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ANTI-INFLAMMATORY ACTIVITY OF *GMELINA ARBOREA* ROXB. FRUIT EXTRACTS

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Abstract

Background: The plant *Gmelina arborea* has been traditionally used in India for several medicinal purposes like anthelmintic, diuretic, anti-inflammatory, antibacterial, antioxidant and antidiabetic.

Aims: The aim of the present study is to explore the anti-inflammatory activity of *G. arborea* fruit extracts using ethanol, ethyl acetate, n-butanol and petroleum ether as solvents.

Material and methods: The anti-inflammatory activity was evaluated by Carrageenan-induced rat paw edema method at a single dose of 300 mg/Kg, b.w. The **Statistical analysis used:** All data are verified for statistically significant by using one way ANOVA at 1 % level of significance ($p < 0.01$).

Results and discussion: All extracts were able to show good anti-inflammatory activity in comparison with standard drug Diclofenac sodium. The anti-inflammatory activity of all the extracts were found in the order of ethanol > n-butanol > petroleum ether > ethyl acetate.

Conclusion: It could be concluded that *G. arborea* fruits possess anti-inflammatory activity.

Key words: *Gmelina arborea*, *Verbenaceae*, Carrageenan, Inflammation.

Introduction

Inflammation is a body defense reaction which represents oedema formation, leukocyte infiltration and granuloma formation. Carrageenan-induced paw oedema 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 and prostaglandins are detectable in the late phase of inflammation.^[1,2]

Gmelina arborea Roxb fruits are oval in shape, ¾ inches in length and are yellow in color. The fruits are sweet in taste and some times astringent.^[6,7] The plant, *G. arborea* was reported to have several medicinal properties such as aphrodisiac, astringent, analgesic, antipyretic, antidiabetic, diuretic, anti-inflammatory and tonic characteristics.^[3-5] The literature survey reveals that fruits of *G. arborea* contain cardiac glycosides and steroids. The ethanol extract contains alkaloids, carbohydrates, anthraquinone glycosides, gums, mucilages, tannins, phenolic compounds and flavonoids. The ethyl acetate extract contains gums, mucilages, proteins and amino acids. The n-butanol extract contains alkaloids, anthraquinone glycosides, gums, mucilages, tannins, phenolic compounds, triterpenoids, saponins and flavonoids. The petroleum ether extract contains alkaloids, carbohydrates, anthraquinone glycosides, proteins, amino acids, triterpenoids and saponins.^[5,6]

Materials and Methods:

Drugs and Chemicals

Diclofenac sodium was procured as gift samples from Cadila Pharmaceutical Ltd., Ahmadabad, India. Petroleum ether AR 40-60°C and n-butanol GR 80°C were procured from Loba Chemie Pvt. Ltd., Mumbai, India. The ethanol AR and ethyl acetate AR 60-80°C (Emsure® ACS) were procured from Merck Pvt. Ltd., Navi Mumbai, Maharashtra, India. All other chemicals and reagents used in present work were procured from authorized dealer.

Collection of plant materials, identification and size reduction

The fruits of *G. arborea* were collected from local area of Koraput district (India) in the month of April and May 2008. The plant was identified and authenticated by the Biju Patnaik Medicinal Plants Garden and Research Centre, Dr. M.S. Swami Nathan Research Foundation, Jeypore, Koraput (District), Orissa (Letter no. MJ/DBT (08)/1067, dated 05.09.2008). The fruits were shade dried under normal environmental conditions. The dried fruits were pulverized to form coarse powder by using electrical grinder and stored in a closed air tight container for further use.

Preparation of solvent extracts

The coarse powder form of dried fruits was extracted by Soxhletion method by using ethanol, ethyl acetate, n-butanol and petroleum ether as solvents. A total amount of 1500 gm coarse powdered fruits was extracted with 1200 ml of each solvent. For each solvent, 10 cycles were run to obtain thick slurry. Each slurry was then concentrated under reduced

pressure to obtain crude extract. All crude extracts were kept in closed air tight containers under cool and dark place for further study.

Acute toxicity studies

To study the toxic effect (if any) of *G. arborea* extracts, Albino mice of either sex (20-25 g) were used. The animals were kept in the standard polypropylene cages at $25\pm 2^{\circ}\text{C}/60\%$ relative humidity in normal day and night photo cycle (12: 12 h). The animals were acclimatized for a period of 14 days prior to performing the experiments. Prior to the study, the experimental protocols were approved by the Institutional Animal Ethics Committee of Gayatri College of Pharmacy, Gayatri Vihar, Jamadarpali, Sambalpur, Odisha (Ethical Committee No 1339/ac/10/CPCSEA).

