



## Histological and biochemical studies on the effect of tetrodotoxin extracted from Puffer fish (*Lagocephalus sceleratus*) against carbon tetrachloride induced hepatotoxicity in albino mice.

Khalil E. A.<sup>1</sup>; Swelim H. H.<sup>1</sup> and Zaky M. A.<sup>2</sup>

1- Department of Zoology, Faculty of Science, Ain Shams University, Cairo, Egypt

2- National Institute of Oceanography and Fisheries, Suez, Egypt.

### ARTICLE INFO

#### Article History:

Received: May 5, 2018

Accepted: June 27, 2018

Available online: July 2018

#### Keywords:

*Lagocephalus sceleratus*

Puffer fish

Carbon tetrodotoxin

Hepatotoxicity

Liver function

histological examination

Biochemical studies

### ABSTRACT

The aim of this study was to investigate the effect of tetrodotoxin (TTX) extracted from puffer fish *Lagocephalus sceleratus* on hepatotoxicity induced by carbon tetrachloride (CCl<sub>4</sub>). Adult male albino mice were divided into four groups: 1) control group treated with saline; 2) CCl<sub>4</sub> group; 3) mice injected with 5 doses of TTX (1 µg/kg) and 4) mice injected with 5 doses of TTX (1 µg/kg) then treated with CCl<sub>4</sub> for 6 weeks. The results showed that injection with 5 doses of TTX extract significantly reduced the impact of CCl<sub>4</sub> toxicity on the serum markers of the liver, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and albumin (Alb). However, the biochemical results of TTX group were significantly different from the control group. On the other hand, the histologic examination of liver sections from mice given CCl<sub>4</sub> showed hyperplasia of bile ducts, excessive fibrosis in portal areas associated with detachment from parenchyma and lymphocytic infiltration. Other damaging features were microvesicular fatty change, pyknotic nuclei, karyolytic nuclei, irregular dilated sinusoids with active Kupffer cells and some hepatocytes showed necrosis. On the other hand, mice injected only with TTX showed an almost normal pattern with few random sites of hydropic degeneration. While the liver sections from TTX + CCl<sub>4</sub> group showed noticeable hydropic degeneration, lymphocyte infiltration, and hemorrhage especially in the portal area. However, no signs of fatty degeneration or excessive fibrosis were observed like those noticed in case of CCl<sub>4</sub>. Moreover, no signs of coagulate necrosis or lytic necrosis were noticed. It can be concluded that treating with TTX extracted from puffer fish, *Lagocephalus sceleratus* decreased the damaging effect of CCl<sub>4</sub> on the liver of albino mice.

### INTRODUCTION

The metabolism and the relationship of the liver to the gastrointestinal tract make it the major target in preclinical toxicity studies of drugs and xenobiotics. The agents which cause acute and chronic hepatic injury have been a great concern for hepatologists. Both animal experiments and *in vitro* studies using cultured hepatocytes are useful models for studying the metabolism and toxicity of hepatotoxins, and the effects of various hepatoprotective agents (Victor and Eric, 2004).

Hepatotoxins, such as ethanol, acetaminophen, and carbon tetrachloride (CCl<sub>4</sub>), sparked off the liver injury which is characterized by varying degrees of hepatocyte degeneration and cell death (Wu *et al.*, 1999). Vitaglione *et al.* (2004) suggested that reactive oxygen species (ROS) including superoxide and hydroxyl radicals are known to play an important role in liver disease's pathology and progression moreover, they have been proven to associate the intoxication by CCl<sub>4</sub>. Documented evidences suggested that CCl<sub>4</sub> has been commonly used as a hepatotoxin in experimental hepatopathy (De-Groot and Noll, 1986; Hsu *et al.*, 2008). Covalent binding of the metabolites of CCl<sub>4</sub>, trichloromethyl free radicals, to cell proteins is the initial step in a chain of events that eventually lead to membrane lipid peroxidation and finally to cell death (Weber *et al.*, 2003).

Many metabolites were extracted from fish, sea snakes and aquatic mammals but their biomedical uses are scanty. Various fish species are used to extract fish oil, rich in omega-3 fatty acids, which are used in the preparation of various kinds of drugs for the remedies of human beings, such as arthritis. Throughout the world about 500 species of fishes are considered toxic, the most spectacular substance with pharmacological importance extracted from them is Tetrodotoxin (TTX) (Blunt *et al.*, 2007).

Tetrodotoxin is a neurotoxin which is found in freshwater and marine species including puffer fish, blue-ringed octopus, Indo-pacific goby, gastropod mollusks, ocean sunfish, triggerfish and boxfish (Yotsu *et al.*, 2007; Huang *et al.*, 2008 and Mebs and Yotsu, 2012). Tetrodotoxin is heat stable, water soluble and a non-protein quinazoline derivative (amino-perhydro-quinazoline) C<sub>11</sub>H<sub>17</sub>N<sub>3</sub>O<sub>8</sub>, one of the strongest marine paralytic toxins known today. It can be absorbed through mucous membranes and the small intestine. TTX named after the order of fish from which it is most commonly associated, the tetradontiformes (tetras-four and odontos-tooth) or the tetraodon puffer fish (Sabrah *et al.*, 2006). The distribution of TTX in puffer fish bodies appears to be species-specific. Furthermore, there is a compartment variability of TTX bioaccumulation, as shown by organs exhibited different toxicities. So, in marine species of puffer fish, liver and ovary generally present the highest toxicity, followed by intestines and skin (Lee *et al.*, 2000). TTX isolated from puffer fish and many other marine organisms has become a useful tool for researchers studying the voltage-gated sodium channel, playing an important role in many biological experiments (Oliveira *et al.*, 2003).

