

## **Role of Green Tea Extract in Reducing Pathological Changes of Induced Diabetes Mellitus in Rats**

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### **ABSTRACT**

Thirty male albino rats were used to investigate the effect of green tea extract on pancreas, renal and hepatic histological parameters in alloxan induced diabetes mellitus.

The animals were randomly distributed into five groups of six animals in each group. The first group was regarded as normal healthy control, the second group was regarded as diabetic control, the third group was treated with insulin, the fourth group was treated with green tea extract, 50 mg/kg body weight (GTE50), and the fifth group was treated with GTE100.

This study showed that functionally diabetes related organs such as pancreas, kidney and liver showed diabetes related pathological changes and these revealed a noticeable tendency for reversion to normal after treatment with green tea extract.

**Keywords:** rat, green tea, alloxan, diabetes.

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## INTRODUCTION

Diabetes mellitus (DM) is an increasingly common, potentially devastating, expensive, treatable, but incurable lifelong disease. The world health organizations (WHO) estimate that more than 220 million people worldwide have diabetes and this number is likely to be more than double by 2030 (WHO, 2009). DM is associated with a number of chronic complications including nephropathy, neuropathy, retinopathy, and cardiovascular disorders (Mahdi *et al.*, 2003).

Many hypoglycemic agents, such as the biguanides and sulfonylureas, are used alone or together with insulin to treat this disease, however these medications can cause serious side effects, motivating a search for safer, more efficacious agents to control diabetes (Huang *et al.*, 2005).

Studies showed that numerous extracts obtained from plants are effective in reducing glycemia, causing fewer side effects and with lower cost than the usual antidiabetic agents (Sohn *et al.*, 2010). The majority of the plants that are used in popular medicine for treatment of DM have been shown to possess biologically active chemical constituents such as alkaloids, flavonoids, phenolic substances, and other constituents, that can be used as new hypoglycemic agents (Negri, 2005).

The polyphenols found in green tea are more commonly known as flavonols or catechins have demonstrated significant antioxidant, anticarcinogenic, anti-inflammatory, thermogenic, and antimicrobial properties in numerous human, animal, and *In vitro* studies (Alschuler, 1998).

Green tea catechins are hypothesized to help protect cells against damaging effects of reactive oxygen species by contributing, along with antioxidant vitamins C and E and enzymes superoxide dismutase and catalase, to the total antioxidant defense system (Abdel-Raheim *et al.*, 2009).

It is reported that oxidative stress is a contributor in the development and progression of DM and its complications. Several mechanisms can contribute to the increased oxidative stress in the diabetic patients, especially the chronic exposure to hyperglycemia (Davi *et al.*, 2005). Hyperglycemia can lead to an elevated reactive oxygen species (ROS) production by respiratory enzyme chains in mitochondria (Nishikawa and Araki, 2007), formation of advanced glycation end-products (AGEs) (Monnier, 2003), and imbalance of glutathione redox status (Maritim *et al.*, 2003).

## Pathological changes of Diabetes Mellitus

### Pancreas

Histological studies using pancreas specimens from patients with Insulin Dependent Diabetes Mellitus (IDDM) demonstrated that  $\beta$ -cell mass is reduced by 70-80% at the time of diagnosis, and beta cells are the target of autoimmune assault and that apoptosis is the major form of beta-cell death, macrophages are found to be the predominant infiltrating immune cells during the early stage of insulinitis together with CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes, natural killer cells and  $\beta$ - cells (Kumar *et al.*, 2005).

Non insulin dependent DM is characterized by combination of insulin resistance and  $\beta$ -cell dysfunction, a 25-50% reduction in beta-cell mass is found at the time of diagnosis, the

remarkably high level of beta-cell loss could result from either an impaired proliferative capability or an elevated rate of cell death (Maedler, 2008).

### **Liver**

The liver is a central and essential organ related to DM, it performs several important functions, and it maintains glucose, fat and amino acid homeostasis (Souhami and Moxham, 1997).

Diabetes related complications in liver include, nonalcoholic fatty liver disease (NAFLD), cirrhosis, hepatocellular carcinoma, and acute liver failure, patients with DM have a high prevalence of liver disease, and numerous studies have confirmed a fourfold increased prevalence of hepatocellular carcinoma in patients with DM, as well as an increased prevalence of diabetes in patients with hepatocellular carcinoma (El-Serag *et al.*, 2004).