Acute oral toxicity study was performed as per OECD-423 guidelines.^[7,8] The animals were kept fasting overnight but allowed free access to water *ad libitum*. The fasted mice were divided into different groups of six animals each. Each solvent extract solution was administered orally at a dose of 10 mg/Kg b.w., using normal saline water as vehicle and mortality in each group was observed for 14 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the same procedure was repeated in each group for each extract with higher doses such as 100, 300, 600, 1000, 2000 and 3000 mg/Kg b.w. One tenth of this lethal dose was selected as the therapeutic dose for the evaluation of anti-inflammatory and antipyretic activities.

Anti-inflammatory activity

Healthy Wistar rats of either sex, weighing 180-250 g were used. They were housed in standard conditions of temperature ($25 \pm 2^{\circ}\text{C}$), and relative humidity of 45-55 % in animal house. The experiments were done at Gayatri College of Pharmacy, Sambalpur, Odisha. They were fed with a standard pellet diet and water *ad libitum*. All the animals were carefully monitored and maintained in accordance with CPCSEA guidelines (Control as well as experimental animals) for 15 days. All operations on animals were done under aseptic condition.

Anti-inflammatory activity of the extracts was evaluated by Carrageenan-induced rat paw edema method.^[9,10] Eighteen rats were divided into six groups of 3 rats each for various treatments.

The first group (I) served as normal control (Vehicle) which received normal saline water (2 ml/Kg b.w.) only. The second group (II) served as standard control which received Diclofenac sodium (5 mg/Kg b.w.). Groups (III) to (VI)

received ethanol, ethyl acetate, n-butanol and petroleum ether extracts respectively at a dose of 300 mg/Kg b.w of each extract. The inflammation inducing agent carrageenan, standard drug and test extracts were administered in solution form using normal saline water as vehicle.

In this study, initially animals were dosed with drugs (Control, standard and four extracts) as per the groups mentioned above. The standard drug and test extracts were administered intraperitoneally. Subsequently 30 min after above treatment, 0.05 ml of 1% solution of carrageenan was injected subcutaneously into the planter region of right hind paw of the animal to induce oedema. The edema was expressed as the increment in paw thickness due to carrageenan administration. The paw volume was measured initially and at 2, 3, 4 and 6 h after carrageenan injection using Plethysmometer (Ugo Basile, Italy).^[11,12] The percentage inhibition of paw thickness was calculated using the following formula. Inhibition of paw thickness (%) = $[1 - (V_t/V_c)] \times 100$, where, V_t Mean relative change in paw volume in test groups and V_c Mean relative change in paw volume in the control group. The reduction in the paw volume compared to the vehicle-treated control animals was considered as anti-inflammatory response.^[13]

Statistical analysis

To determine the statistical significance, standard deviation, standard error mean and one way analysis of variance (ANOVA) at 1% level significance was employed followed by z-test. P values < 0.01 were considered significant.^[14]

Results:

Acute toxicity study revealed that no mortality was found with any solvent extract at any dose in Swiss albino mice. No significant symptoms and side effects were observed with any animal.

All extracts showed anti-inflammatory activities at 300 mg/Kg b.w. (Table 1). The anti-inflammatory effect of the extracts was some what comparable with the standard drug, Diclofenac sodium. All the extracts showed lesser anti-inflammatory activity than the standard drug, Diclofenac sodium.

Table-1: Anti-inflammatory activities of *G. arborea* fruits extracts in Wister rats by carrageenan induced rat paw edema method.

