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Study the Effects of *Annona* sp. Extract on Some Physiological Parameters and Fertility in Diabetic Mice

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Abstract

The current study was conducted to investigate *Annona* fruit pulp effects on the levels of various physiological biomarkers linked with insulin-dependent diabetes mellitus after disease induction in mice, as well as indications of oxidative stress and male hormones. The rats were separated into four groups, three of which were given Alloxan (90 mg/kg body weight) to induce diabetes, while the fourth served as a negative control. The first group of diabetic mice received no therapy, the second received metformin (600 mg/kg body weight) and the third received *Annona* fruit puree. The mice were sacrificed at the end of the experiment, to acquire blood and tissue samples from the liver, kidneys and spleen. The first untreated group showed a drop in insulin, good fats (High-density lipoprotein-HDL), antioxidant markers (Superoxide dismutase (SOD) and catalase –(CAT)), testosterone and follicle stimulating hormone-FSH, as well as an increase in glucose and low-density lipoprotein (LDL), triglyceride (TG), total cholesterol (TC), and luteinizing hormone (LH). Giving diabetic mice in groups 2 and 3 metformin and *Annona* puree improved the physiological markers levels, bringing their values closer to normal. The results indicated that the treatment with *Annona* fruit pulp had more protective effects than metformin. The histological findings confirmed the role of *Annona* fruit in improving the health indicators and the structural condition of the vital organs represented in the liver, kidneys and spleen of diabetic mice.

Keywords: *Annona*, Diabetes, Fertility, Lipids, Antioxidant enzymes and Hormones.

دراسة تأثير مستخلص نبات القشطة في بعض القياسات الفسلجية وخصوبة الفئران المستحث بها داء السكري

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الخلاصة

تناولت الدراسة الحالية تأثير لب ثمرة القشطة على مستويات بعض المؤشرات الفسيولوجية المتنوعة والمرتبطة مع داء السكري المعتمد على الأنسولين بعد تحفيز المرض في الفئران ، وكذلك مؤشرات الإجهاد

التأكسدي والهرمونات الذرية. تم تقسيم الفئران إلى أربع مجموعات ، ثلاثة منها أعطيت Alloxan لتحفيز مرض السكري ، بينما استخدمت المجموعة الرابعة كمجموعة سيطرة سالبة. المجموعة الأولى من الفئران المصابة بداء السكري لم تعطى أي علاج ، والثانية أعطيت الميثفورمين ، أما المجموعة الثالثة فقد أعطيت هريس فاكهة نبات القشطة. في نهاية التجربة تم الحصول على عينات من الدم والأنسجة من الكبد والكلية والطحال. أظهرت المجموعة الأولى غير المعالجة انخفاضاً في مستويات الأنسولين وكوليسترول البروتين الدهني مرتفع الكثافة. HDL ومضادات الأكسدة SOD ، CAT وهرموني التستوستيرون و الهرمون المنشط للحوصلة FSH ، بالإضافة إلى زيادة في مستويات الجلوكوز ، كوليسترول البروتين الدهني منخفض الكثافة LDL ، الدهون الثلاثية TG ، الكوليسترول الكلي TC والهرمون المنشط للجسم الأصفر LH . أدت معالجة الفئران المصابة بداء السكري في المجموعتين 2 و 3 مع الميثفورمين وهريس نبات القشطة إلى تحسين مستويات المؤشرات الفسيولوجية ، مما جعل قيمها أقرب إلى المعدل الطبيعي. أكدت النتائج النسيجية دور ثمرة نبات القشطة في تحسين المؤشرات الصحية و الحالة الهيكلية للأعضاء الحيوية المتمثلة بالكبد ، الكلية و الطحال للفئران المصابة بالسكري.

Introduction:

In the past decade, great progress has been made in the field of alternative medicine which includes the use of various products, whether plants, animals or minerals. In order to meet care needs, such products have become commercially available, with plant products being the most used [1][2]. Natural chemicals and some plants with a long history of usage in traditional medicine have been considered a rich source that provided cures for various diseases during the last century [3]. One of these plants is *Annona sp.* which belongs to the family Annonaceae that has been studied extensively because of its therapeutic potential and traditional uses as an antibacterial, insecticide, parasiticide, for treatment of fever and respiratory diseases, liver, heart, and kidneys and has recently been used to treat hyperglycemia and lowering blood pressure [4][5].

