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Tectona grandis Linn prevent the cardiac dysfunction in alloxan induced diabetic rats.

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Abstract

The bark of *Tectona grandis* Linn. is traditionally used in the treatment of diabetes. The present study was undertaken to study effect of ethanol extract of *Tectona grandis* (TG) in treatment of diabetes mellitus and associated cardiovascular complications in alloxan induced diabetic rats. Diabetes was induced by single intraperitoneal administration of prestandardised dose of alloxan and TG was given orally at 50, 100 and 200 mg/kg. Effect of TG on various biochemical, hemodyamic were observed. Diabetic control showed significant increase ($p<0.01$) in biochemical, hemodyamic parameters (SAP, DAP, MAP), atherogenic index. TG treatment with for 42 days showed significant decrease ($p<0.01$) in biochemical parameters, atherogenic index and hemodyamic parameters when compared with diabetic control. Hence, the results obtained in the present study indicate that *Tectona grandis* may prevent the cardiac dysfunction in alloxan induced diabetic rats.

Key words : Cardiovascular complications, glycemic control, *Tectona grandis*.

1. Introduction

Diabetic cardiomyopathy, a prominent cardiovascular complication, has been recognized as a macrovascular complication that may lead to heart failure. Clinical, epidemiological and experimental data suggests that the pathogenesis of diabetic cardiomyopathy is multifactorial [10]. Diabetic myocardial disease is characterized by hypertrophy of cardiomyocyte, interstitial fibrosis, thickening of capillary basement membrane and reduced capillary density leading to heart failure [11].

In diabetic patients, long term exposure to hyperglycemia induces biochemical manifestations, such as non-enzymatic glycation, sorbitol-myoinositol-mediated changes, redox potential alterations, protein kinase C (PKC) activation, free fatty acid (FFA) metabolism in monocytes and endothelial cells;

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leading to death of cardiomayocyte [4, 29, 9, 16]. These changes lead to particular sequence of events and thereby increasing oxidative stress in diabetes.

Traditionally, *Tectona grandis* is used in the treatment of diabetes, lipid disorders, inflammation, ulcer and bronchitis [34]. *Tectona grandis* Linn is reported to have antiulcer [26], antimicrobial [31], wound healing [22] and anticancer activity [15], antioxidant [37]. Ethanol extract of *Tectona grandis* is found to be effective in dexamethasone induced insulin resistance in mice [13]. Hence, taking into consideration the traditional claims and the reported pharmacological activities, the present study was planned to investigate the effect of ethanol extract of *Tectona grandis* Linn. on alloxan induced diabetic cardiovascular complications in rats.

2. Materials and methods

2.1 Plant material and preparation of extract

Fresh bark of *Tectona grandis* Linn. (Verbenaceae) (TG) was collected from Nanded, Maharashtra, India. The specimen was authenticated at Aghrkar Research Institute, Pune with voucher specimen no. 08-12 and catalogued. 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 ethanol extract of *Tectona grandis* Linn. (TG) was 2.7% w/w.

TG when subjected for phytochemical study showed the presence of beta-sitosterol, terpenoids, phenolic compounds, saponins, glycosides and tannins.

2.2 Animals

Albino Wistar rat of either sex weighing 160-200 g were obtained from National Toxicology Center (NTC), Pune and kept in animal house at ambient temperature. The Institutional Animal Ethics Committee approved the experimental protocol. (IAEC registration no. 198/99/CPCSEA). Animals were provided standard diet and water *ad libitum.*

2.3 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 rat [28]. The diabetic state was confirmed 48 hr after alloxan injection by hyperglycemia. Surviving rats with fasting blood glucose level higher than 250 mg/dl were included in the study [14, 24].

2.4 Treatment schedule

Total of 30 diabetic surviving and 5 nondiabetic rats were divided into 7 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 VII were diabetic. 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 received losartan (2 mg/kg, p.o.) for six weeks. Group V-VII received three different doses of TG (50, 100 and 200 mg/kg/day, p.o., respectively) for six weeks.

