Nada Abd Allah Al-Zunaidy	Assistant Professor	Human Nutrition	

(10/06/1447 H. – 01/12/2025 AD.)

Acknowledgement

'My Lord, inspire me that I should be thankful for Your blessing with which You have blessed me and my parents, and that I may do good works that will please You. Admit me, by Your Mercy, among Your righteous worshipers.' (Sura 27: AL-NAML, Aya: 19). “In the name of Allah, Most Gracious, Most Merciful.” Praise be to Allah, the Cherisher and Sustainer of the Worlds; Allah’s blessings and peace be upon the noblest of prophets and messengers, our Prophet Muhammad, and upon his good relatives and companions. Praise be to Allah for His great favors that He has bestowed upon me. Praise be to Allah; he granted me his generosity and eased the course of knowledge for me and facilitated it to complete my thesis. I would never accomplish anything if it weren't for his blessing and success .

As our prophet Mohammed (peace be upon him) said, "He who does not thank the people is not thankful to Allah." Sahih (Al-Albani). I would like to present my sincere thanks and appreciation to those who helped me through my journey to reach this point. To the light of my path, my supporter, who was the reason—after Allah—for me to start and finish this journey with his unlimited support, to my father, **Saify Eisa Al-Shelaly**. To the source of my happiness, whom I cherish the most, my mother, **Munifah Saud Al_Shelaly**, for her endless encouragement and her readiness to help me anytime. To all my family, my brothers and sisters, **especially Alia, Sumayah and my cousin Ghadah**, for always believing in me and cheering me up. To all my dearest friends, for always being there for me and always praying for me and encouraging me, especially **Rana Mohammed Abu-wardah**, who likewise helped me with equipments and was patient with me through my hardest times.

And I must express my thanks and gratitude to my supervisor, **Dr. Waheeba Alfaki Ahmed**, who helped make this work possible. Her permanent presence, scientific guidance, sincere supervision, and valuable advice carried me through my thesis. I am also extremely grateful for my co-supervisor, **Prof. Dr. Hassan Mirghani Mousa**, for sacrificing his time and always being there to answer any of my questions, his patience when teaching me how to deal with rats and break my fear, guidance, and kind support. My gratitude to all the staff members and classmates of the College of Agriculture and Food at Qassim University and to everyone who helped me by doing a favor and advising me. praise be to Allah for all the blessings in my life.

Protective Effect and Bioactivity of Polyphenolics Extract from Ajwa Dates Against the Drug Prednisolone-induced Antioxidant Disorders and Lipid Peroxidation in Male Rats.

By

Samiyah Saifi Eisa Al-Shelaly.

Prednisolone is a corticosteroid commonly used for short-term treatment. It works on the immune system. However, long-term use may result in serious complications, including hyperlipidemia. which can trigger oxidative stress and lipid peroxidation. Phenolic compounds are known for their antioxidant potential because they break the oxidative reaction chains, which makes them participate in the first line of defense against free radicals. This study aims to evaluate the protective bioactivity of polyphenolics from Awja date extract (30, 60, 90, and 120 mg/kg BW) against the drug prednisolone-induced antioxidant disorders and lipid peroxidation, detect the antioxidant activity and phytochemicals of Awja date extract, and measure the effect of Awja date extract on antioxidant disorders and lipid peroxidation caused by prednisolone on male rats. The evaluation of Awja dates and Awja date extract led to a variety of compounds, including antioxidants like ferulic acid, lactic acid, vanillin, and cinnamic acid, and carbohydrates found in both Awja dates and Awja extract, such as .beta.-D-Glucopyranose, 5TMS derivative, D-Glucopyranose, 5TMS derivative, and D-Fructose, 5TMS derivative, only D-Glucose, 5TMS derivative found in Awja, fatty acids in both Awja dates and Awja extract comprise palmitic acid, linoleic acid, oleic acid, and stearic acid. Ajwa dates and Awja extract contained minerals; calcium was higher in Awja dates than Awja extract (1290 mg/kg, 380 mg/L respectively), sodium was lower in Awja dates than Awja extract (100 mg/kg, 930 mg/L respectively), potassium was the highest in both Awja dates and Awja extract among all minerals, while in Awja dates it was lower than Awja extract (4600 mg/kg, 5900 mg/L respectively), phosphor found higher in Awja dates than Awja date extract (700 mg/kg, 360 mg/L respectively), zinc was also higher in Awja dates than Awja date extract (4.5 mg/kg, 3.6 mg/L respectively), and lastly, magnesium was observed only in Awja extract (400 mg/L). After one week of acclimatization, thirty male Wistar albino rats were divided into six groups.

Group 1 was the negative control, group 2 was given (0.88 mg) prednisolone as the positive control, and groups 3, 4, 5, and 6 were treated with Ajwa extract (30, 60, 90, and 120 mg/kg) for seven days, followed by administration of the same doses of Ajwa to the same group with prednisolone (0.88 mg). Results after prednisolone showed a significant ($P < 0.001$) increase in blood glucose and lipid profile and decreased high density lipoprotein (HDL) levels, increased malondialdehyde (MDA) and superoxide dismutase (SOD) levels, and decreased antioxidant defense system reduced glutathione (GSH), glutathione reductase (GR), glutathione peroxidase (GPx), total antioxidant capacity (TAC), and catalase (CAT) compared to the negative control. Moreover, there was an increase in liver cholesterol and triglycerides, as well as a reduction in GSH levels, compared to the negative control. A discernible rise in collagen fibers was also observed in the histopathology of the liver hepatocytes in response to persistent inflammation. Conversely, Ajwa extract (30mg, 60 mg, 90 mg, and 120 mg) showed a significant ($P < 0.001$) enhancement of blood glucose levels and lipid profile by decreasing them and increasing HDL levels. Ajwa extract also lowered MDA and catalase levels while boosting the body's antioxidant defenses (SOD, GSH, GPx, GR, and TAC). Furthermore, compared to the positive control, the liver hepatocytes exhibited significant improvements. Ajwa extract with its antioxidant contents significantly lessens the oxidative dysregulation, high glucose, and high lipid profile caused by prednisolone, particularly at the 120 mg/kg dose. Its antioxidant properties suggest it may be beneficial as a dietary supplement alongside corticosteroids to reduce oxidative stress and maintain overall health.

List of Contents

List of Tables

List of Figures

List of Abbreviations

No	Abbreviation	Definition
1	AASE	Aqueous Ajwa Date Seed Extract
2	ADE	Ajwa Date Extract
3	ADP	Ajwa Date Pit Powder
4	AOAC	Association of Official Analytic Chemist
5	ATP	Adenosine Triphosphate
6	CAT	Catalase
7	CE	Cholesteryl Ester
8	CVD	Cardiovascular Diseases
9	DLP	Dyslipidemia
10	DM	Diabetes Mellitus
11	DNA	Deoxyribonucleic Acid
12	FA	Fatty Acids
13	FC	Free Cholesterol
14	GPx	Glutathione Peroxidase
15	GR	Glutathione Reductase
16	GSH	Glutathione Reduced
17	HCC	Hepatocellular Carcinoma
18	HDL	High-Density Lipoprotein
19	HPLC	High Performance Liquid Chromatography
20	LDL	Low-Density Lipoprotein
21	MCF7	Human Breast Adinocarcinoma
22	MDA	Malondialdehyde
23	MEAD	Methanolic Extract of Ajwa Dates

24	NCD	Non-Communicable Diseases
25	RNA	Ribonucleic Acid
26	RNS	Reactive Nitrogen Species
27	ROI	Reactive Oxygen Intermediate
28	ROS	Reactive Oxygen Species
29	RT	Retention Time
30	SD	Standard Deviation
31	SOD	Superoxide Dismutase
32	T2D	Type 2 Diabetes Mellitus
33	TAC	Total Antioxidant Capacity
34	TC	Total Cholesterol
35	TCA	Trichloroacetic Deviation
36	TFC	Total Flavonoid Content
37	TG	Triglycerides
38	TPC	Total Phenolic Content
39	US	United States
40	VLDL	Very Low-Density Lipoprotein
41	WHO	World Health Organization

Chapter 1
Introduction

1. Introduction:

Prednisolone is a synthetic glucocorticoid used over the last recent decades routinely as a pharmacotherapy against several diseases for its anti-inflammatory and immunosuppressive potentials **Quinkler et al., 2017.**

Prednisolone is metabolically interconvertible, and it's considered to be the pharmacologically active species **Pickup, 1979**.

Prednisolone is one of corticosteroid classes with short-term use in connection with patients with bacterial meningitis, tuberculous meningitis, tuberculous pericarditis, severe typhoid fever, tetanus, or pneumocystis pneumonia with moderate to severe hypoxemia, treatment with corticosteroids improved patient survival. Generally mild side effects such as: cutaneous effects, electrolyte abnormalities, hypertension, hyperglycemia, pancreatitis, hematologic, immunologic, and neuropsychologic effects were noticed. However, long-term corticosteroid use may lead to more serious consequences including osteoporosis, aseptic joint necrosis, adrenal insufficiency, gastrointestinal, hepatic, and ophthalmologic effects, hyperlipidemia, growth suppression, and possible congenital malformations **Buchman, 2001**. However, hyperlipidemia is one of the prominent side effects associated with corticosteroid treatment **Pirsch et al.,1992**.

Having too many lipids (fats) in the blood is called hyperlipidemia, sometimes referred to as dyslipidemia or excessive cholesterol. Excess cholesterol is unhealthy because it can obstruct the artery highways that carry blood throughout the body, which harms organs whose arteries don't supply enough blood, leading to the buildup of hardened cholesterol deposits (plaque) inside blood arteries. Low-density lipoprotein (LDL) is

the most harmful kind, making the blood more difficult to pass through and increasing the risk of cardiovascular diseases (CVDs), **Cleveland Clinic, 2021.**

Cardiovascular diseases, including stroke are considered the leading cause of illness and death in the United States. In Saudi Arabia, the total prevalence of cardiovascular diseases (CVDs) was 1.6% in all regions, with 1.9% of males and 1.4% of females affected. **Alqahtani and Alenazi, 2024**.

Oxidative stress arises with the existence of an imbalance between free radical formation and the antioxidants in the body, lipid peroxidation for example came from excess hydroxyl radical and peroxynitrite, thus damaging cell membranes and lipoproteins. This in turn will lead to malondialdehyde (MDA), which is known as cytotoxic as well as mutagenic. As a radical chain reaction, lipid peroxidation extends very quickly, which impacts a large amount of lipidic molecules **Pizzino et al., 2017**.

Antioxidants are found in certain foods and may prevent some of the damage caused by free radicals by neutralizing them, it may reduce the risk of many diseases including heart, liver disease and some cancers (such as oral, esophageal, stomach and bowel cancers)., antioxidants scavenge free radicals from the body cells and prevent or reduce the damage caused by oxidation **Better Health Channel, 2021**.

Plant-based foods naturally contain polyphenols; these compounds have wide range of complex structures. It's generally these are classified as phenolic acids and phenolic alcohols. Depending on the strength of phenolic ring, polyphenols can be classified in many classes, but the main

classes in the polyphenols are phenolic acids, flavonoids, stiblins, phenolic alcohols, and lignans. Bioactive compounds are the phytochemicals involved in protection of human health against the chronic degenerative ailments. Polyphenols are the group of biologically active compounds in plant-based foods **Abbas et al., 2016**.

Date fruit is well- regarded for its nutraceutical parcels in Middle East and Africa. still, its significance in Western countries has not been explored yet owing different culture and eating habits. Scientific community now has realized its nutritive value in diet and has started to explore further avenues for development in this order of fruits **Khalid et al., 2017**.

Ajwa, Date fruit (Phoenix dactylifera L. var. Ajwa) is a special variety of Saudi dates (Phoenix dactylifera L.) is a rich source of nutrients, fiber and bioactive molecules. **Bhatti et al., 2019**.

It contains amounts of polyphenols, flavonoids, glycosides, and sterols **Khalid et al., 2017**. Dates are believed to promote health and treat many diseases.

Several studies have shown that Ajwa dates have antioxidant, antiviral, antifungal, antibacterial, antidiabetic, anti-inflammatory, anticancer, hypolipidemic, gastrointestinal protective, and cardioprotective properties **Khalid et al., 2017**. As a result, research on the potential of Ajwa dates and their extracts in the treatment of diseases has been constantly increasing in recent decades.

1.1 Statement of the Problem:

With developed changes in living standards and dietary habits which lead to chronic diseases that require medicines to control it. Prednisolone is one of these medicines that may lead to serious problems such as antioxidant disorders affecting the body or its organs. Lipid peroxidation is a metabolic process that causes oxidative deterioration of lipids by Reactive oxygen species (ROS). This process can degrade the lipids within the cell membrane leading to cell damage and eventually, cell death.

Oxidative stress leads to lipid peroxidation, which damage cells, proteins, and DNA, which can contribute to aging. It may also play a role in development of a range of health conditions, including diabetes, cancer, and neurodegenerative diseases such as Alzheimer's. At cardiovascular levels, oxidative stress is highly implicated in myocardial infarction, ischemia/reperfusion, or heart failure. The body naturally produces antioxidants to counteract these free radicals, antioxidants also come from food like polyphenols.

Our natural food we use for healing is much better than using chemical medications that is unforgiving. And Ajwa dates that containing the nutrients had the potential of healing and nourishing the body.

In our study we will study the effect of Ajwa dates' extract affecting the Antioxidants Disorder and Lipid Peroxidation Caused by Prednisolone.

1.2 Significance of the Study:

With the progress in the field of pharmaceutical industry, there is no way to inhibit the side effects caused by the medications, side effects can grade from mild to moderate to severe according to the body condition, age, allergies etc.

Therefore, the present study can contribute the knowing of the capability of polyphenol to prevent side effects caused by the prednisolone associated with serious side effects and how can polyphenol improve body health.

1.3 Study Objectives:

1. Detecting the phytochemicals and minerals of Ajwa date extract.

2. Measuring the protective effect of different doses of (30, 60, 90 and 120 mg/kg BW) bioactivity of polyphenolic extract from Ajwa dates against glucose, lipid profile, caused by Prednisolone.

3. Evaluate the protective effect of different doses (30, 60, 90 and 120 mg/kg BW) bioactivity of polyphenolic extract from Ajwa dates against the drug Prednisolone -induced antioxidants disorder and lipid peroxidation in male rat.

Chapter 2
Literature Review

2. Literature Review:

2.1. Ajwa Date:

Date Palm (Phoenix dactylifera L.) has long been one of the most important fruit crops in the thirsty regions of the Arabian Peninsula, North Africa, and the Middle East. During the past three centuries, dates were also introduced to new product areas in Australia, India/ Pakistan, Mexico, southern Africa, South America, and the United States. Dates are a main income source and chief food for original populations in numerous countries in which they're cultivated, and have played significant places in the frugality, society, and terrain of those countries. Date palm is one of the oldest types of date palm trees cultivated (5500–3000 BCE), it has a nutritional, environmental, economic and ornamental benefits. the cultivation of date palm thought to be merged with the cultural environmental, social and religious development of people who live in the hot and arid areas especially in the middle east and Africa. The date palm is a monocotyledon tree, and it can grow up to 1500 m in well-drained soils, date palm tree currently is cultivated basically in areas of Iraq, Iran, Saudi Arabia, Algeria, Egypt, Libya, Pakistan, Morocco, Sudan and Oman. Until now date palm considered as a major cultivating crop of above-described areas, making these regions are biggest producer and exporters of date products all around the world **Khalid et al., 2017.**

The date palm fruit has a lot of distinguishing characteristics and one of it is that date fruit can be consumed at three different maturity stages such as Khalal, Rutab and Tamar. However, fresh date fruit is preferred in many countries that produce dates. It's also commercially available in dehydrated form which is prepared by drying the dates through processing techniques to increase the shelf life. thus, reducing the nutrition value for these dates. Taste,

nutritional and phytochemical properties of the dates are vary depending on the maturity stages and variety of the dates, there are nearly 5000 types of dates growing in different regions worldwide and most common ones are Aseel, Zahidi, Majdool, Mabrook, Dhakki, Halawi, Lasht, Deggla and Bamy **Khalid et al., 2017.**

Some virtuous men of AL-Madinah mentioned that Ajwa date may change quality a bit due to the way it saved. Ajwa date also size changes due to environmental factors and circumstanced area, which means that high-quality Ajwa dates are twice the size of low-quality Ajwa dates that are small. Ajwa dates have a special place among other dates thanks to various of feature that gave it an excellence among all different of dates. Rather than its excellence in nutrition value, sunnah had praised its priority in many of hadith told by prophet Mohammad peace be upon him. And if we looked of sunnah we would find that Ajwa date are preferred among all other types of dates because of its provenance sometimes, because of its therapeutic benefits sometimes, because of its nutrition value and prophet Mohammad peace be upon him preference other times **Alghemlas, 2020**.

Several studies have shown that Ajwa dates have antioxidant, antiviral, antifungal, antibacterial, antidiabetic, anti-inflammatory, anticancer, hypolipidemic, gastrointestinal protective, and cardioprotective properties **Khalid et al., 2017.** As a result, research on the potential of Ajwa dates and their extracts in the treatment of diseases has been constantly increasing in recent decades.

2.1.2. Phytochemicals and Chemical Composition of Ajwa Dates:

2.1.2.1. Phytochemicals:

Ajwa dates contain a variety of polyphenols, carotenoids, sterols, and tannins. Dates are known to be rich in fiber and low in calories. The concentration and composition of dates are affected by factors that are date variety, harvesting stage, storing, postharvest production, and the region of origin. Also, dates face a significant switch in their development phase; thus, their chemical composition and functional content are affected, with a lower content of fiber, minerals, and vitamins versus an increased concentration of simple sugar **Alyahya et al., 2022.**

2.1.2.2. Chemical Composition of Ajwa Dates:

A chemical analysis made by Assirey, 2015 to determine the chemical composition of dates from 10 types of palms cultivated in Saudi Arabia, showed the content of Ajwa date flesh in table (1) below:

Table 1: Ajwa Date Flesh Content Determined by Assirey, 2015:

Ajwa Date Flesh Content	Sugar	Protein	Fat	Ash
g/100g dry weight	74.3, 22.8	2.9	0.47	3.43

Although the amount of protein was too small for dates to be considered an important nutritional source, **Ajwa dates content of essential amino acids and Minerals described in table (2) below:**

Table 2: Ajwa Date Flesh Content of Amino Acids and Minerals Determined by Assirey, 2015:

Essential Amino Acids	mg/100g dry weight	Essential Amino Acids	mg/100g dry weight
Alanine	82	Lysine	73
Arginine	93	Methionine	27
Aspartic acid	186	Phenylalanine	45
Glutamic acid	205	Proline	86
Glycine	83	Serine	59
Histidine	26	Threonine	53
Isoleucine	44	Tryptophan	44
Leucine	57	Valine	65
Minerals			
calcium	187	Phosphorus	27
Potassium	476.3	Sodium	7.5
Magnesium	150		

2.1.3. Therapeutic Roles of Ajwa Dates:

2.1.3.1 Anti-Inflammatory and Antimicrobial Activities:

The immune system is considered one of the most significant parts of the human body since it allows us to action in the surrounding area. It's like the immune guard and several kinds of infection stopper. The immune system works as an exposer and engager against pathogens that attack the body, like viruses, bacteria, fungi, parasites and toxins. It also fights cancer, that's why it's very important to human health when it functions adequately **Mucha et al., 2021.**

Inflammation, which includes number of biological processes (such as phagocytosis, chemotaxis, mitosis, and cell differentiation), has emerged as one of the main research topics for biomedical researchers in recent years. Inflammation is a lengthy series of molecular reactions and cellular activity that are meant to heal a variety of burn injuries, mend tissue following childbirth, or heal a small skin cut. Increased blood artery permeability, venule and arteriole dilatation, and blood flow with leukocyte percolation into the tissues are all examples of the inflammatory process at the cellular and tissue levels. If the inflammation cascade does not reach a resolution state, an organ dysfunction and mortality occur **Arulselvan et al., 2016.**

When mentioning exogenous antioxidants that the body can't produce, it's needed to mention a very complex group that are widely extended in the plant world: Polyphenols. The plant secondary metabolized with different biological attributes to which these compounds belonged. Anti-bacterial and anti-inflammatory characteristics the polyphenols had are substantial components to the diet **Mucha et al., 2021.**

Mauludiyana et al., 2023 in their research count on a various reference from the online database, including journals, reports, and all references for the last 10 years that talked about Ajwa date extract and their potential as anti-inflammatory and antibacterial in burn infection. Flavonoids, glycosides, and phenolic acids were among the polyphenolic compounds found in Ajwa dates. With the help of its unique molecules, polyphenol can interact with one or more immune cell receptors, transmitting intracellular messages and affecting the host's immune response. Ajwa dates with their rich polyphenol content show an anti-inflammatory effect which modulates immune cell responses. The polyphenols found in Ajwa dates inhibit the nuclear factor kappa B (NF-κB) signaling pathway, resulting in reducing the production of cytokines that triggers inflammation such as TNF-α, IL-1β, and IL-6. Moreover, they adjust macrophage behavior and suppress pathogen-associated molecular pattern receptor expression, which contribute to immune regulation and tissue repair.

Arwa Ahmad, 2011 in her research the miracle of dates in healing and protection against harmless and pathogenic microbes studied the effects of dates with different concentrations on isolated microbes from 29 fasting male aged 21-23. Samples were taken in the evening, about an hour before Iftar. Arwa found that dates, in their various concentrations have an effective effect in inhibiting and eliminating these bacteria isolated from the mouths of fasting people. Moreover, Arwa studied the effect of water extracts of dates on bacterial strains isolated from tonsillitis that 30 patients (21 female and 9 male) who were clinically diagnosed as having tonsillitis. Some of these patients had severe inflammation such as abscesses on the tonsils before they were removed, other section suffered from tonsillitis after they underwent

surgery to remove them, but their ASO was high. And comparing water extract of dates with other medicinal plants such as lemon, garlic, and others. The study showed that date extract has the highest effect on all bacteria isolated from tonsillitis patients.

2.1.3.2. Anticancer Effect:

As stated by WHO 2024, cancer is a huge group of diseases that can begin nearly in any tissue or organ of the body when the abnormal cells grow uncontrollably and go far their regular boundaries to diffuse to other organs. It is the second major cause of mortality globally, showing an estimated 9.6 million mortalities, or 1 in 6 mortalities in 2018. The cancer load keeps growing globally, which strive massive emotional, financial, and physical strain on individuals, families, communities and the health system. In strong health system countries, the rates of survival of many types of cancer are improved due to the available early detection, the quality of curing the disease and survivorship care.

By contributing their own electrons, antioxidants—which can be produced endogenously or exogenously—play a part in neutralizing free radicals and shielding cells from the damaging effects of ROS **Didier et al., 2023.** It has been shown that certain antioxidants help the body produce its own natural antioxidants, which are frequently ingested after chemotherapy is finished. This increases the survival time of chemotherapy patients and lessens their negative effects. Antioxidants have therefore been found to be helpful in reducing the harmful effects of drugs in nutritional therapies; hence, the therapeutic efficiency will be enhanced **Singh et al., 2018.**

Rich in flavonoids, antioxidants, and fiber, Ajwa dates may be an effective anticancer treatment for breast cancer. utilized in conventional treatments for cancer treatment and possible cell damage prevention **Khan et al., 2021.**

As stated by **Bhosale et al., 2020**, there are several ways that these polyphenols and flavonoids exert anticancer effects through several mechanisms. Some of these mechanisms include changing signaling pathways to kill cancer cells, stopping cell cycle events and starting apoptosis. Ajwa dates, being rich in polyphenols and flavonoids, are believed to possess an anticancer potential, which can induce apoptosis and autophagy, inhibit tumor growth, angiogenesis, metastasis, phosphoinositide 3-kinase (PI3K), protein kinase B (AKT), mammalian target of rapamycin (mTOR), and signal transducer and activator of transcription 3 (STAT3) pathways in various types of cancer.

A study conducted by **Khan et al., 2017** of aqueous extract of Ajwa dates (ADE) and its effect against hepatocellular carcinoma (HCC). Thirty-two male rats were divided into four groups of eight. First group is the negative control, the second group is a positive control treated with diethylnitrosamine (DEN) (180 mg/kg body weight) to induce liver cancer, the third group was treated with DEN (180 mg/kg bw) + ADE 0.5 g/kg bw, and the fourth group was treated with DEN (180 mg/kg bw) + ADE 1.0 g/kg bw. The results showed a significant increase after the treatment with ADE in antioxidant enzymes SOD, GR, and GPx, while CAT increased only in ADE (1.0 g/kg bw) compared to the positive control. Oxidative stress was decreased by low MDA levels. Liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in addition to the tumor marker alpha-fetoprotein (AFP) were all significantly decreased in the

third and fourth groups compared to the positive control. Moreover, ADE showed an inhibited reverse effect of DEN in the histopathological and also contributes to restoring partial to complete histological features. The results indicated that Ajwa dates exert an anticancer activity, anti-inflammatory, and hepatoprotective through their antioxidant properties to scavenge free radicals due to their richness of polyphenols and flavonoids compounds.

Human breast adenocarcinoma (MCF7) cells treated with a methanolic extract of Ajwa dates (MEAD) at different concentrations (5, 10, 15, 20, and 25 mg/ml) for 24, 48, and 72 hours in vitro demonstrate anticancer effects. It demonstrated that MEAD induced cell cycle arrest and death, hence inhibiting MCF7 cells in vitro **Khan et al., 2016.**

2.1.3.3. Antidiabetic Properties:

Diabetes is a chronic disease that affects the way the body cells receive the glucose or the amount of insulin that the pancreas secretes, which causes an abnormal increase or decrease in blood sugar level. Type 1 diabetes, type 2 diabetes, and gestational diabetes are common types of diabetes. Type 1 diabetes is when a patient counts on insulin for life. Type 2 diabetes can be treated or prevented by a healthy diet, exercise, and lifestyle changes. Gestational diabetes is a change in blood sugar levels that was first diagnosed during pregnancy, whether it continues after delivery or not **Ministry of Health, 2024**. Diabetes increases the risk of long-term issues with the heart, brain, feet, nerves, kidneys, and eyes **Cleveland Clinic Abu Dhabi, 2017**. Studies and research show a large increase in people with diabetes in Saudi Arabia. A survey conducted by the Ministry of Health with Washington University cooperation in the United States in 2014 found that the diffusion rate is 13.4% for people with diabetes above the age of 15 years, and this

percentage is increased with age. Also, studies show that over the age of 65 years, people with diabetes have a percentage that reaches approximately 51%. Several people, 43.6%, were diagnosed with diabetes, weren't aware of it, and 15.2% of Saudis had a pre-diabetes phase **Saudi Health Council, 2021**.

Although the exact mechanism is yet unknown, oxidative stress contributes to the development of type 2 diabetes (T2D) and exacerbates the disease and consequences by disrupting regulatory mechanisms linked to insulin resistance and β-cell malfunction. Hyperglycemia, inflammation, and dyslipidemia are linked to oxidative stress and type 2 diabetes. By promoting glucose metabolism, enhancing insulin secretion and lowering insulin resistance, enhancing vascular functions, controlling HbA1c levels, and reducing oxidative stress indicators, dietary antioxidants with anti-diabetic effects help improve diabetes status. A well-planned diet that includes easy access to foods high in antioxidants and regular exercise may help prevent several diseases, especially diabetes, obesity, and cardiovascular issues **Fatima et al., 2022.**

Ajwa dates contribute to lower levels of diabetes due to their flavonoid content, as reported by **Dewanti et al., 2024**. Flavonoids can lower blood glucose levels in several mechanisms, such as modulating glucose metabolism by inhibiting the key enzymes of carbohydrate digestion (α-glucosidase and α-amylase), which slow absorption of glucose and prevent elevated blood sugar after meals. Flavonoids also improve insulin sensitivity by GLUT-4 expression enhancement and translocation, which promote glucose absorption in muscle and tissues, and through adenosine monophosphate activated protein kinase (AMPK) activation, resulting in an increased insulin response. Additionally, flavonoids reduce the inflammation through (NF-kB) inhibition, thus reducing oxidative damage and protecting the functions of pancreatic B-

cells and improving endothelial health, as discussed by **Caro-Ordieres et al., 2020**.

A reviewed study done by **Mirghani 2024** using databases on humans and animals to detect the effect of dates on blood glucose and lipid profiles suggested that consuming dates can reduce blood glucose, total cholesterol, and triglycerides and raise HDL levels. Dates also enhance the abundance of beneficial gut microbiota that play a role in inflammation and oxygen free radicals' inhibition. Thus, consuming dates by diabetic and dyslipidemia patients reduces their blood glucose, cholesterol, and triglycerides.

By lowering blood glucose levels and raising plasma insulin levels in T2D patients in the rat experimental model, aqueous Ajwa dates seeds extract (AASE), administered in two doses (200 and 400 mg/kg, p.o.), seems to have a potential role as a therapeutic candidate for T2DM **Mani et al., 2022.**

2.1.3.4. Cardiovascular Protective Effects:

According to the CDC, 2023 chronic diseases are defined as conditions that last 1 year or more and require continuous medical care or narrow activity of daily life or both, chronic diseases such as heart diseases, diabetes, and cancer are the main cause of disability and death in the US. They are also cost 4.1 trillion of the nation's annual health care.

Many chronic diseases are caused by a short list of risk behaviors:

- Tobacco use and exposure to secondhand smoke.

- Poor nutrition, including diets low in fruits and vegetables and high in sodium and saturated fats.

Physical inactivity and excessive alcohol use.

People of all age groups, regions, and countries are affected by Noncommunicable diseases (NCDs). These conditions are frequently associated with aged age groups, but substantiation shows that 17 million NCD deaths occur before the age of 70 times. Of these unseasonable deaths, 86 are estimated to do in low- and middle-income countries. Children, grown-ups, and seniors are each vulnerable to the threat factors contributing to NCDs, whether from unhealthy diets, physical inactivity, exposure to tobacco banks or the dangerous use of alcohol or air pollution. These conditions are driven by forces that include rapid-fire unplanned urbanization, globalization of unhealthy cultures, and population aging. Unhealthy diets and a lack of physical exertion may show up in people as raised blood pressure, increased blood glucose, elevated blood lipids, and rotundity. These are called metabolic threat factors and can lead to cardiovascular complaints, the leading NCD in terms of unseasonable deaths **WHO, 2023**.

