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ANAESTHETIC, ANALGESIC AND ANTIPYRETIC ACTIVITIES OF *MICROCOSMUS EXASPERATUS* HELLER, 1878

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ABSTRACT

The vast marine environment is a very rich source of most biologically active compounds. *Microcosmus exasperatus* is a simple ascidian belonging to the family Pyuridae. The present study aims to assess the anaesthetic, analgesic and antipyretic activities of the ethanolic extract of *Microcosmus exasperatus* using intracutaneous wheal method, aminobarbitone induced sleeping time, muscle relaxation, Eddy's hot plate, heat conduction and Brewer's yeast induced pyrexia methods on Wistar albino rats. A dose dependent increase was noted in local and general anaesthetic activity. Maximum response was observed in the group treated with the highest dose of the extract. Significant analgesic activity by increasing threshold potential of pain compared to diclofenac (standard drug) was registered in the extract administered groups in both the methods. An effective antipyretic Activity by reducing the rectal temperature from 1 hr to 6 hr was noted in all the extract treated groups similar to that of paracetamol. Hence, the results suggest that the ethanolic extract of *Microcosmus exasperatus* possess significant anaesthetic, analgesic and antipyretic activities.

KEY WORDS: *Microcosmus exasperatus*, anaesthetic, analgesic, antipyretic.

INTRODUCTION

Introduction of new drugs to relieve pain has revolutionized the medical world. Recent research done on analgesics has shown its common therapeutic categories. Pain is considered as a protective and direct response of the body to any damaged tissue. Tissue injury may

stimulate the primary afferent neurons called nociceptors which are found in skin, muscle joints and some visceral tissues. That is why clinically, pain is labeled as "nociceptive". Drugs that are commonly used to relieve pain are opiods or non opiods but they have potential toxic effects. Hence there is a need to select alternative drugs.

Loss of sensation with or without loss of consciousness is termed as anaesthesia. A number of compounds such as cocaine, tetrodotoxin, saxitoxin, chloroform and cyclopropane with local and general anaesthetic activities are commonly used for therapeutic purposes. At excessive doses they cause hypertension, coronary ischemia and CNS toxicity.

Pyrexia or fever is a natural phenomenon which occurs in the body to fight against infectious agents that cause damage to the tissues. Available antipyretic drugs such as paracetamol, aspirin, nimusulide etc., inhibit the PgE2 biosynthesis and inturn impairs the hepatic cells, glomeruli, cortex of brain and from Marine start in a new paragraph environment offers a source of most biologically active compounds. *Microcosmus exasperatus* is a simple ascidian found attached to the barnacles and under water marine structures. Various studies such as antibacterial, acute, sub chronic oral toxicity, antidiabetic, hepato protectivity, antifertility, antitumour, nutritional value, and biochemical components have been reported from *Microcosmus exasperatus*.[1-8] But there are no specific reports on the activities of *Microcosmus exasperatus* as anaesthetic, analgesic and antipyretic which prompted this investigation.

MATERIALS AND METHODS

Collection of animal material

Fresh samples of *Microcosmus exasperatus* (Family : Pyuridae) were collected from Tuticorin harbour area, by SCUBA diving. They were identified and authenticated using key to identification of ascidians.[9] A voucher specimen No: AS 2240 have been deposited in the museum of the Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin.

Preparation of extract

Collected samples were washed thoroughly with sea water to remove the adhering epibionts, dried under shade and homogenized. The moderate coarse powder obtained was stored in an airtight container. 100 g powder was extracted with ethanol in a soxhlet apparatus, cooled to room temperature, concentrated in a rotary evaporator to get residue which was used for further study.

Experimental animal

For experimental study Wistar Albino rats weighing about 180-200 g were selected. They were hoaxed in a ventilated cage, maintained under standard conditions of temperature at 26± 2° C and 12 hrs of dark, light schedule. All animals had access to clean water and standard pellet diet (Hindustan lever Ltd., India) "ad libitum".

