Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(6): 1277 – 1289 (2024)
www.ejabf.journals.ekb.eg

The Anticancer Effects of Tetrodotoxin Derived from *Lagocephalus sceleratus*, a Masked Puffer Fish, on the Muscle of Albino Mice with Ehrlich Solid Carcinoma

Alaa H.H. Edris, Ahmad M. Azab, Mohamed M. Abu- Zaid, Mohamed H. Ghanem, Fathy M. Elshaer*
Zoology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt

***Corresponding Author: Shaer82@gmail.com, Fathi_elshaer@azhar.edu.eg**

ARTICLE INFO
Article History:
Received: Oct. 22, 2024
Accepted: Nov. 26, 2024
Online: Dec. 10, 2024

Keywords:
Tetrodotoxin,
Puffer fish,
Lagocephalus sceleratus,
Ehrlish Ascites Carcinoma,
Ehrlish Solid Carcinoma,
Histopathology,
Muscle

ABSTRACT

The objective of the current study was to evaluate the anticancer effect of tetraodotoxin (TTX), which was isolated from the masked puffer fish *Lagocephalus sceleratus*, on the muscle tumor of albino mice that were carrying Ehrlish Solid Carcinoma (ESC). Commercial captures at the Attaka fishing harbor in the Suez Governorate, Egypt, provided samples of *L. sceleratus*. Ten days after Ehrlich Ascites Carcinoma (EAC) cells were injected into the mice's thigh, Ehrlich Solid Carcinoma (ESC) tumors developed in the mice. ESC-bearing mice were given extracted TTX in nine equal doses over the course of three weeks. Results showed an abnormal histological structure of the skeletal muscle's thigh of ESC bearing mice at zero day of experiment, increasing of aggressive colonies of carcinoma neoplastic cells forming tumor masses in the connective tissue between muscle bundles leading to atrophy in these muscle fibers, increasing the space between fibers and degeneration of these muscle fibers. These histopathological alterations increased leading to a complete damage in muscular tissues and forming multi-tumor masses of neoplastic cells, which distributed and colonized along all the tissue. The treatment of ESC bearing mice with TTX for 21 days after zero day of experiment showed well defined improvement in muscular tissues, recovery of muscle fibers, reduction in the number of carcinoma neoplastic tumor's cells, and disappearance of tumor mass colonies. This study concluded that TTX administration had a strong inhibitory effect on the growth of Ehrlich Solid Carcinoma tumors in mice over a short term (three weeks). However, TTX may have a reversible effect with prolonged exposure.

INTRODUCTION

Tetrodotoxin (TTX) is a very potent neurotoxin that is found in a variety of marine organisms **(Rodríguez *et al.*, 2012)** and also in some terrestrial ones. Its toxicity is often emphasized by referring to the fact that it is over a thousand times more toxic to humans than cyanide; TTX has no known antidote **(Noguchi & Ebeso, 2001; Saoudi *et al.*, 2010; Yotsu-Yamashita *et al.*, 2012)**. One naturally existing toxin that has caused intoxications and deaths in humans is TTX **(Abu-Amra *et al.*, 2002; Fouda, 2005)**. According to the

current research, TTX has migrated to parts of the Pacific and the Mediterranean, refuting the notion that it was limited to South East Asia **(Bane *et al.*, 2014)**.

A helpful technique for identifying, isolating, and characterizing voltage-gated sodium channels is TTX. Because of its strong capacity to attach to the transmembrane glycoprotein and construct the Na+ channel, TTX can block it **(Tosteson, 1992)**.

TTX has inhibitory effect on the invasion of metastatic prostate cancer, so it has the potential to be considered as an anticancer drug **(Prasad, 2004)**. It was also found that as a potential drug of anesthesia **(Schwartz *et al.*, 1998)**, aces dyne is to cure chronic cancer pain and neuro-protective drug **(Narahashi, 2001; Bucciarelli *et al.*, 2021)**.

In Canada and China, a team of Chinese researchers conducted clinical trials utilizing tetraodotoxin as an analgesic that lessens the severe agony those patients with advanced cancer experience. Pain was reduced for two to three weeks after tiny doses of TTX were administered. When administered in extremely small dosages, the purified TTX proved safe, according to the clinical trials. It has a long duration of action and operates swiftly. With fewer adverse effects and no addictive properties, TTX was 3000 times more potent than morphine **(Alonso *et al*., 2003)**.

A broad variety of applications are increasingly interested in bioactive compounds (geo-medicine, plant-science, modern pharmacology, agrochemicals, cosmetics, food industry, nano-bio-science) aimed at diversifying the resources of bioactive compounds and enhancing their salvage or synthesis pathways **(Abdelkarim *et al*., 2014; Kumar *et al.*, 2019; Khalil *et al*., 2020)**.

Over 6 million people worldwide lose their lives to cancer each year, making it the leading cause of death and a serious public health concern in most regions of the world **(Gupta *et al.*, 2004; Jemal *et al*., 2008)**. The use of Ehrlich Ascites Carcinoma (EAC) as a model in anti-cancer research was proven by many authors to give accurate and reliable results **(Ramnath *et al.*, 2002; Gupta *et al.*, 2004; Ozaslan *et al.*, 2011)**. Its ability to evaluate the effectiveness of any anticancer medication by prolonging the experimental animal's life, as well as increasing the quantity and viability of the cell line itself and the volume of fluid produced by the tumor inside the peritoneal cavity, is what makes such a test reliable **(Maity *et al.*, 1999)**.

