ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Preliminary studies on the effects of crude Venom extracts of *Conus Musicus* in isolated Frog Heart preparation and Hematological Parameters in Rats

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ABSTRACT

In this paper we report the effects of crude venom of Conus musicus on the isolated frog heart preparation and hematological parameters of rats. In the episode of administration of the Conus musicus toxin as 10, 20 µg dose attenuated the contractions of the frog heart in a dose dependent manner indicating the depressant action. When toxin co-administered with $Cacl_2$ there was no increase in heart rate. When toxin co-administered with double dose of $Cacl_2$, the action of toxin was partially blocked. From which it could be speculated that the Conus musicus toxin consists of directly acting depressing principle (calcium channels blocking) because the venom is blocking the action of $Cacl_2$ at normal dose and it is reversible when the dose of $Cacl_2$ was doubled the dose of toxin. The hematological parameters study was conducted on 8-weeks old male Wister rats and crude extract (250 µg of Conus musicus) was orally administered (50, 100, and 200 µg /kg) for 28 consecutive days. Our results demonstrated that experimental groups receiving both 100 and 200 mcg/kg Conus musicus treatment led to significant dose-dependent decrease in some hematological parameters RBC, WBC counts, haematocrite and mean corpuscular hemoglobin values at the end of the experiment.

Key words: Conus musicus, heart rate, depressant, RBC counts.

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ISSN: 0975-8585

INTRODUCTION

Predatory marine snails of the genus Conus (family conidae) with over 500 species may comprise the largest single genus of marine animals living today. These species inhabit in tropical reef environments throughout the world. According to their prey preference, cone snails can be classified into three major groups: the piscivorous preying upon fish (e.g. Conus striatus, C.geographus), the molluscivours eating mollusk (e.g.,C.textile , C.pennaceus) and the vermivorous feeding upon polychaete annelids (e.g. C. imperialis, C.vexillum) [10]. All cone snails are venomous predators and have developed a sophisticated biochemical arsenal to rapidly immobilize their prey. Their venoms are complex mixtures of small, disulfide-bridged polypeptide toxins (conotoxins) that inhibit the function of ion channels and neurotransmitter receptors [9]. In addition to their vital role in prey capture and defence against predators, conotoxins are useful tools in neuroscience to characterize receptors and receptor subtypes due to their high binding affinity and specificity [11]. Conotoxins also offer great potential as leads in drug development, and indeed the N-type calcium channel blocker from Conus magus, ω-conotoxin MVIIA, is currently in clinical trial for the treatment of stroke and chronic pain [4]. It is anticipated that the discovery of new toxins displaying characteristically high specificities will increase our understanding of the physiology, pharmacology, biochemistry and structure of their receptors, and may provide leads to new pharmaceuticals [16]. Several classes of conotoxins have been characterized from Conus venoms to date including the α-, α A- and ψ-conotoxins which block nicotinic acetylcholine receptors; μ- and (μO-conotoxins which block voltage-sensitive sodium channels. ω-conotoxins which block voltage-sensitive calcium channels; δ-conotoxins which delay the inactivation of sodium channels; and K-conotoxins which block voltage-sensitive potassium channels [5].

In this attempt, we have extracted the crude venom of C.musicus and studied the effect of crude duct venom on the isolated frog heart preparation and hematology of rats.

MATERIALS AND METHODS

Venom Extraction:

Specimens of Conus musicus were collected from Portonova, Chidambaram, Tamilnadu, Southern India, dissected and crude extract were prepared from the venom duct material as previously described. Briefly, ground dried ducts were extracted with 30% acetonitrile /water acidified with 0.1% trifluoroacetic acid, centrifuged, and the supernatants retained. Crude venom extract was lyophilized and stored at -20 °C. [1,2].

