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Role of Date Palm Seeds Extract in Modulating Inflammatory Mediators, Apoptotic Pathways, and Cellular Signaling in Alloxan-Induced Diabetic Male Rats

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Abstract

Hyperglycaemia is a result of genetic, environmental, and immunological factors. These factors lead to the destruction of pancreatic beta cells, resulting in insulin deficiency. This study aimed to investigate the effect of date seed extract on inflammatory, cellular, and tissue changes in male rats with alloxan-induced diabetes. Thirty rats were housed in the College of Veterinary Medicine at Tikrit University and divided into five groups. The control group was given distilled water, the second group was the diabetes induction group, while the third and fourth groups were induction groups treated with date seed extract and metformin, respectively. As for the fifth group, they were given only date seed extract. The results showed a significant increase in the levels of caspase-3, fetuin-A, IL-6, and STAT-3, with a decrease in IL-12 levels in the diabetic group compared to the control group. In contrast, the diabetic rats treated with date seed extract showed a reduction in the levels of caspase-3, fetuin-A, IL-6, and STAT-3, and an increase in the level of IL-12. Alloxan also showed detrimental effects on liver tissue, manifested in the infiltration of inflammatory cells, congestion of the central vein, and haemolysis. The chemical analysis of date seeds revealed the presence of phenols, flavonoids, and tannins, which possess antioxidant and anti-inflammatory properties. The current study demonstrated the vital role of date seed components in reducing levels of Caspase-3 and Fetuin-A, as well as inflammatory mediators, and improving the liver tissue structure in diabetic-induced rats.

Keywords: Phoenix dactylifera, Diabetes mellitus, Fetuin-A, Caspase-3.

دور بذور نخيل التمر في تعديل الوسائط الالتهابية، ومسارات موت الخلايا المبرمج، والإشارات الخلوية لدى ذكور الفئران المصابة بداء السكري المُستحثّ بالألوكسان

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الخلاصة

إن ارتفاع سكر الدم ناتج عن عوامل وراثية وبيئية ومناعية. هذه العوامل تؤدي إلى تدمير خلايا بيتا البنكرياسية ونقص الأنسولين. هدفت الدراسة الحالية لمعرفة تأثير نوى التمر على المؤشرات الالتهابية والخلوية والنسجية في ذكور الجرذان المصابة بداء السكري المستحث بالالوكسان. تم إيواء 30 حيوان في كلية الطب البيطري–جامعة تكريت وتم توزيعها إلى خمس مجاميع. تم اعطاء مجموعة السيطرة الماء المقطر بينما تم معاملة المجموعة الثانية بالالوكسان. اما المجاميع الثالثة والرابعة اعطيت الألوكسان لإحداث داء السكري ثم تم علاج المجموعتين بمستخلص بذور التمر والميتفورمين على التوالي. اما المجموعة الخامسة اعطيت مستخلص بذور التمر فقط. أظهرت النتائج زيادة كبيرة في مستويات , IL–6 , Fetuin–A , Caspase–3) (STAT–3مع انخفاض مستوى IL–12 في الفئران المصابة بالسكري غير المعالجة مقارنة بالمجموعة الضابطة. . بينما أظهرت الجرذان المصابة بالسكري والمعالجة بمستخلص بذور التمر انخفاضاً في مستويات (STAT–3 , IL–6 , Fetuin–A , Caspase–3)و ارتفاعاً في مستوى . IL–12 كما أظهر الألوكسان تأثيرات ضارة على نسيج الكبد تمثلت في حدوث ارتشاح الخلايا الالتهابية واحتقان الوريد المركزي وتحلل دموي. اظهر التحليل الكيميائي لبذور التمر وجود فينولات وفلافونيدات فضلاً عن التانينات.

