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Anti-tumor activity of tetrodotoxin extracted from the Masked Puffer fish *Arothron diadematus*

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ABSTRACT

Anti-tumor activity of tetrodotoxins extracted from the skin of the Masked Puffer fish (*Arothron diadematus*) from the Red Sea was evaluated using the Ehrlich ascite carcinoma tumor model in mice. Activity was assessed using a variety of cellular and liver biochemical parameters. Experimental mice were divided into 4 equal groups and injected intra-peritoneally with: saline (control); a sub-lethal dose of the toxin (1\10 LD_{50}); 1 ml of a solution containing 2 million ECA cancer cells; and both (1 ml of a solution containing 2 million ECA cancer cells and a sub-lethal dose of toxin). Subsets of mice from each group were dissected after 3, 6, 9, and 12 days.

Statistical analyses demonstrated the following:

- the anti-tumor activity of the toxin increased lifespan by 46%, in addition to decreasing the number of tumor cells.
- There was also an obvious cytotoxic effect of tetrodotoxins on cells, leading to apoptosis and a decrease in the volume of the peritoneal fluid.
- The negative effects of tumor cells on the biochemical processes of liver was illustrated by an increased release of MDA & GGT enzymes and fat oxidation, and a decreased release of both enzymes and anti-oxidation agents. These negative effects were relieved for 6 days after injection by toxin.

KEYWORDS: Cancer, Ehrlich Ascite Carcinoma, Anti-tumor, TTX, Sodium channel blockers, MDA, GSH, SOD, GSHpx and GGT.

INTRODUCTION

Cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives every year (Abdullaev *et al.* 2000). An extremely promising strategy for cancer prevention today is chemoprevention, which is defined as the use of synthetic or natural agents (alone or combination) to block the development of cancer in humans (Gupta *et al.* 2004).

During the last decade, the search for new anti-cancer drugs has taken many different approaches. Taken in consideration the new information available about the mode of action of the anti-neoplastic drugs and information about cancer cell's kinetics, scientists were able to provide chemotherapeutic drugs to be used more comprehensively (Ulakoglu & Altun 2004). The basic aim in the use of these drugs is to inhibit the proliferation of tumor cells or kill them without damaging the normal cells. Among the most effective methods of treatment are the use of natural products which proved in many cases to have much less side effects than the chemical or radioactive treatments. A large number of natural products has been studied for anti-cancer activity on various experimental models. This has resulted in the availability of nearly 30 effective anticancer drugs (Ramakrishna *et al.*1984; Ramnath *et al.* 2002).

Despite the fact that the tetraodotoxins are among the most dangerous marine natural products, their unique mode of action attracts many scientists to study in depth how to put such mode in use for the benefit of mankind. Tetrodotoxin (TTX) is a naturally occurring potent toxin, and a highly selective sodium channel blocker. The toxin is named after the puffer fish (Tetraodontidae), the most commonly available source of TTX. The biosynthesis pathway of

TTX is still totally unknown and as a result biogenesis of the toxin represents a puzzling question remained to be solved (Narahashi 2000).

During the last decades, TTX has been a useful tool in identification, isolation and characterization of voltage-gated sodium channels. Tosteson (1992) demonstrated that TTX has a great ability to bind to the trans-membrane glycoprotein forming the Na^+ channel which resulted in blocking it. Several research papers have been published during the last five to ten years concerning the effect of TTX on cells of different size, type and shape. Recently, a group of Chinese scientists performed clinical trials in Canada and China using tetraodotoxin as an analgesic which reduces the intense pain caused by advanced cancer in patients. Administration of small doses of TTX resulted in pain relief lasted 2 to 3 weeks. The clinical trials indicated that the purified TTX, Tetrodin was safe when given in very small doses. It acts quickly and has a long duration of action. Compared to morphine, TTX was 3000 times more powerful with fewer side effects and no addictive qualities (Alonso *et al.* 2003).

The involvement of ionic channels in cell proliferation as well as in tumor invasiveness was demonstrated in several cancers. Roger *et al.* (2004) reported some dramatic changes in the electrophysiological properties of three breast cancer cell lines exposed to different concentrations of TTX. The results showed that at a concentration of 30 μM which fully blocks sodium ions input to the cells, reduces the proliferation, migration and invasive properties of the cell line by about 30%.

The use of Ehrlich Ascite Carcinoma (EAC) as a model in anti-cancer research was proven by many authors to give accurate and reliable results (Clarkson & Burchenal 1965; Kuttan *et al.* 1990; Sheeia *et al.* 1997; Ramnath *et al.* 2002; Gupta *et al.* 2004). The reliability of such test lies in its ability to determine the value of any anticancer drug through prolongation of experimental animal lifespan in addition to the changes in number and viability of the cell line itself in addition to the volume of the liquid generated by the tumor inside the peritoneal cavity (Maity *et al.* 1999). It is now well recognized that apoptosis is a mode of cell death used by multi-cellular organisms to eradicate cells in diverse physiological and pathological settings. Recent evidence also shows that suppression of apoptosis by tumor-promoting agents in pre-neoplastic cells is an important mechanism in tumor promotion. In this context, it is noteworthy that apoptosis-inducing ability seems to have become a primary factor in considering the efficacy of anti-tumor agents (Ramnath *et al.* 2002; Huo *et al.* 2004).

Bhattacharyya *et al.* (2003) demonstrated the apoptogenic effect of Black tea administration to EAC-bearing Swiss albino mice. They showed that treatment with black tea caused a significant decrease in the tumor cell count in a dose dependent manner.

