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Effect of poisoning with tetrodotoxin extracted from the fish *Takifugu Porphyreus* on the behavior of rats

Ettoubi Elmokhtar[1,2*],Akif Fouad[2,3],Genten Franck[4],Blaghen Mohamed[1],Bennis Faïza[1],Chegdani Fatima[1]

[1]*Laboratory of Immunology & Biodiversity, Hassan II University of Casablanca: Faculty of Sciences, Ain Chock, Km 8 Route d'ElJadida, B.P 5366 Maarif 20100, Casablanca 20000, Morocco*

[2]*Centre Régional des Métiers de l'Éducation et de la Formation Souss Massa, B.P. 106 My. Abdellah Av. Inezegane, Morocco*

[3]*Faculty of Medicine, Free University of Brussels, 808 Route de Lennik, 1070 Brussels, Belgium*

[4]*Laboratoire de BiopathologieVétérinaire, Faculté de Médecine, Université Libre de Bruxelles, 808 Route de Lennik, 1070Bruxelles, Belgique*

Abstract

Food poisoning from pufferfish consumption poses a significant food safety risk, particularly with *Takifugu Porphyreus*, highly toxic due to its tetrodotoxin (TTX) content, known for blocking Voltage-Dependent Sodium Channels (VGSC), leading to poisoning and potential fatality. Despite health risks, this fish is illegally sold in Morocco. This study aims to assess the impact of *Takifugu Porphyreus* consumption on neurotoxicity and physical development using a rat model.Four groups of adult male *Rattus norvegicus* were force-fed TTX extracted from *T. Porphyreus*. Groups included one control (n=10, receiving distilled water), two sub-chronic treatment groups (n=10 each) for 21 days with doses of 4.56 and 9.12 mg/kg/day, and one acute treatment group (n=10) with a single exposure to the highest dose. Doses were determined post TTX extraction and quantification from various fish organs using HPLC and LC-MS/MS. The chosen doses in this study aim to mimic real consumption conditions, taking into account the contamination levels observed in pufferfish sold on the market. The results showed the highest concentration of TTX in the gonads of the fish, followed by the liver, flesh, and viscera, with an average of 9.12 mg/kg. Treated rats exhibited reduced weight gain, severe diarrhea, decreased locomotor activity, anxiolytic and depressive behaviors, and memory disorders, assessed through behavioral tests including the Rotarod, elevated plus maze, tail suspension, and novel object recognition. This study underscores the toxic potential of TTX in this fish, emphasizing the need for enhanced dietary monitoring.

Keywords:*Takifugu Porphyreus*, Tetrodotoxin, Fish extracts, Food poisoning, Marine toxin.

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1. Introduction

The pufferfish, commonly known as *Takifugu*, is renowned for its high toxicity due to its significant content of tetrodotoxin, a potent neurotoxin of non-protein nature with low molecular weight. TTX is soluble in water and acidic environments, and it is colorless, odorless, and tasteless[1-2]. It is an extremely potent neurotoxin, being recognized as 275 times more lethal than cyanide and 50 times more toxic than strychnine or curare[3]. Its mechanism of action involves blocking voltage-gated sodium channels in both muscular and nervous systems, disrupting the propagation of action potentials and ultimately leading to paralysis[4-5]. Among the 21 species of Takifugu, 14 have been found to be toxic, with *Takifugu porphyreus* considered one of the most toxic fish globally, particularly in Asia[6-7]. Recent research has indicated that marine intestinal bacteria, including 20 species of *Pseudoalteromonas spp., Pseudomonas spp., and Vibrio spp.*, serve as the origin of this toxin. Some of these bacteria are known as symbiotes and are transmitted throughout the food chain to pufferfish[8].

