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Research Article

ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF TECTONA GRANDIS LINN. IN ALLOXAN INDUCED ALBINO RATS

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ABSTRACT

Objective: To evaluate the *in vivo* antioxidant and antidiabetic activity of the methanolic bark extract of *Tectona grandis* Linn. (Family: Verbenaceae). Methods: The albino rats divided into four groups (Normal, Diabetic control, Diabetic+TGM (150mg) and Diabetic+TGM(300mg)) and the diabetic was induced by alloxan (120mg/kg i.p) and treated with TGM for 20 days. Then the antioxidant and antidiabetic parameters were evaluated by standard protocol. Results: The altered parameters i.e., blood glucose, glycosylated hemoglobin, protein, total cholesterol, urea, Serum creatinine, Aspartate Transaminase (AST), Alanine Transaminase (ALT), Lactate Dehydrogenase (LDH), Thiobarbituritic acid resactive substances (TBARS), Superoxide dismutase (SOD), Catalase and Glucose 6 phosphatase (G6P) were significantly ($p<0.05$) significantly ($p<0.05$) controlled by TGM (300mg/kg). Conclusion: The methanolic bark extract of *Tectona grandis* Linn. showed potent antioxidant and antidiabetic in alloxan induced diabetic rats and it can be used for the drug discovery development.

Keywords: antidiabetic, antioxidant, alloxan, glucose and *T.grandis*.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders that result in hyperglycemia due to decreased insulin production or inefficient insulin utilization. The World Health Organization predicted that DM affects approximately 171 million people worldwide and the number is expected to reach to 366 million in 2030 [1]. In diabetes, hyperglycaemia generates reactive oxygen species (ROS) which in turn cause lipid peroxidation and membrane damage and thus, plays an important role in the production of secondary complications in diabetes mellitus such as kidney, eye, blood vessel, and nerve damage. Antioxidants have been shown to prevent the destruction of β-cells by inhibiting the peroxidation chain reaction and thus they may provide protection against the development of diabetes. Plants containing natural antioxidants (tannins, flavonoids, vitamins C and E) can preserve β-cells function and prevent diabetes induced ROS formation [2, 3].

In general, current type 2 diabetic drugs are not only expensive but also they have their limitations and are known to produce serious side effects, therefore, the search for safer, specific and effective hypoglycemic agents has continued to be an important area of investigation with natural extracts from readily available traditional medicinal plants offering potentials for discovery of new antidiabetic drugs[(4, 5].

The medicinal values of *T.grandis* are not much known to the scientific world. (Family: Verbenaceae) Traditionally, *T.grandis* (TG) is used in the treatment of diabetes, lipid disorders, inflammation, ulcer, and bronchitis [6]. The bark of TG reported for the antihyperglycemic activity [7] and the root of TG reported for the hypoglycemic activity [8]. However, there is lack of information regarding antioxidant related to antidiabetic activity, hence the present study designed to investigated the antidiabetic and antioxidant activity of TG bark methanol extract in alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant collection and Extract preparation

Tectona grandis L. bark was collected from the medicinal garden at the JJ College of Arts and Science, Pudukkottai, Tamil Nadu, India. The bark was shade dried, coarsely powdered and then extracted with Methanol (20g in 80mL Solvent) by using soxhlet apparatus at 65°C. Then the TG bark methanol (TGM) extract was concentrated and the solid solvent free extract was stored in an airtight container at -4°C until use.

Animals

Male *Albino Wistar* strain rats (50-60 days old) were obtained from "Sri Venkateswara Enterprises", Bangalore, India. They were housed in plastic cages under controlled conditions (28±2^{0}C, 50% humidity and 12 h light/12h dark cycle) fed with normal rat chow marketed by Hindustan Lever Limited, Mumbai, India and were provided with clean drinking water *ad libitum*. The animals care and procedure of the whole experiment followed as per the principles and guidelines of the ethical committee of Bharathidasan University (Tamil Nadu, India) and Indian National Law on animal care and use (CPCSEA).

