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ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC (SEED) EXTRACT OF *ACACIA NILOTICA* L.) DEL MEDIATED THROUGH CYCLOOXYGENASE (COX) INHIBITION

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Abstract

The current study was aimed to evaluate the *Acacia nilotica* for anti-inflammatory, activity against carrageenan induced paw model. The anti-inflammatory effects were assessed in rodents using rat paw edema tests. The intraperitoneal (i.p.) administration of the methanolic extract (50–200 mg/kg i.p.) produced marked anti-inflammatory effect in carrageenan-induced rat paw oedema assay comparable to diclofenac and produced a dose-dependent (0.5–2.5 mg/mL) inhibitory effect against, therefore for mechanistic evaluation, we also check the inhibitory potential of methanolic extract of *Acacia nilotica* against COX1 and COX2, important enzyme for inflammatory reactions. The results indicated that methanolic extract at the concentration of 200 mg/kg inhibit the activity of COX2 but negligibly altered the COX1 activity. Furthermore we also check the toxicity of methanolic extract of an and we found that the extract at all the three tested doses (500, 1000, 1500) did not alter the lipid profile, liver function parameter and kidney function parameter in comparison to vehicle control. The data suggests that *A. nilotica* possesses peripheral anti-inflammatory action. Pharmacological investigations of various species of genus Acacia have revealed hypoglycemic effect of inflammatory properties, with analgesic effects partially associated with the opoid system.

Key words : *Acacia nilotica*, edema, Anti-inflammatory, Carrageenan-induced paw oedema.

Introduction

Acacia nilotica tree colloquially called is the Babbula of Sanskrit writers, who mention the use of young leaves and pods as an astringent. *Acacia nilotica* contains acid, m-digallic acid, (+)-catechin, chlorogenic acid, gallolyated flavan-3, 4-diol, robidandiol (7, 3, 4, 5-tetrahydroxyflavan-3-4-diol), androstene steroid, D-pinitol carbohydrate, catechin-5-galloyl ester. Acacia nilotica has anticancer and antimutagenic, anti-inflammatory, antiplasmodial, antidiarrhoeal, antihypertensive, antiplatelet aggregatory, molluscicidal, antifungal, antimicrobial activity and inhibitory activity against Hepatitis C (Meena *et al*., 2006, Kirira *et al*., 2006 and Agunu *et al*., 2005). Various parts of *A. nilotica* are utilized for treating gonorrhea, leucorrhea, bleeding ulcers, wounds and dysentery (Kapoor 1990). Plants happiness to genus Acacia area unit wide employed in the management of pain and inflammation in folks drugs system. *A. farnesiana* roots, bark and leaves are used in inflammatory conditions, ulcer and wounds (Wiart, 2002). *A. pennata* is utilized as a remedy for headache, rheumatism and fever (Dongmo *et al*., 2005). At least five species of Acacia are used in traditional system of Kenya in stomachache, boils, swelling and eye infections (Geisler *et al*., 2003). Pharmacological investigations of various species of genus Acacia have revealed hypoglycemic effect of A. modesta (Singh *et al*., 1975), anti-inflammatory (Dafallah and Al-Mustafa 1996) and anti-platelet (Shah *et al*., 1997) activities of *A. nilotica*, analgesic and anti-inflammatory (Dongmo *et al*., 2005) effects of *A. pennata*. Some species of Acacia such as *A. adsurgens, A. ancistrocarpa* and *A. catechu* have been shown to exhibit cyclooxygenase inhibitory effects (Li *et al*., 2003). Flavans from genus Acacia are reported for their inhibitory effects against cyclooxgenase and 5-lipoxygenase (Jia *et al*., 2003). The genus Acacia is a rich source of bioactive terpenoids and flavonoids (Garai and Mahato 1997, Seigler 2003).

In view of the wide use of the genus Acacia in pain and inflammation the current study was undertaken to investigate anti-inflammatory activity of *A. nilotica* plant extract.

