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THE SYNERGISTIC IMPACTS OF URTICA DIOICA AND VITAMIN E ON HYPERGLYCEMIA, OXIDATIVE STRESS AND HISTOLOGICAL CHANGES IN DIABETIC ALBINO RATS

ABEER MANSOUR ABDEL RASOOL[1] and SAIF KHALID YAHYA [2]

[1] Nineveh University, College of Pharmacy. abeer.mansour@uoninevah.edu.iq
[2] Nineveh University, College of Pharmacy. saif.khalid@uoninevah.edu.iq

Received: 17 July 2024; **Accepted:** 30 September 2024

ABSTRACT

Diabetes mellitus is a chronic metabolic condition clinically characterized by hyperglycemia, with excessive production of free radicals and the formation of advanced glycation end products playing major roles in the pathogenesis and progression of this disease. Antioxidants, increasingly recognized for their ability to minimize damage caused by diabetes-related oxidation, have been gathering attention. This study aimed to use *Urtica dioica* extract, combined with vitamin E, to investigate its possible beneficial synergy on fasting blood glucose (FBG), antioxidant levels, and histomorphology evaluation of the pancreatic tissues in diabetic rats. By applying three phytochemical methods, we obtained *Urtica dioica* extract and tested its toxic effects to ensure its safety on male albino rats. The rats were distributed into six groups: one diabetes-induced experimental group and groups administered metformin, vitamin E, Urtica, a vitamin E-Urtica combination, while the last group was the control group. The use of these compounds was established in the management of type-2 diabetes. Serum and fasting blood glucose were measured at each follow-up. The concentration of MDA in the adipose tissue and the total antioxidant activity in the mouse serum were prepared and measured. The combined therapy of *Urtica dioica* and vitamin E produced an increase in body weight and a reduction in FBG levels, compared to the other treated groups. Furthermore, an enhancement in the antioxidant activities manifested as a reduction in levels of MDA and an improvement in the total antioxidant status in both pancreatic tissue and serum. Therefore, the mutual use of *Urtica dioica* extract and vitamin E may provide a promising supplementary choice in the treatment of diabetes and its associated complications.

Keywords: Oxidative stress, *Urtica dioica***,** Diabetes mellitus**.**

INTRODUCTION

Diabetes is a chronic or persistent metabolic disorder characterized by high levels of glucose in the blood. Free radicals are reactive oxygen species (ROS) that play a significant role in the progression and development of diabetes. One of the most notable characteristics of free radicals is that they are highly reactive molecules. When their levels are not appropriately controlled, they can harm cells and tissues (Mrowicka, 2005). The amount of free radicals produced and the body's antioxidant systems can be affected by the disease, potentially leading to an impaired antioxidant system (Pizzino *et al., 2017*).

These free radicals increase with oxidative stress, which is frequently a type of

Corresponding author: Abeer Mansour
E-mail address: abeer.mansour@uoninevah.edu.iq
Present address: Nineveh University, College of Pharmacy.

imbalance between the formation and removal of free radicals.

Antioxidants are substances that can neutralize free radicals and protect cells from their damaging effects. In individuals with diabetes, the endogenous antioxidant defence system often fails, resulting in an imbalance or even excessive production of free radicals and a deficiency in eliminating these radicals (Losada-Barreiro *et al.,* 2022). Antioxidants play a crucial role in counteracting the harmful processes caused by free radicals in diabetes. They help restore equilibrium between free radical generation and elimination, thereby preventing the oxidative stress that can occur consequently. Numerous animal trials have provided experimental models of diabetes that demonstrate the capability of antioxidants to reverse diabetes-related changes, as measured by markers of oxidative stress (Chaudhary *et al.,* 2023).

Glucose hyperglycemia has been associated with raised free radical formation in diabetic patients through several mechanisms. This occurs during auto-oxidation of glucose-oxidative degradation, which results in glucose oxidation, and further reactions give rise to reactive keto-aldehydes (González *et al.,* 2023).

Medicinal applications of *Urtica dioica*, known as stinging nettle, attempted centuries back for its traditional remedy against various health conditions. It is well documented to possess anti-inflammatory, analgesic, and diuretic action. During the last decades, much research has been conducted to study the therapeutic uses of *Urtica dioica* either alone or in conjunction with other agents.

Vitamin E is well-acknowledged for its antioxidant and anti-inflammatory action (Bhusal *et al.,* 2022). Nettle has an antioxidant potential; studies have recognized that the leaves of nettle and its aqueous extracts provide a rich source of Vitamin C and Vitamin E, which play an important role in the body's antioxidant capacity. Predictably, nettle essential oils such as flavonoids and total polyphenol content may be attributed to antioxidants, antiproliferative action, and anti-inflammatory activities. (Jaiswal and Lee, 2022; Bhusal *et al.,* 2022).

Despite both *Urtica dioica* and Vitamin E representing an important source of antioxidants, the combination of them has not been studied thoroughly. Therefore, combining these two antioxidants should synergistically enhance their antioxidant activities. However, more complete investigations are required to evaluate the overall antioxidant effect of this combination both in *vivo* and in *vitro* (Bhusal *et al.,* 2022; Flórez, Cazón, and Vázquez, 2022).

Stinging nettle was historically used in the management of several medical conditions, such as diabetes, osteoarthritis, benign prostatic hyperplasia (BPH) and hay fever. Besides that, it has been studied for its possible action of showing adenosine deaminase movement in prostate tissues and its consequences on insulin excretion. (Randall *et al.,* 2008; Baumgardner, 2016).

Vitamin E is a fat-soluble vitamin. Its numerous purposes in the body are frequently connected to its antioxidant consequences, which assist protect cells in contradiction to the free radicals that commonly produce cellular and other biological damage. Free radicals are generated when the body changes its food into energy. Free radicals can also be generated by environmental influences, such as cigarette smoke, and air pollution (Sibo *et al.,* 2023).

