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ARTÍCULO ORIGINAL

Antidiabetic and Nephroprotective effect of Tectona grandis linn. In Alloxan induced Diabetes

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ABSTRACT

In the present study, effect of ethanolic extract of bark of Tectona grandis Linn. (TG) was evaluated using alloxan induced diabetes and associated renal complication. The diabetes was induced by administration of alloxan to the rats at the dose of 140 mg/kg, i.p. TG was administered to diabetic animals for six weeks and various biochemical parameters in blood and urine (plasma glucose, serum albumin, total protein, and creatinine, urine total protein, urine albumin), tissue parameters (cholesterol and triglyceride in kidney homogenate) and % change in body weight were evaluated along with histopathological study. In present study diabetic animals treated with TG showed significant reduction in the elevated level of plasma glucose ($p<0.01$) when compared with diabetic control. While considering renal parameters, diabetic animals treated with TG showed significant decrease in serum creatinine ($p<0.05$), urine albumin and urine total protein levels ($p<0.01$) and significant increase in serum albumin, total protein and % change in body weight ($p<0.01$) when compared with diabetic control. Diabetic control showed significant increase in total cholesterol and triglyceride accumulation in kidney, while diabetic animals treated with TG showed significant decrease in levels of total cholesterol ($p<0.01$) and triglyceride ($p<0.05$) in the kidney when compared with diabetic control. Diabetic control showed significant mark of glomerulosclerosis and hyalinization which occurs because of severe diabetic condition (diabetic nephropathy). Diabetic groups treated with TG showed absence of the sclerotic lesions produced by diabetic condition. Hence, the results obtained in the present study indicate that Tectona grandis has the potential to treat diabetes mellitus and prevent the associated renal damage.

KEYWORDS: Alloxan, creatinine, glucose, nephropathy, *Tectona grandis*

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INTRODUCTION

Diabetes has become a major health issue in South-East Asia. It has been estimated by the international diabetes federation (IDF) that 23 million people have diabetes, which accounts for a sixth of the world's diabetic population. India has the largest diabetic population and one of the highest diabetes prevalence rates in the world. The prevalence rates for type II diabetes in India are still increasing sharply with the number of sufferers predicted to rise from 19.4 million in 1995 to 80.9 million in 2030. With the current high mortality and morbidity rates associated with diabetes, this represents a real threat to the economic productivity of countries such as India[1].

Diabetic nephropathy (DNP) is a major cause of illness and premature death in diabetic patients, largely through accompanying cardiovascular diseases and end-stage renal failure[2]. Diabetes induced by alloxan in rats results in development of nephropathy similar to early stage clinical diabetic nephropathy[3].

Diabetes mellitus is not a single disease entity, but rather a group of metabolic disorders sharing a common underlying feature of hyperglycemia. Hyperglycemia in diabetes, results from defects in insulin secretion, insulin action or most commonly both4.

Traditionally Tectona grandis is used in treatment of diabetes, lipid disorders, inflammation, ulcer, and bronchitis[5]. Tectona grandis Linn. is reported to have antiulcer[6], antimicrobial[7], wound healing[8], and anticancer activity[9]. The present study was aimed to evaluate the effect of Tectona grandis Linn. in the treatment of diabetes and associated renal damage in alloxan induced diabetic rats.

MATERIALS AND METHODS

EXPERIMENTAL

Animals

Albino rats of Wistar strain weighing 160-200 g and Albino mice weighing 20-30 g were obtained from National Toxicology Centre (NTC), Pune. Animals of either sex were housed under standard laboratory conditions of 22 ± 3 0C temperature and relative humidity 30% and 12 h light and dark cycle, free access to standard pellet diet and water ad libitum. The Institutional Animal Ethics Committee approved the experimental protocol. IAEC registration no (198/99/CPCSEA).

Collection and Authentication of Plant Material

The bark of Tectona grandis Linn. (Verbenaceae) was collected in the month of June 2007 from Nanded, Maharashtra, India. The plant was authenticated by Dr. A. M. Mujumdar (Head, Plant Sciences Division) Agharkar Research Institute, Pune as Tectona grandis Linn.

