



International Journal of Pharmacy and Natural Medicines

Journal Home Page: www.pharmaresearchlibrary.com/ijpnm



Research Article

Open Access

Pharmacological Evaluation of Anti-Inflammatory activity of methanolic extract of cordial *subcordata.lam*

C. Madhavi Latha*^{1,2}, K. Bhaskar Reddy³

¹Research Scholar, Pacific University of Higher Education and Research University, Udaipur, Rajasthan, India.

²Department of Pharmacology, Jagan's College of Pharmacy, Nellore, Andhra Pradesh, India.

³Department of Pharmaceutics, Sri Venkateswara College of Pharmacy, RVS Nagar, Chittoor, India.

ABSTRACT

The main object of the present work is to find out good pharmacological activities in herbal source with their preliminary phytochemical study, and also it is aimed to investigate of methanol extract of whole plant cordia subcordata. The carrageenan induced hind paw edema, cotton pellet induced granuloma model was used to study *in-vivo* anti inflammatory activity and membrane stabilization test, inhibition of albumin denaturation models for *in-vitro* anti-inflammatory activity of methanolic extract of whole plant. Indomethacin (4mg/kg) Was used as standard drug for carrageenan induced paw oedema model, diclofenac sodium (5mg/kg) Was used as standard drug for cotton pellet induced granuloma model, aspirin (200 µg/ml) was used as standard drug for membrane stabilization, inhibition of albumin denaturation model. Acute edema in the left hind paw of the animal was induced by subplantar injection of 0.1ml (1%) carrageenan suspension in normal saline. The methanolic extract of the plant of cordial subcordata very significantly ($p < 0.001$) reduced the paw edema in carrageenan treated rats. The effect was maximum at 3 hr after carrageenan injection. The significant suppression of inflammation during the whole experimental period indicates the long duration action of the methanolic extract of the plant.

Keywords: Perindopril, Synthetic polymers and sustained release tablets.

ARTICLE INFO

CONTENTS

1. Introduction	309
2. Materials and Methods	309
3. Results and Discussion	310
4. Conclusion	311
5. Acknowledgement.	311
6. References	311

Article History: Received 29 April 2015, Accepted 18 June 2015, Available Online 15 December 2015

*Corresponding Author

C. Madhavi Latha
Department of Pharmacology,
Jagan's College of Pharmacy,
Nellore, Andhra Pradesh, India.
Manuscript ID: IJPNM2719



PAPER-QR CODE

Citation: C. Madhavi Latha. Pharmacological Evaluation of Anti-Inflammatory activity of methanolic extract of cordial *subcordata.lam*. *Int. J. Pharm. Natural Med.*, 2015, 3(2): 308-312.

Copyright© 2015 C. Madhavi Latha, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

1. Introduction

Inflammation is caused by infection. Although infection is caused by a microorganism, inflammation is one of the responses of the organism to the pathogen. However, inflammation is a stereotyped response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen. Without inflammation, wounds and infections would never heal. Similarly, progressive destruction of the tissue would compromise the survival of the organism. However, chronic inflammation can also lead to a host of diseases, such as hay fever, periodontitis, atherosclerosis, rheumatoid arthritis and even cancer (e.g., gallbladder carcinoma). It is for that reason that inflammation is normally closely regulated by the body. Inflammation is a complex reaction in the vascularised connective tissue that occurs due to exogenous and endogenous stimuli. It is a protective response and this is a part of the host defence, but when the response become too severe, it may be far worse than the disease state, which is counteracted, and in extreme cases, it may be fatal. In the inflammatory process, blood vessels react and leak out leukocytes and other cells into vascular tissue. The cells involved in the inflammatory response can be divided into two categories according to their location. Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.

2. Materials and method

Albino rats of Wister strain weighing 120-200g from the department of pharmacy, JCP were used for the experiment.

