

Histological and Biochemical Changes in Diabetic Albino Male Rats Treated with Soybean *Glycine max* (L.) Seeds

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Abstract: Soybean [*Glycine max*(L.)] seeds is used in folklore medicine. This study was designed for improving blood glucose level and preventing long-term complications in diabetes mellitus by soybean seeds and clarify their role on histological changes in kidney, islet of Langerhans, adrenal gland and changes in the Kidney Functions diabetic rats. 24 males Sprague-Dawley Albino rats were divided into 4 groups, each group included 6 rats. First group was considered as a control, feed ad Libitum with conventional diet second group was treated with soy bean seeds was addition to conventional, diet (30%) third group was induced-diabetic rats using alloxan and fourth group was induced-diabetic rats treated with soybean seeds was addition to conventional diet for 6 weeks. The main histological changes in kidney was massive inflammatory which appeared around blood vessels, vacuolar degeneration in tubular epithelial cells, karyolysis and Karyorrhexis and some glomerular degeneration. The islets of Langerhans showed severe necrotic changes, especially in the center, karyolysis dilatation of large vessels and marked increase in connective tissue component. The adrenal glands also showed cytoplasmic fat vacuolation and pyknotic nuclei (Necrosis) in the cells of zona glomerulosa-alloxan-induced diabetes. That resulted many changes in kidney functions such as a significant increase [$P<0.05$] in plasma levels of creatinine and urea. Moreover the significant decrease [$P<0.05$] in body weight of diabetic rats was observed. In current study it can be Concluded that the treatment of diabetic rats with soybean seeds resulted amelioration of histological changes in kidney, islets of Langerhans, adrenal glands and changes in kidney Functions.

Keywords: alloxan-kidney-pancreas-adrenal-diabetes-soybean

1. Introduction

Diabetes mellitus (DM) is one of the most common endocrine diseases in the world characterized by the state of hyperglycemia. It is systemic disease caused by defect in the Insulin secretion, Insulin action or even both [1] interesting, this common metabolic disorder has a prevalence varying between 1-50% [2].

DM patients are prone to some long-term complications like nephropathy, retinopathy and neuropathy [3]. The long-term complications resulted in diabetic patient life expectancy accounting to only two-thirds of the general population [4].

Alloxan is hydrophilic and unstable substance. Its half-life at neutral pH and 37°C is about 1.5 min and is longer at lower temperatures (5). On the other hand, when diabetogenic dose is used, the time of Alloxan is decomposition sufficient to allow it to reach the pancreas in amount that is deleterious [6].

The mechanism of Alloxan action has been intensively studied, predominantly in *vitro*. It was demonstrated that Alloxan areevokes sudden rise in insulin secretion in the presence or absence of glucose.

Alloxan - induced insulin release. However, of short duration and is followed by complete suppression of the islet response to glucose. Even with high concentrations of this sugar [7].

Alloxan is induces damage and death of pancreatic islet-cells in several experimental animal models

Thus, it is causing diabetes mellitus and decreasing the secretion of insulin. The cytotoxic effect of this diabetogenic agent is mediated by reactive oxygen species, Alloxan and the products of reduction dialuric acid, establish aredox cycle with the formation of superoxide radicals.

These radicals undergo mutability to hydrogen peroxide. Thereafter, highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction in β -cells [6]. Many traditional plants treated for diabetes are used throughout the world but most of the evidence for their beneficial effects is anecdotal. After introduction of insulin therapy using of traditional treatment for diabetes greatly declined although some traditional practices are continued for prophylactic purpose and adjuncts to conventional therapy. In some of the societies there is strong desire to use herbs or plants for treatment, due to less side effects, easier consumption or availability. However, very few of the traditional treatments for diabetes have received scientific or medical scrutiny and several have been shown to assist glycemic control in non-insulin dependent from of diabetes [8].

Plants may act on blood glucose through different mechanism; some of them may have insulin-like substances [9]. Some may inhibit insulins activity, others may cause increase β cells in pancreas by activating regeneration of these cells [10]. The fiber of plants may also interfere with carbohydrate absorption thereby affecting blood glucose [11].

Soybean [*Glycine max* (L.)] seeds are in use for more than 5000 years in china and south-East Asia as food.

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Epidemiological studies show its importance in prevention of several diseases [12].

Soybean seeds contain 36% proteins, 19% oil, 35% carbohydrate (17% of which dietary fiber) 5% minerals and several other components including vitamins [13].

Several years of rigorous scientific and clinical research has established that most of the components of soybean have beneficial health effects as characterized by its preventive potential for the so-called life-style-related disease. In addition, this impact present of most of the nutritionally and physiologically functional components of soybean [14]. Soybean seeds contain its own unique combination of phytonutrients- including the flavonoids [agycone, daizein, gemistein and glycitein each of them present in three glycosidic forms that give it strong antioxidant activity.

Soybean also contains 2% soy saponins [triterpene glycosides]. Soybean saponins have unique chemical structures and biological activities such as hepatoprotective, anti-hyperlipidemic, anti-cancer, anti-oxidative and anti-HIV.[15].

Soybean seeds are a better source of B-vitamin and C-vitamin [13]. C-Vitamin is the body's primary water-soluble anti-oxidant able to neutralize free radicals in all aqueous environments of the body [16]. The present study was undertaken to observe the histological changes on the kidney, pancreas, adrenal gland and changes of kidney function of Alloxan - induced diabetic Fed with soybean seeds and then compared to those which were either normal or untreated. The study on the histology of the kidney, pancreas, and adrenal gland damage can help that to understand the damage that happen in kidneys and function of pancreas and adrenal glands in diabetes mellitus and highlight the protective action of the soybean seeds.

