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Efficacy of Ellagic Acid on Biochemical Parameters and Histopathological Description in Streptozotocin Induced Diabetic Rats

Ganesan Kanchana*[1], Palanisamy Malini[1], Wilson Jerine Shyni[1]

1. Muthayammal College of Arts & Science, Rasipuram - 637408, Tamil Nadu, India.

ABSTRACT

The present study was aimed to evaluate the effect of ellagic acid on physical and biochemical parameters and histopathological changes in tissues of streptozotocin (STZ) - induced diabetes in wistar rats. Diabetes was induced in wistar rats by single intraperitoneal injection of STZ (45mg/kg body weight). STZ-induced diabetic rats showed a significant increase in kidney weight, food intake, water intake, blood urea, serum uric acid and creatinine and a significant decrease in body weight, liver weight, plasma total protein and albumin when compared to normal rats. Oral administration of ellagic acid (50 and 100 mg/kg) in STZ-induced diabetic rats for a period of 35 days significantly improved body weight and reduced food intake and water intake when compared with diabetic control rats. Ellagic acid administration in STZ-induced diabetic rats restored the liver and kidney weight and also the biochemical parameters to near normal when compared to diabetic control rats. The results of the present study clearly show the preventive role of ellagic acid in STZ-induced diabetic rats. Results obtained from histopathological studies also supported the anti-diabetic effect of ellagic acid in STZ- induced diabetes.

Keywords: Diabetes Mellitus, Flavonoids, Ellagic Acid, Antioxidant, Insulin.

*Corresponding Author Email: kanchmoorthi@yahoo.co.in

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INTRODUCTION

Diabetes mellitus is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fats and proteins. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin. Several drugs are currently available to reduce hyperglycemia in diabetes mellitus[1]. In particular, hyperglycemia, the primary clinical manifestation of diabetes is thought to contribute in diabetic complications by altering vascular metabolism, vascular matrix molecules and circulating lipoproteins. Diabetes mellitus remains a major health problem and prevention of diabetes still lies in the realm of future and until then tens of millions will continue to suffer from this disease.

Streptozotocin (STZ) (2-deoxy-2- ({[methyl (nitroso) amino] carbonyl} amino)-β-D-glucopyranose) is a naturally occurring compound produced by the bacterium *Streptomyces achromogenes*, that exhibits broad spectrum antibacterial properties[2]. STZ is a mixture of α- and β-stereoisomers that appear as a pale yellow or off-white crystalline powder. STZ is very soluble in water, ketones and lower alcohols and only slightly soluble in polar organic solvents[3].

STZ is an agent widely employed to induce experimental diabetes due to its ability to selectively targets and destroys insulin producing pancreatic islet β-cells[4,5]. STZ causes DNA strand breaks in pancreatic islets and stimulates nuclear poly (ADP ribose) synthetase and thus depletes the intracellular NAD and NADP levels. NAD depletion by STZ inhibits pro-insulin synthesis and thus induces diabetes[6.]. STZ is used to induce both insulin dependent and non-insulin dependent diabetes mellitus. STZ enters the pancreatic β-cell via glucose transporter-$GLUT_2$ and causes alkylation of deoxyribonucleic acid (DNA).

Most of the plants have been found to contain substance like glycosides, alkaloids, terpenoids, flavonoids etc, that are frequently implicated as having on the specific mode of action of these plants drug or herbal formulation used for treating diabetes[7]. Phenolic compounds are a biologically active group of phytochemicals. They are classified according to their chemical structure into flavonoids, phenolic acids, coumarins, and tannins [8,9].

Flavonoids are a group of naturally occurring polyphenolic compounds ubiquitously found in fruits and vegetables[10,11]. The biological activities of the flavonoids have been extensively reviewed. Some were found to possess antiischemic, anti-inflammatory, antioxidant, anti-diabetogenic properties and other effects have also been described. It is suggested that most of these biological effects are related to their antioxidant activity by various mechanisms, e.g. by scavenging or quenching free radicals, by chelating metal ions, or by inhibiting enzymatic

systems responsible for the generation of free radicals. Considerable attention has been placed on understanding the pathophysiology of diabetes mellitus because of its importance in human health [12].

