Effect of Green Tea Extract on Histological Structure of Kidney, Pancreas and Adrenal Gland in Alloxan-Induced DiabeticMale Albino Rats

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Abstract

Histological structure of kidney, pancreas and adrenal gland in alloxan-induced diabetic rats was investigatedafter daily oral administration of green tea water extract for 4 weeks. Forty adultmale albino rats *Rattusnorvegicus*, 12 weeks old, weighing 150-160 g were divided into 4 experimental groups: The first group was considered as negative control group. The second groups were affected by inducing experimental diabetes by intraperitonial injection of (150 mg/kg b.w) alloxan. The third group was considered as positive control and treated with water extract of the green tea (200 mg/kg b.w), and the fourth group was affected by inducing diabetes and treated with green teaextract. Kidney, adrenal gland and pancreatic samples were obtained and processed for microscopic evaluation after staining the prepared sections with both Heamatoxylin and Eosin. No histological changes were found in the examined organs belong to rats treated with green tea, (+ve control) while there was a severe histopatholoigical changes like aggregation of inflammatory, neutrophil and monocytes around the blood vessels in kidney tissue, severe fatty degenerative in the adrenal gland and destruction of Island of Langerhans of diabetic rats group. The results showed less extensive histological changes like degeneration of the cells lining the kidney tubules, vaculation of glumerules, and weak fatty degeneration in cellslining of kidney tubules, also moderate fatty degenerative in pancreas tissue and adrenal gland were found in diabetic rat treated with green tea group. From these results it can be conclude that the consumption of green tea extract reduced the degenerative effects of alloxan in histological structures.

Keywords: Green Tea, Diabetic, Alloxan, Rats, Histological Structures.

Introduction

Diabetes, a disorder of metabolism, is increasingly affecting more and more individuals in the world and the number of individual ssuffering from diabetes worldwide is predicted to reach 325 million by the year 2025[1].

There are two forms of diabetes mellitus: type 1 and 2. In type 1 diabetes or insulin-dependent diabetes mellitus the pancreatic b-cellsare regressively destroyed and secrete little or no insulin.

Type 2 diabetes or non-insulin-dependent diabetes mellitusis a heterogeneous disorder of insulin resistance and pancreatic β-cells dysfunction [2].Green tea, from the plant *Camellia sinensis*, is one of the mostpopular beverages consumed worldwide. Tea is rich in antioxidantpolyphenolic flavonoids (catechins, flavonols, theaflavinsand thearubigins) that possess various pharmacological effectssuch as anti-hypertensive, anti-arteriosclerotic, hypoglycaemicand hypocholesterolaemic activity. Other compounds of interest in dried green tea leave include gallic acid, quercetin, kaempferol, myricetin, saponins, caffeic acid and chlorogenic acid [3].

Recently green tea is being widely studied for its beneficial effects in the treatment and prevention of human diseases. It has been expected that an intake of green tea extract will prevent or delay the onset of diseases such as diabetes. Flavonoids are known to be effective in removing individual oxygen and free radicals from the lipids peroxidation step [4]. In vitro rat studies suggest that EGCG and other catechins and the aflavins help prevent hyperglycemia by enhancinginsulin activity and possibly by preventing damage to β-cells [5]. GT significantly decreased the blood level, serum creatinine, serum malondialdehyde and kidney excretion of glucoseand proteins and oxidative stress in the kidney [6].

The aim of the present study was to investigate the effects of daily oral consumption of green tea extract for 4 weeks on histological structures of kidney, pancreas

and adrenal gland in alloxan-induced diabetic rats to show thepreventive effects of green tea.

Materials and Methods

Animals and experimental design

Animals used in this study were obtained from animal's house of the College of Science, University of Baghdad, Iraq. Forty adult, 12 weeks old, male albino rats *Rattusnorvegicus*, weighing 150-160 g were housed in standard plastic cages, and kept in a well ventilated room, temperature of 24- 28°C with 12 hrs natural light and 12 hrs darkness. The rats had free access to tap waterand dry rat pellets obtained from localmarket*ad libitum*. The rats were divided into 4 groups; each group contained 10 animals: untreated (-ve control) group, green tea group were given GT extract at a dose of 200 mg/kg b.worally for 4 weeks (+ve control), diabetic group injected intraperitoneally with (150mg/kg b.w of alloxan), anddiabetic rats treated with green tea group (the rats first were injected with alloxan then given green tea 200 mg/kg b.w for 4 weeks).