1. Introduction

Alloxan is a well-known compound for its role as a diabetes-inducing agent in experimental models of diabetes, as it selectively targets pancreatic beta cells, leading to their destruction through mechanisms that involve the generation of reactive oxygen species (ROS) and increased calcium [1] . Diabetes is a chronic metabolic disorder characterized by elevated blood glucose levels [2] . The American Diabetes Association defines diabetes as a chronic and complex disease characterized by hyperglycaemia due to impaired insulin secretion, with insulin action, or both [3]. One of the most important reasons hyperglycaemia causes many complications is that glucose is an aldohexose type of ROS. Consequently, it leads to a moderate spread of oxidative stress in the body [4]. Many experimental studies have found that liver damage caused by diabetes and non-alcoholic fatty liver disease is associated with hepatitis, oxidative stress, and the accumulation of free fatty acids, all of which are the result of metabolic disorders due to insulin resistance and high blood sugar [5]. Some studies have also shown that elevated blood sugar levels contribute to the spread of oxidative stress, which in turn leads to the apoptosis of pancreatic beta cells. This process leads to the overexpression of protein kinase C and NF-κB, resulting in an increase in lipid peroxidation [6].

Diabetes can be managed using conventional pharmacological treatments or complementary approaches such as herbal medicine. In recent years, medicinal plants with antihyperglycemic properties have gained increased attention due to their lower cost and reduced side effects compared to standard therapies [7]. The date palm (*Phoenix dactylifera* L) is distinguished by a variety of medicinal properties due to its content of many active chemical compounds [8]. Date palm is a perennial, woody, monocotyledonous plant belonging to the Arecaceae family, which includes 200 genera and 3000 species [9]. One of the benefits of dates is that they can help treat metabolic diseases because they contain antioxidant compounds. One of the metabolic diseases is diabetes, which is still prevalent among many people who cannot maintain a healthy diet [10]. Studies have proven that date seeds contain a wide range of antioxidants, and the chemical composition of date seeds has been intensively studied through numerous reports that revealed many phenols and flavonoids responsible for free radical scavenging and antioxidant activities [11].

This study aims to explore the protective role of date seed components in reducing levels of Caspase-3 and Fetuin-A, modulating inflammatory mediators, and improving liver histopathology in alloxan-induced diabetic rats.

2. Materials and Methods

2.1. Plant seeds collection

The date seeds were collected from the date palm (Barhi) in the Al-Alam district, Salah al-Din Governorate, Iraq. Seeds were collected, washed, and dried at room temperature (25±2 °C) and with a humidity level of 30-50% for three weeks. They were then ground using an electric grinder to obtain the powder.

2.2. Extraction methods

The cold water method was used for the extraction procedure [12]. Ten grams of date seed powder mixed with 100 millilitres of distilled water and sit in a shaking incubator (20 RPM, temperature 25 Cº for 24 hours) to homogenize the powder. The mixture was filtered through multiple layers of medical gauze to remove any ingredients that had not been sufficiently ground. To obtain a pure filtrate free of contaminants and insoluble plant residues, the filtrate was filtered using filter paper (Whatman No. 1) after being placed in plain tubes and centrifuged for seven minutes at 4400 RPM.

2.3. Chemical analysis of Phoenix dactylifera seeds

The dry powder of the seeds was analyzed to assess the total alkaloid, glycoside, phenol, and flavonoid content.

The method by Manjunath Ajanal *et al.* [13] was used to assess the total alkaloid content. The approach of Tofighi *et al*. [14] was used to estimate the total glycosides, following the method of Laouini *et al.*, [15] for estimating total phenolic compounds. The approach of Habibatni *et al.*, [16] was used to estimate the total flavonoid content.

2.4. Animals of the study

In this investigation, thirty male Sprague Dawley albino rats, aged 10–12 weeks and weighing 190–210 grams, were used. The animals were housed in the animal facility of the College of Veterinary Medicine, Tikrit University, Iraq. They were maintained under standard laboratory conditions in plastic cages (13 × 28 × 46 cm) at a temperature of 25°C, with a 12:12-hour light/dark cycle and adequate ventilation. Throughout the 30-day experimental period, the rats were fed a standard laboratory diet.

