

ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF BARK OF *FICUS RACEMOSA* L. AND ROOT OF *CISSAMPELOS PAREIRA* L. VAR. *HIRSUTA* (DC) FORMAN

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ABSTRACT

The present work was designed to assess the anti-inflammatory activity of the methanolic extracts of the bark of *Ficus racemosa* and root of *Cissampelos pareira*. *Ficus racemosa* an annual tree belongs to family Moraceae and *Cissampelos pareira* a woody, climbing rainforest vine belongs to family Minispermaceae were investigated at the dose of 200 and 400mg/kg using Carrageenan induced hind paw edema model for their anti-inflammatory activity. Both the methanolic extracts of bark of *Ficus racemosa* and root of *Cissampelos pareira* were compared to reference drug Diclofenac. The extracts at different doses showed good anti-inflammatory activity, while studying with Carrageenan induced hind paw edema model. Anti-inflammatory activity effects were monitored in different time intervals. The edema was expressed as an increase in the volume of paw. The results from the present study indicate the efficacy of the extracts as therapeutic agents in acute as well as chronic inflammatory conditions. These useful effects can be resulted from inhibitory interaction of plant extract with cyclooxygenase-2 and subsequently reduction in prostaglandins production.

Keywords: Anti-inflammatory, *Ficus racemosa*, *Cissampelos pareira*, Diclofenac, Carrageenan.

INTRODUCTION

The search for new pharmacologically active agents obtained by screening natural sources and plant extracts has led to the discovery of many clinically useful drugs that play a major role in the treatment of human diseases. In India, a small proportion of wild plants have been investigated both phytochemically and pharmacologically. As medicinal plants have been inducted as a common source of alternative remedy for treating human diseases because they contain numerous bio-active constituents of therapeutic values¹. In the present study two plants have been chosen for their effective anti-inflammatory activity which was being traditionally used by untrained civilians of Malkangiri district of Odisha. Thus, in the present study methanolic extracts of bark of *Ficus racemosa* and root extract of *Cissampelos pareira* have been

investigated for anti-inflammatory activity and interesting results have been obtained.

Phytography analysis of the Herbals

1. *Ficus racemosa* Linn

Morphological profile

Ficus racemosa Linn. Locally known as 'Dimiri' (Odia) belongs to family Moraceae. It is a tree, highly cosmopolitan in occurrence, grows all over India especially in habitats like forests and hills. The tree is of medium height up to 10-16 meters; bark reddish grey, often cracked at outer surface with easily removable translucent flakes, greyish to rusty brown; uniformly hard and non-brittle. Many ancient scriptures of Ayurveda like Susruta Samhita described the properties of its bark as astringent, promotes healing process of fractured wounds by formation of callus (*bhagna sandhaniya*), alleviates hematemeses

(*Rakta pitta*), burning sensation, obesity and useful in vaginal disorders.

Phytochemical profile

Bark of *Ficus racemosa* contain chemicals like two new anthocyanin: leucocyanidin-3-O- β -glucopyranoside, leucopelarogonidin-3-O- α -L-rhamnopyranoside, β -sitosterol unidentified long chain ketone, cerylbehenatelupeol, it's acetate, α -amyrinacetate².

Phytotherapeutic profile

The plant is used both, internally and externally as well, to meet many therapeutic remedies² as:

External use

The latex is applied externally on chronic infected wounds to alleviate oedema, pain promoting the healing process. The decoction of its bark is used as an effective gargle against stomatitis and sore throat. Application of latex alleviates the oedema in adenitis, parotitis, orchitis, traumatic swelling and tooth ache.

Internal use

It incorporates vast range of maladies. The decoction of bark is useful in diarrhoea, dysentery and ulcerative colitis in gastrointestinal tract. In children, the latex is given along with sugar to combat diarrhoea and dysentery. In diabetes, the ripe fruits or decoction from bark is useful as it works well as antidiuretic. In uterine bleeding due to abortion, leucorrhoea and vaginitis, the decoction of its bark is given orally or in form of pessaries/suppository (*basti*) as well. The latex admixed with sugar removes sexual debility in males. The powdered bark works well as an anorexient.

2. *Cissampelos pareira* L. var. *hirsuta*(DC) Forman

Morphological profile

Cissampelos pareira locally known as 'Akanabindi'- Oriya; belongs to family Minispermaceae. Herbal of softly tomentose, herbaceous climbers; petiole to 2.5 cm long; lamina ovate to orbicular; inflorescence dioecious, subtended with many conspicuous bracts imbricate arranged; pistillate inflorescence longer than staminate ones; flowers greenish white.

Phytochemical profile

Cissampelospareira contains a group of phytochemicals called isoquinoline alkaloids³. Out of thirty-eight alkaloids so far discovered; one, called *tetrandrine* is the most well

documented⁴. Protoberberine alkaloids have been found in the roots.

