Trop J Nat Prod Res, February 2021; 5(2):304-308

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Toxicity Effect of *Phaleria macrocarpa* (Mahkota Dewa) on Liver Function in Sprague Dawley Rats

Mohammad A. Dollah[1], Nur I. Rozak[2], Pooya Nazari[3], Saadat Parhizkar[4]*

[1]*Department of Biomedical Science, Faculty of Medicine and Health Sciences, University Putra Malaysia, Malaysia*
[2]*Department of Pharmacology, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia*
[3]*Students Research Committee, Yasuj University of Medical Sciences (YUMS), Yasuj, Iran*
[4]*Medicinal Plants Research Centre, Yasuj University of Medical Sciences (YUMS), Yasuj, Iran*

ARTICLE INFO

Article history:
Received 07 December 2020
Revised 11 January 2021
Accepted 05 February 2021
Published online 01 March 2021

ABSTRACT

Phaleria macrocarpa is used as a traditional medicine for a wide range of diseases including cancer, diabetes, liver disease, kidney disease, allergies and male infertility in Southeast Asia. The study evaluated the possible toxic effect of *Phaleria macrocarpa* fruit on the liver function by measuring liver enzymes and histopathological examination of liver tissue. Twenty-four male Sprague Dawley rats were allotted randomly to four groups including: control (no treatment), while the other three groups were administered *Phaleria macrocarpa* aqueous extract (750, 1500 and 3000 mg/kg bodyweight respectively). All administration was through intragastric gavage for 28 days. The liver enzymes measurement and histological study were done at the end of the administration using standard procedures. The results showed that the significant increase ($p < 0.05$) in body weight at the end of study compared to beginning of experiment was due to the normal growth. There was no significant difference in plasma aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) levels among groups. However, there was a significant increase ($p < 0.05$) in plasma alanine aminotransferase (ALT) and alkaline phosphatase (ALP) compared to control. Histopathological study showed that there were mild to severe changes of vacuolization and degeneration of tissues in treated groups which indicated the liver damage had occurred. *Phaleria macrocarpa* extract at dose of 3000 mg/kg body weight for a period 28 days caused mild toxicity effect to the liver tissue but its effect did not contribute to the changes in liver function of the rats.

Keywords: *Phaleria macrocarpa*, Liver enzymes, Histology, Toxicity evaluation.

Introduction

The use of natural products with therapeutic properties is as ancient as human civilization and for a long time, mineral, plant and animal products were the main sources of drugs used for therapeutic purpose.[1] In recent years, there has been a growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants.[2] *Phaleria macrocarpa*. (Mahkota Dewa*)* is a plant that belongs to the *Thymelaeaceae* family and commonly known as crown of god.[3] It originated from Papua Island, Indonesia and it grows in tropical areas. The plant is one of the most popular medicinal plants in Indonesia.[4] Since a long time ago, *Phaleria macrocarpa* has been used as a traditional medicine for a wide range of therapeutic purposes including cancer, [5] male fertility, [6] impotency [7], hemorrhoids, diabetes mellitus, allergies, liver and heart diseases, kidney disorders, acne, migraine, and various skin diseases. [8] *Phaleria macrocarpa* is reported to be beneficial which is attributed to the presence of several components such as phenolic compounds, terpenes (isoprenoids), alkaloids, and benzophenone compounds.[9]
There are a lot of products like cosmetics, teas and drugs in the market that had been formulated with *Phaleria macrocarpa* due to its outstanding health-enhancing properties, but research on the plant is still limited. Previous study showed that the administration of *Phaleria macrocarpa* at dose up to 360 mg/kg body weight did not show any toxic effect on liver function in rats.[10]
This study determined the toxic effect of *Phaleria macrocarpa* consumption at different doses on the liver function of rat. Liver contains enzymes which help the body detoxify poisonous substances. [11] Therefore, high dose of *Phaleria macrocarpa* might cause damage to the liver tissue and this was evaluated by measuring several liver enzymes levels and microscopic examination of liver tissue after receiving *Phaleria macrocarpa* supplementation for 28 days. The study determined the toxic effect of *Phaleria macrocarpa* on the rats' liver function which was evaluated by Alanine aminotransferase (ALT), aspartate aminotransferase (AST) *Alkaline Phosphatase* (ALP) and *Gamma-glutamyl transferase* GGT) levels as well as histopathological examination of liver tissue.