Thus, the goal of this study was to evaluate the anticancer effect of tetraodotoxin (TTX), which was isolated from the Red Sea masked puffer fish, on the development of tumors in the muscular tissues of albino mice that were harboring ESC.

MATERIALS AND METHODS

1- Animals

A- Fish: Five large specimens of masked puffer fish, *L. sceleratus*, identified according to **Gohar and Clark (1953)**, were obtained from commercial catches at Attaka fishing harbor, Suez Governorate, Egypt. Their total lengths were 30 ± 1.5cm.

B –mice:

80 healthy male albino mice (22± 3g in body weight) were obtained from the National Researches Center in Dokki, Cairo. They were divided into 4 groups (each of 20 male mice). These animals were exposed to 12 hours of regular light/dark periods, fed daily with vegetable, commercial pellets rich of protein.

2- Induction of Ehrlich Ascite Carcinoma (EAC)

Ehrlich Ascites Carcinoma (EAC) 87032503 line cells were obtained from the National Cancer Research Institute, via 22±3g female albino mice. They were maintained by weekly interperitoneally inoculation of saline solution containing 10^6 cells/mice.

3- TTX extraction

To prepare the stock for TTX extraction, the puffer fish samples were dissected, and the liver samples were extracted, homogenized, and soaked in a 10% acetic acid-methanol mixture. These samples were then stored for 3-5 days according to **Kawabata (1978)**. The crude TTX was purified by filtration through a cotton paper funnel, and the excess methanol was evaporated to concentrate the TTX. The TTX was then dissolved in a saline solution (0.9% sodium chloride). Toxicity tests were performed by injecting different concentrations of the TTX intraperitoneally into male albino mice (20-25g). The survival time of the mice was recorded, and the mouse units were calculated according to **Kawabata (1978)**.

4- Experimental groups

A - Control group (G_1): It contains 20 male mice, which were injected with 0.1ml of saline solution (0.9% NaCl) every 48 hours for 21 days, starting from day zero.

B – Positive control group for TTX (G_2): It contains 20 males of mice, which were injected with the crude TTX extracted from *L. sceleratus* with a dose of 65 microns (equal 1\20 MU), dissolved in 0.1ml of saline solution every 48 hours for 21 days, after zero-day **(Kawabata, 1978; Noguchi & Mahmud, 2001)**.

C – Positive control group for ESC (G_3): It includes 20 male mice, which were injected with 0.1 ml of EAC diluted in saline solution into the femoral muscle to induce a solid ESC tumor. Tumor cell inoculation occurred on day 10, which is considered the zero-

day of the experiment. Subsequently, the mice were injected with 0.1ml of saline solution (0.9% NaCl) every 48 hours for 21 days **(Badr El-Din *et al*., 2007)**.

D – Experimental group (G_4): It includes 20 male mice, which were treated as in group G3 until the zero-day. Then, they were injected with crude TTX extracted from *L. sceleratus* at a dose of 65 micrograms (equivalent to 1/20 MU), dissolved in 0.1ml of saline solution every 48 hours for 21 days.

5- Histopathological studies:

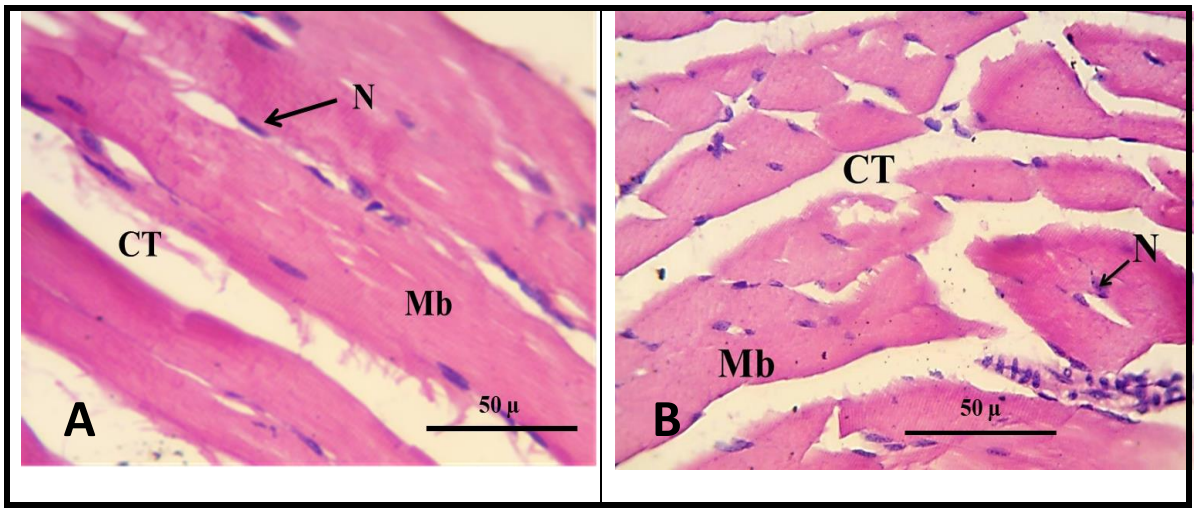
The mice from both the control and treated groups were decapitated at zero time, and then at 7, 14, and 21 days after the experimental injection period. Their muscles were excised and immediately fixed in alcoholic Bouin's solution for 24 hours. Routine histological procedures using Hematoxylin and Eosin staining were then performed. Finally, the slides were microscopically examined and photographed using a camera mounted on a light microscope, and the results were described.

