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التأثير العلاجي لمستخلص الزعتر البري مقارنة بالزعتر المزروع (*Thymus serpyllum*) في الفئران المصابة بداء السكري المستحث بالالوكسان

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الملخص

يعد داء السكري من الاضطرابات الاستقلابية الشائعة التي تؤدي الى مضاعفات خطيرة من بينها تلف الكلى الناتج عن الاجهاد التأكسدي . هدفت الدراسة الحالية الى تقييم التأثير الوقائي والعلاجي للمستخلص الكحولي لنبات الزعتر البري Thymus Serpyllum مقارنة بالزعتر المستزرع في تحسين وضائف الكلى ومؤشرات الاجهاد التأكسدي لدى الجرذان المصابة بالسكري المستحث بالالوكسان . استخدم في التجربة 40 جرذاً ابيضاً ذكور ، قسمت عشوائياً الى ثمانية مجاميع تضمنت مجموعة السيطرة السليمة ، ومجموعة السيطرة المصابة وثلاث مجاميع عولجت بمستخلص الزعتر المستزرع بثلاث تراكيز (200,300,400) ملغم/كغم وثلاث مجاميع عولجت بمستخلص الزعتر البري بالتراكيز نفسها لمدة 30 يوماً . أظهرت النتائج حدوث انخفاض معنوي في اوزان الحيوانات المصابة وارتفاع معنوي في مستويات اليوريا والكرياتين وزيادة ملحوظة في المالونداياالديهايد (MDA) مع انخفاض في مستويات الغلوتاثيون (GSH) وانزيم السوبر أوكسيد ديسميوتاز (SOD)مقارنة بمجموعة السيطرة السليمة بالقابل أدت المعالجة بمستخلص الزعتر وبالأخص الزعتر البري الى تحسين معنوي في اوزان الحيوانات وانخفاض معنوي في مؤشرات وضائف الكلى وتحسن ملحوظ في مضادات الاكسدة بالإضافة الى تحسن في انسجة الكلى ، نستنتج من ذلك ان لمستخلص الزعتر البري فعالية اعلى من الزعتر المستزرع في التخفيف من الضرر الكلوي والاجهاد التأكسدي المصاحب للسكري المستحث بالالوكسان ، مما يشير الى إمكانية اعتماده كمصدر طبيعي مساعد في التخفيف من المضاعفات الكلوية لداء السكري

الكلمات المفتاحية : داء السكري ، الزعتر البري ، الكلى ، الاجهاد التأكسدي

The therapeutic effect of wild thyme extract compared to the cultured (*Thymus serpyllum*) in rats with diabetes induced alloxan

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ABSTRACT

Diabetes is a common metabolic disorder that leads to serious complications, including kidney damage resulting from oxidative stress. The current study aimed to evaluate the preventive and therapeutic effect of the alcohol extract of the wild thyme plant *Thymus Serpyllum* compared to cultured thyme in improving kidney function and oxidative stress indicators in rats with alloxan-induced diabetes. The experiment used 40 white male rats, which were randomly divided into eight groups that included a proper control group, an infected control group and three groups treated

with cultured thyme extract with three concentrations (200,300,400) mg/kg and three groups treated with wild thyme extract in the same concentrations for 30 days. The results showed a significant decrease in the weights of infected animals, a significant increase in urea and creatine levels and a marked increase in malondialdehyde (MDA) with a decrease in glutathione levels (GSH) and the enzyme superoxide dismetase (SOD) compared to the proper control group of treatment with thyme extract, especially wild thyme, led to a moral improvement in animal weights, a significant decrease in kidney function indicators and functions, a marked improvement in antioxidants, in addition to an improvement in kidney tissue. We infer from this, wild thyme extract has a higher effectiveness than cultured thyme in alleviating renal damage and oxidative stress associated with oxygen-induced diabetes, which indicates the possibility of adopting it as a natural auxiliary source in Alleviate kidney complications of diabetes.

