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Anti-diabetic Activity of *Terminalia catappa* Bark Extracts in Alloxan Induced Diabetic Rats.

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Research Article

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ABSTRACT

The present study was carried out to evaluate the antidiabetic activity of *Terminalia catappa bark* extracts in alloxan induced diabetic rats for 21 days period of study. The bark extract at a dose of (400mg/kg) exhibited significant anti-hyperglycemic activity and improvement in parameters like body weight and lipid profile which shows significant $p < 0.001$, on compared with normal control rats during treatment period

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by high blood glucose levels that result from defects in insulin secretion, action, or both. This dreadful disease is found in all parts of the world and is becoming a serious threat to mankind health. There are lots of chemical agents available to control and to treat diabetic patients, but total recovery from diabetes has not yet been reported. Alternative to these synthetic agents, plants provide a potential source of hypoglycemic drugs to prevent diabetes. The synthetic hypoglycemic agents produce serious side effects whereas drug derived from medicinal plants are frequently considered being safe and cost effective so traditional methods using medicinal plants to control diabetes is gaining momentum. Thus the present study is an attempt to test the antidiabetic activity of *Terminalia catappa bark* extract [1,2,3,4,5].

MATERIALS AND METHOD

The plant material was collected from the locally growing area of Kottayam district, Kerala. The plant was identified and authenticated by Raju Thomas, H.O.D, Department of Botany, Baselius College, Kottayam, Kerala. Herbarium sheet presented with UCP Cheruvandoor.

Preparation of extract

The bark was collected and shade dried. The shade-dried bark was subjected to pulverization to get coarse powder. The coarsely powdered *Terminalia catappa* bark 120 gm was used for extraction with ethanol and aqueous solvents in Soxhlet apparatus. The extracts obtained were collected and concentrated by using rotary evaporator and were stored in desiccators. An yield of 14.31% w/w and 9.62% w/w was obtained. These extracts were subjected to phytochemical screening to identify various chemical constituents [6].

Animals

Twenty four numbers of healthy female rats of Wistar albino strain weighing 150-200gms and three female albino mice weighing between 20-25gms were obtained from the animal house of Department of Pharmacology, UCP Cheruvandoor. They were housed in polycarbonate cages under standard conditions of temperature ($25 \pm 2^{\circ}$ C) and relative humidity (50-70%) with a 12:12 cycle. The animals were fed with standard pellet diet and water *ad libitum*. The procedure was approved by the Institutional Animal Ethics Committee (IAEC) of UCP Cheruvandoor was taken for conducting antidiabetic activity. After procurement, all the animals were divided into different groups and were left for one week for acclimatization to room and were maintained on standard conditions.

Acute toxicity study

This was performed to ascertain safe dose by the Organization of Economic Cooperation and Development (OECD) 423 guidelines. Swiss Albino Mice weighing between 20-25 g. They were kept fasting four hours prior to the treatment. A single administration of starting dose of 2000 mg/kg body weight/ p.o of the extract (ethanolic extract and aqueous extract was suspended in 1% CMC solution) was administered to three female mice. They were noted individually after dosing, at least once during the first 30 minutes, with special attention given during the first four hours and thereafter for a total of 14 days. There was no considerable change in body weight before and after treatment and no sign of toxicity was observed. When the experiment was repeated again with same dose level, 2000 mg/kg body weight/ p.o of plant extract for 7 more days and observed for fourteen days, no change was observed.

Experimental procedure

Diabetes was induced in rats by single intraperitoneal injection of alloxan monohydrate 120 mg/kg dissolved in sterile distilled water to over-night fasted rats. After 72 h of injection, rats with marked hyperglycemia (fasting blood glucose > 250 mg/dl) were selected and used for the study [7,8].

Experimental design

The animals were divided into five groups of six rats each.

- Group I** : Control normal rats administrated with 1ml 1% w/v c.m.c vehicle.
- Group II** : Diabetic control administrated with 1ml 1 % w/v c.m.c vehicle.
- Group III** : Diabetic rat received with glibenclamide 5 mg/kg/day p.o.
- Group IV** : Diabetic rat received with E.E.T.C 400mg/kg body weight p.o.
- Group V** : Diabetic rat received with A.E.T.C.400mg/kg body weight p.o.

