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Evaluation of analgesic activity of crude methanolic extract of Sciaenidae fishes. (Nibea maculata and Johnius dussumieri)

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ABSTRACT

The main objective of the present investigation is to evaluate the analgesic activity of methanolic extract of Nibea maculata and Johnius dussumieri on mice. Analgesic activity of methanolic extract of Nibea maculata and Johnius dussumieri at a dose of 50 mg/Kg and 100 mg /Kg were evaluated against drug pentazocine at a dose of 5 mg/Kg. Adult Swiss albino mice of either sex of six numbers in each group were under taken for study and evaluated by tail flick and tail immersion method. The both doses of Nibea maculata crude methanolic extract and Johnius dussumieri crude methanolic extract were found to produce significant ($P<0.05$) analgesic activity. In tail flick method the extract at 50 mg/Kg showed significant activity ($P<0.05$) after 45 minutes, but in tail immersion method, the extract showed significant activity at all tested dose levels after 30 minutes interval. The result showed significant analgesic activity against stimuli.

Keywords: Nibea maculate, Johnius dussumieri, Crude methanolic extract, Tail flick method, Tail immersion test, Pentazocine.

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INTRODUCTION

Fish constitute almost half the number of vertebrates on earth and approximately 31000 species of fishes are contained in some 50 orders and 445 families. Sciaenid fishes are one of the earliest known groups of fishes. In India 37 species of sciaenids are known at present. They are found worldwide Fishes, like many other forms of life are of immense value to human beings in various ways. They have been a staple food item in the diet for many people and they also serve as a curative source of various ailments and vitamins' deficient diseases. Fish oils are particularly high in fatty acids, viz eicosapentaenoic acid(EPA) and docoschexaenoic acid (DHA) functions as anti–inflammatory and have been useful in reducing symptoms of arthritis, lowering cholesterol and triglyceride levels, reducing platelet sickness and symptoms of ulcerative colitis[1].

Epidemiological studies have shown a correlation between a low incidence of coronary heart disease and a high consumption of fish products conversely[2]. Therefore fishes still represent a source of pharmacological compounds. A extensive search of the literature reveals on the analgesic activity of the sciaenid fishes. Thus the fish present investigation was planned to find out the therapeutic level of crude methanolic extract of Nibea maculate and crude methanolic extract of Johnius dussumieri in analgesic activity.

MATERIALS AND METHODS

Two Sciaenidae species of Johnius dussumieri3 (Valenciennes, 1833), and Nibea maculata (Schneidea, 1801) were collected from Solai Nagar landing centre of Puducherry (11° 46' and 12° 03'N & 79° 36'and 79° 53'E), South East Coast of India from Jan'2011 to Dec'2011. Fishes were identified based on meristic and morphometric characters with the help of FAO sheets[3].

Preparation of extracts

Samples were collected and kept in ice and brought as soon as possible to the laboratory. Body tissues were removed, cut into small pieces and homogenized (REMI, RQ-127A) and extracted with petroleum ether and methanol using soxhlet apparatus for 6 hours adapted by Shiomi, 1980. Then the methanolic extract was centrifuged to collect the supernatant and concentrated under vacuum in a rotary evaporator (LARK, Model: VC-100A) at low temperature. The dried extract was dissolved in a solution of 2% gum acacia is distilled water (vehicle) for the evaluation of analgesic activity.

Animals Used

Adult Swiss albino mice weighing between 25-30 g of either sex were used for the studies. The

animals were maintained under normal laboratory condition & kept in standard polypropylene cages at room temperature of 30°±2° and 60 to 65% relative humidity and provided with standard diet & water ad libitum. All the animal experiments were approved by IAEC, numbered 409/01/a-CPCSEA. All the test doses were administrated in the mice by the intraperitoneal route, which are 10 times lower than LD 50 dose. Analgesic Activity[4,5,6]

Tail flick method

Before the study, Swiss albino mice were screened for a sensitivity test by placing the tip of the tail on the radiant heat source. Any animals that held to withdraw its tail in 5 second were rejected from the study. The selected animals were divided into four groups of six mice each. Each animal of the groups received one of the following extract (50mg/kg &100mg/kg). Pentazocine (5mg/kg) and 2% w/v of Gum acacia (2ml/kg) in normal saline intraperitonially. Analgesia was assessed with tail flick apparatus (Analgesiometer). The basal reaction time was measured initially and another set of four measures was taken as 15, 30, 45 and 60 minutes interval and the reaction of the animals considered as the post-drug reaction time. A cutoff period of 10sec was observed to prevent tissue damage of the tail of the animals. The results are tabulated in Table. No.1&2.

