Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.7, No.18, 2017

Extraction and Purification of Neurotoxin (Anatoxin-a) From Blue-green Alga *Pesudoanabaena limnetica* and Indicating Its Histopathological Effects on The Brain of Male Laboratory Mice (Mus Musculus L.)

Emad Yousif Awad AL-Sultan[1*] and Marwa Abid AL-Khareen Aubaed[2]
1*, 2: College of Education for pure Sciences, University of Basra /Iraq
* E-mail of the corresponding author: emad_yousif2000@yahoo.com

Abstract
The current study included isolating, purifying and cultivation the blue- green alga *P.limnetica* and recording for the first time in the province of Basra and Iraq and was diagnosed for its ability to produce neurotoxin (Anatoxin-a) .The toxin was extracted and purified from alga and quantify its quantitative and qualitative concentration using ELISA-KITS as it reached 1.179 µg/L for 50mg D.W of (=23.58µ/L for 1gm D.W). The diagnosis of algae as the product of this type of toxins study is the first of its kind on this specific species and the scarcity of species known for the production of neuropathy. The physical properties of the purified toxin were studied using ultraviolet radiation techniques with a single absorbance peak on the wavelength 226 nm and infrared spectrum as most of the active groups in the standard toxin composition. The study also examined for the first time locally and globally the statement of the histopathological effects of the purified toxin on the brain of the laboratory male mice in few concentrations, for a period of 15 days administration, where many of the histopathological effects were observed, which increased with increased concentration and represented decay in the thickness of the gray matter and the karyopicknosis of the nuclei of the glial cell nuclei and shrinkage of neurons at the concentration 0.5 µg/L and by increasing the concentration to 1 µg/L there was significant and clear decay in the thickness of the gray area and a decrease in the number of glial cells and the disappearance of most of them with increased shrinkage of neurons. The results showed that there was congestion of the capillaries at the concentration 0.5µg/L and the decay of the The disappearance of the endothelial cells lining the blood vessel and may disappear completely with the expansion and congestion more when the concentration of neurotoxin increased to 1µg/L and the white matter was seen as spongiform at the end of the period of exposure to the toxin and the emergence of cases of hyperpigmentation and edema compared with the control group. The study also showed that physiological effects of anatoxin-a on male laboratory mice represented a significant loss of weight, hair loss and loss of sight within a short period of 7-10 days of exposure to toxin which indicates the seriousness of this type of toxins, So far there were very few concentrations.
Key words: Blue-green algae, *P. limnentica, Purification and Extraction, Anatoxin-a, Histopathological effects*

1-Introduction:

Blue-green algae are a large variety of prokaryotic organisms (Bullerjahn and Post, 2014) was described as toxic organisms that produce a wide range of toxins known as cyanotoxins (Harke *et al*., 2016) and that these compounds have a detrimental effect on both the aquatic and wild environment, domestic animals and humans, which cause severe damage to the structure of the water ecosystem (Huisman *et al*., 2005). The most common toxic compounds produced by blue -green algae are hepatotoxins, neurotoxins, cytotoxins, dermatotoxins, and irritant toxins (Wiegand and Pflugmacher, 2005). Since these toxins have distinct chemical properties, neurotoxins are characterized by alkaloids, whereas hepatic toxins have a peptide nature (Sivonen and Jones, 1999). The anatoxin-a is one of the strongest neurotoxins produced by blue-green algae, is a calcareous cyclone with a low molecular weight of 156 dl (Duy *et al*., 2000). Its chemical form is 2-acetyl-9-azbicyclo [4: 2: 1] Non- . While the chemical formula of homoanatoxin-a (propan-1-oxo-1-y1) is 9-azabicyclo [4: 2: 1] non-2-ene and its molecular weight 179 Dalton (Mann *et al.,* 2012) (**Fig-1)**. Both the metabolite anatoxin-a and homoanatoxin-a have the same chemical composition, biologic characteristics and biological effect (Lewis, 2002), in addition both of them target the nervous system and the difference between them is the acquisition of (CH3) homoanatoxin-a Carbon atom No. 11 (Funari and Testai, 2008).

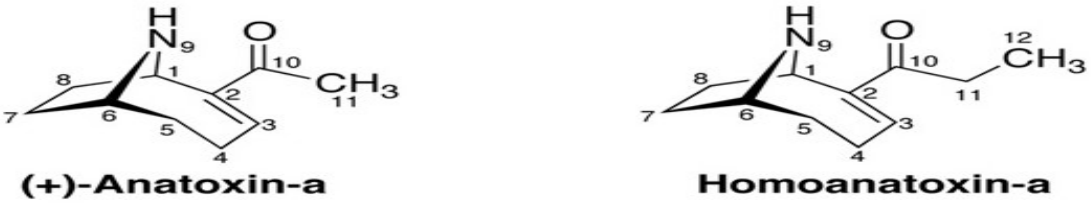

Figure1: Chemical structure of neurotoxin (Anatoxin-a and Homoanatoxin-a)

The neurotransmitter anatoxin-a targets the peripheral and central nervous system of the organism (Huber, 1972), It is found to perform the neurotransmitter action acetylcholine and its ability to bind to its receptors nicotinic receptors (Wonnacott and Gallagher, 2006), The biological effect and mechanism of action of neurotoxin is associated with the receptors of the acetylcholine, which opens the channels of sodium and potassium to the membranes of the nerve cells causing the disappearance of the polarity of the membranes and generate nerve cells in the area of neuromuscular link and that this link causes a deficit in the return of the polarization of the neuron repolarization because of the inability of the enzyme acetylcholinesterase of the neurotransmitter of the acetylcholine for the analysis of neurotoxin anatoxin-a, which causes a continuous stimulus occurs, known as the case of hyperpolarization causes short-term deficit and muscle paralysis (Carmichael, 1994). Soliakov and Wonnacott (1997) showed the effect of neurotoxin-a on the neurotransmitter activity of the rat brain if it was found to affect the brain by increasing the stimulation of nerve cells in the area of the black substance substantial nigra, an area located in the leg of the brain, thus increasing the secretion of the carrier nervous dopamine is about the normal limit. The stimulation and release of the carrier occurs because of the high concentrations of potassium and sodium ions in the extracellular space. The neurotoxin stimulates the neuromuscular membrane to open channels and pump sodium and potassium ions. This increase in the concentration of ions stimulates the presynaptic nerve endings to release the neurotransmitter dopamine with high concentrations (Soliakov *et al.,* 1995). The study of Campos *et al.,* (2006) in the first experiment on animal tissue farms to demonstrate the effect of anatoxin-a on animal cell cultures demonstrated that the antoxin-a stimulates cells to secrete the neurotransmitter dopamine.