Cardiovascular diseases (CVDs), including stroke, are considered the leading cause of illness and death in the United States. In Saudi Arabia, the total prevalence of CVDs was 1.6% in all regions, with 1.9% of males and 1.4 of females affected. According to age, sex, and area in Saudi Arabia, there are significant variations in the prevalence of CVDs, according to this study **Alqahtani and Alenazi, 2024.**

CVDs have been the world's top cause of death for many years. Cardiovascular disease claimed the lives of 20.5 million people in 2021, accounting for over one-third of all deaths worldwide and marking a sharp rise from the 12.1 million deaths from CVD reported in 1990. Nowadays, the main cause of early death for men in 146 nations and for women in 98 countries is

ischemic heart disease **Di et al., 2023**. Since then, it has been acknowledged that most cardiovascular illnesses can be described as exhibiting an imbalance between the production of reactive oxygen species (ROS) and antioxidant mechanisms that break down ROS. Superoxide, hydrogen peroxide, and other chemicals build up as a result of this imbalance, deviating from the steady state. In summary, oxidative stress plays a crucial part in the emergence of cardiovascular disease **Steven et al., 2019.**

2.1.3.4.1. Ajwa Date Role in Cardiovascular Diseases:

Antioxidant therapy is a viable alternative because oxidative stress is a major factor of atherogenesis. Consuming antioxidant vitamins has not been linked to a lower risk of CVD, according to certain intervention trials. As a result, attention has been focused on additional bioactive substances, specifically polyphenols, that are present in fruits and vegetables and may mediate the advantages without the help of the plentiful antioxidants. Additionally, polyphenols in coffee, cocoa, and berries have been linked to a slower progression of CVD, according to epidemiological research **Goszcz et al., 2017**.

Ajwa dates are known for their rich content of polyphenolic compounds, including flavonoids and phenolic acids, that have an antioxidant potential, and also cardiovascular protective activity. Polyphenols exert mechanisms to protect the cardiovascular system, as reported by **Michaleska et al., 2010**, that dietary polyphenols act as an antioxidant, reducing oxidative stress by neutralizing free radicals ROS and RNS and prevent LDL oxidation which plays a role in developing atherogenesis and cardiovascular diseases. Polyphenols also enhance nitric oxide (NO) synthesis leading to vasodilation which improves blood flow and reduce blood pressure. Anti-inflammatory

properties of polyphenol contribute to cardiovascular health by inhibiting production of pro-inflammatory cytokines like Interleukin-1 (IL-1), Interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) resulting in inflammation reduction in blood vessels and therefore improving atherogenesis and cardiovascular health.

These mechanisms of polyphenols on CVDs are supported by studies demonstrating the role of Ajwa dates in hyperlipidemia, obesity, and blood pressure which are connected to cardiovascular diseases. In their study, **Jubayer et al., 2020** evaluated the role of Ajwa date seed powder in improving the lipid profile in humans and reducing the risk of CVDs in a randomized, double-blind, placebo-controlled clinical trials. The Ajwa date seed powder yielded a 19.4 %, 22.5 %, and 25.78 % decrease in total cholesterol (TC), low-density lipoprotein (LDL), and triglycerides (TG) levels respectively along with an increase in high-density lipoprotein (HDL) levels by 23.81 % in the intervention group, these values were much better than the placebo group. Ultimately, Ajwa date seed powder was found to have a positive effect on improving serum lipid profile in the intervention group compared to the placebo group.

The research done by **Nasrullah et al., 2023** to study the impact of Ajwa date pits powder (ADP) 2.7g on obesity and lipid profile pressure of 40 patients with hyperlipidemia. The results showed that APD supplementation improved the nutritional status and lipid profile of the patients. The determination of ADP's effect on total cholesterol (TC), TG, LDL, and VLDL) and HDL, they observe a significant reduction in TC and LDL after 40 days of consuming 2.7g of ADP. Although the reduction in the TG and VLDL and the increase of HDL was there but it was not considerable. In addition, a significant reduction

in weight, fat mass, body fat percentage, visceral fat area, and waist circumference was observed after 40 days of intervention in the ADP group compared to the control group.

2.2. Hyperlipidemia:

The shape and function of cell membranes, the storage, metabolism, and generation of energy, and the propagation of signals inside and between cells are all significantly influenced by lipids, which are molecules with hydrocarbon skeletons. Lipids are fat-soluble substances that fall into one of two categories: simple or complicated. Free cholesterol (FC) and fatty acids (FAs) are examples of simple lipids, while cholesteryl esters (CEs) and triglycerides (TG) are examples of complex lipids, which are mixtures of simple lipids. The complex lipids CE and TG seem to be the lipids with the most pathogenic importance. High-density lipoproteins (HDL), low-density lipoproteins (LDL), intermediate-density lipoproteins (IDL), very low-density lipoproteins (VLDL), and chylomicrons are among the lipoproteins that carry lipids between organs. They are categorized based on their densities and electrophoretic mobilities **Grasso and Rocco, 2016.**

Based on **Cleveland Clinic, 2021**, having too many lipids (fats) in the blood is called hyperlipidemia, sometimes referred to as dyslipidemia or excessive cholesterol. In order to produce hormones and aid in food digestion, the liver produces cholesterol. It is possible to consume cholesterol through foods that contain dairy and meat. Because the liver can produce as much cholesterol as the body requires, there is an excess of cholesterol in diet. Excessive cholesterol is unhealthy because it can obstruct the artery highways that carry blood throughout the body (200–239 mg/dL is borderline high, and 240 mg/dL is excessive). This harms organs whose arteries don't supply enough blood.

Because it leads to the buildup of hardened cholesterol deposits (plaque) inside blood arteries, bad cholesterol (LDL) is the most harmful kind. The blood finds it more difficult to pass through, increasing the risk of a heart attack or stroke. An inflammatory or irritated plaque itself may result in a clot forming around it. A heart attack or stroke may result from this, depending on the location of the obstruction.

An assessment of hyperlipidemia as a risk factor for stroke and CVD is the main objective of a review study conducted by **Alloubani et al. in 2021**, all potential studies referencing hyperlipidemia as a risk factor for stroke and CVD were identified using a database search. concluded that most of the research supported the idea that hyperlipidemia is associated with CVD patients and a risk factor for stroke. Observational studies in many populations show a consistent positive correlation between the incidence of coronary heart disease and LDL cholesterol levels. Furthermore, lowering LDL cholesterol in certain groups can lower the risk of vascular illnesses, particularly when beginning cholesterol is considered. Nevertheless, the use of statins, a lipid-reducing medication, has been shown in numerous studies to have a significant influence on lowering the mortality and morbidity rates of patients with CVD and stroke.

Based on a review studied the efficacy of polyphenols in the management of dyslipidemia, a study on the impact of polyphenols on patients with three dyslipidemia components the most trials in this group showed an improvement of TG, TC, LDL-C and HDL-C in response to polyphenols **Feldman et al, 2021, Zhango et al., 2016.**

Several types of antioxidant compounds in dietary interventions with food with antioxidants showed an important effect on blood lipid level

concentration, as explained in a reviewed studies made by **Khutami et al., 2022.**

In metabolic disorders, dyslipidemia happens very often, and it's related to high oxidative stress. Lipids and lipoproteins, especially LDL, could generate ROS based on some available clues. In both animal models and clinical trial models, increasing HDL-C while decreasing circulatory cholesterol and lipid peroxides is one of polyphenols potential roles. Moreover, polyphenols may protect against oxidative stress with direct or indirect antioxidant mechanisms. Also, renewing additional exogenous antioxidants is a potential role of polyphenols for protection enhancement. Solubilization and transport of PLPs by lipoproteins such as chylomicron or HDL/LDL particles may specifically prevent oxidative stress derived from dyslipidemia (DLP) and cardiometabolic complications, notably atherosclerosis **Feldman et al., 2021**.

2.3. Lipid Peroxidation:

Lipid peroxidation happens in conditions where Reactive Oxygen Species (ROS) readily interact with vulnerable lipids on cell membranes. Polyunsaturated fatty acids are highly exposed to lipid peroxidation due to their unstable double bonds. Because the cell membranes are rich especially with polyunsaturated fatty acids, it's mostly the site where a lot of lipid peroxidation chain reactions. Lipid peroxidation is considered the ultimate catalyst of ferroptosis, an iron-dependent form of non-apoptotic cell death **Mao et al., 2022.**

2.3.1. Mechanism of Lipid Peroxidation:

The process of lipid peroxidation has three steps that form a free radical chain reaction and include initiation, propagation, and termination.

The initiation step includes producing a fatty acid radical when reactive oxygen species are united, such as a hydroxyl radical combined with a hydrogen atom to form water and a fatty acid radical. unstable fatty acid radical unites fast with molecular oxygen in the propagation step to form a peroxyl-fatty acid radical. This unstable radical also reacts with a free fatty acid to make hydrogen peroxide or cyclic peroxide and another fatty acid radical. The chain reaction of free radical reactions continues until forming non-radical species by combining two free radicals in the termination step. Free radicals' reactions can also be stopped by antioxidant molecules inside the living body. Antioxidants can bond with free radicals and prevent lipid peroxidation and mostly come in the form of lipid-soluble vitamins **Stoakes, 2019.**

2.4. Free Radicals and Oxidative Stress:

Oxygen is an element indispensable for life. When body cells use oxygen to generate energy, free radicals are produced as an outcome for ATP (adenosine triphosphate) by the mitochondria. These byproducts, called reactive oxygen species (ROS) and nitrogen species (RNS) are generated as a result of the cellular redox process. The ROS and RNS play a double role as they are both toxic and helpful compounds. The precise balance between ROS and RNS' opposite impact is clearly an important aspect of life. At the low and moderate levels of ROS and RNS, they exert beneficial effects on cellular responses and immune function, High concentrations lead to something called Oxidative Stress, a harmful process can damage the cell structures **Pham-Huy et al., 2008.**

Free radical damage can make low-density lipoprotein (LDL) called bad cholesterol more likely to get trapped in artery walls. The excessive chronic

amount of free radicals causes a case called oxidative stress which may damage cells and results in chronic diseases **Harvard Chan, 2023.** Oxidative stress arises with the existence of an imbalance between free radical formation and the capability of cells to clear them. For example, lipid peroxidation came from excess hydroxyl radical and peroxynitrite, thus damaging cell membranes and lipoproteins. This in turn will lead to malondialdehyde (MDA) and conjugated diene compound formation, which are known as cytotoxic as well as mutagenic. As a radical chain reaction, lipid peroxidation extends very quickly which impacts a large amount of lipidic molecules **Pizzino et al., 2017.**

2.5. Corticosteroids and Glucocorticoids:

Corticosteroids are among the most widely prescribed drug classes globally and are generally used to refer drugs with glucocorticoid effects **Hodgens and Sharman, 2023**. Glucocorticoids are p-prescribed drugs that are steroid hormones used to reduce inflammations; they are synthetic and similar to cortisol, which is a hormone produced by the adrenal gland **Cleveland Clinic, 2024**. From the time corticosteroids were discovered, they have been applied across nearly all medical fields. The indications of corticosteroids used in the treatment of many cases include allergic and autoimmune cases, lower hyperglycemia and treatment of pathologic hypoglycemia, infectious and inflammatory disorders, neurological, hematologic, and skin disorders, corticosteroid therapy replacement, suppression of excess adrenocortical secretion, shock, promotion of water excretion, and prevention of transplant rejection **Hodgens and Sharman, 2023**.

2.5.1. Prednisolone:

Prednisolone is a synthetic glucocorticoid used over the last recent decades routinely as a pharmacotherapy against several diseases for its anti-inflammatory and immunosuppressive potentials **Quinkler et al., 2017.** The active metabolite of prednisone is prednisolone. Which works on the immune system to reduce inflammation **Juhi, 2023.** Prednisolone is one of the corticosteroid classes with short-term use in connection with patients with bacterial meningitis, tuberculous meningitis, tuberculous pericarditis, severe typhoid fever, tetanus, or pneumocystis pneumonia with moderate to severe hypoxemia, treatment with corticosteroids improved patient survival **Buchman, 2001.**

2.5.1.1. Prednisolone Mechanism:

Prednisone reduces inflammation via repression of the migration of polymorphonuclear leukocytes and reversing increased capillary permeability. It also suppresses the vulnerable system by reducing the exertion and the volume of the vulnerable system. After attachment of cell face receptor and enters the cell, prednisone enters the nexus, binds, and activates specific nuclear receptors, performing in altered gene expression and inhibition of proinflammatory cytokine product. This agent decreases the number of circulating lymphocytes, converting cell isolation, and stimulating apoptosis in sensitive excrescence cell populations **Puckett et al., 2023**.

2.5.1.2. Prednisolone Dosage:

According to Mayo Clinic, the dose of prednisolone is different for different patients. Generally, the number of medicines a patients take depends on the strength of the medicine. Also, the number of doses patients take each day, the

time between doses and the period patients take the medicine depends on the medical problem for which patients using the medicine. Prednisolone comes in oral dosage form (solution, suspension, syrup and tables), for adults, the doses begin from 5 to 60 mg/day and as for children, the dose based on body weight usually 0.14 to 2mg per kilogram (kg) of body weight /day and must be determined and adjusted by the doctor **Mayo Clinic, 2023.**

2.5.1.3. Prednisolone Side Effect:

Consumed medicines exert health benefits yet have side effects, which called also adverse reactions, meaning that the effects are unwanted or undesirable that are related to a drug. Side effects can start from a small problem like a runny nose to big problems which life-threatening like heart attacks or liver damage. Factors can affect who is most likely to have a side effect when consuming medicines such as age, another drug use, vitamins, dietary supplements or other diseases that weaken the immune system or effect kidneys and liver functions **FDA, 2022**. Prednisolone general mild side effects include cutaneous effects, electrolyte abnormalities, hypertension, hyperglycemia, pancreatitis, and hematologic, immunologic, and neuropsychologic effects were noticed. However, long-term corticosteroid use may lead to more serious consequences including osteoporosis, aseptic joint necrosis, adrenal insufficiency, gastrointestinal, hepatic, and ophthalmologic effects, hyperlipidemia, growth suppression, and possible congenital malformations **Buchman, 2001.**

2.5.1.4. Previous Studies of Prednisolone Side Effects:

Studies have shown that glucocorticoids were associated with multiple side effects, glucocorticoid drug are often related with the appearance of hyperglycemia as mentioned by **Suh & Park, 2017.** As effectively incredible

in restraining inflammation, prednisolone sometimes can also raise (LDL) levels and reduce (HDL) levels as stated by **Jennifer, 2023**. A study made by **Quinkler et al., 2017** showed that patients with adrenal insufficiency (AI) indicated a significantly higher total cholesterol and LDL levels in patients receiving prednisolone (3 -6 mg/day) in the comparison of those who received hydrocortisone (15-30 mg/day). Moreover, a study by **Tiwari et al., 2024** examined the long-term effects of corticosteroid treatment (deflazacort and methylprednisolone) on oxidative stress markers in patients with arthritis. Resulted in an increase in (MDA) levels with a decrease in antioxidant enzymes (GSH, SOD, and CAT). Suggesting that corticosteroid usage may promote oxidative stress and lipid peroxidation. Glucocorticoid therapy's most important cardiovascular side effects are dyslipidemia, hyperglycemia, and hypertension; yet there is a lack of understanding of the mechanistic. Increased levels of VLDL, TG, and LDL cholesterol, as well as either increased or decreased HDL cholesterol in human lipid profiles on various prednisolone dosages, are documented variations **Ross and Marais, 2014.**

The great health benefits of prednisolone as a drug against several diseases in the recent decade gave its importance. Although, side effects of long-term use shouldn't be ignored. The present study evaluates the role of prednisolone consumption side effect in lipid peroxidation and antioxidants defense system in male rats.

2.6. Antioxidants:

2.6.1. Antioxidants:

Oxygenic photosynthesis by ancient cyanobacteria, enabled the development of aerobic respiration, which paved the way for the evolution of complex

eukaryotic organisms. Molecular oxygen became central to aerobic metabolism in all living aerobic species. However, some derivatives of oxygen known as reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive oxygen intermediates (ROI) are highly toxic to cells due to their reactive nature **Nonell and Flors, 2016**.

(ROS) are those contain reactive oxygen ions and peroxides, which in high concentrations lead to a harmful effect on biomolecules like DNA, RNA, protein and fats, causing pathological cases to humans. In the regular circumstances ROS are produced as necessary intermediates and participate as a secondary messenger in cell signaling in picomolar concentrations. When excessive ROS production and uncontrolled regulated lead to hurtful effects. Singlet oxygen is the most reactive and all other reactive oxygen can be formed from singlet oxygen which includes electron transfer procedures in single step. A superoxide radical is the reduction of one electron of the singlet oxygen whereas hydrogen peroxide, another mischievous ROS is one electron reduction of the superoxide radical. Reactive oxygen species are basically produced in organelles such as mitochondria, peroxisomes and endoplasmic reticulum and mainly through mitochondrial respiratory complex over high rates of ATP (Adenosine Tri Phosphate) production, fatty acids oxidation and detoxification of xenobiotic processes respectively. The balance in ROS production and scavenging by antioxidants rules a health system, while unbalanced ROS led to the oxidative stress **Sarangarajan et al., 2017**.

Trillions of body cells are facing massive threats, starting from the lack of food to virus infection. Another continual threat comes from a chemical called free radicals, at the high levels of the free radical it can damage cells and genetic materials. The body produces free radicals as a byproduct to convert food to energy, it also forms after cigarette smoke, air pollution, sunlight, and

exercise. Antioxidant is a molecule stable enough to donate an electron to neutralize a rambunctious free radical, thus reducing the capability to damage. Antioxidants help prevent or reduce cell damage primarily through their ability to scavenge free radicals. Some antioxidants such as glutathione, ubiquinol, and uric acid are produced by natural metabolism in the body. Major antioxidants like vitamin E (α-tocopherol), vitamin C (ascorbic acid), and β-carotene are principal micronutrient (vitamins) antioxidants, body cannot create it, so they must be supplied in the diet **Lobo et al., 2010**.

2.6.2. Antioxidant Classifications:

2.6.2.1. Antioxidant Classifications in respect of Activity:

Antioxidants are of two types: endogenous, which are produced by the human body, and exogenous, which can be found in the diet from natural sources like fruit, vegetables, fish sources, and meat or in synthetic supplements **Panova and Tatikolov, 2023**. Endogenous enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx) act as a first line of defense **Mirończuk-Chodakowska et al., 2018**. Endogenous non-enzymatic antioxidants include glutathione (GSH), uric acid, melatonin, and bilirubin **Moussa et al., 2019**. Exogenous antioxidants are compounds found naturally and synthetically, and they are enormous assortments including vitamin C, carotenoids which are converted to retinoids (vitamin A), polyphenols, anthocyanins, and sulforaphanes **Korczowska-Łącka et al., 2023**.

2.6.2.1.1. Endogenous Antioxidants:

2.6.2.1.1.1. Superoxide Dismutase (SOD):

Superoxide dismutase (SOD) is an antioxidant enzyme that is present widely in animals, plants, and microorganisms, and it contains copper, manganese,

zinc, and other metal ions. SOD acts as an antioxidant through oxidation/reduction cycles, scavenging oxygen radicals at a high reaction rate that catalyzes superoxide radical ($O_2^{\bullet-}$) into hydrogen peroxide (H_2O_2) and releases an oxygen molecule, which requires cofactors like iron, copper, manganese, and zinc to extend the maximum enzyme activity when metabolizing toxic intermediates. The protection of DNA and cell membranes from the damage caused by oxygen free radicals through SOD participation in the disproportionation of superoxide free radicals intracellularly and extracellularly, also SOD balances biological oxidants' concentration, resulting in protecting all aerobic organisms from the damage caused by biological oxidants **Zheng et al., 2023).**

2.6.2.1.1.2. Catalase (CAT):

Catalase is an enzyme found in all living creatures that are exposed to oxygen. Catalase is a very important enzyme that contributes to many physiological functions, affects several metabolic processes, and plays a role in protecting cells from the damage caused by ROS. Catalase neutralizes H_2O_2 into H_2O and an oxygen molecule, O_2. H_2O_2 is a byproduct of different cellular processes, whose toxic effect is related to their accumulation causing oxidative stress. Catalase helps in maintaining the balance side-by-side with other antioxidant enzymes, protecting the body from oxidative damage that causes various diseases such as aging, inflammation, cancer, neurodegenerative disorders, and cardiovascular diseases. It acts as the defense enzyme against the harmful effect of ROS and influences the cellular response to signals and environmental stresses by affecting redox-sensitive signaling molecules such as protein kinases and transcription factors **Rasheed, 2024**.

2.6.2.1.1.3. Glutathione Reduced (GSH):

Glutathione reduced (GSH) is a tripeptide of the three amino acids glutamine, cysteine and glycine, and it's water-soluble. GSH is an important antioxidant that plays a role in the detoxification of peroxidase and electrophilic compounds and other cellular reactions **Townsend et al., 2003**. GSH is important to protect the cells from oxidative damage and counts as a defense line key role. GSH maintains redox homeostasis, and good ROS levels don't just require GSH enhancement to maintain redox status, but they also require increased energy and substance to replace the consumed GSH. The antioxidant function of GSH is described as it is binding or interacting directly with ROS, RNS, and other reactive species or working as a source of reductive power for some antioxidant systems and participating in detoxifying products derived from lipid peroxidation. Moreover, GSH is a co-substrate of glutathione peroxidase (GPx) to reduce hydrogen peroxide (H2O2) and organic peroxides (LOOH - lipid peroxides) **Basil, 2023**.

2.6.2.1.1.4. Glutathione Peroxidase (GPx):

Glutathione peroxidase (GPx) belongs to the significant family of endogenous antioxidant enzymes in mammals. GPx converts hydrogen peroxide (H_2O_2) into water (H_2O) as a part of its mechanism with SOD and CAT cooperation and other antioxidants, which, in their presence as a catalytic component, can help with the speed of the reaction. Moreover, GPx reduces and limits ROS toxicity through enzymatic antioxidant system formation with SOD and CAT. GPx plays a role in vital functions in the body by reducing harmful peroxides such as hydrogen peroxide, cholesterol peroxide, and long-chain fatty acid peroxide. GPx requires GSH to be active and uses GSH as a reducing agent **Wibawa et al., 2025.**

2.6.2.1.1.5. Glutathione Reductase (GR):

Glutathione reductase (GR) is an antioxidant enzyme that has the same importance; it plays a crucial role in GSH metabolism. GR transfers (GSSG) the disulfide form into (GSH) the sulfhydryl form in a mechanism dependent on NADPH. The GR function is to maintain reduced glutathione high in the cellular concentration and GSSG, the oxidized form, low **Patlevič et al., 2016.**

2.6.2.1.2. Exogenous Antioxidants:

2.6.2.1.2.1. Plant Antioxidants:

Plant antioxidants are a natural reservoir of bioactive compounds and play an important beneficial role in human health. As a phytochemical, the antioxidant content of nutrients is important for health improvement. Over the last decades, research has been increased on natural antioxidants, considering the plants' high nutritional value, richness in nutrients, vitamins, minerals, and polyphenols, to approach industrial products, such as functional foods, nutraceuticals, dietary supplements, and cosmetics, which connect antioxidants between nutrition and medicine **Maury et al., 2020**.

2.6.2.1.2.2. Vitamin (C, A, and E):

Complex chemical substances found in food, vitamins play a crucial function in a healthy metabolism, and deficiencies may result in illnesses. Because they are organic, vitamins differ from other nutrients. They are divided into two classes depending on their chemical makeup and functions: fat-soluble vitamins and water-soluble vitamins. Vitamin A is a fat-soluble vitamin derived from both plants and animals. It is known as provitamin A carotenoids in plant sources and as retinoid in animal sources. It plays a part in antioxidant activity, cell growth and development, and vision. In addition to being fat-soluble, vitamin E exists in four classes of tocopherols and tocotrienols: alpha, beta, gamma, and delta. This results in eight naturally occurring forms of

vitamin E referred to as "tocochromanols," which protect bodily structures from oxidative damage. Water-soluble vitamin C, often known as ascorbic acid, is a common dietary supplement. Because of its antioxidant properties, vitamin C shields cells from the damaging effects of free radicals **Olorunnisola et al., 2019.** Free radicals can develop CVD and cancer through lipid peroxidation and DNA damage. Antioxidant vitamins contribute to protecting the body against these effects by neutralizing agitated ROS and free radicals, thereby reducing LDL-C oxidation, thrombotic potential, platelet activity, and vascular reactivity. This contributes to the prevention of atherosclerotic plaque formation **Hamishehkar et al., 2016**. Due to their abundance in a wide variety of fruits, vegetables, and oils, these vitamins are referred to as plant-based antioxidants, although they are available in animal sources.

2.6.2.1.2.3. Flavonoids:

Flavonoids are a wide group of phytochemicals, and in polyphenol classification they present a major group. Various types are natural antioxidants, mostly phenolics, such as flavonoids, coumarins, carotenoids, and cinnamic derivatives. Different parts of plant sources containing flavonoids possess outstanding antioxidant potential; their antioxidant potentials are present through scavenging free radicals and ROS, preventing LDL oxidation, and chelating metals **Hassanpour and Doroudi, 2023**. Flavonoids are abundant in the plant kingdom and found in a wide variety of plant sources, such as fruits, vegetables, tea, cocoa, and wine. Flavonoids are classified into different families, such as flavones, isoflavones, flavonols, flavanones, and anthocyanidins. These classifications are not only a part of flavonoids but also have special main sources like flavonols in onions and flavanols in tea **Panche et al., 2016**.

2.6.2.1.2.4. Polyphenols:

A lot of studies mentioned the role of medical plants as an antioxidant substitute of manufactured antioxidants, medicines, and chemical treatments. Curing with plants and medical herbs came in a big place in medical science and pharmacology, making it a safe source of medicine industry. Phenolic compounds count one of the top antioxidants to break the oxidative reaction chains that make it participate in first line of defense against free radicals **Cardoso and Fassio et al., 2019**.

Polyphenols are natural compounds that are found in food, including fruits, vegetables, cereals, and beverages. 200-300 mg per 100 mg of fresh weight polyphenol content can be found in apples, pears, grapes, cherries, and berries; manufactured products of these fruits contain a high percentage of it. In the last decade, there has been a a lot of focus on the possible health benefits of dietary polyphenols as antioxidants. Polyphenols can also help with food by bitterness, astringency, color, flavor, odor, and oxidative stability. Plant polyphenols have a plethora of health benefits in the diet. Cancer, cardiovascular disease, diabetes, osteoporosis, and neurological diseases can be prevented by the long-term consumption of high plant polyphenol diets **Rahman et al., 2021.**

2.6.2.2. Antioxidant Classification by Solubility and Mechanism:

Antioxidants can be classified based on their solubility into hydrophilic (water-soluble) or hydrophobic (liposoluble). In general, the hydrophilic antioxidants are active in the cytosol or cytoplasm where they neutralize ROS in body fluids like blood serum, extracellular fluid, and seminal plasma; the liposoluble ones, on the contrary, are found in cell membranes and are more

likely to protect cell membranes from lipid peroxidation caused by ROS mediation. They act in various mechanisms, including donating a hydrogen and donating an electron, inhibiting enzymes, decomposing a peroxide, and being a factor in chelating metal **Albano et al., 2022**.

2.6.3. Impact of Antioxidants on Oxidative stress:

Nutritional antioxidants can adjust oxidative stress on several levels: by reducing the production of ROS and fixing the oxidized membranes, neutralizing the free radicals, or through fat metabolism in which cholesteryl esters and short-chain free fatty acids neutralize ROS. Fruits, vegetables, beverages, green tea, cereal products, coffee, spices and nuts are main sources of plant-derived antioxidants: polyphenols (phenolic acids, flavonoids, anthocyanins, lignans, and stilbenes), carotenoids (xanthophylls and carotenes), and vitamins (vitamins C and E, **Petrovic et al., 2020**.
Even though the endogenous antioxidants protect the cells from ROS, alone they are not enough to scavenge free radicals that are generated in the body. However, fruits and vegetables contain a large content of dietary antioxidants. Dates in general showed a comparable antioxidant activity to that found in vitamin C, vitamin E, and β-carotene. Moreover, the total phenolics in dates are found to be in higher concentration among all dried fruits, which is related to sun drying and high temperature. The polyphenolic compounds scavenge ROS and enhance the endogenous antioxidant enzymes that work as a defense system and also regulate key antioxidant enzymes, such as SOD, GPx, and CAT, which help neutralize free radicals, thereby reducing oxidative damage at the cellular level **Al-Alawi et al., 2017.**

2.6.4. Previous Studies on the Role of Antioxidants in Health:

Numerous studies have investigated the effects of dietary and natural antioxidants on chronic diseases by preventing or managing them through their anti-inflammatory and oxidative stress-modifying properties.

A nested case-control study made by **Naziri et al., 2024**. The study included 156 individuals with first-time MI and 312 matched healthy controls aged 35–70 years; data were derived from the Fasa Adults Cohort Study (FACS) in Iran. The results showed that there is an inverse relationship between dietary antioxidant index and the risk of myocardial infarction, which means that increased dietary antioxidant consumption related to the reduction of myocardial infarction risk, indicating the importance of antioxidant-rich diets in protecting against cardiovascular diseases.

Roşian et al., 2025, conducted a review study about bioactive compounds as antioxidants and anti-inflammatory effects in atherosclerosis. The study explores how various antioxidant compounds reduce the risk of atherosclerosis. Polyphenols can enhance endogenous antioxidant defenses and reduce the production of reactive oxygen species (ROS), flavonoids can scavenge free radicals and modulate inflammatory pathways, contributing to vascular health; carotenoids show antioxidant and anti-inflammatory properties, potentially reducing the risk and progression of atherosclerosis; and curcumin has been shown to inhibit pro-inflammatory mediators and scavenge ROS, thereby reducing oxidative stress and inflammation. These compounds work in different ways, including inhibiting ROS production, suppressing nuclear factor-κB (NF-κB), modulating inflammatory cytokines, enhancing endogenous antioxidant enzymes, and modulating gut microbiota.

The study demonstrates that these compounds possess a potential role in preventing and managing atherosclerosis through their antioxidant properties.

A double-blind placebo study done by **Salehi et al., 2021**, to evaluate the effect of curcumin supplementation engaged 80 healthy females and physically active aged 20-30 years with no history of illness. The participants divided into two groups and consumed 500 mg/day encapsulated curcumin or 500 mg/day encapsulated cornstarch as a placebo for 8 weeks. The results showed that oral administration of curcumin 500 mg/day for 8 weeks was tolerated and reduced levels of MDA giving a positive reduction in oxidative stress and improve the muscle damage and inflammation induced by exercises.

In addition, a study conducted by **Senaphan et al., 2015** to study the effect of ferulic acid in high-carb, high-fat diet in rats. Male Sprague Dawley divided into a control group, the second group was fed a high-carb, high-fat diet (HCHF) with 15% fructose in drinking water for 16 weeks to induce metabolic syndrome, third group were induced with metabolic syndrome and treated with orally 30 mg/kg or 60 mg/kg of ferulic acid at the final 6 weeks. The results showed that the ferulic acid administration significantly reduced high blood pressure, insulin resistance, elevated glucose, and triglyceride levels with better results showed in the 60 mg/kg of ferulic acid. Moreover, ferulic acid enhanced arterial responses, lowered wall thickness and prevent vascular remodeling in mesenteric vessels. In addition, Ferulic acid administration prevented the production of superoxide, MDA levels related to HCHF-diet which means reducing the oxidative stress and inflammation.