However, the source of TTX remains a subject of debate among researchers, with arguments for it being symbiotic, endogenous, or exogenous[9]. Initially observed in Asian countries, the recent detection of TTX along the European Atlantic coast, in the Mediterranean Sea, and even in Morocco suggests geographical expansion, possibly due to the migration of toxin-bearing animals through the Suez Canal and global water temperature increases.TTX is easily absorbed by the gastrointestinal tract and stored in various tissues, including the skin, gonads, liver, ovaries, intestines, and possibly muscles[10]. Symptoms of TTX poisoning, depending on the ingested concentration, include oral paresthesia, dizziness, headache, sweating, gastrointestinal disturbances, neurological and motor disorders, paralysis, muscle coordination disturbances, respiratory depression, circulatory failure, nausea, and potentially death[11]. These symptoms are observed in humans following the ingestion of fish contaminated with TTX. Effects can occur within 10 minutes to 4hours. It is estimated that a dose of approximately 10 mg per kg constitutes a serious toxic dose for humans, with the oral LD50 for mice being 334 mg per kg[10]. Food poisoning associated with the consumption of TTX-bearing organisms mainly occurs when the fish's flesh and organs are improperly prepared and consumed, as the toxin cannot be destroyed by cooking or freezing, leading to the prohibition of the sale of these species[12]. Unfortunately, there is currently no known antidote or antitoxin to effectively treat TTX-poisoned patients[1,13]. Between 2010 and 2015, a total of 430 cases of poisoning and 52 deaths related to pufferfish were reported worldwide[14]. More recently, approximately 38% of these deaths occurred in Europe, as well as in the Middle East and Africa (21.4% and 16.7%, respectively)[15]. Despite the increasing number of TTX poisoning cases, data on its toxicity remain scarce[16]. According to Moroccan legislation (Decree No. 2-97-1003, Article 7), any product deemed unfit for consumption must be removed from the market. However, accidental consumption of TTX-bearing organisms or illegal practices, such as selling *T. porphyreus* in Morocco under the name of another non-toxic and more expensive fish, like *Lophius piscatorius*, continues to deceive consumers and jeopardize their health. This deceptive behavior has also been reported in European countries despite Regulation (EC) No. 854/, which aims to prevent the sale of mislabeled products on the domestic market[17]. Despite the alarming prevalence of TTX poisoning and its impact on public health, it is noteworthy that no study has yet been conducted to evaluate the actual effect of consuming toxic fish in their natural environment. Existing research typically focuses on the toxin alone, neglecting the possibility that the fish may contain other elements that could modulate its toxic effects. This knowledge gap underscores the crucial importance of conducting comprehensive studies to fully understand the risks associated with consuming these fish, considering their environment and overall chemical composition, and the urgent need to establish strict regulations in Morocco and globally, as well as to implement specific monitoring for the marketing of Takifugu fish. This research is the first to document TTX levels in various tissues of T. porphyreus sold in Morocco. It aims to improve our understanding of the toxic effects following acute and sub-chronic consumption of *Takifugu porphyreus* extracts, with a particular focus on physiological disruptions and neurobehavioral alterations.

2. Materials and methods

2.1. Fish sample

Specimens of *Takifugu Porphyreus* were collected from the Mediterranean coast of Morocco and were stored at -20°C for later use.A TTX standard with a concentration of 25.7 ± 2.1 µg/mL and a purity of 96% was obtained from Latoxan in France

2.2. Preparation of liposoluble extracts from fish organs

2.2.1. Extraction of tetrodotoxin

The preparation of liposoluble extracts from the fish organs involved the extraction of Tetrodotoxin (TTX). This extraction process followed previously published protocols from 2015 by Boundy et al. and from 2017 by Turner et al. The various organs of *Takifugu Porphyreus*, including the flesh, liver, and viscera, were used for this extraction, utilizing 1% acetic acid. The raw extracts were then diluted using a solution composed of water/acetonitrile/acetic acid in a ratio of 88.9:11.1:0.167 (v/v/v). Analysis of the extracts was performed using a C18 High-Performance Liquid Chromatography (HPLC) column[18-19].

2.2.2. Liquid chromatography conditions

Liquid Chromatography (LC) conditions were employed to quantify Tetrodotoxin (TTX) in the organs of *T. porphyreus*, following previously published protocols in 2015 by Boundy & al. and Turner & al. in 2015[19-20].

2.2.3. Mass Spectrometry Conditions

Mass Spectrometry conditions using a TSQ Vantage triple quadrupole mass spectrometer from Thermo Fisher Scientific were applied to analyze the TTX concentrations in each organ.

2.3. Animal preparation

Forty adult males Wistar rats (60 days), 300-350 grams were used. These rats were housed in Plexiglas cages with wood shavings as bedding, under controlled environmental conditions, including a 12-hour light/12-hour dark cycle and a temperature of 20 ± 2°C, with lights turning on at 7AM (ZT 0). The rats were provided with unlimited access to standard food and water. The study followed approved institutional protocols and ethical standards and adhered to the provisions of care and use of animals prescribed in the European Council Directive (EU2010/63).

2.3.1. Experimental design

The experiment involved a total of 40 rats divided into four distinct experimental groups, each consisting of 10 rats treated with the pufferfish tetrodotoxin solution. The choice of doses administered to the rats was based on the results of the quantification of TTX in the fish organs, revealing an average amount of TTX of 9.12 mg/kg of body weight [BW]. To reflect potential TTX levels in consumers, two doses were selected: 4.56 mg/kg of body weight and 9.12 mg/kg of BW, corresponding to the detected TTX levels. These doses were administered for sub-chronic treatment over a period of 21 days, once a day.

Additionally, an acute treatment group received a single dose of 9.12 mg/kg of BW of the TTX solution, while the

control group received distilled water. All treatments were administered by force-feeding.

2.3.2. Observation of intoxication signs

Intoxication signs were observed daily, including monitoring for diarrhea, vomiting, tremors, possible blue discoloration in the limbs, and mortality. Additionally, the rats' weights were measured once a week for 21 days to assess any impact on their weight gain. The observed signs of intoxication allowed for evaluating the potential effects of the treatments on the rats, while weight monitoring provided information on their growth and development.

