

Research Article

Phytochemical Evaluation and Anti Inflammatory Activity of *Boswellia ovalifoliolata*A.Tamil Selvan*, Anusha Ravella¹, R.Suthakaran²

Department of Pharmacology, Teegala Ram Reddy College of Pharmacy, Meerpeta, Saroor nagar (M), Hyderabad, AP, India.

*Corresponding author's E-mail: tamilselvanpharmacologist@gmail.com

Accepted on: 15-06-2013; Finalized on: 31-08-2013.

ABSTRACT

Antiinflammatory drugs presently available for the treatment of various inflammatory disorders have diverse and undesirable side effects. *Boswellia ovalifoliolata* was a deciduous medium sized tree; its parts were used in curing various disorders and diseased conditions. Methanolic extract of the leaves of *Boswellia ovalifoliolata* was screened for antiinflammatory activity against carrageenan, histamine and egg white induced inflammation in rat paw oedema. Acute toxicity study reveals that 200mg/kg as the therapeutic dose. Indomethacin, Diclofenac sodium (10mg/kg) and piroxicam gel were used as the standard drug to test against various screening models. The methanolic extract produced significant ($p<0.001$) antiinflammatory action against all the models and can be concluded the methanolic extract of *Boswellia ovalifoliolata* posses antiinflammatory activity.

Keywords: *Boswellia ovalifoliolata*, Carrageenan, Histamine, Egg white, methanol, Indomethacin, Diclofenac sodium, Piroxicam.

INTRODUCTION

Nature always stands as golden mark to exemplify the outstanding phenomena of symbiosis. Nature serves humans with medicines which were used to maintain health, to treat and heal many ailments. Natural products from plant, animal and minerals have been the basis of the treatment of human disease.¹ Medicinal plants is of great importance to the health of individuals and communities. Medicinal plants has a potential source of therapeutic aid has attended a significant role in health system all over the world for both human and animals not only in the diseased condition but also has potential material for maintaining proper health.² Man ever since his first appearance on earth, has used plant throughout his historical development as a source of medicines. Herbal medicine is a triumph of popular therapeutic diversity.³ Tribal healers in most of the countries, where ethno medical treatment is frequently used to treat cut wounds, skin infection, swelling, aging, mental illness, cancer, asthma, diabetes, jaundice, scabies, eczema, venereal diseases, snake bite and gastric ulcer, provide instructions to local people as how to prepare medicine from herbal. Inflammation is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of Inflammation.⁴

Boswellia ovalifoliolata is deciduous medium sized tree. Leaves are imparipinnate. Alternate or crowded at the ends of branches, leaflets sessile, ovate-oblong unequal at base, glabrous beneath, veins reddish. The leaves are generally alternate, spiral, and odd pinnately compound with opposite, frequently long petiolate, entire to serrate, pinnately veined leaflets whose symmetry is distinctive in

some genera. The plant is over exploited for its medicinal uses. The fresh leaf juice used to prevent throat ulcers. Decoction of the stem bark 10 – 25 mL per day reduces rheumatic pains. The gum obtained from the trunk which is highly medicated. This gum is sold in the local market by the native tribals as Konda sambrani in Telugu language. Small lumps of fresh light yellow coloured liquid oozes out from the stem and hardens on exposure.⁵ Amyrins are the chief constituents of the gum together with resin acids and volatile acids. Shade dried gum is powdered dissolved in water and mixed with curd and given orally to cure amoebic dysentery. Gum powder of *Boswellia ovalifoliolata* and *Boswellia serrata* and fruit powder of *Pedalium murex* mixed in equal parts and made into paste and apply externally on the affected part of the testicle to cure hydrocoel.⁶ From the above studies it is quite apparent that the ethanolic extract possesses significant anti-inflammatory activity. The study justifies its use in inflammation as suggested in the folklore medicines.

MATERIALS AND METHODS

Extraction

About 200 g of powder of *Boswellia ovalifoliolata* was packed into a Soxhlet apparatus and extracted with 2 – 2.5 liters of petroleum ether at 40°C by continuous hot percolation. The extract was subjected to distillation and it was stored on desiccators and the % yield value was determined. The same mark was continued with different organic solvents like ethyl acetate, methanol, ethanol and water according to their order of polarity. Percentage yield of extracts were determined.⁷⁻⁹

Phytochemical Screening

The methanolic extract of the medicinal plants were subjected to chemical test for identification.