Grabež et al., 2022, made a randomized, double-blind, placebo-controlled study to evaluate the effect of pomegranate peel extract on inflammation and

oxidative stress in T2DM patients. The study involved 60 patients, 40–65 years old, who were divided into two groups. The first group received capsules containing 250 mg of pomegranate peel extract twice a day, and the placebo group received visually identical capsules containing a placebo of 250 mg twice a day for 8 weeks. The results showed a reduction in glycosylated hemoglobin (HbA1C), a reduction in inflammatory markers and oxidative stress markers, and an increase in total antioxidant capacity (TAC) levels. Moreover, lipid profiles were enhanced, and that was represented in a reduction of total cholesterol, triglycerides, and LDL-C with elevated HDL-C. These results support the therapeutic potential of dietary polyphenols as antioxidants against inflammation, oxidative stress, HbA1C, and lipid profile.

These previous studies have demonstrated the health benefits of plant antioxidants in protecting against oxidative stress and lipid peroxidation and enhancing dyslipidemia, insulin resistance, and inflammation, all of which contribute to the prevention and management of various chronic diseases. Therefore, Ajwa dates and their rich content of bioactive antioxidants are associated with their health benefits. The current study aims to evaluate the protective effect of Ajwa date phenolic extract against lipid peroxidation and antioxidant disorders induced by prednisolone in rats. By looking at the antioxidant markers SOD, CAT, GPx, GG, and GSH, as well as the lipid peroxidation marker MDA, the current study aims to assess whether Ajwa date extract can restore antioxidant activity and protect cells from damage caused by oxidative stress through different dosages of Ajwa date extract to determine the most effective dose among the selected doses.

Chapter 3
Materials and Methods

3. Materials and Methods:

3.1 Materials:

3.1.1 Ajwa Dates:

Ajwa Dates were obtained from Al-Madinah Al-Munawara Farms (10 kg) during harvesting season 2023.

3.1.2 Prednisolone:

Prednisolone medicine was purchased from Adel Pharmacy in Buraydah.

3.1.3 Chemicals and Diagnostic Kits:

Chemicals were purchased from Bio Diagnostic Co. (Egypt). As for diagnostic kits.

3.1.4 Rats:

Thirty male Albino Wistar (120-150 g) were obtained from animal house in College of Pharmacy King Saud University.

3.1.5 Basal Diet:

standard commercial rat chow was from (General Organization for Grain Silos and Flour Mills – Riyadh).

3.2 Methods:

3.2.1. Preparation of Ajwa Date Extract:

Ajwa dates (10kg) obtained from a farm in Al Aliyah village in Medinah. Date fruit of the same size, color and stage of maturity, weighting 6-8 grams had been selected. Date fruit washed well with water; pulp separated manually from the seeds. The collected pulp (5kg) is then dried in an oven at 60°C for

two days. The dried pulp is then ground until homogeneous using an automatic grinder. After that, the powder was kept in brown polyethylene at 25°C until using it in biochemical analysis and preparing the extract as described by **Al-Faris and Lee, 2008**.

3.2.2. Extract Compounds from Ajwa Date Pulp:

Date pulp extract was prepared according to the method described by **Al-Farsi and Lee, 2008.**

Briefly, 2 grams of ground date pulp was extracted with 100 ml (1:50 w/v) of solvent (double distilled water, 50% ethanol) at 45°C for 1 hour with continuous stirring. The extracts were then filtered through Whatman No. 4 filter paper and centrifuged at 4000 × g for 10 minutes. The residue was then re-extracted twice, and the extracts were combined. The solvents were then removed from the combined extracts using a rotary evaporator under reduced pressure at 60°C. The extracts were then frozen and stored in a black bottle at -80°C until use in the rat feeding experiment.

Figure 1: Preparation of Ajwa Date Extract.

3.2.3 Chemical Analysis of Ajwa dates and Ajwa Date Extract:

3.2.3.1. Mineral element determination of date and extract samples:

Mineral element determination was performed according to the **AOAC, 1990** method. 1 gram of dried samples was placed in a Chinese combustion crucible and placed in a muffle furnace at 550°C for 5 hours, until all organic substances were decomposed. In the ash sample crucible, the resulting ash was dissolved by adding 10 ml of a solution consisting of 5 ml of hydrochloric acid (HCl) and 5 ml of distilled water. The solution was then heated, and the process of adding water and hydrochloric acid 1:1 (v/v) was repeated until the samples were pure and clean (ash-free). The samples were then left to cool and filtered thoroughly using ashless paper, which is considered ash-free. The paper was then washed with a 9:1 (v/v) diluted hydrochloric acid solution,

which was diluted to 50 ml in a volumetric flask and stored until ready for use. Quantitative analysis of samples to identify minerals was performed using an Atomic Absorption Spectrophotometer.

3.2.3.2. Analysis of Secondary Compounds in Dates and Date Extract Using High-Performance Liquid Chromatography (HPLC):

Phenols, flavonoids, sugars, were analyzed using high-performance liquid chromatography (HPLC) according to **Tsao et al., 2003** and **AOAC, 2005**. HPLC analysis was performed using an Agilent 1260 series. Separation was performed using a ZORBAX SB-C8 (4.6 mm × 150 mm inner diameter, 5 μm).

3.2.4. Experimental design:

3.2.4.1. Rat Diet and Solutions Preparation:

1. Basal Diet, a standard commercial rat chow was from (General Organization for Grain Silos and Flour Mills – Riyadh). Table (3) shows the composition of the standard provender.

Table3 : The composition of the standard provender is approximate to the provender that is based on Reeves et al., 1993:

Nutritional Components	Quantity
Crude Protein	20.00%
Crude Fat	4.00%
Crude Fiber	3.50%
Ash	6.00%
Salt	0.50%
Calcium	1.00%
Phoshphorus	0.60%
Vitamin A	20.00 IU/g
Vitamin D	2.20 IU/g
Vitamin E	70.00 IU/kg
Energy	2850 kcal/kg
Trace Elements Added	Cobalt, Copper, Manganese, Selenium and Zinc

2. Preparation for Ajwa date extract by dissolving 3 g of Ajwa extract with 30 ml of distilled water, turn it into a solution administrated orally in (30, 60, 90, and 120 mg/kg).

3. preparation of prednisolone by dissolving one tablet (25 mg) with 10 ml of distilled water, each ml contains 2.5 mg/ml, with mean rats' weight 222.32 g. Dividing 2.2232\2.5 equals the final dose of 0.88928 mg/kg.

3.2.4.2. Experimental Animals:

Thirty male Wister albino rats weighted about (120 - 170 g) had been purchased from the animal house in the college of pharmacy King Saud University. Rats were housed in an acrylic cage in a room with a temperature (23 ± 2 Celsius), a humidity about (40-45 %) and 12 hours of a light-dark cycle. Each cage has 6 rats, each cage contains sawdust as bedding. The rats were in an adaptive laboratory environment for one week. A standard commercial rat chow was from (General Organization for Grain Silos and Flour Mills – Riyadh) Food and water were given continually to the rats with complete freedom for the rats to eat and drink. The Qassim University Ethics Committee authorized the experiment protocol. The research was conducted in compliance with the standards for animal testing set forth by Qassim University College of Agriculture and Veterinary Medicine.

Figure 2: Experimental Groups.

3.2.4.3. Experimental Groups:

After one week of the adaptation, rats were weighted and then divided into 6 groups: 1-2-3-4-5-6. As group one will be the negative control diet, the second group will be given Prednisolone dose (0.88 mg/kg Body Weight) **(Mayo Clinic, 2023)**, as a positive control diet, and the groups 3-4-5-6 will be given phenol extract (30, 60, 90 and 120 mg/kg Bwt) respectively **(Alqarni et al., 2019)** for 7-8 consecutive days. After that, the phenol extract dosage and the prednisolone dosage will be given to each group as described. then evaluating the protective effect from damage or increased blood lipids caused by the Prednisolone dose (0.88 mg\kg Bwt) **(Mayo Clinic, 2023)**, then the groups had been divided as follows:

The first group = negative control, basal diet.

The second group = Positive Control (Prednisolone) (0.88 mg/kg Bwt) **(Mayo Clinic, 2023)**.

The third group = Prednisolone (0.88 mg/kg Bwt) **(Mayo Clinic, 2023)** + 30 mg/kg Bwt phenols extract **(Alqarni et al., 2019)**.

Fourth group = Prednisolone (0.88 mg/kg Bwt) **(Mayo Clinic, 2023)** + 60 mg/kg Bwt of phenols extract **(Alqarni et al., 2019)**.

Fifth group: Prednisolone (0.88 mg/kg Bwt) **(Mayo Clinic, 2023)** + 90 mg/kg Bwt phenols extract **(Alqarni et al., 2019)**.

Sixth group = Prednisolone (0.88 mg/kg Bwt) **(Mayo Clinic, 2023)** +120 mg/kg Bwt of phenols extract **(Alqarni et al., 2019)**.

Dates phenolic extract is added in different concentrations (30, 60, 90 and 120 mg/kg Bwt) to sodium chloride solution (9 g/kg) and giving daily oral to the rats.

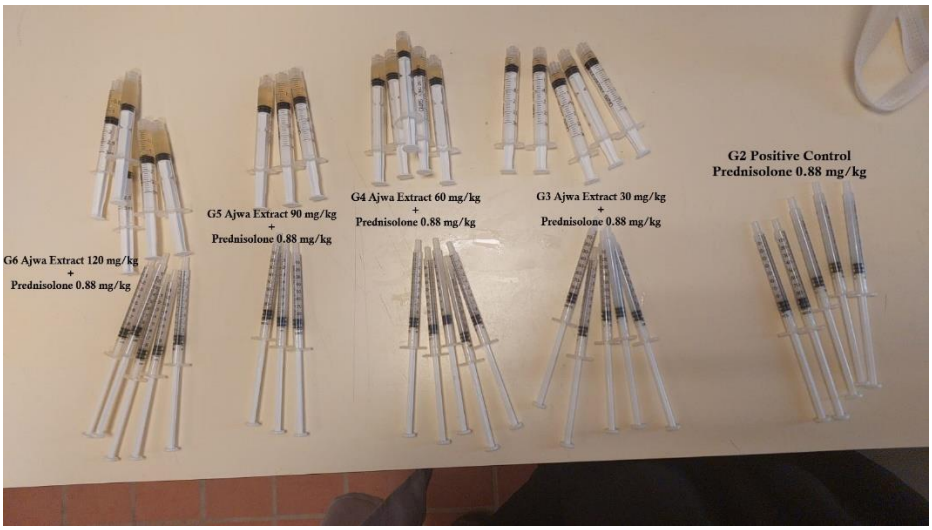


Figure 3: The Dosages of Ajwa Extract (Above) and Prednisolone (Under).

Figure 4: Administrating Doses Orally in Rats.

3.2.5. Blood Sample Collection:

At the end of the experimental period (28 days), the rats were fasted for 10-12 hours, then slaughtered under anesthesia using diethyl ether, which is a way to ensure less harm to the rats. Blood samples were collected in red tubes and then centrifuged at 4000× g for 15 minutes under cooling to separate the serum. The serum was then collected in clean Eppendorf tubes and stored at -20°C until biochemical analysis. The rats had been dissected, and livers of experimental rats from different groups were removed, washed with 0.9% saline solution, and dried. The livers were placed in plastic bags, tightly wrapped in aluminum foil, and put in a deep freezer at -20°C. Some of the liver tissues were placed in a 10% formalin solution until used in histological examination according to the method of **Humason, 1979**. The rats and their remaining parts had been disposed of by transporting them in plastic bags to the veterinary hospital in Buraydah and burning them in the incinerator.

Figure 5: Collected Blood Samples in Red Tubes.

3.2.6. Biochemical Analysis:

Blood serum taken from experimental rats was used to measure various biochemical estimations using chemical reagent kits from Biodiagnostic (Diagnostic and Research Reagents, Egypt) and a Genway 6305 UV-Vis spectrometer.

3.2.6.1 Glucose Level:

Glucose was measured according to **Trinder, 1969**. Glucose, in the presence of glucose oxidase, is oxidized to gluconic acid and hydrogen peroxide .

$$\text{Glucose} \xrightarrow{\text{Glucose oxidase}} H_2O_2 + \text{Gluconic acid}$$

The hydrogen peroxide then is oxidatively coupled with 4-amino-antipyrine and a phenol derivative like p-hydroxybenzenesulfonate in the presence of peroxidase to form a stable red quinoneimine dye. The quinoneimine dye has an absorption maximum at 510 nm. The intensity of the color formed is proportional to the glucose concentration in the sample .

$$2H_2O_2 + \text{phenol} + 4 - \text{amino} - \text{antipyrine} \xrightarrow{\text{peroxidase}} \text{Coloredquinonemine} + 4H_2O$$

Three tubes were used during the determination of glucose: the blank tube, the standard tube, and the sample tube. The procedures were as follows:

1- The blank tube was added by 1.0 ml of working reagent (a mixture of the same amounts of R2: phenol and R3: phosphate buffer, glucose oxidase, peroxidase, and 4-amino antipyrine).

2- The standard tube was added by 0.01 ml of R1 (reagent) and 1.0 ml of working reagent.

3- The sample tube was added by 0.01 ml of serum and 1.0 ml of working reagent.

The tubes were mixed well and incubated for 10 min. at 37°C. The absorbance of the sample (A_{Sample}) and the standard ($A_{Standard}$) were measured against the blank at 510 nm (490 nm - 530 nm). The results were expressed in (mg/dl) and the concentration of the sample was calculated using the following equation:

Concentration of glucose (mg/dl) = $\frac{\Delta A_{Sample}}{\Delta A_{Standard}} \times \text{Standard Conc.}$

$$\frac{\Delta A_{Sample}}{\Delta A_{Standard}} = \frac{A_{Sample} - \text{Blank}}{A_{Standard} - \text{Blank}} \times 100$$

3.2.6.2 Lipid Profiles:

3.2.6.2.1. Total Cholesterol Level (TC):

Cholesterol was determined using the method of **Allain et al., 1974**. The cholesterol is determined after enzymatic hydrolysis and oxidation.

$$\text{cholesterol} - \text{ester} + H_2O \xrightarrow{\text{cholestero esterase}} \text{cholesterol} + \text{fatty acid}$$

$$\text{cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholestene} - 3 - \text{one} + H_2O_2$$

The quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase .

$$2H_2O_2 + \text{phenol} + 4 - \text{Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

Three tubes were used in the cholesterol analysis: a blank tube, a standard tube, and a sample tube, as in the following procedure:

1- In the blank tube, working reagent, which is an equal mixture of R2 (buffer, phenol, and surfactant), and R3 (cholesterol esterase, cholesterol oxidase, peroxidase, and 4-aminoantipyrine) was added by 1.0 ml.

2- The standard tube was added with 0.01 ml of R1 (standard) and 1.0 ml of working reagent.

3- The sample tube was added with 0.01 ml of serum and 1.0 ml of working reagent.

All tubes were mixed well and incubated for 10 min. at 37°C. The absorbance of the sample (A_{Sample}) and the absorbance of the standard ($A_{Standard}$) were measured against the blank at 500 nm (492 nm - 550 nm). Results were expressed in (mg/dl) following the equation below:

$$\text{TC concentration (mg/dl)} = \frac{\Delta A_{Sample}}{\Delta A_{Standard}} \times \text{standard conc.}$$

$$= \frac{A_{Sample} - \text{Blank}}{A_{Standard} - \text{Blank}} \times 200$$

3.2.6.2.2. Triglycerides Level (TG):

Triglycerides were measured based on the method of **Fossati and Prencipe, 1982**. Triglycerides are broken down into glycerol and fatty acids through the action of lipoprotein lipase.

$$\text{triglycerides} \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{fatty acid}$$

Glycerol is then converted into glycerol-3-phosphate by glycerol kinase in the presence of ATP.

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerokinase}} \text{Glycerol} - 3 - \text{phosphate} + \text{ADP}$$

The resulting glycerol-3-phosphate is oxidized by glycerol-3-phosphate oxidase, simultaneously producing hydrogen peroxide .

$$\text{Glycerol} - 3 - \text{phosphat} \xrightarrow[\text{oxidase}]{\text{Glycerol}-3-\text{phosphate}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

The resulting hydrogen peroxide induces oxidative condensation of 4-aminoantipyrine and DAOS through the action of peroxidase, producing a blue dye, and its absorbance is measured to determine the concentration of triglycerides in the sample.

$$2H_2O_2 + 4 - \text{chlorophenol} + 4 - \text{Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O + HCL$$

Three tubes were used in triglycerides analyses: a blank tube, a standard tube, and a sample tube. The procedure was as follows:

1- The blank tube was added with 1.0 ml of working reagent, which was an equal mixture of R2 (buffer and 4-chlorophenol) and R3 (lipase, glycerokinase, glycerol-3-phosphate oxidase, peroxidase, 4-aminantipyrine, and ATP).

2- The standard tube was added with 0.02 ml of R1 (standard) and 1.0 ml of working reagent.

3- The sample tube was added with 0.02 ml of serum and 1.0 ml of working reagent.

The tubes were mixed well and incubated for 10 min. at 37°C. The absorbance of the sample (A_{Sample}) and the standard ($A_{Standard}$) were measured against

the blank at 505 nm (429 nm - 550 nm). The results were expressed in (mg/dl) based on the following equation.

TG concentration (mg/dl) = $\frac{\Delta A_{Sample}}{\Delta A_{Standard}} \times$ Standard Conc.

$$= \frac{A_{Sample} - Blank}{A_{Standard} - Blank} \times 200$$

3.2.6.2.3. High-density lipoprotein cholesterol level (HDL-c)

HDL-C was determined according to **Lopez-Virella et a., 1977** method. Phosphotungstic acid and magnesium ions selectively precipitate lipoproteins except the HDL fraction—cholesterol present in the supernatant can be determined by the same method used for total cholesterol. The process began by adding 0.20 ml of the serum in a tube, then 0.02 ml of R1 (precipitating reagent), the mixture was vortexed and let stand for 10 min., after that centrifuged for 15 min. at 3000 rpm. The HDL-C in the supernatant was measured using the same way for total cholesterol. Three tubes were used for the determination of HDL-C: a blank tube, a standard tube and a sample tube. The procedure is as follows:

1 -The blank tube was added with 0.05 ml of distilled water and 1.00 ml of working reagent (same reagent in cholesterol assay).

2 -The standard tube was added with 0.05 ml of R2 (same reagent in cholesterol assay) and 1.00 ml of working reagent.

3 -The sample tube was added by 0.05 ml of the supernatant and 1.00 ml of working reagent. Tubes were mixed and incubated at 37°C for 10 min. The absorbance of the sample (A_{Sample}) and the standard ($A_{Standard}$) was read

against the blank at 500 nm (495 nm- 550 nm). The results were expressed in (mg/dl) in the following equation:

$$\text{HDL-c concentration (mg/dl)} = \frac{\Delta A_{Sample}}{\Delta A_{Standard}} \times 55$$

$$\frac{A_{Sample} - Blank}{A_{Standard} - Blank} \times 55$$

3.2.6.2.4. Low-density lipoprotein cholesterol level (LDL-c)

Serum low-density lipoprotein cholesterol level was determined according to the method described by **Friedewald *et al*., 1972**, using the following equation:

$$\text{LDL-c concentration (mg/dl)} = \text{Total Cholesterol} - \text{HDL} - \frac{(\text{Triglycerides})}{5}$$

3.2.6.2.5. Very low-density lipoprotein cholesterol level (VLDL-c)

Serum very-low-density lipoprotein cholesterol level was determined according to the method described by **Friedewald *et al*., 1972**, using the following equation:

$$\text{VLDL-c concentration (mg/dl)} = \frac{(\text{Triglycerides})}{5}$$

3.2.6.2.6. Total Lipid Level:

Total lipids were done according to the method of **Zöllner and Kirsch, 1962**. Lipids react with sulfuric and phosphoric acids and vaniline to form pink colored complex. Two tubes of standard and sample were prepared; the standard tube contained 0.025 ml of R1 (standard 1000 mg/dl) and 1.00 ml of sulfuric acid conc.; the other tube was added 0.025 ml of the serum and 1.00 ml of sulfuric acid conc., all tubes were mixed well, covered with a glass bead, and let stand in a boiling water bath for 10 minutes, cooled down, and pipetted

into dry test tubes. The first tube (the blank tube) contained 0.05 ml of the sulfuric acid conc., and 1.5 ml of color reagent R2 (phosphoric acid and vanillin). The solution of the standard tubes was added into a second clean tube by 0.05 ml, and color reagent R2 was added by 1.5 ml. The solution of the sample was added by 0.05 ml to a third clean tube, and 1.5 ml of R2 was added. All three tubes were mixed and then let stand at room temp. for 30 minutes in the dark. Poured into a cuvettes. The absorbance of the sample (A sample) and standard (A standard) were read against the reagent blank within 30 min. at 545 nm (530 nm - 560 nm), results expressed in (mg/dl) according to the equation:

$$\text{Total Lipids Concentration (mg/dl)} = \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times 1000$$

$$\frac{A_{\text{Sample}} - \text{Blank}}{A_{\text{Standard}} - \text{Blank}} - 1000$$

3.2.6.3. Atherogenic index of Plasma (AIP):

To predict atherosclerosis, atherogenic index of plasma (AIP) was calculated according to the method described by **Niroumand *et al*., 2015** using the following formula:

$$\text{AIP} = \log\left(\frac{\text{Triglycerides}}{\text{HDL-c}}\right)$$

3.2.6.4. Antioxidant Activity:

3.2.6.4.1. Measurement of Total Antioxidant Capacity (TAC):

Total antioxidant capacity was measured in plasma according to **Koracevic et al., 200.** The determination of antioxidant capacity is performed by the reaction of antioxidants in the sample with a defined amount of exogenously

provided hydrogen peroxide (H_2O_2). The antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. The residual H_2O_2 is determined colorimetrically by an enzymatic reaction which involves the conversion of 3,5, Dichloro-2-hydroxy benzensulphonate to a colored product.

Two test tubes were used in the determination of TAC the blank tube and the sample tube, as follows:

1- The blank tube was added by 0.02 ml of distilled water and 0.50 R1 (substrate H_2O).

2- The sample tubes were added by 0.02 ml of serum and 0.50 ml of R1.

Tubes were mixed well and incubated for 10 min. at 37°C. After that, all tubes were added by the same working reagent (a mixture of the same amount of R2 chromogen and R3 enzyme-buffer) by 0.5 ml. Mixed well again and then incubated for 5 min. at 37°C. The absorbance was read immediately of blank (A_B) and sample (A_{SA}) against distilled water at 505 nm (500 nm - 510 nm). The results were expressed in (mM/L) as the following equation:

$$\text{Total Antioxidant Concentration (mM/L)} = A_B - A_{SA} \times 3.33$$

3.2.6.4.2. Measurement of Catalase (CAT) Levels:

Catalase was determined according to the method of **Aebi, 1984**. Catalase reacts with a known amount of H2O2. The reaction is stopped after exactly one minute using a catalase inhibitor.

$$H_2O + O_2 \xrightarrow{Catalase} 2H_2O_2$$

In the presence of peroxidase (HRP), the remaining H_2O_2 reacts with 3,5-dichloro-2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with a color intensity inversely proportional to the amount of catalase in the original sample.

$$H_2O_2 + \text{DHBS} + \text{AAP} \xrightarrow{HRP} \text{Quinoneimine Dye} + 4H_2O$$

Four tubes for the procedure of catalase assay were prepared: a sample blank tube, a sample tube, a standard blank tube, and a standard tube. The reagents were added as follows:

1- Serum of 0.05 ml was added to the sample blank with 0.05 ml distilled water and 0.50 ml R1 (buffer, phosphate buffer pH 7.0, and detergent).

2- Serum was also added to the sample tube by 0.05 ml, with 0.50 ml of R1 and 0.10 ml of R2 (H_2O_2 was diluted 1000 times before use).

3- The standard blank tube contained 0.10 ml of distilled water and 0.50 ml of R1.

4- The standard tube contained 0.05 ml of distilled water, 0.50 of R1, and 0.10 ml of R2.

All tubes were incubated for exactly 1 min. at 25°C, then all the tubes were added the same amount of 0.20 ml of R3 (chromogen- inhibitor) and 0.50 ml of R4 (enzyme: peroxidase, 4-aminoantipyrine, and preservative). All tubes were again incubated for 10 min. at 37°C. The sample tube (A_{sample}) was read against the sample blank, and the standard tube (A_{standard}) was read against

the standard blank at 510 nm (500 nm - 520 nm). The results were expressed in (U/L) by the following equation:

$$\text{Catalase activity (U/L)} = \frac{A_{standard} - A_{sample}}{A_{standard}} \times 1000$$

3.2.6.4.3. Glutathione Reductase (GR) Levels:

Glutathione reductase was measured according to **Goldberg and Spooner, 1983**. Glutathione reductase catalyses the reduction of glutathione (GSSG) in the presence of NADPH, which is oxidized to NADP+. The decrease in absorbance at 340 nm is measured.

$$NADPH + H^+ + GSSG \xrightarrow{GR} NADP^+ + 2GSH$$

Serum was added by 0.050 ml, RG1 containing (buffer, potassium phosphate 100 mmol/L, and EDTA 1 mmol/L) was added by 1.00 ml, R2 (subtrate and GSSG) was added by 0.10 ml, and lastly R3 (NADPH) was added by 0.10 ml. The solution was mixed, and the assay is carried out at 25°C, the initial absorbance was read against the air at 340 nm, the timer started simultaneously. After one minute, another reading was taken. Over a period of 5 minutes, the change was obtained in absorbance per min, (ΔA340 nm/min). The results were expressed in (U/L) according to the equation:

$$\text{Glutathione Reductase Activity (U/L)} = 4019 \times \Delta A\ 340\ \text{nm/min}$$

3.2.6.4.4. Measurement of Reduced Glutathione (GSH):

To determine GSH according to the method of **Beutler et al., 1963**. The method is based on the reduction of 5,5 dithiobis (2-nitrobenzoic acid) (DTNB) with glutathione (GSH) to produce a yellow compound. The reduced chromogen directly proportional to GSH concentration and its absorbance can

be measured at 405 nm. Two tubes were used, the serum tube and the blank tube as follows:

-1 The first tube was added by 0.1 ml of serum, 0.5 ml of distilled water, and 0.5 ml of Reagent 1 (Trichloroacetic acid TCA).

2 -The blank tube was added by 0.5 ml of distilled water and 0.5 ml of reagent.

Tubes were mixed well and allowed to stand for 5 minutes at room temperature and then centrifuged at 3000 rpm for 15 minutes to form a supernate.

3 -In a clean tube for the serum, 0.5 ml of the supernatant from the serum was added with 0.1 ml of each R2 (buffer) and R3 (DTNB).

4 -In a clean tube for the blank, 0.05 ml of supernatant from the blank was added with 0.1 ml of each of R2 and R3.

Tubes were mixed well, the absorbance of the produced yellow color was measured after 5-10 minutes at 405 nm of sample (A_{Sample}) against the blank, and the result was expressed in (mg/dl) according to the equation:

Glutathione concentration $mg/dl = A_{Sample} \times 66.66$

3.2.6.4.5. Measurement of Glutathione Peroxidase (GPx):

(GPx) was measured by **Paglia and Valentine1967** method. By an indirect measuring of the activity of c-GPx. Oxidized glutathione (GSS), produced upon reduction of an organic peroxide by c-GPx, is recycled to its reduced state by the enzyme glutathione reductase (GR):

$$R-O-O-H + 2\,GSH \xrightarrow{c-GPx} R-O-H + GSSG + H_2O$$

$$GSSG + NADPH + H^+ \xrightarrow{GR} 2GSH + NADP^+$$

To assay c-GPx, a serum is added to a solution containing glutathione, glutathione reductase, and NADPH. The enzyme reaction is initiated by adding the substrate, hydrogen peroxide, and the A340 is recorded. The rate of decrease in the A340 is directly proportional to the GPx activity in the sample. The procedure started by adding 1.0 ml of buffer contained (assay buffer PH 7.0, phosphate buffer 50 Mm, and triton X-100 for 0.1%, then RG2 was added by 0.1 ml containing NADPH reagent (lyopholized), glutathione (GSH), glutathione reductase (GR), B-nicotinamide-adenine dinucleotide, and phosphate reduced (NADPH). After that, the serum was added by 0.01 ml, and the hydrogen peroxide H_2O_2 was added by 0.1 ml. The solution was mixed well; the decrease of absorbance was recorded at A340 nm/min (A340/min) over a period of 3 minutes against deionized water. The results were expressed in (mU/mL) according to the equation:

$$\text{Enzyme Activity (mU/mL)} = \frac{A_{340}/\text{min}}{0.00622} \times 121 \times \text{dil. Factor}$$

Dill.Factor = 1 → the serum was added without dilution.

3.2.6.4.6. Measurement of Superoxide Dismutase (SOD) Levels:

The SOD activity was measured based on the method by **Nishikimi et al., 1972**. The assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated of nitro blue tetrazolium.

Two clean tubes, one for control and the other for the serum. Working reagent, which is a mix of R1 (phosphate buffer pH 8.5), R2 (nitroblue tetrazolium NBT), and R3 (NADH) in a ratio of (10 + 1 + 1 ml) was added by 1.0 ml to

each tube. The sample group then gets 1.0 ml of the serum added, while the control tube doesn't; the distilled water was added to the control tube only by 1.0 ml. The tubes were mixed well, the reaction was initiated by adding R4 (phenazine methosulphate PMS), the increase of absorbance was measured at 560 nm for 5 min. for the control ($\Delta A_{control}$) and for the sample (ΔA_{sample}) at 25 degrees Celsius. The results were expressed in (U/ml) by the equation:

The inhibition percentage was calculated first as followed:

$$\text{Percent Inhibition} = \frac{\Delta A_{control} - \Delta A_{sample}}{\Delta A_{control}} \times 100$$

Where:

$\Delta A_{control} =$

The change in absorbance at 560nm over 5min. followed the addition of PMS to the reaction mixture in the absence of sample.

$\Delta A_{sample} =$

The change in absorbance at 560nm over 5min. followed the addition of PMS to the reaction mixture in the presence of the sample.

Then SOD activity was calculated as followed:

$$\text{SOD Activity (U/ml)} = \%\ \text{inhibition} \times 3.75$$

3.2.6.5. Determination of Malondialdehyde (MDA), Lipid Peroxidation Marker:

Lipid peroxide was determined in the form of malondialdehyde (MDA) according to the method described by **Satoh, 1978**. The thiobarbituric acid (TBA) reacts with MDA in an acidic medium at 95 Celsius for 30 minutes to

form thiobarbituric acid reactive product. The absorbance of the resultant pink product is measured at 534 nm. Three tubes were used for the MDA assay: the sample tube, the standard tube, and the blank tube, as follows:

1- In the sample tube, 0.2 ml of the serum was added and 1.0 ml of R2 (chromogen, TBA, detergent, and stabilizer).