It is well documented that excessive production of free radicals results in oxidative stress, which leads to damage of macromolecules such as lipids this in turn can induce lipid peroxidation which will lead eventually to degeneration of tissues (Yagi 1991; Shibata *et al.*1996). Lipid peroxides formed in the primary site would be transferred through the circulation and provoke damage by propagating the process of lipid peroxidation (Sinclair *et al.* 1990). MDA, the end product of lipid peroxidation, was reported to be higher in carcinomatous tissue than in non-diseased organs. However, glutathione, a potent inhibitor of neoplastic process, plays an important role as an endogenous antioxidant that is found particularly in a high concentration in liver and is known to have key function in the protective process (Sinclair *et al.* 1990, Gupta *et al.* 2004). Because antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to humans against infection and degenerative diseases, their levels in the body can also be used in expressing the status of the diseased body.

The present study was carried out to explore the antitumor activity of tetraodotoxins extracted from the Masked Puffer Fish of the Red Sea against EAC cells in Swiss albino mice. The potential effects of TTX on lipid peroxidation and the antioxidant status is also evaluated.

MATERIALS AND METHODS

Adult Swiss female albino mice (20-25 g) were procured from the animal house of the National Cancer Research Institute (Kasr El-Ainy St., Cairo, Egypt). About 196 mice were used in the study. They were housed in stainless steel boxes in a controlled environment (temperature 20 ± 2 °C and 12 h dark/ light cycle) with standard laboratory diet and water *ad libitum*. The study was conducted at the animal facility of the Women's College, Ain Shams University.

Induction of EAC: Ehrlich Ascite Carcinoma (EAC) ECACC 87032503 cells were obtained from the National Cancer Research Institute, via a 25-g female albino mouse. They were maintained by weekly interperitoneal inoculation of saline solution containing 10^6 cells/mouse.

Preparation of TTX: The skins of five Masked Puffer Fish *Arothron diadematus* (Rüppell 1829) (Tetraodontidae) was prepared using acidified methanol extract solution according to the method of Kawabata (1978). The extract was then boiled for 10 min, cooled, centrifuged at 1000 rpm for 3-5 min., and the supernatant containing the TTX stored at -4^0C until used.

The toxicity of the TTX extracted from the fish skin was determined (in mouse units) according to the method described by Kawabeta (1978). A series of concentrations were injected intra-peritoneally into 20-g male albino mice obtained from the animal house of Theodore Belharis Institute. One mouse unit is defined as the amount of toxin required to kill a 20-g mouse within 30 min. The LD_{50} of the extracted TTX was also determined according to the method of Behreus & Karbeur (1953) (determined after 96 h).

Two experiments were performed, the first to assess the impact of TTX on EAC cells, and the second to measure the impact of TTX on biochemical parameters.

Impact of TTX on EAC: In the first experiment, animals were divided into four groups each of 20 mice. One (the control) was treated with the same carrier as in all the treated groups (injection of 0.9% sodium chloride solution). All three treated groups were inoculated with with 1 X 10^6 EAC cells/mouse on the first day of experiment. Two of these groups also received an injection of TTX after EAC inoculation, one group at a dose of 1/10 and the other 1/20 of the LD_{50}. Three animals of each group were sampled on each of days 3, 6, 9, and 12 after inoculation (n=12 in each group). The peritoneal fluid was collected from each animal, measured to the nearest cm^3 and a smear prepared for detection of the status of EAC cells. The remaining animals from each group (n=8) were monitored for determination of survival time.

The anti-tumor efficacy of TTX was compared with that of inoculated control group using survival time (expressed as a percentage change: 100*(T-G)/G, where T = number of days the treated animals survived, and G = average number of days control animals survived). To determine the effect of TTX on changes in the status of inter-peritoneal tumor cells, three indicators of EAC-cell activity were measured in both control and the 1/10-LD_{50} TTX-treated groups: (i) the volume of the fluids inside the peritoneal cavity; (ii) the numbers of EAC cells found in the fluid (using a haemocytometer); and (iii) the size-frequency distribution of cells determined using image analysis software (*Image-pro*-Plus, using 6 fields from each smear slide). In case of finding a very limited amount of the fluid the peritoneal cavity was washed with 2 ml of normal physiological saline

Impact of TTX on biochemical parameters: In the second experiment, to detect the influence of TTX on biochemical variables, animals were divided into four groups each of 24 mice, treated in a 2 x 2 factorial design with two treatments: EAC (- = absent and + = present) and TTX (- = absent and + = present) (at a does of 1/10 LD_{50}). The first group (EAC^-, TTX-) is equivalent to the control; then there are the single dose treatments (EAC^+ TTX^- and EAC^- TTX^+), and finally the double dose (EAC^+ TTX^+). Samples from the livers of 6 mice from each group were collected on days 3, 6, 9 and 12 after inoculation. Samples were then homogenized in 10% w/v physiological saline and stored frozen until analysis.

A number of variables were measured. Gamma-glutamyltransferase (GGT) activity was determined in liver homogenate according to the method of Young (1990). Hepatic lipid peroxidation was determined by measuring malondialdehyde (MDA) according to the method of Ohkawa *et al*. (1979). Reduced glutathione (GSH) content was determined in the liver homogenate using the method of Beutler *et al.* (1963). Glutathione peroxidase (GSHpx) activity was measured using the method of Gross *et al*. (1967). Liver superoxide dismutase (SOD) activity was measured by the method of Minami & Yoshikawa (1979).

