Malaysian Applied Biology (2023) 52(2): 61-69
https://doi.org/10.55230/mabjournal.v52i2.2646

Research Article

In Vivo Toxicity Study on The Effects of Aqueous Propolis Extract From Malaysian Stingless Bee (*Geniotrigona thoracica*) in Mice

Nurul Alina Muhamad Suhaini[1], Mohd Faeiz Pauzi[1]*, Siti Norazlina Juhari[1], Khamsah Suryati Mohd[2] and Noor Azlina Abu Bakar[1]

1. Faculty of Medicine, Medical Campus, Universiti Sultan Zainal Abidin, 20400, Kuala Terengganu, Terengganu
2. Faculty of Bioresources and Food Industry, Besut Campus, Universiti Sultan Zainal Abidin, 22200 Besut, Terengganu
Corresponding author: faeizpauzi@unisza.edu.my

ABSTRACT

Geniotrigona thoracica is a stingless bee species of *Trigona* genus. Propolis resinous is a natural product obtained from a honeybees hive with geographical and floral specifications or exudate as such by-products resulting from a variety of botanical processes. Despite its long use for a variety of health conditions, the toxicity profile of propolis sourced from Malaysian stingless bees has not been sufficiently evaluated. For *in vivo* toxicity assessment, the acute oral toxicity on the effects of aqueous propolis extracts (APE) was examined. Male mice swiss strain, were subjected to acute toxicity testing for 14 days. The APE at doses of 400, 1000 and 2000 mg/kg body weight was supplemented daily to the mice through oral gavage. The clinical signs of toxicity and general behaviour, body weight, relative organ weight, and histopathology changes were investigated. *In vivo* study was focused on the acute toxicity testing group consisting of 4 groups including Normal (NS), 400 mg/kg (APE 400), 1000 mg/kg (APE 1000) dan 2000 mg/kg (APE 2000). Regarding the toxicity profile, it is proposed that APE supplementation did not induce any mortality and no visible signs of toxicity. No significant changes in the body and relative organ weight were recorded. All the internal organs of the mice were macroscopically healthy with no gross lesion. Likewise, histopathological examinations of the kidney showed mild to moderate histological lesions. Interestingly, the lesion was adverse with an increased dosage of the extract supplementation. This study proposed APE has considerable anti-inflammatory activities. It also demonstrated that the propolis extract is relatively safe to be consumed orally at a dose of 2000 mg/kg body weight.

Key words: *Geniotrigona thoracica*, histopathology, Malaysian stingless bee, polyphenols, propolis, toxicity study

Article History
Accepted: 27 May 2023
First version online: 30 June 2023

Cite This Article:
Muhamad Suhaini, N.A., Pauzi, M.F., Juhari, S.N., Mohd, K.S. & Abu Bakar, N.A. 2023. *In vivo* toxicity study on the effects of aqueous propolis extract from Malaysian stingless bee *Geniotrigona thoracica* in mice. Malaysian Applied Biology, 52(2): 61-69. https://doi.org/10.55230/mabjournal.v52i2.2646

INTRODUCTION

Bees are natural pollinators for many wild and cultivated tropical plants. It plays a significant role in creating a balanced ecosystem and ensuring food security (Slaa *et al*., 2006; Ismail, 2016). More than 500 species of stingless bees thrive in the tropical and subtropical regions of the world (Ruttner, 1988; Heard, 1999). The most common genera of these stingless bees were *Trigona* and *Melipona*, with *Trigona* being the largest and most widely distributed genus. Approximately 17-32 known species of stingless bees have been discovered in Malaysia, depending on the study area. Most of them belonged to the genera *Trigona*, as it can easily be found and widely used in meliponiculture (Kelly *et al*., 2014).

Propolis is a complex resinous substance produced by stingless bees to line the inner layer of nest cavities, thus narrowing the size of hive entrances. It contains over 300 identified biochemical constituents, mostly polyphenols, flavonoid glycosides, flavonoid aglycones, phenolics, and ketones (Bankova *et al*., 2014). The proportion of phenolic acids is higher in all these compositions than that of flavonoids (Araújo *et al*., 2011).

Malaysian propolis has a specific chemical composition, and new compounds have never been reported in other propolis varieties, including 20-Hydroxy-24-dammaren-3-one and β-panasinsene from sesquiterpene esters (Hossain *et al*., 2022). The health-promoting capabilities of propolis depend mainly on the constituent of phenolic compounds and their composition. In the last decade, studies have investigated the biological activities of propolis, a variety found in Argentina, Iranian, Morocco, Indonesia, Chinese, and Malaysia (Isla *et al*., 2001; Mohammadzadeh *et al*., 2007; Mountassir *et al*., 2014; Sabir & Sumidarti, 2017; Sun *et al*., 2018; Nna *et al*., 2018, 2021; Fikri *et al*., 2019; Brodkiewicz *et al*., 2020). Studies have attributed some

pharmacological properties to the complex phenolic compounds in propolis. The Malaysian stingless bee propolis extract has been shown to have antioxidant activity (Ahmed *et al*., 2017; Asem *et al*., 2020; Yeoh *et al*., 2021), anti-hyperglycaemic (Usman *et al*., 2016; Yeoh *et al*., 2021), anti-inflammatory (Nna *et al*., 2021), cardioprotective properties (Ahmed *et al*., 2017), and healing properties (Jacob *et al*., 2015). Nevertheless, its safety has not been appropriately evaluated, and the variability in reported toxicity would be expected.