(Verbenaceae) with a voucher specimen no Auth08-012.

Preparation of Extract

The bark was washed with distilled water and shed dried and latter powdered. This powder was then defatted with petroleum ether and then macerated with ethanol for 72 h with occasional shaking. It was then filtered and the solvent was evaporated under vacuum. The yield of ethanolic extract of Tectona grandis Linn. (TG) was 2.7% w/w.

Acute Toxicity Study[10].

The acute toxicity study for ethanolic extract of bark of Tectona grandis Linn. (TG) was performed using albino mice. The animals were fasted overnight prior to the experiment and maintained under standard conditions. TG was found safe up to dose of 2,000 mg/kg, p.o.

Alloxan Induced Diabetes in Rats[11-14].

Induction of Diabetes

Diabetes was induced by a single intraperitonial injection of alloxan monohydrate in citrate buffer (pH 4.5) at a dose of 140 mg/kg, body weight of the rat. The diabetic state was confirmed 48 h after alloxan injection by hyperglycemia. Surviving rats with fasting blood glucose level higher than 250 mg/dl were included in the study.

Treatment Schedule

Total of 25 diabetic surviving and 5 nondiabetic rats were divided in to 6 groups (n=5) as follows-

Group-I nondiabetic animals: received only 1% gum acacia (1 ml/kg/day, p.o.) for six weeks, and served as control. Group-II to VI were rendered diabetic by single intraperitonial dose of alloxan monohyderate 140 mg/kg, in citrate buffer (pH 4.5). Group II received 1 % gum acacia (1 ml/kg/day, p.o.) for six weeks and served as diabetic control. Group-III received glimepride (0.09 mg/kg/day, p.o.) for six weeks. Group-IV-VI received three different doses of TG (50, 100 and 200 mg/kg/day, p.o.) for six weeks respectively.

Biochemical Parameters from Blood and Urine

On 1st day and at the end of each week of treatment, blood was withdrawn from all the animals under light ether anaesthesia by puncturing retro-orbital plexus. Estimation of plasma glucose (GOD/POD Method), serum albumin, total protein (Biuret and Dye binding method) and Creatinine (Alkaline picrate method) using standard diagnostic kits from BIOLAB Diagnostics (P) Ltd., India.

At the end of the study urine was collected and centrifuged. Levels of total protein and

albumin were estimated (Biuret and Dye binding method) using standard diagnostic kits from BIOLAB Diagnostics (P) Ltd., India.

Study of Morphometric Parameters

Body weight was recorded throughout the study period.

Study of Biochemical Parameters from Kidney

At the end of experimental period, the animals were sacrificed with overdose of urethane. The kidney was removed. 10% homogenate of kidney was prepared in 50 mM phosphate buffer (pH 7.4) and centrifuged. The supernatant was used for the estimation of total cholesterol (COD/POD Method) and triglyceride (GPO/POD Method) using standard diagnostic kits from BIOLAB Diagnostics (P) Ltd., India.

Histopathological Studies

The kidney was removed from single animal of each group, washed with distilled water and kept in 10% formalin solution; and stained with H&E and then examined for the microscopic morphology.

