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The Role of Baobab in Controlling Oxidative Stress on induced diabetic Male Albino Rats.

Dhuha waleed Salih [1] Husamuldeen Slim Alnajar [2] Dakheel Hussein Hadree[3]

[1,3]Deprtment of Physiology, Pharmacology and Biochemistry , Coll. Vet. Med. Tikrit University
[2] Department of pharmacology College of Medicine – Tikrit University.

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Corresponding Author:
Name: Dhuha waleed Salih.
E-mail: dhuhaaldoury@gmail.com

Tel: :

ABSTRACT

This study has been conducted in order to examine the effects of baobab on oxidative stress in a diabetic model. Including seven groups, involved 10 albino rat for each group the first group is the control group that no treatment and positive group diabetic group which treated with alloxan was used to induce diabetes in the experimental group 100 mg/kg. Blood samples were taken to confirm diabetes, and after stabilization, the experimental group was distributed into subgroups. Different treatments, including baobab crude 500mg/kg , metformin 100 mg/kg, and combination(baobab 500 mg/kg + metformine 100 mg/kg) , were administered orally until day 30 . Kidney and liver samples isolated collected for histological analysis. Statistical analysis using ANOVA and tests was be performed, and the results was presented as mean ± SEM

Baobab effectively reduced malondialdehyde MDA levels and increased total antioxidant status TAOS, indicating its potential as an antioxidant in managing oxidative stress associated with diabetes. Histological examination showed changing in liver tissue structure. Baobab has promising potential as a natural option for decrease the oxidative stress and preventing complications in diabetes.

In conclusion, Baobab, has antioxidant properties and has shown promise in managing oxidative stress In the context of rat diabetes, the application of baobab has demonstrated effective reductions in MDA levels, concurrent increases in TAOS, and notable enhancements in liver tissue structure. The utilization of baobab's crude plant form emerges as a potential natural strategy to bolster overall health and preempt complications linked to diabetes. However, it is imperative to underscore that a more comprehensive investigation is required to gain a thorough grasp of its therapeutic capabilities.

1. Introduction

In recent years, there has been growing interest in the use of natural compounds with antioxidant properties to combat oxidative stress and its associated complications (4). Baobab (Adansonia digitata) is a well-known African tree that has been traditionally used for its medicinal properties(11). Various parts of the baobab tree, including its fruits, leaves, bark, and seeds, have been reported to possess significant antioxidant activity(12).

Baobab fruit is rich in bioactive compounds such as polyphenols, flavonoids, and vitamin C, which are known to exert potent antioxidant effects(13,15). These compounds scavenge and neutralize ROS, thereby protecting cells and tissues from oxidative damage(16). The potential role of baobab in controlling oxidative stress has been investigated in several studies, particularly in the context of diabetes. the potential benefits of baobab in combating oxidative stress in diabetes can contribute to the development of novel therapeutic strategies or dietary interventions to alleviate the burden of diabetic complications(17). The findings of this study may provide valuable insights into the use of baobab as a natural antioxidant agent in the management of diabetes-related oxidative stress and its associated complications.

Baobab acts as an antioxidant through its polyphenols, flavonoids, and vitamin C content. It scavenges harmful reactive oxygen species (ROS), inhibits lipid peroxidation, boosts antioxidant enzyme activity, chelates metal ions, regenerates endogenous antioxidants, and reduces inflammation. These actions help protect cells from oxidative damage(18).

These mechanisms collectively contribute to the protective effects of baobab against oxidative stress and its associated health implications(19).

Oxidative stress is a condition that arises when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize and eliminate them through antioxidant defense mechanisms(1)(2). It plays a significant role in the development and progression of various chronic diseases, including diabetes mellitus(3)(4).

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both(5)(6). It has been linked to increased oxidative stress due to the generation of excessive ROS and reduced antioxidant capacity in the body(7). Chronic oxidative stress contributes to the development of diabetic complications such as neuropathy, nephropathy, retinopathy, and cardiovascular diseases(8)(9)(10).

Additionally, baobab has the ability to inhibit lipid peroxidation. Lipid peroxidation is a process in which free radicals attack and oxidize lipids in cell membranes, leading to cellular damage. Baobab's antioxidant compounds interrupt this chain reaction and stabilize lipid radicals, preventing further damage to cell membranes.(20)

This study aims to investigate the role of Adansonia digitata in controlling oxidative stress in induced diabetic male albino rats. It will explore the effects of baobab supplementation on oxidative stress markers, such as antioxidant enzyme activities, such as TAOS and measure oxidative stress such as MDA and liver of diabetic rats. Additionally, histopathological examination will be conducted to assess the protective effects of baobab of liver organs.