Diabetes mellitus (DM) is well-defined as “an assembly of metabolic diseases” described by hyperglycemia, resulting from a deficiency in insulin secretion or insulin action or both [6]. Hyperglycemia is a common effect of diabetes and leads to severe impairment of several organs, particularly nerves and blood vessels such as retinopathy, heart illness, liver, and kidney impairment, and sexual dysfunction [7] [8] [9]. Chronically elevated blood sugar also causes general oxidative stress and an elevation in the level of free radicals that cause damage to hepatocytes and eventually lead to cirrhosis [10]. Besides, serum dyslipidemia is commonly seen in diabetic populations irrespective of insulin deficiency or insulin resistance [11].

As for the diabetes-induced structural and functional changes in reproductive organs, a decrease in testicular blood flow velocity has been detected in diabetic rats [12]. Type 2 DM patients tend to have significantly lower testosterone level when compared with non-diabetic individuals [13]. Low testosterone level is associated with higher a risk for T2DM among men [14] .

Annona muricata has been used as a therapy to improve the quality of sperm [15], and to prevent Sertoli cell loss in alloxan-induced Swiss Webster mice [16]. By reducing cellular oxidative stress caused by the generation of reactive oxygen species, *A. muricata* leaf extract has potent antioxidant potentials and cytoprotective capacity against the deleterious effects of CdCl₂ on testicular tissue [17].

Considering the scientific interests in this matter, this study aimed to evaluate the effects of *Annona* fruit pulp on some biochemical and fertility parameters in alloxan-induced diabetic male mice.

Materials and Methods:

Annona Fruit Puree

The fruits of the *Annona* plant were obtained from the local markets of the species *Annona Squamosa* imported from India to be administered orally to experimental mice for two weeks. The fruits were peeled, seeds removed, and the pulp was weighed which was then pressed without exposure to heat to obtain puree. that was finally filtered through a sieve and kept in the refrigerator in a dark bottle [18].

Chemicals

Alloxan monohydrate was obtained from Sigma-Aldrich-USA, and metformin (Glucophage obtained from (Merck Sante SASU-France).

Induction of Experimental Diabetes

Diabetes mellitus was provoked in all mice (except the normal control group) by intraperitoneal injections of alloxan (90 mg/kg body weight) dissolved in normal saline. Blood glucose levels (baseline) were established before the usage. After 72 hours of alloxan application, blood glucose was measured by a glucometer in the blood collected from the mice's tail vein. Mice showing fasting blood glucose levels (>250 mg/dl) were selected for the study [18][19].

Experimental Animals:

Fifty adult male mice (age: 6-8 weeks; Wt.: 25-30 g) were obtained from the animal house of Biotechnology Research Center, Al-Nahrain University, and kept in the same house. They were placed in separated cages at 25°C and nourished with a suitable diet and water. The ethical standard procedures of the College of Science Research ethics committee at the University of Baghdad were followed in the care and use of laboratory animals.

Experimental Design and Biometry:

Following the induction of diabetes, a glucose test was done to confirm the drug's impact. Tail vein blood samples were taken after 8 hours of fasting to evaluate blood glucose levels and basal insulin. All diabetic mice were assigned to one of five groups. The following were the groups:

Group 1: Healthy control group included 10 mice (Negative control).

Group 2: Diabetic group, without any treatments, included 10 mice (Positive control)

Group 3: Diabetic group was treated with 600 mg/kg b.w. of metformin for two weeks including 15 mice.

Group 4: Diabetic group was fed 600 mg/kg b.w. of *Annona* puree for two weeks, including 15 mice.

Diets and water were given orally ad libitum during the dietary intervention period. After 8 weeks, the animals were sacrificed after 12 hours of fasting to collect blood and organ tissue. A cardiac puncture was used to obtain blood samples. The serum samples were produced by centrifuging the blood samples, 1.5 mL for 10 minutes at 3000 g. The serum was frozen at -80 degrees Celsius before being used to measure the levels of the following hormones and biomarkers: luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone hormone (TH), and insulin (ELISA kit /Orgmetric/Germany). Glucose (England/Randox kit).

Using enzymatic colorimetric kits (Cloud-clone corp. / USA), total cholesterol (TC), triacylglycerols (TG), and high-density lipoprotein cholesterol (HDL-ch) were measured in blood samples. Friedewald equation was used to determine VLDL and LDL cholesterol.

$$\text{VLDL (mg/dl)} = (\text{serum TG} - \text{cholesterol}) / 5$$

$$\text{LDL (mg/dl)} = (\text{serum TC} - \text{VLDL}) - \text{HDL}$$

Superoxide dismutase (SOD) KIT (Cohesion Biosciences/China). Catalase Assay Kit (Millipore Sigma). The biochemical tests were quantified using the manufacturer's protocol.

