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EVALUATION OF ANTI-DIABETIC AND HEPATO PROTECTIVE ACTIVITY OF 95% METHANOLIC EXTRACT OF TERMINALIA TOMENTOSA BARK BY USING ALBINO RATS

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ABSTRACT

The anti-diabetic and hepatoprotective activity of the Methanolic extract of the bark of *Terminalia tomentosa* (family: combrataceae) was investigated on alloxan induced diabetic albino rats. A comparison was made between both plant extract and a known antidiabetic drug glibenclamide (5mg/kg^{-1}). The dried bark of *Terminalia tomentosa* was subjected to extraction by continuous hot extraction method using methanol as a solvent. Phytochemical estimation was done for the presence of phytoconstituents. Dose selection was made on the basis of acute oral toxicity study (250mg/kg^{-1}, 500mg/kg^{-1} bodyweight) as per OECD guidelines. Oral administration of extract of *Terminalia tomentosa* for 21days resulted in significant reduction in blood glucose level. Alloxan induced diabetic rat model and oral glucose tolerance test (OGTT) model was used for evaluation of antidiabetic activity. The biochemical parameters were analysed. All rats in the diabetic groups had FBG levels well within the diabetic range (>150 mg dL^{-1}) at the initial stage of the experiment but after three weeks of treatment with extracts or glibenclamide the FBG significantly dropped in dose-dependent manner, and also correct the lipid profile and liver enzymes. The results suggest that the Methanol extracts of the bark of *Terminalia tomentosa* restored the metabolic changes in alloxan-induced diabetic rats**.**

Key words: *Terminalia tomentosa*, glibenclamide, alloxan, anti-diabetic activity

INTRODUCTION

Diabetes mellitus is a metabolic disorder initially characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. In 2005, an estimated 1.1 million people died from diabetes with a projected rise in deaths of 50% over the next 10 years. It is estimated that at least 1 in 20 deaths, globally and across all ages, are attributable to diabetes. Diabetes induced by alloxan, it produce oxidative free radicals. This free radicals damage the β-Cells of pancreas. In present study *Terminalia tomentosa* bark was selected for anti diabetic activity because it is having anti oxidant property. Due to this anti oxidant property the oxidative species were reduced so that β-Cells were recovered.

MATERIALS AND METHODS

Chemicals: Alloxan monohydrate, glibenclamide, glucose, all other chemicals and reagents used were analytical grade.

Plant material: The stem bark of *Terminalia tomentosa* (Roxb.) Wight & Arn. Belonging to Combrataceae were collected from Tirumala Hills, Chithoor district (A.P). The plant material was identified and authenticated by Dr. Prathibha (Professor and H.O.D. of Department of Botany Osmania University, Hyderabad, India.

Preparation of plant extraction: The collected bark was shade dried and powdered in mixer grinder to get coarse powder. The powdered plant material (100gms) was extracted with methanol (95%v/v) by using soxhlet apparatus. The extract was air dried to evaporate solvent.

Phytochemical screening: The preliminary phytochemical screening of methanolic extract of *Terminalia tomentosa* was carried by using standard procedures.

Acute Toxicity Study: toxicity studies were performed according to OECD-425guidelines.

Experimental model: Alloxan monohydrate was weighed individually for each animal according to their body weight and solubilised with saline just prior to injection. Diabetes was induced by injecting it at a dose of 150 mg/kg body weight intrapertonially. The animals were kept under observation and after 48 hrs blood glucose level was measured by One-touch glucometer. The diabetic rats (glucose level 200-300 mg/dl) were separated and divided into five different groups for experimental studies, with each group containing six animals. Present study has confirmed that the treatment of methanolic extract of *Terminalia tomentosa* for a period of 3weeks caused a significant decreased in BGL (Blood glucose level) of diabetic rats. 250&500 mg/kg of plant extract were screened for anti diabetic activity against alloxan induced diabetic rats. It produced significant anti diabetic activity in a dose dependent manner. The animals treated with alloxan had high BGL. The anti diabetic activity exhibited by extract was compared with that of standard drug (glibenclamide).

