Journal of Medical Sciences

ISSN 1682-4474

science alert

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OPEN ACCESS Journal of Medical Sciences

ISSN 1682-4474
DOI: 10.3923/jms.2019.69.74

Research Article
Acute Toxicity of Indonesian Natural Food Colorant *Tectona grandis* Leaf Extract in Wistar Rats

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Abstract

Background and Objective: Teak (*Tectona grandis*) belonging to family Verbenaceae, is commonly used in Indonesian cuisine as natural food colorant. However, there remains limited information regarding its toxicity effects. The purpose of this study was to examine *Tectona grandis* leaf extract (TGLF) acute toxicity in Wistar rat animal models through oral route administration. **Materials and Methods:** Acute oral toxicity of TGLF was carried out in single dose of 2 and 5 g kg^{-1} b.wt., (dissolved in NaCMC). Animal models were grouped into control (NaCMC 0.5%), TGLF 2 g kg^{-1} b.wt., TGLF 5 g kg^{-1} b.wt. and 2 satellite groups of each doses. All the animals were individually studied for the mortality, wellness parameters, body weight and histopathology for 14 days. The experiments were performed under OECD guidelines 423. **Results:** No mortality or any significant physiological changes in the animals were noticeable even up to high dose of 5 g kg^{-1} b.wt. Although, histological profiles showed toxicity indication induced by high dose of TGLF (5 g kg^{-1} b.wt.) in vital organs such as liver and stomach. **Conclusion:** Current results showed the safety nature of TGLF as natural food colorant. However, applied dose should be considered as toxicity indications are noticeable at high dose administration.

Key words: Histological profiles, food colorants, Indonesian cuisine, *Tectona grandis* leaf extract, acute oral toxicity

Citation: Candra Dwipayana Hamdin, Eka Sunarwidhi Prasedya, Sinta Wahyu Utami, Dandiko Galanova, Dheny Cahyo Saputro, Arief Nurrochmad, Retno Murwanti, Ahmad Jupri and Haji Sunarpi, 2019. Acute toxicity of Indonesian natural food colorant *Tectona grandis* leaf extract in Wistar rats. J. Med. Sci., 19: 69-74.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Usually, the properties of foods such as shapes, colors, tastes, smells and textures are improved to satisfy consumer expectations. Color is among the most significant factors that directly affect the food choice and eating desires of consumers[1]. Color additives are often used as dye or substance that are capable of coloring when added to food, medicament, cosmetics or applied to the human body[2]. Natural food colorants, which continue to be used worldwide are known to have health benefits compared to synthetic colorants[3]. Consumers perceive natural colorants are safer than synthetic colorants which are thought to be harmful.

The young teak leaf (*Tectona grandis*) are commonly used as food colorants in several regions in Indonesia. The plant contains various chemical constituents in all of its parts such as leaf, bark, roots and seeds[4]. The leaf are reported to contain anthraquinones, lignin derivatives, anthratectone and naphthatectone[5]. Bark contains betulin-aldehyde, lupeol, ursolic acid, eicosanyl-eicosanoate and betulinic acid[6]. Seed contains fatty acids, amino acids and xanthones[7].

The leaf of *T. grandis* are known to contain anthocyanin pigment which gives the color blue, purple, violet, magenta, red and orange in food products[8]. A number of studies have already shown that anthocyanins have positive therapeutic properties and their various biological activities have been extensively reviewed[9]. They are claimed to provide beneficial effects in reducing the incidence of cardiovascular diseases, cancer, hyperlipidaemia and other chronic diseases[10,11].

Despite the immense amount of *T. grandis* usage in Indonesia as food colorants, different aspects regarding their potential toxicity still remains unclear. Before promoting them as safe food additives, some more evaluations about possible toxic effects at certain doses should be performed. Hence, the objective of this study was to evaluate the acute toxicity effects of *T. grandis* in animal models.

MATERIALS AND METHODS

Plant material: The plant materials were received from the Faculty of Pharmacy, Gadjah Mada University. Plants were identified by Department of Biology, Mataram University.

Preparation of plant extracts: The leaf of young teak trees (*Tectona grandis*) were washed with running water in order to remove any dust impurities and dried under shade. They were powdered as coarse particles with a grinder and were extracted by ethanol solvent method by using soxhlet apparatus. The extracts were concentrated in a rotary evaporator under reduced pressure.

