2023, 20(6 Suppl.): 2373-2384
https://dx.doi.org/10.21123/bsj.2023.8785
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Cytotoxic Activities, Determining Toxin, and Molecular Docking of Ovary Pufferfish (*Tetraodon leiurus*) in Singkarak Lake as Cancer Chemoprevention Candidate

Dewi Imelda Roesma**, Djong Hon Tjong, Monica Mulnia Hanif

Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Limau Manis, Padang, West Sumatra, 25163, Indonesia.
*Corresponding Author.

Received 22/03/2023, Revised 22/09/2023, Accepted 24/09/2023, Published 05/12/2023

Abstract

The primary toxin class discovered in freshwater pufferfish is a category of neurotoxins called PSTs (Paralytic shellfish toxins) and pufferfish toxin has been observed to have biological, biochemical, and cytotoxic effects on cancer cell lines. Therefore, it is crucial to determine the cytotoxic activity, toxins present in the ovary of *T. leiurus*, and interaction between ligand (toxin compound) and receptors test. This study used the MTT method in the T47D cell lines, liquid chromatograph-tandem mass spectrometry (LC-MS/MS), and analysis of the molecular interaction using molecular docking. The ovary of *T. leiurus* had cytotoxicity on the T47D cell, having an IC50 value of 229.535 µg/ml, and generated a chromatogram with a retention duration of 1.25 min that was similar to the Decarbamoylneosaxitoxin (dcNEO) standard solution. In molecular interactions between the dcNEO ligand to receptors, the lowest ΔG value was -9.29 kcal/mol at the Na_v 1.7 receptor, and the lowest KI value was 1.23 µM at the Mcl-1 receptor. These findings indicate that the ovary of *T. leiurus* is cytotoxic to the T47D cell line and contains dcNEO toxin. It is more stable for the dcNEO ligand to engage with the Na_v 1.7 receptor than with other receptors, and it inhibits the Mcl-1 receptor more potently than with other receptors. These findings indicate that the ovary of *T. leiurus* may be chemotherapy for the prevention of cancer strategy.

Keywords: Buntal fish, Cancer, Decarbamoylneosaxitoxin, Molecular docking, Singkarak lake.

Introduction

Chemoprevention refers to the reversal, inhibition, or prevention of a premalignant lesion's transformation into a malignant form using synthetic or natural substances[1]. Natural cancer treatment is effective in preventing malignancies, which can be found in terrestrial or marine sources, as well as in animals, plants, and microbes[2]. Several researchers have researched the use of natural substances as anticancer, the leaf extracts (*Anchusa strigosa*) have shown potential in suppressing the proliferation against various cancer cell lines including MCF-7, T47D, MDA-MB-231, and Caco-2[3]. Peptides from the skin of Iraqi frogs (*Rana ridibunda*) exert remarkably selective effects on the cell cultures of leukemia patients and have low toxic effects on human red blood cells[4].

Pufferfish toxin has also been reported as an anticancer and chemoprevention agent. The ovaries and liver of several pufferfish species are particularly toxic organs[5]. TTX (tetrodotoxin) from the liver extract pufferfish (*T. fluviatilis*) caused apoptosis in HeLa cells[6]. In Singkarak lake, West Sumatra, a toxic pufferfish (*T. leiurus*) is known

2023, 20(6 Suppl.): 2373-2384
https://dx.doi.org/10.21123/bsj.2023.8785
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

locally as Buntal or Jabuih fish[7]. The ovary pufferfish of Singkarak lake (*T. leiurus*) has chemopreventive action in invasive ductal carcinoma (MCF-7 cell lines)[8], and also the ovary elevated the expression of the promoter of apoptosis, *Bax* gene, and also declined the expression of the inhibitor of apoptosis, *Bcl-2* gene. These findings raise the possibility of alternate chemotherapy for the prevention of cancer using the ovary of *T. leiurus*, Singkarak lake[9].

In the Mekong River, in pufferfish (*T. leiurus*), STX (saxitoxin) is the dominant toxin, dcSTX (decarbamoylsaxitoxin) and NeoSTX (neosaxitoxin) are secondary, and TTX is absent[10]. Furthermore, experimental evidence for STX's apoptosis-inducing effect elevated pro-apoptotic and reduced anti-apoptotic gene expression. Additionally, STX had negative impacts on zebrafish embryonic development that may have been brought on by oxidative stress[11].

Neurotoxins that are present in pufferfish include TTX and STX[12]. STX can affect muscle and nerve conduction and selectively block voltage-gated sodium (Na_V) channels, it may cause death and respiratory paralysis. In terms of molecular weight, toxicity, and intoxication mechanism, it is comparable to TTX[13]. STX is a member of the PST subgroup, the carbamate PST group. PST is divided into four subgroups from a collection of chemically related tetrahydropurines: decarbamoyl, deoxydecarbamoyl, carbamate, and N-sulfocarbamoyl. Decarbamoyl analogs include dcNEO, and Deoxydecarbamoyl analogs include doGTX2. Carbamate includes NeoSTX, GTX1-4, and STX. The N-sulfocarbamoyl includes C1-C4[14].

The freshwater pufferfish naturally harbors PSTs and selectively accumulates PSTs. Marine pufferfish naturally harbor TTX and selectively accumulate TTX[15]. PSTs and TTX are produced by the food that pufferfish consume both marine and freshwater. Marine dinoflagellates produce TTX, PSTs whereas freshwater cyanobacteria produce PSTs[16]. Pufferfish toxins may vary between individuals. Geographical niches, bacterial buildup, seasonal fluctuations, and biological food chains all have an impact on these variances[17].

To our knowledge, cytotoxic activity in the T47D cell line, the discovery of a toxin, and molecular docking in the ovary of *T. leiurus*, a pufferfish in Singkarak Lake, have not yet been confirmed and published. Thus, using the ovary of *T. leiurus* as a breast cancer treatment and the evaluation of the effect of the toxin on proteins that play a role in apoptosis (sodium channel and B-cell lymphoma-2 protein family).

Materials and Methods

Collection of pufferfish samples

Pufferfish (*T. leiurus*) samples were taken using nets in August 2021 in Singkarak Lake, located in the Tanah Datar Regency of West Sumatra. After dissecting the pufferfish, the collected ovary and liver were stored in film bottles, briefly placed in liquid nitrogen tubes, and then transferred to a -20°C freezer.