RESULTS

1. Histopathological examination of muscles at zero time

Examination of thigh' muscle in the control mice at zero day of the experiment (G_1 & G_2) showed normal histological structure of the skeletal muscle fibers of thigh and no histopathological alterations were observed (PLATE I A&B).

Examination of thigh's muscles of male albino mice implanted with ESC cells at the zero day of the experiment (G_3 & G_4) showed abnormal histological structure of the skeletal muscle fibers of thigh. An increasing of aggressive colonies of carcinoma neoplastic cells forming tumor masses in the connective tissue between muscle bundles leading to atrophy in these muscle fibers, increasing the space between fibers and degeneration of muscle fibers (PLATE I C&D).



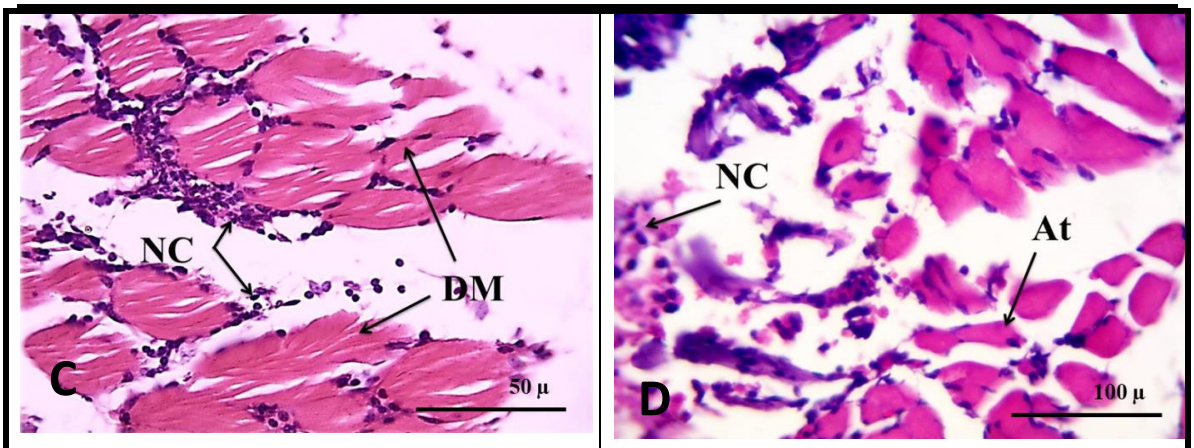


PLATE I

Photomicrographs of histological sections in thigh muscle of male albino mice at zero-day: **A:** Control group (**G_1**); **B:** TTX injected group (**G_2**); **C:** ESC-bearing group (**G_3**); **D:** ESC-bearing group and treated with TTX (**G_4**). **(At:** Atrophied muscle; **CT:** Connective tissue**; DM:** Degenerated muscle; **Mb:** Muscle bundle**; N:** Nucleus**; NC:** neoplastic carcinoma cells; **H&E)**

2. Histopathological examination of muscles after 7 days

Sections of thigh's muscle in mice of G_2 showed a moderate effect after injection with TTX for 7 days. Normal skeletal muscle fibers were observed and almost the structures were still normal like those of the control sections (PLATE II A).

After 7 days of experiment, examination of thigh's muscle of male albino mice in G_3 showed histopathological alterations of muscle fibers bundles. They degenerated and showed severe atrophy in muscles fibers. Increasing in number of neoplastic carcinoma cells was observed, forming many tumor masses. More increasing of spaces between fibers were distributed and colonized by the neoplastic carcinoma cells (PLATE II B).

After 7 days of the experiment, examination of thigh's muscles in mice of G_4 showed reduced number of tumor carcinoma neoplastic cells, some limited improvements in the degenerated muscle tissues. But also, colonies of neoplastic cells in the connective tissue between bundles of muscle fiber were still distributed (PLATE II C).

3. Histopathological examination of muscles after 14 days

After 14 days of experiment, examination of the muscles in mice thigh of G_2 showed that some lymphocytes were observed in connective tissue between muscle bundles; but the histological structure of muscle fibers still like that of the control group (PLATE II D).

After 14 days of exposure to ESC in specimens of G_3, histological examination of thigh's muscles showed that it has severe atrophy in the muscle's fibers. Also, aggressive increasing in number of carcinoma cells forming multi-tumor masses of neoplastic cells

which distributed and colonized along the tissue. Disappearance of the spaces between fibers was observed, leading to acute destroying of thigh's muscles fiber (PLATE II E).