Due to the lack of studies related to the isolation, purification and diagnosis of blue-green algae and neurogenic toxins, especially anatoxin-a, locally and globally and the scarcity of studies on the effects of tissue neurotoxicity on the brain of laboratory mice, the current study was concerned with the attempt to isolate, purify and diagnose new algal species and to show their ability to produce neurotoxins and their histological effects on the brain tissue of laboratory mice with very few concentrations.

2. Material and Methods

2.1. Collection of aquatic samples

Water samples were collected in November of 2015 from Shatt al-Arab in the area opposite Corniche / Basra Governorate by direct collection using 500 ml sterile plastic bottles.

2.2. Isolation and purification of algae

Chu-10 liquid medium was used for isolation, purification and cultivation of alga. Two method dilution and centrifuge at 3000 rpm were used for purification (Weidman *et al.,* 1984).

2.3. Classification: Gong Liang *et al.,* (2015) were adopted in the classification of isolated blue-green alga *Pseudanabaena limnetica*.

2.4. Extraction and purification of neurotoxin(anatoxin-a).

The methods of Harada et al., (1988, 1989, 1993) was used to extract and purify the neurotoxin from the biomass of purified alga *P. limnetica,* which extracted about 50 milligrams of biomass with acetic acid at a concentration of 0.05 molar and 10 milliliters repeated three times for the purpose of purification, adjust the pH of the extract to pH 10 using the NH4OH base at 7% concentration. Then purification was performed using the column of separation and wash with 10 mL distilled water and the same volume wash with 100% methyl alcohol respectively. The method of purification of neurotransmitter anatoxin-a was modified by the use of the trichloroacetic acid-methanol 0.01% and 20 ml instead of the trifluoroacetic Acid-methanol compound. The toxin is then collected in glass containers that are light-proof and sealed and kept in the refrigerator under -18 ° C.

2.5. Quantitative and qualitative diagnosis of Anatoxin-a

2.5.1: Infra-red and Ultra violet spectrum analysis.

Anatoxin-a was diagnosed by infrared spectroscopy, the infrared spectrum of anatoxin-a was measured using the Fourier Transform Infra Red Spectrophotometer in the Department of Chemistry/College of Education for Pure Sciences/Basra Universities/Iraq. The method involved mixing the poison sample after drying from the purification compounds by exposing it to a dry air stream with the KBr (potassium bromide) material. It was well grinded by a small ceramic mortar for this purpose. It was pressed in the form of tablets and then placed in the machine and recorded the infrared spectra In the confined area between 600-3600 cm -1, While the absorption peaks of the UV rays of the anatoxin-a were determined by T80 + UV / VIS Spectrophotometer and within the wavelength 200-400 nanometers interpreted the results based on Silverstien *et al.,* (1991).

2.5.2: Determination of concentration and type of neurotoxin.

Anatoxin-a was quantified and quantified diagnose from alga *P.limnetica* using enzyme-linked immunosorbent assay (ELISA-KITS) technique. The KSA ELSA-manufactured solutions were manufactured by Abraxis Company and by using the Reader ELISA Biotech measuring device. The method included adding 100 µl to each well of the Microtiter Plate of each of the standard toxin solutions manufactured and the purified anatoxin-a from the alga, followed by the rest of the additives and according to the times included in the working paper of each measurement and measurement was done by adopting replicates and was measured at a wavelength of 450 nm according to the method of Fischer *et al.,* (2001).

2.6. Histopathological effects of neurotoxin-a purified from *P. limnetica* on the brain of laboratory mice Mus Musculus L.

2.6.1. La Laboratory animals

In the current study, male *Mus muculus L* mice Balb / C strain, which were obtained from the animal house of the department of Biology, University of Basra. The animals were kept under controlled conditions from 20-20 ° C and at a constant light system of 12 hours of darkness and 12-hour light (AL-Maliki, 2000). The experimental mice were divided into three groups in three plastic cages of standard sizes 30 x 12 x 11 cm, manufactured by north Kent plastic Kent UK. The floor of each cage was replaced with wood sawdust and each box contains a group of six mice weighing 25 grams.

2.6.2. Preparation of the neurotoxin Anatoxin-a purified alga *P. Limnetica* and treatment of laboratory mice.

Anatoxin-a was extracted and purified from 50 mg of live biomass of cyanobacterial alga *Pseudanabaena limnetica*, as in (2.5). The purified toxin with a concentration of 1.179 µg / L was dried using a dry air stream .The toxic substance was then dissolved in 3 mL and the following concentrations 0.5 and 1 µg / L. was prepared.The laboratory mice were divided into three groups, each group included six mice: A) control group composed of six male mice and injected with 0.1 ml of normal saline solution , B) The first treatment group (T1) combined with six male replicates and injected daily for two weeks with the anatoxin-a at 0.5 µg/L. The second treatment group (T2) composed of six male replicates and injected daily for two weeks with anatoxin-a at a concentration of 1 µg / L. Balanchand *et al.,* (1987) was followed by I.P injection of mice and for 15 days for both concentrations..

2.6.3 Preparation of histological sections

The Humason (1972) method was used to prepare tissue sections measuring 7 microns of the brain for all the treatments. The eosin and hematoxylin dyes were used to stain the sections and were examined under a light microscope type type Olympus CX21 and at a 400x magnification force and the sections were photographed using a microscope type Olympus with digital camera.