Ellagic acid is a polyphenol antioxidant found in numerous fruits and vegetables including blackberries, raspberries, strawberries, cranberries, walnuts, pecans, pomegranates, wolfberry and other plant foods[13]. Ellagic acid has antiproliferative, anti-inflammatory and antioxidant properties. The antiproliferative properties of ellagic acid are due to its ability to directly inhibit the DNA binding of certain carcinogens, including nitrosamines[15,16] and polycyclic aromatic hydrocarbons[14]. As with other polyphenol antioxidants, ellagic acid has a chemoprotective effect in cellular models by reducing oxidative stress. The interactions of ellagic acid and quercetin, which occur in muscadine grapes, interact synergistically in the induction of apoptosis and reduction of cell growth in human leukemia cells[17].

In the present investigation, the effect of ellagic acid on certain physical and biochemical parameters and histological alterations in liver and kidney of streptozotocin (STZ) - induced diabetic rats were studied.

MATERIALS AND METHODS

Experimental Animals

Female albino wistar rats (150-200 g) obtained from Venkateswara Enterprises, Bangalore were used in this study. They were housed in polypropylene cages (47cm x 34cm x 20cm) lined with husk. It was renewed every 24 hours under a 12:12 hour light: dark cycle at around 22º C and had free access to water and food. The rats were fed on a standard pellet diet (Pranav Agro Industries Limited., Maharashtra, India). The pellet diet consisted of 22.02% crude protein, 4.25% crude oil, 3.02% crude fiber, 7.5% ash, 1.38% sand silica, 0.8% calcium, 0.6% phosphorus, 2.46% glucose, 1.8% vitamins and 56.17% nitrogen free extract (carbohydrates). The diet provided metabolizable energy of 3600 kcal. The experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Drug and Chemicals

Ellagic acid was purchased from Sigma- Aldrich, St. Louis, USA. Streptozotocin (STZ) was purchased from Himedia Laboratories Private Limited, Mumbai. Uric acid, creatinine, total protein and albumin kits were purchased from Agappe Diagnostics, Kerala, India. All other chemicals used in the study were of analytical grade.

Induction of Experimental Diabetes

The animals were rendered diabetes by a single intraperitoneal injection of STZ (45mg/kg body weight) in freshly prepared citrate buffer (0.1M, pH 4.5) after an overnight fast. STZ injected animals were given 10% glucose solution for 5 days to prevent initial drug induced hyperglycemic mortality. After 48 hours of STZ administration, rats with moderate diabetes having glycosuria and hyperglycemia were taken for the experiment[18].

Experimental Design

A total of 36 rats randomly divided into 6 groups of 6 rats in each group were used in the present investigation. Ellagic acid was dissolved in 0.2% dimethyl sulfoxide and administrated to rats orally using an intragastric tube daily for a period of 35 days.

Group 1: Normal control rats

Group 2: Normal rats + Ellagic acid (50 mg/kg)

Group 3: Normal rats + Ellagic acid (100 mg/kg)

Group 4: Diabetic control rats

Group 5: Diabetic + Ellagic acid (50 mg/kg)

Group 6: Diabetic + Ellagic acid (100 mg/kg)

Sample Collection

After 35 days of treatment, the animals were fasted for 12 hours, anaesthetized with pentobarbital sodium (35 mg/kg) and sacrificed by cervical decapitation. The blood was collected in tubes containing a mixture of potassium oxalate and sodium fluoride as anticoagulant for the biochemical analysis. Liver and kidney was dissected out, washed in ice-cold saline, and patted dry and weighed.

Biochemical Estimations

Blood urea was estimated by the method of Chaney and Marbach (1962) and Searcy *et al*., (1967)[19,20]. Serum creatinine was estimated by the method of Henry *et al*., (1974)[21]. Uric acid in serum was estimated by the method of Fossati *et al*., (1980)[22]. Total Protein, and albumin in plasma was estimated by the method of Doumas *et al*., (1971) and Webster, (1977)[23,24].

Tissue sampling for histological study

The liver and kidney were quickly dissected out and washed in ice-cold saline to remove the blood. After dissection, the tissues were preserved in 10% formalin and stained with haematoxylin and eosin and subjected to microscopical examination.

Statistical Analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by

Duncan's Multiple Range Test (DMRT) using Statistical Package from the Social Sciences (SPSS) software package version 9.05. Results were expressed as mean ± S.D for six rats in each group. *P*-values < 0.05 were considered as significant.