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Materials and Methods

Plant Material

The seed of *Acacia nilotica* were procured in the month of December 2016 from Kisan Seed company, Lucknow India. The plant material was taxonomically identified by the National Botanical Research Institute (N.B.R.I.) Lucknow (Uttar Pradesh), India and the Voucher Specimen (LWG) was retained in Department for future reference.

Preparation of Extract

The plant material was shade dried at room temperature and coarsely powdered by a pulveriser. The powdered plant material was successively extracted in a Soxhlet apparatus with methanol (MeOH) solvent to get respective extract. The extract was filtered concentrated to dryness under vacuum on a rotary evaporator to give the dried residues of extract. The crude extract thus obtained, as a semisolid mass, was stored in the refrigerator for use in the various experimental protocols.

Animals

All the experiments were carried out using any sex, Wistar rats (150-200 g) were obtained from animal house, CDRI, Lucknow U.P., India. On arrival the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C and relative humidity of 30-70%. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial rat chew pallets (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (Register Number: 1585/PO/E/5/11/CPCSEA) and were in accordance with the guidelines of the CPCSEA, Ministry of Forests and Environment, Government of India.

Phytochemical analysis

The methanolic extract of *A. nilotica* was screened for the presence of various phytochemical constituents such as alkaloids, flavonoids, saponins, glycosides, proteins, steroids and tannins according to the previously described method (Edeoga *et al*., 2005, Sofowora, 1993).

Table 2: Qualitative analysis with methanol extract.

S. No.	Test	Inference
1	Alkaloids	+ve
2	Tannins	+ve
3	Carbohydrates	+ve
4	Flavonoids	+ve
5	Glycosides	-ve
6	Steroids	-ve
7	Protien	-ve
8	Foam test	+ve

Investigation of crude extracts for COX inhibitory activity

The methanolic extracts of *A. nilotica* was subjected to preliminary screening for *in vitro* COX1 and COX2 inhibition in a COX catalyzed prostaglandin biosynthesis assay at 100 µg/mL concentration. Of extract three were short-listed for further detail studies based on the significant COX inhibitory activity (Table 1).

Acute toxicity test

Different doses of the methanolic extract of *A. nilotica* (500, 1,000, 1,500, mg/kg) were injected intraperitoneal (i.p.) to mice (20–25g) divided into separate groups, each consisting of six animals. The animals were observed for 7 days after administration of the extract for any acute toxicity symptoms, *e.g.*, behavioral symptoms. After seven days, the blood was collected from retero-orbital plexus. The serum was separated for biochemical analysis.

Investigation of crude extracts for COX inhibitory activity

The results of the preliminary screening of extracts were compared with the curcumin & MeOH extracts of *A. nilotica* showed comparable COX1 and COX2 inhibitory activity as that of curcumin. Therefore, these were subjected to further investigation.

Carrageenan induced rat paw edema assay (anti-inflammatory activity)

The carrageenan-induced hind paw edema test was conducted according to Winter *et al*., (1962). Rats divided randomly into different groups of 5–8 animals were injected subcutaneously into the plantar surface of the hind paw with 0.05 mL of freshly prepared 1% carrageenan (prepared in distilled water). Different doses of plant extract (50–200 mg/ kg i.p.) or diclofenac sodium (20 mg/kg) were injected i.p., 30 min before the administration of carrageenan. The control animals received the same volume of

Table 1: Screening of crude extracts for *in vitro* COX-1 and COX-2 inhibitory activity

S. No.	Plant name	Extracts	Percentage Inhibition at 100 µg/mL extract/standard	
			COX-1 inhibition[a]	COX-2 inhibition[a]
1	*A. nilotica*	MeOH	63.58 ± 1.42	78.94 ± 1.52
2	Curcumin[b]	Dichloromethane	84.42 ± 1.22	46.20 ± 0.96

[a] Percent inhibition expressed as mean ± S.E.M. (n=3); [b] Reference standard.

Table 3: Lipid profile parameters.

