Al Azhar Bulletin of Science Vol. 27, No. 2 (December) 2016, pp. 67-74

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

Abd-Allah Elsyed Ali[1], Mohamed H. Mourad[2]; Mohamed A. Hamed[2]; Emad H. Abou El-Naga and Ali Abd-Elrheem Ibrahim,[2]

1 Marine Biology department Faculty of Science, Suez Canal., Ismailia, Egypt.
2 National Institute of Oceanography and Fisheries, Egypt.

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 (LD_{50}) 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' trigrycerides, urea and createnine 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 river and kidney of treated rats. Histochemical 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. 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 in 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 ar., 2002; Khoo, 2002). Tay et ar., (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 yerrucosa* 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 determinate 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. Historogical 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 LD_{10}). 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 (LD_{50}) 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 l.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 parlays 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 ll7 .O + 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 l20 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. 097D; Mohamed, et al., (1980), Ramadan et al., (1980), Ali et al., (1989) and Balasubashini et al., (2OO6a). 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 l20 min. Then started to decrease (31.0+3.2 mg/dl) at 180 min. There was signification difference ($p<0.05$) at all time intervals except at 180 min ($P>0.05$). This result agrees with Saminathan et al., (2006). Also, Cholesterol level was increased gradually from 75.0 +19.8 mg/dl (control value) to reach the maximum amount at 120., (131.0+1 6.l mg/dl) after injection with LD_{10} of fish venom. Then it declined to 82.0+1.2 mg/dl at 180 min. There is significant difference ($P<0.05$) at time intervals 60, 90 and 120 min. On the other side, triglyceride level was decreased gradually from 168+89.1 mg/dl (control level) to reach the lowest value 85.0+19.9 mg/dl at 180 min after injection with LD_{10} of fish venom. There was insignificant differences only at 30 minutes ($P>0.05$). Finally, the level of creatinine in serum of rats was increased from 0.60+0. 12 mgldl (control value) to 2.5+0.30 mg/dl at 180min. There were significant differences among time intervals after injection with LD_{10} of fish venom ($P<0.05$).

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 enzyrire 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 1ead 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 euzyme activity of the control was 38.0+1 4.4 U/L" it increased at 30 min. (33.0+9.8lU/L) and reached to the highest activity 63.0+12.7U/L at l20min 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 execratory 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. (1 975); Haprper. (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' Historogicalry, the treated river 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 proximar convoluted tubures 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 epithelium 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 pathclogical 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 *petrois 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 *Tharassophryne 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 pterios volitans to estimate the histopathological changes due to *Pterios Volitans* venom on the vital organs (liver, kidney and brain tissues)

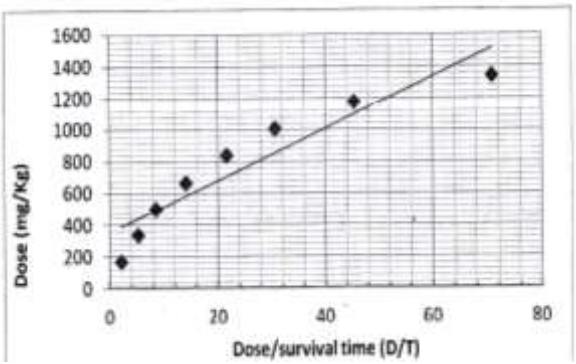


Fig. 1. Linear regression equation of extracted venom of *Synanceia verrusosa.*

Table (2) changes in some biochemical parameters of rats after injection with LD_{10} venom of *Synanceia verrucosa*

Average of 8 observation±S.D.