Animals: The female Wistar rats (3-4 months) were purchased from UPHP-LPPT Gadjah Mada University. Animal models weighing 150-200 g were used for this study. All animals were maintained in standard ventilated room at 20-23°C and 12 h light/dark cycles. Animals had free access to tap water and pellet. The methods of this study were approved by animal ethic committee of Faculty of Medical, Mataram University (285/UN18.8/Ethic/2017).

Acute toxicity studies: A single oral dose (based on OECD 423) of the extracts was administered at 2 and 5 g kg^{-1} b.wt., to male and female rats each with an oral gavage needle[12]. Mortality and general behaviour of the animals were observed for a further period of 14 days for toxic symptoms of piloerection, as well as lachrymatory, locomotor and respiratory activities. Two satellite groups (2 and 5 g kg^{-1} b.wt., dose) were also maintained to determine the delayed onset toxicity of both doses.

Histological observation: Organs histological observations were conducted at the end of the experimental period (day 15). Organs (heart, liver, kidney, stomach, small intestine, spleen) of the Wistar rats were isolated, fixated with 10% formalin and stained with Haematoxylin Eosin (HE) for observation of islet Langerhans morphology. Histology samples were observed under light microscope (Nikon H600L).

Statistical analysis: Statistically significant difference of hypo-glycaemic effect by ANOVA followed with Least Significant Difference (LSD) analysis. Significances were set at $p<0.05$ for all tests.

RESULTS

General sign and behavioural analysis: The results of general sign and behavioural analysis from TGLF acute toxicity treatment indicated the parameters observed in 14 days treatment based on OECD guidelines 423 (Table 1). The present study conducted revealed that TGLF did not cause any mortality in rats throughout the study period of 14 days even at highest administered dose 5 g kg^{-1} b.wt. Administration of TGLF at 2 g kg^{-1} b.wt., resulted in no toxicity symptoms. The writhing reflux was observed at 5 g kg^{-1} b.wt., dose in 1st day treatment. However, administered rats remained alive until end of treatment period.

Table 1: Qualitative acute toxicity symptoms of per-oral administered female Wistar rats in 14 days with TGLF

			Toxicity symptom (days)													
Groups	Treatments	Animal	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	Na CMC 0.5%	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		b	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		c	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I	TGLF 2000 mg kg^{-1} b.wt.	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		b	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		c	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I	TGLF 2000 mg kg^{-1} b.wt. (replication)	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		b	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		c	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II	TGLF 5000 mg kg^{-1} b.wt.	a	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		b	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		c	^	-	-	-	-	-	-	-	-	-	-	-	-	-
II	TGLF 5000 mg kg^{-1} b.wt. (replication)	a	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		b	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		c	+>	-	-	-	-	-	-	-	-	-	-	-	-	-

-: No symptom, +: Shouted loudly, ^: Hiccupped, >: Aggressive, the values of the dose administered to the animals are expressed as mean±SD if 6 samples in each group

Table 2: Histopathological observation of vital organs in per-oral administered female Wistar rats at 14th with single dose of TGLF

			Organ histopathology					
Groups	Treatments	Animal	Heart	Liver	Kidney	Stomach	Small intestine	Spleen
Control	Na CMC 0.5%	a	Normal	Normal	Norma	O	Normal	Normal
		b	Normal	Normal	Norma	n.d.	Normal	Normal
		c	Normal	Normal	Norma	O	Normal	Normal
I	TGLF 2000 mg kg^{-1} b.wt.	a	Normal	Normal	Norma	O	Normal	Normal
		b	Normal	Normal	Norma	n.d.	Normal	Normal
		c	Normal	Normal	Norma	N	I	Normal
I	TGLF 2000 mg kg^{-1} b.wt. (replication)	a	Normal	Normal	Norma	U, K, N	Normal	Normal
		b	Normal	Normal	Norma	O, N	I	Normal
		c	Normal	C	Norma	N, R	Normal	Normal
II	TGLF 5000 mg kg^{-1} b.wt.	a	Normal	Normal	C	N	Normal	Normal
		b	Normal	Normal	Norma	O, N	I	Normal
		c	Normal	HD	Norma	O,N	Normal	Normal
II	TGLF 5000 mg kg^{-1} b.wt. (replication)	a	Normal	Normal	Norma	O, N	Normal	Normal
		b	Normal	Normal	Norma	O, N	G	Normal
		c	Normal	DH	Norma	Normal	Normal	Normal