2.5 Biochemical parameters from blood

Blood was withdrawn under light ether anesthesia by puncturing retro-orbital plexus. Estimation of plasma glucose (GOD/POD

method), serum total cholesterol (COD/POD method), triglyceride (GPO/POD method), HDL-cholesterol (COD/POD method) was done using standard diagnostic kits from Biolab Diagnostics (P) Ltd., India. LDL-cholesterol was calculated using Friedwald formula [12].

2.6 Atherogenic index

Atherogenic index was calculated by using formula as described previously [25].

Atherogenic index = {Total Cholesterol / HDL-cholesterol} - HDL-cholesterol

2.7 Study of hemodyamic parameters

At the end of the six-week treatment, blood pressure was measured according to the procedure described previously [2] Balaraman et al., 1989. Rats of all the experimental groups were anaesthetized by urethane (1.75 gm/kg, i.p.) and the temperature was maintained at 37^0C throughout the experiment. The left carotid artery was cannulated with polyethylene tube which was filled with 0.9% v/v heparinized saline. Systolic and diastolic blood pressure was directly measured from left common carotid artery by connecting the polyethylene tubing to precalibrated pressure transducer SS13L (BIOPAC system, Inc., CA, USA) connected to Biopac MP-30 data acquisition system (BIOPAC Systems, Inc).

2.8 Study of biochemical parameters from heart

The animals were sacrificed with overdose of urethane. The heart was removed. 10% homogenate of heart 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).

2.9 Histopathological studies

Heart 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.

2.10 Statistical analysis

The results were expressed as mean ± SEM and statistically analyzed by ANOVA followed by Dunnett test, with level of significance set at $p<0.05$.

3. Results

3.1. Effect of TG on biochemical parameters

Diabetic control showed significant increase ($p<0.01$) in blood glucose level, cholesterol, LDL-cholesterol and decrease in HDL-cholesterol when compared with normal control group. Glimipride showed significant decrease ($p<0.01$) in plasma blood glucose level from day 15 onwards when compared with diabetic control. It also showed significant decrease in serum cholesterol, LDL-cholesterol along with significant increase ($p<0.01$) in HDL-cholesterol as well as atherogeinc index. Treatment with TG for six weeks showed significant dose dependant decrease ($p<0.01$) in plasma glucose level when compared with diabetic control. From day 30 onwards, Losartan treated group showed decrease ($p<0.1$) in blood glucose level. Diabetic animals treated with TG showed significant decrease ($p<0.01$) in levels of serum cholesterol and serum LDL-cholesterol along with significant increase ($p<0.01$) in HDL-cholesterol levels and hence showed significant decrease ($p<0.01$) in atherogenic index.

3.2. Effect of TG on hemodyamic parameters

In the diabetic control group there was significant increase ($p<0.01$) in systolic, diastolic and mean blood pressure when

compared with control. Losartan treated group showed decrease ($p<0.01$) in blood glucose level. Treatment with TG for 42 days showed significant reduction ($p<0.01$) in systolic and diastolic blood pressure in a dose dependant manner, when compared with diabetic control.

3.3. Effect of TG on tissue parameters

There was significant increase ($p<0.01$) in the heart triglyceride and cholesterol content in the diabetic control group when compared with control. Glimipride showed significant decrease ($p<0.01$) in heart triglyceride and cholesterol content when compared with diabetic control. Losartan treated group amelioration of heart triglyceride and cholesterol content ($p<0.05$) when compared with diabetic control. Treatment with TG for 42 days showed significant reduction ($p<0.01$) in the heart triglyceride and cholesterol content, in a dose dependant manner, when compared with diabetic control.