Acute toxicity studies

The minimum lethal dose of the ethanolic extract of *Microcosmus exasperatus* was performed as per OECD guidelines 2002.[10] To the overnight fasted rats, a dosage of 2000 mg/kg bw of the extract was given orally using intra gastric catheter. The animals were observed continuously for the first 3 hrs for any gross behavioral changes and toxic manifestations like hypersensitivity, grooming, convulsions, sedation, hypothermia and mortality. After 24 hrs, the numbers of dead and surviving animals were recorded. With the same dose of the extract, the experiment was repeated for 7 more days. Thereafter the animals were continuously monitored at regular intervals for 14 days.

ANAESTHETIC ACTIVITY

Local anaesthetic activity

Intracutaneous wheal method

Intracutaneous wheal method [11] was used to study the local anaesthetic activity. The animals were divided into five groups of six each. On the previous day of the experimental study, the hair on the back of albino rats, about 4 cm^2 on 4 different areas near the mid line were clipped and removed. Group I, II were administered with 0.5 and 1% standard drug xylocaine and group III, IV and V received 5, 10 and 15% of the extract. Equal volumes of the drug were injected intracutaneously into the shaved areas and wheals were marked with ink and the time of injection was noted. By applying pin pricks in the mid line the normal responses of the animal which is usually a localized skin twitch accompanied by a squeak was observed at first. Ten pin pricks for every 5 min on the wheal areas after every 4s was given and the responses were recorded for 30 min. Any alteration in the response either by twitching of the muscles or squeaking following a pin prick, a negative response was recorded.

General anaesthetic activity

Aminobarbitone induced sleeping time and muscle relaxation

Animals were divided into four groups, of six each. Group I received 10 mg/kg of standard Drug- aminobarbitone and group II, III and IV were administered 50,100 and 150 mg/kg bw

of the extract. The mean sleeping time and muscle relaxation (% of rats unable to grasp the board with fore paws) were noted.

ANALGESIC ACTIVITY

Eddy's Hot plate method

Eddy's hot plate method was used to measure response latencies.[12] The animals were divided into five groups of Six each Group I which received 1% saline was treated as control. Group II was administered with the standard drug diclofenac sodium (9 mg/kg). Group III, IV and V were given the ethanolic extract at 50, 100 and 150 mg/kg bw respectively. The animals were placed on Eddy's hot plate kept at a temperature of 55± 0.5^{o} C. The paws of the rats are very sensitive to heat and give response in the form of jumping, flicking, withdrawal or licking of the paws. To avoid any damage to paw, a cut off period of 15s was observed. Reaction time and type of response were recorded 90 min after treatment with the extract.

Heat conduction method

Healthy albino rats were screened for the sensitivity test using tail immersion method.[13] The animals were divided into five groups having 6 each. Group I received 1% saline solution. Group II was administered with diclofenac sodium (9 mg/kg) and group III, IV and V received the ethanolic extract at 50, 100 and 150 mg/ kg bw respectively. The tail of the rats were gently immersed up to 5 cm in hot water maintained at 55 ± 0.5^{o} C. The reaction time was noted as soon as the animal withdraws its tail from the hot water. A cut off period of 10s was observed to avoid damage to the tail. The response time was measured after an interval of 90 min and the results were tabulated.

ANTIPYRETIC ACTIVITY

For the present study the experimental module selected was the yeast induced pyrexia method in Wistar strain albino rats.[14] The animals were divided into five groups containing 6 in each. They were kept under fasting for 18 hrs before the start of experiment. The body temperature of the rats was recorded by measuring the rectal temperature using digital thermometer at pre determined time intervals. After measuring the initial basal rectal temperature, the experimental rats were injected subcutaneously with 20 % w/v Brewer yeast suspension (10 ml/kg bw) in normal saline. The extract at various doses and standard drug were administered to respective groups after the initial rectal temperature was recorded (18 hr before pyrexia induced (−18 hr) then rise in temperature after 18 hr (0 hr), 1 hr to 6 hr were measured). For the experiment, rats which showed an increase in temperature of at least 0.7^{o}

C were selected. Group I served as control and was given 1% saline. Group II was treated with the standard drug paracetamol (10 mg) and group III, IV and V received 50, 100 and 150 mg/kg bw of ethanolic extract. All the treatments were given orally using intra gastric catheter. The rectal temperature was recorded at an interval of one hour for the first 3 hrs and then at the end of 6 hrs.

