ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Effects Chitosan and Calcium Nanoparticles Mouthwash from *Xylotrophes gideon* In the Liver And Kidney Rat.

Ade Komariah[1*], Widagdo A Laksono[2], Del A Bustami[3], Bambang S Trenggono[3]

[1*] Department of Histology , Faculty of Dentistry, University of Trisakti Jakarta 11440, Indonesia
Email: akomariah67@gmail.com
[2,3] Department of Oral Biology, Faculty of Dentistry, University of Trisakti Jakarta 11440, Indonesia

ABSTRACT

Xylotrupes gideon is one of the harmful insects that damage coconut crops, one alternative is the use of harmful insects by processing into nano chitosan and nano calcium. Physical modification of chitosan into nano chitosan can increase the activity of chitosan as an antimicrobial, so it can be used as a raw material in mouthwash formulations, but the use of nano materials in consumer and industrial products have a global concern effects in biological systems. This study was done to take advantage of nano materials as a basic ingredient in mouthwash formulations that are safe on the liver and kidney organs. The study used 24 *S. dawley* male rats, which were divided into 4 groups. Group 1 as a control group. 2-4 group given mouthwash formulations nano materials at a concentration of 750ppm, 1500ppm and 3000ppm for 28 days. The observation of body weight showed body weight gain, but did not show any differences in each group. For a macroscopic overview showed that the mouthwash formulations nano chitosan and nano calcium did not show any difference between the groups. Microscopic feature showed the changes in liver and kidney cells, however do not show significant differences between the groups.

Keywords: *Xylotrupes gideon*, nano chitosan, nano calcium, liver, kidneys

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Herbs medicine as alternative medicine or traditional medicine that is recognized as the most common forms used by the public [1]. The World Health Organization (WHO), states that 80% of the population in the world, especially in developing countries use traditional medicine as an alternative treatment [2].

Traditional medicine is a drug ingredient or ingredients derived from plants, animals and minerals, or mixtures of these materials [3]. The use of natural biomaterials as an alternative medicine is more widespread in the community, so further study is needed, in order to make it appropriate to the rules of health care, which is scientifically proven of efficacy, safety and quality standards [4].

The size of the biomaterial was very important for an active ingredient to be working. On a scale of size, the particles can have much different properties and function compared to the same particle [5]. Nanotechnology is the manipulation of material at the level of atoms, molecules and supramolecular scale which involving design, characterization and application of different nano [6]. The use of nanoparticle materials offer great advantages because of their unique size and physicochemical properties. Nanoparticle study is growing rapidly because it can be widely applied in various fields such as the environment, electronics, optical and biomedical [7]. The increasing use of nanomaterials in consumer and industrial products lead to concern of global effects of nanomaterials in biological systems [8].

Chitosan is a natural biopolymer, linear polysaccharide form thaht composed of ß-(1-4)-linked Dglucosamine and N-acetyl-D-glucosamine [9]. Chitosan can be isolated from the shells of crabs, shrimp, and from the exoskeletons of insects cuticle [10], such as beetle [11]. One of the harm beetle is *X. gideon* [3]. *X. gideon* is an insect that is found in Southeast Asia, including Indonesia. One alternative attempt to use the insect to become a high economic value product is processing into chitin and chitosan [12].

Chitosan can be applied widely in a variety of purposes, as it has several advantages [13], including biocompatible, biodegradable and non-toxic properties [14]. Chitosan is obtained from natural sources with stage of process is slightly longer than in obtaining chitin [15]. Chitosan demineralization process can produce calcium in the nanoparticles form. Chitosan and calcium a very promising biomaterials, if we use in the nanoparticles form [16]. But the the problem is in the development of nano-technology is a potential risk to health, safety and the environment as a result of the use of nano materials [17].