Detection of apoptotic cells: Apoptosis of cells was detected using UV micrography according to the method used by Bhattacharyya *et al. (*2003). Slides carrying smears of the peritoneal fluid were stained using Annexin V and then examined and photographed using a special digital camera mounted on a Nikon microscope. Normal cells were golden yellow while those with apoptosis appeared greenish. The photographs were analyzed using *Image-pro*-Plus software, with data from 6 fields captured from each slide.

Statistical analysis: All values are expressed as mean ± SE. The data were statistically analyzed by ANOVA. P values less than 0.05 were considered to be significant. In most cases only the highest-order significant interaction is presented because of the consequent problem in interpreting lower-order ones and main effects. The analysis was performed using Statistica software Ver. 5.0 and SPSS version 12.

RESULTS

During the preparation of TTX , extraction of 365 g of fish skin yielded 20.75 ± 2.82 g of the crude extract. This means that each gram of skin contains about 56.8 mg of the toxin. The results of the toxicity experiment showed that 3 mg of extract was sufficient to cause death of a 20-g mouse in 30 min (one mouse unit). The results of the 96-h LD_{50} experiment showed that 0.35 ± 0.02 mg was sufficient to kill 50% of the animals. Accordingly, the doses used in the experiments were 0.0350 and 0.0175 mg, representing 1/10 and 1/20 LD_{50} .

The application of TTX significantly extends the lifespan of tumor-bearing mice ($F_{2,57} = 3.97$, $p<0.05$) by up to almost 50% (Table 1). There were differences also among treatments in the rate of increase of tumor cells in the peritoneal fluid (Figure 1), which was large without TTX (from 2.5 to about 95 million cells after 12 days of incubation), but much slower when TTX was injected: by day 12, treatment with 1/20 LD50 resulted in about 40% inhibition, whereas a dose of 1/10 LD_{50} caused about 60% inhibition.

Table 1: Effect of TTX treatment on the mean survival time (MST) and the increase in the lifespan of EAC-tumor-bearing mice.

Treatment	MST (days)	Increase in lifespan %
EAC only	14 ± 1 days	-
EAC + 1/10 LD_{50}	20 ± 2 days	46.6 ± 4.2
EAC + 1/20 LD_{50}	18 ± 1 days	26.7 ± 2.6

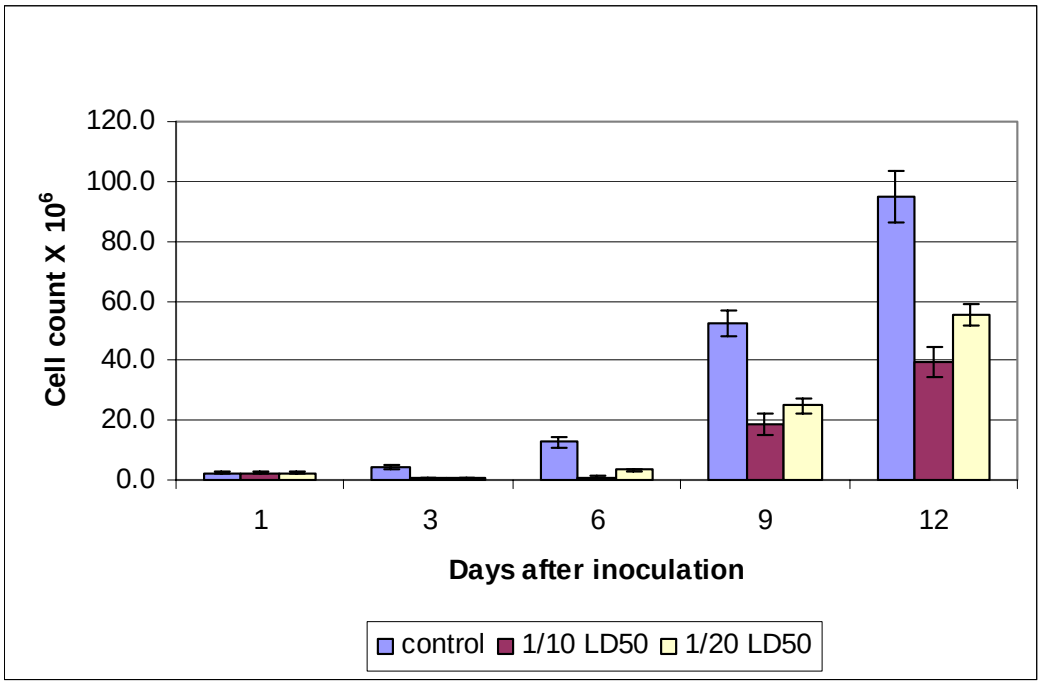


Figure 1: Development EAC cell counts in tumor-bearing mice and after treatment with different doses of TTX

One of the most obvious features of the growth and development of EAC tumors is the volume of inter-peritoneal fluid in which the cells proliferate and move to invade other organs. Figure 2 shows that treatment with TTX suppressed fluid development until day 6 post-injection. There was almost no change in fluid volume for the lower TTX dose (1/20 LD_{50}), with a slow increase afterwards.