2. Material and method

a. Expermental Design

The research employed an interventional study design to investigate the anti-inflammatory effects of baobab on induced diabetic male albino rats.

b. Study Setting and Period

The study was conducted at Mosul University's College of Veterinary Medicine. The research duration spanned from [1/11/2022] to [30/12/2022].

c. Study Sampling and Sampling Methods

Seventy adult albino rats (8-10 weeks old, weighing 250-300g) were obtained from the animal house at Mosul University's College of Veterinary Medicine. They were divided into seven groups, each with ten rats. Alloxan-induced diabetes was induced in specific groups, while others were assigned control conditions

d. Experiment Design

The study was conducted at the Veterinary Medicine College of Mosul University with approval from the Postgraduate Studies Committee. An interventional study design was utilized to investigate the oxidative stress effects of baobab on induced diabetic male albino rats(27).

Baobab is The plant material used in this study, Adansonia digitata (baobab), was sourced from local regions known for its growth .Rats received 500 mg/kg/day of Adansonia digitata through oral gavage(15).

Wistar albino rats were sourced from the Animal House at Mosul University. They were accommodated in a controlled environment room, maintaining conditions at approximately 22 ± 2 °C temperature, around 60% relative humidity, and following a 12-hour light-dark cycle. These rats were given unrestricted access to a standard diet devoid of additives, supplied by Mosul University veterinary collage . Furthermore, they had continuous access to tap water(28) . Metformin, obtained from Samara Company, was given at a dose of 100 mg/kg/day dissolved in water via oral gavage. Dosages for both substances were based on body surface area(29).

Alloxan was induced in the experimental rat group through the administration of alloxan at a dose of 100 mg/kg/day based on the body weight of the rats after an overnight fast. The determination of this dose involved several pilot studies to establish an appropriate and effective dosage. Alloxan was dissolved in 1 ml of normal saline for administration.

Alloxan was used to induce diabetes in the experimental group consisting of 40 rats each group contain ten rats .After the injection, blood samples were taken at 48 and 72 hours to confirm diabetes based on blood glucose levels the level is excess than 200 mg /dl (30)

e) General Experimental group:

The study included multiple groups:

(G1) 10 rats receiving a standard lab diet healthy control.

(G2) 10 diabetic rats induced with alloxan monohydrate100 mg/kg.

(G3) 10 rats treated with baobab crude Baobab received 500 mg/kg/day through oral gavage that dissolve by water

(G4) 10 rats treated with metformin was given at a dose of 100 mg/kg/day.

(G5) 10 diabetic rats group receiving only baobab crude 500 mg/kg for 4weeks

(G6)10 diabetic group receiving only metformin100mg/kg for 4weeks.

(G7) 10 diabetic group receiving baobab crude 500 mg/kg and metformin 100 mg/kg for 4 weeks.

The treatments were administered orally for four weeks to assess their effects on diabetic conditions. For histological examination, the kidney and liver samples were collected from the rats and fixed in 10% buffered neutral formalin for 48 hours to preserve their structure. After washing, the samples underwent a dehydration process using increasing concentrations of ethanol. They were then cleared with xylene and infiltrated with molten paraffin wax. The tissue blocks were embedded in fresh paraffin wax, and thin sections were obtained using a microtome. The sections were mounted on glass slides, deparaffinized, and stained with hematoxylin and eosin. After staining, the sections were examined under a light microscope. This protocol was adapted from Suvarna et al. (2018) for the preparation and analysis of liver tissue sections. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by post-hoc tests. The data will be presented as mean ± standard error of the mean (SEM). A p-value less than 0.05 will be considered statistically significant.

3. Result

Table(1)demonstrate the Impact of Baobab Crude Extract on MDA Levels (ng/dl) in Diabetic Rat Model . This table illustrates the effects of baobab crude extract on malondialdehyde (MDA) levels within a diabetic rat model over different time Control (G1) MDA levels remain consistent. .Diabetic (G2, G5, G6, G7) MDA levels increase.Normal Baobab (G3) and Metformin (G4). MDA levels stay relatively stable.Combination Treatments (G5, G6, G7MDA levels decrease when baobab and metformin are combined in diabetic rats.Significant differences among groups and time points are indicated by low p-values. Letters highlight noteworthy MDA level disparities within the same group. In essence, this table presents how baobab extract affects MDA levels over time in a diabetic rat model, revealing trends and significant differences between different treatments and time intervals.