Reagent and standard toxins

Analysis of toxins pufferfish used standard solutions of STX, NeoSTX (National Research Council Canada), dcNEO, GTX1&4 (Cifga Laboratory).

Toxin extraction of the ovary pufferfish *(T. leiurus)*

Toxin extraction from the pufferfish[18]. Accurately weigh 5 g of pufferfish ovary and liver, respectively and 0.1 N hydrochloric acid (HCl), 5 ml, was used to homogenize the mixture, for every 500 µl of extract sample, add 25 µl of 30% trichloroacetic acid (TCA), vortex until evenly mixed and centrifuged for five min at 7000×g. After that, add 20 µl of 1.0 M sodium hydroxide (NaOH), and vortex until evenly mixed and centrifuged. Next, the solution was filtered using an Advancetec 0.2 m syringe filter. The filtered solution was heated for 10 min and centrifuged at 4 °C for 10 min, 7000×g. In the final stage by adding 0.1% acetic acid to the

2023, 20(6 Suppl.): 2373-2384
https://dx.doi.org/10.21123/bsj.2023.8785
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

supernatant up to a 10 ml capacity, then, a 2 ml sample was put through a syringe filter for analysis.

Cytotoxicity test

Cytotoxicity testing was carried out using the MTT method. The ovary and liver extract were plated in 96-well plates containing T47D cells (10000 cells) at six different concentrations (25; 50; 100; 150; 200; and 250 µg/ml) in DMEM media with three replications and incubated for 24 and 48 h. After discarding the medium in each well, 20 µl of MTT is added and incubated for 4-6 h, then adding 100 µl of DMSO, and the MTT reaction was stopped. Finally, the absorbance of each well is then calculated.

Liquid chromatograph-tandem mass spectrometry (LC-MS/ MS)

At the National Police Criminal Investigation Agency, Forensic Laboratory Center, Bogor, Indonesia the LC-MS/MS test was conducted. Liquid Chromatography coupled to a triple-quadrupole mass spectrometry (Waters Alliance 2695 Quatro Premiere) was used for detection and quantification and set to condition: column XTerra® MS C18 100 mm x 2,1 mm ID, flow rate 0.3 ml/min, particle size 5 µm, injection volume 5 µL. Acquisition mode: positive polarity, resolution analyzer mode, extended dynamic range. Mobile phases with gradient concentrations are summarized in Table 1.

Table 1. Chromatographic gradient condition

Time (min)	Water (%)	Methanol (%)
0	97	3
5.00	75	25
6.10	97	3

Molecular Docking

The pufferfish toxin ligands used in this study were from the PubChem database, using Discovery Studio Visualizer 2021, the Spatial Data File (SDF) format ligand was transformed into a Program database (PDB) file format. The Protein Data Bank database provided the receptors for this study, namely Na_v 1.1 (PDB-ID: 7dtd), Na_v 1.2 (PDB-ID: 4JPZ), Na_v 1.3 (PDB-ID: 7W7F), Na_v 1.4 (PDB-ID: 6AGF), Na_v 1.5 (PDB-ID: 4DJC), Na_v 1.7 (PDB-ID: 6J8H), Bcl-2 (PDB-ID: 4IEH), Bcl-w (PDB-ID: 1O0L), Bcl-XL (PDB-ID: 4QVF), Mcl-1 (PDB-ID: 6QFM), Bak (PDB-ID: 7M5A) and Bax (PDB-ID: 1F16). Using Discovery Studio Visualizer 2021 and AutoDock 4.2.6, the macromolecular crystal structure was generated by eliminating native ligands and water molecules, and polar hydrogen atoms and the Kollman partial charge are added. Docking research using the docking tool provided by AutoDock 4.2.6, and an RMSD value of < 2 Å was obtained by re-docking. A maximum radius of 0.375Å restricts the separation between the receptor's surface, in the x, y, and z axes, 126 Å x 126 Å x 126 Å grid boxes are present and using 10 different conformations of the Lamarckian Genetic Algorithm. PyMOL was used to display the docked complex and BIOVIA Discovery Studio Visualizer for the 2D Interaction of the molecular docking data.

Results and Discussion

Results

Cytotoxic effect of the ovary and liver of *T. leiurus*

The cytotoxic test objective is to calculate the IC50 value using the linear regression equation. The ovary and liver of *T. leiurus* elevated cytotoxicity in T47D for 24 and 48 h in a concentration- and time-dependent manner. Treatment with 250 µg/ml ovary extract reduced the number of cells by 17.988% and 56.764% in 24 and 48 h of incubation, respectively, in Fig. 1(a). At these time points, a liver extract treatment with the same concentration resulted in 17.304% and 33.91%, respectively, in Fig. 1(b).

The morphological differences between control cells and cell induced by the ovary of *T. leiurus* in Fig. 2(a) and 2(b). Control cells displaying live cells

2023, 20(6 Suppl.): 2373-2384
https://dx.doi.org/10.21123/bsj.2023.8785
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

are identified as cells with a flattened epithelium and a distinct membrane or medium border in Fig. 2(a). In contrast, Fig. 2(b) shows that the extract-induced cells had detached from the bottom of the plate and had a spherical cell shape with a torn cell membrane.

The ovary of *T. leiurus* at 48 h of incubation had the highest cytotoxicity on the T47D cell with an IC50 value of 229.535 µg/ml. The classification of compounds as cytotoxic included three categories: active (IC50 < 100 µg/ml), moderately active (100 µg/mL < IC50 < 1000 µg/ml), and nontoxic (IC50 > 1000 µg/ml)[19]. To reduce the negative effects of the chemotherapeutic treatment, extracts with moderate activity might be helpful in combination with chemotherapy medications. Moreover, it might stop the carcinogenesis route[20]. Based on several evaluated variables, the ovary of *T. leiurus* may be used in chemoprevention that can stop the T47D cell from growing and inhibit it.

