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A Subchronic Toxicity Test of *Dillenia ochreata* Leaves Extract on Wistar rats

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ABSTRACT

Dillenia ochreata were used traditionally to treat wounds and scabies. *D. ochreata* leaves have also been reported to have antibacterial, anti-inflammatory, and analgesic activities. This study aimed to determine the subchronic toxicity of an ethanol extract of *D. ochreata* leaves in Wistar rats. Tests were performed 28 days after administration of the extract. This study used 50 rats (25 males and 25 Females), each consisting of five groups: one control group (I) and four treatment groups (II-V) with doses of 100, 200, 400 and 800 mg/kg body weight (BW). Haematological parameters, biochemistry and histopathology of the liver, kidneys and heart were evaluated. The results showed that the treatment groups' haematological levels and blood biochemical levels were still within normal, except for group V, which showed a significant difference ($p < 0.05$) from the group I. The organ histopathology results in group V showed liver and kidney damage with fat degeneration and moderate necrosis in both male and female rats. Administration of the ethanol extract of *D. ochreata* leaves up to a dose of group IV showed no toxic symptoms, but toxic effects were shown at a dose of group V. The dose of 800 mg/kg BW (group V) of the ethanol extract of *D. ochreata* was found unsafe for use.

***Keywords*:** *Dillenia ochreata*; subchronic toxicity; hematology; biochemistry; histopathology

Introduction

Indonesia is rich in various traditional medicinal plants and has opportunities for development in the pharmaceutical industry.[1] The use of plants in traditional medicine requires scientific evidence regarding the efficacy, quality standards, and safety of these plants so that traditional medicines are used in accordance with established quality and safety standards. Drug safety is an important factor to consider in developing herbal medicines and is required for preclinical trials.[2] Traditional medicine safety evaluation approaches can be performed using toxicity tests.[3] Toxicity tests on animals are useful for observing biochemical, physiological and pathological reactions that may occur before use by humans.

The leaves of *D. ochreata* are used in Indonesia, especially in Musi Banyuasin, South Sumatra, as a medicine for scabies and wounds.[4] The Dilleniaceae family is usually known as Sempur or Simpur .[5] Muharni et al.[6] reported that *D. ochreata* leaves showed antibacterial activity and healing properties for burns and incision wounds.[7] *D. ocheata* leaves have been reported to contain sentulic acid and 3β-glucopyranosyl–lup-20(29)-en-28-oat, which are active antibacterial agents against *E. coli* and *S. aureus* bacteria. Sentulic acid has been reported to have cytotoxic effects on human promyelocytic leukaemia HL-60 cells.[8] Further analysis of the *D. ochreata* leaf extract must be performed by testing its toxicity.

In this study, a subchronic toxicity test was performed to determine the dose that did not cause toxic effects. This research was conducted by observing the safety of organs evaluated through blood biochemistry haematological and histopathological examinations of the organs.

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Materials and Methods

Plant collection

D. ochreata leaves (2 kg) were collected from Musi Banyuasin, South Sumatra (-3^{0}34'1.434"N 104^{0}77'18.19"E), Indonesia, in April 2021 and identified at the Indonesian Institute of Sciences, Cibinong, Indonesia as *Dillenia ochreata* (Miq.), registered B-82/IV/D1.01/i/2021. The leaves (2 kg) were dried and ground into powder (1.2 kg).

Extraction

D. ochreata leaves powder (1.2 kg) was extracted by maceration method using 96% ethanol (6 L) for 3x24 h and then filtered. Maceration was performed in triplicate. The pooled macerate was concentrated, and the crude extract was obtained from 160.25 g (8.02 % of fresh leaves).

Animals

The animals used were Wistar rats, aged 2-3 months and weighing 150-250 g 25 male and 25 female). The experimental protocol was approved by the Ethics Committee of Ahmad Dahlan University (No.022210065.)

