ejpmr, 2015,2(4), 682-692

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF ANTI–INFLAMMATORY ACTIVITY OF *MICROCOSMUS EXASPERATUS*

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Article Received on 18/05/2015 **Article Revised on 12/06/2015** **Article Accepted on 04/07/2015**

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ABSTRACT

Marine organisms have long been a source for the discovery of new drugs because of their vast occurrence. The present study aims at investigating the anti-inflammatory activity of simple ascidian *Microcosmus exasperatus* by using carrageenan induced paw edema and cotton pellet induced granuloma in Wistar albino rats. Groups administered with the extract showed a dose dependent percentage reduction in paw volume as early as the first hour of experiment and more or less equal to the standard drug treated group at the end of the experimental duration. A highly significant and significant percentage reduction in the wet weight of granuloma was recorded in groups II and V. Dry weight of granuloma in extract treated and standard drug received group showed highly significant percentage reduction. No marked changes were noted in serum albumin. A significant reduction in the percentage of lipid peroxide was observed in the highest dose treated group. In groups IV and V serum acid phosphatase was brought back to normal similar to that of group II. A dose dependent decrease in the level of GGTP and ALP was observed in the extract treated groups. The results on anti-inflammatory activity obtained in the present study are indicative of the presence of certain phytochemicals in the extract.

KEY WORDS: *Microcosmus exasperatus,* indomethacin, carrageenan, cotton pellet.

INTRODUCTION

Marine organisms with active biological compounds have been the subject of intense pharmacological studies in the last few decades. They gain much importance in the field of medicine to treat various diseases, as the traditional drugs show side effects by affecting many organs. *Microcosmus exasperatus* is one of the medicinally important marine organisms belonging to the family Pyuridae, found on the hull of ships, piers, pilings and materials used for aquaculture operations in the Tuticorin harbor area. Various studies such as antibacterial, acute, sub chronic oral toxicity, antidiabetic, hepato protectivity, antifertility, CNS depressant, myocardial ischemia protectivity, antihyperlipidemic, anaesthetic, analgesic, antipyretic, antitumour, nutritional value, and biochemical components have been reported from *Microcosmus exasperatus.*[1-12] But no pharmacological study for its anti-inflammatory activity have been attempted so far. Hence the present investigation was undertaken to evaluate the effect of ethanolic extract of *Microcosmus exasperatus* on anti-inflammatory activity in carrageenan induced paw edema and cotton pellet induced granuloma.

MATERIALS AND METHODS

Animal material

Microcosmus exasperatus were collected from the Tuticorin harbour area with the help of SCUBA diver. The animal material was taxonomically identified and authenticated using key to identification of ascidians.[13] In the Department of Zoology, A.P.C. Mahalaxmi College for Women, Thoothukudi, a voucher specimen AS 2240 is preserved for future reference.

Systematic position

Microcosmus exasperatus is placed under the Phylum: Chordata, Subphylum: Urochordata, Class: Ascidiacea, Order: Pleurogona, Suborder: Stolidobranchia, Family: Pyuridae, Genus: *Microcosmus*, Species: *exasperatus*.

Preparation of powder and extract

The specimen collected were washed several times with sea water to remove barnacles, shade dried, homogenized to moderate coarse powder and kept in an air tight container. 100 g powder was extracted with ethanol in a soxhlet apparatus then cooled to room temperature, concentrated in a rotary evaporator to get a residue which was used for further investigations.

Chemicals

Normal saline, ethanolic extract of *Microcosmus exasperatus*, standard drug indomethacin.

Experimental animal

Mature male Wistar albino rats weighing 180-200 g were selected. In a well ventilated house, the animals were kept with constant 12h of dark and light schedule, room temperature (24 ± 2^0 C) and humidity (60-70%). Clean water and standard pellet diet (Hindustan Lever Ltd., India) were given to them "ad Libitum". The animals were kept under fasting for at least 16h before the commencement of the experiment.

Acute toxicity studies

The minimum lethal dose of the ethanolic extract of *Microcosmus exasperatus* was performed as per OECD guidelines 2002.[14] To overnight fasted rats, a dosage of 2000 mg/kg body weight (bw) of the extract was given orally using intra gastric catheter. Then the animals were observed continuously at an interval of 3h for any gross behavioral changes and toxic manifestations like hypersensitivity, grooming, convulsions, sedation, hypothermia and mortality. After 24h, the number of dead and surviving animals was recorded. With the same dose of the extract, the experiment was repeated for 7 more days. Thereafter they were continuously monitored at regular intervals for 14 days.