S. No.	Dose	Triglyceride (mg/dl)	HDL (mg/dl)	Bilirubin (mg/dl)	Cholesterol (mg/dl)
1	Control	283.83 ± 16.25	14.58 ±1.28	0.389 ±0.023	105.26 ± 8.26
2	500mg	305.58 ± 22.62	17.48 ±1.32	0.419 ±0.061	100.62 ± 5.04
3	1000mg	314.68 ± 28.52	16.56 ±1.39	0.437 ± 0.028	90.56 ± 8.29
4	1500mg	298.52 ± 22.42	13.84 ±2.21	0.384 ± 0.015	95.67 ± 7.24

the vehicle. Rat paw puffiness was assessed by volume displacement technique (plethysmometerUgoBasile 7150) before and when gum injection at 1, 2, 3 and 4 h. Difference in paw volume, determined before and after injection of the phlogistic agent indicated the severity of oedema. The % inhibition of the inflammation was determined for each animal by comparison with controls and calculated by the following formula:

%I ¼ 1 — ðdt=dcÞ × 100

where "dt" is that the distinction in paw volume within the drug- treated cluster and "dc" the distinction in paw volume up to the mark cluster. "I" stands for inhibition.

Lipid profile estimation

The lipid profile was estimated by using four measurements *i.e.* Triglycerides, Cholesterol, HDL-Cholesterol. The Siemens kits were used for the estimation and the experiments were carried out as per the manufacturer's protocol. The results expressed in terms of mg/dl.

Table 4: Liver Function Parameters.

S. No.	Dose	SGOT(U/L)	SGPT(U/L)	ALKP(U/L)
1	Control	39.85 ± 3.26	90.84 ±3.46	97.42± 8.28
2	500mg	42.45 ± 1.79	94.28 ±6.25	99.47± 5.23
3	1000mg	47.62 ± 4.38	97.82 ±8.51	104.76 ± 8.
4	1500mg	50.26 ± 7.84	102.25 ± 5.94	112.21± 9.76

Table 5: Kidney function Parameters.

S. No.	Dose	Creatinine (mg/dl)	Blood Urea Nitrogen (mg/dl)	Uric Acid (mg/dl)
1	Control	1.34 ± 0.024	13.54 ±1.06	2.84 ± 0.015
2	500mg	1.46 ± 0.018	14.08 ±1.14	2.91 ± 0.022
3	1000mg	1.56 ± 0.031	14.86 ±0.84	3.04 ± 0.007
4	1500mg	1.79 ± 0.076	15.69 ± 2.23	3.48 ± 0.032

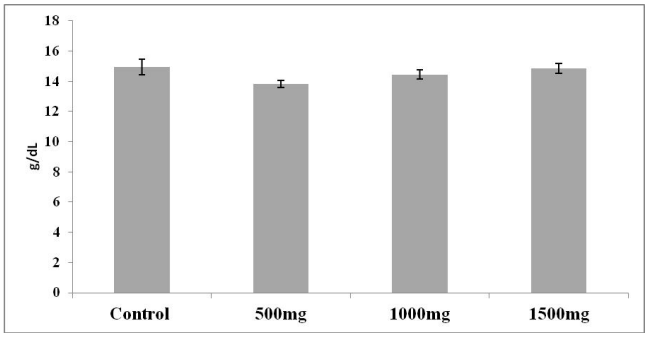

Fig.1: Total Hemoglobin data

terms of mg/dl.

Liver function test

The liver function was estimated by using four measurements *i.e.* SGOT, SGPT, ALKP. The Siemens kits were used for the estimation and the experiments were carried out as per the manufacturer's protocol. The results expressed in terms of U/L.

Kidney function test

The kidney function was estimated by using four measurements *i.e.* Creatinine, BUN, Uric acid. The Siemens kits were used for the estimation and the experiments were carried out as per the manufacturer's protocol. The results expressed in terms of mg/dl.

Results

Phytochemical analysis

The results of the qualitative phytochemical screening of *A. nilotica* methanolic seed extract are summarized in table 1. Previous phytochemical studies on *A. nilotica* have revealed some commonly known compounds such as quercetin, kaempherol and b-sitosterol (Khan, 2004).