O: Oedema, Normal: No changes, C: Congestion, HD: Hydropic, I: Inflammatory, N: Necrotic, G: Increase goblet cell, $p<0.05$ is considered significant between groups based on ANOVA followed by Least Significant Difference (LSD) analysis

Organ and body weight statistical analysis: The body weight as well as weight of vital organs of the animals were measured and recorded in Fig. 1. There were no significant changes in body weights. All animal groups exhibited a normal increment in body weight without drastic difference between both control and treated groups. Table 2 showed the effect of TGLF leaf extract on principle organ weights relative to body weight. There were no significant differences in the changes of each weight. However, significantly lower organ weight index of stomach was noticeable in satellite group of high dose (5 g kg^{-1} b.wt.). This implied a potential toxicity response in high dose administration of TGLF leaf extract in stomach organ. Nevertheless, the results revealed that, the essential organs such as kidney, liver, heart, lung, spleen and stomach were not adversely affected throughout the treatment. The absolute and relative organ weight of mice between extract treated and control groups showed no statistical differences ($p<0.05$).

Histo-pathological analysis: Histo-pathological analysis revealed no alterations of the vital organs from control animals (Table 3). Overall, all organs apart from stomach in administered groups also experienced no toxicity effects from 14 days administration of TGLF. Stomach of animal groups treated with both doses (2 and 5 g kg^{-1} b.wt.) demonstrated indication of congestion (C), Oedema (O) and necrotic (N)

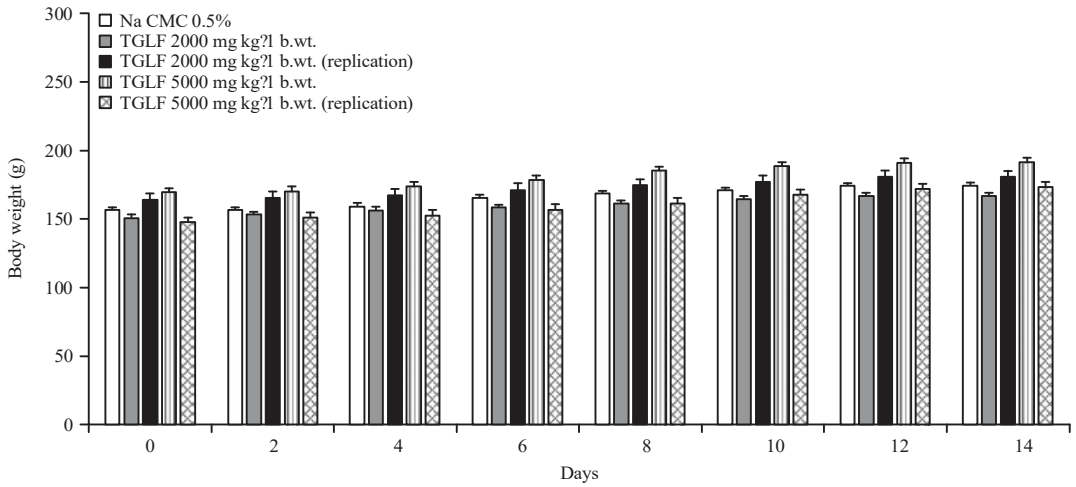

Fig. 1: Body weight patterns of female Wistar rats after per-oral administration of single dose TGLF
p<0.05 is considered significant between groups based on ANOVA followed by Least Significant Difference (LSD) analysis

Table 3: Index organ weight of per-oral administered females Wistar rats with single dose of TGLF

		Index organ weight					
Groups	Treatments	Heart	Lung	Liver	Kidney	Spleen	Stomach
Control	Na CMC 0.5%	0.400±0.04	1.065±0.1	3.860±0.10	0.840±0.03	0.366±0.0	1.010±0.10
I	TGLF 2000 mg kg^{-1} b.wt.	0.437±0.05	1.305±0.34	4.114±0.96	0.850±0.08	0.341±0.0	1.012±0.07
I	TGLF 2000 mg kg^{-1} b.wt. (satellite group)	0.403±0.05	1.105±0.14	4.420±0.46	0.790±0.03	0.320±0.08	0.950±0.05
II	TGLF 5000 mg kg^{-1} b.wt.	1.148±0.34	3.829±0.09	0.736±0.05	0.350±0.05	0.835±0.05	0.835±0.05
II	TGLF 5000 mg kg^{-1} b.wt. (satellite group)	0.409±0.05	1.146±0.28	4.088±1.17	0.887±0.18	0.420±0.07	0.426±0.07*

The values of the dose administered to the animals are expressed as Mean±SD if six samples in each group, *p<0.05

response. In addition, TGLF administration induced slight congestion and inflammatory characteristics in liver and small intestine at both doses.