3.4. Effect of Tectona grandis *on Histopathology of heart*

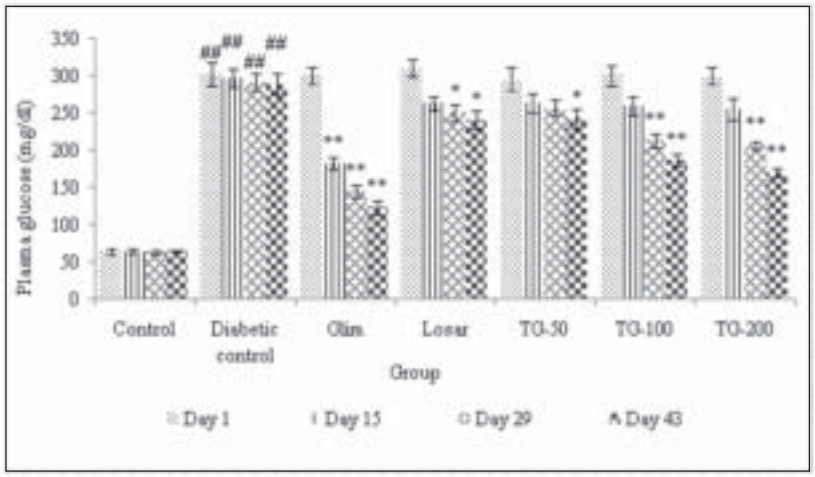
Histopathological examination showed significant myocardial necrosis and infiltration of inflammatory cells along with vacuolization in the D-control group as compared to Control group. Also extensive myonecrosis with fibroblastic proliferation and presence of chronic inflammatory cells were observed in the Diabetic control group compared to that of Control group. In the present study, TG (100 and 200 mg/kg,) and glimepride treatment significantly prevent myonecrosis as indicated by significant reduction in the infiltration and inflammatory cells, vacuolar changes as compared to D-control group. Nondiabetic groups treated with TG showed normal structure of myocardial fibers.

Table 1: Effect of *Tectona grandis* Linn. on atherogenic index in alloxan-induced diabetic rats.

Groups	Atherogenic index on			
	Day-1	Day-15	Day-29	Day-43
Control	0.21±0.003	0.20±0.004	0.20±0.002	0.22 ± 0.003
Diabetic control	0.25±0.003	2.45±0.008$^{\#\#}$	3.08±0.006$^{\#\#}$	4.36 ± 0.007$^{\#\#}$
Glim	0.21±0.005	1.37±0.009**	1.22±0.003**	1.13 ± 0.005**
Losar	0.24±0.006	1.47±0.006**	1.76±0.004**	1.50 ± 0.002**
TG-50	0.21±0.002	2.69±0.006	2.55±0.002**	2.51 ± 0.003**
TG-100	0.21±0.004	1.91±0.007**	1.78±0.004**	1.64 ± 0.002**
TG-200	0.24±0.003	1.47±0.006**	1.44±0.003**	1.16 ± 0.002**

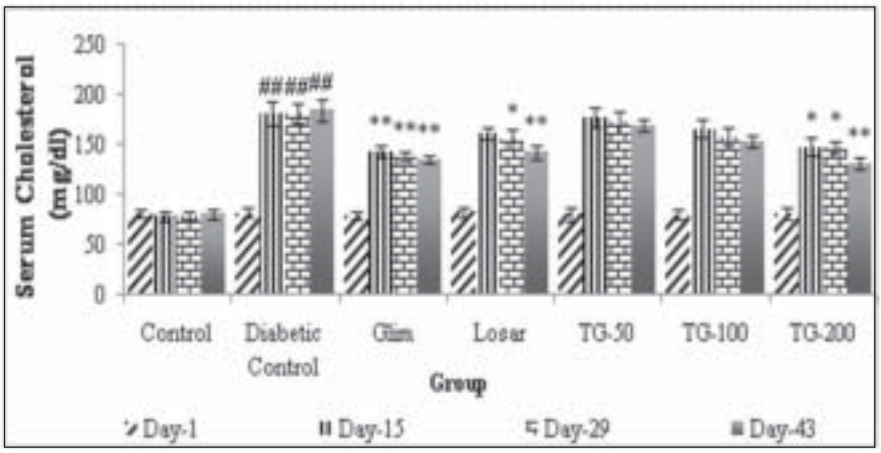
Values are expressed as mean ± SEM. (n=5)
ANOVA followed by Dunnett test.
$^{\#}p<0.05$, $^{\#\#}p<0.01$ when compared with control.
$^{*}p<0.05$, $^{**}p<0.01$ when compared with diabetic control.