Plant material and extracts:

The whole plant of *Cordia subcordata Lam.* will be collected from Tirumala hills and was authenticated by the department of botany, the authenticated plant material where shade dried and powdered coarsely. It will be passed through the 40 mesh sieve. The coarsely powdered drug were extracted separately in Soxhlet apparatus in sufficient volume of redistilled water and methanol at 48-50°C for 24 hrs. The filtrate were collected and evaporated to dryness at 45°C on rotary evaporator. The solid masses were collected carefully and weighed. Their yield were calculated and then stored in sealed glass bottles at 5°C for further experimental work.

Methodology:

Carrageenan-Induced Paw Edema in Rats:

This model is based on the principle of release of various inflammatory mediators by carrageenan. One hour after dosing, the rats will be challenged by a subcutaneous injection of 0.1ml of 1% solution of carrageenan into the

sub-plantar side of the left hind paw. The paw volume will be measured again at 1, 2, 3, 4 & 5 hours after challenge. The increase in paw volume will be calculated as percentage compared with the basal volume. The difference of average values between treated animals and control group will be calculated for each time interval and evaluated statistically.

Cotton Pellet-Induced Granuloma in Rats:

This model is based on the foreign body granuloma which is provoked in rats by subcutaneous implantation of pellets of compressed cotton. One hour after the first dosing, the animals will be anesthetized with anesthetic ether and 20 mg of the sterile cotton pellet will be inserted one in each axilla and groin of rats by making small subcutaneous incision. The incisions will be sutured by sterile catgut. The animals will be sacrificed by excess anesthesia on the 8th day and cotton pellets will be removed surgically. Pellets will be separated from extraneous tissue and dried at 60°C until weight become constant. The net dry weight, i.e. after subtracting the initial weight of the cotton pellet will be determined. The average weight of the pellet of the control group as well as of the test groups will be calculated. The percent change of the granuloma weight relatively with vehicle control will be determined and statistically evaluated. The percentage inhibition increase in the weight of the cotton pellet will be calculated.

Glass Rod induced Granuloma:

Glass rods with a diameter of 6 mm are cut to a length 40 mm and the ends rounded off by flame melting. They will be sterilized before implantation by boiling in water. Albino wistar rats with an initial weight 150g will be anaesthetized with ether, the back skin will be shaved and disinfected. From an incision in the caudal region a subcutaneous tunnel will be formed in cranial direction with a closed blunted forceps. One glass rod will be introduced into this tunnel finally lying on the back of the animal. Treatment with drugs will be either during the 7 days. At the end the animals will be sacrificed and extracted the granuloma sac inverted forming a plain piece of pure connective tissue. Wet weight of the granuloma tissue will be recorded. The specimens will be kept in a humid chamber until further analysis. Finally, the granuloma tissue will be dried and the dry weight is recorded.

In-vitro anti inflammatory activity:

Membrane Stabilization Test: The anti-inflammatory activity of various extracts will be assessed by in vitro HRBC membrane stabilization method. Blood will be collected from healthy volunteers. The collected blood will be mixed with equal volume of Alsever's solution (Dextrose 2%, Sodium citrate 0.8%, Citric acid 0.05%, Sodium chloride 0.42% and Distilled water 100 ml) and will be centrifuged with isosaline. To 1 ml of HRBC suspension equal volume of test drug in three different concentrations will be added. All the assay mixtures were incubated at 37°C for 30 minutes and centrifuged. The haemoglobin content in the supernatant solution will be estimated by using spectrophotometer at 560nm. The percentage of haemolysis will be calculated by using the following formula,

The percentage of haemolysis = OD of test/ OD of Control X 100.

The percentage of HRBC membrane stabilization or protection was calculated by using the following formula,

The percentage of haemolysis = 100 - OD of test/ OD of Control X 100

Inhibition of albumin denaturation: The reaction mixture will be consisting of test extracts and 1% aqueous solution of bovine albumin fraction. P^H of the reaction mixture was adjusted using small amount at 37⁰C HCL. The test extracts will be incubated at 37⁰C for 20 min and then heated to 51⁰C for 20 min after cooling the samples the turbidity will be measured spectrophotometrically at 660nm. The experiment will be performed in triplicate percent inhibition of protein denaturation was calculated as follows.