Preparation of green tea extract

Green tea was obtained from a local market and stored in dry atmosphere and identified by the herbarium staff of Biology Department, College of Science, University of Baghdad, Iraq. Green tea aqueous extract were prepared by dissolving amounts equivalent to 200 mg tea leaf powder per kg body weight in glass ware containing 1ml boiling distilled water (equivalentto 6 cups of tea). The solution was kept to stand for 10 min before being filtered, cooled to room temperature, and dispensed in clean drinking bottles; the extract was kept at 4°C [7].

Induction of diabetes

Diabetes was induced in fasting rats 12hrs by a single intraperitoneal dose of 150 mg/kg b.w. of alloxan dissolved in0·9% saline, and the diabetic state was assessed by measuring the fasting plasma glucose concentration 72 hrs after alloxan treatment in fasting rats, and the rats with a plasma glucose level above 250 mg/dl were selected for the experiment and considered as diabetic [8].

Histological examination

Animals were killedandsmall piece of kidney, adrenal gland and pancreas tissues taken from experimental animals were fixed in 10% neutral formalin, alcohol-dehydrated, paraffin-embedded and the section to mean thickness of 4 μm. The histological examination was evaluated by assessing the morphological changes with Hematoxylin and Eosin (H&E) stains [9].

Results

The present study showed some histopathologicl effects in pancreas, kidney and adrenal gland tissues, histological examination of the normal control (-ve control) kidney, tissues showed normal histology (Fig. (1)). Kidney of diabetic rats showed aggregation of inflammatory, neutrophil and monocytes around the blood vessels (Fig. (2)). While kidney of GT treated diabetic rats showed degeneration of the cells lining the kidney tubules, vaculation of glumerules, and weak fatty degeneration in cells of kidney tubules (Fig. (3)). Kidney tissue belongs to rat treated with green tea extract (+ve control) showing no histological changes (Fig.(4)). Also the results showed normal histology of the normal control (-ve control) pancreas tissue (Fig.(5)), and destruction of Islets of Langerhans in diabetic rats (Fig.(6)). In pancreas tissue belongs to diabetic rat treated with green tea moderate fatty degenerative was observed (Fig.(7)), and no histological changes were found in rat treated with green tea (+ve control) (Fig.(8)). In adrenal gland tissue the histological examination showed normal appearance in (-ve control) control rats (Fig.(9)), and severe fatty degenerative in diabetic rats (Fig.(10)), whereas moderate fatty degenerative was observed in cortex of adrenal gland tissue belongs to diabetic rat treated with green tea (Fig.(11)), and no histological changes were found in rat treated with green tea (+ve control) (Fig.(12)).

Discussion

Several studies reported that alloxan produces oxygen radicals, which destroy pancreatic β-cells and cause severe hypoinsulinaemia (type I diabetes) that is responsible for the hyperglycemia seen in alloxan-treated animals. However, its action is

not directed to pancreatic β-cells only, as other organs such as the liver, kidney and bone marrow are also affected by alloxan administration as seen from the elevation of plasma markers reflecting renal cell damage (urea and creatinine levels) and the reduction of hematological parameters [10]. Alloxan-induced diabetes caused increasing plasma levels of creatinine, and urea, and also alloxan produces oxygen radicals and oxidative stress in the body [11]. Catechins present in green tea possess antioxidant potency and enables the kidney malfunctions resulting from diabetes to return to normal state [12]. GT reduced lipid peroxidation and increasedserum superoxide dismutase, suggesting that catechins influenceglucose metabolism and improve kidney function byreducing oxidative stress in alloxan-treated diabetic rats [10]. It has been demonstrated that catechins can reduce cellular oxidation [13].

In addition, green tea inhibits lipid peroxidation and induces the activity of anti-oxidant enzymes such as SOD, catalase, and GPX. GTE has been found to quench reactive oxygen species such as singlet oxygen, superoxide and hydroxyl radicals [14]. The anti-platelet cohesion action of catechin enables the kidney malfunctions resulting from diabetes to return to a normal state [15]. Hyperglycemia is the principal factor responsible for structural alterations at the renal level. Anonymous has made it clear that hyperglycemia is directly associated with diabetic micro vascular complications, particularly in the kidney [16]. Administration of GT catechins in diabetic animals drastically improved kidney function as a result of its anti-thrombogenicaction, which in turn controls the arachidonic acid cascade system [17]. The catechin in green tea is clearly effective in reducing oxidative stress and inflammatory reactions in kidney tissue [18]. This may be interpretated as a result of the protective effects of green tea in studied tissues and reduced the oxidative stress of alloxan that causes the fatty degenerative and the aggregation of the inflammatory cells in these tissues. The treatment of diabetic rats with green tea reduced the oxidative stress but not prevent completely the effects, and this may be interpreting the present of some histological changes in the tissues of diabetic rats treated with green tea extract. In conclusion the protective effects of green tea may be due to the presence of some kinds of components that available in the extract that have antioxidant activity and reduced the oxidative stress in the cells, and this led to normal structures and functions.