Toxicity Studies

The acute pharmacological studies are carried out at Teegala Ram Reddy College of Pharmacy (Reg.No.1447/a/11/CPCSEA) the form B was approved by IAEC members for the animal species, and further parameters of the study has been evaluated. The acute toxicity of petroleum ether extracts of *Boswellia ovalifoliolata Linn.* Leaves were determined as per the OECD guideline no.423 (Acute Toxic Class Method). It was observed that the test extract was not lethal to the rats even at 2000mg/kg 2000 mg/kg doses. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study. Here to confirm the dose a small trial has been performed in my lab with various concentration of the plant extract and the lethal was confirmed with 2000mg/kg.¹⁰

Pharmacological Screening

*Carrageenan induced paw oedema*¹¹

The anti-inflammatory activity was performed by dividing the animals into three groups (n=6). Group I served as control received the vehicle only (1% CMC, 10 ml/kg, p.o.). Group II served as standard, received Diclofenac Sodium at dose of 10 mg/kg b.w. Group III served as test, received methanolic extract at dose of 200 mg/kg b.w. p.o. The test was used to determine the anti-inflammatory activity of the extract by the method of Winter *et al.* (1962). The animals pre-treated with extract or Diclofenac sodium one hour before were injected with 0.1 ml of 1% carrageenan (in 1% CMC) solution into the sub-plantar region of right hind paw. Paw volume was measured by dislocation of the water column in a plethysmometer immediately after carrageenan application at 0, 1, 3, 6 and 12 h after the stimulus. Reduction in the paw volume compared to the vehicle-treated control animals was considered as anti-inflammatory response.

*Histamine induced paw oedema in rats*¹²

These extracts were tested for anti-inflammatory activity by histamine⁶³ (inflammogens) induced paw oedema method in rats. Different group of animals taken for experiments are as follows: Group I: Control: CMC (p.o.), Group II: Standard Indomethacin 10 mg/kg (p.o.), Group III: Test Methanolic extract 200mg/kg (p.o.). The animals were pre-treated with/Indomethacin/extracts 30 minutes before the injection of 0.1 ml of 1% histamine into the sub-plantar region in right hind paw to the rats⁶⁴. Paw volumes were measured by the dislocation of water column in a plethysmometer at 1, 2, 3, 4 and 5 hours after the administration of test materials. Reduction in the paw volume compared to the control animals were considered as anti-inflammatory response.

*Egg white induced paw oedema*¹³

The Swiss albino rats weighing between 150-200g were divided into three groups. Each consists of 6 animals, one group served as negative control. The second group served as standard (received piroxicam gel). The third

group served as test received methanolic extract and by administration of 0.005 ml of undiluted fresh egg white in the sub-plantar region to both test and the standard group. The paw volume was measured at 0 hr-3 hr after the injection of fresh undiluted egg white using plethysmograph.

RESULTS AND DISCUSSION

The extractive value indicates the amount of phytoconstituents soluble in the particular solvents used and the percentage yield in given Table -1. Phytochemical screening showed the presence of phytosterols, saponins, alkaloids, flavonoids, phenolic compounds, gums and mucilage in the extract. In toxicity study at the dose of 2000mg/kg the extract produces mortality and the therapeutic dose was fixed from the 1/10th of the lethal dose. Hence 200mg/kg was fixed as the therapeutic dose. In Carrageenan induced paw edema model the result obtained indicates that the extract found to have significant ($p<0.0001$) anti-inflammatory activity in rats. The extract at the test doses 200mg/kg reduced the oedema induced by carrageenan by 41.26% at 3 h, whereas the standard drug showed 47.35% of inhibition as compared to the control group. In histamine induced model the results obtained indicate that extract had significant anti-inflammatory activity in albino rats when compared with that of the control groups ($p<0.001$). The potency was found to be inversely proportional to the time taken for reduction in paw volume. The methanolic abstract showed the dose dependent activity. The methanolic abstract of *Boswellia ovalifoliolata* reduced oedema 61.76%, when compared with untreated control groups and thus it is considered as a significant treatment. Plant extract showed a potent anti-inflammatory activity against the paw oedema in Swiss albino rats when compared with reference standards ($p<0.001$). The potency was found to be inversely proportional to the time taken for reduction in the paw volume. It is well known that carrageenan induced paw oedema is characterized by biphasic event with involvement of different inflammatory mediators. In the first phase (during the first 2 h after carrageenan injection), chemical mediators such as histamine and serotonin play role, while in second phase (3-4 h after carrageenan injection). Kinin and prostaglandins are involved. Our results revealed that administration of methanolic extract inhibited the oedema starting from the first hour and during all phases of inflammation, which is probably inhibition of different aspects and chemical mediators of inflammation¹⁴. Histamine which acts as a vasodilatory agent easily produces the inflammatory response. But by our study was observed that histamine is not as effective as carrageenan in producing the inflammation to the paw of rats. Although it is less efficacious in producing the inflammation to the paw of rats. Although it is less efficacious in producing inflammation, it is beneficial as an inflamogen in particular cases. The methanolic extract of *Boswellia ovalifoliolata* showed significant reduction in paw at 2nd