Table (1): Effect of Baobab Crude on MDA Levels (ng/dl) Over Time in a Diabetic Model

Time / Groups	Mean ± S.E MDA Levels (ng/dl) N(10) /Group				
	Day zero	**Two days after treatment with alloxan**	**Day15**	**Day30**	***p-value***
Control group (G1)	2.44 ± 1.31 (A,a)	2.44±0.17 (A,a)	2.49 ± 0.10 (A,a)	2.51 ± 0.09 (A,a)	0.343
Diabetic group G2	2.33 ± 0.10 (A,a)	6.76 ± 0.18 (A,a)	7.24 ± 0.27 (A,a)	7.17 ± 0.15 (A,a)	**0.000****
Normal Baboab(G3)	2.33 ± 0.10 (A,a)	2.30±0.09 (A,a)	2.34 ± 0.09 (A,a)	2.42 ± 0.06 (A,a)	0.450
Metformin group (G4)	2.37± 0.08 (A,a)	2.34±0.09 (A,a)	2.42 ± 0.07 (A,a)	2.38 ± 0.03 (A,a)	0.129
Diabetic +Baboab(G5)	2.56 ± 0.04 (A,a)	6.84 ± 0.17 (C,b)	4.18 ± 0.19 (B,a)	2.98 ± 0.15 (A,b)	**0.000****
Diabetic+metformin (G6)	2.43 ± 0.89 (A,a)	6.83 ± 0.83 (C,b)	4.33 ± 0.30 (B,b)	3.17 ± 0.14 (B,b)	**0.000****
Diabetic+metformin +baobab (G7)	2.42 ± 0.69 (A,a)	6.62 ± 0.20 (C,b)	3.67 ± 0.15 (B,b)	2.36 ± 0.08 (A,a)	**0.000****
p-value		**0.000****	**0.000****	**0.000****	

Horizontally mean superscript capital letter blue color same letter significant difference while different letter non - significant

*** Significant differences at p<0.05, ** highly significant level <0.01.**

Table (2)demonstrate the Baobab Crude Effect on TAOS Levels over Time in Diabetic Rats This table depicts the influence of baobab crude extract on total antioxidant status (TAOS) levels within a diabetic rat model over distinct time intervals.

(G1):TAOS levels remained stable throughout

(G2):Significant TAOS decrease post-alloxan treatment, sustaining low levels.

(G3) and Metformin (G4Minor TAOS fluctuations observed.

Combination Treatments (G5, G6, G7Substantial TAOS increase compared to diabetics. Baobab extract correlates with increased TAOS levels. Combination treatments display noteworthy improvements in TAOS compared to diabetes alone. The results support baobab's potential to enhance antioxidant capacity. Combining baobab with metformin shows particularly promising outcomes. Baobab extract holds promise for bolstering antioxidant defenses in diabetic rats

this table(2) underscores baobab's positive influence on TAOS levels in a diabetic rat model, with significant implications for potential therapeutic applications.

Table (2): Effect of Baboab Crude on TAOS Levels(ng/dl) over Time in a Diabetic Model.

Time / Groups	Mean ± S.E TAOS Levels (ng/dl) N(10) /Group				
	Day zero	**Two days after treatment with alloxan**	**Day15**	**Day30**	***p- value***
Control group (G1)	33.32 ± 0.79 (A,a)	34.62 ± 1.17 (A,b)	33.08 ± 0.93 (A,c)	33.26 ± 0.74 (A,c)	0.915
Diabetic group G2	31.60 ± 0.24 (B,a)	11.78±0.21 (A,a)	11.40 ± 0.09 (A,a)	11.46 ± 0.32 (A,a)	**0.000****
Normal Baboab(G3)	31.84 ± 0.30 (A,a)	31.62±0.25 (A,b)	31.10 ± 0.28 (A,c)	32.86 ± 0.54 (A,c)	0.835
Metformin group (G4)	31.58 ± 0.57 (A,a)	31.25±0.60 (A,b)	30.40 ± 0.27 (A,c)	33.98 ± 0.57 (A,c)	0.805
Diabetic +Baboab(G5)	31.02 ± 0.38[a] (B,a)	14.38 ± 1.16 (A,a)	25.78 ± 1.09 (B,b)	30.96 ± 0.74 (B,c)	**0.000****
Diabetic+metformin (G6)	31.18 ± 0.21 (C,a)	12.62 ± 1.21 (A,a)	24.34 ± 0.67 (B,b)	30.98 ± 0.57 (C,c)	**0.000****
Diabetic+metformin +baobab (G7)	31.32 ± 0.33 (C,a)	10.44 ± 0.89 (A,a)	25.12 ± 0.72 (B,b)	31.00 ± 0.35 (C,c)	**0.000****
p- value	0.875	**0.000****	**0.000****	**0.000****	

✓ Horizontally mean superscript capital letter blue color same letter significant difference while different letter non - significant

✓* Significant differences at p<0.05, ** highly significant level <0.01.

The histological features observed in Figure (1) of the negative control healthy liver tissue are indicative of normal liver histology. The polygonal liver cells, intact blood sinusoids with Kupffer cells, lymphocytic diffusion, and the presence of a portal vein with blood all reflect the typical architecture and cellular composition of a healthy liver tissue section.