Diabetes induction in rats

The alloxan is widely used to induce diabetes (type I) in the experimental laboratory animal model [9]. The rats were injected (intraperitonelly) with alloxan monohydrate dissolved in sterile normal saline (120mg/kg b.w.). The rat were observed for four days, then the moderate diabetes of rats observed by Benedict's test [10] for urine glucose and then the blood was collected from tail vein for glucose estimation (Strip method). After a week the blood glucose range 250-300mg/dl rats were used for the experiment.

Experimental design

The rats were divided into four groups with 6 rats in each as follows: Group I: Normal rats; Group II: Diabetic Control rats**;** Group III: Diabetic + TGM (150mg/Kg body weight) for 20days; Group IV: Diabetic + TGM (300mg/Kg body weight) for 20 days.

At the end of the experimental period all rats were sacrificed by cervical decapitation and the serum was collected from the blood. Immediately liver was removed and washed with ice-cold physiological saline. They were homogenized in 0.1M tris-Hcl buffer pH 7.4 to give a 10% homogenate. This homogenate was used for the appropriate parameter estimation.

Evaluation of Marker Enzymes, Biochemical Assays and Antioxidant levels

The Blood glucose was determined by the method of O-toluidine using the modified reagent [11], Glycosylated hemoglobin was determined by the method of Sudhakar and Pattabiraman [12] with modifications according to Bannon [13], Protein in the enzyme extract was determined by the method of Lowry et al.[14], total Cholesterol [15], Serum urea was estimated by using the diagnostic kit based on the method of Tomas(a) [16], the level of uric acid is by the method of Fossati et al. [17], Serum creatinine was estimated using the diagnostic kit based on the method of Tomas(b) [18], Activities of Aspartate Transaminase (AST) and Alanine Transaminase (ALT) were assayed by the method of Moss and Henderson [19], the Lactate Dehydrogenase (LDH) [20] the levels of Thiobarbituritic acid reactive substances (TBARS) in tissues were estimated by the method of Ohkawa et al.[21], Superoxide dismutase (SOD) activity was determined by the method of Kakkar et al. [22]. The activity of Catalase was determined by the method of Sinha [23]. The Glucose 6 phosphatase (G6P) was quantified by Baginsky et al. [24].

Statistical Analysis

All values are given as means±standard error (S.E.) for each group. Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple-range test (DMRT) using the SPSS software (version 16.0). P values of less than 0.05 were considered to indicate statistically significant differences.

RESULTS

A significant changes ($p<0.05$) were observed in the concentration of serum glucose in alloxan induced diabetic rats. The elevated glucose level controlled by TGM (300mg/kg b.w.) significantly ($p<0.05$) (Table 1).

Table 1: Effect of TGM extract on biochemical parameters (blood glucose, glycosylated hemoglobin, urea, uric acid, creatinine, total protein and cholesterol) in serum

Groups	Biochemical parameters in Serum (mg/dl)						
	Glucose	Gly.Hb	Urea	Uric acid	Creatinine	Total protein	Cholesterol
Group I	74.25 ± 2.98 d	6.42 ± 0.34 c	29.25 ± 2.62 d	3.52 ± 0.29 d	0.67 ± 0.17 c	6.02 ± 0.09 a	115.52 ± 2.88 d
Group II	299.34 ± 9.05 a	10.63 ± 0.55 a	64.75 ± 2.87 a	6.09 ± 0.18 a	1.72 ± 0.19 a	2.91 ± 0.16 d	258.54 ±1.48 a
Group III	210.72 ± 4.08 b	9.82 ± 0.29 ab	45.75 ± 3.59 b	5.32 ± 0.20 b	1.33 ± 0.03 b	3.47 ± 0.33 c	213.75 ± 2.73 b
Group IV	107.25 ± 5.56 c	6.75 ± 0.28 b	35.83 ± 1.63 c	4.04 ± 0.06 c	0.62 ± 0.25 cb	5.60 ± 0.59 b	130.25 ± 2.50 c

Values are expressed in Mean ± S.D; n=6; In each column, values (a,b,c & d) that do not share a common superscript differ significantly at P < 0.05 (ANOVA followed by DMRT).