hours or more with histamine injection, suggesting that *Boswellia ovalifoliolata* shows reduction in oedema which was caused due to the vasodilatory action of the histamine and here it can be better compared with that of the indomethacin¹⁵.

The inflammatory response is a physiological characteristic of vascular tissue. Increased permeability seen in the inflammatory reaction leads to exudation of fluid rich in plasma proteins, coagulation factors and injured tissues with subsequent oedema at the site¹⁵.

Exudation which is a consequence of vascular permeability is considered as major features of acute inflammation¹⁴. Histamine and other mediators of inflammation increase vascular permeability at various times after injury. Chemically induced vascular permeability can causes an immediate reaction and its inhibitions suggests that the topical administration of test extract may effectively suppress the exudative phase of acute inflammation induced by undiluted fresh egg white.

Table 1: Extractive values of *Boswellia ovalifoliolata* leaves

Solvents	Percentage yield (w/w)	Colour	Consistency
Petroleum ether	12%	Greenish- Black	Dry mass
Methanol	50%	Greenish- Black	Dry mass

Table 2: Effect of methanolic extract of *Boswellia ovalifoliolata* on carrageenan induced rat paw oedema

Groups	Dose mg/kg	Paw volume (ml)				
		0 h	1 h	3 h	6 h	12 h
Control	1% CMC	1.205±0.009916	1.730±0.03759	2.302±0.01138	2.060±0.01571	1.637±0.02028
Standard	100	1.192±0.02212	1.590±0.0550 ^a	1.212±0.01537 ^c	1.135±0.01544 ^c	1.173±0.009888 ^c
<i>Boswellia ovalifoliolata</i>	200	1.185±0.02487	1.640±0.04074	1.352±0.01621 ^c	1.288±0.007923 ^c	1.267±0.01256 ^c

n=6, each value is the mean ± S.E.M. for 6 rats ^ap < 0.05; ^bp < 0.01; ^cp<0.0001 compared with control.

Table 3: Effect of *Boswellia ovalifoliolata* extract on histamine induced paw oedema in rats

Treatment	Dose mg/kg	Paw oedema (ml) Mean ± S.E.M (% inhibition)				
		1 st hour	2 nd hour	3 rd hour	4 th hour	5 th hour
Control	5ml	0.68±0.005	1.02±0.010	1.18±0.011	0.96±0.008	0.85±0.08
Standard	10	0.30±0.003 [*] (55.88)	0.39±0.003 [*] (61.76)	0.49±0.003 [*] (58.47)	0.49±0.005 [*] (48.95)	0.36±0.003 [*] (57.64)
<i>Boswellia ovalifoliolata</i>	500	0.30±0.003 [*] (55.88)	0.39±0.005 [*] (61.76)	0.49±0.012 [*] (58.47)	0.47±0.008 [*] (51.04)	0.39±005 [*] (54.11)

n=6, p<0.001(significant) when compared to c-II; statistical analysis was done by one way analysis of variance (ANOVA) followed by Bonferroni's test.