Statistical analysis

The analysis of the data was done using one way ANOVA for individual comparison of group with control. The results are presented as Mean ± SEM. P values less than 0.05 were considered moderately significant, 0.01 as significant and 0.001as highly significant.

RESULTS AND DISCUSSION

ANAESTHETIC ACTIVITY

The local anaesthetic activity of the ethanolic extract of *Microcosmus exasperatus* is given in Table - 1. Rats treated with highest dose 15% of extract showed 51 positive responses out of 60 stimuli whereas 1% standard drug xylocaine showed 31 responses. A dose dependent gradual increase in the percentage of local anaesthesia was noted with 85 in the highest dose treated group compared to that of xylocaine with 51.66.

Table – 1: The local anaesthetic effect of *Microcosmus exasperatus* extract

Data represented as mean of 10 observations, (n=6). Significance between control low dose and extract treated groups *p<0.05; **p<0.01. Significance between control high dose and extract treated groups [a] p<0.05; [aa] p<0.01

Groups	Dose (%)	Number of negative responses over time (min)							Total out of 60	Anasethesia (%)
		0	5	10	15	20	25	30		
Xylocaine - I	0.5	0	8	5	9	4	2	1	29	48.33
Xylocaine II	1	0	7	8	4	3	5	4	31	51.66
ME -III	5	0	9	8	7	6	6	5	41	68.33*
ME - IV	10	0	8	9	8	7	8	9	49	81.66**[aa]
ME -V	15	0	9	8	9	8	9	8	51	85.00**[aa]

The result of general anaesthetic activity in rats is shown in Table - 2. A dose dependent increase in the aminobarbitone induced sleeping time and muscle relaxation was observed. It was noted that a maximum sleeping time of 183±5.46 and 192±7.64 min was observed in groups treated with 100 and 150 mg/kg bw of extract, compared to the standard drug

176±5.86 min. Percentage of muscle relaxation was also found to be high in group III and IV showing 73 and 91 compared to that of 63 in standard drug, aminobarbitone. Intracutaneous wheal model is the most suitable method for estimating the degree of anaesthesia and its duration simultaneously. Earlier studies have reported that the potent local anaesthetic activity could be due to the presence of alkaloids and saponins.[15] The chemical screening of the extract showed the presence of alkaloids which might function as strong anaesthetics and pain killer.[16] Recent studies in other ascidian species have also supported this observation.[17,18]

Table - 2: Effect of the *Microcosmus exasperatus* on aminobarbitone induced sleeping time and muscle relaxation in rats

Groups	Sleeping Time (Min ±SD) Mean time	Muscle Relaxation (% of rats unable to grasp board with fore paws)
I-Aminobarbitone 10 mg/kg	176±5.86	63 %
II- ME 50 mg/kg	173±6.24	41%
III- ME 100 mg/kg	183±5.46*	73 %
IV -ME 150 mg/kg	192±7.64**	91 %

Data represented as mean ±SEM, (n=6). Significance between standard and extract treated groups. *p<0.05; **p<0.01.

ANALGESIC ACTIVITY

The results of study on analgesic activity are given in Table - 3. Significant analgesic activity by increasing the threshold potential of pain compared to normal control and standard drug was observed in the extract administered groups in both the methods. Analgesic activity in group V (150 mg/kg bw) was found to be more effective than the standard treated group.

Table – 3: Analgesic activity of ethanolic extract of *Microcosmus exasperatus*

Groups	Response Time in sec (Mean ± SEM)	
	Eddy Hot Plate Method	Heat Conduction Method
I- Saline1%	3.15±0.295	2.16±0.031
II- Diclofenac 9 mg/kg	16.35±0.316***	11.34±0.124***
III-ME 50 mg/kg	8.30±0.216*	6.36±0.117*
IV-ME100 mg/kg	14.26±0.488**	10.31±0.284**
V- ME 150 mg/kg	19.15±0.325***	16.50±0.166***

Data represented as mean ±SEM, (n=6). Significance between control and extract treated groups. *p<0.05; **p<0.01; ***p<0.001.