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY

Carrageenan induced paw edema model

Carrageenan induced paw edema model was used for the determination of acute anti-inflammatory activity.[15] The experimental rats were divided into five groups of six in each. 0.1 ml of 1% carrageenan was administered in the subplantar region of the right hind paw to the entire group including the control. Group I received normal saline, group II was treated with standard drug indomethacin, group III, IV and V were administered with extract at various concentrations of 50, 100 and 150 mg/kg bw along with 2 ml of 1% vanillin. The drugs were given to the rat orally using intra gastric catheter. The paw volume measurement was carried out using a plethysmometer at 0, 60, 120, 180 and 240 min respectively. The average paw oedema in the extract treated group was compared with the control group.

$$\text{Percentage inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where,

Vc represents paw volume in control group and

Vt represents paw volume in drug treated group.

Cotton pellet induced granuloma

For the evaluation of chronic inflammation, cotton pellet induced granuloma method was employed.[16] Wistar albino rats weighing 180-200 g were selected and divided into five groups containing six in each. They were anaesthetized after shaving off the fur. Granulomatous lesions were induced by surgically implanting two cotton pellets weighing 30±1 mg subcutaneously on both sides of the groin region of the rat. Group I received 1% saline. Group II was administered 10 mg of standard drug indomethacin. Group III to V received 50, 100 and 150 mg/kg bw of the ethanolic extract of *Microcosmus exasperatus* respectively. The extracts were given orally and the experiment was continued for a period of seven days. On the eighth day, the rats were sacrificed and the pellets together with the granuloma tissues were dissected out carefully, dried in an oven at 60^{o} C. The percentage of reduction in the wet and dry weight of granuloma in the extract treated groups were compared with that of the control.

Biochemical parameters of liver, exudates and serum

Experimental animals were sacrificed on the eighth day by cervical dislocation for the analysis of chemical parameters. The collected blood was centrifuged to get the serum. After profusing with 0.86% cold saline to remove all the red blood cells the liver was cut into small pieces, suspended in 10% (w/v) ice cold 0.1M phosphate buffer (pH 7.4) and homogenized between 0 and 4^{o}C. By adopting the standard procedures serum albumin, alkaline phosphatase (ALP), lipid peroxide of liver and exudates, acid phosphatase in the serum and exudates, γ-glutamyl transpeptidase (GGTP) in the exudates of granuloma was estimated.[17-21] The results of enzyme activity present in the serum and exudates are expressed as units of enzyme/mg of protein.

Statistical analysis

For anti-inflammatory activities the values are expressed as "mean increase or decrease in paw volume ±SEM". The level of significance was determined by students "t" – test values with *$p<0.05$, **$p<0.01$, ***$p<0.001$, considered moderately significant, significant and highly significant.[22]

RESULTS AND DISCUSSION

Carrageenan induced paw edema

The results obtained for the acute anti-inflammatory activity are presented in Table - 1. Group I (control) showed an elevation in the paw volume during 4h of observation. A highly

significant decrease was observed in indomethacin treated group after 3h and 4h (60.05% to 65.51%). In group V which received the highest concentration of the extract, the percentage reduction in paw volume was evident as early as the first hour of experiment and was more or less equal to that of standard drug treated group. The groups administered with the ethanolic extract of *Microcosmus exasperatus* showed a dose dependent anti-inflammatory activity starting from the first hour to the fourth hour of experiment.