Liver, kidney and spleen were directly removed and well-preserved in 10% formalin for histological study which was directed along with the technique used by [20].

Statistical Analysis:

Statistical Analysis System (SAS) (2018) [21] program was used to detect the effects of diverse features in the study parameters. The statistical differences between groups were analyzed using the means of the one-way ANOVA table. The data was expressed as mean \pm standard error of the mean (SEM). A *P*-value of 0.05 was considered statistically significant.

Results and Discussion:

Biochemical Study:

As it appears from Table 1 that the level of glucose increased in diabetic mice and reached 299.25 ± 12.57 mg/dl, while the groups treated with metformin and *Annona* fruit showed a significant decrease (131.50 ± 6.95 , 135.25 ± 7.27 mg/dl) when compared with the level of glucose in the control group. On the other hand, the diabetic mice showed a significant decrease in the level of insulin that reached 12.56 ± 1.43 IU/ml, and in the two groups of diabetic mice treated with metformin and *Annona*, a significant increase in the level of insulin was observed (18.06 ± 1.43 , 18.86 ± 0.96 IU/ml respectively), compared to its level in the control group. The statistical differences between the groups for both parameters were highly significant, and it was noted that the results of insulin were complementary to the results of glucose and parallel to it which indicated the effective role of the *Annona* fruit in regulating the level of glucose in the blood.

Table 1: Comparison between the studied groups in Glucose and Insulin level

Groups	Mean \pm SE	
	Glucose (mg/dl)	Insulin (IU/ml)
Group I (Control)	94.50 \pm 5.44 c	24.84 \pm 1.83 a
Group II (Diabetic)	299.25 \pm 12.57 a	12.56 \pm 1.43 c
Group III (Metformin)	131.50 \pm 6.95 b	18.06 \pm 1.43 b
Group IV (<i>Annona</i>)	135.25 \pm 7.27 b	18.86 \pm 0.96 b
LSD value	13.099 **	2.231 **
P-value	0.0001	0.0001

This means having the different letters in the same column varied significantly. ** ($P \leq 0.01$).

There is a boundless request to elucidate some epidemic diseases for example Hyperglycemia and Hyperlipidemia by examining some medicinal plant extracts that the country flourishes and contain important active ingredients that may be an elucidation to such cases [22]. Most of the studies conducted on the *Annona* tree included investigating the effects of extracts of the leafy part of the tree, especially as it is a source of alternative medicines commonly used in many tropical countries. It is given to diabetics to reduce its harmful effects and the reason is often due to its high content of antioxidants [23][24] [25].

The glucose level was detected using the fasting blood sugar (FBS) test after disease induction in laboratory animals, and they showed a decrease in blood glucose level when they were given the leaf extract of *Annona*. Among the vital indicators affected by *Annona* leaf extract; are reducing weight loss resulting from diabetes, raising the level of insulin hormone, improving lipid levels and neutralizing the activity of antioxidant enzymes [26]. It has been revealed that the alcoholic leaf extract of the *Annona* plant is safe up to a dose of 2500 mg/kg of body weight, and no lethal or toxic reactions occur during the 12-day period of the experiment on laboratory rats [27]. Ethyl acetate and n-butanol compounds in *A. muricata* extracts were found to inhibit α -amylase, α -glucosidase, and pancreatic lipase enzymes resulting in control of blood glucose concentration, advanced glycation of the lipid peroxidation as end product, and reduced cytotoxicity [6], through indirectly increasing the manufacture of endogenous antioxidants. *Annona* plant conserves pancreatic β -cell integrity [24].

The results in Table 2 show that the highest concentration of TC was recorded in the untreated diabetes group (128.19 ± 6.28 vs. 72.76 ± 3.93 mg/dl), and the statistical difference was highly significant between the diabetic group and the healthy mice (negative control group). It was also found that the use of metformin and *Annona* as a treatment caused a significant decrease in the general cholesterol level when compared with its levels in the diabetes mice group (positive control), (93.34 ± 5.51 vs 128.19 ± 6.28), (100.00 ± 4.17 vs. 128.19 ± 6.28) mg/dl respectively.