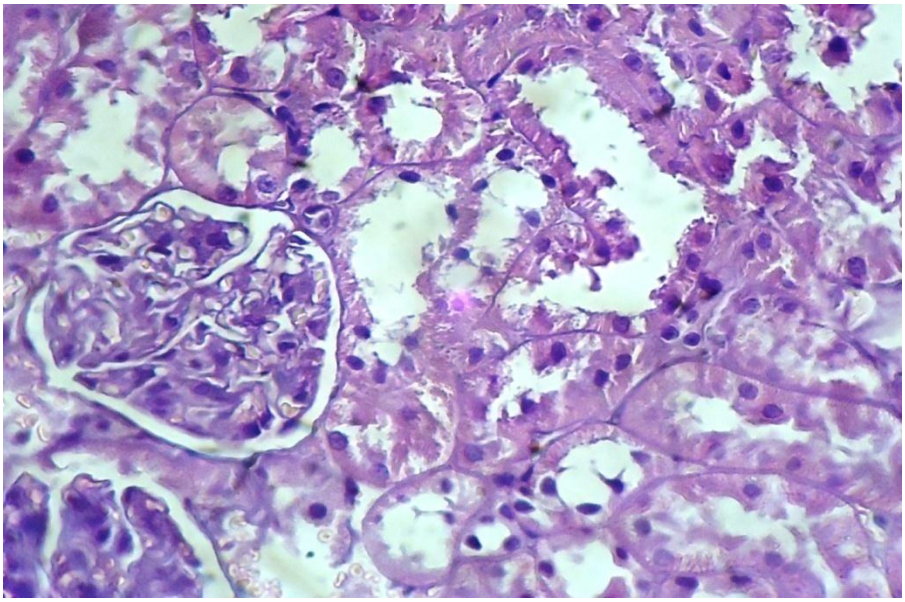

Fig. (1) Section in kidney tissue belongs to normal control rat showing normal histology of the kidney (H&E) 400X.

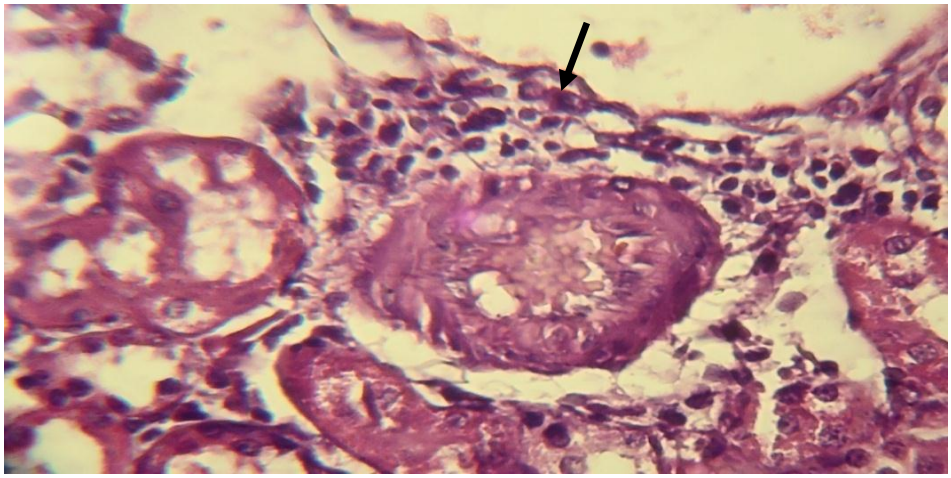

Fig. (2) Section in kidney tissue belongs to diabetic rat showing aggregation of inflammatory, neutrophil and monocytes around the blood vessels (⟵) (H&E) 400X.

