Estimation of the Anti-Oxidant Activities and Histopathological Changes of Leaves Extract of *Morus nigra* on Male Wistar Rats Affected with Induced Diabetes Mellitus

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ARTICLE INFO
Submission date: 23 / 4/ 2018
Acceptance date: 21 / 5 / 2018
Publication date: 24/ 12 /2018

Abstract

The present study was employed to investigate the abilities of leaves extract of *Morus nigra* to alleviate the oxidative stress and histopathological changes associated with induced diabetes mellitus of adult males wistar rats. The present study was included 24 male rats with average age of 5 weeks and 150-210 g weight were used classified into four groups and each group included six (6) male rats. The first group was used as a control group and administered normal diet. The second group was injected intraperitoneally (IP) with alloxan 120mg/kg for 3days. The third group was also injected IP with alloxan 120mg/kg and administered orally with leaves extract of *Morus nigra* (500mg) for six weeks. Finally, the fourth group was administered with leave extract of *Morus nigra* (500mg) orally for six weeks. At the end of experiment blood samples were taken and the blood serum was separated by centrifuge to measure glucose concentration and anti-oxidant level **(**superoxide dismutase (SOD) levels) than the animals were sacrificed and liver was taken for changes of the hepatic tissues study.The results obtained from this study indicated that the extract of *Morus nigra* has ability to increase anti-oxidant activity significantly ($p<0.05$) in affected animals when compared with non-treated affected animals. The result of glucose showed significant decrease($p<0.05$) in affected animals when compared with non-treated affected animals.Concerning histopathological changes of liver the result showed improvement of pathological changes of the hepatic tissues of affected animals receiving leave extract of *Morus nigra* in a comparison with other tested groups.

Keywords: *Morus nigra*, diabetes mellitus, anti-oxidant**,** leaves

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion and action, or both Numerous pathogenic processes are included in the development of diabetes[1]. Excessive oxidative stress has been concerned in the pathology and diabetic pregnancy complications[2].During pregnancy, diabetes causes reproductive defects that increase spontaneous abortion, neonatal morbidity, congenital anomalies and mortality[3].The spontaneous abortion rates are three times more frequent in diabetes and pregnancy Association . Despite the fact that obstetric care and diabetes management are in continued advancement, the risks for morbidity and mortality are raised towards human with diabetes and their offspring [4] Type II diabetes is a multi-factorial disease that is described by declining in the ability of the pancreas to produce enough insulin to possess normal glucose homeostasis, due to impairment of function in pancreatic B-cells[5].It is usually associated with a collection of

pathologies for instance impaired glucose tolerance, obesity, hypertriglyceridemia, dyslipidemia, cardiovascular diseases, insulin resistance, and raised indicators of oxidative stress[6]. Langerhans pancreatic islets release crucial hormones that are essential for controlling blood glucose. Recently, Langerhans pancreatic islets have become one of the main goals of the researchers in the field of diabetes treatment[7].It is rare to obtain perfect glycemic control although there are many drugs are used to control diabetes[8]Historically, plants have been used for cure of Diabetes mellitus[9].Medicinal plants have generally become alternative therapy especially in the developing countries, where they have restricted medical assistance. The effects of such plants have previously been demonstrated experimentally in animals and humans . However, others need advance studies [10]. Theblackberry *(Morus nigra)* is the fruit of the blackberry plant . There is an considerable consumption of this fruit in Spain as well as in other countries of the world. It is native to southwestern Asia, where it has been cultured for long time that its precise natural range is unknown.The use of medicinal plants such as Morus nigra L. (family:Moraceae), well-known as black mulberry, and other members of its genus can be found in numerous countries . Nevertheless, almost all parts of the tree are utilised for pharmacological actions across the world [11].Its berries, bark and leaves are medicinally used, thereby the berries act as anti-inflammation and also to stop bleeding, the bark is for the treatment of toothache, and the leaves for snakebites and as an antidote to action poisoning . Black mulberry comprises "soluble plant substances called as bioflavonoids. These powerful antioxidant could be responsible for their medicinal properties [12]. Thus, it is obviously essential to study the effect of *Morus nigra* extract on oxidative stress.