### **Kidney**

The most important glomerular lesions seen in IDDM are capillary basement membrane thickening, diffuse mesangial sclerosis, and nodular glomerulosclerosis, the glomerular capillary basement membranes are thickened throughout their entire length, but lesions can also be seen in the tubular structures and the surrounding tissue (Perrin *et al.*, 2006). The disease advances at an individual rate over the course of several years, but ultimately most of the glomeruli will be destroyed and the patients will suffer from insufficient filtration ability (Bloomgarden, 2008).

Renal atherosclerosis and arteriolosclerosis constitute part of the macrovascular disease in DM; however, the changes in the arteries and arterioles are similar to those found throughout the body. (USRDS, 1999).

Pyelonephritis, both the acute and chronic forms of this disease occur in nondiabetics as well as in diabetics, but are more common in diabetics than in the general population, one special pattern of acute pyelonephritis, necrotizing papillitis (or papillary necrosis), is much more prevalent in diabetics than in nondiabetics (Kumar *et al.*, 2005)

The aim of this study is to investigate the effectiveness of the green tea extract in the reduction of pathological changes of alloxan induced diabetes on rat pancreas, liver and kidney.

## **MATERIALS AND METHODS**

Thirty male albino rats were used in this experiment. The animals were housed under standard laboratory conditions (12 hrs. light: 12 hrs. dark photoperiod,  $22 \pm 2$  °C, with free access to food and water.

Aqueous green tea extract was prepared by dissolving fifty grams of mortar crushed green tea leaves in 500ml of distilled water, after boiling for (20) minutes, the extract was left standing for one hour till cooling. (Zhang *et al.*, 2004).

The extract was filtered then centrifuged at 3000 rpm for 15 minutes, the supernatant has been concentrated by rotary evaporation, and then the dried aqueous extract was dissolved in normal saline and stored at -20 °C as the stock solution of the extract. In stock solution two doses of green tea extract (50, 100) mg/kg body weight were prepared.

Diabetes mellitus was induced in rats using diabetogenic substance alloxan monohydrate (Sigma-Aldrich Corp, St. Louis, MO, USA) by subcutaneous injection of freshly prepared

solution of alloxan (100 mg/kg) body weight in 24 hrs fasting animals.

One month later, rats with blood glucose more than 200 mg/dl were considered as diabetic and the animals were divided into 5 groups (6 rats in each):

**Group I-normal control:** rats of this group received tap water and normal food.

**Group II-diabetic control:** rats of this group received tap water and normal food.

**Group III-insulin treated diabetic group:** rats of this group received 6 units insulin per rat per day, 2 units every eight hours. The insulin was injected intraperitoneally by using insulin syringe.

**Group IV-green tea extract (50 mg/kg) treated diabetic group:** rats of this group received 50 mg/kg b.w. of green tea extract.

**Group V- green tea extract (100 mg/kg) treated diabetic group:** rats of this group received 100 mg/kg b.w. of green tea extract.

The extract was administered once per day by gavage needle for twenty eight days. After (28) days of the treatment, then the animal scarified and pancreas, liver, and kidney were harvested and immediately fixed in 10% formalin, washed with tap water, dehydrated through a series of ethanol concentrations, cleared by xylol, embedded in paraffin, sectioned by microtome at 5 micron thickness and stained with Hematoxylin and Eosin. (Bancroft and Gamble, 2008).

All sections examined and photographed by Motic digital microscope (Motic Corp, DMWB series, China).