2- In the standard tube, 0.2 ml of R1 (standard) was added, and 1.0 ml of R2 was added.

3- The blank tube contains only the R2 by 1.0 ml .

After that, all tubes were mixed well, covered with glass beads, and heated in a boiling water bath for 30 min. The tubes were cooled down, and then in the blank tube only, serum was added by 0.2 ml and mixed. The absorbance of the sample (A_{sample}) was read against the blank, and the absorbance of the standard against distilled water at 534 nm. Results were expressed in (nmol/ml) in the following equation:

$$\text{Malondealdehyde (nmol/ml)} = \frac{\text{A}_{\text{sample}}}{\text{A}_{\text{standard}}} \times 10$$

3.2.7. Homogenates and Supernatants:

3.2.7.1. Liver Lipids Extraction:

Liver lipids extraction was according to **folch, 1957** way, described by **Mopuri et al., 2021**.

1. About 1 gram of the liver was taken and put between two filter papers to dry it for 10 minutes at room temperature 25 °C. After that is cut into small

pieces and placed in a clean-dry glass cans adding 25 ml of chloroform : methanol (1:2) and homogenate by (homogenizer) for 2 minutes and leave it .for 24 hours at room temperature 25 °C

2. The next day, the mixture is centrifuged at 3000 x g for 10 minutes. After the separation, the supernatant is taken and put in a clean-dry bottle and adding 4 ml of 0.9% NaCl, then shaking the mixture using (Vortex). Then put the mixture again in the centrifuge at 2500 x g for 10 minutes. After the separation we got rid of the supernatant. The remaining infranatant is handled by adding twice the 4 ml of methanol 50%.

3. When supernatant and infranatant are formed, the infranatant is harvested (methanol room) which contains the lipids (triglycerides and cholesterol), then evaporated using (Rotary Evaporator), and weight the remaining sediment. As for the supernatant, it's dried at 45°C for two and a half hours to remove any remaining humidity. All lipids are weighted and related to 1 gram of liver's weight after slaughter.

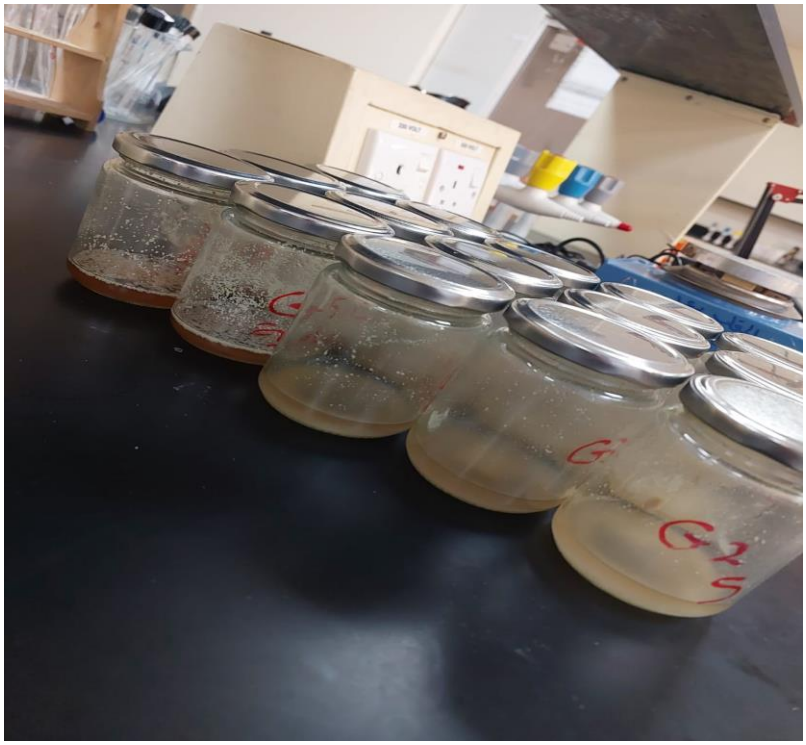


Figure 6: The Process of Liver Lipids Extraction; 1 g of Liver After the Homogenation Using a Homognizer.

3.2.7.1.1. Measurement of hepatic reduced glutathione (GSH):

Hepatic glutathione (GSH) concentration was measured by spectrophotometric based on **Beutler et al., 1963**. Approximately 1 g of liver tissue was perfused with a PBS (phosphate buffered saline) solution, containing 0.16 mg/ml heparin to remove any red blood cells and clots. The tissue then homogenized in 5 ml cold buffer (i.e. 50 mM potassium phosphate, 1 mM EDTA) per gram of tissue, using a tissue homogenizer. centrifuged at 4000 rpm for 15 minutes at 4°C. The supernatant was measured following the same procedure to determine the serum GSH with only a difference in the sample volume (0.1 ml for serum and 0.5 ml of the supernatant); the results were expressed in (mmol/g tissue) following the equation:

$$\text{GSH Concentration (mmol/g.tissue} = \frac{A_{Sample} \times 2.22}{\text{g.tissue used}}$$

The g.tissue used = 0.1

3.2.7.1.2. Measurement of Triglycerides:

Triglycerides were measured based on the method of **Fossati and Prencipe, 1982**.

3.2.7.1.3. Measurement of Total Cholesterol:

Cholesterol was determined using the method of **Allain et al., 1974**.

3.3. Histological Examination of the Liver for Experimental Groups:

The preparation of the histological examination slices of the liver for the experimental rats, which were affected by prednisolone and treated with Ajwa date extract in comparison to the control group, was done according to the method of **Humason, 1979**, as follows:

1 .The stages of preparing tissue slides began with washing the liver samples after their removal and washing them with cold physiological solution (0.9%) sodium chloride, then drying them, and immersing them in a 10% formalin solution to preserve the cells and fix them in their current natural shape.

2. The samples were then subjected to several sequential processes, starting with washing the residual formalin solution, followed by using serial dilutions of ethyl alcohol for dehydration. The samples were then clarified in xylene and immersed in paraffin wax for drying at 55-60°C for 24 hours to make the tissue samples transparent before sectioning using a rotary microtome (Leica) of 5 μm thickness. The small pieces were then collected, attached to glass slides, and stained with special stains such as hematoxylin and eosin, which

stain the cytoplasm pink, red, and blue, for study and microscopic examination after covering them with a suitable transparency and reading them by a person specialized in the field of histopathology.

3.4. Ethical Approval:

Ethical Approval for the study obtained from the local ethics committee at Qassim University Number: 25 – 25 – 11.

3.5. Statistical Analysis:

The study results were analyzed statistically by using the non-parametric ANOVA Kruskal-Wallis Test- Bonferroni multiple comparison- Statistical Descriptive using Mean ± standard deviation (SD). The test revealed a statistically significant difference with a p-value of less than <0.001, **Kruskal and Wallis, 1952.**

Chapter 4
Results and Discussion

4. Results and discussion:

4.1. Results and Discussion of Chromatographic Analysis of Secondary Compounds in Ajwa Dates and Ajwa Date Extract:

4.1.1. Ajwa Dates and Ajwa Date Extract's Content of Antioxidants:

Table 4: Ajwa Dates and Ajwa Date Extract Content of Antioxidants:

No	Compounds Name	Ajwa Dates		Ajwa Extract	
		RT	Area Sum%	RT	Area Sum%
1	4-Phenoxybutyric acid, TMS derivative	8.867	0.11	8.57	0.04
2	4-tert-Octylphenol, TMS derivative	13.514	0.12	13.396	0.22
3	Lactic Acid, 2TMS derivative	14.29	0.47	14.166	0.17
4	Boric acid, 3TMS derivative	ND	ND	14.472	0.16
5	Vanillin, TMS derivative	34.136	0.12	34.286	0.14
6	Cinnamic acid, TMS derivative	34.463	0.08	34.462	0.06
7	4-Coumaric acid, 2TMS	ND	ND	48.576	0.07
8	Isoferulic acid, 2TMS	51.381	0.56	ND	ND
9	Ferulic acid, 2TMS derivative	53.429	0.74	53.403	0.72
10	Sinapinic acid, 2TMS derivative	58.209	0.09	58.203	0.05
11	Glyceryl ferulate, 3TMS	67.308	0.19	ND	ND

Note: RT = retention time (min). ND = not detected

Table (4) Show the various compounds of antioxidants content of Ajwa dates and Ajwa date extract, nine compounds of antioxidants were found during analysis of Ajwa dates and Ajwa date extract, suggesting the high content of

antioxidants which have a health properties. Nutritional antioxidants can adjust oxidative stress on several levels: by reducing the production of ROS and fixing the oxidized membranes, by neutralizing the free radicals, or through fat metabolism in which cholesteryl esters and short-chain free fatty acids neutralize ROS **Pohl et al., 2018, Petrovic et al., 2020**. Fruits, vegetables, beverages, green tea, cereal products, coffee, spices and nuts are main sources of plant-derived antioxidants: polyphenols (phenolic acids, flavonoids, anthocyanins, lignans, and stilbenes), carotenoids (xanthophylls and carotenes), and vitamins (vitamins C and E) **Petrovic et al., 2020.** In table (4) between all antioxidants, ferulic acid was the most plentiful in both Ajwa dates and Ajwa date extract (0.74% and 0.72% respectively). Ferulic acid is one of the phenolic groups and known to be an outstanding antioxidant. It's absorbed easily and stays for much longer time than any other phenolic acids in the body. Ferulic acid as a phenolic acid has a low toxicity and own a lot of physiological impacts, such as anti-inflammatory, anti-cancer, showed an anti-diabetic, anti-microbial and many more functions. Furthermore, a study showed that using ferulic acid ointment in healing wounds had a speed in shrinking them by lipid peroxidation inhibition, and catalase, superoxide dismutase, and glutathione levels increasing **Zduńska et al., 2018**. Vanillin, found in both Ajwa dates and Ajwa date extract, is not only known as an aromatic and flavor compound used in many different food processes and industrial products, but it also has antioxidant health potential. And it's considered that its activity is more useful in daily health care, as a rich diet with antioxidants could reduce free radicals that promote cancer **Bezerra et al., 2018**. Although vanillin is found in lower content, it can contribute with other antioxidant compounds to promote antioxidant capacity. Cinnamic acid was found in a small amount in Ajwa dates (0.08%) and Ajwa date extract

(0.06%), Cinnamic acid and its derivatives are phenolic acid compounds that have antioxidant, anti-inflammatory, anti-cancer, neuroprotective, and antidiabetic properties **Ruwizhi and Aderibigbe, 2020**. The most popular derivatives of cinnamic acid are reported by **Adisakwattana, 2017**, are cinnamic acid, ferulic acid, isoferulic acid which all were reported in table (4) above except for isoferulic acid which was reported only in Ajwa dates. Isoferulic acid was found only in Ajwa dates (0.56%) and was the second most abundant after ferulic acid. Isoferulic acid is a ferulic acid containing trans-cinnamic acid; it plays a role as ametabolite, an antioxidant and a biomarker **PubChem, 2025**. These results of ferulic acid, 2TMS derivative in both Ajwa dates and Ajwa date extract and 4-coumaric acid, 2TMS which was found only in Ajwa date extract are consistent with **Benmeddour, et al., 2013** who determined a variety of phenolic compounds in ten algerian date cultivars which are ferulic acid, gallic acid caffeic acid and p-Coumaric acid. Comaric acid, ferulic acid, and cinnamic acid were also observed in Ajwa dates. In addition, **Aljohani et al., 2025** found that Ajwa date crude extract showed an existence of five phenolic compounds which are coumaric acid, ferulic acid, gallic acid, caffeic acid, and chlorogenic acid. Additionally, total phenolic content (TPC) was measured in previous studies **Arshad et al., 2019** reported that four varieties of dates (Hallawi, Ajwa, Khudravi, and Aseel) contained (TPC) with the highest result found on aseel (291.36 ± 0.04 mg/100 g) followed by (282.65 ± 0.04 mg/100 g) in hallawi, Ajwa dates (252.65 ± 0.05 mg/100 g) with the lowest found on Khudravi (232.64 ± 0.07 mg/100 g). Another study conducted by **Kharal et al., 2023** studied the (TPC) and total flavonoid content (TFC) on Ajwa extraction among different ethanol concentrations (ethanol 0%, ethanol 20%, ethanol 40%, ethanol 60%, ethanol 80%, and absolute ethanol 100%) in Ajwa flesh, Ajwa pits and Ajwa whole

(flesh and pits), determined that (TPC) in whole Ajwa was significantly higher (120 ± 2 g/100 g GAE) in 60% ethanol, wherein pits, the highest was in ethanol 100% with (90±1 g/100 g GAE) and Ajwa flesh was high in ethanol 60% with (56±1 g/100 g GAE). The results in (TFC) also revealed that whole Ajwa had a higher amount of TFC (867±1 mg/g GAE) in ethanol 60%, followed by pit (821±2 mg/g GAE) in ethanol 100%, and flesh (766±2 mg/g GAE) in 0% ethanol. Furthermore, a study on six different varieties of dates in sudan (madini, bittamoda, mishrig, jaw, gondaila, and barakawi) made by **Mohamed et al., 2014** suggested that (TPC) observed in all varieties and was the highest value in madini (199.34 ± 9.51 mg GAE/100 g DW) and the lowest was in barakawi (35.82 ± 5.01 mg GAE/100 g DW). On the other side (TFC) was also observed but lower than (TPC), the highest was with gondaila (3.39 ± 0.09 mg CE/100 g) and the lower value observed in mishrig and madini (1.74 ± 0.00 mg CE/100 g, 1.74 ± 0.04 mg CE/100 g respectively) with no significant difference. The variation between the present study's results of antioxidants compounds and the previous studies mentioned above, suggest that the concentration of polyphenols rely on date variety and extraction solvent as stated by **Saleh et al., 2011**, the environment, soil type, different types of date fruit and maturity stages as reported by **Arshad et al., 2019**, and finally, processing, such as drying, fermentation, and storage also affect phenolic content **Al-Farsi et al., 2005.**

4.1.2. Ajwa dates and Ajwa Date Extract's Content of Carbohydrate:

Table 5: Ajwa Dates and Ajwa Date Extract Content of Carbohydrates:

No	Compounds Name	Ajwa Dates		Ajwa Extract	
		RT	Area Sum%	RT	Area Sum%
1	Glycerol, 3TMS derivative	24.106	0.26	24.164	0.21
2	5-Hydroxymaltol, 2-O-TMS	32.46	0.08	32.525	0.22
3	D-(-)-Fructofuranose, pentakis(trimethylsilyl) ether (isomer 1)	38.376	0.08	ND	ND
4	D-(-)-Fructopyranose, 5TMS (isomer 1)	38.963	9	ND	ND
5	.alpha.-l-Galactofuranoside, methyl 6-deoxy-2,3,5-tris-O-(trimethylsilyl)-	40.026	0.08	ND	ND
6	D-(-)-Ribofuranose, tetrakis(trimethylsilyl) ether (isomer 1)	40.346	1.33	44.337	0.44
7	β-D-Fructofuranose, 1,2,3,4,6-pentakis-O-(trimethylsilyl)-	40.593	0.17	40.587	0.42
8	D-Ribose, 4TMS derivative	40.991	0.27	40.887	0.06
9	Xylitol, 5TMS	41.774	0.27	41.761	0.2
10	Talose, 5TMS derivative	43.404	8.88	ND	ND
11	Arabinofuranose, 1,2,3,5-tetrakis-O-(trimethylsilyl)-	43.867	0.24	43.861	0.36
12	D-(-)-Fructofuranose, pentakis(trimethylsilyl) ether (isomer 2)	45.25	0.22	45.341	2.23
13	D-Fructose, 5TMS derivative	45.654	18.6	45.615	14.28
14	D-Mannitol, 6TMS	45.772	0.54	ND	ND
15	L-Sorbopyranose, (1S,2R,3S)-, 5TMS	46.698	0.16	46.685	0.69
16	.beta.-D-Galactofuranoside, ethyl 2,3,5,6-tetrakis-O-(trimethylsilyl)-	46.815	0.19	46.802	0.16
17	D-Lyxose, 4TMS derivative	ND	ND	47.833	0.65
18	D-Glucose, 5TMS derivative	48.094	14.22	ND	ND
19	D-Glucopyranose, 5TMS derivative	48.218	12.42	48.185	19.36

20	D-Glucitol, 6TMS	50.944	0.85	49.705	0.75
21	.beta.-D-Glucopyranose, 5TMS derivative	51.381	19.3	51.335	19.61
22	Myo-Inositol, 6TMS	54.192	0.13	ND	ND
23	Deoxyglucose, 4TMS derivative	68.951	0.07	68.932	0.16
24	Sucrose, 8TMS derivative	73.491	0.13	73.49	0.08

Note: RT = retention time (min). ND = not detected

Table (5) shows the amount of carbohydrates content found on Ajwa dates and Ajwa date extract. Twenty three compounds found on Ajwa dates through chromatographic analysis which are: Glycerol, 3TMS derivative, 5-Hydroxymaltol, 2-O-TMS, D-(-)-Fructofuranose, pentakis(trimethylsilyl) ether (isomer 1), D-(-)-Fructopyranose, 5TMS (isomer 1), .alpha.-l-Galactofuranoside, methyl 6-deoxy-2,3,5-tris-O-(trimethylsilyl)-, D-(-)-Ribofuranose, tetrakis(trimethylsilyl) ether (isomer 1), β-D-Fructofuranose, 1,2,3,4,6-pentakis-O-(trimethylsilyl)-, D-Ribose, 4TMS derivative, Xylitol, 5TMS, Talose, 5TMS derivative, Arabinofuranose, 1,2,3,5-tetrakis-O-(trimethylsilyl)-, D-(-)-Fructofuranose, pentakis(trimethylsilyl) ether (isomer 2), D-Fructose, 5TMS derivative, D-Mannitol, 6TMS, L-Sorbopyranose, (1S,2R,3S)-, 5TMS, .beta.-D-Galactofuranoside, ethyl 2,3,5,6-tetrakis-O-(trimethylsilyl)-, D-Glucose, 5TMS derivative, D-Glucopyranose, 5TMS derivative, D-Glucitol, 6TMS, .beta.-D-Glucopyranose, 5TMS derivative, Myo-Inositol, 6TMS, Deoxyglucose, 4TMS derivative, and Sucrose, 8TMS derivative respectively according to retention time (RT). Ajwa date extract on the other side contained seventeen compounds, which are less than Ajwa dates. Ajwa date extract are Glycerol, 3TMS derivative, 5-Hydroxymaltol, 2-O-TMS, β-D-Fructofuranose, 1,2,3,4,6 pentakis-O-(trimethylsilyl)-, D-Ribose,

4TMS derivative, Xylitol, 5TMS, Arabinofuranose, 1,2,3,5-tetrakis-O-(trimethylsilyl)-, D-(-)-Ribofuranose, tetrakis(trimethylsilyl) ether (isomer 1), D-(-)-Fructofuranose, pentakis(trimethylsilyl) ether (isomer 2), D-Fructose, 5TMS derivative, L-Sorbopyranose, (1S,2R,3S)-, 5TMS, .beta.-D-Galactofuranoside, ethyl 2,3,5,6 tetrakis-O-(trimethylsilyl)-, D-Lyxose, 4TMS derivative, D-Glucopyranose, 5TMS derivative, D-Glucitol, 6TMS, .beta.-D-Glucopyranose, 5TMS derivative, Deoxyglucose, 4TMS derivative, and Sucrose, 8TMS derivative respectively according to retention time (RT).

Dates are known to have a sweet taste. The major chemical constituents of dates are carbohydrates, about 50%-88% of total weight according to the date type, the maturity stage, and water content. Dates are rich in sugar, foremost glucose and fructose; these sugars compose two-thirds of total dates. Ajwa dates sugar total content is 74.3 g/100g **Aljaloud et al., 2020**. In the human body, carbohydrates are essential. They serve as a source of energy, aid in the regulation of insulin and blood glucose metabolism, take part in the metabolism of triglycerides and cholesterol, and support fermentation. Carbohydrates break down into glucose that is used for energy. The extra levels of glucose in the bloodstream are stored in the liver and muscle tissue until energy is needed **Holesh et al., 2023**. the Dietary Guidelines for Americans recommend that carbohydrates make up 45% to 65% of total daily calories. That's between 225 and 325 grams of carbs a day, not forgetting that 1 gram of sugar equals 4 calories **Mayo clinic, 2025**. Table (5) present the most abundant carbohydrates in both Ajwa dates and Ajwa date extract is (.beta.-D-Glucopyranose, 5TMS derivative) with area sum% values of 19.3% and 19,61% respectively, as for (D-Glucopyranose, 5TMS derivative), observed an abundance in Ajwa date extract 19.36% of area sum%, not quite

for Ajwa dates which was 12.42% of area sum% value. (D-Fructose, 5TMS derivative) found to be the second most abundant component in Ajwa dates 18.6%, and in Ajwa date extract it came third with 14.28%. (D-Glucose, 5TMS derivative) observed only in Ajwa dates 14.22% and was absence in Ajwa date extract. These compounds recorded a profusion above 10% in both Ajwa and Ajwa date extract. However, other components had been significantly plentiful in Ajwa dates such as D-(-)-Fructopyranose, 5TMS (isomer 1) was 9%, Talose, 5TMS derivative which is unnatural monosacchride derived from galactose was 8.88%. (D-(-)-Ribofuranose, tetrakis(trimethylsilyl) ether (isomer 1)) was found in both. but was found higher in Ajwa dates than Ajwa date extract (1.33% and 0.44% respectively). D-(-)-Fructofuranose, pentakis(trimethylsilyl) ether (isomer 2) a component found to be higher in Ajwa date extract than in Ajwa dates (2.23% and 0.22% respectively). Varieties of other components found on Ajwa dates and Ajwa date extract found to be less than 1%. Although the total sugar or carbohydrate percentage in the present study wasn't quantified, the presence of compounds such as D-Fructose, B-D-Glucopyranose, and D-Glucopyranose in Ajwa dates and Ajwa date extract, as well as D-Glucose in Ajwa dates, provides clear evidence of the sugar content in both samples. These findings align with previous studies that reported high levels of carbohydrate, total sugar, or monosaccharides (glucose and fructose) in date fruit. **Saleh et al., 2011** who studied the sugar content of three varieties of dates (Ajwa, khalas, and sukari). Reported that the highest concentrations of glucose and fructose were found in Ajwa dates followed by khals, while the lowest concentrations of glucose and fructose were found in sukari with it being the only variety containing sucrose. Similarly, **Al-Harrasi et al., 2014** who reported the carbohydrate content in the twenty-two date varieties growing in different locations in Oman ranged

between 74.5% and 82.4%. In addition, **Arshad et al., 2019** reported total sugar in four varieties of dates (Ajwa, hallawi, khudravi, and aseel) showing that Ajwa dates contain the highest total sugar of 86.24% followed by hallawi, khudravi and aseel (83.58%, 80.46%, and 71.96% respectively). Despite the fact the high contents of carbohydrates among different varieties, differences can occur due to the moisture levels which are influenced by maturity stages of the fruits. As the moisture content decreases during maturity, higher simple sugar concentrations like glucose and fructose increase. Which aligns with the variety nature of sugar solubility and in date species as discussed by **AlShwyeh and Almahasheer, 2022**. The varieties of compounds between monosaccharides such as (glucose, galactose, and fructose), disaccharides such as (sucrose and lactose) and their derivatives as described by **Hastings et al., 2016**, found in Ajwa dates and Ajwa date extract. Despite the compounds observed in Ajwa date extract being less than in Ajwa dates. This indicates that Ajwa dates contain a magnificent amount of carbohydrates. Which indicates that dates are rich in natural sugar. Referencing the applications in food industries as a natural sweetening product, such as date paste, date fiber, date sugar, date syrup, and powdered dates, promotes potential benefits on overall health and preserves dental health **Almuzaini et al., 2024**.

4.1.3. Ajwa Dates and Ajwa Date Extract Content of Fatty Acids:

Table 6: Ajwa Dates and Ajwa Date Extract Content of Fatty Acids:

No	Compounds Name	Ajwa Dates		Ajwa Extract	
		RT	Area Sum%	RT	Area Sum%
1	Pentadecanoic acid, TMS derivative	48.896	0.62	48.798	0.35
2	Undecanoic acid, ethyl ester	ND	ND	50.128	0.28
3	Palmitic Acid, TMS derivative	51.909	0.99	51.929	10.09
4	Heptadecanoic acid, TMS derivative	ND	ND	54.844	0.15
5	Linoleic acid ethyl ester	55.098	0.46	55.177	0.58
6	Ethyl 9-octadecenoate	55.333	0.27	55.379	0.64
7	9,12-Octadecadienoic acid (Z,Z)-, TMS derivative	56.859	0.64	56.931	13.46
8	Oleic Acid, (Z)-, TMS derivative	57.055	0.35	57.12	8.71
9	Stearic acid, TMS derivative	57.949	0.17	57.948	0.95
10	1-Monopalmitin, 2TMS derivative	69.982	0.46	69.975	1.51

Note: RT = retention time (min). ND = not detected

Table (6) shows Ajwa dates and Ajwa date extract fatty acid contents, in this table there are eight compounds found on Ajwa dates, and ten compounds found on Ajwa date extract. Ajwa dates compounds are Pentadecanoic acid, TMS derivative, Palmitic Acid, TMS derivative, Linoleic acid ethyl ester, Ethyl 9-octadecenoate, 9,12-Octadecadienoic acid (Z,Z)-, TMS derivative, Oleic Acid, (Z)-, TMS derivative, Stearic acid, TMS derivative and 1-Monopalmitin, 2TMS derivative respectively based on (RT). Ajwa date extract compounds are Pentadecanoic acid, TMS derivative, Undecanoic acid, ethyl

ester, Palmitic Acid, TMS derivative, Heptadecanoic acid, TMS derivative, Linoleic acid ethyl ester, ETHYL 9-OCTADECANOATE, 9,12-Octadecadienoic acid (Z,Z)-, TMS derivative, Oleic Acid, (Z)-, TMS derivative, Stearic acid, TMS derivative, and 1-Monopalmitin, 2TMS derivative respectively based on (RT). Results show that Ajwa dates contain less compound in comparison to Ajwa date extract.

Fatty acids individually or as a part of molecules, have different functions in cells, between being a structural building block of cell membranes to being a provider of energy and molecule signals. The essential fatty acids compounds are needed in some organisms, these essential fatty acids cannot be synthesized nor sufficiently synthesized to meet the organisms needs for physical growth, general metabolism functions and reproduction **de Carvalho and Caramujo, 2018**. In original, dates are poor in fat **according to USDA, 2018** each 100 g of dates contain 0.39 g. Palmitic acid, oleic acid, linoleic acid and stearic acid are an outstanding among the fatty acids in theses tables. saturated fatty acids are palmitic and stearic acids, while monosaturated fatty acid is oleic acid (omega-9 fatty acid), Oleic acid is an ingredient in cellular membranes, hormone production and has several therapeutic impacts **Farag and Gad, 2022**. In table (6), all fatty acid compounds established in Ajwa dates and Ajwa date extract as well. Except for two more compounds which are undecanoic acid ethyl ester, and Heptadecanoic acid, TMS derivative were observed only in Ajwa date extract and were absent from Ajwa dates. The most abundant compound to be in Ajwa dates and Ajwa date Extract was Palmitic Acid, TMS derivative. Though in Ajwa date extract found in a significant amount (10.09%) of area sum, in Ajwa dates it was less for (0.99%) of area sum%. The most common saturated fatty acid found in the

human body is palmitic acid. Palmitic acid can be obtained by diet, and it can also be produced endogenously by amino acids, fatty acids, and carbohydrates. Twenty to thirty percent of the total fatty acids in adipose triacylglycerols (TAG) and membrane phospholipids (PL) are palmitic acid. Palmitic acid is found in large quantities in meat and dairy products (50–60% of total fat), cocoa butter (26%), olive oil (8–20%), and breast milk (20–30%). Palm oil also contains a significant quantity of palmitic acid (40% of total fat). Various physiopathological disorders, including cancer, neurological illnesses, and atherosclerosis are related to the disruption of palmitic acid homeostatic balance, an uncontrolled endogenous biosynthesis of palmitic acid, whatever its dietary contribution **Carta et al., 2017**. (Oleic Acid, (Z)-, TMS derivative) observed to be numerous in Ajwa date extract (8.71% of area sum%) but not the same as Ajwa dates which was much less (0.35% of area sum%). Oleic acid a monounsaturated fatty acid considered one of the most common types of (omega 9 fatty acid). Oleic acid is found on olive, nuts, macademia, sesame seeds and is found abundantly in 74.8% of olive oil, 40.7% of sesame oil, flaxseed oil, 58.8% of rapeseed oil, 32.7% of pumpkin seed oil, and 17.5% of flaxseed oil. Diet rich with oleic acid can contribute positively to disorders related to inflammatory by activating various immune cell pathways resulting in modifying immune system make it engaged in several anti-inflammatory actions like, eye inflammation, skin inflammation, lung inflammation and liver inflammation **Farag and Gad, 2022**. (Linoleic acid ethyl ester) and (9,12-Octadecadienoic acid (Z,Z)-, TMS derivative) in table (6) showed in Ajwa dates and Ajwa date extract. In Ajwa dates, both compounds were less than 1% (0.46% and 0.64% of area sum%), while in Ajwa date extract, the same was less than 1% of linoleic acid (0.58% of area sum), as for 9,12-Octadecadienoic acid was plentiful (13.45% of area sum). Linoleic acid (9,12-

octadecadienoic acid) is the most highly consumed polyunsaturated fatty acid found in the human diet. Lenoleic acid has four primary outcomes when consumed. Likewise, all fatty acids that used as a source of energy. It can be esterified to create neutral and polar lipids like phospholipids, triacylglycerols, and cholesterol esters. Linoleic acid functions as a structural component of phospholipid membranes, maintaining a specific degree of membrane fluidity in the epidermis' transdermal water barrierFurthermore, it can undergo enzymatic oxidation to produce a range of derivatives shared in cell signaling after being liberated from membrane phospholipids **Whelan and Fritsche, 2013**. Linoleic (omeg-6 fatty acid) the polysaturated fatty acid, which has been confirmed that the adequate linoleic acid intake as a saturated fatty acid partial substance is associated with a reduction in total blood cholesterol and (LDL-c) concentration improving the effect on cardiovascular health **Djuricic and Calder, 2021**. In Ajwa dates, the compound Stearic acid, TMS derivative shown 0.17% of area sum%. But Ajwa date extract nearly hit 1%. Prior research has demonstrated that dietary stearic acid actually lowers LDL levels and is not linked to an increased risk of atherosclerosis. Furthermore, higher stearic acid levels are linked to improved heart health, lower blood pressure, and a lower risk of cancer as stated by **Senyilmaz-Tiebe et al. 2018**. These results of Ajwa dates containing small amounts of fats similarly to the findings of **Khalid et al., 2016** who reported that the flesh part of Ajwa dates contains (0.47%) of crude fat, which was lower than zaidy flesh dates (0.50%) and not significantly different from aseel (0.46%). While these variety dates' pits recorded higher amounts of crude fat that was higher in Ajwa dates (7.8%), followed by aseel (7.5%) and lastly zaidy pits (4.4%). Another study conducted by **Assirey, 2021** studied fat content in four date varieties (Ajwa, khodari, anabarah, and suqaey) reported that fat content ranged between (0.18

- 0.51 g/100 g dry matter), and in Ajwa dates was (0.48 g/100 g dry matter. In addition, **Alqarni et al., 2019** reported a very small amounts of fat (0.09 g/100 g). As discussed by **Ibrahim et al., 2024**, the content of protein and fats upon dates drying increases due to the moisture content loss. However, agro-climatic differences also contribute to these variations. The content of fat and protein in dates is not restricted to the flesh only; pits also contain considerable amounts of fat and protein that can surpass the flesh in some date types. With a good value of oleic acid and significant amounts of protein and fat, promote the ability to extract oil from the seeds with several nutritional values and health benefits, or even industrial uses like cosmetic products **Alsarayrah et al., 2023**. While specific studies about Ajwa extract are limited, research on Ajwa extract by **Alqarni et a., 2019** studied Ajwa extract on lipid profile of rats fed high-cholesterol diet, reported the sufficient Ajwa extract on lowering LDL-c, VLDL-C, cholesterol and triglycerides, with HDL-C increasing. This suggests that Ajwa extract can be beneficial to lipid profile, even though it is not high in fat.