The present study was undertaken to investigate the activity of TTX extract against CCl<sub>4</sub>-induced oxidative stress and hepatotoxicity in mice. Hepatic activities of AST, ALT and content of Alb in serum were measured to monitor liver injury. The extent of the CCl<sub>4</sub>-induced liver injury and TTX were also analyzed through histopathological examination.

## MATERIALS AND METHODS

### Preparation of TTX extract

Twenty frozen Puffer fishes (*Lagocephalus sceleratus*) weighing (60-70 g each) and their length reach (18 – 30 cm) and width (5 - 7 cm) collected from (Red Sea) during May 2016 were transported to histology laboratory in Department of Zoology, Faculty of Science, Ain Shams University. They were immediately washed thoroughly under tap water until they were completely cleaned then they were dissected out for the preparation of their extract through the following procedures. The extraction technique was followed according to Zaki *et al.* (2001) with slight

modification. Tissues from ovary, liver, and skin were obtained after dissection. The dissected tissues were weighed and extracted with 5 volumes of 1 % acetic acid in methanol. 1 gm in 5 ml was homogenized and heated at 70 °C for 10 minutes. Then the mixture was centrifuged at 10000 RPM for 15 minutes. The supernatant was separated, concentrated and defatted with an equal volume of chloroform. The aqueous layer was concentrated by rotary evaporator working under vacuum and then freeze-dried at -50 °C for 36 hours.

### **Experimental Animals**

Adult male Swiss Albino mice weighing 25-30 g were obtained from Theodor Bilharz Research Institute (TBRI). Animals were grouped and housed in polyacrylic cages (six animals per cage in the well-ventilated animal house of the Department of Zoology, Faculty of Science, Ain Shams University. Animals were maintained at 12 h light/dark cycle, given a commercial pellet diet (protein, fibers, minerals, and vitamins) and tap water *ad libitum* for one week before the start of the experiment as an acclimatization period. All animal experiments were performed under protocols approved by the local institutional animal ethics committee of Ain Shams University.

### **Toxicological test**

Overnight-fasted mice received five intraperitoneal doses of Tetrodotoxin (TTX) extracted from puffer fish at dose level of (1 µg/kg of body weight dissolved in saline) every next day for 10 days. Animals were observed carefully for 24 hours after the first injection and then were followed for next 14 days after the last dose to record the death case. The implemented dose was chosen depending on the calculations of lethal dose (LD<sub>50</sub>) and the sub-lethal (1/10 LD<sub>50</sub>) dose according to the method of **Saganuwan (2011)**.

### **Experimental design**

Twenty-four adult male Swiss albino mice were randomly assorted into four groups: 1) control mice injected i.p. with saline (control group, n=6); 2) mice injected i.p. with CCl<sub>4</sub> (1 ml/kg of body weight, dissolved in olive oil to reach a final concentration of 20%) twice a week for 6 weeks (CCl<sub>4</sub> group, n=6) according to Fan *et al.* (2013); 3) mice administered i.p. 5 doses of TTX extract (1 µg/kg of body weight dissolved in saline) every next day for 10 days (TTX group, n=6); and 4) mice received i.p. TTX extract (1 µg/kg of body weight dissolved in saline) every next day for 10 days then intoxicated with CCl<sub>4</sub> in the same way as in CCl<sub>4</sub> group (TTX + CCl<sub>4</sub> group, n=6).

### **Blood collection and biochemical assays**

At the end of the experimental period, all the animals were anesthetized using diethyl ether and sacrificed after 48 hours from the last dose to collect blood samples. Blood samples were collected in non- heparinized tubes and were allowed to clot at room temperature for 1 hour. Serum samples were obtained by centrifugation at 3000 rpm for 20 min. The level of serum ALT, AST, and albumin concentrations were measured by Automated Biosystems analyzer A25 using commercial available specific kits (Spain; Biosystems S.A.) and the method followed was according to Gella *et al.* (1985) and Doumas *et al.* (1997).

### **Histological examination**

The livers of dissected mice were firstly washed in 0.85 saline solutions to remove the blood. Small pieces (5mm × 5mm) were fixed in 4% paraformaldehyde for 24 hours, dehydrated with ascending series of ethanol. Then cleared in terpineol for at least 48 hours and infiltrated with paraplast at 60 °C and finally embedded using L-shape metallic mold. Sections of 5–6 µm thick were cut using electronic digital microtome then affixed on clean glass slides using dilute egg albumin solution

and dried in incubator for at least 24 hours at 38 °C. Paraffin sections were deparaffinized, hydrated to distilled water and stained with Mayer's hematoxylin and counter stained with 1 % Eosin. Then dehydrated, cleared, mounted using DPX. Microscopic examination was done using Philips photographic microscope and photography was achieved with the digital camera Optika B5.