Acute toxicity test

The animals treated with high doses of plant extract (1,000 mg/kg) showed did not significantly altered the normal profiling of liver and safe upto1000mg/kg.

Anti-inflammatory activity

The subplantar injection of carrageenan produced a localized edema that reached to its maximum at the third hour after injection. The localized inflammatory response illustrated by increase in paw volume was sustained for 4 hrs. and gradually declined after this time.

Table 2 demonstrate that methanolic extract of *A.nilotica* (50–200mg/kg i.p.) reduced the paw edema at third hour after carrageenan administration, with maximum inhibition being 58.33% at 200mg/kg. The early phase of the carrageenan-induced edema was not affected by the plant extract (data not shown). The difference between the paw volume of the control and extract-treated animals was statistically significant ($P \setminus 0.01$) at the third hour of observation. The standard drugs diclofenac sodium (20 mg/kg i.p.) produced 83.34% inhibition of the carrageenan-induced edema.

Values represent mean ± SEM. Different doses of *A. nilotica* or diclofenac sodium were injected i.p., 30 min prior to the administration of carrageenan. Animals were injected subcutaneously into the plantar surface of the hind paw with 0.05 ml of freshly prepared 1%

Table 6: Effect of the methanolic extract of *A. nilotica*and diclofenac sodium on carrageenan-induced paw edema in rats

Treatment	Dose (mg/kg i.p.)	Initial paw volume (ml)	Paw volume at 3rd hour	Increase in paw volume	% Inhibition
Control	Saline (1ml/kg)	0.89±0.03	1.01±0.01	0.12	–
Acacia nilotica	50	0.91±0.01	1.02±0.02	0.11	8.33
	100	0.94±0.03	1.01±0.04	0.07**	41.66
	200	0.99±0.02	1.04±0.03	0.03**	58.33
Diclofenac sodium	20	1.01±0.03	1.03±0.02	0.03***	83.34

carrageenan (prepared in distilled water). The control animals received same volume of the vehicle. Rat paw oedema was assessed by plethysmometer (UgoBasile 7150) before and at third hour after carrageenan injection. Difference in paw volume, determined before and after carrageenan injection indicated the severity of oedema. $^{**}P \setminus 0.01$ compared with control.

Discussion

Large numbers of plants belonging to genus Acacia are used in folk medicine in pain and inflammatory conditions (Geisler *et al*., 2003, Dongmo *et al*., 2005). This prompted us to investigate an indigenous medicinal plant. Peripherally, analgesic drugs such as diclofenac and aspirin and medicinal plants with folkloric use in the management of pain and inflammation such as Asparagus pubescens and Quasiaamara (Okpo *et al*., 2001, Nwafor and Okwuasaba, 2003, Toma *et al*., 2003) have shown analgesic effect in acetic acid-induced writhing in mice.

Based on above findings and folkloric use of various species of genus Acacia in inflammatory conditions, we decided to investigate its anti-inflammatory effect in carrageenan-induced rat paw edema model. The plant extract of A. nilotica exhibited marked anti-inflammatory effect similar to that observed with diclofenace sodium used as a reference compound. The carrageenan-induced acute inflammation is believed to be biphasic the early phase in which the edema production is mediated by histamine and serotonin and the late phase in which the vascular permeability is maintained by bradykinin and prostaglandins (Di Rosa *et al*., 1971, Burch and DeHaas, 1990). It has been reported that second phase of the oedema is sensitive to clinically effective anti-inflammatory drugs and has been frequently used to assess the anti-phlogistic effect of the natural products (Della Loggia *et al*., 1986, Saeed *et al*., 1995). In the present investigation the plant extract produced anti-inflammatory activity predominantly in the late phase of carrageenan induced edema test, indicating that the effect is possibly mediated via inhibition of the activity of prostaglandin. Similarly, the standard drug, diclofenac sodium, produced significant anti-edematous effect in the test. It is known that NSAIDs such diclofenac sodium reduce inflammation, swelling and arthritic pain by inhibiting prostaglandin synthesis and/or production (Skoutakis *et al*., 1988). There are evidences that compounds inhibiting the carrageenan-induced edema are effective in inhibiting the enzyme cyclooxygenases (Selvam and Jachak, 2004). Based on these reports, it can be inferred that the inhibitory effect of the plant extract on the carrageenan-induced inflammation at the third hour is possibly mediated via these mechanisms. The anti-inflammatory effect of the extract and the reference drug in carrageenan induced paw edema model in rats is shown in Illustration 5. After carrageenan administration, paw edema in rats reached to a peak value at 4 h. and various doses of methanolic extract of A. nilotica produced a significant inhibition in the edema volume ($P<0.001$) at the end of 3 h. maximum percent inhibition of edema exhibited with 400 mg/kg of methanolic extract of *Acacia nilotica* at was 58.33% and the effect was comparable to that of the standard drug 83.34%. It is well-known that in chronic and sub-acute inflammation, reactive oxygen species (ROS) play an important role in modulating the extent of inflammatory response and consequent tissue and cell injury (Corner *et al*., 1996).