RESULTS AND DISCUSSION

Effect of ellagic acid on physical parameters

Table 1 represents the effect of ellagic acid on body weight and organ weight in STZ-induced diabetic rats. Rats induced with STZ, showed a significant decrease in body weight, liver weight and increase in kidney weight. The reduction in body weight may be attributed to insulin depletion provoking a loss of adipose tissues and due to changes in carbohydrates and protein metabolism that occur in rats with streptozotocin-induced diabetes[25]. A decrease in body weight of diabetic rats is possible due to catabolism of fats and protein, even though the food intake is more in diabetic rats than control. Following injection with streptozotocin, these animals displayed the expected symptoms of insulin dependent diabetes mellitus, i.e., hyperglycemia, polydypsia, depression of body mass gain, the increase in food and water intake and the decrease in insulin concentration, as previously observed. This could be due to improved glycemic control[26]. Rats treated with ellagic acid significantly improved body weight which might be due to the ability of ellagic acid to enhance glucose metabolism and thus, improves body weight in STZ-induced diabetic rats.

A decrease in the liver weight observed in diabetic animals might be due to enhanced catabolic processes such as glycogenolysis, lipolysis and proteolysis, which is the outcome of lack of insulin and/or cellular glucose in liver cells[27,28]. It was reported that insulin therapy could increase the accumulation of glycogen in diabetic liver[29,30]. A significant increase in the whole kidney weight observed in STZ induced animals might be due to the glomerular cell proliferation accompanying glomerular enlargement in the early phase of STZ induced diabetes. Oral administration of ellagic acid (50 and 100 mg/kg) in STZ-induced diabetic rats restored the liver and kidney weight to near normal.

Food intake and water intake was found to be significantly increased in STZ-induced diabetic rats as compared to normal control rats (Table 2). Rats induced with STZ, showed a significant increase in food intake and water consumption. It is well known that STZ provoked hyperglycemia is accompanied by symptoms like loss of weight, polydypsia and polyphagia[31]. Oral administration of ellagic acid in STZ-induced diabetic rats caused significant reduction in food and water intake when compared with diabetic control rats.

Table 1: Effect of Ellagic acid on body weight, liver and kidney weights in normal and STZ-induced diabetic rats

Groups	Body weight (g)		Organ weight (g)	
	Initial	Final	Liver Weight	Kidney Weight
Normal control	175.16 ± 2.4	195.96 ± 4.6 [a]	6.20 ± 0.33 [a]	2.26 ± 0.26[a]
Normal + EA (50mg/kg)	174.85 ± 2.2	195.44 ± 4.5 [a]	6.23 ± 0.15 [a]	2.25 ± 0.18[a]
Normal + EA (100mg/kg)	175.08 ± 18.3	196.16 ± 12.6[a]	6.29 ± 1.50 [a]	2.25 ± 0.21[a]
Diabetic control	179.39 ± 23.7	165.16 ± 7.2 [b]	4.05 ± 1.80 [b]	3.39 ± 0.41[b]
Diabetic + EA (50mg/kg)	175.00 ± 20.1	174.83 ± 2.1 [c]	5.17 ± 0.18 [c]	2.93 ± 0.52[c]
Diabetic + EA (100mg/kg)	177.00 ± 19.8	185.83 ± 4.6 [d]	5.92 ± 0.41 [d]	2.64 ± 0.28[d]

Each value is mean ± S.D. for six rats in each group.
Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Table 2: Effect of Ellagic acid on food intake and water intake in normal and STZ-induced diabetic rats

Groups	Food intake (g)	Water intake (ml)
Normal control	67.70 ± 0.89[a]	125.42 ± 4.8[a]
Normal + Ellagic acid (50mg/kg)	67.16 ± 1.09[a]	125.69 ± 5.1[a]
Normal + Ellagic acid (100mg/kg)	67.55 ± 0.70[a]	126.27 ± 4.9[a]
Diabetic control	83.16 ± 3.20[b]	198.50 ± 8.0[b]
Diabetic + Ellagic acid (50mg/kg)	74.50 ± 3.09[c]	158.10 ± 5.2[c]
Diabetic + Ellagic acid (100mg/kg)	70.33 ± 2.10[d]	142.89 ± 5.3[d]

Each value is mean ± S.D. for six rats in each group.
Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Effect of ellagic acid on blood urea, serum creatinine and uric acid