Subchronic toxicity test

The rats were acclimatised for seven days and then were divided into five groups of male rats and five groups of female rats, with five rats per group. Group I, as a control, Groups II–V were treated with ethanol extract of 100, 200, 400 and 800 mg/kg body weight (BW). The treatment was administered orally, and each rat was given 2 ml of the extract for 28 days.[9] Toxicity symptoms were observed through gait changes, salivation, diarrhoea, seizures, tremors and death. Observations were made every day for 28 days, and weighing was performed every week. On day 29, haematological and biochemical analyses of blood and histopathology of the organs were performed.[10,11]

Haematology and blood biochemistry analysis

Haematological blood analysis included haemoglobin, erythrocyte, leukocyte and haematocrit values, and blood biochemistry, including SGOT, SGPT, creatinine and urea values.[12] Blood was collected

through the retroorbital plexus in the rat eye (2 mL) and then placed in a vacutainer tube EDTA-2K (Ethylenediaminetetraacetic Acid, 2 K) and the other with heparin. Blood was examined using a haematoanalyser for clinical chemistry analysis using the Swelab Coulter blood cell counter.[13,14] The heparin tubes were centrifuged (5 min, 4000 rpm), and the serum was used for biochemistry analysis. The haematological parameters were measured using an α Swelab Coulter blood cell counter.[14] Serum parameters were determined using biochemical kits: SGOT, SSGP, Urea and Creatinine. Determination of SGOT and SGPT levels was carried out by kinetic enzymatic methods based on the IFCC ((International Federation of Clinical Chemistry) method and creatinine levels using the Jaffe creatinine method.[15]

Macroscopic observation and histopathology of organs

The macroscopic observation of the organs (liver, kidneys and heart) included organ colour, shape, surface and weight. Organ weight was compared with body weight to obtain the relative organ weights (ROWs). Each organ was subjected to histopathological examination.[16]

Histopathological examination

The experimental animals were executed, and surgery was performed to remove the vital organs (liver, kidneys, and heart). The organs were fixed using a solution of 10% formalin (in buffer). Thin organ tissues (5 μm) were prepared and stained with hematoxylin and eosin. Observing the histopathological image using light microscopy, 100 times magnification was first used and then 400 times.[17]

Data analysis

Data were analysed using SPSS® 25.0 with normality test analysis, then the data were analysed with homogeneity tests, one-way ANOVA parametric tests, and post hoc LSD (Least Significant Difference) analysis.

Results and Discussion

During treatment, the body weights of the rats were measured (Table 1). The body weights in both the normal and treatment dose groups slightly increased, except for the 800 mg/kg group. The weight change indicates side effects from chemicals or drugs and is significant if a weight loss is more than 10%.[18] The weight loss may be caused by sensitivity to treatment. The weight loss percentage in the 800 mg/kg BW group did not exceed 10% of the initial body weight. There was no significant difference ($p > 0.05$) in body weight before and after treatment in all groups.

Toxicity symptoms were observed in the test animals every day after four hours of treatment (Table 2). The parameters of toxicity symptoms observed consisted of changes in behaviour, such as seizures, diarrhoea, tremors, salivation, walking backwards and death.[19] The toxicity symptom observations showed that no toxic symptoms appeared up to a dose of group IV. At a dose of group V, there were symptoms of toxicity in the form of diarrhoea in two male rats and one female rat. On the 17th day, one male rat died.

Haematology evaluation

The haematological parameters included haemoglobin, erythrocytes, leukocytes and haematocrit.[20] The normal range for haemoglobin levels in rats aged 2–4 months in male rats is 10.4–165.5 g/dL and 8.6 -15.3 g/dL in Female rats.[21] As shown in Table 3, the haemoglobin levels of all rats were within the normal range with no significant difference ($p > 0.05$). The administration of the extract did not affect haemoglobin levels in either male or female rats, indicating homeostatic processes under normal conditions. Erythrocyte levels in rats normally range from $7–11 \times 10^6$ μL and leukocyte is $2–10 \times 10^3$ μL.[22] Erythrocyte levels the treatment group no significant difference ($p > 0.05$) with the control groups. This indicates that the ethanol extract of *D. ochreata* leaves did not affect polycythaemia in the test animals.[23] All treatment groups showed normal leukocyte values, except for the female group, 800 mg/kg BW, with leukocyte values above the normal range (Table 3). Analysis of leukocyte levels in female rats at a dose of 800 mg/ kg BW significantly differed ($p<0.05$) from the control group. Leukocytes function in the body's defence system against antigens.[24] The normal blood for rat haematocrit levels is 37.6–51.0%.[22] The average values of the haematocrit levels for all rats were within the normal range, with no significant difference ($p > 0.05$). There was a marked increase in female rats in the group V, but the levels remained within the normal range.