Vitamin E originates in numerous forms: natural and synthetic. Most practitioners recommend natural Vitamin E, either d-alpha-tocopherol or natural interspersed tocopherols. It is produced as soft gels, tablets, capsules, and topical oils. The suggested daily amount is 4 mg for men and 3 mg for women. It is usually recommended to get Vitamin E mainly from food rather than supplements. In some conditions, such as

Alzheimer's disease and liver disease, a potential advantage of Vitamin E has been realized, though the impact of high-dose Vitamin E supplementation was less clear on prostate cancer and preeclampsia and requires further research (Shahidi *et al.,* 2021; Sibo *et al.,* 2023).

Shahidi *et al.,* (2021) studied the metabolic parameters in patients with type 2 diabetes mellitus after treatment with *Urtica dioica*. Their study showed that the administration of *Urtica dioica* resulted in improvements in both FBG, HbA1c, as well as the inflammatory marker CRP, with no significant changes in insulin level, cholesterol profile and BMI.

The current study discussed the medical benefits of using a combination of *Urtica dioica* and vitamin E, and their effects on several parameters including body weight, diet and fasting blood glucose and their antioxidant properties; which were assessed in the blood and liver using CAT and MDA indicators and supported by histological studies.

MATERIALS AND METHOD

1. Extraction of *Urtica dioica*

Stirring, non-stirring extractions, and ultrasound-assisted extraction methods were used in this study. Exactly 1 gram of nettle powder was weighed into a screw-capped tube and mixed with 15 mL of water as a solvent. Ultrasound-assisted extraction was conducted at 65°C in an ultrasonic bath. Extraction without stirring was carried out in a warm bath at 65°C. Stirring extraction took place in an orbital shaker at 65°C and 150 rpm. The extraction process lasted 4 hours, with hourly sampling for all three methods. The extraction temperature was chosen based on the findings of prior studies (Bucić-Kojić *et al.,* 2009), which demonstrated a higher extraction yield of antioxidants at elevated temperatures and considered the temperature constraints of the equipment used. Additionally, considering a potential industrial application of the process, it could be more practical and less energy-intensive to operate at higher temperatures (65°C) for shorter durations (≤4 hours). Subsequently, the extract was filtered through Whatman No. 1 filter paper in a funnel to eliminate nettle residues from the liquid extract. A 1/100 dilution of each extract was then prepared, and the diluted extracts were stored in darkness at 5°C until analysis.

2. Ethical Approval

The project for the patent research program was approved by the Ethical Committee of Animal Sciences at the Al-Razi Center before the experimental work began.

3. Animals

All animals were checked frequently and provided with adequate food and water. The living environment inside the cage was kept clean with continuous removal of feces. The weight of the animals in each group was monitored regularly. The current study was conducted at the animal shelter of the Faculty of Veterinary Medicine at the University of Mosul. Male albino rats were housed in plastic cages measuring (30 × 20 × 17) cm. The rats weighed between 175 and 250 g and were kept at a temperature of (23 ± 2°C) with a relative humidity of 55%, following a 12-hour light/dark cycle for 30 days. All animals were vet-checked to ensure they were safe, healthy, and free of disease during the 1-week incubation period. Every procedure was performed under the ethical guidelines of medical research.

3. Toxicity Test

Under the OECD Guidelines for the Testing of Chemicals (2000) (OECD, 1994), a toxicity test was conducted at the Al-Razi Center for Research and Production of medical kits for diagnostic, precisely in the shelter and attention of animal's responsibility. Albino rats were euthanized for the test. The ideal conditions of this setting suggest keeping the animal's ability for a temperature of 22°C, relative humidity

of 30%, and 12 hours of light per day, with food and water provided. The animals used in this test weighed an average of 250 grams and were non-pregnant females.

A 3-hour deprivation period of food was used before administering the doses, which were as follows: The first dose was a single dose of 50 mg/kg (n=3 per sample). The second dose was 300 mg/kg animal, (n=3). The third dose was 2000 mg/kg b.w; (n=3).
For a full understanding of the results and any possible toxicity effects, a reference should be prepared to the complete test report permitted at the Al-Razi Center (Siouda and Abdennour, 2015).

4. Experimental Procedure

4.1. Experimental design

A total of 40 rats were used in the study, and were divided into six groups, each consisting of six rats, and all animals lost during the experiment were replaced. All animals were provided with food and water except during the fasting period before labour induction.

Control group (G1) received distilled water
Diabetic group (G2) received alloxan (200 mg/kg) injections and were kept in cages without treatment.
Diabetic group with metformin treatment (G3) received a daily dose of metformin at 100 mg/kg.
Diabetic group with U. dioica (G4) received a daily dose of *U. dioica* at 500 mg/kg.
Diabetic Group (G5) received a combination of *Urtica dioica* (500 mg) and vitamin E at 500 mg/kg.
Diabetic Group with Vit E500 mg (G6) received Vit E at 500 mg\kg.

5.2. Body weight measurement:

Changes in body weight for each group over time were measured, and the difference between initial and final body weights was determined. Throughout the study period, abnormal signs were observed in the experimental animals.

5.3. Measurement of food and water intake and fasting glucose level

Rats were housed in each cage, and 200 g of food was weighed and placed in each cage. The next day, the remaining food was removed from the bowl, and the amount was measured. The total food intake of a rat is equal to the amount of food added minus the amount left. To calculate the average food intake per rat, divide the total food intake by the number of rats in each cage, and then divide by the average.

A drinking bottle containing 150 mL of tap water was placed in each cage. The next day, the remaining water was collected in a graduated cylinder to measure the water level. The amount of water added minus the remaining amount equals the total amount of water consumed by the rats in the cage. The total water volume in the cage divided by the number of rats represents the average water consumption per rat.
Determination of Fasting Blood Glucose Method: Serum glucose was estimated utilizing the enzymatic method (glucose–oxidase–peroxidase), which is highly specific for D-glucose, using a kit supplied by Randox Laboratories Ltd (England).

Principle: Glucose is determined after enzymatic oxidation in the presence of glucose oxidase (GOD).

Calculation: n = 5.55 mmol/L (Standard concentration) or (99 mg/dL).

5.4. Samples Collection

The blood and tissue samples were collected from the pancreas. The tissues were analyzed for the biochemical parameters collected over 30 days. The tissue and serum samples were maintained at -80°C. The samples were placed in a mixture of xylene and paraffin wax in a ratio of 1:1 at 60 °C, and then the samples were subjected to two stages of molten wax after being impregnated with paraffin wax. Pathological change scores were assessed by a pathologist blinded to the animal groups. Table 1 presents the scores for

pathological pancreatic tissue groupings, arranged according to the order of progression of lesion severity.

