

IN-VIVO SCREENING OF ANTI-INFLAMMATORY ACTIVITY IN METHANOLIC EXTRACT OF *CORBICHONIA DECUMBENS* (FORSK.) USING VARIOUS ANIMAL MODELS OF PAW OEDEMA

UMA.G* BALASUBRAMANIAM. V AND JAGATHES KUMAR. S

PG and Research Department of Botany, Kongunadu Arts And Science College(Autonomous), Coimbatore-29,Tamil Nadu,India.

*Email: umaphd430@gmail.com

Received: 29 Aug 2013, Revised and Accepted: 7 Oct 2013

ABSTRACT

Aims: The present work was decided to evaluate the *in-vivo* anti-inflammatory activity of crude extracts of *Corbichonia decumbens* in order to characterize the role of this extract in affecting the inflammatory process.

Study Design: Extraction of *c.decumbens* leaf and root evaluation of *invivo* anti inflammatory activity.

Methodology: methanol extracts of *c.decumbens* leaf and root were prepared by maceration technique. The *in-vivo* anti-inflammatory activity of all extracts (200mg/kg b.w) was assessed by Carrageenan and histamine induced paw oedema method.

Results:Leaf methanol extract showed significant ($p<0.001$) response followed by Root methanol extract ($p<0.01$) when compared with standard, Indomethacin (5mg/kg).

Conclusion: The study suggests that the extracts possess enough potential to reduce inflammation by *in-vivo* and directs the importance of further research and development of novel anti-inflammatory agents.

Keywords: Anti-inflammatory, Carrageenan, Histamine, Paw oedema, *Corbichonia decumbens*

INTRODUCTION

Medicinal plant is defined as any substance with one or more of its organ containing properties that can be used for therapeutic purposes or which can be used as precursors for the synthesis of various drugs [1]. Plants have contributed lot of medicinal compounds being used today to treat diseases like cancer, hormonal imbalances, jaundice, diabetes, inflammation etc. plants are very commonly available in abundance especially in the tropics [2]. They are the vital sources of wide variety of chemicals from which novel anti-inflammatory agents can be discovered. This attracted the attention of several botanists that lead to an array of reports on ethnomedicine [3]. At present nearly 30% or more of the modern pharmacological drugs are derived directly or indirectly from plants and in homeopathic or ayurvedic medicines, medicinal plants, their parts and extracts dominate the scenes[4].

Inflammation is a complex biological response of vascular tissues against aggressive agents such as pathogens, irritants, or damaged cells. It can be classified as either acute or chronic, and involves a cascade of biochemical events comprising the local vascular system, the immune system, and different cell types found in the injured tissue. Acute inflammation is the initial response and is characterized by the increased movement of plasma and innate immune system cells, such as neutrophils and macrophages, from the blood into the injured tissues. Chronic inflammation concerns a progressive change in the type of cells present at the site of the inflammatory reaction and is characterized by simultaneous destruction and healing of the injured tissue [5].

Mulloginaceae family comprises about 100 species, and was previously included in the larger family Aizoaceae. They are commonly known as stone plants or carpet weeds. *Corbichonia decumbens* (Family:Mulloginaceae) is a prostrate, glabrous, succulent and annual weed found throughout India, is cultivated and waste land. Distribution of this plant is Africa, W. Asia, India and W. Pakistan. It is found in rocky or sandy places, in dry hot areas up to 1000 m alt. sml.This plant is used in kidney stone problems.It is used as a tonic and also used in gonorrhoea [6].

MATERIALS AND METHODS

Plant Material

Plant material of *Carbichonia decumbens*. was collected from Vijayamangalam, Erode district, Tamilnadu, during the month of

December 2012. The plant specimens was identified with Gambles Flora of the Presidency of Madras and the identity is confirmed with the herbarium specimen deposited in Kongunadu Arts and Science college herbarium, Coimbatore

Preparation of extract

The leaf and root of *Corbichonia decumbens*. Were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve no. 30 and stored in an airtight container for further use.

Extraction Procedure

The dried powders of leaf and root of *C.decumbens* were defatted with methanol (60-80°C) in a Soxhlet Apparatus by continuous hot-percolation. The solvent was removed by distillation under low pressure and evaporation. The resulting semisolid mass was vacuum dried by using rotary flash evaporator. The resultant dried extracts were used for further study.

