

Pharmacological activities of extracts of some marine animals and plants on isolated tissues of the guinea-pig

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Two hundred and sixty extracts from marine organisms collected from the western and eastern coasts of India, Lakshadweep and the Andaman and Nicobar Islands have been screened for their effects on three isolated tissues of the guinea pig namely, the ileum, the uterus and the atrium with the aim of detecting any anti-spasmodic, oxytotic, uterine relaxant, inotropic and antiarrhythmic activity. Activity was observed in 236 samples (90.76%) with antispasmodic activity being observed in 22 extracts (8.46%), ecboic activity in 59 samples (22.69); uterine relaxant activity in 16 samples (6.15), anti-histaminic and anti-5HT activity in six samples.

The sea has immense biomedical potential which can be exploited not only as a source of drugs for treatment of disease but also of new and novel structures with useful biological activity. A few drugs have already found a place in therapy like the antibiotic cephalotin from the marine fungus *Cephalosporium acremonium*¹ and the anticancer agent arobinoside from the gorgonian *Eunicella covaloni*². Various marine toxins like tetrodotoxin and saxitoxin³ are not only important pharmacological tools but can be used in terminal cases of cancer for relief of pain.

Several laboratories and institutions around the world have been taking increasing interest in marine pharmacology over the last three decades. In our country, however, there was only sporadic work in this field, with mainly the institutions in the coastal areas doing preliminary studies on the marine organisms they come across^{4,5}.

A systemic effort towards pharmacological exploration of marine wealth of our country began in the nineteen eighties with the collaboration of the National Institute of Oceanography Goa; Central Drug Research Institute, Lucknow and Bose Institute, Calcutta. Their study involved broad based pharmacological screening of approximately 500 marine samples in which various activities like antifertility, antiviral, hypotensive, CNS stimulant etc. were detected⁶. The leads were so promising that it was decided to have a broad based project and thus a National Multicentric Project on "Development of Potential Drugs from the Ocean"

funded by the Department of Ocean Development, Government of India, New Delhi, began in 1990. We are one of the centers of this project and we have screened around 260 marine organisms for pharmacological activities on isolated tissues of the guinea-pig with a view to detect any antispasmodic, cardiogenic, antiarrhythmic, tocolytic and ecboic activities besides other pharmacologically promising activities like antihistaminic, anticholinergic, antitryptaminergic, etc.

Marine organisms both flora and fauna from the western and eastern coasts of India, Lakshadweep and the Andaman and Nicobar Islands were collected and immersed in 90% methanol. The methanolic extract was then concentrated under high vacuum by cold percolation method to obtain the crude extract. The crude extract was tested for bioactivity and if it was showing promising pharmacological activity, it was fractionated using solvents with increasing polarity to obtain fractions such as petroleum ether, chloroform, butanol and aqueous; each of which was again tested for the same pharmacological activities as the crude extract. All the above procedures have been carried out at room temperature. Various pharmacological tests were carried out on the guinea pig. Female virgin guinea-pigs weighing around 300-350g, housed under uniform husbandry conditions (temperature $\pm 25^{\circ}\text{C}$) were used in this study. The animals were starved 24 hours prior to the experiment. Only water was provided *ad libitum*.

The plant/animal extracts were initially dissolved in a few drops of methanol and diluted further in distilled water before use, and were used in doses of 50 and 250 µg/ml on ileal and uterine smooth muscle and 3 and 10 µgms/ml on cardiac muscle. Histamine acid phosphate (Blenkinsop & Co. Ltd), acetylcholine chloride (Hopkin & Williams Ltd), 5-Hydroxytryptamine creatinine sulphate (Sigma Chemicals Co), barium chloride (Apex Chemicals) and nicotine sulphate (BHD Chemicals Ltd) were used as standard drugs on isolated guinea-pig ileum. Oxytocin (Parke Davis India Ltd) and Prostaglandin PGF_{2α} (Astra Idl Ltd) were used on isolated guinea-pig uterus.

