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Full Length Research Paper

Evaluation of anti-inflammatory and analgesic effects of aqueous extract obtained from root powder of *Inula racemosa* Hook. f.

Ponnan Arumugam[1]*, Marudhamuthu Murugan[2] and N. Thangaraj[1]

[1]Centre for Advanced Studies in Botany, School of Life Science, University of Madras, Guindy Campus, Chennai 600 025, Tamil Nadu, India.
[2]Department of Microbial Technology, School of Biological Science, Madurai Kamaraj University, Madurai – 625 021, Tamil Nadu, India.

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Root extract of *Inula racemosa* is widely used in Ayurvedic and folk medicine. Particularly in China and India, it is used for treating human diseases including cardiovascular, inflammation and abdominal pain. However, the root extracts are not scientifically investigated. This study was undertaken to evaluate the anti-inflammatory and analgesic activities of aqueous extract obtained from root powder of *I. racemosa* along with screening of secondary metabolites. The anti-inflammatory activity was evaluated in carrageenan induced rat paw edema model. The analgesic effect was measured in mice using acetic acid-induced writhing test and tail-immersion test. The treatment regime consisted of distilled water for Group I as control and three doses of aqueous extract (100, 200 and 400 mg/kg) and standard drugs, indomethacin (10 mg/kg) or aspirin (100 mg/kg) for the Groups, 2, 3, 4 and 5, respectively, fed orally. Respective groups were injected carrageenan or acetic acid and their endpoints was measured based on time periods. Saponins, terpenes, phenolics, flavonoids and glycosides were detected in aqueous extract. Anti-inflammatory and analgesic activities of aqueous extract were found to be highest at 60 and 63%, respectively, at the dose of 400 mg/kg and comparable to the respective standard drugs. The aqueous extract effect in tail immersion test was also found to be dose dependent. The effective anti-inflammatory and analgesic activities of aqueous extract of *I. racemosa* positively correlate with respect to their dose.

Key words: *Inula racemosa*, aqueous root extract, secondary metabolites, anti-inflammation, analgesic.

INTRODUCTION

The genus *Inula* consisting of 100 species belongs to the family Asteraceae. This genus plants are used as traditional medicines in East Asia and Europe. *Inula racemosa* Hook. f. is a perennial herb distributed in East Asia, Europe and Africa. In India, it is also known as Pushkarmula and grows in the hilly regions of north-western Himalayas (Alan and Miller, 1998; Zhao et al., 2010).

The root extracts of *I. racemosa* has been prescribed as medicine in China for various human diseases such as abdominal pain, acute enteritis, bacillary dysentery, to relieve the depression of the liver qi, alleviate pain especially between the neck and the shoulders and to prevent abortion. The plant root is considered as antimicrobial agent for nearly a thousand years. It is also prescribed as a tonic in veterinary medicine (Liu et al., 2006). In India, the plant root powder is reported to have shown potential effect on cardiovascular system and also used as Ayurvedic medicine for angina and dyspnea (Alan and Miller, 1998). Native Americans utilized the root extract for the treatment of tuberculosis and proved that it

*Corresponding author. E-mail: ponnanarumugam@gmail.com. Tel: 91 - 44 – 22352498. Fax: 91- 44 – 22352498.

blocks the adrenaline induced hyperglycemia. In addition, root extract along with guggul (*Commiphora mukul*) is also employed for curing myocardial ischemia. Previous report indicated that the root extract has potential for the insulin sensitivity and efficiently control steroid induced diabetes (Lokhande et al., 2007). The various biological properties of this plant root are mainly through a variety of bioactive phytochemicals such as alantolactone, isoalantolactone, dihydroalantolactone, dihydroisoalantolactone, sitisterol, daucosterol, inunolide, aplotaxene, phenylacetonitrile and isoinunal (Tan et al., 1998; Wang et al., 2000). Isoalantolactone possessed antimicrobial activity including human pathogenic fungi and strong phytotoxic effects on seed germination and seedling growth (Stojakowska et al., 2005; Liu et al., 2006; Zhao et al., 2010).

The aim of the present study was to find out the efficacy of the herbal extract which has been traditionally used to treat inflammation, allergy and infectious diseases but not scientifically proven yet. Therefore the present investigation was undertaken to evaluate the anti-inflammatory and analgesic activities of aqueous root extract of *I. racemosa.*

MATERIALS AND METHODS

Collection of plant material

The dried roots of *I. racemosa* were purchased from the Ramaswamy Country Drug Merchants & Co. Ltd., Khandhasami Street, Chennai, India. The plant root was authenticated by Dr. Sasikala Ethirajulu, Research officer (Pharmacognosy), Central Research Institute of Siddha, Chennai, India and the Herbarium deposited to the same institute.

