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EVALUATION INFLUENCE OF EXTRACT OF *Terfezia claveryi* DESERT TRUFFLE, PEZIZACEAE, AGAINST *Streptococcus pneumoniae*, *IN VIVO*

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Abstract

This study aims to use extract of desert truffle *Terfezia claveryi* for treatment the infected mice by *Streptococcus pneumoniae.* The bacterium *S. pneumoniae* isolated from patients infected with Pneumonia. Sputum diagnosis of bacteria depended on serological, biochemical and hemolysis tests to identify *S. pneumoniae*. Aqueous extract of *T. claveryi* was used as an antibacterial agent *in vivo* and studied all the changes in the liver and kidney. The changes in kidney function tests, lipid profile and the liver Enzymes also have studied after taking place the infection by this bacterium and compared with the treatment by aqueous extract of *T. claveryi*. Using extract of this truffle was amazing in the cases of Blood Urea, Serum Creatinin, cholesterol, triglyceride, HDL, LDL, GOT, GPT, and ALP which returned significantly (p<0.05) to the normal state like in the control. The infected liver and kidney tissues by the bacterium exhibited degeneration, necrosis and hemorrhage while they returned to normal state after the treatment by aqueous extract of *T. claveryi.* Hence, the introduction of *T. claveryi* in the pharmacological field is remarkable, especially in the treating of *S. pneumoniae* infections.

INTRODUCTION

Desert Truffles represented a complex family of hypogeous fungi. Desert Truffles mainly are including some famous genera *Terfezia*, *Tirmania*, *Picoa* and *Tuber* [1] and they are classified to the class Ascomycetes, order of Pezizales [2]. The geographical distribution of Desert Truffles was found in arid and semi-arid areas as in countries around the Middle East like zones of Iraq, Iran, Syria, Turkey, Kuwait, Saudi Arabia, Libya, Tunisia, Algeria, Morocco, Qatar, Jordan, Bahrain, United Arab Emirates, Palestine, Lebanon and Egypt [3]. Also, some species of truffle have been found in the desert regions of Africa, Asia, and southern Europe [1] and in North America, South Africa, China and Japan [4,5]. *Picoa*, *Tirmania* and *Terfezia* as excellent edible macrofungi with a considerable traditional and economic importance in Turkey and Iraq and they grew with the host-plants *Helianthemum* sp. [3,6,7].

In fact, truffles are a rich source of amino acids, protein, carbohydrates, fatty acids, and minerals [8]. Desert truffles have medicinal and nutritional value because of their composition of amino acids, proteins, fatty acids, carbohydrates, crude fibers, and low energy [3]. Also, Silver nanoparticles synthesized from *Terminia* sp. showed remarkable antibacterial activity [9] due to their compounds like phenol, tannin, flavonoid, glycoside, ascorbic acid, ergosterol, anthocyanin and carotenoid [3].

The bacterium *Streptococcus pneumoniae* belongs to Streptococcaceae family, commonly found in the oropharyngeal and nasopharyngeal mucosa of healthy human [10]. *S. pneumoniae* is the human pathogenic bacterium most commonly in cases of acute otitis media and pneumonia in adults and meningitis in children [11]. At the end of the last century, the introducing antibiotics as drugs like penicillin and sulphonamide led to decrease mortality from pneumococcal infections [12]. Therefore, this study aims to treat mice infected with *Streptococcus pneumoniae* by using aqueous extract of *Terfezia claveryi* and studies the histopathological and biochemical sides.

MATERIALS AND METHODS

Isolation of Bacteria

The bacterium, *Streptococcus pneumoniae*, isolated from sputum of patients infected with Pneumonia. About 8 grams of Nutrient Broth medium was suspended in 1 L D.W. (distilled water), and dissolved using magnetic stirrer hot plate. About 5 ml of Nutrient Broth was poured into suitable plastic tubes and sterilized by

Autoclave at 121 ℃ for 15 min [13]. The prepared broth was stored at 2-8 ℃.

Diagnosis of Bacteria

Diagnosis of bacteria was depended on serological, biochemical and hemolysis methods to identify *Streptococcus pneumoniae*.

The DNA primers

The primers were lyophilized and dissolved in ddH_2O to give a final concentration of 100 pmol/µl and used as stock solution and kept at -20 ℃ to get the concentration of 10 pmol/µl as a working primer. Ten µl of the stock solution was suspended in 90 µl of the ddH_2O water to reach a final volume 100 µl. It was synthesised by Integrated DNA Technologies com., Canada.

