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Anti-inflammatory Activity of *Boswellia Ovalifoliolata* Leaves

Vasantha Kumar P[*,1], Sri Harsha NS[2], Siddaiah M[3], Muralidhar S[1] and Yashwanth Kumar D[4]

[*,1]Vikas institute of Pharmaceutical Sciences, Rajahmundry, Andhra Pradesh, India.
[2]Biocon Health Care, Bangalore, Karnataka, India.
[3]P.Rami Reddy College of Pharmacy, Kadapa, Andhra Pradesh, India
[4]United Health Group, Mind Space, Hyderabad, Andhra Pradesh, India.

ABSTRACT

Medicinal plants play an important role in health care. There are many valuable medicinal plants which are unique in action. By studying the plant drugs we can develop new drugs. There are number of plants used for treating diseases which are not comprehensively documented due to a lack of communication and low frequency of their use. Such a group of medicinal plants are some endemic plants of Tirumala, Tirupati. These plants have medicinal properties and are used in herbal therapy to cure a wide range of health problems. There is a need to bridge the gap for data access on these plants to the scientific community. Very small amount of work has been down on these plants and a lot is to be done. We select endemic medicinal plants of tirupati flora *boswellia ovalifoliolata* member of a burseraceae family and grow in subtropical temperature. This study was intended to evaluate the anti-inflammatory activity of ethyl acetate and methanol extracts of *boswellia ovalifoliolata* leaves in carrageenan induced paw edema in wistar rats at the dose level of 200 and 400 mg/kg administrated orally. Both the extracts exhibited significant anti-inflammatory activity, which supports the traditional medicinal utilization of the plant. This study established anti-inflammatory activity of leaves of *Boswellia ovalifoliolata*.

Key words: Anti-inflammatory activity, *Boswellia ovalifoliolata*, Traditional medicine, Ethyl acetate, Methanol extracts.

INTRODUCTION

Boswellia ovalifoliolata Bal&Henry is a narrow endemic endangered and threatened medicinal tree species. It is deciduous medium sized tree belongs to the family Burseraceae. This tree harbours on Tirumala hills of seshalam hill range of Eastern Ghats of India. The plant used by tribal's like Nakkla, sugali and chanchu and indigenous community to treat number of aliments (Madhava chetty *et al*., 2008). The plant is over exploited for its medicinal uses. Especially the leaves are used to reduce pains inflammation the leaf decoction using as antibacterial, antiulcer, and anti- rheumatoid.

MATERIALS AND METHODS

Plant materials

The fully mature *Boswellia ovalifoliolata* (BO) leaves were collected in June-July 2011from foot slops of Tirumala hills forest chittoor district Andhra Pradesh state India. Leaves were identified and authenticated by Botany department S.V University, Tirupati. Methanol Ethyl acetate was procured from S.D fine chemicals, Ltd, Mumbai. Carageenan procured from Himedia labs Bombay.

Preparation of extracts

The *Boswellia ovalifoliolata* leaves were first washed several times with distilled water. The leaves were dried at room temperature and coarsely powdered by using mechanical grinder. The powder was successively extracted with ethyl acetate, and methanol using cold soxhlet apparatus method. The percentage yields were 2.3% in ethyl acetate and 10.4% in methanol. The extract was stored in air tight container for further studies.

Phyto-chemical Studies

The following qualitative phytochemical

***Corrosponding author**

Vasantha Kumar P
Email id: pvasanthtpt@gmail.com

screening was carried out for leaf extract of BO ie. triterpeniods, saponins, tannins, steroids and alkaloids. Biomolecules are screened by using the standard qualitative procedures as described by Trease and Evans (1989).

a) Test for alkaloids

1 ml of 1% HCL was added to the 3ml of extract in a test tube. Then it was treated with a few drops of Meyer`s reagent .A creamy white precipitate indicated the presence of alkaloids.

b) Test for flavonoids

A few drops of 1%NH_3 solution was added to the 2ml of extract in a test tube. A yellow coloring was observed for the presence of flavonoids.

c) Test for terpenoids

5ml of extract was mixed with 2ml of $CHCl_3$ in a test tube .3ml of concentrated H_2SO_4 was carefully added along with the wall of the test tube to form a layer. An interface with a reddish brown coloration indicated presence of terpinoids.

d) Test for Steroids

0.5ml of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated H_2SO_4 was added slowly. Bluish green color was observed for steroids.

e) Test for Tannins/phenols

Gelatin test: The test solution was evaporated to dryness and the resulted residue was dissolved in 1% liquefied gelatin. To this 10%Nacl solution was added. A white precipitate was obtained indicated presence of Tannins.

Table.1 Results of Preliminary Qualitative Phyto-chemical Studies of *boswellia ovalifoliolata* Leaves

S.No	Test	EAEBO	MEBO
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Triterpenoids	+	+
4	Steroids	+	+
5	Tannins/phenols	+	+

Animals

Wistar rats of either sex weighing 160-180 g were purchased from S.V. medical college Tirupati for experimental study. They were acclimated to animal house conditions fed with commercial pelleted rats chow (Hindustan Lever Ltd., Bangalore, India), and had free access to water. The experimental protocol was approved by the IAEC (Institutional Animal Ethical Committee) of CPCSEA (Committee for the purpose of control and supervision of experiments on Animal).

Oral acute toxicity studies

The oral acute toxicity of ethylacetate and maethanol extract of the *boswellia ovalifoliolata* Leaves was carried out as per the OPPTS guidelines up and down procedure. Colony breads either sex of albinorats wistar strain weighing between200-250gms were selected and maintained under controlled animal house condition with access to food and water *adlibitum*. The limit test was carried out first at 5000mg/kg body weight. All animals were observed for toxic symptoms and mortality for 72 hours and the results were presented in table1.