Extraction procedure

The extraction of dried roots was done by following the method of Zhao et al. (2010). The dried roots were cut into small pieces and made into powdery form with an electric blender. Three hundred grams of the powdered roots sample of *I. racemosa* was weighed and soaked in 1 L of distilled water for 72 h at room temperature. Finally, the extract was filtered and centrifuged. The aqueous extract freeze dried to yield 6 g was stored at -20°C until further use.

Screening of secondary metabolites

Major phytochemicals such as alkaloids, tannins, saponins, terpenes, phenols, flavonoids, glycosides, sterols and resins of the crude extract were determined using the protocol described by Allen (1974) and Harbone (1976).

Experimental animals

Both sex of Wistar albino rats (150 to 180 g) and Swiss albino mice (20 to 25 g) were obtained from King Institute, Chennai, India. The animals were housed in standard environmental conditions (12 h light/12 h dark; 22±2°C) for one week prior to the experiments to acclimatize to the laboratory conditions. They were allowed free access to tap water and pellet rodent diet. The animal care and experimental protocols were in accordance to the Guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. Ethical approval was obtained from Institutional Animal Ethics Committee.

Carrageenan induced paw edema in rats

Thirty albino rats were divided into five groups of six in each and the following treatment regimen was followed. Control group received distilled water orally. Three test doses of aqueous extract (100, 200 and 400 mg/kg) and indomethacin as a positive control (10 mg/kg in distilled water) were administrated orally to the groups 2, 3, 4 and 5 prior to carrageenan injection, respectively. After 1 h, 0.1 ml of 1% solution of freshly prepared carrageenan in normal saline was injected in the sub plantar surface of the right hind paw of the rats. The paw volume was measured (as cm) before and after injection of carrageenan at the time periods of 1, 2, 4 and 8 h by mercury displacement Plethysmograph. The difference between left and right paw volumes indicated the degree of inflammation and it is expressed as % inhibition of paw volume for the standard and aqueous extract of *I. racemosa* over the control value (Arumugam et al., 2008).

Acetic acid induced writhing method

The writhing test in mice was carried out using acetic acid according to the method of Ferreira et al. (2000). The treatment regimen was as follows. Control, Group 1 received distilled water; three doses of aqueous extract (100, 200 and 400 mg/kg) for the Groups 2, 3 and 4, respectively were fed orally. Similarly, Group 5 received aspirin (100 mg/kg) orally as positive control. All the test doses and standard drug were fed one hour before chemical stimulus. The writhes were induced by intraperitoneal injection of 1% acetic acid (v/v, 10 ml/kg) in all the groups except control. After ten minutes, the number of writhe was recorded over a period of ten minutes. A writhe is indicated by abdominal constriction and full extension of hind limb.

Tail immersion method

Thirty mice were divided into five groups of six in each and held in position suitably restrained with the tail extending out. The marked tail (1 to 2 cm) was immersed in the water bath thermo-statistically maintained at 55±1°C. The withdrawal time of the tail from hot water (in seconds) was noted as the reaction time or tail flick latency. The maximum cut off time for immersion was 15 s to avoid injury to the tissues of the tail. The animals were fasted for 16 h with water *ad libitum* before drug administration. The same experimental design such as writhing test was also used for tail immersion method. The tail flick response was measured after administration of test and standard drugs at the time period of 60, 120 and 180 min. Tail flick latency difference or mean increase in latency after drug administration was used to indicate the analgesia produced by test and standard drug (Kumar and Shankar, 2009).

Statistical analysis

The results were expressed as mean ± SD of six animals per group. All the data were analyzed statistically by one-way ANOVA followed by Tukey's multiple comparisons using SPSS software student's version-12. A p value <0.05 was considered statistically significant.

Table 1. Preliminary phytochemicals screening of aqueous extract of *I. racemosa.*

Secondary metabolites	Aqueous extract
Alkaloids	-
Tannins	-
Saponins	+
Terpenes	+
Phenols	+
Flavonoids	+
Glycosides	+
Sterols	-
Resins	-

+, Presence; -, absence.

Table 2. Protective effects of aqueous extract of *I. racemosa* against carrageenan-induced paw edema in rats.