3. Results

3.1. Isolation of blue-green alga *P.limnetica*

Purification, diagnosis and propagation of the blue-green alga *Pseudanabaen limnetica* (Lemmermann) Komárek was isolated from Shatt al-Arab water basin in Basra Governorate. This alga is shown as a single thread, not relatively long, 10 to 30 cells long, extended or slightly curved; its cells are rectangular, 3.5 microns in length and 2.5 microns wide. The culture is bright green or transparent. during the microscopic examination and follow-up, it was observed that some of the strands formed in an advanced stage of growth are circular shapes and that the alga is active or moving trembling movement if the strands move in front or lateral direction as the circular shapes move around (Gongliang *et al.,* 2015), this species is the first recorded in Iraq, according to the checklist (Maulood *et al.,* 2013) (Figure2).This alga belonging to following taxa:

Division: Cyanophyta
Class : Cyanophyceae
Order: Pseudanabaenales
Family: Pseudanabaenaceae
Genus: *Pseudanabaena limnetica* (Lemmermann) Komárek

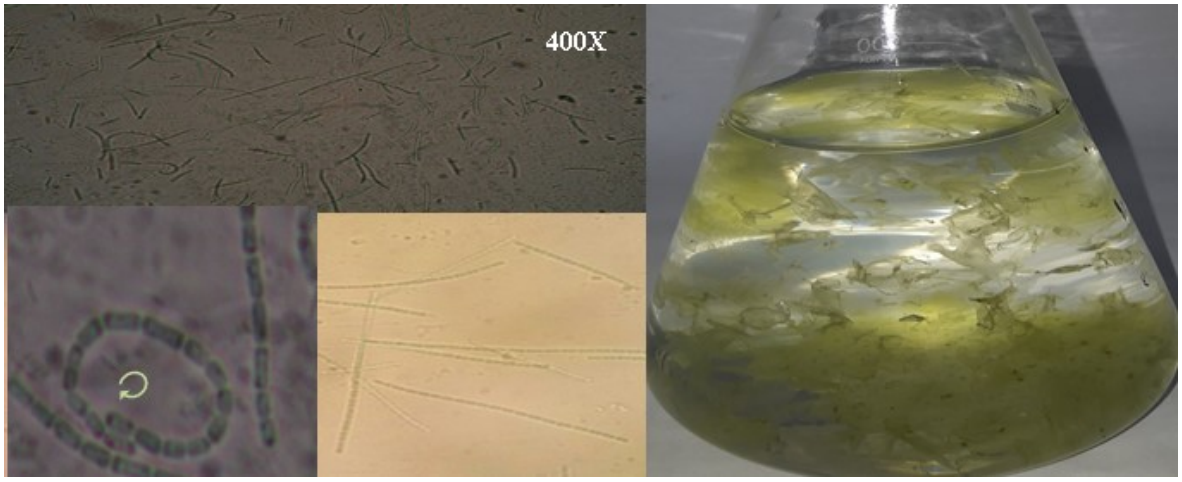

Figure2: A- Liquid culture of Blue-green alga *Pseudanabaena limnetica* B- Picture taken from a video .recording showing rotational algae movement (After zoom)

3-2.Quantitative and Qualitative Diagnosis of Anatoxin-a

Anatoxin-a was first diagnosed locally and globally for *P. limnetica* using enzyme linked immunosurbent assay technique (ELISA-kits), with the concentration of the anatoxin-a reach 1.179 µg / 50 mg (= 23.58 µg/g). for each 50 milligrams of the alga biomass . A qualitative diagnosis using the ultraviolet spectrum showed that there was a single absorption peak of the purified anatoxin-a from alga *P. lymnetica* at the wavelength of 226 nm (Figure 3). The absorption results of infrared radiation showed the most significant absorption bands for the wavelengths of the groups which composed and confined between wavelengths 4000-500 cm $^{-1}$ Table 1and (Figure 4).

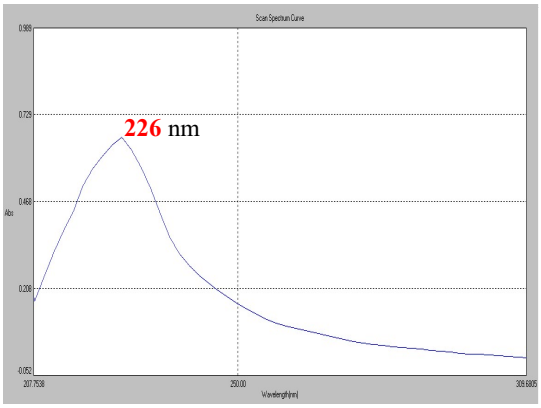

Figure 3: Ultra violet spectrum of purified anatoxin-a from alga *P. lymnetica*

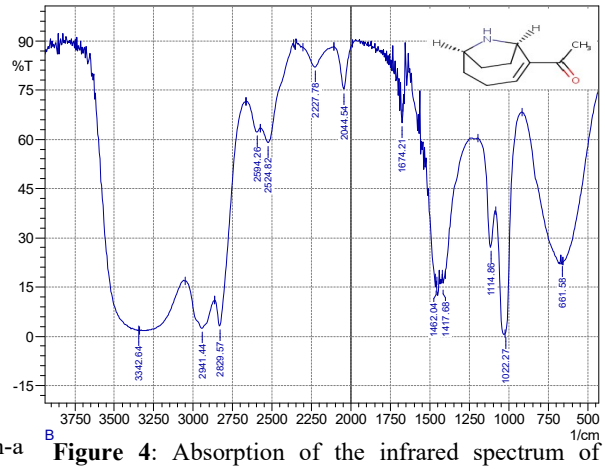

Figure 4: Absorption of the infrared spectrum of anatoxin-a purified from moss *P. lymnetica*

Table 1. Absorption of the infrared spectrum of anatoxin-a purified from alga *P. limnetica*

Chemical groups	Wave length cm^{-1}
N-H	3342.64
(Amide A)	2941.44
C-H	2829.57
CH3	1674.21
C=O	1462.04
(Amide I)	1114.86

3.3. Histopathological effects of neurotoxin-a purified from *P. limnetica* on the brain of laboratory mice *Mus Musculus* L.

The results showed that the low concentrations of neurotoxin (anatoxin-a) purified from the blue green alga *P. limnetica* showed significant and clear histopathological effects on the brain of mice after a 15-day exposure to concentrations 0.5 and 1 µg / L. **Fig-5** shows a longitudinal section of control treatments in the gray matter and white matter regions of the brain of mice showing neurons in the gray area with normal thickness and density of neurons and normal thickness of the white area its containment of homogeneous nerve fibers, density Glial cells and their clear cytoplasm. The results showed that exposure to the low concentrations of neurotoxin-a purified anatoxin-a has caused many obvious pathological effects, especially in the gray and white matter regions and their components of cells and fiber and these effects increased with concentration increase.