Keywords: diabetes, *thymus serpyllum*, kidney, oxidative stress

Introduction

The Thyme Plant, Also Known As The Creeping Thyme, Is An Aromatic Herbaceous Plant Belonging To The Oral Family (Lamiaceae) That Thrives Throughout Eurasia, In Addition To North Africa, The Canary Islands And Northwes And Northwest Greenland [1] And Is Usually Found On Sandy Or Rocky Lands, Granting German Titles Sand Thyme And Field Thyme And Is Found Specifically In Calious Soil [2] In Traditional Medicine, Wild Thyme, Wild Thyme Is Used For Many Herbal Formulas Such As A Cough Drink, Socoffinges, Oils And Dyes [3] Wild Thyme Oil Has Body-Stimulating, Antispasm, Disinfectant, Phlentum-Repellent, Gas Repellent And Antifungal [4] As Well As The alcohol extract of the aerobic parts of the wild thyme plant has the ability to capture free radicals, especially hydroxyl radicals, and that the plant has good activity as an antioxidant. This activity is attributed to the presence of anti-oxidant phytochemical compounds in it [5]

Alloxan is a common cause of sugar widely used to stimulate experimental diabetes in rodent models, which leads to the destruction of beta cells (β) when given to vertebrates and thus developing an insulin-based type of sugar in rodents [6] The action of alloxan in the pancreas is preceded by rapid absorption by beta cells in the pancreas, as these cells perform the reductase process in the presence of various factors such as gutathione (GSH), cysteine acid (Cysteine), ascorbate) and sulphahydryl groups, where alloxan interacts with sulphyryl groups associated with the glucose enzyme in beta cells, leading to the formation of disulphide bonds that disrupt enzyme activity, which hinders glucose and inhibits the secretion Insulin thus damage to beta cells and the appearance of diabetes [7]

Type 2 Diabetes Mellitus Type 2 Diabetes Diabetes is one of the most common disorders, as the spread of type 2 worsens alarmingly in the world, especially among adults and young people in low-income countries [8] Type 2 diabetes results from the interaction of genetic factors that have caused poor insulin production with environmental and behavioural factors. This is usually a multifactorial disease that combines multiple genes and varying environmental factors [9] Type 2 diabetes is also characterised by insulin resistance and abnormal insulin secretion to maintain the stability of blood glucose levels [10] is one of the most important metabolic disorders What characterises and is associated with this pattern is obesity (Obesity) and (dyslipidaemia) and high blood pressure (Hypertension) and these disorders contribute to increasing the likelihood of cardiovascular disease [11] and that the majority of patients with this type suffer from insulin resistance as a result of obesity [12]

materials and methods of work

Collection of *Thymus Serpyllum* and use of the drug:

The wild thyme plant was collected from the city of Al-Qaim and Haditah in Anbar Governorate during the month of July 2025 in the afternoon to ensure its saturation with sunlight, then the roots were removed and the plant parts were removed in a well-ventilated place and away from light and sunlight, taking into account the continuous stirring. After it was completely ensured that the plant was completely dry, it was ground using an electric mill and then the powder was kept in special cans until the time of use.

Alloxan, a substance that helps stimulate experimental sugar, was obtained from the Indian company Thomas Baker at a concentration of 98% and a molecular weight of 160.09 g/mol

Preparation of the extract

The alcohol extract was prepared for the plant by soaking 50 g of plant powder in 500 ml of ethyl alcohol at a concentration of (70% ethanol + 30% distilled water). Then put the mixture in a glass cup (Beaker) and close it with wire completely and then put the mixture in a vibrating water bath for 24 hours at a temperature of 35 m, then filtering the mixture using several layers of medical gauze in order to get rid of the deposits and then take the solution after it became free of impurities and put it in the ultrasonic device (Ultrasonic) with a capacity of 65 Hz for 30 minutes, then take the product and put it on a magnetic motor until the alcohol evaporates The water and the product was concentrated in the form of a gel substance and then poured into glass dishes until it dried completely, and then a dry layer was formed that was scraped and scraped until use [13]

Experimental design

In this experiment, 40 white rats of the Swiss male type were used, similar in age and weight, ranging in age from 8 to 10 weeks, and their weights were from 200-260 grams, and were obtained from the Faculty of Veterinary Medicine at the University of Tikrit.