Statistical Analysis

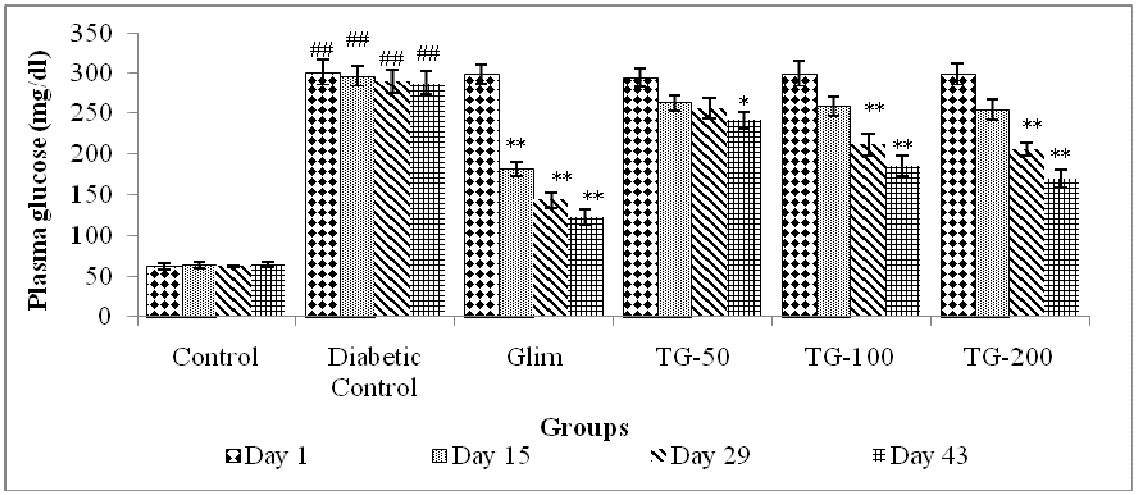
Results were expressed as Mean ± SEM and analysed by ANOVA followed by Dunnett test $p<0.05$ considered as statistical significant.

RESULTS

Effect of Tectona grandis on Biochemical Parameters

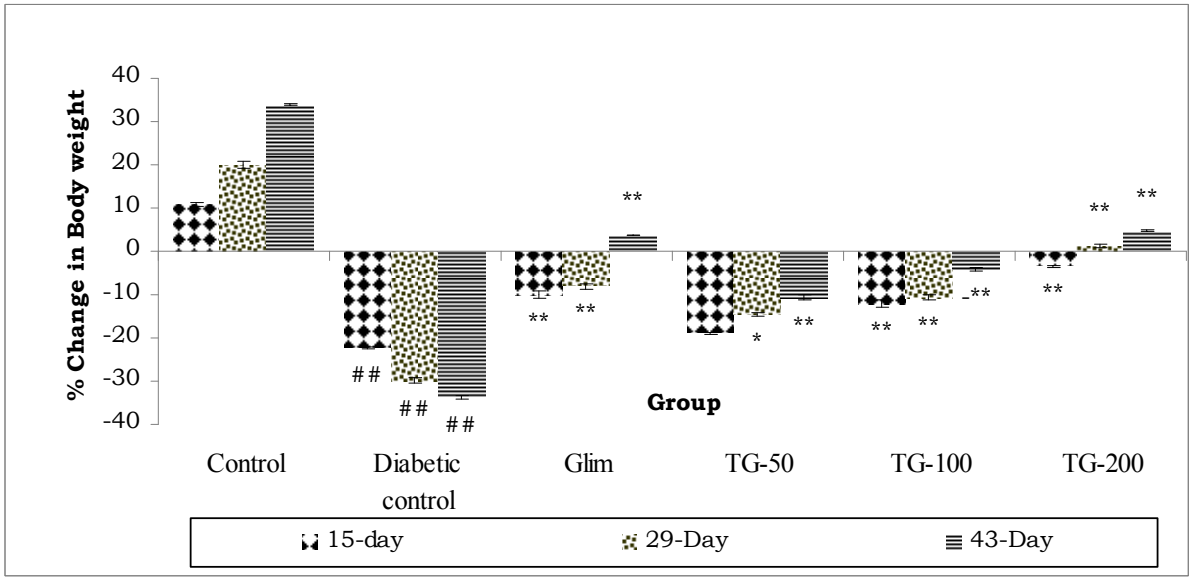
Diabetic control showed significant increase in plasma glucose level, serum creatinine, urine albumin and total protein levels ($p<0.01$) (Figures 1-3) and significant decrease in serum albumin, total protein levels and % change in body weight ($p<0.01$) (Figures 4-6) when compared with control animals. In the present study diabetic animals treated with TG showed significant reduction in the elevated level of plasma glucose ($p<0.01$) when compared with diabetic control (Figure 1). While considering renal parameters, diabetic animals treated with TG showed significant decrease in serum creatinine ($p<0.05$) (Figure 2) and urine albumin and total protein levels ($p<0.01$) (Figure 3) and significant increase in serum albumin, total protein and % change in body weight ($p<0.01$) when compared with diabetic control (Figures 4-6). Diabetic control showed significant increase in total cholesterol and triglyceride accumulation in kidney, while diabetic animals treated with TG showed significant decrease in levels of total cholesterol ($p<0.01$) and triglyceride ($p<0.05$) in the kidney homogenate when compared with diabetic control (Figure 7).