2. Material and Methods

2.1 Study Groups of Animals

A total of 24 male albino rats sexually mature laboratory breed males' Sprague-Dawley Albino rats (*Rattus norvegicus*) of an average body weight of 198± 3.37gm and 15-16 weeks old.

Animals were kept in the department of biology, collage of science, University of Baghdad under the laboratory conditions (12h light condition: 12h Dark condition) photo period with controlled room temperature (25-28°C), good ventilation and feed normal rodent pellets, and tap water ad Libitum.

2.2 Experiment Design

The albino male rats were randomly divided into four groups each group (n=6) were put into 2 plastic box cages (three rats per cage) Domination of each box were 40x 25x 15 cm, both group one and group three feed ad Libitum with conventional diet only, but both group two and group four feed ad Libitum with conventional diet addition 30% soy bean seeds from the weight of feeding.

Group 1: was kept as control (healthy control).

Group 2: non- diabetic rats treated with soybean

Group 3: diabetic rats untreated with soybean

Group 4: diabetic rats untreated with soybean orally each group comprising six (n=6) rats.

2.3 Preparation of Alloxan - induced diabetic rats

The animal must be starved over night before injection of Alloxan 150mg/kg [17]. Alloxan tetrahydrate (sigma) was injected in 12 adult male albino rats intraperitoneally [18].

2.4 Treatment and Sample Collection

Treatment of the rats began 10 days after the induction of diabetes. The treated group received 50 mg/kg body weight of soybean orally daily for 6 weeks. During the experimental period the blood glucose, creatinine and urea were checked. Kidneys, adrenal glands and pancreas of treated rats were collected on 42th day following treatment. The collected tissues were fixed in 10% formalin dehydrated through graded alcohol series (50-100%) cleared by xylene and embedded in paraffin wax. Sections of 5-µm thickness were made and stained with hematoxylin and eosin [19].

2.5 Collection of Blood Sample and Biochemical Analysis

At the end of the experiment, blood samples were taken by cardiac puncture and blood was collected in clean EDTA tubes. Then plasma was collected by centrifugation (3000 rpm for 15 min) and stored at -20°C. Glucose test is a quantitative reagent system to determine glucose quantity in blood serum depending absorbency on color intensity. This method depends on the wave absorbency of light at the extinction of (500 nm) by spectrophotometry. All methods and materials used depend on the information which proceeds from lab kit Company J.T Barcelona (Spain) kits of creatinine and urea were purchased from spin react, S.A. Ctra. Pain. Creatinine was determined by kinetic method described previously by [20], Determination of urea was done according to the enzymatic method [21].

2.6 Statistical analysis

Statistical analysis (standard deviation SD) was carried out according to fisher [22] LSD (least significant difference) test was used to compare the significant treatment [23].

3. Result and Discussion

3.1 Histological Results

No pathological changes could notice in pancreas, kidney and adrenal glands of rat treated with soybean.

3.1.1 Pancreas

The normal histological structure of the pancreas was observed in (Figure- 1). The pancreas of untreated diabetic rats showed necrotic changes of pancreatic islets especially, in the center of the islets such as pyknotic, karyolytic with vacuolation (Figure-2)

The relative reduction of the size of islets, dilation of the blood vessels and marked increase in connective tissue component of the gland (interlobular and intralobular) were observed (Figure -3). These findings are in agreement with previous study [24], who found that the treatment of male rat with Alloxan at dose level of 120 mg/kg induced severe degenerative changes of islets cells with different grades of unclear degeneration most of cells appeared ballooned (pyknotic) with marked vacuolation.

These changes were occupying mostly the center of islet. Moreover, the dilation of blood vessels was noticed with dense connective stroma of the gland; in coinciding with [25]. They reported that pancreatic lesions are consisting of diffuse vacuolation of islets of Langerhans in both acute and chronic onset of diabetes.

In the present study, the alloxanate diabetic rats also showed exocrine part of the gland (serous- acini) flattening of their nuclei that were pushed to the bottom of the cells (Figure- 4) coinciding with previous study [26], they reported that the Pancreatic lesions also consisting of flattening nuclei in the cells and pushed towards the bottom of exocrine portion of islets of Langerhans apparent reduction of size and number islets.

These results were in agreement with another study, which affirmed that islet inflammation was dissociated from onset of clinical diabetes [27]. The pathological changes observed in the present work has been explained by another study [28], who stated that Alloxan is believed to confer its diabetogenic effect by inhibiting pancreatic glucokinase activity, leading to pancreatic β -cell death, which have particularly low antioxidant defense capacity. In the present work, the pathological changes observed in the pancreas of alloxanate diabetic rats may be attributed to increased production of reactive oxygen species and free radicals or by impaired antioxidant defenses [29] , [30] which is widely accepted as important in the development and progression of diabetes complications [31] , [32].

Sections of pancreas from alloxanate rat treated with soybean seeds showed marked decrease in vacuolar degeneration of islets of Langerhans cells (figure-5) and showed the connective tissue component of the gland and dilatation of blood vessel, this result went in line with earlier study [8], they stated that the hypoglycemic effect of plants may be due to the presence of insulin- like substances in plants stimulation of β cells to produce more insulin high level of soluble fiber which interferes with carbohydrate absorption or the regenerative effect of plants on pancreatic tissue [12]

Antioxidant's direct effect on the regeneration of the islets of pancreas was also evidenced by the restoration histopathological studies [26].