Table 1: Rat Pancreatic Histology Scoring System

Score 0-2: Normal rat pancreatic histology shows that pancreatic islets behave normally and have no pathological changes.
Score 2-3: Mild changes in the appearance of the pancreatic islets remain largely unchanged. There are some slight histological alterations, but not enough to suggest pathology.
Score 4-5: Pathological changes include necrotic changes, small vacuoles, and swelling on the slide.

5.5. Assessment of Antioxidant Activity

Antioxidant Activity Measurement: A methanol-DMSO mixture (9:1 v/v) solution was prepared by adding 9 volumes of methanol to 1 volume of DMSO. DPPH Radical Solution: The DPPH radical was dissolved in a DMSO-methanol mixture to prepare a 0.1 mg/ml DPPH radical stock solution. The biochemical assessment of malondialdehyde (MDA) was performed using an ELISA kit designed to determine the levels of MDA in serum, plasma, and other biological fluids. In the current study, serum from rats was used. The characteristics of the MDA ELISA kit include a sensitivity of 18.75 ng/ml and a detection range of 31.25–2000 ng/ml. This kit recognizes MDA in samples (Catalog No: E-EL-0060, Elabscience, USA). Additionally, TAOS levels were measured using ELISA kits (Cato.SL1402Ra) with an assay range of 0.1 U/ml to 7 U/ml and a sensitivity of 0.01 U/ml, as per the manufacturer's protocols (Sun Long Biotech Co., LTD).

5.6. Histological examination

Tissue collection: Rats were euthanized by cervical decapitation at each time point. The pancreas was then removed and separated. The fat was immediately excised, and the tissue was carefully washed. The pancreas was divided into two parts, with one part fixed in 10% buffered formalin. Samples were cut into thin sections (5 μm) and stained with hematoxylin and eosin (H&E) for analysis. Histological tissues were maintained in buffered formalin for evaluation, with pathologist approval, based on descriptive histology for oxidative assessment. One gram of cleaned tissue was weighed and prepared in phosphate-buffered saline (PBS, pH 7.4) according to the kit protocol. Excess blood was removed, and the tissue was weighed before homogenization. Rat tissue was homogenized in phosphate-buffered saline (PBS, pH 7.4), a buffered salt solution mixed with phosphates, which is often used in biological research. The homogenate was frozen on ice at -20°C using a glass homogenizer, or thawed at 2–8°C. The samples were then centrifuged for about 20 minutes at 2000-3000 rpm to preserve serum samples and tissues, which were stored at -80°C.

5.7. Statistical Analysis

SPSS version 19 and GraphPad Prism were used for statistical analysis in this study. Quantitative analysis was performed using the ANOVA test with post hoc analysis via Duncan's test and independent t-tests, along with the non-parametric Kruskal-Wallis test.

RESULT

1. Toxicity Test

Three different doses were administered, ranging from 50 mg/kg to 2000 mg/kg, (Table 2).

Table 2: Toxicity test conducted on Albino rats at the Al-Razi Center, following OECD Guidelines.

Dose	Dosage (mg/kg)	Sample Size
First	50	3
Second	300	3
Third	2000	3

- OECD: The OECD Guidelines for the testing of chemicals are a collection of the most relevant internationally agreed testing methods used by governments, industry, and independent laboratories to assess the safety of chemicals.

2. Body weight measurement.

This study investigated the effects of various treatments on body weight in diabetic rats compared to a control group. The data presented in the table shows the mean weight and standard error (S.E.) of the different groups at day 0 (baseline) and day 30, as shown in Figure 1.

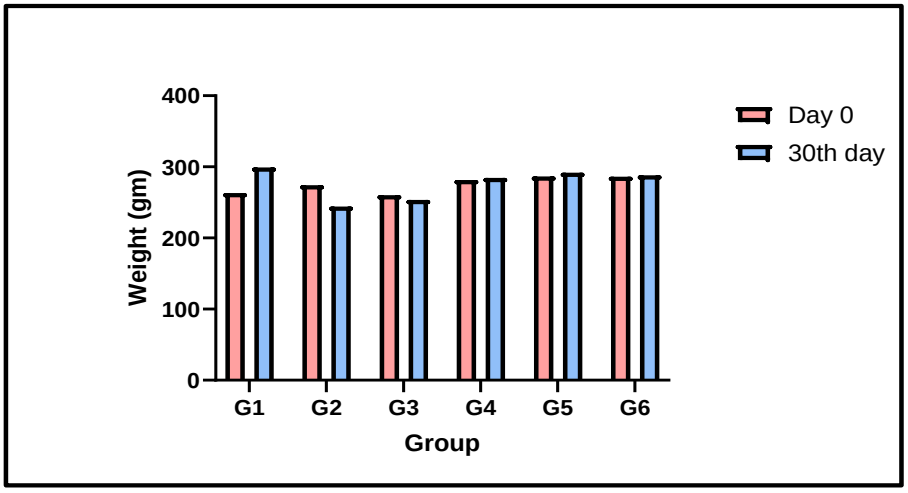


Figure (1): Body weight changes in diabetic rats following treatment with metformin, *Urtica dioica*, vitamin E, or control

3. Food and Fluid Measurement.

Group G2 stands out significantly, with a much higher fluid intake compared to the other groups (G1, G3, G4, G5, G6). The differences were highly significant based on the p-value. Group G2 also exhibits a significantly higher food intake compared to the other groups (G1, G3, G4, G5, G6). Again, the differences were highly significant based on the p-value, as shown in Table 3.

Table 3: Effect of Experimental Treatment on Fluid and Food Intake in Rat Groups.