Figure 1. Effect of Tectona grandis Linn. on plasma glucose level in alloxan induced diabetic rats



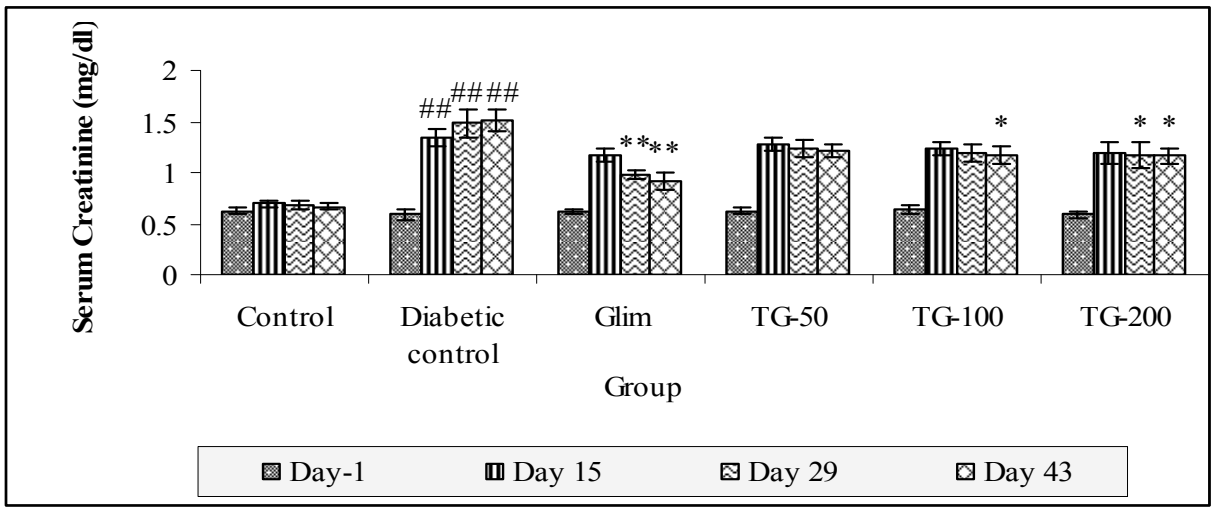
Values are expressed as Mean ± SEM. (n=5), ANOVA followed by Dunnett test. ##p<0.01 when compared with Control; *p<0.05, **p<0.01 when compared with Diabetic control.

Figure 2. Effect of Tectona grandis Linn. on % Change in Body weight in alloxan-induced diabetic rats