4.1.4. Other Contents of Ajwa Dates and Ajwa Date Extract:

Table 7: Ajwa Dates and Ajwa Date Extract Other Contents:

No	Compounds Name	Ajwa Dates		Ajwa Extract	
		RT	Area Sum%	RT	Area Sum%
1	4-Phenoxybutyric acid, TMS derivative	ND	ND	8.57	0.04
2	Heptane, 2,4-dimethyl-	ND	ND	13.468	0.23
3	Ethanamine, 2TMS derivative	13.905	0.15	ND	ND
4	Kynurenic Acid, 2TMS derivative	ND	ND	13.911	0.07
5	Ethanolamine, 3TMS derivative	17.244	0.14	17.27	0.03
6	2-Ketobutyric acid eo-tms	19.058	0.18	ND	ND
7	Pentatriacontane	32.519	0.09	ND	ND
8	Propanedioic acid, 2TMS derivative	ND	ND	33.21	0.04
9	Cyclohexanol, TMS derivative	36.334	0.16	36.55	0.03
10	AZELAIC ACID-DITMS	44.096	0.15	ND	ND
11	D-Psicofuranose, pentakis(trimethylsilyl) ether (isomer 1)	45.25	0.22	ND	ND
12	Methyl .alpha.-Lyxofuranoside, 3TMS derivative	46.241	0.12	ND	ND
13	.beta.-D-Tagatopyranose, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-	46.359	0.97	ND	ND
14	(+)-Alloaromadendrene	ND	ND	48.446	0.07
15	1,2-Benzenedicarboxylic acid, dibutyl ester	49.711	0.09	ND	ND
16	Per(trimethylsilyl)-L-sorbose	ND	ND	49.991	0.05
17	Nonadecane	ND	ND	54.394	0.13
18	Suberic acid, 2TMS derivative	ND	ND	64.001	0.28
19	5-(4-Hydroxyphenyl)-3-methyl-5-phenylhydantion glucronide, methyl ester	71.717	0.33	ND	ND
20	1-Heptanol, TMS derivative	72.108	0.41	72.095	0.16
21	Mannonic acid, γ-lactone, 4TMS	72.388	0.11	ND	ND
22	Glycerol monostearate, 2TMS derivative	76.406	0.22	76.393	0.67

Note: RT = retention time (min). ND = not detected

Table (7) shows that according to the analysis, more compounds were shown in the extract than the whole Ajwa dates. The compounds observed are: Pentadecanoic acid, TMS derivative, Undecanoic acid, ethyl ester, Palmitic Acid, TMS derivative, Heptadecanoic acid, TMS derivative, Linoleic acid ethyl ester, ETHYL 9-OCTADECANOATE, 9,12-Octadecadienoic acid (Z,Z)-, TMS derivative, Oleic Acid, (Z)-, TMS derivative, Oleic Acid, (Z)-, TMS derivative, 1-Monopalmitin, 2TMS derivative and Glycerol monostearate, 2TMS derivative respectively. At the stage of rutab, dates can be used as a natural sweetener in pastry and dairy products; they also contain a higher content of sugar and a lower content of phenolic compounds. As for the stage of tamr, date fruits are processed into a various of products including jam, date paste and date syrup, these products can add a value to different types of food due to their low glycemic index, likely effect of anti-diabetes and antioxidants advantages **Ghnimi et al., 2017**. Several studies have shown that Ajwa dates have antioxidant, antiviral, antifungal, antibacterial, antidiabetic, anti-inflammatory, anticancer, hypolipidemic, gastrointestinal protective, and cardioprotective properties **Khalid et al., 2017**. Ajwa dates contain a variety of polyphenols, carotenoids, sterols, and tannins. Dates are known as rich in fiber and low in calories. The concentration and composition of dates are affected by factors that are date variety, harvesting stage, storing, postharvest production, and the region of origin. Also, dates face a significant switch in their development phase; thus, their chemical composition and functional content are affected, with a lower content of fiber, minerals, and vitamins versus an increased concentration of simple sugar **Alyahya et al., 2022**.

A chemical analysis made by **Eman, 2015** to determine the chemical

composition of dates from 10 types of palms cultivated in Saudi Arabia, showed that Ajwa date flesh contains a high amount of sugar and a very low-fat content. Although the amount of protein was too small for dates to be considered an important nutritional source, Ajwa dates contain essential amino acids.

4.1.5. Mineral Content of Ajwa Dates and Ajwa Date Extract:

Table 8: Comparison of minera Content of Ajwa Dates and Ajwa Date Extract:

No	Parameter	Sample Result of Ajwa Dates mg/kg	Sample Result of Ajwa Date Extract mg/L
1	Calcium	1290	380
2	Sodium	100	930
3	Potassium	4600	5900
4	Phosphor	700	360
5	Magnesium	ND	400
6	Copper	6	2
7	Aluminum	100	10
8	Zinc	4.5	3.6
9	Chromium	4	0.4
10	Lead	<0.05	0.1
11	Cadmium	<0.05	<0.001
12	Nickel	0.2	0.2

Note: ND = not detected.

Table (8) shows the mineral content of Ajwa dates and Ajwa date extract, we can see the content of Ajwa dates of minerals. Potassium content was the highest of 4600 mg\kg, followed by Calcium 1290 mg/kg, phosphor 700 mg/kg, sodium and aluminum with the same content 100 mg/kg, copper 6 mg/kg, zinc 4.5 mg/kg, chromium 4 mg/kg, nickle 0.2 mg/kg and lastly lead and cadmium with same content <0.05 mg/kg respectively, and no magnesium presence. In Ajwa date extract, potassium also comes the highest content of 5900 mg/L, followed by sodium 930 mg/L, magnesium 400 mg/L, calcium 380 mg/L, phosphor 360 mg/L, aluminum 10 mg/L, zinc 3.6 mg/L, copper 2 mg/L, chromium 0.4 mg/L, nickle 0.2 mg/L, lead 0.1 mg/L and cadmium

<0.001 mg/L respectively. Potassium is an essential mineral for the human body. We found that potassium is an essential electrolyte that can be consumed from the diet. When the body can't maintain the dietary intake from this mineral, then exogenous potassium is needed. Potassium is very beneficial in inhibiting the development of hypertension; it's also recommended to patients with hypertension to control blood pressure. Potassium as well recommended in arrhythmia status, when a loss of this mineral can cause cardiac glycoside toxicity or tachyarrhythmias after cardiac surgery and other useful functions **Sur and Mohiuddin, 2024**. Potassium intake according to National Institutes of Health **NIH, 2024** for men +19 is 3400 mg\day and for women +19 is 2600 mg\day which Ajwa dates' content of potassium covers more than these needs. In table (8) potassium content in Ajwa date extract was higher compared to Ajwa dates. Calcium was the second high result in Ajwa dates for 1290 mg\kg. Calcium is a mineral that is plentiful in the body. found in bone and teeth for 99%; it helps the human body to build strong bones and maintain them; calcium plays a part in cardiovascular function and muscle movement; and it's also important to the brain and other parts of the body to communicate with each other **Brown, 2020.** Calcium intake based on Mayo Clinic 2022, for men 19-70 is 1000 mg\day and over 71 is 1200 mg\day, as for women 19-50 is 1000 mg\day and over 51 is 1200 mg\day and Ajwa dates content of calcium is good for human needs.

As for phosphor that came in third position of mineral content in Ajwa dates by 700 mg\kg. Phosphor is an essential mineral for the human body; it's one of the ingredients of the bones, teeth, DNA, and RNA. Phosphor is also considered one of the cell membrane structures and the main energy source of the body in its phospholipid form, which is adenosine triphosphate (ATP). It plays a major part in maintaining a normal pH in extracellular fluid and

storing the energy in the intracellular. Hydroxyapatite is a form of union phosphorus and calcium, which is the major structural component in bones and tooth enamel. Phosphor intake for adults 19 years and older is 700 mg\day **NIH, 2023,** which results that the Ajwa dates content of phosphor is adequate for human needs in a day.

Sodium came in thire position of mineral content in Ajwa dates by 100 mg\kg. Sodium is a mineral found naturally in food; these include all vegetables and dairy products, meat, and shellfish. Factories also add sodium to processed foods like bread. It used to add flavor to condiments like soy sauce. Salt is the combenation of sodium and chloride. Sodium plays a role in balancing fluid in the body and the way muscles and nerves work. Kidneys maintain sodium levels in the body, keeping it in the body when it's low, and release some of it in the urin when sodium levels are high. If high levels of sodium weren't removed, it began to build up in the blood, causing an increase in blood volume, which made the heart need an effort to pump blood. That in time leads to hypertension, strokes, heart diseases, and kidney diseases **Mayo Clinic, 2023**. **WHO, 2023** recommends that adults should consume less than 2000 mg of sodium per day. In this table, Ajwa dates' content of sodium is 100 mg\kg which is very low to cause any harm on the body so it's healthy to consume. Zinc, in this table came in fourth position in this table by 4.5 mg\kg. Due to its significance for numerous processes in the body, such as gene expression, DNA synthesis, enzymatic reactions, immune function, protein synthesis, growth and development, and wound healing, zinc is considered an important nutrient. Zinc also may minimize inflammation and age-related diseases' risk. Moreover, some older research suggests that zinc supplements significantly lower the risk of infections and improve immune responses in elders. And this mineral is necessary for proper healing because it plays

critical roles in collagen synthesis, immune function, and inflammatory response. Zinc decreases oxidative stress and reduces levels of certain inflammatory proteins in the body. Zink daily intake for adult males 11 mg/day and for adult females 8 mg/day **Kubala, 2022.**

Sodium 100 mg\kg, Phosphor 700 mg\kg and Zinc 4.5 mg\kg, these results agree with the report made by **Alqarni et al., 2019** which were 83.96 mg/kg, 696.67 mg/kg and 4.74 mg/kg respectively. In addition, results are also similar to the findings of **Arshad et al., 2019** who reported high content of potassium 482.00 mg/100g, magnesium 146.93 mg /100g, calcium 191.00 482.00 mg/100g, sodium 9.50 and zinc 1.30 482.00 mg/100g. Furthermore, **Otitolaiye et al., 2021** reported potassium 2902.00 mg/kg, calcium 0.52 mg/kg and magnesium for 0.22 mg/kg in dried date fruit. These different content of minerals in the outcomes in the previous studies may be due to the environment, soil type, different types of date fruit and maturity stage **Arshad et al., 2019.**

4.2. Effect of Ajwa Dates Extract on Blood Serum Samples:

4.2.1. Impact of Ajwa Date Extract on Different Blood Serum Samples:

Table 9: Impact of Ajwa Date Extract on Different Serum-tests:

Dependent: GROUP	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-ch (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)	Total lipids (mg/dl)
Negative Control	**89 ±2c**	**102.3 ±3.2c**	**78.0±5.0b**	**40.0 ±1.0a**	**46.7 ±5.2c**	**15.6 ±1.0b**	**405.3 ±15.5c**
Positive Control 0.88mg	**162.4 ±5.2a**	**152.2 ±2.9a**	**100.0±2.0a**	**27.7 ±2.9 c**	**104.5 ±5.2a**	**20.0 ±0.4a**	**671.4 ±5.9a**
0.88mg + 30mg	**112.7 ±10.4b**	**118.9 ±3.5b**	**80.4 ±2.7b**	**33.1 ±2.5b**	**69.7 ±4.7b**	**16.1 ±0.5b**	**454.5 ±7.0b**
0.88mg + 60mg	**101.8 ±7.7b**	**115.5 ±2 b**	**80.3±4.6b**	**34.9 ±1.5b**	**64.5 ±2.0b**	**16.1 ±0.9b**	**432.7 ±10.5b**
0.88mg + 90mg	**98.2 ±8.6b**	**104.7 ±6c**	**83.5±3.2b**	**35.7 ±1.4b**	**52.4±4.9c**	**16.7±0.6b**	**421.7±14.1c**
0.88mg + 120mg	**93.7 ±9.3b**	**75.1 ±5.0abc**	**62.0±4.3c**	**39.4±0.9a**	**23.3 ±4.9abc**	**12.4 ±0.9c**	**396.2 ±7.5abc**
Total	**109.6 ±26.3**	**111.5±23.9**	**80.7±11.8**	**35.1±4.5**	**60.2 ±25.7**	**16.1 ±2.4**	**463.6 ±97.9**
P*	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Dependent: GROUP	Total antioxidants (mM/L)	Catalase (U/L)	Glutathione reductase (U/L)	Glutathione reduced (mg/dl)	Glutathion peroxidase (mU/ml)	SOD (U/ml)	MDA (nmol/ml)
Negative Control	**1.8 ±0.2a**	**344.7 ±5.0a**	**158.4 ±1.8b**	**24.0 ±1.0a**	**118.1±3.3a**	**50.0 ±2.0c**	**7.7 ±0.8c**
Positive Control 0.88mg	**0.5 ±0.0c**	**136.9 ±4.1abc**	**127.9 ±2.7c**	**11.5 ±1.5c**	**101.2 ±1.9b**	**64.5±2.1b**	**12.3±0.4a**
0.88mg + 30mg	**1.0 ±0.1b**	**156.9±6.2 c**	**159.3 ±3.2b**	**20.4 ±1.7b**	**117.7±4.3a**	**78.4±1.8a**	**9.7 ±0.7b**
0.88mg + 60mg	**1.3 ±0.3b**	**158.9 ±6.0c**	**157.0±5.5b**	**23.0±1.7b**	**121.1 ±3.9a**	**79.7 ±2.9a**	**8.9±0.1b**
0.88mg + 90mg	**1.3 ±0.1b**	**172.4 ±4.6 c**	**168.5±10.0a**	**24.6±1.0a**	**121.7±2.3a**	**78.8±1.0a**	**8.2±0.1b**
0.88mg + 120mg	**1.4 ±0.2a**	**248.4 ±1.7b**	**183.0 ±3.8a**	**28.3±2.0a**	**126.0 ± 3.0a**	**80.8±2.4a**	**7.6±0.6c**
Total	**1.2 ±0.4**	**203.0 ±74.7**	**159.0±17.6**	**22.0 ±5.5**	**117.6±8.5**	**72.0 ±11.8**	**9.0 ±1.7**
P*	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Note: *Kruskal Wallis (Kruskal .test), values are expressed as mean ± SD.

Means with different letters in the same column are significantly different at P≤0.001.

The results in table (9) show a summarizes various biochemical measures in blood serum across different groups, including a negative control, a positive control, and several treatment groups with varying dosages. All p-values of non-parametric ANOVA (kruskal.test) are less than <0.001, indicating that there are statistically significant differences among the groups for all measured parameters. The positive control shows elevated levels across most measures compared to the negative control, indicating the effect of Prednisolone treatment. As the dosage increases in some parameters, there are varying responses, particularly noted in cholesterol and triglycerides. Antioxidant measures generally decrease in the positive control compared to the negative control, suggesting oxidative stress.

4.2.2. Impact of Ajwa Date Extract on Glucose Levels:

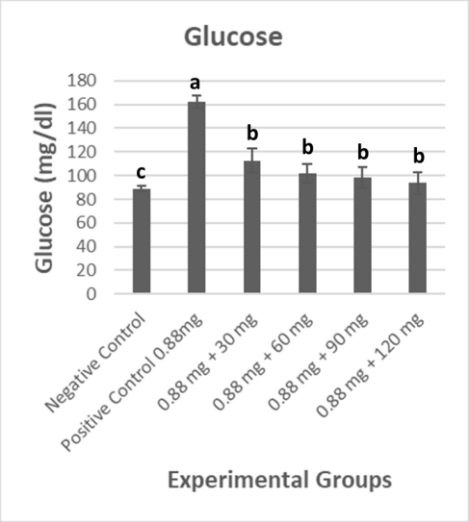


Figure 7: The Impact of Ajwa Date Extract on Glucose Level in Prednisolone-treated rats. Values are expressed as mean ± SD. significannce was accepted at P≤0.001.

In figure (7) results show that all treatment groups (30mg + 60mg + 90mg + 120mg) had a significantly lower glucose levels compared to the positive control and, there is no significant difference among the treatment group and the negative control, results also showed that the best effect was within the highest dosage (0.88mg + 120mg), followed by the (90mg, 60mg, and 30mg) respectively.

High blood sugar (Hyperglycemia) is not a serious matter if it gets high sometimes. However, high blood sugar for a long time or reaching a very high levels can cause serious problems, which may lead to peripheral neuropathy (constant damage to the nerves in hands and feet), diabetic retinopathy (permanent damage to your eyes and problems with your sight), and life-

threatening conditions such as diabetic ketoacidosis **NHS, 2022**. Through the table above, our findings indicate that prednisolone (0.88mg) was associated with high blood glucose in the positive control compared to the negative control. Glucocorticoid drugs are known for its anti-inflammatory and immunosuppressive potential, which used widely for many diseases treatment, glucocorticoid drug group are often related with the appearance of hyperglycemia or diabetes mellitus (DM), glucocorticoid-induced diabetes mellitus is a common and potential harmful condition in clinical practice **Suh & Park, 2017.** Prednisolone, a corticosteroid is connected with elevated blood sugar levels, our results agree with those of **Wu et al., 2020** who after analyzing data from 100,722 adults with six immune-mediated inflammatory diseases to evaluate (T2DM) reported that after 1 year of the follow-up, the cumulative risk of diabetes was 0.9% during the period with no use glucocorticoid, went up to 2.1% in equivalent-prednisolone dose less than 5mg and, a 5.0% when consuming a dose of 25mg or more. Ajwa date extract significantly reduced blood glucose levels across all tested dosages (30mg – 60mg – 90mg and 120mg), suggesting it's potential hypoglycemic effect. These results are consistent with the study made by **Mani et al., 2022**, which reported that aqueous Ajwa date seed extract enhanced plasma insulin levels and reduced hypoglycemia in type 2 diabetes mellitus (T2DM) in rats.

4.2.3. Impact of Ajwa Date Extract on Lipid Profile Levels:

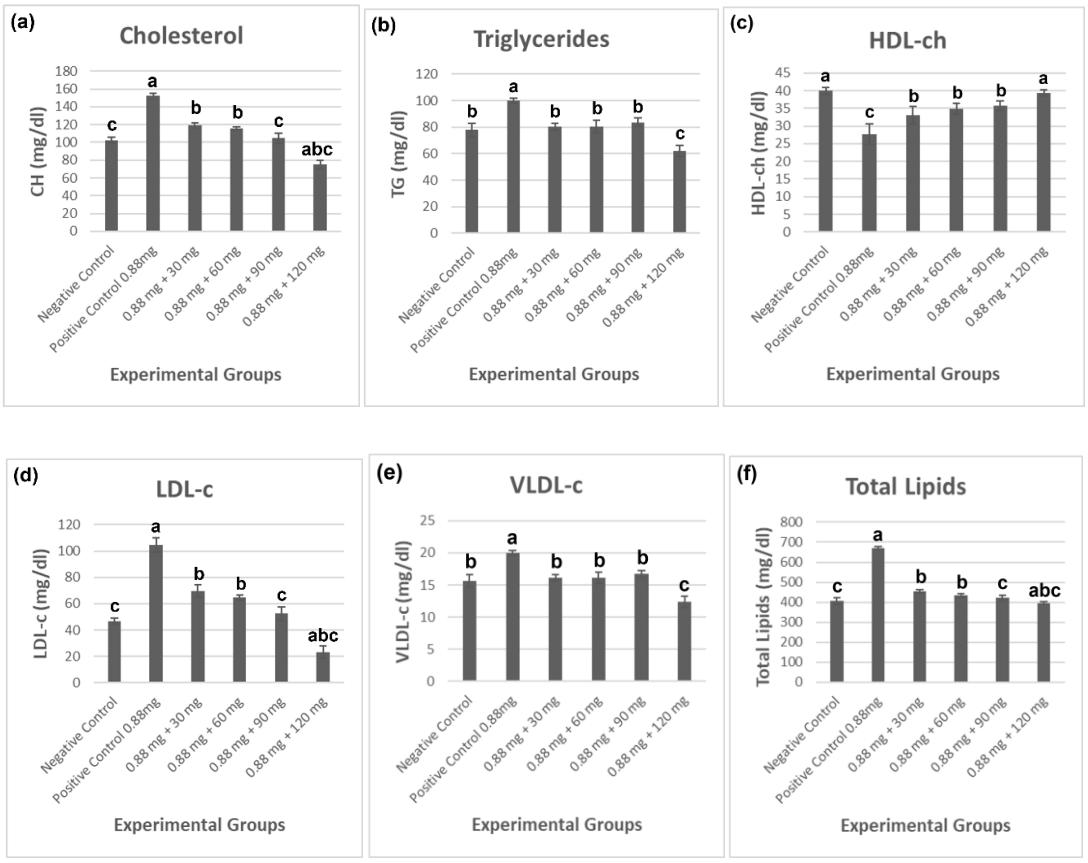


Figure 8: The Impact of Ajwa Date Extract on Lipid Profile Parameters in Prednisolone-treated rats: (a) total Cholesterol, (b) Triglycerides, (c) HDL-ch, (d) LDL-c, (e) VLDL-c, and (f) Total Lipids. Values are expressed as mean ± SD. significannce was accepted at $P \leq 0.001$.

The result in figure (8) shows a summary of the additional biochemical measures aiming on lipid profile; Cholesterol, Triglycerides, HDL-cholesterol (HDL-c), LDL cholesterol (LDL-c), VLDL cholesterol (VLDL-c), and Total lipids across different groups. Cholesterol in the positive control shows a significant higher level compared to the treatment groups (30mg – 60mg –

90mg and 120mg) and the negative control, the levels among the treatment groups were different with a lowest level from the highest dosage (0.88 mg + 120 mg) even much lower than the negative control. The dose (0.88mg) treatment is associated with significantly higher triglyceride levels in the positive control compared to the treatment groups (30mg – 60mg – 90mg and 120mg) and the negative control, suggesting a potential dose-response relationship or differing effects of the treatments on triglyceride levels. Additionally, higher doses of the combination treatments appear to have varying impacts on triglyceride levels, with some comparisons yielding significant differences, particularly with the group receiving (0.88mg + 120mg). HDL-c levels in positive control was lower in comparison of the treatment groups (30mg – 60mg – 90mg and 120mg) and the negative control, and no significant difference between treatment groups and negative control with the higher HDL-c observed in the group (0.88 + 120mg), indicates that the (0.88mg) treatment in positive control is associated with a significantly lower level of HDL cholesterol compared to the (0.88mg + 30mg) treatment group. However, no other comparisons yielded significant differences. The negative control shows significantly lower levels of LDL cholesterol in treatment groups (30mg – 60mg – 90mg and 120mg) compared to the positive control and the negative control, indicating that the treatment or condition in the positive control may be associated with increased LDL-c levels, the various doses of treatment appear to reduce LDL-c levels compared to the positive control, with the lowest level observed in the group receiving the highest dosage (0.88mg + 120mg) followed by the dosages (90mg – 60mg and 30mg) respectively. VLDL-c levels are also significantly higher in the positive control compared to the negative control and the treatment groups (30mg-60mg -90mg and 120mg). The values for VLDL-c are relatively stable across

the treatment groups, with a slight decrease noted in the group receiving the highest dosage (0.88mg + 120mg). Total lipids had a significant high level in positive control compared to the treatment groups (30mg – 60mg – 90mg and 120mg) and the negative control, and no significant between treatment group and negative control with noted decrease in the group receiving highest dosage (0.88mg + 120mg).

The expression "high cholesterol" refers to blood cholesterol levels that are higher than what the body requires. Excessive cholesterol can cause fatty deposits in blood vessels, which eventually make it harder for enough blood to pass through the arteries, raising the risk of cardiovascular diseases like heart attacks and strokes. (LDL) and (HDL) are a spherical particle in a varying size called lipoproteins, which are a cholesterol carrier into the bloodstream. (LDL) is associated with the increasing risk of arteriosclerosis, while in the opposite side, (HDL) protects from the arteriosclerosis by picking up overflowing cholesterol from arteries lining **MOH, 2020**. Prednisone is a drug that belongs to the corticosteroids and is used to reduce inflammation to treat widely inflammatory conditions. As effectively incredible in restraining inflammation, sometimes it can also raise (LDL) levels and reduce (HDL) levels. Side effects risk increases with the dose and duration of the prednisone treatment **Jennifer, 2023**. The active form of prednisone is called prednisolone. Prednisone lowers the body's inflammatory response by acting on the immune system when it is transformed to prednisolone **Juhi, 2023**. Through the results in figure (8), after four weeks of orally prednisolone dosages (0.88 mg) the levels of (Cholesterol, Triglycerides, LDL-c, VLDL-c and total lipids) were elevated in positive control compared to the negative control, attached to low level of HDL-ch in positive control compared to the

negative, suggesting that prednisolone is related to high lipid profile in blood serum. This aligns with the results of **Quinkler et al., 2017** which showed that patients with adrenal insufficiency (AI) who received prednisolone (3 - 6 mg/day) or hydrocortisone (15-30 mg/day), indicated a significantly higher total cholesterol and LDL levels in patients with prednisolone in the comparison of those who had hydrocortisone. A retrospective study in children with chronic diseases assessed the correlation between steroid treatment and lipid profile. The study found that increased triglyceride levels were significantly related to the duration of steroid use, as for the increased (LDL) and cholesterol levels were significantly related to the steroid doses **Lubis and Deliana, 2023**. Unlike the study conducted by **Jeries et al., 2019**, which showed that prednisone and prednisolone reduced cholesterol and triglyceride accumulation in macrophages, the current study found that prednisolone elevated lipid levels. This conflict suggests that while corticosteroids may reduce lipid buildup in immune cells, possibly lowering the risk of foam cell formation and atherosclerosis, they can also contribute to increased lipid levels in the body. Ajwa dates, a type of date fruit known for its potential health benefits, which contribute to body health with their inclusion of antioxidants, fiber, vitamins, minerals, and essential nutrients. Antioxidant content in Ajwa dates helps neutralize free radicals, leading to oxidative stress reduction **Parvez et al., 2021**. Oxidative stress can damage all cell structures and contribute significantly to the development of chronic and degenerative diseases, including cancer, aging, autoimmune disorders, cardiovascular and neurological diseases, and more **Pham-Huy et al., 2008**. The results show the efficiency of Ajwa dates in fighting the increased levels of cholesterol, triglycerides, LDL, and total lipids by decreasing them and increasing the decreased level of HDL by prednisolone in the oral dosages (30 mg, 60 mg, 90

mg, and 120 mg) for four weeks, which indicates the efficiency of Ajwa dates in improving cardiovascular health. These findings are consistent with the previous research by **Alqarni et al., 2019** which found that Ajwa date pulp phenolic extract reduced total cholesterol, triglycerides, LDL-C, VLDL-C and increased HDL-C levels in plasma lipids in albino rats fed with hypercholesterolemic diet.

4.2.4. Impact of Ajwa date extract on atherogenic index:

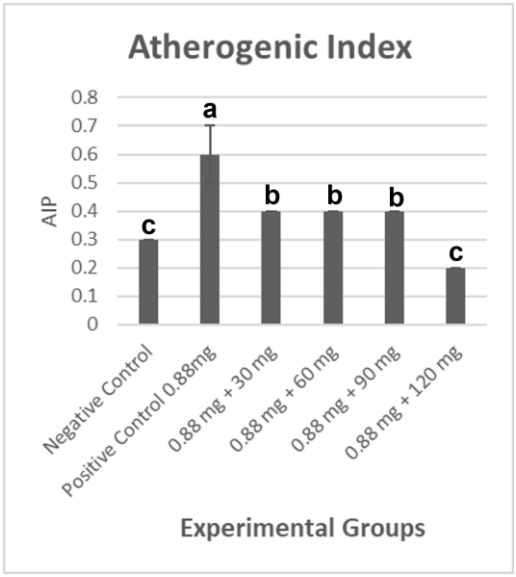


Figure 9: The Impact of Ajwa Date Extract on Atherogenic Index in Prednisolone-treated rats. Values are expressed as mean ± SD. significannce was accepted at P≤0.001.

Figure (9) shows that the atherosclerosis index was significantly higher in the positive control group receiving prednisolone compared to the negative control suggesting a greater risk for atherogenic conditions. Treatment with Ajwa extract in dosages (30mg – 60mg – 90mg and 120mg), led to a

significant reduction in the Atherogenic Index (AI) compared to the positive control group (0.88mg), with the most substantial reduction occurs with the (0.88mg + 120mg) dose showing the most marked reduction.

