

Full Length Research Paper

Role of green tea extract in the improvement of liver and renal functions in alloxan induced diabetes mellitus in male albino rats

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Accepted 18 April, 2014

In the current study, fifty male albino rats weighing between 150-200 g, and 10 weeks old were used to investigate the effect of green tea *Camellia sinensis* extract on liver and renal function related biochemical parameters in alloxan induced diabetic rats. Diabetes was induced experimentally in rats by a single subcutaneous injection with alloxan (100 mg/kg). Rats with blood glucose more than 200 mg/dl were considered as alloxan induced diabetes after 30 days. The animals were randomly distributed into five groups each of six animals in each group. The first group was considered as normal control rats, the second group was regarded as diabetic control rats, the third group was treated with insulin, and the 4th and 5th groups were treated with green tea extract (50 and 100 mg/kg body weight). Alloxan induced diabetes mellitus in rats for four weeks caused significant increase in the levels of serum glucose, liver function parameters, alkaline phosphatase (ALP), glutamic acid transaminase (GPT), and glutamic oxalo acetic acid transaminase (GOT), renal function parameters, creatinine, urea, uric acid and lipid profile. Diabetic rats treated with 50 and 100 mg/kg of green tea showed significant decrease in the levels of serum glucose, triglycerides, cholesterol, liver function tests parameters such as ALP, GPT, and GOT; creatinine, uric acid as compared to non treated diabetic rats. Study of histological changes in functionally related organs such as pancreas, kidney, and liver showed structural changes. In pancreas, distortion of exocrine portion, and inflammatory cell infiltration were seen. In kidney, thickening of glomerular basement membrane, increased mesangial cellularity, deposition of extracellular matrix and dilation in renal tubules were detected. In liver, necrosis, inflammation and perivascular lymphocytic infiltration were apparent. Treatment with high dose (GTE100) of green tea extract was found to almost restore the normal histological architecture of the pancreas, kidney, and liver of diabetic rats.

Key words: Induced diabetes mellitus, green tea, liver and renal functions.

INTRODUCTION

Diabetes mellitus (DM) is associated with a number of chronic complications including nephropathy, retinopathy, and cardiovascular disorders (Mahdi et al., 2003). β cell glucose sensor, glucokinase (GK) is an enzyme that phosphorylates and regulates glucose metabolism in beta-cells, hepatocytes, allowing it to play a central role in glucose homeostasis (Lynedjian et al., 1989).

An association exists between diabetes and liver injury and liver pathology among diabetics including fatty liver (steatosis), steatohepatitis, fibrosis, and cirrhosis (Erbey et al., 2000). Elevated serum activity of the two

aminotransferases, aspartate aminotransferase and alanine aminotransferase, is the most frequently measured indicator of liver disease and occurs in diabetics more frequently than in the general population (Everhart, 1995). The same spectrum of liver injury and enzyme changes in DM has also been described among overweight individuals without diabetes. Nearly 70 to 80%

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of the diabetic subjects have been reported to have hepatic fat accumulation (Gupte et al., 2004). Furthermore, studies showed that activities of liver enzymes such as AST, ALT have increased in NIDDM patients (Wannamethee et al., 2005).

Diabetes mellitus is characterised by hyperglycaemia, which has been strongly linked to nephropathy; diabetics are at risk for end-stage renal disease (Held et al., 1991). Control of the hyperglycaemia by insulin treatment has been shown to avert hypertrophy and hyperfiltration and the subsequent rise in urinary protein excretion. Many studies have suggested that administration of GT catechins in diabetic animals drastically improves kidney function parameters, by improvement in the glomerular filtration rate (Rhee et al., 2002).

Yokozawa et al. (1999) examined glomerular filtration in cisplatin (a nephropathy inducer)-treated rats and demonstrated that GT significantly decreases the blood nitrogen level, serum creatinine, serum malondialdehyde and kidney excretion of glucose and proteins and oxidative stress. Another study has shown that GT reduces serum glucose, creatinine levels and serum lipid peroxidation and increases serum superoxide dismutase, suggesting that catechins influences glucose metabolism and improves kidney function by reducing oxidative stress in alloxan-treated diabetic rats (Sabu et al., 2002).

Urea, creatinine, uric acid, ammonia, and amino acids are the most important nitrogen components of blood serum and they have implications in clinical biochemistry. In DM, protein catabolism is increased due to deficiency of carbohydrate-derived energy in connection with low-serum insulin (Nair et al., 1987). Diabetic male rats showed high levels of serum urea, uric acid, creatinine and serum electrolytes and supplementation of ginseng to diabetic rats greatly improves kidney functions and reversal of these changes (Sawiress, 2011).

Alloxan induce diabetes mellitus introduce histological changes in pancreas, liver and kidney, the most effective pathophysiology of pancreas is β -cell mass loss and destroy β -cells (Akirav et al., 2008) and cause a lack of insulin production, this situation leads to a IDDM (Lowe, 1998).

Pancreatic beta cells are the target of autoimmune assault and that apoptosis is the major form of beta-cell death. β -cell apoptosis is triggered by either direct contact with activated macrophages and T-cells or exposure to soluble cytokines, and ROS (Piemonti et al., 2002).

NIDDM (Non insulin dependant diabetes mellitus) is not only characterized by pancreatic histological changes but also with combination of insulin resistance and β -cell dysfunction. Studies suggest that the hyperglycemia in NIDDM is in large part a consequence of a deficit in β -cell mass. Although a 25-50% reduction in beta-cell mass is found at the time of diagnosis of NIDDM, 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).

The liver is a central and essential organ and DM related complications of liver includes, abnormal liver enzymes, nonalcoholic fatty liver disease (NAFLD), cirrhosis, hepatocellular carcinoma, and acute liver failure. Patients with DM have a high prevalence of liver disease, and patients with liver disease have a high prevalence of DM (Trombetta et al., 2005).

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). The pathogenic sequence of events leading to hepatocellular carcinoma appears to be insulin resistance, increased lipolysis, lipid accumulation in the hepatocytes, oxidative stress, and cell damage followed by fibrosis and cell proliferation, which are procarcinogenic (Kazachkov et al., 1998).

The kidneys are the main part of the human excretory system as they regulate homeostasis by excretion of waste products of metabolism. 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, 2006). Reduced survival of the podocytes that embrace the glomerular capillaries may also accompany the early progressive nephron damage (Wolf, 2005). 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).

MATERIALS AND METHODS

Fifty male albino rats, *Rattus norvegicus* were used in the current study. The animals were fed on standard rat pellets and tap water *ad libitum*. The animals were housed under standard laboratory conditions (12 h light: 12 h dark photoperiod and kept at $22 \pm 2^\circ\text{C}$) in the animal house of Science and Health in Koya University.

The required amount of extract was obtained from local market. Green tea *Camellia sinensis* extracts were prepared by the method of Zhang-Mu (1990). The extract was filtered using gauze and then centrifuged at 3000 rpm for 15 min. The supernatant was concentrated by rotary evaporation and the dried aqueous extract was dissolved in normal saline and stored at -20°C . This was considered as the stock solution of the extract for preparing both experimental doses (50 and 100 mg/kg B.W.).

DM was induced in rats using diabetogenic substance alloxan monohydrate (Sigma-Aldrich Corp, St. Louis, MO, USA). 100 mg of alloxan / kg BW was dissolved in 10 ml normal saline immediately before injection

Table 1. The effects of different doses of green tea on the level of the serum glucose in normal rats.

Variable	Serum glucose						
	Green tea extract concentration (mg/kg body weight)						
	10	25	50	75	100	150	200
Before treatment	85±2.24						
After treatment	85±2.24	83±1.73	78±1.00	77.5±0.58	69±0.29	67±0.29	66±0.7
Lowering effect	No difference	-2	-7	-7.5	-16	-18	-19

subcutaneously. Before induction of IDDM, the rats were starved for 24 h.

Alloxan treated animals were allowed to drink 5% of D-Glucose (Merck KGG, A Darmstadt Germany) overnight to prevent the potentially fatal hypoglycemia occurring as a result of massive insulin release following alloxan injection (Wohaieb and Godin 1987). Diabetes mellitus was confirmed in induced rats by testing for glucosuria using glucose indicator sticks (Exi-check Roche Diagnostics GmbH Mannheim, Germany). Symptoms of diabetes were observed within a week of single alloxan injection. After one month of alloxan injection, animals with blood glucose level ≥ 200 mg/dl were considered diabetic and included in the study.

The experimental doses of the green tea extract doses used in this experiment were determined by oral administrations of group of three normal male rats with different concentrations of the plant extract (10, 25, 50, 75, 100, 150, 200 mg/kg body weight) in 1 ml of normal saline. After 2 h of the administration, the levels of serum glucose were measured, the most effective lowering doses were selected as doses of the experiments.