Analgesics increase the mean basal latency to thermal stimulus and significantly reduce flicking time. Diclofenac sodium is a non-steroidal Anti-inflammatory drug.[19] It inhibits

cyclooxygenase enzyme (COX) and decrease prostaglandin production, an inflammation mediating agent from arachidonic acid there by attenuating the response of nervous system to noxious stimuli and reduce the pain.[20] An increase in the analgesic activity observed on treatment with the extract suggests a mechanism of action similar to that of the standard, diclofenac sodium. Several studies have indicated the presence of compounds such as flavonoids, tannins, steroids, glycosides and terpenoids which are known to inhibit the synthesis of prostaglandins.[21] Earlier studies on the analgesic activity of the simple ascidian *Phallusia arabica* has been attributed either to the inhibition of phospholipase A2 or by blocking cyclooxygenase.[22] GC- MS studies and HPTLC analysis of the ethanolic extract revealed the presence of flavonoids, alkaloids, terpenoids, quinones, anthraquinones and steroids.[23,24] This shows the ethanolic extract of *Microcosmus exasperatus* possess potent analgesic activity.

ANTIPYRETIC ACTIVITY

Antipyretic activity of the ethanolic extract of *Microcosmus exasperatus* was carried out at three dose level i.e. 50, 100 and 150 mg/kg bw and the results are depicted in Table - 4. All the extract treated groups showed effective antipyretic activity as to that of paracetamol after 2 hr of post dosing in Brewer's yeast induced pyrexia.

Table – 4: Effect of ethanolic extract of *Microcosmus exasperatus* on the Antipyretic activity in Brewer's yeast induced pyrexia rats

Groups	Rectal Temperature in ^{0}C after 18hrs of Yeast Injection (Mean± SEM)					
	-18 hr	0 hr	1 hr	2 hr	3 hr	6 hr
I- Saline 1%	37.16±0.24	39.01±0.46	39.24±0.34	39.46±0.28	39.56±0.17	39.89±0.39
II- Paracetamol 10 mg/kg	37.36±0.18	39.16±0.34	38.13±0.43	37.81±0.26*	36.26±0.39*	35.13±0.28**
III- ME 50 mg/kg	37.43±0.26	39.42±0.18	38.64±0.36	35.65±0.34**	35.43±0.28**	34.98±0.36**
IV- ME 100 mg/kg	37.78±0.19	39.76±0.11	37.21±0.83	35.83±0.68**	35.51±0.19**	34.24±0.23**
V- ME 150 mg/kg	37.89±0.26	39.26±0.29	37.06±0.18	35.46±0.23**	34.86±0.46**	34.18±0.19**

Data represented as mean ±S.E.M, (n=6). Significance between control and extract treated groups. *p<0.05; **p<0.01.

The present study revealed a significant reduction in the rectal temperature from 1hr to 6 hr. It is known that hypothalamus a region in the brain is responsible for the elevation or fall in

the normal body temperature ensuring a thermoregulatory function. Any disturbance in hypothalamus leads to rise in body temperature resulting in pyrexia or fever.[25] It occurs when the concentration of prostaglandin E_2 (PGE_2) increases in the hypothalamic region of brain, there by altering the thermoregulatory process. Antipyretics suppress fever by blocking the synthesis of prostaglandin. The extract of *Microcosmus exasperatus* also caused a significant notable decrease in body temperature in yeast provoked rise in body temperature. Presence of flavonoids are known to block prostaglandin synthetase as they are pre dominant inhibitors of cyclooxygenase.[26,27] Phytochemical analysis of *Microcosmus exasperatus* extract have shown the presence of flavonoids, alkaloids, steroids and tannins.[16] The antipyretic activity of the extract observed can be attributed to the presence of these phytochemicals.

CONCLUSION

The present study suggests that the ethanolic extract of *Microcosmus exasperatus* shows significant anaesthetic, analgesic and antipyretic activities due to the presence of certain bioactive compounds. Further study is needed to establish the exact mechanism of action of the extract of *Microcosmus exasperatus.*

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