Table 2: Comparison between the studied groups in the lipid profile

Groups	Mean \pm SE (mg/dl)				
	Total Cholesterol	Triglyceride	HDL-CH	VLDL-CH	LDL-CH
Group I (Control)	72.76 \pm 3.93 c	67.87 \pm 2.86 c	39.17 \pm 3.24 a	14.55 \pm 0.78 c	14.15 \pm 4.30 c
Group II (Diabetic)	128.19 \pm 6.28 a	130.67 \pm 3.46 a	20.03 \pm 1.64 c	25.84 \pm 1.25 a	84.79 \pm 1.10 a
Group III (Metformin)	93.34 \pm 5.51 b	98.94 \pm 3.74 b	30.13 \pm 2.75 b	18.67 \pm 1.10 b	50.13 \pm 5.83 b
Group IV (<i>Annona</i>)	100.00 \pm 4.17 b	97.88 \pm 7.41 b	28.56 \pm 2.16 b	20.01 \pm 0.83 b	49.32 \pm 6.96 b
LSD value	7.815 **	7.281 **	3.889 **	1.563 **	7.793 **
P-value	0.0001	0.0001	0.0001	0.0001	0.0001

This means having different letters in the same column differed significantly. ** (P \leq 0.01).

This explanation applies to other forms of lipid types (TG, LDL-CH, and VLDL-CH), as they showed a significant increase in the diabetes group and a significant decrease in the groups treated with Metformin and *Annona*, for TG (98.94 ± 3.74 vs 130.67 ± 3.46) (97.88 ± 7.41 vs 130.67 ± 3.46) mg/dl respectively; for LDL-CH (50.13 ± 5.83 vs 84.79 ± 1.10) (49.32 ± 6.96 vs 84.79 ± 1.10) mg/dl respectively; for VLDL-CH (18.67 ± 1.10 vs 25.84 ± 1.25) (20.01 ± 0.83 vs 25.84 ± 1.25) mg/dl respectively.

On the other hand, a decrease in the level of good fats (HDL-ch) was observed in the diabetic mice group that reached 20.03 ± 1.64 mg/dl, with a highly significant difference compared to the control group. Whereas, its level increased in the groups treated with metformin and *Annona* when compared to its level in the diabetic mice (30.13 ± 2.75 vs 20.03 ± 1.64) (28.56 ± 2.16 vs 20.03 ± 1.64) mg/dl respectively (Table 2).

The results of the current research were consistent with the results obtained by Adewole and Ojewoli, 2009 [28] that STZ-induced diabetic rats showed hyperglycemia, hypoinsulinemia, an increase in the levels of thiobarbituric acid reactive substances (TBARS), reactive oxygen species (ROS), TC, TG and LDL.

Annona Muricata (Graviola) has been used to alleviate human chronic diseases through two mechanisms, the first providing antioxidants and the second stimulating immune modulation. It was found that treatment of AML12 liver cells with steam (SGE) and ethanol extracts (EGE) of *Annona* leaves worked to reduce the regulation of the expression of oxidative stress genes fatty acids (FA), counting (ACOX1, CPT1, and PPAR α), deprived of an alteration in the appearance of genes accountable for the production of FA. However, genes involved in FA oxidation and the discharge of VLDL were decreased. [29].

The antioxidant agents were measured in the serum of the experimental groups represented by the SOD enzyme which inhibits the activity of superoxide (O_2^-), and the CAT enzyme which inhibits the effectiveness of hydrogen peroxide (H_2O_2). The level of both enzymes decreased significantly when compared to their level in the healthy mice (-ve control group), for the SOD (7.08 ± 0.87 vs 18.68 ± 2.09 U/mg), and for the CAT (207.51 ± 10.67 vs 389.38 ± 4.09 U/mg). On the other hand, the level of these two enzymes (SOD and CAT) increased significantly in the groups treated with either metformin or *Annona* when compared to their levels in the diabetic mice (+ve control). For the SOD in the metformin and *Annona* groups, it was 12.42 ± 1.65 vs 7.08 ± 0.87 U/mg, and 16.20 ± 0.80 vs 7.08 ± 0.87 U/mg respectively. The same thing applies to the CAT which was 249.01 ± 14.78 vs 207.51 ± 10.67 U/mg, and 299.88 ± 4.09 vs 207.51 ± 10.67 U/mg respectively.

Table 3: Comparison between the studied groups in SOD and Catalase

Groups	Mean \pm SE	
	SOD (U/mg)	Catalase (U/ml)
Group I (Control)	18.68 ± 2.09 a	389.38 ± 4.09 a
Group II (Diabetic)	7.08 ± 0.87 d	207.51 ± 10.67 d
Group III (Metformin)	12.42 ± 1.65 c	249.01 ± 14.78 c
Group IV (<i>Annona</i>)	16.20 ± 0.80 b	299.88 ± 4.09 b
LSD value	2.254 **	14.742 **
P-value	0.0001	0.0001

This means having the different letters in the same column differed significantly. ** ($P \leq 0.01$).