### Statistical Analysis

All values were expressed as mean  $\pm$  SD. Results of biochemical studies were statistically analyzed using one-way analysis of variance (ANOVA) followed by Post-hoc Bonferroni's test for multiple comparisons. All statistics were processed by using SPSS software (Statistical Package for Social Science). Statistically significant differences between groups were defined as  $p < 0.05$ .

## RESULTS

### Toxicological results

The data presented in Table (1) illustrate the mortality rate of TTX crude extract to determine The lethal dose (LD<sub>50</sub>) of *Lagocephalus sceleratus* was obtained from the following equation. The LD<sub>50</sub> was 0.019 mg/Kg.

$$LD_{50} = \frac{\text{The sum of all doses administered}}{\text{The sum of all the mice that died in each group}} \times 5$$

Table 1: Determination of LD<sub>50</sub> of TTX crude extract of pufferfish (*Lagocephalus sceleratus*)

Dose (mg/Kg)	No. of animals	Survivals (S)	Death (D)	Total		% Mortality
				S	D	
0.01	10	9	1	21	1	4.5%
0.015	10	5	5	12	6	33%
0.02	10	4	6	7	12	63%
0.03	10	2	8	3	20	86.9%
0.04	10	1	9	1	29	96%

### Biochemical results

**Table (2)** illustrate the results of statistical analysis as Mean  $\pm$  SD and percent of change of ALT, AST and total albumin for all groups respectively. ALT (alanine aminotransferases): The results obtained from the present study showed that administration of TTX 1  $\mu$ g/kg only significantly increases the serum activity of ALT as compared to the control. Also, administration of CCl<sub>4</sub> causes highly significant increase in the serum level, while the TTX 1  $\mu$ g/kg + CCl<sub>4</sub> group exhibited significant increase as compared to the control and significant decrease compared to CCl<sub>4</sub> group. This indicates that TTX has a suppressive effect on the induced toxicity of CCl<sub>4</sub>.

AST (aspartate aminotransferases): Mice injected with CCl<sub>4</sub> revealed the highest significant increase in serum AST activity among all groups, while the control group showed the lowest serum enzyme activity. Tetrodotoxin group featured significant increase in the AST level but also showed significant decrease as compared to CCl<sub>4</sub> group. Administration of TTX 1  $\mu$ g before CCl<sub>4</sub> significantly decrease the rise in serum AST as compared to CCl<sub>4</sub> treated mice in the same pattern that happened with the ALT.

Albumin: Administration of TTX only significantly decreases the albumin content as compared to the control group. CCl<sub>4</sub> group displayed significantly the lowest serum level of albumin when compared to all other groups, while the control group demonstrated the highest level. Administration of Tetrodotoxin 1  $\mu$ g/kg before

CCl<sub>4</sub> caused insignificant change in response to the CCl<sub>4</sub> treated mice while showed significant decrease as compared to the control.

Table 2: Effects of treatment with Puffer fish extract on serum ALT, AST and Albumin.

	Control	CCl <sub>4</sub>	TTX 1 µg/kg	TTX 1 µg/kg + CCl <sub>4</sub>	F - ratio
<b>ALT (U/L)</b> Mean ± S.D	49 ± 5.48 <sup>b</sup>	149.67 ± 22.88 <sup>a</sup>	79.5 ± 6.3 <sup>a,b</sup>	112.67 ± 10.48 <sup>a,b</sup>	79.70
<b>% change</b>		205.44	62.24	129.94	
<b>AST (U/L)</b> Mean ± S.D	108.83 ± 5.74 <sup>b</sup>	276.67 ± 32.22 <sup>a</sup>	151.16 ± 6.33 <sup>a,b</sup>	179.16 ± 5.67 <sup>a,b</sup>	125.60
<b>% change</b>		154.21	38.89	64.62	
<b>Alb (g/dL)</b> Mean ± S.D	3.18 ± 0.12 <sup>b</sup>	2.8 ± 0.14 <sup>a</sup>	3.02 ± 0.08 <sup>a,b</sup>	2.91 ± 0.08 <sup>a</sup>	13.60
<b>% change</b>		-11.95	-5.14	-8.49	

Data are expressed as Mean ± SD (n=6 mice per group), Values sharing the superscripts (a) and (b) are significantly different.(a) Significantly different from the control group; p<0.05.(b) Significantly different from the CCl<sub>4</sub> model group; p<0.05.