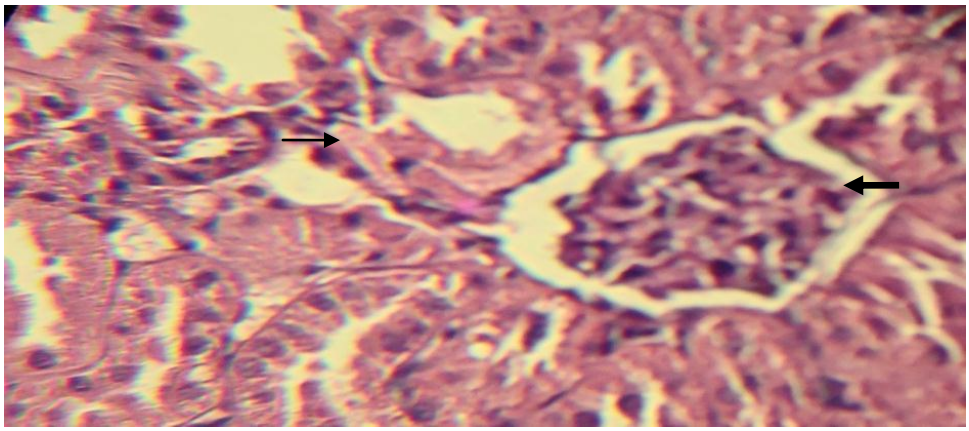

Fig. (3) Section in kidney tissue belongs to diabetic rat treated with Green tea showing degeneration of the cells lining the kidney tubules(⟶), vaculation of glumerules (⟵), weak fatty degenerative in cells of kidney tubules (H&E) 400X.

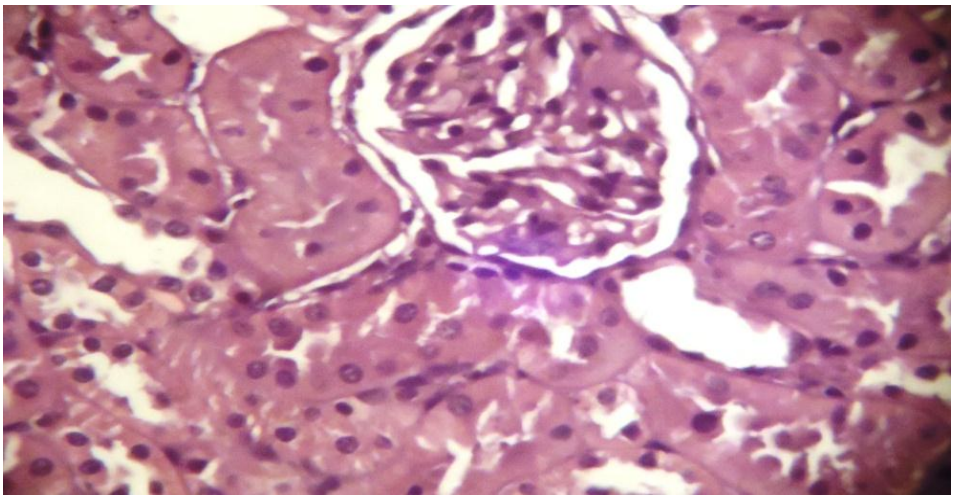

Fig. (4) Section in kidney tissue belongs to rat treated with green tea extract showing no histological changes (H&E) 400X.

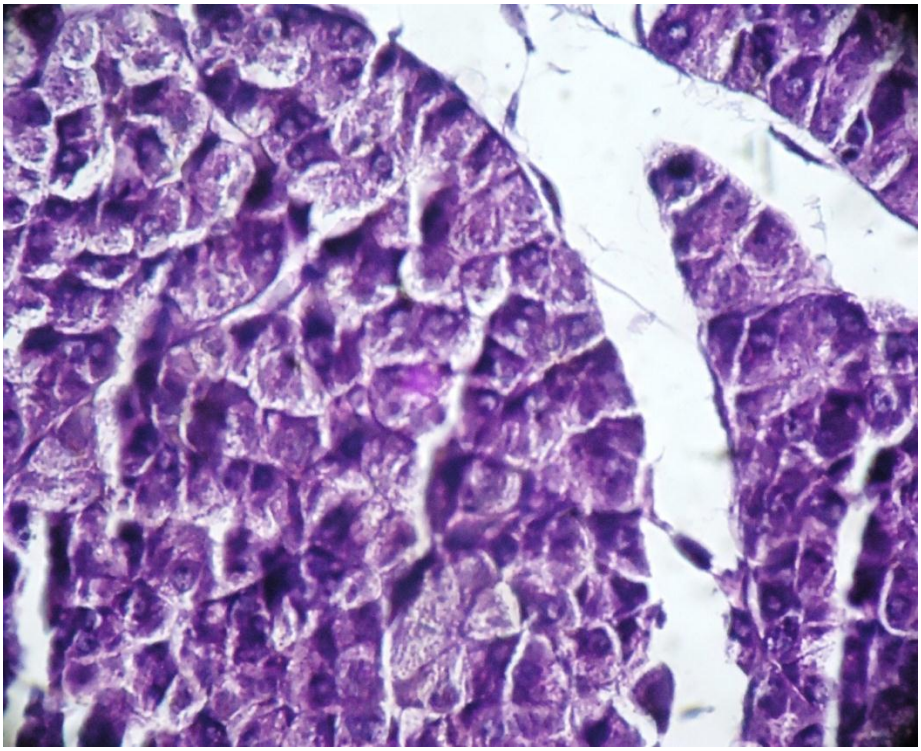

Fig. (5) Section in pancreas tissue belongs to normal control rat showing normal histology of the pancreas (H&E) 400X.