To test the safety of a drug, it can be done with a toxicity test. Toxicity tests conducted to determine the toxic effects caused to the animal's vital organs, including the liver and kidneys [18]. Liver is an organ that has a major and complex role includes a metabolic, detoxification, excretion, secretion and storage functions [19]. While the kidney has the function including excrete foreign compounds such as drugs, food, pesticides and other exogenous materials non-nutrients that enter the body [20].

The use of a specific time interval doses expected to give a different effect or indication to the body's organs including the liver and kidneys. Therefore, this study was designed to investigate the sub accute effect of administering nano-chitosan and c-calcium mouthwash formulations with repeated administration for 28 days [21], the growth performance of animals model, as well as the feature of macroscopic and microscopic liver and kidney structure.

MATERIALS AND METHODS

Nano Material

Calcium and chitosan as raw material drug formulation from the exoskeletons of *X. gideon* nature from West Bogor, namely; Cangkurawok, Damaga, Balumbang Jaya **(Fig. 1).** Nano calcium obtained from demineralized chitosan with precipitation method. Chitosan is obtained through a demineralization (HCl 3N, Merck), deproteinization (NaOH 3N, Merck), decolorization (H2O2, Merck) and deacetylation (NaOH 50%, Merck) process. Physical modification of nano chitosan into chitosan obtained by ionic gelation method using a magnetic stirrer.

ISSN: 0975-8585

Figure 1: *Xylotrupes gideon* from West Bogor

Study of Sub-Acute Toxicity

Study using *S. dawley* rats, with a body weight of 120-150gr. The number of subjects required in the study of 24 male, which is calculated using the formula Federer, 1963. The study was divided into 4 groups. Group 1 was given distilled water as a control group. Group 2-4 given nano chitosan and nano calcium mouthwash formulations at a concentration of 750ppm, 1500ppm and 3000ppm for 28 days **(Table 1)**. Mouthwash formulations are given 1 ml/100g [21]. Animal growth performance by measuring the observed body weight every week. Observation of macroscopic and microscopic feature of liver and kidney organs was done on day 28 [22]. The experiment was approved by the Health Research Ethics Committee of the Faculty of Dentistry, University of Trisakti No:339/KE/FKG/8/2016.

Table 1. Treatment Group

Group	Treatment	Time (Day)	Number of Rats
1	Standard feed + add libitum drink +1 ml / 100g distilled water	28	6
2	Standard feed + add libitum drink + 1 ml/100g mouthwas formulation 750ppm	28	6
3	Standard feed + add libitum drink + 1 ml/100g mouthwas formulation 1500ppm	28	6
4	Standard feed + add libitum drink + 1 ml/100g mouthwas formulation 3000ppm	28	6

Measurement of Body Weight

Measurement of body weight is evaluated weekly on day 0, 7 , 14, 21 and 28, using the scale with a sensitivity of 0,01g [21].

Sample Collection

At the end of the study period, the animals model were sacrificed using anesthetic ketamine and xylazin, ketamine offer analgesic effects while xylazin result in muscle relaxation [23].

Macroscopic and Microscopic Observation of Kidney and Liver Organ

Macroscopic observation is done by observing the color, size and weight of animals model organs. For microscopic observation, preparations histology was made for observation of the liver and kidneys cells. Preparations histology was made through several stages, including sampling, fixation (preservation) and the stopping point, dehydration to the withdrawal of water molecules from inside the tissue, clearing (purification) using xylol to take the place of alcohol in a tissue with a medium purification, embedding, the attachment tissue into paraffin blocks, sectioning (cutting), and staining using hematoxylin-eosin (HE). HE staining used for interpret morphological changes in the tissue cells [24]. Histopathological changes parameter for the liver and kidneys to be done with observation of tissue 5 times in each group replications, with assessment of the

ISSN: 0975-8585

damage degree as follows: (0) indicates no change, and (I) (II) and (III) shows minimum, moderate and maximum changes, respectively **(Tabel 2)** [25,26].