Oxidative stress plays a pivotal role in the development of diabetes complications. Hyperglycemia causes tissue damage through five major mechanisms: increased flux of glucose through the polyol pathway, increased intracellular formation of advanced glycation end-products (AGEs), increased expression of the receptor for advanced glycation end products and its activating ligands, activation of protein kinase C (PKC) isoforms and overactivity of the hexosamine pathway [30].

Biological activities and antioxidant effects of various *Annona* sp.-derived extracts have been described in the past, administration of aqueous *Annona* leaves extract to streptozotocin-induced diabetic rats led to an enhancement of the levels of SOD, CAT activities, malondialdehyde (MDA) and nitrites up to the level of nondiabetic rats [31].

Introduction of 50% ethanol leaf extract into Hep G2 cells causes upregulation of nuclear factor erythroid 2–related factor 2 (Nrf2) which is one possible explanation to increase antioxidant enzyme activity [32]. Besides, a strong positive correlation was observed for the estimated total phenolic contents and radical scavenging potentials and DNA protective activity of both aqueous and methanol extract of *Annona* [33].

Among the important biochemical indicators that were measured in this study, are male hormones, since the experiment was conducted on male mice to investigate the effectiveness of *Annona* fruits particularly in maintaining fertility from the adverse effects of diabetes.

Table 4: Comparison between the studied groups in hormones level

Groups	Mean ± SE		
	Testosterone (ng/ml)	FSH (mIU/ml)	LH (mIU/ml)
Group I (Control)	12.57 ±0.53 a	4.59 ±0.50 b	2.21 ±0.43 c
Group II (Diabetic)	5.87 ±0.21 c	5.80 ±0.52 a	4.69 ±0.40 a
Group III (Metformin)	10.26 ±1.67 b	5.24 ±0.39 ab	3.68 ±0.33 b
Group IV (<i>Annona</i>)	9.28 ±0.36 b	5.09 ±0.22 b	2.66 ±0.66 c
LSD value	1.391 **	0.658 **	0.737 **
P-value	0.0001	0.010	0.0001

This means having the different letters in the same column differed significantly. ** (P≤0.01).

According to Table 4, there were highly significant differences in the testosterone level between the treated groups, whether with metformin or *Annona*, compared to the untreated diabetes group and the control group. The lowest level of testosterone in the diabetes group reached 5.87 ± 0.21 ng/ml, and the concentration increased significantly in the groups treated with metformin or *Annona* reaching 10.26 ± 1.67 and 9.28 ± 0.36 respectively. Accordingly, the same applies to the FSH hormone, as its level was high in the diabetic group compared to its level in the control group, with no significant difference in the level of this hormone between the diabetic group treated with metformin and the untreated group. As well as there was no significant difference in the level of the FSH between the group treated with metformin and the group treated with *Annona*. However, a significant difference was observed when the group treated with *Annona* was compared to the untreated diabetic group. There is a strong association between sex hormones and the occurrence and development of diabetes. It has been found that diabetic males suffer from low sex hormone-binding globulin (SHBG) [34]. In addition, reductions in the levels of (FSH), LH, and (TH in the diabetic group when compared with the control or non-diabetic group confirmed that the diabetic condition affects reproductive activities negatively [35].

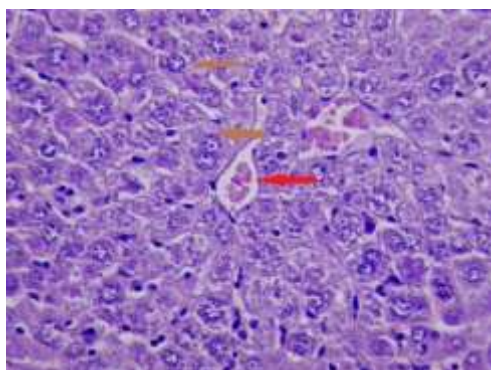
Studies related to the effects of *Annona* on hormone levels are not available. However, a study on morphometric parameters and weight of the reproductive organs in female rabbits fed with *Annona*, showed that they were not affected and continued a normal reproductive process [36].

