International Journal of Herbal Medicine
Available online at www.florajournal.com

I J H M International Journal of Herbal Medicine

E-ISSN: 2321-2187
P-ISSN: 2394-0514
IJHM 2016; 4(3): 40-44
Received: 11-03-2016
Accepted: 12-04-2016

Gummalla Pitchaiah
Associate Professor, Department of Pharmacology, Hindu College of Pharmacy, Amaravathi Road, Guntur- 522002, Andhra Pradesh, India. India.

Shaik Samiya
Hindu College of Pharmacy, Amaravathi Road, Guntur-522002, Andhra Pradesh, India.

KNSV Lakshmi
Hindu College of Pharmacy, Amaravathi Road, Guntur-522002, Andhra Pradesh, India.

Sailaja P
Hindu College of Pharmacy, Amaravathi Road, Guntur-522002, Andhra Pradesh, India.

Correspondence
Gummalla Pitchaiah
Associate Professor, Department of Pharmacology, Hindu College of Pharmacy, Amaravathi Road, Guntur- 522002, Andhra Pradesh, India. India.

Anti-Inflammatory and wound healing activity of hydroalcoholic extract of *Murraya koenigii* fruits in rats

Gummalla Pitchaiah, Shaik Samiya, KNSV Lakshmi, Sailaja P

Abstract
Traditionally, a large number of plants are used for the treatment of inflammation and wounds. *Murraya koenigii* is popular Indian herb and different parts are used in traditional medicine for the treatment of various diseases. However, many studies have not conducted on its fruit potential. Hence, the present study was carried out to investigate anti-inflammatory and wound healing activities of *Murraya koenigii* fruits in rodents. The anti-inflammatory activity of the *Murraya koenigii* was based on its effects on carrageenan-induced paw ooedema in rats. Its wound healing effect was investigated using an excision wound model. Four hours after administration, the reduction in carrageenan-induced rat paw ooedema by low dose (100 mg/kg) and high dose (200 mg/kg) of *Murraya koenigii* was 42.6 and 50.3%, respectively, while ooedema reduction by indomethacin (10 mg/kg) was 55.5%. Topical application of *Murraya koenigii* fruit extract ointment showed significant (p<0.05) wound closure and epithelialization compared to control. This study demonstrates that the hydroalcoholic extract of *Murraya koenigii* fruits has significant anti-inflammatory and wound healing properties.

Keywords: *Murraya koenigii*, Anti-inflammatory, Wound healing

1. Introduction
Inflammation is a host defence mechanism in response to various infections, injury or metabolic stimuli. The function of inflammation is to eliminate the foreign bodies or injurious agents. Furthermore, it removes damaged tissue components, so that the body can begin to heal and recover [1]. Although it is a defence mechanism, the complex events and mediators involved in inflammatory reaction can induce, maintain or aggravate many diseases, if the inflammation is left untreated or uncontrolled [2]. Wound healing is a natural and fundamental histopathological process that restores the function and integrity of damaged tissues [3]. The cells lost are replaced by the proliferative activity of those remaining, while in other cases, the healing of the wounds of skin and subcutaneous tissue occurs by the formation of fibrous scars [4]. Unhealed wounds and ulcers have a great impact on the public health and economy [5]. Even though great advancements in the chemical drug industry, the availability of substances with anti-inflammatory property and are capable of stimulating the wound repair process is still limited. Thus, an intervention that exhibits therapeutic effects on achieving significant anti-inflammatory and wound healing is of great value and is necessary.
Herbal drugs play an important role in health care programs especially in developing countries like India. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers 'all plant parts to be potential sources of medicinal substances'. Further, several lines of evidence have shown the benefits of a wide variety of plants for their anti-inflammatory and wound healing capacity. Thus, plant products are considered to be the best and cost-effective substitutes for the treatment of inflammation and healing of wounds [6].
Murraya koenigii is one of such medicinal plant, belonging to the family Rutaceae, commonly known as curry-leaf tree, is a native of India, Sri Lanka and other South Asian countries. The whole plant is considered to be a tonic and stomachic. The leaves are used extensively as a flavoring agent in curries and chutneys. Almost every part of this plant has a strong characteristic odour. It was found that reduction in total serum cholesterol, increase in the HDL and lower release of lipoproteins into the circulation take place when rats were fed with a standard diet along with curry leaves [7]. Curry leaves also exhibited strong antioxidant and antidiarrheal properties [8]. Hypoglycemic & lipid lowering activity of *Murraya koenigii* was reported [9]. Significant wound healing property is also reported for leaves in diabetic rats [10]. There is no scientific report on anti-inflammatory and wound healing effect of *Murraya koenigii* fruits.