Table 2. Criteria for the classification at the cellular level of the liver and kidneys

Assessment degree	Liver	Kidney
0	No morphological changer	No morphological changes
I	Shaped cell and nucleus was enlarged and irregular, nucleus is lie in lateral . there are granular eosinophil in cytoplasm.	Cell and nucleus was enlarged, pale cytoplasm, enlarged of glomerular capillary and glomerulus.
II	Predominance vacuolization in cytoplasm, and nucleus (hydrophic degeneration)	Reduced in Bowman space and blood exist in Bowman space
III	Necrosis	Necrosis

Statistic analysis

The results were analyzed by looking at the means ± standard deviation (SD). Statistical analysis of body weight and organ weights using one-way ANOVA for comparison between groups with a significant level of 95% ($p < 0.05$).

RESULTS AND DISCUSSION

Observation of Growth Performance of Animals Models

The observation of the growth performance in mice by measuring body weight at day 0, 7, 14, 21, and 28 of each treatment can be seen in **Fig. (2)**. The results of body weight observation of mice showed improvement every week, either in the control group and the treatment group were given nanoparticles of chitosan and calcium mouthwash formulations, with p-value <0.05. For comparison the increase in body weight in each group showed no significant difference with p-value> 0.05.

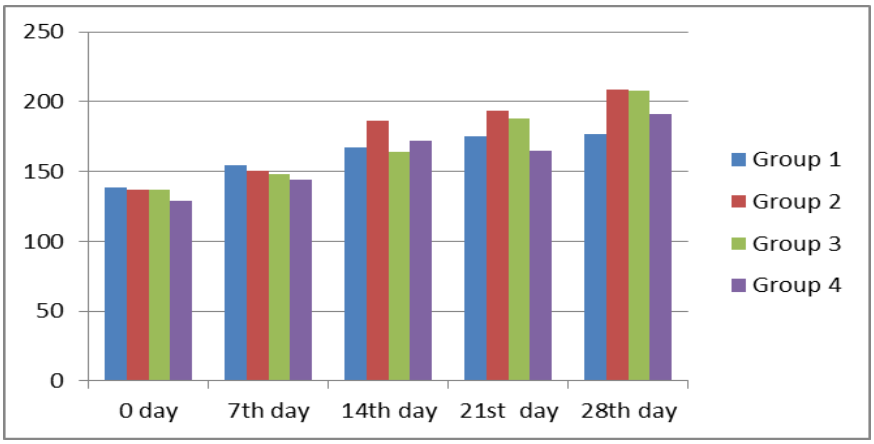

Figure 2. Comparison of rats body weight between of control group and experimental groups

The observation of the growth performance shows the weight in the treatment group those given nanoparticles-based mouthwash formulations showed an increase in the concentration of each group. On the control group, mean of body weight gain of 138.75±9.02 to 177.00±26.51 on day 28. At low concentrations (750ppm) mean of body weight gain of 137.25±9.61 to 209.01±17.91 on day 28 . For moderate concentrations (1500ppm) mean of body weight gain of 137.02±12.62 to 208.25±21.72 on the day 28. At high concentrations (3000ppm) mean of body weight gain of 129.10±12.91 to 191.25±15:36 on the day 28.

ISSN: 0975-8585

Observations of Macroscopic Structure

Macroscopic and microscopic observation of the liver and kidneys was done, because the both of organs are very important in the process of detoxification in the body [27]. Macroscopic observations include color, size and organ weights, observed by the comparison between the control group and the treatment group at concentration (750ppm, 1500ppm and 3000ppm, respectively). The observation of the color and size of both the liver and kidneys in the treatment group showed a fresh red color and the same size with the control **(Fig. 3)**. From macroscopic observation that found no abnormalities or significant difference from both the control group and the treatment group.