2.4. Neuro-behavioural evaluation tests

2.4.1. Rotarod Test

The Rotarod Test was employed to assess the rats' ability to maintain their balance on a rotating rod during a trial period of 300 seconds. The test apparatus consisted of a textured horizontal roller with a diameter of 5 cm, positioned at a height of 28 cm above the ground. The roller rotated at a constant speed of 15 revolutions per minute. Each rat participated in two trials, with a 15-minute resting interval between them. The main measure recorded during the trials was the elapsed time before a rat fell from the rotating rod. This test provides valuable information on the rats' motor coordination and balance abilities. After each trial, the apparatus was thoroughly cleaned in preparation for the next rat's assessment.

2.4.2. The Elevated Plus Maze

The Elevated Plus Maze Test [EPMT] utilized a maze with two open arms measuring 50 × 10 cm and two closed arms measuring 50 × 10 × 40 cm, all connected to a central platform of 10 × 10 cm. The maze had transparent Plexiglas sidewalls and end walls, each 15 cm in height. It was positioned 50 cm above the ground and illuminated to approximately 200 lux. In the test, each rat was placed in the center of the maze, facing one of the open arms. The rat was allowed to explore the maze during a single recorded session lasting for 300 seconds. The percentage of time spent in the open arms was analyzed by calculating the ratio of "time spent in open arms" to the "total time spent in open and closed arms." Additionally, the time spent in the center of the maze was recorded. These neurobehavioral evaluation tests were designed to provide a better understanding of the effects of the treatments on rat behavior and motor abilities and to help identify any behavioral alterations related to the consumption of Takifugu.

2.4.3. Tail Suspension Test

In the Tail Suspension Test [TST], the rats were suspended individually from a plastic rod fixed 50 cm above the surface. Their tails were attached to the rod using adhesive tape. The test aimed to measure immobility, which was defined as the absence of any movement of the rats' limbs or body. The duration of immobility was recorded and measured for a total of 360 seconds. This test helps assess the depressive state of the rats and their behavioral responses to the treatments they received.

2.4.4. Novel object recognition test

The Novel Object Recognition Test is designed to evaluate cognition, with a particular focus on recognition memory. The test spans three days of experimentation:

- Day 1 - Habituation Phase: Rats were allowed to freely explore an empty arena for 5 minutes. This phase served to familiarize the rats with the testing environment.
- Day 2 - Training Phase (T1): After a 24-hour interval, the rats were introduced to the same arena for 10 minutes. During this phase, two identical objects were placed in the arena at an equal distance. The rats' interaction with these objects was observed.
- Day 3 - Test Phase (T2): On the third day, the rats were once again placed in the open field arena for 10 minutes. This time, they encountered one familiar object (the same as in T1) and one novel object. The aim was to assess long-term recognition memory.

During all three phases, the time spent exploring each object was recorded. To quantify the rats' behavior, several metrics were calculated:

- Recognition Index: This is calculated as the time spent exploring the novel object divided by the total time spent exploring both objects, expressed as a percentage.
- • Discrimination Index: It is calculated by subtracting the time spent exploring the familiar object from the time spent exploring the novel object, then dividing the result by the total exploration time.
- Latency Time: This measures the time spent exploring the familiar object in T2 compared to the time spent exploring the same object in T1.

Exploration of an object was defined as the rat placing its nose within 2 cm of the object. After each trial during the three phases, the rats were removed from the arena and placed back in their retention cage, and the apparatus was cleaned with 70% ethanol. This test helps assess the rats' recognition memory and cognitive abilities in response to the experimental treatments they received.

3. Results and Discussions

3.1. LC-MS/MS Mass Spectrometry of TTX extracted from fish organs

The results reveal significant variations in TTX distribution across fish tissues. The gonads of *T. porphyreus* show the highest TTX concentration, averaging 23.40 mg/kg of BW, indicating substantial toxin accumulation in this region. Additionally, fish flesh also displays significant levels of TTX, with an average of 5.80 mg/kg of BW. The liver and viscera of the fish exhibit intermediate TTX concentrations, with respective averages of 6.02 mg/kg and 1.25 mg/kg of BW. On average, the concentration of TTX in all examined tissues is 9.12 mg/kg of BW (Table 1).

3.2. Effect of T. porphyreus TTX on rats' physical development

3.2.1. Evaluation of intoxication signs

Each rat was closely monitored for signs of food intoxication. The ten rats treated with a 9.12 mg/kg of BW dose showed signs of intoxication, characterized by significant diarrhea starting on day 3 of the treatment, persisting until day 10, reappearing on day 14, and continuing until the end of the exposure. In the sub-chronic group, four rats treated with the 4.56 mg/kg of BW dose were manifested milder diarrhea. All treated rats reported an increase urinary micturition during their treatment.