3.1.2 Kidney

The normal histological structure of the kidney was observed in Figure-6. The kidneys of untreated diabetic rats showed massive infiltrated with inflammatory cells (mononuclear cells) a round blood vessels Figure-7 degeneration in glomeruli with thickening of Bowman's

capsule and increase the urinary space and notes signs of vacuolar degeneration in some tubular epithelial cells (Figure-8). The narrowing of lumen with deformed renal tissue architecture (Figure-9). In agreement with previous study [37], who noticed that in diabetic rats the glomerular tufts were obviously contracted degeneration and infiltrated by chronic inflammatory cells. The urinary space become wide and change of architecture [34]. The treatment of rats with Alloxan showed some histopathological changes in the Kidney such as form of degeneration, inflammation, necrosis and deformed renal tissue architecture. Earlier study [35] reported that in diabetic rats the kidney showed degenerative changes in cortex and medulla and necrosis of tubules. In addition other investigator [36], observed that in diabetics kidney sections, damaged glomeruli, proximal tubules and interstitial inflammation. However other found in kidney of Alloxan induced diabetic rats; the histopathological studies showed early nephropathic changes and revealed vacuolar degeneration in tubular epithelial cells [37].

Vacuolation may be due to altered permeability of the cells membrane that would allow increasing fluid uptake [38]. These vacuoles were demonstrated by other scientists [39] they stated that the vacuoles were formed because of lactate accumulation in the tubules of the kidney resulting in increased osmotic pressure and subsequent water influx.

Oxidative stress plays an important role in chronic complications of diabetes mellitus and hence the regulation of free radicals is essential in the treatment of diabetes. The oxidative stress was related to decrease glutathione content and superoxide dismutase activity in tissue of Alloxan diabetic rats [40], [32]. Alloxanatediabetic treated with soybean seeds showed protective effect as compared to diabetic group of rats in form disappear of inflammatory infiltrate cells in kidney (Figure 10). This finding in agreement with other scientists [12], they reported that isoflavones may act like anti-inflammatory action. The isoflavones in soybean seeds prevents activation of potentially strong gene-altering and inflammation triggering molecule called NF-Kappa B also noted diminution of glomerular degeneration and vacuolar degeneration in some tubular epithelial (Figure-10) and showed the renal tissue architecture become normal.

These side effects may be neutralized by antioxidant substances. Antioxidants scavenge superoxide radical lipid peroxide hydroxyl radical [41]. According to others, [12], isoflavones in soybean seeds act as antioxidant activity and diminution the damage in the kidney.

3.1.3 Adrenal gland (suprarenal gland).

The normal histological structure of the adrenal gland was observed in Figure-11. The cell of Zona glomerulosa of the alloxanate diabetic rats group were seen disturbed in the arrangement. Showed cytoplasmic fat vacuolation (Figure-12) and pyknotic nuclei (Necrosis) (Figure-13) these findings were in agreement with previous report of some investigators [42], [43] these organelles are actively involved in the aldosterone synthesis [44].

Cholesterol acts as precursor to various hormones associated with the endocrine organs [44]. The lower use of cholesterol in aldosterone synthesis may account for the notable rise in the lipid droplets that were observed in Zona glomerulosa cells in Alloxan induced diabetic rats. In fact, cholesterol is stored in the lipid droplets. Quantity of aldosterone at least in the rat, depends on the balance between exogenous uptake of cholesterol from serum high-density lipoproteins and its utilization in steroidogenesis

[45], [46], steroid synthesis take place in the smooth endoplasmic reticulum and the mitochondria are the source of energy [47],[48]. Therefore, the degeneration of the mitochondria could lead to decrease energy levels and reduce the secretion of aldosterone [49]. Lack of insulin in diabetes could directly affect aldosterone affecting metabolism leading to impairment of steroidogenesis in zona glomerulosa [50], [51].

The alloxanate diabetic rats treated with soybean seeds showed protective effect as compared to diabetic group in the form diminution in fat vacuolation and pyknotic nuclei (necrosis) in the zona glomerulosa figure-14.

Soybean seeds act as antidiabetic plant, that can act through supplying β cells with the necessary elements (Cu^{++} , Mg^{++} , Ca^{++}). On the other hand, soybean seeds may act on glucose homeostasis in diabetic group treated with plant. They stimulate insulin secretion from β cells by induce regeneration of the β cells present insulin leading to increase of steroidogenesis [52].

3.1.4 The effect of soybean seeds on glucose level in diabetic rats

Presents study showed a significant increasing [$P < 0.05$] levels of glucose in blood of the alloxanate diabetic rats group as compare with control rats. The contrast administration of soybean seeds to diabetic rats recorded a significant reduction [$P < 0.05$] in the level of plasma glucose when compared with diabetic rats but they still significant higher than the control rats [table-1].+

Alloxan - induce diabetes caused increasing plasma levels of glucose and also Alloxan produces oxygen radicals and oxidative stress in the body [53] proposed that reactive oxygen species produced from Alloxan cause DNA strand breaks, and the damaged DNA activates nuclear poly [ADP ribose] synthetase, which deplete the cellular pool of NAD^+ , resulting in b-cells damage [54].

