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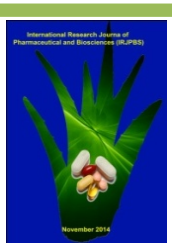

Screening of Streptozocin induced Diabetic activity of *Boswellia ovalifoliolata*

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Abstract

The hypoglycemic effect of ethanolic extract of stem bark *Boswellia ovalifoliolata* (EEBO) was evaluated in normal glucose fed and Streptozocin induced diabetic rats. Oral administration of extract (200 and 400mg/kg body wt.) for 7 days resulted in a significant reduction in blood glucose level. The effect was compared with 10mg/kg (p.o) glibenclamide.

Keywords: Ethanolic extract of *Boswellia ovalifoliolata* (EEBO), Streptozocin, Blood sugar lowering activity, Glucose.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic disease caused by insufficient production of insulin by pancreatic gland and decrease in absorption glucose by the muscles in the systems there by increasing the concentration of glucose in blood [1]. Diabetics is also depends on hereditary characters. Due to increase of glucose level in blood causes various deficiencies and hampers the normal physiological effects of the human system such as blood vessels nerves system etc [2]. Asian committee rate in next projected that the diabetic is the main disease which can increase the death rate in next coming 25 years in Asia. [3] Now a days there are many allopathic drugs available to treat this disease. But all these agents causing serious side effects after prolonged use. Hence to overcome these adverse effects such as Hematological effects, coma, malfunction of liver kidney etc [4]. Hence many traditional plants medicines are used throughout the world to treat the diabetic diseases [5]. When compared with synthetic drugs, the plant drugs have less toxic effects with fewer side effects [6]. *Boswellia ovalifoliolata* belongs to family burseraceae. In Indian system of Medicine, the various plants parts like leaves, stem, stem bark , roots, fruits etc are used treat the diabetes, mouth ulcers etc [7]. Hence the present investigation was under taken to evaluate the anti-diabetic activity of

ethanolic extract of stem bark of *Boswellia ovalifoliolata* in streptozocine induced diabetic rates to confirm the pharmacological evidence in support of Folklore claim [8].

MATERIALS AND METHODS

Plant Materials

Fresh stem barks were collected from Tirumala hills, Chittoor district, Andhra Pradesh, India and authentified by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany S.V. University, Tirupati, and Andhra Pradesh, India. Voucher specimen No. 1295 is kept for further future reference at S.V. University, Andhra Pradesh, India.

Extraction of Plant Materials

The collected fresh stem bark were in shade dried and powdered in a mechanical grinder to get coarse powder and then passed through sieve 60 mesh to get uniform powder granules. The Powdered stem bark (100g) were defatted with hexane and later extracted with ethanol by using Soxhlet extractor at 1000c for about 18 hours. The solution was subjected to evaporation by using rotary evaporator until becomes reddish residue.

The extract was evaporated to dryness, and the residue 15% W/W was kept in desiccators for further studies and to remove moisture content completely [9]. Phytochemical screening of preliminary phytochemicals of EEBO was carried by using standard procedures [10].

Animals

Wistar albino rats (200-250g) of both sexes were purchased from Sri Venkateswara Enterprises, Bangalore. Before and during the experiment rats were fed with standard diet (Gold Mohr, Lipton India Ltd). After randomization in to various groups and before initiation of experiment, the rats were acclimatized for a period of 14 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived off food and water for 16 hours. Ethical clearance for animal study was obtained from the institutional animal ethics committee. (IAEC/ACP/1220/a/08/CPCSE 08).

Chemicals

Streptzocin was procured from sigma chemical company USA. Glucose strips procure from M/S Boechringer Mannherim India private limited. The solvents and reagents of analytical grade were purchased and used as such.

Experimental Design

Acute oral toxicity study

An acute toxicity study was carried out to determination of LD50 values by using different doses 5, 50,300 and 2000mg/kg body weight of the extract in healthy adult female Swiss albino mice weighing between 250-25 body weights were selected for oral acute toxicity study. This study was carried out as per the OECD guidelines Number 401 [11]. From the

toxicity study, it was indicated that the extract is safe up to dose 2.0g/kg body weight. It is very safe for further studies at different doses.

Hypoglycemic effect of EEBO on blood glucose levels in Normoglycemic rats

In this study the entire groups of animals were fasted over Night and administered with respective drugs as per the mentioned dosage schedule.

Animals were divided in to three groups of six rats in each group. Group – I, II and II receives 1% sodium carboxy methyl cellulose (2ml/kg) 200 and 400 mg/kg orally of ethanolic extract of BO respectively. Blood glucose levels were determined at zero hour (before drug administration) 60, 120 min, after drug administration [12].