Phytotherapeutic profile

Clinical research over the years has found tetrandrine to have pain-relieving, anti-inflammatory, and fever-reducing properties. Used in menstrual problems (pain, cramps, excessive bleeding, fibroids, and endometriosis) as a female tonic (hormonal balancing, menopausal libido loss, hormonal acne, premenstrual syndrome, childbirth) for heart problems (irregular heartbeat, high blood pressure, heart tonic) as a general antispasmodic and muscle-relaxer (asthma, stomach cramps, muscle pain/strains, irritable bowel syndrome [IBS], diverticulitis) for kidney support (kidney stones, kidney/urinary infections and pain)⁶.

Materials and Methods

Plant materials

Ficus racemosa (barks) and *Cissampelos pareira* (roots) were freshly collected from local habitat of Tanginiguda of Malkangiri district in Odisha, the herbaria, so prepared from both the herbal species were identified, confirmed and duly authenticated by Dr. S. K. Dash, Professor and Head, P.G. Department of Biosciences, CPS, Mohuda, Berhampur (Odisha) and were preserved in the institutional museum of College of Pharmacy (Poly), Pandharpur of Solapur district, Maharashtra for future reference.

Extraction

The plant parts were separately washed, shade dried and extracted with 90% methanol. The extracts so collected air dried at 50⁰ to 60⁰ C; proceeded further for preliminary phytochemical analysis.

Preliminary phyto-chemical screening

These two plants were subjected for its presence of different phytoconstituents like tannins, phytosterols and flavonoids found in both; exerted the physiological effect (Table-1).

Pharmacological screening

Animals

Healthy adult cross-breed albino male rats (150–200 g) divided into five groups were used in the study. The animals were kept in plastic cages (six per cage) under standardized animal house conditions with continuous access to pellet feed and tap water. Every effort was made to minimize animal suffering and to reduce the number of animals used in this study. Carrageenan induced hind paw

oedema model was used in the study and the oedema was expressed as an increase in the volume of paw. Animal study was performed in the division of Pharmacology, B.R.Nahata College of Pharmacy, Mandasaur, with permission from the Institutional Animal Ethical Committee (CPCSEA No- 1019/C/06/CPCSEA & Reg. no.- 009/Ph.D./2012/IAEC/MIP/Mandasaur.)

Acute toxicity study

The acute toxicity test of the extracts was determined according to the OECD guidelines No. 420 (Organization for Economic Co-operation and development). Female Wistar rats (150–180 g) were used for this study. After the sighting study, starting doses of 2000 mg/kg (P.O.) of the test samples were given to various extracts of 5 groups containing 6 rats in each group. Rats were randomly selected for the study and marked to provide individual identifications. Rats were observed immediately after dosing during first 30 minutes, periodically during the first 24 hours, with special attention given during first 4 hours, and daily thereafter for 14 days (OECD guidelines, 2001). During the first four hours rats were tested for following various responses.

Assesment of anti-inflammatory activity Procedure

Carrageenan induced hind paw oedema model was used in the study. Rat right hind paw oedema was induced by subplantar injection of 0.1 ml of 1% (w/v) carrageenan suspension in normal saline. The animals were divided in to five groups (n=5), fasted for 12 hours and deprived of water only during the experiment. The deprivation of water was to ensure uniform hydration and to minimize variability in oedematous response. The extracts of *C. pareira* and *F. racemosa* were suspended in 1% Tween 80 at doses of 200 and 400 mg/kg and administered through oral route. The control groups were treated with 0.2 ml of Tween 80 (negative control) and 100 mg/kg of Diclofenac (positive control). Immediately after injection of the phlogistic agent, readings of oedema volume in the drug treated group (Vt) were obtained for each rat at 0, 30, 60, 120, 180, 240 and 300 min, with the aid of a Plethysmometer⁵ (Fig-1 & 2). The oedema was expressed as an increase in the volume of paw.

RESULTS

The qualitative phytochemical test^{9,10} results of both the methanolic extract of *Cissampelos*

pareira and *Ficus racemosa* are summarized in Table 1.

Evaluation of anti-inflammatory activity of extracts

Both the plants tested for anti-inflammatory activity exhibited the said activity. Dose depended effect was not observed in *C. pareira*, as 200 mg/kg was equally active as 400 mg/kg. *F. racemosa* exhibited dose dependent effect till the end of the study. The effect of higher doses of the plants was as effective as the standard drug Diclofenac (Table-2).

DISCUSSION

Inflammation is a complex physiopathological response to different stimuli. It can be treated and resolved by acting on the different mediators, enzymes, and pathways implicated in the process. This can include influencing the known arachidonate metabolism, inhibiting either certain transcription factors or the production and/or scavenging of the free radicals produced during the process, and by acting on the cells implicated in the process, such as macrophages and lymphocytes. For this reason, the study of the anti-oxidant capacity of plant extracts and their potential effects on pro-inflammatory cells to induce apoptosis could provide useful insight into the mechanisms of action of their anti-inflammatory activity¹¹. To this end, we selected two species, *Ficus racemosa* and *Cissampelos pareira* which are used in folk medicine in the South Odisha region to treat several inflammatory diseases. The results from the present study show that the extract of *Cissampelos pareira* exhibited activities in various degrees against inflammation, pain and fever. By activating the cyclooxygenase, the levels of prostaglandin, especially PGE₂, increases markedly and its production provokes inflammation¹², pain and fever⁷. Therefore, we assume that some active metabolites of the extract in this study could inhibit cyclooxygenase activity. The most widely used primary test to screen anti-inflammatory agent¹³, is to measure the ability of a compound to reduce local oedema induced in rat paw following the injection of irritants such as carrageenan⁸.