2.5. Induction of diabetes mellitus by alloxan

After 18 hours of fasting, the animals received an injection of 150 mg/kg body weight of alloxan [1]. Before being used immediately, the alloxan was dissolved in normal saline and administered as a single injection intraperitoneally To avoid a decrease in blood glucose levels and to lessen the risk of hypoglycaemia shock after alloxan treatment, the animals were fed and given a 10% glucose solution for 24 hours after the injection. To verify that the condition was present, the rats' blood glucose levels were checked every two days for 10 days

2.6. Experimental design

Thirty rats weighing between 190 and 210 grams and between 10 and 12 weeks of age were kept at the animal house section of Tikrit University's College of Veterinary Medicine in Iraq. The practical study period was conducted under standard laboratory conditions. Alloxan 150 mg/kg was used to cause diabetes in 20 of the 30 rats. The rats were split into five groups: the diabetic group (DM), the diabetic group treated with 50% v/v date seed

extract, and the diabetic group given metformin [17]. The healthy control group (CTRL) was given distilled water. The last group was given a simple extract from date seeds, 50% v/v.

2.7. Collection of blood samples

Rats were anesthetized using chloroform, and then blood samples were taken via heart puncture following the 30-day trial period. The serum was extracted by centrifuging the blood in test tubes at 3000 RPM for 15 minutes after it had been sitting in a water bath at 37°C for 30 minutes.

2.8. Measurements of biochemical parameters

Serum was utilized to estimate the concentrations of caspase-3, fetuin-A, interleukin-6 (IL-6), interleukin-12 (IL-12), and signal transducer activator of transcription 3 (STAT-3). The concentrations were determined using an enzyme-linked immunosorbent assay ELISA kit following the manufacturer's instructions, made by Sunlong Biotech, China.

2.9. Histological study preparation

After being immediately preserved in 10% formalin for 24 hours, the liver tissues were rinsed with tap water and dried using varying alcohol concentrations. Following purification and infiltration, the samples were embedded in paraffin at 58-60 Cº. A rotary microtome was used to slice the paraffin-embedded tissues to a thickness of 5 micrometres. Following hematoxylin and eosin staining, the sections were viewed under a light microscope [18].

3. Results

3.1. Biochemical results

As indicated in Table 1, the date palm seed extract contains various antioxidants, including phenols, flavonoids, alkaloids, glycosides, tannins, and saponins. These have all been identified through chemical examination of the seeds.

Results in Table 2 revealed that the levels of caspase-3, fetuin-A, IL-6, and STAT-3 in the untreated diabetic group were significantly increased ($P < 0.05$), whereas the levels of IL-12 were significantly decreased ($P < 0.05$). Compared to the untreated diabetic group, the groups treated with date palm seed extract exhibited improvements in their caspase-3, fetuin-A, IL-6, and STAT-3 levels ($P<0.05$). Compared to the untreated diabetic group, metformin treatment also showed improvements in IL-12, fetuin-A, IL-6, and STAT-3 levels. However, there were no discernible changes in caspase-3 levels ($P<0.05$).

Table 1: Chemical content and antioxidants of the date palm seed extract

Contents	Concentration
Total phenolic content (mg Gallic / 100 g)	452.2
Total flavonoid content (mg Rutin / 100 g)	241.9
Total alkaloid content %	0.85
Total glycoside content %	0.41
Total tannin content %	1.1
Total saponin content %	0.06

Table 2: The effect of the studied treatments and different concentrations in all study groups

	control	*DM*	*DM+Seeds*	*DM+Metformin*	*seeds*
Caspase-3	**1.092 ± 0.049**	**1.429 ± 0.053**	**0.64 ± 0.021**	**1.308 ± 0.074**	**0.953 ± 0.061**
Fetuin-A	**264.56 ± 2.59**	**347.591` ± 6.257**	**298.576 ± 2.811**	**255.394 ± 3.964**	**303.455 ± 5.921**
STAT-3	**9.568 ± 0.015**	**10.975 ± 0.061**	**7.554 ± 0.287**	**9.776 ± 0.256**	**10.345 ± 0.166**
IL-6	**145.04 ± 2.828**	**391.373 ± 3.055**	**182.04 ± 1.414**	**237.04 ± 2.828**	**279.04 ± 1.414**
IL-12	**155.013 ± 1.341**	**136.584 ± 3.673**	**148.922 ± 2.388**	**154.117 ± 0.811**	**130.957 ± 5.249**

