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Original Article

BIOACTIVITY GUIDED FRACTIONATION AND ANTI-INFLAMMATORY ACTIVITY OF *ACACIA NILOTICA* PODS

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ABSTRACT

Objective: The present study aims at screening the methanol extract and the tannin fraction of the pods of *Acacia nilotica* for anti-inflammatory activity.

Methods: The methanol extract and its tannin fraction were evaluated for acute toxicity using rats as per the Organization for Economic Co-operation and Development (OECD) guidelines. They were found to be safe up to a dose of 2000 mg/kg. The anti-inflammatory activity of the extract and its fraction was evaluated using the carrageenan-induced rat paw edema method for determining the acute phase of inflammation. The activity against sub-acute inflammation was evaluated using the cotton pellet granuloma pouch method. Diclofenac sodium was used as the reference drug.

Results: The results of pharmacological screening indicated the methanol extract to possess statistically significant anti-inflammatory activity at 100 mg/kg dose. The tannin fraction of methanol extract was found to be more potent than the methanol extract with maximum response at 25 mg/kg dose.

Conclusion: The methanol extract and tannin fraction of pods of *Acacia nilotica* possess significant anti-inflammatory activity the latter one being more potent.

Keywords: *Acacia nilotica*, Anti-inflammatory, Phenolics and HPTLC.

INTRODUCTION

Inflammation is caused by a variety of stimuli including physical damage, ultra violet irradiation, microbial invasion and immune reactions. 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. The classical key features of inflammation are redness, warmth, swelling and pain. Inflammation cascades can lead to the development of diseases such as chronic asthma, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease and psoriasis. Many of these diseases are debilitating and are becoming increasingly common in our aging society. Rheumatoid arthritis and osteoarthritis are the major inflammatory diseases affecting people worldwide. Several classes of drugs, such as corticosteroids, nonsteroidal anti-inflammatory drugs (NSAID) and biologics are used to treat the inflammatory disorders. All these drugs possess several adverse effects and biologics are expensive to be used. Natural products or natural product-derived compounds represent great structural diversity, which is not commonly seen in synthetic compounds. Of the 1184 new chemical entities reported during 01/1981 to 06/2006, 60% are derived from or based on natural products. Thus, natural products play a dominant role in the discovery of leads for the development of drugs for treating human diseases. Natural products (and traditional medicines) offer great hope in the identification of bioactive compounds and their development into drugs for the treatment of inflammatory diseases. Plants have been the basis of many traditional medicine systems throughout the world for thousands of years and continue to provide mankind with new remedies [1, 2]. Biomolecules can be oxidized by free radicals. This oxidative damage has an important etiological role in aging and the development of diseases like cancer, atherosclerosis and other inflammatory disorders. Therefore, there is an increasing interest in searching for antioxidants of natural origin. As a part of this effort, we evaluated the methanol extract of *Acacia nilotica* for potential antioxidant activity. The results of these assays indicated that the methanol extract exhibited a significant antioxidant activity and this led us to extend our research and evaluate the potential of this extract for anti-inflammatory activity.

Traditionally *Acacia nilotica* (Family: Mimocaceae) has a versatile medicinal value and has been used in folk medicine for treating cold, cough, dysentery, ulcers, tuberculosis, leprosy etc. It is rich in tannins. The tannin content of the bark varies from 12-20 %. Several polyphenolic compounds have been reported to be present in the bark viz. catechin, epicatechin, quercetin, gallic acid and leucocynidin gallate [3, 4]. The present study aims at screening the methanol extract and its tannin fraction of *Acacia nilotica* pods for anti-inflammatory activity. The existing battery of NSAID is associated with gastro-intestinal disturbances that reduce patient compliance. Additionally, patients with chronic inflammatory diseases look for alternative systems of medicines and plants are the single largest source of medicaments used in alternative systems (Ayurveda, Unani and Homeopathy) of medicine. These pods are easily and abundantly available in our country and can serve as a rich source of anti-inflammatory compounds.

MATERIALS AND METHODS

Plant material

The ripe pods of *Acacia nilotica* were collected from the outer region of Pune, India. The plant specimen was authenticated at the Agharkar Research Institute, Pune, India.

Preparation of extracts

Shade dried pods of *Acacia nilotica* were ground to coarse powder and extracted with Soxhlet using methanol for 18 hours. Concentration of the methanol extract (ME) was done using a rotary evaporator under vacuum. This resulted in a thick brown extract which were refrigerated and requisite quantity used for conducting the experiments.

