AN INVESTIGATION OF THE ANALGESIC AND ANTI-INFLAMMATORY EFFECTS OF AERIAL PARTS OF FLACOURTIA JANGOMAS

C. JOTHIMANIVANNAN, P. LALITHA, K. MEENA, A. MEENAJESILIYA, J. C. MOGANAPRIYA, P. MANIMEKALAI

Swamy Vivekanandha College of Pharmacy, Elayampalayam Namakkal 637205
Email: mekalaivel@gmail.com

Received: 30 Jan 2021, Revised and Accepted: 15 May 2021

INTRODUCTION

Flacourtia jangomas is an important member of the family Flacourtiaceae showing a variety of medicinal uses and found in the lowland and mountain rain forest tree [1]. It is widely cultivated in southeast and East Asia and escaped cultivation in number of places [2]. The family includes 87 genera and about 900 species and the genus Flacourtia includes 7 species. The species under study is very commonly found in lowland and mountain rain forest tree. It is widely cultivated in the southeast and East Asia and as escaped cultivation in a number of places [2] Aerial parts of the plant is used in the treatment of diabetes, asthma, anemia and antibacterial, antidiarrheal, antioxidiant activities [3]. Fruits are widely eaten as pickles, jams, juice [4] Dried roots are used to suppress toothache [5]. Phytochemical studies of F. jangomas revealed several bioactive constituents, including carbohydrates, protein, lipids, alkaloids, glycosides, tannins, etc [6]. Flacourtia Montana, a related species used as hepato Protectives, anti-inflammatory and antioxidiant activities [7].

The plant has also been investigated pharmacologically for antidiabetics, antibacterial, antiantioxidant; analgesic, antifungal activities [8]. The related species of F. Montana, F. spure, F. ignorumas and F. romance have been reported with various pharmacological activities like antibacterial, antidiabetic, anti-inflammatory and hepatoprotective [9]. The analgesic and anti-inflammatory activity of Flacourtia jangomas on the methanolic extract was already reported with methanolic extract. So we curious know the same effect with high polar solvent to know the analgesic and anti-inflammatory activity in aqueous extract of Flacourtia jangomas.

The current study aimed to validate the traditional use of F. jangomas in the management of inflammatory condition and pain. In the present study, we have chosen the plant Flacourtia jangomas used in herbal medicine to determine its anti-inflammatory activity and Analgesic activity.

MATERIALS AND METHODS

Animals

Swiss albino mice (20-25 g) and Wistar rats (200-250 g) of both sexes were obtained from the animal house facility Department of Pharmacology, Swamy Vivekanandha College of Pharmacy, Elayampalayam, Tiruchengode, Namakkal. The animals were housed in plastic cages under standard conditions with 12 hrs light: dark cycle with free access to food and water. The study was conducted according to good animal practice (Reg. No. 1158/PO/AC/18) and Institutional Animal Ethics Committee and was performed in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

Chemicals

The following chemicals were used in the experiments: Carrageenan, diclofenac sodium, Carboxymethylcellulose (CMC), Pentazocine, Acetic acid were purchased from (India).

Plant materials

The whole plant of Flacourtia jangomas (Flacourtiaceae) was collected from Pallikkara, Thiruvalla, Pathanamthitta (Dt), Kerala in November, 2017 and identified by Dr. S. Senthil Kumar (Botanist) at the department of botany, Vivekanandha College of Arts and Sciences for women, Namakkal.

Phytochemical procedure

Extraction

Leaves and stem of Flacourtia jangomas were washed thoroughly with water to remove the soil particles, shade dried and grounded. About 800 g of leaf powder was extracted with 2500 ml of distilled
water by cold maceration. About 150 g of stem powder was extracted with 500 ml of distilled water by cold maceration. After completion of the extraction, it was filtered and dried to produce a semisolid mass. The dried extract was stored in a desiccator until use.

Preliminary phytochemical analysis

**Flacourtia jangomas** extract was subjected to preliminary phytochemical screening through qualitative chemical analysis for confirmation of the phytoconstituents [10, 11].