Ficus racemosa (barks) and *Cissampelos pareira* (roots) were collected from local habitat after authentication and were washed, shade dried and powdered. The powdered materials of both plant parts were passed through sieve number 60 separately and stored in hygienic conditions. The materials were subjected to extraction (Soxhlet) in

methanol. After complete extraction the solvent was filtered and concentrated on a water bath. The filtrate was dried at 50°C to 60°C. and subjected to preliminary phytochemical analysis. The preliminary phytochemical screening of extracts shows the presence of tannins, phytosterols and flavonoids in both the plant extracts whereas alkaloids and triterpenoids found in *C. pareira* and saponins in *F. racemosa*. Thus the said anti-inflammatory activity may be due to the presence of constituents like tannins, phytosterols and flavonoids as were common to both plants.

The acute toxicity test of the extracts was determined according to the OECD guidelines No. 420 (Organization for Economic Co-operation and development). Female Wistar rats (150–180 g) were used for this study. After the sighting study till the end of 14th day toxic symptoms were not observed in the treated groups. All the animals were found healthy and the extracts were found to be safe up to a dose of 2000 mg/kg.

From Table No. 2, it is observed that the extracts from both the species administered in low (200mg /kg) and/or high (400mg/kg) concentration are exhibiting inflammatory activities. However *Ficus racemosa* is comparatively less effective than *Cissampelos pareira*. It seems *Cissampelos pareira* in low concentration exhibits comparatively better anti-inflammatory than the control. However in

higher concentration (400mg/kg) results the anti-inflammatory effect between 1 hour to 4 hours administration beyond which high concentration is comparatively less effective than low concentration. Similarly high concentration of *F. racemosa* seems to be less effective than that of low concentration.

CONCLUSION

The use of plants and plant preparations has been in existent since prehistory. The World Health Organization (WHO) reported that about 80% of the world's population depend mainly on traditional medicine and the traditional treatment involve mainly the use of plant extracts (WHO, 1993). In the present study, the root extract of *C. pareira* and bark extracts of *F. racemosa* showed promising activity to reduce oedema induced by Carrageenan. The result of this study confirmed that *Ficus racemosa* bark and *Cissampelos pareira* root could be beneficial in the management of inflammations and pains. These activities may be due, in part, to the presence of phyto-chemicals such as tannins, flavonoids, alkaloids and /or terpenoids.

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Table 1: The result of preliminary phytochemical screening of *F. racemosa* and *C. pareira*

Plants Constituents	<i>F. racemosa</i>	<i>C. pariera</i>
Carbohydrates	(--)	(--)
Alkaloids	(--)	(+)
Tannins	(+)	(+)
Flavonoids	(+)	(+)
Saponins	(+)	(--)
Proteins	(--)	(--)
Fats	(--)	(--)
Steroids	(+)	(+)
Triterpenoids	(--)	(+)

(+)Indicate Present and (--) indicate Absent

Table 2: Results of anti-inflammatory activity of extracts

Treatment	0 min.	30 min.	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.
Control	1.30 ± 0.20	2.20 ± 0.25	3.06 ± 0.29	2.90 ± 0.18	2.86 ± 0.09	2.36 ± 0.21	1.90 ± 0.10
Extract-A (200mg/kg)	1.10 ± 0.10	1.16 ± 0.16***	2.50 ± 0.15*	1.94 ± 0.11**	1.90 ± 0.11***	1.34 ± 0.10***	1.24 ± 0.11***
Extract-A (400mg/kg)	1.10 ± 0.10	1.40 ± 0.16**	1.74 ± 0.15**	1.46 ± 0.20***	1.44 ± 0.16***	1.14 ± 0.09***	1.28 ± 0.09**
Extract-B (200mg/kg)	1.26 ± 0.16	1.50 ± 0.15*	2.10 ± 0.27*	1.74 ± 0.21***	1.40 ± 0.10***	1.50 ± 0.15**	1.40 ± 0.10**
Extract-B (400mg/kg)	1.30 ± 0.12	1.24 ± 0.11**	1.86 ± 0.18**	1.60 ± 0.10***	1.76 ± 0.11***	1.50 ± 0.15**	1.34 ± 0.10**
Standard Diclofenac 100mg/kg	1.20 ± 0.18	1.20 ± 0.16***	1.64 ± 0.11**	1.54 ± 0.20***	1.38 ± 0.16***	1.32 ± 0.09***	1.22 ± 0.09**

Each value represents the mean ± S.E.M. (n=5); **P* < 0.05, ** *P* < 0.01, *** *P* < 0.001 vs. control. (Dunnet's test); Extract A –*Cissampelos pareira*, Extract B- *Ficus racemosa*.



Fig. 1: Administration of the extracts through oral route



Fig. 2: Anti-inflammatory Studies using Plethysmometer

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