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BIOTOXICITY IN ECHINODERMS

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ABSTRACT

Ten species of echinoderms *viz. Holothuria atra, H. scabra, Bahadschia marmorata, Actinocucumis typicus, Pentoceraster regulus, Tropiometra carinata, Astropecten indicus, Gontodiscaster scaber* and *Stomopneustes variolaris* have been screened for the biotoxicity of their various organs. Four series of bioassays were carried out. They were tested for toxicity to *Chanos* and *Tilapia* fingerlings, toxicity to mice and hemolytic activity. The gradation strongest to weakest toxic species is *H. atra* (both body wall and viscera), *B. marmorata* (both body wall and cuvierian tubules), *H. spinifera* (both body wall and viscera), *H. scabra* (body wall alone), *P. regulus* (body wall alone), *A. typicus* (body wall alone), *A. indicus, H. scabra* (viscera), *P. regulus* (viscera), *S. variolaris* (viscera), *G. scaber, T. carinata* and *A. typicus*. Further detailed analysis is being pursued on isolation and characterisation in order to determine the variation in toxicity and the causative factor responsible for the same.

INTRODUCTION

IT IS well known that certain marine organisms exhibit toxicity. While certain species may or may not be toxic and lethal, there is every possibility for these to contain pharmocologically active substances such as anti-carcinogenic, anti-helminthic, anti-biotic, anti-viral anti-ulcer, growth promoting or inhibiting, anti-fertilitic, hemolytic, analgesic, anti-spasmodic, hypotensive and hypertensive agents. The liver oil of some fish has been utilised as sources of vitamin A and D ; insulin has been extracted from whales and tuna ; the red algae *Digenia simplex* has been used as anti-helminthic. Agar-agar and alginic acids are also widely used in pharmaceutical preparations. The powdered oyster and the gall bladder of cod were used in some proprietory medicines. The sea has again been searched as a potential source of drugs because of the vast and diverse range of marine life. The marine organisms seem to be far more attractive as sources of drugs than the terrestrial plants and animals with which we have long been familiar. Recently, the attention of scientists all over the world has been attracted to this vast and potential resources of the sea which can be made use of for the understanding, developing and synthesizing of new hitherto unknown chemical compounds which are pharmacologically active. The marine organisms are being screened for these activities and the chemical compounds responsible for the same are isolated and studied in detail.

Sea cucumbers, starfishes and sea urchins are well known to exhibit toxictiy. The primitive man cut bits of certain species of holothurians (sea cucumbers) and put them in rock pools to stupefy fish and catch them (Frey, 1951). Crude starfish meal contains factors

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which inhibit the growth of chickens (Baughinan, 1951). It has also been known that sea cucumbers evoke nausea in humans (Yoshiro Hashimoto, 1979). The ovaries of the sea urchin *Paracentrotus lividus* during their reproductive period are as lethal as puffer poison and the ovaries of the white sea urchin, *Tripneustes ventricosus* produce severe allergic symptoms when eaten (Yoshiro Hashimoto, 1979). Hippocrates stated that ingestion of sea urchins may produce diarrhoea (Halstead, 1965). The pedicellariae of sea urchins have been reported to produce swellings of the lips or mouth if the ovaries of *T. gratilla* are not sufficiently washed before consuming (Yoshiro Hashimoto, 1979). There is also an old record that dogs and cats died from eating cooked star fish (Fürth, 1903). Dried star fish meal is reported to have long been used for extermination of harmful insects and fly maggots in Hakkaido (Yoshiro Hashimoto, 1979). Ando and Hasegawa (1955) found that dried starfish meal inhibits ecdysis of fly maggots. The toxicity in holothurians to fishes has recently been studied by Bakus (1968 and 1974) and Bakus and Green (1974). They have noted that the toxicity is inversely related to latitude. James (1986) has tried the toxin of *H. atra* to eradicate undesirable organisms from fish farms successfully at Mandapam. A preliminary account of bioactivity in echinoderms is given by Rao *et al.* (1985). Recently Rajendran and Kasinathan (1987) studied the effect of cone toxins on fishes and crabs.

This paper deals with the screening of 10 species of echinoderms collected from Gulf of Mannar area for toxicity (lethality) to fishes and mice and also for hemolytic activity. Out of the 10 echinoderms, 5 were holothurians *viz. Holothuria atra. H. scabra. H. spinifera, Bohadschia marmorata* and *Actinocucumis typicus*; three starfishes *Pentaceraster regulus, Astropecten indicus* and *Goniodiscaster scaber*; one sea feather *Tropiometra carinata* and one sea urchin *Stomopneustes variolaris.*

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Material and Methods

The specimens were collected mostly by hand picking by using mask and snorkel in shallow waters and also by using dredge. The specimens collected were kept in the sea water of the locality from where they were obtained in different plastic containers. In all cases the experiments were started within one hour of collection.