Treatment groups	Oral dose (mg/kg)	Paw volume at different time intervals (h)			
		1	2	4	8
Control	DW	0.40±0.14	0.53±0.10	0.48 ± 0.08	0.45 ± 0.13
Indomethacin	20	0.30±0.09(25%)	0.35±0.11*(34%)	0.27 ±0.08*(44%)	0.14 ±0.18*(69%)
Aqueous extract	100	0.40±0.11(0%)	0.50±0.09(5.7%)	0.42 ±0.12(12.5%)	0.35 ±0.11(22%)
	200	0.37±0.09(7.5%)	0.46 ±0.08(11.3%)	0.35 ±0.14(27%)	0.27 ±0.14*(40%)
	400	0.33 ±0.10(17.5%)	0.38 ±-0.10(28.3%)	0.30 ±0.09*(37.5%)	0.18 ±0.09*(60%)

Values expressed in centimeters are mean ± standard deviation computed over six rats per group. Percentages indicated to the change in edema size with respect to the negative control (DW: distilled water) group. Comparisons were made between control and drug treated groups. * $p< 0.05$.

RESULTS

The presence of secondary metabolites in the aqueous extract of root powder of *I. racemosa* was determined with a preliminarily screening for the following phytochemicals: Alkaloids, tannins, saponins, terpenes, phenolics, flavonoids, glycosides, sterols and resins using conventional methods. Among the secondary metabolites, saponins, terpenes, phenolics, flavonoids and glycosides were observed to be present in the aqueous extract of *I. racemosa.* In contrast, other metabolites such as alkaloids, tannins, sterols and resins were found to be absent (Table 1).

Anti-inflammatory effect of aqueous extract of *I. racemosa* was evaluated against carrageenan induced paw edema in rats. The increased edema paw volume in rat was measured at time periods of 1, 2, 4 and 8 h and the results are presented in Table 2. The results revealed that the aqueous extract of *I. racemosa* effectively prevents the carrageenan induced paw edema. An increased paw volume in the control group was observed ranging from 0.53 ± 0.10 to 0.40 ± 0.14 at a time period of 1 to 8 h. Standard drug and aqueous extract treated groups were observed to decrease the paw volume based on the dose with respect to the time period. Indomethacin decreased the paw volume (25 to 69%) with respect to the control group for a time period of 1 to 8 h. However, 100 mg/kg dose of aqueous extract treated groups showed the least decrease in paw volume about to be 5.7%. At the dose of 200 mg/kg, the reduction of the paw volume was observed ranging from 7.5 to 40% for the time period of 1 to 8 h. The significant reduction of paw volume by aqueous extract of *I. racemosa* was found to be as equal as standard anti-inflammatory drug, indomethacin at their maximum dose. The significantly decreased carrageenan induced paw volume in rats by aqueous extract of *I. racemosa* was observed to be dose dependent.

The anti-nociceptive effect of aqueous extract of *I. racemosa* was evaluated using both chemical and thermal methods of nociception in mice. The acetic acid induced abdominal writhes were observed to be 52.33 ± 08.14 over the period of 10 min in the control group (Figure 1). The number of abdominal writhes were significantly ($p0<0.5$) inhibited by both standard drug and aqueous extract of *I. racemosa*. Standard drug, acetyl salicylic acid significantly inhibited writhes by about 74% over the control. Aqueous extract of *I. racemosa* effectively inhibited ($p0<0.5$) abdominal writhes in mice induced by acetic acid. The highest inhibition of writhes, about 63% was observed at the dose 400 mg/kg. At the dose of 200 mg/kg, the writhes inhibition effect was about40% over the control. In contrast, the writhes inhibition effect was found to be not significant at the

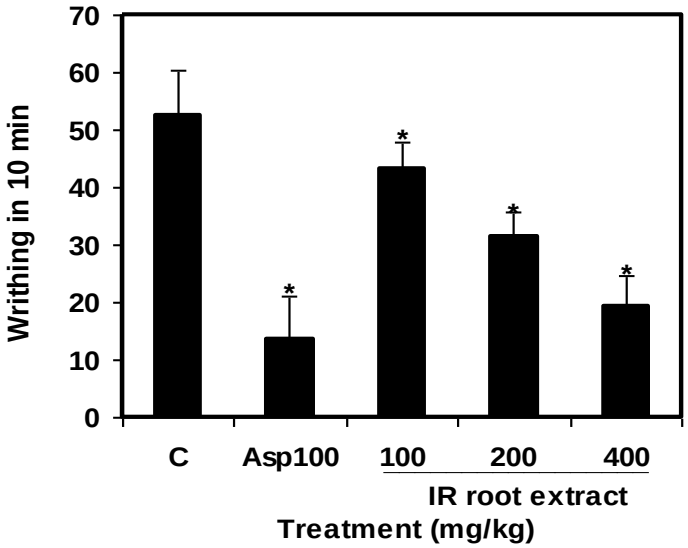

Figure 1. Preventive effect of aqueous extract of *I. racemosa* on acetic acid-induced writhing in mice (C, control; Asp, aspirin; IR, *I. racemosa*; *p<0.05; Comparisons were made between control and drug treated groups).