The rats were distributed randomly in plastic cages with dimensions of (43 x 28.5 x 20) cm, equipped with metal covers, and at the rate of 5 animals per cage, and were placed in the animal house of the Department of Life Sciences at the Faculty of Education for Girls, Anbar University.

The animals were placed under standard laboratory conditions that included a lighting cycle of 11 hours compared to 13 hours in the dark with the temperature stabilization at 22±2°C. Water and food were provided freely throughout the trial period. The cages were cleaned and sterilised weekly. The animals were given an adaptation period of 10 days before the start of the experiment in order to ensure that they adapt to the new conditions and ensure that they are free of diseases.

The animals were divided into 8 aggregates as follows:

G1: a group of proper control and given distilled water and normal food throughout the duration of the experiment and without any inducitation or treatment.

G2: the diseased control group, injected with Alloxan and no treatment was treated later.

G3: Injected with alloxan to induce diabetes and then dosed orally with cultured thyme at a dose of 200 mg/kg for 30 days.

G4: Injected with alloxan to induce diabetes and then dosed orally with cultured thyme at a dose of 300 mg/kg for 30 days.

G5: Injected with alloxan to induce diabetes and then dosed orally with cultured thyme at a dose of 400 mg/kg for 30 days.

G6: Injected with alloxan to induce diabetes and then dosed orally with wild thyme at a dose of 200 mg / kg for 30 days.

G7: Injected with alloxan to induce diabetes and then dosed orally with wild thyme at a dose of 300 mg/kg for 30 days.

G8: Injected with alloxan to induce diabetes and then orally dosed with wild thyme at a dose of 400 mg/kg for 30 days.

Blood sample collection

Blood samples were withdrawn directly from the heart using a sterile syringe with a capacity of 5 ml to obtain as much blood as possible. After that, the samples were transferred directly to sterile and anticoagulant-free test tubes, then placed in the centrifuge at a speed of 3000 rpm/minute for a quarter of an hour, then the serum was collected and stored at a temperature of20 ◌̊- for the purpose of estimating the indications and functions and functions of the kidneys (urea and creatine), as well as insulin, and antioxidants were also measured (Malondaldehyde (MDA), glutathione (GSH) and the enzyme superoxide desmiutase (SOD))

Measurement of biochemical standards

In this study, the standard kits of the French company Biolabo were used to estimate the concentrations of urea, creatin and insulin using the Spectrophotometry method.

Measuring of MDA, SOD, GSH

The concentrations of these indicators in the blood serum were measured using the enzyme-linked immunoassay (ELISA) technique, where the reaction plates were pre-coated with specific antibodies for both MDA, SOD, and GSH. The concentrations of MDA, SOD,GSH in the samples were determined depending on the intensity of the colour formed in the substrate solution, where the levels of MDA, SOD, GSH showed a direct relationship with the development of the colour, and the reaction was terminated by adding an acid stop solution, and then the absorbance was measured at a wavelength of 450 nm

Histological examination for rat Kidney

After withdrawing blood samples, the animals were explained directly by making an incision in the mervical cavity that extends from bottom to up in the direction of the heart, then the kidneys were removed after the adipose tissue and the surrounding macrophages were removed, and washed with distilled water to remove blood residue and preserved the tissue in a formalin solution at a concentration of 10% for the purpose of histological fixation. After the fixation process, 5 micrometre-thickness of tissue sections were prepared for each tissue and dyed with haematoxylene and eosin (H&E). The pathological changes were evaluated by a pathologist blindly (Blind assessment) [14]

Ethical consent

Experimental protocols and animal utilisation were approved by the Scientific Research Ethics Committee at Anbar University by virtue of the approval letter No. (3/14) dated 13/01/2025

Statistical analysis:

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The data was statistically analysed using the Statistics Program for Social Sciences (SPSS, 2019 edition) in order to study the impact of different coefficients in the studied qualities according to a complete random design (CRD) and the averages were compared using the minimum moral difference test (LSD) to verify the moral differences between them.

Results:

Comparison of animal weights before and after the experiment

The results of the current study Table No. (1) revealed moral differences at the probability level (P≤0.05) in the weight rate of animals before and after the experiment, where a clear moral decrease was observed in the sugar-infected control group compared to the healthy group. In return, the groups treated with different concentrations (200, 300, 400) showed a clear improvement in the body weight rate compared to the sugar-infected control group, as these groups showed an improvement close to the proper control group with minor moral differences, depending on the concentration of the treated substance.