Physico-chemical and phytochemical analyses

The ME was standardized with respect to physico-chemical parameters viz. color, consistency, pH and extractive value. The powder of the pods was standardized with respect to alcohol and water soluble extractive values and ash values as per the Indian Pharmacopoeia. Preliminary phytochemical analysis of the methanol extract was performed to identify the major class of phytoconstituents present according to standard reported methods.

High performance thin layer chromatography (HPTLC) fingerprint

HPTLC fingerprints of the ME were generated after development of a suitable mobile phase-toluene: ethyl acetate: formic acid (6:3:1) for the separation of different phytoconstituents in the extract to be used as a tool for standardization and quality control of this ME.

Fractionation of ME

The ME was fractionated based on the nature of phytoconstituents into tannin fraction and non-tannin fraction. An aqueous solution of the ME was treated with 10 % lead acetate solution and the solution was filtered. The filtrate was evaporated to dryness. This fraction was termed as the non-tannin fraction. The resultant precipitate of lead tannates was subsequently treated with hydrogen sulfide to remove the lead as lead sulfide. The resultant filtrate was subsequently evaporated to dryness and this was termed as the tannin fraction (TFME) of the ME.

Animals

Male and female wistar rats approximately the same age, weighing about 175-250 g were obtained from Haffkine Institute for conducting the experiments. They were housed in polypropylene cages and fed with standard pellet diet and water ad libitum. The animals were exposed to alternate cycle of 12 h of darkness and light each. Before each test, the animals were fasted for at least 12 h. The experimental protocols were subjected to the scrutiny of the Institutional Animal Ethics Committee and were cleared by the same (CPCSEA/IAEC/BNCP-02/2011 and CPCSEA/IAEC/BNCP-03/2011).

Acute toxicity studies

The acute toxicity studies were carried out as per the Organization for Economic Co-operation and Development (OECD) guidelines. The animals were divided into control and test groups containing six animals each. The control group received the vehicle (1% sodium carboxy methyl cellulose suspension in water) while the test groups received different doses of methanol extract (as a suspension in water using 1% sodium carboxy methyl cellulose) and the fractions thereof per orally.

Carrageenan induced rat paw edema assay [5]

The rats were divided into nine groups of six animals each. The control group received 0.2 ml of vehicle (1% sodium carboxy methyl cellulose suspension in water), the standard group received 10 mg/kg of diclofenac sodium (DFS) and test groups received ME (50, 100, 200 and 400 mg/kg) and TFME (25, 50 and 100 mg/kg) per orally. After 1 h the rats were challenged with subcutaneous injection of 0.1 ml of 1 % w/v solution of carrageenan into the plantar side of the left hind paw. The paw was marked with ink at the level of lateral malleolus and immersed in water up to the mark. A plethysmometer was used for the measurement of rat paw volume. The paw volume was measured immediately after injection (0 h) and at 1, 2 and 3 h after injection of carrageenan to each group. The percentage reduction in edema volume was calculated using the formula:

$$\% \text{ reduction} = (Vo - Vt)/Vo \times 100$$

where,

Vo = Volume of the paw of control group at time't'

Vt = Volume of the paw of test group treated at time't'

Cotton pellet induced granuloma assay [6]

In cotton pellet granuloma model, the rats were divided into six groups of six animals each. The animals were anaesthetized with ether. The back skin was shaved and disinfected with 70 % ethanol. An incision was made in the lumbar region. Subcutaneous tunnels were formed by a blunted forceps and a sterilized, pre weighed cotton pellet was placed on both sides in the scapular region. The control group received 0.2 ml of vehicle (1% sodium carboxy methyl cellulose suspension in water), the standard group received 10 mg/kg of DFS and test groups received ME (100 and 200 mg/kg) and TFME (50 and 100 mg/kg) per orally for six days. The animals were sacrificed on the seventh day and the cotton pellets along with the granuloma mass were collected, dried and weighed. The results of the assay were calculated as % inhibition of dry weight of granuloma formation by using the formula:

$$\% \text{ inhibition} = (A - B)/A \times 100$$

where,

A= gain in dry weight of control pellet (mg)

B= gain in dry weight of extract treated pellet (mg)

Statistical analysis

Assessment of statistical significance for *in vivo* experiments was done using one way analysis of variance (ANOVA) followed by Dunnetts test. All the values are expressed as mean±SEM. The level of significance was fixed at $P<0.05$.

RESULTS

Physico-chemical analysis

The results of the physico-chemical analysis and standardization of the *Acacia nilotica* powder sample are given in Tables 1 & 2.