Histopathological study:

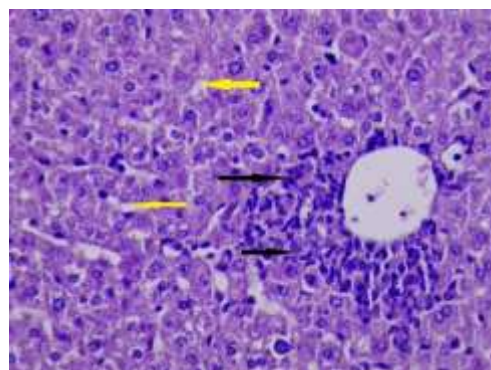
Liver Results:

As shown, the control group section of the liver shows a central vein (CV) with threads of hepatocytes (CV red, hepatocyte brown) according to Figure (1-a). The diabetic group shows liver damage, necrosis of hepatocytes (black arrow), inflammatory cell infiltration, and

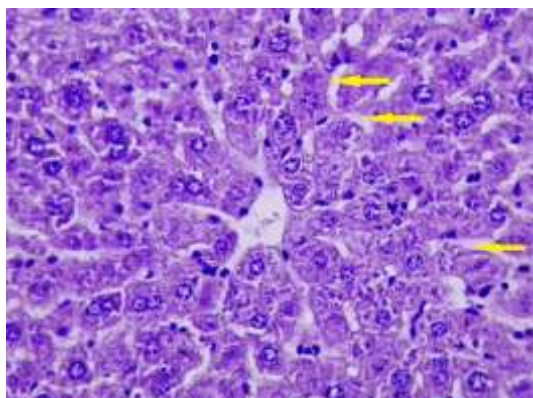
dilation of sinusoids (yellow arrow) as in Figure (1-b). The metformin group showed how the liver look like in a normal shape but still had sinusoid dilation (yellow arrow) and widening of the white pulp and reduction of the red pulp according to Figure (1-c). While in the *Annona* group liver looks normal with normal CV and normal hepatocytes as in Figure (1-d).



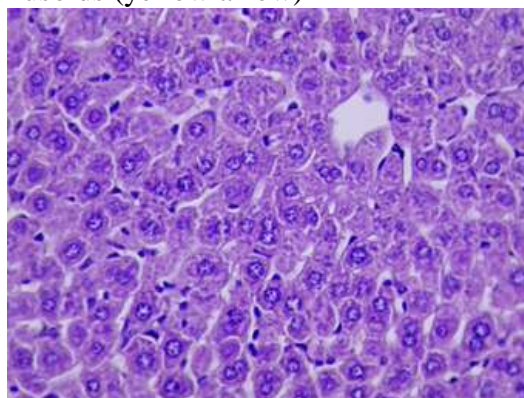
(a): Section of the liver showing central vein with normal threads hepatocytes (Brown arrow) (cv red arrow).



(b): Section of the liver showing tissue necrosis of hepatocytes, Inflammatory cell infiltration (Black arrow), and Dilation of sinusoids (yellow arrow)



(c): Section of the liver shows the liver look-like a normal shape but still has sinusoid dilation (yellow arrow).

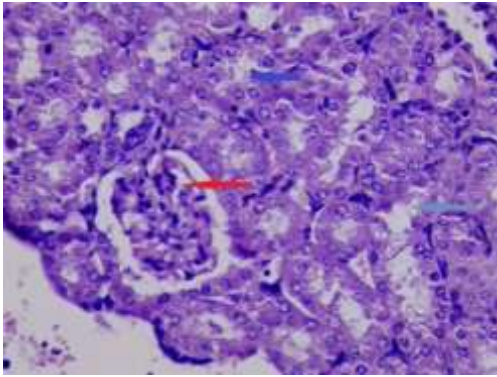


(d): Section of the liver shows the liver look-like normal shape, normal CV, normal hepatocyte.

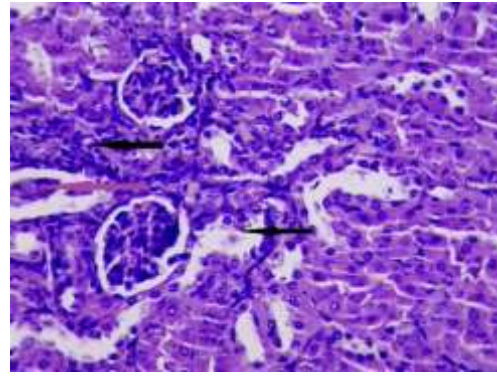
Figure 1: Sections of the liver in the studies groups, (a) G1-Control, (b) G2-Diabetic, (c) G3-Metformin, (d) G4-*Annona*. (X400) (H &E).