Parameters / Groups	**Mean + S.E**	
	Food Intake (gm) /rat	**Fluid Intake(ml)/ rat**
G1	19.18 ± 0.42 a	20.00 ±1.154 a
G2	31.96 ± 0.98 b	149.33 ±25.86 b
G3	21.26 ± 0.81 a	34.66 ±0.88 a
G4	21.00 ± 0.57 a	35.45±0.41 a
G5	19.00 ±0.32 a	31.00±0.23 a
G6	21.00±0.54 a	34.33 ± 0.49 a
***P*- value**	**0.000****	**0.000****

- One-way ANOVA test and Post hoc Duncan.
- Similar letters mean non-significant difference (P> 0.05). Different letters mean a significant difference (P< 0.05). * means significant and ** means 0.01 highly significant.

4. Result of fasting blood glucose (mg/dl)
The results revealed that metformin is effective in lowering blood sugar in diabetic rats. *Urtica dioica* extract may have some potential as a complementary therapy. The combination of *Urtica dioica* and vitamin E might offer slightly better blood sugar control than *Urtica dioica* alone. Vitamin E alone seems to have minimal effects on blood sugar control in this study, as shown in Figure 2.

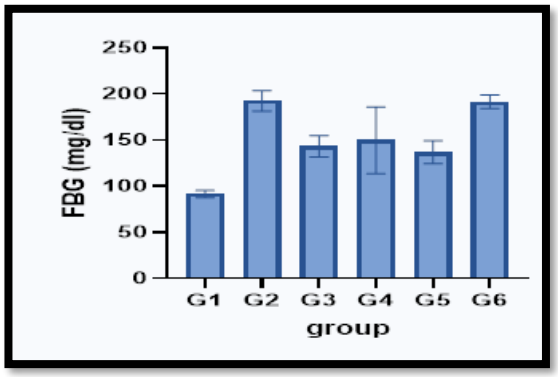


Figure (2): Effect of fasting blood glucose (mg/dl)

5. Assessment of Antioxidant Activity

5.1. Assessment of Antioxidant Activity Measurement for *Urtica dioica* alone and combination with vit E

Table 4 demonstrates that the highest percentage of DPPH radical scavenging activity was exhibited by the combination of vitamin E and *Urtica dioica*, suggesting a synergistic effect between the two antioxidants.

5.2. Assessment of Antioxidant Activity Measurement in vivo by Assay of *Urtica Dioica* with Vit E in blood and pancreas tissue on serum rat Malondialdehyde (ng/mL) Level
Figure 3 compares MDA levels in pancreas tissue and serum blood levels across various groups, analyzing measurements in both pancreas tissue and serum blood over a specified period. The results are summarized as mean values with standard errors (Mean ± S.E.) for each group under the two conditions.

Table 4: Antioxidant Activity of *Urtica dioica* alone and combination with vit E.

Groups	Radical Scavenging Activity (%) DPPH
Urtica dioica alone	**49.56%**
Vit E alone	**54.10%**
Urtica dioica + Vitamin E combination	**65.28%**

6. Total Antioxidant Status (TAOS) Assay Of *Urtica Dioica* With Vit E in blood and pancreas tissue
Based on the table, the group with the highest mean TAOS in pancreas tissue was G5, while the group with the lowest mean TAOS in serum blood was G2. This table displays antioxidant levels (TAOS) in pancreas tissue and blood serum across different groups over time. Generally, pancreas tissue has higher antioxidant levels than blood serum.

7. The Impact on Pancreatic Tissue (Histological Study)

The control group:

The histological section of the healthy control group illustrates the structural organization of the pancreas. Microscopic examination of the pancreatic tissues in this group did not reveal any significant histopathological lesions, as shown in the microscopic image of the pancreas for the healthy control group (A). The natural structure of the pancreatic tissues is depicted, including pancreatic acini (A), Langerhans islets (B), and the ducts between lobules (C) (photomicrograph 1).

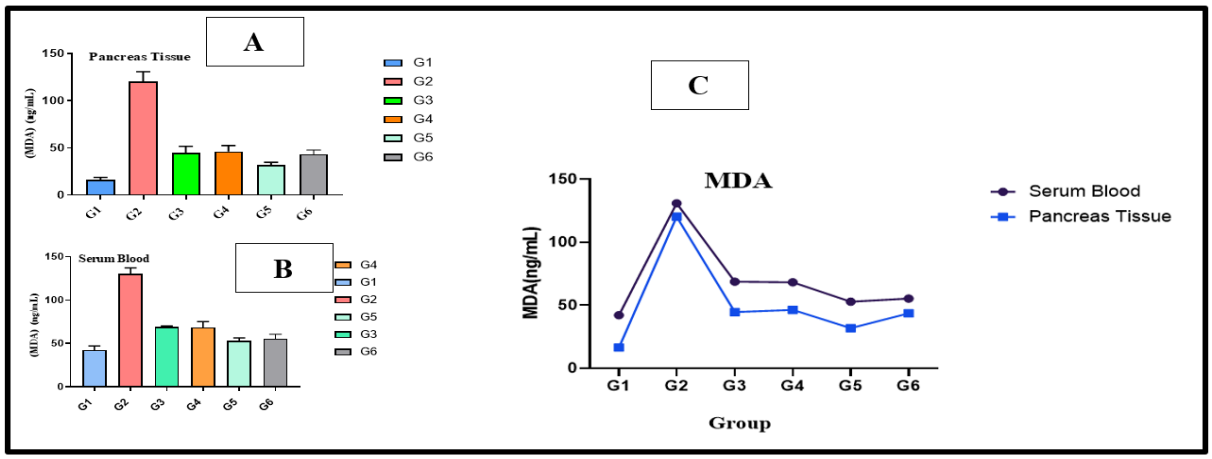


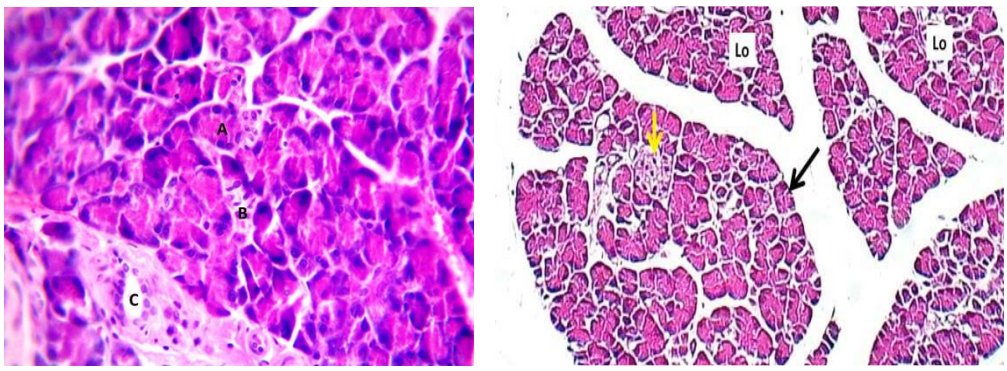
Figure (3): Comparison of MDA (ng/mL) levels **A** MDA level in pancreas **B** MDA level in blood tissue **C** compare between blood and pancreas MDA level

- One-way ANOVA test and Post-hoc Duncan between group
- Independent t-test between blood and pancreas MDA level.

