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Neurotoxicity and immunotoxicity effects in mice Brain induced by oral administration of saxitoxins extracted from the cockles *Acanthocardia tuberculatum*

Nadia Takati[1,2], Houssine Azzedoug[1], Mohammed Nabil Benchekroun[2], Mohamed Blaghen[3], Moulay Mustapha Ennaji[2*]

Laboratory of BioGeosciences and Materials Engineering, Ecole Normale Supérieure, University Hassan II of Casablanca, Ghandi, 50069, Casablanca, Morocco[1]
Team Research of Virology, Oncology, and Biotechnologies, Laboratory of Virology, Oncology, Biosciences, Environment and New Energies, Faculty of Sciences and Techniques Mohammedia, University Hassan II of Casablanca, Casablanca, Morocco[2]
Laboratory of Immunology and Biodiversity, Faculty of Sciences Ain cock, Hassan II University of Casablanca, 5366, Casablanca, Morocco[3]

Corresponding author: 2*

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ABSTRACT

Saxitoxins (STXs) are a highly marine neurotoxins derived from harmful algal blooms and cause paralytic shellfish poisoning (PSP) that pose a significant risk to public and environmental health. The study of STXs toxicity has been carried out but little and is known about the histopathological responses on mice. The purpose of this study was to evaluate immunotoxic and histological responses induced by STXs extracted from *Acanthocardia tuberculatum*. To this end, daily, mice were treated orally during 7 days with sublethal concentrations (10 mg /100 g mouse). Lymphocyte proliferation and brain histopathology were analysed after treatment. The results showed a significative increase of lymphocytes level and a decrease of polynuclears level. The histological study in brain mice showed an increase of the number of the nucleus as well as a hypercondensation of the chromatin brain, Also, we observed the presence of some multinucleated giant cells that indicate the inflammation in brain. We conclude that STXs induce inflammation and cells necrosis in brain mice and causes the importation of the immunizing cells and the development of the inflammatory reactions.

1. INTRODUCTION

Harmful algal blooms (HABs) show range expansion and increased frequency in coastal areas since the 1980s in response to both climatic and non-climatic drivers, and have had negative impacts on food security, tourism, local economy, and human health [1]. Biotoxins produced by HABs can efficiently be bioaccumulated by filter-feeders, such as bivalves and small zooplankton, and travel up the trophic chain and

ultimately cause mass mortality events of aquatic fauna [2- 4].

Human consumption of contaminated shellfish can also cause lethal neurotoxic syndrome named Paralytic shellfish poisoning (PSP) with a variety of deleterious syndromes (headache, vomiting, numbness, and respiratory paralysis) that, in some cases, can lead to death [5- 7].

STXs are a water-soluble neurotoxins that bind to the voltage-dependent sodium channels in excitatory cells leading to hyperpolarization of the cell [8]. STXs are one of the most potent neurotoxins that were first reported in butter clam and mussel tissues in 1957 [9]. To date, More than 57 STX isomers with different toxic abilities and sodium channel affinities have been identified [10], [11]. STXs are also known as paralytic shellfish toxins (PSTs) and are mostly produced by marine dinoflagellates and freshwater cyanobacteria [12], [13].

Acanthocardia tuberculatrum, is a red cockle living in the western Mediterranean coast of Morocco, was chosen for extraction of STXs for the following reasons; this cockle is an appropriate organism to study immunological and histological effects in mice of the red cockle STXs extracted due to its remarkable retention high persistent levels of STXs for several years in its tissues even when the potentially toxin producing microalgae are not present [14], [15]. In effect, the highest levels of STXs were registered in cockle from Oued Laou and Kaa Srass, while in other regions STXs levels were lower but remained continuously higher than the regulatory level. Toxicity data also showed a very irregular distribution in samples from the same area that could be related to the size of sampled specimen [16]. Also, this type of cardid bivalve is found in southern Spain and on the Northorn coast of Morocco for the Spanish canning industry [17], [18].

The aims of this sudy were to determine whether the observed toxic effects after intraperitoneal administration also occur when the same extracts are administered orally and to demonstrate that the toxicity induced by the oral administration of the saxitoxins extracted from the cockles *Acanthocardia tuberculatum* to mice that can affect the histological aspects of vital organs like brain. Hence, immunological and histopahological studies were assessed after experimental exposure to sublethal concentrations of STXs.

2. Materials and methods

2.1 Samples

Specimens of the cockle (*Acanthocardia tuberculatum*) were collected from kaa Srass on the Mediterranean coast of Morocco. The cockle tissues were kept at -20°C until use. All other chemicals were of analytical grade.

