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Observation of acanthotoxicity effect of freshwater catfish *Mystus gulio* from spine and mucus extract

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Abstract
The freshwater catfish *Mystus gulio* was studied in the present investigation with reference to the biotoxic nature of the spine sheath and epidermal secretions of mucus. The study was carried out in the Biotoxinology laboratory of the Central Institute of Fisheries Education, Mumbai. Live specimens of *M. gulio* was procured from the Mahalakshmi Fish Market at Mumbai and brought to the laboratory. The study was carried out in the year September, 1997 to August, 1999. The extracts were obtained by direct extraction into petroleum ether and lyophilized. The amount of protein in the crude spine extract and crude mucus extract toxin were found to be 0.42 µg/ml and 0.30 µg/ml respectively. The spine toxin was purified in HPLC fractionated sample injected to mice observed 3 fractions (F2-F4) were lethal whereas obtained from mucus secretion toxin extract did not elicit any lethality to the albino mice. Toxicity of crude spine extracts and crude mucus extract were observed lethal to male albino mice MLD 8±2 min and MLD 120 min at 1 ml dose. Hemolytic activity of crude spine extract indicates induced spontaneous hemolysis of chicken blood. The skin extracts had no hemolytic properties and both the extracts failed to elicit hemagglutination activities. Analgesic activity exhibited moderate level of toxicity when compared to paracetamol. The spine sheath venom appears to have synergistic effect primarily on the cardiovascular system and peripheral neuromuscular transmission. The observed symptoms of toxicity indicate the potential application of biomedicinal utilities on albino mice model. Analgesic activity was measured by Analgesia meter. The reaction time of the animal is the displayed and noted down. Mice without administration of any toxic or known painkillers were used as controls while these injected i.p. with Paracetamol (Crocin 0.25ml and normal saline @0.25ml/20gm, mice served as reference standards. The experiment was conducted in triplicates, each mice receiving a dose of 0.25ml of the toxin i.p, and subjected to a light intensities of 4 units (3.0, 3.5, 4.0, 4.5) Ampheres. And the knowing the optimum dose at this intensity, mice injected i.p, with the same dose were subjected to various light intensities between 5 and 10 with increment of 1 unit. Analgesic activity was expressed as a ratio of reaction time of envenomated mice against those of the control as also those of the mice injected with paracetamol.

Keywords: *Mystus gulio*, analgesic effect, spine, mucus, Ip (intraperioneal injection)

1. Introduction
The fishes have number of accessory structures interspersed among their skin connective tissues that greatly extend the apparent simple function of body covering [1]. Among the most evident of these structures are the mucus cells which produce slimy lubricating secretion so evident when handling the fish. The range of roles includes respiration, ionic exchange and osmotic regulation, reproduction excretion, disease resistance, communication, feeding, nest building and protection which make mucus highly multifunctional material [2]. Indeed the term cuticle is often the public health aspects of this biotoxication or icthyotoxism are complicated by the lack of adequate public health facilitates the many of the localities where these fishes are found, which the incidences of fish poisoning are very little in India, while it were compared with Japanese incidences [3] The fishermen have always known that certain species of fishes are not compatible with others and cause death if kept together[4].The first explanation of this phenomenon known as due to the toxic effect of skin secretions of these fish was made by Prokhoroff [5]. Halstead and Courville [6] classified the species of fish that have glands that secrete toxins, but lack the traumatogenic organs to deliver them as ichthyocrinotoxic. These secretions known as ichthyocrinotoxics of fish skin toxins are thought to be a part of their defense mechanism to repel predators. Skin toxins (Crinotoxins) have been demonstrated for at least some species of the following families of tropical marine fishes like trunk fishes (Ostraciidae), soap fishes (Grammistidae), gobies (Gobidae),

soles (Soleidae),toadfishes (Batrachoididae) and Clingfishes (Gobiessocidae) [7]. Possible toxic or repelling substance have also been reported the skin of puffers (Tetradodontidae), venomous marine catfishes (Plotosidae and Ariidae), file fishes (Monocanthidae), scorpion fishes (Scorpaenidae), Moray eel *Lycodontis nudivomer* [8] and described many other fish toxins as biodynamic substances [9].