Table 3 depicts the level of blood urea, serum creatinine and uric acid in normal and STZ-induced diabetic rats. A significant increase in the levels of blood urea, serum creatinine and uric acid were observed in STZ-induced diabetic rats when compared to normal rats. The significant increase in serum urea concentration of diabetic rats may be due to depletion of serum protein, increase in the rate of circulating amino acids and deamination takes place that consequently leads to the formation of large amount of ammonia which is eventually converted to urea. Due to insulin deficiency, protein content is decreased in muscular tissue by proteolysis[32]. The breakdown of amino acids during gluconeogenesis in the liver results in increased production of urea, fostering negative nitrogen balance[33]. Catabolism of the protein and nucleic acid results in the formation of non-protein nitrogenous compounds, urea and creatinine. Diabetic rats treated with ellagic acid caused significant reduction in the levels of blood urea, serum creatinine and uric aicd suggesting an improvement in kidney function[34].

Table 3: Effect of Ellagic acid on blood urea, serum creatinine and uric acid in normal and STZ - induced diabetic rats

Groups	Urea (mg/dl)	Creatinine	Uric acid (mg/dl)
Normal control	25.84 ± 1.62[a]	3.54 ± 0.64[a]	1.75 ± 0.14[a]
Normal + Ellagic acid (50mg/kg)	25.38 ± 1.50[a]	3.56 ± 0.68[a]	1.76 ± 0.08[a]
Normal + Ellagic acid (100mg/kg)	25.88 ± 1.58[a]	3.49 ± 0.55[a]	1.67 ± 0.09[a]
Diabetic control	36.50 ± 1.88[b]	5.95 ± 1.20[b]	3.15 ± 0.57[b]
Diabetic + Ellagic acid (50mg/kg)	30.99 ± 2.0 [c]	4.57 ± 0.14[c]	2.30 ± 0.65[c]
Diabetic +Ellagic acid (100mg/kg)	28.02 ± 2.2[d]	4.02 ± 0.30[d]	1.96 ± 0.21[d]

Each value is mean ± S.D. for six rats in each group.
Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Effect of ellagic acid on plasma total protein and albumin

The effect of ellagic acid on the levels of total protein and albumin in normal and STZ-induced diabetic rats are shown in Table 4. Rats induced with STZ, showed a significant decrease in the level of total protein and albumin when compared to normal rats. The decrease in serum total protein observed in diabetic rats coincides with the earlier report[35]. This decline may be due to the inhibited oxidative phosphorylation processes, which lead to decrease in protein synthesis, increase in the catabolic processes and reduction of protein absorption. The metabolism of protein is abnormal in diabetes due to defects in insulin secretion, leading to various metabolic disorders[36,37]. Total protein and albumin level reduction may be due to increased protein catabolism caused by streptozotocin[38]. Oral administration of ellagic acid in STZ-induced diabetic rats significantly increased the levels of total protein and albumin when compared with diabetic control rats.

Table 4: Effect of Ellagic acid on plasma total protein and albumin in normal and STZ - induced diabetic rats

Groups	Total protein (g/dl)	Albumin (g/dl)
Normal control	8.45 ± 0.67[a]	4.71± 0.31[a]
Normal + Ellagic acid (50mg/kg)	8.58 ± 0.74[a]	4.48 ± 2.5[a]
Normal + Ellagic acid (100mg/kg)	8.61 ± 0.80[a]	4.63 ± 2.8[a]
Diabetic control	5.75 ± 0.58 [b]	2.45 ± 0.18[b]
Diabetic + Ellagic acid (50mg/kg)	7.15 ± 0.49 [c]	3.15 ± 0.15[c]
Diabetic + Ellagic acid (100mg/kg)	7.92 ± 0.40 [d]	3.97 ± 0.21[d]

Each value is mean ± S.D. for six rats in each group.
Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT)

Effect of ellagic acid on histopathology of liver

The effect of ellagic acid on histological changes in liver of normal and STZ-induced diabetic rats is shown in Figures 1-6. Normal control rat liver showed the normal architecture of hepatic

parenchyma (Figure. 1). The liver of normal rats treated with ellagic acid (50 mg/ kg) group showed the hepatic parenchyma within normal limits (Figure. 2). The liver of normal rats treated with ellagic acid (100 mg/ kg) showed dilated vessel and mild sinusoidal congestion (Figure. 3). Diabetic control rat liver showed the severe congestion and vacuolar degeneration of hepatocytes with accumulation of inflammatory cells (Figure. 4). The liver of diabetic rats treated with ellagic acid (50 mg/ kg) showed moderate venous and sinusoidal congestion (Figure. 5). The liver of diabetic rats treated with ellagic acid (100 mg/ kg) showed mild dilation with a few inflammatory cells and near normal architecture (Figure. 6).