Materials and Methods

Experimental design

A repeated measure randomized block experimental design was used with two level of periods (day 0 and day 28) and 3 dose levels of *phaleria macrocarpa* extract (0, 750, 1500 and 3000 mg/kg bodyweight). Six rats were used in each experimentalgroup. The animals were administered in the morning with 2.0 ml of *Phaleria macrocarpa* extract in various dosages, whereas control group received normal saline.

*Corresponding author. E mail: parhizkarsa@gmail.com
Tel: +98-7433223505

Citation: Dollah MA**,** Rozak NI, Nazari P, Parhizkar S. Toxicity Effect of *Phaleria macrocarpa* (Mahkota Dewa) on Liver Function in Sprague Dawley Rats. Trop J Nat Prod Res. 2021; 5(2):304-308. doi.org/10.26538/tjnpr/v5i2.15

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Extraction of Phaleria macrocarpa

Phaleria macrocarpa (Voucher no. SK1929/11) fresh fruits were supplied by Dr. Mohammad Aziz bin Dollah, Department of Biomedical Science, Faculty of Medicine and Health Sciences, University Putra Malaysia in 2010. 250 g of dried *Phalera macrocarpa* fruits slices were macerated in 4L of hot water boiled until the water became half. Subsequently, the extract was filtered and centrifuged at 3000 rpm for 15 minutes.

In order to obtain a crystal or powder form of the extract, the supernatant was freeze-dried and the powder was weighted and kept at -20^{o}C to be used later.

Experimental animal

The protocol of the study was approved by Animal Care and Use Committee (ACUC), Faculty of Medicine and Health Sciences, University Putra Malaysia (UPM) with UPM/FPSK/PADS/BR/UUH/F01-00321 reference number for notice of approval. Twenty four male Sprague Dawley rats with 220-250 g body weight were supplied by Faculty of Medicine and Health Sciences, University Putra Malaysia. The study was carried out in the Animal House of the Faculty. They were housed individually in cages under standard laboratory conditions with a period of 12 h light/dark at 29 to 32℃ and 70 to 80% relative humidity in the Animal House, Faculty of Medicine and Health Sciences, University Putra Malaysia. The animals were allowed to acclimatize for at least 10 days before the start of the experiments. The rats were fed with a standard rat chow pellet and allowed to drink water *ad libitum*. All animals were handled according to the criteria outlined in the "Guide for care and use of laboratory animals" prepared by the ACUC of Faculty of Medicine and Health Sciences, University Putra Malaysia. The administrations were conducted between 08.00 and 10.00 am. The treatments were given to the rats for 4 weeks. The body weight was measured once a week.

Animals were assigned into four treatment groups and were administered different dosages of *Phaleria macrocarpa* extract (750, 1500, 3000 mg/kg bodyweight) and control group was given normal saline. The treatments were given to the rats through intragastric gavage for 4 weeks.

Blood collection

The blood samples were collected at the end of study. The rats were fasted for 12 h before blood collection. Prior to blood sampling, the rats were anesthetized with diethyl ether to ease handling. The blood samples were collected by cardiac puncture using 25 G, 1" needle. Approximately 5 ml of blood were taken and dispensed into labeled plain tubes. The blood samples were then centrifuged at 3000 rpm for 10 min to separate the serum. The serum was stored at -40℃ until enzyme assays were carried out.[12]

Biochemical analysis

Stocks and working solutions were maintained at 0℃ in a refrigerator. Serum was obtained by high speed centrifugation. The hepatic enzymes were measured by an automatic analyzer (Hitachi 902) using Roche Liver Enzyme Kits based on the manufacturer's instructions and standard methods.[13]