3.2.2. Impact of TTX treatment on rat weight

The obtained results were analyzed with ANOVA a two-factor, taking into account the exposed dose and treatment period as the main factors.Our results revealed a significant impact of these factors on body weight ($F(2, 108) = 1123$, $P < 0.0001$; $F(3, 108) = 82.41$, $P < 0.0001$; $F(6, 108) = 180.9$, $P < 0.0001$, respectively) (Table 2). Bonferroni post hoc analysis showed a significant loss in body weight in treated rats compared to controls on day 7 ($t = 12.76$, $P < 0.0001$; $t = 15.96$, $P < 0.0001$, respectively), day 14 ($t = 27.57$, $P < 0.0001$; $t = 34.14$, $P < 0.0001$, respectively), and day 21 ($t = 34.35$, $P < 0.0001$; $t = 37.51$, $P < 0.0001$, respectively). Furthermore, the group treated with 9.12 mg/kg of BW also weighed less than the group treated with 4.56 mg/kg of BW on day 7 ($t = 3.200$, $P < 0.01$), day 14 ($t = 6.566$, $P < 0.0001$), and day 12 ($t = 3.163$, $P < 0.01$). No significant difference was observed on day 1 of treatment (Table 2).

3.3. Effect of T. porphyreus TTX on rats sensorimotor development

3.3.1. Motor Coordination

Figure 1 illustrates the impact of *T. Porphyreus* extract on motor coordination. We meticulously scrutinized the performance of different rat groups in the first trial compared to the second. On the whole, the control group and the acute treatment group demonstrated superior performance when contrasted with the sub-chronically treated group. Following the inaugural trial, the Rotarod test scores consistently reflected an upswing in performance for all tested rats. Notably, the control group consistently displayed markedly better motor coordination than both the sub-chronically treated groups and, to a lesser extent, the acute treatment group. The findings are visually depicted as follows:A two-factor analysis of variance for the Rotarod test unveiled that motor coordination, as assessed by the Rotarod test, was significantly influenced by the trial ($F (1, 72) = 130.0$, $P < 0.0001$), treatment ($F (3, 72) = 296.0$, $P < 0.0001$), and the interaction between treatment and trial ($F (3, 72) = 11.10$, $P < 0.0001$). Our meticulous Bonferroni post hoc analysis disclosed a notable decline in fall latency during the first trial for the sub-chronically treated groups in comparison to the control group [(4.56 mg/kg of BW vs. control: $t = 18.52$, $P < 0.0001$; 9.12 mg/kg of BW vs. control: $t = 8.59$, $P < 0.0001$; 9.12 mg/kg of BW acute vs. control: $t = 2.619$, NS; Treated 4.56 mg/kg of PC vs. 9.12 mg/kg of BW: $t = 0.07047$, NS; treated 4.56 mg/kg of PC vs. 9.12 mg/kg of BW acute: $t = 15.90$, $P < 0.0001$; treated 9.12 mg/kg of BW vs. 9.12 mg/kg of PC acute: $t = 15.97$, $P <0.0001$)] (Figure 1). A marked distinction was also observed between the sub-chronically treated groups and the acutely treated group [($t = 8.498$, $P < 0.0001$; $t = 9.721$, $P < 0.0001$; respectively)]. No significant variance was noted between the control group and the acutely treated group or between the two sub-chronically treated groups.

3.3.2. Anxiety

We probed anxiety-like traits subsequent to exposure to *T. porphyreus* extract employing the Elevated Plus Maze Test (EPMT). Evidently, the exposure significantly precipitated a surge in anxiety-like behaviors in the treated rats, as manifested by the duration spent in the open arms of the EPM ($F (3, 36) = 74.55$, $P < 0.0001$) (Figure 2). Furthermore, a meticulous Bonferroni post hoc analysis unveiled that the sub-chronically exposed groups, subjected to doses of 4.56 mg/kg of BW and 9.12 mg/kg of PC, devoted conspicuously more time to the closed arms of the EPM [($t = 11.05$, $P < 0.0001$; $t = 12.27$, $P < 0.0001$; respectively)] in contrast to the control group. Additionally, a marked differentiation was discerned between the sub-chronically treated groups and the acutely treated group [($t = 8.498$, $P < 0.0001$; $t = 9.721$, $P < 0.0001$; respectively)]. No substantial divergence was detected between the control group and the acutely treated group or between the two sub-chronically treated groups.

3.3.3. Depression

The evaluation of depressive-like behaviors following treatment with Takifugu solution unfolded through the utilization of the Tail Suspension Test (Figure 3). In this context, the sub-chronically treated rats unequivocally displayed prolonged periods of immobility in comparison to the control and acutely treated rats (one-way ANOVA: $F (3, 36) = 125.3$, $P < 0.0001$); in-depth Bonferroni post hoc analysis revealed the disparities (4.56 mg/kg of BW vs control, $t = 13.71$, $P < 0.0001$; 9.12 mg/kg of BW vs control, $t = 14.40$, $P < 0.0001$;) between the sub-chronically treated groups (4.56 mg/kg of BW and 9.12 mg/kg vs 9.12 mg/kg of BW acute: ($t = 12.98$, $P < 0.0001$); $t = 13.67$, $P < 0.0001$) respectively. There was a conspicuous absence of noteworthy distinction between the control group and the acutely treated group, as well as between the two sub-chronically treated groups.