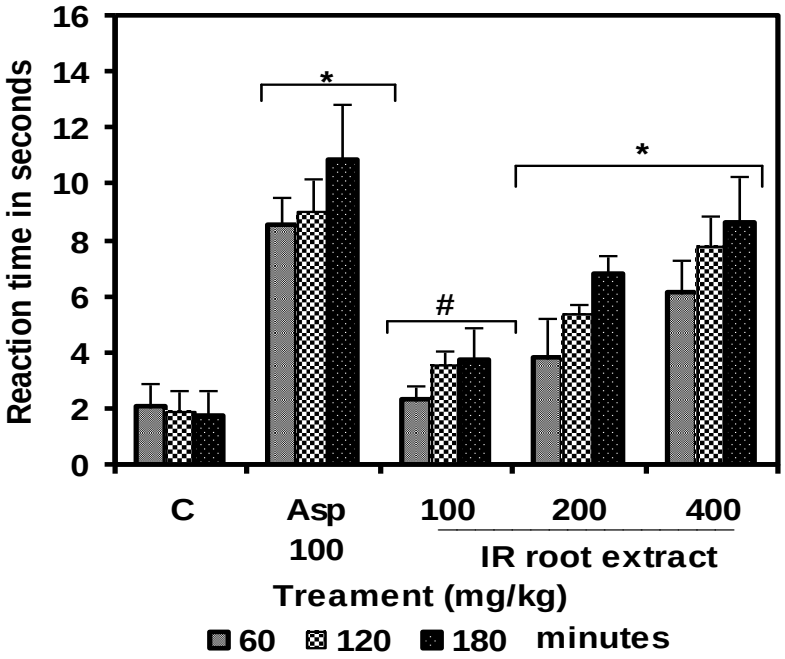

Figure 2. Preventive effect of aqueous extract of *I. racemosa* on tail flick latency time of mice exposed to hot water both test (C, control; Asp, aspirin; IR, *I. racemosa*; #, not significant; *, p<0.05; Comparisons were made between control and drug treated groups).

dose of 100 mg/kg (p0<0.5) which has shown about 18% inhibition over the control. Hence, the writhes effect of aqueous extract of *I. racemosa* was found to be dose dependent. The analgesic effect of *I. racemosa* aqueous extract on the tail immersion-test in mice has been shown in Figure 2. All the doses of aqueous extract were effective in significantly (p<0.05) reducing the pain response as indicated by the increase of reaction time over the control, 2.05 ± 0.89 to 1.73 ± 0.75 at time period of 1 to 3 h. Acetyl salicylic acid significantly enhanced the

reaction time in the range of 8.56 ± 0.96 to 10.85 ± 2.01. Thus, the reduction of pain response seems to be better than control and aqueous extract of *I. racemosa*. Among the dose of aqueous extract, the highest enhanced reaction time was observed at 400 mg/kg (8.65 ± 1.63 at 3 h) and least at the dose 100 mg/kg (2.31 ± 0.53 at 1 h). At the dose of 200 mg/kg, the enhanced reaction time was observed to be in the range of 3.84 ± 1.36 to 6.76 ± 0.66 after 1 to 3 h of oral feeding which is better than control value ($p<0.05$). The aqueous extract effect in tail immersion test was found to be dose dependent and its effect at highest dose (400 mg/kg) showed to be comparable to that of acetyl salicylic acid.