3. Results and Discussion

Carrageenan- induced paw oedema in rats:

During the search for anti-inflammatory efficacy of selected extracts of the plant using Carrageenan induced Rat paw oedema method, it was quite evident that, a gradual increase in paw volume was observed after carrageenan administration and which reached maximum at 3hour and then declined. The standard drug Indomethacin at a dose level of 4mg/kg body weight showed highly significant activity (P<0.0001) as compared to control group at 1, 2, 3,4 and 5 hours. Methanolic extract at a dose of 200mg/kg showed highly significant anti-inflammatory activity (P<0.0001) as compared to control group at 3, 4 and 5 hours respectively. The methanol extract at 100mg/kg was found to have significant activity (P<0.001) at 3 hour. There were no significant differences (P<0.05) in paw edema volume when standard was observed along with methanolic extract 200mg/kg dose level (Table 1 & 2).

Cotton pellet induced granuloma inflammation:

The extract has been found to reduce the weight of cotton pellet granuloma in a dose dependent manner (Table:3 in the cotton pellet induced model of inflammation in rats. The reduction in the weight of cotton pellet granuloma with different doses of extract 200, 400 and 600 mg/kg was found 17, 26 and 51% respectively, (Fig. 1). However, the decrease in inflammation by *C.subcordata* 600 mg/kg was comparable to diclofenac sodium, which reduced the weight of cotton pellet granuloma by 64% (Figure:1). In pharmacological studies, number of medicinal plants demonstrated antinociceptive and ant inflammatory properties, which helped into the management of inflammatory diseases, especially, rheumatism through inhibition of synthesis of cellular prostanes The cotton pellet-induced granuloma is widely used to assess the transudative and proliferative components of chronic inflammation. The weight of the wet cotton pellets correlates with transude material and the weight of dry pellet correlates with the amount of granulomatous tissue. In the present study, administration of *C.subcordata* extract has been observed to inhibit the weight of wet cotton pellet in a dose dependent manner and the higher dose of *C.subcordata* exhibited inhibition of inflammation very close to the inhibitory effect of diclofenac sodium. It is well

known fact that diclofenac sodium act by inhibiting the prostaglandins synthesis at the late phases of inflammation. This effect may be due to the cellular migration to injured sites and accumulation of collagen, an important mucopolysaccharide Decreasing granuloma tissue, prevention of occurring of the collagen fiber and suppression of mucopolysaccharids are indicators of the anti proliferative effect by NSAIDs. In preview of this, results of present study demonstrate that ethanol root extract of *C.subcordata* has potential to inhibit sub-acute inflammation by interruption of the arachidonic acid metabolism. Present study finding supports the traditional claims and provides a scientific basis for anti-inflammatory effect of *C.subcordata* in inflammatory diseases.

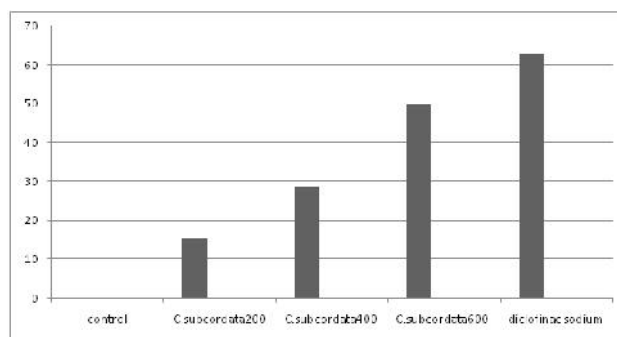


Figure 1: Percentage inhibition in mean weight of cotton pellet in different groups

In-vitro anti inflammatory activity:

Membrane stabilization test:

Stabilization of RBCs membrane was studied for further establishes the mechanism of anti-inflammatory action of different methanolic extract of *cordia subcordata*. All the extracts were effectively inhibiting the heat induced hemolysis. These results provide evidence for membrane stabilization as an additional mechanism of their anti inflammatory effect. This effect may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. The extracts inhibited the heat induced hemolysis of RBCs to varying degree (Table 1). The maximum inhibitions 78.11% from whole plant extract the aspirin standard drug standard drug showed the maximum inhibition 85.92%.