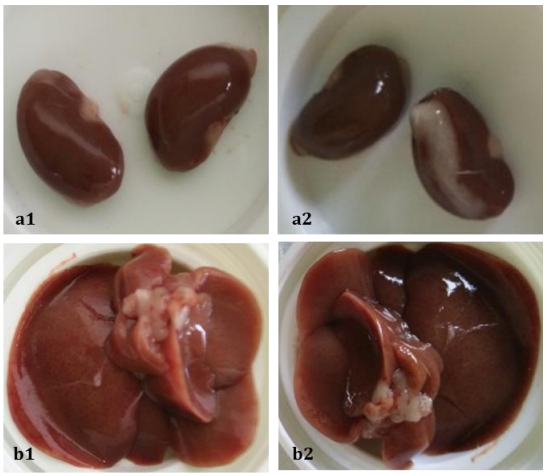

Figure 3. Structure of macroscopic; a1. Kidney control; a2.Kidney treatment; b1.The liver controls; and b2. The liver treatment

Examination of liver and kidney organ weights aims to determine the changes in organ weights after administration of the subchronic test preparation which will be correlated with histopathological examination. The mean of the liver and kidney organ weights at day 28 after the administration of the test preparation can be seen in **Table (3)**. The observation of measurements calculations using statistical analysis One Way Annova. From the test of hepatic organ weights, we obtained significance of 0.326, meaning that no differences in liver organ weights in all five groups. For kidney significance value is 0.366, which means there is no difference in renal organ weights between the control and treatment group.

Table 3. Measurement of Liver and Kidney weight result

Group	Mean of organ weight	
	Liver	Kidney
1	3.84 ± 0.73	0.68 ± 0,22
2	2.74 ± 0.73	0.82 ± 0.18
3	3.31 ± 0.54	0.93 ± 0.18
4	3.6 ± 1.18	0.94 ± 0.15

Observation of Microscopic Structure

Observations result based on the assessment of the degree of damage to the liver and kidney cell after administration of mouthwash formulations at different concentrations for 28 days (**Table 4**). The results of microscopic observation of liver cells showed changes in liver cells with the maximum degree of damage, in the group with a concentration of 3000ppm, but the degree of damage showed no significant with p values> 0.05, so that the test results showed no difference between the control group treatment group. Microscopic feature of liver cells that can be seen in **Fig. (4)** and **Fig. (5)**.

ISSN: 0975-8585

Table 4. Degree of Damage Presentation Liver Cells In Each Group

Degree Damage	Liver								Kidney							
	1		2		3		4		1		2		3		4	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
0	14	47	15	50	18	50	16	53	25	83	27	90	24	80	26	87
I	9	30	7	23	5	30	9	30	5	17	3	10	6	20	4	13
II	7	23	8	27	7	20	5	17	0	0	0	0	0	0	0	0
III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	30	100	30	100	30	100	30	100	30	100	30	100	30	100	30	100

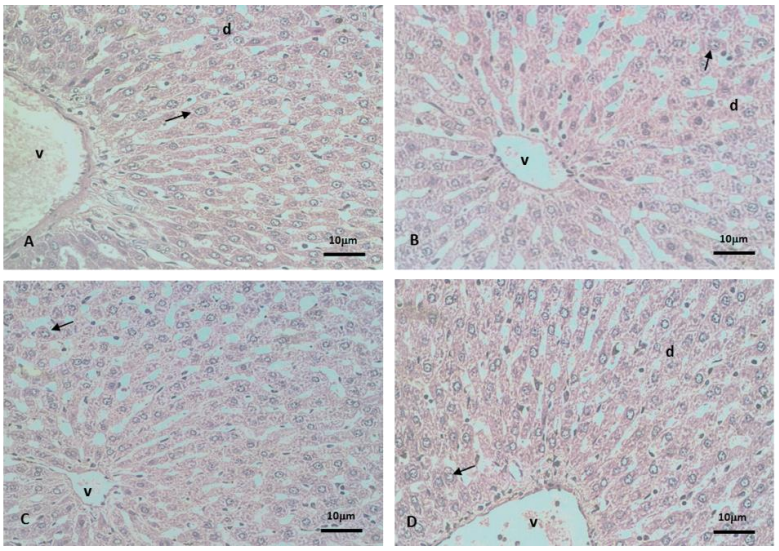

Figure 4. Microscopic Observations of stained (H & E X20) Rat Liver Microscopic Structure. (A) Control; (B) Low concentrations of 750 ppm; (C) moderate concentration of 1500ppm; (D) a high concentration of 3000ppm. There is a feature of hepatocyte cell enlargement (arrow); (D) Hepatocytes hydropic degeneration; (V) Vein centralis.

