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Purposeful scientific research as a pillar of sustainable development

The effect of organic extract of the plant Zand al-Arus on fatty acids in the cholesterol ester fraction of rats with induced diabetes

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ABSTRACT

This study dealt with the extraction of a number of active compounds from the Ammi majus plant through three extracts (organic, alcoholic, and aqueous). Then, these extracts were used on five groups of rats infected with alloxan-induced diabetes. These groups were (normal control, positive control, and three groups treated with the extracts). The percentage of fatty acids of all types, saturated, monounsaturated, and polyunsaturated, in the blood serum of the five groups above was monitored. Significant differences were observed in the percentage of fatty acids of all types in the three parts of the blood serum: cholesterol ester, triglycerides, and phospholipids, when comparing the control groups with the groups of infected rats treated with the extracts. The results showed that the organic extract was the best type of extract due to its content of several important compounds, such as apigenin, p-coumaric acid, sinapic acid, and ferulic acid. These compounds had a positive effect on improving the percentage of unsaturated fatty acids in the three parts of blood serum.

Keywords: Zand al-Arous, diabetes, fatty acids

Introduction

Medicinal Plants

Medicinal plants are widely popular for producing health-related products, especially medicines, due to their content of biologically active chemical compounds such as terpenoids, anthocyanins, flavonoids, tannins, and alkaloids, which have been extensively used in traditional medicine (Rojas et al., 2022). According to the World Health Organization, about 80% of the global population relies on traditional medicine to meet their healthcare needs, mainly because of its low cost and easy accessibility (Alemu et al., 2024). Compounds extracted from plants are considered highly safe for therapeutic use due to their minimal side effects and high efficacy. These active components, also known as secondary metabolites or secondary receptors, are plant metabolic products that humans cannot easily synthesize (Anwar et al., 2019; Máthé & Khan, 2022). More than 1200 species of medicinal plants are known to be used in the treatment of diabetes (Tran et al., 2020). Furthermore, for many people living in rural areas, medicinal plants are the most commonly used source of therapy. The use of medicinal herbs by indigenous populations is related to

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ethnobotany, a branch of botany that studies the importance of plants based on their traditional medicinal uses for various health complications (Nazir et al., 2021). Until the twentieth century, most of the therapeutic properties of medicinal plants were derived from plant extracts, usually prepared as ethanol or water infusions. The therapeutic effects of these extracts were likely due to a complex mixture of biologically active natural products, although the presence of harmful substances could not be excluded. Later efforts focused on identifying individual bioactive components within these complex plant extracts to be used as single-component regulatory drugs, such as paclitaxel, an anticancer drug derived from Taxus brevifolia, and artemisinin, an antimalarial drug (Zotchev, 2023).

Experimental Plant

The plant known as Ammi majus L. is a wild medicinal plant belonging to the genus Ammi in the Umbelliferae family. It is also known as bishop's weed or local khalla. This plant is characterized by its ability to grow in tropical, subtropical, and temperate regions and is considered an annual or biennial plant (Fathallah et al., 2023; Al-Jumaili et al., 2023). Ammi majus has been used in The bride's forelock has been used in folk medicine long before it gained acceptance in modern medicine. It is rich in biologically active compounds such as coumarin, flavonoids, and essential oils, which are found in all its parts.Recent studies indicate that this plant possesses a wide range of therapeutic benefits. It has been used as a diuretic, immune stimulant, and antioxidant, in addition to its role in treating diabetes, dizziness, spastic bronchitis, abdominal cramps, and kidney stones (Al-Jumaili et al., 2023). Recent research also demonstrates that the bride's forelock possesses multiple therapeutic properties that qualify it as an effective component in traditional and modern treatments for a variety of ailments (Chishti et al., 2024).

Diabetes Mellitus (DM):

DM is a chronic disease with a rapidly increasing prevalence in the 21st century, with incidence rates more than quadrupling since 1980, making it a leading cause of premature death (Yari et al., 2023; Lazić et al., 2024). Diabetes is defined as a chronic metabolic disorder characterized by persistently high blood glucose levels, resulting from impaired insulin production, insulin resistance, or both (Yedjou et al., 2023). It is one of the most commonly diagnosed metabolic problems in both humans and animals and is a major health concern due to its negative effects on overall health and long-term well-being (Díaz et al., 2023; Vallianou et al., 2021). Diabetes has multiple causes, including genetic factors, poor nutrition, environmental influences, and some infectious agents (Dahiya and Nigam, 2023). The increasing prevalence of this disease is due to societal and environmental factors, including rapid population growth, aging, diets rich in calories and fats, and a sedentary lifestyle. According to the latest statistics, approximately 463 million people worldwide suffer from diabetes, with this number expected to increase significantly in the near future. In light of this, researchers have begun to study and use medicinal plants as alternative or complementary treatment options, as these natural remedies have gained increasing attention as a strategy for the prevention and management of diabetes (Mustapha et al., 2023).