Conclusion

The methanol extracts of *A. nilotica* excellent anti-inflammatory activities, which might be attributed to the presence of high concentration of phenolic compounds in this plant. This study supports the traditional use of this plant in inflammations. Further studies for isolation and identification of the phytochemicals responsible for the activities are needed to establish it as a herbal medicine for therapy of inflammation.

References

Agunu, A., S. Yusuf , G.O. Andrew, A.U. Zezi and E.M. Abdurahman (2005). Evaluation of five medicinal plants used in diarrhea treatment in Nigeria. *Journal of Ethnopharmacology*., **101:** 27-30.

Burch, R.M. and C. DeHaas (1990). A bardykinin antagonist inhibits carrageenan edema in rats. *Nuanyn Schmiedebbergs Arch. Pharmacol.,* **342:**189.

Conner, E.M. and M.B. Grisham (1996). Inflammation, free radicals and antioxidants. *Nutrition*., **12(4):**274-7.

Dafallah, A., Z. Al-Mustafa (1996). Investigation of the anti-

inflammatory activity of *Acacia nilotica* and *Hibiscus sabdariffa*. *Am. J. Chin. Med* ., **24:** 263-269.

Della Loggia, R., A. Tubaro, P. Dri, C. Zilli and P. Del Negro (1986). The role of flavonoids in the antiinflammatory activity of *Chamomilla recutita*. *Prog. Clin. Biol. Res.,* **213:** 481-484.

Di Rosa, M., J.P. Giroud and D.A. Willoughby (1971). Studies on the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. *J. Pathol*., **104:**15-29.

Dongmo, A.B., T. Nguelefack and M.A. Lacaille-Dubois (2005). Antinoci-ceptive and anti-inflammatory activities of *Acacia pennata* wild (Mimosaceae). *J. Ethnopharmacol.,* **98:** 201-206.

Edeoga, H.O., D.E. Okwu, B.O. Mbaebie (2005). Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol.,* **4:** 685-688.

Garai, S. and S.B. Mahato (1997). Isolation and structure elucidation of three triterpenoidsaponins from *Acacia auriculiformis*. Phytochemis- try., **44:**137-140.

Geisler, P.W., S.A. Harris, R.J. Prince, A. Olsen, R.A. Odhiambo, H. Oketch- Rabah, P.A. Maidiega, A. Andersen and P. Molgaard (2003). Medicinal plants used by Luo mothers and Children in Bondo district, *Kenya. J. Ethnopharmacol.,* **83:** 39-54.

Jia, Q., T.C. Nichols, E.E. Rohden and S. Waite (2003). Isolation of dual COX2 and 5-lipoxygenase inhibitor from Acacia. US Patent., **180:** 402.

Kapoor, L.D. (1990). Handbook of ayurvedic medicinal plants. CRC Press Inc, *Boca. Raton. Selangor.*, 130-131.