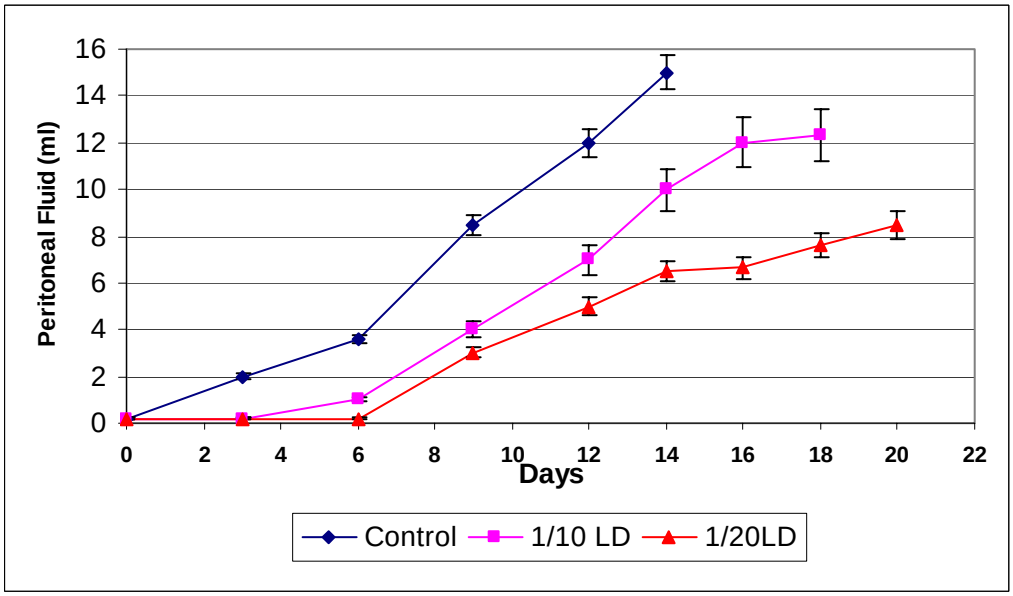


Figure 2: Changes in inter-peritoneal fluid volume in tumor-bearing mice in relation to treatment with two doses of TTX.

The size-frequency distribution of EAC cells without TTX showed early dominance by cells 30 - 40 µ in diameter, with only a very small percentage exceeding this size. Later the cell population became dominated by 50-µ cells, and then by cell diameters up to 70 µ, accompanied by the appearance of small sized-cells reflecting the cell renewal due to division. Finally at the end of 12 days, the size range was characterized by a great increase in small sizes (20 - 40 µ) relative to large ones (70-80 µ). After treatment with a single dose (1/10 LD_{50}) of TTX, the smaller cells remained dominant for longer, with 45% of cells belonging to the 30-µ diameter class after 6 days. There was then a rapid increase in the larger size classes, but at the end after 12 days, all the large-sized cells had disappeared and the 30 - 50 µ cells represented almost 98% of the cell population, mainly (59%) the 40 µ size class.