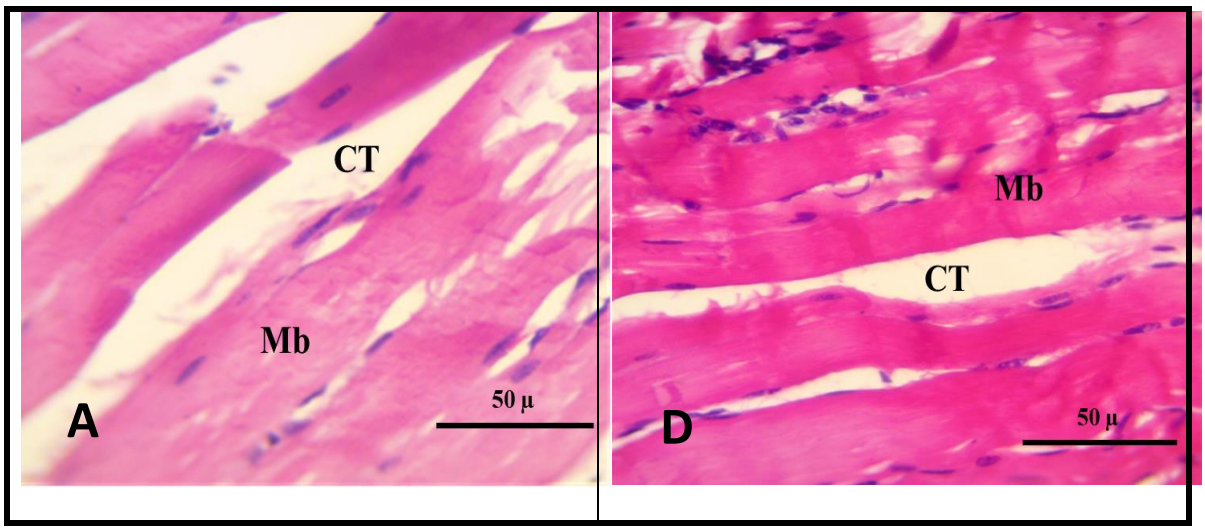
The histopathological examination of G_4 specimens after 14 days of treatment with TTX showed highly reduced number of carcinoma neoplastic tumor's cells, moderate improvements of interstitial material of connective tissue and regeneration of atrophied muscle fibers which brought back nearly as normal control group, except some of neoplastic cells in the connective tissue between muscle fibers (PLATE II F).

4. Histopathological examination of muscles after 21 days

Examination of the muscles of thigh in G_2 specimens after injection with TTX for 21 days showed generally well-defined histological structure, except some disorganized effects in connective tissue, but muscular structures still nearly normal like those of the control sections (PLATE III A&B).

After 21 days of exposure to Ehrlich Solid Carcinoma (ESC) tumor in specimens of G_3, histological examination of thigh's muscles showed acute destroying muscles and completely damaged fibers of thigh leading to absence of muscular fibers structure of thigh. Additionally, an aggressive increase was detected in the number of carcinoma cells forming multi-tumor masses of neoplastic cells, which distributed and colonized along all the tissues leading to the disappearance of the spaces between fibers (PLATE III C).

The histopathological examination of G_4 specimens after 21 days of treating the ESC with TTX showed well defined improvements of muscular fibers tissues with convenient state of interstitial material of connective tissue, and recovered muscle fibers which brought back nearly as normal structure of control group. Furthermore, highly reduction was recorded in the number of carcinoma neoplastic tumor's cells along connective tissues, aligned with the disappearance of tumor mass colonies, except some of neoplastic cells in the connective tissue between muscle fibers (PLATE III D).