The normal and diabetic animals were distributed into five groups, with each group consisting of 6 rats. The rats of the 1st and 2nd groups received tap water and normal food, rats of third group received 6 units insulin per rat per day, 2 units every eight hours. The soluble short action insulin was injected intraperitoneally by using insulin syringe. Rats of the fourth group received 50 mg/kg BW of green tea extract while rats of the fifth group received 100 mg/kg B.W. of green tea extract.

The extract was administered once per day using gavage at 7.00 p.m for twenty-eight days. After 28 days of the treatment, blood samples were taken for serum, and the tissue samples from pancreas, liver, and kidney were taken and preserved in 10% formalin.

After starving for 24 h, all animals were anaesthetized by intraperitoneal Ketamine injection in the dose of 100 mg/kg of body weight (Keane et al., 1999). Blood samples were withdrawn by puncturing the heart and the serum was separated at once by a long Pasteur pipette and was stored at -4°C for further analysis.

Biochemical analysis

Reflotron Plus automatic analyzer was used to determine

the biochemical parameters. 32 μL of serum was applied to the red mesh of the reagent strip and was analyzed by using automatic analyzer (Reflotron Plus, Roche, Germany).

For histological processing, the animals were sacrificed; liver, kidney, and pancreas were aseptically retrieved, washed in saline and immediately fixed in 10% formalin. Then the tissues were dehydrated in ethanol, cleared in xylene and embedded in paraffin wax. Tissue paraffin blocks of pancreas, liver and kidney were cut at (4) microns thickness by rotary microtome (Leitz 1512, Germany 46194). Tissue sections were stained by routine Hematoxylin and Eosin and examined by specialist histopathologist. The study was conducted by permission of the Faculty of Science and Health, Koya University.

Statistical analysis

Complete Randomized Design (CRD) was employed to compare between measurements. Statistical analysis was done by using computer software: Statistical Package for Social Sciences (SPSS) version 16.

RESULTS

The results of the experiments concerned with the dose are shown in Table 1. As shown in this table, the more effective dose for lowering glucose was 50 and 100 mg/kg.

Improvement role of green tea extracts in some diabetes related biochemical parameters

Induction of DM by alloxan caused significant increase in the levels of serum glucose, cholesterol and triglycerides respectively as compared to normal non-diabetic rats respectively. After 28 days of treatment with green tea extracts (50, 100 mg/kg) by oral administrations daily caused significant ($P<0.05$) lowering in the levels of serum glucose, cholesterol and triglycerides respectively as compared to their levels in diabetic non-treated rats respectively (Table 2). In GTE100 treated group, the higher dose of green tea extract (100 mg/kg B.W) produced more effective results, in comparison with the insulin and GTE50 treated groups, and lowered the levels of glucose, cholesterol, triglyceride significantly ($P<0.05$) to normal level.

Table 2. Effect of green tea extracts (50, 100 mg/kg) on biochemical parameters (glucose, cholesterol, and triglycerides) in normal and diabetic male rats.

Parameter	Glucose mg/dl	Cholesterol mg/dl	Triglyceride mg/dl
Animal groups			
Normal control	85.00±2.441 ^{ab}	83.20±0.970 ^a	118.98±8.378 ^a
Diabetic control	482.8±12.68 ^c	130.75±4.0286 ^b	243.3±30.45 ^b
Alloxan diabetic rats+insulin	105.25±8.004 ^b	83.50±1.322 ^a	180.5±17.63 ^{ab}
ALX diabetic rats+GTE50	94.50±2.5000 ^{ab}	90.00±1.000 ^a	145.5±1.500 ^{ab}
Alloxan diabetic rats+ GTE100	77.33±1.452 ^a	82.67±1.201 ^a	122.3±4.631 ^{ab}

The different letters mean significant difference (P<0.05).

Table 3. Effect of green tea extracts (50, 100 mg/kg) and insulin on serum liver function test parameters (APL, GOT, and GPT) in normal and diabetic male rats.

Parameter	ALP U/L	GOT U/L	GPT U/L
Animal groups			
Normal control	42.00±1.483 ^a	34.80±1.715 ^a	23.80±1.356 ^a
Diabetic control	107.3±2.428 ^c	83.75±1.974 ^d	78.00±1.581 ^d
Alloxan diabetic rats+insulin	40.13±2.240 ^a	52.75±1.797 ^c	43.03±1.703 ^c
Alloxan diabetic rats+ GTE50	49.50±1.500 ^b	51.00±1.000 ^c	35.00±3.000 ^b
Alloxan diabetic rats+ GTE100	38.00±0.577 ^a	42.00±1.000 ^b	23.67±1.453 ^a

The different letters mean significant difference (P<0.05).

Table 4. Effect of green tea extracts (50, 100 mg/kg) on renal function test parameters (creatinine, uric acid) in normal and diabetic male rats.

Parameter	Creatinine mg/dl	Uric acid mg/dl	Urea mg/dl
Animal groups			
Normal control	0.430±0.018 ^a	2.378±0.188 ^a	22.2000±1.828 ^a
Diabetic control	0.678±0.013 ^c	4.115±0.187 ^b	27.7500±0.854 ^b
Alloxan diabetic rats+insulin	0.505±0.023 ^b	2.700±0.000 ^a	24.7500±1.548 ^{ab}
Alloxan diabetic rats+ GTE50	0.540±0.040 ^b	2.700±0.000 ^a	24.5000±0.500 ^{ab}
Alloxan diabetic rats+GTE100	0.427±0.176 ^a	2.647±0.032 ^a	23.0000±0.577 ^{ab}

Role of green tea extract in improvement of diabetes related liver disorders

Alloxan induced diabetes mellitus in rats showed significant increase (P<0.05) in the levels of serum ALP, GOT, GPT compared with their concentrations in normal control rats. Four weeks treatment of alloxan induced DM rats with insulin and 50 mg/kg of green tea extract, caused lowering in the levels of studied parameters significantly (P<0.05). Treatment with 100 mg/kg of green tea extract caused reduction in the levels of ALP, GOT and GPT to normal levels (Table 3).

Improvement role of green tea extract in diabetes related renal disorders

DM induced by alloxan caused significant increases (P<0.05) in the levels of serum urea, creatinine, and uric

acid in diabetic rats fed with normal diet. Four weeks of treatment by 50 mg/kg and 100 mg/kg of green tea extract caused significant lowering in the levels of creatinine, uric acid and non significant change in level of urea in the treated rats, as compared with the levels in non-treated rats respectively (Table 4).

Effect of green tea extracts on histological parameters

Pancreas

The rat normal islets of Langerhans appeared as masses of cells with round uniform nuclei having an amphophilic cytoplasm surrounded by exocrine portion with glandular configuration lined by single layer of polygonal cells with basally located nuclei and deep acidophilic cytoplasm as shown in Plate 1. Pancreas of Alloxan induced diabetic



Plate 1. Section from normal rat pancreas showing normal islet of Langerhans surrounded by exocrine pancreas (Stain: Haematoxylin-Eosin.400X).

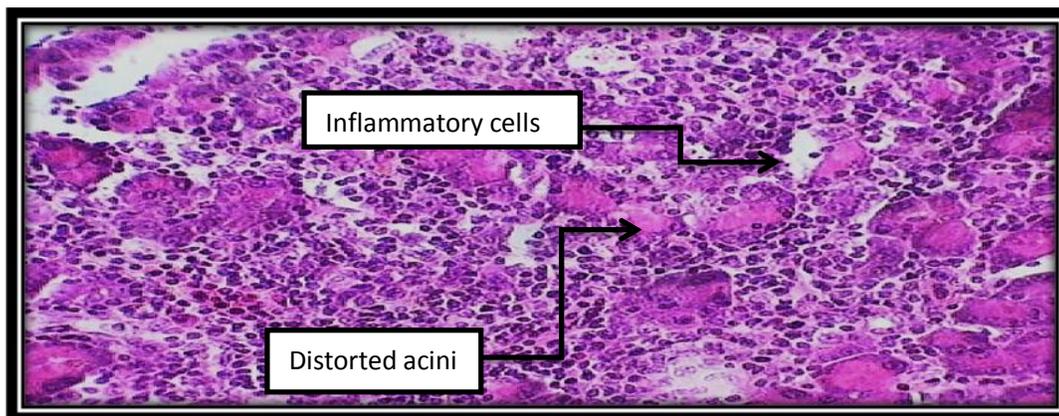


Plate 2. Section from diabetic control pancreas showing distorted exocrine pancreas with marked chronic inflammatory cell infiltration (Hematoxylin and eosin. 400X).

rat showed distortion of exocrine pancreas and inflammatory cell infiltration (Plate 2). Pancreas of insulin treated rat shows relatively normal islet of Langerhans (Plate 3). GTE100 treated rats showed marked reduction in islets vascular congestion and the severity of inflammation infiltration, despite the fact that the cellularity of islets of Langerhans was still reduced (Plate 4).