### Histological results

Histologic examination of liver sections of the control group that were stained with hematoxylin and eosin showed a normal hepatic architecture which was covered by fibrous capsule called Glisson's capsule (Fig. 1 A). The hepatocytes contact blood in sinusoids, which are distensible vascular channels lined with highly fenestrated endothelial cells and populated with phagocytic Kupffer cells. The wall of the sinusoid is very thin while its lumen is always filled with blood cells (Fig. 1 B). In contrast, liver sections of CCl<sub>4</sub> intoxicated mice showed hyperplasia of bile ducts and excessive fibroplasia with numerous spindle nuclei of fibroblasts in portal areas associated with detachment from parenchyma and lymphocytic infiltration (Fig. 1 C). Other damaging features were microvesicular fatty change, pyknotic nuclei, karyolytic nuclei, irregular dilated sinusoids with active Kupffer cells and some hepatocytes showed necrosis with increased eosinophilia and cell swelling (Fig. 1 D). Conversely, liver sections from mice injected only with 1 µg/kg TTX showed hepatocytes intact and arranged in the form of strands around central veins with slight hydropic degeneration in the center (Fig. 1 E and F). While liver sections from mice that were treated with 1 µg/kg TTX then injected with CCl<sub>4</sub> showed noticeable hydropic degeneration, lymphocyte infiltration, and hemorrhage especially in the portal area (Fig. 1 G and H). However, it should be recorded that no signs of fatty degeneration or excessive fibrosis were observed. Also, no signs of coagulative necrosis or lytic necrosis were noticed.

