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Evaluation of antidiabetic activity of *Excoecaria agallocha* L. in alloxan induced diabetic mice

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ABSTRACT

Excoecaria agallocha (L.) Leaves extract (ethanolic and aqueous 500 mg/ kg body wt.) were evaluated for its effect on blood sugar level in normal and alloxan induced wistar albino mice at various time points comparing it with standard drug metformin (75 mg/ kg). The studies indicated that the crude ethanolic extract exhibited significant hypoglycemic ($P < 0.01$) and anti- hyperglycemic ($P < 0.001$) activities in normal and alloxan – induced diabetic albino mice respectively. The study reports for the first time the hypoglycemic acivity of *Excoecaria agallocha* (L.) in mice.

KEYWORDS

Excoecaria agallocha;
Alloxan;
Antidiabetic activity;
Metformin.

INTRODUCTION

There are an estimated 143 million people worldwide suffering from diabetes and this number is expected to increase to 333 million by the year 2030[1]. Therefore, the human population worldwide appears to be ion the midst of an epidemic of diabetes, can have several adverse effects. No satisfactory effective therapy is still available in modern medicine to cure diabetes. There is a growing interest in herbal remedies due to perceived effectiveness, minimal side effects in clinical practice and relatively low cost, herbal drugs are widely prescribed even when their biologically active compounds are unknown. Many Indian medicinal plants are reported to be useful in diabetes[2,3]. However, search for new anti-diabetic drugs continue.

Mangrove plant *Excoecaria agallocha* (L.) was found throughout the river areas of India, particularly in Krishna river. *Excoecaria agallocha* (L.) is distributed on the seashores and edge mangroves, sometimes cultivated for wind- and sea – breaks in tropical Africa and East Asia. They are well-known as extreme skin irritants and tumor promoters[4]. The leaves and latex of *E.agallocha* have been used as a dart poison and fish Poison in India[5]. The piscicidal constituent, excoecariatoxin and some related daphnane diterpene esters known as skin irritants and Tumor promoters,were isolated from the twigs, bark And latex of *E. agallocha* from Thailand. Interestingly, a novel phorbol ester was also isolated as an anti-HIV principle from the leaves and stems of *E.agallocha*, collected in Northwest Australia[4]. The present work was undertaken to study the effect of leaves extracts of *Excoecaria agallocha* (L.) plant on blood glucose following administration to normal and hyperglycemic mice.

MATERIALS AND METHODS

The leaves of *Excoecaria agallocha* (L.) were collected from Machilipatnam, Krishna district, AP, India.

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An authenticated voucher specimen was deposited in GIET School of Pharmacy, Rajahmundry, A.P. India.

Preparation of extract

The dried powdered leaves were extracted with petroleum ether (40-60º C) to remove lipids and waxy materials and then successively extracted with ethanol 50% and water in soxhlet extractor[6]. The extract was concentrated under vaccum to get residue. The residue was dried in vaccum dessicator. Both aqueous and ethanolic extract were selected for hypoglycemic screening.

Animals used

Wistar Albino mice of both sexes, weighing between 20-30 gms, were used. They were housed in polypropylene cages and were fed on standard laboratory diet and water ad libitum[7]. Animals described as fasted were deprived of food for 16hr, but had free access to water. All the drugs (standard and test) as well as vehicle were administered per-orally, using infant feeding tube. The project was undertaken after the prior approval from the Animals Ethics Committee.

Acute and short-term toxicity study

The ethanol and aqueous extracts were tested for its acute and short- term toxicity of different doses of the drug (1.0, 3.0 and 5.0 g/kg) were administrated to different groups of mice (2 mice were used for each group; control mice received 0.5% CMC). Mortality and general behavior of the animals were observed periodically for 48 h. The animals were observed periodically for 48 h. The animals were observed continuously for the initial 4 h and intermittently for the next 6 h and then again at 24 h and 48 h following drug administration. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion[8].

To study short-term toxicity, 3 groups of mice used. 3 male mice (20-25 g, body weight) were used in each group. Group I was kept as control and group II and III received 500 mg/kg ethanol and aqueous extracts respectively in 0.5%CMC. The drug was administrated daily for 7 days (p.o.). Control group received 0.5% CMC in an identical manner. The behavior of the animals was observed daily for 1 h in the forenoon (10 to 11 A.M) for 7 days.

Acute toxicity study and determination of test dose

During preliminary toxicity study, no adverse effect or mortality was observed in albino mice with oral administration of ethanol and aqueous. Extract up to a high of 5 gm/kg body weight observed for 24 hr. Hence a high dose of 500 mg/kg body weight was selected as a test dose.

Standard drug and basis of selection of its dose

Metformin tablet (500 mg/ tab.) "Glycomet" manufactured by USV Limited was used as a standard drug. The dose was selected on the basis of adult human effective dose. (75 mg/kg body weight)[9].

Determination of blood glucose level

For blood glucose determination, blood was obtained by snipping tail with the help of sharp razor. Blood glucose level was monitored by using Hypo guard Advance Blood Glucose Meter, (Nicholas Piramal Ltd. India) Each time the tail of the mice was sterilized with spirit.