Kidney

Normal rat kidney showed round regular glomeruli with normal cellularity surrounded by regular tubules lined by cuboidal epithelium having centrally located nuclei and eosinophilic cytoplasm as shown in Plate 5. Kidneys of Alloxan induced diabetic rats showed histological features such as increase in glomerular mesangial cellularity, deposition of proteinaceous material,

glomerular capillary dilation, tubular dilation and interstitial hemorrhages (Plates 6 and 7).

Insulin therapy relatively protected the glomeruli, despite tubular dilation and interstitial hemorrhage (Plates 8 and 9). GTE elicited a protective effect to the kidney despite some tubular dilation and necrotic changes (Plates 10 and 11) and higher dose of GTE (GTE100) produced a better renal protective effect for both glomeruli and tubules (Plates 12 and 13).

Liver

Plate 14 shows normal section of liver hepatocytes and its matrix. Diabetes induced a subtle histological effect in our experiment manifested as mild disruption of hepatocytes plate arrangement, focal hepatocytic necrosis, mild focal interstitial and perivascular inflammatory cell infiltration (Plates 15 and 16). Insulin

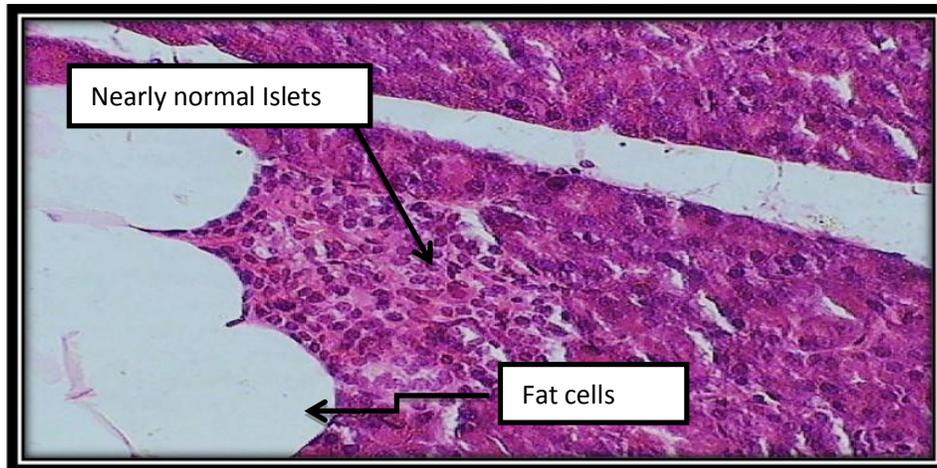


Plate 3. Section from insulin treated pancreas showing relatively normal islet of Langerhans, and mild distortion of exocrine pancreas and focal fatty changes (Hematoxylin and eosin. 400X).

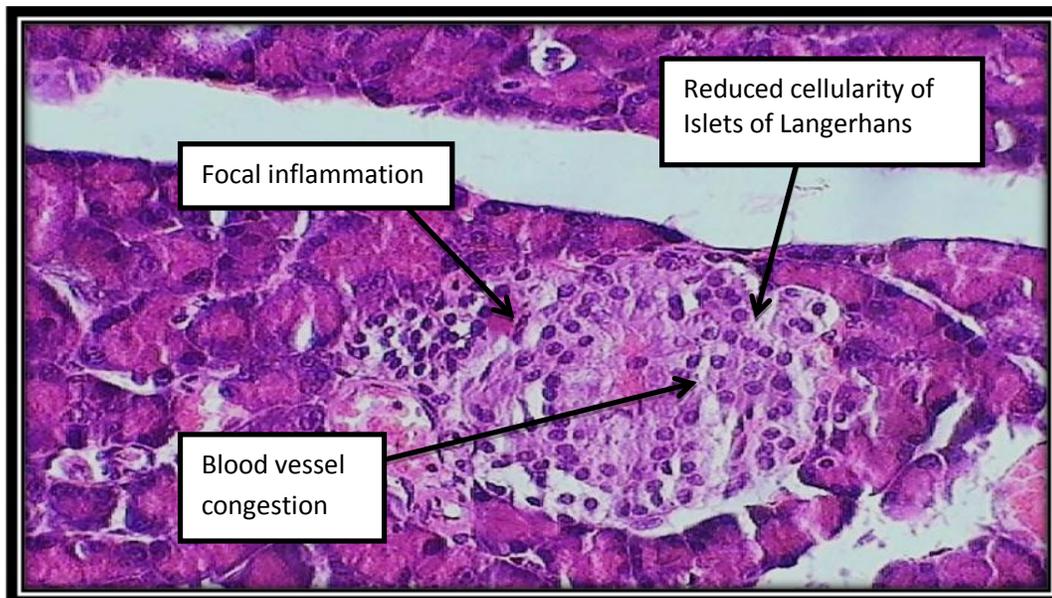


Plate 4. Section from GTE100 treated pancreas showing reduction in the cellularity of Islets of Langerhans, vascular congestion and focal inflammation (Stain: haematoxylin-eosin.400X).

relatively preserves hepatic arrangement (Plate 17), but GTE showed a normal hepatocyte arrangement despite mild vascular congestion (Plates 18 and 19).

DISCUSSION

The present study has demonstrated that alloxan induced diabetes mellitus rats showed a significant increase in the levels of serum glucose, triglycerides, cholesterol (Table 2). These findings are in agreement with previous

experimental diabetes studies. Kasetti et al. (2010) reported that streptozotocin which is a diabetogenic substance produces insulin dependent diabetes mellitus (IDDM) which causes significant increase in the serum levels of glucoside, triglycerides and cholesterol in male rats. Also, they demonstrated significant reduction in the levels of serum insulin in these diabetic rats.

The present study indicates that GTE has hypoglycemic, hypolipidemic, hyperproteinemic effects (Table 2). In a study by Al-kakey et al. (2009), they

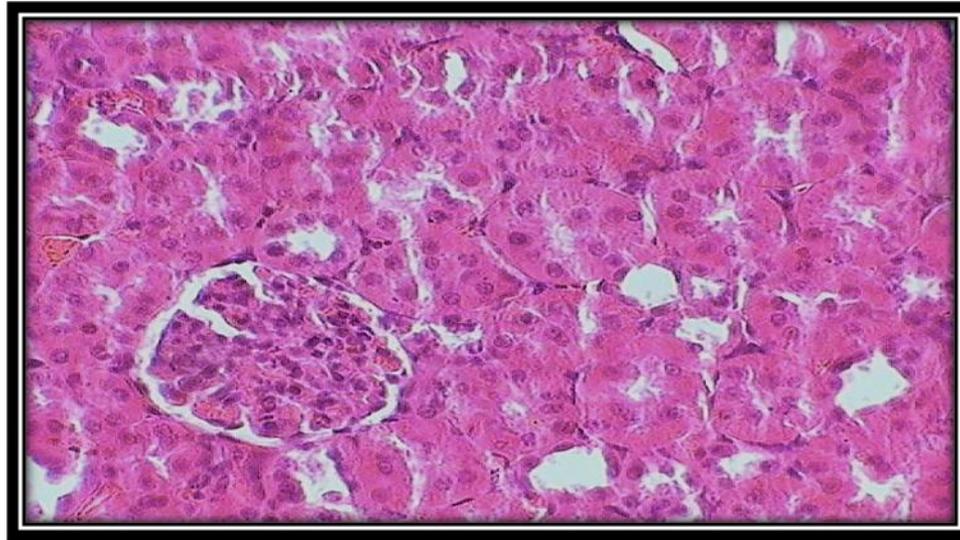


Plate 5. Section from normal rat kidney showing glomerulus and tubules (Hematoxylin and eosin. 400X).