**Acute toxicity tests**

Swiss Albino rats weighing 200-250 g selected by random sampling were used in this study. Acute oral toxicity was performed as per OECD-423 guidelines. The animals were fasted overnight, provided only with water. The extract was administered orally at the dose level of 5 mg/kg body weight by gastric intubation and the animals were observed for 14 days. The animals were observed for toxic symptoms such as pain, fur movement, lacrimal secretion, nasal fluid secretion, allergy, pupil size and diameter, eye colour, body weight, convulsion, and mortality for 72h [12].

**Pharmacological activity**

**Anti-inflammatory activity (Carrageenan induced paw oedema in rat)**

Anti-inflammatory activity of the aqueous plant extract of *Flacourtia jangomas* was assessed by using a carrageenan induced acute paw oedema model. The albino Wistar rats of both sexes were divided into 6 groups of 4 animals each. Food was withdrawn overnight, but adequate supply of water was given to the rats before the experiment. Group 1 serving as control received 1% w/v carboxy methyl cellulose in syrup orally. Group 2 receiving 20 mg/kg Diclofenac sodium, Group 3 and 4 receiving 200 and 400 mg/kg of *Flacourtia jangomas* leaves extract respectively. Group 5 and 6 receiving 200 and 400 mg/kg of *Flacourtia jangomas* stem extract respectively. The drugs were given orally with the help of an oral catheter. After 1 hr a sub-planter injection of 0.1 ml of 1% carrageenan was administered in the right hind paw to all the 6 groups. The paw volume was measured with the help of plethysmograph immediately after injection. The paw volume observed after 1, 2 and 3h [13]. Mean increase in the paw volume was measured and percent inhibition was calculated [14].

Percentages of inhibition were obtained using the following ratio:

\[
\text{Percentage inhibition} = \left( \frac{Vt - Vo}{Vo} \right)_{\text{control}} \times 100
\]

Vt is the average volume for each group after treatment, Vo is the average volume for each group before any treatment.

**Analgesic activity**

**Hot plate method**

Analgesic activity of the aqueous plant extract of *Flacourtia jangomas* was assessed by heat. The Hot plate method was performed by Eddy and Leimbach. The pre-screened Swiss albino mice showed the reaction time of 3 to 5 Sec and were selected and randomly divided into six groups of four mice per group. Group 1 were given 1% CMC solution 10 ml/kg (control), Group 2 were given pentazocine 4 mg/kg (standard), while Group 3 and Group 4 received 200 and 400 mg/kg of leaf extract of *Flacourtia jangomas*, Group 5 and Group 6 received 200 and 400 mg/kg of stem extract of *Flacourtia jangomas*. Extract respectively all by gastric gavage. Animals were placed on Eddy’s hot plate maintained at 55 ± 1°C. The reaction time in control and treated animals was recorded till they showed licking or jumping movements [15]. The cut-off time was considered as 10 Sec. The reaction time was recorded at 0, 30, 60, 90, and 120 min following administration of the test drug.

**Acetic acid-induced writhing method**

Acetic acid-induced writhing model was performed by the method of koster et al., with slight modification. Twenty four albino mice of both sexes were randomly divided into six groups of four mice per group. Group 1 were given 1% CMC solution 10 ml/kg (control), Group 2 were given diclofenac sodium 20 mg/kg i.p. (Standard), while Group 3 and Group 4 were received 200 and 400 mg/kg of leaf extract of *Flacourtia jangomas*, Group 5 and Group 6 received 200 and 400 mg/kg of stem extracts of *Flacourtia jangomas*. Extracts respectively all by gastric gavage. One hour after administration of drug and extract, 0.6% glacial acetic acid (10 ml/kg) was given i.p. to all the mice to induce pain characterized by abdominal constrictions are writhes. The number of wriths observed in each mouse was counted for 10 mins and recorded [16-18]. The percentage protection against abdominal writhing was used to assess the degree of analgesia and was calculated using the formula,

\[
\% \text{ inhibition of writhing} = \frac{\text{No. of wriths in control} - \text{No. of wriths in treated group}}{\text{No. of wriths in control group}} \times 100
\]