Groups	Dose mg/kg	Carrageenan induced rat paw edema volume in mL (% inhibition) (X±S.D.)				
		0 h	2 h	3 h	4 h	6 h
I	2 ml/kg	1.75±0.67	1.86±0.92	1.89±0.48	1.79±0.88	1.75±0.56
II	5	1.84±0.97	1.44±1.06 (22.6)	1.25±1.11 (33.9)	1.10±0.76 (38.5)	1.05±1.03 (40.2)

III	300	1.80±1.12	1.70±1.02 (8.6)	1.48±0.71 (21.7)	1.38±0.89 (22.9)	1.31±0.90 (25.2)
IV	300	1.72±0.82	1.78±0.66 (4.3)	1.74±0.91 (7.9)	1.60±0.58 (10.6)	1.55±0.98 (11.4)
V	300	1.91±1.01	1.72±1.03 (7.5)	1.59±0.93 (15.87)	1.55±0.80 (13.4)	1.44±1.06 (17.7)
VI	300	1.83±0.66	1.75±0.86 (5.9)	1.75±0.95 (7.4)	1.64±0.56 (8.3)	1.57±0.48 (10.3)

Each value represents as mean \pm standard deviation (n = 3). Standard error of mean < 0.647. Group I – Control (Normal saline water), group II - Standard (Diclofenac sodium - 5 mg/Kg b.w.), groups III to VI – ethanol, ethyl acetate, n-butanol and petroleum ether extracts (300 mg/Kg b.w.) respectively.

Discussion:

The result of acute toxicity study confirmed that *G. arborea* fruits extract would be non-toxic in living body and the LD₅₀ values of the extracts were found to be 3000 mg/Kg body weight. One tenth of this lethal dose that is 300 mg/Kg b.w. was selected as the therapeutic dose for the evaluation of pharmacological activities.

The ethanol extract exhibited better anti-inflammatory activities when compared to other extracts. The anti-inflammatory activities of the extracts were found in the order of ethanol > n-butanol > petroleum ether > ethyl acetate extract.

The activities shown by all the extracts are of considerable importance and have justified their use in controlling the pyrexia as suggested in the folklore medicine. It will be worth mentioning that although different constituents were extracted in different solvents as per their polarities, the petroleum ether extract is more effective when compared to other solvent extracts.

Conclusion:

It can be concluded that the extracts of *G. arborea* fruits possess anti-inflammatory activity. The petroleum ether and ethanol extracts showed better anti-inflammatory activity. However, the components responsible for the anti-inflammatory activity are currently unclear. Therefore, further investigation is needed to isolate and identify the constituents present in the fruits extracts.

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References:

1. A.V. Krishnaraju, C.B.M. Rao, D. Sundararaju, K.S.G. Trimurtulu, Am J Inf Dis, 2009, Vol 5, issue 2, pp 68-73.
2. K.C. Preethi, G. Kuttan, R. Kuttan, Indian J Exp Biol, 2009, Vol 47, pp 113-120.
3. A.C. Smith, Flora Vitiensis nova: a new flora of Fiji. National Tropical Botanical Garden, Lawai Kauai, Hawaii 1991.
4. J.O. Adegbehin, J.O. Abayomi, L.B. Nwaigbo, Forestry Review, 1988, Vol 67, issue 2, pp 159-166.
5. B.S. Nayak, P.K. Jena, S.C. Dinda, P. Ellaiah, J Pharmacy Res, 2011, Vol 4, issue 5, pp 1-2.
6. B.S. Nayak, P.K. Jena, S.C. Dindha, P. Ellaiah, Asian J Chem, 2012, Vol 24, issue 8, pp 3445-3348.
7. R.A. Tunner, Acute Toxicity studies. 4th ed., Academic Press, an Imprint of Elsevier, Haryana 2009.
8. S.C. Dinda, B. Mukharjee, Acta Pharm Scientia, 2009, Vol 51, pp 189-198.
9. L.G. Chen, L.L. Yang, C.C. Wang, Food Chem Toxicol 2007;doi:10.1016/j.fct.2007.09.096.
10. A.M. Mujumdar, D.G. Naik, C.N. Dandge, H.M. Puntambekar, Indian J Pharmacol, 2000, Vol 32, pp 375-377.
11. J. Prakash, P. Tyagi, Anti-inflammatory agents. In: S.K. Gupta (Eds.), Screening Methods (Preclinical Evaluation of new Drugs), Jaypee Brothers Medical Publishers (P) Ltd, New Delhi 2009, pp 480-482.
12. N.S. Parmer, S. Prakash, Screening Methods in Pharmacology, Narosa Publishing House Pvt. Ltd., New Delhi 2006.
13. S. Patil, S. Anarthe, R. Jadhav, S. Surana, Iranian J Pharm Res, 2011, Vol 10, issue 2, pp 253-259.
14. S. Bolton, Pharmaceutical Statistics-Practical and Clinical Application, Marcel Dekker, New York 2002.

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