BIOCHEMICAL AND HISTOCHEMICAL CHANGES IN RATS EXPOSED TO CRUDE VENOM OF SYNANCEIA VERRUCOSA

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ABSTRACT

This study aimed to determine the effect of crude venom of Synanceia verrucosa on some biochemical and histochemical changes in rats. The median lethal dose (LD50) of crude venom of Synanceia verrucosa was 351.3 µg/kg body weights. Some behavioral characters were observed as quick and irregular movement with the quick breathing, distinct relatively fast heart beats parlays which finally leachable from nose and internal bleeding and death. The results revealed that an elevation of glucose, cholesterol, aspartate and alanine transaminases, alkaline phosphates enzymes' triglycerides, urea and creatinine within 30-90 min. with significant differences (p<0.05) after injection by the toxin, while, the level of triglyceride was decreased gradually.

Histochemical studies were carried out on liver and kidney of treated rats. Histochemical sections of the treated liver by the crude venom of Synanceia verrucosa showed leucocytes infiltration, swelling of hepatocytes and enlarging in size of their nuclei and chromatin elements. Treated kidney by the crude venom of synanceia verrucosa showed that, the Bowman's capsule and epithelium cells of cortex and medulla of the kidney are shrunken. Some tubular kidney cortex showed a dilatation while they were atrophy. In addition, parenchymatous degeneration of cells of renal tubules, vaculation and filtration between the proximal tubules were observed.

Key words: Synanceia verrucosa, Lethal dose, behavioral, histochemical, biochemical, AST, ALT, ALP, glucose, cholesterol, urea' triglycerides, and creatinine.

1. INTRODUCTION

Stonefish are found throughout tropical, temperate regions and marine waters of the Indo-pacific. They prefer calm, water, estuaries and sheltered bays. The venom of the stonefish is a protein stored in the dorsal fin spines. The stings produced by the spines induce extreme pain, inflammatory, damage to the cardiovascular system, respiratory arrest, skeletal muscle paralysis and convulsion, sometimes leading to death (Saunders, 1959a; Breton et al., 2002; Khoo, 2002). Tay et al., (2016) reported that stonefish is one of the most venomous fish in the world with potential fatal local and systemic toxicity effects to human.

It is well known that, biochemical and histopathological alterations have been used as markers to understand animal hearth exposed to contaminants in lab (wester and canton, 1991; Pawan Kumar et al., 2013) and in field (Hinton et al., 1992; Schwaiger et al., 1997; Teh et al., 1997). These markers are necessary to monitor the target organs, including brain, heart, kidney and liver which are responsible for important functions, such as excretion and the deposition and bio-magnifications of toxins in the fish (Gernhofer et al., 2001).

Therefore, the main objective of this study is to determine the median lethal concentration of Synanceia verrucosa venom as well as its effects on some behavioral biochemical and histopathological changes of rats.

MATERIALS AND METHODS

Collection of fish samples

The specimens of synanceia verrucosa fish were collected from three sites on the Red Sea. (Altour city: Newibah and Hurgada). The three collection sites are rocky shores. Eleven collected fish were put in plastic containers containing sea water and it had to be washed in running tap water to remove these algae before the skin and spines could be seen clearly.
Crude extraction

The poison from the glands of synanceia verrucosa were absorbed by a needle of injector excised and stored in ice box. The venom is whitish color and transformed to milky color. The poison was ryophilized to transform to powder. The powder is whitish color and coarse which can be easily dissolved in saline (0.9 Meier) before use.

Determination of the tethal dose

Nine mare rats Swiss albino strain of about 100 gm body weight were used and one rat was kept as control. Eight rats were injected intraperitoneally with the crude venom which was extracted from synanceia verrucosa to determine the lethal dose according Meier, (1986).

Histological examination

The dissected liver and kidney of the rats were removed and placed into Bouin’s sorution in sea water for 24 hours. Fixed parts were then passed to the graded series of alcohol from 30 to 100%. They were cleared in toluene three times each for 5 minutes then embedded in paraffin wax. Sectioning was made by microtome at 5-7 pm thickness. Histological stains used were Harris hematoxylin and eosin combination (H&E), (Steedman, 1950) and Mercuric Bromophenol blue for demonstration of general proteins (Masia et al., 1953).