Effect upon Isolated Perfused Frog Heart:

A large size frog was pithed. The heart was exposed cutting open the thoracic cage on the ventral side. The pericardium was carefully removed without injuring the heart. The post caval vein was identified by lifting the heart and placing it on the other side. A wet thread was passed below the post caval vein and silt was made in the vein. The syme's cannula was inserted in the post caval venin and tied firmly. The entire heart was dissected out carefully from the animal's body. The side tube of syme's tube of syme's cannula was connected to the perfusion bottle containing frog ringer solution. The apex of the heart was hooked and tied with a thread joined to a sterling heart leaver extended to kymograph. The level of perfusion fluid was maintained constant throughout the experiment to provide a perfusion pressure of 6 to 8cms of water. The force of contraction and the heart rate recorded on the smoked glazed paper fitted on the Kymograph. The venom extracts 1.0ml, 2.0ml, (10µg, and 20µg respectively) apart from other standard drugs were added on the upper end of the syme's cannula and their effects were recorded on the smoked paper. [8].

Effects on hematological parameters:

Crude extract of Conus musicus was used for this study. It was diluted 50, 100, and 200 µg in water in order to reach the test concentrations. The intraperitoneal LD50 for Conus musicus in male rats was 425.20. Therefore, 50 µg /kg as low dose, 100 µg /kg (two fold of low dose) as medium dose, and 200 µg /kg (two fold of medium dose) as high dose were selected in the study. Solutions were freshly made immediately before usage. All the other reagents used were of analytical reagent grade and obtained from Sigma Chemical Co. Mumbai, India. [7].

Animals and Experimental Design:

The Institutional Animal Ethics Committee of Annamalai University Faculty of Medicine approved the protocol. This experiment was conducted on 72 adult male (8-week old) Wistar rats (120-160 g in weight) obtained from Breeding Center of Experimental Animals in Annamalai University. After 10 days of acclimation, the animals were randomly assigned to either the experimental groups (low dose group, 50 mcg/kg; medium dose group, 100 µg /kg; and high dose group, 200 µg/kg) or the control group, each containing 10 rats and housed individually in labeled cages with solid plastic sides and stainless-steel grid tops and floors. Animals were orally fed daily with a normal diet in standard laboratory chow (10 g/rat/day). In this study, Conus musicus was intraperitonially administered for 28 consecutive days as described in OECD guideline 407.They were maintained in controlled laboratory conditions of 12 hr dark/light cycle, 25 ± 2˚C temperature and 45-75% humidity.

Tap water was also given ad libitum. All animals were weighed weekly throughout the study.

Hematological Analysis:

At the end of the experiment, 6 rats of each group were anaesthetized with ether and blood samples were drawn from the heart of each animal. Blood samples were taken with EDTA, and were used for hematological parameters namely red blood cell counts, white blood cell counts, haemoglobin , haematocrite , mean corpuscular volume (MCV), mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration [3].

Statistical Analysis:

The results of hematological analysis were presented as the mean SEM. Comparisons were made between control and treatment groups using one-way analysis of variance (ANOVA) followed by Dennett's test. Values of $p< 0.05$ were regarded as statistically significant.

RESULTS

Effect on Isolated perfused Heart Preparation

On the episode of administration of the Conus musicus toxin as 0.1ml (100 µg), 0.2ml (200 µg) dose showed the depressant action. After the inhibitory action, the stimulant adrenaline 0.2ml (20 µg) was administered, the action of venom was completely blocked and it was repeated once again in the same concentration, which confirmed the depressant action of the toxin. When toxin co-administered with $Cacl_2$ there is no increase in heart rate or contraction, which is a characteristic feature of $Cacl_2$, confirmed the direct depressant action of the toxin on the heart. When toxin co-administered with double dose of $Cacl_2$, the action of toxin was partially blocked. And when toxin co-administered with acetylcholine, propranolol, and potassium chloride respectively the action of toxin was increased. From this it could be speculated that the Conus musicus toxin consists of directly acting depressing principle because the venom is blocking the action of $Cacl_2$ at normal dose and then it is reversible when the dose of $Cacl_2$ was increased (fig.1).

