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Anti Inflammatory, Wound Healing and Analgesic Activities of Fresh Water Mollusc *Parreysia Cylindrica* in Albino Rats

KEYWORDS: Analgesic, anti inflamatory, wound healing

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ABSTRACT *Objective: New trends in drug discovery from natural sources emphasize on investigation of the fresh water ecosystem to explore numerous complex and novel chemical entities. Molluscs are very promising source for wide range of bioactive compounds. Among the mollusc P.cylindrica shell has been used as medicine from ancient days. But no systematic study has been done on its efficiency as analgesic, anti-inflammatory and wound healing effect. In the present study, the protective action of processed shell powder of P.cylindrica was evaluated by us in an animal model for the above treatments. Method: P.cylindrica shell powder was prepared by standard method. Then the analgesic, anti-inflammatory, and wound healing effect of the processed powder was tested in Wister albino rats. Result: The analgesic, anti-inflammatory and wound healing effect of the P.cylindrica shell powder suspension was experimented to albino rats shown promising results. The shell powder suspension at the concentration of 100 and 200mg/kg decreased the pain in analgesic activity which was compared with pentazocine for narcotic type by tail immersion method. The same dose in the anti-inflamatory activity against carragennan induced paw edema was compared with diclofenac sodium reduced the paw edema in albino rats. The wound healing effect of the powder was very effective. In the thigh region 2cm wound was made and the different dosage of shell powder (C- Control, SD-Single, DD-double dosage, TD-Trible dosage/day) was applied externally as ointment. The scar was produced in eighth day onwards in DD and TD. Conclusion: The present observation suggested processed P.cylindrica medicine and it has analgesic, anti-inflammatory as well as wound healing properties.*

Introduction:

In the search of new antioxidants, analgesic, anti inflamatory properties of aquatic habitats has led to the discovery that aquatic plants and invertebrates also contain antioxidants, analgesic, anti inflamatory properties. Among the invertebrates, the discovered bioactive compounds in mollusks were identified essentially as peptides, depsi-peptides, sterols, sesquiterpenes, terpenes, polypropinates, nitrogenous compounds, macrolides, prostaglandins and fatty acid derivatives and alkaloids which presented specific types of activities [1]. Reactive oxygen species and free radicals are formed in the body as a consequence of normal metabolic reactions, exposure to ionizing radiation and by the influence of many xenobiotics. Reactive oxygen species are an important part of the defense mechanisms against infection, but excessive generation of free oxygen radicals may damage tissue [2]. Free radicals are known to be generated through biological and environmental interactions [3] and they have been implicated in more than one hundred disease conditions in humans, including cardiovascular, brain and ocular dysfunctions, arthritis, ischemia and reperfusion carcinogenesis, and AIDS [4].

The cost of manufacture of synthetic drugs is also high and they may also produce adverse side effect, compared to bioactive naturally derived drugs. Therefore, naturally originated agents with very little side effects are required to substitute chemical therapeutics. The knowledge acquired in the past two decades and the discovery of new groups of drugs makes natural antibiotics the basic element of a novel generation of drugs for the treatment of human diseases [5].

In recent years, more and more researchers have come to the realization that aquatic organisms hold immense potential as a source of novel molecules and new drugs. Over the past decade intense efforts were made to more effectively explore new drug clinical and preclinical agents from the rich chemical diversity offered by aquatic life [6]. The core reason behind the production of highly active compounds from marine organisms can be justified by the so called dilution effect of fresh water. Thus any chemical warfare agent must be extremely potent, as it has to overcome dilution en route to its target.

Comprehensive research into the medicinal properties of aquatic organisms in 1960s examined a large number of shellfish products, particularly with the aim to isolate possible natural compounds for various disease treatments. Molluscs represent good candidates for anti inflamatory, wound healing, and analgesic, natural products research considering their evolutionary and ecological significance. Considering the importance of the aquatic natural products, in the present study, an attempt was made to investigate the analgesic, anti-inflammatory and wound healing properties of a drug prepared from the shell of P.cylindrica.

Material and methods:

Preparation of P.cylindrica shell powder:

Live bivalves (P.cylindrica) were collected from Waddepally Lake and brought immediately to the laboratory for further studies. The shells were broken and the soft tissues were removed and the shells were washed thoroughly with distilled water. The shell powder was prepared according to the method of [7]. About 500g of shell was broken into small pieces, washed thoroughly with fresh water and then immersed in fresh limejuice for 4h 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 pudam, heated to a very high temparature (approximately 450^{o}C and locally it was obtained by burning 300 dried cow dung cakes), mixed with 500ml 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^{o}C and locally

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it was obtained by burning of 100 dried cow cakes) and the shell powder obtained was higher pure than 90%.