Experimental design: The rats were divided into five groups each consist of six rats. Significant hyperglycaemia was achieved within 48 hrs after alloxan (150 mg/kg body weight i.p) injection.

Group I- Served as normal control and did not receive any treatment
Group II- Served as diabetic control and received Inducer (alloxan-150mg/kg) and vehicle
Group III- Alloxan + Glibenclamide (5 mg/kg p.o.) and served as standard
Group IV- Alloxan + 95% MTT extract (250 mg/kg, p.o.)
Group V- Alloxan + 95% MTT extract (500 mg/kg, p.o.)

The results obtained were comparable and satisfied the standard **Statistical Analysis:** The result of the study were subjected to one way analysis of variance (ANOVA) fallowed by Dunnet's test for multiple comparisions. Values with $p<0.05$ were consider significant.

RESULTS AND SISCUSSION

Phytochemical Screening: Phytochemical screening of methanolic extract of *Terminalia tomentosa* showed the presence of various chemical constituents mainly alkaloids, Proteins, glycosides, phytosterols and saponins. Extract may be responsible literature.

Acute oral toxicity studies: In the present study the MTT was subjected for toxicity studies. For the LD50 dose determination MTT was administered the dose level of 1000 mg/kg and 2000 mg/kg body weight and both doses did not produce any mortality. Hence one-fourth of the dose tested i.e. 250mg/kg and 500mg/kg body weight was selected for the study in order to ascertain a scientific base for the useful of this plant in the treatment of diabetes. It was decided to evaluate experimental design of antidiabetic activity by Alloxan induced model.

Anti-diabetic activity:

Alloxan induced diabetic model: The anti-diabetic effect of MTT in alloxan induced diabetic animals is presented. The results showed that after single dose treatment of the extract in individual group of alloxan induced diabetic rats, there was a significant reduction in serum glucose levels throughout the entire period of study (21 days) as compared to diabetic control group. *Terminalia tomentosa* bark extract were screened for antidiabetic activity in rats Where Alloxan (150 mg/kg, i.p.) used as the diabetogenic agent.

In an alloxan induced diabetic rats (Gr. II) significantly increased serum glucose level at '0'day ($P<0.001$), 1st day ($P<0.001$), 7th day ($P<0.001$), 14th day ($P<0.001$) and 21st day ($P<0.001$) were shown in the Table No.1.

Glibenclamide at an oral dose 5 mg/kg reduced serum glucose level at '0'day ($P<0.001$), 1st day ($P<0.001$), 7th day ($P<0.001$), 14th day ($P<0.001$) and 21st day ($P<0.001$) significantly when compared with control respectively.

Administration of MTT 250 and 500 mg/kg orally reduced significantly serum glucose level at '0' day ($P>0.001$), ($P>0.001$), at 1st day ($P<0.001$), ($P<0.001$), at 7th day ($P<0.001$), ($P<0.001$), 14th day ($P<0.001$), ($P<0.001$) and 21st day ($P<0.001$), ($P<0.001$) when compared to (Gr. II) control respectively.

Oral glucose tolerance test (OGTT) model: The anti-diabetic effect of MTT in glucose induced diabetic animals is presented. The results showed that after single dose treatment of the extract in individual group of glucose induced diabetic rats, there was a significant reduction in glucose levels throughout the entire period of study (1440 min) as compared to diabetic control group.

In a glucose induced diabetic rats significantly increased serum glucose level at '0'min ($P<0.01$), 30 min ($P<0.001$), 60 min ($P<0.001$), 90 min ($P<0.001$), 120 min ($P<0.01$) and 1440 min ($P<0.001$) were shown in the Table No.2.