3.3.4. Recognition memory

For the evaluation of recognition memory subsequent to treatment with Takifugu solution, we employed the Novel Object Recognition Test (Figures 4a, b & c). Notably, the sub-chronically treated rats at 9.12 mg/kg of BW exhibited a marked decline in both the recognition index and discrimination index in comparison to the other groups. Intriguingly, all rats in the three treated groups devoted increased time to re-exploring the familiar object during T2 in contrast to the control group, which displayed negative values indicative of memorization (one-way ANOVA: $F (3, 36) = 4.798$, $P < 0.01$; $F (3, 36) = 4.798$, $P < 0.01$; $F (3, 36) = 46.80$, $P < 0.0001$, respectively); the meticulous Bonferroni post hoc analysis corroborated these findings (Recognition index and discrimination index 9.12 mg/kg of BW sub-chronic vs control, $t=3.585$, $P < 0.01$). In this context, no noteworthy differences were evident between the control group and the other treated groups, nor between the rest of the treated groups.

In terms of latency time, the sub-chronically treated groups (4.56 mg/kg of BW vs control, $t = 9.427$, $P < 0.0001$; 9.12 mg/kg of BW vs control, $t = 10.28$, $P < 0.0001$; and acute 9.12 mg/kg of BW vs control, $t = 9.186$, $P < 0.0001$) exhibited a pronounced elevation in re-exploration time. Remarkably, there was no substantial variation detected between the treated groups.In this study, our primary objective is to underscore the potential risks associated with the consumption of Takifugu, a fish often sold under alternative names, which can create confusion among consumers regarding its origin and unique characteristics. To achieve this goal, we conducted a comprehensive assessment of the effects of sub-chronic and acute exposure to a Takifugu solution on male Wistar rats. We employed various behavioral tests to evaluate its impact on weight gain, motor coordination, anxiety, depression, and recognition memory.In terms of weight gain, our findings revealed a significant reduction in body weight among rats exposed to Takifugu solution when compared to the control group. Furthermore, we observed a significant difference between groups treated with varying doses, indicating a dose-dependent relationship in the effects of Takifugu solution on weight gain. This response could be attributed to a normal physiological adaptation mediated by TTX extracts, leading to a decrease in appetite and subsequent reduction in caloric intake in the treated animals. Concerning motor coordination, our study unveiled a noteworthy impairment in motor coordination among rats exposed to Takifugu solution. Specifically, the sub-chronically treated rats exhibited a decreased fall latency during the Rotarod test, indicating a disruption in motor coordination induced by exposure to Takifugu solution. This outcome aligns with the well-established impact of TTX on motor activity. The administration of *T. Porphyreus* solution led to a reduction in locomotor activity, characterized by reduced spontaneous movement, exploration of the environment, and motor coordination. This effect arises from TTX's specific action on voltage-gated sodium channels, essential for the generation and propagation of action potentials in neurons. TTX binds to these channels, blocking their opening and preventing the entry of sodium ions necessary for membrane depolarization and signal transmission along neurons. Consequently, this sodium channel inhibition by TTX leads to paralysis and disruption of neuronal functions, including motor activity[20-22]. Turning to anxiety, our results indicated a substantial increase in anxiety-like behaviors in rats treated with Takifugu solution compared to the control group. Specifically, rats exposed to sub-chronic doses of Takifugu solution spent more time in the closed arms of the elevated plus maze, suggesting heightened anxiety levels among these animals. This observation aligns with previous studies that have linked tetrodotoxin intoxication, found in Takifugu, with anxiety symptoms in humans. One possible underlying mechanism for this anxiogenic effect could be attributed to Tetrodotoxin's action on voltage-dependent sodium channels. By blocking these channels, Tetrodotoxin disrupts action potential conduction, affecting neuronal functions and behavioral responses, including anxiety[23]. In the realm of depression, our research demonstrated a significant increase in immobility time during the tail suspension test among sub-chronically treated rats compared to the control group. These results suggest that exposure to Takifugu solution may induce depressive behaviors in rats. Although limited studies have directly investigated the impact of TTX or the consumption of Takifugu fish on depression, several studies have reported the inhibitory effect of TTX on serotonin secretion, a critical neurotransmitter in mood regulation[24]. Moreover, another study has shown the sensitivity of cerebral serotonin receptors (or 5-hydroxytryptamine (5-HT)) to tetrodotoxin. However, it's crucial to emphasize that further research is essential to establish a direct link between this toxin or Takifugu fish consumption and depression, whether at the molecular or cellular level. Our study results suggest an association between exposure to Takifugu solution and depressive behaviors in rats, but additional investigation Proposed reviewer’s ns are necessary to comprehensively explore the underlying mechanisms and substantiate this relationship[25]. Moving on to the recognition memory test, our results revealed a significant decrease in recognition memory among rats treated sub-chronically with 9.12 mg/kg of BW of Takifugu solution. These rats exhibited a significant reduction in the novel object recognition index and discrimination index, indicating a deficit in recognition memory. Additionally, all three groups treated with *T. Porphyreux* solution spent more time re-exploring the familiar object during T2 compared to the control group, signifying a disruption in memory retention. Subsequent studies have suggested that exposure to TTX induces alterations in the CA1 and CA3 regions of the hippocampus in treated rats, resulting in significant deficits in memory, particularly spatial memory.