Oral glucose tolerance test

Effect of ethanolic extract of BO on blood glucose level on glucose fed hyperglycemic rats was carried out in animal models**.** In this study, the entire groups of animals were fasted overnight and administered with respective drugs as per the mentioned dosage schedule. Animals were divided into four groups of six rats in each group. Group-I, II, III and IV. Group I receives glucose 2g/kg only, Group II receive glibenclamide 10mg/kg (p.o.). Group III and group IV received 200 and 400mg/kg. Glucose 2g/kg body weight administered orally, half an hour before administration of standard and test extract respectively. Blood glucose levels were determined at (before glucose challenge) 0,60,120th minutes after glucose administration [12].

Anti-diabetic study

Effect of ethanolic extract of BO on blood glucose level in streptozocine induced diabetic rats**.** Different groups of rats were used to study the effects of EEBO. The rats were divided into five groups each consisting of six rats.

Group-I Normal control animals received 1% sodium carboxy methyl cellulose 2ml /kg body wt. per orally.

Group-II streptozocine (40 mg/kg body wt) induced diabetic animals received 2ml/kg body wt. i.v

Group-III diabetic animals received glibenclamide 10mg/kg body wt. per orally and served as standard.

Group-IV diabetic animals received 200 mg/kg body wt of EEBO and

G**roup-V** diabetic animals received 400 g/kg, body wt. of EEBO per orally.

Significant hyperglycemia was achieved within 48 hours after Streptzocin (40mg/kgb.w. i.v.) injection induced diabetic rats with more than 200mg/dl of blood glucose were identified as to be diabetic and used for the study. In acute study all the surviving diabetic animals and normal animals were fasted overnight blood samples were collected from the fasted animals prior to the treatment with above scheduled and after administration, at each day up to 7 days . For glucose determination, blood was obtained shipping tail with sharp razor. Then the

blood glucose levels were determined by using Haemo-Glukotest (20-800R) glucose strips supplied by M/S Boechringer Mannherim India Limited. These methods which permit the measurement of blood glucose levels with minimum injury to rat, was previously validated by comparison with glucose oxidase method [13,14].

Statistical Analysis

The data were analyzed as mean +_ SEM and statistical significance between extract treated and diabetic control groups analyzed by using one way analysis of variance followed by Turkey-Kramer multiple comparison test. $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Ethanolic extract of plant material gave residue of 15%w/w. Preliminary phytochemical studies of EEBO revealed that the presence of alkaloids, tannins flavanoids, proteins, carbohydrates saponins, steroidal, glycosides [15].

Acute oral toxicity study

The results of the acute oral toxicity indicated that the ethanolic extract of *Boswellia ovalifoliolata* stem bark were not lethal up to a dose of 2000mg/kg body weight. From the toxicity study it was observed and concluded to select 1/8th and 1/4th of 200mg/kg dose ie.200 and 400mg/kg orally was selected for anti-diabetic. The results presented in Table 1.

Normoglycemic rats

Effect of aqueous EEBO on blood glucose in normoglycemic rats at dose 200mg/kg and 400mg/kg of EEBO on fasting blood sugars level were determined in normal rats at various times interval is shown in table-2. The mean blood glucose level decrease from 77.83 , 76.83.06 mg/dl to 77.06mg/dl at dose of 400mg/kg body weight of ethanolic extract of BO and 77.60, 74.50, mg/dl to 76.67mg/dl at dose of 400mg/kg body weight.

Oral Glucose Tolerance Test

Effect of EEBO on blood glucose level in glucose fed hyperglycemic rats at dose 200mg/kg and 400mg/kg level were assessed and they were presented table3 at various time intervals. The blood glucose levels decreased from 80.50+0.99mg/dl to 78.50 + 0.62mg/dl at 400mg/kg body weight and 81.50+0.94 mg/dl to 78.16 + 0.96 mg/dl at 400mg/kg body Weight.

Anti-diabetic study

Effect of EEBO on blood glucose level in Streptozocin induced diabetic rats was carried out. The blood sugar lowering effect of the extracts on the blood sugar level on diabetic rats is shown in table – 4. The blood glucose level of diabetic animal significantly ($P<0.05$) reduced from 210.15 mg/dl to 105.18 mg/dl at 300mg/kg body wt.of ethanolic extract of BO and 209.01 mg/dl to 99.73 mg/dl at 400mg/kg body wt. of ethanolic extract of BO. These results were in good agreement with 10mg/kg of glibenclamide.