**Statistical analysis**

The results of the study were expressed as Mean ± SEM and statistical significance between control and treated groups, standard and treated groups evaluated by one-way ANOVA followed by post hoc Dunnett’s multiple comparison test by using SPSS V.15 (Student trail version). P<0.05 was considered significant.

| S. No. | Test | Aqueous extract of leaf | Aqueous extract of stem |
|-------|------|-------------------------|-------------------------|
| 1     | CARBOHYDRATES | + | + |
| a. Mallich test | + | + |
| b. Fehling’s test | + | + |
| c. Benedict test | + | + |
| 2     | ALKALOIDS | + | + |
| a. Dragondrof’s test | + | + |
| b. Mayer’s test | + | + |
| c. Hager's test | - | - |
| 3     | SAPONINS: | + | + |
| 4     | GLYCOSIDES | - | - |
| a. Legaf’s test | - | - |
| b. Balget’s test | - | - |
| c. Bontrager’s test | + | + |
| 5     | FLAVONOIDS | + | + |
| 6     | PROTEINS and AMINO ACIDS | + | + |
| 7     | STEROIDS | + | + |
| a. Salkowski test | + | + |
| 8     | TANNINS and PHENOLIC COMPOUNDS | + | + |

+Presence - Absence
RESULTS

Preliminary phytochemical analysis

The aqueous extract of Flacourtia jangomas was subjected to a preliminary phytochemical screening revealed the presence of carbohydrates, alkaloids, saponins, glycosides, flavonoids, proteins, tannins and steroids.

Acute toxicity test

The aqueous extract of Flacourtia jangomas produced no toxic symptoms or mortality up to a dose level of 2000 mg/kg body weight orally in rats. Hence the drug was considered safe for further pharmacological screening. So 1/10th and 1/5th (200 mg and 400 mg, respectively) of toxic dose were selected for all in vivo experiments submaximal and maximal dose.

Anti-inflammatory activity

The anti-inflammatory effect of aqueous extract of Flacourtia jangomas was assayed in the carrageenan-induced paw edema in rat. The injection carrageenan when injected into a sub-plantar region of the rat paw produced localized edema that reached to its maximum at the 3rd h after injection. The localized inflammatory response to carrageenan was sustained for 4 h and gradually declined after this time.

As shown in table 2, Flacourtia jangomas produced a marked reduction in carrageenan-induced paw edema (55.6% at 200 mg/kg leaf extract) at the 3rd h. The difference between the paw volume of the control and extracts treated animals was statistically significant (p<0.001) at the 2nd h of the observation. The standard drug Diclofenac sodium at 20 mg/kg produced about 44.4% inhibition of the carrageenan-induced edema as shown in table 1.

Table 2: Anti-inflammatory effect of leaf and stem part of Flacourtia jangomas on carrageenan-induced acute paw oedema in Wistar albino rats

| Treatment                   | Increase in paw volume in ml (Mean ± SEM) | % inhibition after 3h |
|-----------------------------|------------------------------------------|-----------------------|
|                            | 0h | 1/2 h | 1h | 2h | 3h | 4h |
| 1% CMC                      | 0.25 ± 0.03 | 0.55 ± 0.03 | 0.65 ± 0.03 | 0.55 ± 0.03 | 0.45 ± 0.03 | - |
| Diclofenac sodium 20 mg/kg/p. o + 0.1 ml Carrageenan | 0.25 ± 0.03 | 0.35 ± 0.03 | 0.30 ± 0.01 | 0.30 ± 0.01 | 0.25 ± 0.03 | 44.4% |
| ALE-200 mg/kg/p. o + 0.1 ml Carrageenan | 0.20 ± 0.01 | 0.33 ± 0.03 | 0.23 ± 0.03 | 0.23 ± 0.03 | 0.20 ± 0.01 | 55.6% |
| ALE-400 mg/kg/p. o + 0.1 ml Carrageenan | 0.20 ± 0.01 | 0.35 ± 0.03 | 0.23 ± 0.03 | 0.23 ± 0.03 | 0.23 ± 0.03 | 48.9% |
| ASE-200 mg/kg/p. o + 0.1 ml Carrageenan | 0.23 ± 0.05 | 0.33 ± 0.03 | 0.25 ± 0.03 | 0.23 ± 0.03 | 0.25 ± 0.03 | 44.4% |
| ASE-400 mg/kg/p. o + 0.1 ml Carrageenan | 0.25 ± 0.06 | 0.40 ± 0.01 | 0.30 ± 0.01 | 0.30 ± 0.01 | 0.25 ± 0.03 | 44.4% |