The present study has been undertaken to examine the anti-inflammatory and wound healing potential of hydroalcoholic extract of *Murraya koenigii* fruits in rodents.

2. Materials and Methods

2.1. Chemicals

Ketamine hydrochloride was purchased from Park-Davis Company (France). Carrageenan lambda (Sigma, USA) was used for induction of paw oedema. Indomethacin and the other chemicals were purchased from Merck Company (Germany) and Nitrofurazone ointment (0.2% (w/w) from GSK Pvt. Ltd

2.2. Collection of plant material

The ripen fruits of *Murraya Koenigii* were collected from local area of Guntur, Andhra Pradesh. The fruits of the plant were authenticated by Department of Botany, Hindu College, Guntur, Andhra Pradesh.

2.3. Preparation of hydroalcoholic extract

The ripen fruits were dried under shade and were ground to form the smooth powder. The powdered fruits of *Murraya Koenigii* (80 gm each) were macerated with ethanol (50% v/v) as solvent for 7 days and after every 24 hours; the mixtures were stirred with a sterile glass rod. The extract was filtered by whatmann filter paper no.1 to obtain the filtrate. The filtrates were kept on water bath to obtain the crude extract [11].

2.4. Preliminary phytochemical evaluation

The extract was then subjected to phytochemical screening for the qualitative identification of the various phyto-constituents [12].

2.5. Animals

Male Wistar albino rats (150 - 175 g) were used for the evaluation of anti-inflammatory and wound-healing activities. They were obtained from the animal house of Hindu College of Pharmacy, were maintained under standard environmental conditions, and fed with standard pellet diet supplied by Hindustan Lever Ltd., Kolkata, India and water *ad libitum*. The guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India were followed for handling of the animals during the experiments. The research proposal was approved by the Institutional Animal Ethical Committee.

2.6. Acute oral toxicity

The acute toxicity study was done by "fixed dose" method in healthy adult female albino mice according to CPCSEA recommended "OECD guidelines 420. In brief, female Albino mice of, weighing 20-25 gm were used to determine the lethal dose. The animals were fasted overnight prior to the actual experimental procedure. Six mice were administered with a dose of 2000 mg/kg hydroalcoholic extract of *Murraya koenigii* by oral route. Animals were observed for clinical signs and mortality, continuously for first 2 hr. and then for every forth hr up to 14 days [13].

2.7. Assessment of anti-inflammatory activity

The activity was evaluated by using carrageenan induced paw oedema in rats. Albino rats were divided into four groups of six animals each. Group I served as toxicant control, received carrageenan (0.1 ml of 1% solution), group II served as standard, received indomethacin (10 mg/kg, p.o), group III and IV were treated with 100 mg / kg & 200 mg/kg hydroalcoholic extract of *Murraya Koenigii* fruits respectively by oral route. One hour after drug or test compound (extract) administration, 0.1 ml. of 1% carrageenan in distilled water was injected into the sub plantar region of right hind paws of all groups. The paw oedema volume was measured with the help of plethysmometer at zero hr (immediately after injecting carrageenan). The same procedure was repeated at 1, 2, 3 and 4 h after carrageenan injection. The difference between the initial and subsequent reading gave the actual ooedema volume. Reduction in paw volume compared to the control animals was considered as anti-inflammatory response [14].