Figure (2) shows hypertrophy of liver cells (A), a lymphoblastic mass around the bile duct branch (B), and normal liver cells (C) in the positive group. These histological findings indicate potential liver pathology, such as cell enlargement and an immune response in the liver tissu

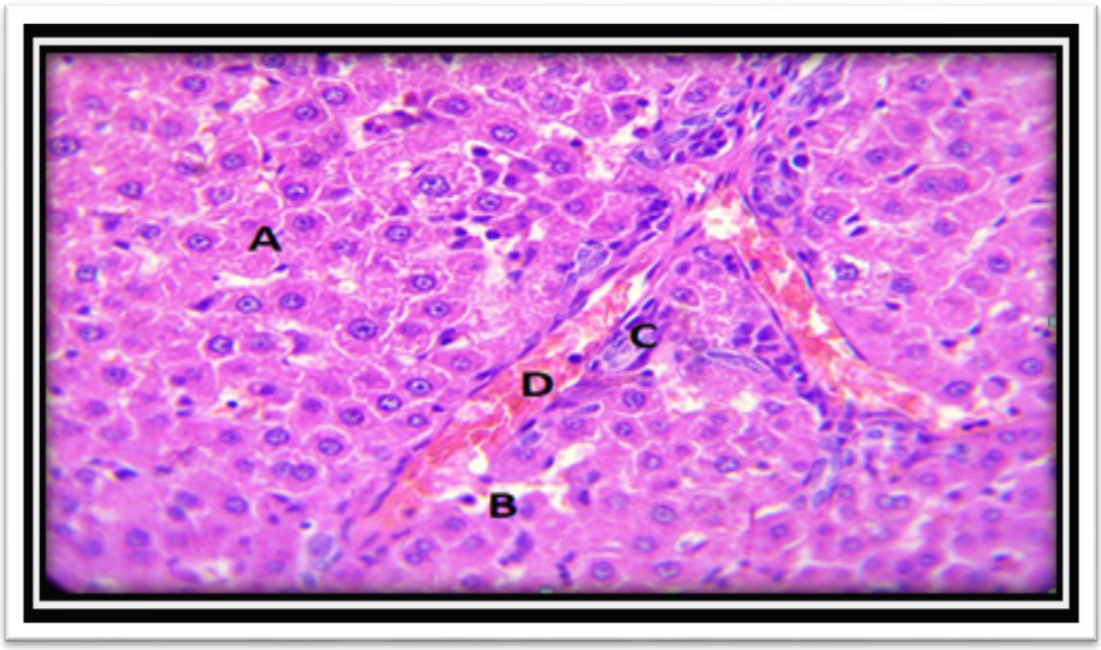


Figure (1): healthy negative control group healthy Polygonal liver all's (A) Blood Sinusoid with Kuepfer cells (B) lymphocytic diffusion (c) portal vein with blood (D) (H2EX 40).

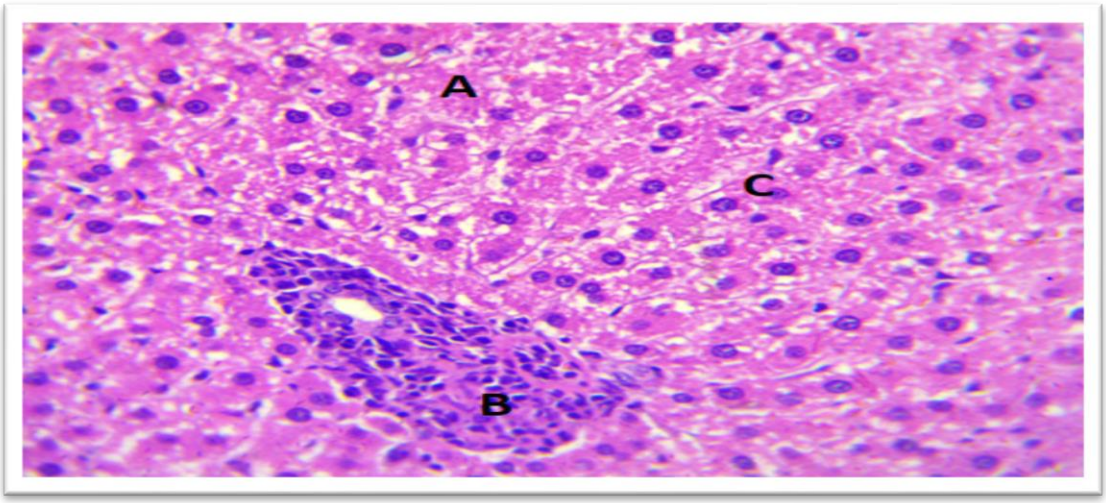


Figure (2): Positive group diabetic group that received Alloxan **.** Hypertrophy of liver cells(A), lymphoblastic mass around the bile duct branch (B), Normal liver cells (C). (H2EX40)

In figure 3 this histological image of the Normal Baobab group provided a glimpse into the liver tissue's normal architecture, including the portal area, bile ducts, hepatic artery branches, hepatocytes, lymphocytic diffusion, and Kupffer cells.