A decrease in the thickness of the gray matter and the appearance of vaculation in the white area were observed. Karyopiknosis increased the number of helper cells (glial cells) and shrinking of neuron after a 15 day exposure to 0.5 µg / L of purified neurotoxin anatoxin-a (**fig-6**) and with increase of toxin to1 µg / l and for the same period of time, significant and clear decay in the thickness of the gray area and the lack of number of glial cells or disappearance of most of them were observed with increased shrinkage of nerve cells (**fig- 7**). The results showed congestion of capillaries in the treatment with 0.5 µg /L of neurotoxin to observe the increase in numbers compared to the control group (**fig. 8, 9**). Also the endothelial epithelial lining of the capillaries was observed. It was clear that some glial cells showed nuclei surrounded by peripheral area (Necrosis around blood vessel). As a result of increased concentration, the disappearance of the endothelial cells lining the blood vessel in whole or in bloating was observed, with the observation of the expansion of the dilation and the congestion of these vessels clearly in (**fig-10**). Emergence of vaculation significantly and clearly in the white matter of the brain and increase with increase of toxin concentration from 0.5 to 1 µg/L which appears as spongiform shape and also noted with the increased of toxin concentration the necrosis was significantly increased and appear of hyperpigmentation and edema compared with the control group (**fig-11, 12, 13**) respectively.

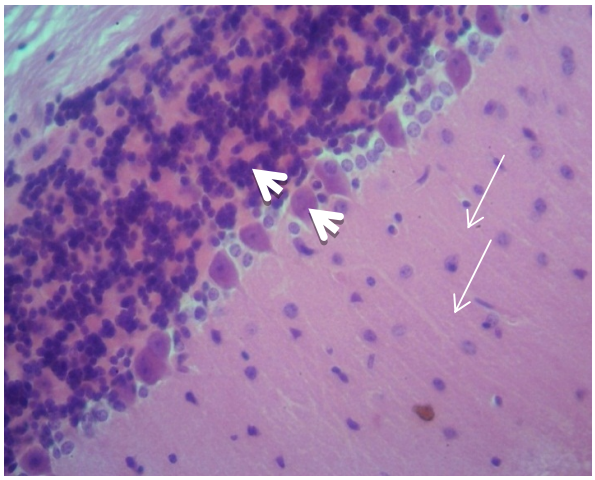

Figure 5. Histological structure of mice brain of control group show neuron bodies in gray matter, neuron density and numbers (head arrow) and White matter show nerve fibrils (arrow) and glial cells (long arrow) 400x.

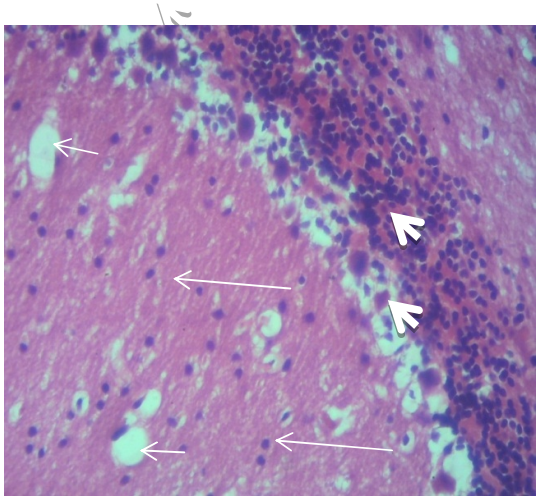

Figure 6. Treatment with 0.5 µg / L concentration of purified anatoxin-a from alga *P.lymnetica* of 15 days Showing the beginning of the decay of gray matter and shrinkage of nerve cells (head arrow) and the vaculation in the white area(short arrow) and karyopicknosis of glial cells (long arrow) 400x**.**

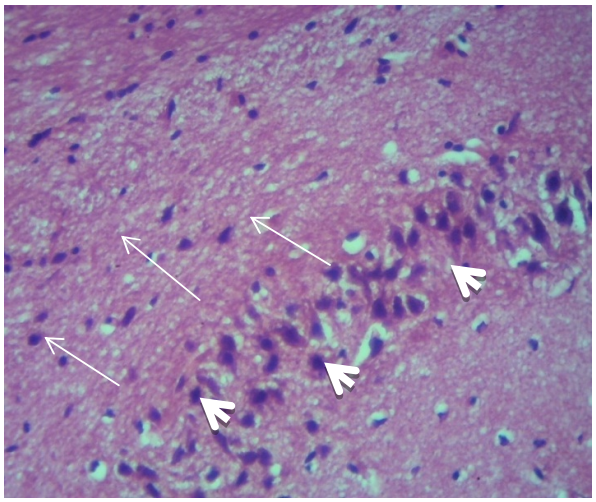

Figure7. Treatment with 1 µg / L concentration of purified anatoxin-a from alga *P.limnetica* of 15 days Showing Significant and clear decay in gray matter thickness and low cell density with increased shrinkage of neurons(head arrow) and Clear damage in the white matter area and lack of help cells or disappearance of most of them (long arrow) 400x.

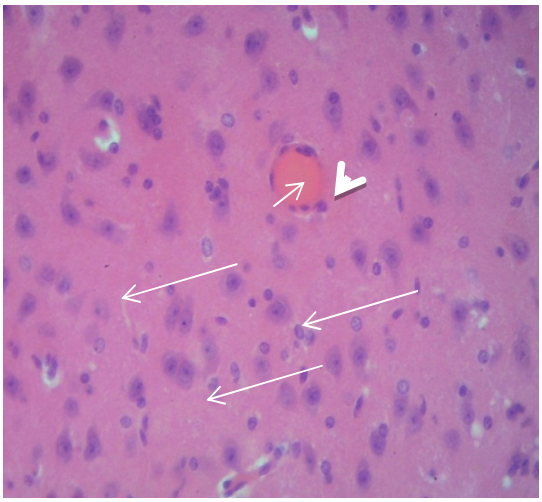

Figure8. Treatment of control show normal structure of capillaries (head arrow), cells of the endothelial epithelial tissue of the capillaries (short arrow) , Natural structure of the white matter and its homogeneity, the density of the glial cells and the observation of its cytoplasm (long arrow) 400x.