Table 1: Comparison of different aggregates in the body weight rate of rats before and after the experiment

Average ± standard error of body weight(g)		Group
After the experiment	Before the experiment	
1.28± 238.20a	7.60± 223.33ab	G1
1.84± 175.00e	3.57± 218.33b	G2
1.18± 231.00ab	8.43± 238.33a	G3
1.02± 227.80bc	6.14± 226.67ab	G4
1.18± 221.00bc	4.95± 232.50ab	G5
4.20± 209.60d	3.27± 230.83ab	G6
1.69± 215.60cd	6.02± 222.50ab	G7
1.70± 210.00d	3.82± 227.50ab	G8
* 10.788	* 16.47	L.S.D.
Different letters in the same column indicate statistically significant differences at the *(P ≤ 0.05)		

Comparison of the effect of coefficients in kidney function (urea and creatine)

The results in Figures 2 and 3 showed a significant increase in urea and creatine concentrations at the probability level (P≤0.05) in the infected control group compared to the proper control group, where the values amounted to 68.76, 0.546 respectively, respectively, the aggregates treated with wild thyme extract (wild and cultured) showed a significant decrease in urea and creatine levels compared to the infected group as these values approached normal limits and the aggregates treated with wild thyme showed a concentration of (200,300,400) a greater improvement as the values approached the normal group compared to the cultured thyme.