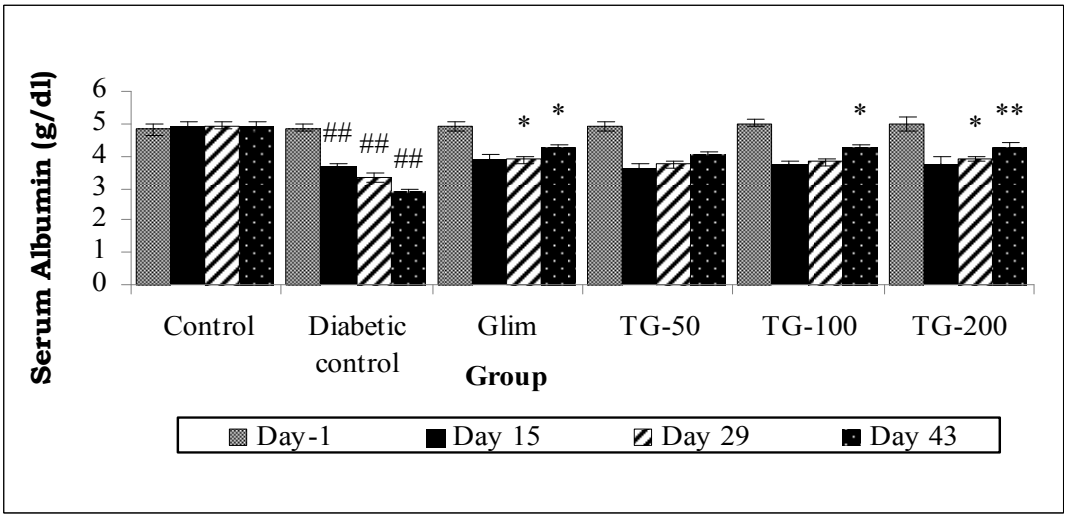
Values are expressed as Mean ± SEM. (n=5), ANOVA followed by Dunnett test. ##p<0.01 when compared with Control; *p<0.05, **p<0.01 when compared with Diabetic control.

Figure 3. Effect of Tectona grandis Linn. on serum creatinine level in alloxan-induced diabetic rats.



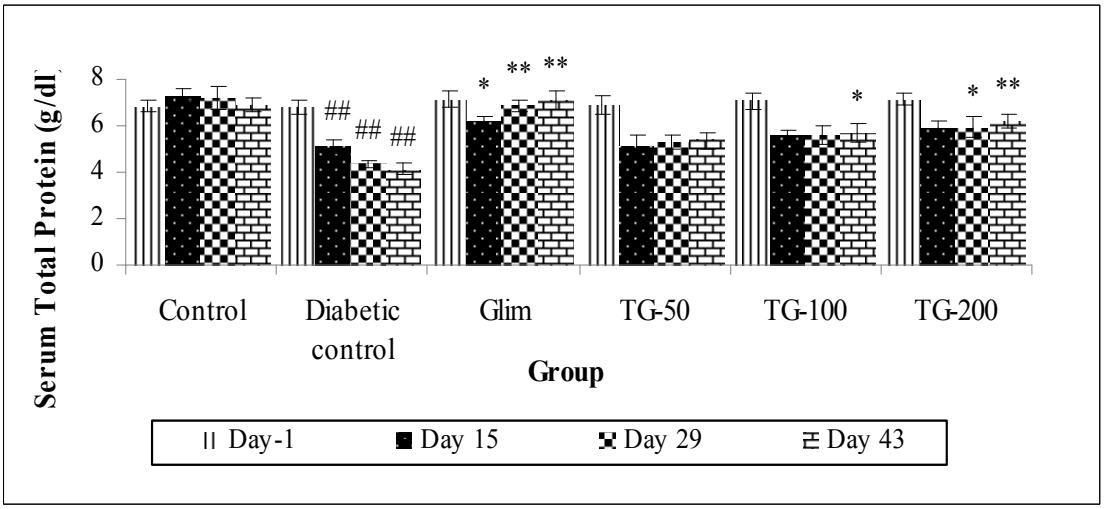
Values are expressed as Mean ± SEM. (n=5), ANOVA followed by Dunnett test. ##p<0.01 when compared with Control; *p<0.05, **p<0.01 when compared with Diabetic control.

Figure 4. Effect of Tectona grandis Linn. on serum albumin level in alloxan-induced diabetic rats.