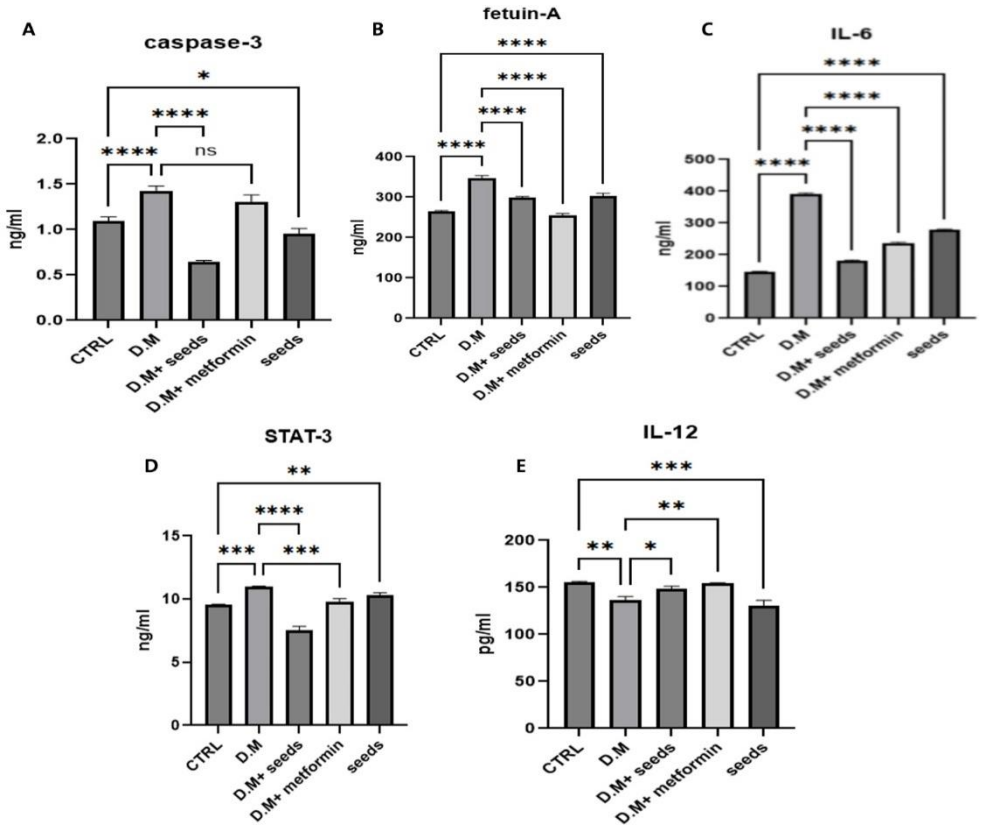

Figure 1: Effects of date seed extract and metformin on serum levels of (A) Caspase-3, (B) Fetuin-A, (C) IL-6, (D) STAT-3, and (E) IL-12 in diabetic rats. Diabetic rats (DM) exhibited increased levels of Caspase-3, Fetuin-A, IL-6, and STAT-3, while IL-12 levels were reduced. Treatment with date seed extract and metformin significantly reduced levels of caspase-3, fetuin-A, IL-6, and STAT-3, with the seed extract showing a more pronounced effect than metformin. Conversely, IL-12 levels, which were lower in diabetic rats, increased following treatment. Statistical significance is indicated as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

In Figure 1, the diabetic rats demonstrated the ability to increase the blood level of caspase-3. On the other hand, the date seed extract treatment group experienced a decrease in caspase-3 concentration. The seed extract showed the most substantial reduction in caspase-3 content compared to metformin. It also showed that the amount of fetuin-A in the serum had increased. However, following treatment with date seed extract and metformin, fetuin-A levels dropped. Metformin significantly reduced the concentration of fetuin-A compared to the seed extract. It also exhibits the ability to raise IL-6 levels in the blood. However, following treatment with date seed extract and metformin, IL-6 levels dropped. The seed extract was more important in proving this than metformin.

