Chitra A. *et al.* / *International Journal of Phytopharmacology.* 4(5), 2013, 301-304.

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

ANTI-INFLAMMATORY, ANALGESIC AND ANTIPYRETIC ACTIVITY OF METHANOLIC EXTRACT OF *CATUNAREGUM SPINOSA* THUNB.

Chitra A*[1,2], Senthil Kumar N[2], Asraf Ali M[1]

[1]Sunrise University, Alwar, Rajasthan, India.
[2]JKKMMRF's College of Pharmacy, Ethirmedu, Komarapalayam, Salem (Dist.), Komarapalayam, Tamil Nadu, India.

ABSTRACT

To evaluate the anti-inflammatory, analgesic and antipyretic activity of methanolic extract of *Catunaregum spinosa* (MECS), using different models in rats. Methanolic extract of *Catunaregum spinosa* (100, 300, 1000 and 2000 mg/kg body weight) was given to rats orally to observe acute toxicity, and observed for 14 days. Analgesic activity was evaluated using tail immersion and anti-inflammatory activity was evaluated using carrageenan induced paw edema model in rats. Antipyretic activity was evaluated using Brewer's yeast induced pyrexia model in rats. Methanolic extract of *Catunaregum spinosa* were given at dose of 100, 200 and 500 mg/kg *p.o.* Results demonstrated that the no mortality was reported even after 14 days. This indicated that the methanol extract was safe up to a single dose of 2000 mg/kg body weight. Methanolic extract of *Catunaregum spinosa* (100, 200 and 500 mg/kg *p.o.*) significantly increased the latency period in the tail immersion test. Methanolic extract of *Catunaregum spinosa* (100, 200 and 500 mg/kg *p.o.*) significantly prevented increase in volume of paw edema. Methanolic extract of *Catunaregum spinosa* (100, 200 and 500 mg/kg *p.o.*) significantly decreased the rectal temperature of the rats. The study exhibits that methanolic extract of *Catunaregum spinosa* possesses analgesic, anti-inflammatory and anti-pyretic activity which may be mediated by the central and peripheral mechanisms.

Key words: Pain, Inflammation, fever, Nociception, *Catunaregum spinosa*, Analgesic, Antipyretic, Methanol.

INTRODUCTION

Catunaregum spinosa Thunb (Family-Rubiaceae) is a rigid shrub or tree, up to 9 m in height and 1.2 m in girth found throughout India up to an altitude of 1,900 m. the species is common as undergrowth in the sub-Himalayan tract and elsewhere. The flowers and fruits are edible; the flowers are eaten as a vegetable in Nasik district. The raw fruits have a highly astringent taste due to high tannin content (Sasikala E *et al*., 2008; Sharma PC *et al*., 2000). The fresh fruits contain high amount of carbohydrates and saponins. The seeds contain essential oil and organic acid. The main uses of *Catunaregum spinosa* are nauseant, expectorant, anthelmintic and abortifacient properties. It also showed hypoglycaemic, insecticidal and anti cancer activities. The seeds are tonic to induce appitite. Their decoction is taken for relief from headache. The bark is used to diarrhea and dysentery (Guangchun G *et al*., 2008; Karki S *et al*., 2011; Mukherjee PK, 2002).

Corresponding Author

A. Chitra
Email: chitrampharm@gmail.com

MATERIALS AND METHODS

Plant material

The aerial parts of the plant *Catunaregum spinosa* Thunb. belongs to the family Rubiaceae was collected and authenticated by the botanist Dr.P.Jayaraman, M.Sc., Ph.D. Director Plant Anatomy Research Center (PARC), Thambaram, Chennai. The aerial parts of the plant dried in shade were powdered and subjected to soxhlet extraction with methanol at 40-60^{o}C for 72 h. the extract collected was evaporated (yield 25.6% w/w), and stored in vacuum desiccators. The

preliminary phytochemical investigations with the methanolic extract revealed the presence of flavonoids, alkaloids, glycosides, saponins, carbohydrates, tannins, phenols and mucilage.