Catfishes in general have high levels of epidermal mucus secretion. Many catfishes have prominent venom elaborated in their skin secretions [10], described six species of catfish which produce crinotxins and had been defined by Halstead and Courville [11] as toxins in fish epidermal secretions not associated with any venom apparatus. Most catfishes are probably crinotoxic. The Arabian Gulf Catfish (*Arius thalassinus*), produce a moderate flow of epidermal, mucus under normal conditions. In addition, they secrete a thick gel coating form the proteinaceous cells when threatened, thermally shocked or injured [12]. The chemical and pharmacological properties of this material indicate that it may promote the healing of wounds. Studies also suggest that abrupt secretion of mucus like gel in response to a perceived hazard is an externally expressed defense response and may have primary function in healing injury beyond the commonly postulated roles of deterring predators or facilitating escape. The skin toxin of the oriental catfish is protenious in nature is composed of hemolytic lethal and edema forming factors [13]. Considerable attention on these lines has been paid with reference to Indian marine catfishes [14, 15], while preliminary studies were conducted as to the crinotoxicity of freshwater fishes such as *H. fossils* [16] and *B. dentatus* [17]. However, many freshwater fishes of gastronomic importance are not studies with reference to their potential health hazards or their biomedicinal and neuropharmacological utilities.

2. Materials and methods

2.1 Collection of fishes

Live specimens of *Mystus gulio* were procured from the Mahalakshmi Fish Market, Mumbai, and brought to the Biotoxinology laboratory. Taxonomic identification of the fish was done on the basis for Talwar and Jingaran [18]. The fish were maintained frozen at 20 ºC soon after bringing to the laboratory. The average size of fish was 10.5 cm and average weight 20g.

2.2 Extraction of crude spine toxin

The dorsal spine was removed by making a small cut at the base of the spine with a one-sided surgical blade and uprooting the spine with forceps. The integumentary sheath of the spines was scraped off and homogenized in phosphate buffer saline. The resulting mixture was centrifuged at 15,000 rpm for 15 minutes at 4 ºC. Again the supernatant was centrifuged at 15,000 rpm for 15 minutes at 4 ºC. In both cases the residue was collected, pooled up and stored at 20 ºC for further use as crude venom [19]

2.3 Extraction of crude mucus toxin

Extraction of crude mucus toxin was done by slightly modifying by immersing 12 fishes into 250 ml of petroleum ether in a glass jar. The fishes were swirled about the solvent for 5 minutes. The fish became stressed under the conditions and secreted foamy mucus. The fishes were then removed and the solvent mucus mixture was evaporated to dryness in a serological bath at 45 ºC and redissolved in phosphate buffer saline and stored at 20 ºC [20].

2.4. HPLC Fraction of the crude toxin

20 mg of the crude toxin was dissolved in 1 ml of phosphate buffer saline. This was filtered through a 0.45µl Millipore filter paper and 20µl aliquots were used for fractionation on HPLC method of Peterson [21]. The run charecteristies were as follows:

1. Column used: HP hypersil BDS (C – 18)
2. Mobile phase: Phosphate buffer pH 7.5 & M NaCl. Linear gradient
3. Flow rate: 1 ml/min
4. Wave length: 400 nm

The fractions were collected manually at 1-minute intervals.

2.5 Estimation of protein

Protein estimation was done by Peterson [21], using bovine serum albumin (BSA) as standard ranging from 0.1 - 1.0 ml were taken and made up 1 ml with distilled water and 1 ml reagent 'A' was added. Reagent A was prepared by mixing equal parts of copper tartarate carbonate (CTC), 10% sodium dodecyl sulphate (SDS), 0.8 N NaOH and triple filtered water and allowed to stand for 10 min at room temperature. After 10 min 0.5 ml of reagent B (Folin Ciocaltey phenol) was added. The test tubes were shaken gently to mix the solution incubated for 10 min in the dark. The absorbance at 750 nm was read spectrophotometrically.

2.6 Mice bioassay

Kasauli strain male albino mice of 20±2 gm weight were procured from M/S Haffkine Biopharma Ltd. Mumbai, and were maintained in the laboratory. Two sets of bioassays were conducted, one using crude toxin and the other one using the fractions. Triplicate sets of mice were injected intraperitoneally with varying doses of crude spine toxin (1mg/ml).