Preparation of the drug for the experimental study

Extracts and the standard drugs were administrated in the form of suspension suspending to distribute drug uniformly in water with 1% sodium carboxy methyl cellulose (SCMC) as suspending agent.

Anti-inflammatory activity

The anti-inflammatory activity of B*oswellia ovalifoliolata* and was carried out by using 1%v/v carrageenan and dextran 1%v/v in experimental rats. The animals either sex was divided into six groups each composed of six animals for carrageenan induced paw oedema.

Carrageenan induced paw oedema

Group I - Control animals received 1% SCMC 10 ml/kg p.o.
Group II – Animals received ethyl acetate extract at the dose of 200 mg/kg p.o.
Group III – Animals received ethyl acetate extract at the dose of 400 mg/kg. p.o.
Group IV – Animals received methanolic extract at the dose of 200 mg/kg p.o.
Group V – Animals received methanolic extract at the dose of 400 mg/kg p.o.
Group VI – Standard diclofenac sodium 5 mg/kg, p.o.

Dextran induced paw oedema

Group I - Control animals received 1% SCMC 10 ml/kg p.o.
Group II – Animals received ethyl acetate extract at the dose of 200 mg/kg p.o.
Group III – Animals received ethyl acetate extract at the dose of 400 mg/kg. p.o.
Group IV – Animals received methanolic extract at the dose of 200 mg/kg p.o.
Group V – Animals received methanolic extract at the dose of 400 mg/kg p.o.
Group VI – Standard diclofenac sodium 5 mg/kg, p.o.

Percentage inhibition of edema = $\frac{(V_c - V_t)}{(V_c)} \times 100$

Where, Vc is the inflammatory increase in paw volume in control group of animals and Vt is the inflammatory increase in paw volume in drug-treated animals. Paw oedema was induced injecting 0.1 ml of 1% carrageenan in physiological Saline into the sub plantar tissues of the left hind paw of each rat. The extracts are given orally 30 min prior to carrageenan administration. The paw volume was measured at interwals of 60, 120, 180, and 240 min by the mercury displacement method using a plethysmograph. The percentage inhibition of paw volume in drug treated group was compared with the carrageenan control group group-I. Diclofenac sodium (5 mg /kg/ p.o.) was used as reference drug.

Statistical analysis

Data obtained from pharmacological

experiments are expressed as mean ± SEM. Difference between the control and the treatments these experiments were tested for significance using ANOVA followed by Dunnet's t-test.

RESULT AND DISCUSSION

The extractive value of ethylacetate is 2.3% and Methanol 10.4%. The preliminary phytochemical screening indicates the presence of alkaloids, flavonoids, triterpinoids, steroids, tannins. The plant extracts did not exhibit any mortality up to ten dose level of 5000 mg/kg. So, the extracts safe for long term administration. The ethyl acetate and methanol extracts of *Boswellia ovalifoliolata* levels at the dose level 200 and 400 mg/kg decreased the oedema significantly ($p < 0.001$) at 3rd and 4th after administration of the extract. When compared to the control group. The effect was compared to the activity ($p < 0.001$) produced by standard drug diclofenac sodium at 3rd and 4th after administration.

In the present study, the anti-inflammatory activity of the ethyl acetate and methanol extracts of *Boswellia ovalifoliolata* leaves has been established. The extracts were found to significantly inhibit the carrageenan-induced rat paw edema, a test which has significant predictive value for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation (Bhuijan MA *et al*., 1996). Carrageenan induced inflammation is useful in detecting orally active anti-inflammatory agents (Crunkhon P *et al*., 1971; Di Rosa M *et al*., 1971). Oedema formation due to carrageenan in the rat paw is a biphasic event (Vinegar R *et al*., 1969; Winter CA *et al*., 1969). The initial phase is attributed to the release of histamine and serotonin. The extracts of *boswellia ovalifoliolata* leaves possessed varying degree of anti-inflammatory activity (Jain SC and B Singh, 1998) when tested at various doses of 20 and 400 mg/kg. the methanol extract at the dose of 400 mg/kg showed high significant anti-inflammatory activity at 4 h, where it was shown 62.6% inhibition, as compared to that of 5 mg/kg of diclofenac sodium.

Table.2 Assesment of anti-inflammatory activity of MEBO and EAEBO

Group	Paw oedema volume (ml)				% inhibition
	60 min	120 min	180 min	240 min	
I	0.35 ± 0.04	0.49 ± 0.02	0.65 ± 0.01	0.67 ± 0.01	..
II	0.27 ± 0.06**	0.38 ± 0.02*	0.39 ± 0.02***	0.30 ± 0.02***	54.7
III	0.25 ± 0.01**	0.37 ± 0.02*	0.38 ± 0.0***	0.27 ± 0.03***	59.7
IV	0.28 ± 0.02**	0.38 ± 0.03*	0.38 ± 0.0***	0.31 ± 0.03***	55.2
V	0.24 ± 0.01 ***	0.37 ± 0.01**	0.35 ± 0.0***	0.26 ± 0.03***	61.6
VI	0.22 ± 0.01***	0.36 ± 0.01**	0.27 ± 0.02***	0.17 ± 0.10***	74.6

Values are shown in the table mean ± SEM.
Comparisons were made between Group I Vs II, III, IV, V and VI.P-values: *p<0.05, **p<0.01, ***p<0.001

CONCLUSION

It was concluded that the results of the present study support to the traditional use of *boswellia ovalifoliolata* in inflammation. *boswellia ovalifoliolata* Methanolic leaves extract, possessing significant anti-inflammatory activity. This may be due to the presence of triterpenoids, saponins and tannis which deserves further studies to establish its therapeutic value as well as its mechanisim of action.

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