Table 1: Standardization of the *Acacia nilotica* pod powder

Parameter	Percentage
Alcohol soluble extractives	42±0.25
Water soluble extractives	45±0.22
Total ash	4.98±0.20
Water insoluble ash	2.25±0.11
Acid insoluble ash	0.65±0.12
LOD	8.96±0.15

Values are mean±SD of triplicates

Table 2: Physico-chemical evaluation of the ME of *Acacia nilotica* pods

Physico-chemical	Parameters
Nature	Semisolid
Color	Brown
pH	3
Extractive value	44.36%

HPTLC fingerprint

HPTLC fingerprints serve as a tool to standardize the extract thereby assuring the quality of the same. The HPTLC densitometric data of the ME at 254 nm and 366 nm are given in table 3 & 4. The ME was found to show characteristic bands for gallic acid, catechin and epicatechin including other phenolic compounds as confirmed from chemical derivatization of the bands using Folin Ciocalteau reagent.

Table 3: HPTLC data of ME at 254 nm

Peak	Maximum Rf	Maximum height (mm)	Maximum area
1	0.30	140.3	5777.2
2	0.39	185.0	6336.5
3	0.50	618.3	25162.3
4	0.56	672.4	25322.1

Table 4: HPTLC data of ME at 366 nm

Peak	Maximum Rf	Maximum height (mm)	Maximum area
1	0.16	42.0	1069.5
2	0.22	58.3	4010.8
3	0.35	81.0	3140.1
4	0.44	141.7	6698.8
5	0.53	81.4	1351.0
6	0.60	152.9	14931.6

Phytochemical investigation

The results indicated the presence of tannins, phenolics, saponins, flavonoids, steroids and polysaccharides in the methanol extract.

Acute toxicity studies

Acute toxicity studies revealed that the ME and TFME were found to be non-toxic on oral administration; the LD50 value was higher than 2 g/kg. No mortality was observed and no toxic reactions were observed during and at the end of the study.

Carrageenan induced rat paw edema

The anti-inflammatory effect of the ME of *Acacia nilotica* pods against carrageenan induced inflammation is shown in table 5. The ME was found to reduce the inflammation at doses of 50-200 mg/kg with the best response being observed at 100 mg/kg. The ME at a dose of 100 mg/kg was found to reduce the inflammation by 39.01 %. The TFME was found to reduce inflammation at doses of 25-100 mg/kg with the best response being observed at 50 mg/kg. The TFME at a dose of 25 and 50 mg/kg was found to reduce the inflammation by 45.29 % and 48.43 % respectively. DFS was found to reduce the inflammation by 59.64 %.

Cotton pellet induced granuloma assay

The ME showed a significant inhibition in cotton pellet granuloma formation as compared to the control group. The percentage inhibition at doses of 100 and 200 mg/kg was found to be 33 and 38 % respectively. The TFME exhibited a significant inhibition in cotton pellet granuloma formation (36 and 41%) at doses of 50 and 100 mg/kg respectively. The TFME was found to be more potent than the ME at the doses tested.

Table 5: Carrageenan induced rat paw edema

Treatment	Dose (mg/kg	Paw volume in ml		
		1 h	2 h	3 h
Control	Vehicle	1.25±0.22	2.03±0.20	2.23±0.19
ME	50	0.94±0.20 (24.80%)	1.25*±0.32 (38.42%)	1.66*±0.38 (25.56%)
	100	0.88±0.13 (29.60%)	1.24*±0.09 (38.92%)	1.36*±0.05 (39.01%)
	200	0.86±0.06 (31.20%)	1.17*±0.12 (42.36%)	1.39*±0.13 (37.67%)
	400	1.22±0.10 (2.40%)	1.56±0.14 (23.15%)	1.68±0.22 (24.66%)
TFME	25	0.99±0.03 (20.80%)	1.12*±0.08 (44.83%)	1.22*±0.09 (45.29%)
	50	0.86*±0.10 (31.20%)	1.01*±0.08 (50.25%)	1.15*±0.10 (48.43%)
	100	1.05±0.08 (16.00%)	1.49±0.04 (26.60%)	1.69±0.03 (24.22%)
DFS	10	0.82*±0.11 (34.40%)	0.86**±0.08 (57.64%)	0.90**±0.09 (59.64%)

Values are mean±SEM (n=6). Fig. in parentheses indicates % reduction in edema, *Denotes significance at the level of P<0.05 *versus* control group, **Denotes significance at the level of P<0.01 *versus* control group.