Table 1. The specific primer 16s RNA of gene

Primer	Sequence	Tm (°C)	GC (%)
Forward	5'- AGAGTTTGATCCTGGCTCAG- 3'	54.3	50.0
Reverse	5'- GGTTACCTTGTTACGACTT- 3'	49.4	42.1

Preparation of watery extract of *Terfezia claveryi*

T. claveryi was purchased from a local market in Baghdad in 2017. The truffle sample was washed carefully then made the aqueous extract. Fruiting bodies of *T. claveryi* was soaked in D.W. at ratio of 1:10 (w/v) and boiled with agitation at 60±2 ℃ for 30 minutes. The boiled *T. claveryi* was then left covered for 30 minutes. Residues were removed by filtration through gauze and further centrifuged at 10,000 rpm for 30 min at 4 ℃. Supernatants were collected and filtered using the filter paper (Whatman No.1) then stored at 4±2 ℃ until the use according to the method of [14] with some modifications.

Experimental Design

A total number of 40 albino mice males with ages range between 8-10 weeks were obtained from National Control Center For Drugs and Researches (NCCDR), Baghdad. Mice were put in plastic clean cages, mice reared at temperatures from 22-25 ˚C. Animals were fed on standard diet provided from the National Center of Drug Control. The animals were used to determine the antibacterial activity for aqueous extract of *Terfezia claveryi*. Animals were divided into five groups, each group contains 8 mice. The groups were as follows: Group 1: Animals were received 2 ml/kg body wt. of D.W. daily for 14 days by oral as control. Group 2: Animals were received 2 ml/kg body wt. of bacterial broth daily for 14 days by oral without any treatment. Group 3: Animals were received 2 ml/kg body wt. of bacterial broth daily for 14 days by oral also received 2 ml/kg body wt. for aqueous extract of *Terfezia claveryi* as treatment.

Biochemical tests

Plasma and serum of mice' blood has isolated using special tubes by centrifuge for 5 min. The biochemistry tests include kidney function tests (Blood Urea and Serum Creatinin), liver Enzymes tests (GOT, GPT, and ALP) and total lipid profile (triglyceride, cholesterol, and low and high density lipoprotein). They were calculated using standard methods according to instructions of kits from linear company (France).

Histopathological study

All mice were sacrificed after 16 days of the treatment. Liver were dissected out and fixed in plastic containers containing 100 ml of formalin (10%). After that, the organ sample was dehydrated in progressively more concentrated alcohols. The samples were clearing by xyloel twice for 10 minutes then embedded in paraffin and cut into section of 4-5 µm thickness and stained with Haematoxylin and Eosin [15] as follows:

The sections were deparaffinized by hot xylene for 5-10 min; this step was repeated twice. Graded alcohol (100 %, 90 % and 70 %) was used for dehydration; 5 min in each alcohol grade. The section was stained with haematoxylin for 2-3 min, washed with tap water for 5-10 min and differentiated a few second in 1% acidic alcohol (1% HCl in 70% alcohol) until the section looks red, usually 5-15 seconds. The section was washed well in running tap water for 3-5 min to remove the acid, then stained in 1% eosin for 10 min. Graded alcohol (70 %, 90 %, 100 %, and100%) was used for dehydration, (5) min in each alcohol grade. The section was cleared by xylene through three changes (15, 15 and 30 min). Mounting was done by using Disterne Plastcizer Xylene (DPX) and cover slide for the microscopical examination.

RESULTS AND DISCUSSION

Identification of *Streptococcus pneumoniae*

Serological, biochemical and hemolysis has been used to identify Streptococcus pneumoniae. Also, Molecular test was used to know species of Streptococcus exactly as in **Figure 1** which referred to gel electrophoresis of genomic DNA extraction from this bacterium. **Figure 2** exhibited PCR product the band size 1250 bp. The product was electrophoresis on 2% at 5 volt – cm2.1× TBE buffer for 1:30 hours. N: DNA ladder (100). **Table 2** reported the positive biochemical and serological results for Inulin, Optochin and Bile salt solubility, and negative results such as Glucose and Lactose fermentation, Manitol, Bacitracin, streptokinase, CAMP test and growth at 6.5% NaCl. Also, this bacterium leads to α-hemolysis when grown on Blood Agar [16].

