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## Antipyretic, wound healing and antimicrobial activity of processed shell of the marine mollusc *Cypraea moneta*

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## ABSTRACT

**Objective:** Some of the products derived from marine organisms have been recommended in alternative system of medicine especially Siddha medicine for several treatments. Among the marine molluscs, *Cypraea moneta* shell has been used as siddha medicine from ancient days. But no systematic study has been done on its efficacy as antipyretic, wound healing and as antimicrobial agent. In the present study, the protective action of processed shell powder of *C. moneta* was evaluated by us in an animal model for the above treatments. **Methods:** *C. moneta* shell powder was prepared by standard method described in Siddha medicine. Then the antipyretic, wound healing as well as antimicrobial effect of the processed powder was tested in Wister albino rats. **Results:** By the intravenous injection of yeast cell suspension into albino rats, the antipyretic effect of the shell powder given orally was studied by various concentrations of 0, 10, 20 and 30 mg/ml. The body temperature of the albino rat became normal within a short duration (3h). The wound healing effect of the shell powder was very effective. In the thigh region 2 cm wound was made and the different dosages of shell powder (C –Control, SD – Single dose, DD –Double dosage and TD –Triple dose/day) were applied externally as ointment. The scar was produced in eighth day onwards in DD and TD. Antimicrobial activity was studied in three different opportunistic human pathogens such as *Micrococcus* sp., *Proteus vulgaris* and *Salmonella abory* in different concentrations (2, 3, 4 and 5% w/v) of *C. moneta* shell powder extract. Among these, *Proteus vulgaris* showed the maximum zone of inhibition (15mm size) against 5% w/v concentration, followed by *Micrococcus* sp. (12mm) and *S. abory* (10mm) against the same concentration. **Conclusions:** The present observation suggested that, processed *C. moneta* shell powder can be used as an alternative medicine, and it has antipyretic, wound healing as well as antimicrobial properties.

### 1. Introduction

In recent years, human pathogenic microorganisms have developed resistance in response to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases [1]. Moreover, cost of production of synthetic drugs is also high and they cause adverse effect when compared to bioactive naturally derived drugs [2]. Hence, intense research is under progress towards search for natural remedies with potent biological activities from marine organisms. In terms of biodiversity, marine environments is the richest and most complex ecosystem, harsh chemical and physical conditions of the environment have been important drivers for the production of variety of bioactive substances with unique

structural features. These substances exhibit various types of biological activities [3]. Marine invertebrates offer a rich source of potential drugs with excellent biological activities [4]. From marine invertebrates, so far approximately 7000 marine natural products have been reported, among these 33% from sponges, 18% from coelentrates (sea whips, sea fans and soft corals) and 24% from representatives of other invertebrate phyla such as ascidians (also called tunicates), opisthobranch molluscs (nudibranchs, sea hares, etc.), echinoderms (starfish, sea cucumbers, etc.) and bryozoans [5]. Among invertebrates, molluscs are widely distributed throughout the world and have many representatives such as slugs, whelks, clams, mussels, oysters, scallops, squids and octopods in the marine and estuarine ecosystem. Many classes of bioactive compounds exhibiting anti-tumor, anti-leukemic, antibacterial and antiviral activities have been reported worldwide [6–9].

The materia medica of India provides a great deal of information on the folklore practices and traditional aspects

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of therapeutically important natural products [10]. For instance, in traditional Indian medicine, especially Siddha and Ayush medical preparations, the opercula of gastropods are used as an ingredient to combat different diseases [5]. Likewise, in traditional system of medicine the shells of mollusc *Cypraea moneta*, have been used as medicine to cure various ailments mainly related with stomach and in the treatment of dyspepsia, jaundice, enlarged spleen, liver, asthma, cough and also reported to be externally used as caustic in various forms of ointments [11,12]. Considering the importance of the marine natural products, in the present study, an attempt was made to investigate the antipyretic, wound healing and antimicrobial properties of a drug prepared from the shell of *C. moneta*.