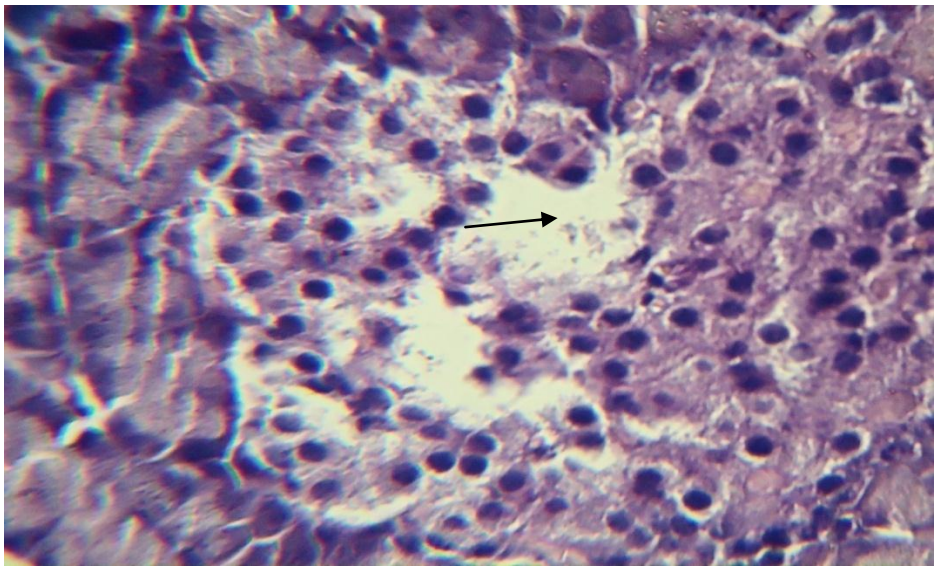

Fig. (6) Section in pancreas tissue belongs to diabetic rat showing destruction of Islets of Langerhans (⟶) (H&E) 400X.

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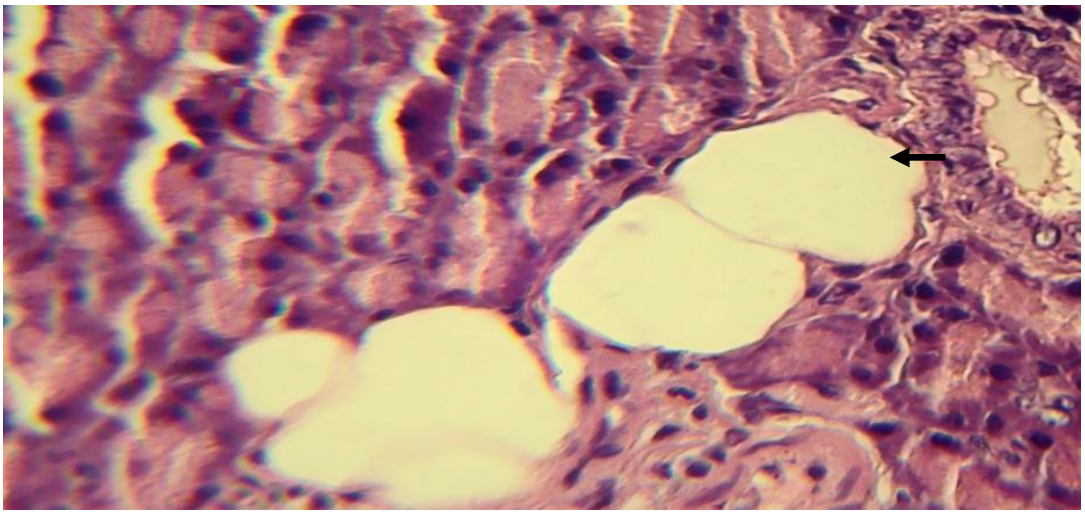

Fig. (7) Section in pancreas tissue belongs to diabetic rat treated with green tea showing fatty degenerative (⟵) (H&E) 400X.