Table 5: Comparison of Total Antioxidant Status (TAOS) (U/ml) levels in pancreas tissue and serum blood across different groups over time

Parameter Groups	TAOS (U/ml) Mean + S.E		
	Serum Blood	Pancreas tissue	P –value
G1	6.42 ± 0.14 (A,b)	7.40 ± 0.59 (A,b)	0.061
G2	1.41 ± 0.17 (A, a)	0.89 ± 0.43 (A, a)	0.198
G3	4.26 ± 0.29 (A,b)	4.41 ±0.13 (A,b)	0.23
G4	4.46 ± 0.60 (A,b)	4.16 ± 0.96 (A,b)	0.08
G5	4.23 ±.054 (A, b)	7.56 ±0.71 (B,b)	**0.043***
G6	2.80 ±.039 (A, ab)	3.16 ± 0.08 (A, ab)	0.232
p-value	**0.000*****	**0.000*****	

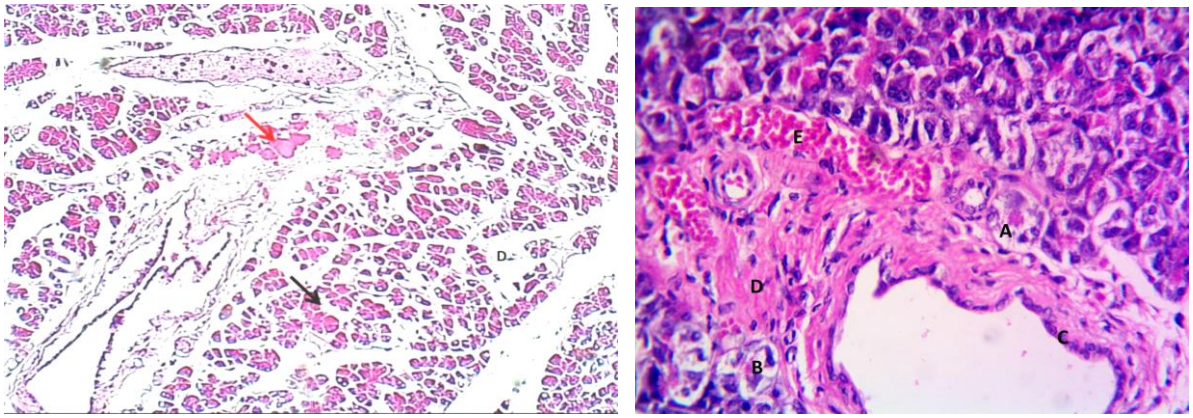
- One-way ANOVA test and Post-hoc Duncan's.
- Independent t-test between blood and pancreas MDA level capital letter.
- Similar letters mean non-significant difference (P> 0.05). Different letters mean a significant difference (P< 0.05). * means significant and ** means 0.01 highly significant.



Photomicrograph (1): Shows a section of pancreatic tissue for the healthy control group at magnifications of 100x and 400x, stained using Hematoxylin and Eosin (H&E) G1

Diabetic Control Group:

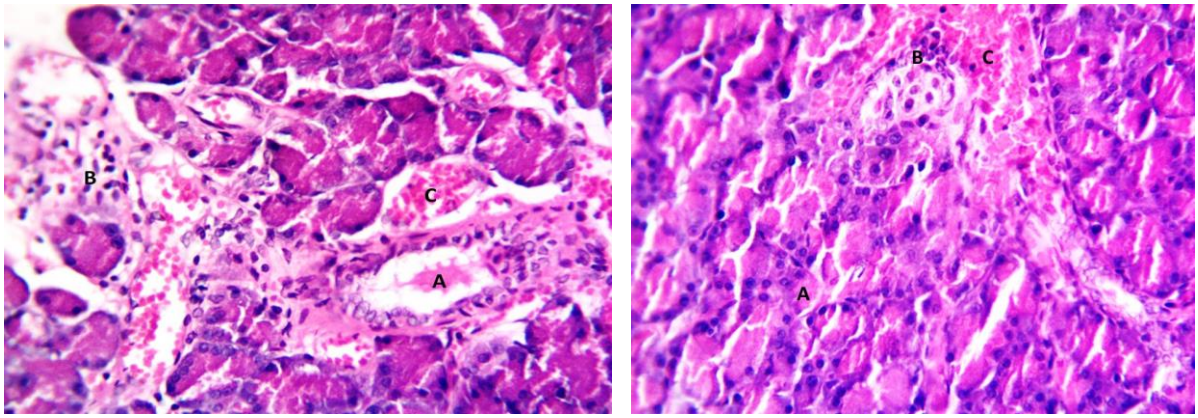
Through the illustrated histological section, it is evident that diabetes has led to excessive inflammatory lesions in pancreatic tissue, resulting in atrophy and degeneration, particularly in the Langerhans islets. Irregularities in pancreatic acini are noted, associated with tissue breakdown and infiltration, as well as the atrophy of some acinar cells. This is primarily attributed to the potent cytotoxicity of alloxan, which generates reactive oxygen species (ROS). Pancreatic beta cells exhibit a low antioxidant defence capacity. The microscopic image of the pancreas from the diabetic control group reveals necrosis (A) and atrophy (B) of pancreatic acinar cells, surrounding squamous cells of ducts (C), fibrosis of the surrounding duct (D), and vascular congestion (E). H&E staining, photomicrograph (2).