Table 1: Quantification of TTX extracted from *T. porphyreus*by LC-MS/MS.

Tissues	**Concentration mg/kg of BW**	**+/- Mean SD**
Flesh	5.80	0.71
Gonads	23.40	3.25
Liver	6.02	2.15
Viscera	1.25	0.32
Average	**9.12**	**1.61**

Table 2: Rats' weight during the experiment.

Body weight	Takifugu treatment		
	Control	**4.56 mg/kg of BW**	**9.12 mg/kg of BW**
D1	338.40 ± 7.32	335 ± 6.67	339.67± 6.89
D7	374.90 ± 7.90	333.72 ± 7.46****	323.34 ± 9.11****##
D14	425.80 ± 9.56	336.697 ± 6.78****	315.48 ± 5.33****####
D21	440.00 ± 8.26	329 ± 5.14****	318.78 ± 4.25****##

(**P < 0.01 compared to the control, ****P < 0.0001 compared to the control. ##P < 0.01 and ####P < 0.0001 compared to the treated group).

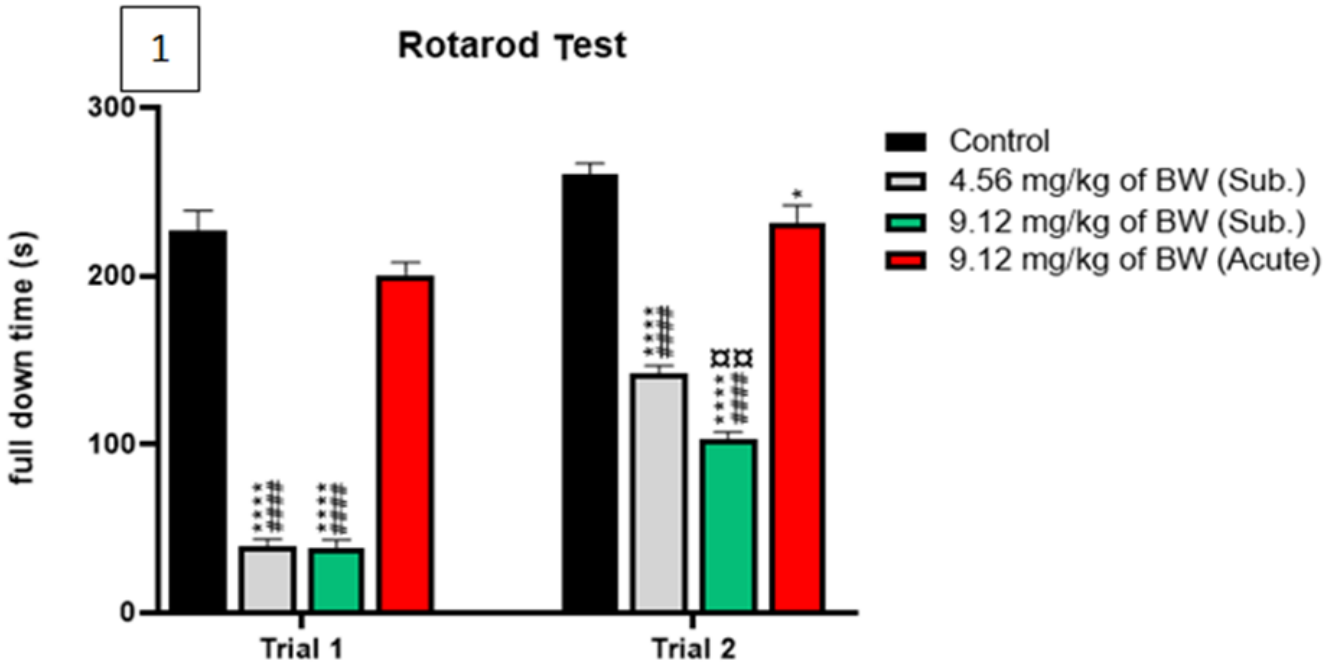

Figure 1: Effect of *T. porphyreus*solution on locomotor activity: Fall down latency during both trials calculated in the Rotarod test. The results are presented as mean ± SEM (N = 10/ Each group). *P < 0.05, and ***P < 0.001 compared to the control group. ¤¤P < 0.001 compared to the Sub-chronic 9.12 mg/kg of BW group, and ###P < 0.001 compared to the Acute group.