Materials and methods

The Plant Materials

Morus nigra leaves were bought from a local market in Al-Qassim city of Iraq. Voucher specimens of plants were collected to be classified and Authenticated at University of Al-Qassim Green/Veterinary medicine collage. Department of Physiology

Prepatation of the leave extract

To separate the leaves extract of the leaves were air dried at 50 °C and powdered. Approximately 1000 g of the dried powder was extracted with distilled water and 24 h at room temperature by vacuum filtration. This concentrate had a dark green color and a sweet smell . The *Morus nigra* leaves extract as dissolved in filtered water and administered at 500 mg kg[13 .

Animals: 24 adult males wistar rats of Sprague Dawley strain weighting 150-210g body weight and 5weeks old were used in this study . Rats were obtained from the laboratory animal house, University of Al-Qadysiaa/Veterinary medicine collage .

Groupings and Experimental Design 24 adult males wistar rats of Sprague Dawley strain weighting 150-210g body weight and 6 weeks used in this Study.Rats were obtained from the laboratory animal house, university of Al-Qadysiaa/veterinary medicine collage.

The animals were divided into four groups. each group included six animals as follow: .

Group I : Normal control(the animals were given normal saline only) .

Group II : In this group were administration alloxan (120 mg.kg/day for 3 days) by intraperitoneal Injection to induce diabetes mellitus.

Group III: This group were administration alloxan (120 mg.kg/day for 3days) by intraperitoneal injection for induction of diabetes mellitus and treated with *Morus nigra* extract at 500 mg.kg b. wt. for 6 weeks.

Group IV: The animals were administration *Morus nigra* extract at 500 mg.kg b.wt. for 6 weeks without diabetes mellitus .

Alloxan: It was purchased Alloxan-monohydrate from Sigma chemical company Ltd.England.

At the end of the drug treatment period, all the animals were anaesthetized by application of light chloroform and blood samples were collected from a group of animals from dorsal aorta by heparinized syringe in vacutainer tubes . Plasma was separated from the collected blood by centrifugation of 3000rpm for 5 minutes . Separated blood samples were collected from another group of anaesthetized animals in glass test tubes and allowed to coagulated for 30 mins. Serum was separated by centrifugation at 3000 rpm for 15 min . Plasma and samples were kept at -20°C for biochemical Analysis .

Biochemical Kits: The sample or standard acts to reduce Cu^{++} to Cu^{+} is combined action of the antioxidants . This reduced from of copper will selectively appearance a 2:1 complex with the chromogenic reagent . This complex is stable and has an absorption maximum at ~450nm . A known concentration of Trolox is used to generate are ference curve to compare those readings obtained by the samples . Data can be expressed as mM copper reducing equivalents or in mM Trolox equivalents [14].

Blood glucose measurement: Blood glucose was measured by enzyme colorimetric method [15].GLUCOSE MR kit was used for this purpose.

Histopathological Examination:

Samples were taken directly from rats and fixed in 10% formalin to left for 72 hours. After fixation, the specimens were washed by tap water for 3-4 hours to remove the formalin solution and transferred to the following steps: dehydration, clearing and embedded and finally cutting and staining by using the rotary microtome and stained with H & E, Van-Geson and PAS stains [16].

Statistical Analysis:

The data were expressed as a means ± standard error (mean^{-} ± SE) and ($p<0.05$)was used least significant different (LSD) [17].

Results and Discussion

Intraperitoneal Injection of alloxan (120mg. kg b.wt) to rats causes asignificant ($p<0.05$) enlarged in the administration of *Morus nigra* extract in doses of 500mg.kg b.wt. to diabetic rats for 6 weeks significantly Increased ($p<0.05$) the antioxidant levels.