2.2 Extraction of paralytic shellfish toxins and mouse bioassay

Toxicity analysis was carried out by mouse bioassay according to AOAC method (1990) [19]: 100g homogenized tissues collected from toxic cockles (Kaa Srass) were mixed with 100ml 0.1M hydrochloric acid and boiled gently for 5min, pH adjusted to 2–3. The volume of mixture was brought to 200 ml with double-distilled water, stirred and centrifuged at 3000rpm for 10min. The PSP mouse bioassay involves acidic aqueous extraction of selected organs. One milliliter of the supernatant was injected intraperitoneally into each of three albino mice (20± 2g). The mice are observed for classical PSP symptoms, such as jumping in the early stages, ataxia, ophtalmia, paralysis, gasping and death by respiratory arrest. The time from initial injection to mouse death is recorded and the values of toxicity are expressed in terms of STX. The time taken for the mice to die (death time) is taking into account for determination of sample toxicity expressed in mouse unit (MU) by Sommer Table [20]. A conversion factor (CF) is obtained by the calibration test using the

saxitoxin standard (STX). The CF allows to convert the results from MU to µg STXeq. / Kg of meat. The calculation of this factor is detailed in AOAC, 2000. In our case the CF found is 0.2, determined with ideal concentrations of 0.322, 0.33 and 0.34 µg/ml of saxitoxin.

2.3 Animals and administration of STXs

Swiss albino's mice were adapted to laboratory conditions at a temperature of 22°C with food and water *ad libitum*. The light cycle during the entire experiment was set to 14 h light and 10 h dark.

Forty-five animals were randomized into five groups of nine mice each, and the STXs extract was administered daily by oral injection during 7 days. Mice were given STXs at 10 µg /100 g of mice's weight while corresponding groups were given sterile double distilled water serving as control in each treatment.

2.4 Immunological study

In order to explore the impact of STXs extract on immunological cells, the number of lymphocytes, monocytes and polynuclears were calculated in the swiss albinos mice before and after orraly injection (7 days) with 10 mg/100g mouse of STXs extract.

2.5 Histopathological Analysis

After 7 days of treatment, brain samples were preserved in fixative solution (ethanol 80%; formaldehyde 40% and glacial acetic acid 5%) for 16 h, dehydrated in a graded series of ethanol baths, and embedded in Paraplast Plus resin. Sections (3– 5 mm) were stained in Hematoxylin and eosin (H&E) [21], then were examinated under light microscopy (Olympus-BH-2, Olympus, Southall, UK).

3. Results and Discussion

Cyanotoxins are a diverse group of natural compounds, such as anatoxins (ATXs) and STXs, These toxins have been implicated in the deaths of wild and domestic animals as well as in incidents of human illness. The liver is the most affected organ in humans but the exposure to the toxin is likely to affect organs such as the kidney, colon, gonads, and brain as evidenced by in vivo and in vitro studies. The neurotoxicity of cyanotoxins is a multipathway process, and despite recent achievements, the molecular mechanisms underlying neurotoxic effects remain elusive [22]. Acute and chronic mice intoxication symptoms and histopathological effects of the most common cyanotoxins have already been described in the literature. The signs that indicate the presence of alkaloid neurotoxin cyanotoxins, such as STXs and ATXs are: muscle progressive paralysis, overrated abdominal breath, cyanosis, convulsions, respiratory arrest, and death in a few minutes after sample administration, and absence of organ alterations in the post-mortem examen [23].

In our study, immunotoxic and histological responses in mice can be found after orally administration to STXs extracted from *Acanthocardia tuberculatum*. The general state and the mice mortality were followed with the amount 10mg/100 g mice during the 7 days of treatment. No sign of stress or difficulties on breathing, neither mortality nor visible disease signals in the treated orally mice STXs were observed.

Lymphocyte proliferation is a very sensitive test and is used as a potential biomarker for toxic exposures. While STX is notorious for its neurotoxicity, immunotoxic effects have also been described. Previous study investigated the role of STX in altering immune function, specifically T lymphocyte proliferation. In the present study, figure 1 showed that exposure to STXs increase significantly lymphocyte level (140%) and decrease significantly (P 0.05) polynuclear level (66%). No change was observed for monocytes level. The decrease of polynucelars can be explained by the fact that STXs can damage directly or indirectly immune system cells especially polynuclears, disrupting their production in the bone marrow, or inhibiting their

activity in the immune response. These effects can reduce the body's ability to fight infections and increase the risk of diseases. Our results were consistent with the toxicological studies that showed that STX caused an increase in harbor seal (Phoca vitulina concolor) lymphocyte proliferation at 10 ppb and exposure to STX significantly increased the amount of virus present in lymphocytes [24].