Drugs and chemical

The following drugs namely, aspirin (Disprin) and Paracetamol (Crocin), and chemicals, methanol (Merck) and acetic acid (Fisher Scientific) were used during the experimental study.

Animals

Albino rats of either sex (150-200 g) were used for the experimental study. The animals were maintained under standard conditions in polypropylene cages and provided with food and water *ad libitum.* The animals were kept on fasting overnight prior to the experimentation and all the procedures used in these studies were approved by the institutional Animal Ethics Committee.

Acute toxicity studies

The acute toxicity was performed according to OECD guidelines. The selected female albino rats were used for toxicity studies. The animals were divided in to four groups of three in each. The animals were fasted overnight prior to the acute experimental procedure. Extract was given orally to rats at the graded doses like 100, 300, 1000 and 2000 mg/kg body weight. Immediately, after dosing, the animals were observed continuously for first four hours for behavioral changes and for mortality at the end of 24 h, and daily for 14 days for any behavioral change or mortality.

Tail immersion test

The tail withdrawal response was determined by immersing the lower 3.5 cm of the animals tail into a cup freshly filled with water from a large bath at a constant temperature of 50^{o} C until the typical response was observed. A 25 s cutoff was imposed to avoid tail damage by heat. A control group received vehicle while the aspirin 100 mg/kg *p.o.* administered to group II and methanolic extract of *Caatunaregum spinosa* (100, 200 and 500 mg/kg *p.o*) was given to III, IV and V groups. Analgesic activity was measured at 0, 30, 60, and 90 mins after administration of methanolic extract of *Catunaregum spinosa,* aspirin and distilled water.

Carrageenan induced paw edema

Five groups of six animals each were used. Paw swelling was induced by sub-planter injection of 0.1 mL 1% carrgeenan in saline in to the right hind paw. Methanolic extract of *Catunaregum spinosa* were administered at 100, 200 and 500 mg/kg orally 60 mins before Carrageenan administration. Aspirin 100 mg/kg *p.o* was used as reference drug. Control group received the vehicle only. The inflammation was quantified by measuring the volume displaced by the paw, using plethysmometer at time 0, 1, 2, 3 and 4h after carrageenan injection. The percent inhibition of edema was calculated in comparision to the control animals (Niazi J *et al*., 2009; Nisar M *et al*., 2008).

Yeast-induced hyperpyrexia in rats

Before experimentation rectal temperature of rats were recorded by inserting a well lubricated bulb of a thermometer in the rectum. Hyperpyrexia was induced in rat by subcutaneous injection of 10 mL/kg b.w. of a 15% aqueous suspension of brewer's yeast in the back below the nape of the rat. Pre-drug control temperatures were taken at 24 h after the yeast injection to determine the pyretic response of yeast. Methanolic extract of *Catunaregum spinosa* (100, 200 and 500 mg/kg body weight) served as the reference drug given orally 24 h after the yeast injection. The temperatures were recorded at 1-4 h after the drug treatment (Aiyalu R *et al*., 2010; Devi K. Jyotsna and Swetha K, 2013).

RESULTS

Acute toxicity study

In toxicity study four groups of rats were administered with methanolic extract of aerial parts of *Catunaregum spinosa* in graded doses of 100, 300, 1000 and 2000 mg/kg *p.o.*, respectively. The animals were kept under observation for the change in behavior or death up to 14 days following the plant extract administration. The extract administration neither caused any significant change in the behaviours nor the death of animal(s) in all the test groups. This indicates that the methanolic extract of safe up to a single dose of 2000 mg/kg body weight. Hence we had selected 200 to 500 mg/kg oral doses of methanolic extract of *Catunaregum spinosa* to evaluate analgesic, anti -inflammatory and antipyretic activity in rats.

Tail immersion test

Methanolic extract of *Catunaregum spinosa* at doses of 100, 200 and 500 mg/kg *p.o.* significantly increased pain threshold after 30 mins. Methanolic extract of *Catunaregum spinosa* exhibited a dose dependant increase in the reaction time at various time intervals of observation exhibited powerful analgesic activity were comparable to the reference standard.

