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Research Article

OPIOID AND NON-OPIOID ANALGESIC ACTIVITY OF *GMELINA ARBOREA* ROXB. FRUIT EXTRACTS

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ABSTRACT

Gmelina arborea Roxb. is found in the tribal areas of Koraput district. It is extensively used traditionally by the tribal people as anthelmintic, analgesic, antimicrobial, antifungal, antidiabetic and hepatoprotective. The present study is an attempt to explore the analgesic activity of different extracts of fruits of plant *Gmelina arborea* using ethanol, ethyl acetate, n-butanol and petroleum ether as solvents. Preliminarily the extracts were evaluated for non-toxic properties examined by acute toxicity study in Swiss albino mice. The analgesic activity was determined by tail immersion (opioid analgesic) and acetic acid induced writhing response (non-opioid analgesic) method using Swiss albino mice as animal model. The extracts were found as non-toxic. The ethanol and petroleum ether extracts showed significant analgesic activity as compared to standard drugs Pentazocine and Indomethacine. It could be concluded that *G. arborea* fruits possess analgesic activity. The data were verified as statistically significant by using one way ANOVA at 5% level of significance ($p < 0.05$) followed by z-test.

Keywords: *Gmelina arborea*, *Verbenaceae*, Tail immersion, Pentazocin, Indomethacin.

INTRODUCTION

Poly modal nociceptors (PMN) are the main types of peripheral sensory neurons that respond to thermal stimulus. The thermal stimuli acting on PMN to cause pain, include substance-P, neurokinin-A and B, bradykinin, 5-HT and histamin, prostaglandins, proton (H^+), ATP and vanilloids[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[3,4]. The plant, *G. arborea* was reported to have several medicinal properties such as aphrodisiac, astringent, analgesic, antipyretic, antidiabetic, diuretic, anti-inflammatory and tonic characteristics[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 saponinns[6].

The literature survey reveals that there were no reports scientifically on the analgesic activity of the fruit extracts of *G. arborea*. This prompted us to investigate the analgesic activity of *G. arborea* fruit extracts.

MATERIALS AND METHODS

Drugs and Chemicals

Pentazocine hydrochloride was procured as gift sample from Ranbaxy Laboratories Limited, New Delhi, India. Indomethacine was procured as gift sample from Glennmark Pvt. Ltd., Mumbai, 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±2°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.

Opioid analgesic activity by Tail immersion method

The Tail immersion method was used to evaluate the analgesic activity of plant extracts in Swiss albino mice[9-13]. The mice tail

withdrawal reflex can be elicited by immersion of the tail in hot water at 55°C. This test is specific for opioid like central analgesics and is used to differentiate them from peripheral analgesics[11]. Swiss albino mice weighing 20-25 g were divided into ten groups (3 each). 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 Pentazocine (10 mg/Kg b.w.). Groups (III) to (X) received ethanol, ethyl acetate, n-butanol and petroleum ether extracts respectively at doses of 200 or 400 mg/Kg b.w of each extract. The standard drug and test extract were administered subcutaneously (S.C) in solution form using normal saline water as vehicle.

The young Swiss albino mice were placed into individual cylindrical mice holders leaving the tail hanging out freely. The animals were allowed to get acclimatized to mice holders for 30 min before testing. The lower 5 cm portion of the tail was marked. This part of the tail was immersed in a cup of freshly filled water maintained exactly at 55°C temperature. The reaction time (Tail flicking latency) was recorded using a stop watch of 0.5 s accuracy. After each measurement, the tail was carefully dried. The reaction timings were determined before and after 0.5, 1, 2, 3, 4 and 6 h, the administration (S.C) of the test extracts. The cut off time of immersion was 15 s. The withdrawal time (Normal reaction time) of control group usually lies between 1 and 5.5 s. Centrally acting analgesic drugs are capable of causing prolongation of tail withdrawal reflex and hence a withdrawal time of more than 6 s in test animals was regarded as appositive analgesic response.

Non-opioid analgesic activity by acetic acid induced writhing response method.

The acetic acid induced writhing response method was used to evaluate the non-opioid analgesic activity of plant extracts in Swiss albino mice[14-16]. Swiss albino mice weighing 20 to 25 g were divided into six groups (3 each). 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 Indomethacine (10 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 for each extract. The standard drug and test extracts were administered subcutaneously (S.C) in solution form using normal saline water as vehicle.