Guinea-pig ileum: A piece (2-3 cms) of the terminal ileum from a freshly killed animal was suspended⁷ in an organ bath containing aerated Tyrode soln, at 37°C. The contractions were recorded on a GRASS Polygraph (Model 7). The spasmolytic activity of the extract/fraction was detected by its ability to inhibit the spasm induced by a submaximal contraction of the five standard spasmogens.

Guinea - pig uterus: One of the uterine horns of a stunned guinea-pig was suspended in an organ bath containing de Jalon soln. at room temperature of 32 degrees C, bubbled with fresh air⁸. The tocolytic effect of the extract/fraction was assessed by its ability to inhibit submaximal contractions induced by oxytocin and prostaglandin PGF_{2α} on a polygraph.

Guinea - pig auricles : The spontaneously beating pair of auricles a stunned guinea-pig was suspended in an organ bath containing Ringer Locke soln, aerated⁹ with pure oxygen at 34°C, at a resting tension of 1g. The isometric contractions were recorded through a force transducer on a physiograph. Arrhythmias were induced electrically and the antiarrhythmic activity of the extract/fraction was evaluated by its ability to inhibit the electrically induced arrhythmias. Any positive inotropic effect was also noted.

Out of the 260 samples of marine organisms screened, 236 (90.76%) showed some pharmacological activity, some of them exhibiting more than one activity. This is in contrast with the findings of the collaborative work done by CDRI, NIO, and Bose Institute in which 34% were found to be active⁶.

Table 1 shows the profile of pharmacological activity of various crude extracts, fractions and

pure compounds. We use the guinea-pig ileum to detect any antispasmodic activity as well as antihistaminic, anti 5-HT, atropine-like and general intestinal relaxant activity. The guinea-pig uterus can detect oxytocic as well as tocolytic actions, whereas the atrium can pick up positive inotropic and antiarrhythmic actions. Out of the 260 samples studied, 22(8.46%) inhibited contractions produced by all five spasmogens, suggesting a non specific smooth muscle relaxant activity. Four extracts selectively inhibited only histamine induced contractions and one fraction inhibited both histamine and 5-HT, showing promise as potential drugs (Table 2). On the other hand, 109 samples (41.92%) produced contractions by themselves which were blocked by pretreatment with antihistamine. So also, 59 samples (22.69%) produced contractions by themselves on guinea-pig uterus.

Thus a surprisingly large number of samples screened showed contractile activity (64.61%) either on guinea-pig ileum or uterus. We feel that this could be an artefact perhaps because of the procedures adopted during the process of chemical extraction. Thirty eight samples (14.61%) increased the contractions produced by oxytocin on guinea-pig uterus though by themselves they did not produce any contraction. Though it is tempting to speculate that this could be because of inhibition of the enzyme oxytocinase, it is unlikely that such an

Table 1- Pharmacological activities of some marine animals and plants

Activity	Crude extracts	Fraction	Pure compounds
Antiarrhythmic	1	—	—
Antihistaminic	4	—	—
Antihistaminic/ Anti 5-HT	—	1	—
Anti 5-HT	1	—	—
Antispasmodic	20	—	2
Cardiodepressant	4	—	—
Ecbolic	50	6	3
Histaminergic	96	13	—
Potentiation of Oxytocin/PGF _{2α}	28	6	4
Tocolytic	11	5	—

Table 2—Percentage of samples showing potentially useful activity

Antispasmodic	8.46%
Antihistaminic	1.53%
Anti-5HT	0.76%
Ecbolic	22.6%
Tocolytic	6.15%

activity could be present in so many samples. Sixteen samples inhibited oxytocin as well as $\text{PGF}_{2\alpha}$ contractions, suggesting a tocolytic action on the uterus. Experiments conducted on guinea-pig auricles showed that four extracts have a cardiodepressant activity while one showed antiarrhythmic activity. To conclude, though 236 samples out of 260 samples studied by us were active, only a few show promise as potential drugs.

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