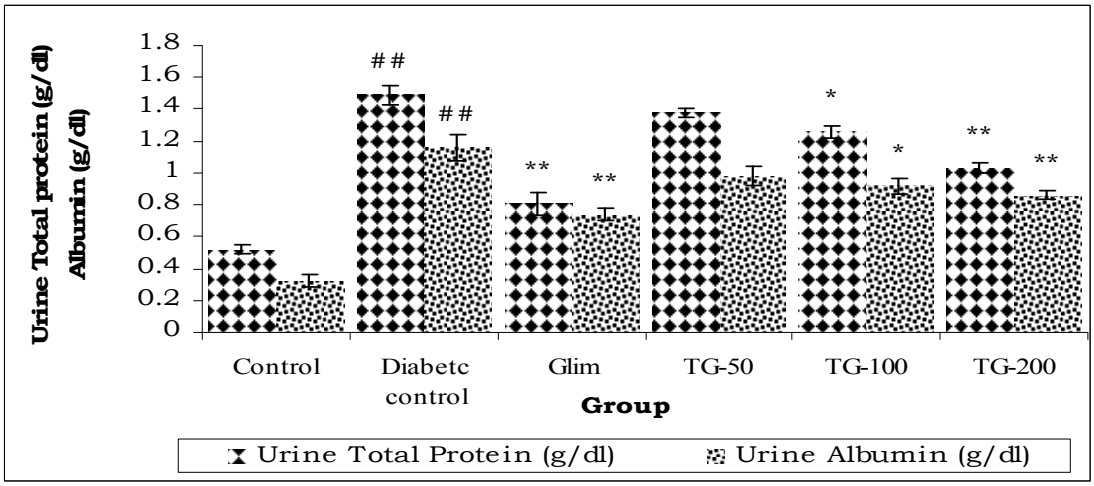
Values are expressed as Mean ± SEM. (n=5), ANOVA followed by Dunnett test. ##p<0.01 when compared with Control; *p<0.05, **p<0.01 when compared with Diabetic control.

Figure 5. Effect of Tectona grandis Linn. on serum total protein level in alloxan-induced diabetic rats



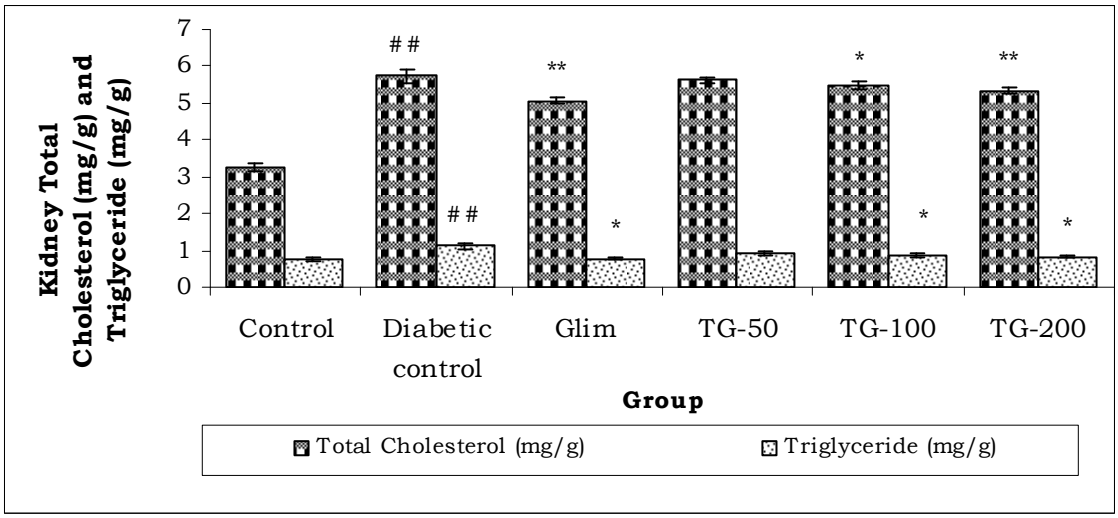
Values are expressed as Mean ± SEM. (n=5), ANOVA followed by Dunnett test. ##p<0.01 when compared with Control; *p<0.05, **p<0.01 when compared with Diabetic control.

Figure 6. Effect of Tectona grandis Linn. on urine total protein and urine albumin level in alloxan-induced diabetic rats



Values are expressed as Mean ± SEM. (n=5), ANOVA followed by Dunnett test. ##p<0.01 when compared with Control; *p<0.05, **p<0.01 when compared with Diabetic control.