Percentage Inhibition = [Vc - Vt/Vc] x 100

Where, Vt = mean paw volume of test group & Vc = mean paw volume of control group.

2.8. Assessment of wound healing activity

The rats were divided randomly into three groups of six rats each and inflicted with excision wounds. The animals were starved for 12 h prior to wounding. Before creation of wound, the area was cleaned with disinfected with 70 % alcohol. A circular wound of about 500 sq. mm diameter was made on depilated thoracic of rats under light anesthesia in aseptic conditions. The animals were housed individually and observed throughout the study. The test sample (herbal extract) was formulated as an ointment in simple ointment base. Formulated ointment (0.5 g) was applied on the wound once daily for 15 days starting from the day of wounding. The observation of wound closure was made on 3rd, 6th, 9th, 12th and 15th post-wounding days. The period of epithelialization was calculated as the number of days required for falling away of the dead tissue remnants of the wound without any residual raw wound [15, 16]. The percent wound contraction was calculated using the following formula:

[(Initial wound size – specific day wound size)/ Initial wound size] × 100

2.9. Histopathology

At the end of experimental period, wound area was removed for histological examination. The tissue samples were fixed in 10% neutral formalin. The materials were processed by conventional paraffin embedding method. Microtome sections were prepared at 6 µ thicknesses, mounted on glass slides, stained with hematoxylin and eosin stain followed by observation for histopathological changes under light microscope [17].

2.10. Statistical analysis

The results were expressed as mean + SEM. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by student's t' test. P values less than 0.05 were considered to be statistically significant, when compared with control.

3. Results

3.1. Preliminary phytochemical evaluation

The preliminary phytochemical screening of hydroalcoholic extract of *Murraya koenigii* fruits shows presence of triterpenoids, alkaloids, flavonoids and phenolic compounds.

3.2. Acute oral toxicity

Animals did not show any signs of toxicity during the observation period (14 days). Hence, hydroalcoholic extract of *Murraya koenigii* was safe to use up to a dose level of 2000 mg/kg in animals.

3.3 Anti-inflammatory activity

Carrageenan injection induced skin inflammation in rat hind paw which increased paw diameter in all the animals. However, Carrageenan-induced paw ooedema was significantly ($p < 0.05$) reduced by both standard and *Murraya koeingii* compared to control time dependently (Table 1). Further, the anti-inflammatory effect at high dose (200 mg/kg) was comparable to the standard drug, indomethacin. The reduction in carrageenan induced paw ooedema by 100 mg/kg & 200 mg/kg of *Murraya koenigii* after 4 h were 42.6 and 50.3 respectively, while ooedema reduction by indomethacin (10 mg/kg) at the same time was 55.5%.

Table 1: Effect of hydroalcoholic extract of *Murraya koenigii* fruits on carrageenan-induced rat paw oedema**.**

Group	Dose (mg/kg, p.o.)	Paw oedema volume in ml (% Inhibition of paw oedema)				
		0 hr	1 hr	2 hr	3 hr	4 h
Vehicle Control	--	0.50±0.02	1.25±0.05	1.33±0.03	1.42±0.06	1.55±0.04
Indimethacin	10	0.51±0.03	1.01±0.01 (19.2)	0.89±0.01 (33.1)	0.82±0.02 (42.3)	0.69±0.03*** (55.5)
HAEMK (Low dose)	100	0.48±0.05	1.10±0.07 (12)	1.0±0.04 (24.8)	0.94±0.07 (33.8)	0.89±0.06* (42.6)
HAEMK (High dose)	200	0.50±0.05	1.04±0.04 (16.8)	0.94±0.06 (29.3)	0.87±0.08 (38.7)	0.77±0.04** (50.3)

Values are expressed as mean ± SEM. Number of animal used 6 in each group;*p<0.05, **p<0.01, ***p<0.001 as compared to control.