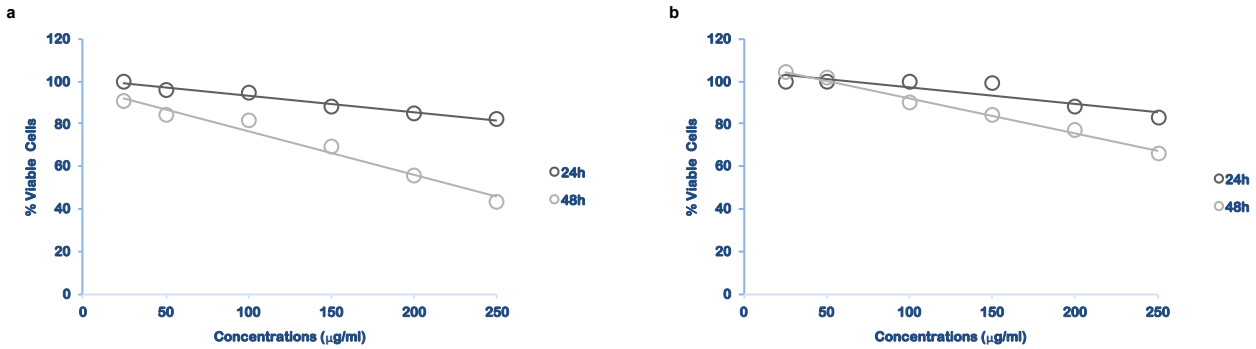

Figure 1. Cytotoxicity in the T47D cell line induced by (a) ovary extract, (b) liver extract of *T. leiurus* with various concentrations for 24 and 48 h

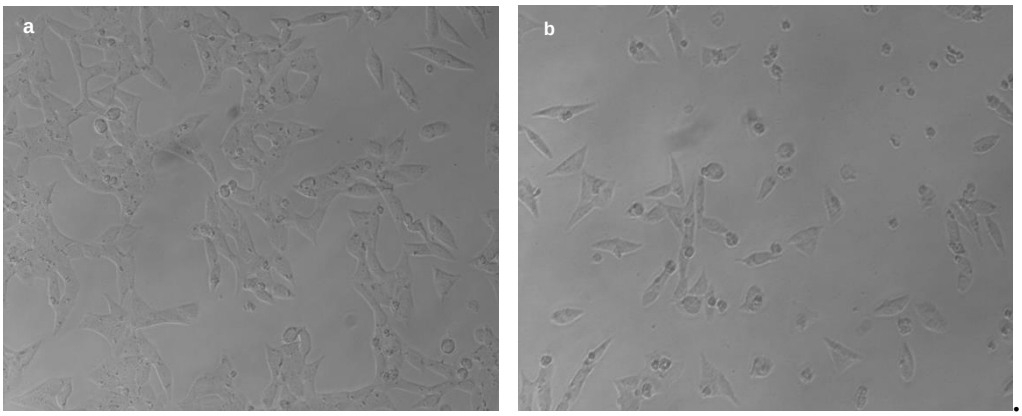

Figure 2. Microscopic the T47D cell line. (a) control cell, (b) induced by the ovary of *T. leiurus* 250 µg/ml for 48 h incubation.

Identification of toxin in the ovary of *T. leiurus*

This study uses LC-MS/MS to identify the toxin pufferfish in the ovary of *T. leiurus* as a chemoprevention. The standard solution is pufferfish toxin from the PSTs subgroup, namely STX, NeoSTX, dcNEO, and GTX1&4.

The standard solution showed that all toxins were separated within 0.50-1.36 min. The retention times for STX at 1.36 min, NeoSTX at 0.50 and 1.29 min, dcNeo at 1.25 and 1.33 min, and GTX1&4 at 1.29 min are shown in Fig. 3(a and d). As a result, a chromatogram from the ovary of *T. leiurus* matched the dcNEO standard solution exactly at a retention duration of 1.25 min in Fig. 4. The liver extract of *T. leiurus* did not produce identical chromatograms with standard solutions STX, NeoSTX, dcNEO, and GTX1&4.

2023, 20(6 Suppl.): 2373-2384
https://dx.doi.org/10.21123/bsj.2023.8785
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