Procurement of experimental

Animals

Swiss albino mice (20-25 g) and Wister albino rats (150-200 g) of either sex and of approximate same age are used in the present studies were procured from listed suppliers of animal breeding center veterinary college trissur, kerala India. The animals were fed with standard pellet diet and water ad libitum. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The animals were fasted for at least 12 hours before the onset of each activity. The experimental protocols were approved by **Institutional Animal Ethics Committee (659/02/a/CPCSEA)** after scrutinization. The animals received the drug treatments by oral gavage tube.

Oral Acute Toxicity Study

The LD50 was determined using the graphical method [7] in mice. Briefly, geometric doses of the extract (100–200mg/kg) were administered i.p. to 4 groups of mice each consisting of six animals. Control group received normal saline (5 mL/kg i.p.). Signs of toxicity and mortality within 24-72 h were noted. Confirmatory test was

carried out and the LD50 was calculated from the graph of percent mortality against profit log dose of the extract. The lethal dose (LD 50) of the methanolic extract of dried leaf and root of *C. decumbens* were determined by OECD guideline [8]. The LD50 of methanolic extract was found to be 200 mg/kg therefore the LD50 value is 200mg/kg.

Determination of anti-inflammatory activity

Carrageenan Induced Hind Paw Oedema

The anti-inflammatory activity of the extract was determined using carrageenan induced rat paw oedema assay [9]. Wister Albino rats of either sex weighing 150-200 grams were divided into five groups of six animals each. The dosage of the drugs administered to the different groups was as follows. Group I - Control (normal saline 0.5 ml/kg), Group - II Negative control (Carrageenan) Group III and IV Leaf and Root extract of *C. decumbens* 200 mg/kg, Group V - Indomethacin (5mg/kg). All the drugs were administered orally. Indomethacin served as the reference standard anti-inflammatory drug. After one hour of the administration of the drugs, 0.1 ml of 1% W/V carrageenan solution in normal saline was injected into the sub plantar tissue of the left hind paw. The paw volume of the rats were measured in the digital plethysmograph (Ugo basile, Italy), at the end of 0 min., 1 hrs, 2 hrs, 3 hrs, 4 hrs, 5 hrs. The percentage increase in paw edema of the treated groups was compared with that of the control and the inhibitory effect of drugs was studied. The relative potency of the drugs under investigation was calculated based upon the percentage inhibition of the inflammation.

Histamine Induced Hind Paw Oedema

The anti-inflammatory activity of the extract was determined using histamine induced rat paw oedema assay [9]. Wister Albino rats of either sex weighing 150-200 grams were divided into five groups of six animals each. The dosage of the drugs administered to the different groups was as follows. Group I - Control (normal saline 0.5 ml/kg), Group - II Negative control (histamine) Group III and IV Leaf and Root extract of *C. decumbens* 200 mg/kg, Group V - Indomethacin (5 mg/kg,). All the drugs were administered orally. Indomethacin served as the reference standard anti-inflammatory drug. After one hour of the administration of the drugs, (histamine 1mg/ml) histamine solution in normal saline was injected into the sub plantar tissue of the left hind paw. The paw volume of the rats were measured in the digital plethysmograph (Ugo basile, Italy), at the end of 0 min., 1 hrs, 2 hrs, 3 hrs, 4 hrs, 5 hrs. Mean increase in Paw volume and percentage of anti-inflammatory activity were calculated. The results were statistically analysed by analysis of variance.

Statistical analysis

The values were represented as mean \pm SEM. and the data obtained from this study was subjected to one-way analysis of variance

(ANOVA) followed dunnet's test. The values of *** $p < 0.001$, * $p < 0.01$, ** $p < 0.05$ were considered to indicate the significant levels.

RESULT AND DISCUSSION

In recent years, the search for phytochemicals possessing anti-inflammatory properties has been on the rise due to their potential use in the therapy of various chronic and infectious diseases. Epidemiology and experimental studies have implicated oxidative cellular damage arising from an imbalance between free radical generating and scavenging systems as the primary cause of cardiovascular, diseases, cancer, aging etc [10].

The present study establishes the anti-inflammatory activity of *C. decumbens* methanol extract of leaves and root in different experimental animal models. Pain and inflammation are associated with the pathophysiology of various clinical conditions such as arthritis, cancer and vascular diseases. Inflammatory reactions are not only the response of living tissues to injury and infection, but also are relevant to disease developments, such as asthma, multiple sclerosis, colitis, inflammatory bowel disease and atherosclerosis. Carrageenan-induced oedema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic.

Oral Acute Toxicity Study

In this assay, neither deaths nor symptoms associated with toxicity such as convulsion, diarrhoea or increased diuresis occurred during the 72 hour observation period. These results indicate the effectiveness and relative safety of all extracts for the treatment of conditions associated with inflammation.