The soybean seeds contain isoflavones and saponins which act as Insulin- like substances [9], and inhibit insulins activity. Others may cause increase in β cells in pancreas by activating the regeneration of these cells [10]. Soluble fiber from soybean seeds may be useful because of its insulin-moderating effect. Particularly soluble fiber is important to control plasma glucose concentration in diabetic. Soybean fiber intake has also been implicated for the improvement of the blood glucose levels of diabetic [12]

Table 1: Effect of soybean treated on the kidney functions, blood glucose and body weight of diabetic male rats

Characteristic	Control G1	Soy bean treated G2	Diabetic G3	Soy bean diabetic G4
Body weight (g)	198±3.37	220±3.14	157±3.13	173±3.37
Blood glucose mg/dl	109±6.37	115±6.56	290±8.78	190±7.88
Urea mg/dl	16±2.36	18±2.76	58±4.38	33±2.97
Creatinine mg/dl	0.4±0.17	0.6±0.36	2.3±0.83	1.6±0.37

3.1.5 Effect of soybean seeds on the creatinine and urea levels in diabetic rats

Table 1 showed the effect of soybean seeds treatment on plasma creatinine and urea levels under conditions of diabetes. The plasma creatinine and urea level were significant higher ($P < 0.05$) in diabetic animals than control rats. The administration of soybean seeds to diabetic rats recorded a significant reduction ($P < 0.05$) in the levels of plasma creatinine and urea as compared to diabetic rats but they still significantly higher ($P < 0.05$) than normal rats. While, no significant differences were observed in the level of plasma creatinine and urea in soybean seeds treated rats when compared with normal control but they still significantly lower than the diabetic rats.

Alloxan -induced diabetes caused increase plasma levels of creatinine and urea. Alloxan is produces oxygen radicals and oxidative stress in the body [55]. Another interpretation made by previous study [15] reported that soybean seeds contains ~2% soyasaponin have unique chemical structures and physiological functions such as hepatoprotective anti-hyperlipidemic, anti-cancer, anti-oxidative and anti-HIV etc.

The saponin enables the kidney malfunctions resulting from diabetes to return to normal state by decreasing renal hyperfiltration proteinuria and renal acid load therefore reduced the risk of renal disease [56]. In diabetes soybean seeds contain phenolic category called flavonoids therefore plasma creatinine and blood urea levels were found to be reduced by the administration of soybean seeds in Alloxan - induced diabetic rats [57]. The soybean seeds reduced serum glucose, creatinine levels and serum lipid peroxidation and increased serum superoxide dismutase suggesting soluble fiber from soybean may be useful because of its insulin-moderating effect. It's generally accepted that a high fiber diet, particularly soluble fiber is useful to control plasma glucose concentration in diabetics. Soybean of the blood glucose levels of diabetics. Soybean fiber intake has also been implicated for the improvement of the blood glucose levels of diabetic [58]. This may interpreted the result obtained in this study it is concluded from the current study that the taken dose of soybean seeds affected positively in reduced glucose levels and improving the impaired kidney functions in diabetic rats.

3.1.6 Effect of soy bean seeds on the Body weight

There was a significant decrease ($P < 0.05$) in body weight of the rats in diabetic group in comparison to the control group. In contrast, the administration of soybean seeds to diabetic rats recorded a significant increase ($P < 0.05$) in body weight, but they still significantly higher than the diabetic rats.

Induction of diabetes with Alloxan is associated with the characteristic loss of body weight which is due to increased muscle wasting [59] and due to loss of tissue proteins [60]. Diabetic rats treated with the soybean seeds increase in body weight, which may be due its protective effect in controlling muscle wasting reversal of gluconeogenesis and may also be due to the improvement in insulin secretion and glycemic control.

The effect of soybean protein on enzymes involved in fatty acid accumulation and accumulation of body fat has been reported in sprague-Dawley rats [61]; while, there are numerous reports of the health benefits of soybean proteins [61, 62].

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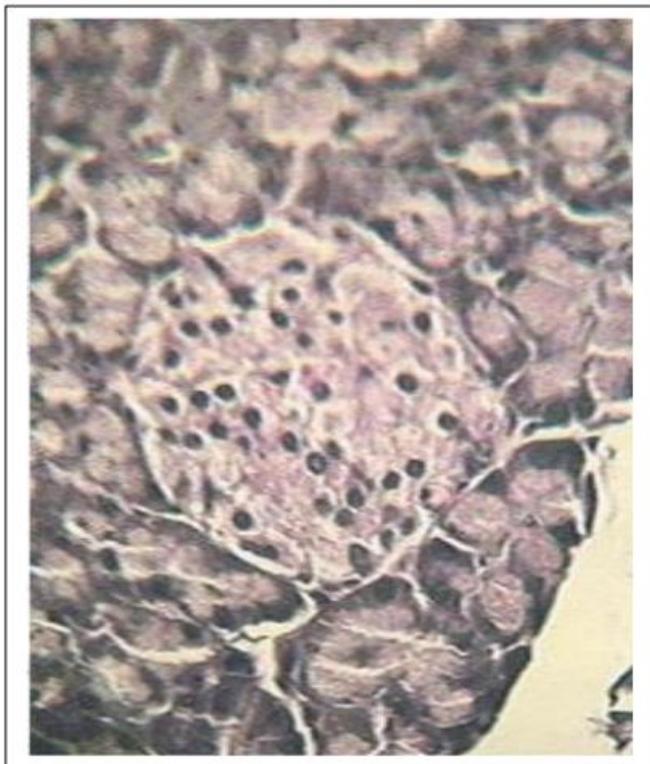