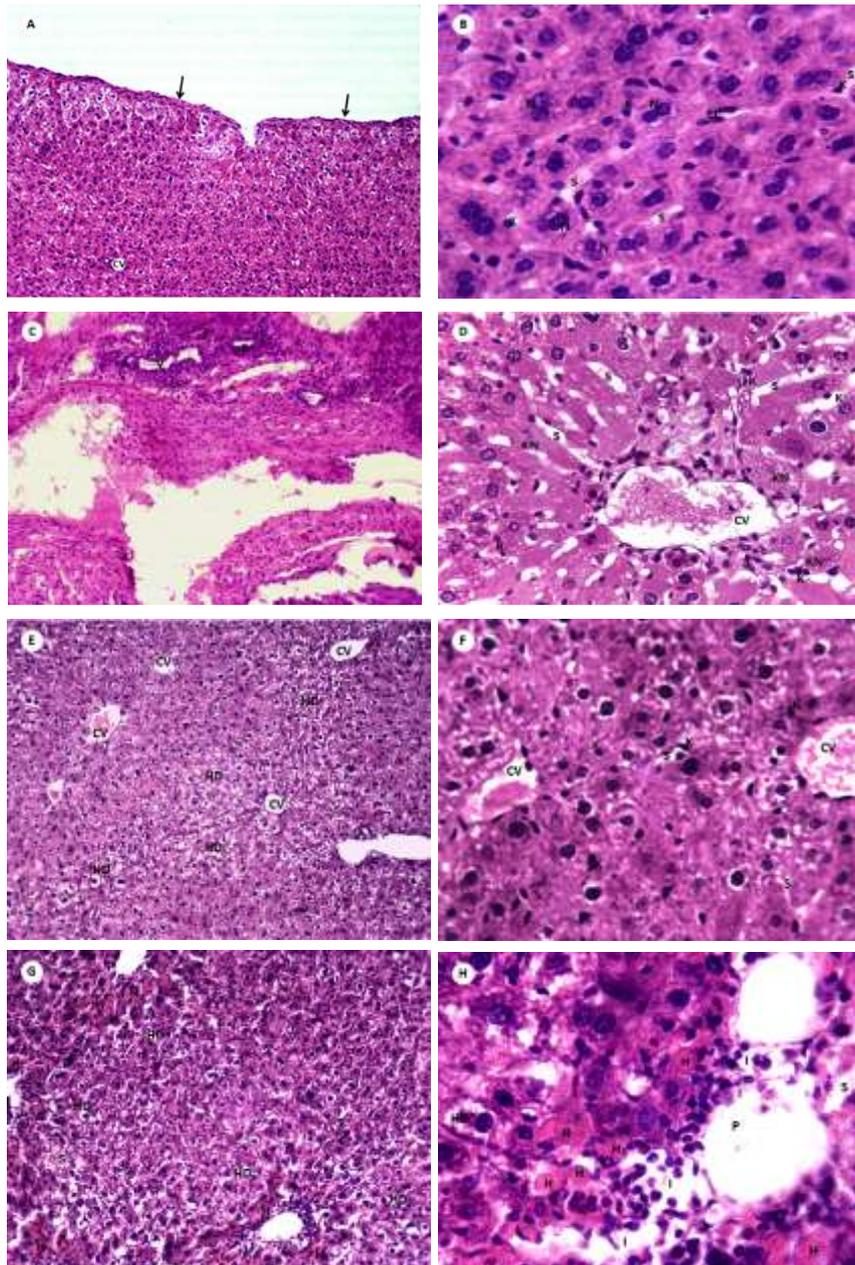


Fig. 1. Photomicrographs of liver sections stained with hematoxylin and eosin from (A) control mouse showed Glisson's capsule (arrow) (magnification 100) and (B) control mouse showed the hepatocytes with two nuclei. Kupffer cells which bulge into the sinusoidal lumen and endothelial cells form the wall of the sinusoids (magnification 400). Liver sections of  $\text{CCl}_4$ -intoxicated mice showed (C) hyperplasia of bile ducts and excessive fibroplasia in portal areas associated with detachment from parenchyma, lymphocytic infiltration and edematous features on the left side (magnification 100), (D) microvesicular fatty degeneration with faintly stained karyolytic nuclei, moreover others were devoid of their nuclei, irregular dilated sinusoids with active Kupffer cells and necrotic cells (magnification 400). Liver sections of the TTX group showed (E) hepatocytes appear intact and arranged in the form of strands around central veins with slight hydropic degeneration (magnification 100), (F) nuclei with halo, necrotic cells and increased active Kupffer cell (magnification 400). Liver sections of the TTX +  $\text{CCl}_4$  group showed (G) lymphocytic infiltration with hemorrhage and significant hydropic degeneration (magnification 100) and (H) hemorrhage and a lot of lymphocytes in the portal area (magnification 400). A, portal artery; B, bile duct; CV, central vein; H, hemorrhage; HD, hydropic degeneration; I, inflammatory cells; K, Kupffer cells; KN, karyolytic nuclei; N, two nuclei; P, portal tract area; S, sinusoids; V, portal vein.

## DISCUSSION

It is well recognized that the carbon tetrachloride (CCl<sub>4</sub>) is a lipid-soluble toxic chemical. This makes it difficult to be excreted from the body. Fat soluble chemicals have a high affinity for fat tissues and cell membranes and are thus well distributed throughout the body (Chenoweth and Hake, 1962). The biochemical mechanisms involved in the development of CCl<sub>4</sub> hepatotoxicity have long been investigated. CCl<sub>4</sub> is metabolized by drug-metabolizing enzyme system (cytochrome P-450) in the hepatic cell into trichloromethyl free radical (CCl<sub>3</sub>\*) which either bind covalently with lipoproteins or react with oxygen to form trichloromethyl peroxy radical (CCl<sub>3</sub>OO\*) inducing peroxidation of polyunsaturated fatty acids in the hepatic cell membrane. Destructive lipid peroxidation products lead to breakdown of cellular membrane structure as well as its function (Drury and Wallington, 1980; Kuzu *et al.*, 2007). The disturbance in transport function of hepatocytes because of CCl<sub>4</sub> effect causes the leakage of cytosolic enzymes from hepatocyte to the serum due to altered permeability that leads to rapid increase in the serum enzyme levels (Zimmerman and Seeff, 1970; Karthikeyan *et al.*, 2010).

The present study revealed that intraperitoneal injection with CCl<sub>4</sub> to male albino mice at a dose of 1 ml/kg dissolved in olive oil, twice/week for six weeks following the method of Fan *et al.* (2013) resulted in an increase in the activity of serum ALT and AST. Also, the level of Alb decreased. This result is in coincidence with many other results obtained by different authors who used CCl<sub>4</sub> in their experiments (Hassan *et al.* 2003; Hsiao *et al.* 2003; Hismiogullari *et al.* 2014; Kang and Koppula, 2014 and Lu *et al.* 2016).

In the present study, the intraperitoneal injection of Puffer fish extract to normal mice caused a significant increase in serum activities of ALT by (62.24%) and AST by (38.89%) and decrease serum Alb by (5.14%) compared with normal control mice. In contrast, hepatocellular necrosis and fibrosis caused by exposure to CCl<sub>4</sub> caused a dramatic increase of serum AST by (154.21%) and by ALT (205.44 %) activities and decrease of Alb serum by (11.95%) compared with control mice leads to an increased incidence and severity of histopathological hepatic lesions in mice. These results are supported by the findings of Yeh *et al.* (2012), who reported a significant increase in serum ALT, AST in mice intoxicated with CCl<sub>4</sub> (0.1 ml/100g) for 8 weeks. Similarly, Meenakshi *et al.* (2013) affirmed a significant increase in serum ALT and AST and a dramatic decrease in serum Alb level in mice intoxicated with CCl<sub>4</sub> (2.5 ml/kg) for 6 weeks.

In the present study, mice injected with Puffer fish extract at dose level 1 µg/kg showed free movement and no toxic symptoms. These are in good agreement with the results of Marcil *et al.* (2006) who reported that administration of Tetrodotoxin extract to the mice showed no behavioral disorders, while Hagen *et al.* (2005) reported that animals injected with TTX exhibited some symptoms of toxicity such as slow movement, hair erection and loss of appetite.

Administration of Puffer fish extract (5 doses of 1 µg/kg) before intoxication with CCl<sub>4</sub> attenuated the increased levels of the serum enzymes AST, ALT and the decreased level of albumin as compared to CCl<sub>4</sub> group. However, it caused a noticeable hydropic degeneration, lymphocyte infiltration, and hemorrhage especially in the portal area comparable to the control group. In line with our findings, Fouda (2005) showed an anti-tumor effect of TTX against Ehrlich ascites carcinoma (EAC) in Swiss albino mice expressed in the form of reduction in tumor peritoneal cell count and fluid volume and an increase in its lifespan by about 28-42 % depending on the