Table(1) All groups were compared to the control group

Groups and Treatments	Antioxidant levels superoxide dismutase (SOD) levels (U/ mL)
	Mean± Standder error
Normal control Distilled water 1ml	20.79±745.35
Diabetic control Alloxan(120 mg/kg)	5.47±786.95*
Mours nigra ext.(500mg/kg)without Alloxan	9.34±814.31*
Mours nigra ext.(500 mg/kg) with Alloxan(120mg/kg)	13.76±805.55*
L.S.D$_{(0.05)\ value}$	**32.418**

N=6 rats* Significant at p<0.05

*** refers to significant difference at p<0.05**

Table(1) showed The main discovery of the present study is that treatment with leaves extracts from *Mours nigra* antioxidants can decrease the hepatic insult under hyperglycemic conditions, and it can affect the glucose metabolism Pathways Plants containing flavonoids and other polyphenolic compounds have been exposed to have antioxidant potential and may effectively reduce hyperglycemia mediated by oxidative stress and liver injury [18]. Studies have been conducted to identify plants with hypoglycemic and anti-diabetic activities that may ameliorate the complications of diabetes mellitus The superoxide dismutase enzyme constitute the first line of cellular antioxidantdefense. superoxide dismutase activity increased in untreated diabetic rats in our study. superoxide dismutase is a key enzyme of the antioxidant defense system, catalyzing the dismutation of superoxide radicals to produce H2O2.[19] Its overexpression, however, is harmful to cellsAn increase in SOD activity has been associated with elevated levels of H2O2 and accompanying oxidative damage.[20]

Figure(1) showed increased serum glucose which was done with destroying islets of Langerhans β-cells and with alloxan [21]. One of hydro-alcoholic extract effects of *Morus nigra* leaves is controlling α-amylase and α-glycosidase and by this way it lowers the blood sugar content . This activity is associated to existing antioxidant compounds such as flavonoids, ellagic*acid*, and antocianine in mentioned extract [22]. By increasing blood sugar content in diabetic rats following injecting alloxan, function of capillaries and typically controls blood pressure, recovery of body antioxidant system and blood sugar [23].

