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ANTIDIABETIC ACTIVITY OF ETHANOLIC ROOT EXTRACT OF *TRAGIA BICOLOR* IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

The aim of present study to evaluate antidiabetic activity of ethanolic extract of Tragia Bicolor MIQ. (Family: Euphorbiaceae) roots in streptozotocin induced diabetic rats. The alcoholic extract of Tragia Bicolor MIQ was studied for antidiabetic activity in streptozotocin induced diabetic rats by oral administration of extract 200&400mg/kg body weight for the period of 28 days. The preliminary phytochemical study showed the presence of flavonoids, carbohydrates and glycosides, phenolic compounds and tannins. From the toxicity study it was observed that ethanolic extract of Tragia Bicolor MIQ was nontoxic up to the dose of 2000mg/kg body weight. The determination of blood glucose level by GOD-POD kit method. The effect was compared with oral dose of 0.6mg/kg Glibenclamide. The result shows the alcoholic extract of Tragia Bicolor MIQ level significantly lowered the blood glucose of hyperglycemic rats in the dose dependent manner and it was also comparable to that of the standard drug glibenclamide.

KEYWORDS

Anti-diabetic, Tragia Bicolor MIQ, Glibenclamide and Streptozotocin.

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INTRODUCTION[1]

Diabetes mellitus (DM) is a group of metabolic disorder characterized by elevated blood glucose level resulting from the defects in insulin secretion, insulin action, or both. The world prevalence of diabetes among adults is expected to be 6.4%, affecting 285 million adults, in 2010, and will increase to 7.7% i.e. about 439 million adults by 2030. Between 2010 and 2030, there will be a 69% increase in numbers of adults with diabetes in developing countries and a 20% increase in developed countries. However, among the two major

types of diabetes i.e. Type 1 and type 2, type 2 DM is the commonest form of diabetes constituting 90%-95% of the diabetic population. It was also documented that the number of people diagnosed with type 2 DM globally is estimated to be at 2%-3% of the world population and is rising at a rate of 4%-5% per year. Currently available oral hypoglycemic drugs for the treatment of DM have characteristic profile of adverse effects[2]. Hence, research is focused to screen the medicinal plant that are used traditionally for the treatment of DM to find a newer lead drug molecule from phytoconstituents with more potential and lesser side effects than the existing hypoglycemic agent. Many review articles research paper appeared in the journals and book showed that many plant used in the traditional system of medicine for the treatment of DM proved to be scientifically effectives[3].

Tragia Bicolor MIQ belongs to the Family: Euphorbiaceae is found in commonly in tropical forest of south India. Densely hispid twiners, young parts fulvous-tomentose. Leaves to 13 x 8 cm, ovate, acuminate, cordate at base, regularly serrate, densely hispid below, sparsely above; lateral nerves 5 or 6 pairs, nervules reticulate; stipule 8 mm long, lanceolate, acuminate, 1 or 2-toothed on sides, ciliate. Racemes to 7 cm long, hispid; bracts 4 x 1 mm, lanceolate, glandular-ciliate; pedicels 3 mm long, glandular-hispid. Male flowers 2.5 mm across; sepals 3, orbicular, glabrous; stamens 3, filaments free. It is used in the Ayurvedic system of medicine. It possess the same medicinal properties as that of *Tragia involucrata* whose root are used for the treatment of pruritic skin eruption, veneral disease, haemorrhoid, diabetes, vomiting, stomach troubles, guinea worms, to purify blood and in relieving giddiness, melalgia, and brachialgia. No scientific reports are available on the antidiabetic activity of Tragia Bicolor MIQ roots. Hence, the present study focuses on the scientific investigation of antidiabetic activity of alcoholic extract of Tragia Bicolor MIQ roots in type 2 diabetic rats[4-7].

MATERIALS AND METHODS

Collection & Authentication of Plant Material

The roots of *Tragia bicolor miq.* were collected locally from the market of Kannur district in the month of November-2014. The plant has been taxonomically identified and authenticated by the Botanist Dr. Balasubhramaniyam, ABS botanical garden. The authenticated plant was used for preparation of extract.

Preparation of Extract

The roots of *Tragia bicolor miq* were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve no. 40 and stored in an airtight container for further use. The dried powdered roots of *Tragia bicolor miq* was defatted with petroleum ether (60-80°c) in a Soxhlet apparatus. The defatted powder material thus obtained was further extracted with chloroform, acetone, ethanol and water. The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vaccum dried using rotary flash evaporator. The extractive values are represented in Table No.1.

