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Original Article

ANTI-ARTHRITIC AND ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *ORMOCARPUM SENNOIDES*

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ABSTRACT

Objective: The study focuses on evaluating the anti-inflammatory and anti-arthritic activity of ethanolic extract of *Ormocarpum sennoides* (Os), a herb belonging to the family fabaceae. Both *in vitro* and *in vivo* methods were employed to study the anti-inflammatory activity of Os extract.

Methods: The *in vitro* analysis of Os extract was performed by HRBC membrane stabilization and protein denaturation methods. The *in vivo* analysis was done by Formalin-induced paw edema and acetic acid induced writhing test using wistar male albino rats as an animal model.

Results: The *in vitro* analysis revealed the inhibition of hemolysis and protein denaturation in a dose-dependent manner in concentrations ranging from 50-500 µg/ml. The *in vivo* study showed that Os extract reduced the number of paw lickings and abdominal writhing significantly at dosage concentrations of 200 mg/kg body weight and 300 mg/kg body weight compared with standard diclofenac sodium ($p<0.05$).

Conclusion: These results support the application of Os extract in ailing arthritis and treatment of painful inflammatory conditions.

Keywords: Anti-inflammatory activity, Anti-arthritic activity, *Ormocarpum sennoides*, Rheumatoid arthritis

INTRODUCTION

Inflammation is the body's natural response against antigens like parasites, pathogenic microorganisms, toxic chemicals, autoantigens and tissue injury. During cell metabolism, free radicals like reactive oxygen species and reactive nitrogen species are released which eventually involves in signal transduction, receptor activation, and gene expression [1]. The problem arises when excess free radicals are produced leading to many diseases like age-related disorders, neurodegenerative disorders, atherosclerosis, cancer and inflammation [2, 3]. There are two kinds of inflammation (a) acute inflammation where the vascular permeability is increased leading to infiltration and emigration of leukocytes (b) chronic inflammation where infiltration of macrophages, monocytes, neutrophils and mononuclear immune cells occurs. Following infiltration, activation and proliferation of fibroblast with fibrosis occurs.

Inflammation being a clinical condition exhibits four cardinal signs namely redness, swelling, heat and pain. Rheumatoid arthritis (RA) is an autoimmune disorder in which accumulation of autoantigens occurs in the synovial joints leading to inflammation of the joint [4]. There are steroidal and non-steroidal anti-inflammatory drugs available to treat inflammatory conditions. Unfortunately, these drugs exhibit severe side effects like gastric lesions, renal failure, cardiovascular failure [5] and GI tract damage [6, 7].

Traditionally herbal medicines are used for treating various ailments, and now it has become necessary to explore these herbs scientifically due to its low side effects and cost effectiveness. Various herbs are employed for treating rheumatism and inflammation. In this order, *Ormocarpum sennoides* a new herb is introduced in proving its anti-inflammatory and anti-arthritic activity which reflects the novelty of this study. *Ormocarpum sennoides* (Os) a shrub belongs to fabaceae family found growing all over Tamilnadu, and also in northern districts of Eastern Ghats [8-10]. This plant is being known to the villagers as bone-knit or elumboti and used for fracture healing [11]. Traditionally, the leaf powder of Os is consumed orally by mixing with honey or milk and also the leaf paste is applied topically on the fracture site and covered. The phytochemicals of Os leaf extract have shown the presence of phenols and flavonoids which possess excellent antioxidant and osteogenic property [12, 13]. In addition to these properties, this article is keen in bringing out the anti-inflammatory and anti-arthritic property of *Ormocarpum sennoides* as an alternative for NSAIDs with proper scientific evidence. In the present study, ethanolic leaf extract of Os was collected and subjected to *in vitro* and *in vivo* anti-inflammatory and anti-arthritic activity using animal models.

MATERIALS AND METHODS

Preparation of the plant extract

Healthy leaves of *Ormocarpum sennoides* (Os) were collected from pachakuppam hills near Ambur, Tamilnadu and authenticated in CAS Botany, University of Madras. The collected leaves were washed with double distilled water, dried under shade and powdered in an electric blender. The dried powder was extracted using 95% ethanol in a Soxhlet apparatus. Using rotary evaporator the extract was concentrated, stored at 4 °C and the dried crude ethanolic extract was used for further study.