Table 6: Cotton pellet induced granuloma

Treatment	Dose (mg/kg)	Average weight of cotton pellet (mg)	Average weight of cotton pellet with granuloma (mg)	Percent inhibition
Control	Vehicle	50±0.01	272.67±4.55	-
IME	100	50±0.01	181.67*±2.55	33.37
	200	50±0.01	168.33*±3.79	38.27
IME-TF	50	50±0.01	174.67*±2.50	35.94
	100	50±0.01	160.33*±2.13	41.20
DFS	10	50±0.01	121.33**±2.03	55.50

Values are mean±SEM (n=6), *Denotes significance at the level of P<0.05 *versus* control group, **Denotes significance at the level of P<0.01 *versus* control group.

DISCUSSION

The present study involved the evaluation of anti-inflammatory activity of *Acacia nilotica* pods in different experimental models of inflammation. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Edema formation, leukocyte infiltration and granuloma formation represent such components of inflammation [7]. Edema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow [8]. Carrageenan-induced rat paw edema model is widely used for determining the acute phase of inflammation. Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation whereas prostaglandins are detectable in the late phase of inflammation [9, 10]. Sub-plantar injections of carrageenan provoked marked time-related increase in the hind paw diameters of the "untreated" control rats. Maximal swelling and/or edema occurred approximately 90 min following carrageenan administration. It is known that the third phase of the edema induced by carrageenan, in which the edema reaches its highest volume, is characterized by the presence of prostaglandins (PGI_2) and other compounds of slow reaction [11]. It has been found that the injection of carrageenan into the rat paw induces the liberation of bradykinin, which later induces the biosynthesis of prostaglandin and other autacoids, which are responsible for the formation of the inflammatory exudates [12]. Besides, in the carrageenan-induced rat paw edema model, the production of prostanoids has been through the serum expression of COX-2 by a positive feedback mechanism [13]. Therefore, it is suggested that the mechanism of action of the methanol extract may be related to inhibition of prostaglandin synthesis. The cotton-pellet granuloma is widely used to evaluate the transudative and proliferative components of the chronic inflammation. The moist weight of the

pellets correlates with transude, the dry weight of the pellet correlates with the amount of granulomatous tissues [14, 15]. Chronic inflammation occurs by means of the development of proliferate cells. These cells can be either spread or in granuloma form. Non-steroidal anti-inflammatory drugs decrease the size of granuloma which results from cellular reaction by inhibiting granulocyte infiltration, preventing generation of collagen fibers and suppressing mucopolysaccharides [16, 17]. The ME and the TFME of *Acacia nilotica* pods showed significant anti-inflammatory activity in cotton pellet induced granuloma and thus could be effective in chronic inflammatory conditions. There are many reports of whole extracts, extract fractions and phytoconstituents exhibiting anti-inflammatory activity. Dion *et al.* (2015) have reported the anti-inflammatory and antioxidant potential of fern extracts from *Matteuccia struthiopteris*, *Osmunda japonica*, *Matteuccia orientalis* and *Pteridium aquilinum* traditionally been used both for dietary and therapeutic purposes. They have also suggested the positive correlation between antioxidant and anti-inflammatory activities observed for these edible ferns [18]. A similar analysis is reported by Tomovic *et al.* (2015) where in there is a good correlation between the antioxidant and anti-inflammatory activities of *Potentilla reptans* rhizome aqueous extract. Their study suggests that these activities are due to the high phenolic content in the extracts which is in tandem with previous reports [19]. Kapewangolo *et al.* (2015) have demonstrated promising anti-inflammatory and antioxidant properties for *Ocimum labiatum* ethanolic extract attribute to the inhibition of the production of pro-inflammatory cytokines IL-2, IL-4, IL-6 and IL-17A [20]. *Acacia nilotica* pods are generally eaten by cattle and are a good source of nutrients for them. The pods have demonstrated significant antioxidant activity in our earlier experiments [21]. In view of the above reports, it can be said that the pods hold excellent promise as a natural source of anti-inflammatory compounds.

CONCLUSION

Inflammation plays a major role in many diseases, for instance in arteriosclerosis, rheumatoid arthritis, autoimmune disorders and cancer. The methanol extract *of Acacia nilotica* pods was found to possess a significant anti-inflammatory activity. The tannin fraction of the methanol extract was found to be more potent than the methanol extract. This is corroborated by literature that indicates that the major categories of compounds that modulate the inflammatory pathways are polyphenolics. Several polyphenolic compounds such asflavonoids, lignans, phloroglucinols, quinones, stilbenes, phenylpropanoids, and diarylheptanoids have been reported to exhibit significant *in vitro* and *in vivo* anti-inflammatory activity. This could thus add to the armamentarium of natural product research exploring safe and effective anti-inflammatory molecules derived from natural resources.

CONFLICT OF INTERESTS

The authors have declared no conflict of interest.

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