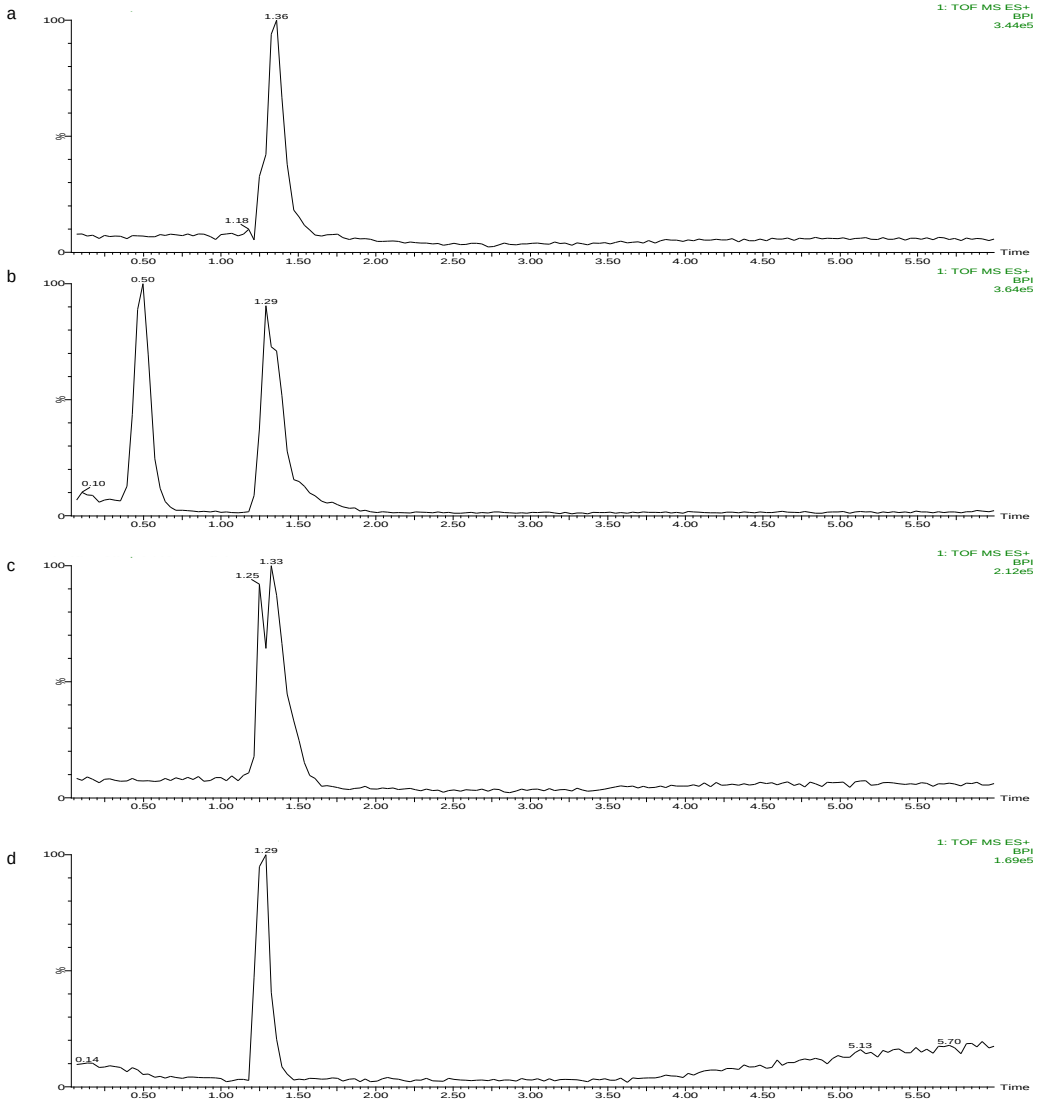

Figure 3. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) chromatograms of a standard solution. (a) STX, (b) NeoSTX, (c) dcNEO, (d) GTX1&4

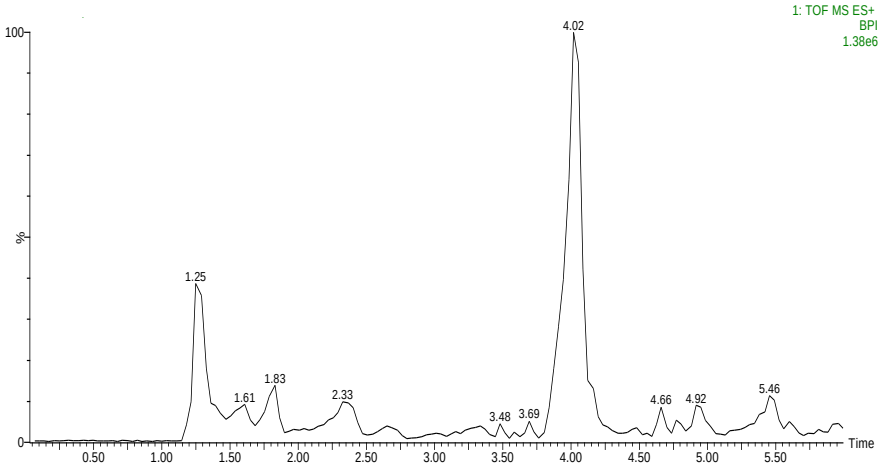

Figure 4. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) chromatograms of ovary extract of *T.leiurus*

2023, 20(6 Suppl.): 2373-2384
https://dx.doi.org/10.21123/bsj.2023.8785
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Molecular interaction of the ligand and receptors

As a result of the identification of toxin in the ovary of *T. leiurus*, obtained dcNEO as a ligand for analysis of molecular interaction against receptors test. The receptors used in this study are sodium channel proteins (Na_v 1.1; Na_v 1.2; Na_v 1.3; Na_v 1.4; Na_v 1.5; and Na_v 1.7), apoptotic protein, Bcl-2 protein family; antiapoptotic (Bcl-2; Bcl-w; Bcl-X_L; and Mcl-1) and proapoptotic (Bak and Bax).

All the molecular interaction has RMSD values <2Å. In molecular interactions between the dcNEO ligand to receptors, the lowest ΔG value was -9.29 kcal/mol at the Na_v1.7 receptor in Table 2 and Fig. 5. According to the ΔG value, the dcNEO ligand's interaction with the Na_v1.7 receptor is more stable than that of other receptors. At the Mcl-1 receptor, the lowest KI value was 1.23 M in Table 2. The Mcl-1 receptor was more effectively inhibited by the interaction of the dcNEO ligand in a small concentration than the other receptors, according to the KI value.

Table 2. Binding affinities (ΔG) and inhibition constants (KI) of dcNEO ligand to receptors

Receptors	ΔG (kcal/mol)	KI (μM)
Na_v 1.1	-8.28	853.93
Na_v 1.2	-5.01	212.42
Na_v 1.3	-7.22	5.08
Na_v 1.4	-8.62	478.3
Na_v 1.5	-7.55	2.91
Na_v 1.7	-9.29	154.02
Bcl-2	-5.98	41.59
Bcl-w	-6.81	10.27
Bcl-X_L	-6.14	31.82
Mcl-1	-3.97	1.23
Bak	-7.19	5.43
Bax	-6.11	33.4

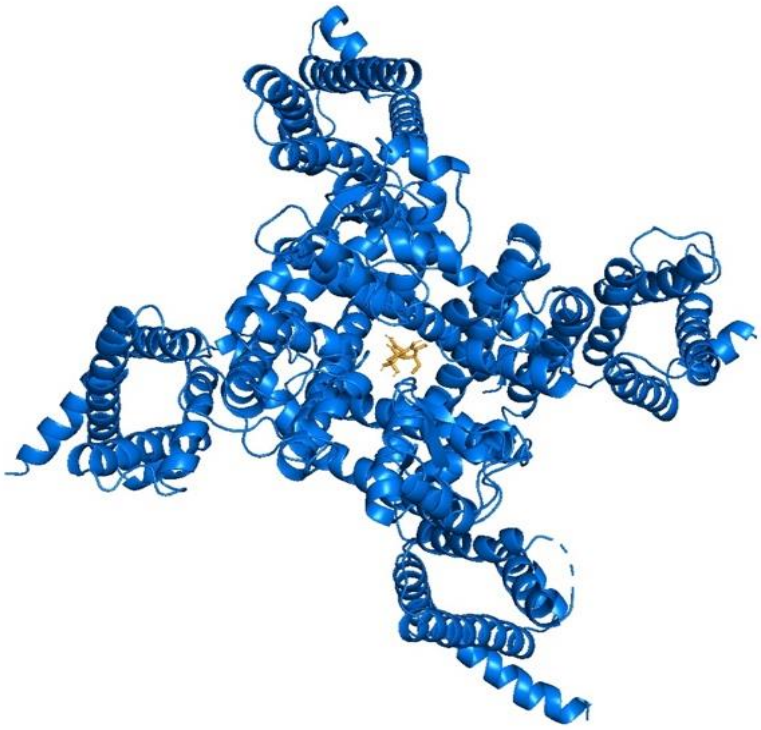

Figure 5. Structure of Na_v1.7 receptor (blue) and its binding site for the dcNEO ligand (yellow)