Selection of Experimental Animals:
Adult Wister albino rats of either sex weighing between 150 1nd 180gm maintained in Laboratory of Eco-physiology and Toxicology lab at Dept. Of. Zoology, Kakatiya University. The selected animals were housed under standard environmental conditions (temperature of 22±1°C) maintained by giving uniform pelette diet, water ad libitum with an alternating 12 hrs light dark cycle and relative humidity of 60± 5%.

Acute toxicity (LD50): The shell powder suspension in 0.5%(W/v) suspension of sodium hydroxide was administered orally in graded doses (100 mg to 800mg body weight) to albino rats. They were observed continuously for the first 2hour for toxic symptoms and 24 hours for mortality.

Analgesic testing:
Wister albino rats were screened for its sensitivity by placing the tip of the tail (last 1-2cm) gently in warm water maintained at (55°C±2°C). Any albino rat flicking the tail within 5 sec were selected for six animals each. Group-I and Group-II received distilled water (Control) 1ml/kg and Pentazocine (Standard) 4 mg/kg i.p respectively. Group III and Group IV received shell powder suspension at concentration of 100mg 200mg/ml respectively. After drug treatment the basal reaction time of all groups of animals was noted at different time intervals such as 1hr, 2hrs, 3hrs, and 4hrs.

Anti -Inflammatory Testing:
Anti - inflammatory activity was assessed by the method suggested by [8] using Carrageenin as phlogestic agent. The adult Wistar albino rats of either sex weighing between 150 & 180 gm were housed in groups of six animals each. They were starved overnight during the experiment but had free access to water. The volume of paw of each animal was determined before giving any drug. Group I and II were given orally the distilled water (Control) 1mg/gm and suspension of Diclofenac Sodium (Standard) (10 mg/kg b.w) respectively. III and IV group of animals received the shell powder suspension at concentration of 100 and 200 mg/kg respectively. The extract was suspended in 0.5% W/v Sodium Hydroxide and administered orally 30 minutes before injection of Carrageenin (0.1 ml of 1% W/v solution) in normal saline into sub planter region of left hind paw of each rat [9].

The degree of edema formation at the hind paw volume was measured by plethysnographically at each hour, for 4 hour after Carrageenin was injected. The percentage inhibition of edema has been calculated by the following formula.

$$A\text{-}B = \frac{C}{A} \times 100$$

Where A represents the average increase in paw volume of control and B represents the average increase in paw volume after the administration of shell powder suspension.

Wound healing testing:
A 15% of P.cylindrica shell powder ointment was prepared by mixing 3g of powder with 20ml poultry egg albumin, the mixture was there after stored in refrigerator until use. wound healing effect was analyzed following the method of [7]. Four groups of healthy and pre-acclimatized albino rats, each group with six rats with the mean weight of 185±12g were selected. Wounds of 2cm length were made in the thigh region of the hind limb of four groups of albino rats with help of sharp sterilized blades. Rats were 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 up to formation of scar over the wound.

Statical analysis: The results obtained in the present study were expressed as mean ± SE statical significance of the difference between control and treated groups accessed by the method of analysis of one way ANOVA.

RESULTS:
Analgesic effect: The analgesic effects of the shell powder suspension on albino rats were presented in table 1. The shell powder suspension at the concentration of 200mg and 300mg/kg showed significant decrease in pain when compared to that of control, at a four hour of experiment. The results were comparable with that of control and the drug was a good analgesic activity, with larger reaction time values of the shell powder suspension (11±0.31, 6±0.31) than control group (3.5±0.5). The difference between the shell powder suspension and control group was significant at P<0.005 and P<0.001.

Anti-inflammatory testing: The anti-inflammatory effect of the P.cylindrica shell powder was experimented an albino rats and the result was depicted in table 2. The shell powder suspension of 100 and 200 mg/kg of the test animals showed significant decrease in paw thickness when compared to that of control, at 4th hr of experiment. The results were compared with that of control and the percentage inhibition of paw thickness was found to be 55.66% and in P.cylindrica at concentration of 100 & 200 mg/kg respectively. In the present investigation 200mg of P.cylindrica shell powder suspension controlled the inflammation maximum than that of 100mg/kg. The suspension of all test animals exhibited a significant (P<0.001) reduced in paw thickness at 4th hour in Carrageenan induced paw edema when compared to control.