While figure 4 the histological results showed that Metformin treatment in the normal liver group does not significantly affect the observed tissue components, supporting its safety and lack of notable alterations in liver histology.

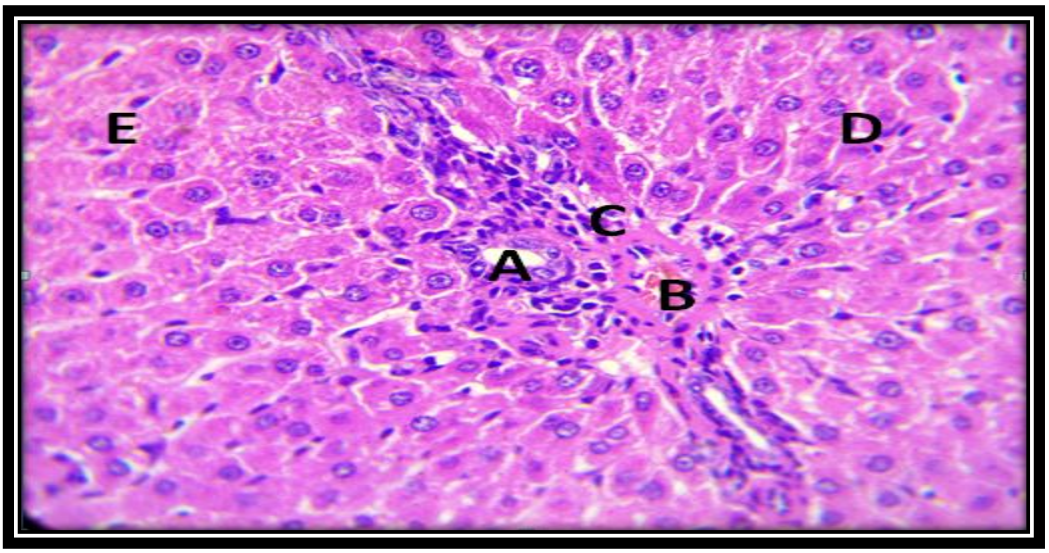
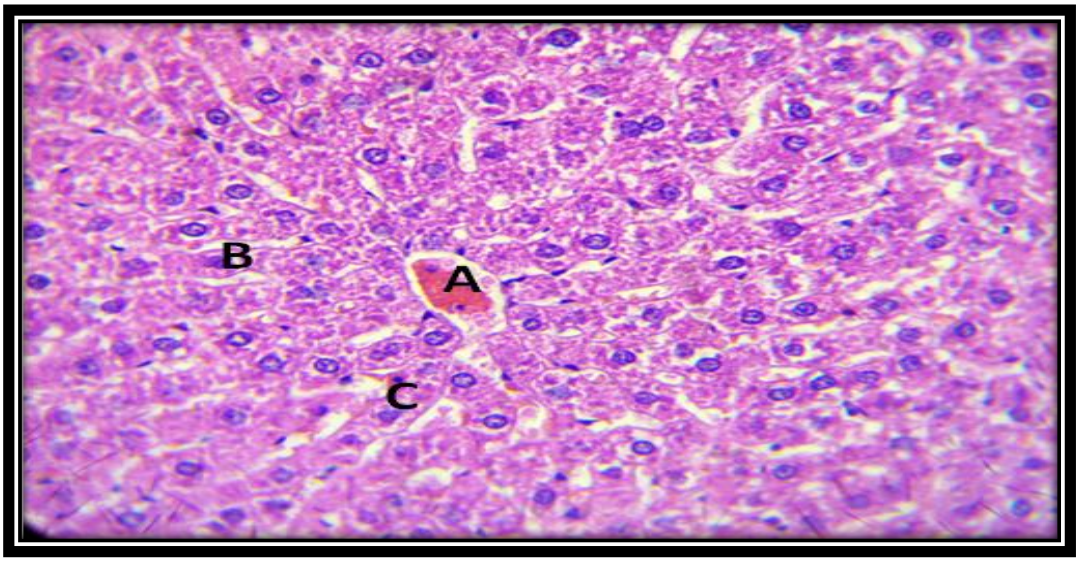


Figure (3): portal area (A), branches of bile duct Branch of hepatic artery (B) lymphocytic diffusion (C) Groups of polygonal liver cells (D) Kupffer cells(E). (H2EX 40)