Histological examination

All rats were sacrificed by cervical dislocation under chloroform anesthesia and then midline laparotomy was performed. Resected liver specimens of each rat in all groups were fixed in 10% buffered formaldehyde for 24 hours and embedded into paraffin of alcohol process in which tissues were equilibrated in turn in 70% (v/v) ethanol (1 h), then 80% ethanol (1 h), 90% ethanol (1 h), 99% ethanol (2 × 1 h), absolute ethanol (1 h and overnight), xylene (3 × 1 h), and paraffin (3 × 1.5 h) (Wako Pure Chemical Industries, Osaka, Japan).[14] 5 μm thick sections were obtained from the paraffin blocks and stained with hematoxylin and eosin. Each slide was examined under a light microscope by the same pathologist, who was blinded to the study group allocations. Central venous congestion, congestion and dilation of the hepatic sinusoids and inflammation of the portal tracts were noted and graded from 0 to 3, with "0" indicating no change, "1" slight change, "2" moderate change and "3" severe change. A sum of all grades was regarded as total score, which ranged between 0-9.[15]

Statistical analysis

Data were expressed as means ± standard deviation. The data were analyzed using statistical package for social science (SPSS, v18). Differences between groups were compared using analysis of variance, ANOVA followed by Duncan's multiple range tests. A p-value of ($P < 0.05$) was considered to be significant.

Results and Discussion

Body weight

The body weight of the animals treated with *Phaleria macrocarpa* fruit extract at various doses for 4 weeks period is presented in Figure 1. Measurement of the body weight was used to evaluate the health status of the rats during the treatment period. There was a significant ($p < 0.05$) weight increment in treated rat groups compared to control. This is indicating the healthy status of rats following *Phaleria macrocarpa* supplementation.

Liver enzymes

The study investigated the toxicity effect of *Phaleria marocarpa fruit extract* on liver function evaluating Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) Alkaline Phosphatase (ALP), Gamma-glutamyl transferase (GGT) and histopathological changes of liver. Liver plays an important role in digestive and excretory functions, stores and process nutrients, synthesize new molecules and detoxifies harmful chemicals. [16] Liver produces many enzymes which contribute to its function. Some of these enzymes are useful in the diagnosis of liver disorder which includes; ALT, AST, ALP and GGT. These enzymes are indicators of liver damage. If the level of some hepatic enzymes elevated from its normal range, this may indicate liver damage.[17]

The results of liver function tests are shown in Table 1. The finding revealed a significant difference between ALT levels among rats in different treatment groups compared to control group. The concentration of AST for *Phaleria macrocarpa* treated groups was higher than those control groups and difference was significant ($p < 0.05$) at the dose of 3000mg/kg bw group. The finding demonstrated a significant difference ($p < 0.05$) in ALP level among the animals in the 750 and 1500 mg/kgdoses of *Phaleria macrocarpa* in comparison to control group. In 750 mg/kg *Phaleria macrocarpa* group, although the level of ALP was much higher than those of control, but the difference was not significant. There was no significant difference in GGT concentration among all groups.

The results showed no significant difference in serum ALT following treatment with *Phaleria macrocarpa* in various doses and although in all treated rats upon to supplementation period ALT concentration increased but increment was not significant. On the other hand, control group showed a slight reduction in ALT level which did not differ significantly from baseline. ALT is an enzyme produced within the cells of the liver and is the most sensitive marker for hepatocyte damage. The level of ALT is increased out of normal range in conditions where cells of the liver have been inflamed or have undergone cell death.[15] The normal range of ALT level is 20 to 71.86 U/L. [18] From the biochemical study, it showed that the treatment doses (750, 1500 and 3000 mg/kg bw) of *Phaleria macrocarpa* fruit extract increased ALT level in treated rats compared to control group of rats, but the values were still in normal range of serum ALT level. However, there was no significant difference between various dosages. These results agree with previous study reporting that *Phaleria macrocarpa* fruit extract were able to protect the liver from damage due to exposure to Cadmium.[19]