Blood Biochemical Levels

The analysis of blood biochemical levels (Table 4) included parameters such as SGOT, SGPT, creatinine and urea. Liver function was observed by examining SGOT and SGPT. Meanwhile, the function of the kidney is urea and creatinine serum parameters.[25] Normal SGOT levels in male rats are 74–143 and 65–203 U/L in female rats, SGPT levels in male rats are 18–45 and 16–48 U/L in female rats, urea levels in male rats are 12.3–24.6 and 13.2–27.1 mg/dL in female rats and creatinine levels in male rats are 0.2–0.5 mg/dL and 0.2-0.6 mg/dL in female rats.[26]

SGOT levels in all rats were within the normal, except for the group IV and V which exceeded the normal range but no significant difference ($p > 0.05$) for group IV (Table 3). This indicated that the ethanol extract of *D. ochreata* had an effect at a dose of the V group. Increased SGOT levels are associated with toxic effects on cellular organs, such as disturbances in liver function.[27] Different hormone levels caused differences in SGOT levels obtained between male and female rats.

SGPT is more specific to liver function. Statistical analysis showed that SGPT levels were significantly different between the control group and the V group. Increased SGPT levels result in decreased liver activity.[28] When liver damage occurs, hepatocyte cells are more permeable, so the SGOT and SGPT enzymes leak into the blood vessels and cause their levels to increase.[29] Creatinine and urea levels are the primary indicators of kidney function,[25]. There was no significant difference in creatinine levels between the control and all treatment groups. This shows that the ethanol extract of *D. ochreata* did not affect creatinine levels.

Table 4 shows that the average values of urea levels were within the normal range for both the control and treatment groups, but the IV and V dose groups had levels above the normal. Based on the analysis of just female rats in the V group, there was a significant difference ($p <0.05$) with the control group. Increased urea levels indicate hypovolemia or a lack of kidney fluid volume. Urea and creatinine levels are often used to determine the state of the kidneys.[30]

The normal relative organ weight (organ index) of a rat's liver is 2.3–3.1% of body weight, a rat's heart is 0.26–0.58%, and the kidney is 0.4–0.9%.[31,32] Table 5 shows the relative organ liver, kidney and hearts of rats. Organ macroscopy showed no differences in colour or morphology with the control, except for the dose in the V group. The relative liver and heart weights of all rats were within the normal range, except for the liver weight of female rats at a dose in the V group, which exceeded the normal range. The relative weights of the kidneys of female rats at a treatment dose IV also exceeded the normal range. Exposure to toxic compounds in the kidneys can cause changes in their weight.[33] The liver, kidney and heart organ weights showed no significant differences with the control group. The increase or decrease in the relative weights of these organs was within the normal range.

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Table 1: Effect of extract on Body weight increase of the rats

Groups	Gender	Body weight increase/week (%)	Body weight decrease/week (%)
I (Control)	Male	4.84 ± 2.30	-
	Female	3.65 ±1.96	-
II (Dose 100 mg/kg BW)	Male	4.81 ± 1.95	-
	Female	2.85 ± 1.04	-
III (Dose 200 mg/kg BW)	Male	2.97 ± 1,15	-
	Female	3.96 ± 4.52	-
IV(Dose 400 mg/kg BW)	Male	4.54 ± 3.34	-
	Female	4.68 ± 7.31	-
V (Dose 800 mg/kg BW)	Male	-	2.49 ± 1.33
	Female	-	1.17 ± 1.83