Materials and Methods

The thorny plant (Ammi majus L.) was collected during its ripening period from the forests within Mosul city and the green spaces within the University of Mosul campus. Following collection, the plant underwent a thorough cleaning process using distilled water to remove any impurities or suspended dust. The samples were then dried in a shaded environment at moderate temperatures to avoid damage to the active ingredients. They were placed on large-sized filter papers with periodic stirring to ensure uniform drying and prevent mold. After the drying process was complete, the plant was ground into a fine powder using a specialized mill. The resulting product was then passed through a fine sieve to separate the particles and standardize the powder size. Finally, appropriate extraction processes were performed to extract the active

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chemical compounds from the prepared powder, in preparation for subsequent research and laboratory studies. Extraction using a continuous Soxhlet apparatus

A continuous Soxhlet apparatus was used in the extraction process, using a petroleum ether solvent (60-40) to extract the plant compounds that dissolve in the organic extract. After obtaining the extract, it was dried using a rotary vacuum evaporator, which efficiently removes the solvent under low pressure and moderate temperature, resulting in a high-quality crude extract while preserving the biological activity of the components. (Al-Assaf, 2023).

Experimental design

Fifteen male rats, aged between two and three months and weighing between 250 and 300 grams, were raised. The animals were placed in designated plastic cages and divided into three groups, with five animals per group. Food and water were provided daily throughout the month-long experiment. (Kayode, 2023).

Group 1: (1) Negative control group (uninfected).

Group 1: (2) Positive control group (infected but untreated).

Group 1: (3) Infected and treated with the organic extract.

Then, diabetes was induced in rats by injection of alloxan at a dose of 100 mg/kg body weight (Ibrahim, 2023). After confirming the infection, the rats were monitored for two weeks. Then, Group 3 was treated with the treated extracts by oral administration at a dose of 150 mg/kg. Treatment continued for an additional two weeks (Ragab et al., 2023). After the treatment period ended, blood samples were drawn from the animals via the ophthalmic vein for the required analyses (Ibrahim, 2023). The analysis included measuring the concentration and types of fatty acids in the lipid fraction (cholesterol ester) of the serum.

Lipid Extraction

Extraction of Blood Lipids

1 ml of blood serum was taken for lipid extraction using the following steps: 2 ml of methanol was added to the blood serum, stirring well to ensure complete mixing. 4 ml of chloroform was then added while continuing to shake, and the resulting solution was filtered. The filtered solution was concentrated to 1 ml using nitrogen gas, leaving the lipids dissolved in 1 ml of methanol. These samples were then used to separate lipid components using thin-layer chromatography (TLC) (Folsom et al., 1996).

Analysis and separation of lipids using thin-layer chromatography (TLC).

Separation of Lipid Components Using Thin Layer Chromatography (TLC)

Basic Characteristics of Thin Layer Chromatography (TLC) Plates:

Preparing Chromatography Plates:

Thin-layer chromatography plates consist of a solid base, often made of glass, aluminum, plastic, or synthetic plastics, with standard dimensions of 20 x 20 cm. This base is covered with a thin layer of silica gel (0.25 mm thick). These plates are usually obtained ready-made from specialized companies such as SILICYCLE/Canda.

Solvent System

Several solvent systems can be used in the mobile phase to separate different types of lipids. However, the most stable and suitable mixture for this study is a mixture of hexane, ether, and formic acid in a volume ratio of 2:20:80 (Al-Tamer and Mahmood, 2004).

Sample Applications:

50 µL of serum extract solutions were placed on a chromatography plate using a micropipette. The plate was then allowed to flow upwards for 60 to 120 minutes inside the technology bath. After the flow was complete, the plate was removed from the bath, dried, and then sprayed with a developer to detect the separated compounds (Touchstone and Sherma, 1979).

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Visualization:

This study relied on the dye 2,7-dichlorofluorescein as a developer (Ma et al., 1995). When the plate was exposed to ultraviolet light at a wavelength of 310 nm, the lipid regions turned a yellow-green color (Touchstone and Sherma, 1979). After development, spots representing the major lipid types (phospholipids, cholesterol ester, triglycerides, and free cholesterol) appear. Each of these spots is removed by scraping off the silica layer, and the samples are then placed in separate tubes to convert the fatty acids into methyl esters.