2023, 20(6 Suppl.): 2373-2384
https://dx.doi.org/10.21123/bsj.2023.8785
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Hydrogen bonds and hydrophobic interactions are present between the amino acid residues in these interactions in Fig. 6(a and l). Interaction, dcNEO ligand to Na_v 1.1 receptor, has eight conventional hydrogen bonds (Asp382, Glu385; 951 and Phe1431), two carbon-hydrogen bonds (Glu954 and Lys1432), one pi-alkyl hydrophobic Interaction (Phe383), one unfavorable donor-donor bond (Trp384). At Na_v 1.2 receptor, interaction dcNEO ligand has six conventional hydrogen bonds (Asp33; 37 and Thr35). Na_v 1.3 receptor and dcNEO ligand have eight conventional hydrogen bonds (Asp 1421; 1712, Glu386, Gly1710, and Tyr384), one pi-alkyl hydrophobic Interaction (Met1420), and one carbon-hydrogen bond (Gly1713). Interaction ligand to Na_v 1.4 receptor has eight conventional hydrogen bonds (Arg756, Asp1539, Glu761; 764, and Tyr 407). For the Na_v 1.5 receptor, the dcNEO ligand has five conventional hydrogen bonds (Glu88;85). At Na_v 1.7 receptor has eight conventional hydrogen bonds (Asp1701, Glu364;930, Gly1407, Phe1405) and two carbon-hydrogen bonds (Glu927 and Lys1406).

Interaction dcNEO ligand to Bcl-2 protein family, for antiapoptotic, at Bcl-2 receptor has six conventional hydrogen bonds (Asp70, Gln77, and Glu73;95). At Bcl-w receptor has six conventional hydrogen bonds (Asp16; 40, Glu35, Pro33), one pi-alkyl hydrophobic Interaction (His43), and one carbon-hydrogen bond (Ala12). Interaction with Bcl-X_L has one carbon-hydrogen bond (His177) and six conventional hydrogen bonds (Asp176, Glu124, Trp169, and Tyr120). dcNEO ligand interaction with Mcl-1 receptor has six conventional hydrogen bonds (Asp296, Pro289, Thr293 and Tyr175), and one carbon-hydrogen bond (Glu292).

Proapoptotic protein interaction with dcNEO ligand, Bak receptor has five conventional hydrogen bonds (Asp30, Gln66, Ser69, and Pro67) and one alkyl hydrophobic interaction (Met71). The Bax receptor has two alkyl hydrophobic interactions (Ala183 and Val180) and seven conventional hydrogen bonds (Asp98; 102 and Ser184).

Based on the ligand-receptor interaction, the dcNEO ligand has the greatest hydrogen bonds with the Na_v 1.5 and Na_v 1.7 receptors, making this association more stable than that with other receptors.

2023, 20(6 Suppl.): 2373-2384
https://dx.doi.org/10.21123/bsj.2023.8785
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