Tail immersion Test

Prior to analgesic experiments, the animals were screened for the sensitivity test by immersing the tail of the mice gently in hot water maintained at 55°C-55.5°C. The animal immersing the tail from hot water within 5 seconds was selected for the study. The selected mice were then divided into four groups of six mice each. Group III & Group IV received the extract in 2% w/v of Gum acacia in normal saline intraperitoneally at a dose of 50mg/kg & 100mg/kg respectively. Group II received Pentazocine (10mg/kg) and Group I received 2% w/v of Gum acacia (2ml.kg) in the normal saline manner. After administration of the drugs, the reaction time was measured at 0, 15, 30, 45 and 60 minutes. The cutoff time of the immersion is 15 Sec. The with a drawl theme of untreated animals is between 1 and 5.5 Sec. A with a drawl time of more than 6 Sec, therefore is regarded as a positive response[7]. The results are tabulated in Table. No. 3&4.

Statistical Analysis

The mean value ± SEM was calculated for each parameter. The results were analyzed statistically by ANOVA. The minimum level of significant was fixed at $p < 0.05$. The results of experiments by proper statistical analysis are tabulated in table.No.1 to 4 respectively.

RESULT AND DISCUSSION

In analgesic studies, the extracts showed significant analgesic activity of all tested dose levels. In tail flick method the methanolic extract of Nibea maculata at a dose of 50 mg/Kg showed significant activity (4.250± 0.550) after 45 minutes whereas at a dose of 100mg/Kg showed significant analgesic activity (50±.612) after 30 minutes (Table.1) but in tail immersion method, the methanolic extract of both species showed significant activity after 30 minute interval of the experiment at all tested dose levels (Table.3&4). The results showed significant analgesic activity against thermal stimuli.

Table.1: **Analgesic activity of crude methanolic extract of Nibea maculata by Tail Flick Method.**

Group	Treatment	Dose	Basal Reaction time after drug administration (sec)M+_SEM				
			0 min	15min	30 min	45 min	60 min
I	2%W/V Gum acacia	2ml/Kg	2.000±0.000	2.100±0.004	2.260±0.000	2.300±0.004	2.350±0.000
II	Pentazocine	10mg/Kg	2.600±0.280	4.100±0.410	5.600±0.310	7.000±0.230	8.500±0.210
III	Sample- I	50mg/Kg	2.280±0.007	3.000±0.000	3.700±0.240	4.250±0.550	5.000±0.785
IV	Sample-II	100mg/Kg	2.200±0.050	4..000±0.930	5.000±0.612	6.000±0.790	6.000±0.250

Result expressed as mean±SEM ,p<0.05(ANOVA).

Table 2:**Analgesic activity of crude methanolic extract of Johnius dussumieri by Tail Flick Method.**

Group	Treatment	Dose	Basal Reaction Time after drug administration (Sec) M±SEM				
			0 min	15min	30 min	45 min	60 min
I	2%W/V Gum acacia	2ml/Kg	2.000±0.000	2.100±0.004	2.260±0.000	2.300±0.004	2.350±0.000
II	Pentazocine	10mg/Kg	2.600±0.280	4.100±0.410	5.600±0.310	7.000±0.230	8.500±0.210
III	Sample- I	50mg/Kg	3.000±0.000	3.250±0.450	4.000±0.004	5.000±0.785	5.400±0.004
IV	Sample-II	100mg/Kg	3.500±0.410	4..000±0.930	5.000±0.620	6.000±0.790	6.000±0.250

Result expressed as mean±*SEM* p<0.05(ANOVA).