## RESULTS

### Pancreas

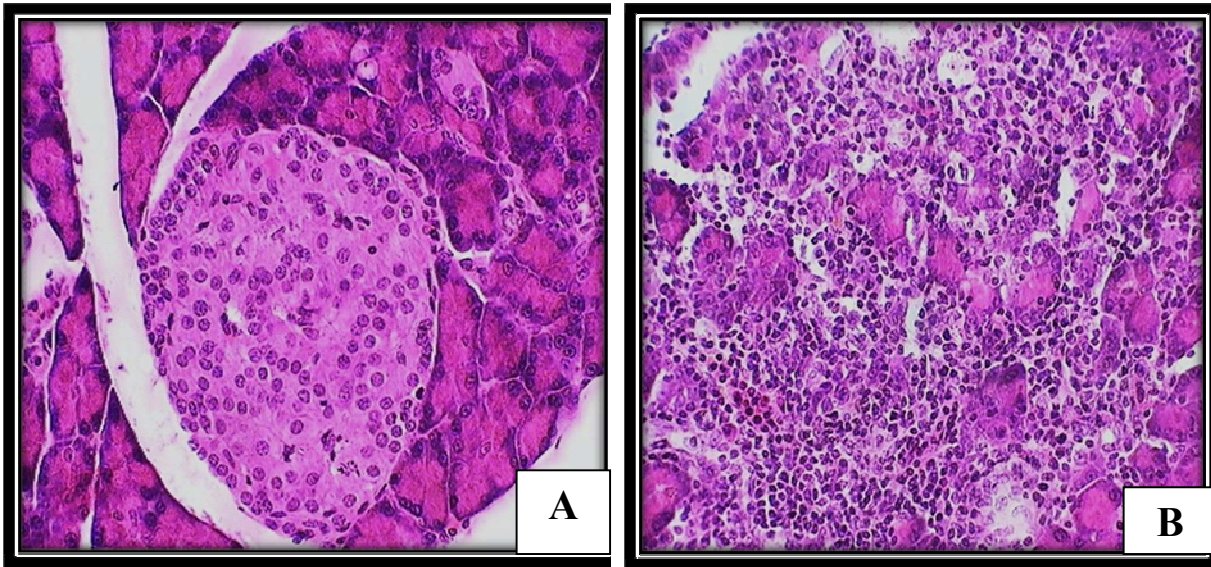
In alloxan induced diabetes, pancreas showed the distortion of exocrine pancreas and inflammatory cell infiltration. Insulin had a relative protective role for islets of Langerhans with the effect on the exocrine portion. GTE100 treated rats, showed marked reduction in islets vascular congestion and the severity of inflammatory infiltration, despite the fact that the cellularity of islets of Langerhans was still reduced (Figs. 1 and 2).

### Kidney

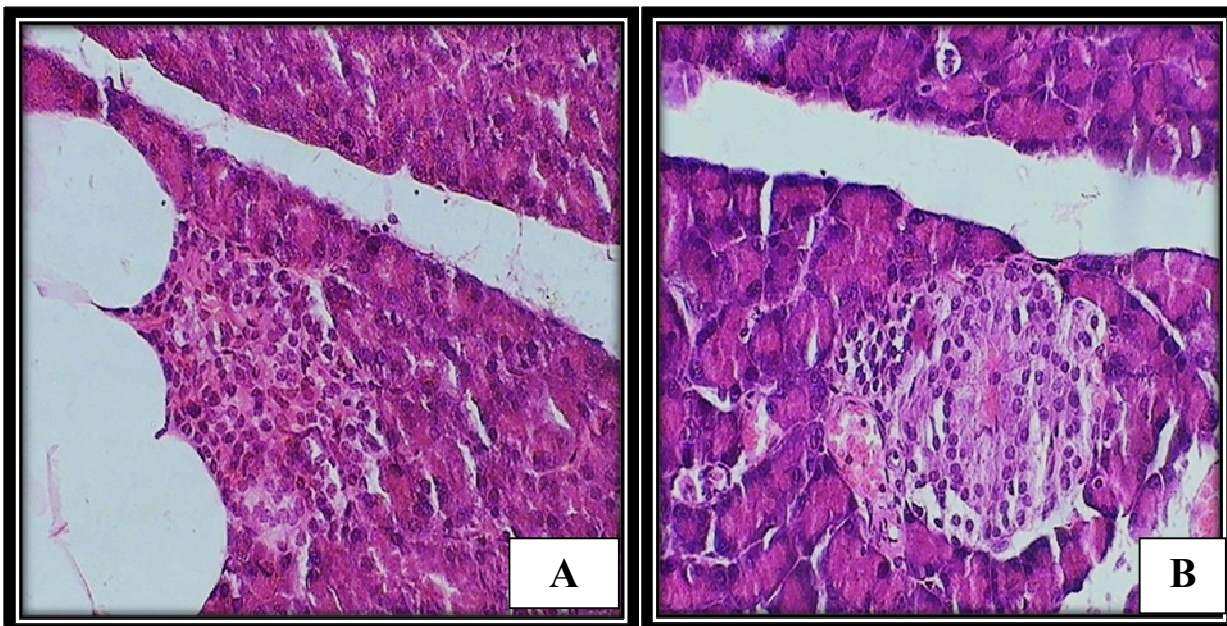
Diabetes showed marked histological changes in rat kidneys deposition of proteinaceous material, glomerular capillary dilation, tubular dilation and interstitial hemorrhage.