Table 2: The toxic symptoms after extract administration

Group	Gender	Rats	Symptom					
			1	2	3	4	5	6
I	Male	5	-	-	-	-	-	-
	Female	5	-	-	-	-	-	-
II	Male	5	-	-	-	-	-	-
	Female	5	-	-	-	-	-	-
III	Male	5	-	-	-	-	-	-
	Female	5	-	-	-	-	-	-
IV	Male	5	-	-	-	-	-	-
	Female	5	-	-	-	-	-	-
V	Male	5	-	-	-	++	-	+
	Female	5	-	-	-	+	-	-

1. Seizures 2. Tremors 3. Salivate 4. Diarrhoea 5. walk backwards 6. Dead
(-) no symptoms (+) showed symtoms

Table 3: Effect of extract on the haematological parameters of rats

Parameters	Group	Gender	
		Male	Female
Hemoglobin (g/dl)	I	14.60 ± 2.04	11.83 ± 4.30
	II	14.20 ± 1.59	15.57 ± 1.24
	III	12.97 ± 2.50	14.30 ± 0.60
	IV	13.27 ± 2.97	12.53 ± 3.41
	V	13.90 ± 0.46	13.13 ± 2.25
Erythrocyte (10^6/mm^3)	I	9.23 ± 0.47	7.20 ± 2.85
	II	8.43 ± 0.90	8.03 ± 1.47
	III	7.53 ± 0.98	8.13 ± 0.55
	IV	7.62 ± 1.81	7.05 ± 1.30
	V	8.57 ± 0.25	8.88 ± 0.75
Leukocyte (10^3/mm^3)	I	4.10 ± 0.50	1.87 ± 1.23
	II	4.56 ± 0.94	3.14 ± 1.16
	III	5.11 ± 0.75	5.95 ± 4.55
	IV	4.48 ± 4.81	9.57 ± 5.00
	V	8.41 ± 6.07	13.39 ± 7.13[a]
Hematocrit (%)	I	49.33 ± 4.04	39.00 ± 14.42

II	46.67 ± 5.13	44.00 ± 7.00
III	42.00 ± 7.00	47.67 ± 3.21
IV	43.00 ± 10.15	42.33 ± 8.14
V	45.00±3.61	50.00 ± 3.61

Data are expressed as mean ± SD (n=3), [a] significant difference compared to the control group ($p<0.05$) at the *Post Hoc* LSD test.

Organ Histopathology

Histopathological examination of vital organs was undertaken (Figure 1). The liver, kidneys and heart histopathology of rats in the control group and at doses until 400 mg/kg BW were normal, while those in the V group showed moderately severe damage. Organ macroscopy showed no differences in colour or morphology with the control, except for the V group. Kidneys also showed colour differences in male rats treated with a dose of IV. Observations of the liver structure in rats at a dose of IV showed white lumps (nodules) on the surface of the liver in several lobes.[34,35] Changes in the macro anatomical structure at a dose of IV were thought to be due to the influence of compounds contained in the extract of *D. ochreata* leaves. This is in line with the values obtained for blood biochemical parameters, which had a high value at a dose of the V group.

Histopathological organs in the V groups in the liver (Figure 1) showed moderate fat degeneration and necrosis. Fatty degeneration is characterised by the formation of round, clear vacuoles in liver tissue. The degeneration was in the moderate category, and the livers were considered to be functioning normally because this degeneration is reversible.[35] The data for blood haematology, blood biochemistry, clinical symptoms and macroscopic observations showed that the treatment groups of up to IV did not experience liver tissue damage. Moderate damage to the liver tissue in the V groups was thought to be caused by the administration of the extract, which can inhibit the Na^+/K^+ ATPase enzyme.[36] The large of tannins in the extract may cause liver and kidney damage.[37, 38]