Figure [1]: Across section in Pancreas (control) of rat (HRE 40X)



Figure [3]: Across section in Pancreas of rat, treated with 150 mg/Kg alloxan showing dilation of blood vessel (→) Increase in connective tissue component, ← the nuclei of exocrine part pushed to the bottom of acinus → (H&E 40X)

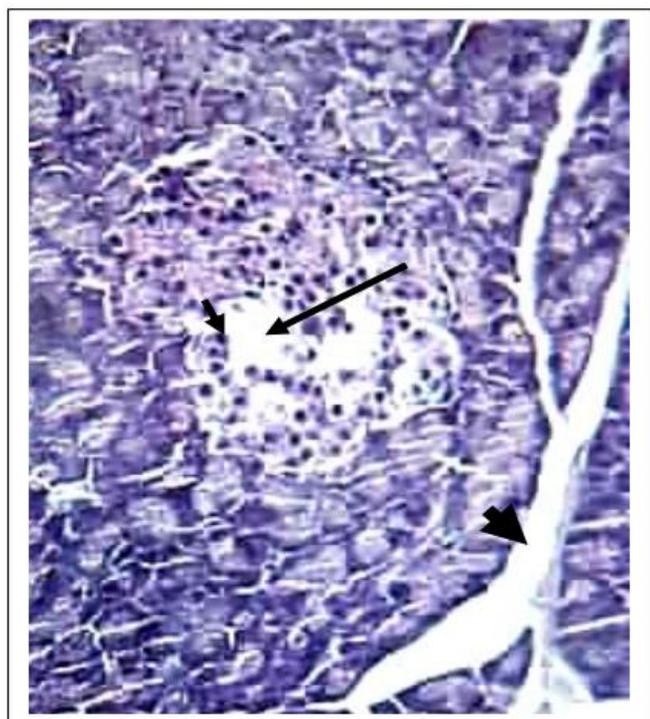


Figure [2]: Across section in Pancreas of rat, treated with 150 mg/Kg alloxan showing degeneration in central part of Langerhans (→) and increase connective tissue component ► (H&E 40X).

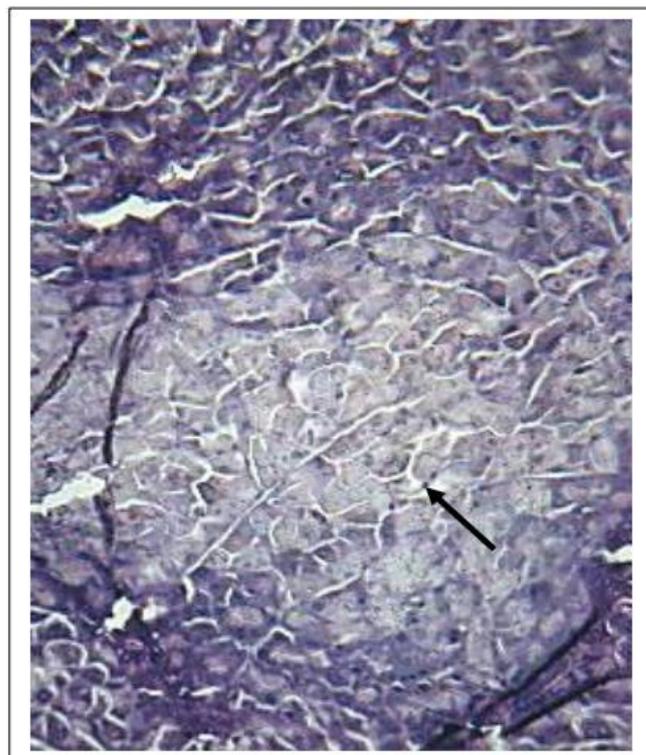


Figure [4]: Across section in Pancreas of rat, treated with 150 mg/Kg alloxan showing flattening of serous acini and Pushed to the bottom and degeneration (→) (H&E 40X)

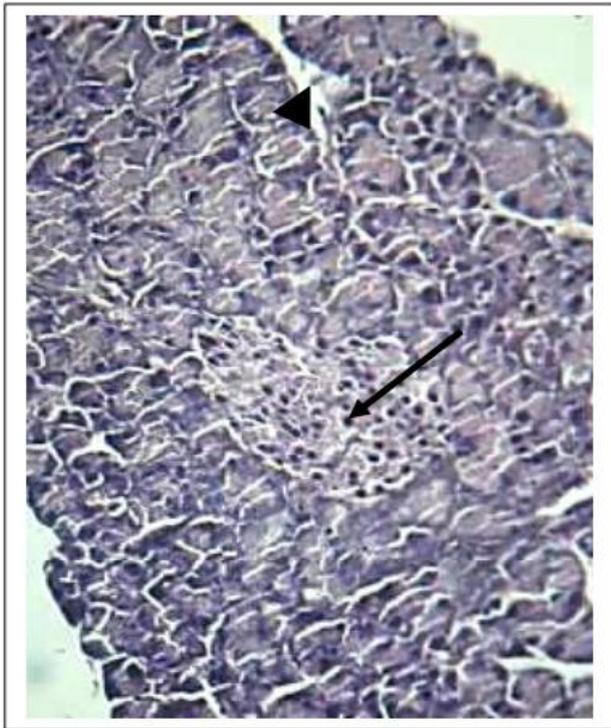


Figure [5]: Across section in Pancreas of alloxanated rat treated with 50 mg/Kg soy bean seeds showing marked reduced vacuolation in central part of Langerhans islets (→), with presence connective tissue component ▶ (H&E 40X)