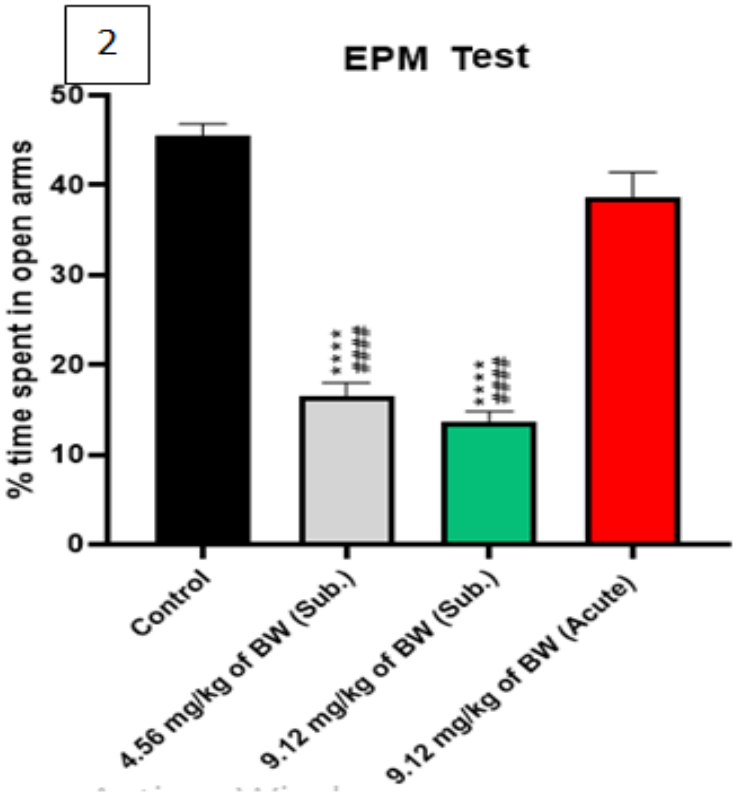

Figure 2: Effect of T. *Porphyreus solution o*n anxiety-like behavior: Ratio of time spent in open arms in the elevated plus maze. The results are presented as mean ± SEM (N = 10/ Each group). ***P < 0.001 compared to the control group and ###P < 0.001 compared to the Acute group.

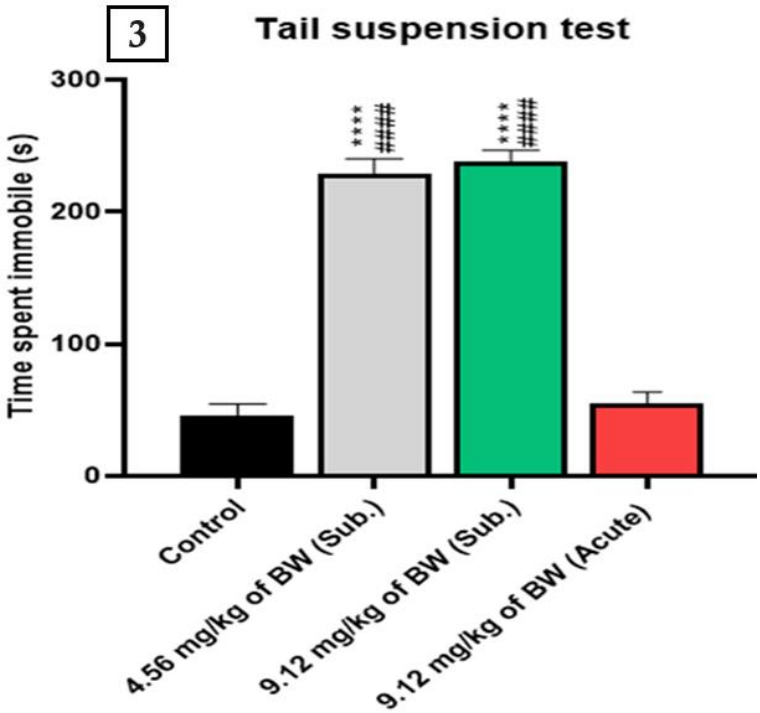

Figure 3: Effect of *T. Porphyreus* solution on depress if like behavior: Immobility time measured in the tail suspension test. The results are presented as mean ± SEM (N = 10/ Each group). ***P < 0.001 compared to the control group. and ###P < 0.001 compared to the Acute group.