Insulin therapy relatively protected the glomeruli, despite tubular dilation and interstitial hemorrhage. GTE elicited a protective effect to the kidney despite some tubular dilation and necrotic changes and higher dose of GTE (GTE100) produced a better renal protective effect for both glomeruli and tubules (Fig. 3, 4, 5 and 6).

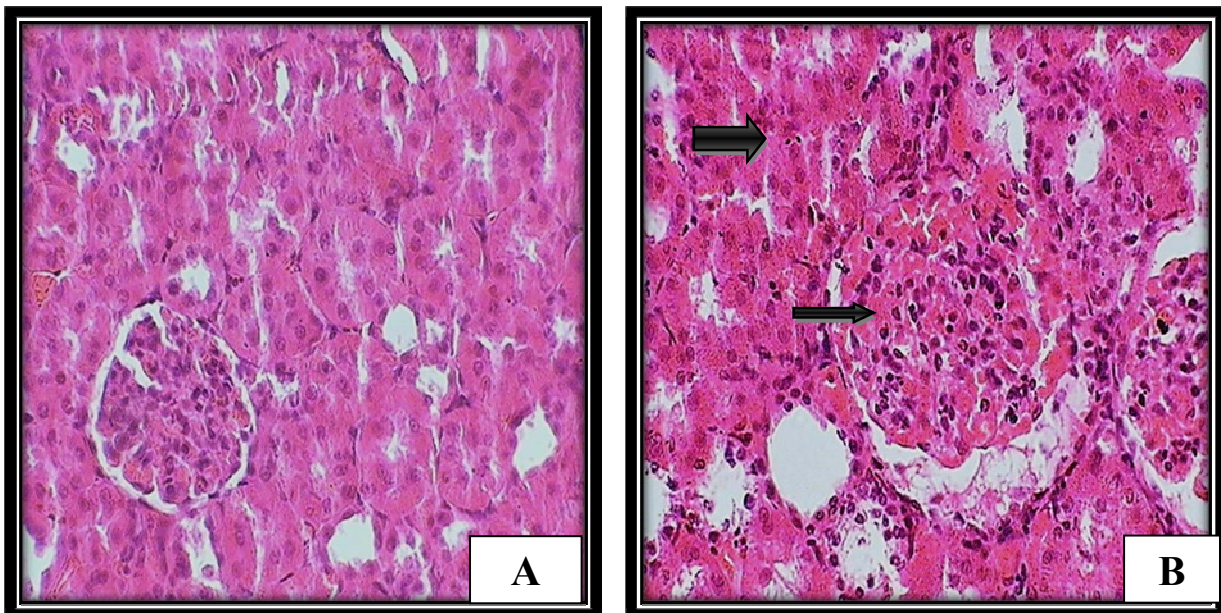




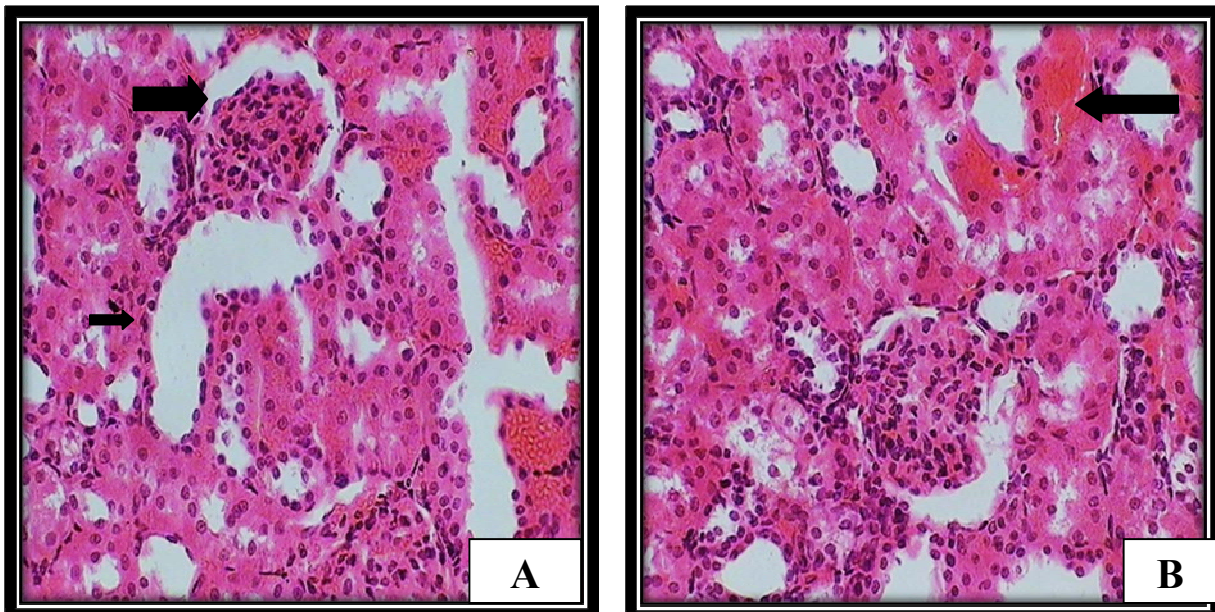
**Fig. 1: Rat pancreas: A, normal islet of langerhans and surrounding exocrine pancreas; B, Untreated diabetes (control) pancreas, showed distorted exocrine pancreas with marked chronic inflammatory cell infiltration. (H and E. 400X)**