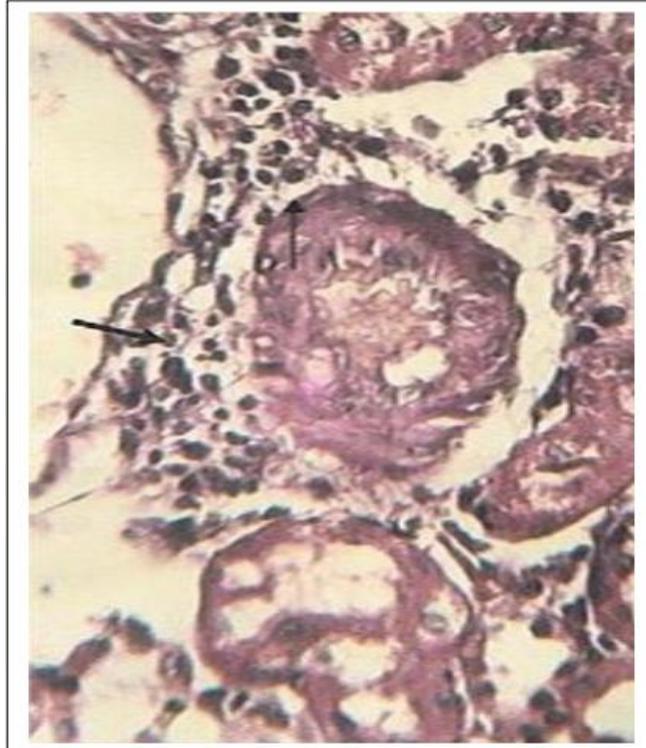


Figure [7]: Across section in kidney of rat treated with 150 mg/Kg alloxan showing massive infiltrated by the inflammatory cells (Mononuclear cells) around blood vessels (→) (H&E 40X)

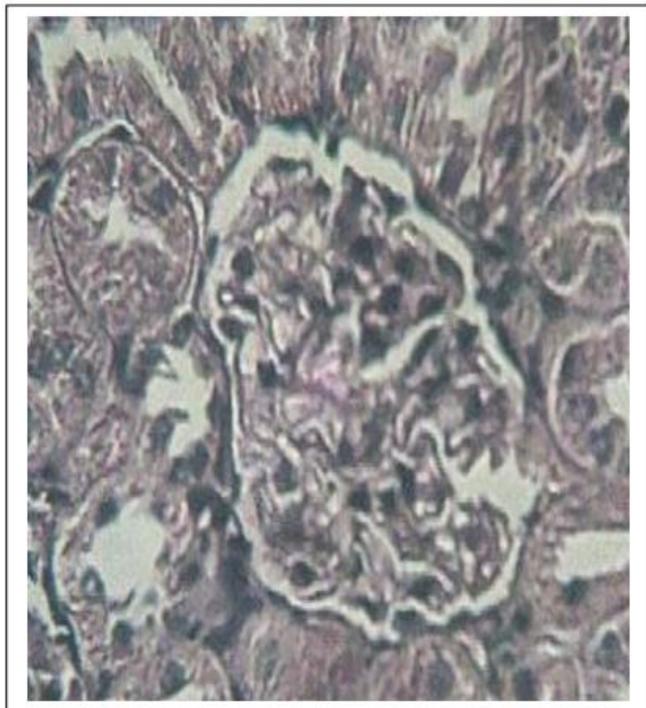


Figure [6]: Across section in kidney (control) of rat (H&E 40X)

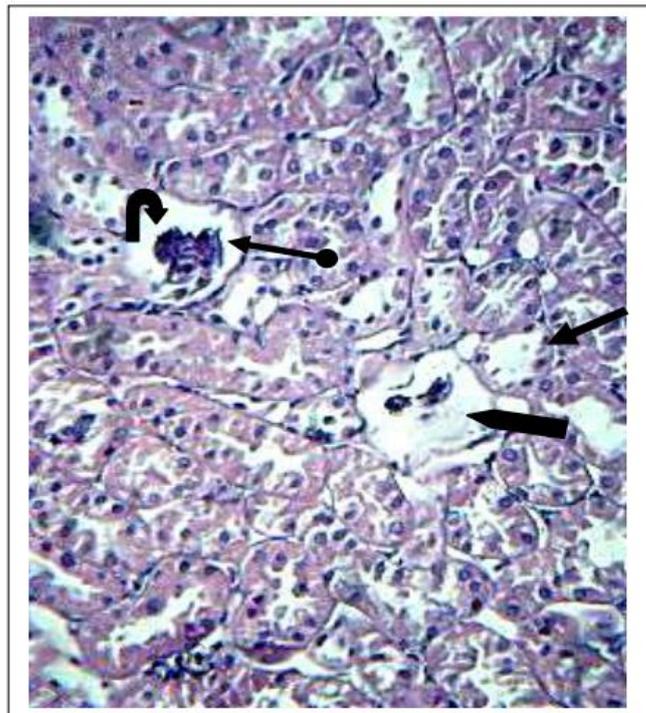


Figure [8]: Across section in kidney of rat treated with 150 mg/Kg alloxan showing degeneration in glomeruli (█), thickening of Bowman's capsule), (●→) increase the urinary space, (P) with vacuolar cells (→)(H&E 40X)