Carrageenan induced paw edema

Pre-treatment with methanolic extract of *Catunaregum spinosa* at doses 100, 200 and 500 mg/kg significantly prevented increase in volume of paw edema in a dose dependent manner (Table 2). However, a maximal effect is observed at 500 mg/kg *p.o.* dose and

was comparable to aspirin (100 mg/kg *p.o.*). The 500 mg/kg of methanolic extract of *Catunaregum spinosa* was able to effectively inhibit increase in paw value.

Brewer's yeast induced hyperpyrexia

Subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 24 h administration. Treatment with the methanolic extract of *Catunaregum spinosa* at the doses of 100, 200 and 500 mg/kg significantly decreased the rectal temperature of the rats in a dose dependent manner. The antipyretic effect started as from the first hour and the effect was maintained for 4 h, after administration of the extract. The result obtained from both the standard (paracetamol) and methanolic extract of *Catunaregum spinosa* treated rats were compared with that of control and a significant reduction in the yeast induced elevated rectal temperature was observed.

Table 1. Analgesic effect of methanolic extract of *Catunaregum spinosa* (MECS) by tail-immersion test

Treatment	Dose mg/kg	Mean Response time after drug treatment (sec)			
		0min	30min	60min	90min
Control		1.42±0.05	1.53±0.02	1.58±0.07	1.61±0.04
Aspirin	100	3.42±0.01	8.35±0.07	9.48±0.07	10.40±0.07
MECS	100	2.58±0.14	4.35±0.07	5.21±0.01	5.58±0.07
MECS	200	3.47±0.07	3.91±0.14	5.83±0.07	6.23±0.01
MECS	500	4.15±0.01	5.26±0.04	6.36±0.04	8.79±0.06

All values are expressed as mean (n=6), $p<0.01$, Experimental animals compared with control.

Table 2. Anti-inflammatory activity of methanolic extract of *Catunaregum spinosa* (MECS) by carrageenan induced rat paw edema

Group	Dose Mg/kg	Paw volume (ml)			
		1 hour	2 hour	3 hour	4 hour
Control		1.76±0.12	1.53±0.08	1.63±0.08	2.42±0.11
Aspirin	100	1.5±0.05	1.45±0.17	1.4±0.06	0.92±0.05
MECS	100	1.53±0.03	1.7±0.21	1.63±0.12	1.22±0.03
MECS	200	1.68±0.06	1.54±0.07	1.5±0.14	1.18±0.04
MECS	500	1.86±0.12	1.6±0.06	1.44±0.05	1.02±0.06

All values are expressed as mean (n=6), $p<0.01$, Experimental animals compared with control.

Table 3. Effect of methanolic extract of *Catunaregum spinosa* (MECS) on yeast induced pyrexia in rats

Treatment	Dose mg/kg	Rectal temperature ^{o}C		Rectal temperature after administration of drug ^{o}C		
		Normal (A)	18h after yeast administration (B)	1h (C_1)	2h (C_2)	3h (C_3)
Control		36. 82±1.17	39.71±1.13	38.79±1.12	38.62±1.23	38.25±1.21
Paracetamol	100	36.99±1.58	39.85±1.21	38.92±1.15	37.22±1.13	37.02±1.71
MECS	100	36.78±1.18	39.64±1.34	38.85±1.86	38.34±1.15	37.82±1.83
MECS	200	36.75±1.17	39.67±1.26	38.56±1.32	38.26±1.12	37.64±1.26
MECS	500	36.84±1.17	39.31±1.13	38.28±1.14	37.75±1.11	37.15±1.59

All values are expressed as mean (n=6), $p<0.01$, $p<0.05$, Experimental animals were compared with control.