180min	120min	90min	60min	30min	Control	Parameters
130.0±19.9	185±18.1*	195.0±15.3*	177.0±14.2*	140.0±12.5	117.0±23.1	**Glucose**
82.0±17.2	131.0±16.1*	116.0±1.3*	101.0±15.1*	88.0±17.1	75.0±19.8	**Cholesterol**
5.0±19.9*	99.0±20.2*	107±19.1*	123±22.6*	146.0±31.0	168.0±89.1	**Triglyceride**
31.0 ±3.2	52.0±3.1*	48.0±.03*	37.0±2.2*	26.0±2.81*	20.0±3.20	**Urea**
2.5±0.30*	2.2±0.20*	2.0±0.31*	1.34±0.22*	0.92±0.21*	0.601±0.12	**Creatinine**
187±20.0*	210±25.2*	256.0±22.7*	210.0±19.8*	131.0±15.7	97.0±28.4	**AST**
53.0±14.2*	63.0±12.7*	58.0±11.11*	47.0±10.2	43.0±9.81	38.0±14.4	**ALT**
190±23.9	225±26.8	262±28.7*	210±35.0	193.0±42.1	174.0±81.6	**ALP**

*P<0.05

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

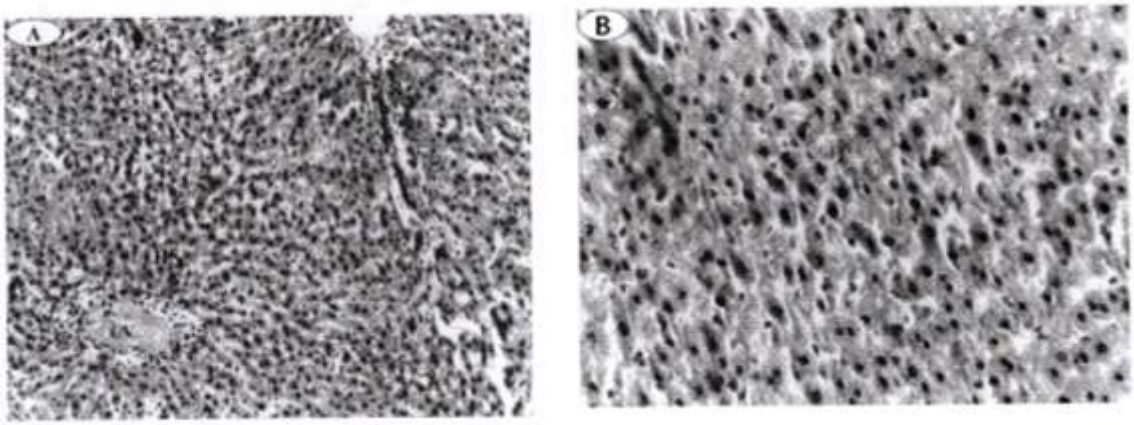
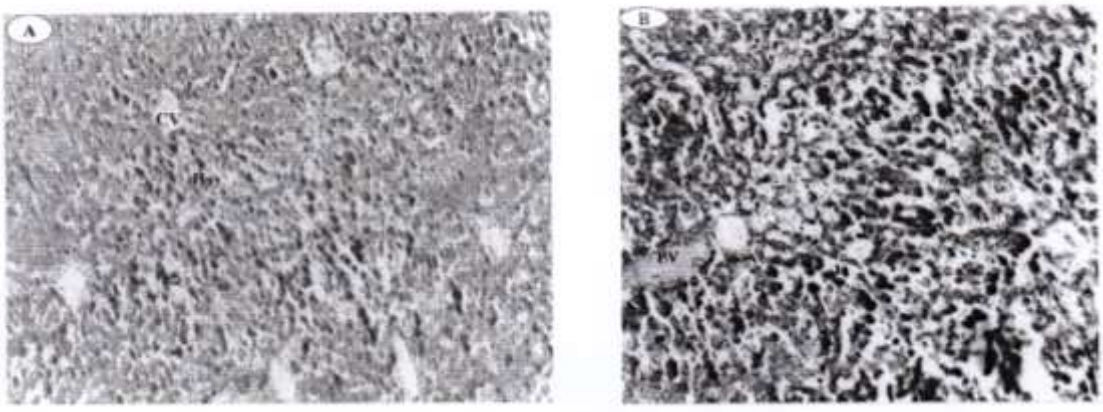