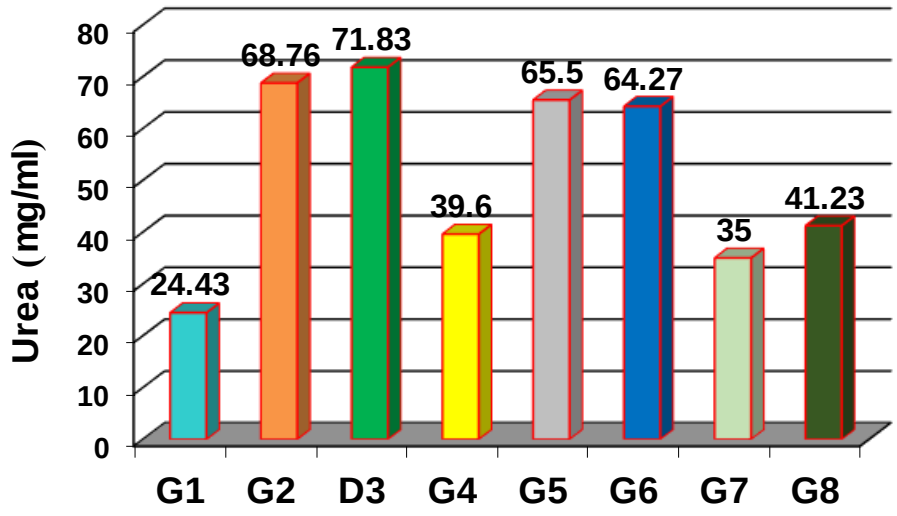

Figure 2: Comparison of different aggregates in urea values

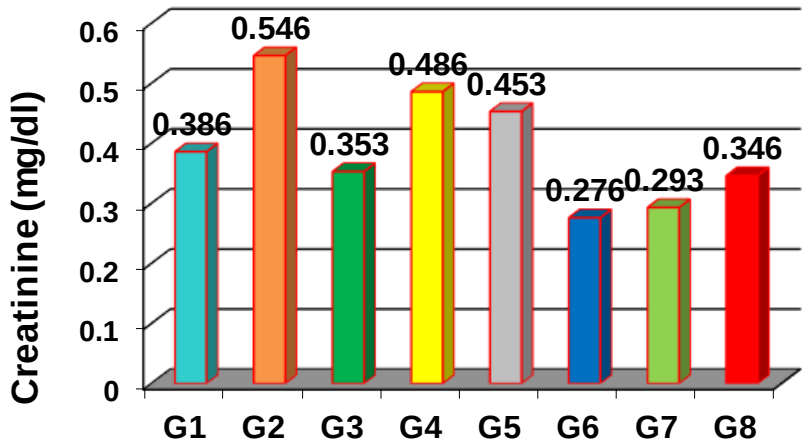

Figure 3: Comparison between different aggregates in creatine values

Antioxidants, stress and oxidants

The results of this study described in Figures (4,5,6) showed moral differences between the study aggregates at the probability level (P≤0.05). The sound control group showed the lowest concentration of the MDA level and the highest levels of SOD and GSH concentration compared to the rest of the aggregates. The sugar control group showed a significant rise in the MDA level and a clear decrease in GSH and SOD levels. This is evidence of oxidative stress resulting from increased free radical production and weakness in antioxidant Defence. The treated aggregates showed a gradual improvement in antioxidant levels, including a decrease in MDA levels and a rise in SOD and GSH levels. Compared to the infected diabetes group, the groups treated with wild thyme in concentrations (200, 300, 400) showed our better improvement than cultured thyme as the values approached the normal control group.