3.4. Wound healing activity

The studies on excision wound-healing model revealed that significant wound-healing activity was observed in rats treated with *Murraya koenigii* fruit extract ointment (test group) and 0.2% w/w nitrofurazone ointment (standard group) compared with the control group. There was a reduction in wound area from day 3 onwards in both standard and test drug-treated rats; also on subsequent days, the wound contraction was much faster than for control rats (Table 2). By the end of experimental period, percentage of wound contraction by *Murraya koenigii* was 90.3%, whereas in control 59.7%. Epithelialization time in *Murraya koenigii* treated animals was significantly less when compared with control.

Table 2: Effect of hydroalcoholic extract of *Murraya koenigii* fruits ointment on the excision wound rat model.

Group	Wound area in mm^2 (% Wound contraction)					Epithelialization period (Days)
	Day 3	Day 6	Day 9	Day 12	Day 15	
Control	459.6±5.6 (8.1)	398.3±3.3 (20.3)	326.2±6.0 (34.8)	257.5±4.1 (48.4)	201.3±5.1 (59.7)	27.3±2.4
Standard (0.2% (w/w) nitrofurazone ointment)	386.3±3.7 (22.7)	238.8±3.1 (52.2)	158.5±3.6 (68.3)	91.5±1.9 (81.7)	28.2±1.9 (94.4)	17.17±1.3**
Murraya koenigii (10% w/w hydroalcoholic ointment)	401±6.1 (19.8)	277.6±6.2 (44.5)	203.9±5.1 (59.2)	121.5±4.2 (75.7)	48.13±3.7 (90.3)	20.5±2.9*

Values are expressed as mean ± SEM of n=6 animals in each group.*p<0.05, **p<0.01 as compared to control.

3.5. Histopathology

The histopathological results (Figures 1, 2 and 3) revealed that skin of control rats showed poor epithelialization, indistinguishable collagen fibres with increased number of macrophages. Animals treated with 0.2 %w/w nitrofurazone ointment (standard group) revealed higher epithelialization and well-formed collagen fibres. On the other hand, animals treated with *Murraya koenigii* (test group) also showed good epithelialization and well-formed collagen fibres.

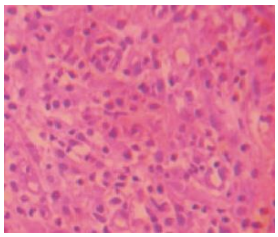

Fig 1: Histopathological evaluation of skin in control rats shows poor epithelialization and lesser collagen formation.

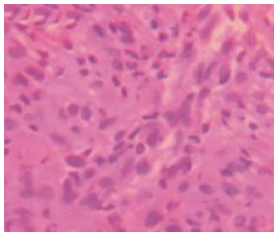

Fig 2: Histopathological evaluation of skin in standard ointment treated rats shows higher epithelialization and good collagen formation.

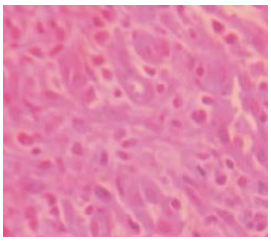

Fig 3: Histopathological evaluation of skin in *Murraya koenigii fruits* ointment treated rats also shows good epithelialization and better collagen formation.

4. Discussion

Carrageenan-induced inflammation is a useful model for acute inflammation which been extensively used to determine the anti-inflammatory effect of new investigational agents. The development of ooedema in the paw of the rat after injection of carrageenan is biphasic. First phase is due to release of histamine, serotonin and the second phase is caused by the release of bradykinin and prostaglandin-like substances [18, 19]. In our present study, there was significant inhibition of paw oedema in the early hours of study by hydroalcoholic extract. Hence, it can be concluded that there is inhibition of histamine and serotonin release. Similarly, there is a significant percentage of inhibition of paw oedema, at doses of 100 and 200 mg/kg at 3rd and 4th hour by hydroalcoholic extract of *Murraya koenigii* fruits. Therefore, it can be inferred that the inhibitory effect of hydroalcoholic extract on carrageenan-induced inflammation may be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis. This establishes the efficacy of

Murraya koenigii as an efficient therapeutic agent in acute inflammatory conditions.