Biochemical parameters

Swiss albino strain of about 100 gm. was divided into two groups, normal control and treated groups (exposed to by LD10). The control rats (eight rats) and treated groups (40 rats) were sacrificed at 30, 60, 90,120 and 180 minutes. Serum glucose (mg/dl) was measured colorimetric by kits of spin reaction (Glucose-TR, Trinder.GOD-POD) according to Kaplan, (1984). Serum cholesterol (mg/dl) was measured colorimetric by kits of spin reaction (CHOD-POD, Liquid) according to Natio et al., (1984).Serum triglycerides (mg/dl) was measured colorimetric, by Kits of spin reaction (GPO-POD, Liquid) according to Buccolo, (1973). Serum urea (mg/dl) was measured colorimetric by kits of Biosystems (UREA/BUN_COLORUREAS/SALICYLATE) according to Chaney and Marabach, (1962). Serum creatinine (mg/dl) was measured colorimetric by kits of Biosystems (CREATININE/ JAFFE) according to Bartels, (1971). Aspartate and alanine transaminases activities (U/L) were kinetic measured by kits of spin reaction (NADH.Kinetic. UV. IFCC rec. Liuid) according to (Munay, 1984). Alkaline phosphatase activities (U/L) was measured kinetic by kits of EliTech Clinical systems (ALP (DEA) SL) according to Henderson and Donald (2001).

Statistical analysis

Mean, standard error, student test and significant difference were calculated by statistic a version, 12. P< 0.05 was regarded as statistical significant difference.

RESULTS AND DISCUSSION

As shown in Fig., (1), the median lethal concentration (LD50) of Synanceia verrucosa venom on rats was 351.3 g/kg body weights. The obtained results is in agreement with Kooh (1992) who worked on stonefish synanceia horrida and recorded lethal dose equal to 300 g/kg of body weight. Balasubashini et al., (2006a) reported that the toxicity value of Pterois volitans equal 42.5 ug/kg body weight. Also, the toxicity value of separated fraction from crude venom of the Egyptian scorpion was estimated by Abd-El-Rhim (1990) and found to be 1.392g/kg. On the other hand, the behavioral characters were observed as quick and irregular movement with the quick breathing, distinct relatively fast heart beats parleys which finally leachable from nose and internal bleeding and death. Gwee et al., (1994) reported that venoms from stonefish (genus Synanceia) have marked effects on the neuromuscular and cardiovascular systems and on vascular permeability. Also, Wang et al., (2007) decideil that the Verrucotoxin is the major component of venom from the stonefish (Synanceia verrucosa). Stings from the dorsal spines of the stonefish produce intensive pain, convulsions, hypotension, paralysis, respiratory weakness and collapse of the cardiovascular system, and some time leadings to death. These findings help for enhance our understanding of the toxic effects of verrucotoxin from the stonefish.
Biochemical parameters in serum of rats:

Changes in some biochemical parameters of rats after injection of LD_{10} crude venom of Synanceia verrucosa are shown in Table (2). The main findings are:

Glucose concentration was elevated from 117.0 ± 23.1 mg/dl (control value) to 140.0 ± 12.5 mg/dl at 30 min, after injection with LD_{10} of venom from fish Synanceia verrucosa with continues glucose level elevation to reach the highest value at 90 min (195.0 ±15.3 mg/dl). Further it showed a decrease and reached 130.0 + 19.9 mg/dl at 180 min. There are significant differences at the time intervals of 60, 90 and 120 min. (P<0.05). An increase in glucose level after rats exposure to venom may be due to an increase in catecholamine's which cause glycogenlysis in liver. Many studies reported similar results, serum hyperglycemia and liver glycogen depletion post venom injection (Mohamed et al. (1972); El-Asmer et al. (197D; Mohamed, et al., (1980), Ramadan et al., (1980), Ali et al., (1989) and Balasubashini et al., (2006a). Control value of urea in serum of rats was 20.0+3.20 mg/dl. After injection by LD_{10} of fish venom the urea level was elevated gradually to reach the highest value 52+ 3.1 mg/dl at 120 min. There was significant difference (p<0.05) at all time intervals except at 180 min (P>0.05). This result agrees with Saminathan et al., (2006).