Thirty minutes later, 0.7% acetic acid (10 ml/Kg) solution was injected intra-peritoneally to all the animals in the different groups. The number of writhes (abdominal constrictions) occurring between 5 and 20 min after acetic acid injection was counted. A significant reduction of writhes in tested animals compared to those in the control group was considered as a non-opioid analgesic response.

The percent inhibition (% analgesic activity) was calculated by using the following formula, % inhibition = $[(N - N^t)/ N] \times 100$, where, N is the average number of stretchings or writhes of control per group and N^t is the average number of stretchings or writhes of test group.

Statistical analysis

For determining the statistical significance, standard deviation, standard error mean and one way analysis of variance (ANOVA) at 5% level significance was employed followed by z-test. P values < 0.05 were considered significant[17].

RESULTS AND DISCUSSION

Acute toxicity study

Acute toxicity study revealed that no mortality was found with any solvent extract at any dose in Swiss albino mice, which confirmed that *G. arborea* fruits extract would be non- toxic in living body and the LD_{50} 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.

Opioid analgesic activity

The ethanol and petroleum ether extracts were able to show analgesic activity at 200 mg/Kg b.w., as represented in Table 1. The ethyl acetate and n-butanol extracts did not show analgesic activities at two different doses. The analgesic effects of ethanol and petroleum ether extracts are some what comparable with the standard drug. The ethanol extract showed lesser analgesic activity than the standard drug, Pentazocine HCl. An increase in the dose of the extracts showed increased analgesic activity. It will be worth mentioning that although different constituents were extracted with different solvents as per their polarities, the petroleum ether extracts is more effective as compared to other solvent extracts, as the tail flicking latency (Reaction time) was much delayed. The activity shown by petroleum ether extract is of considerable importance and has justified its use in controlling the pain as suggested in the folklore medicine. By employing one-way ANOVA, all data were found to be statistically significant (F value < F crit) at 5 % level of significant ($p < 0.05$ that is $p = 0.019397$) followed by z-Test. Analgesic effect against thermal noxious stimuli may be elicited through opioid receptors or through modulation of several neurotransmitters involved in relevant phenomena but the extent of activity shown by the petroleum ether extract (300 mg/Kg b.w.) is more than that of the standard drug, Pentazocine HCl, which justifies its activity (Fig 1).

Table 1: Opioid analgesic activity of *G. arborea* fruits extracts in mice by Tail immersion method.

Groups	Tail flicking latency period (s) (X±S.D.)						
	B.S.T.	**0.5 h**	**1 h**	**2 h**	**3 h**	**4 h**	**6 h**
I	1.6±0.34	1.8±0.22	1.7±0.29	1.8±0.12	1.7±0.21	1.6±0.14	1.9±0.11
II	1.7±0.22	7.4±0.15	8.7±0.12	9.9±0.23	6.4±0.34	6.0±0.29	5.2±0.15
III	1.2±0.41	3.0±0.72	4.8±1.01	5.1±0.90	7.3±1.1*	5.5±0.55	2.6±0.82
IV	1.3±0.18	5.3±0.78	5.6±0.92	6.1±0.7*	12±0.4*	8.4±0.5*	3.0±0.68
V	1.4±0.11	1.8±0.90	2.7±0.77	2.2±0.81	3.1±0.92	1.9±0.87	1.7±0.91
VI	1.7±0.45	2.5±0.93	4.6±0.89	3.18±1.1	4.6±0.77	2.7±0.77	2.2±0.82
VII	2.1±0.37	1.5±0.69	2.1±0.92	4.1±1.05	2.5±0.92	1.9±1.03	1.1±1.02
VIII	1.6±0.66	2.7±0.67	3.1±0.72	4.2±0.36	2.5±0.58	1.9±0.65	1.9±0.97
IX	1.1±0.20	2.9±0.49	4.1±0.8	8.8±0.9*	6.1±0.6*	5.6±1.2	2.5±0.69
X	2.9±0.30	4.7±0.88	10.2±0.6*	13±0.7*	8.3±0.7*	8.3±1.1*	5.1±0.8
ANOVA							
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***	
Between Groups	127.94743	6	21.324571	2.253003	0.019397	2.37178	
Within Groups	252.74613	35	7.2213181				
Total	380.69356	41					

B.S.T. – Basal reaction time. Each value is represented as mean ± standard deviation (n = 3). Standard error of mean < 0.698. Group I – Control (Normal saline water), group II - Standard (Pentazocine HCl - 10 mg/Kg b.w.), groups III to X – ethanol, ethyl acetate, n-butanol and petroleum ether extracts (At doses of 200 & 300 mg/Kg b.w.) respectively. *P<0.05 (Test of significance between two proportions by z-Test) in comparison to standard. Data are found to be significant (*F value < F crit*) by testing through one way ANOVA at 5 % level of significance ($p < 0.05$ that is $p = 0.019397$).