3.2 Histological results

Hemolysis, central venous congestion, and inflammatory cell infiltration were all signs of alloxan's detrimental effects on the liver. On the other hand, the date palm seed extract showed the capacity to protect liver tissues from the harm caused by alloxan, compared to both the metformin group and the untreated diabetic group.

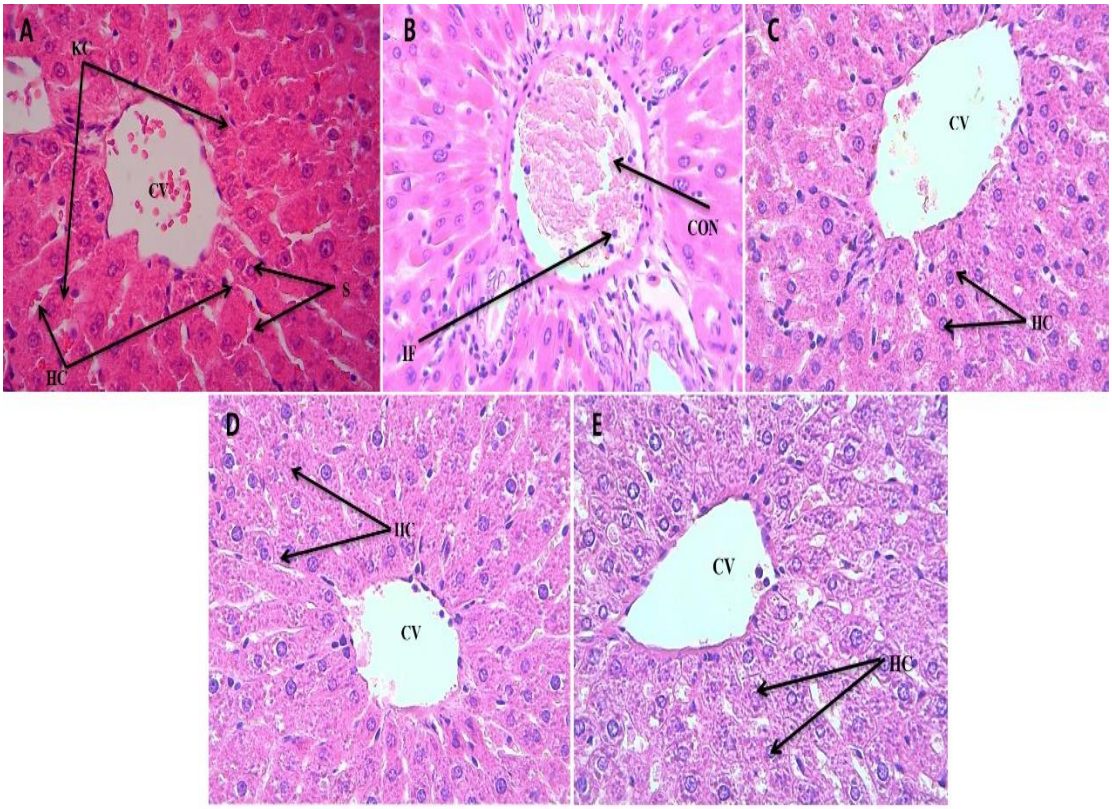

Figure 2: Liver sections A: liver section from the control group showing the central vein (CV) and hepatocytes (HC) arranged around it in hepatic cords, with sinusoids (S) visible and Kupffer cells (KC) observable. H & E 400X; **B**: The liver section of the group treated with alloxan shows congestion of the central vein (CON) and infiltration of inflammatory cells (IF). H & E 400X; **C**: The liver section of the group is treated with alloxan, and the aqueous extract of date seeds shows the central vein (CV) and hepatocytes (HC) in their standard form. H & E 400X; **D**: The liver section of the group treated with alloxan and metformin shows the central vein (CV) and hepatocytes (HC) in their standard form. H & E 400X; **E**: section of the liver from the group treated with the aqueous extract of date seeds shows the central vein (CV) and hepatocytes (HC) in their standard form. H & E 400X,