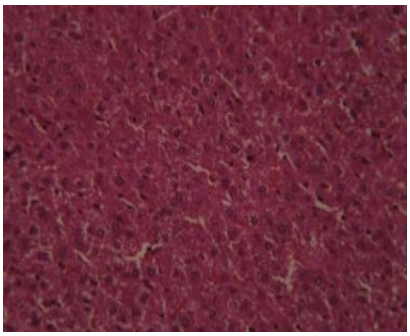

Figure 1 Control rat liver show normal architecture

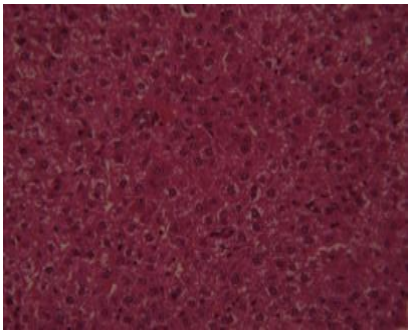

Figure. 2 Liver from normal rat treated with ellagic acid 50 mg shows hepatocytes with normal appearance

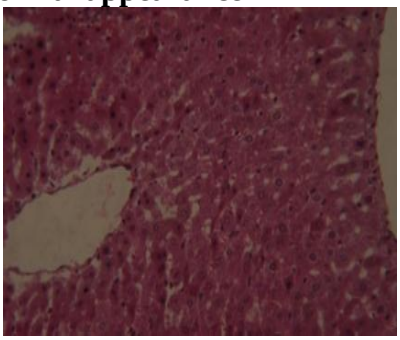

Figure 3 Liver from normal rat treated with ellagic acid 100 mg shows near normal architecture with mild sinusoidal congestion

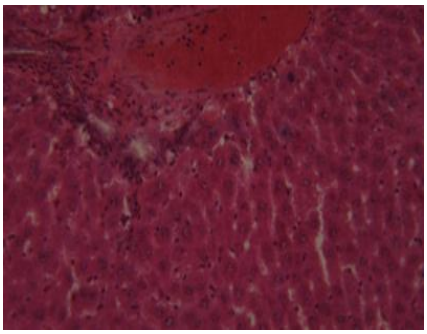

Figure. 4 Diabetic control rat liver shows severe hepatic necrosis and accumulation of inflammatory cells

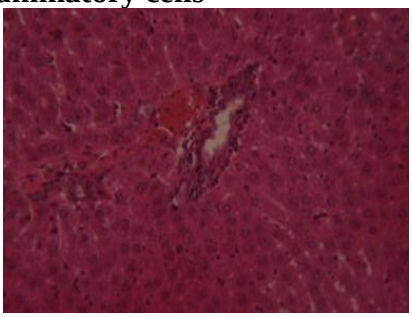

Figure. 5 Diabetic rat liver treated with ellagic acid 50 mg shows moderate sinusoidal congestion

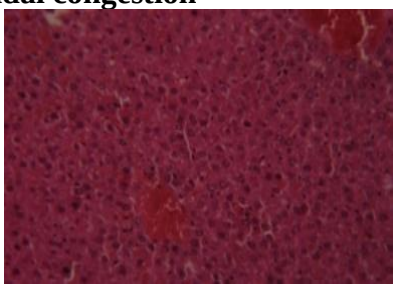

Figure. 6 Diabetic rat liver treated with ellagic acid 100 mg shows mild dilation and near normal architecture

Effect of ellagic acid on histopathology of kidney

The effect of ellagic acid on histological changes in kidney of normal and STZ-induced diabetic rats is shown in Figures 7-12. Normal control rat kidney showed the normal glomeruli and tubular structure (Figure. 7). The kidney of normal rats treated with ellagic acid (50 mg/ kg) showed the intact tubules with lining epithelium (Figure. 8). The kidney of normal rats treated with ellagic acid (100 mg/ kg) showed apparently normal pattern of parenchyma with mild aggregation of mononuclear cells (Figure. 9). Diabetic control rat kidney showed swollen tubules resulting in narrowing of lumen and disintegrated glomeruli (Figure. 10).). The kidney of diabetic rats treated with ellagic acid (50 mg/ kg) showed moderately dilated tubules with inflammatory cells (Figure. 11). The kidney of diabetic rats treated with ellagic acid (100 mg/ kg) showed normal glomeruli with normal tubules (Figure. 12).