2.7 Hemolytic and Hemoaggulination activities assay

2.7.1 Preparation of Erythrocyte Suspension

Chicken blood were procured from a nearby slaughter house in glass vials treated with 2.7% EDTA as anticoagulant @ 5mg/ml blood and brought to the laboratory. The blood sample was mixed with normal saline and centrifuged at 5,000 rpm for 7 minutes at 4 ºC, the supernatant was discarded and the process of mixing with normal saline and centrifugation was repeated twice. 3 ml or 4ml of the residue was taken and 97 or 96 ml of normal saline added to get a 3 or 4% of erythrocyte suspension respectively.

2.7.2 Hemolytic test

The test was carried out following the method of Pani Prasad and Venkateshvaran [22] in "V" shaped 96 well micro titrate plates. µl of the toxin was added to the first will in a row, mixed thoroughly and from this 100 µl was transferred to the next well and from the last well 100µl was discarded. 100µl of 4% erythrocyte suspension was added to all the wells. The plates were incubated at room temperature for 2 hours. Appropriate positive and negative controls were set up using distilled water and normal saline respectively. Formation of button in wells was taken as negative and complete red coloration was taken as positive. Reciprocal of the highest dilution showing the hemolytic pattern was taken as 1 hemolytic unit (HU).

2.7.3 Hemagglutination assay
The hemagglutination activity of crude toxin and HPLC fraction were tested following the method of Liang and Pan [23] in U bottom micro agglutination plates 96 welled 25 µl for the sample were used for the test on 3 % erythrocyte suspension of chicken blood. The plates were shaken for 1 min after addition if the erythrocytes. The plates were subsequently incubated for 1 hr at 4 ºC.

2.8 Determination of Analgesic Activity
Analgesic activity was measured according the D'Amour and Smith [24]. Test using a Tail flick Analgesia meter with a variable 150 W24 Quartz lamp s the heat source. The mouse was held in an improvised holder in such a way that it's tail covers the photocell of the meter the heat source was turned on using a foot switch starts an electronic timer. When the mouse feels the pain tilt flicks the tail and therefore uncovers the photocell. This action switches off the bulb and stops the counter. The reaction time of the animal is then displayed and noted down. Mice without administration of any toxic or known painkillers were used as controls while these injected i.p. with Paracetamol (Crocin @ 0.25ml/20g mice served as reference standards.

The experiment was conducted in triplicates, each mice receiving a dose of 0.25ml of the toxin i.p. and subjected to light intensities of 4 units (3.0, 3.5, 4.0, 4.5 amperes. Upon knowing the optimum dose at this intensity, mice injected i.p with the same dose was subjected to various light intensities between and 5 and 10 with increment of 1 unit. Analgesic activity was expressed as the ratio of reaction time of envenomated ice against those of the control and also those of the mice were injected with paracetamol.

2.9 Statistical Analysis
The values are expressed as the mean ± SEM from 5 mice in each group. The experiments were arranged in triplicate sets of mice were injected intraperitoneally with varying doses of crude spine toxin. Statistical analyses were performed using by one-way analysis of variance (ANOVA), followed by t-test. All groups were compared with control group. Microsoft Excel spreadsheet was used for the statistical and graphical evaluation.

3. Results
3.1 Protein Estimation
Amount of protein in the crude spine and crude mucus toxin of *M. gulio* were found to be 0.42 µg/ml and 0.30 µg/ml (Table 1).

Table 1: Amount of protein in the crude spine and crude mucus extracts @2mg/ml.

Sample	Quantity (ml)	Protein content (µg/ml)
Crude Spine Extract	0.10	0.42
Crude Mucus Extract	0.10	0.30

3.2 Partial Purified
Partial purification yielded seven residues (F1-F7) of the spine sheath extract and five (F1-F5) residues for the skin. The chromatograms are presented for spine sheath extract and mucus secretion extract of *M. gulio* (Fig 1). The fractions F2, F3, and F4 were observed to be lethal to male albino (20±2g) at 45 min, 60 min and 50 min respectively and crude mucus venom fractions were observed to be non-lethal and the mice recovered in 4 hours (Table 2). This indicates a powerful synergistic effect, the toxic nature becoming more pronounced when the three factors act in combination.