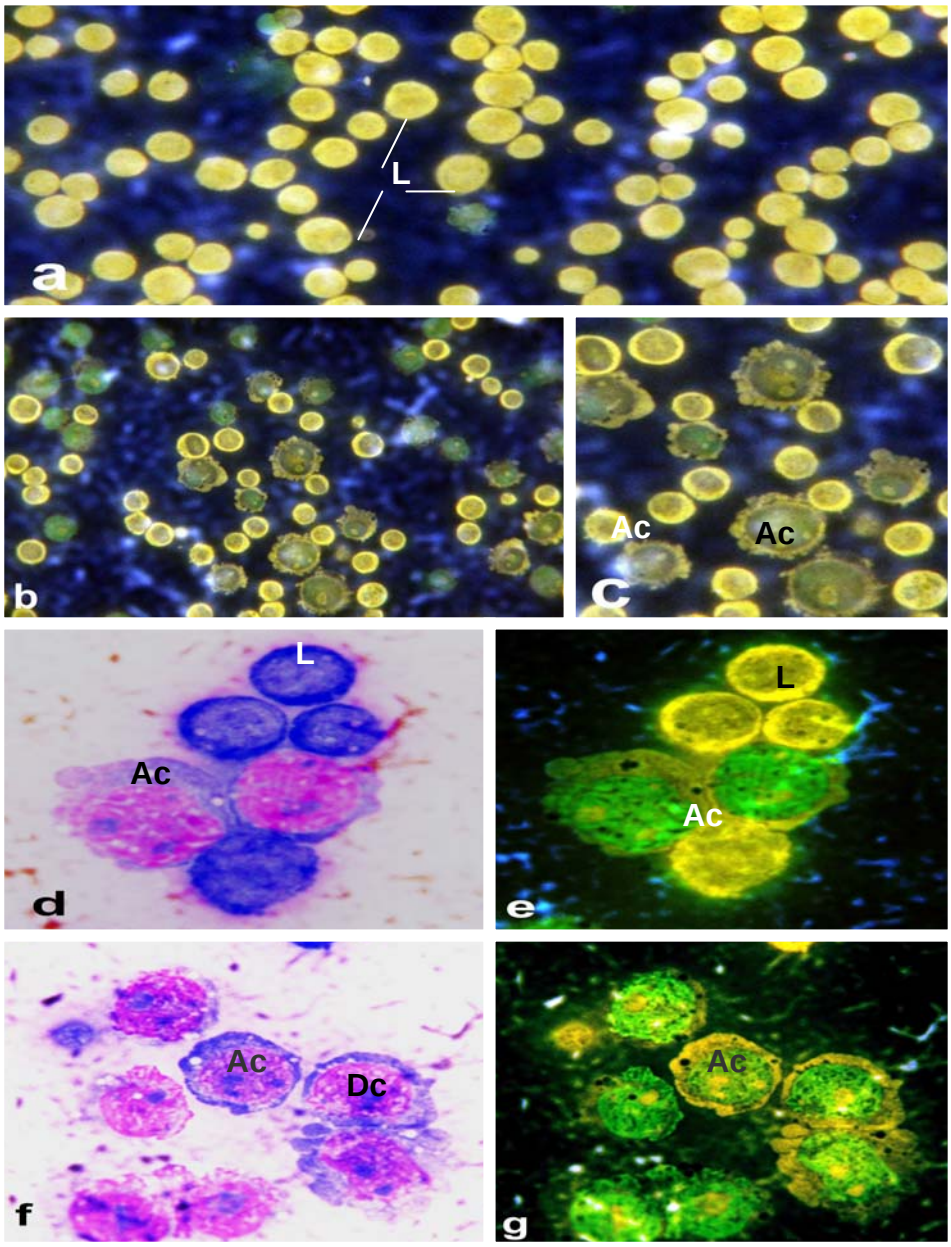


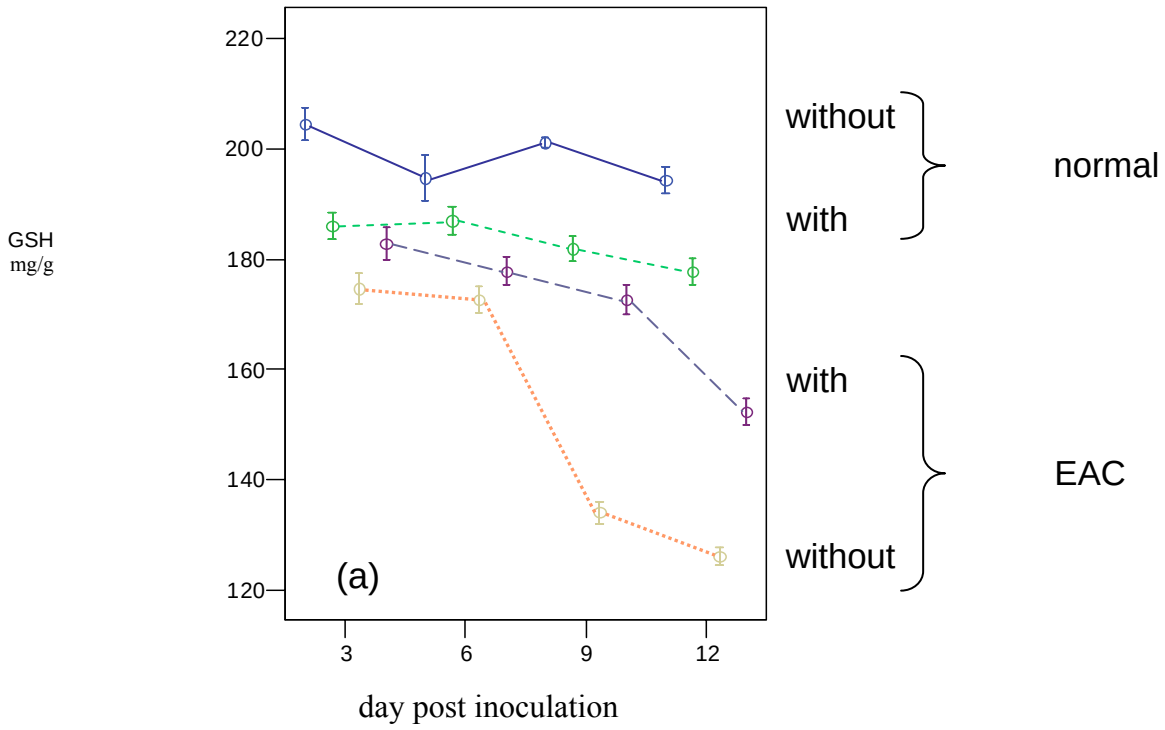
Plate I: Photomicrographs showing the reflection image of EAC cells (a) X200, and after exposure to 1/10 LD_{50} of TTX and appearance of apoptotic cells (b X200) and (CX400). Photos (d-f) represent the different stages of cells as demonstrated by trypan blue stain (d and f) and after treatment with Annexin V stain (e & g) (X1000). Notice that the green reflection represents the cell content of apoptotic cells. (L= life cell; Ac= apoptotic cell; Dc= degenerative cell).