Glibenclamide at an oral dose 5 mg/kg reduced serum glucose level at '0'min ($P<0.01$), 30 min ($P<0.001$), 60 min ($P<0.001$), 90 min ($P<0.001$), 120 min ($P<0.001$) and 1440 min ($P<0.001$) significantly when compared with control respectively.

Administration of MTT 250 and 500 mg/kg orally reduced significantly serum glucose level at '0'min ($P<0.001$) ($P<0.001$), 30 min ($P<0.001$) ($P<0.001$), 60 min ($P<0.001$) ($P<0.001$), 90 min ($P<0.001$) ($P<0.001$), 120 min ($P<0.001$) ($P<0.001$) and 1440 min ($P<0.001$) ($P<0.001$) when compared with control respectively.

The results obtained from the present investigation demonstrated that the bark extract of *Terminalia tomentosa* constantly maintained significant reduction of the glucose level in alloxan and oral glucose mediated diabetic rats throughout the experimental period suggesting the antidiabetic property of the title plant. Diabetes mellitus causes failure to use of glucose for energy that leads to increased utilization and decreased storage of protein responsible for reduction of body weight essentially by depletion of the body proteins. In the present study, it was observed that the extract reversed the weight loss of the diabetic rats. Alloxan has been shown to induce free radical production and cause tissue injury. The pancreas is especially susceptible to the action of alloxan induced free radical damage. The dose of 150mg/kg of alloxan can induces an autoimmune process that results in the destruction of the β-cells of islets of Langerhans; it also results in the toxicity of beta cells with emergence of clinical diabetes within 2-4 days.

Table.1.Effect of 95% MTT extract on fasting blood glucose level in Alloxan induced diabetic rats

	Normal	Control (Allaxon-150mg/kg)	Standard (Glibenclamide-5mg/kg)	MTT (250mg/kg)	MTT (500mg/kg)
0 Day	83.5±0.84	371.83±0.72 [a]	254.17±0.64***	283.17±0.77***	260.83±0.64***
1st Day	79.83±0.44	355±0.82 [a]	240.17±0.64***	249.67±0.77***	235.83±0.86***
7th Day	74.83±1.01	298.33±1.05 [a]	179±0.67***	185.17±0.76***	154.5±0.87***
14th Day	71±0.47	232.16±0.89 [a]	175.5±0.61***	179.83±0.83***	149.5±0.87***
21st Day	79.5±0.39	234.5±2.10 [a]	120.33±0.77***	131.5±0.51***	121±0.47***

All the values are mean± SEM, n=6, One way ANOVA followed by multiple compression Dennett's test ***P<0.001 as compare to control. [a] P<0.001 when compared with normal.

Table.2. Effect of 95% MTT extract on ogtt in oral glucose induced diabetic rats

	Normal	Control (glucose-gm/kg)	standard (Glibenclamide-5mg/kg)	MTT (250mg/kg)	MTT (500mg/kg)
0 min	68±0.666	72±0.5270[b]	73.33±0.6085**	73.16±0.8633***	78.66±0.6526***
30 min	72±0.666	122±0.7817[a]	94.33±0.4513***	96.16±0.5485***	80±0.7453***
60 min	75.66±0.5610	140±0.5270[a]	80.5±0.6972***	91.33±0.6526***	79.33±0.4513***
90 min	77.66±0.5610	92.66±0.7698[a]	71.66±0.6085***	83.16±0.5485***	71.83±0.4356***
120 min	77±0.4082	80.16±0.6419[b]	61.66±0.6085***	71.5±0.6972***	66.16±0.6419***
1440 min	75.33±0.3849	70.5±0.6972 [a]	70.5±0.6971***	71.33±0.4513***	71.16±0.4356***

All the values are mean± SEM, n=6, One way ANOVA followed by multiple compression Dennett's test ***P<0.001, **P<0.01 as compare to control, [a] P<0.001, [b]P<0.01 when compared with normal.