Photomicrograph (2): Illustrates a section of the pancreas in the diabetic control group(G2)

Metformin Group (100 mg/kg/day)

The microscopic image of the pancreas from the metformin-treated group reveals the natural structure of pancreatic acini (A), with mild infiltration of inflammatory cells (Grade 1) (B) and vascular congestion (Figure 3).



Photomicrograph (3): Illustrates the appearance of the natural structure of pancreatic tissues, represented by pancreatic acini (A), Langerhans islets (B), and the duct inside the lobule (C) after the use of the drug metformin**.**

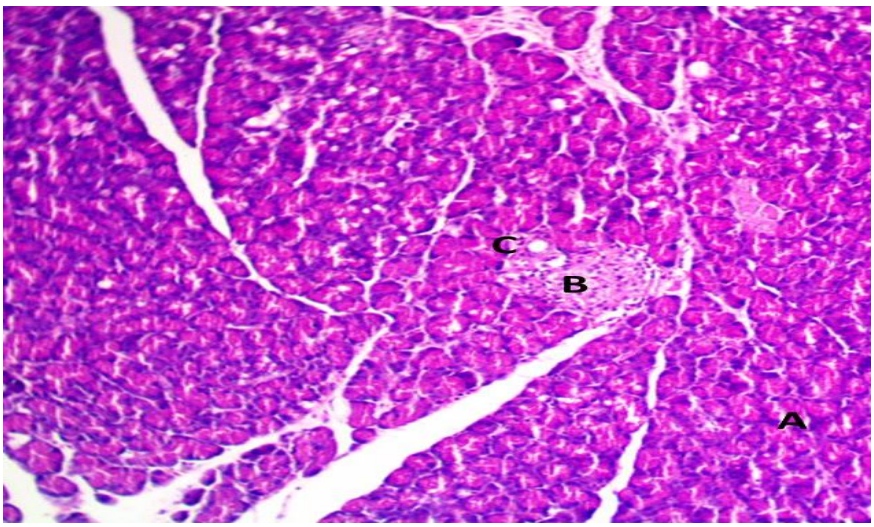
Group Treated with *Urtica dioica* Only:

Microscopic examination of the histological section of the pancreas from rats treated with the gourd plant alone reveals the disruption of acinar cells and an irregular tissue structure in a lobular arrangement. This disruption is attributed to the intensity of induced diabetes.

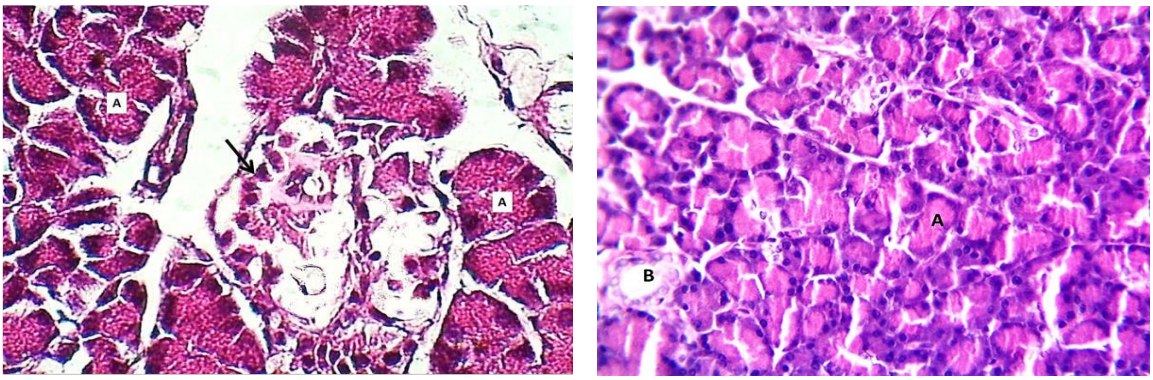
The examination displays the uneven shape of some pancreatic lobules, with certain lobules exhibiting moderate to mild disruption in acinar cells. This indicates an improvement compared to the diabetic control group (Photomicrograph 4).

Group Treated with *Urtica dioica* & Vit E

The histological section of the pancreas in animals from the treated groups represents a combination of the gourd plant and vitamin E. The image reveals the natural appearance of pancreatic acini (A), pancreatic islets, and hair-like structures. It highlights a condition that resembles the healthy control group in terms of pancreatic lobules and the tissue structure of acinar cells. A microscopic image of the pancreas from the treated group (combination of the plant with vitamin E) displays the natural structure of pancreatic tissues, represented by pancreatic acini (A) and the duct inside the lobules (Photomicrograph 5).



Photomicrograph (4): Illustrates the appearance of the natural structure of pancreatic tissues by treatment group with *Urtica dioica*, represented by pancreatic acini (A), Langerhans islets (B) and (black arrow), and the duct inside the lobule (C) after treatment with plant extract.

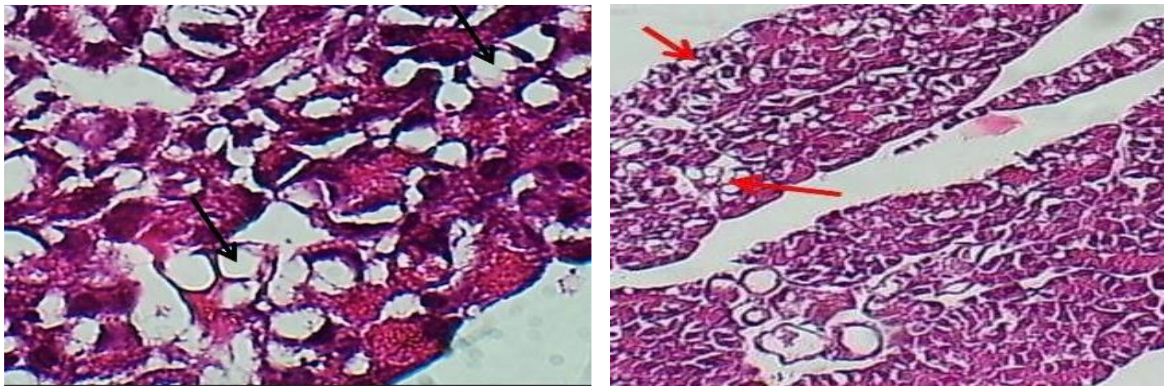


Photomicrograph (5): Illustration of the appearance of the natural structure of pancreatic tissues, represented by pancreatic acini (A), Langerhans islets (B), and the duct inside the lobule (C) after using a combination of gourd plant with Vitamin E

Group Treated with Vit E

The microscopic examination in Figure (6) illustrates the histological section of the rat pancreas treated with vitamin E. The figure shows morphological changes (red arrow) occurring in pancreatic tissue, including acinar cell disruption and irregular tissue

structure in a lobular arrangement, reflecting the severity of induced diabetes. The figure also demonstrates the uneven appearance of some pancreatic lobules, with certain lobules exhibiting moderate acinar cell disruption. Additionally, there are signs of atrophy and irregular tissue organization in the arrangement of lobules.