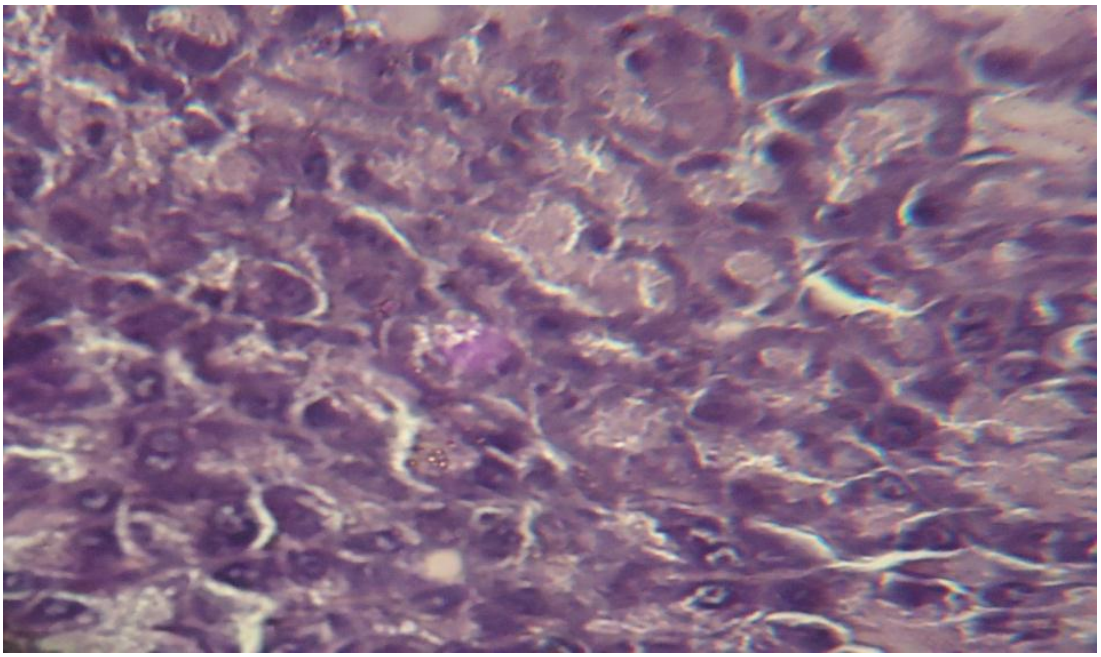

Fig. (8) Section in pancreas tissue belongs to rat treated with green tea extract showing no histological changes (H&E) 400X.

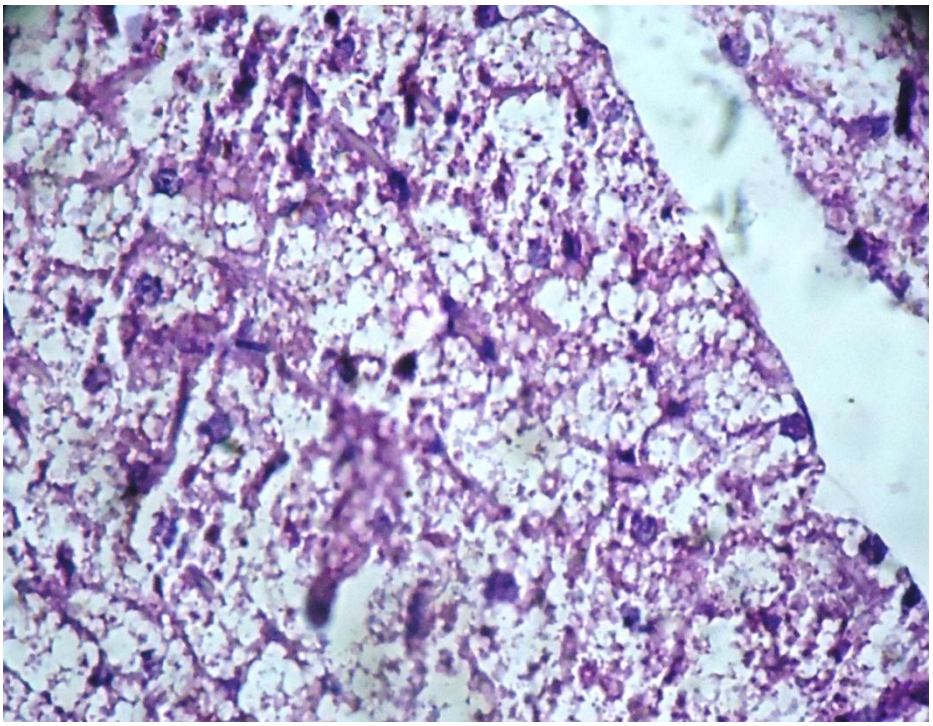

Fig. (9) Section in adrenal gland tissue belongs to normal control rat showing normal histology of the gland (H&E) 400X.