Preliminary Phytochemical Screening

The phytochemical examination of the selected extracts showed the presence of various constituents. From that ethanolic and aqueous extracts showed maximum phytoconstituent especially flavonoids, tannins and phenolic compounds. The phytochemical screening of the alcoholic and aqueous root extracts *Tragia bicolor miq.* showed the presence of flavonoids, carbohydrates and glycosides, phenolic compounds and tannins. The results were representented in Table No.2.

Experimental Animals

Studies were carried out using male Wistar albino rats (150 - 200g) and Swiss albino mice (20 - 25kg). They were procured from Sri Venkateswara Enterprises, Bangalore, India. The animals were grouped and housed in polyacrylic cages (38 × 23 × 10cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light cycle (12/12h). The animals were fed with standard pellet supplied by Hindustan Lever Ltd. Bangalore, India

and fresh water *ad libitum.* All the animals were acclimatized to laboratory condition for a week before commencement of experiment. All procedures described were reviewed and approved by the University Animal Ethical Committee.

Acute oral toxicity studies

An acute oral toxicity study was performed as per OECD guidelines 423. By acute toxic class method Swiss albino mice of female sex weighing 20-25gms were used for the study. The alcoholic and aqueous root extracts of *Tragia bicolor miq* did not showed any lethal effect on the animals up to the doses of 2000 mg/kg and the animals were observed for further 14 days for any sign of delayed toxicity. The LD_{50} value considered as 2000mg/kg. So, the ED_{50} dose 200mg/kg[8].

Induction of diabetes

Streptozotocin (STZ) induced hyperglycaemia has been described as a useful experimental model to study the activity of hypoglycaemia agents. After overnight fasting (deprived of food for 16 hours, had been allowed free access to water), diabetes was induced in rats by intraperitoneal injection of STZ dissolved in 0.1 M sodium citrate buffer pH 4.5 at a dose of 50mg/kg body weight. After the injection they have free access to food and water. The animals were allowed to drink 5 % glucose solution overnight to overcome the hypoglycaemic shock. The development of diabetes was confirmed after 48 h of the streptozotocin injection. The animals having fasting blood glucose levels more than 200mg/dl were considered as diabetic rats and used for experimentation[9-11].

Experimental protocol

In the diabetic rat, 5 days after the induction of diabetes, animals were divided into four groups each having 6 rats. Non-diabetic animal are grouped for control. Total of 5 groups of animal of six each were used for study.

Group I: Vehicle control received 1% DMSO + 1% tween 80 (10ml/kg) orally (non-diabetic control).

Group II: Served as STZ induced diabetic control received 1% Tween 80 orally for 28 days.

Group III: Streptozotocin induced diabetic animal received the standard drug Glibenclamide 0.6 mg/kg body weight once daily orally for 28 days.

Group IV: Streptozotocin induced diabetic animals received ethanol extract of *Tragia bicolor miq* roots 200 mg/kg body weight once daily orally for 28 days.

Group V: Streptozotocin induced diabetic animals received ethanol extract of *Tragia bicolor miq* roots 400 mg/kg body weight once daily orally for 28 days.

All the group of animal received the treatment for 28 days. Rats were fasted overnight and the blood was withdrawn from the retro-orbital plexus on the 7^{th}, 14^{th}, 21^{st} and 28^{th} day of induction of diabetes to determine the blood glucose level by glucose oxidase - peroxidase (GOD/POD) method. The change in body weight was observed throughout the treatment period in experimental animals.

Statistical analysis

All the grouped data were expressed as mean ± SEM. Difference between the control and treatment groups were tested for significance using ANOVA followed by Dunnet's test.$P<0.05$ were considered significant.

RESULTS

The alcoholic and aqueous root extracts of *Tragia bicolor miq.* yielded 4.64% and 7.30% w/w respectively. The phytochemical screening of the alcoholic and aqueous root extracts *Tragia bicolor miq.* showed the presence of flavonoids, carbohydrates and glycosides, phenolic compounds and tannins. The results were represented in Table No.1 and Table No.2.

DISCUSSION

Any drug that is effect in diabetes will have the ability to control the rise in glucose level by different mechanisms and the ability of the extracts to prevent hyperglycaemia could be determined by hyperglycaemic animal model. In animals, diabetes can be induced by partial pancreatectomy or by the administration diabetogenic drugs such as streptozotocin, alloxan, ditizona and anti-insulin

serum. Streptozotocin is a naturally occurring nitrosourea product of Streptomyces achromogenes, and it is widely used to induce diabetes in experimental animals. Usually, the intraperitoneal injection of a single dose (50 mg/kg body weight) of it exerts direct toxicity on β cells resulting in necrosis 48-72 h and causes a permanent hyperglycemia. Streptozotocin was used in the present study for the induction of diabetes in rats.