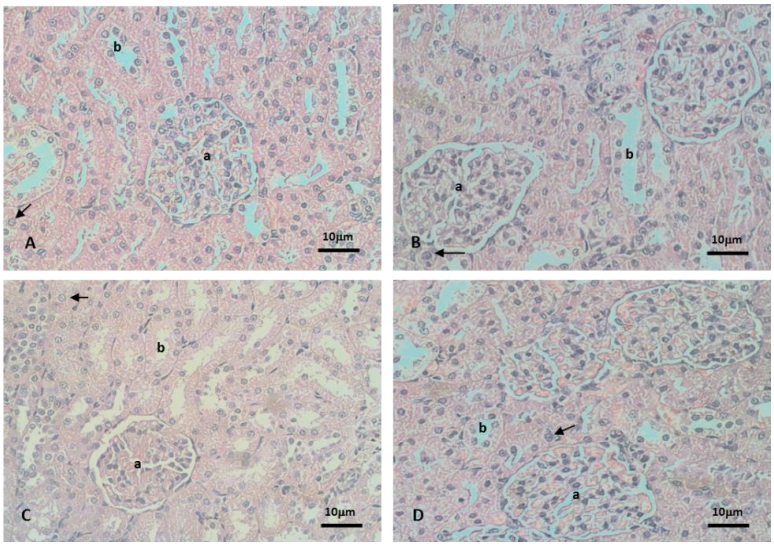

Figure 5. Microscopic Observations of stained (H & E X20) Rat Liver Microscopic Structure. (A) Control; (B) Low concentrations of 750 ppm; (C) moderate concentration of 1500ppm; (D) a high concentration of 3000ppm. There is a feature of hepatocyte cell enlargement (arrow); (D) Hepatocytes hydropic degeneration; (V) Vein centralis.

ISSN: 0975-8585

DISCUSSION

Growth Performance of Animals Models

Increased body weight in the concentrations group, the concentration at 3000ppm showed the lowest body weight gain compared with low and medium concentrations, this is due to the baseline weight of the animals model to high concentrations have an average lower than the other concentration. The use of nano calcium in mouthwash formulations which are expected to affect the growth performance of animals model, but the results did not show an increase in body weight compared to the control treatment group. We measure the body weight performance because, calcium was needed for bone mineral formation and important for the regulation of physiological and biochemical processes. Calcium is needed to maximize bone mass including to maintain bone density and body mass index, it means the greater of body mass index, bone mass more dense [28].

Macroscopic structure of kidney and liver

Macroscopic observation conducted on the condition of both liver and kidneys organ to see the change in the morphology organ [21]. Observations made to the macroscopic structure of each treatment group, and then compared with the control group. Macroscopic feature of the liver [29], and kidney [30], may exhibit the toxicity of a given material in animals model. From macroscopic observation, we are not found abnormalities or significant difference from both the control and treatment groups.

Drug intoxication can result in damage to the function of various organs, including kidney damage (nephrotoxicity) [31], and damage to the liver (hepatotoxicity) [32]. The liver is the multifunctional organ which plays an important role in metabolism, biosynthesis, excretion, secretion and detoxification [33]. The liver plays a role in primary metabolism that will detoxify and eliminate all toxins whether from toxic metabolites and xenobiotics [34]. Damage to liver cells due to exposure to the toxic drug will cause liver cells into necrosis. The most common necrosis is oncotic necrosis [33]. Kidneys play a role in the excretion of metabolic waste products, kidneys produce urine which is the main route of excretion toxicant [35]. Damage to the kidneys causing kidney failure and chronic kidney disease [36]. Toxic material is influenced by several factors, including other types of chemicals, the dose given, and the length of exposure to these substances [37]. The higher concentration of a given compound, the greater toxic response was caused. Generally, macroscopic changes in an organ occurs in a chronic condition, such as the degeneration of the cells of the liver, and kidneys. However, mild degeneration in the cells of the liver and kidneys do not affect their macroscopic appearance.