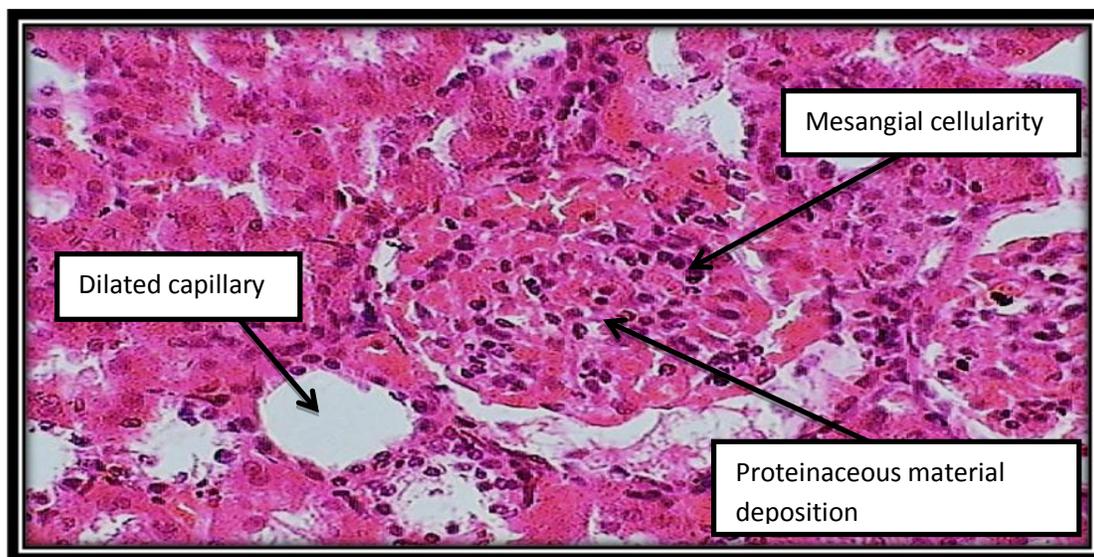


Plate 6. Section from diabetic rat kidney showing increased glomerular mesangial cellularity, deposition of proteinaceous material and capillary dilatation. The interstitium shows a dilated renal tubule and focal necrotic changes (Hematoxylin and eosin. 400X).

demonstrated that green tea extract treatments of alloxan induced diabetic rats for three weeks reduces serum glucose and cholesterol significantly. Anandh et al. (2006) showed that administration of green tea extract to diabetic rats resulted in significant decrease in cholesterol, triglycerides, free fatty acids levels, and lipoprotein lipase activity in the myocardium of diabetic rat. Further they reported that these beneficial effects of green tea extract are ascribed to its anti-hyperglycemic and hypolipidemic activity. The hypoglycemic role of GTE

may be related to its antioxidant effects. The traditional tea infusion is characterized by a high content of flavonoids. Flavonoids are a large group of phenolic products of plant metabolism with a variety of phenolic structures that have unique biological properties and may be responsible for many of the health benefits attributed to tea (Pushparaj et al., 2000).

Alloxan induces highly significant increases in the levels of serum GPT, GOT, and ALP (Table 4). The present study indicates that GTE provoked protective

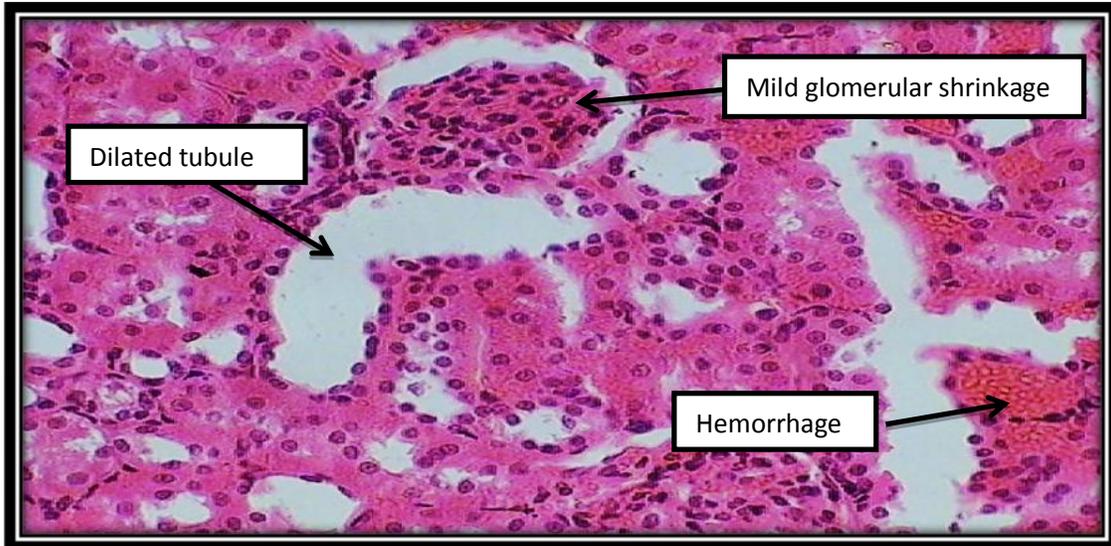


Plate 7. Section from diabetic rat kidney showing mild glomerular shrinkage, dilated tubules and interstitial hemorrhage (Hematoxylin and eosin. 400X).

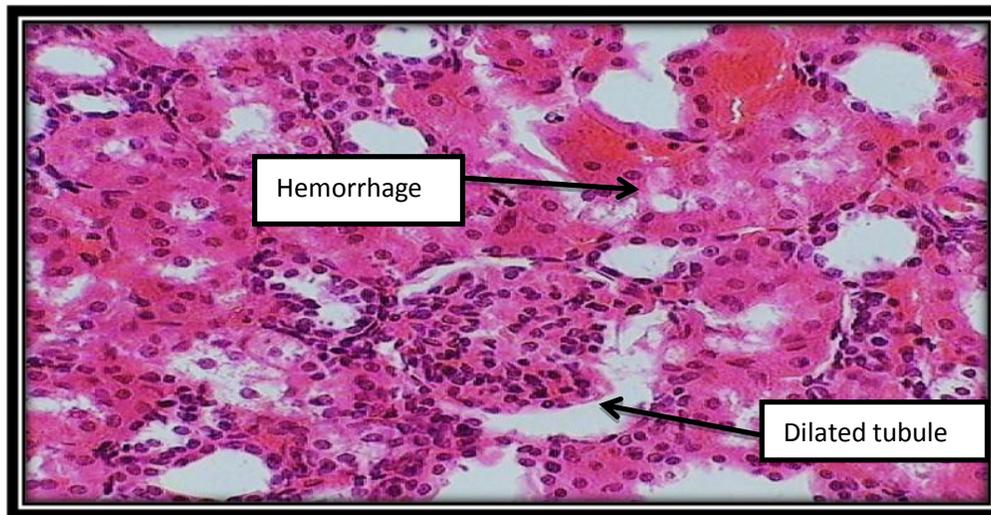


Plate 8. Section from insulin treated rat kidney showing tubular dilation and interstitial hemorrhage (Hematoxylin and Eosin. 400X).

effect to liver and attenuation of liver serum enzymes activities (GPT, GOT and ALP) (Table 3).

Tea is an important source of flavonoids in the diet and the flavonoids found in tea are known to be strong antioxidants (Rietveld and Wiseman, 2003). Cai et al. (2002) studied the antioxidative effects of the principal polyphenolic components extracted from green tea leaves and they showed that the kinetic analysis of the antioxidation process demonstrates that these green tea polyphenols are good antioxidants for liver.

The present study has demonstrated that alloxan

induced diabetes mellitus rats showed significant increases in the levels of serum creatinine, urea, uric acid (Table 4); these results are in agreement with previous results of experimental diabetes studies (Sellamuthu et al., 2009; Salahuddin et al., 2010).

The present study indicates protective effect of GTE on kidney as indicated by reduced levels of serum creatinine and uric acid (Table 4). A study by Sabu et al. (2002) showed that green tea polyphenol administration to normal rats caused significant reduction in liver function test enzymes and renal function test.

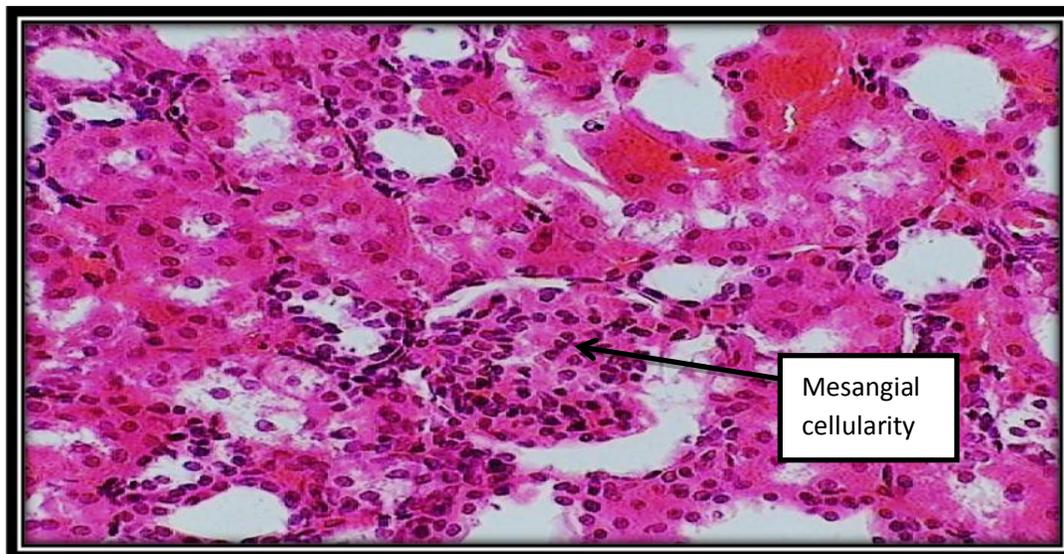


Plate 9. Section from insulin treated rat kidney showing increased mesangial cellularity and interstitial hemorrhage (Hematoxylin and Eosin.400X).