Table 2. Biochemical, serological and hemolysis tests of *Streptococcus pneumoniae*

Tests	*Streptococcus pneumoniae*
Inulin, Optochin	+
Glucose and Lactose fermentation., Manitol, Bacitracin, CAMP, Streptokinase	-
Growth at 6.5% NaCl	- no growth
Bile salt solubility	+ growth
Hemolysis	α-hemolysis

Biochemical characteristics

Results of biochemical analysis showed significant (p<0.05) effect when use *Terfezia claveryi* truffle extract as a drug against bacterium of Pneumonia (*Streptococcus pneumoniae*) compared with the infected mice with bacteria without treatment as in **Table 3**. The biochemistry tests include kidney function tests (Blood Urea and Serum Creatinin), liver Enzymes tests (GOT, GPT, and ALP) and total lipid profile (triglyceride, cholesterol, high and

low density lipoprotein). Kidney function tests are Blood Urea and Serum Creatinin. The infected mice with *Streptococcus pneumoniae* exhibited significant (p<0.05) high blood urea 24.5±3.8 mg/dl compared with the control 18.5±2.3 mg/dl, while the blood urea decreased to 23.0±4.0 mg/dl after the treatment by *T. claveryi* extract. Serum Creatinin of all mice did not showed any significant effect and was 0.80±0.01 mg/dl with the control and increased to 0.77±0.02 mg/dl after the infection by bacteria, while it decreased to 0.74±0.01 mg/dl the treatment. Total Lipid profile exhibited significant differences (p<0.05) especially total Cholesterol, HDL (high density lipoprotein) and LDL (low density lipoprotein) which were 228.0±5.7 mg/dl, 78.0±6.8 mg/dl and 133.7±3.3 mg/dl with the infected mice without treatment in comparison with the treated mice by truffle extract 208.1±8.5 mg/dl, 71.5±5.2 mg/dl and 121.0±4.9 mg/dl respectively. However, the values of total Cholesterol, HDL and LDL for the control were 206.1±6.0 mg/dl, 69.0±7.4 mg/dl and 121.7±3.5 mg/dl respectively. The infected mice with *Streptococcus pneumoniae* showed significant (p<0.05) high triglyceride 81.2±5.4 mg/dl compared with the control 76.6±3.4 mg/dl, while the triglyceride decreased to 78.0±4.9 mg/dl after the treatment by *T. claveryi* extract.

Figure 1. Gel electrophoresis of genomic DNA extraction from bacteria, 1% agarose gel at 5 volume /cm for 75 min

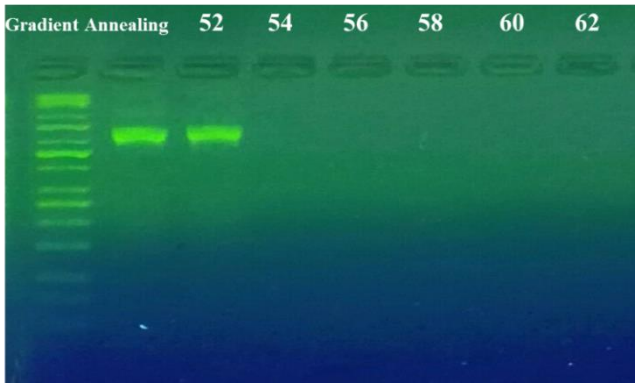

Figure 2. PCR product with the band size 1250 bp. The product was electrophoresis on 2% at 5 volt –cm2.1× TBE buffer for 90 min .N: DNA ladder (100bp).

Also, liver enzymes functions [GOT (Glutamate Oxaloacetate Transaminase), GPT (Glutamate Pyruvate Transaminase) and ALP (Alkaline Phosphatase)] showed significant differences (p<0.05) as in table 6 reached 1.86±0.17 U/ml, 1.02±0.14 U/ml and 2.01±0.21 U/ml with the infected mice without treatment in comparison with the control 1.26±0.09 U/ml, 1.26±0.09 U/ml and 1.42±0.12 U/ml respectively. However, the values of GOT, GPT and ALP decreased to 1.29±0.08 U/ml, 0.80±0.05 U/ml and 1.56±0.10 U/ml after the treatment by truffle extract respectively. However, the results of biochemistry agree with some studies which indicated of the importance of health and nutritional value of the desert truffle *Terfezia claveryi* in countries of Middle East. The beneficial impact in this study may be related to results of researchers who demonstrated that the desert truffles contain amino acids, protein, saturated and unsaturated fatty acids, carbohydrates, crude fibers, carotenoids, ascorbic acid, phenolic compounds [17], and macro-elements like phosphate, potassium and iron [18]. It was found that extracts of this truffle have remarkable antibacterial activity against the eye disease trachoma which caused by *Pseudomonas aeruginosa* compared with antibiotics, and that leads to inhibit growth of *Sterptococcus pneumoniae* in the infected mice after the treatment using this desert truffle. No known toxic compounds have been detected yet [19]. These antibacterial results agree with the results of which was used the biosynthesized AgNPs to treat *Pseudomonas aeruginosa* [9].