Table 4: Effect of *Boswellia ovalifoliolata* extract on egg white induced paw oedema in rats

Drug	Time in intervals (min)				
	0 min	30 min	60 min	120 min	180 min
Control drug	0	0.34±1.4	0.42±1.03	0.49±2.15	0.58±2.15
Standard drug	0	0.36±0.85	0.39±0.75	0.35±0.70	0.32±1.4
<i>Boswellia ovalifoliolata</i>	0	0.37±0.85	0.34±0.9	0.30±1.03	0.28±1.25

The data obtained were expressed as mean ±SEM. Statistical analysis were performed by one way analysis of variance (ANOVA) followed by student's t test. p values<0.001 were considered significant.

CONCLUSION

Thus it can be concluded that the methanolic extract of *Boswellia ovalifoliolata* possess anti-inflammatory activity. Determination of anti-inflammatory activity is based on plethysmograph measurement of oedema produced by carrageenan induced histamine induce and

egg white induced methods. The increase in oedema in animals treated with the various chemicals in all the groups were compared with increase in oedema of untreated control animals at constant intervals. Thus percentage inhibition of oedema at known intervals in treated animals was used for the purpose of calculating percent inhibition of oedema of control. Further studies



involving the purification of the chemical constituents of the plant and the investigations in the biochemical pathway may result in the development of a potent anti-inflammatory agent with low toxicity and better therapeutic index.

REFERENCES

1. Chopra RN, Nayar SL, Chopra IC, In Glossary of Indian Medicinal Plants, CSIR, New Delhi, 1st ed, 1956, 197.
2. The Ayurvedic Pharmacopoeia of India , Ministry of health and family welfare Department and Indian system of Medicine and Homeopathy, New Delhi, 11(1), 1999, 137-140.
3. Yue- Zhong Shu, Recent natural products based drug development: A Pharmaceutical Industry Perspective, *J.Nat. Prod.* 61, 1998, 1053-71.
4. Mukeshwar pandey, Mousumi Debnath, Shobit Gupta, Surender K, Chikara, Phytomedicine: an Ancient approach turning into future potential source of therapeutics, *J. Pharmacog. Phytotherapy*, 3(3), 2011, 27-37.
5. Mitchell RN, Cotran RS. In: Robinsons Basic Pathology, Edtn 7. Harcourt Pvt. Ltd., New Delhi, India, 2000, 33 - 42.
6. Phytochemical methods: a guide to modern techniques of plant analysis. Harbone, 3rd edition. *Chapman & Hall Pub.* London, UK, 1998, 129 – 138.
7. Ialenti A, Ianaro A, Moncada S, Di Rosa M. Modulation of acute inflammation by endogenous nitric oxide. *Eur. J. Pharmacol.* 211, 1995, 177-184.
8. Di Rosa M, Willoughby DA. Screening for anti-inflammatory drugs. *J. Pharm. Pharmacol.* 23, 1971, 297-303.
9. Kokate CK, Purohit A.P and Gokhale S.B, "Herbal Medicine Introduction". *Pharmacognosy*, Nirali Prakashan, Pune, 2, 2010, 1-6.
10. DE S, Dey YN, Ghosh AK, "Phytochemical investigation and chromatographic evaluation of the different extracts of tuber of *Amorphophallus sapeondifolius* (Aracea)", *Int.J.Pharma Bio. Res.*, 1(5), 2010, 150-157.
11. Ghosh MN. Fundamentals of Experimental Pharmacology. 2nd ed. Calcutta: Scientific book agency, 1994, 153-158.
12. Perianayagam JB, Sharma SK, Pillai KK. Anti-inflammatory activity of *Trichodesma indicum* root extract in experimental animals. *J Ethnopharmacol.* 104, 2006, 410-414.
13. Otimenyin SO, Uguru MO, Atang BL. Anti-inflammatory and analgesic activity of *Ficus thonningi* and *Pseudocdrela Kotschy*i extracts. *Nig.J. Pharm.Res.*, 3(1), 2004, 82-85.
14. Hernandez PM, Rabanal Gallego R. Evaluation of the anti-inflammatory and analgesic activity of *Sideritis anariensis* Var. pannosa in mice, *J. Ethanol.*, 81, 2002, 43-47.
15. Doppalapudi Sandeep, Sandya.L, Chandra Kalyan Reddy, Y. Nagarguna, S.Padmanabha Reddy, Y.Sabashafeen. Anti-inflammatory avtivity of cicer *Arietinum* seed extracts. *Asian Journal of Pharmaceutical and Clinical research*, 5(1), 2012, 101.

Source of Support: Nil, Conflict of Interest: None.