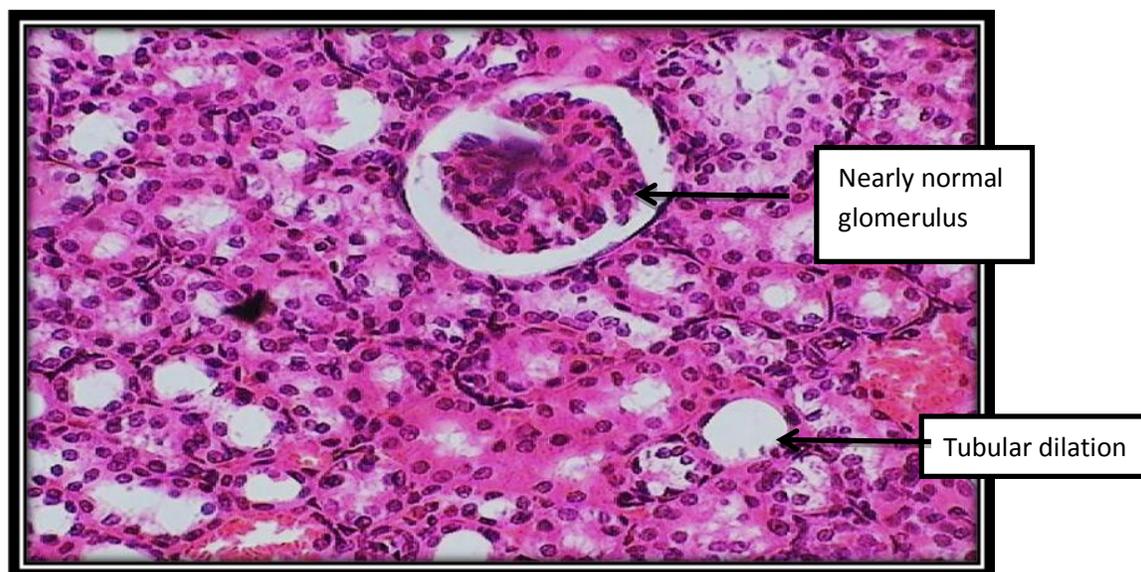


Plate 10. Section from GTE50 rat kidney, nearly normal looking glomerulus, despite mild tubular dilatation (Stain: haematoxylin-eosin.400X).

Treatments of Alloxan induced diabetes mellitus with 100 mg/kg BW of GTE showed a protective effect in the Pancreas histology (Plate 4). In this plate, the section of pancreas showed reduced cellularity and vascular congestion, also the focal inflammation with normal appearance of β -cells.

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.

Studies reported that epigallocatechin gallate which promotes pancreatic β -cells regeneration in alloxan-treated rats, has insulin-like and insulinotropic activities, and inhibits gluconeogenesis through inhibition of liver phosphoenolpyruvate kinase synthesis (Walter-Law et al., 2002; Chemler et al., 2007; Islam and Choi, 2007).

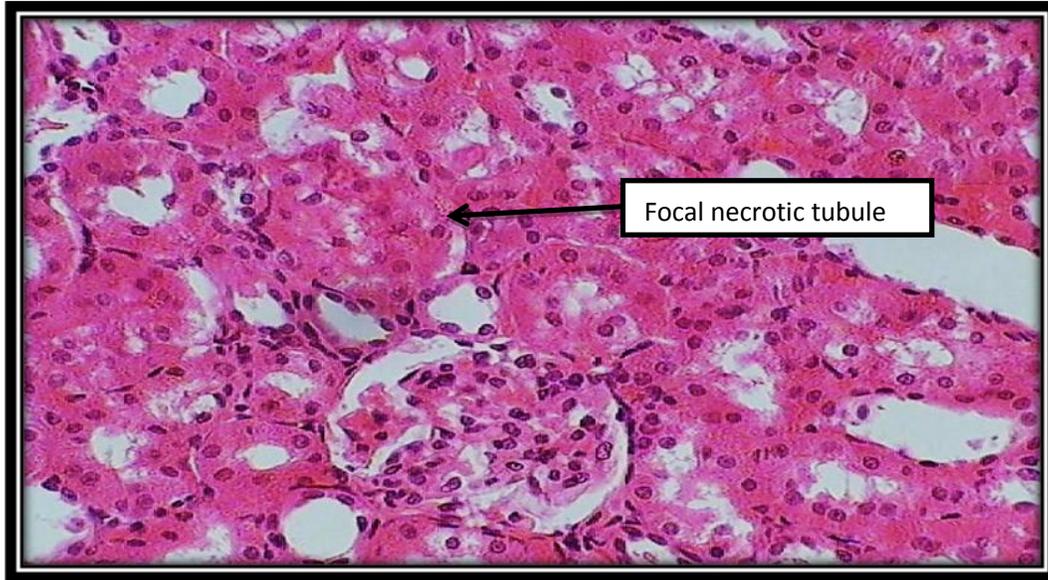


Plate 11. Section from GTE50 rat kidney showing normal looking glomerulus and some tubules show dilatation and focal necrotic changes (Stain: haematoxylin-eosin.400X).

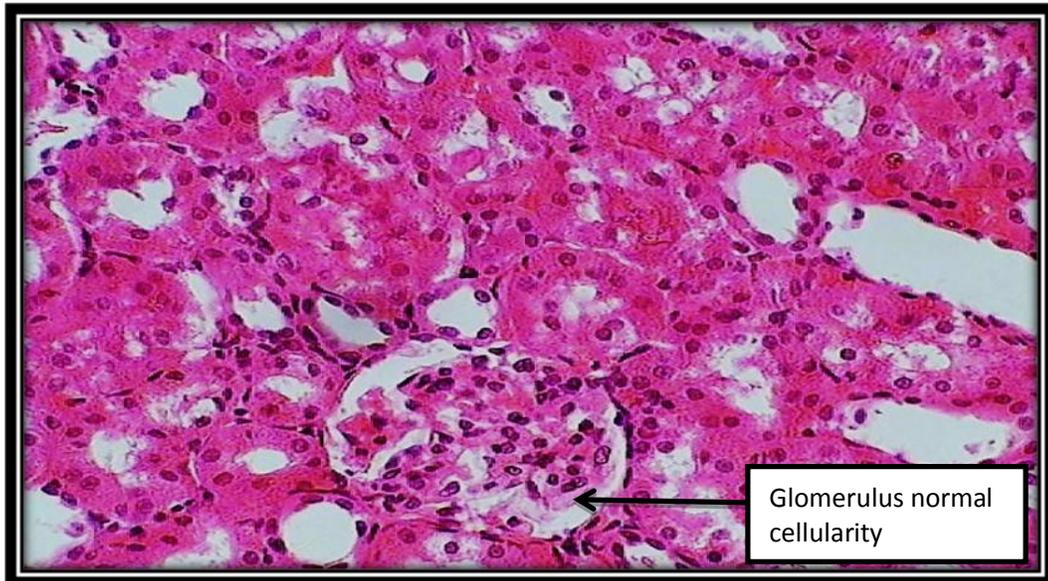


Plate 12. Section from GTE100 treated rat kidney, glomerulus showing normal cellularity despite some tubular dilatation (Stain: haematoxylin-eosin.400X).

A study by Karaca et al. (2010) showed that green tea extract significantly increases levels of serum insulin in the diabetic rats. Green tea extract treatment protected the cells in the islets of Langerhans. Necrotic degeneration was observed in the peripheral parts of the islets of Langerhans in diabetic rats.

Coksun et al. (2005) and Cemek et al. (2008) reported that the numbers of insulin-immunoreactive β -cells in the

islets of Langerhans were increased by treatment with *Matricaria chamomilla* and *Nigella sativa*, respectively.