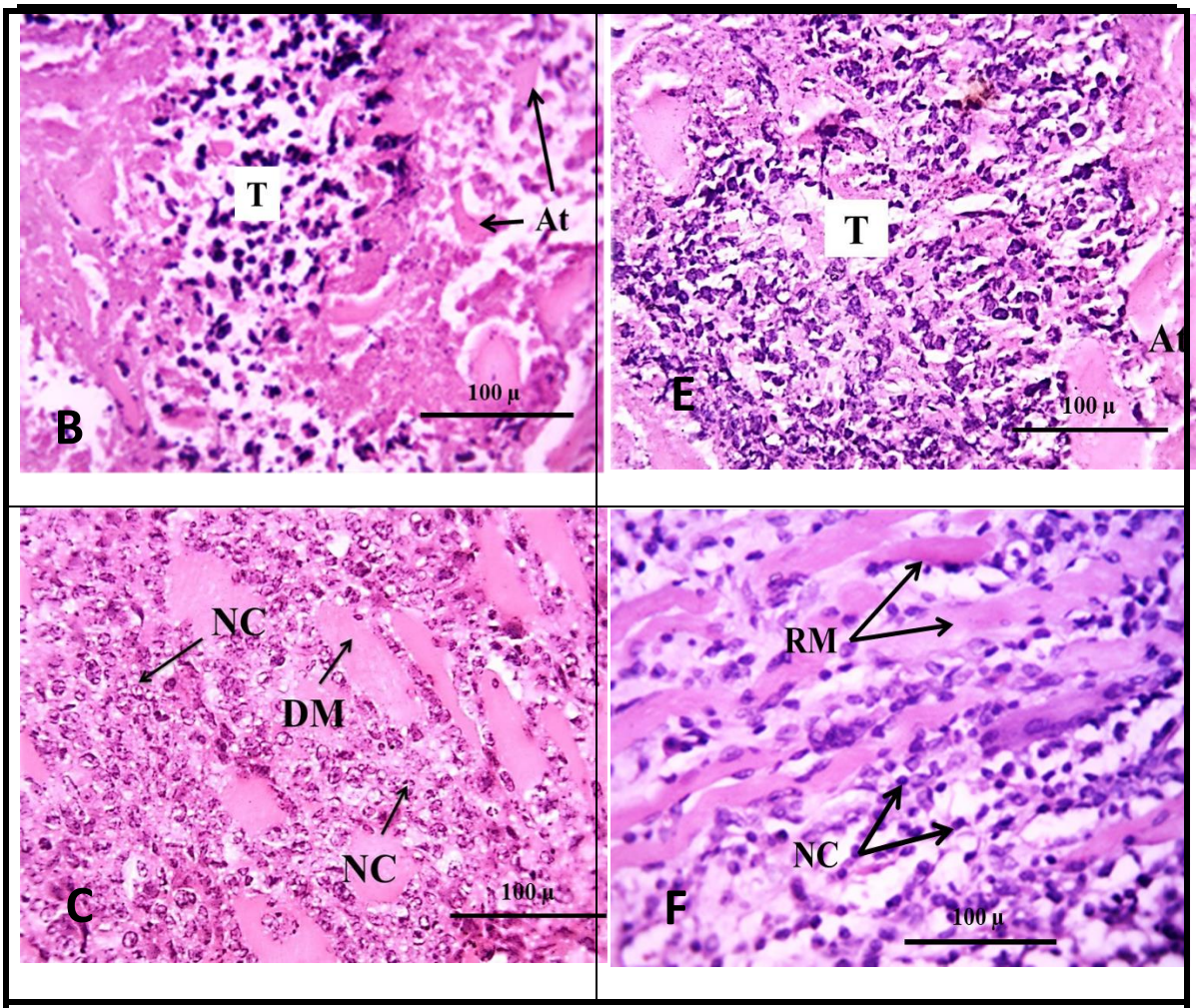
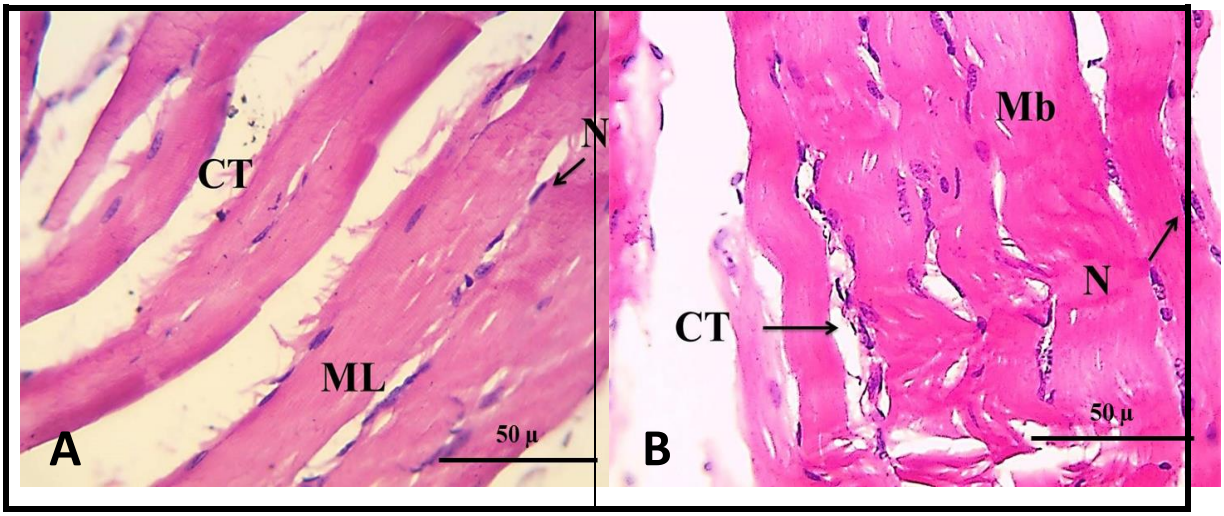


PLATE II

Photomicrographs of histological sections in thigh muscle of male albino mice: **A, B & C:** After 7 days of experiment in **G_2, G_3 & G_4**, respectively. **D, E & F:** after 14 days of experiment in **G_2, G_3 & G_4**, respectively. **(At:** Atrophied muscle; **CT:** Connective tissue**; DM:** Degenerated muscle; **Mb:** Muscle bundle**; NC:** Neoplastic carcinoma cells; **RM:** Recovered muscle**; T:** Tumor; **H&E)**