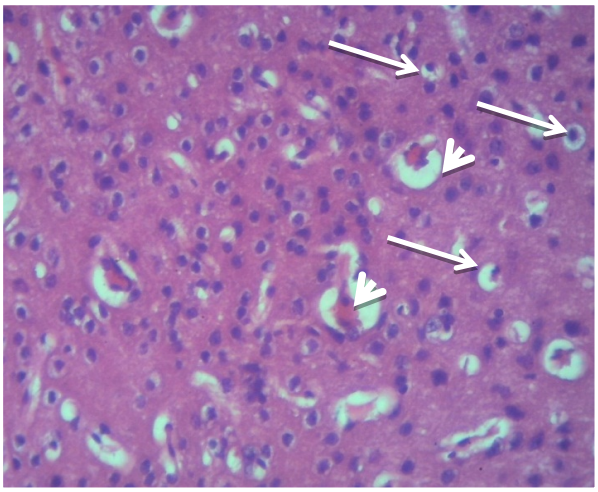

Figure9. Treatment with 0.5 µg / L concentration of purified anatoxin-a from alga *P.limnetica* of 15 days Showing congestion and decay of the endothelial epithelial tissue of capillaries and necrosis around the blood vessel (head arrow), The helper cells show the nuclei in them surrounded by an empty peripheral area that appears as a gap (long arrow) 400x.

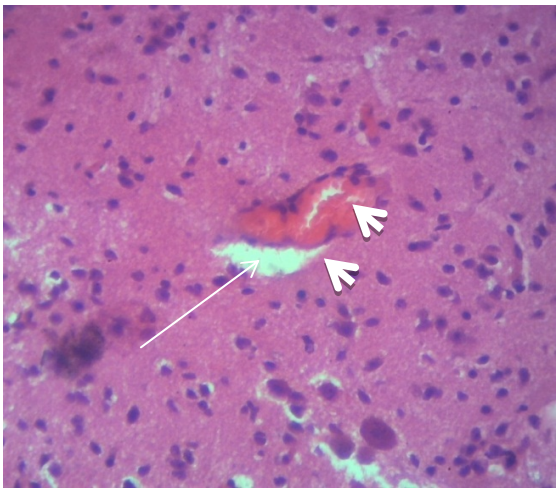

Figure10. Treatment with 1 µg / L concentration of purified anatoxin-a from alga *P.limnetica* of 15 days Showing Increased capillary congestion and expansion (dilation) of blood capillaries in the white area (head arrow) , The disappearance of the endothelial cells lining the blood vessel completely or bloating (long arrow) 400x.

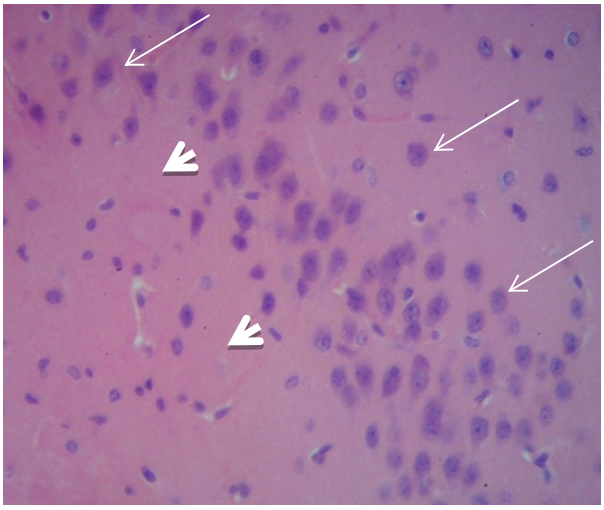

Figure11. Treatment of control show the normal homogenous structure of the white matter tissue of the mice brain, which is composed of nerve fibers (head arrow) Glial cells are present in the white matter by a large percentage, noting the clear nature of cytoplasm and the clarity of the nucleus, (long arrow) 400x.

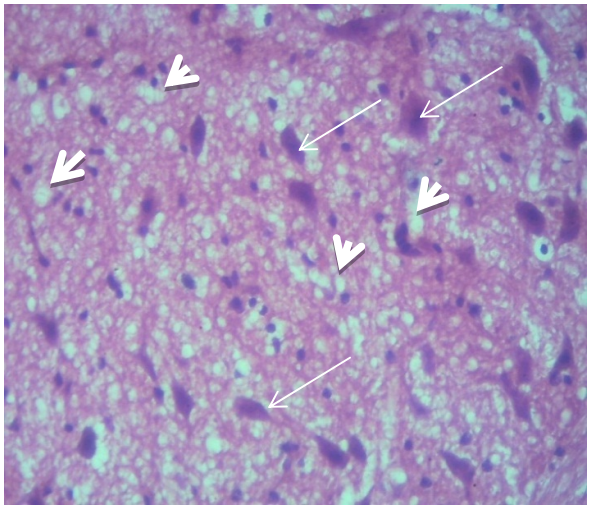

Figure12. Treatment with 0.5 µg / L concentration of purified anatoxin-a from alga *P.limnetica* of 15 days showing the apparent vaculation condition appears in the white area (head arrow), the beginning of the decay of the helper cells in the white area and the decline in numbers with the observation of hyperpigmentation and the occurrence of change in nature (long arrow) 400x.