Kidney Results:

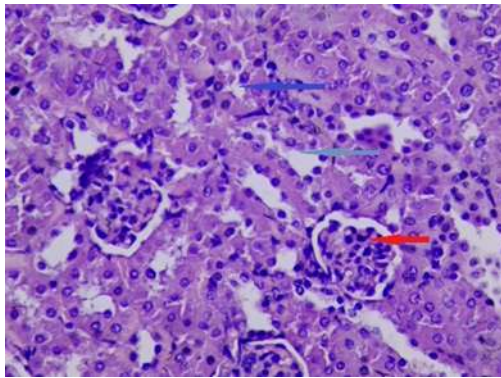
Section of kidney in the control mice showed the normal-looking appearance of renal tissue, normal proximal convoluted tubule (dark blue arrow) and distal convoluted tubule (blue arrow), glomeruli (red arrow) according to Figure (2-a). Section of kidney in the diabetic mice showed the aggregation of inflammatory cells in the interstitial tissue, necrotic cells (black arrow), and presence of renal cellular cast (purple arrow). The diabetic group showed kidney real dreary inflammatory cells in the interstitial tissue necrosis (black arrow) presence of renal cellular cast purple arrow as in Figure (2-b). The section of the kidney in the diabetic mice treated with metformin showed the normal-looking appearance of renal tissue which consists of glomeruli (red arrow) and renal tubules (blue arrow) according to Figure (2-c). Section of kidney in diabetic mice fed with *Annona* puree showed the normal-looking appearance of renal tissue which consists of glomeruli (red arrow) as in Figure (2-d).



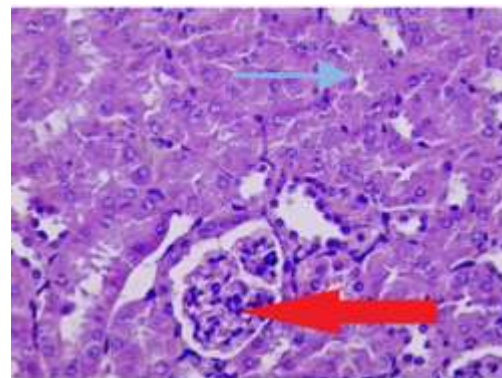
(a): Section of kidney shows the normal-looking appearance of renal tissue, normal proximal convoluted tubule (dark blue arrow) and distal convoluted tubule (blue arrow), glomeruli (red arrow).



(b): Section of kidney shows the aggregation of inflammatory cells in the interstitial tissue, necrotic cells (black arrow), and presence of renal cellular cast (purple arrow).



(c): The section of the kidney shows the normal-looking appearance of renal tissue which consists of glomeruli (red arrow) and renal tubules (Blue arrow).

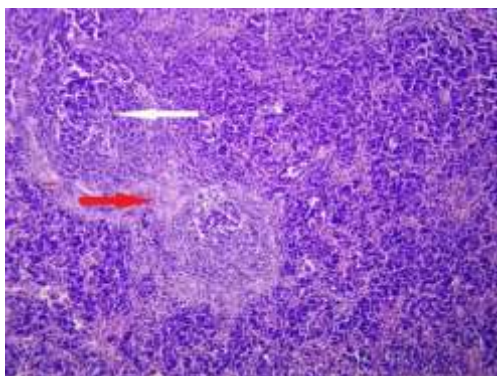


(d): Section of the kidney shows the normal-looking appearance of renal tissue which consists of glomeruli (red arrow) and renal tubules (Blue arrow).

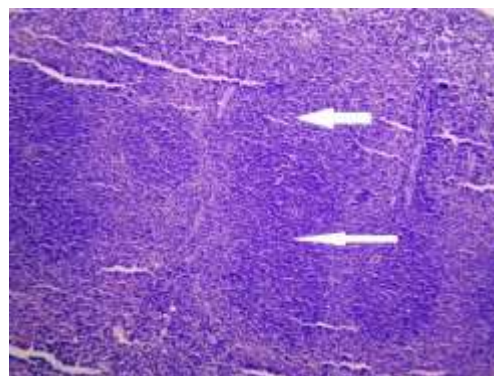
Figure 2: Sections of the Kidney in the studies groups, (a) G1-Control, (b) G2-Diabetic, (c) G3- Metformin, (d) G4- Annona. (X400) (H &E).