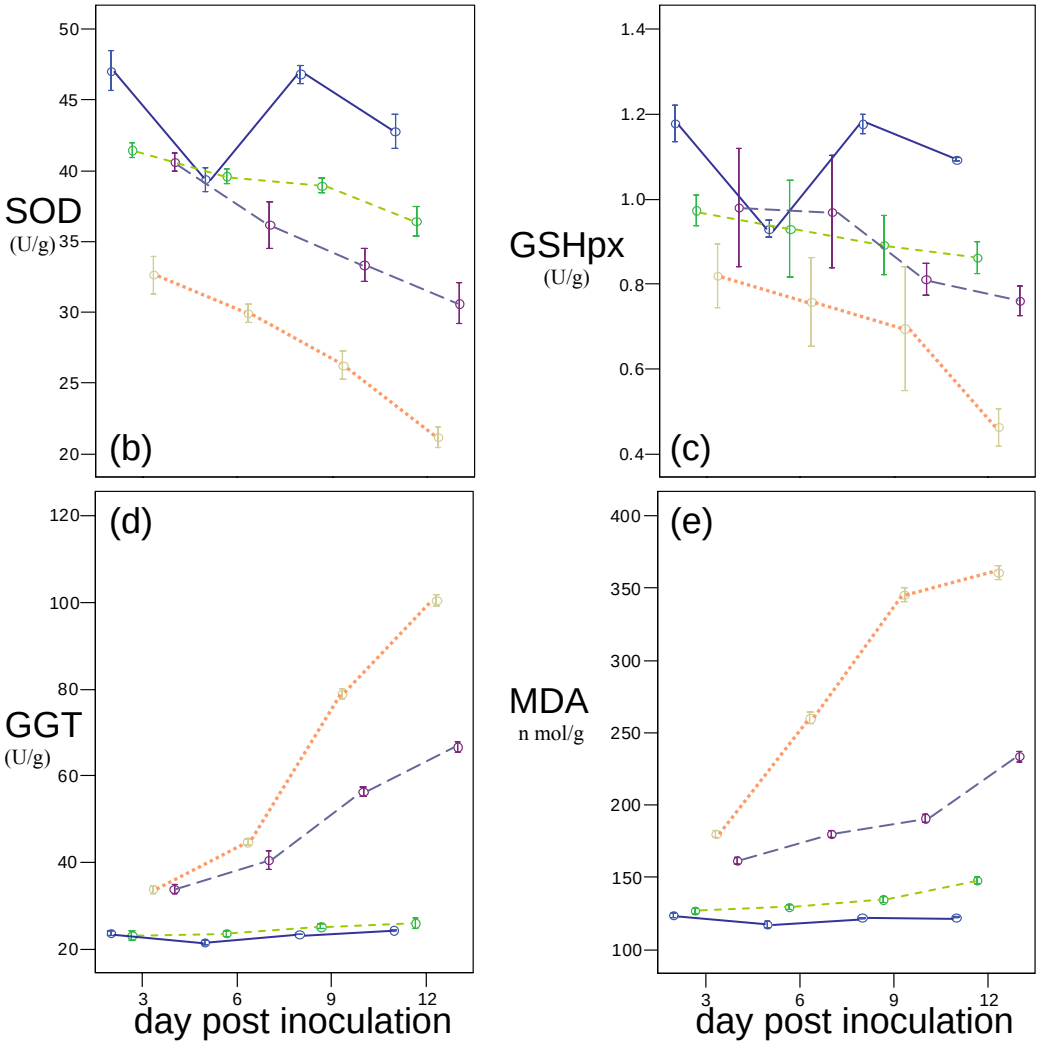
During the examination of EAC cell smears, it was noticed that some of the cells were different in shape and size (Plate 1a). The application of the viability test by staining with trypan blue revealed that most of the abnormal cells were in a certain stage of cell death. To understand the nature of cell death, slides stained with Annexin V were examined to distinguish between apoptotic and normal cells. During the early stages of apoptosis, cell membranes were still intact, but the cell contents were not recognizeable (Plate 1b). Close examination revealed successive stages of death starting with changes in cell shape and volume, followed by blebbing of the external surfaces (Plate 1b & c). The next stage included degradation of cell contents and loss of nucleus integrity, which left the cell as an unrecognizeable mass. During the last stage cells disintegrate and appeared as smaller masses with different size and shapes (Plate 1, d-g).

The examination of slides prepared on days 3, 6, 9 and 12 post inoculation showed that the number of apoptotic cells was higher during the first 6 days of the experiment, especially in the group injected with TTX. However, the number was relatively reduced during the rest of the experimental period. Most of the cells that underwent apoptosis during the first 6 days were between 40 to 70 µ in diameter, while later on apoptotic cells tended to be larger (60-70 µ) in diameter.

The responses of the physiological variables to the treatments (Fig 3) showed a highly significant effect of TTX in all cases, usually interacting with time as well as EAC. In all cases it is clear that the effect of TTX is to act as a poison to normal cells, but as an anti-cancer agent to EAC cells, restoring values to a great extent towards normal values. In some enzymes this means that TTX reduced activity levels in normal cells (anti-oxidants GSH, GSHpx, SOD: Fig 3a-c), whereas in others it increased them (GGT, MDA: Fig 3d-e). In normal TTX-treated subjects, all injected animals exhibited some symptoms of toxicity such as slow movement, hair erection and loss of appetite.

Figure 3: The changes in the mean values (± SE) of different physiological variables measured in the factorial experiment (EAC treatment by TTX treatment by days). The first graph (a) is annotated, and the remaining ones (b-e) have the same symbols.





(a) Glutathione (GSH). The 3-way interaction EAC x TTX x day was very highly significant ($F_{3,80}$ = 14.6, p<0.001) and the overall effect of TTX is very clear in the EAC x TTX interaction ($F_{1,80}$ = 187.2, p<<0.001) because TTX reduced GSH values in normal cells (acting as a poison) but increased them in EAC cells (acting as an anti-cancer agent).

(b) Superoxide dismutase (SOD). The 3-way interaction EAC x TTX x day was highly significant ($F_{3,80}$ = 30.0, p<0.01)

(c) Glutathione peroxidase (GSHpx). The 2-way interaction EAC x TTX was very highly significant ($F_{1,80}$ = 22.0, p<0.001): again TTX reduced GSHpx values in normal cells (acting as a poison) but increased them in EAC cells (acting as an anti-cancer agent).

(d) Gamma-glutamyltransferase (GGT). The 3-way interaction EAC x TTX x day was very highly significant ($F_{3,80}$ = 66.4, p<<0.001).