## 2. Materials and Methods

### 2.1. Preparation of *C. moneta* powder

*C. moneta* (marine molluscs) shells were graded according to their weight and only shells of about 25–50 g were used. The shell powder was prepared according to the method of Narayanasamy [13]. About 500 g of shell was broken into small pieces, washed thoroughly with fresh water and then immersed in fresh limejuice for 4 h for purification (to remove unwanted materials). It was ground well and mixed with limejuice to prepare pellets. The pellets were put into a special heating apparatus locally called as pudam, heated to a very high temperature (approximately 450°C and locally it was obtained by burning 300 dried cow dung cakes), mixed with 500 ml of cow milk, and made into pellets. The milky made pellets were again put into the special heating apparatus (pudam) and heated for some extent (approximately 150°C and locally it was obtained by burning of 100 dried cow dung cakes) and the shell powder obtained was higher pure than 90%.

### 2.2. Antipyretic effect

Shell powder of *C. moneta* in 100, 200 and 300 mg was suspended in 10 ml of distilled water to obtain final concentration of 10, 20 and 30 mg/ml. Four groups of healthy and pre-acclimatized albino rats, each group with six rats with the mean weight of 185±12g were selected and made hyperthermic by sub-cutaneous injection of a 15% w/v suspension of bakers yeast, *Saccharomyces cerevisiae* at a dose of 10 mg/100g of body weight. The prepared test suspensions were administered orally to the experimental rats [13]. One of the four groups of rats, which received distilled water was maintained as control (group A) whereas, the remaining rats received the shell powder suspension at concentration of 10 (group B), 20 (group C) and 30 mg/ml (group D). After the treatment, the body (rectal) temperature was measured in 1 h intervals by using clinical thermometer (0.01°C accuracy) until recovery of normal body temperature (36°C).

### 2.3. Wound healing effect

A 15% (w/v) *C. moneta* shell powder ointment was prepared by mixing 3 g of powder with 20 ml poultry egg albumin, the mixture was thereafter stored in refrigerator until use. Wounds of 2 cm length were made in the thigh region of the hind limb of four groups of albino rats (each group with six rats) with the help of sharp sterilized blades. Rats were

then allocated into four groups according to the ointment administration as control (C), single time (SD), two times (DD) and three times (TD) per day. Egg albumin was applied instead of ointment in the control group. Each day the length of the wound was measured upto the formation of scar over the wound [13].

### 2.4. Antimicrobial activity

Three different human pathogens viz., *Micrococcus* sp., (S1), *Salmonella abory* (S2) and *Proteus vulgaris* (S3) were taken in the study of antimicrobial activity against the *C. moneta* shell powder. Shell powder suspension was prepared in different concentrations (2, 3, 4 and 5% w/v) using sterilised distilled water. The inhibiting activity of these suspensions was tested by the disc diffusion method [14]. Petri dishes containing nutrient agar medium (Himedia) were labelled corresponding to the shell powder suspensions for individual test organisms. Then 0.1 ml each of the test organisms were taken from the previously prepared respective stoc (broth) and swabbed on the agar medium by using sterilised buds. Sterile paper discs (5mm diameter) previously impregnated with the respective shell powder concentrations for 7 days to obtain maximum product impregnation were placed on the agar medium using sterilised forceps (simultaneously triplicates were maintained for each concentration and each bacterial strain). The plates were then incubated at 37°C for 24h. The antimicrobial activity of the shell powder was observed through zone of inhibition (in mm) on the plates.

### 2.5. Statistical analysis

The results obtained in the present study were expressed as mean ± SD and were analysed using Two-way ANOVA test at 5% significant level using computer software Statistica 6.0 (Statsoft, UK).