Spleen Results

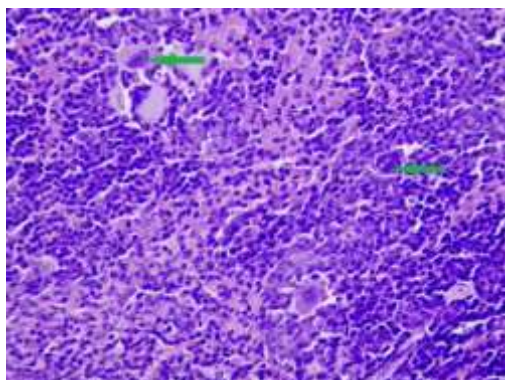
The control group of the spleen showed the white pulp (white arrow) and red pulp (red arrow) as in Figure(3-a). The diabetic group spleen showed a widening of the white pulp, and a reduction of the red pulp, according to Figure (3-b). Metformin group spleen showed the presence of megakaryocytes (green arrow), and inflammatory cells as in Figure (3-c). *Annona* group spleen looked normal in general with a slight widening in the white pulp with the presence of many megakaryocytes according to Figure (3-d).



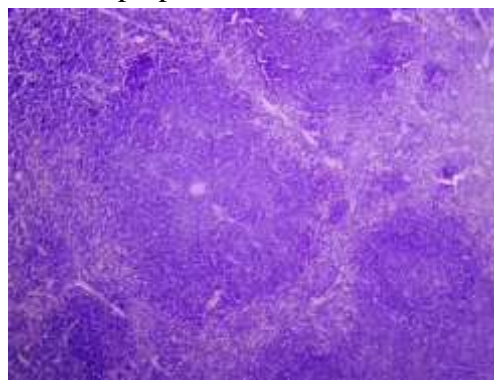
(a): Section of the spleen showing white pulp (white arrow), Red pulp (red arrow).



(b): Section of the spleen showing widening of the white bulb and reduction of the red pulp.



(c): Section of the spleen showing the presence of megakaryocyte (green arrow), inflammatory cells.



(d): Section of the spleen look normal in general with a slight widening in the white pulp with the presence of man megakaryocytes.

Figure 2: Sections of the Spleen in the studies groups, (a) G1-Control, (b) G2-Diabetic, (c) G3- Metformin, (d) G4- *Annona*. (X100) (H &E).

In the present study, we found that in the group with *Annona* supplementation, the liver looked normal, with normal CV, and normal hepatocyte which agrees with findings of Florence *et al.* [37] who found that *Annona* is anti-diabetic, hepatoprotective. Foong and Hamid [38] found that *Annona* has anti-inflammatory criteria.

The results of histological sections of the spleen indicated clear immunological effects for both the diabetic group and the metformin-treated group. The spleen showed the same characteristics of expansion of the white pulp and aggregation of inflammatory cells. While the *Annona* treated group showed a somewhat restoration of the normal properties of the tissue which may be due to the immune modulation, and anti-cancer and immunomodulation activity, since the phytochemical profile of *Annona sp.* leaf implies it to be a rich source of immunomodulatory agents [39].

In samples of the kidney with *Annona* extracts supplementation, the section of the kidney in diabetic mice fed with *Annona* puree showed the normal-looking appearance of renal tissue which consisted of glomeruli as found by Stenzel *et al.*, 2016 [40], normal distribution of left renal glomerular tubularization was reported in this study. The leaves of the *Annona* plant contain antioxidant components that are highly effective in scavenging the excessive reactive oxygen species (ROS), like alkaloids, flavonol glycoside and monoterpenoid lactone derivatives. There is a significant difference in Bowman's space width of alloxan-induced mice after administration of the *Annona* extract. Renal glomerular tubularization of alloxan-

induced mice was reduced following the administration of the *Annona* extract in a dose-dependent manner, which indicated a therapeutic potential of *Annona* in kidney restoration in DM[41].

Conclusion

According to the findings of the present study, the *Annona* fruit was found to have a role in reducing the adverse consequences of diabetes by reducing the level of LDL and TG fats and raising the level of antioxidants in the body, as well as improving the level of fertility hormones. The use of *Annona* fruit is the ideal choice for diabetics in terms of the lack of harmful compounds, unlike the rest of the tree parts.

Ethical clearance

All institutional and national guidelines for the care and use of laboratory animals were followed and approved by the Scientific Research Ethics Committee of Baghdad University, College of Science, Biology department, and numbered CSEC/0123/0004.

Conflict of interest

The authors declare that they had no conflict of interest.

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