The V group's histopathological analysis of the kidneys (Figure 1) dose showed moderately severe damage. This is thought to be due to the constituents in the extract increasing the permeability of the lipid bilayer of red blood cells, which can cause haemolysis of these cells.[39]

Histopathological examination of the heart (Figure 1) in the V treatment group showed changes in cardiac histopathology, especially in the female rat group. This was thought to have been triggered by the treatment, which was too high and caused a toxic effect. It could also be caused by an excessive immune response, resulting in histopathological changes in inflammation and necrosis. Another factor differentiating the damage between male and female rats was hormones. Female rats have the hormone oestrogen, which is a driving factor for the onset of an autoimmune reaction or hyperthyroidism.[40]

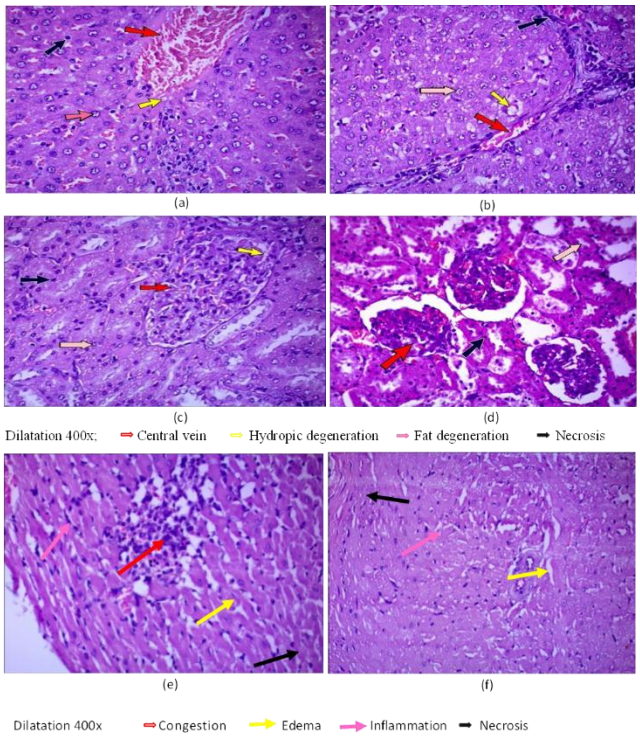

Figure 1: Histopathology of liver of male rat (a) and female rat (b), kidney of male rat (c) and female rat (d) and heart of male (e) and female (f) at dose 800 mg/kg BW.

Table 4: Effect of extract on the biochemical parameters of rats

Parameter	Group	Gender	
		Male	Female
SGOT (U/L)	I	133.96 ± 8.52	163.75 ± 10.94
	II	133.16 ± 11.20	165.81 ± 7.54
	III	132.35 ± 17.02	181.74 ± 14.20
	IV	145.35 ± 15.25	220.53 ± 16.69
	V	166.34 ± 13.92[a]	239.92 ± 12.06[a]
SGPT (U/L)	I	29.31 ± 11.02	41.64 ± 9.40
	II	37.98 ± 3.30	46.84 ± 17.86
	III	42.04 ± 8.44	48.71 ± 16.02
	IV	42.77 ± 3.65	50.10 ± 10.01
	V	54.57 ± 7.85[a]	78.43 ± 5.01[a]
Creatinine (mg/dL)	I	0.42 ± 0.03	0.41 ± 0.06
	II	0.38 ± 0.04	0.49 ± 0.05
	III	0.43 ± 0.07	0.45 ± 0.06

	IV	0.46 ± 0.05	0.39 ± 0.04
	V	0.39 ± 0.04	0.46 ± 0.07
Urea (mg/dL)	I	19.28 ± 3.20	21.68 ± 5.96
	II	23.25 ± 4.28	21.77 ± 8.69
	III	25.89 ± 19.44	22.38 ± 3.42
	IV	27.98 ± 4.78	30.36 ± 12.40
	V	30.54 ± 11.95	36.78 ± 6.69[a]

Data are expressed as mean ± SD (n=3), [a] significant difference compared to the control group ($p<0.05$) at *the Post Hoc* LSD test.