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



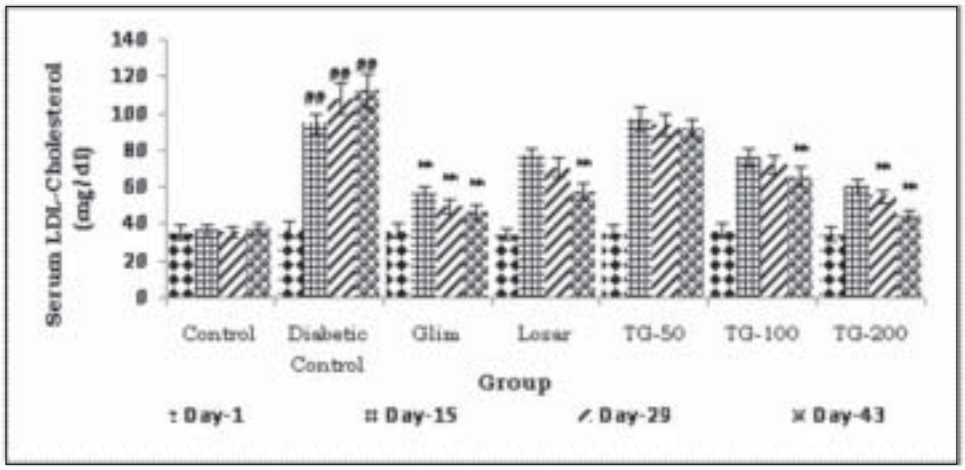
Values are expressed as mean ± SEM. (n=5),
ANOVA followed by Dunnett test.
$^{\#}p<0.05$, $^{\#\#}p<0.01$ when compared with control.
$^{*}p<0.05$, $^{**}p<0.01$ when compared with diabetic control.

Figure 2: Effect of *Tectona grandis* Linn. on serum cholesterol level in alloxan induced diabetic rats.



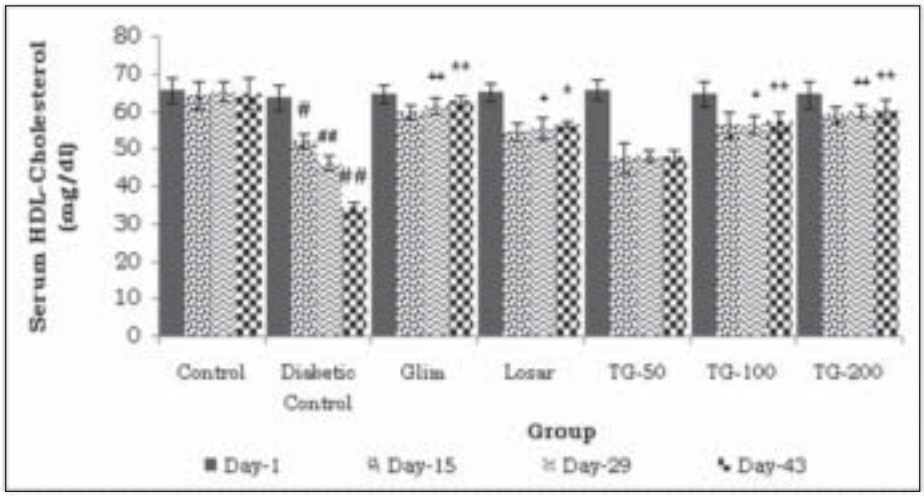
Values are expressed as mean ± SEM. (n=5),
ANOVA followed by Dunnett test.
$^{\#}p<0.05$, $^{\#\#}p<0.01$ when compared with control.
$^{*}p<0.05$, $^{**}p<0.01$ when compared with diabetic control.

Figure 3: Effect of *Tectona grandis* Linn. on serum LDL-cholesterol level in alloxan induced diabetic rats.