The method of Bakus (1974) was generally followed. 2 g weight of the tiny pieces of the body wall, viscera and cuvierian glands (wherever available) were placed in 250 ml R.B. flask and were refluxed with 100 ml of pure ethanol for 30 minutes. In the case of sea urchin, *S. variolaris*, only the viscera was used and in the case of star fishes and sea feather, only the pieces of the body itself were digested with ethanol. The ethanol extracts were filtered, distilled to recover ethanol and finally evaporated at the boiling temperature of the water bath. The residues were either dissolved in sea water, distilled water or phosphate buffer saline according to the type of bioassay conducted. For each type of bioassay the residue obtained from 2 g of the animal part was used wherever possible, in cases where sufficient quantity of the animal

part was not available the weights used were less.

Bioassay for Fish Toxicity (Lethality)

The ethanolic residue from each part of each animal was dissolved in 250 ml of sea water or the water to which the test fish had been acclamatised and placed in a finger bowl (Bakus, 1974). One test fish is placed in the finger bowl and the time at which it dies is recorded. Experiments were terminated when fish showed consistently normal behaviour or had died. Violent escape behaviour, paralysis and loss of equilibrium indicated the presence of toxin. A strongly toxic echinoderm is defined as causing death in fishes within 15 minutes (often 10 minutes), a weakly toxic echinoderm causes death in fishes from 20 to 45 minutes. The temperatures under which the experiments were conducted, were at laboratory temperature (35.3° to 35.7° C). Control experiments were conducted simultaneously with every test experiment. These consisted of placing fishes in the same volume of clean sea water or water as that used in the experiments.

Two species of test fishes were used for the bioassays on fish toxicity experiments *viz. Chanos* and *Tilapia* fingerlings. The mean size of *Chanos* fingerlings used for the experiments was 96.5 mm and the mean weight 6 g. The mean size of *Tilapia* fingerlings used were 40 mm and the mean weight 0.9 g.

In the cases of holothurians, the sea water in which they were collected and kept in the plastic container was used for cleaning them first and then with fresh sea water. These collective washings were found to contain the mucous of the body of the animal and were ; in most cases, having a stale odour, slippery, soapy to touch and foaming. Therefore, separate fish toxicity bioassays were conducted using the washings of each animals with *Chanos* and *Tilapia* fingerlings along with control, at laboratory temperature. In a few cases these washings were also tested on cuttle fish fingerlings (*Sepia* sp.) with control.

Bioassay for Mice Toxicity (Lethality)

The ethanolic extract residue equivalent to 2 g from each part of each animal was dissolved individually in 10 ml of distilled water and 1 ml of this is injected intraperitoneally into white (albino) male mice. The average weight of the animal used in these experiments was 20 gms. The time of injection and the time taken for death was noted. Here the toxicity was defined as causing death in mice in less than 15-20 minutes and the experiments were terminated when mice showed consistently normal behaviour or died. Paralysis and respiratory difficulty was taken as indicative of toxin. Control experiments were conducted simultaneously by injecting 1 ml of distilled water intraperitoneally in mice with every test experiment.

Bioassay for Hemolytic Activity

The hemolytic effect was studied using the method of Zvi Paster (1973). For this blood is freshly withdrawn from an adult rabbit heart into a capped Erlenmeyer flask and shaken for 3 minutes with glass beads to prevent coagulation. The blood is then centrifuged at 1500 rpm for 2 minutes to separate the erythrocytes from the whole serum. The erythrocytes are washed with saline at pH 7 until they are free from serum, discarding the supernatant. They are then stored in an asceptic bottle in Alsevers and DGV solution at a cell concentration of 30% below 15°C.

The ethanolic extract residue obtained from 2 g of each part of each animal was dissolved individually in 10 ml. of phosphate-buffered saline (PBS), (Disodium hydrogen phosphate 0.028 M, adjusted to pH 7 by addition of 0.084M potassium dihydrogen phosphate, this mixture is mixed with an equal volume of 0.286 M sodium chloride).

2.5 ml of the 30% erythrocytes concentrate was mixed with 7.5 ml of saline at pH 7 and

centrifuged for 2 minutes at 1500 rpm and the supernatant discarded. The erythrocytes were then washed thrice with saline at pH7 each time discarding the supernatant. The final washed erythrocytes were made up to 50 ml with phosphate-buffered saline (PBS) at pH7 and this freshly prepared 5% suspension was kept over ice in an ice box ; and used within 3 hours.