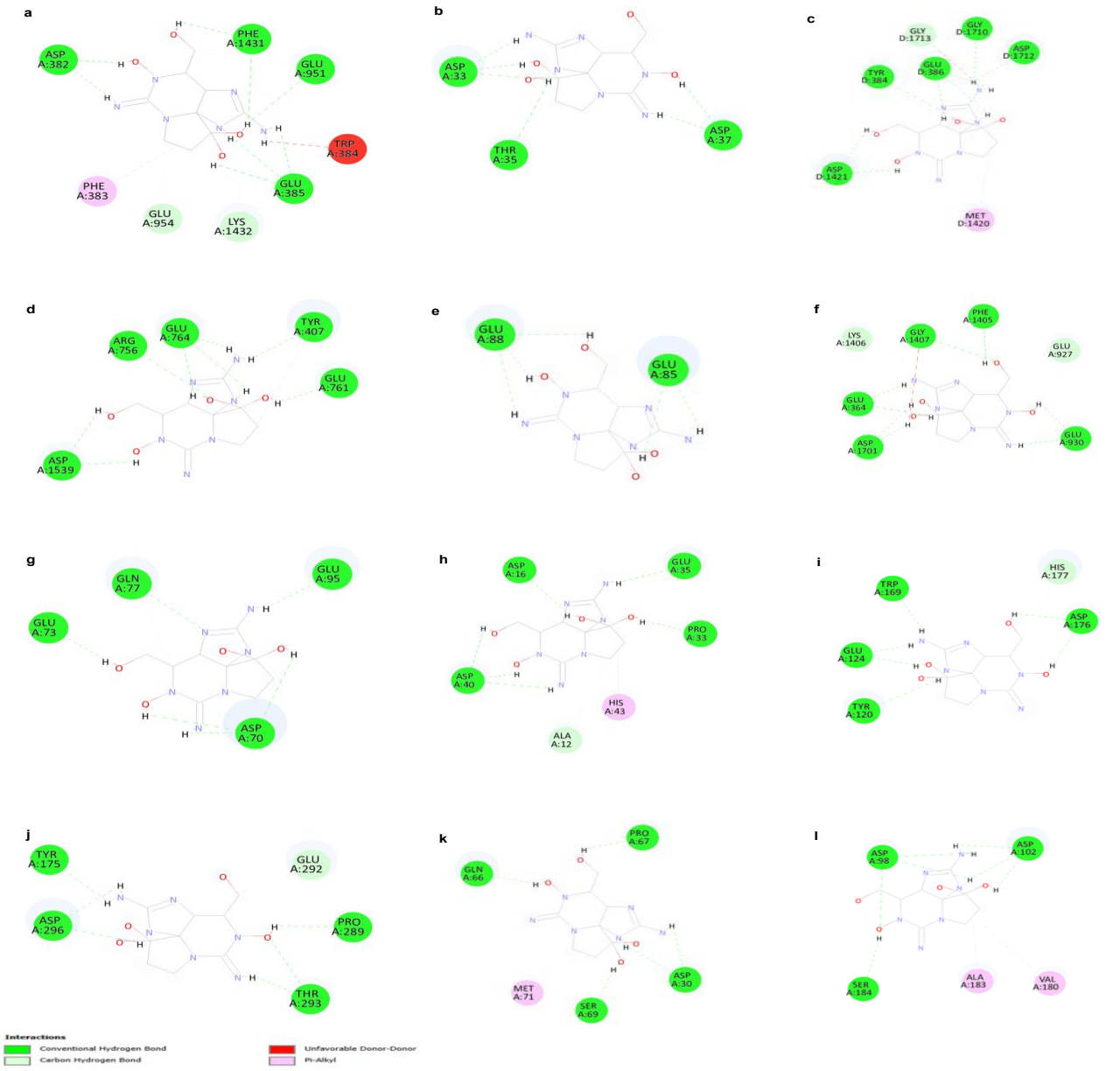

Figure 6. The interactions of dcNEO ligand with receptors. (a) Na_v 1.1 (b) Na_v 1.2 (c) Na_v 1.3 (d) Na_v 1.4 (e) Na_v 1.5 (f) Na_v 1.7 (g) Bcl-2 (h) Bcl-w (i) Bcl-X_L (j) Mcl-1 (k) Bak (l) Bax

Discussion

The freshwater and marine pufferfish are endowed with contrasting TTX/PST selectivity[15]. In *Takifugu pardalis* and *Takifugu nipholes* as marine pufferfish, the two types of pufferfish samples detected TTX and PST components (NEO, dcGTX2, dcGTX3, C1, C2, GTX1, GTX2, GTX3, GTX4, and GTX5)[18]. Others reported that *T. pardalis* had a few amounts of dcSTX, and in *P. suvattii*, freshwater pufferfish had a few amounts of TTX[15]. PST components (GTX2, GTX3, STX, dcSTX, dcGTX2, and dcGTX3) were found in prior research of two freshwater pufferfish species from Bangladesh (*T. cutcutia* and *Chelonodon patoca*)[21].

PSTs can be structurally altered by different biological agents, leading to the production of brand-new PST that cannot be produced by cyanobacteria or dinoflagellates by themselves. Moreover, PSTs that are less harmful may be changed into harmful analogs or the other way

2023, 20(6 Suppl.): 2373-2384
https://dx.doi.org/10.21123/bsj.2023.8785
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

around[22]. On the other hand, it has been discovered that *Placopecten magellanicus* (homogenate of the scallop) converts the GTXs and neoSTX to STX by reducing the O22-sulfate and N1-hydroxyl groups, respectively[23] and STX is the most common toxin in freshwater pufferfish, followed by dcSTX and NeoSTX[10]. If the selective absorption includes internal component conversion of the toxin and/or pufferfish tissues, or whether the composition of the toxin corresponds to that of harmful prey organisms, more research is required to answer these questions[12].

Pufferfish toxin content varies between individuals and tissues, and the underlying molecular pathways are still under investigation[12]. A more thorough examination of the expression, distribution, and use of PSTBP/TBT-bp2 isoforms in conjunction with TTX/STX distribution patterns is required[10]. PSTBP and its analogs are caused for the accumulation of TTX in toxic pufferfish[24], and suggests that in freshwater pufferfish, TBT-bp2 is involved in STX accumulation[12].

Guanidinium alkaloids are a class of neurotoxins that includes STX and TTX analogs that have a strong affinity for the Na_V channel and ion flux blockage abilities[25]. Inhibition of Na_V channel expression in astrocytoma cells caused a reduced Na^+ concentration[26] and an elevator in the Ca^{2+} concentration from intracellular storage[27]. Ca^{2+} has a crucial part in intrinsic apoptosis, hence an excessive accumulation in the mitochondria can result in apoptosis. It was discovered that many proteins regulate the Ca^{2+} by controlling Ca^{2+} flux between the endoplasmic reticulum (ER) and the mitochondria[28].

Pufferfish toxin (TTX/STX) is a cytotoxic compound[6] that can trigger the development of apoptosis mechanisms and its application in the treatment of cancer[29]. STX increases reactive oxygen species (ROS)[11], which also activates P53 and JNK, and the promoter of apoptosis such as Bax, Noxa, and Puma, and can interfere with the activity of the inhibitor of apoptosis including Bcl-2 and Bcl-X_L proteins. In addition, cardiolipin oxidation, which is a result of ROS, releases and allows cytochrome c to enter the cytosol. Procaspase 9, cytochrome c, and APAF-1 are the cause of caspase 9 activations[30]. Following the activation of the initiator caspase 9, the executioner's caspases are triggered, which causes a feedback stimulation of the initiator caspases and direct substrate proteolysis, both of which end in cell death (apoptosis)[31].

Conclusion

The ovary of *T. leiurus* is cytotoxic to the T47D cell line and contains decarbamoylneosaxitoxin (dcNEO) toxin. The molecular interaction of the dcNEO ligand with the Na_v 1.7 receptor is more stable than other receptors and was more effective in inhibiting the Mcl-1 receptor than other receptors.

Acknowledgment

We like to convey our gratitude to the Ministry of Education, Culture, Research, and Technology Directorate General of Higher Education, Research, and Technology (190 /E5/PG.02.00.PT/2022) which has provided research grants. We also acknowledge Andalas University's Biology Department for permitting us to do fieldwork and lab work. Finally, we appreciate the participation of Andalas University's Genetic and Biomolecular Laboratory students who helped us with sample collection and lab work.

Author's Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.

- Ethical Clearance: The project was approved by the local ethical committee in Andalas University.

Author's Contribution Statement

D.I.R, D.H.T, and M.M.H. were involved in the conception and planning of the research. M.M.H. has performed the experiments. D.I.R, D.H.T, and M.M.H. developed and designed the experiment, and also evaluated the data, and wrote the report. D.I.R, and D.H.T. supervised the work, revising and approving the paper. The findings were discussed and the text was reviewed by all authors.