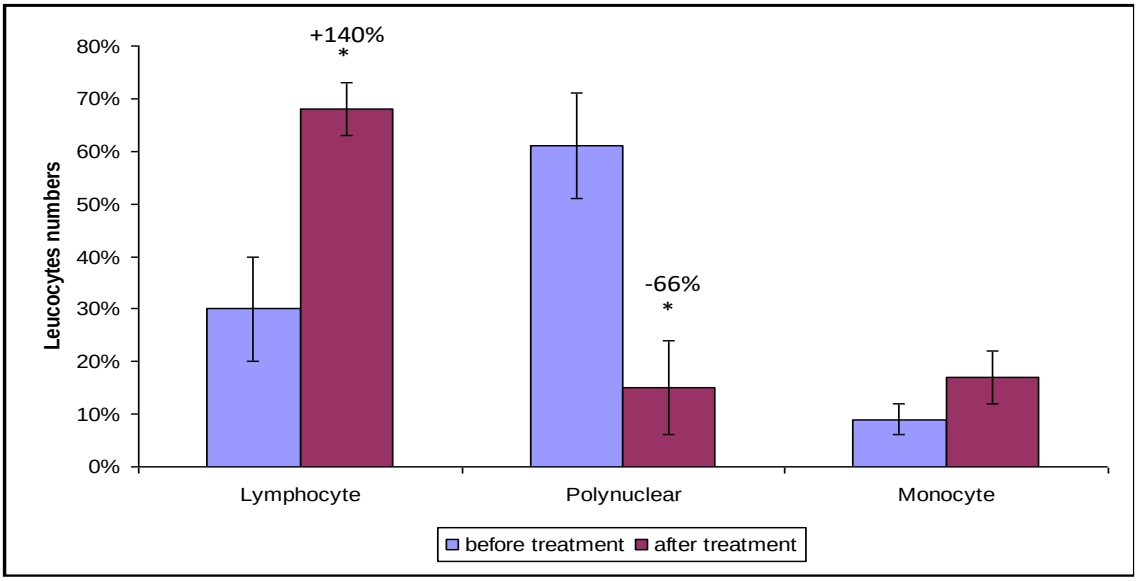

Figure 1: Leucocytes numbers values are means ± standards deviations. P< 0.05 (student t-test). %, indicate the percentage of decrease or increase of the values comparing with the control.

The macroscopic analysis of the removed brain during the dissection did not reveal any changes compared to the control. By performing Hematoxylin and eosin (H&E) staining validated that STXs exposure in brain of the mice treated with 0.3 mg/g compared to control show an increase of the number of the nucleus as well as a hypercondensation of the chromatin. Also, we observed the presence of some multinucleated giant cells that indicate the inflammation in brain (Figures 2 and 3). In effect, the necrosis of the cells causes the importation of the immunizing cells and the development of the inflammatory reactions.

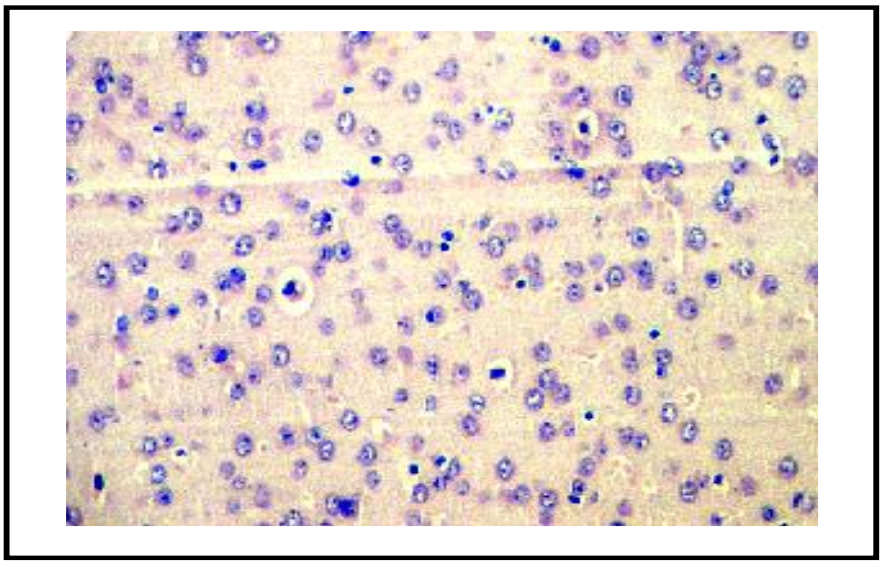

Figure 2: Photomicrograph (100 × H&E) of a section of brain from control mice treated with STXs showing a normal histological appearance.