Atherosclerosis originally is a Greek term that means the interior layer of arteries thickening and fat accumulating. Atherosclerosis results from hyperlipidemia and lipid oxidation, and in developed countries it is a cause of mortality. It is a vascular intima disease; it affects the entire vascular system from the aorta to the coronary arteries. Hyperglycemia and hyperlipidemia are engaged in increased oxidative damage, affecting lipoprotein levels and antioxidants status **Rafieian-Kopaei et al., 2014**. Atherosclerosis originally is a Greek term that means the interior layer of arteries thickening and fat accumulating. Atherosclerosis results from hyperlipidemia and lipid oxidation, and in developed countries it is a cause of mortality. It is a vascular intima disease; it affects the entire vascular system from the aorta to the coronary arteries. Hyperglycemia and hyperlipidemia are engaged in increased oxidative damage, affecting lipoprotein levels and antioxidants status **Rafieian-Kopaei et al., 2014**. Atherogenic index which is a composition of triglycerides and HDL-C, used to measure lipid profile in blood. An optimistic indicator to hyperlipidemia and related diseases such as cardiovascular diseases **Zhu et al., 2018**. The previous figure (9) of lipid profile above after prednisolone administration (0.88 mg) indicates high levels of triglycerides and low levels of HDL-C, translating to an elevated atherogenic index thus increasing the risk of hyperlipidemia and cardiovascular diseases. However, Ajwa dates managed to reduce lipid profile (triglycerides, total cholesterol, LDL-C, VLDL-C), and elevate (HDL-C) levels, therefore decreasing the risk of hyperlipidemia and maintaining cardiovascular health. Various dates

contain different phenolic compounds such as ferulic acid, cinnamic acid, and p-coumaric acid, which function in free radical damage prevention and are effective antioxidants **Khalid et al., 2020**. These findings of the present study consistent with the findings of **Alduwayghiri and Algheshairy, 2023** who reported that lipid profile (TC, TG, VLDL and LDL) due to rich-fat diet resulted in hyperlipidemia and that Ajwa date and germinated barley mixture significantly reduced lipid profile and increased HDL levels, which indicates an anti-hyperlipidemic role contributed by Ajwa dates as they contain a variety of phytochemicals. In addition of **Sa'adah et al., 2017** who reported that high-fat diet on rats for 30 days resulted in increased cholesterol, LDL-C levels which then significantly increased atherogenic index and cause hyperlipidemia, and that the intake of the methanolic extract of (parijoto) containing flavonoid significantly reduces total cholesterol and LDL-C and elevated HDL-C levels resulting that total cholesterol and LDL-C reduced levels by (parijoto) was positively related to atherogenic index reduction, and the risk of atherosclerosis is smaller. Which indicates the contribution role of antioxidants in preventing dyslipidemia and maintaining cardiovascular health.

4.2.5. Impact of Ajwa Date Extract on Antioxidants Enzymes Glutathione Reductase (GR), Glutathione Reduced (GSH), Glutathione Peroxidase (GPx), Total Antioxidants (TAC), Catalase (CAT), Superoxide Dismutase (SOD), and Lipid Peroxidation Marker Malonaldehyde (MDA) Results and Discussion:

4.2.5.1. Impact of Ajwa date extract on Glutathione Reductase (GR), Glutathione Reduced (GSH), and Glutathione Peroxidase (GPx):

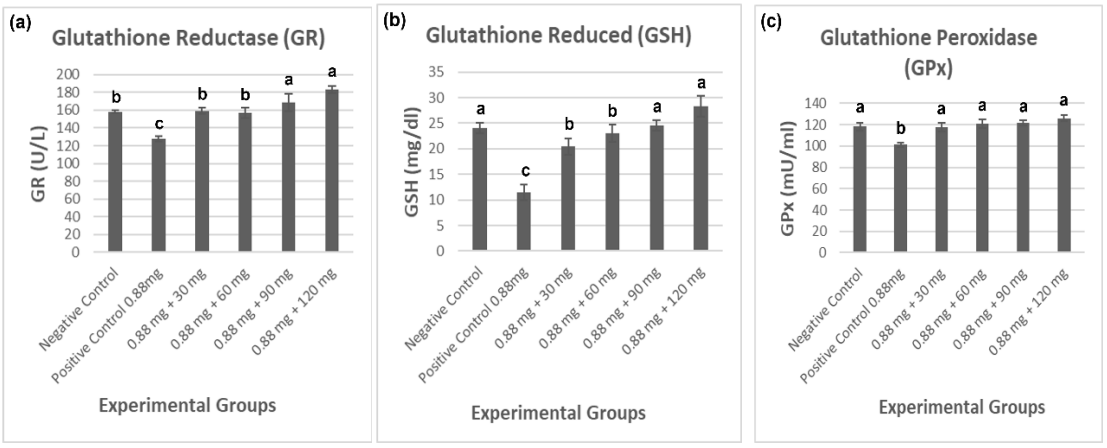


Figure 10: The Impact of Ajwa Date Extract on on Glutathione Parameters in Prednisolone-treated rats: (a) Glutathione Reductase (GR), (b) Glutathione Reduced (GSH), and (c) Glutathione Peroxidase GPx). Values are expressed as mean ± SD. significance was accepted at P≤0.001.

4.2.5.2. Impact of Ajwa date extract on Total Antioxidants Capacity (TAC), Catalase (CAT), and Superoxide Dismutase (SOD):

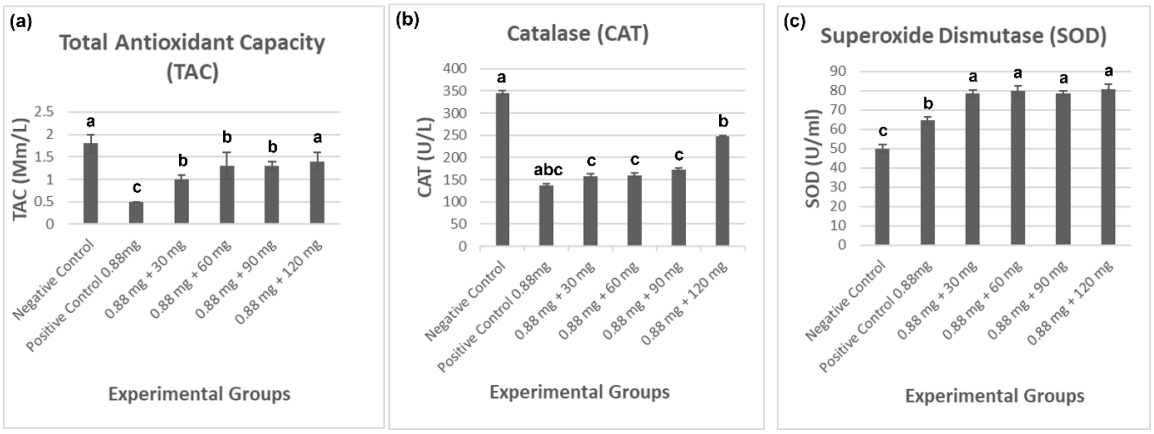


Figure 11: The Impact of Ajwa Date Extract on Antioxidant Parameters in Prednisolone-treated rats: (a) Total Antioxidant Capacity (TAC), (b) Catalase (CAT), and (c) Superoxide Dismutase (SOD). Values are expressed as mean ± SD. significance was accepted at $P \leq 0.001$.

4.2.5.3. Impact of Ajwa Date Extract on Lipid Peroxidation Marker Malondialdehyde (MDA):

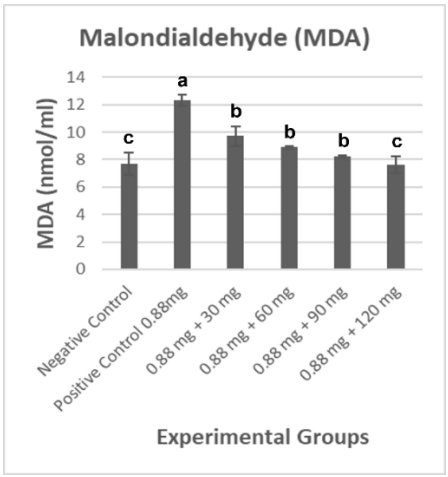


Figure 12:The Impact of Ajwa Date Extract on Lipid Peroxidation Marker in Prednisolone-treated rats: Malondealdehyde (MDA). Values are expressed as mean ± SD. significance was accepted at P≤0.001.

The results in figure (10) shows the administration of prednisolone (0.88 mg) in positive group reduced (GR, GSH, and GPx) compared to the control group. And the oral administration of Ajwa extract in all treatment groups (30mg + 60mg+ 90mg+ 120mg) show a higher significant level of (GR, GSH, and GPx) in comparison with the positive control, and with no significant difference in treatment groups and the negative control group. The best result showed in the highest dosage of Ajwa extract (0.88mg + 120mg) in (GR, GSH, and GPx), followed by the treatment dosages (90mg, 60mg and 30mg) respectively, with no significant different between them.

The result in figure (11) show that all Ajwa extract groups (30mg + 60mg + 90mg +120mg) had a significant higher level of total antioxidants (TAC)

compared to the positive control, no significant difference with negative control, it also showed that the best result was with the highest dosage (0.88mg + 120mg), followed by the results in the treatment groups (90mg + 60mg) then (30mg) respectively. Catalase (CAT) showed a significantly lower level in the positive control compared to the negative control. Ajwa date extract (30mg - 60mg - 90mg and 120mg) didn't restore CAT fully; in fact, it kept declining even more as the dosages increased, and the lowest CAT level was within the highest dosage of Ajwa date extract (0.88mg + 120mg). As for superoxide dismutase (SOD) results showed that prednisolone increased SOD in positive group compared to the negative, and Ajwa date extract dosages (30mg - 60mg - 90mg and 120mg) showed significantly high levels of SOD compared to the positive control and negative control. Levels of SOD kept elevating respectively as the dosage intakes increased, and the highest level was with the highest dosage (0.88mg + 120mg).

Figure (12) MDA levels were increased after the administration of prednisolone (0.88 mg) compared to the negative control, Ajwa date extract (30mg + 60mg + 90mg + 120mg) significantly lower MDA levels compared to the positive control and no significant difference with the negative control, results showed that the lowest MDA level was with the highest dosage (0.88mg + 120mg), trailed by the treatment dosages (90mg – 60mg and 30mg) respectively.

The results in figure (12) showed that oral administration of prednisolone (0.88 mg) for four weeks significantly increased MDA level compared to the negative control. Elevated MDA levels suggest the presence of lipid peroxidation, as MDA is the major and widely studied compound derived from lipid peroxidation, known for its toxic and mutagenic effect **Cordiano et**

al., 2023. MDA is commonly used as a biomarker of oxidative stress, used in clinical trials and environmental epidemiological studies, especially in evaluating oxidative stress in response to air pollution **Cui et al., 2018**. In inflammatory diseases caused by oxidative stress, the cellular antioxidant defense system explores severe modifications, exposing the cells to excessive free radical levels. This free radical oxidation leads to lipid peroxidation, which unabated continuously directs the generation of more free radicals **Yadav, 2015**. Massive risks to trillions of bodily cells range from viral infections to starvation. Another ongoing danger is posed by a chemical known as free radicals, which can harm cells and genetic elements in excessive concentrations. As a result of converting food into energy, the body also creates free radicals in response to exercise, sunlight, air pollution, and cigarette smoke **Lobo V. and others, 2010**. The excessive and chronic free radical production leads to a case called oxidative stress, which contributes to atherosclerosis by making (LDL) more likely to get trapped in artery walls **Harvard T.H Chan, 2023**. Oxidative stress can damage all cell structures and significantly contribute to the development of chronic and degenerative diseases, including cancer, aging, autoimmune disorders, cardiovascular and neurological diseases, and more **Pham-Huy et al., 2008**. The imbalance between (ROS) and the body's antioxidant results in oxidative stress **Jeeva et al., 2015**. Effective neutralization of oxidative stress is enhanced by cellular defenses in antioxidant form. Inside the body, certain compounds act as antioxidants, raising self-antioxidant defenses. Antioxidants can be categorized in several ways: enzymatic and non-enzymatic, solubility in water or lipids, and by size (small and large molecules). Small molecules neutralize (ROS) in a radical scavenging process and eliminate them from the system. These antioxidants include (vitamins C and E, carotenoids, and GSH). While

large molecules prevent (ROS) from attacking other essential proteins by absorbing them, including (SOD, CAT, and GPx) and sacrificial proteins (albumin) **Nimse and Pal, 2015**. Glutathione reduced (GSH) is a tripeptide of the three amino acids glutamine, cysteine and glycine, and it's water-soluble. GSH is an important antioxidant that plays a role in the detoxification of peroxidase and electrophilic compounds and other cellular reactions. Glutathione peroxidase (GPx) works in company with catalase (CAT) and superoxide dismutase (SOD) to protect the cell from the damage caused by (ROS), GSH works as an electron donor in the reduction reaction, which allows GPx to detoxify peroxides, providing glutathione disulfide (GSSG) as a final product **Townsend et al., 2003**. GPx plays a role in neutralizing hydrogen peroxide (H_2O_2), while glutathione reductase (GR) regenerates GSH from GSSG **Pandey and Rizvi, 2010**. Studies have shown the importance of supplying glutathione to prevent cell damage caused by oxidative stress, while low glutathione levels contribute to the loss of the glutathione-dependent enzyme pathway and develop various diseases. Glutathione is considered one of the most plentiful thiols intracellularly. Yet, the dysregulation in glutathione synthesis or variation in its concentrations is related to number of different pathological statuses **Kwon et al., 2019**. Superoxide dismutase (SOD), an enzymatic antioxidant generally found in the body, catalyzes the dismutation of superoxide, producing hydrogen peroxide as a by-product in this reaction, thereby reducing oxidative stress caused by free radicals **Bulugahapitiya, 2020**. Catalase (CAT) is the important part of enzymatic defense, which neutralizes hydrogen peroxide (H_2O_2), which is not radical but can be promptly converted by fenton reaction into a very reactive (·OH) radical—and turns it into H_2O **Pandey and Rizvi, 2010**. CAT works side by side with GPx in neutralizing H_2O_2 and converting it into water (H_2O). The exposure to

prednisolone (0.88 mg) in the positive group significantly decreased antioxidant enzyme levels. These finding are consistent with **Torres et al., 2014** who reported that chronic administration of methylprednisolone (6 mg/kg per day) to rats for 30 days, as measured by lipid peroxidation and total reactive antioxidant potential (TRAP) to determine oxidative stress. Resulted in a significance greater in the degree of pulmonary oxidative damage of the chronic treatment compared to the chronic control group with 38% and no significant difference with (TRAP). In addition, a study by **Tiwari et al., 2024** examined the long-term effects of corticosteroid treatment (deflazacort and methylprednisolone) on oxidative stress markers in patients with arthritis. Resulted in an increase in MDA levels with a decrease in antioxidant enzymes GSH, SOD, and CAT. Suggesting that corticosteroid usage may promote oxidative stress and lipid peroxidation. Furthermore, it can lead to liver damage due to altering liver enzymes such as aspartate aminotransferase (AST) and alanine transaminase (ALT) levels. These studies provide evidence that methylprednisolone, a corticosteroid of which prednisolone is a part, can be linked to lipid peroxidation and oxidative stress. Also, **Ebru et al., 2020** reported that intramuscular injection with 100 mg/kg prednisolone showed an effectiveness reducing GPx, GSH, CAT, and SOD in rat models. The enzyme activity of GPx activity started to decrease at 4 hours after administration of the drug, falling to 35% at 8 hours, GSH levels decreased steadily, reaching 51% after 12 hours, the CAT activity decreased constantly up to 24 hours, after which it decreased to about 43% all compared to the control group. While **(Ebru et al., 2020)** reported the SOD level showing a constant decrease by 24 hours, to about 71% compared to the control group. Interestingly, in the present study SOD levels were elevated in the positive control following prednisolone (0.88 mg) administration compared to the negative control,

alongside decreased levels of GR, GSH, and GPx in figure (10), CAT and TAC figure (11) as well as an increased level of MDA figure (12), suggesting that the balance between the antioxidant mechanisms and the ROS production was disorganized, leading to oxidative stress, as stated by **Ramjibhai et al., 2025**. Since SOD is the first line of antioxidant defense against free radicals **Wang et al., 2018**, the elevated levels of SOD figure (11) could represent a compensatory response to the accumulation of free radicals. A similar observation was reported by **Tang et al., 2013** who found that the activity of antioxidant defense TAC and SOD increased during corticosterone treatment at concentrations of (00.31 and 0.063 mmol/L) indicating that antioxidant defenses showed adequate increases to neutralize the oxidative threat. However, the highest dose (0.125 mmol/L) resulted in a decreased level of SOD, suggesting that high doses of glucocorticoids can decrease antioxidant defense, while corticosterone treatment had no effect on CAT activity.

In response to the lipid peroxidation and oxidative stress induced by prednisolone, the present study investigated the potential effective role of Ajwa date extract against lipid peroxidation induced prednisolone. Several studies have shown that Ajwa dates have antioxidant, antiviral, antifungal, antibacterial, antidiabetic, anti-inflammatory, anticancer, hypolipidemic, gastrointestinal protective, and cardioprotective properties **Khalid et al., 2017**. Ajwa dates contain a variety of polyphenols, carotenoids, sterols, and tannins **Alyahya et al., 2022**. Polyphenols are natural compounds that are found in food, including fruits, vegetables, cereals, and beverages. There has been a lot of focus on the possible health benefits of dietary polyphenols as antioxidants in the last decade; polyphenols can help with oxidative stability. Plant polyphenols have a plethora of health benefits in the diet. Cancer,

cardiovascular disease, diabetes, osteoporosis, and neurological diseases can be prevented by the long-term consumption of high plant polyphenol diets **Rahman et al., 2021**. figures (10) and (11) showed an increased levels of antioxidants GR, GSH, GPx, TAC, CAT, and SOD after Awja date extract administration in dosages (30 mg, 60 mg, 90 mg, and 120 mg) compared to the positive control, the best improvement observed with the highest dosage of Awja date extract (0.88 mg + 120 mg). The increased levels of endogenous antioxidants correspond to the decrease in MDA levels compared to the positive group shown in figure (12) with the best result of MDA reduction found to be with the highest dose (0.88 mg + 120 mg). Suggesting the potential role of natural antioxidants found in Awja dates in enhancing the endogenous antioxidant defense system and protecting the body from the harmful effects caused by lipid peroxidation and oxidative stress. Because nutritional antioxidants can adjust oxidative stress on several levels: by reducing the production of ROS and fixing the oxidized membranes, by neutralizing the free radicals, or through fat metabolism in which cholesteryl esters and short-chain free fatty acids neutralize ROS **Petrovic et al., 2020**. These results support the findings of **Baothman et al., 2023** who reported a substantial reduction in antioxidants enzymes GR, GPx, SOD, and CAT. In addition, MDA was massively increased due to oxidative stress and ROS production induced by Doxorubicin a chemotherapeutic drug (DOX) injection nephrotoxicity, and oral administration of Awja date aqueous extract generated improvements in the antioxidant enzyme activities besides decreasing MDA levels, attributing these positive effects to the active bioconstituents that remove free radicals and prevent lipid peroxidation. In addition to the study conducted by **Alqarni et al., 2019** who reported that a high-fat diet reduced antioxidant enzymes GPx, SOD, and CAT in rat serum and livers compared to

the negative control, suggesting the body faces the risks of various diseases due to the ROS formation, and that Ajwa date extract helped restore these enzymes in liver and serum, indicating the enhancement of Ajwa date extract to antioxidant enzymes to destroy (ROS) and prevent diseases. These findings support the therapeutic potentials of Awja dates in enhancing endogenous antioxidant enzymes, preventing lipid peroxidation and oxidative stress occurrence, thus protecting the body from various diseases.

4.3. Impact of Ajwa Date Extract on Liver Fats Induced by Prednisolone:

Table 10: Impact of Ajwa Date Extract on Liver Fats (Cholesterol, Tryglecerides, and GSH induced by prednisolone:

	Liver Lipids			
Dependent: GROUP	**Cholesterol (mg/dl)**	**Triglycerides (mg/dl)**	**Liver Fat %**	**GSH (mmol/g tissue)**
G1 Negative Control	6.21±0.19 ^{d}	6.18±0.18 ^{c}	6.12±0.03 ^{c}	9.43±0.38 ^{bc}
G2 Positive Control Prednisolone 0.88 mg	11.82±0.25 ^{b}	11.65±0.70 ^{a}	11.49±0.02^{a}	7.78±0.04 ^{d}
G3 Prednisolone 0.88 mg + ADE 30 mg	8.86±0.29 ^{c}	8.89±0.49 ^{b}	9.11±0.01^{b}	8.61±0.26 ^{cd}
G4 Prednisolone 0.88 mg + ADE 60 mg	7.44±0.79 ^{cd}	4.87±0.14 ^{cd}	8.5±0.2^{cd}	10.54±0.04 ^{b}
G5 Prednisolone 0.88 mg + ADE 90 mg	5.75±0.22 ^{d}	4.08±0.19 ^{d}	5.01±0.01^{d}	12.86±0.29 ^{a}
G6 Prednisolone 0.88 mg + ADE 120 mg	5.68±0.24 ^{d}	4.05±0.17 ^{d}	4.5±0.1^{d}	12.99±0.29 ^{a}

Note: *Kruskal Wallis (Kruskal .test), values are mean ± SD (standard deviation).

Means with different letters in the same column are significantly different at P≤0.001.

The positive control group that received prednisolone in table (10) showed a significant increase in liver cholesterol and triglycerides compared to the negative control group. In contrast, different doses of Awja date extract (30 mg, 60 mg, 90 mg, and 120 mg) showed a reduction of both cholesterol and triglyceride levels, the highest doses of G5 and G6 (90 mg and 120 mg respectively) were close to the values of the negative group. Liver fat percent

showed a significant increased level in G2 after prednisolone administration compared to the negative control, after the administration of Ajwa date extract, the percents started to decrease compared to the positive control, the lowest fat liver percent was observed with the highest doses of (90 mg and 120 mg respectively). Regarding GSH levels, prednisolone administration in G2 showed decreased levels compared to the negative group. Treatment with Awja date extract doses resulted in an increase in GSH levels, with the highest effect observed in the (90 mg and 120 mg) groups of Awja date extract.

Hepatic steatosis without any indication of hepatocellular damage, such as hepatocyte ballooning, is referred to as "Fatty Liver" or Non-alcoholic Fatty Liver Disease (NAFLD). According to **Antunes et al., 2023,** NAFLD is typically associated with metabolic syndrome, obesity, diabetes, and hyperlipidemia. Table (10) showed an increase in liver fat (cholesterol and triglycerides) in positive control compared to the negative, indicating that prednisolone administration is associated to elevated liver fats leading to fatty liver. **Rahimi et al., 2020** reported that glucocorticoid-induced increase in hepatic deposition of lipids (triglycerides) in most cases is mediated by multiple mechanisms, such as food intake increasing, stimulation of gluconeogenesis, and de novo fatty acid synthesis by high glucose, insulin, and glucocorticoid levels, and increased release of free fatty acids from adipose tissue and their uptake and deposition in the liver as triglycerides. Prednisolone administration is also associated with the reduction of hepatic GSH in the positive control compared to the negative control. GSH has a key function in protective processes. GSH participate in detoxification reactions of electrophilic substances like carcinogen-epoxide, detoxifies hydrogen peroxide, and lipid peroxidation **Kaplowitz, 1981.** The low GSH levels can further contribute to liver injury by lowering the antioxidant defense, allowing

inflammatory cytokines to be released and fibrosis to progress **Lu, 2020**. Through the results above, Awja date extract showed a decreased levels of cholesterol and triglycerides with an increase GSH levels compared to the positive group, with the best results observed with the doses (90 and 120 mg/kg). Based on their chemical structure, polyphenols can be broadly classified as flavonoids or nonflavonoids, and they may have a role in reducing oxidative stress and inflammatory processes. A natural antioxidant found in large quantities in the human diet are flavonoids. These chemicals exhibit a variety of positive and antioxidative effects through various methods; in fact, they can target many pathways that may be involved in the pathophysiology of liver illnesses. By reducing lipogenic proteins and raising lipolytic proteins, they can regulate de novo lipogenesis. According to **Ferramosca et al., 2017**, they are also efficient scavengers of ROS and RNS (superoxide, hydrogen peroxide, and hydroxyl radicals), which are increased in pathological conditions and metabolic disorders such non-alcoholic fatty liver disease (NAFLD).

4.4. Liver Histopathology in Rats:

4.4.1. Evaluation of Protective Role of Ajwa Date Extract Against Prednisolone-Induced Damage in Liver Histology:

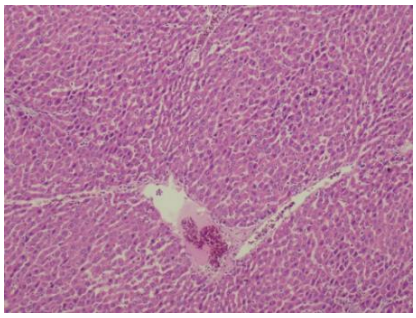


Figure 13: Liver Histology of Negative Control Group (H&E, Original magnification 100×). Scale bar: 50 μm.

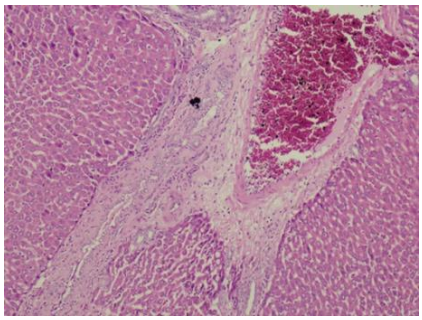


Figure 14: Liver Histology of Positive Control Group Prednisolone (0.88 mg/kg) (H&E, Original magnification 100×). Scale bar: 50 μm.

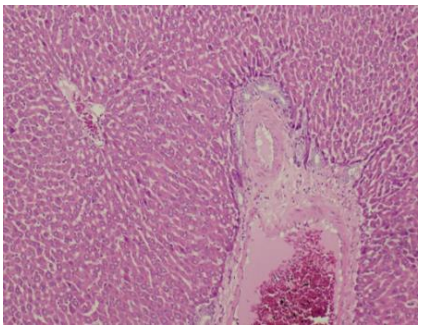


Figure 15: Liver Histology of Third Group Prednisolone (0.88 mg/kg) + (Ajwa Date Extract 30 mg/kg) (H&E, Original magnification 100×). Scale bar: 50 μm.

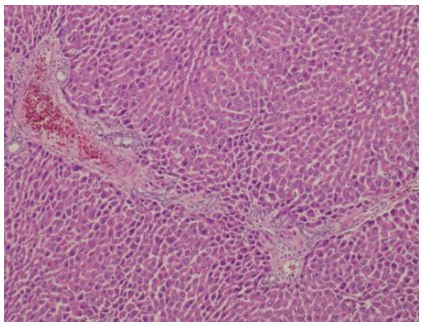


Figure 16: Liver Histology of Fourth Group Prednisolone (0.88 mg/kg) + (Ajwa Date Extract 60 mg/kg) (H&E, Original magnification 100×). Scale bar: 50 μm.

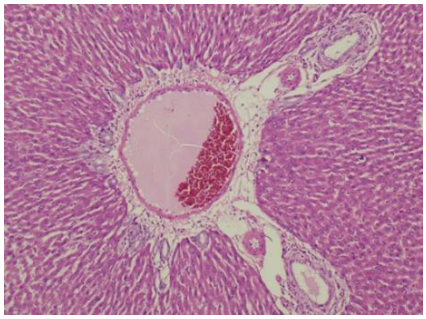


Figure 17: Liver Histology of Fifth Group Prednisolone (0.88 mg/kg) + (Ajwa Date Extract 90 mg/kg) (H&E, Original magnification 100×). Scale bar: 50 μm.

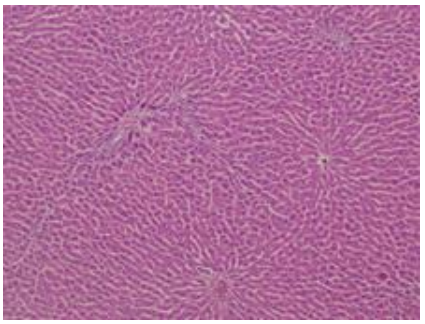


Figure 18: Liver Histology of Sixth Group Prednisolone (0.88 mg/kg) + (Ajwa Date Extract 120 mg/kg) (H&E, Original magnification 100×). Scale bar: 50 μm.

The previous figures show hepatocytes with their normal, sinusoidal, central vein, and hepatic architecture are all present in figure (13) the normal control rat group. Figure (14) exhibits evidence of a large portal tract-central vein dilatation with a noticeable increase in collagen fibers in response to chronic inflammation and collagen fiber deposition surrounding the portal tract. The third group in figure (15) receiving (prednisolone 0.88 mg/kg + Ajwa date extract 30 mg/kg) shows evidence of a moderate dilatation of the central vein and portal area with a noticeable increase in hemorrhage, edema, and moderate fibrosis. The fourth group in figure (16) displays a mildly dilated central vein with minimal fibrosis, congestion, inflammation, and normal hepatic architecture. Group five in figure (17), general lobular architecture preservation, moderate leukocytic infiltration is observed along with scanty congestion, edema, and portal system swelling. The sixth group in figure (18) reveals typical sinusoids, hepatocytes with regeneration features, and normal hepatic architecture. The results indicate that prednisolone (0.88 mg/kg) exhibit negative affect on liver fat as supported by the previous liver

fats, which shown in table (10) an increase levels of cholesterol and triglycerides compared to the negative control group. The administration of Ajwa dates extract in doses (30, 60, 90, and 120 mg/kg) respectively reduced prednisolone side effects compared to the positive control group.

Chapter 5
Recommendations and Conclusion

5. Conclusion and Recommendations:

5.1. Conclusion:

The present study focused on prednisolone administration and its relationship to antioxidant disorders, which resulted in dysregulation of the antioxidant defense system GPx, GSH, GR, SOD, CAT, and TAC, leading to oxidative stress and lipid peroxidation, which is connected to various diseases such as cardiovascular disease, cancer, diabetes. Moreover, prednisolone increased blood glucose, lipid profile levels and decreased HDL levels, leading to atherosclerosis, which is the major cause of cardiovascular diseases. Interestingly, Ajwa dates contain high amounts of antioxidants like polyphenols and flavonoids, sugar, minerals, vitamins, and fiber. Even though protein and fats are present in small quantities, it means that Ajwa date is a rich source of nutrients. The study demonstrated that Ajwa date extract in 30 mg, 60 mg, 90 mg, and 120 mg dosages significantly improved glucose levels, lipid profile, and the antioxidant defense system by increasing GPx, GR, GSH, TAC, CAT and SOD levels and decreasing MDA levels, resulting in the reduction of lipid peroxidation induced by prednisolone. These outcomes highlight the potential therapeutic value of natural antioxidants, such as those found in Ajwa dates, to protect against antioxidant disorders and lipid peroxidation-related disorders. Ajwa date extract could be a promising intervention with the c, with its natural contents to prevent oxidative damage and maintain health.

5.2. Recommendations:

1. Including plant food in the diet may provide a protective benefit against oxidative stress-induced conditions due to the high content of plant antioxidants.

2. Considering Ajwa date extract as an intervention with corticosteroid drugs to safeguard the antioxidant defense system of dysregulation caused by lipid peroxidation.

3. Ajwa date extract can make a good supplement to help reduce corticosteroid side effects.

4. Future studies should evaluate Ajwa date extract on long-term administration.

5. Further studies are needed to detect the mechanism of different antioxidant parameters influenced by Ajwa date extract.