Hyperglycemia is the principle factor responsible for structural alterations at the renal level, and 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

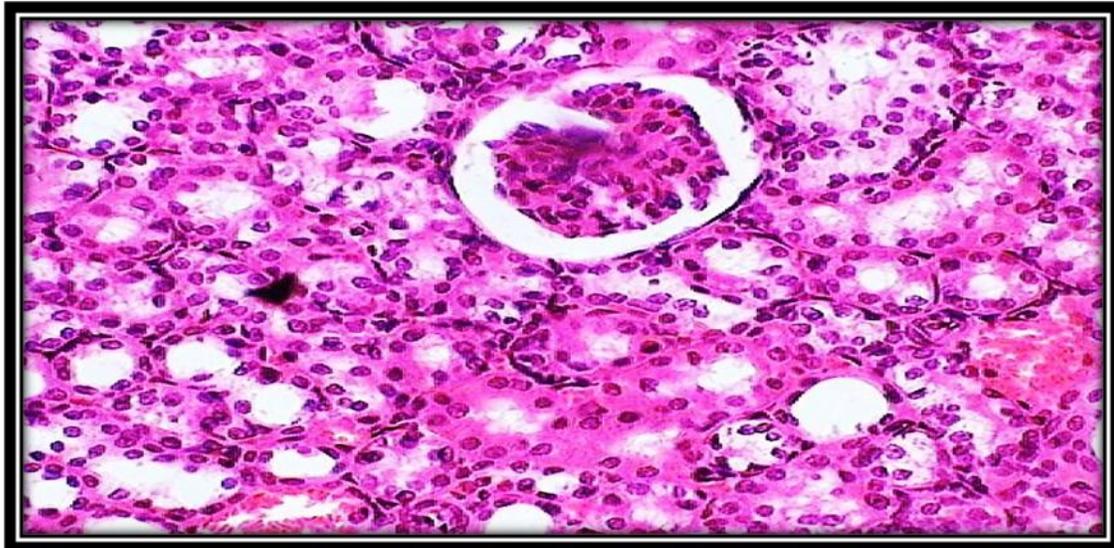


Plate 13. Section from GTE100 treated rat kidney showing normal looking glomerulus and tubules (Stain: haematoxylin-eosin.400X).

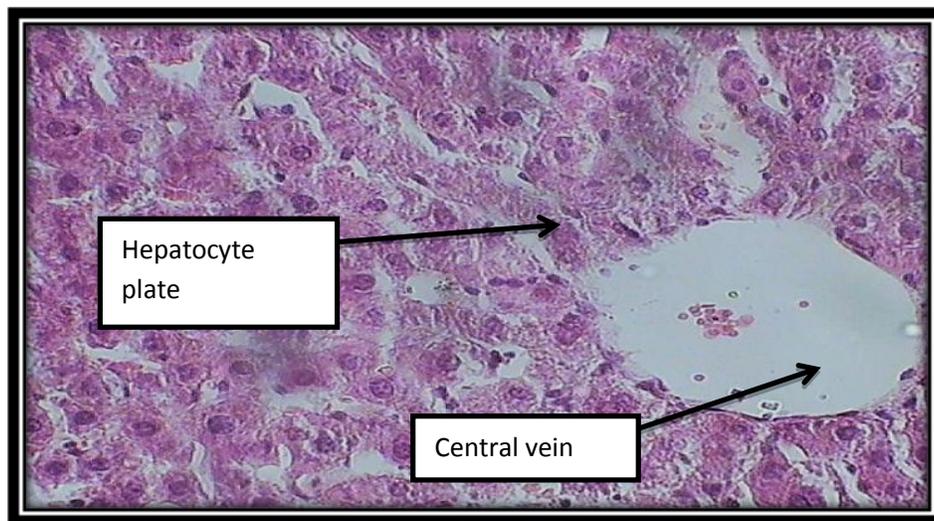


Plate 14. Section from normal rat liver (Stain: haematoxylin-eosin.400X).

target of therapy. In this study, the glucose level of diabetic rats showed approximately a 5-fold significant increase; however green tea extracts reduced this increase (Table 4). In support of the present results, recent reports have shown that epigallocatechin 3-O-gallate (EGCG) reduces the level of mRNA for gluconeogenesis enzymes (Koyama et al., 2004) and causes many similar effects to insulin, including expression of glucose production phosphoenolpyruvate and glucose-6-phosphatase gene expression in cells (Waltner-Law et al., 2002). Likewise, Yokozawa et al. (2005) have recently shown that green tea polyphenols

(GTP) and partially hydrolyzed guar gum (PHGG) decreases blood glucose levels and attenuates the urinary protein excretion and morphological changes characteristic of diabetic nephropathy when renal dysfunction was already evident. In addition increase in lipids, for example, cholesterol and triglyceride, whose abnormal metabolism has been proven to play a role in the pathogenesis of diabetic nephropathy (Sun et al., 2002) and to enhance lipid peroxidation were all improved by administration of green tea extracts. Thus green tea extracts showed a positive effect on serum glucose and lipid metabolic abnormalities.

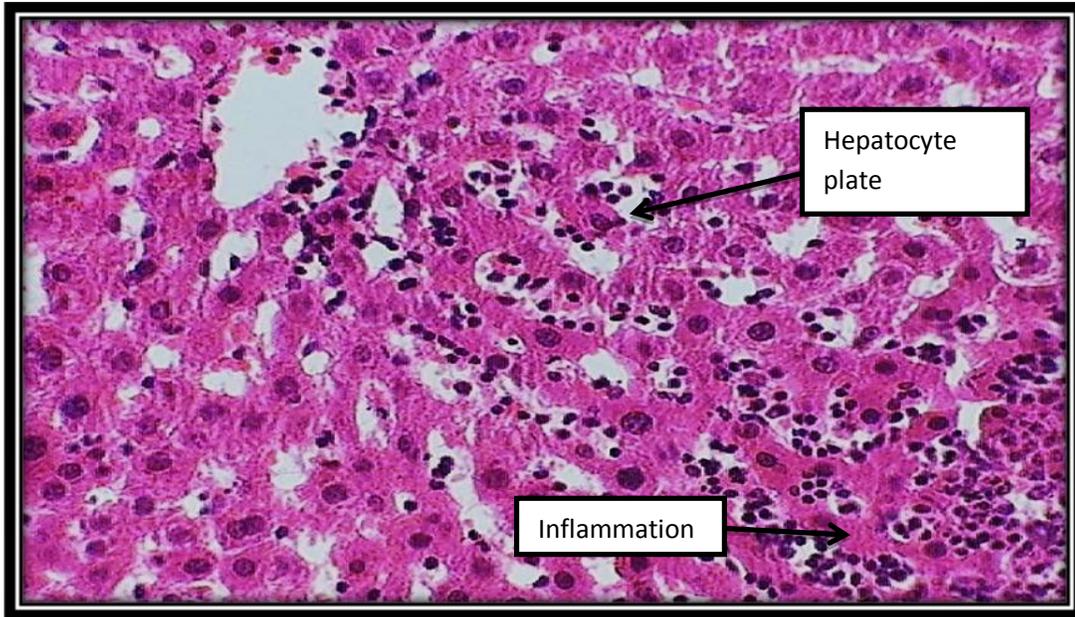


Plate 15. Section from diabetic control liver showing mild disruption of hepatocytes plates' arrangement and focal inflammation (Stain: haematoxylin-eosin.400X).

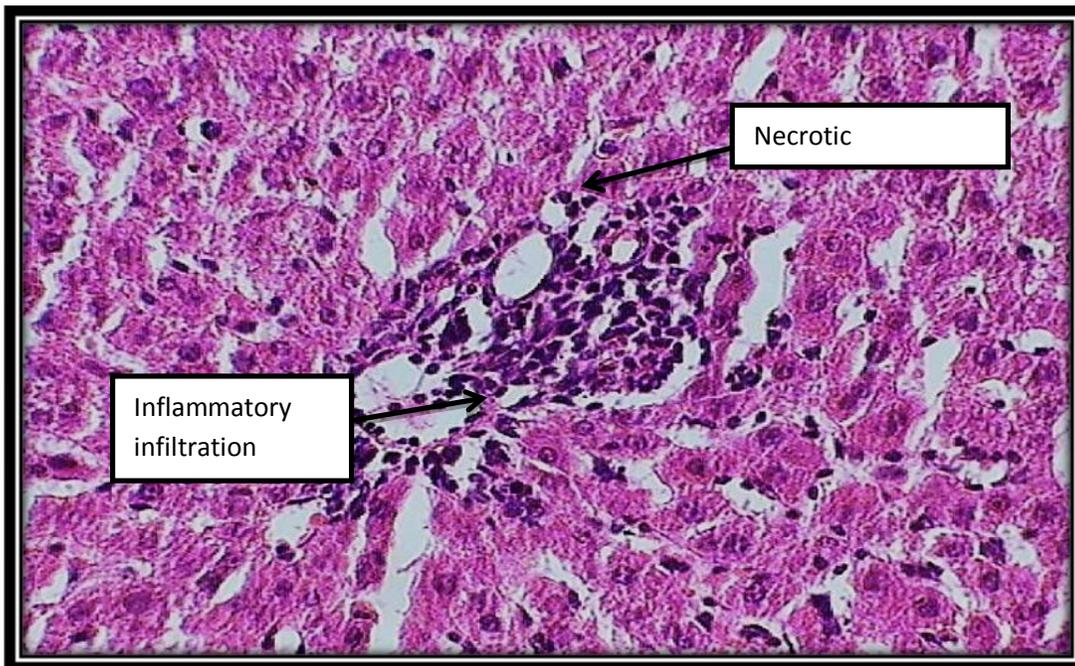


Plate 16. Section from diabetic control liver, necrotic hepatocytes, portal lymphocytic infiltration (Stain: haematoxylin-eosin.400X).

