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ORIGINAL ARTICLE

Possible recovery from an acute envenomation in male rats with LD_{50} of *Echis coloratus* crude venom: I-A seven days hematological follow-up study

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Abstract The effect of an acute LD_{50} dose of *Echis coloratus* crude venom in male albino rats was tested on blood parameters: white blood cells (WBCs), red blood cells (RBCs), platelets count, hemoglobin, hematocrit, mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC), also serum glucose, total protein, triglycerides with alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and γ-glutamyl transferase (GGT) enzyme activities. The effect of the LD_{50} dose was monitored over a period of seven days, with time intervals of 1, 3, 6, 12, 24, 72 h. All of the tested parameters show fluctuations with time and with tendency to regain normal control level after 12 h. At 12–24 h it seems to be crucial for the process of physiological recovery, in spite of the irreversible damage and tissue distraction. The process of physiological adaptation and recovery from the lethal destructive venom effect seems to stabilize after one week, leaving the animal alive with several biochemical altered metabolisms and disturbed physiological profile.

1. Introduction

Echis coloratus is a viper belonging to the family Viperidae, it occurs in rocky deserts, from sea level to altitudes as high as 2500 m and not found in sandy deserts. It is common throughout the central region of Saudi Arabia. It is aggressive and one of the most venomous snakes, responsible for the majority of snake bites in Saudi Arabia. Their bites can cause serious health problems, disturbance in metabolism and even death (Al-Sadoon, 1989). Snake venoms contain a complex mix of components, with biologically active proteins and peptides comprising the vast majority (Casewell et al., 2009). Viperidae venoms typically contain an abundance of protein-degrading enzymes, called proteases, which produce symptoms such as

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pain, strong local swelling and necrosis, blood loss from cardiovascular damage complicated by coagulopathy, and disruption of the blood clotting system. Death is usually caused by collapse in blood pressure. However, this rule does not always apply as some viperid bites produce neurotoxic symptoms (Slowinski, 2000). Proteolytic venom is also dual-purpose: it is used for defense and to immobilize the prey, as with neurotoxic venoms, and also many of the enzymes have a digestive function, breaking down molecules in prey items, such as lipids, nucleic acids, and proteins. This is important, as many vipers have weak digestive systems. The venom from the species of family Viperidae showed moderate levels of proteinase, alkaline phosphomonoesterase, phosphodiesterase, arginine ester hydrolase, L-amino acid oxidase, hyaluronidase and a high nucleotidase and phospholipase A_2 activities. It has been reported that in snakes from family Viperidae venoms exhibited moderate to high alkaline phosphomonoesterase and arginine ester hydrolase activities and were devoid of acetylcholinesterase activity (Alam et al., 1996). The nonprotein fraction of their venoms is similar to those of Elapidae (Wallace, 2007). Due to the nature of proteolytic venom, a viperid bite is often a very painful experience and should always be taken seriously, even though it is not necessarily fatal. Even with prompt and proper treatment, a bite can still result in a permanent scar, and in the worst cases the affected limb may even have to be amputated. A victim's fate is impossible to predict as this depends on many factors, including (but not limited to) the species and size of the snake involved, the amount of venom injected (if any), and the size and condition of the patient before being bitten. The patient may also be allergic to the venom and/or the antivenin (Meier and White, 1995).

Vipers' venoms were reported to exhibit different toxic effects, due to the presence of lipolytic and proteolytic enzymes in their compositions (Tan et al., 1990). The ability of the venom to induce cytotoxicity (Bertke and Atkins, 1961), nephrotoxicity (Ickowiz et al., 1966), muscular dystrophy (Mohamed and Khaled, 1966), diverse immune response (Brando et al., 2000), alteration in general metabolism and above all, inducing hyperglycemia (Abdel-Raheem et al., 1985), also the contrary was reported, hypoglycemia (Abu-Sinna et al., 1993) .

There are relatively few studies on the long term effects of *E. coloratus* crude venom on clinical parameters. Report of biting does not cover the real picture of accidental envenomation, leaving the possibility of self healing and recovery.

This study aims to determine the bio-physiological changes from the first hour of envenomation, with an acute LD_{50} dose of *E. coloratus* crude venom extended to seven days monitoring the changes in some chosen blood parameters in male rats.

2. Materials and methods

2.1. Venom collection

The venom was obtained from *E. coloratus*. Snakes were kept in a serpentarum at the Zoology Department, College of Science, King Saud University, after being collected from central region of Saudi Arabia by a skilled professional hunter. The snakes were kept in large tanks, heat was provided from a 100 W lamp for a daily period of 9 h. Water was always available. Venom was milked from adult snakes, lyophilized and reconstituted in saline solution prior to use.

2.2. Determination of lethal dose (LD_{50})

The LD_{50} value was determined according to Sun (1963) and obtained from a dose mortality curves set up especially for venom. At the end of the experiment only surviving animals were anesthetized with pentobarbital (60 mg/kg body weight) and the entire blood was drawn by heart puncture technique. Dead animals were neglected.

2.3. Experimental design

Adult male albino rats (weight range: 200–250 g) were divided into two groups:

1. The first group (five animals) was considered as a control group treated with physiological saline injection (0.2 ml i.p.).
2. The second group was (35 animals) treated with LD_{50} (0.175 mg/kg, i.p.) of *E. coloratus* crude venom.

Treated animals were sacrificed off and two blood samples were collected into heparinized syringe by heart puncture technique at intervals of 1, 3, 6, 12, 24, 72 h on the seventh day. The first sample was collected in a heparinized tube (2.25 μl heparin/5 ml blood) for blood components count analyzed by an XF9030B Hematology Analyzer. The second non-heparinized blood sample was centrifuged (1000×*g* for 10 min), collected in test tubes with screw caps and stored at −20 °C until analyzed. In serum, creatinine was estimated according to Bartels (1971). Total proteins were measured according to the method of Lowry et al. (1951). Triglyceride level was carried out using kits from Boehringer, Mannheim. The level of glucose was determined according to the method of Howanitz and Howanitz (1984). Transaminase (ALT and AST), alkaline phosphatase (ALP) and γ-glutamyl transferase (γ-GT) activities were determined according to the recommendations of Scandinavian Committee on Enzymes (SCE) and Kits from Sera-Pack (Ames Division, Miles Ltd., England) were used.

2.4. Statistical analysis

In order to compare between the control and envenomated group, a Student's *t*-test was used. The data are presented as means ± S.E. and statistically analyzed using SPSS 10. Significance was set at the level of $P < 0.05$ or $P < 0.001$.

3. Results and discussion

Envenoming by (Viperidae: Echis) species lead to a combination of systemic and local hemorrhagic symptomatologies and up to 20% mortality rates without antivenom treatment (Casewell et al., 2009).

In the present experiment, an acute dose of LD_{50} of *Echis coloratus* caused the number of white blood cells (WBCS) to highly significantly increase for the first 24 h, then drop to control level after 72 h. On the seventh day WBCs were observed to be significantly decreasing (Fig. 1A). Leucocytosis followed by leukopenia was obvious. Such variability, often indicates the presence of opposing activities in crude venom, but may also indicate biphasic activity of a single component of the venom, such as, phospholipase A_2 (Longenecker and Longenecker,