Table 1: Shows in-vitro anti-inflammatory activity of *C.subcordata*

Test sample	ms
Methanolic extract	78.11±0.03
Asprin 200 µg/ml	85.92±0.03

Inhibition of albumin denaturation:

Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti inflammation activity, ability of extract protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation (Table 0).Maximum inhibition 87.14% was observed from whole plant extract of *C.subcordata* .Aspirin, a standard anti-inflammation drug showed the maximum inhibition 75.89% at the concentration of 200 µg/ml.

Table 2: Effect of methanol extracts of *C.subcordata* on albumin denaturation

Test sample	Albumin denaturation
Methanolic extract of <i>C.subcordata</i>	87.14±0.006
Aspirin (200 µg/ml)	75.89±0.006

Table3: Determination of paw volume of rats at different time for *c.subcordata* whole plant extracts.

Groups	Initial paw volume	Paw volume at different time interval (in ml)				
		1hr	2hr	3hr	4hr	5hr
Control (1%DMSO)	1.20±0.175	1.40±0.122	1.53±0.109	1.75±0.370	1.98±0.10	2.01±0.185
Indomethacin (4mg/kg)	1.25±0.300	1.39±0.100	1.49±0.155	1.51±0.110	1.65±0.125	1.62±0.145
CSME (50 mg/kg)	1.25±0.105	1.46±0.096	1.60±0.144	1.81±0.106	2.01±0.206	2.02±0.072
CSME (100 mg/kg)	1.27±0.117	1.49±0.133	1.59±0.079	1.79±0.060	1.94±0.015	1.89±0.015
CSME (200 mg/kg)	1.25±0.106	1.45±0.115	1.58±0.045	1.63±0.045	1.86±0.146	1.71±0.076

CSME = *C.subcordata* methanol extract, All values are expressed in Mean ± SEM, n=6.

All values are expressed in Mean ± SEM, n=6; †p< 0.0001, ‡p< 0.001 and _p< 0.05 compare with control and ‘#’ - Indicates there is no significant difference between standard and test drug at P< 0.05 significant level.

Table 4: Data of % Inhibition of rat paw oedema by *c.subcordata* whole plant extract

Groups	% Inhibition of rat paw oedema at different time				
	1hr	2hr	3hr	4hr	5hr
Indomethacin (4mg/kg)	46.42	43.90	46.26	47.67	50.63
CSME (50 mg/kg)	5.11	6.55	8.15	10.11	12.34
CSME (100 mg/kg)	10.71	12.19	19.40	20.93	24.05
CSME (200 mg/kg)	21.42	29.26	32.83	31.39	35.42

CSME = *C.subcordata* methanol extract, ‘-’ Indicates no inhibition.

Table 5: Changes in mean weight of cotton pellet in different groups.

Treatment	Dose (mg/kg)	Weight of dry cotton pellet (mg)	% Inhibition
Control	-	120.83 ± 2.88	-
<i>C.subcordata</i>	200	95.91±2.90*	15.31
<i>C.subcordata</i>	400	88.55±2.90*	28.79
<i>C.subcordata</i>	600	56.33±2.82*	49.89
Diclofenac sodium	5	43.66±3.31*	62.86

Data represents mean ± SEM of 6 rats. *p < 0.01 vs. control

4. Conclusion

From the above findings it can be concluded that the methanolic extract of cordial subcordata possesses anti-inflammatory activity. The presence of alkaloids, glycosides, phenolic compounds, flavonoids in the methanolic extract of the plant under study may be responsible for these activities.

5. Acknowledgement

We express our sincere thanks to the Management and Madhusudhan Reddy, Honorable Chairman, Jagan's

College of Pharmacy, jangalakandriga, Nellore, for providing necessary facilities and financial support for this study.