dose. Moreover, Fouda (2005) demonstrated that the administration of tetrodotoxin at 1/10 LD<sub>50</sub> dose (0.035 mg) to tumor-bearing mice caused a partial improvement in the level of SOD and GSHpx in a time-dependent manner. As a consequence, the proliferation and invasiveness of EAC cells are suppressed. The mechanism of the potential protective role of tetrodotoxin in ameliorating the toxic effects of EAC cells is presumably because of its antioxidant and free-radical scavenging properties. Similar results have been obtained from treatment of hepatotoxicity with other natural marine animals such as marine brown alga (Makhmoo *et al.*, 2013), *Sepia kobiensis* cuttlebone (Ramasamy *et al.*, 2014) and Sea Cucumber *Holothuria atra* extract (Dakrory *et al.*, 2015). These findings contrast with those of Yamaguchi (1996), who found that liver and kidney sections showed congestion in mice after intraperitoneal injection of tetrodotoxin prepared from the ovary of *Takifugu porphyreus*. Treatment with an anti-tumor dose of tetrodotoxin could replenish the host's antioxidant system, thereby protecting the host's liver and kidney from lipid peroxidation and subsequent degeneration. Thus, unlike many other anti-cancer agents, tetrodotoxin not only has anti-tumor properties but also protects the host liver and kidney from tumor-induced toxicity (Abd El-Wahab and Fouda, 2009).

Even though TTX is well documented as a pain relief agent because of its ability to block the Na<sup>+</sup> channel in the nervous system, it has been demonstrated that the mode of action of TTX on EAC cells is probably not different from that on other cells in the nervous system. It plays an important role in binding to P-glycoprotein of the Na<sup>+</sup> channels, blocking it and preventing Na<sup>+</sup> influx into the cells. This action, in return, prevents the carcinoma cells from getting enough Na<sup>+</sup> ions needed for their various intracellular functions, and above all to maintain the normal distribution of charge across the cell membrane, a process necessary to maintain cell integrity. Consequently, the proliferation and invasiveness of such cells are suppressed (Grolleau *et al.* 2001; Bragadeeswaran *et al.*, 2010).

## CONCLUSION

It can be concluded that treatment with tetrodotoxin (TTX) extracted from puffer fish, *Lagocephalus sceleratus* decreased the damaging effect of CCl<sub>4</sub> on the liver of albino mice both at the level of biochemical parameters and histopathological investigations. Further studies on active compounds isolated from Tetrodotoxin extracted from puffer fish should be carried on to understand the exact mechanism behind Tetrodotoxin ability to reduce the hepatotoxicity caused by CCl<sub>4</sub>.

## REFERENCES

- Abd El-Wahab, S. M. and Fouda, F. M. (2009). Histological and histochemical study on the effect of Ehrlich ascites carcinoma on the liver and kidney of mice and the possible protective role of tetrodotoxin. *Egyptian Journal of Biology*, 11: 13–25.
- Blunt, J. W., Copp, B. R., Hu, W. P., Munro, M. H., Northcote, P. T. and Prinsep, M. R. (2007). Marine natural products. *Natural product reports*, 24(1): 31-86.
- Bragadeeswaran, S.; Therasa, D.; Prabhu, K. and Kathiresan, K. (2010). Biomedical and pharmacological potential of tetrodotoxin-producing bacteria isolated from marine pufferfish *Arothron hispidus* (Muller, 1841). *The Journal of Venomous Animals and Toxins including Tropical Diseases*, 16(3): Botucatu.

- Chenoweth, M. B. and Hake, C. L. (1962). The smaller halogenated aliphatic hydrocarbons. *Annu Rev Pharmacol*, 2: 363-398.
- Dakrory, A. I.; Fahmy, S. R.; Soliman, A. M.; Mohamed, A. S. and Amer, S. A. (2015). Protective and curative effects of the sea cucumber *Holothuria atra* extract against DMBA-induced hepatorenal diseases in rats. *Biomedical research international*. Doi:10.1155/2015/563652.
- De-Groot, H. and Noll, T. (1986). The crucial role of low steady oxygen partial pressure in haloalkanes free radical induced lipid peroxidation. Possible implications in haloalkane liver injury. *Biochem. Pharmacol*, 35:15-19.
- Doumas, B. T.; Watson, W. A. and Biggs, H. G. (1997). Albumin standards and the measurement of serum albumin with bromocresol green. *Clinica chimica acta*, 258(1): 21-30.
- Drury, R. A. D. and Wallington, E. A. (1980). *Carleton's Histological Technique*. New York: Oxford University Press.
- Fan, X.; Zhang, Q.; Li, S.; Lv, Y.; Su, H.; Jiang, H. and Hao, Z. (2013). Attenuation of CCl<sub>4</sub> induced hepatic fibrosis in mice by vaccinating against TGF- $\beta$ 1. *PLoS One*, 8(12): e82190.
- Fouda, F. M. (2005). Anti-tumor activity of tetrodotoxin extracted from the masked puffer fish *Arothron diadematus*. *Egyptian Journal of Biology*, 7: 10–22.
- Gella, F. J.; Olivella, T.; Pastor, M. C.; Arenas, J.; Moreno, R.; Durban, R. and Gomez, J. A. (1985). A simple procedure for the routine determination of aspartate aminotransferase and alanine aminotransferase with pyridoxal phosphate. *Clinica chimica acta*, 153(3): 241-247.
- Grolleau, F.; Gamelin, L.; Boisdron-Celle, M.; Lapiéd, B.; Pelhate, M. and Gamelin, E. (2001). A possible explanation for a neurotoxic effect of the anticancer agent oxaliplatin on neuronal voltage-gated sodium channels. *Journal of Neurophysiology*, 85(5): 2293-2297.
- Hagen, N. A.; Fisher, K. and du Sourich, P. (2005). Safety of intramuscular tetrodotoxin for inadequately controlled, severe cancer pain. In 11<sup>th</sup> World Congress on Pain (IASP), Sydney.
- Hassan, S. A.; Salem, M. M. and Hamam, O. (2003). Antioxidative and antiapoptotic effects of vitamin A and vitamin C against carbon tetrachloride induced hepatotoxicity in mice. *The Egyptian Journal of Hospital Medicine*, 11: 30 – 40.
- Hismiogullari, S. E.; Hismiogullari, A. A.; Sunay, F. B.; Paksoy, S.; Can, M.; Aksit, H.; Karaca, O. and Yavuz, O. (2014). The protective effect of curcumin on carbon tetrachloride induced liver damage. *Revue Méd Vét*, 165(7-8): 194-200.
- Hsiao, G.; Shen, M. Y.; Lin, K. H.; Lan, M. H.; Wu, L. Y. and Chou, D. S. (2003). Antioxidative and hepatoprotective effects of *Antrodia camphorate* extract. *J Agric Food Chem*, 51:3302–3308.
- Huang, H. N.; Lin, J. and Lin, H. L. (2008). Identification and quantification of tetrodotoxin in the marine gastropod *Nassarius* by LC-MS. *Toxicon*, 51: 774-779.
- Hsu, Y. W.; Tsai, C. F.; Chang, W. H.; Ho, Y. C.; Chen, W. K. and Lu, F. J. (2008). Protective effects of *Dunaliella salina*-A carotenoids-rich alga, against carbon tetrachloride induced hepatotoxicity in mice. *Food and Chemical Toxicology*, 46: 3311-3317.
- Kang, H. and Koppula, S. (2014). Hepatoprotective effect of *Houttuynia cordata thunb* extract against carbon tetrachloride-induced hepatic damage in mice. *Indian journal of pharmaceutical sciences*, 76(4): 267-273.