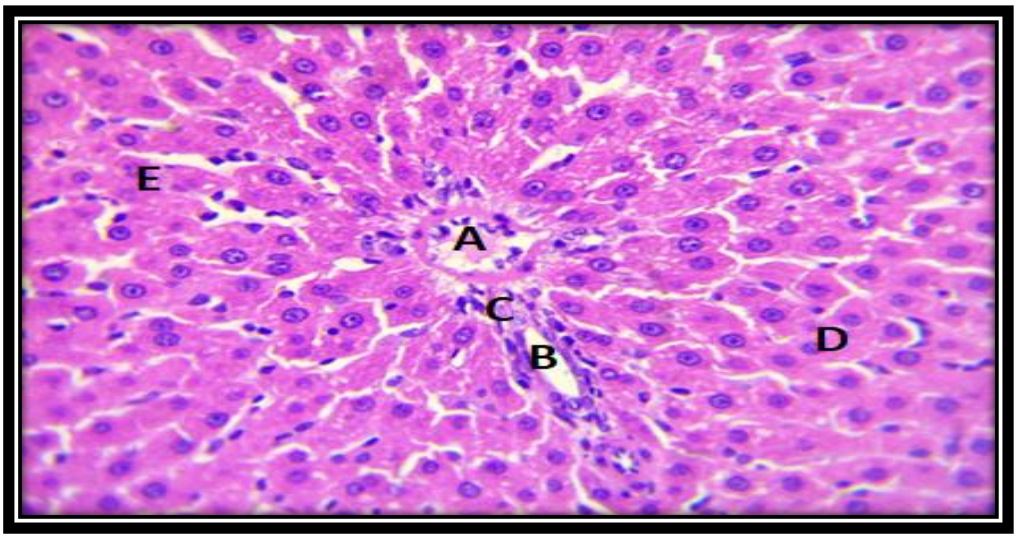


Figur(4):Central vein with hemolyzed blood (A) Columns of liver cells (1) Blood Sinusoids. (B) (H2EX 40).

The histological results of the Diabetic Baobab group, as seen in Figure (5), show the presence of the portal vein, bile duct, lymphocytes, polygonal liver cells (hepatocytes), blood sinusoids, and Kupffer cells. These findings provide insights into the liver tissue's histological features and suggest potential changes or responses associated with diabetes and Baobab treatment.

in Figure (6) capture various features, including a branch of the portal vein with hemolyzed blood, branched bile ducts, lymphocytic infiltration, groups

of liver cells (hepatocytes), and blood sinusoids. These findings provide insights into the tissue's condition, potential inflammation, and liver architecture.



Figure(5): portal vein (A), bile duct(B),lymphocytes(C), polygonal liver cells (D), blood Sinusoid (f), Kupffer Cells (E) (H2EX 40)

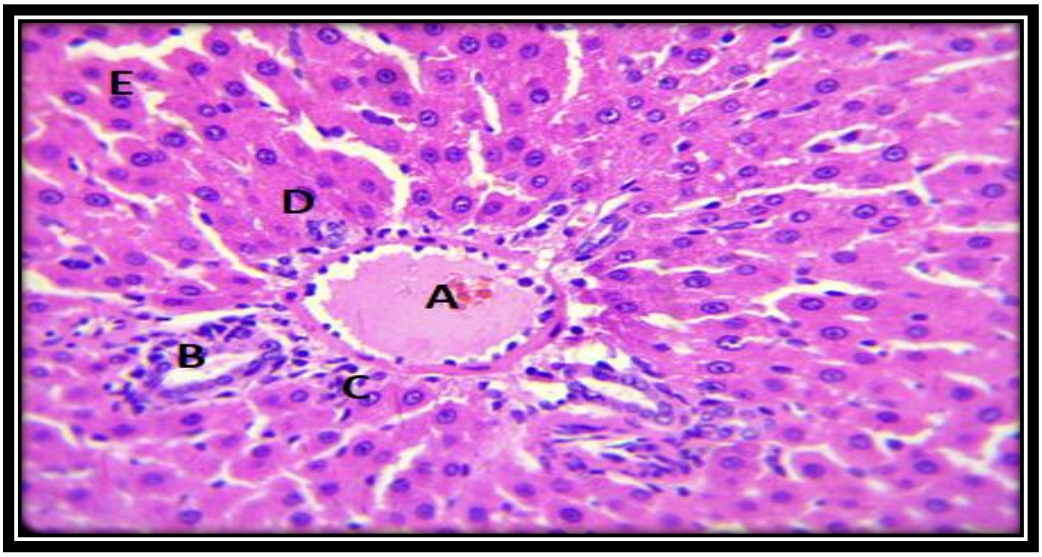


Figure (6): Branch of Portal Vein with Hemolyzed Blood (A), Bile Duct Branched (B), Lymphocytic Infiltration(C), Groups of Liver Cells (D), Blood Sinusoid (E). (H2 EX 40).

The histological results of the Diabetic Metformin Baobab Combination Group showed the presence of normal polygonal liver cells with spherical nuclei, intact blood sinusoids for proper vascular supply, and Kupffer

cells for immune function. These findings suggest the preservation of liver tissue structure and essential cellular components in the diabetic model treated with a combination of Metformin and Baobab as showen in figure (7).