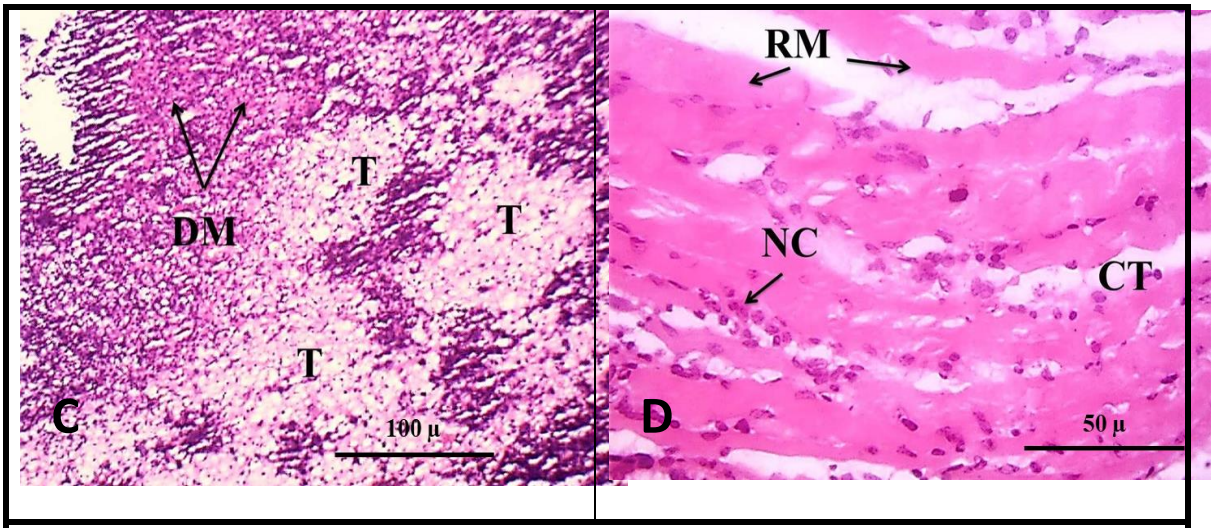


PLATE III

Photomicrographs of histological sections in thigh muscle of male albino mice after 21 days of experiment: **A:** Control group (**G_1**); **B:** TTX injected group (**G_2**); **C:** ESC-bearing group (**G_3**); **D:** ESC-bearing group and treated with TTX (**G_4**). **(CT:** Connective tissue**; DM:** Degenerated muscle; **Mb:** Muscle bundle**; N:** Nucleus; **NC:** Neoplastic carcinoma cells; **RM:** Recovered muscles**; T:** Tumor**; H&E)**

DISCUSSION

There have been various approaches to the search for novel anti-cancer medications. Using the most recent data on the mechanism of action of anti-neoplastic medications and data on the kinetics of cancer cells, scientists were able to develop chemotherapy medications that could be utilized more widely **(Ulakoglu & Altun 2004; Falzone *et al.*, 2018; Ioele *et al.*, 2022)**.

Ehrlish Assites Carcinoma cell **(**EAC) line was the modeling system of ascites tumor to study the effect of TTX. Ehrlish cells were growing carcinoma cells which have a very aggressive behavior **(Nascimento *et al.*, 2006)**. According to numerous researchers, using EAC as a model in anti-cancer research yields accurate and trustworthy results **(Ramnath *et al.*, 2002; Gupta *et al.*, 2004)**. In addition to assessing changes in the number and viability of the cell line itself, the test's credibility rests in its capacity to assess the effectiveness of any anticancer medication by extending the lifespan of experimental animals **(Maity *et al.*, 1999)**.

In the present study, after incubation of implanting EAC in the skeletal muscles of thigh for ten days, solid form of cancer cells was observed which aggressively multiplied, migrated and invaded some other organs like liver and kidney. These cancer cells made neoplasia, which defined as metastasis to cancer neoplastic cells **(Abd El-Wahab & Fouda, 2009; Azab *et al.*, 2017)**.

TTX therapy significantly slowed the growth of tumors in Ehrlish Solid Carcinoma tumor-bearing mice in the current investigation. A greater increase in the

tumor inhibition ratio indicated a more noticeable effect in the TTX treatment plan. The findings of **Roger *et al.* (2004)** and **Fernandes *et al.* (2006)**, who reported that TTX has a great ability to bind to the trans-membrane glycoprotein forming Na channel and block it, thereby reducing the proliferation, migration, and invasive properties of cancer cell lines, are in line with the findings of **Fouda (2005)**.

The impact of TTX on the formation of EAC cells is evident from the present results. Small-sized cells predominated during the three weeks of the experiment, and the overall number of cells decreased significantly. According to **Roger *et al.* (2004)**, the longer lifespan indicates that TTX may have partially inhibited the growth of the cancer cells by preventing them from absorbing Na+ from the surrounding media. According to **Grolleau *et al.* (2001)**, who previously found a similar result, the timing and dosage of Na+ blockers have a significant role in regulating cell metastasis.

The present study showed also that Ehrlich cells which treated with TTX extracted from liver of *Lagocephalus sceleratus* turn to normal sizes instead of abnormal sizes of untreated Ehrlich group at two weeks of exposure. The way that TTX acts on ESC is most likely the same as it is for other nervous system cells. When it binds to the p-glycoprotein of the Na channels, it blocks it and stops the influx of Na into the cells. In turn, this action stops the carcinoma cells from receiving enough sodium ions for the needs of different intracellular processes and, most importantly, to preserve the normal distribution of charges across the cell membrane, which is essential for preserving the integrity of the cell **(Abd El-Dayem *et al.*, 2012).**

According to the histological data, ESC cells underwent apoptosis, most likely as a result of losing their capacity to proliferate. According to **Jia *et al.* (2005)**, the EAC cell cycle typically doubles in size every 36 to 48 hours. However, this rate of cell division was significantly decreased during exposure to TTX, particularly during the first- and second-weeks following therapy. As a result of increased apoptosis, the number of cells has likely decreased. Others have also observed that this experiment clearly inhibits the development rate of tumor cells **(Pal *et al.*, 2001; Bhattacharyya *et al.*, 2003**; **Guicciardi & Gores, 2005; Katikou *et al.*, 2022)**.

Given the prior findings, which are consistent with those of **Abd El-Motelp and Zaazaa (2013)** and **Xiao (2014)**, it can be concluded that TTX may have an inhibitory effect on the invasion of metastatic cancer, which could make it a viable medicine for cancer treatment.

CONCLUSION

This study concluded that TTX administration had a strong inhibitory effect on the growth of Ehrlich Solid Carcinoma tumors in mice over a short term (three weeks). However, TTX may have a reversible effect with prolonged exposure. This suggests that TTX extracted from the liver of *Lagocephalus sceleratus* has the potential to restore or

normalize the size of tumors in comparison with the untreated Ehrlich group after three weeks, as reflected in the increased lifespan of the mice. Therefore, TTX may hold promise as a novel natural cancer treatment.