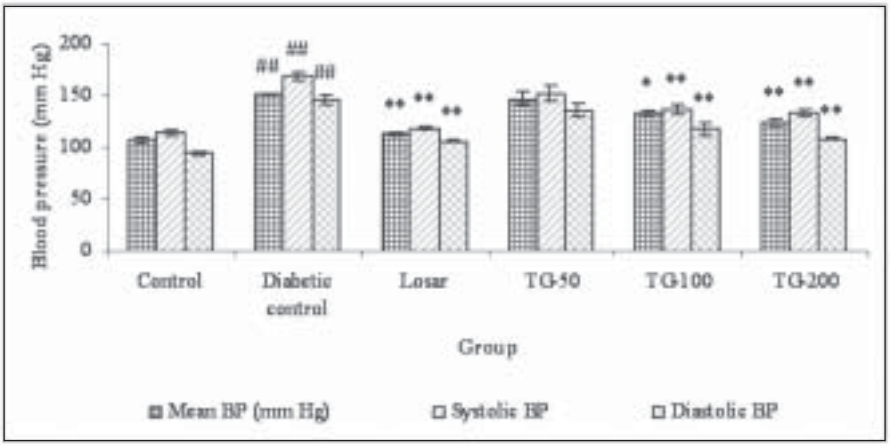
Values are expressed as mean ± SEM. (n=5)
ANOVA followed by Dunnett test.
$^{\#}p<0.05$, $^{\#\#}p<0.01$ when compared with control.
$^{*}p<0.05$, $^{**}p<0.01$ when compared with diabetic control.

Figure 4: Effect of *Tectona grandis* Linn. on serum HDL-cholesterol level in alloxan induced diabetic rats.



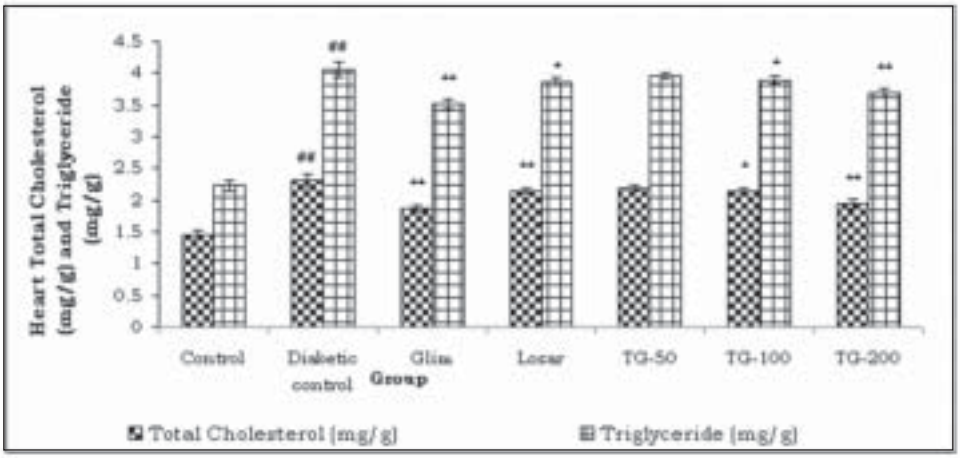
Values are expressed as mean ± SEM. (n=5)
ANOVA followed by Dunnett test.
$^{\#}p<0.05$, $^{\#\#}p<0.01$ when compared with control.
$^{*}p<0.05$, $^{**}p<0.01$ when compared with diabetic control.