4. Discussion

Alloxan increases the production of ROS, which leads to alterations in insulin synthesis and secretion due to its selective toxicity toward pancreatic beta cells. Because of the high levels of interaction between proteins and glucose, protein glycosylation can be triggered by hyperglycemia levels. Advanced glycation end products (AGEs), which are the reaction's byproducts, are a sign that oxidative stress has occurred. Later stages of oxidative stress may worsen the harm to beta cells in the pancreas, resulting in cell death [19].

Due to their immunomodulatory and anti-inflammatory properties, date palm seeds (*Phoenix dactylifera* L.) have a significant influence on inflammation and cellular signalling [20]. The current study's findings demonstrated the efficacy of date seeds by demonstrating a reduction in caspase-3 activity. The chemical compounds of date seeds, which includes phenolics, flavonoids, alkaloids, tannins, and other bioactive compounds, are responsible for this decline, as indicated in Table 1. Antioxidants reduce the activation caspase-3 in various cell types by lowering oxidative stress, which is a primary cause of programmed cell death [21]. Additionally, research indicates that date seed extracts can increase the expression of anti-apoptotic proteins like Bcl-2 [22] and decrease the expression of pro-apoptotic proteins like Bax and caspase-3.

Fetuin-A levels are genetically associated with an increased risk linked to an elevated risk of both diabetes and coronary artery disease, particularly in individuals with diabetes with diabetes [23]. According to the study, date seed extract lowers levels of the protein fetuin-A, which is linked to metabolic diseases. The active compounds in date seeds, primarily phenolics, mainly phenolic ones, improve insulin sensitivity and contribute to their antioxidant qualities. Since elevated fetuin-A levels are frequently linked to poor glycemic control and glucose management, date seeds may indirectly result in a drop in fetuin-A levels [24].

Although its direct impact on insulin-mediated glucose uptake absorption is still debatable, high levels of IL-6 are also linked to insulin resistance in patients with type 2 diabetes. The recent study's findings demonstrated that date seeds help reduce IL-6 levels, thereby attenuating inflammatory responses by inhibiting COX-1 and COX-2 [25], while metformin inhibits cytokine receptor AMPK, which inhibits IL-6 gene expression [26].

Polyphenolic compounds, which are abundant in date seeds, have been shown to suppress pro-inflammatory cytokines, such as IL-6, TNF-α, and IL-1β [27].

One pathophysiological feature of disorders linked to hyperglycemia is the increase in STAT-3. In diabetic patients, higher levels of STAT-3 result in higher amounts of free fatty acids and reduced insulin sensitivity [28]. Increased levels of pro-inflammatory cytokines like IL-6 relate to the activation of STAT-3, which is thought to be implicated in the inflammatory response linked to diabetes [29]. Date seeds contain trace elements like zinc and magnesium. Zinc directly suppresses STAT3 activation through MAPK/EPK pathway, and magnesium may synergize with the effect to improve metabolic parameters and reduce insulin resistance [30].

Findings also demonstrated that the group treated with date seeds had an increased level of STAT-3; however, zinc in healthy states increases STAT3 phosphorylation at ser727 through the ERK pathway, which improves cardiac oxidative phosphorylation and induces astrocyte proliferation , also date seed that indicated can inhibit IL-1β and TGF-β, it showed no significant change in IL-12 levels, while our study showed improvement in IL-12 levels in the diabetic group after date seed extract administration ($P<0.05$). Further research is needed to clarify the precise impact of date seeds on IL-12 and other cytokines [31].