Figure (3) (A, B) control and treated liver stained Bromophenol blue



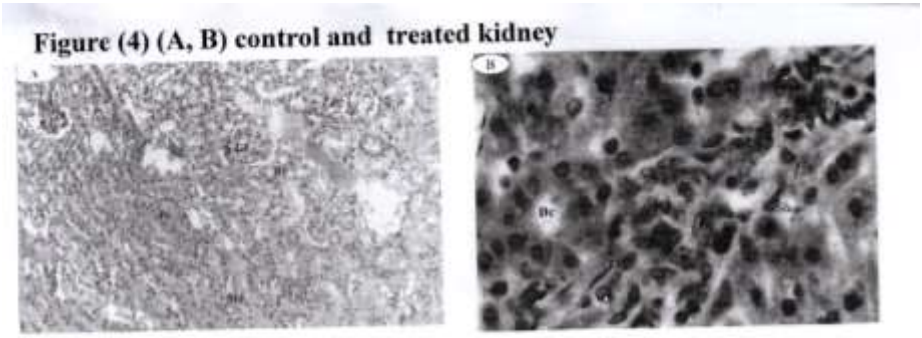


Figure (4) (A, B) control and treated kidney

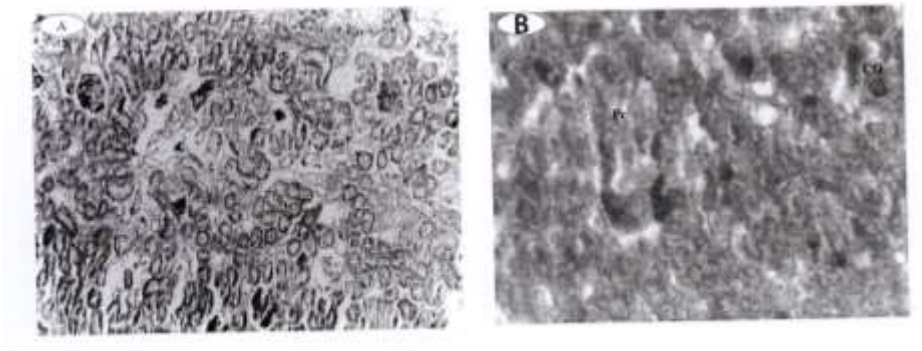


Figure (5)(A,B) control and treated kidney stained Bromophenol blue

REFERENCES

Saunders, P. R. (1959b): 'venom of the stonefish stiminceia horrida (Linnaeus). Arch. Int.pharmacodyn., 123, 195.

Abbd-El-Rhim, S. A. (1990). Physiological studies of the separation fraction from the venom of an Egyptian scorpion of living cell of some organs. PhD thesis zoology department. Faculty of science, Sohage.

Al-Hassan, J. M.; Thomsson, M.; Fatima, T. and Gubler c. J., (1995). Toxic effects of the soluble skin secretion from the Arabian Gulf catfish (Arius thallassinus) on plasma and liver enzymes levels. Toxicon, 23: 532

Ali, M.; Thomson, M.; Al-Hassan, J.M.; Al-Saleh, J.; Fayado S.; Assad, H. and Criddle, R. S. (1989). Comparative biochemical and pharmacological properties of epidermal secretion from Arid catfish of the Arabian Gulf. Comp.biochem. physiol.,928: 205.

Balasubashini, M. S.; Karthigayan, S. T. Somasundaram, and T. Balasubramanian; P. Viswanathan venugopal P. Menon (2006a). in Vivo and in Vitro Characterization of the Biochemical and Pathological Changes Induced by Lionfish (Pterios Volitans) Venom in Mice. Toxicol. Mech. Methods , 16: 525-531.

Balasubashini, M.S., Karthigayan, S., Somasundaram, S. T., Balasubramanian, T., Rukkumani, R., and Menon, V. P., (2006b). FV induces apoptosis in HEp-2 and HeLa cells: an insight into the mechanism of induction. J Carcinog. 5 (1): 27-35.