References

1. Grigolato R, Bizzoca ME, Calabrese L, Leuci S, Mignogna MD, Muzio LL. Leukoplakia and immunology: New chemoprevention landscapes?. Int J Mol Sci. 2020; 21(18): 1-20. https://doi.org/10.3390/ijms21186874
2. Mushtaq S, Abbasi BH, Uzair B, Abbasi R. Natural products as reservoirs of novel therapeutic agents. EXCLI J. 2018; 17: 420–451. https://doi.org/10.17179/excli2018-1174
3. Al-Khatib AO, Khateeb I, Dahab R, Al-Rawi N. The Cytotoxic Effect of the Extract of Anchusa strigosa (Him Him) Grown in Jordan Against Different Cancer Cell Lines. Baghdad Sci J. 2021; 18(1): 70-76. https://doi.org/10.21123/bsj.2021.18.1.0070
4. Al-Khafaji AJO. The Effects of Extracted Peptide from Skin of Iraqi Frog (Rana ridibunda) on Human Leukemic Lymphocytes. Baghdad Sci J. 2019; 16(1): 40-48. https://doi.org/10.21123/bsj.2019.16.1.0040
5. Farrag MMS. Toxicity pattern of pufferfish Lagocephalus sceleratus (Gmelin, 1789), Mediterranean Sea, Egypt: awareness and food safety. AACL BiofluX. 2022; 15(2): 941-962.
6. Untario N, Dewi T, Widodo M, Rahaju P. Effect of Tetrodotoxin from Crude Puffer Fish (Tetraodon fluviatilis) Liver Extract on Intracellular Calcium Level and Apoptosis of HeLa Cell Culture. J Trop Life Sci. 2017; 7(1): 23-29. https://doi.org/10.11594/jtls.07.01.04
7. Roesma DI, Tjong DH, Janra MN, Rahmawati D, Maulidia S, Aidil DR. Scientific name confirmation of freshwater pufferfish (buntal) in Singkarak Lake, West Sumatra, Indonesia, based on cytochrome b and cytochrome oxidase subunit I gene. AACL Bioflux. 2022; 15: 1570-1585.
8. Hanif MM, Putra WD, Wilis L, Roesma DI, Tjong DH. Antitumor potential of ovarian and skin extract Singkarak Lake Pufferfish (Tetraodon leiurus). Int J Biol Res. 2021; 6: 45-49.
9. Hanif MM, Tjong DH, Santoso P, Roesma DI. Expression of Bcl-2 and Bax Genes Induced by an Ovarian Extract of Singkarak Lake Pufferfish (Tetraodon leiurus) in Breast Cancer Cell. Malays Appl Biol. 2022; 51 :63–69. https://doi.org/10.55230/mabjournal.v51i3.2283
10. Zhu H, Yamada A, Goto Y, Horn L, Ngy L, Wada M, et al. Phylogeny and Toxin Profile of Freshwater Pufferfish (Genus Pao) Collected from 2 Different Regions in Cambodia. Toxins. 2020; 12(689): 1-11. https://doi.org/10.3390/toxins12110689
11. Chen G, Jia Z, Wang L, Hu T. Effect of acute exposure of saxitoxin on development of zebrafish embryos (Danio rerio). Environ Res. 2020; 185: 1-12. https://doi.org/10.1016/j.envres.2020.109432
12. Zhu T, Sakai Y, Nagashima H, Doi T, Takatani T, Arakawa O. Tetrodotoxin/Saxitoxins Selectivity of the Euryhaline Freshwater Pufferfish Dichotomyctere fluviatilis. Toxins. 2021; 13(10): 1-11. https://doi.org/10.3390/toxins13100731
13. Katikou P, Gokbulut C, Kosker AR, Campàs M, Ozogul F. An Updated Review of Tetrodotoxin and Its Peculiarities. Mar Drugs. 2022; 20(1): 1-48. https://doi.org/10.3390/md20010047
14. Jen HC, Nguyen TAT, Wu YJ, Hoang T, Arakawa O, Lin WF, et al. Tetrodotoxin and paralytic shellfish poisons in gastropod species from Vietnam analyzed by high-performance liquid chromatography and liquid chromatography-tandem mass spectrometry. J Food Drug Anal. 2014; 22(2): 178–188. https://doi.org/10.1016/j.jfda.2013.09.005
15. Gao W, Kanahara Y, Yamada M, Tatsuno R, Yoshikawa H, Doi H, et al. Contrasting Toxin Selectivity between the Marine Pufferfish Takifugu pardalis and the Freshwater Pufferfish Pao suvattii. Toxins. 2019; 11(8): 1-11. https://doi.org/10.3390/toxins11080470
16. Abdulhussain AH, Cook KB, Turner AD, Lewis AM, Bibby TS, Mayor DJ. The Influence of the Toxin-Producing Dinoflagellate, Alexandrium catenella (1119/27), on the Survival and Reproduction of the Marine Copepod, Acartia tonsa, During Prolonged Exposure. Front Mar Sci. 2021; 8: 1-8. https://doi.org/10.3389/fmars.2021.652225
17. Akbora HD, Kunter I, Erçetin T, Elagöz AM, Çiçek BA. Determination of tetrodotoxin (TTX) levels in

various tissues of the silver cheeked puffer fish (Lagocephalus sceleratus (Gmelin, 1789)) in Northern Cyprus Sea (Eastern Mediterranean). Toxicon. 2020; 75: 1–6. https://doi.org/10.1016/j.toxicon.2019.12.002