Chapter 6
References

6. References:

6.1. Arabic References:

الاستاذ المساعد نورة الغملاس. (2020). تمر العجوة في السنة النبوية مكانته واستخداماته. مجلة جامعة الشارقة للعلوم الشرعية والدراسات الإسلامية, 17(2) , 413-440

من أبحاث المؤتمر العالمي العاشر للإعجاز العلمي في القرآن والسنة بتركيا 1432هـ – 2011م
د/ أروى عبد الرحمن احمد (يرحمها الله)، قسم علوم الحياة – ميكروبيولوجي – كلية العلوم – جامعة صنعاء – اليمن.

أميرة كاظم ناصر، رغدان هاشم محسن و جواد كاظم مهدي، استخلاص وتشخيص المركبات الفينولية من بذور الكينوا وتقدير فعاليتها كمضادات للأكسدة. (2019)، قسم الإنتاج الحيواني- كلية الزراعة و الكلية الطبية التقنية- جامعة البصرة- جمهورية العراق.

.) المزيني, حلا, فقيه, إيمان, الحميد, نوره, ... & جاد الله السيد. (2024). فوائد منتجات تمر الخلاص كبديل طبيعي للسكر والمحليات في تصنيع الأغذية. مجلة الإسكندرية للتبادل العلمى, (Phoenix dactylifera L) .45(4), 661-674

6.2. English References:

Abd Allah, H., Lahmer, R., & Dogman, M. (2020). Chemical and Microbial Properties and Energy Content of Rub Al-Tamr Made by Traditional and Modern Methods. The Scientific Journal of King Faisal University. Basic and Applied Sciences, 21(2).

Abbas, M., Saeed, F., Anjum, F. M., Afzaal, M., Tufail, T., Bashir, M. S., … Suleria, H. A. R. (2016). Natural polyphenols: An overview. *International Journal of Food Properties*, *20*(8), 1689–1699. https://doi.org/10.1080/10942912.2016.1220393

Aebi H. (1984). Catalase in vitro. Methods in enzymology, 105, 121–126. https://doi.org/10.1016/s0076-6879(84)05016-3

Al-Alawi, R. A., Al-Mashiqri, J. H., Al-Nadabi, J. S., Al-Shihi, B. I., & Baqi, Y. (2017). Date palm tree (Phoenix dactylifera L.): natural products and therapeutic options. .Frontiers in plant science, 8, 845

Albano, G. D., Gagliardo, R. P., Montalbano, A. M., & Profita, M. (2022). Overview of the Mechanisms of Oxidative Stress: Impact in Inflammation of the Airway Diseases. Antioxidants, 11(11), 2237. https://doi.org/10.3390/antiox11112237

Alduwayghiri, R., & Algheshairy, R. M. (2023). Effect of Ajwa Date and Germinated Barley on Lipid Profile in Rats Fed High-Fat Diet. Applied Sciences, 13(10), 6043.

Al-Farsi, M., Alasalvar, C., Morris, A., Baron, M., & Shahidi, F. (2005). Comparison of antioxidant activity, anthocyanins, carotenoids, and phenolics of three native fresh and sun-dried date (Phoenix dactylif- era L.) varieties grown in Oman. Journal of

Agricultural Food Chemistry, 53, 7592–7599. https://doi.org/10.1021/jf050579q.

Al-Farsi MA, Lee CY. (2008).Nutritional and functional properties of dates: A review. Crit Rev Food Sci Nutr. 2008;48:877–887.

Al-Harrasi, A., Rehman, N. U., Hussain, J., Khan, A. L., Al-Rawahi, A., Gilani, S. A., ... & Ali, L. (2014). Nutritional assessment and antioxidant analysis of 22 date palm (Phoenix dactylifera) varieties growing in Sultanate of Oman. Asian Pacific journal of tropical medicine, 7, S591-S598.

Ali, B. H, Mousa, H. M and Al Mougy, S. A. (2003). The effect of water extract and anthocyanins of Hibiscus sabdariffa on paracetamol – induced hepatotoxicity in rats. Phytotherapy Research, 117: 56-59.

Aljohani, A. K., Maghrabi, N. A., Alrehili, O. M., Alharbi, A. S., Alsihli, R. S., Alharthe, A. M., ... & Hussein, M. F. (2025). Ajwa date extract (Phoenix dactylifera L.): Phytochemical analysis, antiviral activity against herpes simplex virus-I and coxsackie B4 virus, and in silico study. Saudi Medical Journal, 46(1), 26.

Allain, C. C., Poon, L. S., Chan, C. S., Richmond, W., & Fu, P. C. (1974). Enzymatic determination of total serum cholesterol. Clinical chemistry, 20(4), 470–475.

Alloubani, A., Nimer, R., & Samara, R. (2021). Relationship between Hyperlipidemia, Cardiovascular Disease and Stroke: A Systematic Review. Current cardiology reviews, 17(6), e051121189015. https://doi.org/10.2174/1573403X16999201210200342

Alqahtani, B. A., & Alenazi, A. M. (2024). A national perspective on cardiovascular

diseases in Saudi Arabia. *BMC Cardiovascular Disorders*, *24*(1), 184.

Alqarni, M.M., Osman, M.A., Al-Tamimi, D.S., Gassem, M.A., Al-Khalifa, A.S., Al-Juhaimi, F. and Mohamed Ahmed, I.A., 2019. Antioxidant and antihyperlipidemic effects of Ajwa date (Phoenix dactylifera L.) extracts in rats fed a cholesterol-rich diet. Journal of food biochemistry, 43(8), p.e12933.

Alsarayrah, N. A., Oma, E. A., Alsanad, S. M., Arsad, H., Abudahash, M. M., ALEnazi, F. K., & Alenzi, N. D. (2023). The health values of Phoenix dactylifera (dates): A review. Emirates Journal of Food and Agriculture, 35, 1-16.

AlShwyeh, H., & Almahasheer, H. (2022). Glucose content of 35 Saudi Arabian date fruits (Phoenix dactylifera L.). Journal of the Saudi Society of Agricultural Sciences, 21(6), 420-424.

Alyahya, S., Arif, M., Wasim, R., & Kushwaha, S. P. (2022). Phoenix dactylifera L.(Ajwa Dates): An energetic plant fruit with immense nutraceuticals value for strengthening the power of resistance. Journal of Pharmacognosy and Phytochemistry, 11(3), 151-162.

Antunes C, Azadfard M, Hoilat GJ, et al. Fatty Liver. [Updated 2023 Jan 1]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441992/

AOAC (2005): Official Methods of Analysis of the Association of Official Analytical Chemists.18th Edition, Washington DC

AOAC (1990): Association of Official Analytical Chemist. Official Methods of Analysis. Washington DC, United States: Association of Official Analytical Chemists, 1990; P. 20-26.

Arshad, M. S., Batool, S. M., Khan, M. K., Imran, M., Ahmad, M. H., Anjum, F. M., & Hussain, S. (2019). Bio-evaluation of functional date bars using rats as model organism against hypercholesterolemia. Lipids in Health and Disease, 18, 1-13.

Arulselvan P, Fard MT, Tan WS, Gothai S, Fakurazi S, Norhaizan ME, Kumar SS. Role of Antioxidants and Natural Products in Inflammation. Oxid Med Cell Longev. 2016;2016:5276130. doi: 10.1155/2016/5276130. Epub 2016 Oct 10. PMID: 27803762; PMCID: PMC5075620.

Assirey, E. A. (2021). The chemical composition, total phenolic and antioxidant content of four date palm saudi cultivars. Journal of Taibah University for Science, 15(1), 282–287. https://doi.org/10.1080/16583655.2021.1978805

Assirey, E. A. R. (2015). Nutritional composition of fruit of 10 date palm (Phoenix dactylifera L.) cultivars grown in Saudi Arabia. Journal of Taibah University for science, 9(1), 75-79.

Baothman, O. A., Altayb, H. N., Zeyadi, M. A., Hosawi, S. B., & Abo-Golayel, M. K. (2023). Phytochemical analysis and nephroprotective potential of Ajwa date in doxorubicin-induced nephrotoxicity rats: Biochemical and molecular docking approaches. *Food Science & Nutrition*, *11*(3), 1584-1598.

Barham, D., & Trinder, P. (1972). An improved colour reagent for the determination of blood glucose by the oxidase system. The Analyst, 97(151), 142–145. https://doi.org/10.1039/an9729700142

Basil Ribeiro. (2023). Glutathione: the master antioxidant. Ozone Therapy Global Journal Vol. 13, nº 1, pp 175-197

Benmeddour, Z., Mehinagic, E., Le Meurlay, D., & Louaileche, H. (2013). Phenolic composition and antioxidant capacities of ten Algerian date (Phoenix dactylifera L.) cultivars: a comparative study. *Journal of Functional Foods*, *5*(1), 346-354.

Bethesda, Md. : U. S. Dept. of Health, Education, and Welfare, Public Health Service, National Institutes of Health; 1979

Better Health Channel, Antioxidants, 2021, https://www.betterhealth.vic.gov.au/health/healthyliving/antioxidants

Bezerra, D. P., Soares, A. K., & de Sousa, D. P. (2016). Overview of the Role of Vanillin on Redox Status and Cancer Development. Oxidative medicine and cellular longevity, 2016, 9734816. https://doi.org/10.1155/2016/9734816

Bhatti, S.G., & Bhatti, A.G. (2019). Characterization of Nutritional and Bioactive Compounds in Ajwa in Comparison to other Five Varieties of Palm Dates.

Bhosale PB, Ha SE, Vetrivel P, Kim HH, Kim SM, Kim GS. Functions of polyphenols and its anticancer properties in biomedical research: a narrative review. Transl Cancer Res. 2020 Dec;9(12):7619-7631. doi: 10.21037/tcr-20-2359. PMID: 35117361; PMCID: PMC8798728.

Buchman, Alan L. M.D., M.S.P.H.. Side Effects of Corticosteroid Therapy. Journal of Clinical Gastroenterology 33(4):p 289-294, October 2001.

Bulugahapitiya, V. P. (2020). NATURAL ANTIOXIDANTS THEIR ROLE & ANALYSIS.

BEUTLER, E., DURON, O., & KELLY, B. M. (1963). Improved method for the determination of blood glutathione. The Journal of laboratory and clinical medicine, 61, 882–888.

Cardoso, S. M., & Fassio, A. (2020). The Antioxidant Capacities of Natural Products 2019. *Molecules (Basel, Switzerland)*, *25*(23), 5676. https://doi.org/10.3390/molecules25235676

Caro-Ordieres, T., Marín-Royo, G., Opazo-Ríos, L., Jiménez-Castilla, L., Moreno, J. A., Gómez-Guerrero, C., & Egido, J. (2020). The Coming Age of Flavonoids in the Treatment of Diabetic Complications. Journal of Clinical Medicine, 9(2), 346. https://doi.org/10.3390/jcm9020346

Carta, G., Murru, E., Banni, S., & Manca, C. (2017). Palmitic Acid: Physiological Role, Metabolism and Nutritional Implications. Frontiers in physiology, 8, 902. https://doi.org/10.3389/fphys.2017.00902

Centers for Disease Control and Prevention, United State, Chronic diseases, 2023, https://www.cdc.gov/chronic-disease/about/index.html

Cleveland Clinic Abu Dhabi- Long-Term Problems for People with Diabetes- 2017- https://www.clevelandclinicabudhabi.ae/en/health-hub/health-resource/diseases-and-conditions/long-term-problems-for-people-with-diabetes.

Cleveland Clinic, Corticosteroids (Glucocorticoids) Overview, an article Last reviewed on

10/21/2024, https://my.clevelandclinic.org/health/treatments/corticosteroids-glucocorticoids

Cleveland Clinic, Hyperlipidemia, Overview (What is hyperlipidemia?), An article last reviewed on 08/04/2022, https://my.clevelandclinic.org/health/diseases/21656-hyperlipidemia

Cordiano, R., Di Gioacchino, M., Mangifesta, R., Panzera, C., Gangemi, S., & Minciullo, P. L. (2023). Malondialdehyde as a Potential Oxidative Stress Marker for Allergy-Oriented Diseases: An Update. Molecules, 28(16), 5979. https://doi.org/10.3390/molecules28165979

Cui X, Gong J, Han H, He L, Teng Y, Tetley T, Sinharay R, Chung KF, Islam T, Gilliland F, Grady S, Garshick E, Li Z, Zhang J. Relationship between free and total malondialdehyde, a well-established marker of oxidative stress, in various types of human biospecimens. J Thorac Dis 2018;10(5):3088-3097. doi: 10.21037/jtd.2018.05.92

de Carvalho, C. C. C. R., & Caramujo, M. J. (2018). The Various Roles of Fatty Acids. Molecules (Basel, Switzerland), 23(10), 2583. https://doi.org/10.3390/molecules23102583

Dewanti, A. A. W., Susanto, J., Apriliawati, D., & Yuliawati, T. H. (2024). Effect of Ajwa Date Fruit (Phoenix dactylifera) Methanol Extract on Blood Glucose Levels in Mice with Diabetes Mellitus.

Di Cesare, M., Perel, P., Taylor, S., Kabudula, C., Bixby, H., Gaziano, T. A., McGhie, D. V., Mwangi, J., Pervan, B., Narula, J., Pineiro, D., & Pinto, F. J. (2024). The Heart of the World. *Global heart*, *19*(1), 11. https://doi.org/10.5334/gh.1288

Didier AJ, Stiene J, Fang L, Watkins D, Dworkin LD, Creeden JF. Antioxidant and Anti-Tumor Effects of Dietary Vitamins A, C, and E. Antioxidants (Basel). 2023 Mar 3;12(3):632. doi: 10.3390/antiox12030632. PMID: 36978880; PMCID: PMC10045152.

Djuricic, I., & Calder, P. C. (2021). Beneficial Outcomes of Omega-6 and Omega-3 Polyunsaturated Fatty Acids on Human Health: An Update for 2021. Nutrients, 13(7), 2421. https://doi.org/10.3390/nu13072421

Ebru Bardas, Yusuf Kemal Arslan, Seher Polat, Mine Erisir, Gözde Atila Uslu, Nihal Cetin, Betul Cicek. Vitamin E and Selenium Reduce Prednisolone Side Effects in Rat Hearts. Int. J. Vitam. Nutr. Res. 2020, 90(3-4), 309–317. https://doi.org/10.1024/0300-9831/a000582

Farag, M. A., & Gad, M. Z. (2022). Omega-9 fatty acids: potential roles in inflammation and cancer management. Journal, genetic engineering & biotechnology, 20(1), 48. https://doi.org/10.1186/s43141-022-00329-0

Fatima, M. T., Bhat, A. A., Nisar, S., Fakhro, K. A., & Al-Shabeeb Akil, A. S. (2022). The role of dietary antioxidants in type 2 diabetes and neurodegenerative disorders: An assessment of the benefit profile. Heliyon, 9(1), e12698. https://doi.org/10.1016/j.heliyon.2022.e12698.

Feldman F, Koudoufio M, Desjardins Y, Spahis S, Delvin E, Levy E. Efficacy of Polyphenols in the Management of Dyslipidemia: A Focus on Clinical Studies. Nutrients. 2021 Feb 19;13(2):672. doi: 10.3390/nu13020672. PMID: 33669729; PMCID: PMC7922034.

Feldman, F., Koudoufio, M., Desjardins, Y., Spahis, S., Delvin, E., & Levy, E. (2021).

Efficacy of Polyphenols in the Management of Dyslipidemia: A Focus on Clinical Studies. Nutrients, 13(2), 672. https://doi.org/10.3390/nu13020672

Ferramosca, A., Di Giacomo, M., & Zara, V. (2017). Antioxidant dietary approach in treatment of fatty liver: New insights and updates. World journal of gastroenterology, 23(23), 4146–4157. https://doi.org/10.3748/wjg.v23.i23.4146

FOLCH, J., LEES, M., & SLOANE STANLEY, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. The Journal of biological chemistry, 226(1), 497–509.

Food and drugs Administration, United States, Finding and Learning about Side Effects (adverse reactions), Content current as of 08/08/2022, https://www.fda.gov/drugs/find-information-about-drug/finding-and-learning-about-side-effects-adverse-reactions

Fossati, P., & Prencipe, L. (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clinical chemistry, 28(10), 2077–2080.

Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry, 18(6), 499–502.

Ghnimi, S., Umer, S., Karim, A., & Kamal-Eldin, A. (2017). Date fruit (Phoenix dactylifera L.): An underutilized food seeking industrial valorization. NFS journal, 6, 1-10.

Goldberg, D.M. and Spooner, R.J. (1983) Assay of Glutathione Reductase. In: Bergmeyen, H.V., Ed., Methods of Enzymatic Analysis, 3rd Edition, Vol. 3, Verlog Chemie,

Deerfiled Beach, 258-265.

Goszcz, K., Duthie, G. G., Stewart, D., Leslie, S. J., & Megson, I. L. (2017). Bioactive polyphenols and cardiovascular disease: chemical antagonists, pharmacological agents or xenobiotics that drive an adaptive response?. British journal of pharmacology, 174(11), 1209–1225. https://doi.org/10.1111/bph.13708

Grabež, M., Škrbić, R., Stojiljković, M. P., Vučić, V., Rudić Grujić, V., Jakovljević, V., Djuric, D. M., Suručić, R., Šavikin, K., Bigović, D., & Vasiljević, N. (2022). A prospective, randomized, double-blind, placebo-controlled trial of polyphenols on the outcomes of inflammatory factors and oxidative stress in patients with type 2 diabetes mellitus. Reviews in cardiovascular medicine, 23(2), 57. https://doi.org/10.31083/j.rcm2302057

Grasso, A. W., & Rocco, M. B., The Dyslipidemias, 2016, The Cleveland Clinic Cardiology Board Review.

Hamishehkar, H., Ranjdoost, F., Asgharian, P., Mahmoodpoor, A., & Sanaie, S. (2016). Vitamins, Are They Safe?. Advanced pharmaceutical bulletin, 6(4), 467–477. https://doi.org/10.15171/apb.2016.061

Harvard T.H Chan, School of Public Health, Antioxidants, 2023, https://www.hsph.harvard.edu/nutritionsource/antioxidants/#introduction

Hassanpour, S. H., & Doroudi, A. (2023). Review of the antioxidant potential of flavonoids as a subgroup of polyphenols and partial substitute for synthetic antioxidants. Avicenna journal of phytomedicine, 13(4), 354–376. https://doi.org/10.22038/AJP.2023.21774

Hastings J, Owen G, Dekker A, Ennis M, Kale N, Muthukrishnan V, Turner S, Swainston N, Mendes P, Steinbeck C. (2016). ChEBI in 2016: Improved services and an expanding collection of metabolites. Nucleic Acids Res.

Havel, R. J.; Anderson, D. W.; Kostner, G. M.; Mahley, R. W.; Patsch, J. R.

Hodgens A, Sharman T. Corticosteroids. [Updated 2023 May 1]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554612/

Holesh JE, Aslam S, Martin A. Physiology, Carbohydrates. [Updated 2023 May 12]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459280/

Humason, G.L. (1979) Animal Tissue Techniques. 4th Edition, W.H. Freeman, San Francisco.

Ibrahim, A. S., Sukor, R., Anwar, F., Murugesu, S., Selamat, J., & Siva, R. (2024). Nutritional, nutraceutical attributes, microbiological and chemical safety of different varieties of dates—A review. Future Foods, 100421.

Jeeva, J. S., Sunitha, J., Ananthalakshmi, R., Rajkumari, S., Ramesh, M., & Krishnan, R. (2015). Enzymatic antioxidants and its role in oral diseases. Journal of pharmacy & bioallied sciences, 7(Suppl 2), S331–S333. https://doi.org/10.4103/0975-7406.163438

Jennifer Moll, Medications That Cause High Cholesterol Levels, an article updated on June 19, 2023, https://www.verywellhealth.com/which-drugs-can-raise-cholesterol-

levels-698229?utm_source=chatgpt.com

Jewell, D. E., Motsinger, L. A., & Paetau-Robinson, I. (2024). Effect of dietary antioxidants on free radical damage in dogs and cats. Journal of Animal Science, 102, skae153.

Jubayer, F., Kayshar, S., & Rahaman, M. (2020). Effects of Ajwa date seed powder on serum lipids in humans: A randomized, double-blind, placebo-controlled clinical trial. Journal of Herbal Medicine, 24, 100409.

Juhi Modi, Prednisone vs Prednisolone: What's the Difference?, an article medically reviewed by HaVy Ngo-Hamilton, Pharm.D., Updated Apr 10, 2023, https://www.buzzrx.com/blog/prednisone-vs-prednisolone-whats-the-difference

Juhi Modi, Prednisone vs Prednisolone: What's the Difference?, an article updated on Apr 10, 2023, https://www.buzzrx.com/blog/prednisone-vs-prednisolone-whats-the-difference

Kaplowitz N. (1981). The importance and regulation of hepatic glutathione. *The Yale journal of biology and medicine*, *54*(6), 497–502.

Khalid, S., Ahmad, A., Masud, T., Asad, M. J., & Sandhu, M. (2016). Nutritional assessment of Ajwa date flesh and pits in comparison to local varieties. JAPS: Journal of Animal & Plant Sciences, 26.(4)

Khalid, S., Arshad, M., Saad, B., Imran, M., Ul, H. B., Ain, T. T., ... & Ahmed, S. (2020). Cardio protective effects of dates. International Journal of Biosciences, 6655, 110-123.

Khalid, S., Khalid, N., Khan, R.S., Ahmed, H. and Ahmad, A., 2017. A review on chemistry and pharmacology of Ajwa date fruit and pit. Trends in food science & technology, 63, pp.60-69.

Khan F, Ahmed F, Pushparaj PN, Abuzenadah A, Kumosani T, Barbour E, AlQahtani M, Gauthaman K. Ajwa Date (Phoenix dactylifera L.) Extract Inhibits Human Breast Adenocarcinoma (MCF7) Cells In Vitro by Inducing Apoptosis and Cell Cycle Arrest. PLoS One. 2016 Jul 21;11(7):e0158963. doi: 10.1371/journal.pone.0158963. PMID: 27441372; PMCID: PMC4956039.

Khan MA, Siddiqui S, Ahmad I, Singh R, Mishra DP, Srivastava AN, Ahmad R. Phytochemicals from Ajwa dates pulp extract induce apoptosis in human triple-negative breast cancer by inhibiting AKT/mTOR pathway and modulating Bcl-2 family proteins. Sci Rep. 2021 May 14;11(1):10322. doi: 10.1038/s41598-021-89420-z. PMID: 33990623; PMCID: PMC8121835.

Khan, F., Khan, T.J., Kalamegam, G. et al. Anti-cancer effects of Ajwa dates (Phoenix dactylifera L.) in diethylnitrosamine induced hepatocellular carcinoma in Wistar rats. BMC Complement Altern Med 17, 418 (2017). https://doi.org/10.1186/s12906-017-1926-6.

Kharal, S., Siddique, F., Arshad, M., Iftikhar, K., Akhtar, M. T., Qadir, R., ... & Xia, L. (2023). Assessment of phytochemicals, antioxidant properties, and in vivo antidiarrheal activity of date palm (Phoenix dactylifera L.). International Journal of Food Properties, 26(1), 2243-2255.

Khutami, C., Sumiwi, S. A., Khairul Ikram, N. K., & Muchtaridi, M. (2022). The Effects of Antioxidants from Natural Products on Obesity, Dyslipidemia, Diabetes and Their Molecular Signaling Mechanism. International journal of molecular sciences, 23(4),

2056. https://doi.org/10.3390/ijms23042056

Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S., & Cosic, V. (2001). Method for the measurement of antioxidant activity in human fluids. Journal of clinical pathology, 54(5), 356–361. https://doi.org/10.1136/jcp.54.5.356

Korczowska-Łącka, I., Słowikowski, B., Piekut, T., Hurła, M., Banaszek, N., Szymanowicz, O., Jagodziński, P. P., Kozubski, W., Permoda-Pachuta, A., & Dorszewska, J. (2023). Disorders of Endogenous and Exogenous Antioxidants in Neurological Diseases. Antioxidants (Basel, Switzerland), 12(10), 1811. https://doi.org/10.3390/antiox12101811

Kruskal, W. H., & Wallis, W. A. (1952). Use of ranks in one-criterion variance analysis. Journal of the American statistical Association, 47(260), 583-621.

Kwon, D. H., Cha, H. J., Lee, H., Hong, S. H., Park, C., Park, S. H., Kim, G. Y., Kim, S., Kim, H. S., Hwang, H. J., & Choi, Y. H. (2019). Protective Effect of Glutathione against Oxidative Stress-induced Cytotoxicity in RAW 264.7 Macrophages through Activating the Nuclear Factor Erythroid 2-Related Factor-2/Heme Oxygenase-1 Pathway. Antioxidants (Basel, Switzerland), 8(4), 82. https://doi.org/10.3390/antiox8040082

Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010 Jul;4(8):118-26. doi: 10.4103/0973-7847.70902. PMID: 22228951; PMCID: PMC3249911.

Lu, S. C. (2020). Dysregulation of glutathione synthesis in liver disease. Liver Research, 4(2), 64-73.

Lubis, S. M., & Deliana, M. (2023, September). Association between Steroid Therapy and Lipid Profiles in Children with Chronic Disease. In HORMONE RESEARCH IN PAEDIATRICS (Vol. 96, pp. 219-219). ALLSCHWILERSTRASSE 10, CH-4009 BASEL, SWITZERLAND: KARGER.

Macdonald, J.F., Han, Y., Astafyeva, Y. *et al.* Exploring *Tetraselmis chui* microbiomes—functional metagenomics for novel catalases and superoxide dismutases. *Appl Microbiol Biotechnol* **109**, 6 (2025). https://doi.org/10.1007/s00253-024-13395-w

Mani, V., Arfeen, M., Sajid, S., & Almogbel, Y. (2022). Aqueous Ajwa dates seeds extract improves memory impairment in type-2 diabetes mellitus rats by reducing blood glucose levels and enhancing brain cholinergic transmission. Saudi Journal of Biological Sciences, 29(4), 2738-2748.

Mani, V., Arfeen, M., Sajid, S., & Almogbel, Y. (2022). Aqueous Ajwa dates seeds extract improves memory impairment in type-2 diabetes mellitus rats by reducing blood glucose levels and enhancing brain cholinergic transmission. Saudi Journal of Biological Sciences, 29(4), 2738-2748.

Mao, C., Lei, G., Horbath, A. and Gan, B., 2022. Assessment of lipid peroxidation in irradiated cells. In Methods in Cell Biology (Vol. 172, pp. 37-50). Academic Press.

Mauludiyana S, Aryati, Dachlan YP, Saputro ID. Anti-inflammatory and antibacterial potential of Ajwa date (Phoenix dactylifera L.) extract in burn infection. J Adv Pharm Technol Res. 2023 Jul-Sep;14(3):161-165. doi: 10.4103/japtr.japtr_138_23. Epub 2023 Jul 28. PMID: 37692010; PMCID: PMC10483919.

Maury, G. L., Rodríguez, D. M., Hendrix, S., Arranz, J. C. E., Boix, Y. F., Pacheco, A. O., Díaz, J. G., Morris-Quevedo, H. J., Dubois, A. F., Aleman, E. I., Beenaerts, N.,

Méndez-Santos, I. E., Ratón, T. O., Cos, P., & Cuypers, A. (2020). Antioxidants in Plants: A Valorization Potential Emphasizing the Need for the Conservation of Plant Biodiversity in Cuba. Antioxidants (Basel, Switzerland), 9(11), 1048. https://doi.org/10.3390/antiox9111048

Mayo Clinic, Drugs and Supplements, Prednisolone (Oral Route), 2023, https://www.mayoclinic.org/drugs-supplements/prednisolone-oral-route/proper-use/drg-20075189

Minami, M., & Yoshikawa, H. (1979). A simplified assay method of superoxide dismutase activity for clinical use. Clinica chimica acta; international journal of clinical chemistry, 92(3), 337–342. https://doi.org/10.1016/0009-8981(79)90211-0

Ministry of Health- Diabetes- 2024 - https://www.moh.gov.sa/en/awarenessplateform/ChronicDisease/Pages/Diabetes.aspx.

Ministry of health, Cardiovascular diseases, heart diseases, 2020, https://www.moh.gov.sa/HealthAwareness/EducationalContent/Diseases/Heartcirculatory/Pages/HealthDay-2013-9-25-001.aspx

Ministry Of Health, Cardiovascular Diseases, Hypercholesteremia (High Cholesterol), Last Update : 27 February 2020 09:32 AM, https://www.moh.gov.sa/en/HealthAwareness/EducationalContent/Diseases/Heartcirculatory/Pages/001.aspx

Mirghani H. O. (2024). Effect of dates on blood glucose and lipid profile among patients with type 2 diabetes. World journal of diabetes, 15(6), 1079–1085. https://doi.org/10.4239/wjd.v15.i6.1079

Mirończuk-Chodakowska, I., Witkowska, A. M., & Zujko, M. E. (2018). Endogenous non-enzymatic antioxidants in the human body. Advances in medical sciences, 63(1), 68-78.

Mohamed, R. M., Fageer, A. S., Eltayeb, M. M., & Mohamed Ahmed, I. A. (2014). Chemical composition, antioxidant capacity, and mineral extractability of S udanese date palm (P hoenix dactylifera L.) fruits. Food Science & Nutrition, 2(5), 478-489.

Mopuri, R., Kalyesubula, M., Rosov, A., Edery, N., Moallem, U., & Dvir, H. (2021). Improved Folch method for liver-fat quantification. Frontiers in Veterinary Science, 7, 594853.

Moron, M. S., Depierre, J. W., & Mannervik, B. (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochimica et biophysica acta, 582(1), 67–78. https://doi.org/10.1016/0304-4165(79)90289-7

Moussa, Z., Judeh, Z. M., & Ahmed, S. A. (2019). Nonenzymatic exogenous and endogenous antioxidants. Free radical medicine and biology, 1, 11-22.

Mucha P, Skoczyńska A, Małecka M, Hikisz P, Budzisz E. Overview of the Antioxidant and Anti-Inflammatory Activities of Selected Plant Compounds and Their Metal Ions Complexes. Molecules. 2021 Aug 12;26(16):4886. doi: 10.3390/molecules26164886. PMID: 34443474; PMCID: PMC8398118.

Nasrullah PR, Sehar B, Safdar M, Fatima S, Zeb F, Shah J, Ali Khan Khalil A, Ahmed B, Alsubaie ASR, Zaman MH. Effect of Ajwa date pits powder (*Phoenix dactylifera* L.) on body composition, lipid profile and blood pressure in patients with hyperlipidemia: A randomized clinical trial. Avicenna J Phytomed. 2023 Mar-Apr;13(2):143-152. doi: 10.22038/AJP.2022.21316. PMID: 37333476; PMCID:

PMC10274311.