Table 5: Effect of extract on the relative organ weight of rats.

Sex	Group	Relative organ weight (%)		
		Liver	Kidneys	Heart
Male	I	3.20 ± 0.20	0.79 ± 0.05	0.41 ± 0.06
	II	3.02 ± 0.26	0.74 ± 0.04	0.41 ± 0.02
	III	2.83 ± 0.25	0.70 ± 0.08	0.36 ± 0.02
	IV	3.07 ± 0.31	0.71 ± 0.09	0.43 ± 0.04
	V	3.17 ± 0.74	0.66 ± 0.14	0.45 ± 0.07
Female	I	3.60 ± 0.17	0.78 ± 0.13	0.37 ± 0.06
	II	3.08 ± 0.52	0.89 ± 0.45	0.47 ± 0.03
	III	3.46 ± 0.73	0.82 ± 0.10	0.48 ± 0.10
	IV	3.76 ± 0.72	0.81 ± 0.19	0.44 ± 0.17
	V	4.00 ± 0.50	1.06 ± 0.14	0.48 ± 0.03

Conclusion

Administration of the ethanol extract of *D. ochreata* leaves in rats up to a dose of 400 mg/kg BW for 28 days did not cause a toxic effect, but a dose of 800 mg/kg BW affected haematological and blood biochemical parameters. A dose of 800 mg/kg BW had a damaging effect with the occurrence of fat degeneration and necrosis in liver and kidneys.

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.

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References

1. Sholikhah EM. Indonesian medicinal plants as sources of secondary metabolites for pharmaceutical industry. J Med Sci. 2016;48:226-239.
2. Alshammari TM. Drug safety: The concept, inception and its importance in patients' health. Saudi Pharm J. 2016; 24: 405-412.
3. Jitareanu A, Trifan A, Vieriu M, Caba I-C, Mârțu I, Agoroaei L. Current Trends in Toxicity Assessment of Herbal Medicines: A Narrative Review. Processes. 2023;11:1-83.
4. Yustian I, Muharni S, Zulaicha S, Arbi M. Special research on the exploration of ethnomedicine and local community medicinal plants in Indonesia (Ethnic Musi II Palembang). Ministry of Health Republic of Indonesia 2012.
5. Endress PK. Relationships Between Floral Organization Architecture and Pollination Mode. Plant System Evolution,1996; 206: 99-118.
6. Muharni M, Fitriya F, Farida S. Antibacterial assay ethanol extract Musi tribe medicinal plant in Musi Banyuasin, South Sumatra [Indonesian]. Jurnal Kefarmasian Indonesia 2017; 7: 127–135.
7. Muharni M, Amriani A, Fitrya F, Mukafi A. Wound healing activity of *Dillenia ochreata* leaves ethanol extract in Wistar rats. J Pharm Pharmacogn Res 2022; 10: 896-904.
8. Efdi M, Ninomiya M, Suryani E, Tanaka K, Ibrahim S, Watanabe K, Koketsu, M. Sentulic Acid: A Cytotoxic Ring A-Seco Triterpenoid from *Sandoricum koetjape* Merr. J Bioorganic and Med Chem Lett. 2012; 22: 4242–4245.
9. Kpemissi M, Metowogo K, Melila M, Veerapur VP, Negru M, Taulescu M, Potarniche AV, Suhas DS, Puneeth TA, Vijaya Kumar S, Galedbeku KE, Aklikokou K . Acute and subchronic oral toxicity assessments of *Combretum micranthum* (Combretaceae) in Wistar rats. Toxicol Rep. 2020; 118:162-168.
10. Shukla V, Asthana S, Gupta P, Dwivedi PD, Tripathi A, Das M. Toxicity of Naturally Occurring Anthraquinones. Adv Mol Toxicol. 2017;11:1-50.
11. Fitrya F, Fithri NA, Muharni M. A subchronic toxicity test of ethanol extract from Tunjuk langit rhizome (*Helminthostachys zeylanica*) on albino rats, *Rattus*