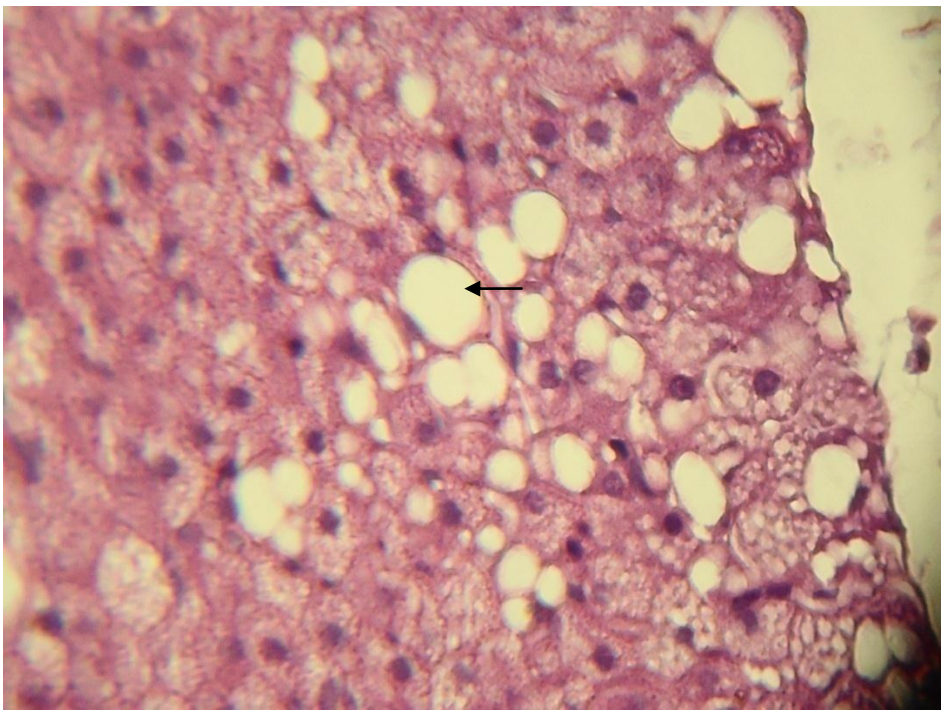

Fig. (10) Section in adrenal gland tissue belongs to diabetic rat showing severe fatty degenerative in the gland (⟵) (H&E) 400X.

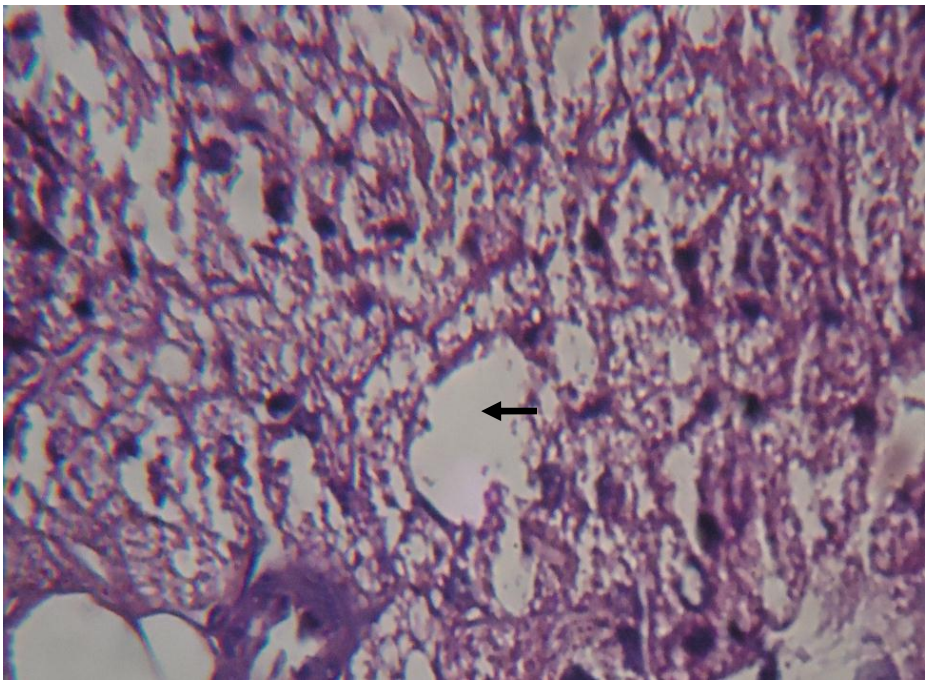

Fig. (11) Section in adrenal gland tissue belongs to diabetic rat treated with green tea showing fatty degenerative in the gland (←) (H&E) 400X.