Khan, A. (2004). Phytochemical and pharmacological studies on *Acacia modesta*. M.Phil. Thesis, University of Peshawar, Peshawar, Pakistan.

Kirira, P.G., G.M. Rukunga, A.W. Wanyonyi, F.M. Muregi, J.W. Gathirwa, C.N. Muthaura, S.A. Omar, F. Tolo, G.M. Mungai and I.O. Ndiege (2006). Anti-plasmodial activity and toxicity of extracts of plants used in traditional malaria therapy in Meru and Kilifi Districts of Kenya. *Journal of Ethnopharmacology*., **106:** 403-407.

Li, R.W., S.P. Myers, D.N. Leach, G.D. Lin and G. Leach (2003). A cross-cultural study: anti-inflammatory activity of Australian and Chinese plants. *J. Ethnopharmacol.,* **85:** 25-32.

Meena, P.D., P. Kaushik, S. Shukla, A.K. Soni, M. Kumar and A. Kumar (2006). Anticancer and antimutagenic properties of Acacia nilotica (Linn.) on 7, 12-dimethylbenz (a) anthracene-induced skin papillomagenesis in Swiss albino mice. *Asia Pacific Journal of Cancer Prevention*., **7:** 627-632.

Nwafor, P.A. and F.K. Okwuasaba (2003). Anti-nociceptive and anti-inflammatory effects of methanolic extract of Asparagus pubes- cens root in rodents. *J. Ethnopharmacol.,* **84:** 125-129.

Okpo S.O., F. Fatokun, O.O. Adeyemi (2001). Analgesic and anti- inflammatory activity of *Crinum glaucum* aqueous extract. *J Ethnopharmacol.,* **78:** 207-211.

Saeed, S.A., R.U. Simjee, G. Shamim and A.H. Gilani (1995). Eugenol: a dual inhibitor of platelet activating factor and arachidonic acid metabolism. *Pytomedicine.,* **2:** 23-28.

Seigler, D.S. (2003). Phytochemistry of *Acacia sensu lato*. *Biochem. Syst. Ecol.,* **31:** 845-873.

Selvam, C. and S.M. Jachak (2004). A cyclooxygenase (COX) inhibitory flavonoids from the seeds of *Semecarpus anacardium*. *J. Ethno. pharmacol.,* **95:** 209-212.

Shah, B.H., B. Safdar, S.S. Virani, Z. Nawaz, S.A. Saeed and A.H. Gilani (1997). The anti-platelet aggregatory activity of *Acacia nilotica* is due to blockade of calcium influx through membrane calcium channels. *Gen. Pharmacol.,* **29:** 251-255.

Singh, K.N., V. Chandra and K.C. Barthwal (1975). Letter to the editor: Hypoglycaemic activity of *Acacia arabica*, *Acacia benthami* and *Acacia modesta* leguminous seed diets in normal young albino rats. *Indian J. Physiol. Pharmacol.,* **19(3):** 167-168.

Skoutakis, V.A., C.A. Carter, T.R. Mickle, V.H. Smith, C.R. Arkin, J. Alissandratos and D.E. Petty (1988). Review of diclofenac and evaluation of its place in therapy as a nonsteroidal anti- inflammatory agent *Drug IntellClin Pharm.,* **22:**850-859.

Sofowora, E.A. (1993). Phytochemical assays. In: Medicinal plants and traditional medicine in Africa, 3rd edn. Spectrum Books Ltd, Nigeria., 150-153.

Toma, W., J.S. Gracioso, C.A. Hiruma-Lima, F.D. Andrade, W. Vilegas and A.R. Souza Brito (2003). Evaluation of the analgesic and antiedematogenic activities of *Quassia amara* bark extracts. *J. Ethnopharmacol.,* **85:** 19-23.

Wiart, C. (2002). Medicinal plants of Southeast Asia, 2nd edn. Pearson Malaysia SdnBhd, Selangor., 130-131.

Winter, C.A., E.A. Risely and G.W. Nuss (1962). Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med.,* **111:** 544-547.