Histology analysis: The histological analysis of administered organs of control animals presented normal aspect (Fig. 2). However, animal groups administered with 2 g kg^{-1} b.wt., dose of TGLF experienced stomach oedema. Whereas, administration with high dose 5 g kg^{-1} b.wt., induced various toxicity effects such as liver hydropic degeneration, stomach and small intestine inflammation.

DISCUSSION

In general, *in vivo* toxicity study is the toxicological analysis of many natural sources and its potency to evaluate qualitatively and quantitatively by histo-pathology and oral acute toxicity studies[13]. Oral acute toxicity testing in animal models could be used to evaluate natural remedies for different pharmacological activities, taking into account the basic premise that pharmacology is simply toxicology at lower dose[14]. A toxic substance might elicit interesting pharmacological effects at a lower non-toxic dose[15]. Toxicity results from animals will be crucial in definitively judging the safety of natural remedies which are used as food colorants[16].

In this oral acute toxicity study, the Wistar rats animal models were employed to observe the toxicity effects of the ethanol extract of Teak (*Tectona grandis*) leaf. Method of administration depends on the dosage form in which the compound was available. Based on previous studies, the oral route administration (per-oral administration) was the most efficient and commonly used method when evaluating acute toxicity[17]. Since the crude extract was administered orally, the animals should be restricted from food consumption before treatment because food and other chemicals in the digestive tracts may affect the reactions of the compounds[18]. All the procedures were performed based on the appropriate OECD 423 guideline[12].

Overall, results from this study revealed the safety profile of TGLF administration in Wistar rats at doses of 2 and 5 g kg^{-1} b.wt. During the 14 days of period acute toxicity evaluation, rats which were orally administrated