## 3. Results

### 3.1. Antipyretic effect

The reduction of body temperature was proportional to the concentration of the drug (Table 1). Body temperature returned to normal (about 36°C), within 3, 4 and 5 hours, in groups D, C and B, respectively. The temperature of the control rats was still high (about 38.5°C) even after 5h. The statistical analysis by Two-way ANOVA on changes in antipyretic effect as a function of different groups of *C. moneta* shell powder and time intervals revealed that these were statistically non significant (  $F(2) = 0.512084$  and  $1.642189$ ;  $P > 0.05$ ).

### 3.2. Wound healing effect

The rats administered shell powder showed healing and scar formation in a dose-dependent manner (Table 2). DD and TD groups produced scars in the wounded region within eight days. The SD groups did not produce any scars but the size of the wound was reduced from 2 to 0.23 cm. Control rats showed only a small reduction in wound length (from 2 to 1.6 cm) and did not produce scar even after 9 days. The statistical analysis (Two-way ANOVA) revealed that the variation in wound healing effect between different dosages and different days intervals were statistically more significant ( $F(2) = 13.44527$  and  $13.2981$ ;  $P < 0.0001$ ).

**Table 1**Variation in antipyretic effect of *C. moneta* shell powder in hypothermal rats

Observation time (hr.)	Body temperature (°C)			
	Group A (Control)	Group B (10 mg/ml)	Group C (20 mg/ml)	Group D (30 mg/ml)
0	39.0	28.83 ± 0.288	39.16 ± 0.288	39.03 ± 0.057
1	39.0	38.50 ± 0.500	38.56 ± 0.115	37.73 ± 0.750
2	39.0	38.16 ± 0.288	38.00 ± 0.000	37.00 ± 0.000
3	38.5	37.50 ± 0.500	36.86 ± 0.230	36.60 ± 0.173
4	38.5	37.06 ± 0.115	36.33 ± 0.288	36.56 ± 0.115
5	38.5	36.16 ± 0.288	36.00 ± 0.00	36.00 ± 0.00

Each value (Mean ± SD) represents mean of six data

Body temperature between observation time and between groups was statistically non significant ( $P > 0.05$ ; Two-way ANOVA).**Table 2**Wound healing effect of *C. moneta* shell powder in rats

Dosage(No/ day)	Wound length (cm) at day of observation								
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Control (C)	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	1.93± 0.057	1.73± 0.057	1.63± 0.057	1.6± 0.057	1.6± 0.057
Single (SD)	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	1.76 ± 0.57	1.40± 0.10	1.13± 0.152	0.7± 0.0	0.5± 0.0	0.23± 0.057
Double (DD)	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	1.53± 0.057	0.96± 0.057	0.76± 0.057	0.13± 0.057	0*± 0.0	0.0*± 0.0
Triple (TD)	2.0 ± 0.0	2.0 ± 0.0	1.76 ± 0.057	1.03± 0.057	0.50± 0.10	0.23± 0.057	0.1± 0.0	0.0*± 0.0	0.0*± 0.0

\* Scar formed

Each value ( Mean ± SD) represents mean of six data;

Wound healing between groups and between days was statistically more significant ( $P < 0.0001$ ; Two-way ANOVA).**Table 3**Antibacterial effect of different concentrations (2 – 5 % w/v) of *C. moneta* powder on selected bacterial strains.

Bacterial strain	Concentrations (%-w/v) and zone of inhibition (mm)			
	2	3	4	5
<i>Micrococcus</i> sp. (S1)	–	–	+(8.33 ± 0.57 mm)	++(12 ± 1.0 mm)
<i>Proteus vulgaris</i> (S2)	–	+(7.66± 0.57mm)	+(12.33 ± 0.57mm)	+++ (15 ± 1.73 mm)
<i>Salmonella abory</i> (S3)	–	–	+(6.0 ± 0.0 mm)	++(10.0 ± 1.0mm)

Each value ( ) represents mean of six data

+ = positive; – = negative; +++ = maximum effect; ++ = moderate effect; + = less effect

### 3.3. Antibacterial activity

Among all the bacterial strains, and shell powder concentration tested, maximum inhibition was observed at 5% (w/v) concentration against the bacterial strain *P. vulgaris* with the zone of inhibition of 15 mm diameter, followed by *Micrococcus* sp and *Salmonella abory* with the zone of inhibition of 12 and 10 mm, respectively. In 4% (w/v) concentratin, *Micrococcus* sp, *P. vulgaris* and *S.abory* showed 8.33, 12.33 and 6.0 mm zone of inhibitin respectively (Table 3).