Table – 1: Effect of ethanolic extract of *Microcosmus exasperatus* and Indomethacin on carrageenan induced paw edema in rats

Groups	Paw volume in ml ± SEM and percentage inhibition				
	0 hour	**+1 hour**	**+2 hour**	**+3 hour**	**+4 hour**
I 1% saline	0.478±0.05	0.731±0.09	0.792±0.05	0.811±0.04	0.893±0.02
II Indomethacin 10 mg/kg	0.508±0.04	0.409±0.05*** (44.05)	0.365±0.07*** (53.91)	0.324±0.06*** (60.05)	0.308±0.07*** (65.51)
III – ME 50 mg/kg	0.493±0.01	0.628±0.05* (14.09)	0.518±0.07*** (34.59)	0.426±0.07*** (47.47)	0.388±0.06*** (56.55)
IV – ME 100 mg/kg	0.511±0.06	0.603±0.06* (17.51)	0.511±0.08** (35.48)	0.413±0.05*** (49.07)	0.362±0.07*** (59.46)
V- ME 150 mg/kg	0.493±0.03	0.458±0.01** (37.35)	0.388±0.06*** (51.01)	0.352±0.01*** (56.59)	0.341±0.05*** (61.81)

Data expressed as mean ± SEM; (n=6). Level of significance: *P<0.05, **P<0.01, ***P<0.001 Control vs. treated. Percentage inhibition is indicated in paranthesis.

The most commonly used methods employed experimentally for the evaluation of anti-inflammatory activity is the carrageenan induced paw edema model. It provides a good predictive value for anti-inflammatory potential of novel compound.[23] Carrageenan, a mucopolysacharide derived from Irish Sea moss *Chondrus*, causes experimental arthritis which is non-antigenic and does not produce any systemic effects. [15] Acute inflammation is found to occur in 2 phases. The first phase occurs 1-2h after carrageenan injection in which edema production is mediated by histamine and serotonin. The second phase is related to the release of prostaglandin, bradykinin with a peak at 4h.[24,25] Several studies have indicated that phospholipase an enzyme responsible for the release of arachidonic acid, the key molecule in the biochemical processes, synthesizes prostaglandins during inflammatory activity.[26-28] It is reported that many marine organisms including ascidian have inhibitors of phospholipase A2, thereby inhibiting prostaglandin synthesis via inhibition of cyclooxygenase in arachidonic acid pathways.[29] In the carrageenan induced paw edema, group V treated with 150 mg/kg bw of the extract showed a significant inhibitory response to

inflammation and after 2h, a highly significant mean reduction was observed in the paw volume of the rats.

Cotton pellet induced granuloma

Table - 2 shows the results of chronic anti-inflammatory activity of ethanolic extract of *Microcosmus exasperatus*.

Table – 2: Effect of the ethanolic extract of *Microcosmus exasperatus* on cotton pellet induced granuloma in rats

Groups	Granuloma Wet wt (mg)	% of Reduction	Granuloma Dry wt (mg)	% of Reduction
I 1% saline	41.92±1.84	-	21.66±1.31	-
II - Indomethacin 10 mg/kg	18.42±1.06***	56.05	9.26±1.84***	57.24
III– ME 50 mg/kg	38.31±1.29	8.61	18.36±1.18	15.23
IV- ME 100 mg/kg	29.36±1.84*	29.96	15.65±1.23*	27.74
V- ME 150 mg/kg	23.56±0.98**	43.79	9.36±1.13***	56.78

Data expressed as mean ± SEM; (n=6). Level of significance: *P<0.05, **P<0.01, ***P<0.001 Control vs. treated.

Wet weight of the cotton pellet correlates with transude material and dry weight of pellet relates with amount of granulomatous tissue.[30] In group I, an increase in the wet weight of cotton pellet showed the absorption of fluid by the pellet. Group II administered with indomethacin and group V treated with 150 mg/kg bw of the extract indicated a highly significant and significant percentage of reduction in wet weight of granuloma. An increase in the dry weight of the control is indicative of the elevation of granulomatous tissue. The standard drug treated group and that which received the highest dose of the extract recorded a highly significant percentage of reduction in dry weight granuloma. The percentage reduction in dry weight granuloma showed a dose dependent increase in the extract administered groups. During the process of inflammation proliferation of macrophages, neutrophils, fibroblasts and multiplication of small blood vessels occur, which are the basic sources of highly vascularized reddish mass, termed as granulation tissue.[31,32] A comparison of the results of extract treated and standard drug indomethacin administered, showed that highest dose of the extract has an inhibitory effect almost equal to that of the standard drug. The

significant anti-inflammatory activity of *Microcosmus exasperatus* observed against cotton pellet induced granuloma may be indicative of its ability to reduce the number of fibroblasts and synthesis of collagen and muco polysaccharide, which occur in the natural proliferative events of granulation tissue formation.