Baron, D. N. (1979). In short text book of chemical pathology 3rd Ed. Hodder& Stoughton, Guild ford, Surey, PP 94

Bartels H, Bohmer M. Eine (1971). Mikromethod zuf kereatininbestimmung. Clin Chem Acta 32:81 - 85.

Breton P, Delamanche I, Buee J, Goudey-Perriere F, and Perriere C (2002). Evidence for a neurotoxic activity in crude venom of the stonefish (Synanceia verrucosa). J Nat Toxins 1 1 : 305-3 13.

Buccolo G. et al (1973). Quantitative determination of serum triglycerides by use of enzymes. CIin. Chem, 19(5): 476- 482.

Chaney, and A.l. Marbach., E.P. (1962). Modified reagents for determination of urea and ammonia. Clin Chem 8;130-132.

Correa, M. M., Sampaio, S. V., Lopes, R. A., Mancuso, L. c., cunha, O. A. B., Franco, J. J. and Gigrio, J. R., (1997). Biochemical and histopathological alterations induced in rats by

Tityusserrulatus scorpion venom and its major neurotoxin tityustoxin- 1. Toxicon,35:1053-1067.

El- Asmer, M. F.; soliman, S. F.; Ismail, M. and osman,O.H. (1974). Glycemic effect of venom from the scorpion Buthus minax (L.koch). Toxicon 12: 249.

Gernhofer, M., Pawet, M., Schramm, M., Miiller, E. and rriebskorn, R., (2001). Ultrastructural biomarkers as tools to chara cterize the health status of fish in contaminated streams. Journal of Aquatic Ecosystem, Stress and Recovery, S: 241-260.

Goodlad, G. A. J. and clark, C. M. (1982). Alterations in hepatic 5

nucleotides in the tumer bearing rat, Enzyme 29:119

Gwee, M. C. E, Gopalkrishnakone, P. yuen, Khoo. R. H. E and Low. K. S. Y. (1994). A review of stonfish venoms and toxins Pharmac. Ther. Vol. 64, pp. 509-528, 1994

Haprper, H. A. (1977). In: Review of physiological chemistry. 17th end large medical publication, Los Altos, california, (1977).

Henderson, A.R., and Donald W.M., (2001). Enzymes Tietz fundemmental of Clinical Chemistery 5th Ed., Burtis ,C.A & Ashwood, E. R.(W.B) Saunders eds. philadelphia USA), 352

Hinton, D. E., Baumann, p. c., Gardner, G. R., Hawkins, W. 8., Hendricks, J. D., Murcherano, R. A. and okihiro, M. S., (1992). Histopathologic biomarkers. In: Hugget, R., R. Kimerle, P. Mehrle& H. Bergman (Eds.). Biomarkers-biochemical, physiological and histological markers of anthropogenic stress. Boca Raton, Lewis Publishers, pp.155-195.

Kaplan L.A. (1984). Glucose. Kaplan A et al Clin chem the C.V. Mosby Co. St Louis Toronto Princeton; 1032-1036.

Khoo HE (2002). Bioactive proteins from stonefish venom. Clin, Exp, Pharmacol. Phys iol' 2002; 29 :802-6.

Mansour. M. A. (1975). Physiological and biochemical studies on the effect of crude toxin extracted from the fish A. Hispidus'Linn" or some mammals. Ph.D thesis Faculty of science, Tantauniversity, Tanta.

Mansour, M. A.1 Abdel-hamid, M. E.; Ramadan, M. A.1 and Al-Nagdy, S. A. (1980). Physiochemical studies on the protein and minerals metabolism and the b100d in rabbits poisoned by Arthron hispidus tetradotoxin ann' Zool., 16;139

Masia, D.; Brewer, P. A and Alfert, M. (1953). The cytochemical staining and measurement of protein with mercuric bromophenol blue Biol. Bull., 104:57-67.