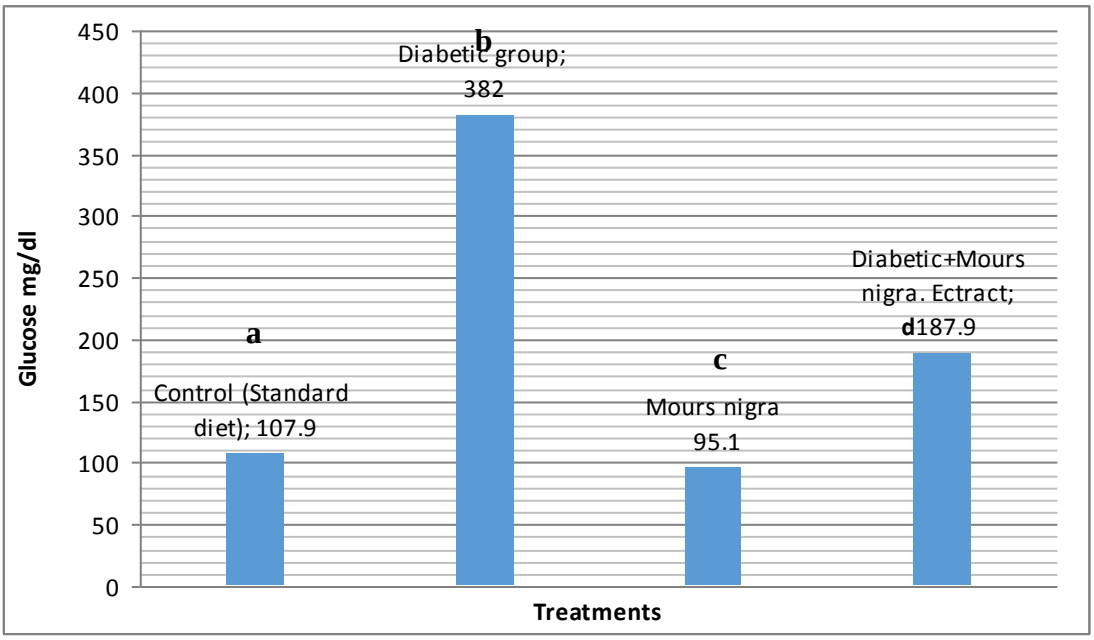

Figure (1) Blood glucose level

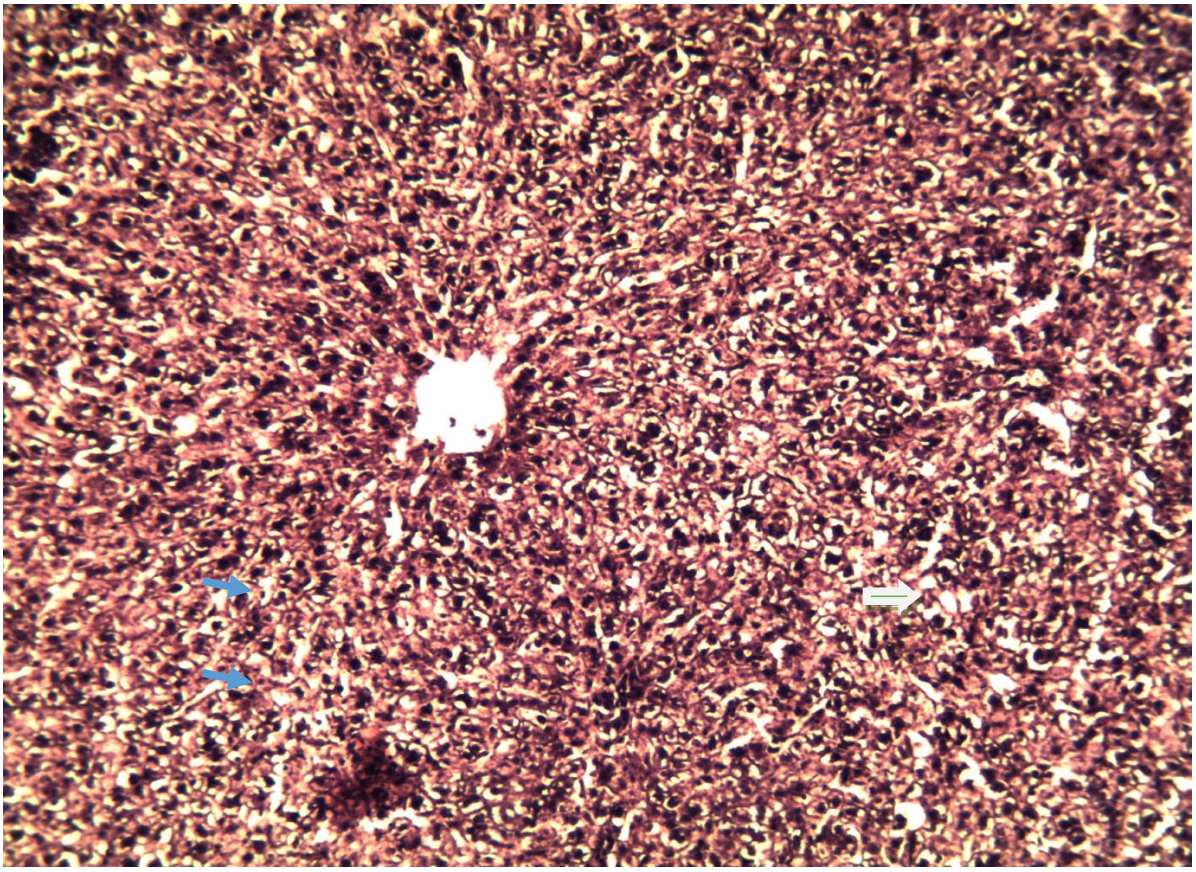

Figure (2) Liver of male rat .it received normal saline for 6 weeks.Normal hepatic architecture(radially arrangement of hepatocytes around normal central vein) → .normal sinusoid ⇒ .10X H&E.