Microscopic Structure of Kidney and Liver

The results of microscopic examination of the kidneys after being given treatment for 28 days can be seen in Table 5. The microscopic feature showed no abnormalities in the cells contained in the units of renal function compared to the negative control. According to Wahyono *et al*., (2012), one of the microscopic abnormalities in the kidneys was the congestion that visually demonstrate more red color (purple) and capillaries in the wide tissue filled with blood. Besides congestion process of inflammation is a protective response directed at the cause of the initial damage to cells, such as enlargement of podosit in the glomerulus, thickening of the media layer in lobular artery and ductal epithelial hyperplasia [39].

Microscopic examination of the liver organ after being given treatment for 28 days can be seen in **Table (5)**. Microscopic observations showed no abnormalities in liver cells compared to the negative control. Wahyono *et al*., (2012), shown that the damage to the liver can be necrosis, congestion and inflammation. Necrosis is the death cells or tissue in a living organism characterized by nucleus shrank and became dark, no longer euchromatin (piknosis), then fragmented (caryorrhexis), then disappears (cariolysis).

The results of macroscopic and microscopic observation of the liver and kidney organs showed no abnormalities. The results of this study was supported by El-Fattah *et al.,* (2013), shown that which states granting chitosan had no impact organ abnormalities in animals model, this is because the chitosan are antioxidative and has the ability to capture the hydroxyl radical. Chitosan can reduce liver damage, because chitosan has the ability biocompatibility, biodegradability and degradation. Besides, chitosan is non-toxic, non-immunogenic and non carcinogenic.

ISSN: 0975-8585

CONCLUSION

Based on the results and discussion of this study, it can be conclude that the chitosan and calcium nanoparticles-based mouthwash formulations from the exoskeletons of *X. gideon* at a concentration of 750ppm, 1500ppm and 3000ppm with repeated doses orally can increase body weight, although it does not show the difference between the treatment groups. Macroscopic and microscopic feature showed no significant difference between control and treatment groups. This suggests that the mouthwash formulations based on nano chitosan and nano calcium showed no toxic effects on the body's systems, especially the liver and kidney organs.