In this study, serum aspartate aminotransferase (AST) activity was measured as myocardial infarction parameter. These enzymes are present in large amount in the muscular tissue and, in particular myocardium. The extent of increase enzyme activity is dependent on the size of infraction; the larger the infraction size, the higher is the activity of these enzymes in serum (Streov, 1989). The enzyme activity of aspartate transaminase (AST) was increased at 30 min. of the venom injection (131.0+15.7) to reach the maximum activity at90min. (256.00+22.7)U/L. Then started to decrease but still higher than control activity (97.0+28.4 U/L). There was a significant difference at all time except at 30 min. (p > 0.05). present results can be explained on the basis of that injection of the crude venom may lead to heart and liver affection (the crude venom is cardio-hepatotoxic). The highly significant increase in the hepatic Asr is compatible with increase in the protein catabolism and urea formation in the first 30 min. post injection. ALT enzymes appear as a cytosolic enzym% present in high concentration in the liver. Although it is absolute amount is less than that of AST, greater proportion is present in liver compared with heart and skeletal muscle. Thus the increase of ALT in serum is more specific for liver damage than AST (Sherlock, 1989). The enzyme activity of the control was 38.0+1 4.4 U/L" it increased at 30 min. (33.0+9.8IU/L) and reached to the highest activity 63.0+12.7U/L at 120min then declined to 53.0+14.2U/L at 180 min but still higher than control. There are significant differences at 90 and 120 min (P<0.05). The obtained results are in accordance with Mansour et al., (1980); Al-Hassan et al., (1985) and Ali et al., (1989). serum alkaline phosphatase (ALP) enzyme is also determined to assess the excretory function of the liver (Wilhelm,1982). This enzyme is mainly located in the plasma membrane lining the bile canaliculated and sinusoids of the liver (Goodlad and clark, 1982).The enzyme activity of Alkaline phosphatase (ALP) was increased gradually after injection with LD_{10} of venom of fish from 174.0+81.6 U/L. (control value) to reach the maximum activity (262.0+28.7 U/L) at 90 min. Then started to decrease and reached around
control level at 180 min (190.0±23.9). There are insignificant differences at all-time intervals (P>0.05) except at 90 min. (P<0.05). This result is in accordance with Wootton, (1964), Mansour. (1975); Harprzer. (1977) and Baron, (1979) and Ali et al., (1989).

On the other hand, it was found that histopathological alterations can be used as markers to understand animal health exposed to contaminants in lab (Wester and Canton, 1991; Pawan Kumar et al., 2013) and in field (Hinton et al., 1992; Schwaiger et al., 1997 and Teh et al., 1997).

**Effect of toxin on Liver**

The normal liver lobes are divided into indistinct lobules with the central vein in the middle and at the corners the portal triad (consisting of branches of the hepatic artery, the portal vein and the bile duct). The normal liver parenchyma consists of large polygonal hepatocytes with large central nuclei and arranged in cords. The blood sinusoids are located between the hepatocytes cords and lined with fenestrated endothelium. The kupffer cells are small in size locating in the sinusoids and associated with the endothelium (Fig., 1). Histological sections of the treated liver by the crude venom of the *Synanceia verrucosa* showed leucocytes infiltration, swelling of hepatocytes and enlarging in size of their nuclei and chromatin elements. Also, apparent increase in numbers of Kupffer cells. The hepatic cords are fused together and lost their architecture. Histologically, the treated liver of mice showed increase in proteins level (Fig., 2-4).