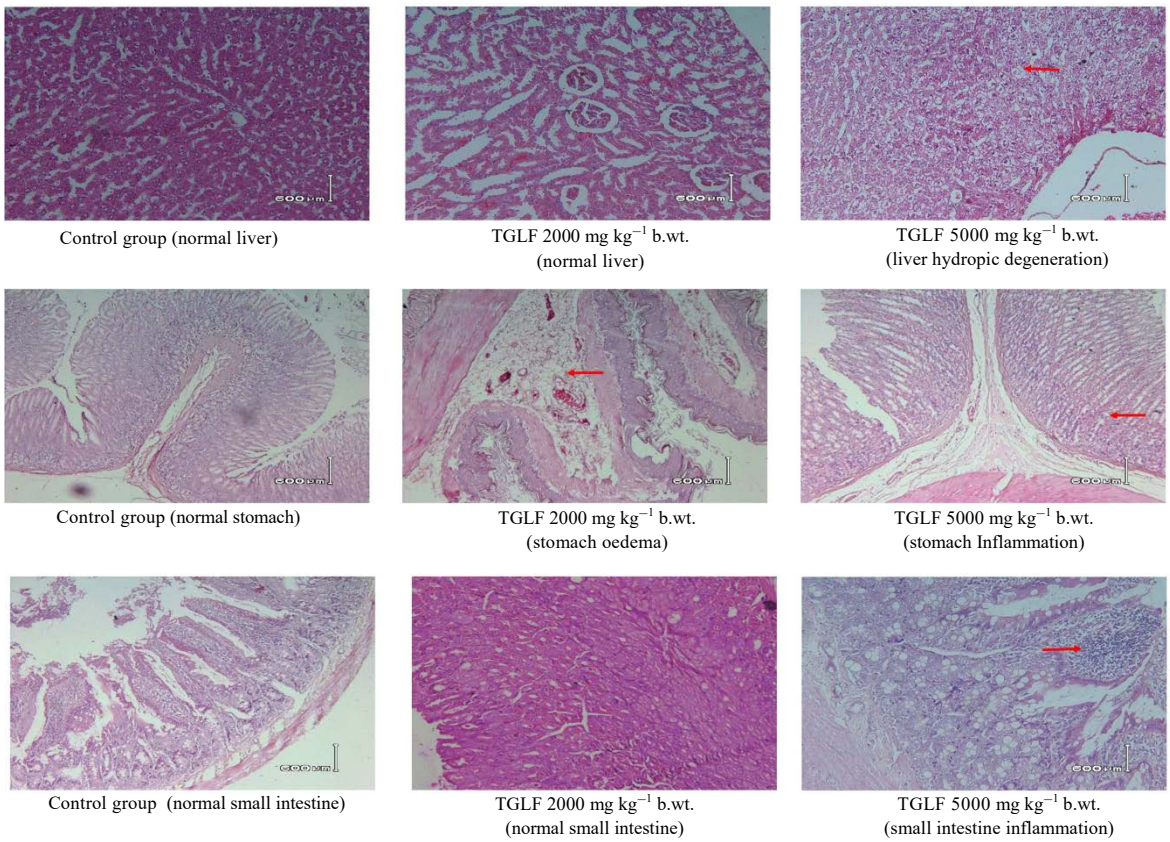

Fig. 2: Histological profiles of stomach, liver and small intestine organs of administered Wistar rats with TGLF for 14 days
Arrows show indication of acute toxicity effects

showed no signs of distress and there were no observable symptoms of either toxicity or deaths. All treated animal models also did not experience any noticeable weight gain during period of administration. This result correlated with study by Kushwah *et al.*[19], which reported that administration of *T. grandis* methanolic extract induced no acute toxicity symptoms and behaviour in rats even up to 14 days. However, apart from physical and behavioural appearance, rats administered with *T. grandis* experienced toxicity effects in vital organs such as hydropic degeneration in liver and inflammation in stomach and small intestine (Fig. 2). Hydropic degeneration is a result of ion and fluid homeostasis that could possibly lead to an increase of intracellular water[20]. The vacuolated swelling of the cytoplasm of the hepatocytes of the treated rats might indicate acute liver injury induced by high dose of TGLFs[21]. Inflammation is a complex biological response of vascular tissues invasion by an infectious agent, antigen challenge, physical, chemical or traumatic damage[22]. Although inflammation is a defence mechanism, the complex events and mediators involved in the inflammatory reactions could lead to organ injury[23].

CONCLUSION

The present results showed that ethanol leave extract of *T. grandis* does not cause any apparent *in vivo* toxicity in an animal model. No death or signs of acute toxicity were observed in Wistar rats treated with TGLF at administered doses (2 and 5 g kg^{-1} b.wt.). This current result established the safety use of TGLF as food colorants. However, histology examination revealed inflammatory effects in vital organs such as stomach and small intestine. This implied high dose of TGLF administration could lead to stomach and intestine injury.

SIGNIFICANCE STATEMENT

Teak (*Tectona grandis*) leaf extract (TGLF) is commonly used in Indonesia as natural food colorants. Despite its immense use as food additives, there remains no information regarding the toxicity profiles of TGLF. The present study evaluates the acute toxicity effects of TGLF in Wistar rats as animal models. Current results revealed that TGLF did not cause mortality or significant physiological changes in the

animals. However, histological profiles show inflammatory responses in vital organ stomach at administered high dose of 5 g kg^{-1} b.wt. This suggests that applied dose of TGLF as food colorants should be considered, as its administration in high dose (5 g kg^{-1} b.wt.) potentially induces stomach and intestine injury.

ACKNOWLEDGMENT

Sincere thanks to Faculty of Veterinary, Gadjah Mada University for assist in histological preparation and analysis.