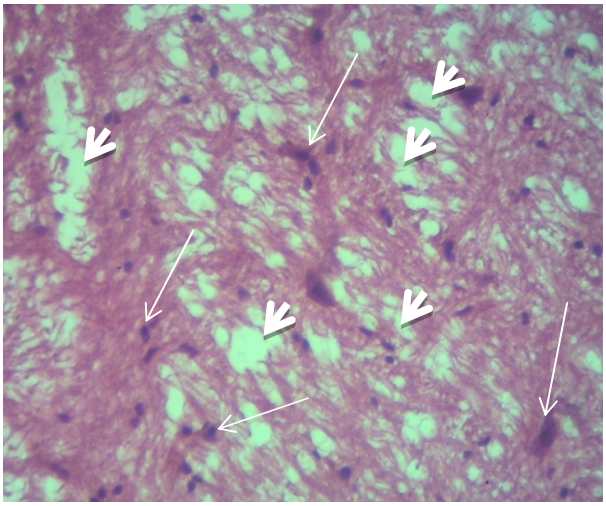

Figure13. Treatment with 1 µg / L concentration of purified anatoxin-a from alga *P.limnetica* of 15 days showing Increase edema With a greater concentration of toxic substance concentration in the white matter area appears through which the shape of the white area spongiform shape (head arrow), Increased concentration of Necrosis, increased hyperpigmentation, and clear and significant dissociation of glial cells (long arrow) 400x**.**

3.4. Behavioral and morphological effects of purified anatoxin-a on laboratory mice.

The study showed the occurrence of some clear behavioral observations during the injection period, especially after 7-10 days. It was noticed that the blindness and hair loss of the treated mice were severe and clear, in addition to a decrease in the weight of the mice tested and significantly below the level of significance $p \leq 0.05$

when treated with concentrations of anatoxin-a compared to control group (**figure 14**).

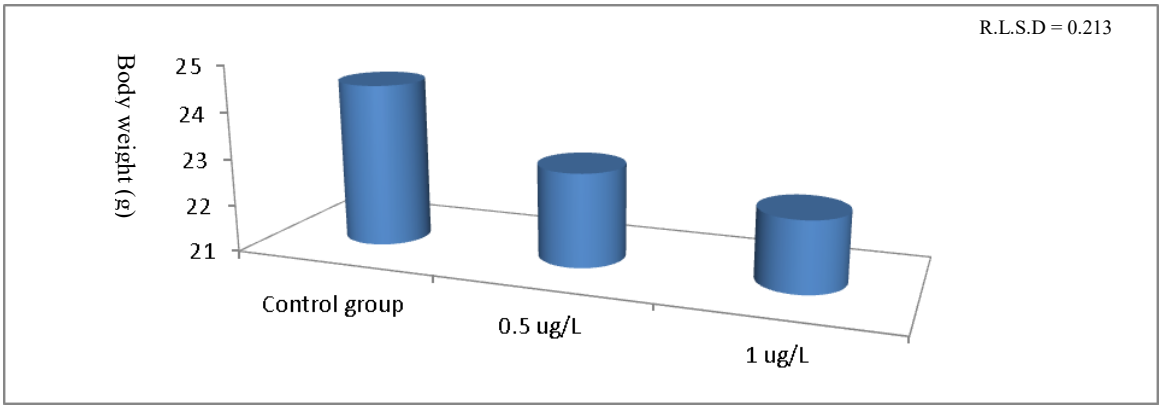

Figure 14. The rate of laboratory rat weights after being treated with the anatoxin-a from alga *P. limnetica* for 15 days. (RLSD= Revise least significant differences P≤0.05)

4. Disscussion:

Cyanobacteria have negative consequences on economic, environmental and social aspects because their production of a wide range of pathogenic toxins in both aquatic and terrestrial environments (Sukenik *et al*., 2015; Carmichael and Boyer, 2016). The remarkable rise in the proportion of blue-green algal blooms all over the world and generated growing concerns about the impact of toxins produced by the human health and the aquatic environment and its economy (Haider *et al*., 2003). Therefore, in an attempt to shed light on the untested or isolated species of blue-green algae from the water bodies of the province of Basra in particular and Iraq in general, which did not diagnose its toxicity, So the blue-green alga *P. limnetica* is considered the first recording in Iraq and also the isolating, purifying an cultivation of this alga in Basra from the Shatt al-Arab river water according to check list in Iraq (Maulood *et al*., 2013). As well as its ability to produce neurotoxin (anatoxin-a) is the first time examined locally and globally for this specific species.

The qualitative and quantitative of antoxin-a were determined for the first time locally and globally at the level of both genus and species. The results of the present study showed that blue-green alga *P. limnetica* was capable of producing anatoxina-a with a concentration of 1.179 µg / L for 50 mg of dry weight . This may be agree with study of Gonzalez-Gil *et al.*, (2010) which showed that some species of this genus are toxic because they have the ability to produce strong neurotoxins. The Ultra violet spectrum of purified anatoxin-a- from alga *P. limnetica* was showed a single absorbance peak of 226 nm, so the results were agreed with the study of Gugger *et al*., (2005) their measured the standard anatoxin-a UV absorption which showed a peak absorption of 227 nm, this indicates the large congruence in absorbance values, which can be attributed to have a highly complex chemical composition. These beams, which appeared in the ultraviolet spectrum of the region and at wavelengths above 200 nm, may be attributed to purify and standard anatoxin-a to the transfers of type π-π*, these transfers are due to the unsaturated or aromatic system of neurotoxin (anatoxin-a) (Silverstien *et al.,* 1991).

The results of table -1 and fig-3 the infra –red spectrum assay of the purified anatoxin-a from blue-green alga *P. limnetica* showed that it characterized by the emergence of the NH group at the amide group at site 3342.64 cm $^{-1}$ and the emergence of a package in situ 1674.21 Cm^{-1}. This is due to vibration of the carbonyl amide group C-O, because this package appears in a position less than the carbonate of ketone due to the electronic double on the nitrogen atom, which enters into a resonance with the double-stranded carbonyl, Carbonyl group of amides at this frequency and confirms the appearance the absorption package of CO at 1114.86 cm $^{-1}$. The appearance of a package at the site 2941.44-2829.57 cm $^{-1}$ was due to the oscillation of the C-H group of the symmetry and symmetry stretching , whereas the emergence of a package at 1462.04 cm -1 was due to the bending vibration of the C-H group (Silverstien *et al.,* 1991)