Antidiabetic screnning

Induction of experimental diabetes

Mice were fasted for 18 hrs and experimental diabetes was then induced by administration of three doses of alloxan monohydrate (150 mg/ kg) each i.p. at intervals of 48 hrs. Then 7 days after the last administration, the animals were fasted for 18 hrs and blood glucose levels were determined. Animals with fasting blood glucose levels ranging from 200-300 mg/ dl (Mild diabetic mice) were considered as diabetic and used for the study[10].

Determination of efficacy of ethanolic 50% and aqueous extract in allaxon induced diabetic mice

The diabetic mice were divided into four groups of six each. Group one received vehicle only (0.5% CMC) and served as control group. Group two received standard drug metformin (75 mg. kg) and served as standard group. Remaining two groups received ethanolic and aqueous extracts at a dose of 500mg/kg body wt. Study for the acute hypoglycemic activity involved determination of blood glucose levels at 0, 1,2,3,5 and 24 hrs after administration of single dose. Effect of various extracts of *Excoecaria agallocha* (L.) leaves on blood glucose level in alloxan induced diabetic Wister mice

were given in TABLE 1.

TABLE 1: Effect of ethanolic and aqueous extracts of Excoecaria agallocha (L.) leaves on blood glucose level in alloxan induced diabetic Wister albino mice.

Groups	0 hr	1 hr	2 hr	3 hr	5 hr	24 hr
	BGL (mg/dL)	BGL (mg/dL)	BGL (mg/dL)	BGL (mg/dL)	BGL (mg/dL)	BGL (mg/dL)
Control	225.67±19.70	227.33±16.80	220.33±13.20	224.5±11.90	213±14.70	233.5±23.4
Std	271.33±22.50	240±10.05	192.8±19.70**	167±17.32*	152±21.03*	274±26.70
Et.E	266.16±21.82	244.16±21.82	201.83±21.82**	162.83±21.24*	156±25.32*	265.33±22.74
Aq.E	271.16±29.13	229.16±19.90	182.167±17.68	177.167±3.20	174.167±5.65	272.667±10.5

Values are mean±SD, n = 5 in each group, *p<0.01 when compared with vehicle treated group (Dunnett's test); BGL- Blood glucose level; Et.E- Ethanol extract; Aq.E- Aqueous extract.

Hypoglycemic study in normal fasted mice

Animal were fasted overnight and were divided in to four groups of five each as follows

Group1- Received 3% CMC suspension as control group

Group2- Received metformin (75mg/kg) body weight as Std.group

Group3- Received aqueous extract (500mg/kg) body weight

Group4- Received ethanolic 50% extract (500mg/kg) body weight

Blood samples were collected after 120 min of drug administration[11].

Results are elaborated in TABLE 2.

TABLE 2: Effect of ethanolic and aqueous extracts of excoecaria agallocha (L.) leaves on blood glucose level in normal fastened Wistar albino mice.

Groups	0 hour	2 hour
Control	107±11.640	98.4±11.104
Standard	102±7.949	64±9.471*
Ethanol extract	89.6±11.88697	51.2±8.927486*
Aqueous extract	98.2±7.1902271	67.8±7.085196

Values are mean±SD, n = 5 in each group, *p<0.01 when compared with vehicle treated group (Dunnett's test)

RESULTS

Preliminary phytochemical screening reveals presence of carbohydrates, glycoside, proteins, alkaloid, Tri-terpenes, saponins, flavanoids and tannins. During Preliminary toxicity study, no adverse effect or mortality was observed in albino mice with oral administration of ethanolic 50% and aqueous extracts up to a high dose of 5gm/kg body weight observed for 24 hr. Hence a high dose of 500mg/kg body weight was selected as a test dose.

Single administration (single dose) of ethanolic 50% extract (500mg/kg) of leaves of *Excoecaria agallocha* (L.) in diabetic Wistar albino mice, showed significant reduction on fall in blood glucose level (FBGL) after 3 and 5hr interval. Maximum reduction in FBGL (39.09%, 41.35 %) was seen after 3 and 5 hr of administration of dose with a significance level of $p<0.01$. Metformin (75 mg/kg) showed maximum reduction (38.37%, 43.91%) after 3 and 5 hr with a significance level of $p<0.01$.

Acute study in normal fasting mice ethanolic 50% extract (500 mg/kg body weight) showed 42.85% reduction in blood glucose level and aqueous extract (500 mg/kg body weight) showed 30.95% reduction in blood glucose level while metformin treatment resulted in 40.78% reduction in blood glucose level at 2 hr interval.

DISCUSSION

The results indicate that the ethanolic extract (500 mg/kg body weight) of leaves of *Excoecaria agallocha* (L.) has significant hypoglycemic activity in both the normal and alloxan induced diabetic mice. Flavonoids, Tri-terpenoids, alkaloids and phenolics are known to be bioactive antidiabetic principles. The antidiabetic effect of ethanolic extract of *Excoecaria agallocha* (L.) leaves may be due to the presence of more than one antihyperglycemic principles mentioned above. Further studies will be focused on determination of the mechanism(s) of action and isolation of bioactive principles responsible for hypoglycemic activity.

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