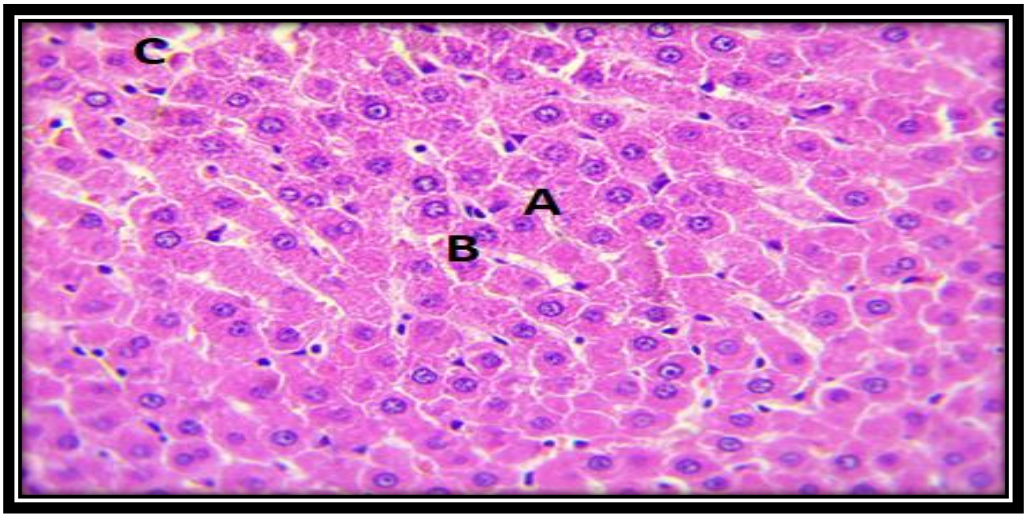


Figure (7): Diabetic Metformin Baobab Combination Group The polygonal liver cells with spherical nuclei (A) Blood Sinusoids (B) Kupffer cells (C). (H2EX40)

4.Discussion

Baobab, alone and in combination with Metformin, effectively reduced MDA levels over time in a diabetic model. This indicates its potential as an antioxidant in managing oxidative stress associated with diabetes(31). Baobab supplementation may help inhibit lipid peroxidation and protect against cellular damage(32–35).this result agree with Garcia et al., 2022 who found that It contains amino acids that possess strong antioxidant properties and can effectively suppress lipid peroxidation. Supplementing one's diet with baobab may provide protection against oxidative stress and its associated detrimental effects(36).another study by Mwangi et al., 2023 found that the addition of baobab fruit pulp, a cultured bovine milk product can be referred to as a baobab-enhanced or baobab-supplemented cultured milk.

The observed variations in TAOS levels in response to baobab crude extract treatment align with established knowledge of the antioxidant properties of baobab. This effect can be attributed to the extract's rich content of bioactive compounds, such as polyphenols and flavonoids, known for their free radical scavenging ability. These compounds contribute to the enhancement of the rats' antioxidant defense system(15). The substantial drop in TAOS levels in the diabetic group (G2) following

alloxan administration corroborates previous research indicating that hyperglycemia, a hallmark of diabetes, leads to oxidative stress and impairs antioxidant mechanisms. This validates the suitability of the diabetic rat model for oxidative stress studies.

(G3) and (G4) groups, displaying minor fluctuations in TAOS levels, are consistent with the idea that baobab's natural antioxidant properties may help mitigate oxidative stress. Metformin, a common antidiabetic medication, may also exert some antioxidant effects. Notably, the combination treatments (G7) exhibit significantly improved TAOS levels compared to the diabetic group (G2), indicating a synergistic effect between baobab and metformin in enhancing antioxidant defenses.

The consistent low p-values across groups and time points reinforce the robustness of the observed differences, substantiating the statistical significance of the findings.

In summary, the outcomes align with existing knowledge of baobab's antioxidant potential and the impact of diabetes on oxidative stress. The combination treatments suggest a promising avenue for future research on potential synergies between natural interventions and standard antidiabetic therapies .

Baobab, alone or in combination with Metformin, significantly increased TAOS levels in a diabetic model compared to the positive control. This indicates the potential antioxidant effects of Baobab in managing oxidative stress associated with diabetes(12,15,37). The findings suggest that Baobab has independent antioxidant properties and may have a synergistic effect when combined with Metformin(12). this result agree with Halilu and Muhammad, 2023 found that Oxidative stress plays a significant role in the development of various diseases, such as cancer and diabetes. It is characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses, resulting in cellular damage. Baobab fruit and leaf have been traditionally used in medicine as an antipyretic, or fever-reducing, agent. These parts of the baobab tree contain compounds with antioxidant properties, which may help combat oxidative stress and potentially contribute to the management or prevention of diseases associated with oxidative damage, including cancer and diabetes(38). Another study by Silva et al., 2023 who demonstrated that Baobab fruit and leaf have been found to exert beneficial effects on diabetes by scavenging free radicals and reducing oxidative stress . This research emphasizes their potential in managing diabetes and suggests promising applications for their use in diabetes treatment. The antioxidant properties of baobab contribute to its ability to counteract the harmful

effects of free radicals, providing potential therapeutic benefits in the context of diabetes.