**Fig. 2: Rat pancreas: A, insulin treated rat, showed relatively normal islet of langerhans and mild distortion of exocrine pancreas; B, GTE100 treated rat pancreas, showed cellularity in Islets of Langerhans is reduced and vascular congestion (Hand E. 400X).**

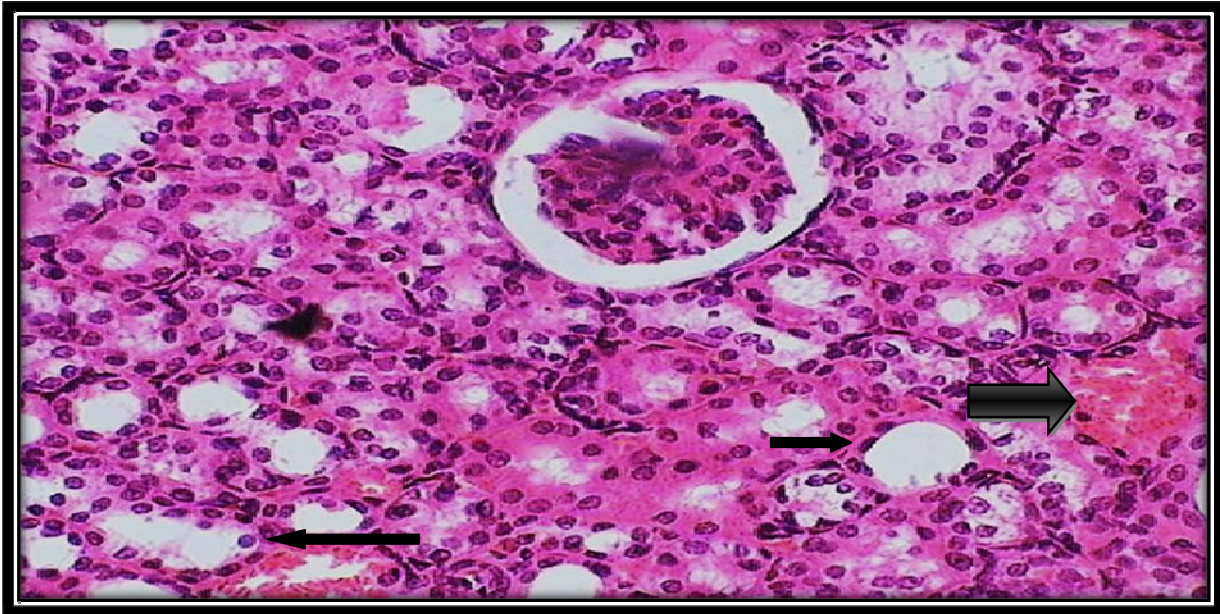


**Fig. 3: Rat Kidney: A, normal glomerulus and tubules; B, diabetic rat kidney, deposition of proteinaceous material (thin arrow) and capillary dilatation, tubular dilatation and focal necrotic changes (thick arrow). (Hand E. 400X).**