National Academies of Sciences, Engineering, and Medicine. 2023. Achieving Whole Health: A New Approach for Veterans and the Nation. Washington, DC: The National Academies Press. https://doi.org/10.17226/26854.

National Center for Biotechnology Information (2025). PubChem Compound Summary for CID 736186, Isoferulic acid. Retrieved April 16, 2025 from https://pubchem.ncbi.nlm.nih.gov/compound/Isoferulic-acid.

National Center for Chronic Disease Prevention and Health Promotion. (2023). About Chronic Diseases, https://www.cdc. gov chronicdisease about/index.htm.

National Health Service (NHS), High blood sugar (hyperglycaemia), 2022, https://www.nhs.uk/conditions/high-blood-sugar-hyperglycaemia/

Naziri, Z., Rahimlou, M., Rezaei, M., Tabrizi, R., Nasr, M., Motazedian, M., & Kardeh, S. (2024). High dietary antioxidant intake linked to lower risk of myocardial infarction: a nested case-control study. BMC Cardiovascular Disorders, 24(1), 485.

Nimse, S. B., & Pal, D. (2015). Free radicals, natural antioxidants, and their reaction mechanisms. RSC advances, 5(35), 27986-28006.

Niroumand, S., Khajedaluee, M., Khadem-Rezaiyan, M., Abrishami, M., Juya, M., Khodaee, G., & Dadgarmoghaddam, M. (2015). Atherogenic Index of Plasma (AIP): A marker of cardiovascular disease. Medical journal of the Islamic Republic of Iran, 29, 240.

Nishikimi, M., Rao, N. A., & Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and biophysical research communications, 46(2), 849-854.

Nonell, S., & Flors, C. (Eds.). (2016). Singlet Oxygen: Applications in Biosciences and Nanosciences, Volume 1. Royal Society of Chemistry.

Olorunnisola Olubukola Sinbad, Ajayi Ayodeji Folorunsho, Okeleji Lateef Olabisi, Oladipo Abimbola Ayoola, Emorioloye Johnson Temitope. Vitamins as Antioxidants. Journal of Food Science and Nutrition Research 2 (2019): 214-235.

Otitolaiye, Catherine & Orji, Blessing & B, Akintade. (2021). Characterization and Nutritional Composition of Dried Date fruit (Phoenix dactylifera). 10.15413/ajmp.2021.0111.

Paglia, D. E., & Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. The Journal of laboratory and clinical medicine, 70(1), 158–169.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. Journal of nutritional science, 5, e47. https://doi.org/10.1017/jns.2016.41

Pandey, K. B., & Rizvi, S. I. (2010). Markers of oxidative stress in erythrocytes and plasma during aging in humans. Oxidative medicine and cellular longevity, 3(1), 2–12. https://doi.org/10.4161/oxim.3.1.10476

Panova, I. G., & Tatikolov, A. S. (2023). Endogenous and Exogenous Antioxidants as Agents Preventing the Negative Effects of Contrast Media (Contrast-Induced

Nephropathy). Pharmaceuticals (Basel, Switzerland), 16(8), 1077. https://doi.org/10.3390/ph16081077

Parvez, R., Gautam, A., & David, J. (2021). Study on antioxidant activity and health benefits of Ajwa dates. The Pharma Innovation Journal, 10(9), 10-13.

Patlevič, P., Vašková, J., Švorc Jr, P., Vaško, L., & Švorc, P. (2016). Reactive oxygen species and antioxidant defense in human gastrointestinal diseases. Integrative medicine research, 5(4), 250-258.

Petrovic, Snjezana, Aleksandra Arsic, Danijela Ristic-Medic, Zorica Cvetkovic, and Vesna Vucic. 2020. "Lipid Peroxidation and Antioxidant Supplementation in Neurodegenerative Diseases: A Review of Human Studies" Antioxidants 9, no. 11: 1128. https://doi.org/10.3390/antiox9111128

Pham-Huy, L. A., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. International journal of biomedical science : IJBS, 4(2), 89–96.

Pickup M. E. (1979). Clinical pharmacokinetics of prednisone and prednisolone. Clinical pharmacokinetics, 4(2), 111–128. https://doi.org/10.2165/00003088-197904020-00004

Pirsch, J. D.; D'Alessandro, A. M.; Sollinger, H. W.; Knechtle, S. J.; Reed, A.; Kalayoglu, M.; Belzer, F. O. paper from 1992, titled "Hyperlipidemia and transplantation: etiologic …" in the Journal of the American Society of Nephrology (JASN).

Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A. Oxidative Stress: Harms and Benefits for Human Health. Oxid Med Cell

Longev. 2017;2017:8416763. doi: 10.1155/2017/8416763. Epub 2017 Jul 27. PMID: 28819546; PMCID: PMC5551541.

Prenesti, E., Berto, S., & Tardito, D. (2021). Total Antioxidant Capacity: A Biomarker of Oxidative Stress in Health and Disease. Antioxidants, 10(1), 58. https://doi.org/10.3390/antiox10010058

Puckett Y, Gabbar A, Bokhari AA. Prednisone. [Updated 2023 Jul 19]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan- Available from: https://www.ncbi.nlm.nih.gov/books/NBK534809./

Quinkler M, Ekman B, Marelli C, Uddin S, Zelissen P, Murray RD; EU-AIR Investigators. Prednisolone is associated with a worse lipid profile than hydrocortisone in patients with adrenal insufficiency. Endocr Connect. 2017 Jan;6(1):1-8. doi: 10.1530/EC-16-0081. Epub 2016 Nov 18. PMID: 27864317; PMCID: PMC5148794.

Rafieian-Kopaei, M., Setorki, M., Doudi, M., Baradaran, A., & Nasri, H. (2014). Atherosclerosis: process, indicators, risk factors and new hopes. International journal of preventive medicine, 5(8), 927–946.

Rahimi, L., Rajpal, A., & Ismail-Beigi, F. (2020). Glucocorticoid-Induced Fatty Liver Disease. *Diabetes, metabolic syndrome and obesity : targets and therapy*, *13*, 1133–1145. https://doi.org/10.2147/DMSO.S247379

Rahman, M. M., Rahaman, M. S., Islam, M. R., Rahman, F., Mithi, F. M., Alqahtani, T., Almikhlafi, M. A., Alghamdi, S. Q., Alruwaili, A. S., Hossain, M. S., Ahmed, M., Das, R., Emran, T. B., & Uddin, M. S. (2021). Role of Phenolic Compounds in Human Disease: Current Knowledge and Future Prospects. Molecules (Basel, Switzerland), 27(1), 233. https://doi.org/10.3390/molecules27010233

Ramjibhai, K. A., Arvindbhai, A. H., Yogeshbhai, D. M., & Firozbhai, B. M. A. (2025). Estimation Of Antioxidant Enzyme Levels in Individuals with Regular and Irregular .Sleep Patterns. European Journal of Cardiovascular Medicine, 15, 789-792

Rasheed Z. (2024). Therapeutic potentials of catalase: Mechanisms, applications, and future perspectives. International journal of health sciences, 18(2), 1–6.

Ross, I L, & Marais, A D. (2014). The influence of glucocorticoids on lipid and lipoprotein metabolism and atherosclerosis. SAMJ: South African Medical Journal, 104(10), 671-674. Retrieved February 12, 2025, from http://www.scielo.org.za/scielo.php?script=sci_arttext&pid=S0256-95742014001000015&lng=en&tlng=en.

Ruwizhi, N., & Aderibigbe, B. A. (2020). Cinnamic Acid Derivatives and Their Biological Efficacy. International journal of molecular sciences, 21(16), 5712. https://doi.org/10.3390/ijms21165712

Sa'adah, N. N., Purwani, K. I., Nurhayati, A. P. D., & Ashuri, N. M. (2017, June). Analysis of lipid profile and atherogenic index in hyperlipidemic rat (Rattus norvegicus Berkenhout, 1769) that given the methanolic extract of Parijoto (Medinilla speciosa). In AIP Conference Proceedings (Vol. 1854, No. 1). AIP Publishing.

Sakai, T., & Morimoto, Y. (2022). The History of Infectious Diseases and Medicine. Pathogens (Basel, Switzerland), 11(10), 1147. https://doi.org/10.3390/pathogens11101147

Saleh, E. A., Tawfik, M. S., & Abu-Tarboush, H. M. (2011). Phenolic contents and antioxidant activity of various date palm (Phoenix dactylifera L.) fruits from Saudi Arabia. Food and Nutrition Sciences, 2011.

Salehi, M., Mashhadi, N. S., Esfahani, P. S., Feizi, A., Hadi, A., & Askari, G. (2021). The Effects of Curcumin Supplementation on Muscle Damage, Oxidative Stress, and Inflammatory Markers in Healthy Females with Moderate Physical Activity: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial. International journal of preventive medicine, 12, 94. https://doi.org/10.4103/ijpvm.IJPVM_138_20

Sarangarajan, R. S. P. G., Meera, S., Rukkumani, R., Sankar, P., & Anuradha, G. (2017). Antioxidants: Friend or foe?. Asian Pacific journal of tropical medicine, 10(12), 1111-1116.

Satoh K. (1978). Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica chimica acta; international journal of clinical chemistry, 90(1), 37–43. https://doi.org/10.1016/0009-8981(78)90081-5

Saudi Health Council- Diabetes Overview- 2021- https://shc.gov.sa/Arabic/NewNDC/Diabetes/Pages/default.aspx.

Senaphan, K., Kukongviriyapan, U., Sangartit, W., Pakdeechote, P., Pannangpetch, P., Prachaney, P., Greenwald, S. E., & Kukongviriyapan, V. (2015). Ferulic Acid Alleviates Changes in a Rat Model of Metabolic Syndrome Induced by High-Carbohydrate, High-Fat Diet. Nutrients, 7(8), 6446–6464. https://doi.org/10.3390/nu7085283

Senyilmaz-Tiebe, D., Pfaff, D. H., Virtue, S., Schwarz, K. V., Fleming, T., Altamura, S., Muckenthaler, M. U., Okun, J. G., Vidal-Puig, A., Nawroth, P., & Teleman, A. A. (2018). Dietary stearic acid regulates mitochondria in vivo in humans. Nature communications, 9(1), 3129. https://doi.org/10.1038/s41467-018-05614-6

Silvestrini, A., Meucci, E., Ricerca, B. M., & Mancini, A. (2023). Total Antioxidant

Capacity: Biochemical Aspects and Clinical Significance. International journal of molecular sciences, 24(13), 10978. https://doi.org/10.3390/ijms241310978

Singh K, Bhori M, Kasu YA, Bhat G, Marar T. Antioxidants as precision weapons in war against cancer chemotherapy induced toxicity - Exploring the armoury of obscurity. Saudi Pharm J. 2018 Feb;26(2):177-190. doi: 10.1016/j.jsps.2017.12.013. Epub 2017 Dec 19. PMID: 30166914; PMCID: PMC6111235.

Stein, E. A., & Myers, G. L. (1995). National Cholesterol Education Program recommendations for triglyceride measurement: executive summary. The National Cholesterol Education Program Working Group on Lipoprotein Measurement. Clinical chemistry, 41(10), 1421–1426.

Steven, S., Frenis, K., Oelze, M., Kalinovic, S., Kuntic, M., Bayo Jimenez, M. T., ... & Daiber, A. (2019). Vascular inflammation and oxidative stress: major triggers for cardiovascular disease. Oxidative medicine and cellular longevity, 2019(1), 7092151.

Stoakes, Shelley Farrar. 2019. Lipid Peroxidation. News-Medical, viewed 15 October 2023, https://www.news-medical.net/life-sciences/Lipid-Peroxidation.aspx.

Suh, S., & Park, M. K. (2017). Glucocorticoid-induced diabetes mellitus: an important but overlooked problem. Endocrinology and metabolism, 32(2), 180.

Tiwari, P., Singh, N. & Sharma, B. Long Term Treatment of Corticostreroids May Cause Hepatotoxicity and Oxidative Damage: A Case Controlled Study. Ind J Clin Biochem 39, 179–187 (2024). https://doi.org/10.1007/s12291-023-01127-2

Torres, R. L., Torres, I. L., Laste, G., Ferreira, M. B., Cardoso, P. F., & Belló-Klein, A. (2014). Effects of acute and chronic administration of methylprednisolone on oxidative stress in rat lungs. Jornal brasileiro de pneumologia : publicacao oficial da Sociedade Brasileira de Pneumologia e Tisilogia, 40(3), 238–243. https://doi.org/10.1590/s1806-37132014000300006

Townsend, D. M., Tew, K. D., & Tapiero, H. (2003). The importance of glutathione in human disease. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 57(3-4), 145–155. https://doi.org/10.1016/s0753-3322(03)00043-x

Tsao R., Yang R., Young J.C., Zhu H. Polyphenolic profiles in eight apple cultivars using high–performance liquid chromatography (HPLC) J. Agric. Food Chem. 2003;51:6347–6353.

Trinder, P. (1969) Enzymatic determination of glucose in blood serum. Annals of Clinical Biochemistry, 6, 24.

Wang, Y., Branicky, R., Noë, A., & Hekimi, S. (2018). Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. The Journal of cell biology, 217(6), 1915–1928. https://doi.org/10.1083/jcb.201708007

Whelan, J., & Fritsche, K. (2013). Linoleic acid. Advances in nutrition, 4(3), 311-312.

Wibawa, J. C., Febrianto, N., Fudin, M. S., Ockta, Y., & Festiawan, R. (2025). The mechanism of physical exercise increases glutathione peroxidase as an endogenous antioxidant: a systematic review. Retos, 63, 610-619.

World Health Orgnization, Noncommunicable diseases, 16 Sep 2023, https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases.

World Heart Report 2023: Confronting the World's Number One Killer. Geneva, Switzerland. World Heart Federation. 2023.

Wu, J., Mackie, S. L., & Pujades-Rodriguez, M. (2020). Glucocorticoid dose-dependent risk of type 2 diabetes in six immune-mediated inflammatory diseases: a population-based cohort analysis. BMJ Open Diabetes Research and Care, 8(1), e001220.

Yadav, U.C.S. (2015). Oxidative Stress-Induced Lipid Peroxidation: Role in Inflammation. In: Rani, V., Yadav, U. (eds) Free Radicals in Human Health and Disease. Springer, New Delhi. https://doi.org/10.1007/978-81-322-2035-0_9

Zango, O., Rey, H., Bakasso, Y., Lecoustre, R., Aberlenc, F., & Pintaud, J. C. (2016). Local practices and knowledge associated with date palm cultivation in Southeastern Niger. Agricultural Sciences, 7(09), 586-603.

Zduńska, K., Dana, A., Kolodziejczak, A., & Rotsztejn, H. (2018). Antioxidant properties of ferulic acid and its possible application. Skin pharmacology and physiology, 31(6), 332-336.

Zheng, M., Liu, Y., Zhang, G., Yang, Z., Xu, W., & Chen, Q. (2023). The Applications and Mechanisms of Superoxide Dismutase in Medicine, Food, and Cosmetics. Antioxidants (Basel, Switzerland), 12(9), 1675. https://doi.org/10.3390/antiox12091675

Zöllner, N., & Kirsch, K. (1962). Colorimetric method for determination of total lipids. Journal of Experimental Medicine, 135, 545-550.

Zhu, X., Yu, L., Zhou, H., Ma, Q., Zhou, X., Lei, T., ... & Lei, S. (2018). Atherogenic index of plasma is a novel and better biomarker associated with obesity: a population-based cross-sectional study in China. Lipids in Health and Disease, 17, 1-6.

Appendix

Appendix:

Appendix A: The Approval of Committee Research Ethics, Deanship of Scientific Research, Qassim University.

Kingdom of Saudi Arabia
Ministry of Education
Qassim University
Deanship of Graduate Studies
and Scientific Research

المملكة العربية السعودية
وزارة التعليم
جامعة القصيم
عمادة الدراسات العليا
والبحث العلمي

Date: Thursday, February 13, 2025 No: 25-25-11

Subject: موافقة اللجنة الدائمة لأخلاقيات البحث العلمي

To:

Name	Position/ department	Contribution
Samiyah Saify Eisa Alshelaly	Master's Student,College of Agriculture and Veterinary MedicineDepartment of Food Science and Human Nutrition	Principal investigator
Dr.Waheeba Alfekki Ahmad Mohammad, Dr. Hassan Mirghani Mousa		Co-investigator

From: Committee Research Ethics, Deanship of Scientific Research, Qassim University.

Research title: "Protection effect of Bioactivity of polyphenolics extract from Ajwa Dates against the drug Prednisolone -induced Antioxidants Disorder and Lipid Peroxidation in male rats"

Study Setting: Qassim University

Study Design: Experimental study

Dear P.I.,
We are pleased to inform you that the Committee of Research Ethics had approved your research proposal.
Upon receiving this approval, you may commence your field work at your convenience.

- You should be responsible for upholding the confidentiality of participant's data.
- **If any work conducted outside Qassim University, a written approval should be obtained from the concerned authority.**
- Kindly, update us on your project advancement every 6 months. On completion of your project, kindly send to us a summary of the project final report.
- Finally, be aware that that this approval embraces no financial, or any other, obligations or responsibilities on Qassim University.

Note: Any corrections and/or alterations of this certificate will make it invalid.
For queries, please call Dr. Osamah Al Rugaie at telephone No. +966163010355, and e-mail: bioethics@qu.edu.sa .
Best regards

د.الرقيعي

Chairman, Committee of Research Ethics
Qassim University

Dr. Ziyad M. Almohaimeed

P.o. Box:6555-Buraidah:51452-Tel:016380170S-0163014004 Fax(016)3801709

وGR وGPx وTAC وCAT مقارنة بالضوابط السلبية. وعلاوة على ذلك، كانت هناك زيادة في نسبة الكوليسترول والدهون الثلاثية في الكبد، بالإضافة إلى انخفاض في مستويات GHS، مقارنة بالضوابط السلبية. كما لوحظ ارتفاع ملحوظ في ألياف الكولاجين في الأنسجة المرضية لخلايا الكبد استجابة للالتهاب المستمر. على العكس من ذلك، أظهر مستخلص العجوة (30 ملغ، 60 ملغ، 90 ملغ، و120 ملغ) تحسنًا ملحوظًا ($P < 0.001$) في مستويات سكر الدم ومستوى الدهون من خلال خفضها وزيادة مستويات البروتين الدهني عالي الكثافة (HDL). كما خفّض مستخلص العجوة مستويات MDA وCAT، مع تعزيز دفاعات الجسم المضادة للأكسدة (SOD، GSH، GPx، GR، وTAC). علاوة على ذلك، وبالمقارنة مع المجموعة الضابطة، أظهرت خلايا الكبد تحسنًا ملحوظًا. يُقلّل مستخلص العجوة، بمحتواه المضاد للأكسدة، بشكل ملحوظ من الخلل التأكسدي، وارتفاع مستوى الجلوكوز، وارتفاع مستوى الدهون الناتج عن البريدنيزولون، وخاصةً عند جرعة 120 ملغ/كغ. وتشير خصائصه المضادة للأكسدة إلى أنه قد يكون مفيدًا كمكمل غذائي إلى جانب الكورتيكوستيرويدات لتقليل الإجهاد التأكسدي والحفاظ على الصحة العامة.

التأثير الوقائي للنشاط الحيوي لمستخلص البوليفينول من تمر العجوة ضد اضطراب مضادات الأكسدة وأكسدة الدهون الناجم عن عقار بريدنيزولون في ذكور الفئران

اسم الباحثة: سامية صيفي عيسى الشلالي.

بريدنيزولون هو كورتيكوستيرويد يُستخدم عادةً للعلاج قصير الأمد. ومع ذلك، قد يؤدي استخدامه على المدى الطويل إلى مضاعفات خطيرة، بما في ذلك ارتفاع مستوى شحميات الدم، مما قد يُحفز الإجهاد التأكسدي وبيروكسيد الدهون. تُعرف المركبات الفينولية بقدرتها المضادة للأكسدة، إذ تُكسر سلاسل التفاعل التأكسدي، مما يجعلها تُشكل خط الدفاع الأول ضد الجذور الحرة. تهدف هذه الدراسة إلى تقييم النشاط الحيوي الوقائي للبوليفينولات من مستخلص تمر العجوة (30، 60، 90، و120 ملغ/كغ من وزن الجسم) ضد اضطرابات مضادات الأكسدة المسببة بدواء بريدنيزولون وأكسدة الدهون المسبقة، واكتشاف النشاط المضاد للأكسدة والمواد الكيميائية النباتية في مستخلص تمر العجوة، وقياس تأثيره على اضطرابات مضادات الأكسدة وأكسدة الدهون المسببة بدواء بريدنيزولون لدى ذكور الجرذان. أدى تقييم تمر العجوة ومستخلصها إلى اكتشاف مجموعة متنوعة من المركبات، بما في ذلك مضادات الأكسدة مثل حمض الفيروليك وحمض اللاكتيك وحمض السيناميك والفانيلين، بالإضافة إلى الكربوهيدرات الموجودة في كلٍّ من تمر العجوة ومستخلصها، مثل بيتا-دي-جلوكوبيرانوز، مشتق TMS5، ودي-جلوكوبيرانوز، مشتق TMS5، ودي-فركتوز، مشتق TMS5، وأما دي-جلوكوز، مشتق TMS5 موجود في العجوة فقط. تتكون الأحماض الدهنية في كلٍّ من تمر العجوة ومستخلصها من حمض البالمتيك، وحمض اللينوليك، وحمض الأوليك، وحمض الستياريك. يحتوي تمر العجوة ومستخلصها على معادن؛ كان الكالسيوم أعلى في تمر العجوة منه في مستخلص العجوة (1290 ملغم/كجم، 380 ملغم/لتر على التوالي)، وكان الصوديوم أقل في تمر العجوة منه في مستخلص العجوة (100 ملغم/كجم، 930 ملغم/لتر على التوالي)، وكان البوتاسيوم أعلى في كل من تمر العجوة ومستخلص العجوة بين جميع المعادن، بينما كان في تمر العجوة أقل منه في مستخلص العجوة (4600 ملغم/كجم، 5900 ملغم/لتر على التوالي)، وكان الفوسفور أعلى في تمر العجوة منه في دبس العجوة (700 ملغم/كجم، 360 ملغم/لتر على التوالي)، وكان الزنك أيضًا أعلى في تمر العجوة منه في مستخلص العجوة (4.5 ملغم/كجم، 3.6 ملغم/لتر على التوالي)، وأخيرًا، لوحظ المغنيسيوم فقط في مستخلص العجوة (400 ملغم/لتر). بعد أسبوع واحد من التأقلم، تم تقسيم ثلاثين من ذكور جرذان ويستار البيضاء إلى ست مجموعات. كانت المجموعة 1 هي الضابطة السلبية، وأعطيت المجموعة 2 بريدنيزولون (0.88 ملغ) كمجموعة ضابطة إيجابية، وعولجت المجموعات 3 و4 و5 و6 بمستخلص العجوة (30 و60 و90 و120 ملغ/كغ) لمدة سبعة أيام، تلا ذلك إعطاء نفس جرعات العجوة لنفس المجموعات مع إضافة البريدنيزولون (0.88 ملغ). أظهرت النتائج بعد تناول البريدنيزولون في المجموعة الإيجابية زيادة كبيرة ($P < 0.001$) في نسبة الجلوكوز في الدم ومستوى الدهون وانخفاض مستويات HDL وزيادة مستويات MDA وSOD وانخفاض نظام الدفاع المضاد للأكسدة GHS

الملخص العربي

شكر وتقدير

﴿ربِّ أوزعني أن أشكر نعمتك التي أنعمت عليّ وعلى والديّ وأن أعمل صالحًا ترضاه، وأدخلني برحمتك في عبادك الصالحين﴾ (سورة النمل، الآية ١٩). ﴿بسم الله الرحمن الرحيم﴾ الحمد لله رب العالمين، وصلى الله وسلم على أشرف الأنبياء والمرسلين، نبينا محمد، وعلى آله وصحبه أجمعين. الحمد لله على نعمه العظيمة التي أنعم بها عليّ. الحمد لله الذي منّ عليّ بكرمه، ويسر لي طريق العلم، ويسر لي إتمام رسالتي. ما كنت لأحقق شيئًا لولا فضله وتوفيقه.

كما قال نبينا محمد صلى الله عليه وسلم: "من لم يشكر الناس لم يشكر الله". صحيح (الألباني). أتقدم بخالص الشكر والتقدير لمن ساعدني في رحلتي للوصول إلى هذه المرحلة. إلى نور دربي، وسندي، الذي كان –بعد الله– سببًا في بدء هذه الرحلة وإنهائها بدعمه اللامحدود، إلى والدي الغالي **صيفي عيسى الشلالي**. إلى مصدر سعادتي، التي أعتز بها كثيرًا، والدتي الحبيبة **منيفة سعود الشلالي**، لتشجيعها المتواصل واستعدادها الدائم لمساعدتي في أي وقت. إلى جميع عائلتي، إخوتي وأخواتي، وخاصةً أخواتي **علياء وسمية وابنة عمي غادة**، لإيمانهم الدائم بي ودعمهم المتواصل. إلى جميع أصدقائي الأعزاء، لوقوفهم الدائم بجانبي ودعواتهم الدائمة لي وتشجيعهم لي، وخاصةً **رنا محمد أبو وردة**، التي ساعدتني أيضًا في شراء المعدات وصبرتني في أصعب الأوقات. ولا يسعني إلا أن أتقدم بالشكر والامتنان لمشرفتي، الدكتورة **وهيبة الفكي أحمد**، التي ساهمت في إنجاح هذا العمل، لقد كان حضورها الدائم، وتوجيهها العلمي، وإشرافها الصادق، ونصائحها القيّمة خيرَ عونٍ لي في إتمام رسالتي. كما أتقدم بجزيل الشكر والامتنان لأستاذي المشارك، الأستاذ الدكتور **حسن ميرغني موسى**، على تضحيته بوقته وتواجده الدائم للإجابة على أسئلتي، وصبره على تعليمي كيفية التعامل مع الفئران وكسر حاجز الخوف، وتوجيهه ودعمه الكريم، كما أتقدم بالشكر الجزيل لجميع أعضاء هيئة التدريس وزملائي في كلية الزراعة والأغذية بجامعة القصيم، ولكل من ساعدني بالمعروف ونصحني.

وأخيرًا، إن أصبت فمن الله، وإن أخطأت فمني ومن الشيطان. والحمد لله رب العالمين، وصلى الله على نبينا محمد وعلى آله وصحبه أجمعين.

المملكة العربية السعودية

وزارة التعليم

جامعة القصيم

كلية الزراعة والأغذية

قسم علوم الأغذية وتغذية الإنسان

" التأثير الوقائي للنشاط الحيوي لمستخلص البوليفينول من تمر العجوة ضد اضطراب مضادات الأكسدة وأكسدة الدهون الناجم عن عقار بريدنيزولون في ذكور الفئران.

إعداد الطالبة:

سامية صيفي عيسى الشلالي.

الرقم الجامعي: 441212013

تقرير اللجنة:

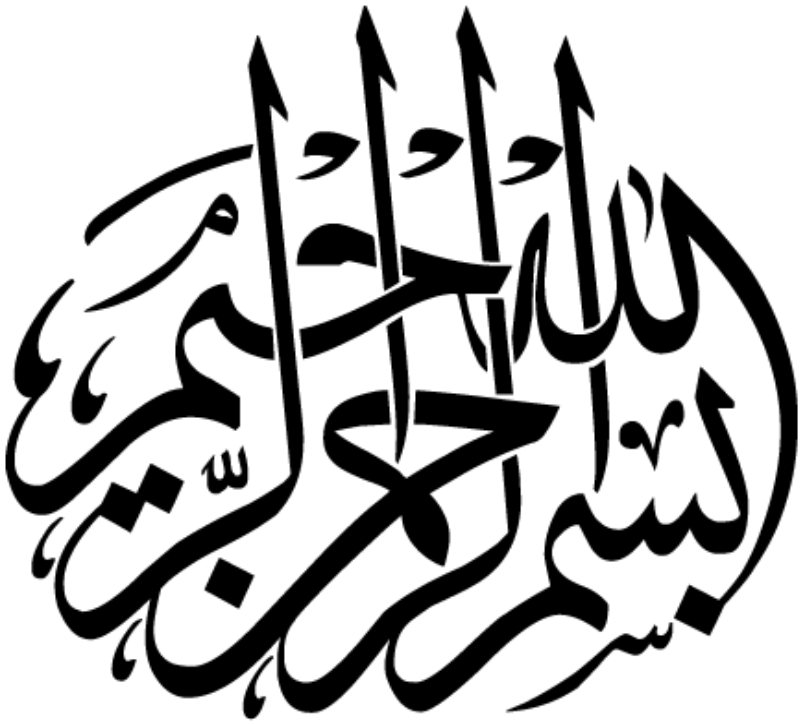
تمت الموافقة على قبول هذه الرسالة استكمالًا لمتطلبات

درجة الماجستير في تخصص تغذية الإنسان

لجنة المناقشة والحكم على الرسالة

أعضاء اللجنة	الاسم	المرتبة العلمية	التخصص	التوقيع
المشرف الرئيس	د. وهيبة الفكي أحمد	أستاذ مشارك	تغذية الانسان	
المشرف المساعد	أ.د. حسن ميرغني موسى	أستاذ	الكيمياء الحيوية	
المناقش الداخلي	د. هاشم سليمان إبراهيم	أستاذ مشارك	التغذية والتغذية العلاجية	
المناقش الداخلي	د. هند فيصل الحربي	أستاذ مشارك	تغذية الانسان	
المناقش الداخلي	د. ندى عبدالله الزنيدي	أستاذ مساعد	تغذية الانسان	

(1447/06/10هـ - 2025/12/01م)


بسم الله الرحمن الرحيم

المملكة العربية السعودية

وزارة التعليم

جامعة القصيم

كلية الزراعة

قسم علوم الأغذية وتغذية الإنسان

" التأثير الوقائي للنشاط الحيوي لمستخلص البوليفينول من تمر العجوة ضد اضطراب مضادات الأكسدة وأكسدة الدهون الناجم عن عقار بريدنيزولون في ذكور الفئران.

رسالة مقدمة لاستكمال متطلبات الحصول على (درجة الماجستير) التخصص العام (علوم الأغذية وتغذية الإنسان) والتخصص الدقيق (تغذية إنسان)

إعداد الطالبة:

سامية صيفي عيسى الشلالي.

الرقم الجامعي: 441212013

مشرف رئيس

الدكتورة / وهيبة الفكي أحمد محمد

أستاذ مشارك في قسم علوم الأغذية وتغذية الإنسان – كلية الزراعة والأغذية – جامعة القصيم

الأستاذ الدكتور/ حسن ميرغني موسى

أستاذ في قسم علوم الأغذية وتغذية الإنسان – كلية الزراعة والأغذية – جامعة القصيم

(1447 هـ - 2025 م)