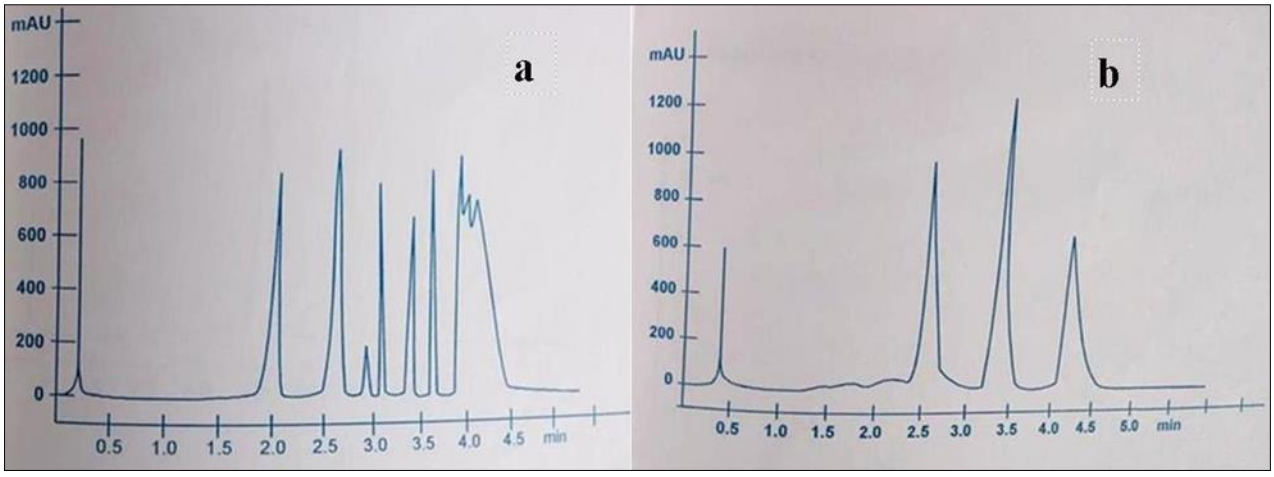

Fig 1: Chromatogram of (a) spine sheath extract and (b) mucus secretion extract of *M. gulio*

Table 2: Toxicity of HPLC fractionated spine toxin and mucus toxin extract of *M. gulio* to male albino mice (20±2g).

Spine sheath toxin				Mucus secretion toxin extract			
Fractions	**Dose (ml)**	**Death time (min)**	**Remark**	**Fractions**	**Dose(ml)**	**Death time (min)**	**Remark**
F1	0.50	-	Non lethal	F1	0.50	-	Non lethal
F2	0.50	45	Lethal	F2	0.50	-	Non lethal
F3	0.50	60	Lethal	F3	0.50	-	Non lethal
F4	0.50	50	Lethal	F4	0.50	-	Non lethal
F5	0.50	-	Non lethal	F5	0.50	-	Non lethal
F6	0.50	-	Non lethal				
F7	0.50	-	Non Lethal				

3.3 Mouse Bioassay

The crude mucus toxin extract and spine toxin of *M. gulio* injected to the male albino mice, exhibited the following symptoms, viz., lethargy, apathy, excessive urination, defection and paralysis of hind limbs. In addition to these general symptoms, the mice when injected with the highest dose of 1ml of extract exhibited excessive scratching, escaping activity and collapsed. The crude spine extract and mucus secretion extract of *M. gulio* were found to be lethality at 1 ml dose mice death 8±2 minutes and 120 minutes respectively from the time of injection. In addition to these general symptoms mice when injected with highest dose of 1 ml of extract, exhibited excessive hyperactivity and scrabbling, very dark coloration of urine and bleeding form mouth (Table 3).

Table 3: Toxicity of crude spine extract and crude mucus extract (concentraion: 2mg/ml) of *M. gulio*, injected to male albino mice (20±2g).

Crude spine extract			Crude mucus extract		
Dose(ml)	**Death time (min)**	**Remarks**	**Dose(ml)**	**Death time (min)**	**Remarks**
0.40	-	Non lethal	0.40	-	Non lethal
0.50	-	Non lethal	0.50	-	Non lethal
0.60	-	Non lethal	0.60	-	Non lethal
0.70	-	Non lethal	0.70	-	Non lethal
0.80	-	Non lethal	0.80	-	Non lethal
0.90	-	Non lethal	0.90	-	Non lethal
1.00	8±2	Lethal	1.00	120	Lethal

3.4 Hemolytic and Hemagglutinating Activities

Crude spine toxin of *Mystus gulio* induced spontaneous hemolysis of chicken blood, the HU being 8. One hemolytic factor F3 was discernible in the partially purified fractions of the spine sheath extract with HU value of 4 (Fig. 2; Table 4).

