OPERCULUM OF *CHICOREUS RAMOSUS* AND *PLEUROPLOCA TRAPEZIUM* - A POSSIBLE SOURCE OF BIOACTIVE SUBSTANCES

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ABSTRACT

Opercula of marine gastropods are exported from India and are presumed to be used in perfume industries. They are also used in traditional medicine in villages of Southern India. The opercula of *Chicoreus ramosus* and *Pleuroploca trapezium* are fetching about Rs. 1,600 (approx. US $46) per kg. This high price is an indication that they are used as raw materials for valuable products. It may be hypothesised that the operculum is a source of bioactive substances due to its use in traditional medicine. Hence, petroleum ether and methanolic extracts of the opercula were studied to reveal possible antibiotic and pharmacological properties. Methanolic extracts were found to produce cardiac inhibition in isolated frog heart.

INTRODUCTION

Molluscs are a potential source of bioactive substances. Many biologically active compounds like toxins and antibiotics have been isolated from the digestive glands of gastropods and are considered to have a role in the chemical defence of the animals against predators. Some marine bioactive substances have been found to posses antimicrobial, antitumor, analgesic, cardiac inhibitory, and growth inhibitory properties. Antibacterial compounds have been isolated from the oyster *Crassostrea virginica*, the clam *Mercenaria mercenaria* and gastropods like *Strombus gigas* and *Tegula gallina*. The tissue extracts of the pulmonate snail *Helix aspersa* was reported to contain cardiac active agents (Baslow 1971).

The family Muricidae has attracted interest due to the royal Tyrian purple of antiquity and murexine from hypobranchial glands containing pharmacologically active choline esters (Whittaker 1960). The pharmacological potential of the hypobranchial gland crude extract of *Chicoreus ramosus* on isolated frog heart and rabbit intestine was studied by Rajakumar & Ayyakkannu (1992). Studies were mainly targeted on endogenous toxins and the role of components from exogenous origin were not assessed. In this context, the opercula of marine gastropods assumes importance as they are exported from India, and as well used in traditional medicines in the villages of Southern India. The cost of one kilogram of operculum of *Chicoreus ramosus* and *Pleuroploca trapezium* is about Rs. 1,600 (approximately US $46). The high price is indicative of its use as raw material for valuable products. Vovells (1967) observed, through histochemical studies of the operculum of *Gibbula magus*, that it is composed of layers of quinone-tanned proteins resulting from a process of secretion containing a protein with aromatic residues, a polyphenol and polyphenol oxidase. Probably it is the presence of such aromatic compounds that has led to its use in the preparation of perfumes. However, no authentic evidence is available regarding this particular aspect of its use. It is hypothesised that the operculum might be a good source of bioactive substances due to its use in traditional medicines. Hence, an attempt was made with petroleum ether and methanolic extracts of the operculum of *C. ramosus* and *P. trapezium* to investigate antibiotic and pharmacological properties.

MATERIALS AND METHODS

Extraction

Opercula of *Chicoreus ramosus* and *Pleuroploca trapezium* were purchased from a shell exporter at Kilakarai, Gulf of Mannar region. The opercula of both species were cleaned, ground to powder and stored for extraction. Petroleum ether was added to a known quantity of powdered operculum, kept for 48 hrs, filtered, and vacuum dried. The filtrate was dried and methanol was added, and the mixture was kept for 48 hrs with vigorous shaking. It was then filtered using Whatman (No. 1) filter paper and vacuum dried (40±3 °C). The concentrated extract was collected, weighed, and used for further analysis. The substance obtained through petroleum ether extraction was very minimal and only material obtained through methanolic extraction was used in this study.