Values are expressed as Mean ± SEM, n=4, the symbol represents statistical significance: a=comparison of Group-1 Vs Group-2,3,4,5 and 6; b=comparison of Group-2 Vs Group-1, 3,4,5 and 6. ***P<0.001, **P<0.01 and *P<0.05, one way ANOVA by Dunnet’s multiple comparison test as compared to control and standard.

Table 3: Central analgesic activity of aqueous extract of Flacourtia jangomas on reaction time to hot plate method in mice

| Treatment                   | Basal reaction time (sec) |
|-----------------------------|---------------------------|
|                            | Basal | 30 min | 60 min | 90 min | 120 min |
| 1% CMC                      | 7.3 ± 0.67 | 6.7 ± 0.88 | 8.3 ± 0.33 | 9.3 ± 0.76 | 9.7 ± 0.32 |
| Pентazocine (4 mg/kg i.p)   | 7.3 ± 0.20 | 9.3 ± 0.88 | 15.0 ± 0.45 | 13.0 ± 0.15 | 13.0 ± 0.23 |
| ALE-200 mg/kg/p. o          | 7.6 ± 0.88 | 8.0 ± 0.15 | 11.0 ± 0.08 | 9.0 ± 0.58 | 9.7 ± 0.76 |
| ALE-400 mg/kg/p. o          | 8.0 ± 0.52 | 8.7 ± 0.32 | 9.3 ± 0.85 | 9.3 ± 0.88 | 8.7 ± 0.32 |
| ASE-200 mg/kg/p. o          | 9.0 ± 0.57 | 8.3 ± 0.23 | 12.7 ± 0.10 | 10.3 ± 0.88 | 10.0 ± 0.45 |
| ASE-400 mg/kg/p. o          | 8.0 ± 0.58 | 9.3 ± 0.67 | 11.0 ± 0.73 | 10.0 ± 0.52 | 7.3 ± 0.88 |

Values are expressed as Mean ± SEM, n=4, the symbol represents statistical significance: a=comparison of Group-1 Vs Group-2,3,4,5 and 6; b=comparison of Group-2 Vs Group-1, 3,4,5 and 6. ***P<0.001, **P<0.01 and *P<0.05, one way ANOVA by Dunnet’s multiple comparison test as compared to control and standard.

Table 4: Peripheral analgesic activity of Flacourtia jangomas on acetic acid induced writhing method in mice

| Treatment                   | Total no. of writhing (in 10 min) | % Inhibition |
|-----------------------------|----------------------------------|-------------|
| 1% CMC                      | 44.33 ± 1.76                     | -           |
| Pентазочине (4 mg/kg i.p + 0.6% Acetic acid) | 25.67 ± 4.84**                  | 42.1%       |
| ALE-200 mg/kg/p. o + 0.6% Acetic acid | 28.00 ± 7.57                   | 36.8%       |
| ALE-400 mg/kg/p. o + 0.6% Acetic acid | 52.00 ± 1.58                   | -           |
| ASE-200 mg/kg/p. o + 0.6% Acetic acid | 29.33 ± 4.18*                  | 33.8%       |
| ASE-400 mg/kg/p. o + 0.6% Acetic acid | 52.67 ± 2.33                   | -           |

Values are expressed as Mean ± SEM, n=4, the symbol represents statistical significance: a=comparison of Group-1 Vs Group-2,3,4,5 and 6; b=comparison of Group-2 Vs Group-1, 3,4,5 and 6. ***P<0.001, **P<0.01 and *P<0.05, one way ANOVA by Dunnet’s multiple comparison test as compared to control and standard.