REFERENCES

[1] Iwuanyanwu K.C.P., Amadi, A.I.A., Ayalogu E.O. EXCLI J 2012; 11:632-640.
[2] Ogbonnia S.O., Mbaka G.O., Anyika E.N., Emordi J E Pak J Nutr 2011; 10:1022-8.
[3] Kumar R., Rajkhowa G., Dhar N.J., Rajan R.K. Ent Zool 2011; 6(1): 173-175.
[4] Izhar T., Chakraborty M. J Pharmacogn Phytochem 2013; 1(60):1-5.
[5] Yah C.S., Iyuke S.E., Simate G.S. J. Pharmaceut Sci 2012; 25: 477-491.
[6] Silva J, Fernandes A.R., Baptista P.V. INTECH. Chapter 4 2012;127-154.
[7] Stern S.T., McNeil S.C. Toxicol Sci 2008;101(1): 4-21.
[8] Dhawan A., Sharma V. Anal Bioanal Chem 2010; 398 :589-605.
[9] Puvvada S.Y., Vankayalapati S., Sukhavasi S. Int Current Pharmaceut J 2012; 1(9): 258-263.
[10] Bansal V., Sharma P.K., Sharma N., Pal P.O., Malviya R. ABR 2011; 5(1): 28-37.
[11] Liu S., Sun J., Yu L., Zhang C. J Molecules 2012;17: 4604-4611.
[12] Komariah A., Astuti L. Proceedings of the National Seminar IX Biology Education FKIP UNS 2012; 648-654.
[13] Yogeshkumar G., Atul G., Adhikrao Y. IJRPBS 2013; 4(1):312-333.
[14] Sun K., Li Z.H. eXPRESS Polymer Letters 2013;5(4):342–361.
[15] Goy R.C., De Britto D., Assis O.B.G. Polímeros Sci Tecnologia 2009;19(3):241-247.
[16] Mardliyat E., Muttaqien S., Setyawati D.R. Proceedings InSiNas 2012;71: 25-30.
[17] Riwayati I. Momentum 2007; 3:17-20.
[18] Atici S., Cinel L., Doruk N., Eskandari G., Oral U. Model J Biosci 2005;30: 245-252.
[19] Chang C.W., Beland F.A., Hines W.M., Fuscoe J.C., Han T. Int. J. Mol. Sci 2011;12: 4609-4624.
[20] Anzai N., Endou H. . J Pharma 2007; 14 :447-452.
[21] OECD, Organisation for economic cooperation and development. repeated dose 28-day oral toxicity test method guideline 407 adopted 23.03.1996. In: OECD, Guidelines for testing of chemicals. Paris: Organisation for Economic Co-Operation and Development, 2006.
[22] Diallo A., Gadegkeku K.E., Agbonon A., Aklikokou K., Creppy E.E, Gbeassor M. Trop J Pharm Res 2010; 9 (5): 463-467.
[23] Yudaniayanti I.S., Maulana E., Ma'ruf A. Vet Medika 2010; 3(1):23-30.
[24] Khalik A.A., Nosseir N., Salah M.K., Tawfik M.K. Afr J Health Sci 2007;14:19-36.
[25] Aydin G., Umen A., Ekrem U., Karahan N., Kalp O. Turk J Vet Anim Sci Neotropical Ichthyology 2003; 5(3):327-336.
[26] Camargo M.M.P., Martinez C.B.R. Neotrop. ichthyol 2007; 5(3):327-336.
[27] Arsad S.S., Esa N.M., Hamzah H. J Cytol Histol 2014;1-6.
[28] Conde D.M., Paiva L.C., Martinez E.Z., Neto A.M. Rev Assoc Med Bras 2012; 58(6):673-678.
[29] Girish C., Koner B.C., Jayanthi S., Rao K.R. Indian J Med Res 2009;129:569-578.
[30] Modaresi M., Pour M.G., Tabeidan S.A., Jalalizand A. IPCBEE 2011; 9: 16-20.
[31] Bicalho D.M., Soares S.M., Botoni F.A. Int. J. Environ. Res. Public Health 2015; 12: 11227-11240.
[32] Pandit A., Sachdeva T., Bafna P. 2012. J of Applied Pharmaceut Sci 2012 ;2(5); 233-243.
[33] Malhi H., Gores G.J., Lemasters J.J. Hepatology 2006;43(2): 31-44.
[34] Xinsheng G., Manautou J.E. Expert Rev Mol Med 2012.
[35] Asli S., Alaattin S.E.N. AFR J Biotec 2007;6(3):273-277.
[36] Singh N.P., Ganguli A., Prakash A. JAPI 2003; 55:970-979.
[37] Raies A.B., Bajic V.B. Comput Mol Sci 2016;6:147–172.
[38] Wahyono S., Hakim L., Nurlaila, Abdulah D. Journal of Pharmacy 2012:1-8.
[39] Mazue G., Newman A.J., Scapini G., Torre P.D., Hard G.C., Latropoulos M.J., Bagnasco S.M. Toxicol Pathol 1993; 21(5):490-501.

[40] El-fattah H.M.A., Abdel-Kader Z.M., Hassnin E.A., El-rahman M.K.A., Hassan L.E. IOSR-JESTFT 2013;7(3):11-17.