DISCUSSION

It is well known that pharmaceutical companies around the world are interested in developing safer and more effective drugs to treat pain, inflammation and fever. The present study evaluated the analgesic, anti-inflammatory and antipyretic effect of methanolic extract of *Catunaregum spinosa* in several animal models. Methanolic extract of *Catunaregum spinosa* prolonged the tail-immersion latency, indicating an increase in the nociceptive threshold. The tail-immersion response is believed to be a spinally mediated reflex.Inflammation induced by carrageenan was observed to have two phases. The early phase was associated with significantly severe inflammation, whereas late phase was observed to have slow increase in volume of paw edema. The initial phase has been attributed to the action of mediators such as histamine, serotonin and bradykinin on vascular permeability (Vane JR, 1987). The result of pre-treatment of methanolic extract of *Catunaregum spinosa* (at all the doses) is effective in the early phase of inflammation which has been reported because of release of histamine

and serotonin primarily. Based on this an assumption can be made that the extract may be showing its effect through inhibition of histamine release (Ramachandran S *et al*., 2011).

Methanolic extract of *Catunaregum spinosa* significantly reduced the pyrexia induced by yeast in rats. The reference drug aspirin also suppressed the yeast-induced fever in rats by inhibiting the synthesis of prostaglandin E2 (Elumalai A *et al*., 2012). These results support the use of *Catunaregum spinosa* as an antipyretic for the treatment of fever. The results of this study exhibited that methanolic extract of *Catunaregum spinosa* possesses analgesic, anti-inflammatory and antipyretic activities which may be mediated by the central and peripheral mechanisms. An activity-guided fractionation of this extract is presently being carried out. The isolation of new and effective analgesic, anti-inflammatory and antipyretic compounds is important for both drug development and establishment of the ethno medicinal use of this plant.

REFERENCES

Aiyalu R, Ramasamy A, Shanmugasundaram M. Evaluation of antipyretic activity of ethyl acetate extract of *Adenema hyssopifolium* G. Don in a rat model. *Asian Paci J Trop Med*, 3, 2010, 523–526.

Devi K. Jyotsna and Swetha K. Antipyretic activity of *Terminalia catappa* (Linn) Gum in Pyrexia Induced Albino Rats. *International Journal of Biological & Pharmaceutical Research*, 4(3), 2013, 172-175.

Elumalai A, *et al*. Acute Toxicity Studies and Antipyretic Activity of a Polyherbal Formulation. *International Journal of Biological & Pharmaceutical Research*, 3 (1), 2012, 130-132.

Guangchun G, Shuhua Qi, Si Zhang, Huo yin, Zhihui Xiao, Minyi Li, Qingxin. Minor compounds from the stem bark of Chinese mangrove associate *Catunaregum spinosa* (Thunb) Tiruven. *Pharmazie*, 63(7), 2008, 542-4.

Karki S, Kumar K. Study on the possible use of Vi polysaccharide typhoid fever vaccine to control endemic typhoid fever in Nepal. *Asian Pac J Trop Dis*, 1(1), 2011, 76–79.

Mukherjee PK. Quality control of Herbal drugs. Edition 1. Kolkata: Business Horizon; Pharmaceutical Publishers; 2002, 23-24.

Niazi J, Singh P, Bansal Y, Goel RK. Anti-inflammatory, analgesic and antipyretic activity of aqueous extract of fresh leaves of *Coccinia indica*. *Inflammopharmacol*, 17, 2009, 239–244.

Nisar M, Khan I, Simjee SU, Gilani AH, Perveen OH. Anticonvulsant, analgesic and antipyretic activities of *Taxus wallichiana* Zucc. *J Ethnopharmacol*, 116, 2008, 490–494.

Ramachandran S, Rajini Kanth B, Rajasekaran A, Manisenthil Kumar KT. Evaluation of anti-inflammatory and analgesic potential of methanol, extract of *Tectona grandis* flowers. *Asian Pac J Trop Biomed*, 1(Suppl 1), 2011, S155–S158.

Sasikala E, Saradha V, Balakrishna K and Veluchamy. Pharmacognosy studies on *Catunaregum spinosa* Root. *J. Econ. Taxon. Bot*, 32(3), 2008, 533-6.

Sharma PC, Yelne MB, Dennis TJ. Database on medicinal plants used in Ayurveda. 2nd ed, New Delhi. Central Council for Research in Ayurveda and Siddha, 2000, 380-8.

Vane JR. The evolution of non-steroidal anti-inflammatory drugs and their mechanisms of action. *Drugs*, 33, 1987, 18–27.