DISCUSSION

Anti-inflammatory activity of Flacourtia jangomas was determined by the carrageenan-induced acute paw edema model, which is one of the most feasible methods of screening anti-inflammatory agents. The carrageenan-induced acute inflammation is biphasic, in the early phase (1-2 h after carrageenan injection), edema production is mediated by histamine, serotonin and kinins while in the late phase...
(after 2 h), the inflammatory response is maintained by Bradykinin and prostaglandins [19]. These mediators are well established for their role in an inflammatory reaction which is measured at 3 h. In the present investigation, Flacourtia jangomas exhibit marked anti-inflammatory activity in the early phase of carrageenan-induced edema test similar to diclofenac, a standard non-steroidal anti-inflammatory drug (NSAID). Aqueous extract of Flacourtia jangomas produced a significant (p<0.001) inhibition of carrageenan-induced paw edema at 2h in a dose-dependent manner. Therefore, it can be concluded that the inhibitory effect of aqueous extract of Flacourtia jangomas on carrageenan-induced inflammation could be due to inhibition of the inflammatory enzyme cyclooxygenase and subsequent inhibition of prostaglandin synthesis, significant inhibition of paw edema in the yearly hours after carrageenan injection by Flacourtia jangomas could be attributed to the inhibition of prostaglandin [20].

Antinociceptive activities of aqueous extract of Flacourtia jangomas were evaluated by acetic acid-induced writhing method and hot plate method. This method allows the analysis of peripheral and centrally mediated antinociceptive responses respectively. The hot plate method is commonly used to assess the centrally acting analgesics. The analgesic activity of Flacourtia jangomas was tested upon adult mice by the hot plate method. The aqueous extract of stem at lower dose of 200 mg/kg shows significant (p<0.05) analgesic activity when compared to standard pentazocine 4 mg/kg. The aqueous stem extract (200 mg/kg) may be activate the opioid receptor at the interneuronal level, which produces hyperpolarisation of the neurons, result in the inhibition of the firing and the release of tachykinin neuropeptides, a neurotransmitter involved in pain transmission, thereby blocking the pain transmission that causes a prolongation of the hot plate latency by this model must be acting centrally [21].

The analgesic activity of Flacourtia jangomas was tested upon adult mice by acetic acid-induced writhing method. The aqueous extract of leaf and stem at low dose of 200 mg/kg shows significant (p<0.05) analgesic activity when compared to standard diclofenac sodium 20 mg/kg. Bradykinin, neurokinins and prostanoids are known mediators for acetic acid-induced writhing [22,24]. The effect of the aqueous leaf and stem extracts of Flacourtia jangomas at low concentration produces antinociceptive activity. It may be depressed the production of irritants and their by reduction in the number of writhes on the mice.

The abdominal contraction induced by acetic acid is a sensitive method to assess peripherally acting antinociceptives. This method allows the analysis of peripheral and centrally mediated antinociceptive activities is also required which will guarantee its clinical worth.

CONCLUSION

In conclusion, our results reveals that among all the extracts of leaves and stem of Flacourtia jangomas aqueous extracts exhibited significant analgesic and anti-inflammatory activities. These findings validated the claim for the traditional use of this plant in the treatment of pain and inflammatory ailments. In addition to this, research regarding the mechanism responsible for these activities is also required which will guarantee its clinical worth.

ACKNOWLEDGEMENT

The study substantiates the traditional use of F. jangomas as a remedy of inflammatory and pain condition. The findings of this study will help natural product researcher to identify the active constituent of this plant and precise underlying mechanism as possible anti-inflammatory and analgesic drug candidate with good safety and tolerability profile.