Photomicrograph (6): illustrates the histological section of rat pancreas treated with vitE

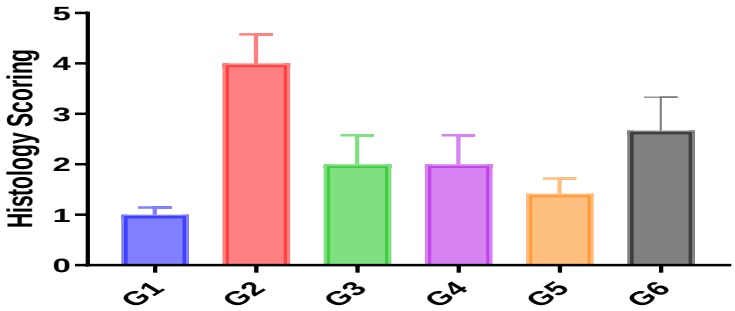


Figure (4): pancreatic pathology score.

- Data was not normally distributed, and statistical analysis was performed by Kruskal-Wallis, non-parametric with descriptive means and standard errors of the means.

Pathological changes of the pancreas

The reported pancreatic pathology scores showed statistical differences between the groups of the experiment (Figure 4).

DISCUSSION

1. Evaluation of animal behaviour, with the determination of fasting blood glucose

Depending on the specific needs of the animal or the research goals, the observations of the rats in the control and diabetic groups were more concise and objective. The control group showed normal behaviour, with active appearances, healthy fur, and good color and shine. The diabetic group, however, was lethargic, slow to respond, and exhibited discoloured fur (Smith *et al.,* 2018). When treated with *Urtica dioica* and vitamin E, there was an enhancement in behaviour, with improved activity and better fur colour.

Alloxan-induced diabetes in mice causes elevated blood sugar levels, and more than 200 mg/dl is defined as diabetic. Alloxan produces oxidants that break down beta cells in the pancreas. (Rasool and Mahmood, 2021).

This study investigated the effects of various treatments on body weight in diabetic rats compared to a control group. Baseline body weights (day 0) showed no statistically significant differences (p-value = 0.919) between the groups. The diabetic group experienced weight loss, which is commonly

seen in diabetes due to the body's inability to use glucose effectively, resulting in the breakdown of muscle and fat for energy and causing unintended weight loss and muscle wasting (Merz and Thurmond, 2020).

The mechanisms by which *Urtica dioica* may influence body weight in diabetic rats are complex and multifaceted. Although the precise pathway is still under investigation, several potential mechanisms have been proposed. One possible mechanism is the curtailment of glucose metabolism. Research has revealed favourable hypoglycemic effects of *Urtica dioica*. While its role in blood sugar regulation is generally inhibitory, it may address the symptoms of diabetes and consequently affect body weight by influencing energy balance (Ahangarpour, Mohammadian, and Dianat, 2012)

Urtica dioica, a species of plant, is categorized as part of the Urticaceae family. It can be either hermaphroditic or dioecious, implying that each individual has the capacity for both genders. This type of plant has irritating hairs that cause the skin to itch when touched. However, although *Urtica dioica* is often regarded as a nuisance plant, people have used it for medicinal purposes for centuries. Traditional applications include relieving arthritis, allergies, and urinary tract infections. In addition to current research concerning the use of *Urtica dioica* in weight control, some other studies have suggested that the effective weight loss properties of the plant may exist. Nevertheless, the exact mechanisms by which these effects take place are yet to be understood.

Remarkably, *Urtica dioica* also contains functional molecules like polyphenols, flavonoids, and triterpenoids, which act as antioxidants and anti-inflammatory components. These compounds play a role in the management of oxidative stress and inflammation. Both inflammation and oxidative stress conditions can be considered complications or causative factors for diabetes. The bioactive compounds also play a role in the modulation of gut microbiota (Peer, 2022).

Vitamin E is part of a group of vitamins that act as important antioxidants, protecting cells from damage caused by free radicals. It is a fat-soluble vitamin found in various diets and vegetable oils. Furthermore, vitamin E is crucial for immune response, DNA repair, and many other biochemical reactions occurring in the body. In addition to these benefits, vitamin E may also play a role in weight control. A previous study indicated that the intake of vitamin E contributed to significant reductions in weight and BMI among obese individuals. In line with that, another study showed that vitamin E supplementation improved insulin sensitivity and glucose metabolism among overweight individuals, reducing the risk of developing type 2 diabetes (Rizvi *et al.*, 2014).

2. Assessment of %DPPH values

The use of a combination of *Urtica dioica* and vitamin E (61.66%) showed a significantly higher percentage of DPPH radical scavenging activity compared to both vitamin E (52.66%) and *Urtica dioica* alone (49.66%). This suggests a stronger ability to neutralize free radicals when these two antioxidants are combined. Various anti-oxidant compounds, such as phenolic acids and flavonoids, are present in *Urtica dioica*. On the other hand, Vitamin E is a well-known lipid-soluble antioxidant, which protects cell membranes from oxidative damage. Thus combined action may provide broader protection against free radicals in different cellular parts.

Vitamin E can be regenerated by other antioxidants, such as vitamin C or certain compounds present in *Urtica dioica*. This regeneration cycle could enhance the overall antioxidant capacity. The hypothesis of the combined effect between vitamin E and other natural antioxidants has been previously studied by Pincemail and Meziane (2022).