The biochemical parameters of serum and exudates are given in Table - 3. In the present study no marked changes were noted in the serum albumin. A significant reduction in the percentage of lipid peroxide in the exudates (91.36%) and liver tissue (54.31%) of group V was noted. The values were more or less similar to that of standard drug treated group II (89.56% and 50.36%). In group III and IV the percentage was lower which may be due to a lag phase occurring during the initiation process of lipid peroxidation and poly unsaturated fatty acids react with reactive oxidation species.[29]

Table–3: Effect of the ethanolic extract of *Microcosmus exasperatus* on various biochemical parameters of cotton pellet induced granuloma in rats

Groups	Albumin (g/dl)	Lipid Peroxide		Acid Phosphatase		Exudates GGTP (U/mg protein x 10^4)	Serum ALP (U/mg protein x 10^4)
		Exudate (%)	Liver (%)	Serum (U/mg protein x 10^4)	Exudate (U/mg protein x 10^4)		
Normal	5.18±0.34	-	62.93±1.36	1.81±0.33	-	-	0.865±0.023
I- Control	5.12±0.27	100.00	100.00	2.39±0.26	63.22±1.63	16.88±0.21	0.924±0.011
II - Indomethacin 10 mg/kg	4.21±0.81	89.56±3.41**	50.36±1.84**	1.43±0.02*	26.93±1.84**	6.93±0.27**	1.284±0.03**
III- ME 50 mg/kg	5.04±0.13	26.92±0.93	30.26±1.33	2.18±0.13	56.88±2.04	9.51±0.28*	1.316±0.067*
IV- ME 100 mg/kg	4.63±0.63	62.92±1.69*	42.92±1.84*	1.74±0.03*	39.22±3.88*	8.06±0.92*	1.293±0.05**
V- ME 150-mg/kg	4.59±0.34	91.36±2.84***	54.31±2.88***	1.36±0.04*	22.66±2.84***	6.24±0.51**	1.184±0.09***

Data expressed as mean ± SEM; (n=6). Level of significance: *P<0.05, **P<0.01, ***P<0.001 Control vs. treated.

Serum acid phosphatase increased in group I (control) 2.39 U/mg protein/10^4 during inflammation (table 3) and this was brought back to normal (1.74 and 1.36 U/mg protein/10^4) in group IV and V similar to that of group II. An elevated level of acid phosphatase in the exudate of group I and a significant reduction in group II was noted. A dose dependent highly significant decrease was observed in the extract treated groups. In the control, a higher level of γ-glutamyl transpeptidase (GGTP) was recorded. The values obtained for group V

was similar to that of the standard drug. The results on anti-inflammatory activity obtained in the present study are an indication of the presence of phytochemicals in the extract. Serum alkaline phosphatase (ALP) level is elevated during chronic inflammation. There was a dose dependent decrease on treatment with different doses of the extract. Group V which received 150 mg/kg bw was highly significant when compared to the standard drug indicating the efficacy of the extract in controlling the inflammation.

The results are suggestive of the presence of potent anti-inflammatory components in the ethanolic extract of *Microcosmus exasperatus*. It is also reported that flavonoids, alkaloids, tannins and phenolic compounds have potent anti-inflammatory activities.[33,34] 26-Nor-5-cholesten-3a-01-25one, cholestan-3-01, 6, 9, 12-octadecatrienoic acid, phenyl methyl ester, (z,z,z)-, and 2-piperidinone, N- [-4-bromo-n-butyl] were reported in the ethanolic extract of *Microcosmus exasperatus* by GC-MS analysis[35] which may also play a role in anti-inflammatory mechanism.

CONCLUSION

The ethanolic extract of *Microcosmus exasperatus* shows significant anti-inflammatory activity in carrageenan induced paw edema and cotton pellet induced granuloma model. This may be due to the presence of flavonoids, alkaloids, tannins, saponins, and phenolic compounds. However, extensive study is needed to establish the exact mechanism of action of the extract of *Microcosmus exasperatus.*

ACKNOWLEDGEMENT

The authors express their gratitude to the UGC, New Delhi –F. No. 39- 588/2010 (SR) for financial assistance and also to Dr. S. Sampath Raj, Samsun Clinical Research Laboratory, Tirupur for providing necessary facilities to carry out the pharmacological studies.

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