Tragia bicoloar has been traditionally used for the treatment of diabetics mellitus (Type-II). The intra peritoneal administration of STZ damages the partially the insulin secreting β- cells of the pancreas by breaking the DNA strand, resulting in decrease endogenous insulin release. The present study, after treatment of STZ induced diabetic rats with EETB (200 and 400mg/kg). There was a significant reduction in blood glucose level and simultaneously and increase in the insulin level. The possible mechanism by which EETB brings about its hypoglycaemic action in diabetic rats may be improving the glycemic control mechanism and by increasing the insulin secretion from regenerated pancreatic β-cells. After 28 days of treatment with plant extract, the decrease blood glucose level was 27, 81% and 31.81% respectively at the dose of 200mg and 400mg/kg compare to initial value. The standard drug glibenclimide (0.6mg/kg) significant reduction blood glucose level by 57.08% after 28 days (Table No.3)[12,13].

Table No.1: Data Showing the Extractive Values of root extracts of *Tragia bicolor miq.*

S.No	Plant Name, and Parts used	Method of extraction	Yield in percentage (w/w)				
			Pet.ether	Chloroform	Acetone	Ethanol	Aqueous
1	*Tragia bicolor miq.* (Dried root)	Continuous hot percolation	2%	1.5%	1.76%	4.72%	7.30% (cold maceration)

Table No.2: Preliminary phytochemical studies of various extracts of root plant of *Tragia bicolor miq.*

S.No	Constituents	Test	Pet. ether	Chloroform	Acetone	Alcoholic	Aqueous
1	Alkaloids	Mayer's test	-	-	-	-	-
		Hager's test	-	-	-	-	-
		Wagner's test	-	-	-	-	-
2	Sterols	Libermann's sterol test	+	+	-	-	-
		Salwoski's test	+	+	-	-	-
3	Carbohydrate and glycosides	Molisch's test	-	+	+	+	+
		Fehling's test	-	+	+	+	+
		Barfoed's test	-	+	+	+	+
		Borntrager's test	-	+	+	+	+
4	Fixed oils and fats	Saponification	-	-	-	-	-
5	Phenolic compounds	Extract+$FeCl_3$	-	-	+	+	+
6	Tannins	Gelatin test	-	+	+	+	+
		$FeCl_3$ test	-	+	+	+	+
7	Protein and aminoacids	Biuret test	-	-	-	-	-
		Ninhydrin test	-	-	-	-	-
		Xanthoprotein test	-	-	-	-	-
		Million's test	-	-	-	-	-
8	Tritepenoids and saponins	Tin+Thionyl chloride	-	-	-	-	-
		Foam test	-	-	-	-	-
9	Gum and mucilage	Ppt. with 95% alcohol	-	-	-	-	-
		Ruthenium test	-	-	-	-	-
10	Flavones and flavonoids	Aq. NaOH	-	-	-	+	+
		Shinoda test	-	-	-	+	+
		Conc. H_2SO_4	-	-	-	+	+

Table No.3: Effect of ethanol ether extract of *Tragia Bicolor* on fasting blood sugar (mg/dl) level in STZ induced diabetic rats

S.No	Groups	Blood glucose levels(mg/dL)				
		0 day	7th day	14th day	21st day	28th day
1	Normal Control	80.30±3.1	85.40±2.80	83.48±5.32	85.58±4.60	84.5±2.18
2	Diabetic control	250.28±6.88	266.03±3.28	275.28±4.76	290.44±6.42	293.03±1.28
3	Glibenclamide (0.6mg/kg)	256.42±1.27	190.38±6.20**	163.37±5.11***	123.40±2.42***	110.03±3.20***
4	EETB(200mg/kg)	270.68±7.27	260.38±7.98**	245.27±3.98**	220.44±5.78**	195.38±4.87*
5	EETB(400mg/kg)	253.62±4.67	244.49±6.76**	228.86±6.99**	185.47±8.59**	162.80±2.24**

All the values are expressed as mean ±SEM, n=6 in each group. Values are significantly different from control. *P<0.05; **P<0.01; ***P<0.001.

CONCLUSION

The ethanolic extract of *Tragia bicolor miq* roots exhibited significant hypoglycaemic activity in streptozotocin induced diabetic rats. From the phytochemical analysis it was found that the major chemical constituents of the roots extract were flavonoids and glycosides. On the basis of above evidence it is possible that the presence of flavonoids may be responsible for the observed antidiabetic activity. In future study is required for the study of isolation and characterization of the anti-diabetic bio active compound and establishment of exact mechanism of action.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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