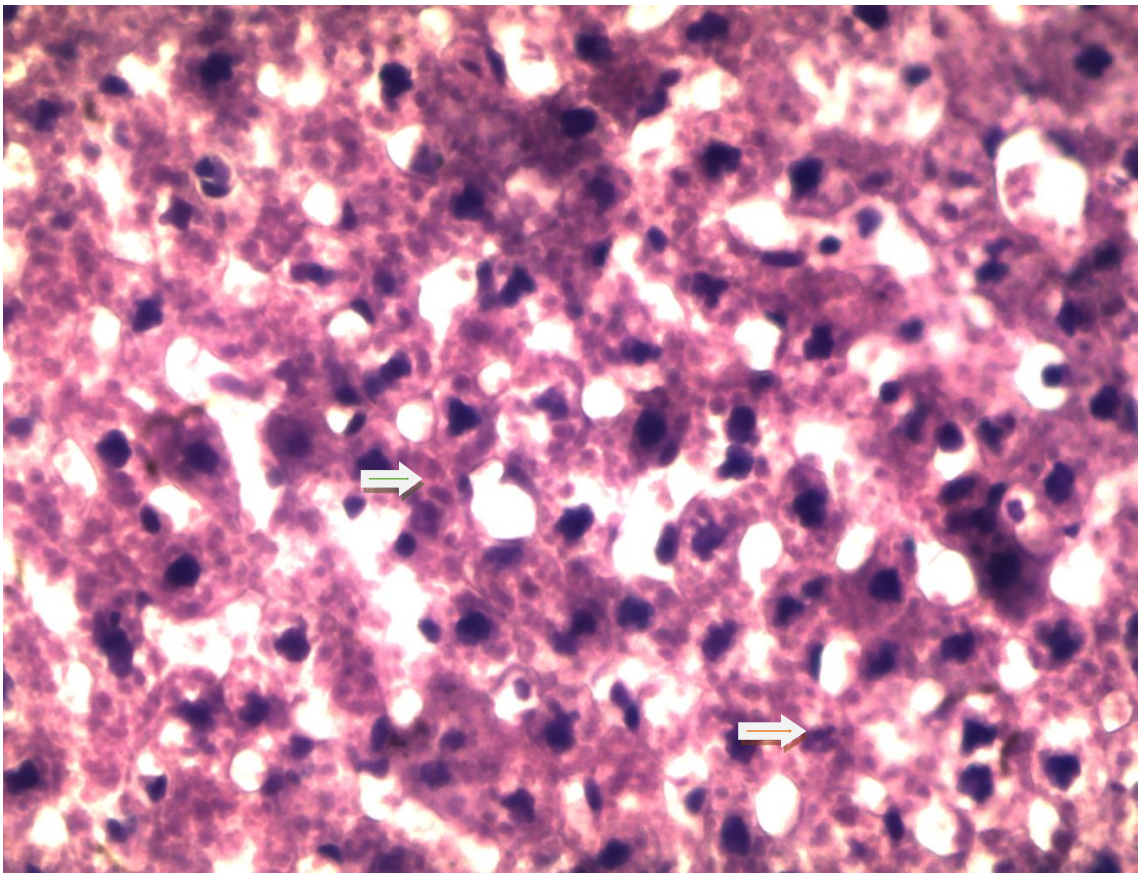

Fig (3) Liver of male rat .it received Alloxan(120 mg/kg B.W)I/P for 3 days.Marked fatty degeneration (fatty change)in which the hepatocytes showed as a signat-like shape (peripheral nuclei) ,with dilation of sinusoids .40X H&E.