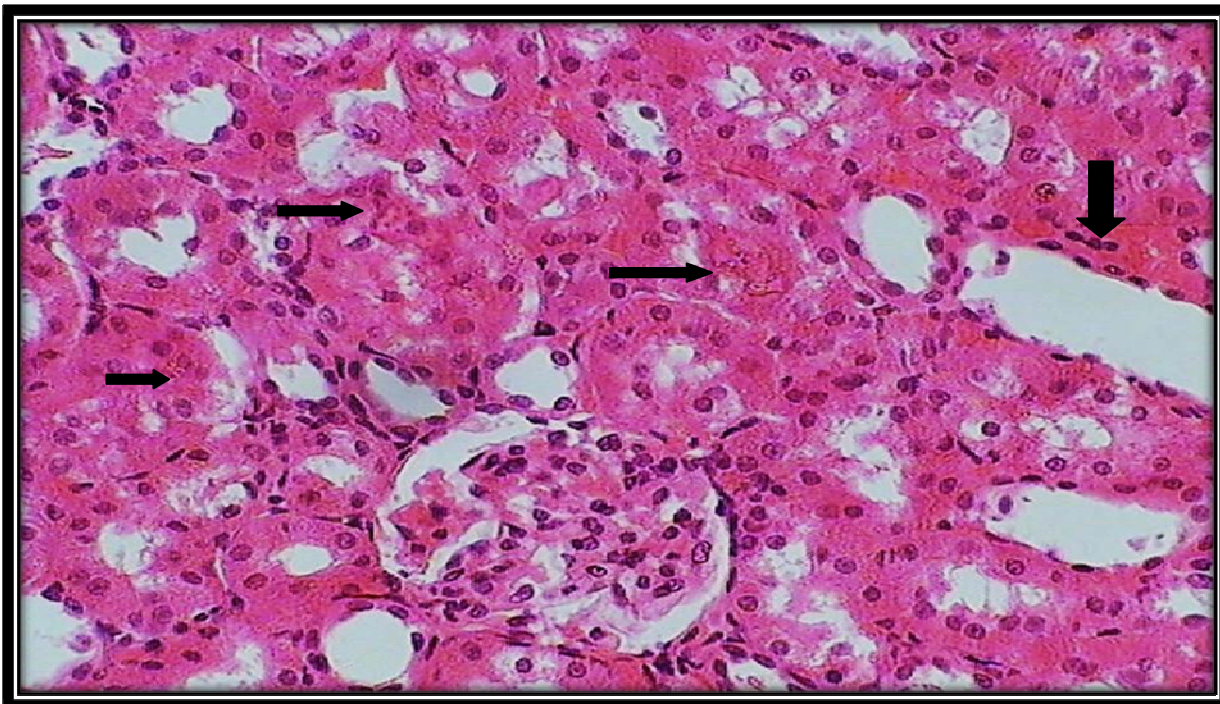


**Fig. 4: Rat kidney: A, diabetic changes with mild glomerular shrinkage (thick arrow) and tubular dilatation (thin arrow); B, insulin was treated with tubular dilation and interstitial hemorrhage . (H and E. 400X).**





**Fig. 5 : Rat kidney : GTE50 treated, nearly normal looking glomerulus, despite mild tubular dilataion(thin arrows) and focal hemorrhage (thick arrow). (H and E.400X).**