As seen in Figure 2 B, the histological analysis revealed that alloxan administration caused significant alterations in the liver tissue of rats resulting in various histological alterations in

their liver tissue. Hemorrhagic necrosis and inflammatory cell infiltration were among these alterations, as were central vein (CON) congestion and inflammatory cell infiltration. Furthermore, an increase in Kupffer cells and the Hepatic sinusoidal dilation were observed. Increased production of inflammatory cytokines like TNF-α and IL-6 results from alloxan activating inflammatory pathways in the liver, including the NF-κB pathway. According to Al-Harbi *et al.,* these cytokines worsen inflammation, promote damage to liver tissue, and cause inflammatory cells to proliferate .In the infected group that received date seed treatment, no histological alterations were seen in the liver tissue, as seen in Figure 2 C. Since date seeds contain various compounds with antioxidant properties that can stop the adverse effects of alloxan, the central vein and liver cells show up in their standard form, demonstrating the protective role of date seeds. Triterpenoids, lutein, total anthocyanins, saponins, oxalates, and caffeine are found in date seeds. Furthermore, every date seed extract exhibited free radical scavenging and antioxidant properties, which prevent lipid oxidation. The chronic activation of STAT-3, linked to several cancer forms, may be inhibited by date seed extract [32]. Numerous bioactive substances with significant biological and pharmacological qualities, such as antioxidants, anti-inflammatory, antidiabetic, antibacterial, and antiviral agents, are abundant in date seeds [33].

Furthermore, date seed extracts have been shown to have numerous potential protective effects against lipid peroxidation and atherosclerosis because these active compounds have antioxidant qualities that are crucial for reducing the adverse effects of free radicals in a variety of illnesses, including diabetes, cancer, obesity, anaemia, neurological disorders, lung diseases, nephropathy, and cardiovascular diseases [34].

However, as seen in Figure 2D, where there is blood congestion and inflammatory cell infiltration surrounding the bile ducts, the study did not detect any histological alterations in the liver tissue of the infected group receiving metformin. As previously stated, this is because of the negative consequences of alloxan. Additionally, the effects of metformin are considered. Metformin is one of the biguanide drugs that bind to the phospholipid group in the cell membrane. It causes thickening by reducing membrane fluidity due to its interaction with the phospholipids' polar head group. Permeability changes as a result, reflecting damage to the cells. Furthermore, metformin is primarily active in the liver, which receives it straight from the intestines through the portal vein. When taken orally, it builds up in the liver and reaches a high concentration [35]. Since the liver is the first organ to metabolize chemicals from the digestive tract, this impacts it and intensifies tissue effects.

In contrast to metformin, the date seed extract showed a superior efficacy to lower the levels of IL-6, Caspase-3, and the STAT-3 pathway. However, as Figure 2 illustrates, the data demonstrated how well metformin worked to lower the level of fetuin-A.

5. Conclusion

This study demonstrates how date seed extract may be therapeutic in controlling important indicators linked to inflammation and apoptosis caused by diabetes. Caspase-3, fetuin-A, IL-6, and STAT-3 levels were higher in diabetic rats, although IL-12 levels were lower. These changes were considerably lessened by date seed extract treatment, showing a more significant reduction in caspase-3, fetuin-A, IL-6, and STAT-3 levels than metformin. The extract also assisted in raising IL-12 levels again. These results imply that date seed extracts have anti-inflammatory and anti-apoptotic qualities, making them a potential supplemental therapy or natural option for treating diabetes-related issues. Future research should examine the potential clinical applications in diabetic management and the molecular mechanisms underlying the immune response.

Ethics approval

The study was approved by the Tikrit University-Iraq Scientific Research Ethics Committee (Approval No. TUA000013; October 10, 2024).

Disclosure and conflict of interest

The authors of this study state that they have no conflicts of interest related to its publication. Every contributor has declared any affiliation or activity that might potentially influence the study's findings.

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