In vitro anti-arthritic activity

In vitro, anti-arthritic activity was performed using protein denaturation method. In this method, 0.45 ml of 5 % bovine serum albumin in distilled water was mixed with 0.05 ml of Os extract (50-250 µg/ml). The pH of the reaction mixture was adjusted to 6.3 using 1N hydrochloric acid. The mixture was incubated at 37 °C for 20 min and further heated at 57 ˚C for 3 min. The mixture was cooled and 2.5 ml of phosphate buffer was added. Control was prepared with 0.05 ml of distilled water instead of plant extract and product control was prepared by adding distilled water instead of bovine serum albumin [14]. The turbidity of the reaction mixture was measured at 600 nm. Protein denaturation inhibition percentage was calculated as follows,

$$\text{Percentage inhibition} = 100 - \frac{(\text{Test OD} - \text{Product Control OD})}{\text{Control OD}} \times 100$$

The control refers to 100% protein denaturation. Diclofenac sodium (250 µg/ml) was taken as a positive control and the results were compared.

***In vitro* anti-inflammatory activity**

In vitro, anti-inflammatory activity of the plant extract was done by Human Red Blood Cell (HRBC) membrane stabilization method. 1 ml of Os extract prepared in normal saline (200 to 1000 µg/ml), 2 ml of hypotonic saline (0.25% NaCl) and 1 ml of 0.15 M phosphate buffer with pH 7.4 were taken in a test tube. To this reaction mixture, 0.5 ml of 10% rat RBC in normal saline was added. Control was prepared in the same way by adding 1 ml of isotonic saline instead of plant extract. Product control was prepared without adding the RBC. The reaction mixture was incubated at 56 for 30 min. The tubes were shown under running tap water and cooled for 20 min. After incubation, the reaction mixture was centrifuged and the supernatant was collected. The absorbance of the supernatant was measured at 560 nm [15]. The membrane stabilization activity was calculated using the formula,

$$\text{Percentage Stabilization}=100-\frac{(\text{Test OD-Product Control OD})}{\text{Control OD}}\text{X } 100$$

100% lysis was shown by the control. Diclofenac sodium (250 µg/ml) was taken as a positive control to compare results.

***In vivo* anti-inflammatory activity**

Adult male wistar albino rats weighing 180-200 g were used for this study. The animals were housed in polypropylene cages at an ambient temperature of 25°C±1°C with a 12:12 h light/dark cycle. Animals were provided with commercial food pellets and water ad libitum. The animals were obtained after the approval from the Institutional ethical committee (IAEC No. 03/008/2014), and the experimental procedures were performed under the guidelines of the CPCSEA. The experiments were conducted in the forenoon between 9 am to 11 am. The extract was dissolved in 5 % tween 80 and administered orally at different doses and Diclofenac sodium was administered 100 mg/kg body weight.

Animals were grouped into 5, each group consisting of 6 animals. Group I controlled (saline treated), Group II was Os extract treated with 100 mg/kg body weight, Group III was Os extract treated with 200 mg/kg body weight, Group IV was Os extract treated with 300 mg/kg body weight, and Group V was positive control treated with diclofenac sodium at 100 mg/kg body weight.

Formalin-induced paw licking in rats

The anti-inflammatory activity in rats using formalin induced paw licking method proposed by Hunskaar and Hole (1977) [16] was done by dividing the animals into five groups mentioned as Control, Positive control and Test group I, Test group II and Test group III, each group comprising of 6 rats. Control group orally received saline (0.9% NaCl), Positive control group orally received Diclofenac sodium (100 mg/kg body weight) 30 min before formalin injection. Test group I, Test group II and Test group III were treated with Os extract orally at the dose of 100, 200 and 300 mg/kg body weight 60 min before formalin injection. 0.2 ml of 3 % formalin was injected into left hind paw on the dorsal surface of all rats. The number of paw licking by the animal on the injected paw was examined in two phases, early (0-5 min) and late (20-30 min). The mean of licking was determined and compared with the control group.

Acetic acid induced writhing test in mice

According to Whittle *et al.* (1964) [17], acetic acid induced writhing test acts as a good diagnostic method to assess the anti-inflammatory activity of the extract in rats. In this study animals were grouped similar to formalin-induced paw licking method in rats and the treatment and drug administration were similar to groups as mentioned in the formalin test. Then 1% acetic acid was administered intra-peritoneally to all the animals. 5 min time was given for animals to absorb the acetic acid and then the number of writhing was counted for 15 min. The percentage inhibition was calculated using the formula,

$$\%\ \text{Inhibition}=\frac{\text{Mean no. of writhing (Control)-Mean no. of writhing (drugs)}}{\text{Mean no. of writhing (Control)}}\text{X } 100$$

Statistical analysis

The results were expressed as mean±SEM of all experiments. The statistical significance was determined by one-way ANOVA using SPSS version 15 and the significance among groups was obtained by Tukey's multicomparison test. A value of $p<0.05$ was considered to indicate a significant difference between groups.