3. Assessment of oxidative parameters and histological outcomes.

MDA is one of the markers that can indicate lipid peroxidation as radicals attack the cell membranes. This provides evidence for the validity of using alloxan, a common diabetogenic agent frequently used to induce diabetes in animals for experimental studies. Alloxan consistently induces increased free radical production, and consequently leads to lipid peroxidation and a decline in the Total Antioxidant Capacity (TAC), as seen in the diabetic group animals being treated. It is recognized that this form of nanoparticles accumulates in the pancreatic beta cells, raising ROS levels through a reductive oxidative cycle. These ROS, in particular, generate oxidative stress specific to islet cells, with hydroxyl radicals causing damage to insulin-producing beta cells, leading to the development of insulin-dependent diabetes (Lenzen, 2008).

In diabetes, the production of reactive oxygen species (ROS) often surpasses the body's ability to neutralize them. It can be assumed that the cause of diabetes involving alloxan is the generation of ROS and the impairment of antioxidants within the body, which are significant mechanisms for increased MDA and decreased TAC. Oxidative stress promoted by alloxan-induced diabetes provokes the formation of lipid peroxides, ultimately resulting in the biomarker MDA leaking into the bloodstream. As the body's TAC reduces, the antioxidant system's capability declines and more free radicals are produced (Lenzen, 2008).

Urtica dioica plant has been used for centuries as a rich source of food and medicines. It is loaded with many phytochemical materials, such as polyphenols, flavonoids, steroids, and lignans. These components possess potent pharmacological actions, and one of them is the antioxidant effects (Taheri et al., 2022).

Several studies have indicated that *Urtica dioica* may reduce oxidative stress and improve antioxidant capacity by different mechanisms, such as cuprizone-induced demyelination and oxidative stress in a mouse model (Namazi *et al.,* 2022). Vitamin E, a fat-soluble and powerful antioxidant, is known to reduce lipid peroxidation and increase the activity of antioxidant enzymes. (Rizvi *et al.,* 2014).

This study suggested that the combination of metformin and *Urtica dioica* with vitamin E provided partial protection against pancreatic damage. The administration of *Urtica dioica* and vitamin E was responsible for the restoration of the damaged pancreatic structure. However, individual vitamin E supplementation did not significantly affect COPD disease progression.

Studies suggest that a potential strategy for managing diabetes with metformin, in combination with natural substances, may effectively preserve the pancreas from drug-induced damage. The synergy observed between *Urtica dioica* and vitamin E is notable, as these natural ingredients could be combined with traditional medication to enhance therapeutic outcomes.

Nonetheless, it is noteworthy that vitamin E alone may be weak and should not be ignored as a preventive measure. It could be included in a combined treatment with other traditional methods. These components can protect against tissue lesions, and the optimal doses and method of administration should be established. Further clinical trials are needed to confirm their effectiveness in human subjects and to detect any possible side effects or interactions with other drugs (Skalska-Kamińska *et al.,* 2023).

CONCLUSION

This study highlighted that the combined administration of vitamin E and *Urtica dioica* extract has potential in the treatment of diabetes-associated disorders. This was

confirmed by measuring the antioxidant activity, ability to reduce histological alterations in diabetic rats, and synergy with other treatment programs. Through these confirmatory studies and investigations into the mechanisms of action, its benefits are scientifically measurable and clinically validated. Further research, which might include human subjects, might be suggested.

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التأثيرات التآزرية لمستخلص نبات القراص وفيتامين E على ارتفاع السكر في الدم، الإجهاد التأكسدي، والتغيرات النسيجية في الجرذان البيضاء المصابة بالسكري

عبير منصور عبد الرسول [1] و سيف خالد يحيى [2]

كلية الصيدلة جامعة نينوى

Email: abeer.mansour@uoninevah.edu.iq Assiut University website: www.aun.edu.eg

داء السكري هو حالة مزمنة تتسم بارتفاع مستويات السكر في الدم. يلعب زيادة إنتاج الشوارد الحرة وتكوين المواد الضارة في الجسم دورًا كبيرًا في ظهور المرض وتفاقمه. مضادات الأكسدة تُعرف بقدرتها على تقليل الأضرار الناجمة عن الأكسدة المرتبطة بالسكري، وهذا جعلها تحظى باهتمام كبير. تهدف هذه الدراسة إلى استخدام مستخلص نبات القراص ومعرفة الفوائد المحتملة عند دمجه مع فيتامين E في التحكم بمستوى السكر في الدم ومضادات الأكسدة والتغيرات النسيجية في الجرذان المصابة بالسكري. تم إعداد مستخلص نبات القراص ، واختبار سميته للتأكد من أنه آمن للاستخدام. تم تحفيز السكري في ذكور الجرذان البيضاء وتقسيمها إلى ست مجموعات: المجموعة الضابطة بدون علاج و مجموعة علاج بالميتفورمين، مجموعة لفيتامينE ، مجموعة القراص، مجموعة مركبة من فيتامين E والقراص، وأخيرًا مجموعة تم إعطاؤها مزيجًا من المواد. كما تم قياس مستوى السكر في الدم خلال كل متابعة. تم تقييم تركيز مادة MDA في الأنسجة الدهنية والنشاط الكلي لمضادات الأكسدة في مصل الجرذان من خلال تجارب مختلفة. كما أجرينا تحليل نسيجي للأنسجة البنكرياسية. كانت النتائج للدراسة الحالية أن العلاج المشترك لمستخلص نبات القراص وفيتامين E له فوائد ملحوظة , حيث أظهرت المجموعة التي تناولت المركب تحسنًا في وزن الجسم، وانخفاض مستويات السكر في الدم، وزيادة في نشاط مضادات الأكسدة، كما تبين من انخفاض مستوى MDA وزيادة نشاط مضادات الأكسدة في أنسجة البنكرياس ومصل الدم.نستنتج ان خلط مستخلص نبات القراص وفيتامين E له تأثير إيجابي في معالجة ارتفاع السكر في الدم ومضاعفات الإجهاد التأكسدي الناتجة عن السكري، مما يجعله علاجًا مكملًا واعدًا في هذا المجال.