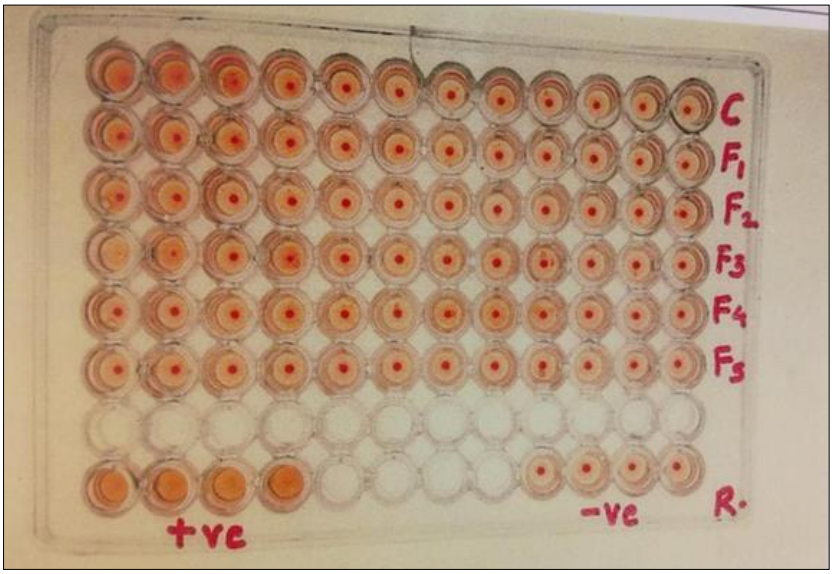

Fig 2: Hemolytic activity of the spine sheath venom of *M. gulio* on chicken blood.

Table 4: Hemolytic activity of crude spine extract and its HPLC fractions.

Toxin	Protein (µg)	Total hemolysis upto dilution	Hemolytic Unit (HU)	Specific hemolytic activity (HU/µg)
Crude	0.420	2^3	8	19.04
F1	0.030	-	-	-
F2	0.037	-	-	-
F3	0.050	2^2	4	80
F4	0.060	-	-	-
F5	0.070	-	-	-

3.5 Analgesic Activity

Analgestic activity of the crude spine extract and crude mucus extract were observed from *M. gulio* on male albino mice at different ampere. Crude spine sheath extract venoms exhibited analgesic properties with analgesic ratio against normal saline values between 1.51 to 1.76 but at the lesser extent compared to paracetamol whose analgesic ratio values ranging between 0.69 to 0.78 whereas, crude mucus extract analgesic ratio against normal saline value ranging between 1.16 to 1.48 and paracetamol analgesic ratio value ranging between 0.54 to 0.63 (Table 5).

Table 5: Analgesic activity of the crude spine extract and crude mucus extract from *M. gulio* on male albino mice (20±2g).

	Reaction time of mice injected with crude spine extract			Analgesic Ratio against	
Amps	**Spine extract (A)**	**Normal saline (B)**	**Paracetamol (C)**	**Normal saline A/B**	**Paracetamol A/C**
3.0	74.0	48.8	95.4	1.51	0.78
3.5	66.3	40.4	86.3	1.64	0.77
4.0	52.0	30.0	75.4	1.73	0.69
4.5	43.3	24.6	57.2	1.76	0.76
	Reaction time of mice injected with crude mucus extract			**Analgesic Ratio against**	
3.0	56.6	48.8	95.4	1.16	0.59
3.5	47.0	40.4	86.3	1.16	0.54
4.0	43.1	30.0	75.4	1.44	0.57
4.5	36.4	24.6	57.2	1.48	0.63