Toxicity testing mainly generates data on the adverse effects of drug or compound doses or examines specific endpoints on animals, such as eye irritation, mutagenicity, genotoxicity, inhalation toxicity, and neurotoxicity (Parasuraman, 2011). In vivo, toxicity testing is classified into acute, sub-acute, or chronic. Since propolis is used as a natural nutraceutical for various human health conditions, its biosafety must be ascertained through acute toxicity testing. Recently, an acute toxicity test performed by Brodkiewicz *et al*. (2020) reported that oral administration of ethanol extracts of propolis at 2000 mg/kg and 5000 mg/kg doses did not cause any adverse effects and consider a safe amount for a short period of 48 hr and a prolonged period of 14 days.

However, current interest and high demand in farming and breeding stingless bee colonies among rural residents, selection of species, subspecies, and varieties of bees have a significant impact on propolis's chemical components and quality. The biological activities of each type of propolis might also be different in matters of pharmacological and toxicological action (Huang *et al*., 2014). Since its safety has been evaluated involving the propolis of stingless bees from different countries, there is not sufficient report about the water extract of Malaysian propolis. In the present study, we assessed the toxic effects of orally administering an aqueous propolis extract (APE) using international protocols as described in OECD Guideline 420 to study acute toxicity testing (OECD, 2001).

MATERIALS AND METHODS

Propolis collection and preparation

The fresh propolis produced by *Geniotrigona thoracica* were collected in November 2021 from Kuala Besut, Terengganu Darul Iman, Malaysia (5º45'16" N). The authentication of propolis from *Geniotrigona thoracica* stingless bee species was obtained from Forest Research and Institute Malaysia (FRIM), Kepong, Malaysia with the reference number ENTO/2022/03. The propolis was stored at -20°C after being washed with tap water to remove dust and foreign particles. The extraction procedure was applied according to previous studies by Salim *et al*. (2018) and Asem *et al*. (2020) with slight modifications. The frozen propolis was ground in a commercial blender (Waring, USA) into powder. Then, the freeze powder form (40g) was dissolved in distilled water (Millipore, France) for 24 hours. The crude extract solution was transferred into a clean volumetric flask. The residue was re-extracted with distilled water for a total of three times. The final total extract was then filtered through No. 41 filter paper (Whatmann®) before lyophilization using a freeze dryer (Christ, GmbH, Germany). The dried brown APE was kept cool at -4 °C. For in vivo acute toxicity assay, the APE was dissolved in 1% dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Germany) and resuspended in distilled water.

Animals

Male *Swiss* strain mice (age, 4-8 weeks; 25-30 g) were randomly selected and used in this study. Three mice were housed per polycarbonate cage for each treatment group in a temperature-controlled room (22 $\pm$ 2 °C) and were maintained under standard environmental conditions (12 h light/dark cycle) with relative humidity does not exceed than 70%. The mice were provided with free access to a normal diet and water. The animals were acclimatized to the housing conditions for 7 days before the toxicological experiments begin. All procedures were evaluated and approved by the Animal Plant Research Ethics Committee of Universiti Sultan Zainal Abidin (UAPREC) with permit number UAPREC/07/005.

In vivo toxicity assay

The experiment was conducted according to the protocols in OECD Guideline 420 (OECD, 2001). The APE extract was dissolved in 1% (v/v) DMSO in an aqueous solution and administered orally at doses of 400 (APE 400), 1000 (APE 1000) and 2000 mg/kg of body weight (BW) (APE 2000) (volume of 10 mL/kg BW), whereas the control group received only the normal saline in the same volume.

The mice were administered orally at a single dose of 400, 1000, and 2000 mg/kg BW at a volume of 0.1 mL/10g of body weight and were observed for 14 days. The initial dose level was selected based on the previous study by Araujo *et al*. (2011) as the dose expected to produce some signs of toxicity without causing severe toxic effects or mortality. The toxicity testing was carried out by the OECD Guidelines for the testing of chemicals (Table of Contents of Part 4: Health Effects; TG 420). All the parameters were utilized as a guideline by the researcher to differentiate any abnormalities seen in all mice during the experimental period. The reporting of clinical signs in laboratory mice are reported by Fentener van Vlissingen *et al*. (2015). Behavioural and physical conditions of the mice were observed continuously during the first 24 hr and daily until day 14 after dosing. Body weight was recorded at baseline Day 0. During the study, the body weight of the mice was measured weekly on Day 1, Day 7, and Day 14. At the end of the experiment, i.e., day 14, all animals were euthanized with pentobarbital (100 mg/mL) (Ceva Sante Animale, France) and their organs were removed and examined macroscopically. Relative organ weights (ROW) for each organ were calculated as below:

$$\text{ROW (\%)} = \frac{\text{Organ weight}}{\text{Body weight}} \text{x } 100 \text{ (\%)}$$

Kidneys were fixed in formalin (R&M Marketing, United Kingdom), dehydrated in ethanol, cleared in toluene, embedded in paraffin wax, and sectioned into 5-µm thick sections. These tissue sections were stained with haematoxylin and eosin (H&E) for histological examination. The microscopic analysis of all kidney tissue samples was carried out as a blind study by a veterinary histopathologist. These included changes in the blood vessels and circulation, changes in tissue parenchyma of the kidneys, and the presence of inflammatory reactions. The identified changes or lesions were initially described before the severity of the lesions was evaluated and scored. Scoring of microscopic lesions was carried out based on the description in Table 1.