The neurotoxin (anatoxin-a) is an alkaloid compound that plays the role of the neurotransmitter acetylcholine as it binds to the receptors of the acetylcholine receptors. The fail of acetylcholine esterase in the analysis of anatoxin-a, as with the neurotransmitter acetylcholine, long-term neuronal and hyperpolarization, which ends with neuronal cell death (Carmichael, 1994; Wonnacott and Gallacher, 2006). Saliakov and Waonnacott (1996) showed that neurotoxin with 1 micromolar concentration had a clear effect on the neural membrane of laboratory rat brain, which increases the stimulation of neurons in the region of black substance substantia nigra a region located in the leg of the brain responsible for movement, which in turn leads to increased excretion of neurotransmitter dopamine and excessively leading to increased nervous cell stimulation

and then death. The purified anatoxin-a caused many of the pathological symptoms in the brain of laboratory mice in both the gray matter, which includes neuron cells bodies, as well as the white matter, which includes the fibers and glial cells according to Kotran (1999). A number of pathogens were observed: white matter represented by vacuolation, karyopyknosis, hyperpigmentation, spongiform shape, and shrinking of neurons in the white matter (Robin *et al.,* 2014). This may be due to the disturbance of the process of balancing the ionic of potassium and sodium ions concentration between the neurons and the outer environment under the continuous influence of the neurotransmitter generated by the toxin which caused the hyperpolarization of the nerve fibers causing the death and necrosis of the neuronal cell and thus the accumulation of fluid inside the cells and nerve fibers causing shrinkage or vice versa. The emergence of fluid outside the nerve cells and fibers which causes of shrinkage as well as causes of hyperpigmentation that because entering of pigments into the cell and the occurrence of hyperpigmentation of chromatin have been observed to increase the effects with increasing of concentration of anatoxin-a (Charmichael, 1994; Roibin *et al.,* 2014). One of the factors contributing to the toxicity of anatoxin-a in the effects of the above mentioned disease may be due to its high solubility in water as well as low molecular weight about165 Dalton, which enables access through the membranes and the nuclear envelope and nuclear bonding material, which may cause hyperpigmentation and other effects (Lewis, 2000, Rubin *et al.,* 2014)In the white area of the brain, congestion of the capillaries has been observed. This can be attributed to the mechanical pressure caused by the accumulation of blood in these capillaries under the influence of neurotoxicity, causing their expansion compared to the control group (Rubin *et al.,* 2014) The results also showed the emergence of other effects in mice treated with anatoxn-a compared to the control group represented by loss of weight of mice treated significantly and loss of vision after 7 days of treatment and hair loss clearly and this may be explained because great damage caused by poison in nerve cells and fiber in the areas gray matter and in different areas of the brain, especially responsible for sight and hair growth, etc., in addition to the cases of cramping is clear because of the mechanism of action of this poison, which was explained earlier, as this poison also affects mainly peripheral nerves, the area of contact the muscular nervous system, as well as the nerves responsible for breathing and this explains the cases of suffocation and convulsions and tremors of mice treated with neuropathy as the nervous system continues to cause the cases of tetanus and tremor and death after a short period of exposure to poison and may depend on the type of animal and concentration of poison and exposure time (Charmichael, 1994 ; Rubin 2014)

5- Conclusion

The study concluded that the water of the Shatt al-Arab in the province of Basra in southern Iraq was considered a rich environment with blue-green algae. The blue-green alga *P.limnetica* , which is diagnosed as a new record of species for the first time locally and globally and proved to have the ability to produce neurotoxin (anatoxin-a) reach to 1.179 µg/L for 50mg D.W of (=23.58µ/L for 1gm D.W) . , the purified anatoxin-a have similar peak value of ultra -violet spectrum with standard toxin reach to 226 nm and also have the same chemical groups in their structures when investigated by infra-red spectrum.The purified toxin causes many physiological effects on male mice represented by significant decrease in mean weights, hair loss and loss of sight compared with control group. and histopathological effects in brain of mice at low concentration 0.5 and 1 µg/l under exposure periods reach to 15 days.

6- Acknowledgment

My thanks and appreciation to the Deanship of the College of Education for Pure Sciences / University of Basra/Iraq and head of the Department of Biology to assist in the completion of this work as well as my thanks and appreciation to Assistant Professor Dr. Qassim Fawzi Abid-Al-Khreem in the College of Pharmacy and Assistant Professor Dr. Ali Abdul Latif Abed Ali University of Basra/Iraq to help us to read the effects of textile, Thanks and gratitude.

7. References:

Al-Maliki, S.J. (2000). A behavioral and some physiological effect of (*Apium graveolens)* seeds in albino mice. J. Sci. Bas., 2:77-88.

Balanchand, R. J., Hori, K. & Blanchard, D.C. (1987). Ethanol effects in aggression of rat selected for different levels of aggressiveness pharmacology, Bio. and Beh. 27: 641.

Bullerjahn, G.S & Post, A.F. (2014). Physiology and molecular biology of aquatic cyanobacteria. Front.J.of Microbiol. 5:359.

Campos, F., Duran R., Vidal L., Faro L.R.F.& Alfonso, M.(2006).In vivo Effects of the Anatoxin-a on Striatal Dopamine Release .J.of Neurochem Res. 31:491–501.

Carmichael, W.W. (1994).The toxins of cyanobacteria. J.of Scientific American. 64–72.

Carmichael, W.W. & Boyer, G.L. (2016).Health impacts from cyanobacteria harmful algae blooms: Implications

for the North American Great Lakes. J.of Harmful Algae 54,194–212.

Duy, T.N., Lam, P.K.S., Shaw, G.R. & Connell, D.W. (2000). Toxicology and risk assessment of freshwater cyanobacterial (blue-green algal) toxins in water. Reviews of Environmental Contamination and J.of Toxicology, 163: 113-186.

Fischer, W. J., Garthwaite, I., Miles, C.O., Ross, K.M., Aggen, J.B., Chamberlin, A.R., Towers, N.A. & Dietrich, D.R. (2001). Congener-Independent Immunoassay for Microcystins and Nodularins. J.of Environ. Sci. Technol. 35, 2001, 4849-4858.

Funari, E. &Testai, E. (2008). Human health risk assessment related to cyanotoxins exposure. Critical Reviews in J.of Toxicol., 38: 97-125.

Gong Liang, Y., Meng ling, Z., Youxin, C., Qianqi an, P., Wenbo, C. & Renhui, L. (2015). Polyphasic characterization of four species of *Pseudanabaena* (Oscillatoriales, Cyanobacteria) from China and insights into polyphyletic divergence within the *Pseudanabaena* genus.J.of Phytotaxa 192 (1): 001–012.