REFERENCES

Abd El-Dayem, S.M.; Fouda, F.M.; Ali, E.H. and **Abd El-Motelp, B.A. (2012).** The antitumor effects of tetrodotoxin and/or doxorubicin on Ehrlich Ascites carcinoma-bearing female mice. Toxicol. Indust. Health., **1**: 1-14.

Abd El-Motelp, B.A. and **Zaazaa, A.M. (2013).** Potential antitumor effect of tetrodotoxins and/or quercetin against Ehrlich Carcinoma in mice. W.J.P and P.S., **2** (6): 6653-6667.

Abd-Elkarim, G.; Saddi, A.; Bouali, R.; Boukhatem, N.; Benchat, N. and **Hamal, A. (2014).** What is a bioactive compound? A combined definition for a preliminary consensus. International Journal of Nutrition and Food Sciences., **3**(3): 174-179.

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.

Abu-Amra, E.; El-Shater, A.R.; Khalifa, M.H. and **Abd-Elsatter, M. (2002).** Effect of the tetrodotoxins extracted from the ovary and liver of puffer fish *Arothron hispidus* on kidney function of male albino rat. J. Egypt. Ger. Soc. Zool., **37**(A): 353-367.

Alonso, D.; Khalilb, Z.; Satkunanthanb, N. and **Livettc, BG. (2003).** Drugs From the Sea: Conotoxins as Drug Leads for Neuropathic Pain and Other Neurological Conditions Mini Reviews in Medicinal Chemistry., **3**: 785-787.

Azab, A.M.; Abu- Zaid, M.M.; Ghanem, M.H. and **Edris, A.H.H. (2017).** Evaluation of antitumor activity of tetrodotoxin extracted from liver of the Red Sea puffer fish, *Lagocephalus sceleratus* against Ehrlich Ascites Carcinoma in albino mice: 1. liver and kidney functions. International Journal of Development., **6** (1): 15-30.

Badr El-Din, NK.; Ghoneum, M; Noaman, E. and Tolentino, L. (2007). Saccharomyces cerevisiae, the Baker's Yeast, suppresses the growth of Ehrlich carcinoma-bearing mice. Canc. Immunol.Immunother., 57(4):581–592.

Bane, V.; Lehane, M.; Dikshit, M.; O'Riordan, A. and **Furey, A. (2014).** Tetrodotoxin: Chemistry, Toxicity, Source, Distribution and Detection. Toxins., **6**: 693-755.

Bhattacharyya, A.; Choudhuri, T.; Pal, S.; Chattopadhyay, S.; Datta, GK.; Sa, K. and **Das, T. (2003).** Apoptogenic effects of black tea on Ehrlich's ascites carcinoma cells. Carcinogenesis., **24** (1): 75–80.

Bucciarelli, G.M.; Lechner, M.; Fontes, A.; Kats, L.B.; Eisthen, H.L. and **Shaffer, H.B. (2021).** From poison to promise: The evolution of tetrodotoxin and its potential as a therapeutic. Toxins.;13:517. doi: 10.3390/toxins13080517.

Gohar, H.F. and **Clark, E. (1953).** The fishes of the Red Sea: Order Plectognathi. Fouad I University Press, Egypt., Pp. 80.

Fernandes, R.; Pires, O.R. and **Schwartz, C.A. (2006).** Eating Habits: Are we safe to Consume Freshwater Puffer Fish from the Amazon Region in Brazil. J Venom Anim Toxins incl Trop Dis., **12** (2): 153-155.

Falzone, L.; Salomone, S. and **Libra, M. (2018).** Evolution of Cancer Pharmacological Treatments at the Turn of the Third Millennium. Front Pharmacol.,**9**:pp.1300. doi:10.3389/fphar.2018.01300.

Fouda, F.M. (2005). Anti-tumor activity of tetrodotoxin extracted from the Masked Puffer fish *Arothron diadematus*. Egyptian Journal of Biology., **7**: 1-13.

Grolleau, F.; Laurence, G.; Michèle, B.; Brunom L.; Marcel, P. and **Erick, G. (2001).** A possible explanation for a neurotoxic effect of the anticancer agent oxaliplatin on neuronal voltage-gated sodium channels. J. Neurophysiol., **85**: 2293–2297.

Guicciardi, M.E. and **Gores, G.J. (2005).** Apoptosis: a mechanism of acute and chronic liver injury. Gut. Jul., **54** (7): 1024–1033.

Gupta, A.; Mazumder, U.K.; Kumar, R.S. and **Kumar, TS. (2004).** Anti-tumor activity and anti-oxident role of Bauhinia racemosa against Ehrlich ascites carcinoma in Swiss albino mice. Act. Pharm. Sin., **25** (8): 1070-1076.

Ioele, G.; Chieffallo, M.; Occhiuzzi, M.A.; De Luca, M.; Garofalo, A.; Ragno, G. and Grande, F. (2022). Anticancer Drugs: Recent Strategies to Improve Stability Profile, Pharmacokinetic and Pharmacodynamic Properties. Molecules., 2022 Sep; 27(17): pp.5436. Published online 2022 Aug 25. doi: 10.3390/molecules2717543.