ISSN: 0975-8585

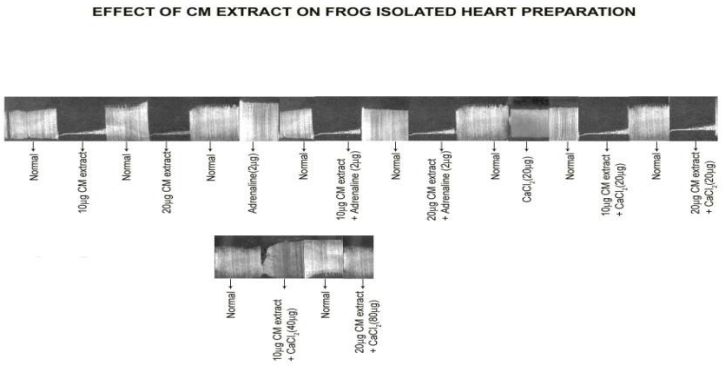

Figure: I Effect of Crude Venom of Conus musicus on the Isolated Frog Heart Preparation

Changes in Hematological Parameters

The results of hematological analysis of rats in control, treatment groups and normal hematological values are given in Table **1**. In rats treated with 50, 100 and 200 µg/kg Conus musicus, RBC, Ht, thrombocyte and MCH values showed a significant dose dependent decrease. In addition, 200 µg/kg Conus musicus treatment caused a significant decrease in Hb value. Conus musicus caused a significant dose dependent decrease in MCV values of all treated groups. There was no statistically significant increase in WBC counts at $p< 0.05$.

Table. I. Results of the haematological parameters of CM extracts in albino rats

Tests/Groups	Normal values	Group-I Control	Group-II 50µg/kg i.p.	Group-III 100µg/kg i.p.	Group-IV 200µg/kg i.p.
RBC(mill/mcl)	7-10	9.56±1.82	8.33±2.01*	8.75±1.19**	7.50±1.59**
WBC(thous/mcl)	6-18	10.25±1.56	14.78±1.56*	16.45±1.88*	17.45±1.22**
Hb(g/dl)	11-19.2	17.45±2.45	16.00±14.2*	15.11±14.2**	13.23±14.2*
HT(%)	35-48	47.92±2.09	46.45±0.89*	44.49±0.56*	40.13±0.98*
MCV(fl)	48-70	65.12±22.0	58.10 ±7.0*	50.40 ±7.0*	46.50 ±7.0*
MCH(pg)	18-29	25.87±6.67	25.41±0.89	23.89±1.47*	20.46±1.45**
MCHC(%)	40-46	45.19±5.44	43.30±1.4*	41.46±1.91*	40.14±1.23**

- Merk manul & veterinary data, Values are mean ± SEM; n=6 in each group. Percentage inhibition when compared to control. ,*Values are statistically significant at $P< 0.05$.,**Values are statistically significant at $P< 0.01$.***Values are statistically significant at $P< 0.001$

DISSCUSSION

The results of our study showed that Conus musicus caused a dose-dependent decrease in heart rate and force of contraction of frog heart. Especially, high dose of Conus musicus treatment in the heart experiment caused a significant decrease in heart rate,

which is reversed by doubling the dose of cacl2. We think that the decrease in heart rate is due to direct action of venom on the voltage sensitive calcium channels present in the frog heart.

The results of hematological parameters showed that Conus musicus caused a dose-dependent decrease in some hematological parameters of the rats such as RBC, Ht and thrombocyte values. Especially, high dose Conus musicus treatment in this experiment caused a significant decrease in Hb concentration. We think that the decrease in Hb value is due to an increase in the rate at which Hb is destroyed. Our results are in accordance by indicating a disruption of erythropoiesis or an increase in destruction of blood cells. The increase in WBC, lymphocyte and monocyte was noted Conus musicus treated rats compared to the control group. This result is consistent with the literature showing Conus musicus have not an immunosuppressive effect on rats, although more parameters than done here have to be examined to talk about this subject. While Hb value decreased significantly in rats treated with high dose crude extract of Conus musicus, MCH values showed a significant dose-dependent decrease in medium and high dose Conus musicus treatment. As a result, it was apparent that Conus musicus caused the negative alteration on some haematological parameters. Indeed, alterations in the haematological parameters due to conotoxins were extensively investigated, but very little attention has been paid to morphological changes induced by Conus musicus.

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