The finding revealed that after 4 weeks supplementation, the AST level did not differ significantly compared to day zero in all groups. Control group and 1500mg/kg *Phaleria macrocarpa* treated group showed a slight reduction compared to baseline. AST is an enzyme that also reflects damage to the hepatic cells but it is less specific for liver disease as it is also produced in muscle and can be elevated in other conditions such as myocardial infarction.[20] From the results obtained, the AST level in the control group was ranged from 49.80 to 71.86 U/L. These values were considered in the normal range which was between 20.18 to 129.34 U/L.[21]. Following treatment with *Phaleria macrocarpa* fruit extract (750, 1500, 3000 mg/kg bw), all the AST levels were similar with the level in the control group.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Furthermore, the value before treatment period was also similar with the value after treatment period. This indicated that the AST level of rats in all groups was maintained throughout the experimental period.

ALP level of all treatment groups reduced except 750 mg/kg *Phaleria macrocarpa* group (750 mg/kg). The finding revealed that this reduction was significant in control and normal dose ($p < 0.05$) as compared to baseline. ALP is an enzyme in all tissues. Tissues with particularly high concentrations include liver, bile ducts and bone cells. If the ALP is elevated, biliary tract damage and inflammation should be considered. Normal range for plasma ALP level is 53 to 184 U/L.[22] The results with *Phaleria macrocarpa* fruit extract are in agreement with results obtained by two studies that found hepatoprotective effect of *phaleria macrocarpa* against ethanol-induced hepatotoxicity and carbon-tetrachloride induced hepatotoxicity.[23, 24]

The total mean of plasma ALP level was within the normal range (125.93 U/L). Following supplementation with *Phaleria macrocarpa* fruit extract (750, 1500, 3000 mg/kg bw) the total mean value of plasma ALP level was significantly higher than total mean in control group. At 1500 mg/kg bw, the plasma ALP level was 168.95U/L that is within the normal range. At 750 and 3000 mg/kg bw, the plasma ALP levels were 199.21 and 227.52 U/L which were out of the normal range. This might be due to the various sources of enzymes ALP that might contribute to the high level of plasma ALP. Alkaline phosphatases are present in many human tissues, including bone, intestine, kidney, liver, placenta and white blood cells. Renal or intestinal damage can also cause an increase of plasma ALP level.[25]

All groups illustrated elevation in GGT concentration which was not significantly differ from the baseline treatment ($p < 0.05$). Similar to alkaline phosphatase (ALP) in detecting disease of the biliary tract, the GGT test is used to help detect liver disease and bile duct obstructions. Measurement of GGT is an extremely sensitive test and it may be elevated in virtually any liver disease and even sometimes in normal individuals. The normal range for plasma GGT level is 2 to 5.72 U/L.[26] From the result obtained, plasma GGT level of control group was similar at the initial and at the end of experiment. The total mean of plasma GGT level in control group was 5.16 U/L which was within the normal plasma GGT level. Following supplementation with *Phaleria macrocarpa* fruit extract (750, 1500, 3000 mg/kg bw) all the GGT level were similar to the level in the control group. Thus, the result indicated that supplementation of *Phaleria macrocarpa* fruit extract up to 3000 mg/kg bw for 28 days did not significantly change plasma level of GGT. The findings are consistent with results from the previous study, in which proven *Phaleria macrocarpa* extract have improved liver function test markers through decreased ALT, AST, GGT and ALP activity.[27]

Histopatological finding

The results of liver histopathologic examination are shown in Table 2. The results of histopathologic examination of the liver of treatments groups showed hepatic vacuolization, degeneration, inflammation with the appearance more pronounce in high dose of *Phaleria marocarpa* fruit extract. However no necrosis was found in the liver tissue of all groups of rats as shown in Figure 2 (A, B, C, and D). Histology examination showed that liver tissue for control group was morphologically normal. No vacuolization and degeneration of tissue was found. Healthy hepatocytes with normal cellular structure were observed. These indicated the rats in control group were healthy and their liver function was normal. Observation on the liver of rats in *Phaleria macrocarpa* fruit extract supplemented groups revealed that there was vacuolization and degeneration of tissue observed in all groups. The degree of vacuolization and degeneration varies according to the dose of supplementation of *Phaleria macrocarpa* fruit extract. There was also some inflammation which was accumulation of lymphocytes observed in liver tissue of supplemented group. In the mild focal inflammation area, lymphocytes were seen accumulating around the inflammation area. These characteristics indicated that the tissue injury had occurred in the liver of supplemented groups.