Histopathological Field

Figure 3a exhibited that the control liver tissue of mice has normal liver tissue, normal central vein and normal hepatocyte while, the liver tissue infected by *Streptococcus pneumoniae* bacterium showed hemorrhage and necrosis (**Figure 3b**), then the liver tissue returned to normal state after the treatment by aqueous extract of *Terfezia claveryi* (**Figure 3c**). **Figure 3d** showed normal kidney whereas, the kidney infected by *Streptococcus pneumoniae* showed degeneration, necrosis and hemorrhage (**Figure 3f**). Figure 3g has normal kidney after treatment by extract of *T. claveryi*. Kidney and Liver are susceptible to damage and useful organs of detoxification, metabolism, and excretion and storage of xenobiotics [20]. The liver tissue consists of hepatocytes that aggregate in masses, separated from each other by blood sinusoids, and arranged in anastomosing laminae and in rings around a central vein, and is light brown in herbivores animals, damage to the liver is the most frequently reported histopathological response to organic compounds, the importance of the liver as a marker for pathological change reflects the central role of mammalian hepatic tissue in nutrition, lipid and carbohydrate storage, synthesis of protein and enzymes, fatty acid metabolism, and biotransformation and elimination of wastes [21]. The vacuole in the cytoplasm in liver tissue can contain glycogen and fats, which are linked to the normal metabolism of the liver [22].

The liver is characterized by a multiplicity of complex functions; excretion of waste products, secretion, synthesis of fibrinogen, globulins, albumin and clotting factors, storage lipids, A and B vitamins, glycogen, phagocytosis foreign particular matter, detoxification lipid soluble and drugs, steroid hormones, conjugation toxic substances, esterification free fatty acids to triglycerides, metabolism of carbohydrates, lipids, proteins, drugs and hemoglobin [23]. Evacuation of liver cells may indicate a defect between the rate of material synthesis in the parenchymal cells and their release rate in the systemic blood circulation, whereas the increased vacuolization of hepatic cells as a reference to the degenerative process that indicates metabolic damage; possibly linked to exposure to contaminated food. Also, vacuole formation was considered as a cellular defense mechanism against injurious substances to hepatocytes and this mechanism

responsible for collecting the injurious elements and preventing them from interring with the biological activity of the cells. The occurrence of fatty changes proposed inhibition of some enzymes of lipid metabolic, causing thereby, a disturbance in metabolic activity required for preservation of tissue. Prominent fatty changes with necrosis in portal areas indicated that some toxic metabolites may be transported from intestine to liver [24]. However, Desert Truffle has hepatoprotective, antioxidant, antiradical, antidepressant and immune-modulating properties [3] due to its pharmaceutical characteristics [25]. These results agree with results of using tuber extract by [26].

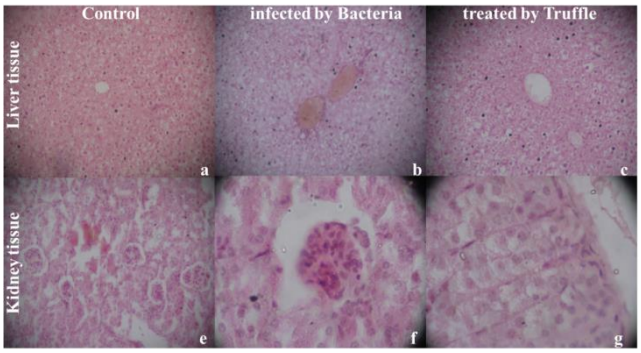

Figure 3. The infected and treated liver and kidney tissues of mice

This study aims to use extract of desert truffle *Terfezia claveryi* for treatment the infected mice by *Streptococcus pneumoniae.* Aqueous extract of *T.claveryi* was used as an antibacterial agent and studied the changes in the liver and kidney tissues. Using aqueous extract of this truffle was surprising in the cases of Blood Urea, Serum Creatinin, cholesterol, triglyceride, HDL, LDL, GOT, GPT, and ALP which returned significantly (p<0.05) to the normal state like in the control. The infected liver and kidney tissues by the bacterium exhibited degeneration, necrosis and hemorrhage while they returned to normal state after the treatment by aqueous extract of *T. claveryi.* Therefore, this work aimed to introduce *T. claveryi* for treating of *S. pneumoniae* infections *in vivo*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