- Karthikeyan, R.; Somasundaram, S. T.; Manivasagam, T.; Balasubramanian, T. and Anantharaman, P. (2010). Hepatoprotective activity of brown alga *Padina boergesenii* against CCl<sub>4</sub> induced oxidative damage in Wistar rats. *Asian Pacific Journal of Tropical Medicine*, 3(9): 696-701.
- Kuzu, N.; Metin, K.; Dagli, A. F.; Akdemir, F.; Orhan, C.; Yalniz, M.; Ozercan, I. H.; Sahin K. and Bahcecioglu, I. H. (2007). Protective role of genistein in acute liver damage induced by carbon tetrachloride. *Mediators of inflammation*, 1:1-6.
- Lee, M. J., Jeong, D. Y., Kim, W. S., Kim, H. D., Kim, C. H., Park, W. W. (2000). A tetrodotoxin-producing *Vibrio* strain, LM-1, from the puffer fish *Fugu vermicularis* radiatus. *Applied and environmental microbiology*, 66(4): 1698-1701.
- Lu, Y.; Hu, D.; Ma, S.; Zhao, X.; Wang, S.; Wei, G.; Wang, X.; Wen, A. and Wang, J. (2016). Protective effect of wedelolactone against CCl<sub>4</sub>-induced acute liver injury in mice. *International Immunopharmacology*, 34: 44-52.
- Makhmoor, T.; Naheed, S.; Shujaat, S.; Jalil, S.; Hayat, S.; Choudhary, M. I.; Khan, K. M.; Alam, J. M. and Nazir, S. (2013). Hepatoprotection by chemical constituents of the marine brown alga *Spatoglossum variabile*: a relation to free radical scavenging potential. *Pharmaceutical biology*, 51(3): 383-390.
- Marcil, J.; Walczak, J. S.; Guindon, J.; Ngoc, A. H.; Lu, S. and Beaulieu, P. (2006). Antinociceptive effects of tetrodotoxin (TTX) in rodents. *BJA: British Journal of Anaesthesia*, 96(6): 761-768.
- Mebs, D. and Yotsu, M. (2012). Tetrodotoxin in North-American newts. *Toxicon*, 60: 1-120.
- Meenakshi, V. K.; Gomathy, S.; Senthamarai, S.; Paripooranaselvi, M. and Chamundeswari, K. P. (2013). Hepatoprotective activity of the ethanol extract of simple ascidian, *Microcosmus exasperatus* Heller, 1878. *European Journal of Zoological Research*, 2(4): 32-38.
- Oliveira, J. S.; Pires Junior, O. R.; Morales, R. A. V.; Bloch Junior, C.; Schwartz, C. A. and Freitas, J. C. (2003). Toxicity of Puffer fish: two species (*Lagocephalus laevigatus*, Linnaeus 1766 and *Sphoeroides spengleri*, Bloch 1785) from the Southeastern Brazilian coast. *Journal of Venomous Animals and Toxins including Tropical Diseases*, 9(1): 76-88.
- Ramasamy, P.; Subhadrappa, N.; Shanmugam, V. and Shanmugam, A. (2014). Extraction, characterization and antioxidant property of chitosan from cuttlebone *Sepia kobeensis* (Hoyle 1885). *International journal of biological macromolecules*, 64: 202-212.
- Saganuwan, S. A. (2011). A modified arithmetical method of Reed and Muench for determination of a relatively ideal median lethal dose (LD<sub>50</sub>). *African Journal of Pharmacy and Pharmacology*, 5(12): 1543-1546.
- Sabrah, M. M.; El-Ganainy, A. A. and Zaky, M. A. (2006). Biology and toxicity of the pufferfish *Lagocephalus sceleratus* (Gmelin, 1789) from the Gulf of Suez. *Egyptian journal of aquatic research*, 32:283-297.
- Vitaglione, P.; Morisco, F.; Caporaso, N. and Fogliano, V. (2004). Dietary antioxidant compounds and liver health. *Critical Reviews in Food Science and Nutrition*, 44: 575-586.
- Victor, M. and Eric, O (2004). Liver. *Pediatrics*, 113: 1097-1106.
- Weber, L. W.; Boll, M. and Stampfl, A. (2003). Hepatotoxicity and mechanism of action of haloalkanes: Carbon tetrachloride as a toxicological model. *Critical Reviews in Toxicology*, 33: 105-136.