(e) Malondialdehyde (MDA). The 3-way interaction EAC x TTX x day was very highly significant ($F_{3,80}$ = 145.6, p<<0.001).

DISCUSSION

Aside from surgery and radiation therapy, traditional chemotherapeutic treatment of cancer is commonly used even though it has detrimental side effects. Current chemotherapeutics are effective to treat cancer because they slow down cell growth or kill cancerous cells outright (necrosis) because the chemical(s) used are toxic. This is disadvantageous because these chemicals also adversely affect normal cells (Jayaram 2005).

Many scientists have agreed that the reliable criteria for judging the value of any anticancer drug are prolongation of lifespan of the tumor bearing animals. The results of the present study showed an anti-tumor effect of TTX against EAC in Swiss albino mice reflecting an increase in its lifespan by about 28-42 % depending on the dose. A significant enhancement of MST and reduction of tumor peritoneal cell count and fluid volume were also observed. Similar results have been obtained from treatment with other natural products such as green tea (Mukhtar & Ahmed 2000), *Indigofera* plant extract (Rajkapoor *et al*. 2004), *Nigella sativa* seeds (Musa *et al*. 2004) and *Bauhinia racemosa* plant (Gupta *et al*. 2004).

Despite the fact that TTX is well documented as a pain relief agent because of its ability to block the Na^+ 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^+ channels, blocking it and preventing Na^+ influx into the cells. This action, in return, prevents the carcinoma cells from getting enough Na^+ 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. As a consequence the proliferation and invasiveness of such cells are suppressed (Grolleau *et al.* 2001).

Nielsen (2004) stated that the ability of EAC cells in resisting drugs depends on the ability of the cell membrane to prevent the drug from penetrating the cell in addition to controlling the surrounding media. He also demonstrated the ability of P-glycoproteins found in the membrane to form adduct with the drug, reducing the chance of drug penetration. Here, the binding of TTX to P-glycoproteins guarding the Na^+ channel may have reduced the influx of Na^+ to the cell, which in return could have reduced its potential for proliferation and curtailed its invasiveness. Simillar results have also been reported for different types of Na^+-channel blockers by Woodfork *et al*. (1995), Ouadid-Ahidouch *et al.* (2000) and Roger *et al.* (2004) on breast cancers.

In this regard, Roger *et al*. (2004) studied differences in the membrane potential of breast-cancer cell line in presence of TTX as a Na^+-channel blocker. Their results proved that there is a continuous entry of sodium into the cells through these channels during proliferation, expressed as an increase in the action potential of the cell membrane. A fast inward sodium current clearly exists in an invasive breast-cancer cell line. They also suggested that this current is involved in the process of invasion, probably through its involvement in the regulation of intracellular sodium homeostasis. The current was blocked by high concentrations of TTX, reducing cell invasiveness by about 30%.

The data clearly demonstrate the effectiveness of TTX on the development of EAC cells. There was a considerable reduction in the number of cells during the first 6 days of the experiment, accompanied by domination of small-sized cells, probably the same size initially injected into the animal. The increase in lifespan suggests that TTX delayed proliferation of the carcinoma cells to a certain extent, probably by denying Na^+ intake from the surrounding medium (Roger *et al.* 2004). However, after 6 days, the effect of TTX was reduced, allowing the EAC cells to divide and increase in number. A similar picture was also reported by Grolleau *et al*. (2001), leading them to suggest that the time and amount of Na^+ blocker is an important factor in controlling cell metastasis.

Nagy *et al.* (1995) and Maity *et al.* (1999) reported that the presence of EAC cells in the inter-peritoneal space tends to generate a liquid medium from the surrounding tissues through hyper-permeability of blood vessels, extravasation of plasma proteins and clotting of extravagated proteins to form cross-linked deposits in the peritoneal lining. This is most probably the case in the present study, where the effect induced by TTX on the EAC cells caused delay in proliferation, reflected in their ability to generate the inter-peritoneal fluid.

Jayaram *et al.* (2002) and Jayaram (2005) stated that the best method of cancer treatment is the one causes cancer cells to kill themselves (i.e. to undergo apoptosis - programmed cell death) instead of overall necrosis. Such an approach will reduce the adverse side-effects of chemotherapy. It was clear from the histological data that EAC cells underwent a process of apoptosis, probably due to the loss of the ability to proliferate. The normal EAC-cell cycle involves population doubling every 36 to 48 hrs (Jia *et al.* 2005): however, exposure to TTX caused this rate of cell division to be reduced greatly, especially in the first 6 days post treatment. The reduction in cell number is probably due to increased apoptosis. The obvious inhibition of tumor-cell growth rate in this experiment has also been noted by others (Levin 1997; Das *et al.* 1999; Pal *et al.* 2001; Bhattacharyya *et al*. 2003).

There are two major lines of investigation in cancer biochemistry: the metabolism of cancer cell, and the effect of cancer on the host metabolism. The present work concentrated on a group of variables indicative of the metabolism of the host. These included GGT, a plasma-membrane-bound liver enzyme strongly affected by liver cellular damage; MDA, the end-product of lipid peroxidation; and glutathione, a potent inhibitor of the neoplastic process as result of its pivotal role in the cellular antioxidant system. The other variables included SOD and GSHpx as integrated links in the antioxidant system. The former, SOD, represents the first line of defense against superoxide anions because it catalyzes the dismutation of superoxid anions to hydrogen peroxide and molecular oxygen. GSHpx catalyzes the conversion of H_2O_2 into water at the expense of reduced GSH. Thereby these two enzymes work in the context of the antioxidant system against the potentially damaging reactivities of superoxide and hydrogen peroxide.