Table 1. Acute oral toxicity study of *Boswellia ovalifoliolata*

Treatment	Dose (mg/Kg body wt)	No. of Animals	No of Survial	No of death	Percentage of Morality	LD_{50} value
Control	1%NaCMC	10	10	0	0	-----
EEBO	5	10	10	0	0	-----
	50	10	10	0	0	-----
	300	10	10	0	0	-----
	2000	10	10	0	0	>2.0/kg,bw

Table 2. Effect of EEBO on blood glucose in normoglycemic rats

Group	Dose mg/kg	Blood glucose Levels (mg/dl)		
		Intial- (0 min.)	60min.	120min.
Group I	2 ml	79.10 + 0.66	78.83 + 0.71	79.66 + 0.53
Group III	200 mg	77.83 + 0.89	76.83 + 0.94	77.06 + 0.54
Group III	400 mg	77.60 + 1.10	74.50 + 0.85	76.67 + 0.95

Table 3. Effect of EEBO on blood glucose in glucose fed hyperglycemic rats

Group	Treatment Dose mg/kg	Blood glucose Levels mg/dl				
		0 min.	30 min.	60 min.	90 min.	120 min.
I	2gm/kg	83.34 + 0.46	116.83 + 1.64	121.16 + 1.28	103.34 + 1.11	83.50 + 0.66
II	GBM	76.50 + 0.81	110.50+ 1.84*	112.67+ 1.84*	99.34 + 1.05*	87.50+ 0.79*
III	EEBO 200	80.50 + 0.99	118.34+ 0.71*	105.67+ 0.88*	92.60 + 0.84*	78.50+ 0.62*
IV	EEBO 400	81.50 + 0.94	117.50+ 0.63*	107.34+ 0.62*	107.00+ 0.34*	78.16+ 0.96*

The values are expressed an mean + SEM. n = number of animals in each group. Statistical Significant test for comparison was done by ANOVA, followed by Turkey-Kramer multiple tests. The 60^{th} and 120^{th} min. values are compared with initial value.

The EEBO at a dose of 200mg/kg body weight per orally did not significantly suppress blood glucose levels in overnight fasted normoglycemic animals. The same effect was observed at a higher dose level of 400mg/kg body weight per orally normoglycemic animals after 1st, 2nd and 3^{rd} hour of oral administration, when compared with control group of animals [16]. The EEBO showed significant improvement in glucose tolerance in glucose fed hyperglycemic normal rats.

Table 4. Effect of EEBO on blood glucose level in Streptozocin induced Diabetic Rats

Group	Treatment Dose mg/kg	Blood glucose Levels mg/dl				
		O Day	1 Day	3rd Day	5th Day	7th Day
Normal	**1% cmc**	83.65+0.62	83.53+0.62	83.53+1.35	83.57+ 0.355	83.84+ 0.27
STZ	**40**	190.54+1.40	209.23+0.85	218.73 + 0.89	232.12+1.84	252.89+1.80
GBM	**100**	181.01+0.39	156.90+0.85	139.41+ 0.65	104.49+ 0.36	98.74+ 0.25
EEBO	**200**	248.42+1.01	254.45+1.35	267.76+ 1.40	250.95+ 0.88	195.28+1.80
EEBO	**400**	193.37+0.33	159.68+ 0.15	127.87+ 0.41	103.57+ 0.39	84.25+ 0.47

The values are expressed as mean + SEM. n = number of animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Turkey-Kramer multiple tests.

Such an effect may be accounted for, in part, by a decrease in the rate of intestinal glucose absorption, achieved by an extra pancreatic action including the stimulation of peripheral glucose utilization or enhancing glycolytic and glycogenic process with concomitant decrease in glycogenolysis and glyconeogenesis [17]. However, the effect was less significant when compared to standard drug glibenclamide. Streptozocin is the most commonly employed agent for the induction of experimental diabetic animal models of human insulin-dependent diabetes mellitus. There is increasing evidence that streptozocin caused diabetes by selective destruction of pancreatic insulin secreting beta cells which makes less active and leads to poor glucose utilization by tissues. This indicates that the extract may possess as insulin like effect on peripheral tissues by either promoting glucose uptake or metabolism, by inhibiting hepatic gluconeogenesis or absorption of glucose in to the muscles and adipose tissues by stimulation of regeneration process and revitalization of the remaining beta cells. From the phytochemical analysis it was found that the major chemical constituents of the extract and some of this active principle including flavanoids are known to be used for the treatments of diabetes [18-20]. On the basis of the above evidences it is possible that the presence of flavanoids and tannins are responsible for the observed anti-diabetic activity.

CONCLUSION

In the recent times many traditionally used medicinally important plants were tested for their anti-diabetic potential by various investigators in experimental animals. These properties were attributed to different formulations, extracts and active principles. Working on the same line, we have undertaken a study on *Boswellia ovalifoliolata* for its anti-diabetic property.Our study proved that BO is having a good anti diabetic property and for the clinical studies can be under taken.

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