Carrageenan induced paw Oedema

The anti-inflammatory activity of *C. decumbens* against carrageenan induced paw oedema has been shown in Table 1 and the results were comparable to that of reference drug indomethacin. The methanol extract of *C. decumbens* showed maximum inhibition time (3.95 \pm 0.09 and 3.53 \pm 0.13 in min) at leaf and root extract dose of 200mg/kg body wt. respectively after 3 hrs of the extract treatment against carrageenan induced paw oedema .

Carrageenan-induced oedema falls in the category of acute inflammation, which involves the synthesis or release of inflammatory mediators at the injured site which further cause pain and fever [11]. The early phase (1 - 2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins. The methanol extract and diclofenac sodium showed inhibitions at both early and late phase. The maximum inhibition of methanol extract was at 4th and 5th hr.

Table 1: Anti-inflammatory activity of methanolic extract of Leaf and Root of *C. decumbens* by Carrageenan induced Paw Oedema

Parameters		Carrageenan induced paw oedema in rats in hours						
Group	Treatment	Dose mg/kg	0 min	1hr	2hr	3hr	4hr	5hr
Group-I	Control	-	4.13 \pm 0.14	4.08 \pm 0.09	3.88 \pm 0.09	4.15 \pm 0.1	4.15 \pm 0.1	3.95 \pm 0.07
Group-II	Negative control (Carrageenan)	1	***2.88 \pm 0.07	***3.28 \pm 0.06	3.43 \pm 0.07	**3.53 \pm 0.07	***3.41 \pm 0.11	3.56 \pm 0.23
Group-III	Positive control (Indomethacin)	5	3.96 \pm 0.06	3.8 \pm 0.08	3.5 \pm 0.09	3.75 \pm 0.11	3.91 \pm 0.06	3.73 \pm 0.1
Group-IV	Leaf extract	200	*3.76 \pm 0.1	3.8 \pm 0.1	3.68 \pm 0.11	3.95 \pm 0.09	3.83 \pm 0.05	3.63 \pm 0.07
Group-V	Root extract	200	***3.3 \pm 0.07	*3.6 \pm 0.08	**3.3 \pm 0.09	**3.53 \pm 0.13	***3.35 \pm 0.07	**3.25 \pm 0.09

Notes: Values expressed as mean \pm SEM, n=6 in each group * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with control.

Carrageenan induced oedema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. Folkloric treatment of inflammation of various etiologies, using medicinal plants, is well known to masters of the art of traditional medicine practice [12]. The development of

carrageenan induced inflammation is a biphasic event, the first phase occurs within an hour of injection is attributed to the release of histamine, 5-HT and kinins, while the second phase which can be measured around 3- 4 hr time is related to the release of prostaglandins [13].

Histamine induced paw Oedema

Histamine induced paw Oedema, the methanol extract produced 49.85 and 53.02% of inhibition (figure-1) at the dose of 200mg/kg body wt., respectively whereas the reference drug indomethacin produced 71.22% of inhibition. Histamine induced paw edema is said to occur in earlier stage of the vascular reaction in the chemically induced inflammation. In this, swelling occurs primarily

due to action of histamine. Generally histamine is released following the mast cell degranulation by number of inflammatory mediators including substances P interleukin-1. This is likely to evoke the release of neuropeptide as well as release of prostaglandins and monohydroxy eicosatetraenoic-acid from endothelial cell leading to hyperalgesia and other pro inflammatory effects. Methanol extract and diclofenac sodium showed significant inhibition against histamine induced oedema[13].