The diabetic rats were treated with test extract and standard drug continuously for 21 days. The effect of extract was determined by measuring the initial and final body weight, blood glucose level. 1, 7, 14, 21st days fasting blood glucose levels from retro orbital plexus puncture were estimated by using glucose estimating strips by a one touch glucometer (one touch horizon glucometer, Johnson and Johnson). During the 21st day blood samples were collected by cardiac puncturing and lipid profile were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided in the kit using Autoanalyser [9,10].

Statistical analysis

Results were expressed as mean \pm SEM, (n=6). Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Tukey Kramer multiple comparison test. *P<0.05, **<0.01 and ***<0.001, show statistical significance when compared treatment group with diabetic control.

RESULTS AND DISCUSSION

The preliminary phytochemical analysis revealed the presence of carbohydrate, alkaloids, tannins and phenolic compounds, carbohydrate, flavanoids. The extracts were found to be safe up to 2000 mg/kg body weight since no death and signs of toxicity were observed while conducting acute toxicity test.

The present work was designed to investigate the antidiabetic effects of *Terminalia catappa* bark extract in Alloxan-induced diabetic albino rats. Fasting blood glucose levels of animals in all groups were

recorded on 1st, 7th, 14th, 21st days of the treatment period Table No.1. A progressive decrease in blood glucose levels were seen during the time period of study. On the 1st day of treatment period a hyperglycemic state is observed in the alloxan induced groups. On the 7th day of treatment glib 5mg/kg , EETC 400 mg/kg ,AETC 400 mg/kg shows significance as compared with diabetic control group in which changes in the blood glucose levels were observed, extract group shows moderate level of significance(p<0.01). At the end of the study the 21st day a higher reduction in blood glucose levels indicating a very high level of significance (p<0.001) is observed in the EETC and AETC group Fig.1.

Induction of diabetes with alloxan is associated with decrease in body weight, loss of body weight is due to defect in glucose metabolism and excessive breakdown of tissue protein is a characteristic condition in diabetics. Body weight of all group animals were recorded on 0th, 1st, 7th, 14th, 21st days of during the treatment period Table No.2. In the treated groups with GLB 5mg/kg, EETC 400 mg/kg, AETC 400 mg/kg on the 21st day shows a significant increase in body weight as compared to diabetic control group (p < 0.001). Fig. 2. The various parameters of blood lipid profile were estimated after 21 days of treatment and compared with diabetic control Table No.3. The enhanced levels of TC, LDL cholesterol and TG, VLDL were brought down significantly (P < 0.001) and HDL levels were improved after 21 days of treatment. Fig. 3.

Table 1: Effect of Terminalia catappa barks extract on blood glucose levels (mg/dl) in diabetic rats

Groups	Fasting blood glucose level(mg/dl)			
	1 st day	7 th day	14 th day	21 st day
Normal	93.16±2.48	89.50±3.69	97.5±3.60	94.5 ±4.36
Diabetic control	394.33±10.04	398.17±10.19	402.33 ± 9.60	413.33±7.20
GLB 5mg/kg	392.67±10.59	288.67±16.25***	176 ±6.66***	120.17±1.79***
EETC 400mg/kg	384.50±12.21	306.17±20.24**	196.6±5.62 ***	123±1.45 ***
AETC400mg/kg	388.67±8.43	317.17±19.67**	201.66±6.45***	131.83±2.42***

All values are Mean±SEM, n=6, **p<0.01, *** P<0.001 represents statistical significance of treatment group Vs Diabetic control.

Figure 1: Effect of Terminalia catappa bark extract on blood glucose levels (mg/dl) in diabetic rats

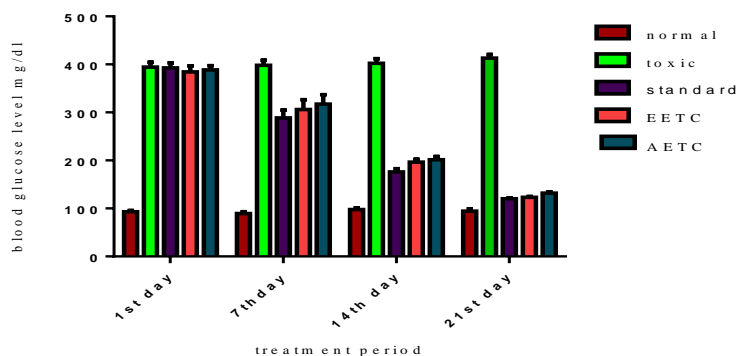


Table 2: Effect of Terminalia catappa bark extract in change in body weight of diabetic rats.