Wound healing effect: The rats administered shell powder showed healing and scar formation in a dose dependent manner (table-3) DD and TD groups produced scars in the wound region within eight size of the wound was reduced from 2 to 0.26cm, control rats showed only a small reduction in wound length (from 2 to 1.3cm) and did not produce scar even after 9 days.

Table 1: Analgesic action of shell powder suspension of P.cylindrica by tail immersion method

Treatment	Dose mg/kg	Basal reaction Time (in seconds)				
		0 hr	1hr	2hr	3hr	4hr
Control	1ml/kg	2.65±0.15	2.65±0.15	3±0.6	3.25±0.62	3.5±0.5
Pentazocine	4 i.p	2.5±0.7	4.5±0.18	7.25±0.37	9.25±0.2	11±0.4
Shell powder suspension	100mg	2.45±0.15	3.5±0.18	4±0.31	5±0.31	6±0.31
Shell powder suspension	200mg	3±0.6	3.65±0.5	6.5±1.7	6.5±1.7	8±1.45

All values expressed as Mean±S.E, P<0.05*

*P<0.001

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Table-2 Anti-inflammatory action of shell powder suspension of P.cylindrica against Carrageenan induced paw oedema in albino rats

Treatment	Dose mg/kg	Basal reaction Time (in seconds)			
		1hr	2hr	3hr	4hr
Control	1ml	0.20±0.013	0.29±0.012	0.63±0.012	0.845±0.008
Diclofenac sodium	10	0.08±0.016	0.175±0.022	0.26±0.022	0.3±0.020
Shell powder suspension	100mg	0.165±0.01	0.24±0.076	0.30±0.08	0.495±0.08
Shell powder suspension	200mg	0.165±0.02	0.285±0.033	0.22±0.04	0.39±0.09

All values expressed as Mean±S.E, P<0.05*

*P<0.001

Table-3 wound healing effect of P.cylindrica shell powder suspension in albino 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	1.63±0.047	1.63±0.047	1.23±0.047	1.4±0.047	1.4±0.047
Sindu (SD)	2.0±0.0	2.0±0.0	2.0±0.0	1.66±0.47	1.33±0.31	1.12±0.342	0.5±0.0	0*±0.0	0.25±0.047
Double(DD)	2.0±0.0	2.0±0.0	2.0±0.0	1.43±0.47	0.86±0.047	0.66±0.047	0.17±0.047	0*±0.0	0.0*±0.0
Treble(TD)	2.0±0.0	2.0±0.0	1.66±0.0235	1.02±0.037	0.49±0.31	0.13±0.047	0.1±0.0	0.0*±0.0	0.0*±0.0

*= Scar formed

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

Discussion: Pharmaceutical industry now accepts the world aquatic environment as a major frontier for medical research. Pharma MAR (Spain and USA) has taken leading position in the development of drugs from the aquatic environment. The results indicated that the extracts of P.cylindrica mollusk was active against all the experimentally induced laboratory models of analgesic, anti inflammation and wound healing the extract at the two doses 100 and 200mg/kg used in the study significantly increases the duration response in albino rats. In the present study for analgesic, anti inflammatory and wound healing of shell powder suspension of P.cylindrica showed significant activity when compared to the standard. The suspension contains alkaloids, steroids, terpenes, amino compounds with sulphur linoleic acid ester and oleic acid ester.

Further P.cylindrica shell powder suspension produced significant analgesic activity at 200mg/kg than that of control. In the earlier reports alkaloid extract found to possess analgesic activity [10]. It is therefore, possible that the shell powder suspension from P.cylindrica analgesic effect by inhibition synthesis or action of prostaglandins. Oral administration of shell powder suspension of the test animal showed a significant (P< 0.01, P< 0.05 and P<0.01) inhibition of Carrageenan induced in the paw inflammation at 100 mg and 200 mg. At the dose of 100 mg/kg orally the P.cylindrica shell powder produced 57.67% inhibition in case of the Carrageenan induced edema (P<0.001).The results showed that the extract of the test animal contains substances capable of inhibiting Carrageenan induced inflammation. In the present study also we examined the efficiency of drug prepared from the shell of P.cylindrica to reduce fever and heal wounds in albino rats the wound healing process ending with the production of scar indicated that tissue generation was completed in drug administered rats. Sometimes pathogenic microbes can enter through the wound and produce pus. In the 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. Results of the present study was corroborated by findings of [11], who emphasized that P.cylindrica was found to be effective in analgesic, anti-inflammatory and wound healing in experimentally induced albino rats.

Conclusion: The shell extracts of P.cylindrica posses bioactive compounds that have potent analgesic, anti-inflammatory and wound healing effect. Further studies are essential to explore those bioactive compounds and convert them into usable drugs.

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