0.2 ml of the solution of the ethanolic residue in PBS is made up to 1 ml. with PBS and to this 4 ml of the 5% erythrocyte suspension added. This mixture is incubated at 37°C for 30 minutes. The resulting mixture is centrifuged and the supernatant collected, diluted to 2.5 times its volume with 1% sodium carbonate and the optical density of the resultant solution measured at 530 mm using the photoelectric Colorimeter AE-IIN, Erma Optical Works Ltd., Tokyo, Japan. A blank was run simultaneously with 1 ml of PBS instead of the toxin extract, to which is added 4 ml of the 5% erythrocyte suspension and experiment conducted as for the extract.

In most cases 2 gm of each part of the animal was used for extraction except were the quantity was less due to non-availability.

Observations and Results

The observations and results of the bioassays carried out are summarised in Tables 1 to 4.

Table 1. *The physical nature of the ethanolic extracts*

Species of echinoderm and its organ	Colour of ethanol extract	odour	Foaming nature during final evaporation	Toxicity on inhaling while pipetting
Holothuria atra body wall	dark orange yellow	Stale which on complete drying is replaced by a fine odour of lemon grass	Foams at the end	—
,, viscera	Yellowish orange	Same as for body wall	Foams at the end	—
H. spinifera body wall	colourless	Stale which turns to a fine mango odour on drying	Foams at the end	—
H. scabra body wall	colourless	Stale	—	—
,, viscera	yellow	Stale	—	—
Bahadschia marmorata body wall	colourless	Stale	—	—
,, Cuvierian tubules	light yellow	Stale	—	-
Actinocucumis typicus (Body wall)	light yellow	Stale	—	—
,, viscera	light yellow	Stale	—	—
Pentaceraster regulus (Body wall)	yellow	Stale	—	—
Astropecten indicus body	yellow	Stale	—	—
Goniodiscaster scaber—body	yellow	Stale	—	—
Stomopneustes variolaris (Body)	Dark red	Stale and peculiar odour	—	Giddiness and momentary fainting
Tropiometra carinata body	Dark red	Stale and peculiar odour	Frothing at the end	Momentary giddiness

TABLE 2. *Toxicity of ethanolic extracts of echinoderms to* Chanos *fingerlings*

Species of echinoderm	Organ used for experiments	Wt. of the organ taken in gm	Duration of Expt. in minutes	Reaction of the fish to the toxin
H. atra	Body wall	2	10	Violent escaping behaviour, loss of Equilibrium and death
H. atra	Viscera	2	11	Same as in *H. atra*
H. scabra	Body wall	2	30	Moderate escaping behaviour, loss of equilibrium and death
H. scabra	Viscera	2	33	Same as in *H. scabra*
H. spinifera	Body wall	2	30	Same as in *H. scabra*
H. spinifera	Viscera	2	30	Same as in *H. scabra*
B. marmorata	Body wall	2	15	Same as in *H. atra*
B. marmorata	Cuvierian tubules	0.35	15	Same as in *H. atra*
A. typicus	Body wall	1.3	35	In distress intermittently, no death
A. typicus	Viscera	0.41	35	Same as in *A. typicus*
P. regulus	Body wall	2	40	Almost normal behaviour except at times restless during the first 30 minutes and then normal
P. regulus	Viscera	2	40	Same as in *P. regulus*
T. carinata	Body	2	40	Same as in *P. regulus* except that it was only restless twice (less number of times)
A. indicus	Body	1.85	40	Same as in *T. carinata*
G. scaber	Body	2	40	Normal
S. variolaris	Viscera	1.35	40	Normal

TABLE 3. *Toxicity of ethanolic extracts of echinoderms to* Tilapia *fingerlings*

Species of echinoderms	Organ used for experiment	Wt. of organ taken in gm	Duration of Expt. in minutes	Reaction of the fish to the toxin
H. atra	Body wall	2	13	Violent behaviour, distress, loss of equilibrium and death
H. atra	Viscera	2	13	Same as in *H. atra*
H. scabra	Body wall	2	41	Loss of equilibrium intermittently and death
H. scabra	Viscera	2	24	Distress, loss of equilibrium and death
H. spinifera	Body wall	2	31	Distress, loss of equilibrium, sinking and death
H. spinifera	Viscera	2	22	Same as in *H. spinifera* but more intense reaction
B. marmorata	Body wall	2	15	Violent behaviour, distress, rising to surface, loss of equilibrium, struggling and death
B. marmorata	Cuvierian tubules	0.35	23	Same as in *B. marmorata*
A. typicus	Body wall	1.3	55	Normal
A. typicus	Viscera	0.41	55	Normal
P. regulus	Body wall	2	55	Normal
P. regulus	Viscera	2	40	Normal
T. carinata	Body	2	55	Normal
A. indicus	Body	1.85	55	Normal
G. scaber	Body	2	55	Normal
S. variolaris	Viscera	1.35	40	Normal