4. Discussion

4.1 Toxicity of Spine and Mucus Extracts

The mice injected with crude mucus and spine extracts showed of lethargy, excessive defecation, urination and convulsion of hind limbs at lower doses. At a dose of 1ml the animals showed pronounced symptoms like escaping tendency jumping and severe paralysis and died in 2 hour time. Similar behaviors of mice to mucus toxin of Ariid catfishes had been reported earlier [15]. The crude mucus toxin extracts from *Heteropneustes fossilis* [16] and *Boleophathalmus dentatus* [17] have been reported earlier to be lethal to mice 20±2g body weight at levels of 1ml dose and the mice died in 2 h. The death time taken were comparable to the toxin study of *Osteogeneiosus militaries* and *Arius dessmieri* [15]. The toxicity of purified protein fractions from *Conus lentigenosus*, *C. inscriptus* and *C. zeylanicus* on male albino mice [25]. The present study of mucus toxin was purified in HPLC and the 5 fractions obtained were injected to mice but did not elicit any lethality. Absuma [17], observed that purified fractions were not lethal to mice, and suggested it may be due to the fact the quantum of toxin per given volume of injected solution was higher in the study. However loss of toxicity upon purification could also have been responsible for the observed results and further detailed studies on this aspect are warranted. The crude spine toxin extract was found to be lethal and the dosage of 1ml was observed to kill mice in 8±2 min. The mice when injected with dose upto 0.9 ml showed stress symptoms, hyperactivity, and escape reaction and collapsed. Birkhead [26] has reported a similar toxicity of extracts from the stings of *Ictalurus melas* and natalis which were found lethal to *Gambusia* sp. Bhimachar [20] observed frogs to collapse in 15 to 20 min after injection of 1 cc glycerin extracted toxic fluid from *Heteropneustus fossils* spines. Deo [16] found that *A. dussumieri* and *O. millitaris* exhibited toxic to mice, resulting in lethargy, apathy, excessive defaecation and urination, paresis of hindlimbs, convulsions, jumping tendency, etc. Analysis of plasma levels of lactate dehydrogenase, glutamate-oxaloacetate tranasminase and glutamatepyruvate transaminase in injected rabbits indicated that the skin toxin caused cardiac and liver damage to the animals [27]. In the case of *Synaceja horrid* and *S. vercucosa* spine venom, ataxia, paralysis of limbs and neck, convulsions respiratory arrest and death were reported in rabbits by Saunders *et al*. [28, 29, 30]. In the case of ictalurid catfishes and one ariid carfish, the injection of spine extract caused necrosis, edema, hemorrhage, and chromatophore expansison in Gambusia [31]. Weiner [31] observations include muscular incardination, paralysis of the hind limbs, irregular respiration, coma and death in mice, when injected with *S. horrid* sting venom. Upon purification on HPLC 3 lethal factors would be identified which had death times higher than that of the crude extract. All the 3 lethal fractions elicited symptoms to those of the crude extract. Shiomi *et al.* [13] presented evidence of the presence of two lethal factors in the crude extract for the skin secretion of *Plotosus lineatus*.

4.2 Hemolytic activity

Primor and Zlotkin [32] reported the presence a hemolytic factor in *Paradachirus* secretion, which was assumed to be related to ichthyotoxicity. Such hemolytic action of crude venom of the mucus secretions of Ariid catfishes has been reported by earlier workers **[15], [17], [33]** observed the crude toxin of *Boleophthalmus dentatus* to elicit hemolysis in chiecken blood and sheep blood at levels of 21 and 27 respectively. The mucus negative results, which confirms with the above said reports. In India similar haemolysis has been reported by Bhimachar [19] who observed that fresh poison squeezed out of specimen of *H. fossilis*, was hemolysis **[9]** identified one hemolytic factor in the epidermal secretions. The crude venom as well as the mucus extracts obtained negative for hemo agglutination test. A1-Hassan *et al.* [33] found the skin toxin of Arabian Gulf catfish to be hemolytic to chicken blood. The spine sheath venom also had one potent hemolytic factor whose specific hemolytic activity was 4 times stronger than that of the crude extract. The strong cytolytic effects of this factor appear to be mediated through membrane break down. Cytolytic activity mediated through rise in blood pressure can also be suspected based on the related symptoms and the hemorrhage observed at autopsy. The pronounced cytolytic activity and the observed analgesic activity point to the potential biomedicinal value of the spine sheath of this fish.

4.3 Analgesic activity

The petroleum ether extracts were evaluated in tail flick test for its analgesic activity from mucus. The study was designed to evaluate the analgesic potential of crude spine sheath and crude mucus extract of *M. gulio* in albino mice at different ampere. The venoms exhibited analgesic properties at the lesser extent compared to paracetamol appears to be a significant finding and suggests that this drug has a slow onset of analgesic action. Kumar *et al*. [34] reported crude proteins extracted from *Conus* species exhibited pronounced analgesic activity. The analgesic ratio decreased with increase in time, in terms of tail flick response observed response time before injection 28.8 sec and after injection 145.5, 185.0 and 190.5 after 30, 60 and 90 min respectively**.** As is widely known, analgesic activity can be induced in rats using formalin, carrageenan, and other methods [35, 36, 37, 38]. Correia *et al* [39] suggest that a change in behavioral responses of zebra fish to acetic acid is a reasonable model to test analgesics and the efficacy of morphine as an analgesic and naloxone as antagonistic substance.