Table 1. Scoring of the severity of microscopic lesions

Severity	Criteria	Score
No lesion	Absence of the lesion that is being described	0
Mild lesion	Presence of the lesion involving between 1% and 20% of the organ	1
Moderate lesion	Presence of the lesion involving between 21% and 60% of the organ	2
Severe lesion	Presence of the lesion involving more than 60% of the organ	3

Statistical analysis

All data are expressed as mean + standard error of the mean (S.E.M) as descriptive statistics. One-way analysis of variance (ANOVA) was used to determine differences between groups. For the mice's body weight, the weight of target organs and severity of lesion scores, mean differences between the treated and control mice groups were analysed by one-way ANOVA. Multiple comparisons test was conducted for mean group comparisons when there was a significant overall difference between the groups. p-values less than 0.05 and 0.001 ($p<0.05$ & $p<0.001$) were considered significant. All statistical analysis was performed using GraphPad Prism version 9.0 for Windows (GraphPad Software, Inc., USA).

RESULTS AND DISCUSSION

Effects of APE on clinical signs and conditions

In the present study, APE oral administration from doses of 400 to 2000 mg/kg of body weight showed the absence of abnormalities as reflected by the no visible changes in the parameters studied as well as the physical and general behaviours of the treated mice (Table 2). Both control and treated male mice showed no visible signs of toxicity throughout 14 days of APE supplementation. Both control and treated male mice showed no visible signs of toxicity throughout 14 days of APE supplementation.

The administration of APE to male mice from a lower dose group that received 400 mg/kg to the higher dose group that received 2000 mg/kg body weight did not cause any deaths for the 14 days of the experiment. All 12 mice survived during or after the experiment. In addition, although reports cited that propolis can cause allergen (Burdock, 1998) and dermatitis but Brodkiewicz *et al*. (2020) remained proven in toxicity studies as a non-toxic natural product with a dosage of over 5000 mg/kg. These differences may somewhat be due to an altered chemical composition of propolis, and probably to season or plant source, thus changing its toxicity profile (Huang *et al*., 2014).

Table 2. Parameter of acute toxicity in male mice for control and treated APE with 400, 1000 and 2000 mg/kg

Treatment		Normal saline	APE		
	Dose (mg/kg)	-	400	1000	2000
Physical and behavioural changes	General appearance (s)	Normal	Normal	Normal	Normal
	Mucous membrane	Normal	Normal	Normal	Normal
	Respiration	Normal	Normal	Normal	Normal
	Eyes	Normal	Normal	Normal	Normal
	Muscles and movements	Normal	Normal	Normal	Normal
	Body incontinence	Normal	Normal	Normal	Normal
	Body condition (BS) score	Normal	Normal	Normal	Normal
	Mortality	Normal	0/3	0/3	0/3

Effects of APE on weekly body weight

In the present study, no significant differences ($p>0.05$) in body weight gain between treated groups at the doses of 400, 1000 and 2000 mg/kg body weight as compared to their control group in male mice throughout the 14 days of treatment period in Figure 1. However, a marked reduction in body weight was observed following the dosing in the treated groups during the 7 days of observation. This observation may be due to the high energy content of the extract which is not determined. Moreover, these might probably be due to the fast metabolic response in the mice that suggests longer duration exposure of treatment to accomplish a better safety associated with the use of medicinal products.

Similarly, oral administration of propolis extracts in mice does not influence body weight as reflected by the insignificant body weight gain (Brodkiewicz *et al*. 2020). The finding is in contrast with Khacha-ananda *et al*. (2018) that found the body weights of mice had increased normally and no abnormalities were found in the histopathology of the liver and the kidney findings. The finding of changes was possibly due to the shorter period of treatment of 14 days, compared to 60 days in the former study.

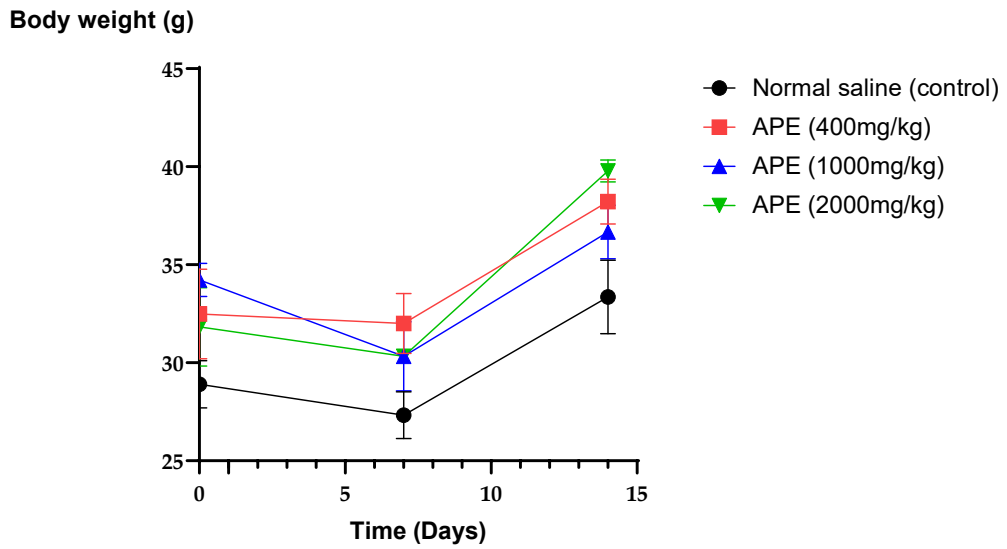

Fig. 1. Body weight of male mice (g) mean ± S.E.M as a function of time after orally administered with a single dose of APE (400, 1000 & 2000 mg/kg BW) in 14 days of treatment.