Levels of GGT showed an increase after introducing EAC into the mice which may indicate the development of primary or secondary carcinoma (Whitby *et al.* 1993). Excessive production of free radicals results in oxidative stress, leading to damage of macromolecules such as lipids, and can induce lipid peroxidation *in vivo* (Sinclair *et al.* 1990; Yagi 1991; Gupta *et al*. 2004). Increased lipid peroxidation causes degeneration of tissues, and lipid peroxides formed in the primary site are transferred through the circulation and provoke damage by propagating the process of lipid peroxidation. Because MDA is the end-product of lipid peroxidation, its level is higher in carcinomatous tissue than in non-diseased organs. The same results were obtained here: an increase in MDA level with time was observed in the group bearing the tumor cells.

The observed reduction of GSH levels in tumor-bearing mice has been previously reported (Esterla *et al.* 1992; Navarro *et al.* 1999), explicable by an increased rate of transformation of GSH to GSSG as a result of GSH consumption to get rid of H_2O_2. The other possible reason could be the inhibition of GSH synthesis or a lack of the amino acids used in making GSH. The decrease in the activity of SOD in tumor-bearing mice has also been reported before (Navarro *et al.,* 1999). This reduction in SOD activity could alter antioxidant defenses, resulting in enhanced oxidation due to the accumulation of H_2O_2.

The current study demonstrates, therefore, that EAC inoculation induces a significant decrease in hepatic GSH content associated with an inhibition of hepatic GSHpx and SOD activity (Farag & Abdel Dayem 2001). The impairment of the cellular redox status may be attributed to an increase in the production of reactive oxygen species and a reduction of the anti-oxidants in liver tissues. SOD acts to trap superoxide radicals, while GSH can chemically

detoxify H_2O_2. This reaction is catalyzed by GSHpx to form oxidized GSSG and H_2O. As a group, these enzymes serve as a defense system to safeguard cells from the toxic effects of reactive oxygen intermediates.

Sun *et al.* (1989) also reported a decrease in SOD activity in EAC-bearing mice which might be due to loss of Mn-SOD activity in EAC cells, and a loss of mitochondria, leading to a decrease in total SOD activity in the liver. The inhibition of SOD and GSHpx activity as a result of tumor growth was also reported by Marklund *et al.* (1982). Here, the administration of TTX at 1/10 LD_{50} dose to tumor-bearing mice caused a partial improvement in the level of both SOD and GSHpx in a time-dependent manner, which may highlight the antioxidant and free-radical scavenging properties of TTX.

The administration of a single acute dose of TTX into tumor-bearing mice caused significant changes to the values of the measured liver physiological variables, not to the level of a cure, but to a possible useful level of amelioration. Further research is needed to determine the exact effective dose of TTX, the duration of its effect, and the best route of administration.

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الملخص العربى

النشاط المضاد للخلايا السرطانية لمادة التيترودوتوكسين المستخلصة من سمكة الفهقة (*Arothron diadematus*)

فاطمة مختار فودة
قسم العلوم البيولوجية – كلية البنات – جامعة عين شمس

يهدف البحث إلى دراسة تأثير السم المستخلص من جلد سمكة الفهقـة ***Arothron diadematus*** والمنتشرة بمياه البحر الأحمر على نمو ونشاط الخلايا السرطانية فى فئران التجارب.
تم دراسة تأثير هذا السم على الفئران الحاملة للخلايا السرطانية من خلال رؤية تأثير هذه السموم علـى الخلايا السرطانية وعلى العمليات البيوكيميائية للكبد. وقد قسمت فئران التجارب إلى أربع مجموعـات متساوية العدد كالآتي:
المجموعة الضابطة: تم حقنها بمحلول ملحي لتكون مجموعة قياسية ضابطة.
المجموعة الثانية: تم حقنها بجرعة غير مميتة من السم (1/10 الجرعة المميتة).
المجموعة الثالثة: تم حقن الفئران بجرعة قدرها 1 مل تحتوي على 2 مليون خليـة سـرطانية نوعهـا ECA في التجويف البريتوني.
المجموعة الرابعة: تم حقن الفئران بجرعة قدرها 1 مل تحتوي على 2 مليون خليـة سـرطانية فـي التجويف البريتوني ثم تم حقنها بجرعة غير مميتة من السم
تم تشريح عدد متساوى من الفئران بعد 3،6،9،12 يوم فى جميع المجموعات السابقة.
ولقد أوضحت النتائج بعد التحليل الإحصائي مايلي:

- زيادة ملحوظة في فترة حياة الفئران بنسبة 46% نتيجة للتأثير المضاد لهذه الـسموم علـى نـشاط الخلايا السرطانية، بالإضافة إلى إنخفاض أعداد الخلايا السرطانية
- لوحظ نقص كبير في السائل البريتوني نتيجة للموت الخلوي المبرمج (Apoptosis).
- الحقن بالخلايا السرطانية له تأثير ضار على العمليات البيوكيميائية للكبد حيث أنه يؤدي إلى زيـادة ملحوظة في إنزيم MDA & GGT، أكسدة الدهون، ونقص ملحوظ فـي الإنزيمـات ومـضادات الأكسدة ولكن عند حقن الفئران بالسم فقد لوحظ أنها تقلل من هذه التأثيرات خصوصا بعد 6 أيام من الحقن.