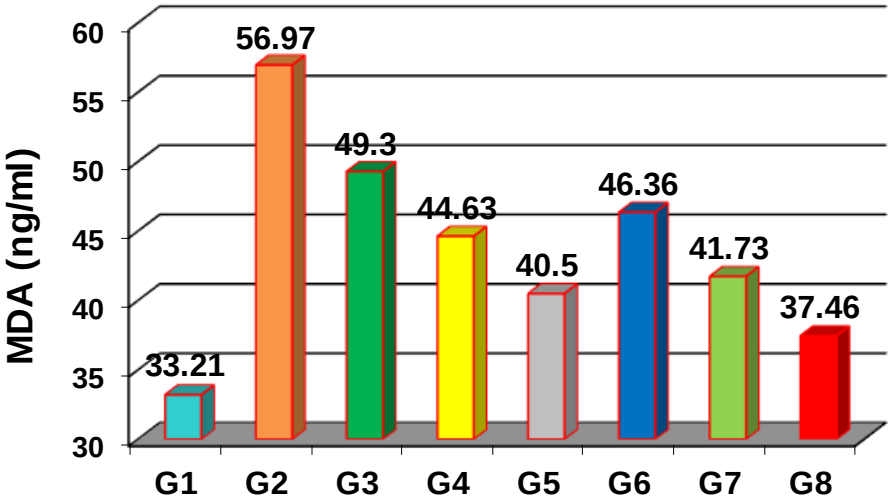

Figure 5: A comparison of different aggregates in MDA values

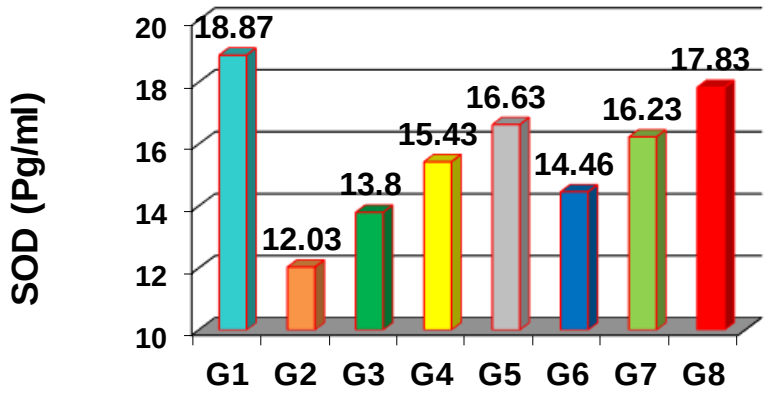

Figure 6: Comparison of different aggregates in SOD values

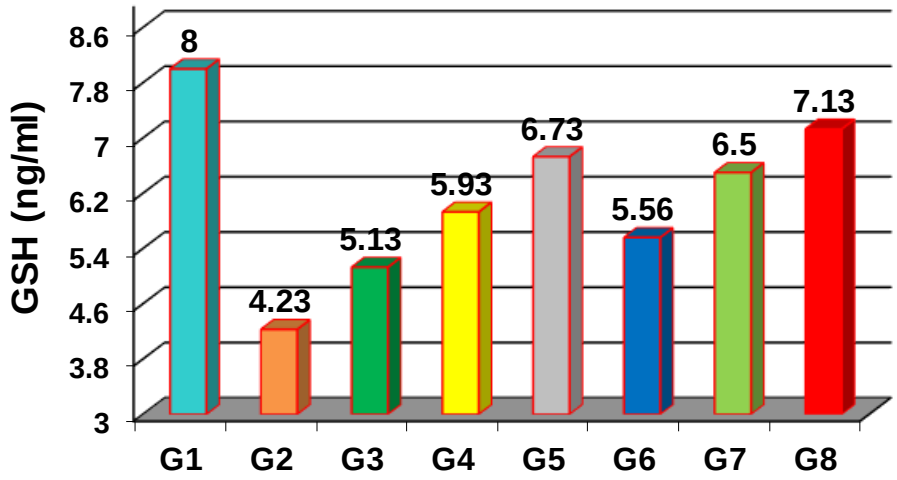

Figure 7: Comparison of different aggregates in GSH values

Histological results

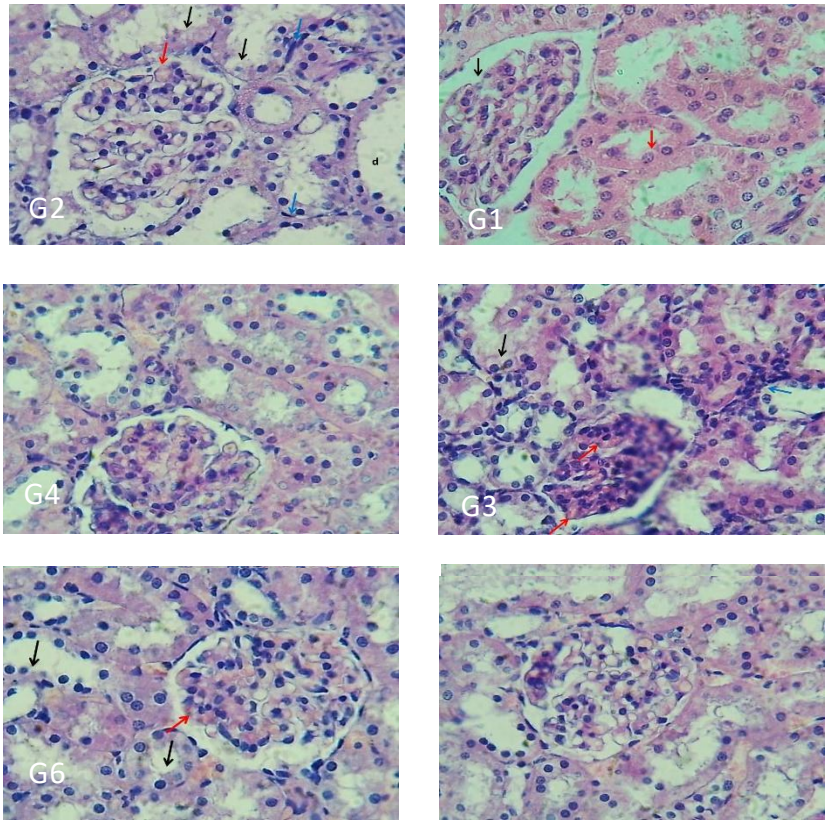

Figure 8: Tissue sections of the kidneys in different aggregates

The normal control group G1 with normal renal tissue consisting of endothelial cells of the normal renal tubules (red arrow) and a normal glombule (black arrow), the haematoxlin and iosine pigment (H&S) was adopted and with a magnification force of 400x, in the tissue of the infected control group G2 interstitial nephritis was observed characterised by the infilation of lymphocytes between the tubes (blue arrow), with degeneration of the renal tube cells (black arrow) expansion of the of the lymphocytes (d) and the infiltration of lymphocytes (blue arow) With the adoption of dye (H&S) and with a 400x magnification force, in the infected G3 group and treatment with cultured thyme, a concentration of 200, thickening and fibrosis (f) appears in the kidney wallet with hyperplasia in the middle cells (blue arrow) with degeneration and swelling of renal tubular cells with infiltration of inflammatory cells (black arrow) and fibrous (red arrow) dye (H&S) and with a force of 400x, in the infected G4 group and treated with cultured thyme Turker 300, a simple vascular congestion appears between the splaves and minor degeneration and without necrosis and fibrous, dye (H&G) and with a strength of 400x, In the infected G5 group and treated with cultured thyme, a concentration of 400 shows in the tissue a simple vascular congestion of the vesels between the tubes, simple degeneration and without necrosis and fibrous, dye (H&S) and with a strength of 400x, the infected group G6, G7 and G8 and treated with wild thyme concentration of (200, 300, 400) shows a normal appearance of the glomelus (red arrow) and normal for the renal int cells (black arrow) dye (H&S) and with a strength of 400x.

The discussion

The results of the current study showed that the development of sugar with aloxan led to a significant decrease in animal weights compared to the proper control group. This decrease in weight is due to the destruction of beta cells in the pancreas and a lack of insulin secretion. This leads to the lysis of fats and proteins and their use as alternative sources of energy. This study is consistent with a recent study that indicated weight loss in animals with oxygen-induced diabetes as a result of imbalances in metabolism and oxidative stress associated with diabetes.[16] Treatment with Thymus Serpyllum extract also showed a clear improvement in the weights of treated animals compared to the infected control group because the plant contains active compounds such as flavonoids and antioxidant compounds. This may be responsible for improving metabolism and preventing general impairment caused by diabetes. This is consistent with the study (Melesie 2020; Taye et al), which used an aeous extract of water and 80% methanol from Thymus schimperi leaves, which showed a marked improvement in the rate of weights of mice with aloxan-induced diabetes.[17]