AUTHORS CONTRIBUTIONS

All authors have contributed equally in this piece of work

CONFLICT OF INTERESTS

The author declared no conflict of interest

REFERENCES

1. Talukdar C, S Saha, S Adhikari, HK Mondal, MdK Islam, Md Anisuzzaman, et al. Evaluation of antioxidant, analgesic and antioxidant activity of Flacourtia jangomas (Lour.) raeusch leaves. Pharmacologyonline 2012;3:20-8.
2. Hanlet, Peter. Institute of plant genetics and crop plant research. Edns. Mansfeld's. Encyclopedia of Agricultural and Horticultural crops [Except ornamentals] springer; 2001. p. 3700.
3. Neelakandan T. International journal of current medical and pharmaceutical research. Print and online Publication; 2016; p. 69.
4. Anonymous. Flacourtia comm. (Flacourtiaeae). In: The Wealth of India: Raw Materials, CSIR, New Delhi; 1956;4:42-4.
5. Ashalata Devi Khumbongmayum AO, ML Khan, RS Tripathi. Ethnomedicinal plants in the sacred groves of Manipur. IJITK 2005;4:21-32.
6. Ghan A. Medicinal plants of Bangladesh: chemical constituents and uses. Dhaka, Asiatic Soc Bangladesh 2003;5:3-16.
7. Ajay Kumar Singh, Jyoti Singh. Evaluation of the anti-diabetic potential of leaves and stem of flacourtia jangomas in streptozotocin-induced diabetics in rats. Indian J Pharmacol 2016;42:301-5.
8. Chinchu Joshy PA, R Thahimon, Arun Kumar, Betty Carla, Christudas Sunil. Hepatoprotective, anti-inflammatory and antioxidant activities of flacourtia monata J. Grah leaf extract in male wistar rats. Bull Faculty Pharm Cario University 2016;5:209-17.
9. Wu Jian, Danielsson Ake, A Zern Mark: Toxicity of hepetotoxins: new insights into mechanisms and therapy. Expert OpinInvestig Drugs 1999;8:585-607.
10. Ferrero Miliani L, OH Nielsen, PS Andersen, SE Girardin. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1 beta generation. Clin Exp Immunol 2007;147:227-35.
11. Venkatesa Perumal R, M Adrai, P Shanmuga Pandiyan. Synthesis, analgesic and anti-inflammatory evaluation of substituted 4-piperidones. Indian Drugs 2001;38:156-9.
12. Ramprasad VR, P Shanthi, P Sachadadamam. Anti-inflammatory effect of Semecarpus anacardium Linn. Nut extract in acute and chronic inflam cond. Biol Pharm Bull 2004;27:2028-31.
13. Eddy NB, D Leimbach. Synthesis analgesics. 2. Dithienyl - butenyl and dithienbutylamines. J Pharmacol Exp Ther 1953;107:385-93.
14. Koster R, M Anderson, EJ De Beer. Acetic acid for analgesics screening; 1959;18:412-8.
15. Uddin SJ, JA Shilpi, J Barua, R Rouf. Antinociceptive activity of ceripes decandra leaf and pneumophoreum. Fitorterapi 2005;76:261-3.
16. Mushshina Ferdous, Razina Rouf, Jamil Ahmad Shilpi, Shaikh Jamal Uddin. Antinociceptive activity of the ethanolic extract of ficus racemosa lin. (Moraceae) 2008;8:93-6.
17. Tambahwagh UU, AD Kandhare, VS Homore, PP Kadam, VM Khedkar. Anti-inflammatory and antioxidant potential of guianolide isolated from sytychone purpurea leaves of COX-2 inhibition. Int Immunopharmacol 2017;52:110-8.
18. Toma W, JS Gracioso, CA Hiruma Lima, FPGA Andrade, W Villegas, ARMS Brita, et al. Evaluation of the analgesic and antiedematogenic activities of qassia amara bark extract. J Ethnopharmacol 2005;101:18-23.
19. Iboronke GF, KI Ajiboye. Studies on the anti-inflammatory and analgesic properties of chenopodium ambrosiodes leaf extract in rats. Int J Pharm 2007;3:111-5.
21. Ikeda Y, A Ueno, H Naraba, S Ohrishi. Involvement of vanilloid receptor VRI and prostanoids in the acid induced writhing responses of mice. *Life Sci* 2001;69:2911-9.

22. Chakraborty AR, RK Devi, S Rita, KH Sharatchandra, TI Singh. Preliminary studies on anti-inflammatory and analgesic activities of *s pilanthes acmella* in experimental animal models. *Indian J Pharmacol* 2004;36:148-50.

23. Laaboudi W, J Ghanam, H Aissam, M Merzouki, M Benlemlih. Anti-inflammatory and analgesic activities of olive tree extract. *Int J Pharm Pharm Sci* 2016;8:414-9.