Glycosylated hemoglobin is increased in alloxan treated diabetic rats. Administration of TGM (300mg/kg b.w.) for 20 days showed significant ($p<0.05$) control in glycosylated hemoglobin, thereby increasing the levels of total hemoglobin in diabetic rats were controlled (Table 1). This could be due to the result of improved glycemic control produced by the TG. Table 1, showed significant ($p<0.05$) increase in the level of serum urea, uric acid and creatinine in the diabetic groups were significantly ($p<0.05$) decreased by TGM and also the treatment of alloxan induced diabetic rats with the TGM extract significantly ($p<0.05$) increased the levels of protein in diabetic group (Table 1).

One of the possible actions of TGM extract may be due to its inhibition of endogenous synthesis of cholesterol and enhancement of the degradation of formed cholesterol by increasing the excretion through intestinal tract (Table 1) and also the serum levels of AST and ALT, the hepatic enzyme markers showed a significant ($p<0.05$) decrease in *Tectona grandis* treatment of diabetes mellitus (Table 2).

Table 2: Effect of TGM extract on liver marker enzymes in serum

Groups	Marker enzymes		
	AST (U min/mg of Protein)	ALT(U min/mg of Protein)	LDH (U min/mg of Protein)
Group I	32.75 ± 8.53d	65.25 ± 3.30d	283.25 ± 6.34d
GroupII	181.75 ± 8.13a	161.75 ± 4.11a	525.37± 2.81a
GroupIII	94.52± 7.18b	102.75 ± 5.73b	383.75 ± 5.43b
GroupIV	36.58± 2.16c	66.52± 4.50c	252.74± 3.74c

Values are expressed in Mean ± S.D; n=6; In each column, values that do not share a common superscript differ significantly at P < 0.05 (ANOVA followed by DMRT).

Table 3: Levels of SOD, CAT and TBARS in liver tissues of control and experimental group of rats

Groups	G6P (U/mg of protein)	SOD (U min/mg of Protein)	Catalase (U min/mg of Protein)	TBARS (nmol/ml)
Group I	2.64 ± 0.09a	45.84 ± 0.26a	35.75 ± 0.34a	1.88 ± 0.50d
GroupII	1.44 ± 0.06c	22.85 ± 0.05d	20.38 ± 0.29d	3.29 ± 0.46a
GroupIII	1.93 ± 0.03b	36.47 ± 0.28c	28.41 ± 0.29c	2.38 ± 0.32b
GroupIV	2.55 ± 0.28ab	41.13±0.08b	33.47 ± 0.37b	1.68 ± 0.32c

Values are expressed in Mean ± S.D; n=6; In each column, values that do not share a common superscript differ significantly at P < 0.05 (ANOVA followed by DMRT).

The treatment of alloxan induced diabetic rats with the TGM extract and significantly ($p<0.05$) decreased the LDH (Table 2) and glucose - 6 - phosphatase (Table 3) levels on diabetic group. Experimental results also reflect that the *Tectona grandis* is capable of reducing the oxidative state associated with diabetes (Table 3). The reduction of thiobarbituric acid levels in tissues in the TGM extract treated diabetic group ensures the antioxidant potential of the *Tectona grandis*. Alloxan produces diabetes by liberating oxygen free radicals, which cause lipid peroxide mediated pancreatic injury. The TGM extract may scavenge free radicals and facilitate reconstruction of pancreatic cells to release more insulin and ultimately produces antidiabetic effect.

DISCUSSION

Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin will be secreted [25]. However, Alloxan is an oxygenated pyrimidine derivative betacytotoxin and is known to induce diabetes mellitus in a wide variety of animal species through the damage of pancreatic β-cells [26]. When there are not enough available beta-cells to supply sufficient insulin to meet the needs of the body, insulin-dependent diabetes results [27].In the present study, results of the experiment indicated the significant antidiabetic and antioxidant activity of TGM (300 mg/kg b.w.). Since, the experiment focused on exploring the

competence of methanol extract of *Tectona grandis* for the treatment of diabetes and relative complications like oxidative stress to substantiate folklore claim.

The elevated glucose level was successfully controlled by the TGM extract and several investigators have recommended that glycosylated hemoglobin to be used as an indicator since glycohemoglobic control of diabetes since glycohemoglobin levels approach normal values in diabetics in metabolic control [28]. So in our case also the TGM controlled the glycosylated hemoglobin.