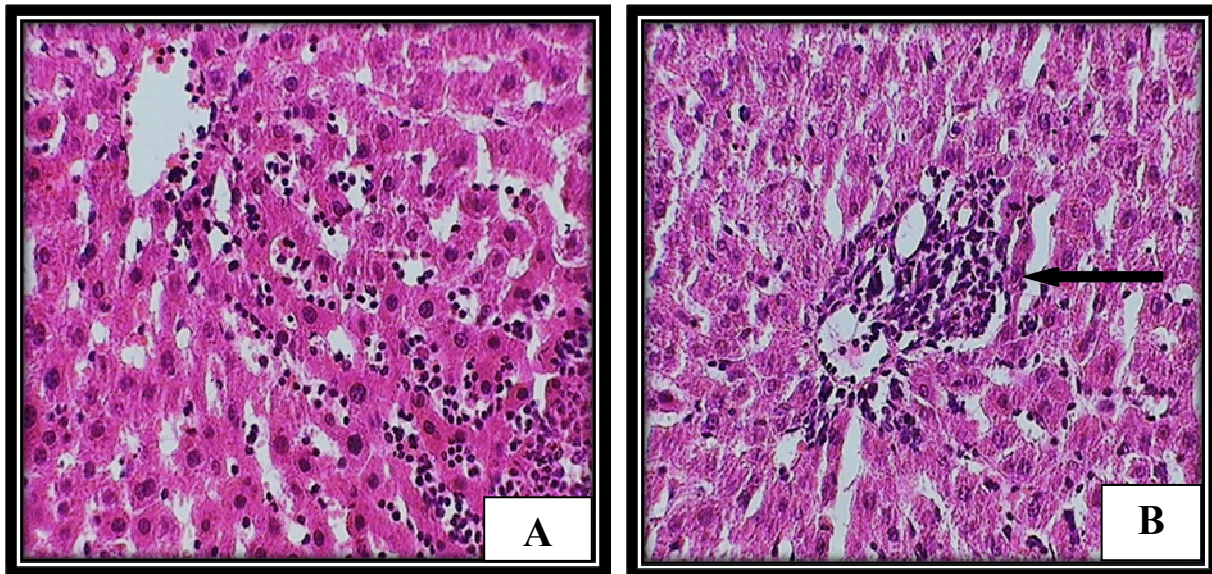


**Fig. 6: Rat kidney :A, GTE100, showed normal looking glomerulus and some tubules show diltation (thick arrow)and focal necrotic changes in tubular epithelial cells(thin arrows). (Hand E.400X).**