Table 3. Biochemical tests of the infected mice

Groups	Kidney functions		Total Lipid profile				Liver Enzymes functions		
	Blood Urea mg/dl	S. Creatinin mg/dl	Cholesterol mg/dl	Triglyceride mg/dl	HDL mg/dl	LDL mg/dl	GOT U/ml	GPT U/ml	ALP U/ml
D.W. Control	A 18.5±2.3	A 0.77±0.02	A 206.1±6.0	A 76.6±3.4	A 69.0±7.4	A 121.7±3.5	A 1.26±0.09	A 0.80±0.02	A 1.42±0.12
Infected by Bacteria	B 24.5±3.8	A 0.80±0.01	B 228.0±5.7	B 81.2±5.4	B 78.0±6.8	B 133.7±3.3	B 1.86±0.17	B 1.02±0.14	B 2.01±0.21
Treated by ***Terfezia***	B 23.0±4.0	A 0.74±0.01	A 208.1±8.5	AB 78.0±4.9	A 71.5±5.2	A 121.0±4.9	A 1.29±0.08	A 0.80±0.05	A 1.56±0.10

Legend: Mean+SD: mean+Standard Deviation, Differences letters A and B are significant at (p<0.05) to comparison each column. S. Creatinin: serum Creatinin. HDL: high density lipoprotein, LDL: low density lipoprotein, GOT (AST): Glutamate Oxaloacetate Transaminase (Aspartate Transaminase), GPT (ALT): Glutamate Pyruvate Transaminase (Alanine Transaminase), ALP: Alkaline Phosphatase.

CONCLUSION

REFERENCES

1. Janakat, S., Al-Fakhiri, S. and Sallal, A. K. (2014) A promising peptide antibiotic from *Terfezia claveryi* aqueous extract against *Staphylococcus aureus* in vitro. *Phytother Res.*, **18**(10), 810–813.
2. Moreno, G., Alvarado, P. and Manjón, J. L. (2014) Hypogeous desert fungi. In V. Kagan-Zur, N. Roth-Bejerano, Y. Sitrit and A. Morte (Eds.), *Desert truffles* (pp. 3–20). Berlin Heidelberg: Springer.
3. Owaid, M. N. (2018) Bioecology and uses of desert truffles (Pezizales) in the middle east. *Walailak Journal of Science and Technology*, **15**(3), 179–188.
4. Trappe, J. M., Claridge, A. W., Arora, D. and Smit, W. A. (2008) Desert truffles of the African Kalahari: ecology, ethnomycology, and taxonomy. *Econ. Bot.*, *62*, 521–529.