Table 2: Non-Opioid analgesic activities of *G. arborea* fruits extracts in mice by acetic acid induced writhing response method.

Groups	Drugs	Dose (mg/kg)	No. of writhings (X±S.D.)	% inhibition
I	Normal Saline Water	2 ml/kg	73±0.57	0
II	Indomethacin (Standard)	10	7±0.81	90.41
III	Ethanol extract	300	18±0.96	75.34
IV	Ethyl acetate extract	300	32±0.92	56.16
V	n-butanol extract	300	38±0.73	47.94
VI	Petroleum ether extract	300	29±1.02	60.27

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4248.54544	1	4248.54544	1.46973	0.00225	5.31766
Within Groups	1745.70292	8	218.212865			
Total	5994.24836	9				

Each value is represented as mean ± standard deviation (n = 3). Standard error of mean < 0.589. Data are found to be significant (*F value* < *F crit*) by testing through one way ANOVA at 5 % level of significance (p < 0.05 that is p = 0.00225).

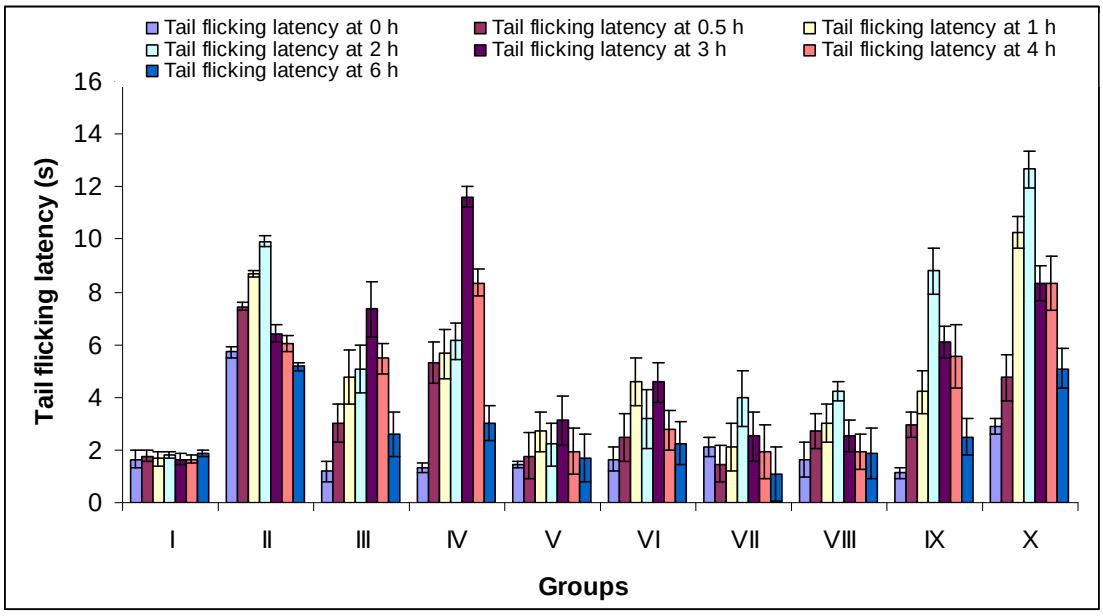

Fig. 1: Comparative study on opioid analgesic activities of *G. arborea* Roxb. fruit extracts.

Each bar represented as mean ± standard deviation (n = 3). Group I – Control (Normal saline water), group II - Standard (Pentazocine HCl - 10 mg/Kg b.w.), groups III to X – ethanol, ethyl acetate, n-butanol and petroleum ether extracts (At doses of 200 & 300 mg/Kg b.w.) respectively.

Non-opioid analgesic activity

All extracts exhibited analgesic (Non-opioid) activities at 300 mg/Kg b.w., as represented in Table 2. The analgesic effects of the ethanol and petroleum ether extracts are better than that of other extracts. All the extracts showed lesser analgesic activities than the standard drug, Indomethacine. By employing one-way ANOVA, all data were found to be statistically significant (*F value* < *F crit*) at 5 % level of significant (p < 0.05 that is p = 0.00225) followed by z-Test. The analgesic activity of the extracts were found to be in the order of ethanol > petroleum ether > ethyl acetate > n-butanol.

CONCLUSION

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

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