In the present study, group II (diabetic control) rats showed histological changes of diabetic nephropathy like mesangial hypertrophy, mesangial extracellular matrix deposition, increased glomerular basement membrane

thickening, capillary dilation and dilated renal tubules (Plate 4). Similar structural changes in kidney have been reported by Vlassara et al. (1994), and they speculated that these structural changes may be directly influenced

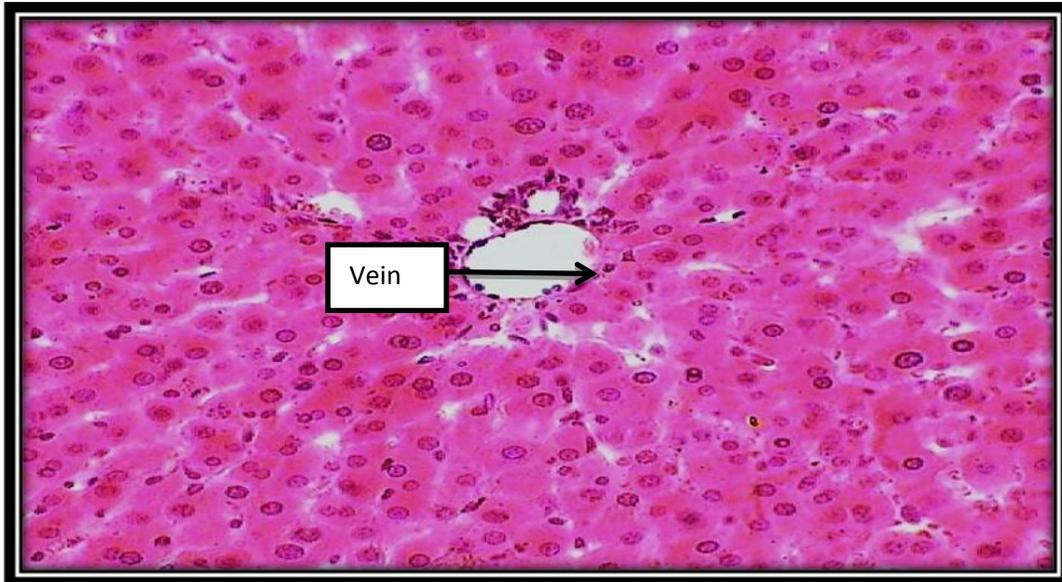


Plate 17. Section from insulin treated rat liver showing portal tract and focal hepatocyte degeneration (Stain: haematoxylin-eosin.400X).

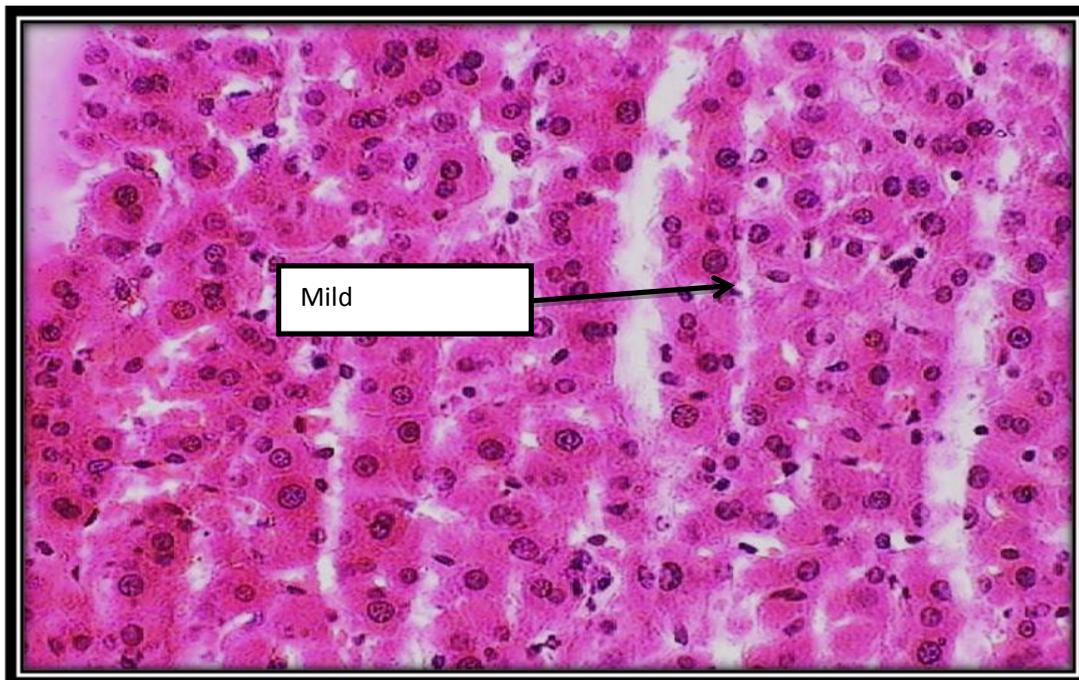


Plate 18. Section from GTE50 treated rat liver: hepatocytes showing mild derangement of architecture and foci of necrotic hepatocytes (Stain: haematoxylin-eosin.400X).

by advanced glycation end-products (AGEs) through excessive cross-linking of matrix molecules in a receptor independent way. Moreover, another pathway exists in the action of AGEs, that is, receptor-dependent processes, whereby AGEs bind to their cognate cell

surface receptor RAGE, resulting in the activation of post receptor signaling, generation of intracellular oxygen free radicals, and activation of gene expression. Particularly, the RAGE promoter contains NF- κ B binding sites, through which AGEs are involved in the activation of

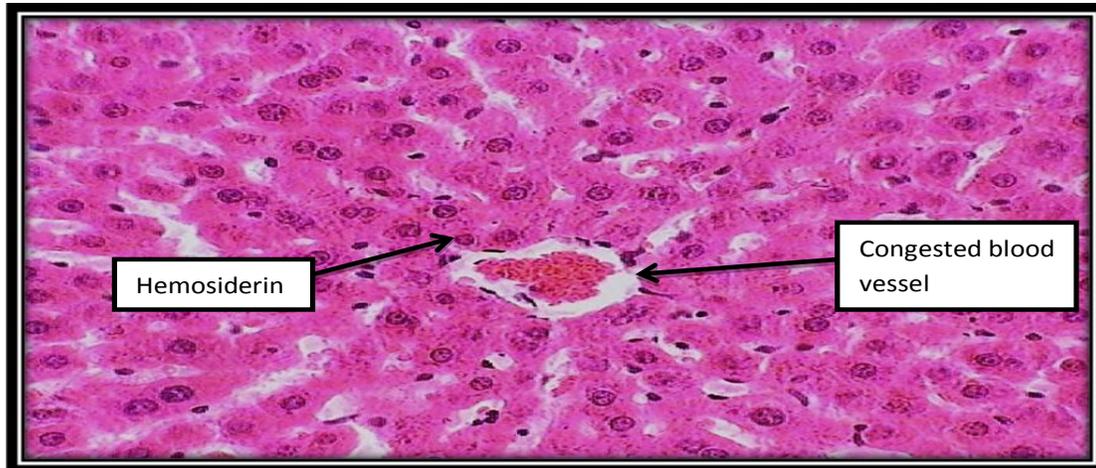


Plate 19. Section from GTE100 treated rat liver showing nearly normal hepatocyte plates and mild vascular congestion (Stain: haematoxylin-eosin.40X).

reactive oxygen species and NF- κ B via AGE-RAGE interaction (Wang et al., 1999), and in turn, up-regulation of RAGE ensures that sustained NF- κ B activation is not only maintained but also amplified (Bierhaus et al., 2001). Furthermore, AGE-RAGE and signaling pathways including NF- κ B and mitogen-activated protein kinase modulate the activation of TGF- β with subsequent effects in inducing the accumulation of matrix in synergistic ways, that is, TGF- β causes renal cell hypertrophy and promotes the production of extracellular matrix (ECM) molecules including type I and IV collagen, fibronectin, and laminin while inhibiting their decomposition, and it induces the expression of receptors for the matrix protein integrin, resulting in renal sclerosis and fibrosis (Fukami et al., 2004).