Jemal, A.; Siegel, R.; Ward, E.; Hao, Y.; Xu, J. and **Murray, T. (2008).** Cancer statistics. A Cancer Journal for Clinicians., **58** (2): 71–96.

Jia, H.; Zhang, Y.; Lan, X.; Qin, G.; Zhang, J. and **Zhi-hong, H. (2005).** An imaging study using laminin peptide 99mTc- YIGSR in mice bearing Ehrlich ascites tumor. Chin. Med. J., **118** (9): 753-758.

Katikou, P.; Gokbulut, C.; Kosker, A.; Campàs, M. and Ozogul, F. (2022). An Updated Review of Tetrodotoxin and Its Peculiarities. Mar Drugs., 2022 Jan; 20(1): 47. doi: 10.3390/md20010047.

Kawabata, T. (1978). Food hygiene examination manual Volume 2, Assey method for Tetrodotoxins. Jap. Food Hyg. Assoc. Tokyo, Japan., 223-241.

Khalil, E.A.; Swelim, H.H. and **Zaky, M.A. (2020).** Histological and biochemical studies on the effect of tetrodotoxin extracted from Puffer fish (*Lagocephalus sceleratus*) against carbon tetrachloride induced hepatotoxicity in albino mice. Egyptian Journal of Aquatic Biology & Fisheries., **22** (2): 137- 147.

Kumar, A.; Ahmad, F. and **Zaidi, S. (2019).** Importance of Bioactive Compounds Present in Plant Products and Their Extraction – A Review. Agricultural Reviews., **40**: 249-260.

Maity, P.; Chakrabory, S. and **Bhattacharya, P. (1999).** Angiogenesis, a putative new approach in glutamine related therapy. Path. Onco. Res. J., **5** (4): 309-314.

Narahashi, T. (2001). Pharmacology of tetrodotoxin. Journal of Toxicology-Toxin Reviews., **20** (1): 67-84.

Nascimento, R.F.; Cruz, G.V.B.; Pereira, P.V.S.; Maciel, C.G.; Silva, L.A.; Azevedo, A.P.S.; Barroqueiro, E.S.B. and **Guerra, R.N.M. (2006).** Ascitic and solid Ehrlich tumor inhibition by *Chenopodium ambrosioides* L. treatment. Life Sciences., **78**: 2650–2653.

Noguchi, T. and Ebesu, J.S.M. (2001). Puffer poisoning: Epidemiology and treatment. Toxin Rev., **20**: 1–10.

Ozaslan, M.; Karagoz, D.I.; Kilic, I.H. and **Guldur, M.E. (2011).** Ehrlich ascites carcinoma. African Journal of Biotechnology., **10** (13): 2375-2378.

Pal, S.; Choudhuri, T.; Chattopadhyay, S.; Bhattacharya, A.; Datta, GK.; Das, T, and **Sa, G. (2001).** Mechanisms of curcumin-induced apoptosis of Ehrlich's ascites carcinoma cells. Biochem. Biophys. Res. Commun., **288**: 658-665.

Prasad, H.S.R. (2004). Potential effects of tetrodotoxin exposure to human glial cells postulated using microarray approach. Toxicon., **44**: 597-608.

Ramnath, V.; Kuttan, G. and **Kuttan, R . (2002).** Anti-tumor effect of abrin on transplanted tumors in mice. Indian J. Physiol. Pharmacol., **46**: 69-77.

Rodríguez, P.; Alfonso, A.; Otero, P.; Katikou, P.; Georgantelis, D. and **Botana, L.M. (2012).** Liquid chromatography-mass spectrometry method to detect Tetrodotoxin and Its analogues in the puffer fish *Lagocephalus sceleratus* (Gmelin, 1789) from European waters. Food Chem., **132**: 1103-1111.

Roger, S.; Le Guennec, J.Y. and **Besson, P. (2004).** particular sensitivity to calcium channel blockers of the fast inward voltage-dependent sodium current involved in

the invasive properties of a metastatic breast cancer cell line. Brit. J. Pharmacol., **141**:610-615.

Saoudi, M.; Abdelmouleh, A. and **El Feki, A. (2010).** Tetrodotoxin: A potent marine toxin. Toxin Rev., **29**: 60-70.

Schwartz, D.M.; Fields, H.L.; Duncan, K.G.; Duncan, J.L. and **Jones, M.R. (1998).** Experimental study of Tetrodotoxin, a long-acting Topical Anesthetic. Am. K. Ophthalmol., **125**: 481-487.

Tosteson, M.T. (1992). Effects of toxins on the voltage-gated Na+ channel, Proc. Third Int. Conf. Ciguatera, Portorico, Tosteson., **2**: 47-54.

Ulakoglu, G. and Altun, S. (2004). The effects of epirubicin on proliferation and DNA synthesis of Ehrlich ascites carcinoma cells in vitro and in vivo. Biologia, Bratislava., **59** (6): 727-734.

Xiao, Z. (2014). Biosynthetic Studies of Tetrodotoxin and its Anticancer Activities Assessment in Vitro. Hong Kong Baptist University., 1-87.

Yotsu-Yamashita, M.; Gilhen, J.; Russell, R.W.; Krysko, K.L.; Melaun, C.; Kurz, A.; Kauferstein, S.; Kordis, D. and **Mebs, D. (2012).** Variability of tetrodotoxin and of its analogues in the red-spotted newt, *Notophthalmus viridescens* (Amphibia: Urodela: Salamandridae). Toxicon., **59**: 257-264.