RESULTS AND DISCUSSION

***In vitro* anti-arthritic activity**

An autoimmune disorder is a condition where there is an overproduction of autoantigens due to the denaturation of tissue proteins. Rheumatoid arthritis is highly inflammatory polyarthritis, where the autoantigens lead to joint destruction, deformity and loss of function [18, 19]. *In vivo* anti-arthritic activity of Os extract showed inhibition of denaturation of bovine serum albumin in a dose-dependent manner (table 1). Os extract at 50 µg/ml was able to show 21.22% inhibition against protein denaturation. As the concentration increases the percentage inhibition of protein denaturation also increases. Os extract at 1000 µg/ml was able to inhibit the denaturation with 80.31% which was nearing the percentage inhibition value of diclofenac sodium at 200 µg/ml. This reveals that Os extract prevents denaturation of proteins *in vitro* [20]. It may be attributed to the presence of bioactive compounds namely alkaloids and flavonoids in the extract.

Table 1: Effects of Os extract on *in vitro* anti-arthritic activity

Concentration (µg/ml) n=3	Os extract (% inhibition of protein denaturation) (%)	Diclofenac sodium (% inhibition of protein denaturation) (%)
50	21.22±1.56	
100	33.53±1.34	
200	51.21±1.02	86.71±0.92
300	58.29±1.64	
400	67.71±1.45	
500	69.79±1.22	
1000	80.31±2.03	

Values are expressed as % inhibition of protein denaturation by Os (*Ormocarpum sennoides*) extract. Diclofenac sodium was used as positive control at a concentration of 200 µg/ml.

***In vitro* anti-inflammatory activity**

Os extract was subjected to *in vitro* anti-inflammatory activity in various concentrations by HRBC membrane stabilization [21]. Protective effect of hypotonic saline induced erythrocyte lysis is known to be a very good index of anti-inflammatory activity of any agent. Os extract with concentration ranging from 50-1000 µg/ml showed a dose-dependent increase in percentage inhibition showing membrane stabilizing potential of Os extract and the results were well in comparison with those of diclofenac sodium (200 µg/ml) (table 2).

Os extract at 50 µg/ml was able to inhibit hemolysis by 22.82%. The percentage inhibition of hemolysis by Os extract gradually increased in a dose-dependent manner. At 1000 µg/ml, the extract was inhibiting the hemolysis by 77.26% which was nearing to the

inhibition of hemolysis by diclofenac sodium at 200 µg/ml. The anti-inflammatory activity exhibited by Os extract could be due to the presence of alkaloids and flavonoids in the extract [20]. This was further confirmed in the *in vivo* study.

Table 2: *In vitro* anti-inflammatory activity by HRBC membrane stabilization method

Concentration (µg/ml) (n=3)	Os extract % inhibition of hemolysis (%)	Diclofenac sodium % inhibition of hemolysis (%)
50	22.82±1.02	
100	34.75±1.34	
200	51.20±0.96	80.73±0.84
300	62.34±1.42	
400	66.12±1.32	
500	71.59±1.08	
1000	77.26±2.03	

Values are expressed as % inhibition of hemolysis by Os (*Ormocarpum sennoides*) extract. Diclofenac sodium was used positive control at a concentration of 200 µg/ml.

***In vivo* animal studies**

***In vivo* anti-inflammatory activity**

Formalin test

The anti-inflammatory activity was evaluated by inducing inflammation produced by sub-aponeurotic injection of 0.1 ml of 2 % formaldehyde in the right hind paw of the rat and the number of paw lickings and the behavior of the rats in the early or neurogenic phase (0-5 min) and late or inflammatory phase (10 min from 20-30 min) were observed. Thus, oral administration of Os extract at doses of 200 and 300 mg/kg body weight insignificantly inhibited the early phase whereas showed significant inhibition of late phase which was the anti-inflammatory phase when compared with diclofenac sodium (100 mg/kg body weight).

As the concentration of Os extract significantly inhibited the paw licking ($p<0.05$) compared with diclofenac sodium, it shows that Os extract at doses of 200 and 300 mg/kg body weight possesses the anti-inflammatory activity (table 3).