Body weight reduction is used as a simple and sensitive indicator of any adversely affected by the significant change in their relative weight (Pingale *et al*., 2021). The sign of progressive body weight loss is an integral part of the conventional safety evaluation of a test material (Ferreira *et al*., 2014). There should be a little variation between animals and only allowed for body weight decrement which is not more than 10% from the initial body weight and internal organ weights (Pingale *et al*., 2021). It is known that the oral administration of propolis extract form in the range of 1000 - 4000 mg/kg does not induce clinical symptoms of toxicity in mice. Similar results were obtained in another acute toxicity study from Brazil and Argentine stingless bee geopropolis (Liberio *et al*., 2011; Brodkiewicz *et al*., 2020).

Effects of APE on relative organ weight

Assessing the clinical sign of abnormalities together with behavioural changes in mice are considered inadequate since treatment-related effects can cause slight changes in their body-to-organ weight ratio. The relative organ weight (ROW) ratio was used as a parameter to detect the effect of administering certain chemicals by weighing the organs with body weight after 14 days as reported by (Farida *et al*., 2022). Oral administration of the APE showed statistically insignificant differences observed in the relative organ weight to body weight ratios compared to the control group (Figure 2).

It is generally known that toxic materials that enter an animal's body would be detoxified by the liver and subsequently excreted through the kidneys. The liver and kidney act as vital organs for detoxification and were useful in toxicity studies which correlate well with histopathological changes. Serum albumin and bilirubin are also useful indicators in accessing liver-cell damage and acute hepatocellular injury respectively. ROW parameters often to be associated with the histopathological examination of the internal organ and liver function test. The present study revealed the ROW in all APE-treated groups for 14 days did not show any significant difference (p>0.05) from the control groups. However, toxic substances(s) may not reach the toxic level due to the destruction in the absorption, distribution, metabolism, and elimination of drugs (Fikri *et al*., 2021).

Effects of APE on macroscopic examinations in the kidneys

The macroscopic examinations of the kidney organs of animals treated with various doses of APE did not show any colour changes and there were no remarkable differences in the kidney weight of the APE extract-treated groups and control group as shown in Figure 3. If APE will cause certain damage to the organ function, of course, it will either enlarge (swelling) or shrunk the size of the organ as can be calculated from the organ-to-body weight ratio. The enlargement of organs is correlated to the body weight increase (Dekanski *et al*., 2018). Thus, strengthened by the fact of the relative weight of the kidneys, APE did not show any evidence of toxicity. The observed effect is in agreement with the literature data by Liberio *et al*. (2011). Furthermore, since there are no macroscopic changes after APE supplementation, which confirms that propolis does not present any toxic effects on the mice.

Effects of APE on microscopic examinations in the kidneys

All treated groups developed mild to moderate microscopic lesions (Table 2). The severity of these lesions appeared to be insignificant differences (p>0.05) in histopathological changes in the APE-treated groups at the doses of 400 and 1000 mg/kg body weight (Table 3). However, vascular congestion, and glomerular and tubule lesions in the kidney were observed in the higher dose group. These results demonstrated that oral administration of APE at (2000 mg/kg) may alter renal function as observed in Figure 4.

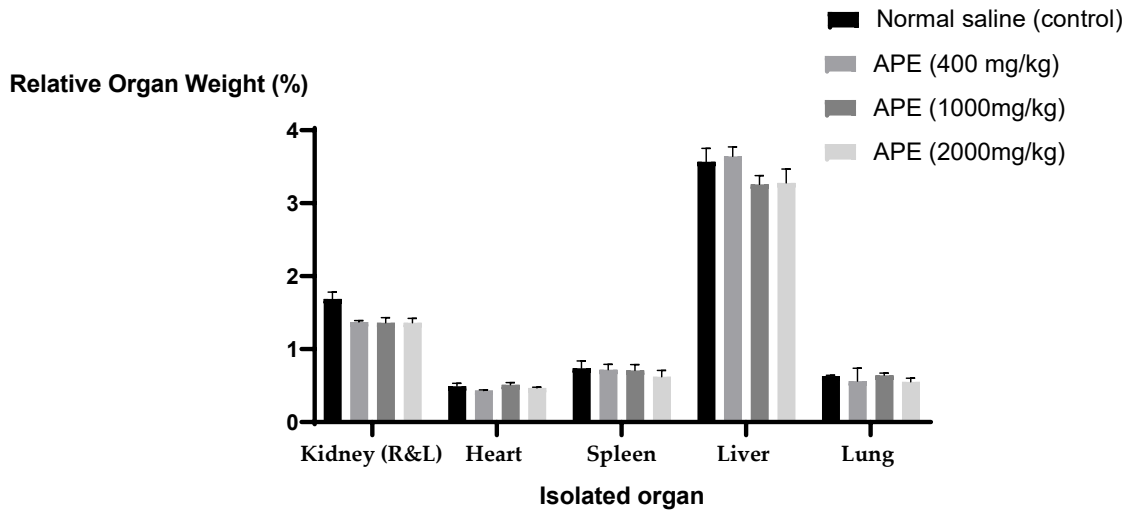

Fig. 2. Effects of APE on the relative organ weight ratio (%) in male mice after oral administration of (400, 1000 & 2000 mg/kg) in 14 days of treatment.