Figure(2) showed liver parenchyma with general structures preserved, including hepatic lobules with normal hepatocytes surrounded by sinusoids and distributed radially towards the centrilobular veins

Figure(3) showed lesions in the livers of diabetic animals in this study reached all structures of the organ, including both portal areas and sinusoids, and hepatocytes, nuclei, and intracytoplasmic organelles, including a progressive enlargement of sinusoids, micro- and macrovesicular fatty degeneration, steatohepatitis and periportal fibrosis. Further, ultrastructural changes in hepatocytes, particularly the mitochondria, the rough endoplasmic reticulum and cell nuclei, were also observed .

However, whether the observed histopathological changes in the livers of alloxan induced diabetic rats were due to the toxic action of the drug or the diabetic state was not certain . Alloxan exert a toxic effect on pancreatic beta cells, which causes type 1 diabetes mellitus but this effect extends to the kidneys and livers of animals of several species [24] . However, the systemic toxicity of this drug is closely related to species, age and body weight of the animals used and the hydration

status, route of administration, infusion rate and duration of fasting for drug administration [25].

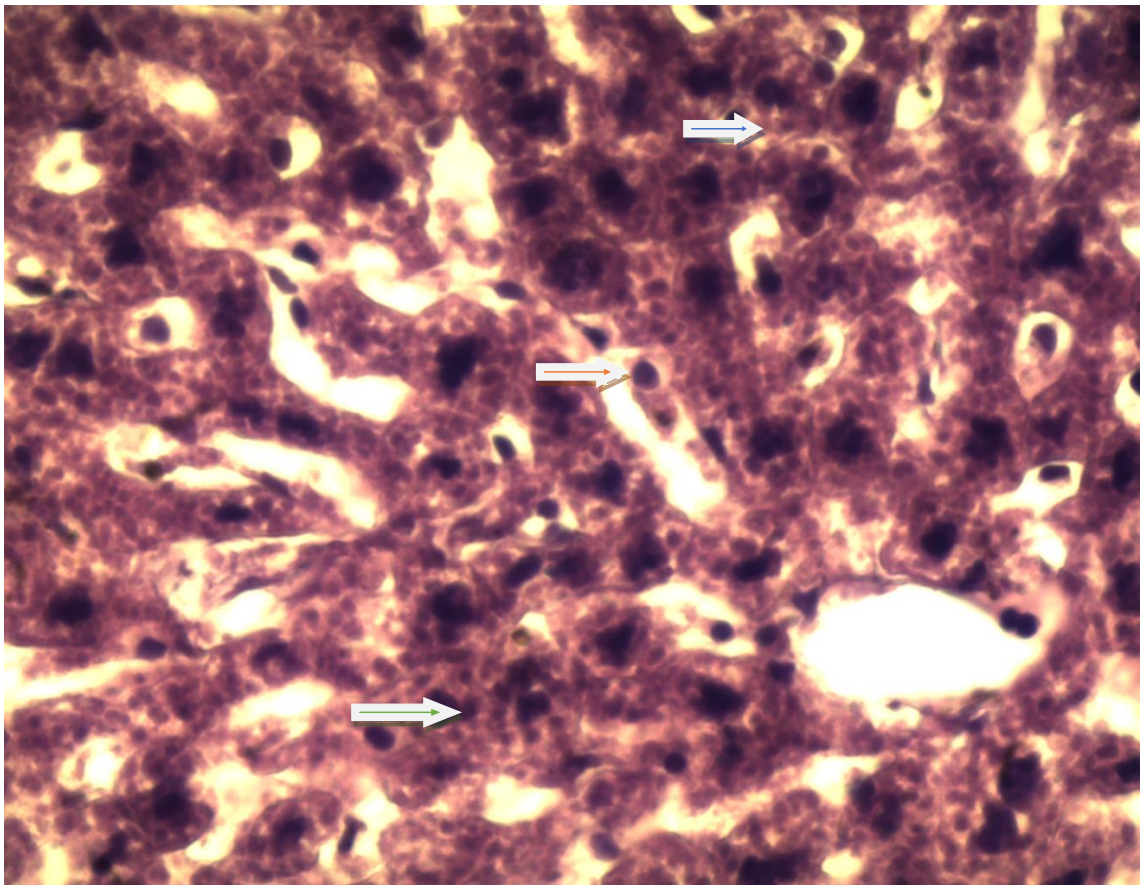

Figure (4)Liver of male rat . it received *Morus nigra* extract in doses of 500 mg.kg b.wt with Alloxan. There is mild fatty degeneration which showed in few hepatocytes ,marked dilation of sinusoids and proliferation of kupher cells .40X H&E.

Histopathology of liver Irregularities in insulin action may be involved in the pathogenesis of invasion of fatty acids to the liver and fatty liver disease conditions that range from clinically benign fatty liver to its more severe form, NASH (Non-Alcoholic Steatohepatitis) [26]. As shown in **Figure(3)** the liver cytoplasm in rats with diabetes was filled with lipid droplets of various sizes and showed fatty degeneration. According to the present results, the fatty degeneration in extract treated rats with diabetes