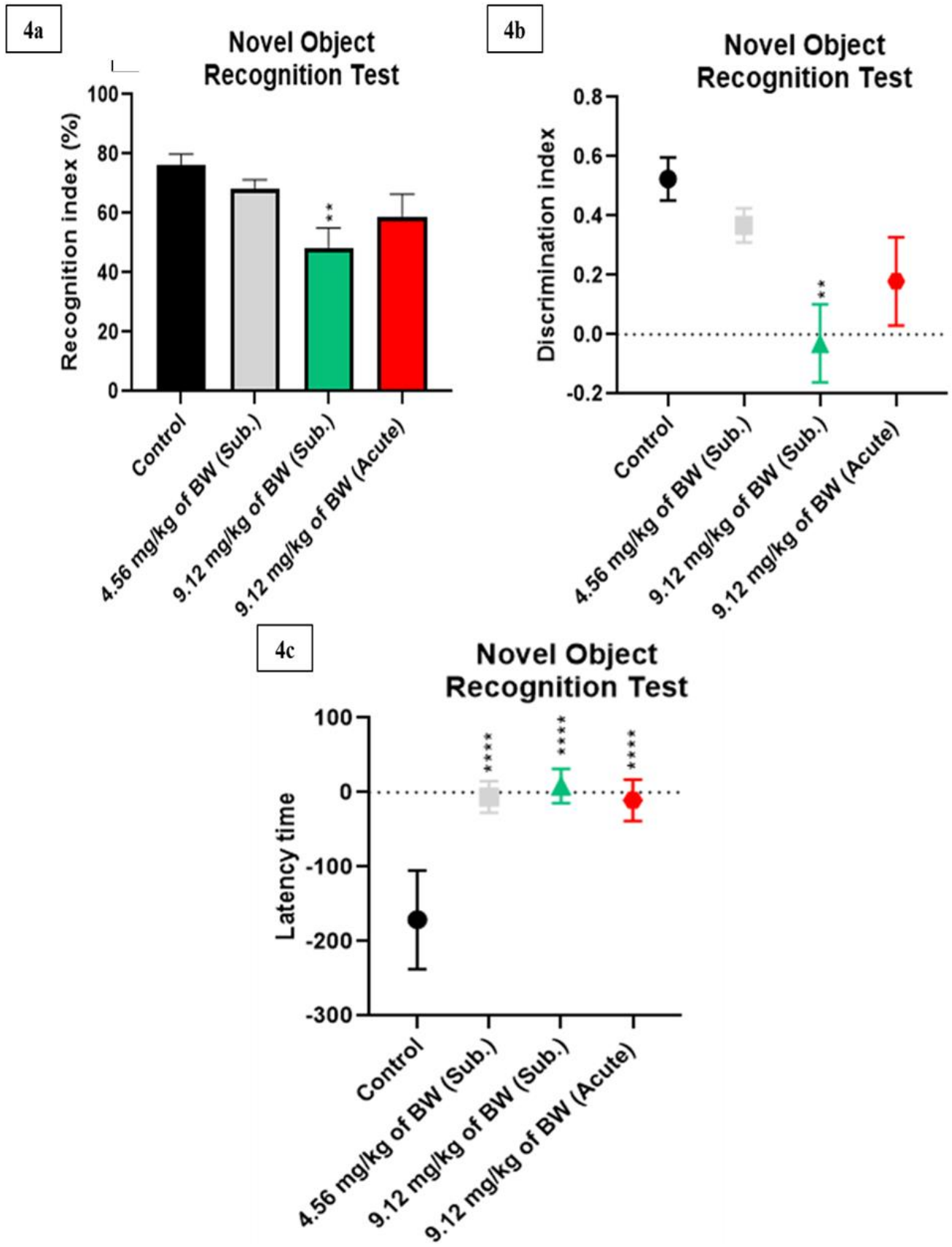

Figure 4: Effect of *T. porphyreus* solution on recognition memory: (a) Novel object recognition index calculated in the novel object recognition (NOR) test. (b) Discrimination index measured in the NOR test. (c) Latency time calculated in the NOR test. The results are presented as mean ± SEM (N = 10/ Each group). *P < 0.05, **P < 0.01, and ***P < 0.001 compared to the control group.

These observations suggest that these alterations may potentially explain the underlying cause of the observed deficits in recognition memory in our research subjects, warranting further investigations in the field of neurobiology[26]. In summary, our results indicate that sub-chronic exposure to Takifugu solution in rats leads to significant alterations in weight gain, motor coordination, anxiety, depression, and recognition memory. These findings are consistent with the well-documented toxic effects of the toxins present in Takifugu. Nevertheless, further studies are needed to gain a deeper understanding of the underlying mechanisms of the observed effects and to assess the long-term consequences of exposure to Takifugu solution. Additional research can also be undertaken to identify specific toxic compounds present in Takifugu and evaluate their individual toxicity.

4. Conclusions

This study, which focuses on the risks associated with consuming Takifugu, underscores the significant consequences that can result from the sale of this fish under different names. The results highlight the paramount importance of raising awareness and educating the public about the potential risks associated with the consumption of marine products. Preventing any confusion and promoting informed and safe consumption of marine products are essential goals, achieved by informing consumers about species that carry risks and encouraging transparent sales practices. Furthermore, the experimental study conducted on rats treated with a solution containing Takifugu has unveiled behavioral changes, shedding light on the potential adverse effects of this fish on health. These findings underscore the necessity for a more in-depth exploration of the toxic mechanisms associated with Takifugu and its impact on other organisms. Looking ahead, it is crucial to continue researching in this field to enhance our understanding of the risks linked to the consumption of potentially hazardous fish. Additional studies on marine biotoxins, heightened monitoring of marine product quality, increasing consumer awareness, and strengthening regulation and labeling of marine products are all important paths to explore. By combining the efforts of researchers, healthcare professionals, regulatory authorities, and industry stakeholders, we can collaborate to ensure the safe consumption of marine products and safeguard consumer health. Ultimately, this research contributes to a better comprehension of the risks connected to consuming Takifugu and underscores the significance of prevention and awareness to guarantee safe food consumption. We hope that these findings will stimulate concrete actions to ensure the safety of marine products and protect the public.

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