- Wu, J.; Danielsson, A. and Zern, M. A. (1999). Toxicity of hepatotoxins: new insights into mechanisms and therapy. *Expert opinion on investigational drugs*, 8: 585-607.
- Yamaguchi, P. (1996). Histopathological and electron microscopic changes in mice treated with puffer fish toxin. *The Journal of Toxicological Sciences*, 1: 1-14.
- Yeh, Y. H.; Hsieh, Y. L.; Lee, Y. T. and Hu, C. C. (2012). Protective effects of *Geloina eros* extract against carbon tetrachloride-induced hepatotoxicity in rats. *Food Research International*, 48(2): 551-558.
- Yotsu, M.; Mebs, D.; Kwet, A. and Schneider, M. (2007). Tetrodotoxin and its analogue 6-epitetrodotoxin in newts (*Triturus* spp.; Urodela, Salamandridae) from southern Germany. *Toxicon*, 50: 306-309.
- Zaki, Z. A.; Mady, E. A.; Ahmed, S. M. and Youssef, N. M. (2001). Effect of tetrodotoxin (TTX) on some brain neurotransmitters in rats. *Journal of natural toxins*, 10(4): 307-316.
- Zimmerman, H. J. and Seeff, L. B. (1970). Enzymes in hepatic disease, *Diagnostic Enzymology*. EL Coodley (ed). Philadelphia, Lea & Febiger, 1-38.

### ARABIC SUMMARY

دراسة نسيجية و بيوكيميائية على تأثير التيدروتوكوسين المستخلص من أسماك البخاخ ( *Lagocephalus sceleratus* ) ضد التسمم الكبدى الناتج عن رابع كلوريد الكربون فى الفئران البيضاء

إيمان عبد العال خليل<sup>1</sup> - حمدى حامد سويلم<sup>1</sup> - محمد احمد زكى<sup>2</sup>

1- قسم علم الحيوان - كلية العلوم - جامعة عين شمس - مصر.

2- المعهد القومى لعلوم البحار و المصايد - السويس - مصر.

الهدف من هذه البحث هو دراسة تأثير التيدروتوكوسين (TTX) المستخلص من أسماك البخاخ على التسمم الكبدى الناتج عن رباعي كلوريد الكربون (CCl<sub>4</sub>). قسمت ذكور الفئران البيضاء البالغين إلى أربع مجموعات : (1) فئران ضابطة تم معالجتها بمحلول ملحي، (2) مجموعة معالجة برابع كلوريد الكربون، (3) فئران تم حقنها بخمس جرعات من TTX (1 ميكروغرام / كغ)، (4) فئران تم حقنها بخمس جرعات من TTX (1 ميكروغرام / كغ) ثم اعطائها CCl<sub>4</sub> لمدة ستة أسابيع. أظهرت النتائج أن الحقن بخمس جرعات من تيدروتوكوسين (TTX) قلل بشكل كبير من تأثير سمية CCl<sub>4</sub> على بعض دلالات وظائف الكبد مثل نشاط الانزيم الناقل للأسبارتات (AST) والانزيم الناقل للألانين (ALT) و مستوى الألبومين (Alb). و كانت النتائج البيوكيميائية للمجموعة TTX مختلفه اختلافاً كبيراً عن المجموعة الضابطة. ومن ناحية أخرى أظهر الفحص النسيجي للكبد من الفئران المحقونة فقط ب TTX نمطاً طبيعياً تقريباً مع القليل من المواقع العشوائية للانحطاط المائي. بينما أظهر الفحص النسيجي لكبد الفئران المعالجة ب TTX و CCl<sub>4</sub> انحطاطاً مائياً جزئياً وتسلل لمفاوي ونزيفى خاصة في المنطقة البابية بالكبد. الا انه لم تظهر الأضرار النسيجية التي ظهرت عند استخدام رابع كلوريد الكربون باقل الضرر واختفت بعض مظاهر التلف.