Figure 7. Effect of Tectona grandis Linn. on total cholesterol and triglyceride levels in kidney homogenate in alloxan-induced diabetic rats.

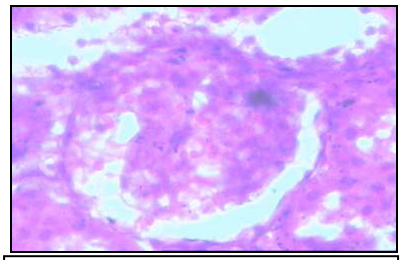


Values are expressed as Mean ± SEM. (n=5), ANOVA followed by Dunnett test. ##$p<0.01$ when compared with Control; *$p<0.05$, **$p<0.01$ when compared with Diabetic control.

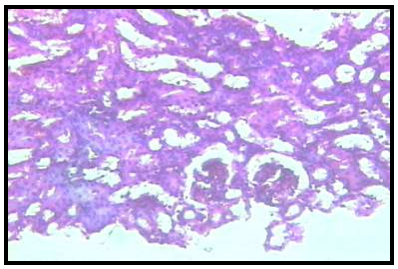
Effect of Tectona grandis on histopathology of kidney

In present study, histopathology of control group showed normal structure of glomerulus, while diabetic control group showed significant mark of glomerulosclerosis and hyalinization which occurs because of severe diabetic condition (diabetic nephropathy). Diabetic group treated with TG 200 mg/kg, p.o. showed absence of the sclerotic lesions produced by diabetic condition. While TG 100 mg/kg, p.o. treated and glimepride treated diabetic groups showed partial prevention of the hyalinization but failed to recover the glomerulosclerosis to the normal condition. Diabetic group treated with TG 50 mg/kg, p.o. did not show any protection against necrosis of kidney produced by diabetic condition.

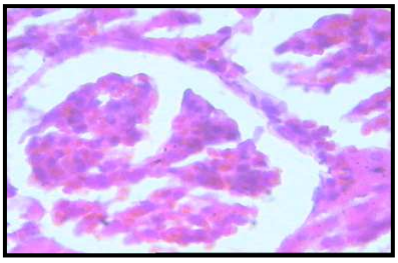
Figure 8. Effect of Tectona grandis Linn. on histopathological studies of kidney in alloxan-induced diabetic rats.



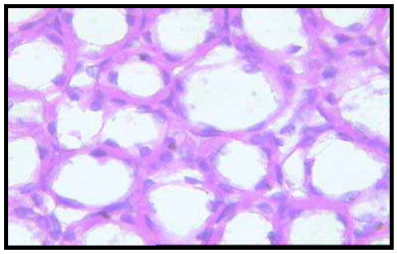
Photomicrograph of Control group kidney showing normal structure of glomerulus showing normal structure of glomerulus (H & E 100X)



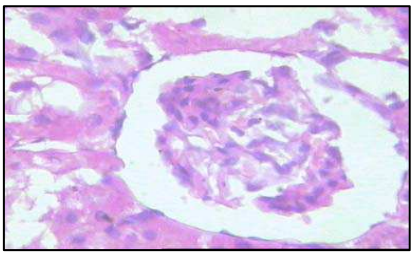
Photomicrograph of Diabetic control group kidney showing significant mark of glomerulosclerosis (nephritis) and Hyalinization (H & E 100X)



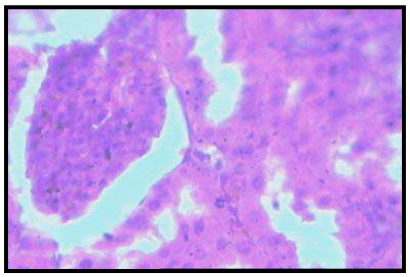
Photomicrograph of Glim group kidney showing mild glomerulosclerosis (nephritis) and Hyalinization (H & E 100X)



Photomicrograph of TG-50 group kidney showing significant glomerulosclerosis (nephritis) and mild Hylinization (H & E 100X)