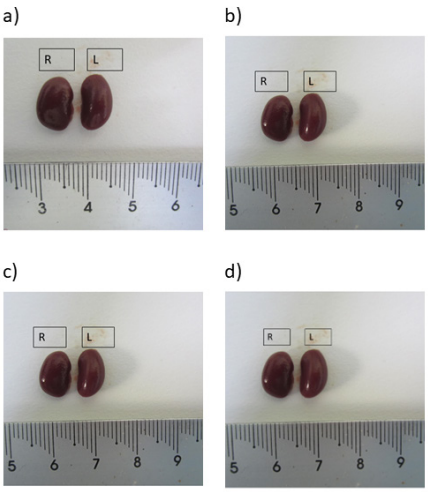

Fig. 3. Macroscopic appearance of the target kidney organ in mice. **(a)**, **(b)**, and **(c)** kidneys from male mice were administered with APE (400, 1000 & 2000 mg/kg) respectively. **(d)** kidney from male mice administered with normal saline (0.1 mL/kg) as a control group. R=right and L=left. **(a)**, **(b)**, **(c)** and **(d)** x5 magnification.

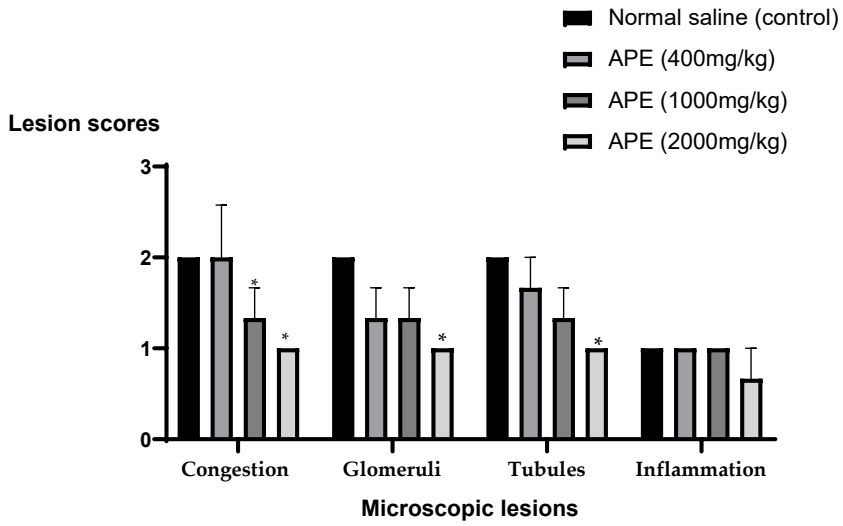

Fig. 4. Lesion scores indicate the severity of microscopic lesions in the kidney. The values are presented as mean ± S.E.M (*n*=3). 1-way ANOVA was applied to compare each of the treated groups with the control group. *p<0.05 represents a significant difference when the control and treated groups (1000 & 2000 mg/kg) were compared.

Histopathological examination of the renal structure of control mice manifested a normal appearance of glomeruli, basement membrane and renal tubules (Figure 5(a)). The kidney of male mice administered with 400, 1000 and 2000 mg/kg show changes as displayed in Figure 5(b), (c) and (d). All treated groups developed mild to moderate microscopic lesions (Table 2). These include mild congestion of blood vessels, involving few vessels of the cortex areas. The epithelial cells of the convoluted tubules were relatively normal with few tubules containing swollen epithelium that blocked the lumen. These changes were seen in both 1000 and 2000 mg/kg groups within the established acute toxicity assay. The significance of this change is uncertain as the animals did not show any sickness or behavioural changes. Fortunately, the changes are mild to moderate and do not give rise to any significant changes in mice's general behaviours, or gross or histopathological examination of internal organs. This view is also strengthened by the fact that the relative weight of the kidneys did not show any evidence of toxicity. However, kidney histology reveals a characteristic progression of treatment-related effects ranging from vascular congestion, swollen tubular epithelium, and atrophied glomeruli. Hence, this treatment-related in the kidneys was responsible for the observed alterations.

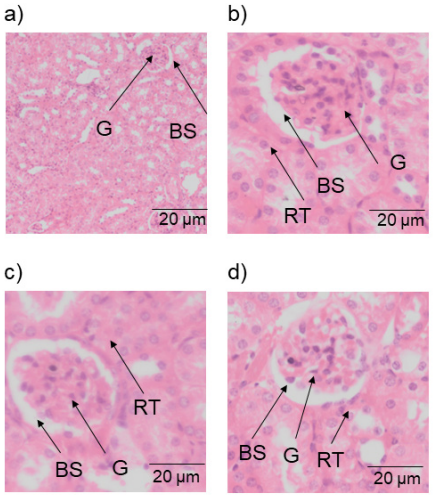

Fig. 5. The microscope histological examination of kidney tissues in control and treated groups (400, 1000 & 2000 mg/kg) administered with APE. **(a)** Male mice of the control group (normal saline (0.1 mL/kg)) while **(b)**, **(c)** and **(d)** male mice of the treated groups that received (400, 1000 & 2000 mg/kg body weight) of APE, respectively. Representative sections from **(a)** the kidney showing atrophy glomeruli, (**b)** the kidney showing atrophy glomeruli, epithelial cells of the convoluted tubules appeared swollen, and a small focus of inflammation within the cortex, **(c)** the kidney showing moderate thrombosis and vascular congestion, and atrophy glomeruli, **(d)** the kidney showing tubular dilation, mild vascular congestion and glomeruli appeared atrophied. BS: Bowman's space; RT: renal tubules; G: glomeruli. Haematoxylin & Eosin staining. **(a)** x10 magnification, **(b)**, **(c)** and **(d)** x40 magnification.