Table.3. Analgesic activity of crude methanolic extract of Nibea maculata by Tail Immersion Method

Group	Treatment	Dose	Basal Reaction Time after drug administration (Sec) M±SEM				
			0 min	15min	30 min	45 min	60 min
I	2%W/V Gum acacia	2ml/Kg	2.000±0.000	2.100±0.004	2.260±0.000	2.300±0.004	2.350±0.000
II	Pentazocine	10mg/Kg	2.430±0.190	4.100±0.410	5.600±0.310	7.000±0.230	8.500±0.210
III	Sample- I	50mg/Kg	3.000±0.000	3.900±0.420	5.000±0.612	6.000±0.250	7.000±0.612
IV	Sample-II	100mg/Kg	3.500±0.410	3..988±0.000	5.280±0.004	6.800±0.004	8.000±0.000

Result expressed as mean±*SEM* p<0.05(ANOVA).

Table.4. Analgesic activity of crude methanolic extract of Johnius dussumieri by Tail Immersion Method

Group	Treatment	Dose	Basal Reaction Time after drug administration (Sec)M±SEM				
			0 min	15min	30 min	45 min	60 min
I	2%W/V Gum acacia	2ml/Kg	2.000±0.000	2.100±0.004	2.260±0.000	2.300±0.004	2.350±0.000
II	Pentazocine	10mg/Kg	2.430±0.190	4.100±0.410	5.600±0.310	7.000±0.230	8.500±0.210
III	Sample- I	50mg/Kg	3.200±0.220	4.000±0.930	5.000±0.612	6.000±0.790	6.000±0.250
IV	Sample-II	100mg/Kg	3.750±0.330	4.250±0.550	5.400±0.004	6.500±0.725	8.200±0.000

Result expressed as mean±*SEM* $p<0.05$(ANOVA).

Pain can be controlled effectively by opioids, which exert their effects by mimicking naturally occurring substances, termed endogenous opioid peptides or endorphins. Endogenous opioid peptides are the naturally occurring ligands for opioid receptors. Three classical opioid receptors types are μ,δ and κ widely distributed in the central nervous system and the peripheral of other tissues[8] Most of the clinically used opioids is relatively selective for either μ receptors or κ receptors. The standard drug used in the present was pentazocine (κ receptor agonist) exerted a significant analgesic effect in all experiments by directly inhibit the ascending transmission of pain[9].

Zinconotide from the mollusc *conus magus* is currently being developed as an analgesic to treat inflammation[10,11] have reported that the water-soluble fraction of shark cartilage had weak anti-inflammatory and significant analgesic activity. Some Ircinia metabolites exhibited activity on phospholipase A2 with subsequent analgesic and anti –inflammatory effects[12]. Malarvannan has reported that the ootoxins from fish possess analgesic activity and exhibited an analgesic ratio above 1.0. The two sea anemone toxins stichodactyla mertensii and Stichodactyla gigantea have exhibited much higher analgesic ratios than fish.

The methods used for the assessment of fish extract for its analgesic activity are based on the enhancement of pain threshold by the active principles present in the crude extracts. As nature of the fish extract were more lipophilic and the probable mechanism of action may be the combination of opioid receptors, which enhance the pain threshold, or due to the potentiation of naturally occurring endogenous opioids like endorphins which also involved in natural pain resistant mechanisms[13].This was evidence from the fact that the crude extracts *of Nibea maculata and Johnius dussumieri* were found to be effective as an analgesic since they significantly enhanced the basal reaction time equally like that of standard drug used in the present study. The analgesic studies revealed that the methanolic extract of Sciaenidae fishes exhibited potent analgesic (Central analgesic activity) effect against thermal stimuli and also revealed that the

extract shows dose dependent analgesic effect.

CONCLUSION

From the above investigation it is quite apparent that a crude methanolic extract of Nibea maculate and crude methanolic extract of Johnius dussumieri possesses the potent analgesic effect against different stimuli. This is evidenced by a significant increase in the reaction time by stimuli in different experimental models.

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