5. Conclusion

The present study clearly establishes the toxic nature of the epidermal secretions of mucus from *M. gulio* appear to be mucus less toxic and to lose their toxic nature upon purification. The spine sheath venom appears to have a synergistic effect preliminary on the cardio vascular transmission. The potential biomedicinal utilities of the spine sheath and mucus secretion cause death of mice were investigated and knowledge about its toxic effect present in the fish. With demand for bioactive compound on an exponential phase, and fish being the most diversified in nature, they are definitely a promising bountiful source of such compounds and various related chemicals.

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7. References

1. Junqueira LCU, Toledo AMS, Porter KR. Observation on the structure of the skin of the teleost, *Fundulus leteroditis* (L). Arch. Histol. Jpn. Niigata. Jpn. 1970; 32(1):15.
2. Shiomi K, Tsuchiya S, Kikuchi T. Occrance of proteinaceous toxin in the skin mucus of the japanes eel *Anguilla japcnoca*. Short paper, Nippon Suisan Gakkaishi. Bulletin of the Japanese Society of Scientific Fisheries. 1990; 56(12):2121.
3. Russel FS. Marine toxins and venomous and poisonous marine animals, Advance marine biology. 1965; 3:255-384.
4. Halstead BW, Courville D, Poisonous A. Venomous Marine Animals of the World, U.S. Govt, Printing Office, Washington D.C, 1967, 1.
5. Prokhoroff P. On poisonous characters of certain lamperys (in Russian) Vrach. St. Peterab. 1884; 5:54-55.
6. Halstead BW, Courville D. A Poisonous and venomous marine Animals of the world U.S. Govt. Printing Office. Washington, D.C. 1970, 3.
7. Hori K, Fusetani N, Hashimoto K, Aida K, Randall JE. Occurrence of a Grammistin like mucus toxin in the Clingfish *Diademichthys lineatus*, Toxicon. 1979; 17:418- 424.
8. Randall JE, Aida K, Oshima Y, Hori K, Hashimoto Y. Occurrence of a crinotosin and hemagglutosin in the skin mucus of the moray eel *Hycodoutes nudivomer*, Marine Biology. 1981; 62(2-3):179-184.
9. Bhakuni DS, Silva M. Biodynamic substances form marine fauna. J Scient Indust Res. 1975; 34:36-53.
10. Cameron AM, Endean R. Epidermal secretions and the evolution of the venom glands in fishes. Toxicon. 1973; 11:401.
11. Halstead BW, Courville DA. Poisonous and venomous marine animals of the World. US. Govt. Printing Office, Washington, D.C. 1970, 3.
12. AI-Hassan JM, Thomson M, Criddle RS. Composition of the proteinaceous gel secretion from the skin of the Arabian Gulf catfish (*Arius thalassinus*). Marine Biology. 1982; 70:27-33.
13. Shiomi Takamiya K, Yamannka MH, Kikuchi T. Purification of a lethal factors in the skin secretion from the oriental catfish *Plotosus lineatus*, Department of food science and techonology. 1986; 24:1015-1018.
14. Nair CV. Bioactive compounds from the mucous of marine catfish *Arius cuelatus*. Un Published M.Sc. Dissertation. Central Institute of Fisheries Education. Mumbai. 1994, 35.
15. Variyath V. Bioactivity of epidermal secretions of three ariid catfishes of Mumbai waters. M.F. Sc. Dissertation. Central Institute of Fisheries Eduation, Mumbai. 1997; 50.
16. Deo AD. Biotoxins from the freshwater catfish *Heteropneustus fossilis* (Bloch 1794). M.F. Sc. Dissetation. Central Institute of Fisheries Education, Mumbai, 1995, 53.
17. Absuma V. Bioitoxicity of epidermal secretions of *Heteropneustes fossilis and Boleophthalmus dentatus*. M.F. Sc. Dissertation. Central Institute of Fisheries Education. Mumbai. 1997, 50.
18. Talwar PK, Jhingran AG. Inland fishes. Oxford and IBM Publication co. pvt ltd., New Delhi. 1991, 2.
19. Bhimachar BS. Poison glands in the pectoral spines of two catfishes, *Heteropneustes fossils* (Bloch) and *Plotosus arab* (Forsk) with remarks on the nature of their venom. Proc. Indian. Acad. Sci. 1944; 19:65-70.
20. Hashimoto Y, Noughi T. Occrance of a tetradotoxin like substance in goby, *Gobius cringer*, Toxicon. 1971; 9:79-84.
21. Peterson GL. A simplification of the protein assay method of Lowry *et al*. which is more generally applicable. Analytical biochemistry. 1977; 83:346-356.
22. Paniprasad K, Venkateshvaran K, Microhemolytic Assay. In: Laboratory manual on advanced techniques in marine Biotoxinology Venkateshvaran, K. and paniprasad, K. (eds). Central Institute of Fisheries Education. CAS in Fishery Science. Mumbai. 1997; 76:46-49.
23. Liang SP, Pan X. A lectin-like peptide isolated from the venom of the Chinese bird spider *Selenocosmia huwena*. Toxicon, 1995; 33:875-882.
24. D'Amour F. Smith DL. A method for determining loss of pain sensation. J Pharmacol Exp Ther. 1941; 72:74-79.
25. Pawan Kumar, Venkateshvaran K, Srivastava PP, Nayak SK, Shivaprakash SM, Chakraborty SK. Toxicity and histopathological observations on albino mice on intra-peritoneal injection of three species of *Conus*. IJARPB. 2013; 3(4):1-11.
26. Birkhead WS. Toxicity of strings of ariid and Ictalurid catfishes: copeia, 1972; 4:790-807.
27. Thomson M, AI-Hassan JM, Fayad S, AI-Saleh, Ali MJ. Purification of a toxic factor from Arabian Gulf Catfish epidermal secretions. Toxicon. 1998; 36(6):859-866.
28. Saunders PR. Venom of the stonefish (*Synanceja horrida*). Federation Proc. 1958; 17(1):1612.
29. Saunders PR, Taylor PB. Venom of the lionfish *Pterois volitans*, Am J Physiol. 1959; 197:437.
30. Saunders PR. Pharmocalogical and chemical studies of the venom of the stone fish (Genus: Synanceja) and other scorpion fishes. Ann N.Y. Acad. Aci. 1960; 90:798-804.
31. Weiner. Observation on the venom of the stone fish *Synanceja trachynis*. Med J Aust. 1959; 46(19):620-627.
32. Primor N, Zlotkin E. On the Ichthyotoxic and hemolytic