Photomicrograph of TG-100 group kidney showing mild glomerulosclerosis (nephritis) but no sign of Hylinization (H & E 100X)



Photomicrograph of TG-200 group kidney showing Very mild glomerulosclerosis (nephritis) but no sign of Hylinization (H & E 100X)

DISCUSSION

Diabetes mellitus ranks highly among the top ten disorders which cause mortality throughout the world. Diabetes mellitus being chronic disorder, treatment without side effect for long term control is important. Present antidiabetic agent possess side effect as risk of hypoglycemia, anemia, choestatic jaundice[15]. There has been growing public interest in herbal medication for treatment of diabetes.

In the present study the periodic estimation of plasma glucose revealed that TG produced significant antihyperglycemic activity which began from 22nd day of treatment and it progressed throughout the study. The antidiabetic effect of the TG could possibly be due to presence of glycosides, terpenoids, tannins and saponins. Substances like glycosides, alkaloids, terpenoids, tannins and saponins are frequently implicated as having antidiabetic effects[16].

Various reports suggest that there is reduction in the body weight in diabetic rats[3]. Loss of body weight could be due to, dehydration and catabolism of fats and protein seen during diabetes mellitus17. It is reported that the recovery in body weight is far less in the poorly controlled diabetic rats as compared to well-controlled diabetic rats. In the present study diabetic control group rats showed significant loss of body weight. All animals treated with TG showed significant prevention of the loss in body weight throughout the study. This prevention of loss in body weight by TG may be due to increasing glucose uptake in peripheral tissues or inhibiting catabolism of fat and protein or by glycemic control.

Diabetes produces qualitative and quantitative changes in the composition of the basement membrane and this altered material undergoes accelerated glycosylation and further rearrangement to form advanced glycation end-products (AGEs), which stimulate protein synthesis, further decrease degradability of the basement membrane, increase its permeability and cause endothelial dysfunction. Hyperglycemia increases the expression of transforming growth factor beta (TGFβ) in the glomeruli and of matrix protein specifically stimulated by cytokine. TGFβ may contribute to both the cellular hypertrophy and enhanced collagen synthesis is observed in diabetic nephropathy[18].

During diabetes, there is increased protein catabolism with inflow of amino acids to liver, which feed gluconeogenesis and accelerate ureagenesis, resulting in hypoproteinemia and hypoalbuminemia[19]. Diabetic hyperglycemia induces elevation of the levels of serum creatinine, urine total protein and urine albumin which are considered as significant markers of renal dysfunction[20].

In the present study, diabetic animals treated TG showed reduction in proteinurea and albuminurea and also showed improvement in the serum total protein and albumin level. Treatment with TG also prevented the rise in serum creatinine levels. These results indicate that TG attenuates the progression of renal damage in alloxan induced diabetic rats.

The use of typical antioxidants alone or in combination may retard or even prevent the

normal progression of diabetic complications[21].

In case of uncontrolled diabetes there is accumulation of lipids in kidney. Excessive production and accumulation of lipids can have devastating effect on renal structure and function[22]. Changes in the fractions of the lipid in renal cortex and medulla readily show its abnormality in diabetes[23]. In the present study it was found that concentrations of total lipids were significantly increased in cortical and medullary region of the kidney; concentration of total cholesterol was significantly increased in cortical region. TG treated diabetic rats showed significant reduction in the total cholesterol and triglyceride level in the kidney homogenate. The glycemic control exerted by TG may have affected the dislipidemia and the subsequent accumulation of the lipids in the kidney.

The histopathological study of diabetic control group showed significant mark of glomerulosclerosis and hyalinization which was probably due to severe diabetic condition (diabetic nephropathy); and the diabetic groups treated with TG showed absence of the sclerotic lesions produced by diabetic condition indicating the protective effect of TG on the kidneys of the diabetic animals.

Hence, the results obtained in the present study indicate that Tectona grandis has the potential to treat diabetes mellitus and prevent diabetes mellitus associated renal damage.

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