The diabetic hyperglycemia induces elevation of the serum levels of urea, uric acid and creatinine, which were considered as significant markers of renal function [29]. The diabetic hyperglycemia induces elevation of the plasma levels of urea, uric acid and creatinine which are significant markers of renal dysfunction and reflecting a decline in the glomerular filteration rate were significantly recovered by *Tectona grandis*.

Protein synthesis is decreased in all tissues due to absolute or relative deficiency of insulin in alloxan induced diabetic rats [30], the TGM increased the protein level in blood. Hypercholesterolemia has been reported to occur in alloxan diabetic rats and marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots [31], the TGM decreased the total cholesterol in the diabetic rats.

Elevated activities of serum aminotransferases are a common sign of liver disease, and are more frequently observed among people with diabetes, than in the general population. Furthermore, diabetic complications such as limited joint mobility, retinopathy and neuropathy are associated with liver enzyme activities, independently of alcohol consumption, body mass index, and metabolic control of diabetes [32]. Besides, it has been shown that the alloxan injection causes a significant increase in the activity of several enzymes, such as beta-glucuronidase, N-acetyl-beta-glucosaminidase, lysosomal acid phosphatase, leucine aminopeptidase, and cathepsin D [33]. Moreover, the activities of the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes, among others, have been used as indicators of tissue toxicity in experimental diabetes. The levels of AST, ALT and alkaline phosphatase (ALP) were higher in streptozotocin-induced diabetic rats, over a 53-day period [34]. So in the present study the liver marker enzymes elevation are controlled by the TGM extract. Higher activities of lipogenic enzymes like G6P (glucose -6- phosphatase) and LDH provides hydrogen, which binds with NADP+ in the form of NADPH and is helpful in the synthesis of fats from carbohydrates. When glycolysis slows down because of cellular inactivity, the pentose phosphate pathway still remains active in liver to break down glucose that continuously provides NADPH which converts acetyl radicals into long fatty acid chains. The high serum and tissue levels of lipogenic enzymes in Alloxan diabetic rats suggests up regulation of NADPH and enhanced lipogenesis [35].

In our study, an increased activity of glucose -6- phosphatase was observed in the liver of alloxan induced diabetic rats. Glucose -6-phosphatase, one of the key enzymes in the homeostatic regulation of blood glucose level, catalyzes the terminal step in both gluconeogenesis and glycogenolysis. Increased activities of glucose-6-phosphatase in the liver may be due to activation or increased synthesis of the enzymes contributing to the elevated glucose production in diabetes, so the TGM extract decreased the activity of glucose -6- phosphatase.

The increase in the levels of lipid peroxidation might be indicative of a decrease in the enzymatic antioxidant defence mechanism [36]. Several studies have indicated that oxygen free radicals are generated in diabetic β-cells, and that the overexpression of antioxidant enzymes, such SOD, CAT, and GPx, plays an important role in protecting cells from oxidative damage [37-39]. In the present study, it was observed that the TGM extract could increase the SOD and CAT activities in the liver tissues of diabetic rats. This indicates that TGM extract could inhibit or reduce the oxidative stress in diabetes [38]. The activities of both SOD and CAT were augmented in diabetic rats which could be attributed to the strong antioxidative properties [40].

Researchers are interested in search of new drugs from medicinal plants for their biological activities like antioxidant and antidiabetic [41, 42]. In this study, we have evaluated the anti oxidant and anti diabetic effect of bark of TGM extract in alloxan induced rats. A new compound (Abeograndinoic acid) and 21 known terpenoids were isolated from the bark of TG [43]. The enriched secondary metabolites may be responsible for the anti-diabetic and anti-oxidant activity of TG. The methanolic extract of TG bark exhibited significant antidiabetic and antioxidant activity in alloxan induced diabetic rats.

CONCLUSION

Methanol extract of *Tictona grandis* has shown optimistic antidiabetic and antioxidant properties in alloxan induced diabetes in rat models. Further investigations are in process to know the active principles. The active principles may be single compound or synergetic activity (group of compounds).

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