was mild and the cytoplasm of hepatocytes is distended by smaller amount of fatty droplets compared with diabetic rats. By controlling inflammation and so regulating insulin action, the leaf extract of mulberry may modulate fat accumulation and degeneration in liver cells which may be due to its high phenolic content.

Conclusion

From the results which are obtained from this study on can be concluded that leave extract can be exerted anti-oxidant activities and reduced their harmful effects on hepatic tissues in male rats affected with induced diabetes mellitus by all alloxan.

CONFLICT OF INTERESTS.

There are non-conflicts of interest.

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الخلاصة

اجريت الدراسة الحالية لتقييم مدى فعالية مستخلص اوراق التوت الخافض لمستوى الضغوط التاكسدية والتغيرات النسجية في ذكور الجرذان الوستر المستحدث فيها داء السكري باستخدام مركب الالوكسان. تضمنت الدراسة الحالية 24 ذكر بالغ من الجرذان المختبرية بعمر 5 اسابيع ومعدل اوزانها 150–210غم والتي قسمت الى اربعة مجاميع متساوية (كل مجموعة تتكون من 6 ذكور). المجموعة الاولى مثلت مجموعة السيطرة ،المجموعة الثانية حقنت بمركب الالوكسان 120 ملغم /كلغم لمدة ثلاثة ايام حقنة داخل البريتون، المجموعة الثالثة بمركب الالوكسان 120 ملغم/كلغم حقنة داخل البريتون وجرعت بمستخلص اوراق التوت 500 ملغم لمدة ستة اسابيع ، المجموعة الرابعة جرعت بمستخلص اوراق التوت 500 ملغم لمدة ستة اسابيع .تم اخذ عنيات الدم من جميع المجاميع بعد ستة اسابيع لاجراء التحاليل الكيموحيوية لتقدير الكلوكوز ومستوى مضادات الاكسدة وبعدها شرحت الحيوانات واخذ الكبد لغرض دراسة التغيرات النسيجية واظهرت نتائج الدراسة الحالية بان مستخلص اوراق التوت له القدرة على حدوث زيادة معنوية في مستوى مضادات الاكسدة في الحيوانات التي تمت معاملتها مقارنة مع الحيوات غير المعاملة أما بالنسبة لنتائج فحص الكلوكوز اظهرت مجموعة الحيوانات المعاملة بمستخلص اوراق التوت انخفاض معنوي في مستوى الكلوكوز مقارنة مع مجموعة الحيوانات غير المعاملة . في ما يتعلق بنتائج التغيرات النسيجية في الكبد اظهرت حدوث تحسن في الانسجة الكبدية المصابة بتغيرات مرضية في الحيوانات المعاملة بمستخلص اوراق التوت مقارنة مع مجاميع الحيوانات الاخر.

الكلمات الدالة: نبات التوت ،داء السكري ، مضادات الاكسدة ، الاوراق