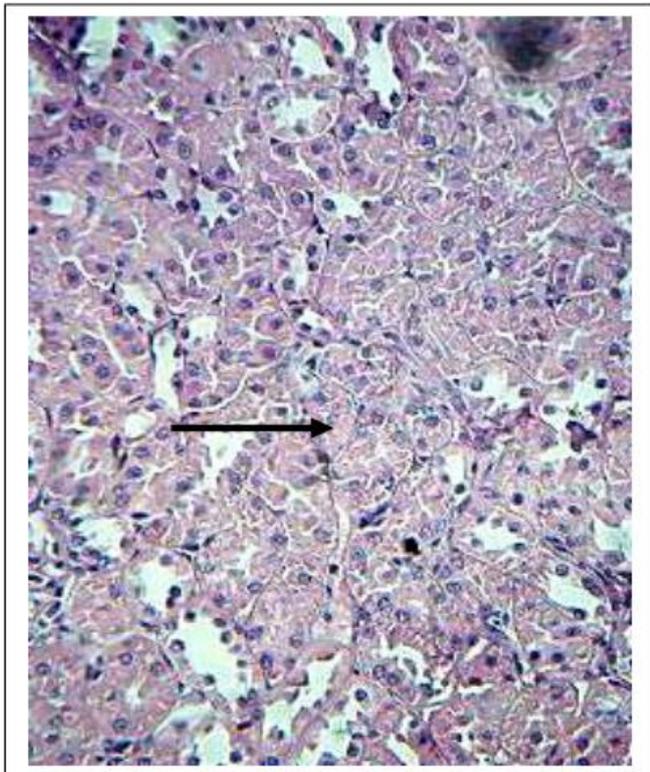


Figure [9]: Across section in kidney of rat treated with 150 mg/Kg alloxan showing deformed renal tissue architecture with narrowing of Lumen tubules (→) (H&E 40X)

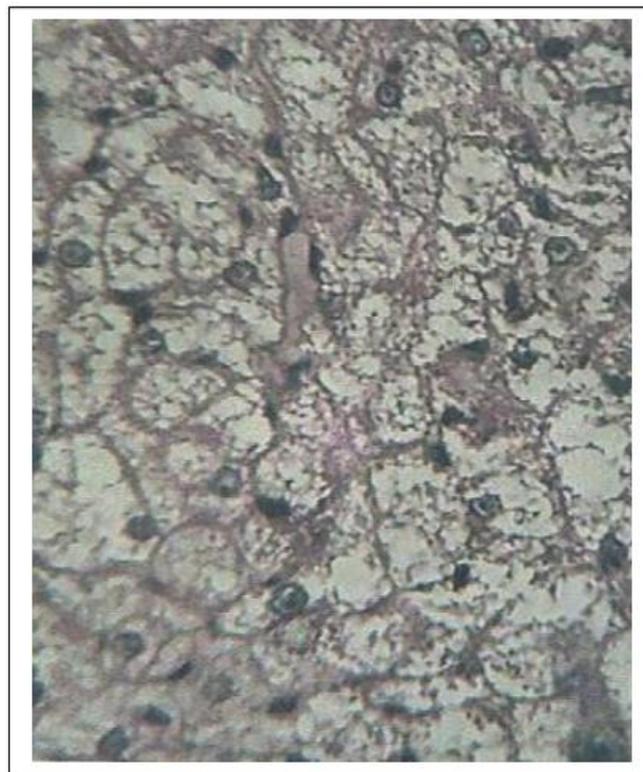


Figure [11]: Across section in adrenal gland [control] of rat (H&E 40X)

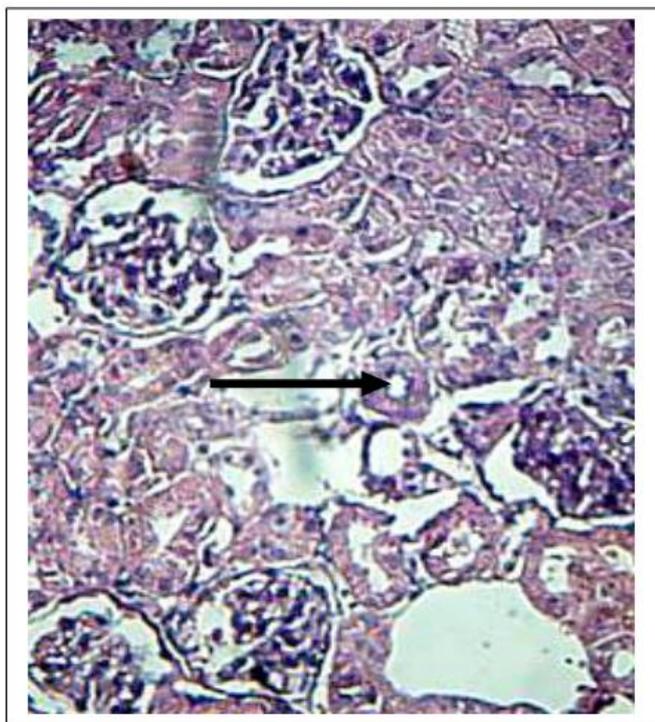


Figure [10]: Across section in kidney of alloxanated rat treated with 50 mg/Kg soy bean showing disappear inflammatory cells (Mononuclear) around blood vessels (→), with marked reduced degeneration in glomerulosa and tubules (H&E 40X)