18. Lee KJ, Ha KS, Jung YJ, Mok JS, Son KT, Lee HC, et al. Paralytic shellfish toxins (PSTs) and tetrodotoxin (TTX) of Korean pufferfish. Fish Aquat Sci. 2021; 24(11): 360-369. https://doi.org/10.47853/FAS.2021.E35
19. Prayong P, Barusrux S, Weerapreeyakul N. Cytotoxic activity screening of some indigenous Thai plants. Fitoterapia. 2008; 79(7-8): 598-601. https://doi.org/10.1016/j.fitote.2008.06.007
20. Nordin ML, Kadir AA, Zakaria ZA, Abdullah R, Abdullah NH. In vitro investigation of cytotoxic and antioxidative activities of Ardisia crispa against breast cancer cell lines, MCF-7 and MDA-MB-231. BMC Complement Med Ther. 2018; 18(87): 1-10. https://doi.org/10.1186/s12906-018-2153-5
21. Zaman L, Arakawa O, Shimosu A, Onoue Y. Occurrence of paralytic shellfish poison in Bangladeshi freshwater puffers. Toxicon. 1997; 35(3): 423–431. https://doi.org/10.1016/s0041-0101(96)00167-5
22. Wiese M, D'Agostino PM, Mihali TK, Moffitt MC, Neilan BA. Neurotoxic Alkaloids: Saxitoxin and Its Analogs. Mar Drugs. 2010; 8(7): 2185-2211. https://doi.org/10.3390/md8072185
23. Shimizu Y, Yoshioka M. Transformation of Paralytic Shellfish Toxins as Demonstrated in Scallop Homogenates. Science. 1981; 212(4494): 547-549. https://doi.org/10.1126/science.7209548
24. Yotsu-Yamashita M, Nagaoka Y, Muramoto K, Cho Y, Konoki K. Pufferfish Saxitoxin and Tetrodotoxin Binding Protein (PSTBP) Analogues in the Blood Plasma of the Pufferfish Arothron nigropunctatus, A. hispidus, A. manilensis, and Chelonodon patoca. Mar Drugs. 2018; 16(7): 1-10. https://doi.org/10.3390/md16070224
25. Cembella AD, Durán-Riveroll LM. Chapter One - Marine guanidinium neurotoxins: Biogenic origins and interactions, biosynthesis and pharmacology. Advances in Neurotoxicology. 2021; 6: 1–47. https://doi.org/10.1016/bs.ant.2021.05.001
26. Xing D, Wang J, Ou S, Wang Y, Qiu B, Ding D, et al. Expression of neonatal Nav1.5 in human brain astrocytoma and its effect on proliferation, invasion and apoptosis of astrocytoma cells. Oncol Rep. 2014; 31(6): 2692-2700. https://doi.org/10.3892/or.2014.3143
27. Boitano S, Woodruff WL, Dirksen ER. Reduction of extracellular Na+ causes a release of Ca2+ from internal stores in airway epithelial cells. Am J Physiol Lung Cell Mol Physiol. 1997; 272(6): 1189-1197. https://doi.org/10.1152/ajplung.1997.272.6.L1189
28. Patergnani S, Danese A, Bouhamida E, Aguiari G, Previati M, Pinton P, et al. Various Aspects of Calcium Signaling in the Regulation of Apoptosis, Autophagy, Cell Proliferation, and Cancer. Int J Mol Sci. 2020; 21(21): 8323. https://doi.org/10.3390/ijms21218323
29. Jensen K, WuWong DJ, Wong S, Matsuyama M, Matsuyama S. Pharmacological inhibition of Bax-induced cell death: Baxinhibiting peptides and small compounds inhibiting Bax. Exp Biol Med. 2019; 244(8): 621-629. https://doi.org/10.1177/1535370219833624
30. Dutordoir RM, Bates ADA. Activation of apoptosis signalling pathways by reactive oxygen species. Biochim Biophys Acta. 2016; 1863(12): 2977–2992. https://doi.org/10.1016/j.bbamcr.2016.09.012
31. Anson F, Thayumanavan S, Hardy JA. Exogenous Introduction of Initiator and Executioner Caspases Results in Different Apoptotic Outcomes. JACS Au. 2021; 1(8): 1240–1256. https://doi.org/10.1021/jacsau.1c00261

2023, 20(6 Suppl.): 2373-2384
https://dx.doi.org/10.21123/bsj.2023.8785
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

الأنشطة السامة للخلايا، وتحديد السموم، والالتحام الجزيئي لسمكة المبيض المنتفخة (*Tetraodon leiurus*)في بحيرة سينغكاراك كمرشح للوقاية الكيماوية من السرطان

ديوي إيميلدا روزما، دجونغ هون تجونغ، ومونيكا مولنيا حنيف

قسم الأحياء، كلية الرياضيات والعلوم الطبيعية، جامعة الأندلس، ليماو مانيس، بادانج، سومطرة الغربية، 25163، إندونيسيا.

الخلاصة

فئة السموم الأولية المكتشفة في أسماك المياه العذبة المنتفخة هي فئة من السموم العصبية تسمى PSTs (سموم المحار المشلولة) وقد لوحظ أن سموم الأسماك المنتفخة لها تأثيرات بيولوجية وكيميائية حيوية وسمية للخلايا على خطوط الخلايا السرطانية. . ولذلك، فمن الأهمية بمكان تحديد النشاط السامة للخلايا، والسموم الموجودة في المبيض من T. ليوروس، والتفاعل بين يجند (مركب السم) واختبار المستقبلات. استخدمت هذه الدراسة طريقة MTT في خطوط الخلايا T47D، ومقياس الطيف الكتلي السائل بالترادف (LC-MS/MS)، وتحليل التفاعل الجزيئي باستخدام الالتحام الجزيئي. . كان لمبيض *T. leiurus* سمية خلوية في خلية T47D، حيث بلغت قيمة IC50 229.535 ميكروغرام/مل، وأنتج مخططًا كروماتوغرافيًا بمدة استبقاء قدرها 1.25 دقيقة كان مشابهًا للمحلول القياسي (Decarbamoylneosaxitoxin (dcNEO). في التفاعلات الجزيئية بين يجند dcNEO والمستقبلات، كانت أدنى قيمة ΔG هي -9.29 كيلو كالوري/مول عند مستقبل Nav 1.7، وكانت أدنى قيمة KI 1.23 ميكرومتر عند مستقبل Mcl-1. تشير هذه النتائج إلى أن مبيض *T. leiurus* سام للخلايا لخط الخلايا T47D ويحتوي على توكسين dcNEO. يعد تفاعل ligand dcNEO مع مستقبل Nav 1.7 أكثر استقرارًا مقارنة بالمستقبلات الأخرى، كما أنه يثبط مستقبل Mcl-1 بشكل أكثر فعالية من المستقبلات الأخرى. تشير هذه النتائج إلى أن مبيض *T. leiurus* قد يكون علاجًا كيميائيًا لاستراتيجية الوقاية من السرطان.

الكلمات المفتاحية: أسماك البونتال، السرطان، ديكاربامويل نيوساكسيتوكسين، الالتحام الجزيئي، بحيرة سينغكاراك.