5. Morte, A. and Bordallo, J. (2006) Biotechnology and cultivation of desert truffles: an agricultural tool for degraded semi arid lands. In *Proceedings of the 5th International Conference on Mycorrhiza*. Granada, Spain.
6. Akyüz, M., Akyüz, H. E. and Kirbağ, S. (2017) Ethnomycological Aspects of Traditional Usage and Indigenous Knowledge about the Arid-Semi Arid Truffles Consumed by the Residents of the Eastern Anatolia Region of Turkey. *Gazi University Journal of Science*, **30**(4), 57–70. Retrieved from http://dergipark.gov.tr/gujs/issue/32802/364461
7. Owaid, M. N. (2016) Biodiversity and bioecology of Iraqi desert truffles (Pezizaceae) during season 2014. *Journal of Aridland Agriculture*, **2**, 22–25. https://doi.org/10.19071/jaa.2016.v2.3046
8. Bokhary, H. A. and Sarwat, P. (1993) Chemical composition of desert truffles *Terfezia clavery*. *J. Food Comp. Anal.*, **6**(3), 285–293.
9. Owaid, M. N., Muslim, R. F. and Hamad, H. A. (2018) Mycosynthesis of Silver Nanoparticles using *Terminia* sp. Desert Truffle, Pezizaceae, and their Antibacterial Activity. *Jordan Journal of Biological Sciences*, **11**(4), 401–405.
10. Hausdorff, W. P., Bryant, J., Paradiso, P. R. and Siber, G. R. (2000) Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, Part I. *Clin Infect Dis*, **30**, 100–121.
11. Brandileone, M. C., de Andrade, A. L., Di Fabio, J. L., Guerra, M. L. and Austrian, R. (2003) Appropriateness of a pneumococcal conjugate vaccine in Brazil: potential impact of age and clinical diagnosis, with emphasis on meningitis. *J Infect Dis*, **187**, 1206–1212.
12. Nascimento-Carvalho, C. M., Freitas-Souza, L. S., Moreno-Carvalho, O. A., Alves, N. N., Caldas, R. M. and Barberino, M. G. (2003) Cepas invasivas de pneumococo isoladas de crianças e adolescentes em Salvador. *J Pediatr (Rio J)*, **79**, 209–214.
13. Hussein, N. M., Owaid, M. N. and Khalaf, B. H. (2018) Effects of Lipopolysaccharides of *Pseudomonas aeruginosa* and Aqueous Extract of *Ginkgo biloba*, Ginkgoaceae, on Cellular Immune Response in Mice Balb/c. *Gazi Medical Journal*, **29**(3), 206–210. https://doi.org/10.12996/gmj.2018.57
14. Chen, X. and Schluesener, H. J. (2008) Nanosilver: a nanoproduct in medical application. *Toxicology Letters*, **176**(1), 1–12.
15. Zhang, H. W., Li, W., Yan, Q. Y., He, L. J. and Su, Y. P. (2006) Impact of Blastocystis hominis on ultrastructure of intestinal mucosa in mice. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi*, **24**(3), 187–191.
16. Burckhardt, I., Panitz, J. and Zimmermann, S. (2017) Identification of streptococcus pneumoniae :Devlopment of astandarized protocol for optochin suscptibility testing using total lab Automation. *BioMed Research International*, *2017*, 4174168. https://doi.org/10.1155/2017/4174168
17. Saddiq, A. A. and Danial, E. N. (2012) Assessment of phenolic content, free radical-scavenging capacity and antimicrobial activities of *Truffle claveryi*. *Wulfenia J*, **19**, 403–422.
18. Han, K. S., Balan, P., Molist Gasa, F. and Boland, M. (2011) Green kiwi fruit modulates the colonic microbiota in growing pigs. *Lett Appl Microbiol*, **52**, 379–385.
19. Janakat, S., Al-Fakhiri, S. and Sallal, A. K. (2005) Evaluation of antibacterial activity of aqueous and methanolic extracts of the truffle *Terfezia claveryi* against Pseudomonas aeruginosa. *Saudi Med J*, **26**(6), 952–955.
20. Metcalfe, C. D. (1998) Toxicopathic responses to organic compounds. In J. F. Leatherland & P. T. K. Woo (Eds.), *Fish Diseases and Disorders: Non-Infectious Disorders* (2 Ed). UK and USA: CABI publishing.
21. Gingerich, W. H. (1982) Hepatic toxicology of fishes. In L. J. Weber (Ed.), *Aquatic toxicology* (pp. 55–105).
22. Yamaguchi, P. (1996) Histopathological and electron microscopic changes in mice treated with puffer fishtoxin. *Journal of Toxicological Sciience*, **1**, 1–14.
23. Camargo, M. M. P. (2007) Martinez kidney and liver of a Neotropical fish caged in an urban stream. *Neotropical Ichthyology*, **5**(3), 327–336.
24. Phansri, K., Sarnthima, R., Thammasirirak, S., Boonchalee, P. and Khammuang, S. (2011) Antibacterial activity of *Bauhinia acuminata* L. seed protein extract with low hemolytic activity against human erythrocytes. *Chiang Mai J Sci*, **38**(2), 242–251.
25. Tel-Çayan, G., Devecİ, E., Çayan, F. and Duru, M. E. (2018) Comparative assessment of phytochemical composition, antioxidant and anticholinesterase activities of two Basidiomycota Truffle Fungi from Turkey. *Marmara Pharmaceutical Journal*, **22**(1), 59–65.
26. Hasan, M.H., Zin, M.M., Wahab, I.A., Ponto T. and Adam A. (2018) *Myrmecodia platytyrea* aqueous Tuber extract inhibits pain. *Malay. J. Biochem. Mol. Biol*., **21**(2), 23-27.