Figure 5: Effect of *Tectona grandis* Linn. on blood pressure in alloxan-induced diabetic rats



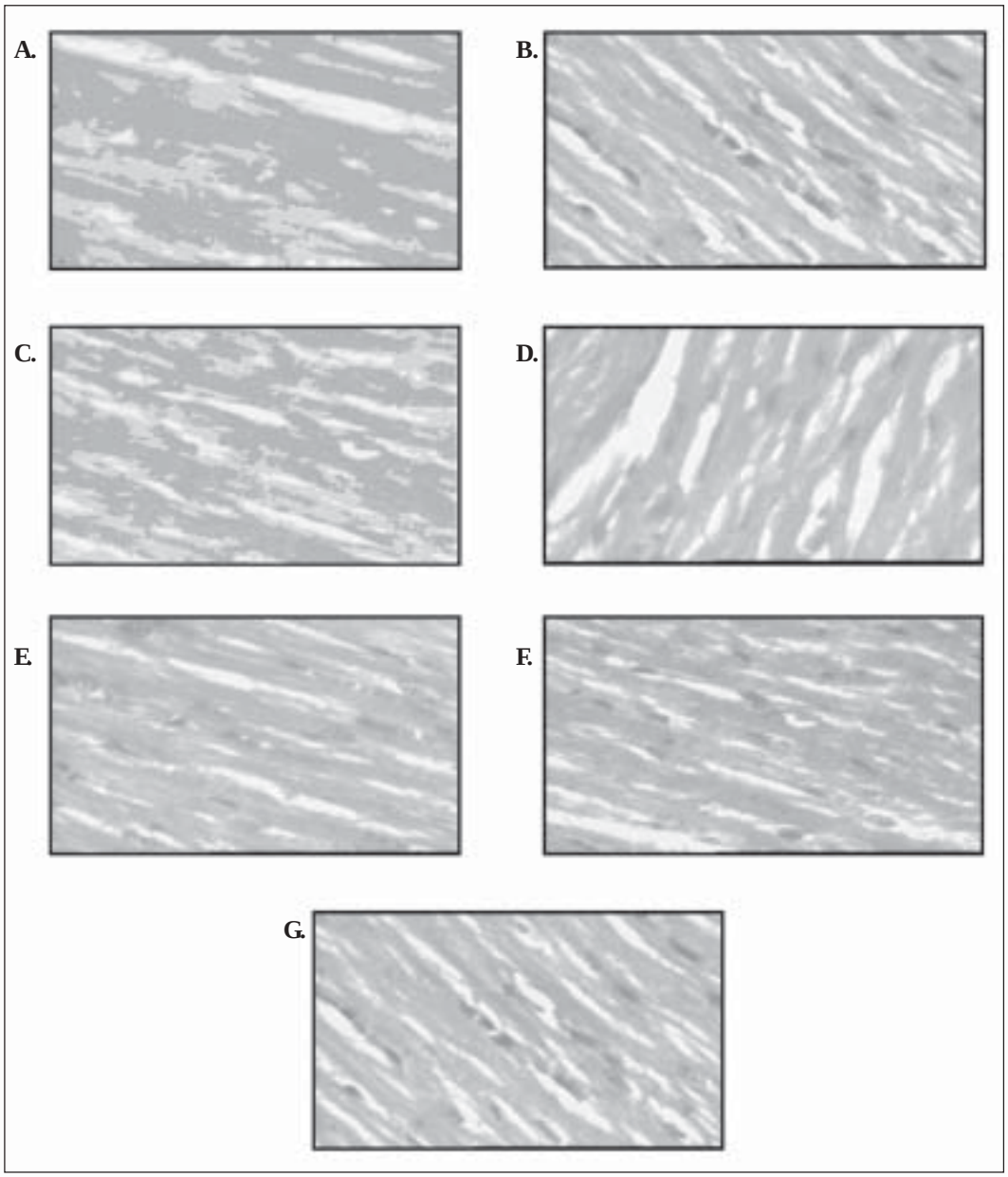
Values are expressed as mean ± SEM. (n=5)
ANOVA followed by Dunnett test.
$^{\#}p<0.05$, $^{\#\#}p<0.01$ when compared with control.
$^{*}p<0.05$, $^{**}p<0.01$ when compared with Diabetic control.

Figure 6: Effect of *Tectona grandis* Linn. on total cholesterol and triglyceride levels in heart homogenate in alloxan-induced diabetic rats



Values are expressed as mean ± SEM. (n=5)
ANOVA followed by Dunnett test.
$^{\#}p<0.05$, $^{\#\#}p<0.01$ when compared with control.
$^{*}p<0.05$, $^{**}p<0.01$ when compared with diabetic control.