**Effect of toxin on Kidney**

The normal kidney is covered by a thin connective tissue capsule and consists of outer cortex and inner medulla. The kidney cortex which comprises a glomerulus surrounded by the Bowman’s capsule and proximal convoluted tubule. The epithelium lining the parietar surface of the Bowman’s capsule is frequently cuboidal in adult male mice. The proximal convoluted tubules are lined by cuboidal epithelium with the microvilli, while the cuboidal epithelium of distal convoluted tubules has no microvilli (Fig.,5). Histopathologically, sections of extracted kidney by the crude venom of *Synanceia verrucosa* showed that, the Bowman’s capsule and epithelial cells of cortex and medulla of the kidney are shrunken, some tubular kidney cortex showed a dilatation and while others one atrophied. In addition, parenchymatous degeneration of cells of renal tubules, vaculation and infiltration between the proximal tubules were observed (5-7). Histochemical, the treated kidney showed increase in protein level (Fig., 8-9).

Histopathologically, the effect of crude extraction of *Synanceia verrucosa* on the liver and kidney tissue of mice showed pathological changes such as infiltration vaculation and swelling of liver hepatocytes. While, the treated mice kidney showed that the Bowman’s capsule and epithelial cells of cortex and medulla are shrunken; some tubular kidney of cortex showed a dilatation and atrophy. Also, parenchymatous degeneration of cells of renal tubules and infiltration between the proximal tubules were observed. Balasubashini et al., (2006a) observed histopathological changes as the effects of *petrios volitanis* venom on the vital organs such as liver, heart, brain, lungs, and kidney of the venom-treated rats. Similar results were observed during administration of venom from *Tharassophyrene nattereri* (Fonseca 2000), *Tityusserrulatcis* (Correa et al., 1997) and *Conus lorrossi* (Saminathan et al., 2006).

The present result agree with Balasubashini., et al, (2006b), who study the effect of venom of *pteros volitans* to estimate the histopathological changes due to *Pterios Volitans* venom on the vital organs (liver, kidney and brain tissues).

![Fig. 1. Linear regression equation of extracted venom of *Synanceia verrusosa.*](image-url)
Table (2) changes in some biochemical parameters of rats after injection with LD_{10} venom of *Synanceia verrucosa*.

Average of 8 observation±S.D.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>180min</th>
<th>120min</th>
<th>90min</th>
<th>60min</th>
<th>30min</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>130.0±19.9</td>
<td>185±18.1*</td>
<td>195.0±15.3*</td>
<td>177.0±14.2*</td>
<td>140.0±12.5</td>
<td>117.0±23.1</td>
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<tr>
<td>Cholesterol</td>
<td>82.0±17.2</td>
<td>131.0±16.1*</td>
<td>116.0±1.3*</td>
<td>101.0±15.1*</td>
<td>88.0±17.1</td>
<td>75.0±19.8</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>5.0±19.9*</td>
<td>99.0±20.2*</td>
<td>107±19.1*</td>
<td>123±22.6*</td>
<td>146.6±31.0</td>
<td>168.0±89.1</td>
</tr>
<tr>
<td>Urea</td>
<td>31.0±3.2</td>
<td>52.0±3.1*</td>
<td>48.0±4.03*</td>
<td>37.6±2.2*</td>
<td>26.0±2.81*</td>
<td>20.0±3.20</td>
</tr>
<tr>
<td>Creatinine</td>
<td>2.5±0.30*</td>
<td>2.2±0.20*</td>
<td>2.0±0.31*</td>
<td>1.34±0.22*</td>
<td>0.92±0.21*</td>
<td>0.691±0.12</td>
</tr>
<tr>
<td>AST</td>
<td>187±29.0*</td>
<td>210±25.2*</td>
<td>256.0±22.7*</td>
<td>210.0±19.8*</td>
<td>131.0±15.7</td>
<td>97.0±28.4</td>
</tr>
<tr>
<td>ALT</td>
<td>53.0±14.2*</td>
<td>63.0±12.7*</td>
<td>58.0±11.11*</td>
<td>47.0±10.2</td>
<td>43.0±9.81</td>
<td>38.0±14.4</td>
</tr>
<tr>
<td>ALP</td>
<td>190±23.9</td>
<td>225±26.8</td>
<td>262±28.7*</td>
<td>210±35.0</td>
<td>193.0±42.1</td>
<td>174.0±81.6</td>
</tr>
</tbody>
</table>

*P<0.05

**Figure (2 A, B):** control and treated liver

**Figure (3) (A, B) control and treated liver stained Bromophenol blue**
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