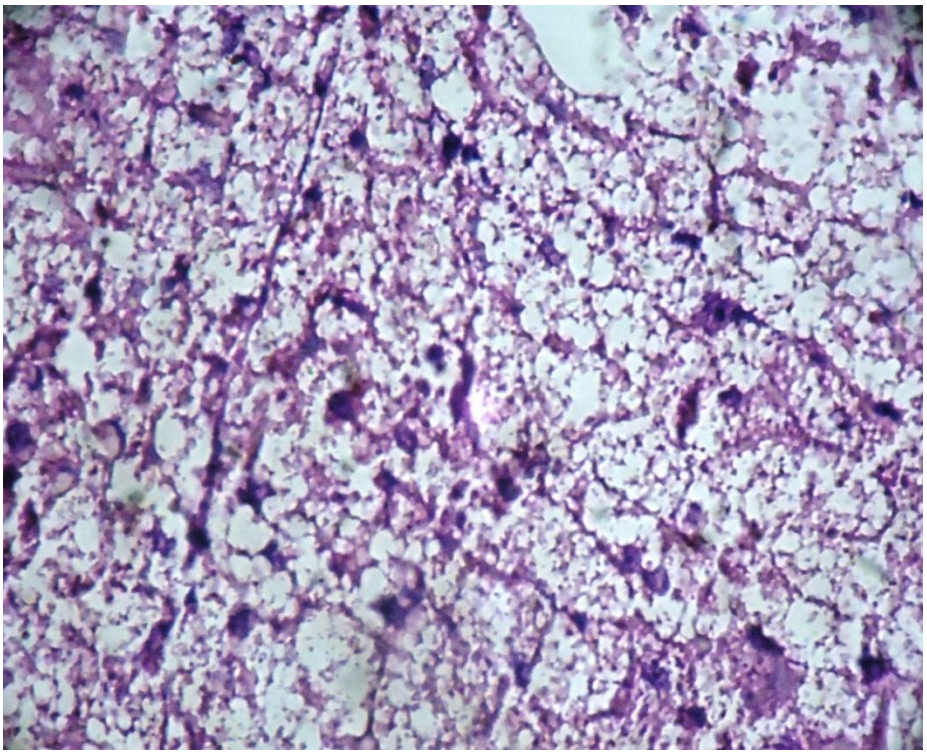

Fig. (12) Section in adrenal gland tissue belongs to rat treated with green tea showing no histological changes in the gland (H&E) 400X.

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الخلاصة

تمت دراسة التركيب النسجي للبنكرياس والكلية والغدة الكظرية في ذكور الجرذان البيض المصابة بالسكري المستحث باللوكسان بعد التناول اليومي للمستخلص المائي للشاي الاخضر لمدة اربعة اسابيع. اربعون جرذا من ذكور الجرذان البيض البالغة بعمر ١٢ اسبوعا، وباوزان تراوحت ما بين ١٥٠ الى ١٦٠ غرام، قسمت الى اربع مجاميع: عوملت المجموعة الاولى كمجموعة سيطرة سالبة وتلقت المجموعة الثانية جرعة من الالوكسان مقدارها ١٥٠ ملغرام/ لكل كيلوغرام من وزن الجسم عن طريق الحقن بالبريتون. وعوملت المجموعة الثالثة (سيطرة موجبة) بمستخلص مائي من الشاي الاخضر بجرعة مقدارها ٢٠٠ ملغرام/ لكل كيلوغرام من وزن الجسم يوميا وعن طريق الفم لمدة ٤ اسابيع والمجموعة الرابعة جرعت بالالوكسان ثم جرعت بمستخلص الشاي الاخضر وكما في المجموعتين الثانية والثالثة. اخذت عينات من انسجة البنكرياس والكلى والغدة الكظرية للتصبيغ والفحص النسجي.لم تظهرالنتائج وجود تغيرات نسجية في الاعضاء المدروسة للجرذان المعاملة بمستخلص الشاي الاحضر فقط،في حين ظهرت تاثيرات امراضية نسجية واضحة مثل وجود تجمعات للخلايا الالتهابية العدلة ووحيدة النواة في نسيج الكلية، ووجود تنكس دهني شديد في نسيج الغدة الكظرية، وتحطم لجزيرات لانكيرهانس في المجموعة المصابة بالسكري، اما في المجموعة المصابة بالسكري والمعاملة بمستخلص الشاي الاخضرا فقد اظهرت النتائج حدوث تغيرات اقل شدة مثل، تنكس لبعض الخلايا المبطنة للانيبيبات البولية، وحدوث تنكس دهني معتدل في خلايا نسيج البنكرياس والغدة الكظرية. ومن نتائج الدراسة يتضح ان المعاملة بمستخلص الشاي الاخضر لها تاثير واضح في التقليل من التاثير الضار للالوكسان في التراكيب النسجية.