In summary the results of the biochemical analysis of liver enzymes showed that the plasma ALT, AST, ALP and GGT levels were considered to be normal for this animal. Furthermore, the increment of body weight of rats in the study indicated that rats were relatively healthy throughout the experimental period. However, the histology study revealed that there are mild to severe changes occurred in liver tissue of supplemented rats, indicating liver damage. Even though, damage was seen on the liver cells of supplemented rats but the liver enzymes were still in the normal range. This might be due to the high regeneration capability of the liver cells. On the other hand this might come from its high content of flavonoids, phenols, tannin, saponin and terpenes which proves its antioxidant activity.[28] The liver had a remarkable capacity to regenerate after injury, thus it could possibly overcome the effect of liver injury.

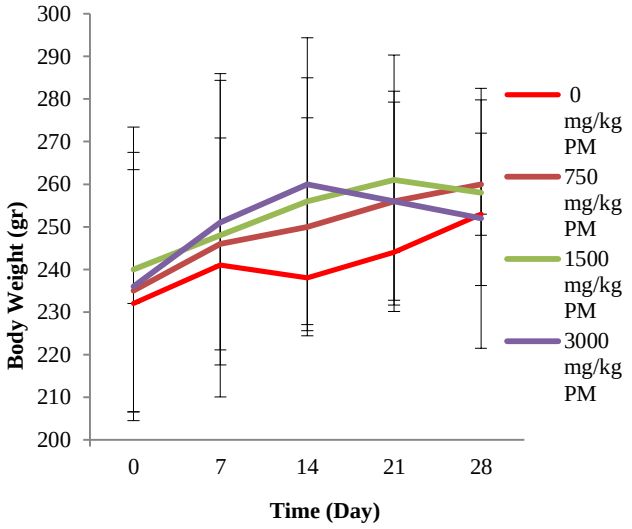

Figure 1: Changes in body weight of rats supplemented with different doses of *Phaleria macrocarpa* (PM) for 28 days.

Table 1: Changes in Liver Enzyme (ALT, AST, ALP and GGT) of rats supplemented with various doses of *Phaleria macrocarpa* extract for 4 weeks

Liver Enzyme	Time	Doses of *Phaleria macrocarpa* (mg/kg bodyweight)			
		0 (Control)	750	1500	3000
ALT	Day 0	36.03 ± 7.32	37.63 ± 5.75	37.33 ± 10.22	48.50 ± 11.92
	Day28	21.36 ± 13.93	49.90 ± 11.48¥	44.71 ± 19.93¥	63.18 ± 18.86¥
AST	Day 0	71.86 ± 15.75	73.15 ± 17.49	72.26 ± 10.39	76.65 ± 11.88
	Day28	49.80 ± 35.56	74.25 ± 15.31	64.57 ± 29.28	95.31 ± 19.35¥
ALP	Day 0	167.22 ± 69.27	161.30 ± 62.43	270.68 ± 51.43	239.37 ± 64.39
	Day28	76.38 ± 43.67*	199.21 ± 50.51¥	168.95 ± 120.42*	227.52 ± 70.60¥
GGT	Day 0	2.24 ± 0.44	4.23 ± 4.22	5.04 ± 4.58	2.89 ± 1.44
	Day28	5.16 ± 2.74	6.54 ± 7.75	7.27 ± 6.68	4.40 ± 5.69

Data is presented as Mean ± SD. *: Indicated significant differences compared to day zero at ($P < 0.05$). ¥: indicate significant differences compared to control at ($P < 0.05$)