TABLE 4. *Toxicity of aqueous washings of echinoderms to* Chanos, Tilapia, *crab and cuttlefish* fingerlings*

Species	Duration of experiments in minutes	Reaction of fish to the toxin
H. atra-chanos	25	Restless, violent behaviour, loss of equilibrium, gasping and death
B. marmorata—body washings—*Tilapia*	9	Violent escaping behaviour, loss of equilibrium, gasping and death
B. marmorata—washings of cuvierian tubules—*—Tilapia*	7	Same as in *B. marmorata*
A. typicus —*Tilapia*	30	Normal and calm
A. typicus —*Chanos*	63	Restlessness, distress, loss of equilibrium and death
A. typicus —crab	30	Normal and calm
A. typicus —*Sepia* sp.	5	Ejection of ink thrice, distress and death
T. carinata —*Tilapia*	33	Normal
T. carinata —*Crab*	33	Normal
T. carinata —*Chanos*	30	Normal
T. carinata —*Sepia* sp.	15	Ejection of ink thrice, distress and death
G. scaber —*chanos*	30	Normal
,, —*Tilapia*	35	Normal
,, —*Crab*	35	Normal
,, —*Sepia* sp.	5	Ejection of ink twice, distress and death
A. indicus —*Tilapia*	30	Normal
,, —*Crab*	30	Normal
,, —*Chanos*	25	Restlessness, distress, loss of balance and death
,, —*Sepia* sp.	8	Ejection of ink thrice, distress and death
S. variolaris —*Chanos*	35	Normal
,, —*Tilapia*	35	Normal

* The length of *Sepia* fingerlings in these experiments was 10 mm size.

DISCUSSION

It is seen from the results that *Holothuria atra, H. spinifera* and *Bahadschia marmorata* exhibit high degree of toxicity to the fish fingerlings, mices and also show strong action on the erythrocyte cells. The cuvierian tubules of *B. marmorata* seems to be highly toxic to *Chanos* and *Tilapia* as only 0.35 g of this organ, was used compared to 2 g of other specimens. The same is true in the case of destruction of erythrocyte cells in the hemolytic bioassay.

In the case of fish bioassay, with *Chanos*, it was observed that all organs of *H. atra* and *B. marmorata* are highly lethal and toxic, and those of *H. scabra* and *H. spinifera* less toxic. It is seen that the toxin from the echinoderms, *Actinocucumis typicus, Pentoceraster regulus, Tropiometra carinata*, and *Astropecten indicus* are only mildly toxic and are not lethal where as *Goniodiscaster scaber* and *Stomopneustes variolaris* do not contain any substance toxic to *Chanos*.

Tilapia fingerlings which are more sturdy and resistant to most of the changes in their environments (except temperature) are acted upon by toxins of Echinoderms more or less in the same way as regards, *H. atra*, *H. scabra*, *H. spinifera* and *B. marmorata*, of which the toxin of *H. scabra* and *H. spinifera* are slightly less toxic. A change noted between *Chanos* and *Tilapia* is that *Tilapia* remains normal to all other species of Echinoderms studied except the four referred to above.

The experiments conducted with the aqueous washings show interesting results. It is seen that the toxins are mostly water soluble. As expected *H. atra* and *B. marmorata* washings are also highly toxic to fishes. Whereas the alcoholic extract of *A. typicus* did not show lethality to *chanos*, the aqueous washings clearly show that this Echinoderm contains some toxin, the concentration of which is more in the body wall. Another interesting phenomenon noted is that the echinoderms which are more or less non-toxic to fishes are lethal to *Sepia* fingerlings except the starfish *Stomopneustes variolaris*. *Sepia*, thus seems to be the most sensitive of all the test fishes used and crabs seems to be more or less sturdy and resistant.

The mice bicassay, shows that only two species of Echinoderms studied are having lethality. They are *H. atra* and *B. marmorata*. All the organs of *H. atra* are lethal to mice whereas, only the body wall of *B. marmorata* shows this effect. As the weight of cuvierian tubules tested is less, it cannot be ascertained fully whether this organ is toxic or not. In all probability, it may also be toxic if more weight of this organ is taken for test. Further, as the toxicity and lethal dose depends on the body weight of the animal tested, there is no wonder that other Echinoderms show little or no toxicity.