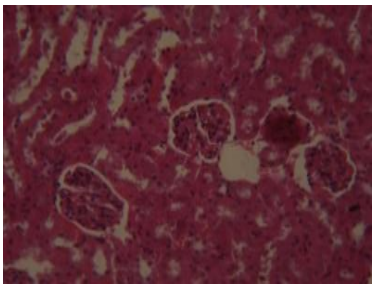

Figure. 7 Normal control rat kidney shows normal glomeruli and tubules

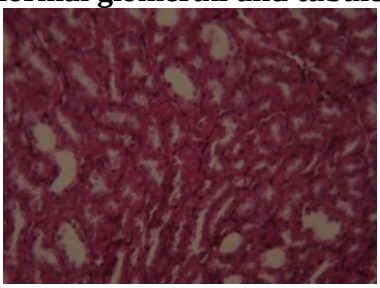

Figure. 8 Kidney from normal rat treated with ellagic acid 50 mg shows intact tubules

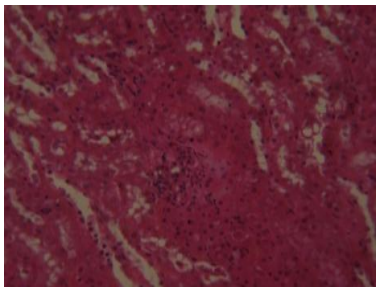

Figure. 9 Kidney from normal rat treated with ellagic acid 100 mg shows near normal architecture with mild aggregation of mononuclear cells

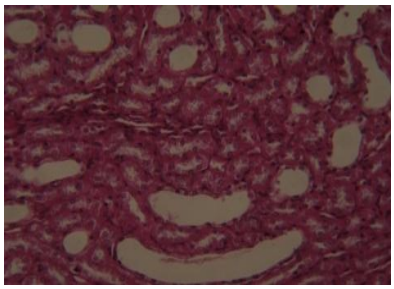

Figure. 10 Diabetic control rat kidney shows vacuolation tubules, glomerular proliferations with fatty vacuoles in the tubules

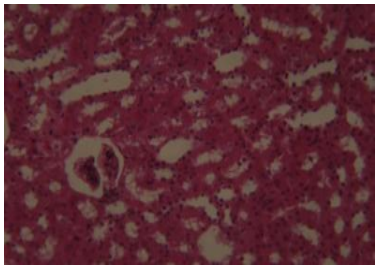

Figure. 11 Kidney of diabetic rat treated with ellagic acid 50 mg shows moderately dilated tubules with inflammatory cells

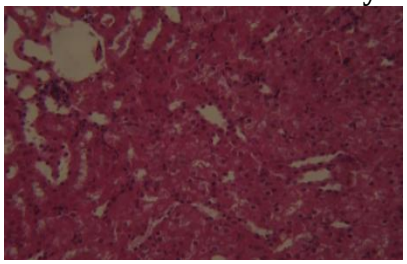

Figure. 12 Kidney of diabetic rat treated with ellagic acid 100 mg shows near normal glomeruli with tubules

The histopathological findings of STZ-induced diabetic rats showed severe congestion and vacuolar degeneration of hepatocytes and swollen renal tubules resulting in narrowing of lumen and disintegrated glomeruli. Normal rats treated with ellagic acid showed normal hepatic parenchyma and normal glomeruli and tubular structure which indicates that ellagic acid does not possess any adverse effect under normal condition. Diabetic rats treated with ellagic acid significantly minimized the histopathological alterations, which could be due to protective effect of ellagic acid in liver and kidney.

CONCLUSION

In conclusion, ellagic acid administration in diabetic rats for a period of 35 days showed significant anti-diabetic effect by minimizing the physical, biochemical and histopathological alterations. Thus our study suggests the potential antidiabetic efficacy of ellagic acid in STZ-induced diabetic rats.

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