Table.3.Effect of 95% MTT extract on lipid profile in alloxan induced diabetic rats

Parameters	TREATMENT				
	Normal	Control (Alloxan-150mg/kg)	Standard (Glibenclamide-5mg/kg)	MTT (250mg/kg)	MTT (500mg/kg)
Cholesterol	152.25±0.50	353.86±1.49[a]	163.58±0.60***	188.32±0.75***	157.74±0.78**
Tri glycerides	144.92±0.95	292.29±1.05[a]	151.07±0.79***	152.04±0.78***	163.73±0.81***
HDL Cholestol	48.87±0.58	19.42±0.65 [a]	65.45±0.59***	62.05±0.71***	62.89±0.73***
LDL Cholestrol	73.89±0.46	275.98±1.91[a]	67.89±0.64***	95.87±1.20***	62.09±1.34***
VLDL cholestrol	28.82±0.22	58.48±0.21 [a]	30.21±0.16***	30.41±0.16***	32.75±0.16***

All the values are mean± SEM, n=6, one way ANOVA followed by multiple compression Dennett's test ***P<0.001, **P<0.01 as compared to control, [a] P <0.001 when compared with normal

Table.4.Effect of 95% MTT extract on liver enzyme levels in Alloxan induced diabetic rats

Parameters	Treatment				
	Normal	Control (Alloxan-150mg/kg)	Standard (Glibenclamide-5mg/kg)	MTT (250mg/kg)	MTT (500mg/kg)
SGPT	15.13±0.23	58.55±0.72[a]	11.68±0.55***	21.77±0.38***	17.00±0.37***
SGOT	20.65±0.50	63.23±0.76[a]	22.45±0.63***	22.51±0.55***	26.05±0.79***
ALP	66.06±0.84	114.06±0.83[a]	107.33±0.77***	123.94±0.92***	106±0.42***
Total bilurubin	0.82±0.02	1.43±0.056[a]	1.28±0.08***	0.85±0.01***	0.43±0.01***
Direct bilurubin	0.25±0.01	0.33±0.01[a]	0.26±0.01***	0.37±0.01***	0.27±0.01***
Total protein	8.50±0.26	6.77±0.15[a]	7.88±0.30***	4.83±0.19***	6.28±0.032***

All the values are mean± SEM, n=6, one way ANOVA followed by multiple compression Dennett's test ***P<0.001 as compared to control. [a] P <0.001 when compared with normal

Table.5. Effect of 95% MTT extract on body weight of alloxan induced diabetic rats

	NORMAL	CONTROL (Allaxon-150mg/kg)	STANDARD (Glibenclamide-5mg/kg)	MTT (250mg/kg)	MTT (500mg/kg)
0 Day	231.66±5.97	210±7.0710[a]	231.66±5.485[***]	236.66±4.513[***]	235±5.1370[***]
1st Day	231.66±5.49	206.66±5.610[a]	230.33±6.0858[***]	233.33±4.5133[***]	231.66±4.953[***]
7th Day	233.33±6.94	205±5.6519[a]	231.66±6.419[***]	235±3.908[***]	231.66±4.356[***]
14th Day	236.66±4.51	208.33±3.664[a]	228.33±6.419[***]	228.33±4.356[***]	228.333±3.664[***]
21st Day	240±6.666	200±8.0794[a]	230±6.666[***]	234.166±6.057[***]	240±3.333[***]

All the values are mean± SEM, n=6, one way ANOVA followed by multiple compression Dennett's test [***]P<0.001,[**]P<0.01 as compared to control. [a] P <0.001 when compared with normal.

CONCLUSION

The results of the present study indicated that *Terminalia tomentosa* bark extract possesses significant antidiabetic activity and hepatoprotective activity against alloxan induced diabetic rats. Thus justifies the traditional use of this plant in the treatment of diabetes mellitus. Bark extract of the title plant possesses almost equipotent antidiabetic activity when compared with reference standard Glibenclamide.

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