Wound healing is a very complex, multifactor sequence of events involving several cellular and biochemical processes which helps in restoration of functional and anatomical continuity [20]. It mainly depends on the repairing ability of the tissue, type and extent of damage and general state of the health of the tissue [21, 22]. Results of the present study revealed that the topical application of *Murraya koenigii* fruit extract promoted the healing of the wound as evidenced by increased wound contraction and reduced epithelialization period compared to control rats suggesting the wound healing potential of the plant. Further, histopathological examination of wounds showed improved collagen synthesis in *Murraya koenigii* treated rats.

The preliminary phytochemical screening with the various qualitative chemical tests revealed the presence of flavonoids, triterpenoids, alkaloids and tannins as active compounds. The wound healing activity of *Murraya Koenigii* fruits may be attributed to the suppression of the production of free radicals at or around the wound bed caused by the anti-oxidant potential of this herb helps in reducing inflammation, increasing angiogenesis and collagen deposition [23, 24]. Further, triterpenoids and tannins are also known to promote the wound-healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialization [25, 26].

5. Conclusion

In conclusion, the present study on hydroalcoholic extract of *Murraya Koenigii* fruits has demonstrated that this plant has significant anti-inflammatory and wound healing properties, and it justifies the traditional use of the plant in the treatment of inflammation and external wounds. These activities may be due to presence of active constituents like flavonoids either alone or in addition to other phyto-constituents like triterpenoids and tannins. Although there are many underlying factors for its anti-inflammatory and healing potential, hence this study has paved the way for delving further to unveil the mechanism of this multifarious medicinal plant.

6. Acknowledgement

Authors are thankful to the Management and Principal of Hindu College of Pharmacy, Guntur for providing the necessary facilities for doing this research work.