Meier, J. and Theakston, R. D. G., (1986). Approximate LD_{50} determination of snake venoms using eight to ten experimental animals Toxicon 24 : 395-401 Pergamon Press UK.

Mohamed, A. H., Foud, F., A. Abdel. Baset, A., Amr Abbas, N., Zahran. F. (1980). Metabolicstudies of the Egyptian and allied African snake venom: toxicon 8:381.

Mohamed, A. H.; Fatmfl, A. H. and Eldamarawy (1972). Diabetogenic actions of Naja nigricollis venom, effect on glucose tolerance, plasma insuline like activity and blood potassium' Toxicon 10: 151.

Murray R (1984). Aspartate aminotransferase. Kaplan Aetal Clin Chem the c.v. Mosby co. st Louis Toronto Princeto"; 1112- 1116.

Natio H. K. (1984). Cholesterol. Kaplan Aetal Clin Chem the C. V Mosby co. St Louis Toronto Princeton;11 94-11206 and 437 .

Pawan Kumar, K., Venkateshvaran, P. P., Srivastava, S' K'' Nayak S. M., Shivaprakash and Chakraborty, S. K., (2013). Toxicity and histopathorogical observations on arbino mice on intra-peritoneal iniection of three species of Conus. IJARPB,3(4),1-11.

Ramadan, M. A.1 Tash, F. M.; Al Nagdy, S. A.1 Mahdy, Z. and Mansour, M. A. (19S0) Effect of toxin extracted from the red sea fishes Arthron hispidus on the metaborism of carbohydrates and lipid metabolism. Zool. Soc. Egypt. Bull. 22:22

Saminathanr R., Babuji, S. Rsethupathy, S., Viswan-athan, P., Balasuharamanian, T., and Gopalakrish-anakone, P. (2006). Clinico toxicological characterization of the acute effects of the venom of the marine snail, Conus loroisii. Acta Trop. 97:75-87.

Schwaiger, J., Wanke, R., Adaffi, S., Pawert, M., Honnen, W. and Triebskorn, R., (1997). The use of histopathological indicators to evaluate contaminant-related stress in fish. Journal of Aquatic Ecosystem, Stress and Recovery, 6:75-86.

Schwaiger, J., Wanke, R., Adafil, S., Pawert, M., Honnen, W and Triebskorn, R., (1997). The use of histopatological indicators to evaluate contaminant-related stress in fish. Journal of Aquatic Ecosystem, Stress and Recovery, 6:75-86.

Sherlock, S. (1989). Diseases of liver and Biliary system. Blackwell scientific publication, Oxford-London.

Steedmar, H. E. (1950). Quart. J. Micr. Sci. 91 ,477.

Stroev, E. A. (1989). Clinical biochemistery in: Biochemistery pp.425-430 first Ed., Mirpublishers, Moscow

Tuy, T. K.; Zhe Chan, H; Ahmad, T.T-, Tze Hau Low K, and AbWahab N. (2016). Stonefish envenomation of hand with impending compartment syndrome. Journal of Occupational Medicine and Toxicology 11:23

Teh, S. J., Adams, S. M. and Hinton, D. E., (1997). Histopathological biomarkers in feral freshwater fish populations exposed to different types of contaminant stress. Aquatic Toxicology,37: 5l-70.

Wang. J., Yazawa K., Ying Hao L., Onoue Y.,rc, Kameyama M.(2007). Verrucotoxin inhibits KATP channels in cardiac myocytes through muscarinic M3 receptor-PKc pathway. European Journal of Pharmacology 563 (2007) 172-179

Wester, P. W. and Canton, J. H., (1991). The usefulness of histopathology in aquatic toxicity studies. Comparative Biochemistry and Physiology (C), 100: 115-117.

Wilhelm, R. F. (1982). In: Human biochemistry. Macmillan publishing Co. Inc New York, Toronto and London.

Wootton, D. P. (1964). Microanalysis in medical biochemistery. J and Churchall livingstone. Ltd, Edenberg and London, 6th ed.