6. References

1. Ararun k B.carlor, Anti inflammatory activity of Triterpene lactones from lactuca sativa, Phytopharmacology, Infore sighty publishing, **2010**, 1(1): 1-6.
2. Bani, S., Kaul, A., Jaggi, B. S., Suri, K. A., Suri, O.P. and Sharma, O. P. Anti-inflammatory activity

- of hydrosoluble fraction of *Euphorbia royleana* latex. *Fitoterapia*. **2000**, 71: 655-662.
3. Ballabeni, V.; Tognolini, M.; Giorgio, C.; Bertoni, S.; Bruni, R.; Barocelli, E. *Ocotea quixos* Lam. Essential oil: in vivo and in vitro investigation on its anti-inflammatory properties. *Fitoterapia*. **2010**, 81, 289-295.
 4. Cashin, C. H., Dawson, W., Kitchen, E. A. The pharmacology of benoxaprofen (2,4-chlorophenylmethyl-5-benzoxazole acetic acid) LRC.L3694, a new compound with anti-inflammatory activity apparently unrelated to inhibition of prostaglandin synthase. *J.Pharm.pharmacol.* **1979**, 29: 330-336.
 5. Damas J, Liegeois JF. The inflammatory reaction induced by Formalin in the rat paw. *Naunyn-Schneidebergs Arch Pharmacol.* **1999**, 359: 200.
 6. Fang SC, Hsu CL Yen GC. Anti-inflammatory effects of phenolic compounds isolated from the fruits of *artocarpus heterophyllus*. *JAgric. Food chem.* **2008**, 5(6): 63-68.
 7. Gabino Garrido *et al*, Gonzalez D, Lemus Y, Garcia D, Lodeirol, *Invitro & Invivo* Anti inflammatory activity of *Mangifera indica* L. Extract, *Pharmacol res*, August. **2004**, 50(2): 143-149.
 8. Halliwell, B. Production of superoxide, hydrogen peroxide and hydroxyl radicals by phagocytic cells, a cause of chronic inflammatory disease? *Cell Biol. Int. Rep.* **1992**, 6: 529
 9. Jung H, Kang H, Song YS, Park E, Kim Y and Lim C, Anti-inflammatory, Anti-angiogenic and anti-nociceptive activities of *Sedum sarmentosum* extract, *J Ethanopharmacol.* **2008**, 116(1): 138-143.
 10. Kalaiarasan A, S. Ahmed John. *In-vitro* screening for Anti-inflammatory activity of *Bulbophyllum kaitense*. Rechib. Pseudobulb extract by HRBC method. Eastern peninsular flora in south india, international journal of scientific and research publications. **2012**, 2(7): 1-5.
 11. Lekshmi R. Nath, K.P Manjunath, R.V Savadi, K.Sakki, Anti-inflammatory activity of *Mirabilis jalapa* leaves, *Journal of Basic & Clinical Pharmacy, Academic journals*, **2010**, 1(2): 93-97
 12. Mavar-manga H, Haddad M, Pieters L, Baccelli C, Penge A and Quetin-Leclercq J, Anti-inflammatory compounds from leaves and root bark of *Alchornea cordifolia* (Schumacher & Thonn.) Mull. Arg., *J Ethanopharmacol*, **2008**, 115(1): 25-29.
 13. Park EK, Ryu MH, Kim YI, Lee YA, Lee SH, Woo DH, Hong SJ, Han JS, Yoo MC, Yang HI and Kim KS, Anti-inflammatory effect of an ethanolic extract from *Clematis mandshurica* Rupr., *J Ethanopharmacol.* **2006**, 108(1): 142-147.
 14. Periyayagam, K. Nirmala Devi, In Vitro Anti Inflammatory Activity of *Plectranthus Amboinicus* (Lour) Spreng By Hrbc Membrane Stabilization, International Journal of Pharmaceutical Studies and Research. **2010**, 1(1): 26-29.
 15. Rao YK, Fang Shilua, Tzeng, Yew-Min. Anti-inflammatory activities of flavonoids and terpenes isolated from *Bauhinia variegata*. *phytotherapy Res.* **2008**, 22: 957-62.