By far the most convenient and sensitive method of assaying toxicity is by studying hemolytic activity of the toxin as it is the action at primary cellular level. The presence of the toxic action in the Echinoderms studied here is clearly brought out by the hemolytic activity, giving the true index of toxicity. It is seen from Table VI that even a low concentration as 0.35 gms of the cuvierian tubules of *B. marmorata* shows an activity equivalent to 2 gms of the body wall of the same animal, thus indicating that the cuvierian tubules of *B. marmorata* are the most highly toxic than all the organs of all other animals tested. The gradation of toxicity is brought out clearly and Table VI gives the same. The gradation from strongest to weakest toxic species is *H. atra* (both body wall and viscera), *B. marmorata* (both body wall and cuvierian tubules), *H. spinifera* (both body wall and viscera), *H. scabra* (body wall alone), *P. regulus* (body wall alone), *A. typicus* (body wall alone), *A. indicus*, *H. scabra* (Viscera), *P. regulus* (viscera), *S. variolaris* (viscera), *G. scaber*, *T. carinata* and *A. typicus*. The finding of high toxicity in *H. atra* body wall and viscera agrees well with results of Bakus (1968). The odour of lemon grass oil (Citronella oil) and that of mango for the ethanolic extract residues of *H. atra* and *H. spinifera* indicate the presence of volatile terpenes. The foaming and frothing towards the end of the evaporation of the extract residues of *H. atra* and *Tropiometra carinata* indicate the presence of Saponins. Some of these extracts were also producing giddness.

A detailed chemical investigation aimed at isolation and characterisation of the bio-active chemical compounds present in each of the species could reveal the causative factor for variation in toxicity.

TABLE 5. *Toxicity of the extracts of the echinoderms on mice*

Species of echinoderm	Organ used for expt.	Weight of the organ taken in gm	Duration of expt. in minutes	Reaction of mice to toxin
H. atra	Body wall	2	37	Instantaneous paralysis of hind legs, falling on side, accelerated respiration and death
H. atra	Viscera	2	42	Same as in *H. atra*
H. scabra	Body wall	2	120	Instantaneous paralysis of hind legs which became free after 90 minutes, alive
H. scabra	Viscera	2	120	Same as in *H. scabra*
H. spinifera	Body wall	2	120	Same as in *H. scabra*
H. spinifera	Viscera	2	120	Same as in *H. scabra*
B. marmorata	Body wall	2	75	First restless, then accelerated breathing and finally death
B. marmorata	Cuvierian tubules	0.35	60	Keeping still and alive. No death
A. typicus	Body wall	1.3	60	Same as in B. *marmorata*
A. typicus	Viscera	0.41	60	Same as in *B. marmorata*
P. regulus	Body wall	2	120	Same as in *B. marmorata*
P. regulus	Viscera	2	120	Same as in *B. marmorata*
P. carinata	Body	2	60	Normal
A. indicus	Body	1.85	60	Normal
G. scaber	Body	2	60	First lying on lateral side and after 15 minutes, continued to be normal
S. variolaris	Viscera	1.35	120	Normal

TABLE 6. *Hemolytic activity of the extracts of echinoderms*

Species of echinoderm	Organ used for experiment	Weight of the organ taken in gm	Optical density	Reaction of erythrocyte cells to the toxin
H. atra	Body wall	2	0.44	strongly + ve
H. atra	Viscera	2	0.44	strongly + ve
H. scabra	Body wall	2	0.30	strongly + ve
H. scabra	Viscera	2	0.13	mildly + ve
H. spinifera	Body wall	2	0.39	strongly + ve
H. spinifera	Viscera	2	0.34	strongly + ve
B. marmorata	Body wall	2	0.42	strongly + ve
B. marmorata	Cuvierian tubules	0.35	0.42	strongly + ve
A. typicus	Body wall	1.3	0.29	Moderately + ve
A. typicus	Viscera	0.51	0.02	mildly + ve
P. regulus	Body wall	2	0.31	strongly + ve
P. *regulus*	Viscera	2	0.10	mildly + ve
T. carinata	Body	2	0.025	Weakly + ve
A. indicus	Body	1.85	0.18	Mildly + ve
G. scaber	Body	2	0.05	Weakly + ve
S. variolaris	Viscera	1.35	0.06	Weakly + ve
Blank	..	..	0.00	..

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