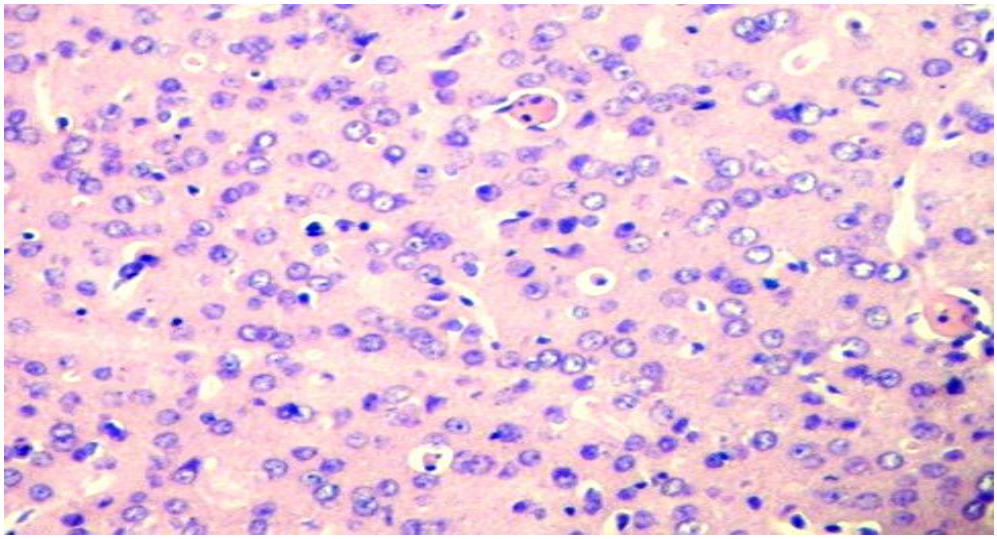

Figure 3: Photomicrograph (100 × H&E) of a section of brain from mice treated with STXs showing an increase of the number of the nucleus as well as a hypercondensation of the chromatin.

Previous studies have reported that STX is able to induce oxidative stress in algae and neural cell cultures through the lipid peroxidation (LPO) pathways [25]. As we know, LPO mechanisms are well established in the pathogenesis of Alzheimer's disease [26].

Another study has shown that examination of brain from zebrafish after 30 μg/L microcystin-LR (MCLR), toxin produced by cyanobacteria, exposure induced severe damage to cerebrum ultrastructure, showing edematous and collapsed myelinated nerve fibers, distention of endoplasmic reticulum and swelling mitochondria. This finding suggested that MCLR showed neurotoxicity in zebrafish which might attribute to the disorder of GABA neurotransmitter pathway [27].

Our results were consistent with the toxicological study that reported that STX-exposed mice exhibited brain neuronal damage characterized by decreasing neuronal cells and thinner pyramidal cell layers in the hippocampal CA1 region compared with control mice. Long-term low doses of STX exposure can cause neuronal inhibition, which is a process related to spatial memory impairment. Additionally, H&E staining validated those long-term low-level exposures did not cause significant gross morphological lesions in hippocampal regions of the brain, but decreased pyramidal cells and neuronal cells in CA1 [28].

4. Conclusions

In this study, a mouse model was used to explore the immunotoxic and histological effects of STXs exposure. The present study demonstrated that STXs exposure for 7 days to 10 mg/100g mice of STXs cause significant increase of lymphocyte level and a decrease of polynuclear level and induce inflammation and cells necrosis in Brain. The necrosis of the cells causes the importation of the immunizing cells and the development of the inflammatory reactions. These findings provide new insights and evidence for STXs neurotoxicity.

Abbreviations:

ATXs, anatoxins; Harmful algal blooms, HABs; H&E, Hematoxylin and eosin; LPO, Lipid peroxidation;

MCLR; microcystin-LR; MU, Mouse unit; Paralytic shellfish poisoning, PSP; PSTs, paralytic shellfish toxins; STXs, saxitoxins; STX, saxitoxin;

5. Author contributions

N. T: Conceived, designed and performed the experiments, analyzed the data, contributed analysis tools, wrote the paper.
H. A: revised the final version of the paper,
M. N. B: revised the final version of the paper,
M. B: Contribute to design and analyzed the data, correction the paper
M. M. E: Conceived, designed and supervised the experiments and the analysis of the data, critical of wrote the paper and coordinate the work

All authors approved the final version of the paper.

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7. Conflict of interest

The authors declare that they have no conflict of interest.

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