Gongliang, Y., Meng ling, Z., Youxin, C., Qianqi an, P., Wenbo, C. & Renhui, L. (2015). Polyphasic characterization of four species of *P. seudanabaena* (Oscillatoriales, Cyanobacteria) from China and insights into polyphyletic divergence within the *P. seudanabaena* genus.J.of Phytotaxa 192 (1): 001–012.

Gonzalez-Gil, S., Aguilera, A., Lopez-Rodas, V. & Costas. (2010). Characterization of morphospecies and strains of *Pseudanabaena* (Cyanophyceae) from laboratory cultures using antibodies and lectins .J.of Phycol.34: 27-33.

Gugger, M., Lenoir, S., Berger, C., Ledreux, A., Druart, J.C., Humbert, J.F., Guette, C. & Bernard, C.(2005). First report in a river in France of a benthic cyanobacterium *Phormidium favosum* producing anatoxin-a associated with dog neurotoxicosis. J.of Toxicon. 45:919-928.

Haider, S., Naithain, V., Viswanathan, P.N. & Kaddar, J. (2003).Cyanobacterial toxins: a growing environmental concern, J.of Chemosphere 52:1 – 21.

Harada, K., Kimura, Y., Ogawa, K., Suzuki, M., Dahlem, A.M., Beasley, V.R. &Carmichael, W.W. (1989). A new procedure for the analysis and purification of naturally-occurring anatoxin-a from the blue–green alga *Anabaena-flos-Aquae*. J.of Toxicon. 27 (12), 1289–1296.

Harada, K.I., Matsuura, K., Suzuki, M., Oka, H.; Watanabe, M.F., Oishi, S., Dahlem, A.M., Beasley, V.R. &Carmichael, W.W., (1988). Analysis and purification of toxic peptides from cyanobacteria by reversed-phase high-performance liquid chromatography.J.of Chromatogr. 448, 275–283.

Harada, K.-i., Nagai, H., Kimura, Y., Suzuki, M., Park, H.-D., Watanabe, M.F., Luukkainen, R., Sivonen, K. &Carmichael, W.W.(1993). Liquid chromatography/mass spectrometric detection of anatoxin-a, a neurotoxin from cyanobacteria. J.of Tetrahedron. 49:9251-9260.

Harke, M., Steffen, M., Gobler, C., Otten, T., Wilhelm, S., Wood, S.A. & Pearl, H. A (2016). Review of the globalecology, genomics, and biogeography of the toxic cyanobacterium, Microcystis. J.of Harmful Algae. 54: 4–20.

Huber,C.S.(1972).The crystal structure and absolute configuration of 2,9-diacetyl-9-azabicyclo[4,2,1]non-2,3-ene.*Acta Crystallo graphica* Section B, B28(8): 2577-2582.

Huisman, J., Matthijs, H. C. P. & Visser, P. M. (2005). Harmful Cyanobacteria. J. of Springer.6:33-70.

Humason, G.L. (1972). Animal tissue techniques. Freeman, W.H. (3th ed.), San Francisco press. UAS.PP.641.

Kotran, A., Kumar, V. & Collins, S. (1999).Pathological of disease. 6thed. Volume 2.

Lewis, R. J. (2002). Sax's Dangerous Properties of Industrial Materials, Vol. 1-3, 10th ed., John Wiley & Sons Inc., New York, NY. 33:20-57.

Mann, S., Cohen, M., Chapuis-Hugon, F., Pichon, V., Mazmouz, R., Méjean, A.& Ploux, O. (2012).Synthesis, configuration assignment, and simultaneous quantification by liquid chromatography coupled to tandem mass spectrometry, of dihydroanatoxin-a and dihydrohomoanatoxin-a together with the parent toxins, in axenic cyanobacterial strains and in environmental samples. J.of Toxicon 60: 1404-1414.

Maulood, K.M., Hassan, F.M., AL-ami, A.A., Toma, J.J. & Ismail, A.M. (2013). Checklist of Algal flora in Iraq.Ministry of Environment.

Rubin, E. & Reisner, H.M. (2014).Essential of Rubin's pathology, 6thed.Wlters klumer, Lippincott Williams and Wilkins.pp.840.

Silverstien , R.M. , Basster , G.C.& Morril , T.C. (1991).Spectrometric identification of organic compounds 5 th ed. Tohn wily and Sons , Inc. USA. 419 PP.

Sivonen, K. & Jones, G.J. (1999). Cyanobacterial toxins. In: Chorus, I., Bartram, J. (Eds.), Toxic Cyanobacteria in Water: A Guide to Public Health Significance, Monitoring and Management. The World Health Organization, E & FN Spon, London, UK, p. 41–111 .

Soliakov, L., Gallagher, T. &Wonnacott, S. (1995). Anatoxin-aevoked [^{3}H] dopamine release from rat striatal synaptosomes. J.of Neuropharmacol. 34:1535-1541.

Soliakov, L. &Wonnacott, S. (1996). Voltage-Sensitive Ca^{2+} Channels Involved in Nicotinic Receptor-Mediated [3H] Dopamine Release from Rat Striatal Synaptosomes .J.of Neuropharmacol.40:567-589.

Sukenik, A., Quesada, A. & Salmaso, N. (2015).Global expansion of toxic and non-toxic cyanobacteria: effect on ecosystem functioning. J. of Biodiversity Conserv.24:889–908.

Wiegand, C. &Pflugmacher, S. (2005). Ecotoxicological effects of selected cyanobacterial secondary metabolites a short review. J. of Toxicol. Appl. Pharmacol. 203: 201–218.

Wonnacott, S. (1997).Presynaptic nicotinic Ach receptors .J.of Trends. Neurosci. 0: 92–98.

Wonnacott, S. &Gallagher, T. (2006).The chemistry and pharmacology of anatoxin-a and related homotropanes with respect to nicotinic acetylcholine receptors. J.of Mar. Drugs 4, 228–254 .

Wonnacott, S. &Gallagher, T. (2006).The chemistry and pharmacology of anatoxin-a and related homotropanes with respect to nicotinic acetylcholine receptors. J.of Mar. Drugs 4, 228–254 .