## 4. Discussion

Among the marine invertebrates, the molluscs are a potential source of bioactive substances. The bioactive compounds isolated from the molluscs, in particular from the gastropods are considered to have a role in the chemical defense of the animals against their predators. Many promising lead compounds have been reported from marine mollusc having anti-inflammatory activity [15]. Nevertheless, in most cases, there has been no scientific research undertaken to substantiate the health benefits derived from molluscs and the active ingredients in the taxa involved are typically unknown [16].

Studies of medicinal animals can assist in pharmacologica screening and may serve both as a source of medicine and as a measure of economic value for these species [17]. In the present study, we examined the efficiency of drug prepared from the shell of mollusc *C. moneta* to reduce fever and heal wounds in albino rats as well as to inhibit microbial activity *in vitro*. This drug efficiently reduced the body temperature of rats that was made hypothermic by yeast-injection. Similarly, the wound healing process ending with the production of scar indicated that tissue regeneration was completed in drug administered rats. Sometimes pathogenic microbes can enter through the wound and produce pus. In this experiment, control rats, which did not receive the drug produced pus about three days after the wound was made. Treated rats, on the contrary, did not produce pus, indicating that drug prevented the entry of opportunistic pathogenic microbes through wound. Both antipyretic and wound healing activities were dose dependent. Results of the present study was corroborated by the findings of Devanathan et al. [18], who emphasized that *C. moneta* was found to be effective in anti-pyretic and anti-inflammatory in experimentally induced albino rats. On the other hand, Badiu et al. [19] evidenced that lipids extracted from two different species of mollusc *Mytilus galloprovincialis* (L.) (Mediterranean mussel) and *Rapana venosa* (hard shell clam) were found to be more efficient in healing induced skin burns in wistar rats. Also in support the results of present

study, Santhi et al. [2] evidenced that 100% chloroform purified extract of gastropod *Purpura persica* reduced the yeast induced pyrexia raised body temperature in albino rats and displayed its significant antipyretic activity. Similarly, a traditional ayurvedic medicinal preparation called Mukta shouktic bhasma (MSB) from mollusc *Pinctada margaritifera* was found to exhibit significant antipyretic activity [20].

Studies of antimicrobial activity provide valuable information for new antibiotic discoveries insights into extraction of bioactive compounds from molluscs. Perusal of literature related to antimicrobial activities from molluscs involves either single body component alone, like haemolymph or egg masses or extracts of whole body tissues have been tested [21]. For instance, antimicrobial activity from the gill extraction of bivalve molluscs *Perna viridis* [22], *Meretrix meretrix* and *M. casta* [23] and sea snail *Cerithidea cingulata* [9] were reported. However, in the present study shell powder suspension (5% w/v concentration) of *C. moneta* was tested against three different opportunistic human pathogenic bacterial strains such as *P. vulgaris*, *Micrococcus* sp and *S. abory* and found that the growth of all the three pathogens was inhibited. In support of the result of the present study, Premanand and Edward [24] investigated the antimicrobial activity of cold methanolic tissue extracts of five species of cowries such as *Cypraea errones*, *C. arabica*, *C. onyx*, *C. tigris* and *C. vitellas* and evidenced that *C. errones* had significant bioactivity against pathogenic bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*) and fungi (*Aspergillus niger* and *Candida albicans*).

In conclusion, it is evident from the study that *C. moneta* shell powder would be a good source of antipyretic, anti-inflammatory and antimicrobial agent which would replace the existing cost effective antibiotics and conventional medicines.

### Conflict of interest statement

We declare that we have no conflict of interest.

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