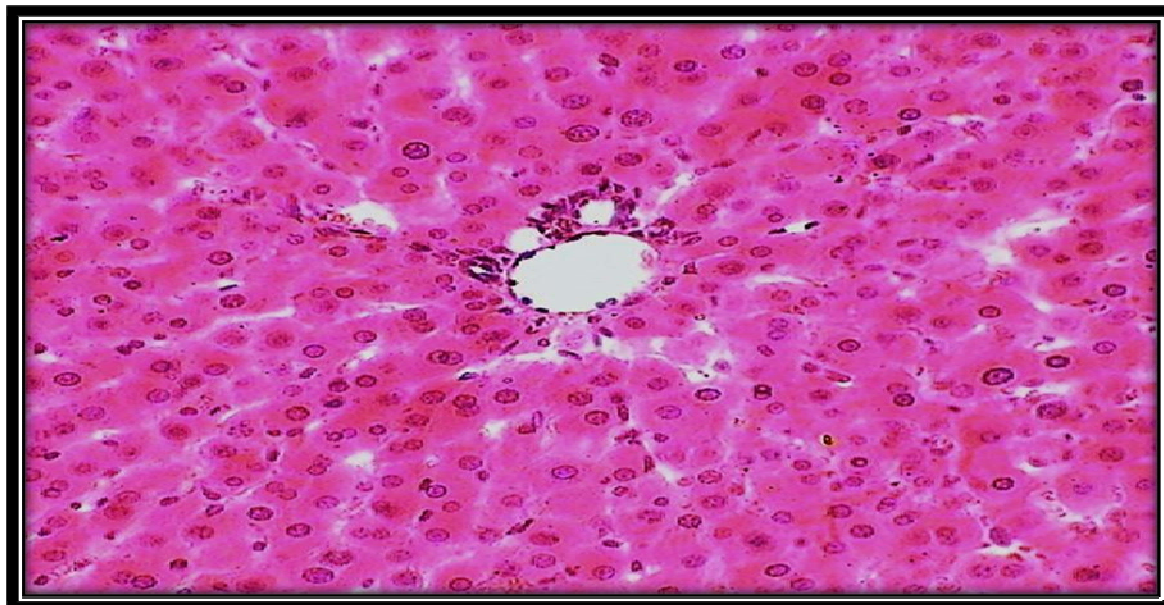


**Liver**

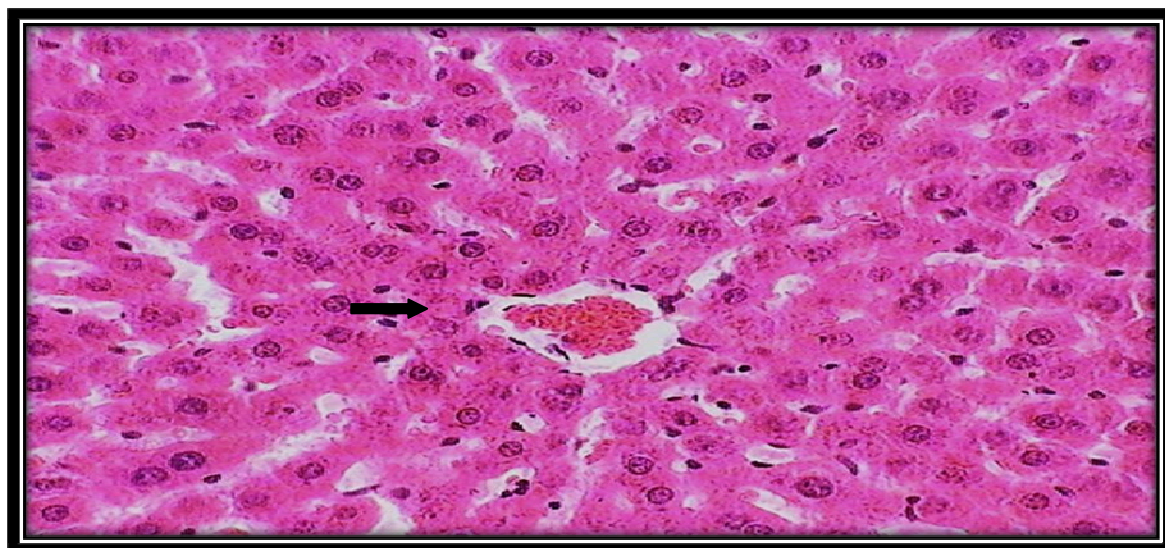
Diabetes induced a subtle histological effect manifested as mild disruption of hepatocytes plate arrangement, focal hepatocytic necrosis, and mild focal interstitial and perivascular inflammatory cell infiltration. Insulin relatively preserved hepatic arrangement, but GTE showed a normal hepatocyte arrangement despite mild vascular congestion (Fig. 7, 8, and 9).



**Fig. 7: Diabetic control rat liver: A, showed mild disruption of hepatocytes plates' arrangement and focal inflammation; B, necrotic hepatocytes, portal lymphocytic infiltration (HandE.400X).**



**Fig. 8: Rat liver: insulin treated, showed a portal tract and focal hepatocyte necrosis. (HandE.400X)**



**Fig. 9: Section from GTE100 treated rat liver, showed nearly normal hepatocyteplates and mild central vein cogestion (arrow). (H and E 400X)**

### DISCUSSION

Wolfram *et al.*, (2006) demonstrated that green tea epigallocatechin gallate possesses pronounced anti-diabetic efficacy in preclinical models of type 2 diabetes mellitus, which is at least partially mediated through reducing hepatic glucose production and enhancing the pancreatic function.

A study by Karaca *et al.*, (2010) showed, green tea extract significantly increased levels of serum insulin in the diabetic rats, and green tea extract(combined with ginseng roots) protected the cells in the islets of Langerhans.

The Diabetes Control and Complications Trial Research Group (1993) has elucidated that hyperglycemia is directly linked to diabetic microvascular complications, particularly in the kidney; therefore glycemic control remains the main target of therapy.

In diabetic nephropathy in group II (diabetic control) rats in this study there is capillary dilation, and dilated renal tubule. Similar structural changes in kidney have been reported by Vlassara *et al.*, (1994), and they speculated that, these structural changes may be directly influenced by advanced glycation end-products (AGEs) through excessive cross-linking of matrix molecules in a receptor independent way. Green tea extract helped in amelioration of renal diabetic changes mainly through the correction of oxidative stress as shown in study by (Pedraza-Chaverri *et al.*, 2003).

Histological examinations indicated that the liver of diabetic control male rats exhibited mild disruption of hepatocytes, focal inflammation, necrotic hepatocytes, and perivascular lymphocytic infiltration although advanced hepatic pathological changes were not seen in this study (Fig. 7).

Treatment of alloxan-induced diabetic rats with green tea extracts (50, 100 mg/kg) resulted in apparent amelioration of most hepatocytes and decreased vascular congestion (Fig. 8 and 9).

Das *et al.*, (2005) study has shown that black tea extract (BTE) supplementation has the ability to prevent hepatocellular damage in rats receiving ethanol with or without a high-fat diet, and to restore the activity of antioxidant enzymes, reduce the generation of free radicals, lipid peroxidation. Moreover, black tea theaflavins has the ability to reduce hepatic lipid accumulation (Lin *et al.*, 2007), and probably by chemical components of black tea that may stabilize the integrity of the cell membrane and keeping the membrane intact and the enzymes enclosed through scavenging free radicals (Oyejide and Olushola, 2005). Thus, the hepatoprotective effect of green tea extracts (GTE50, GTE100) found in the present study may be due to inhibition of the oxidative stress, induced by alloxan, by increasing cellular antioxidant capacity and reducing membrane lipid peroxidation.

Some researchers reported that, green tea extract reduce cholesterol synthesis in liver (Bursill *et al.*, 2007) and decrease the severity of liver injury in association with lower concentration of lipid peroxidation (Chen *et al.*, 2004).

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