However, it must be noted that animal euthanasia using drug overdoses such as barbiturate or similar drugs would result in similar microscopic lesions in the kidneys. This observation agrees with the report by Mohamed *et al*. (2020). These authors reported that animal euthanasia by an overdose drug including barbiturate or sodium pentobarbitone would result in similar microscopic lesions in the kidneys. It was demonstrated by Mohamed *et al*. (2020) that the use of sodium pentobarbital at a minimum dosage (50 mg/kg) showed normal glomeruli and renal tubules in both female and male rats. However, the renal structure treated with 150 mg/kg sodium pentobarbital manifested congested glomerular tuft capillaries and vacuolated renal tubular epithelium was found in kidney organs. In addition, another study demonstrated that the use of 50 mg/kg sodium pentobarbital did not cause any significant difference in the magnitude of blood pressure and heart rate (HR) reductions by plant extracts in both anaesthetized and conscious Winstar rats which were well below the doses reported by Mohamed *et al*. (2020). For the stated reasons, sodium pentobarbital was used in this study at a dose of 100 mg/kg body weight.

Toxicity of certain compound(s) or treatment may indicate changes in the blood vessels in the form of congestion or thrombosis or the form of degenerative change and/or necrosis of cells. Our study revealed that mice developed mild to moderate changes in the blood vessels of the cortex, atrophy of the glomeruli and swollen tubular epithelium. There is documentation reported on the effect of propolis extract on the histopathological analysis of the liver and kidney. Moreover, no specific changes in histopathological examinations probably due to the hepatoprotective effect of propolis were found as previously demonstrated in the in vivo study by Fikri *et al*. (2021) and Khacha-ananda *et al*. (2018). They used a similar histopathological parameter profile in the mice model.

Table 3. Histopathology examination of the kidneys given APE for 14 days

Treatment	Dose (mg/kg)	Description
Normal saline	-	Significant vascular congestion with a thrombus of the cortex; two foci of inflammation in the pelvis area which consisted of macrophages; epithelial cells of the convoluted tubules appeared swollen
APE	400	Moderate to severe vascular congestion with thrombus; only a few glomeruli appeared atrophied; epithelial cells of the convoluted tubules appeared swollen; a small focus of inflammation within the cortex
	1000	Moderate thrombosis and vascular congestion; atrophy glomeruli; two foci of inflammation in the pelvis
	2000	Mild vascular congestion and thrombosis of the cortex; glomeruli appeared atrophied; many epithelial cells of the convoluted tubules appeared swollen with the absence of inflammation in the renal pelvis.

CONCLUSION

In conclusion, the present work has demonstrated important information on the toxicity of aqueous propolis extract in the acute oral toxicity assay that should be very useful for subsequent clinical investigation. The fact that our results signify the safety profile of propolis samples from Malaysian stingless bees based on physical, and behavioural conditions, and gross pathology can be devoid of any toxic effects. Besides, the pathological examination showed abnormalities in mice at a dose of 2000 mg/kg body weight. It could be suggested that the aqueous propolis extract is non-toxic at low doses and has a low toxicity potential in acute oral supplementation. However, the administration of APE at high oral doses evaluated induced toxicity to the renal by histology analysis. Therefore, this is an important confirmation of the suitability of the extract for therapeutic uses.

ACKNOWLEDGEMENTS

The authors thank the Faculty of Medicine, Universiti Sultan Zainal Abidin (UniSZA) for providing the facilities. Financial support was granted by the Ministry of Higher Education (MOHE), Malaysia for the Fundamental Research Grant Scheme (FRGS/1/2019/WAB11/UNISZA/02/1).

ETHICAL STATEMENT

This study was approved by the Animal and Plant Research Ethics Committee from Universiti Sultan Zainal Abidin (UAPREC) with approval number UAPREC/07/005.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Ahmed, R., Tanvir, E.M., Hossen, M.S., Afroz, R., Ahmmed, I., Rumpa, N.-E.-N., Paul, S., Gan, S.H., Sulaiman, S.A. & Khalil, M.I. 2017. Antioxidant properties and cardioprotective mechanism of Malaysian propolis in rats. Evidence-Based Complementary and Alternative Medicine, 2017: 5370545. https://doi.org/10.1155/2017/5370545

Araújo, M.J.A.M., Mattar, N.S., Reis, A.S., Serra, I.C.P.B., Fialho, E.M.S., Assunção, A.K.M., Dutra, R.P., Nogueira, A.M.C., Libério, S.A., Guerra, R.N.M., Lopes, A.S., Ribeiro, M.N.S. & Nascimento, F.R.F. 2011. Pharmacognostic and acute toxicological evaluation of *Scaptotrigona* aff. *postica* propolis extract in pre-clinical assays. Natural Product Research, 25(11): 1037–1046. https://doi.org/10.1080/14786419.2010.482059