REFERENCES

1. Bordagaray, A., R. Garcia-Arrona, M. Vidal and M. Ostra, 2018. Determination of food colorants in a wide variety of food matrices by microemulsion electrokinetic capillary chromatography. Considerations on the found concentrations and regulated consumption limits. Food Chem., 262: 129-133.
2. Harp, B.P., E. Miranda-Bermudez and J.N. Barrows, 2013. Determination of seven certified color additives in food products using liquid chromatography. J. Agric. Food Chem., 61: 3726-3736.
3. Sigurdson, G.T., P. Tang and M.M. Giusti, 2017. Natural colorants: Food colorants from natural sources. Annu. Rev. Food Sci. Technol., 8: 261-280.
4. Vyas, P., D.K. Yadav and P. Khandelwal, 2018. *Tectona grandis* (teak)-A review on its phytochemical and therapeutic potential. Nat. Prod. Res., 6: 1-17.
5. Degbe, M., F. Debierre-Grockiego, A. Tete-Benissan, H. Debare, K. Aklikokou, I. Dimier-Poisson and M. Gbeassor, 2018. Extracts of *Tectona grandis* and *Vernonia amygdalina* have anti-Toxoplasma and pro-inflammatory properties *in vitro*. Parasite, Vol. 25. 10.1051/parasite/2018014.
6. Varma, S.B. and D.L. Jaybhaye, 2010. Antihyperglycemic activity of *Tectona grandis* Linn. bark extract on alloxan induced diabetes in rats. Int. J. Ayurveda Res., 1: 163-166.
7. Jaybhaye, D., S. Varma, N. Gagne, V. Bonde, A. Gite and D. Bhosle, 2010. Effect of *Tectona grandis* Linn. seeds on hair growth activity of albino mice. Int. J. Ayurveda Res., 1: 211-215.
8. Khoo, H.E., A. Azlan, S.T. Tang and S.M. Lim, 2017. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients and the potential health benefits. Food Nutr. Res., Vol. 61, No. 1. 10.1080/16546628.2017.1361779.
9. Kong, J.M., L.S. Chia, N.K. Goh, T.F. Chia and R. Brouillard, 2003. Analysis and biological activities of anthocyanins. Photochemistry, 64: 923-933.
10. Bartikova, H., L. Skalova, J. Drsata and I. Bousova, 2013. Interaction of anthocyanins with drug-metabolizing and antioxidant enzymes. Curr. Med. Chem., 20: 4665-4679.
11. Putta, S., N.S. Yarla, E.K. Kumar, D.B. Lakkappa and M.A. Kamal *et al*., 2017. Preventive and Therapeutic potentials of Anthocyanins in diabetes and associated complications. Curr. Med. Chem. 10.2174/0929867325666171206101945.
12. Jonsson, M., M. Jestoi, A.V. Nathanail, U.M. Kokkonen and M. Anttila *et al*., 2013. Application of OECD guideline 423 in assessing the acute oral toxicity of moniliformin. Food Chem. Toxicol., 53: 27-32.
13. Parasuraman, S., 2011. Toxicological screening. J. Pharmacol. Pharmacother., 2: 74-79.
14. Pariyani, R., I.S. Ismail, A.A. Azam, F. Abas, K. Shaari and M.R. Sulaiman, 2015. Phytochemical screening and acute oral toxicity study of Java tea leaf extracts. BioMed Res. Int., Vol. 2015. 10.1155/2015/742420.
15. Jacob, J., A. Amalraj, C. Divya, S. Janadri, P.M. Manjunatha and S. Gopi, 2018. Oral toxicity study of sports nutritional powder in Wistar rats: A 90 day repeated dose study. Toxicol. Rep., 5: 497-503.
16. Parmar, R.S. and C. Singh, 2018. A comprehensive study of eco-friendly natural pigment and its applications. Biochem. Biophys. Rep., 13: 22-26.
17. Walum, E., 1998. Acute oral toxicity. Environ. Health Perspectives, 106: 497-503.
18. Gowda, B.K.C., B.S. Sundharam, J. Mahadesh and Mukund, 2014. Oral-toxicology. J. Forensic Dental Sci., 6: 3-8.
19. Kushwah, A.S., P. Kaur and T.B. Shivanandappa, 2018. Effect of methanolic extracts of *Tectona grandis* Linn leaves on diabetic neuropathy in streptozotocin-induced diabetic rats. MOJ Drug Design Dev. Ther., 2: 203-209.
20. Abdelhalim, M.A.K. and B.M. Jarrar, 2011. Gold nanoparticles induced cloudy swelling to hydropic degeneration, cytoplasmic hyaline vacuolation, polymorphism, binucleation, karyopyknosis, karyolysis, karyorrhexis and necrosis in the liver. Lipids Health Dis., Vol. 10. 10.1186/1476-511X-10-166.
21. Al-Griw, M.A., R.O. Alghazeer, S.A. Al-Azreg and E.M. Bennour, 2016. Cellular and molecular etiology of hepatocyte injury in a murine model of environmentally induced liver abnormality. Open Vet. J., 6: 150-157.
22. Hunter, P., 2012. The inflammation theory of disease: The growing realization that chronic inflammation is crucial in many diseases opens new avenues for treatment. EMBO Rep., 13: 968-970.
23. Hatakeyama, N. and N. Matsuda, 2014. Alert cell strategy: Mechanisms of inflammatory response and organ protection. Curr. Pharm. Design, 20: 5766-5778.