Histological examination revealed that the liver of diabetic control male rats exhibited mild disruption of hepatocytes, focal inflammation, necrotic hepatocytes, and perivascular lymphocytic infiltration (Plates 15 and 16). 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 (Plates 18 and 19).

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, restores the activity of antioxidant enzymes, and reduces the generation of free radicals and lipid peroxidation (Das et al., 2005). Moreover, black tea aflavins have the ability to reduce hepatic lipid accumulation both *in vitro* and *in vivo* (Lin et al., 2007). The release of liver alanine aminotransferase (GPT), aspartate aminotransferase (GOT) and alkaline phosphatase (ALP) was inhibited in rats 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. On the other hand, it has been found that green tea epigallocatechin gallate inhibits hepatic steatosis, obesity, the metabolic syndrome, and the release of liver enzymes induced by a high-fat diet in mice (Bose et al., 2008). Other studies reported that green tea epigallocatechin gallate inhibited lipid absorption through its ability to form complexes with lipids and lipolytic enzymes and hence interfering with the luminal processes of emulsification, hydrolysis, and subsequent uptake of lipids (Koo and Noh, 2007). Green tea epicatechins and epigallocatechin gallate have been found to modulate the increase in LDL-cholesterol, probably by decreasing the apoB (the principal protein that comprises nearly 90% of total protein mass of LDL), and the decrease in HDL-cholesterol in experimental animals fed a high-fat or high-cholesterol diet (Yee et al., 2002). Flavonols are less affected by tea processing and are present in comparable amounts in both green and black teas (Keith et al., 1997). The aflavins present in black tea possess the same antioxidant potency as catechins present in green tea, and the conversion of catechins to the aflavins during fermentation in making black tea does not alter significantly their free radical scavenging activity (Leung et al., 2001). Besides, EGCG prevents the ethanol induced hepatotoxicity and inhibits the development of the fatty liver and renal failure (Yokozawa et al., 1999; Yun et al., 2007). Many researchers reported that GTE reduces cholesterol synthesis in liver (Bursill et al., 2007) and decreases the severity of liver injury in association with lower concentration of lipid peroxidation (Chen et al., 2004).

Conclusion

The results of the present study indicated that green tea leaves' extracts have a significant lowering effect of glucose, lipids, creatinine, uric acid, ALP, GOT and GPT, in alloxan induced diabetic rats. Also, histological studies showed that liver, kidney and pancreas damage associated with alloxan-induced diabetes in rats were alleviated.

REFERENCES

- Anandh BPV, Sabitha KE, Shyamaladevi CS (2006). Green tea extract impedes dyslipidaemia and development of cardiac dysfunction in streptozotocin-diabetic rats. *Clin. Exp. Pharmacol. Physiol.*, 33, 1184–1189.
- Al-kakey IS, Al-Hassani O, Al-kahef A (2009). Green tea *Camellia sinensis* extract have reversed oxidative stress in alloxan induced diabetes mellitus in rats. The 3rd biological conference, Mousl University.
- Cai YJ, Ma LP, Hou LF, Zhou B, Yang L, Liu ZL (2002). Antioxidant effects of green tea polyphenols on free radical initiated peroxidation of rat liver microsomes. *Chem. Phys. Lipids*. 120: 109–117.
- Erbey JR, Silberman C, Lydick E (2000). Prevalence of abnormal serum alanine aminotransferase levels in obese patients and patients with type 2 diabetes. *Am. J. Med.*, 109: 588–590.
- Everhart JE (1995). *Diabetes in America*. 2nd ed. National Institute of Health. National Institute of Diabetes and Digestive and Kidney Diseases. Washington, DC: GPO: 457-483.
- Gupte P, Amarapurkar D, Agal S, Bajjal R, Kulshrestha P, Pramanik S (2004). Non-alcoholic steatohepatitis in type 2 diabetes mellitus. *J. Gastroenterol. Hepatol.*, 19: 854-858.
- Held PJ, Port FK, Webb RL, Wolfe RA, Garcia JR, Blagg CR, Agodoa LY (1991). The United States Renal Data System's 1991 annual data report: an introduction. *Am. J. Kidney Dis.*, 18, 1–16.
- Kaneto, H., Xu, G., Song, K.H., Suzuma, K., Bonner-Weir, S., Sharma, A., Weir, G.C., (2001). Activation of the hexosamine pathway leads to deterioration of pancreatic b-cell function through the induction of oxidative stress. *J. Biol. Chem.*, 276, 31099–31104.
- Kasetti RB, Rajasekhar MD, Kondeti VK, Fatima SS, Kumar EG, Swapna S, Ramesh B, Rao CA (2010). Antihyperglycemic and antihyperlipidemic activities of methanol:water (4:1) fraction isolated from aqueous extract of *Syzygium alternifolium* seeds in streptozotocin induced diabetic rats. *Food Chem. Toxicol.*, 48, 1078–1084.
- Lynedjian PB, Pilot PR, Nouspikel T, Milburn JL, Quaade C, Hughes S, Ucla C, Newgard CB (1989). *Proc. Natl. Acad. Sci. U.S.A.*, 86, 7838-7842.
- Mahdi A.A, Chandra A, Singh RK, Shukla S, Mishra LC. (2003). Effect of herbal hypoglycemic agents on oxidative stress and antioxidant status in diabetic rats. *Indian J. Clin. Biochem.*, 18:8.
- Nair KS, Ford GC, Ekberg K, Fernqvist-Forbes E, Wahren J (1995). Protein dynamics in whole body and in splanchnic and leg tissues in type I diabetic patients. *J. Clin. Invest.*, 95:2926-2937,
- Pu shparaj P, Tan CH, Tan BKH (2000). Effects of Averrhoa bilimbi leaf extract on blood glucose and lipids in streptozotocin diabetic rats. *J. Ethnopharmacol.* 72, 69–76.
- Rhee SJ, Choi JH, Park MR (2002). Green tea catechin improves microsomal phospholipase A2 activity and the arachidonic acid cascade system in the kidney of diabetic rats. *Asia Pac J Clin Nutr.* 11: 226–231.
- Rietveld A, Wiseman S (2003). Antioxidant effects of tea: evidence from human clinical trials. *J. Nutr.* 133: 3285S–3292S.
- Sabu MC, Smitha K, Kuttan R (2002). Anti-diabetic activity of green tea polyphenols and their role in reducing oxidative stress in experimental diabetes. *J. Ethnopharmacol.* 83:109-116.
- Salahuddin M, Jalalpure SS, Gadge NB (2010). Antidiabetic activity of aqueous bark extract of *Cassia glauca* in streptozotocin induced diabetic rats. *Can. J. Physiol. Pharmacol.*, 88:153–160.
- Sawiress FAR (2011). Effect of Ginseng Extract Supplementation on Renal Functions in Diabetic Rats. *J. Agric. Sci.*, 3(2): 17-31.
- Sellamuthu PS, Muniappan BP, Perumal SM, Kandasamy M (2009). Antihyperglycemic effect of mangiferin in streptozotocin induced diabetic rats. *J. Health Sci.*, 55, 206–214.
- Wannamethee SG, Shaper AG, Lennon L, Whincup PH (2005). Hepatic enzymes, the metabolic syndrome, and the risk of type 2 diabetes in older men. *Diabetes Care*, 28: 2913-2918.
- Wohaieb SA, Godin DV (1987). Alteration in free radical tissue defense mechanisms in streptozotocin-induced diabetes in the rat: effect of insulin treatment. *Diabetes*. 36: 1014-1018 (Abstract).
- Wolf G, Chen S & Ziyadeh FN. (2005). From the periphery of the glomerular capillary wall toward the center of disease: podocyte injury comes of age in diabetic nephropathy. *Diabetes*. 54:1626-1634.
- Yokozawa T, Nakagawa T, Lee KI, Cho EJ, Terasawa K, Takeuchi S (1999). Effects of green tea tannin on cisplatin-induced nephropathy in LLC-PK1 cells and rats. *J. Pharm. Pharmacol.*, 51:1325-1331.
- Zhang-Mu, M., Sakai Y., Ose Y., Nagase H., Kito H., Sato M., Mizuno M., Ono K., and Nakane H., (1990). Antimutagenic activity by the medicinal plants traditional chines medicines shoyakuhak zasshi, 44: 225-229.