Analysis and Identification of Fatty Acids

Re-esterification of Fatty Acids

The esterification method using boron trifluoride (BF3) dissolved in 16% methanol was used to analyze and re-esterify fatty acids.

This layer was transferred to sealed test tubes for subsequent analysis by capillary gas chromatography (CGC) (Ma et al., 1995).

Estimation of Propotion of Fatty Acids

The percentage of cholesterol esterified fatty acids in serum was estimated by injecting 1 µL of the sample prepared for measurement into a capillary gas chromatography (CGC).

Statistical analysis:

The results were analyzed using SPSS 22, with the accepted probability level being ($P>0.05$) (Urbano et al., 2019).

Results and Discussion

1- SFA

The results of this study showed the presence of an effect of the extracts of the Zand al-Arous plant on many biochemical variables, especially fatty acids of all types, in mice with induced diabetes. The results, as shown in Table 1, showed the presence of significant significant differences between the group of mice with diabetes compared to the group of mice treated with the extract. This indicates the role of the Zand al-Arous extract in improving a number of biochemical variables, especially fatty acids, which leads to an improvement in the condition of the group of patients. (Sunday et al., 2025).

These results confirm the role of plant extracts in preventing the deterioration of diabetes or improving health conditions associated with the disease, as well as their role in improving the process of fat metabolism. (Abbas et al., 2022). The results of this study showed a significant decrease in the level of saturated fatty acids, as shown in Table 1, in mice with alloxan-induced diabetes. This was due to the effect of alloxan on the pancreatic beta cells that produce insulin, leading to their destruction, which causes a major imbalance in the process of fat metabolism, including fatty acids. (Miao et al., 2023). It was noted that the organic and alcoholic extracts had a stronger and greater effect than the aqueous extract, due to the nature of the chemical compounds present in the organic and alcoholic extracts, such as flavonoids and cinnamic acids, which greatly affect the level of fatty acids in the blood serum. Organic extract was the best type in terms of its effect on the measured variables, as it contains the apigenin compound, which plays a major role in improving the rate of saturated fatty acids, as well as its role in working as an antioxidant and anti-inflammatory. (Aryal et al., 2024).

2-MUFA and PUFA

The results of this study showed a significant effect of the extract of the Zand al-Arus plant on the percentage of fatty acids in terms of the presence of significant changes in the metabolism of many fats, as the plant extract works to improve the levels of monounsaturated and polyunsaturated fatty acids in rats with newly treated diabetes compared to the group infected without treatment... as these fatty acids have a

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major role in improving the level of fats, reducing inflammation and increasing insulin sensitivity in the group treated with the extract. (Cheng et al., 2025). Studies have shown that the effect of the extract is on the level of Omega-3 and Omega-6 fatty acids, which in turn leads to a balance in the patient's general health, especially in the case of chronic diseases such as diabetes. (Ibrahiem et al., 2024). Increasing the ratio of omega-3 fatty acids to omega-6 fatty acids reduces inflammation, improves insulin sensitivity, and improves cardiovascular health (Kaltalioglu, 2023).

The effect of the organic extract of the Zand al-Arus plant is very significant on the level of polyunsaturated fatty acids in the cells of rats with induced diabetes by protecting the cell membranes and reducing the complications of diabetes. This is due to the compounds present in the composition of the organic extract, which work to improve the percentage of polyunsaturated and monounsaturated fatty acids in rats with diabetes and treated with the extract. (Kim et al., 2008; Abd El-Aleim, 2014).

Table 1. Percentage of fatty acids in blood serum for the three extracts

Groups	SFA	MUFA	PUFA
Group 1	30 ± 3.66*	5.10. ± 1.38*	34.21 ±5.15*
Group 2	27.67 ± 4.33	5.41 ±1.58	32.10 ±3.07
Group 3	18.33 ±1.88	3.66 ±1.81	18.56 ±4.83
Group 4	17.14 ±1.92	3.75 ±1.22	18.22 ±2.94
Group 5	16.93 ±2.44	3.69 ± 2.45	18.50 ±3.87

* Mean ± SD

Group 1- is the positive control group (healthy, uninfected). Group 2- is the negative control group (untreated, infected).

Group 3-is treated with the organic extract. Group4- is treated with the alcoholic extract.

Group 5- is treated with the aqueous extract.

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