Table 3: Effects of Os extract on formalin test

Groups n= 6	Early phase (0-10 min)	P value	Late phase (20-30 min)	P value
Control	16.33±0.32	---	14.83±0.20	
Os extract (100 mg/kg)	13.16±0.20	P=0.949	11.5±0.22*	P=0.05
Os extract (200 mg/kg)	12±0.25	P=0.164	9.33±0.22*	P=0.05
Os extract (300 mg/kg)	13±0.15	P=0.607	9.0±0.19*	P=0.05
Diclofenac sodium (100 mg/kg)	8.66±0.16	P=0.001	8.33±0.19***	P=0.001

The number of paw lickings was expressed as mean±SEM. Diclofenac sodium was used as positive control at a concentration of 100 mg/kg. * indicates significance of *Ormocarpum sennoides* (Os) compared to control at ($p<0.05$) and *** indicates high significance compared to control at ($p<0.001$)

Both the formalin test and acetic acid-induced writhing test are used as an index for finding out the anti-inflammatory activity of any drug. The drug which acts on the early phase was said to have a neurogenic effect and the drug which act in the late phase was said to possess anti-inflammatory effect or analgesic effect [22]. The plant extract was administered to the animal 1 h prior to the formalin injection whereas diclofenac sodium was given 30 min before the formalin administration. This difference is due to the lesser concentration of the active compound in the extract which takes extra time to act when compared to diclofenac sodium. Though it takes longer time the active compound was able to treat inflammation without leaving side effects [16, 23]. Due to this property, herbal extract application has gained momentum in various treatments.

The physical examination (fig.1) of the hind paw reveals that positive control rats treated with diclofenac sodium showed a very minimal or nil erythema in the injected area whereas negative control rats treated with saline showed high inflammation with increased erythema. The rats administered with Os extract showed a good anti-inflammatory activity with reduced erythematous areas on the paw. Thus, Os extract at a concentration of 200 and 300 mg/kg body weight showed a similar activity of diclofenac at 100 mg/kg body weight.

Fig. 1: Images of formalin induced anti-inflammatory activity of Os extract

Acetic acid induced writhing

The anti-inflammatory activity of Os extract was evaluated by writhing induced by acetic acid in rats. Acetic acid solution (1% w/v) was administered intraperitoneal to each animal in the group. After an interval of 5 min, the number of writhes, a response consisting of contraction of the abdominal wall, pelvic rotation followed by hind limb extension, was counted for a period of 30 min after the acetic acid injection. The Os extract had significantly reduced the number of abdominal constrictions or writhings (table 4). The Os extract at the doses of 200 and 300 mg/kg body weight significantly decreased the number of writhings induced by acetic acid which indirectly indicates the control of inflammation performed by Os extract. The activity of the extract at a higher dose (300 mg/kg) was comparable to that of standard diclofenac sodium (100 mg/kg body weight).

Table 4: Effect of Os extract on acetic acid-induced abdominal writhing test

Acetic acid-induced abdominal writhing test	Control	Os extract 100 mg/kg body weight	Os extract 200 mg/kg body weight	Os extract 300 mg/kg body weight	Diclofenac sodium 100 mg/kg body weight
No of abdominal writhings	14.83±0.26	13.5±0.20	11.66±0.20*	9.5±0.12***	5.5±0.18***
P value	P=0.000	P=0.000	P=0.05	P=0.001	P=0.001

The numbers of abdominal writhings were expressed as mean±SEM. Diclofenac sodium was used positive control at a concentration of 100 mg/kg. *indicates significance of *Ormocarpum sennoides* (Os) compared to control at ($p<0.05$) and *** indicates high significance compared to control at ($p<0.001$)

Acetic acid induced writhing test is considered as a model of visceral pain which is employed in analgesic drug development [24]. The mechanism by which acetic acid induces inflammation is by activating phospholipase A2, which in turn causes the release of several chemical mediators like arachidonic acid which eventually forms eicosanoids from cell membrane resulting in the production of prostaglandins and leukotrienes [25].

The anti-inflammatory activity of Os extract could be due to inhibition of phospholipase A2 activity or even blocking cyclooxygenase (COX-1 and/or COX-2) required for eicosanoids synthesis [26]. NSAIDS like aspirin control an inflammatory reaction by inhibition of cyclooxygenases I & II and thereby reduce the production of prostaglandins from arachidonic acids [27-29]. These effects may be due to the presence of bioactive compounds like saponins, alkaloids, and flavonoids present in the Os extract.

CONCLUSION

The present study aims in bringing out the anti-inflammatory and anti-arthritic activity of *Ormocarpum sennoides*. The leaf extract showed good membrane stabilization and inhibition of protein denaturation. Herbs play a vital role in ancient times as well as in the present medicine. Most of the people are affected by inflammation and most of the women suffer from rheumatoid arthritis. Among various herbs, *Ormocarpum sennoides* possesses both anti-inflammatory and anti-arthritic activity which was revealed in this study. The anti-inflammatory activity was attributed by the bioactive compounds present in the ethanolic leaf extract of Os which supports the utilization of Os in treating inflammation and arthritis.

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CONFLICT OF INTERESTS

Declared none

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