Antibacterial activity

Sterile absorbent paper discs (5 mm diameter) were impregnated with 10 mg per disc using methanol as solvent. The human pathogenic bacteria *Salmonella typhi, Klebsiella pneumoniae, Bacillus subtilis, Vibrio cholerae, Staphylococcus aureus* and *Streptococcus faecalis* were transferred from slants to the nutrient broth and grown at room temperature for 24 hours. Solidified nutrient agar in petri dishes was inoculated with the bacterial cells (24 h growth). The impregnated discs were kept at the surface of the seeded agar medium and incubated at room temperature. The antibacterial activity was recorded after 24 h (Murugan *et al.* 1991).

Isolated perfused frog's heart preparation

Perfusion fluid: Clark's frog Ringer; pH: 7.4. The method used was that of Harris *et al.* (1956). Big sized frog was pithed, median incision was made through the skin over the abdomen and chest, opened and the heart was exposed by cutting away the pericardium. A fine silk thread was passed under, from the heart's inferior *vena cava*, a little away and a slit was made in the *vena cava posterior*. The Syme's cannula was introduced and was tied securely. Then the entire heart was dissected out carefully from the frog with Syme's cannula, secured to a vertical stand in upright position with the apex of the heart pointing down. The 'T' end of the Syme's cannula was connected to a perfusion bottle containing frog ringer solution composed of (mM): NaCl (110), KCl (1.9), $MgCl_2$ (1.5), $CaCl_2$ (1.28), $NaHCO_3PO_4$ (0.9), and Glucose (5.5). The apex of the heart was connected by a hook to a starling's heart lever and the cardiac movements were recorded on a smoked kymograph drum. The level of perfusion fluid was maintained con-

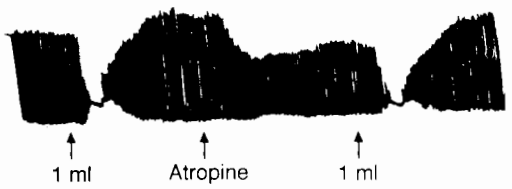

Figure 1. Isolated frog heart kymograph experiment with petroleum ether extract from operculum of *Pleuroploca trapezium*.

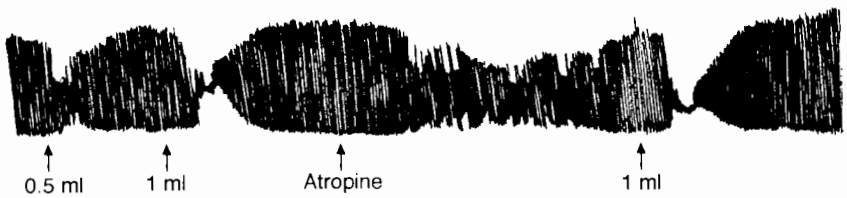

Figure 2. Isolated frog heart kymograph experiment with petroleum ether extract from operculum of *Chicoreus ramosus*.

stant throughout the experiment to provide a perfusion pressure of 6.8 cm of water. The test extracts were injected into the Syme's cannula.

RESULTS AND DISCUSSION

The results obtained with antibacterial tests were not encouraging. Extracts of opercula of *C. ramosus* and *P. trapezium* displayed only mild antibacterial activity and further work is going on using higher dosage and other extraction methods.

One in one methanolic extracts of operculum of *Chicoreus ramosus* and *Pleuroploca trapezium* were administered to the isolated perfused frog heart preparation and it produced the following effects. 1 ml of the extract of operculum from both species of snail caused complete inhibition of the myocardial contraction, and the heart beat was normal, once the administered test drug was washed. This suggest that the extract may contain cardiac inhibitory principles either of cholinergic type or direct acting inhibitory principles of quinidine nature.

In order to ascertain the type of the inhibitory principle, administration of the test extracts was repeated after atropinisation of the heart. Even after atropinisation, the test extracts could produce inhibition of the heart. Therefore, it is inferred that the extracts of opercula may possess some cardiac inhibitory principles not of cholinergic type, but acting directly upon the myocardial cells, like quinidine, verapamil, *etc*. Further work is going on in our laboratory to investigate the antiarrhythmic effect of the test extracts in comparison with that of quinidine (Figs. 1-2).

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