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

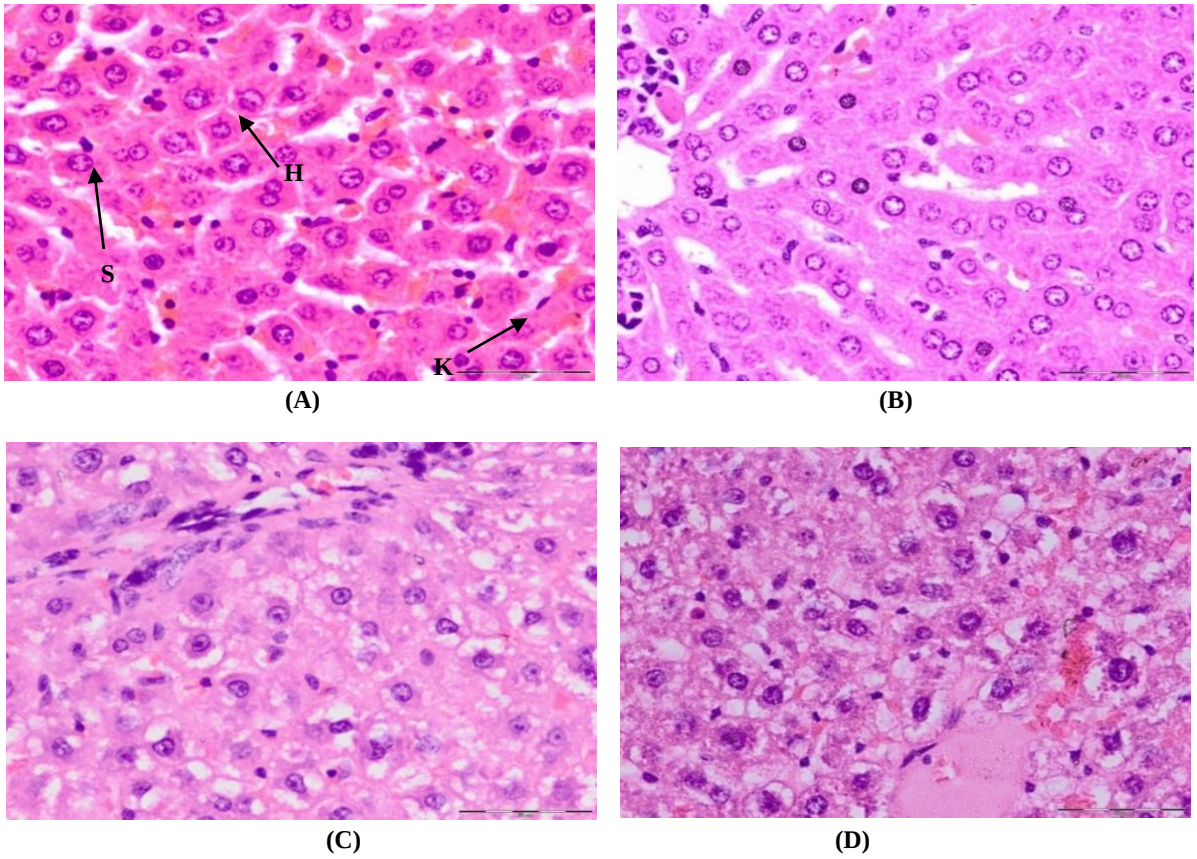

Figure 2: Histopathological section in the liver tissue of rats in different treatment groups for 28 days
(A) control group(0 g/kg *Phaleria marocarpa* fruit extract), displaying hepatocytes (H), kuppfer cells (K) & sinusoids **(B)** Supplemented with Low dose *Phaleria marocarpa* fruit extract (750mg/kg), displaying hepatocyte vacuolization **(C)** Supplemented with Normal Dose *Phaleria marocarpa* fruit extract (1500mg/kg), displaying hepatocyte vacuolization **(D)** Supplemented with High Dose *Phaleria marocarpa* fruit extract (3000mg/kg) displaying hepatocyte vacuolization. (H&E, X40).