7. References

1. Agnel Arul John N, Shobana G. Anti-inflammatory activity of *Talinum fruticosum* L. on formalin induced paw oedema in albino rats. Journal of Applied Pharmaceutical Science. 2012; 2(1):123-127.
2. Sosa S, Balicet MJ, Arvigo R, Esposito RG, Pizza C, Altinier GA. Screening of the topical anti-inflammatory activity of some Central American plants. Journal of Ethanopharmacology. 2002; 8:211-215.
3. Suguna L, Chandrakasan G, Joseph KT. Influence of honey on biochemical and biophysical parameters of wounds in rats. Journal of Clinical Biochemistry and Nutrition. 1999; 14:91.
4. Nayak BS, Anderson M, Pinto Pereira LM. Evaluation of wound-healing potential of *Catharanthus roseus* leaf extract in rats. Fitoterapia. 2007; 78(7-8):540-4.
5. Kogilavanee D, Yoke-Keong Y. Anti-inflammatory and wound healing properties of Malaysia Tualang honey. Current Science. 2016; 110(1):47
6. Raina R, Prawez S, Verma PK, Pankaj NK. Medicinal plants and their role in wound healing. Vetscan. 2008; 3:1-7.
7. Iyer UM, Mani UV. Studies on the effect of curry leaves supplementation (*Murraya koenigii*) on lipid profile, glycated proteins and amino acids in non-insulin dependent diabetic patients. Plants and Food in Human Nutrition. 1990; 40:275.
8. Yukari Tachibana, Hiroe Kikuzaki, Nordin HJL, Nobuji Nakatani. Antioxidative activity of carbazoles from *Murraya koenigii* leaves. Journal of Agricultural and Food Chemistry. 2001; 49:5589.
9. Yadav S, Vats V, Dhunnoo Y, Grover JK. Hypoglycemic and antihyperglycemic activity of *Murraya koenigii* leaves in diabetic rats. Journal of Ethnopharmacology. 2002; 82:111.
10. Anand T, Kalaiselvan A, Gokulakrishnan K. Wound-healing activity of *Murraya koenigii* in male albino rats. International Journal of Current Research. 2011; 3(12):425-427.
11. Alade PI, Irobi ON. Antimicrobial activities of crude leaf extracts of Acalyphawilkensiana. Journal of Ethnopharmacology. 1993; 39:171-174.
12. Kokate KR. Practical Pharmacognosy. Vallabha Prakashan, New Delhi, 1990, 110-117.
13. OECD. OECD Guidelines for Acute Toxicity of Chemicals; Organisation for Economic Co-operation and Development: Paris, France. 2001, 420.
14. Winter C, Risley EA, Nuss GW. Carragennian induced ooedema in hind paw of the rats for anti-inflammatory drugs. Proceedings of the Society for Experimental Biology and Medicine. 1962; 3:544-547.
15. Nagappa AN, Cheriyan Binu. Wound Healing Activity of the Aqueous Extract of *Thespesia populnea* Fruit. Fitotherapia. 2001; 72:503-506.
16. Taranalli AD, Tipare SV, Kumar S, Torgal SS. Wound healing activity of *Oxalis corniculata* whole plant extract in rats. Indian Journal of Pharmaceutical Sciences. 2004; 66:444-6.
17. Chandana Choudhury B, Shameem Ara B, Debesh Chandra P, Rumi Saikia B. Wound healing activity of *Alternanthera brasiliana* Kuntze and its anti-oxidant profiles in experimentally induced diabetic rats. Journal of Applied Pharmaceutical Science. 2013; 3(10):161-166.
18. Manjit Singh, Vijender Kumar, Ishpinder Singh, Vinod Gauttam, Ajudhia Nath Kalia. Anti-inflammatory activity of aqueous extract of *Mirabilis jalapa Linn*. Leaves. Pharmacognosy Research. 2010; 2(6):364-367.
19. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenan ooedema in rats. Journal of Pharmacology and Experimental Therapeutics. 1969; 166:96-103.
20. Karodi R, Jadhav M, Rub R, Bafna A. Evaluation of the wound healing activity of a crude extract of *Rubia cordifolia* L. (Indian madder) in mice. International Journal of Applied Research in Natural Products. 2009; 2(2):12-18.
21. Chitra Shenoy, Patil MB, Ravi Kumar. Wound Healing Activity of *Hyptis suaveolens* (L.) Poit (Lamiaceae). International Journal of PharmTech Research. 2009; 1(3):737-744.
22. Falanga V. Wound healing and its impairment in the diabetic foot. Lancet. 2005; 366:1736-1743.
23. Ramasamy A, Das S, Mani V, Sengottuvelu S, Vinoth Prabhu V. Evaluation of Anti-diarrheal Potential of

Hydro-alcoholic Extracts of Leaves of *Murraya koenigii* in Experimental Animals. Journal of Dietary Supplements. 2016; 13(4):393-401.
24. Atish NW, Sachin VT, Dinesh MS. Phytochemical Analysis and In vitro Antioxidant Properties of *Murraya koenigii* (L.) Fruits. American Journal of Phytomedicine and Clinical Therapeutic. 2015; 3(5):403-416.
25. Ya C, Gaffney Sh, Lilley TH, Haslam E. Carbohydrate-Polyphenol complexation. Chemistry and Significance of Condenced Tannins. New York: plenum, 1988.
26. Scortichini M, Pia Rossi M. Preliminary in vitro evaluation of the antimicrobial activity of triterpenes and terpenoids towards Erwinia amylovora (Burrill). Journal of Bacteriology. 1991; 71:109-12.