Groups	Body weight (gm)			
	1 st day	7 th day	14 th day	21 st day
Normal	154.07± 3.25	155.37±3.26	156.72±3.18	159.12±2.91
Diabetic control	139.33±1.60	133.33±1.94	125.83±1.79	119.67±1.52
GLB 5mg/kg	142.00±2.06	144.17±2.07	146.67±1.66***	149.50±1.33***
EETC400mg/kg	139.17±2.25	138.80±1.53	139.33±0.84***	141.17±1.84***
AETC400mg/kg	132.33±3.51	133.08±3.97	136.00±1.93**	137.92±3.28***

All values are Mean±SEM, n=6, **P<0.01, ***P<0.001 represents stastical significance of treatment group Vs Diabetic control.

Figure 2: Effect of *Terminalia catappa* bark extract in change in body weight of diabetic rats.

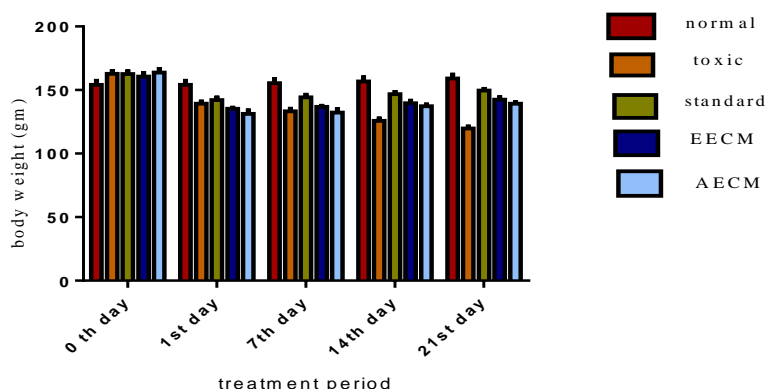
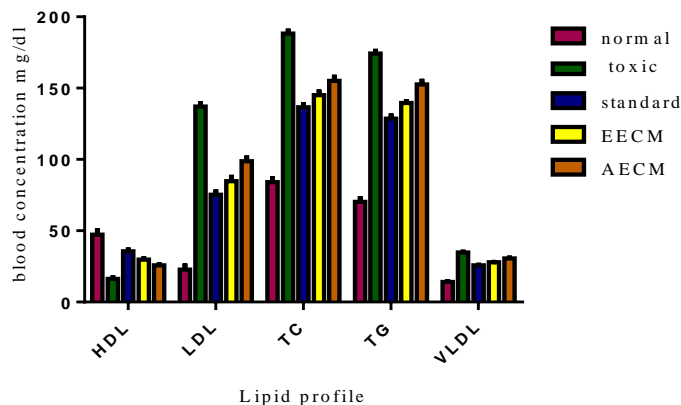


Table 3: Effect of *Terminalia catappa* bark extract on lipid profile of diabetic rats.

Groups	Lipid profile(mg/dl)				
	HDL	LDL	TG	TC	VLDL
Normal	47.16±3.31	22.76±2.84	70.33±2.48	84.00±2.80	14.06±0.49
Toxic	16.33±0.71	137.00±2.47	174.17±1.88	188.17±2.08	34.83±0.37
GLB	35.50±1.20***	75.30±2.09***	128.25±2.23***	136.50±2.29***	25.70±0.44***
EETC	29.66±0.91***	84.76±2.93***	139.50±1.25***	145.00±2.63***	27.90±0.25***
AETC	25.66±0.80**	98.80±2.46***	152.67±2.55***	155.00±2.92***	30.53±0.51***

All values are Mean±SEM, n=6, *P<0.05, **P<0.01 and *** P<0.001 represents stastical significance of treatment group Vs diabetic control group.

Figure 3: Effect of *Terminalia catappa* bark extract on lipid profile of diabetic rats.



CONCLUSION

In conclusion, the results of the present study clearly indicate that the extract of *terminalia catappa* barks have glucose lowering effect on alloxan-induced diabetic rats. Body weight study also showed increase in the body weight during the treatment, which may be due to reduced breakdown of tissue protein indicating the antidiabetic activity. Further studies are in fact currently on the way to isolate the active principle and elucidate the exact mechanisms of action of of *terminalia catappa* barks extract.

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