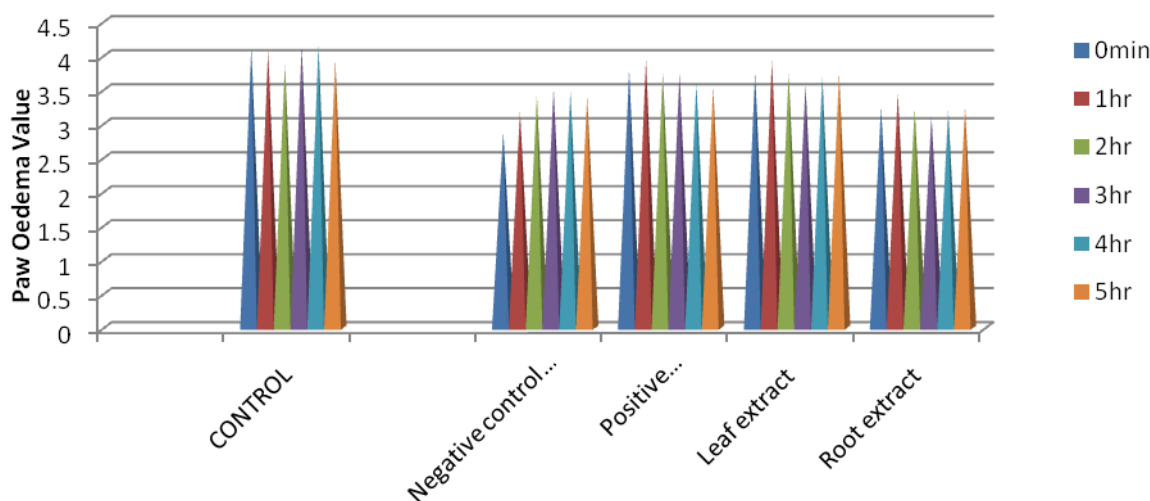


Fig. 1: Anti-inflammatory activity of methanolic extract of Leaf and Root of *C.decumbens* by Histamine induced Paw Oedema

CONCLUSION

C.decumbens methanol extract of leaves and root showed significant anti-inflammatory effects in various animal models. Our results revealed that administration of methanol extract inhibited the oedema starting from the first hour and during all phases of inflammation, which is probably inhibition of different aspects and chemical mediators of inflammation.

These experimental results have established a pharmacological evidence for the folklore claim of the drug to be used as an anti-inflammatory agent. These studies also provide new scientific information about the anti-inflammatory activities of leaf and root extract of *C.decumbens*. These activities may be attributed to the various phytochemical constituents present in the extracts.

REFERENCE

- Sofowora A: Medicinal Plants and traditional medicines in Africa. 2nd Edn. Karthala Ibadan Nigeria. 1993.
- Padmanabhan P, Jangle SN: Evaluation of *in-vitro* anti-inflammatory activity of herbal preparation, a combination of four medicinal plants. *Int J Basic Appl Med Sci*; 2012; 2(1):109-16.
- Anilkumar M: Ethnomedicinal plants as anti-inflammatory and analgesic agents, *Ethnomedicine: A Source of Complementary Therapeutics*; 2010; 267-293.
- Mohammad M, Hassan, Shahneaz A, Khan, Amir H, Shaikat M.d, Emran Hossain M.d, Ahasanul Hoque, Md Hasmad Ullah, Saiful Islam: Analgesic and anti-inflammatory effects of ethanol extracted leaves of selected medicinal plants in animal model. doi: 2013;10.5455/vetworld.68-71.
- Ferrero-Miliani L, Nielsen, O.H, Andersen, P.S., Girardin, S.E: Chronic inflammation: Importance of NOD2 and NALP3 in interleukin-1beta generation. *Clin. Exp. Immunol*; 2007;147, 227-235.
- Katewa S.S. and Galav P.K: Traditional herbal medicines from shekhawati region Rajasthan. *Indian Journal of Traditional Knowledge*; 2005; Vol.4(3), pp 237-245.
- Litchfield, J.T.; Wilcoxon, F. A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther*; 1949; 96, 99-133.
- OECD, 2001. (Organization for Economic co-operation and Development). OECD guidelines for the testing of chemicals /Section 4: Health Effects Test No. 423; Acute oral Toxicity – Acute Toxic Class method. OECD. Paris. 2002
- Winter C.A, Risley E.A, Nuss G.W: Carrageenin-induced oedema in hind paws of the rat as an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med*; 1962; 111:544-547.
- Halliwell B.: Antioxidants in human health and disease. *Annu Rev Nutr*; 1996;6:33-50
- Brooks R.R, Carpenter J.F, Jones S.M, Ziegler, T.C, Pong S.F. Canine Carrageenin-induced acute paw inflammation model and its response to nonsteroidal anti-inflammatory drugs. *J Pharmacol. Methods*; 1991; 25,275-283.
- Jasem Estakhr, Fereshteh Javadian, Zohre Ganjali, Mehdi Dehghani and Abbas Heidari; Study on the Anti-Inflammatory Effects of Ethanolic Extract of *Cynanchum acutum*. *Current Research Journal of Biological Sciences*; 2012; 4(5): 630-632.
- Vane J.R. and Botting B.M: Overview, the mechanism of action of anti-inflammatory drugs. In: Clinical significance and potential of selective COX-2 inhibitors (Ed: Vane Jr Botting). London: William Harvey Press; 1998; pp. 1-18.