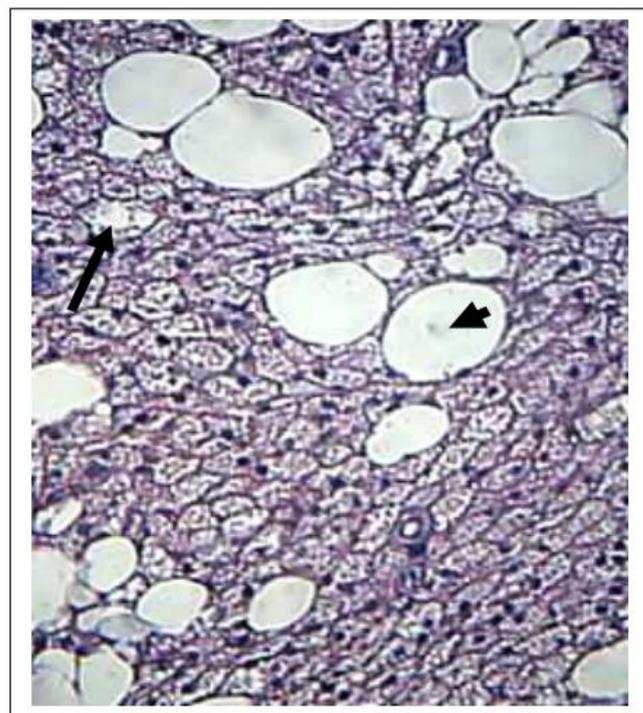


Figure [12]: Across section in adrenal gland of rat treated with 150 mg/Kg alloxan showing massive cytoplasmic Fat vacuolation, with necrosis in cells of zona glomerulosa (→) (H&E 40X)

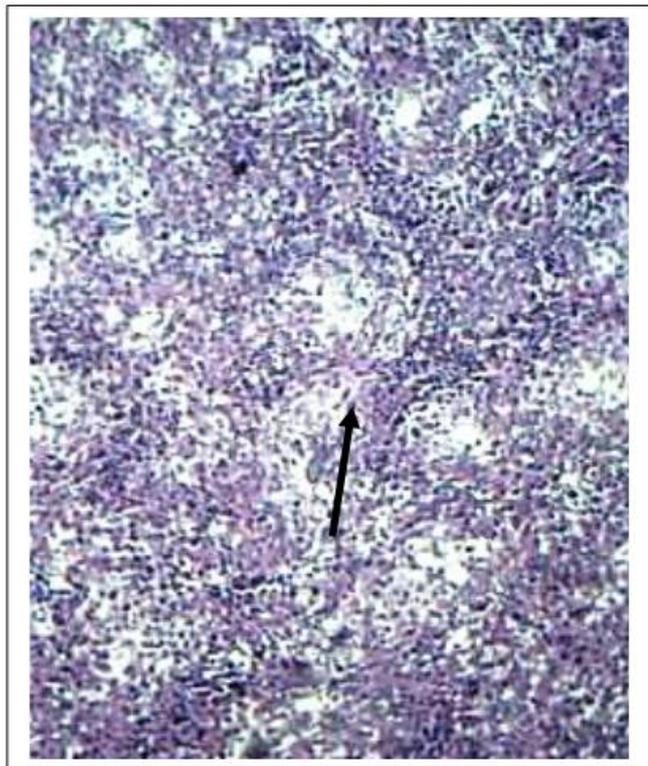


Figure [13]: Across section in adrenal gland of rat treated with 150 mg/Kg alloxan showing massive necrosis [pyknotic nuclei] in cell of zona glomerulosa (→) (H&E 40X)

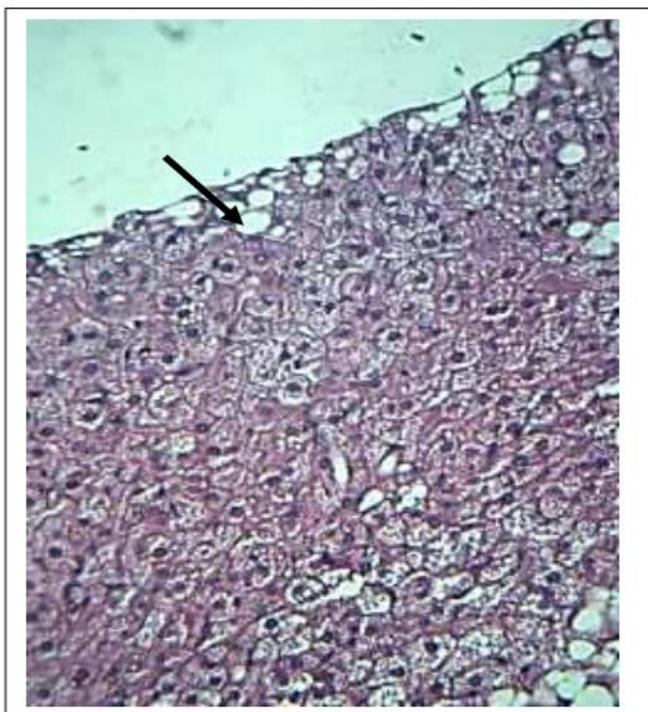


Figure [14]: Across section in adrenal glands of alloxanated rat treated with 50mg/Kg soy bean seeds showing diminution of cytoplasmic fat vacuolation and necrosis in zona glomerulosa cells (→) (H&E 40X)