Asem, N., Abdul Gapar, N.A., Abd Hapit, N.H. & Omar, E.A. 2020. Correlation between total phenolic and flavonoid contents with antioxidant activity of Malaysian stingless bee propolis extract. Journal of Apicultural Research, 59(4): 437–442. https://doi.org/10.1080/00218839.2019.1684050

Bankova, V., Popova, M. & Trusheva, B. 2014. Propolis volatile compounds: Chemical diversity and biological activity: A review. Chemistry Central Journal, 8: 28. https://doi.org/10.1186/1752-153X-8-28
Brodkiewicz, I.Y., Reynoso, M.A. & Vera, N.R. 2020. In vivo evaluation of pharmacological properties of Argentine stingless bee geopropolis. Beni-Suef University Journal of Basic and Applied Sciences, 9: 32. https://doi.org/10.1186/s43088-020-00058-8
Burdock, G.A. 1998. Review of the Biological Properties and Toxicity of Bee Propolis (Propolis). Food and Chemical Toxicology, 36: 347–363. https://doi.10.1016/s0278-6915(97)00145-2
Dekanski, D., Spremo-Potparević, B., Bajić, V., Živković, L., Topalović, D., Sredojević, D. N., Lazić, V. & Nedeljković, J.M. 2018. Acute toxicity study in mice of orally administrated TiO_2 nanoparticles functionalized with caffeic acid. Food and Chemical Toxicology, 115: 42–48. https://doi.org/10.1016/j.fct.2018.02.064
Farida, S., Pratami, D.K., Sahlan, M., Laksmitawati, D.R., Rohmatin, E. & Situmorang, H. 2022. In-vitro antioxidant, in-vivo anti-inflammatory, and acute toxicity study of Indonesian propolis capsule from *Tetragonula sapiens*. Saudi Journal of Biological Sciences, 29(4): 2489–2500. https://doi.org/10.1016/j.sjbs.2021.12.034
Fentener van Vlissingen, J.M., Borrens, M., Girod, A., Lelovas, P., Morrison, F. & Torres, Y.S. 2015. The reporting of clinical signs in laboratory animals: FELASA Working Group Report. Laboratory Animals, 49(4): 267–283. https://doi.org/10.1177/0023677215584249
Ferreira, S.A., Guimarães, A.G., Ferrari, F.C., Carneiro, C.M., de Paiva, N.C.N. & Guimarães, D.A.S. 2014. Assessment of acute toxicity of the ethanolic extract of *Lychnophora pinaster* (Brazilian arnica). Revista Brasileira de Farmacognosia, 24(5): 553–560. https://doi.org/10.1016/j.bjp.2014.09.005
Fikri, A.M., Sulaeman, A., Marliyati, S.A. & Fahrudin, M. 2019. Antioxidant activity and total phenolic content of stingless bee propolis from Indonesia. Journal of Apicultural Science, 63(1): 139–147. https://doi.org/10.2478/jas-2019-0012
Fikri, A.M., Sulaeman, A., Marliyati, S.A., Fahrudin, M. & Handharyani, E. 2021. Effect of Propolis on Maternal Toxicity. Pharmaceutical Sciences Asia, 48(3): 224–230. https://doi.org/10.29090/psa.2021.03.20.056
Heard, T.A. 1999. The role of stingless bees in crop pollination. Annual Review of Entomology, 44: 183–206. https://doi.org/10.1146/annurev.ento.44.1.183
Hossain, R., Quispe, C., Khan, R.A., Saikat, A.S.M., Ray, P., Ongalbek, D., Yeskaliyeva, B., Jain, D., Smeriglio, A., Trombetta, D., Kiani, R., Kobarfard, F., Mojgani, N., Saffarian, P., Ayatollahi, S.A., Sarkar, C., Islam, M.T., Keriman, D., Uçar, A., Martorell, M., Sureda, A., Pintus, G., Butnariu, M., Sharifi-Rad, J. & Cho, W.C. 2022. Propolis: An update on its chemistry and pharmacological applications. Chinese Medicine, 17: 100. Chinese Medicine. https://doi.org/10.1186/s13020-022-00651-2
Huang, S., Zhang, C.P., Wang, K., Li, G.Q. & Hu, F.L. 2014. Recent advances in the chemical composition of propolis. Molecules, 19(12): 19610–19632. https://doi.org/10.3390/molecules191219610
Isla, M.I., Nieva Moreno, M.I., Sampietro, A.R., & Vattuone, M.A. 2001. Antioxidant activity of Argentine propolis extracts. Journal of Ethnopharmacology, 76(2): 165–170. https://doi.org/10.1016/S0378-8741(01)00231-8
Ismail, W.I.W 2016. A review on beekeeping in Malaysia: History, importance and future directions. Journal of Sustainability Science and Management, 11(2): 70–80.
Jacob, A., Parolia, A., Pau, A. & Davamani Amalraj, F. 2015. The effects of Malaysian propolis and Brazilian red propolis on connective tissue fibroblasts in the wound healing process. BMC Complementary and Alternative Medicine, 15(294): 1–11. https://doi.org/10.1186/s12906-015-0814-1
Kelly, N., Farisya, M.S.N., Kumara, T.K. & Marcela, P. 2014. Species diversity and external nest characteristics of stingless bees in meliponiculture. Pertanika Journal of Tropical Agricultural Science, 37(3): 293–298.
Lahlou, S., Figueireido, A.F., Magalhaes, P.J.C. & Leal-Cardoso, J.H. 2002b. Cardiovascular effects of 1,8-cineolle, a terpenoid oxide present in many essential oils in normotensive rats. Canadian Journal of Physiology and Pharmacology, 80: 1125-1131. https://doi.org/10.1139/y02-142
Liberio, S.A., Pereira, A.L.A., Dutra, R.P., Reis, A.S., Araújo, M.J.A.M., Mattar, N.S., Silva, L.A., Ribeiro, M.N.S., Nascimento, F.R.F., Guerra, R.N.M. & Monteiro-Neto, V. 2011. Antimicrobial activity against oral pathogens and immunomodulatory effects and toxicity of geopropolis produced by the stingless bee *Melipona fasciculata* Smith. BMC Complementary and Alternative Medicine, 11(108): 1–10. https://doi.org/10.1186/1472-6882-11-108
Mohamed, A.S., Hosney, M., Bassiony, H., Hassanein, S.S., Soliman, A.M., Fahmy, S.R. & Gaafar, K. 2020. Sodium pentobarbital dosages for exsanguination affect biochemical, molecular and histological measurements in rats. Scientific Reports, 10(1): 378. https://doi.org/10.1038/s41598-019-57252-7
Mohammadzadeh, S., Sharriatpanahi, M., Hamedi, M., Amanzadeh, Y., Sadat Ebrahimi, S.E. & Ostad, S.N. 2007. Antioxidant power of Iranian propolis extract. Food Chemistry, 103: 729–733. https://doi.org/10.1016/j.foodchem.2006.09.014
Mountassir, M., Chaib, S., Selami, Y., Khalki, H., Ouachrif, A., Moubtakir, S., Aboufatima, R., Zyad, A., Benharref, A. & Chait, A. 2014. Antinociceptive activity and acute toxicity of moroccan black propolis. International Journal of Engineering Research & Technology, 3(2): 2393–2397.
Nna, V.U., Abu Bakar, A.B., Md Lazin, M.R.M.L. & Mohamed, M. 2018. Antioxidant, anti-inflammatory and synergistic anti-hyperglycemic effects of Malaysian propolis and metformin in streptozotocin–induced diabetic rats. Food and Chemical Toxicology, 120: 305–320. https://doi.org/10.1016/j.fct.2018.07.028
Nna, V.U., Abu Bakar, A.B., Zakaria, Z., Othman, Z.A., Jalil, N.A.C. & Mohamed, M. 2021. Malaysian propolis and metformin synergistically mitigate kidney oxidative stress and inflammation in streptozotocin-induced diabetic rats. Molecules, 26(11): 1–15. https://doi.org/10.3390/molecules26113441
OECD Guideline for Testing of Chemicals. 2001. Section 4, Health Effects, Test No. 420 Acute Oral Toxicity- Fixed Dose Procedure.
Parasuraman, S. 2011. Toxicological screening. Journal of Pharmacology and Pharmacotherapeutics, 2(2): 74–79. https://doi.org/10.4103/0976-500X.81895
Pingale, S.S., Virkar, P.S., Mahale, R.G. & Wirnkor, V.A. 2021. Toxicity study of *Coriandrum sativum* Linn. International Journal of Modern Pharmaceutical Research, 5(2): 42–48.