Table 2: Histopathology scoring of liver tissue for rats supplemented with different doses of *Phaleria marocarpa* fruit extract supplementation for 28 days

Tissue characteristics	Number of Specimen	Control	Dose 750 mg/kg bw	Dose 1500 mg/kg bw	Dose 3000 mg/kg bw
		0 1 2 3 4	**0 1 2 3 4**	**0 1 2 3 4**	**0 1 2 3 4**
Vacuolization	6	4 2 0 0 0	0 1 2 2 1	0 0 0 3 3	0 0 0 1 5
Degeneration	6	5 1 0 0 0	1 1 3 1 0	0 0 1 1 4	0 0 0 2 4
Inflammation	6	6 0 0 0 0	3 3 0 0 0	2 3 1 0 0	1 4 1 0 0
Necrosis	6	6 0 0 0 0	6 0 0 0 0	6 0 0 0 0	6 0 0 0 0

Conclusion

It can be concluded that supplementation of *Phaleria macrocarpa* up to 3000 mg/kg bw caused some toxic effects to the liver tissue but its effect did not contribute to the changes in liver function and thus the rats grew normally.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors are thankful to the authorities of Faculty of Medicine and Health Sciences, University Putra Malaysia, for providing analytical facilities.

References

1. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecules 2016; 21(5):559.
2. Wangchuk P. Therapeutic applications of natural products in herbal medicines, biodiscovery programs, and biomedicine. *J Bio Active Prod from Nat*. 2018; 8(1):1-20.
3. Hendra R, Ahmad S, Sukari A, Shukor MY, Oskoueian E. Flavonoid analyses and antimicrobial activity of various