Figure 7: Effect of *Tectona grandis* Linn. on histopathological studies of heart in Alloxan-induced diabetic rats.



A- control (1ml/kg, gum acacia); B-diabetic control (1ml/kg, gum acacia); C-glimipride (0.09mg/kg); D-losartan (2 mg/kg); E, F and G-(Ethanol extract of *Tectona grandis* Linn. 50, 100 and 200 mg/kg respectively).

4. Discussion

Clinical trials have identified hyperglycemia as the key determinant in the development of chronic diabetic complications [4]. In the present study TG administration caused significant decrease in plasma glucose level in a dose dependant manner. The glucose lowering effect of TG may be due to its ability to improve insulin sensitivity [13] and the presence of chemical constituents like terpenoinds and tannins which are reported to possess antihyperglycemic activity [23].

Accumulation of triglycerides is one of the risk factors in Coronary Heart Disease (CHD). Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic rats [19]. Diabetes induced hyperlipidemia is attributed to excess mobilization of fat from adipose tissue due to less utilization of glucose [27]. In diabetes, level of serum HDL is decreased and due to decrease in level of HDL, chances of heart disease increases [14]. In the present study, there was hypertriglyceridemia and hypercholesterolemia and decrease in serum HDL in the diabetic control group. Similarly, diabetic control showed significant increase in heart cholesterol and triglyceride level when compared to control. The increase in the triglyceride and total cholesterol in hearts of diabetic rats is attributed to increased lipid mobilization. The treatment with TG significantly reduced the hypertriglyceridemia and hypercholesterolemia while it increased the serum HDL level; along with decrease in the triglyceride and total cholesterol in heart. These results indicate that TG has potential to prevent the diabetes-induced dyslipidemia which may be probably due to the glycemic control exerted by it.

Atherogenic Index is a marker of plasma atherogenicity. Diabetic dyslipidemia is generally characterized by increased serum triglyceride and decreased HDL-cholesterol concentration, a preponderance of LDL-cholesterol, and increased apolipoprotein B concentration which farther leads to formation of atherogenic plaque in the arteries causing cardiovascular complication [32]. In present investigation diabetic control group had high atherogenic index as compared to control. TG treated groups showed significantly lower atherogenic index as compared with diabetic control probably due to increase in HDL-cholesterol which is beneficial for scavenging LDL-cholesterol from periphery towards liver for excretion.

Diabetic dyslipidemia may cause elaboration of vasoactive factors, defective glucose transport, increased myocyte free acid uptake and altered calcium uptake which leads to vascular changes [29]. Hyperglycemia is reported to induce oxidative stress through multiple pathways such as redox imbalances secondary to enhanced aldose reductase activity [36], increased advanced glycation end products [5] and altered protein kinase C activity, particularly ß-isoforms [8], prostanoid imbalances and mitochondrial overproduction of superoxide [18]. These pathways converge together and further cause NF-kB activation and COX-2 up regulation with increased production of vasoconstrcting PGH_2, thromboxane $A_{2,}$ thereby favoring vasoconstriction and ischemia in mayocardium. All these changes lead to hemodyamic and structural alterations in the heart [16]. In the present study, diabetic control group showed significant increase in the systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure when compared with the control. Treatment with TG significantly reduced the SAP, DAP and MAP in a dose dependant manner probably due to antioxidant potential of tannins and saponins in ethanol extract of *Tectona grandis;* which are reported to play a major role

in reducing oxidative stress associated with diabetes [6, 37]. TG is reported to increase in the levels of various antioxidant enzymes, which could be beneficial in countering, the hyperglycemia induced oxidative stress [13].

Hence, from the results obtained in the present study, it can be concluded that *Tectona grandis* Linn. may prevent the diabetic cardiac dysfunction owing to its antioxidant activity along with its ability to exert glycemic control and reduce the atherogenic index due to the control over dislipidemia.

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