Ruttner, F. 1988. Stingless Bees (Meliponinae). In: Biogeography and Taxonomy of Honeybees. F. Ruttner (Ed.). Springer, Berlin, Heidelberg. pp. 13–19. https://doi.org/10.1016/b978-0-12-342203-3.50011-4

Sabir, A. & Sumidarti, A. 2017. Interleukin-6 expression on inflamed rat dental pulp tissue after capped with *Trigona* sp. propolis from south Sulawesi, Indonesia. Saudi Journal of Biological Sciences, 24(5): 1034–1037. https://doi.org/10.1016/j.sjbs.2016.12.019

Slaa, E.J., Chaves, L.A.S., Malagodi-braga, K.S. & Hofstede, F.E. 2006. Stingless bees in applied pollination: Practice and perspectives. Apidologie, 37(2): 293–315. https://doi.org/10.1051/apido:2006022

Sun, L., Liao, L. & Wang, B. 2018. Potential antinociceptive effects of Chinese propolis and identification on its active compounds. Journal of Immunology Research, 2018: 1–6. https://doi.org/10.1155/2018/5429543

Usman, U.Z., Abu Bakar, A.B. & Mohamed, M. 2016. Phytochemical screening and comparison of antioxidant activity of water and ethanol extract propolis from Malaysia. International Journal of Pharmacy and Pharmaceutical Sciences, 8(5): 413–415.

Yeoh, B.S., Omar, N., Mohammad, M., Mokhtar, S.S. & Ahmad, R. 2021. Antioxidative propolis from stingless bees (*Heterotrigona itama*) preserves endothelium-dependent aortic relaxation of diabetic rats: The role of nitric oxide and cyclic guanosine monophosphate. Brazilian Journal of Pharmaceutical Sciences, 57: e19187. https://doi.org/10.1590/s2175-97902020000419187