action of the skin secretion of the flatfish *Pardachirus marmoratus* (Soleidae). Toxicon. 1975; 13:227-231.
33. AI-Hassan JM, Thomson M, Summers B, Criddle RS. Purification and properties of a hemagglutination factor from Arabian Gulf Catfish (*Arius thalassinus*) epidermal secretion. Compo Biochem. Physiol. 1986; 85(1):31-39.
34. Kumar P, Venkateshvaran K, Srivastava PP, Nayak SK, Shivaprakash SM, Chakraborty SK. Pharmacological studies on the venom of the marine snail *Conus lentiginosus* Reeve, 1844. International Journal of Fisheries and Aquatic Studies. 2014; 1(3):79-85.
35. Khodabakhsh P, Shafaroodi H, Asgarpanah J. Analgesic and anti-inflammatory activities of *Citrus aurantium* L. blossoms essential oil (neroli): involvement of the nitric oxide/cyclic-guanosine monophosphate pathway. J Nat Med. 2015; 69:324-331.
36. Segawa T, Miyakoshi N, Kasukawa Y, Aonuma H, Tsuchie H, Shimada Y. Analgesic effects of minodronate on formalin-induced acute inflammatory pain in rats. Biomed Res. 2013; 34:137-141.
37. Srebro DP, Vuckovic SM, Savic Vujovic KR, Prostran MS. Nitric oxide synthase modulates the antihyperalgesic effect of the NMDA receptor antagonist MK-801 on Carrageenan-induced inflammatory pain in rats. Tohoku J Exp Med. 2014; 234:287-293.
38. Zeredo JL, Sasaki KM, Kumei Y, Toda K. Hindlimb withdrawal reflexes evoked by Er:YAG laser and scalpel incisions in rats. Photomed. Laser Surg. 2006; 24:595-600.
39. Correia Sergio, Cunha R, Martin Scholze, Don Stevens E. A novel behavioral fish model of nociception for testing analgesics Ana D. Pharmaceuticals. 2011; 4:665-680.