DISCUSSION

Several inflammatory mediators such as histamine, bradykinin, serotonine, and prostaglandins are released from damaged cells and produce inflammation and nociception by the induction of pain (Garcia-Pastor et al., 1999; Hajhashemi et al., 2010). These mediators are present in tissues with high content of water and plasma during arachidonic acid metabolism via cyclo-oxygenase and lipo-oxygenase enzyme pathways (Moura et al., 2005). The first phase of inflammation begins immediately up to an hour after injection of carrageenan by the release of histamine and serotonin whereas the second phase starts after one hour and up to three hours by the release of bradykinin, protease and prostaglandins (Arumugam et al., 2008; Gupta et al., 2009). In the present study, results revealed that the potential anti-inflammatory activity of *I. racemosa* was found to be 60% at the dose of 400 mg/kg which was comparable with the known anti-inflammatory drug, indomethacin (Table 1). Among the tested dose of *I. racemosa*, anti-inflammatory activity of the highest dose (400 mg/kg) was 5, 3 and 2.7 folds higher than the dose of 100 mg/kg at 2, 4 and 8 h time period, respectively. The second highest dose of *I. racemosa* also showed effective anti-inflammatory activity than that of the lower dose. Hence, aqueous extract of *I. racemosa* showed dose dependent anti-inflammatory activity and its effects clearly indicate being equal to standard drug, indomethacin. The potential activity is mainly due to the presence of secondary bioactive compounds such as saponins, terpenes, phenolics, flavonoids and glycosides. Therefore, effects of aqueous extract of *I. recemosa* on carrageenan induced paw edema could be due to its ability to inhibit the release of inflammatory mediators. Anti-inflammatory mechanism of non-steroidal drugs like indomethacin is also reported to inhibit the inflammatory process induced by carrageenan (Neto et al., 2005; Hajhashemi et al., 2010). Particularly, phenolics and flavonoids were reported to inhibit the cyclo-oxygenase and lipo-oxygenase pathways of arachidonate metabolism (Pelzer et al., 1998; Zheng et al., 2003).

Acetic acid induced writhing test was used for detecting peripheral analgesia, whereas tail flick tests are most sensitive to centrally acting analgesics (Hajhashemi et al., 2010; Neto et al., 2005). Results of writhing test demonstrated that the maximum inhibition effect of *I. racemosa* was found to be about 63% at the dose of 400 mg/kg over the control which was shown to be 3.6 and 1.6 folds higher than that of other doses, 100 and 200 mg/kg. The highest inhibition effect of *I. racemosa* was almost comparable to the effect of aspirin (74%). In writhing test, injection of acetic acid mainly causes the release of prostaglandins mediators such as $PGE_{2\alpha}$ and $PGF_{2\alpha}$ with increased level in the peritoneal fluid (Neto et al., 2005). Writhing test results indicate that the anti-inflammatory activity of *I. racemosa* might also be involved in the peripheral analgesic activity. However, the centrally acting analgesics generally elevate the pain threshold of mice towards heat which was measured by tail immersion method. *I. racemosa* significantly ($p<0.05$) increased the reaction time of animals towards the thermal source in a dose-dependent manner. The plant sample showed significant activity in tail-immersion method but further experimentation is needed to understand the exact mechanism. Centrally acting analgesic effect of *I. racemosa* showed significant pain tolerance over the control. The pain tolerance at highest dose of *I. racemosa* and standard drug were found to be comparable. When compared to control, they varied about 5.6 and 6.3 folds higher over the control (Figure 2). At the dose of 400 mg/kg, pain tolerance shown was ~2 folds higher compared with the dose of 100 mg/kg. The protective action of known analgesic drugs is also mainly either on the central nervous system or on the peripheral nervous system (Planas et al., 2000). Recent report indicates that antioxidants are able to reduce pain induced by chemical and thermal stimulus (Hacimuftuoglu et al., 2006). Antioxidant and cardioprotective effects of *I. racemosa* were recorded earlier (Dwivedi et al., 1988). The genus root contains major constituents such as sesquiterpene lactones, alantolactone and isoalantolactone recorded in *Inula helenium* and *Inula royleana* and possessed antiinflammatory, antimicrobial and anthelmintic activities (Stojakowska et al., 2005). *Inula viscose, I. britanica* and *Inula hupehensis* were reported to be active against various types of diabetes (Zeggwagh et al., 2006; Zhao et al., 2010). Similar to the present results, analgesic and anti-inflammatory activity of aqueous root extract of *Pfaffia glomerata* in carrageenan model, writhing and tail flick tests were reported in animal model (Neto et al., 2005).

Conclusion

The plant, *I. racemosa* used as traditional medicine to treat many human diseases such as stomach ache, relieve the depression of the liver qi, and prevent abortion

including inflammation and analgesia (Liu et al., 2006). However, there was no experimental evidence yet for the anti-inflammatory and analgesic activities of *I. racemosa*. The results clearly revealed that aqueous extract of *I. racemosa* showed potential anti-inflammatory property in carrageenan model and also analgesic effects in writhing by acetic acid and tail immersion tests in a dose dependent manner. These potential activities were found to be as equal as to respective standard drugs. These results provided adequate credit for the use of traditional medicine, *I. racemosa* as a remedy against painful and inflammatory conditions which can also be utilized for modern drug development.

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