parts of Phaleria macrocarpa (Scheff.) Boerl fruit. Int J Mol Sci. 2011; 12(6):3422-3431.
4. Parhizkar S, Zulkifli SB, Dollah MA. Testicular morphology of male rats exposed to Phaleria macrocarpa (Mahkota dewa) aqueous extract. Iran J Basic Med Sci. 2014; 17(5):384.
5. Lay MM, Karsani SA, Banisalam B, Mohajer S, Abd Malek SN. Antioxidants, phytochemicals, and cytotoxicity studies on *Phaleria macrocarpa* (Scheff.) Boerl seeds. Biomed Res Int. 2014; 2014.
6. Parhizkar S, Zainudin CZBC, Dollah MA. Effect of *Phaleria macrocarpa* on sexual function of rats. Avicenna J Phytomed. 2013; 3(4):371.
7. Parhizkar S, Yusoff MJ, Dollah MA. Effect of *Phaleria macrocarpa* on sperm characteristics in adult rats. Adv Pharm Bull. 2013; 3(2):345.
8. Kavitha N, Oon CE, Chen Y, Kanwar JR, Sasidharan S. *Phaleria macrocarpa* (Boerl.) fruit induce G0/G1 and G2/M cell cycle arrest and apoptosis through mitochondria-mediated pathway in MDA-MB-231 human breast cancer cell. J ethnopharmacol. 2017; 201:42-55.
9. Alara O, Alara J, Olalere O. Review on Phaleria macrocarpa pharmacological and phytochemical properties. Drug Des. 2016; 5(134):2169.
10. Andriani Y, Tengku-Muhammad TS, Mohamad H, Saidin J, Syamsumir DF, Chew GS, and Abdul Wahid ME. *Phaleria macrocarpa* Boerl.(Thymelaeaceae) leaves increase sr-bi expression and reduce cholesterol levels in rats fed a high cholesterol diet. Molecules. 2015; 20(3):4410-4429.
11. Okokon JE UJ, Bassey AI, Edem UA, Agu EC. Hepatoprotective and Nephroprotective Activities of Husk Extract of Zea mays Against Paracetamol-Induced Liver and Kidney Injuries In Rats. Trop J Nat Prod Res. 2020; 4(3):67-76.
12. Kumar M, Dandapat S, Sinha MP, Kumar A, Raipat BS. Different blood collection methods from rats: A review. Balneo Res J. 2017; 8(2):46-50.
13. Lamrabat S, Lyagoubi A, Bouayadi O, Elmalki J, Elkouche Kh, Bensalah M, Choukri M. Comparison between Roche Integra 400 plus and Abbott Architect ci8200 in Alanine aminotransferase assay. Pract Lab Med. 2019; 15:e00121.
14. Uchida Y, Sasaki H, Terasaki T. Establishment and validation of highly accurate formalin-fixed paraffin-embedded quantitative proteomics by heat-compatible pressure cycling technology using phase-transfer surfactant and SWATH-MS. Sci rep. 2020; 10(1):1-17.
15. Dollah MA, Parhizkar S, Latiff LA, Hassan MHB. Toxicity effect of Nigella sativa on the liver function of rats. Adv Pharm Bull. 2013; 3(1):97.
16. Reid AHM, Baird R, Workman P. Emerging molecular therapies: Drugs interfering with signal transduction pathways. Principles of Molecular Oncology: Springer; 2008; 317-365.
17. Wolford ST, Schroer RA, Gohs FX, Gallo PP, Brodeck M, Falk HB, and Ruhren R. Reference range data base for serum chemistry and hematology values in laboratory animals. J Toxicol Environ. 1986; 18(2):161-188.
18. Church RJ, Kullak-Ublick GA, Aubrecht J, Bonkovsky HL, Chalasani N, Fontana RJ, Kirby S. Candidate biomarkers for the diagnosis and prognosis of drug-induced liver injury: an international collaborative effort. Hepatology 2019; 69(2):760-773.
19. Berlian G, Tandrasasmita OM, Tjandrawinata RR. Standardized bioactive fraction of Phaleria macrocarpa (Proliverenol) prevents ethanol-induced hepatotoxicity via down-regulation of NF-κB-TNFα-caspase-8 pathway. Asian Pac J trop biomed. 2016; 6(8):686-691.
20. Reichling JJ and Kaplan MM. Clinical use of serum enzymes in liver disease. Digest Dis Sci. 1988; 33(12):1601-1614.
21. Wolf PL. Biochemical diagnosis of liver disease. Indian J Clin Biochem. 1999; 14(1):59-90.
22. Nasution AN, Aziz H, Tjong DH, Zein R. Effect of Phaleria Macrocarpa Flesh Fruits Extract on MDA Level, SGOT and SGPT Activity in Serum of Experimental Rats Contaminated by Cd (II) Ion. Open Access Maced J Med Sci. 2019; 7(23):3950.
23. Sundari N, Soetikno V, Louisa M, Wardhani BW, Tjandrawinata RR. Protective effect of Phaleria macrocarpa water extract (Proliverenol) against carbon tetrachloride-induced liver fibrosis in rats: Role of TNF-α and TGF-β1. J Toxicol. 2018; 2018.
24. Wardhani BW, Sundari N, Tjandrawinata RR, Jusuf AA, Soetikno V, Louisa M. Antifibrotic Activity of Phaleria macrocarpa Extract in Rat Liver-fibrosis Model: Focus on Oxidative Stress Markers, TGF-β1 and MMP-13. Open Access Maced J Med Sci. 2020; 8(A):555-562.
25. Bouyet B. *Akita, treasure of Japan.* Vol 2: Magnum Publishing; 1992.
26. Braun JP, Rico AG, Siest G. Uses of γ-glutamyltransferase in experimental toxicology. Advances in veterinary science and comparative medicine. Elsevier; 1987; 151-172.
27. Wardhani BWK, Sundari N, Tjandrawinata RR, Jusuf AA, Soetikno V, Louisa M. Antifibrotic Activity of Phaleria macrocarpa Extract in Rat Liver-fibrosis Model: Focus on Oxidative Stress Markers, TGF-β1 and MMP-13. Open Access Maced J Med Sci. 2020; 8(A):555-562.
28. Easmin MS, Sarker MZI, Ferdosh S, Shamsudin SH, Yunus KB, Uddin MS, Khalil HPSA. Bioactive compounds and advanced processing technology: Phaleria macrocarpa (sheff.) Boerl, a review. J Chem Technol Biot. 2015; 90(6):981-991.