The Normal Baobab group displayed normal liver architecture, while Metformin treatment in the normal liver group did not significantly affect the tissue components. The Diabetic Baobab group exhibited potential changes associated with diabetes and Baobab treatment. Features suggesting inflammation and alterations in liver architecture were observed. Lastly, the Diabetic Metformin Baobab Combination Group displayed preserved liver tissue structure. These histological findings contribute to our understanding of the effects of treatments on liver histology in the diabetic model.

Baobab may have an impact on liver architecture(39) and inflammation in the diabetic condition(40,34,41), while the combination of Metformin and Baobab may offer benefits in preserving liver tissue structure .

5. conclusion

Baobab, a tree from Africa with antioxidant properties , has shown promising results in managing oxidative stress associated with diabetes. The study demonstrated that baobab effectively reduced MDA levels and increased TAOS, indicating its antioxidant potential. Additionally, histological analysis revealed improvements in liver tissue structure. These findings suggest that baobab supplementation may serve as a natural approach to enhance health and prevent complications in individuals with diabetes. Further research is warranted to explore the full therapeutic potential of baobab and its mechanisms of action in managing oxidative stress in diabetes**.**

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دور الباوباب في السيطرة على الإجهاد التأكسدي لدى ذكور الجرذان البيضاء المصابة بالسكري.

ضحى وليد صالح 1، حسام الدين سالم النجار 2، دخيل حسين حدري 3

فرع الفسلجة والأدوية والكيمياء الحياتية_ كلية الطب البيطري، جامعة تكريت, العراق [1‘3]
فرع الأدوية_ كلية الطب، جامعة تكريت ، العراق[2]

الملخص

إجريت هذه الدراسة لفحص تأثيرات الباوباب على الإجهاد التأكسدي في نموذج سكري. شملت الدراسة سبع مجموعات، تضمنت 10 جرذان في كل مجموعة. كانت المجموعة الأولى مجموعة السيطرة لم تتلق أي علاج، والمجموعة الإيجابية (المجموعة السكرية) التي عولجت بالألوكسان بجرعة 100 مجم/كجم لتحفيز السكري في المجموعة التجريبية. تم أخذ عينات دم لتأكيد الإصابة بالسكري، وبعد الاستقرار، تم توزيع المجموعة التجريبية إلى مجموعات فرعية. تلقت هذه المجموعات علاجات مختلفة، بما في ذلك مطحون الباوباب بجرعة 500 مجم/كجم، وميتفورمين بجرعة 100 مجم/كجم، ومزيج من الباوباب (500 مجم/كجم) والميتفورمين (100 مجم/كجم)، وتم إعطاؤها عن طريق الفم حتى اليوم 30. تم جمع عينات من الكلى والكبد للتحليل النسيجي. تم إجراء التحليل الإحصائي باستخدام اختبار ANOVA، وتم عرض النتائج كمتوسط ± الخطأ المعياري.

أظهرت النتائج أن الباوباب قلل بشكل فعال من مستويات المالونديالديهيد (MDA) وزاد من الحالة الإجمالية لمضادات الأكسدة (TAOS)، مما يشير إلى إمكانيته كمضاد للأكسدة في إدارة الإجهاد التأكسدي المرتبط بالسكري. وأظهر الفحص النسيجي تغيرات في بنية أنسجة الكبد. يعتبر الباوباب واعداً كخيار طبيعي لتقليل الإجهاد التأكسدي ومنع المضاعفات في السكري.

في الختام، يُظهر الباوباب خصائص مضادة للأكسدة وقد أثبت فعاليته في إدارة الإجهاد التأكسدي عند الجرذان المصابة بالسكري. أظهر استخدام الباوباب انخفاضات فعالة في مستويات MDA، وزيادات متزامنة في TAOS، وتحسينات ملحوظة في بنية أنسجة الكبد. يظهر استخدام الباوباب الخام كاستراتيجية طبيعية محتملة لتعزيز الصحة العامة والوقاية من المضاعفات المرتبطة بالسكري. ومع ذلك، من الضروري التأكيد على أن هناك حاجة إلى تحقيق شامل أكثر لفهم قدراته العلاجية بشكل كامل.

الكلمات المفتاحية: الباوباب, مضادات الاكسدة, الاجهاد التاكسدي, داء السكري