The results of the current study also showed a significant improvement in kidney function by lowering the values of urea and creatine in the aggregates treated with wild thyme compared to the infected diabetes group. This is due to the containment of wild thyme on chemical compounds with antioxidant and anti-inflammatory activity and that these compounds reduce the oxidative stress caused by diabetes and oxygen in the kidneys. The results of this study are consistent with the study (Al-Mayali & Aljaefiri, 2022), which showed an improvement in the groups treated with wild thyme extract Thymus vulgaris in kidney functions such as urea, creatine and uric acid [18], and the groups treated with wild thyme also showed a better improvement compared to cultured thyme as the values approaching the group Natural control This is due to the fact that the content of active compounds in wild thyme is higher than in cultivated thyme, and antioxidant activity is stronger in wild species and also because of the high environmental stress that forces wild plants to produce more defensive compounds [19]

The results of the current study indicated a clear improvement in the indicators of AMD, SOD, and GSH values in the treated aggregates compared to the affected control group. The wild thyme showed a significant improvement as the values approach the normal control group. This indicates that wild thyme has antioxidant properties and its ability to capture free radicals and reduce oxidative stress. This is what the previous studies indicated that the use of olive oil and thyme powder affected liver functions and reduced oxidative stress in mice with cirrhosis, where the level of antioxidant indicators of Glutathione GSH and the hyperdismutase enzyme SOD decreased significantly, while the level of malonaldehydA decreased [20]

The results of the current study showed the occurrence of harm in the kidney tissue in the affected control group, represented by internerstitis, characterised by lymphocy inflation, cellular swelling of renal cells, with dilation of the collective tubes and congestion of glomerous vessels, while treatment with wild thyme in various doses led to a clear tissue improvement and the approach of tissue to the normal appearance and the disappearance of inflammatory and degenerative changes, as a result of wild thyme having a therapeutic effect with antioxidant and anti-inflammatory properties. This is consistent with previous studies that proved that the treatment of induced mice with Aloxan with Artemisia herba-alba extract

improved the renal tissue and the treated tissue showed a clear and closer change to normal tissue with the disappearance of many pathological changes [21]

The conclusion

We conclude from this study that wild thyme extract is more effective than cultured thyme in improving kidney function, reducing oxidative stress and repairing tissue damage associated with aloxan-induced diabetes. This indicates that it can be used as a natural source in the treatment of renal complications associated with diabetes.

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Conflict of interest

I declare that there is no conflict of interest related to the publication of this research.

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