noverticus (Wistar strain). Asian J Pharm Clin Res 2017;10:270-273.
12. Kanina L, Solehah M, Plumeriastuti H, Widyowati R, Wardoyo BPE. Acute and Subchronic Toxicity Assessment of 70% Ethanol Extract of Gendarusa Leaves *In Vivo* [Indonesian]. Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia. 2022; 9:39-47.
13. Schmidt BM, Tameris M, Geldenhuys H, Luabeya A, Bunyasi E, Hawkridge T, MCClaim JB, Mohamed H, Scriba TJ, McShane H, Hatherill M. Comparison of haematology and biochemistry parameters inhealthy South African infants with laboratory reference interval. Trop Med Int Health. 2018; 23: 63-68.
14. Nadia M, Rima A, Rima R, khouloud A, Abdelouahab B.Evaluation of the Subacute Toxic Effects of the Alkaloids of the Seeds of *Peganum harmala* L in the Liver, Kidney and Ovary of Female Rats. Trop J Nat Prod Res. 2022; 6(10):1632-1637.
15. Küme T, Sağlam B, Ergon C, Sisman AR. Evaluation and comparison of Abbott Jaffe and enzymatic creatinine methods: Could the old method meet the new requirements? J Clin Lab Anal. 2018 Jan;32(1):e22168. doi: 10.1002/jcla.22168. Epub 2017 Feb 15.
16. Lazic SE, Semenova E, Williams DP. Determining organ weight toxicity with Bayesian causal models: Improving on the analysis of relative organ weights. Sci Rep 2020; 10: 6625
17. Mayanti T, Darwati D, Al Anshori J, Supratman U, Madihah M, Lesmana R, Dinata DI, Gaffar S. Toxicity Evaluation of Ethanol Extract of *Lansium domesticum* cv kokossan Seeds in Female Wistar Rats. Trop J Nat Prod Res. 2020; 4(8):348-354.
18. Traesel GK, de Souza JC, de Barros AL, Souza MA, Schmitz WO, Muzzi RM, et al. Acute and subacute (28 days) oral toxicity assessment of the oil extracted from *Acrocomia aculeata* pulp in rats. Food Chem Toxicol. 2014; 74: 320-25.
19. Nalimu F, Oloro J, Peter EL, Ogwang PE. Acute and sub-acute oral toxicity of aqueous whole leaf and green rind extracts of *Aloe vera* in Wistar rats. BMC Complement Med Ther 2022; 22: 1-14.
20. Chanda S, Parekh J, Vaghasiya Y, Dave R, Baravalia Y, Nair R. Medicinal plants-from traditional use to toxicity assessment: A review. Int J Pharm. 2015; 6(7): 2652-2670.
21. Delwatta SL, Gunatilake M, Beuman V, Seneviratne MD, Dissanayaka MLB, Batagoda SS et al. Reference values for selected haematological, biochemical and physiological parameters of Sprague- Dawley rats at the Animal House, Faculty of Medicine, University of Colombo, Sri Lanka. Animal Model Exp Med. 2018; 1: 250–4.
22. Douglas JW, Wardrop KJ. Schalm's Veterinary Hematology. 6th ed. Wiley-Blackwell; 2010: 852-887 p.
23. Holopainen S, Laurila HP, Lappalainen AK, Rajamäki MM, Viitanen SJ. Polycythemia in dogs with chronic hypoxic pulmonary disease. J Vet Intern Med 2022; 36:1202-1210.
24. Tigner A, Ibrahim SA, Murray IV. Histology, White Blood Cell. StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK563148/
25. Warditiani NK, Sari PMNA, Yustiantara PS, Wirasuta IMAG. Acute and Sub-Acute Oral Toxicity Profile of Purified Tomato Extract on the Liver and Kidney Functions of Male Wistar Rats. Trop J Nat Prod Res. 2021; 5(11):1962-1965.
26. Giknis M L, Clifford CB. Clinical Laboratory Parameters for Crl: Wl(Han), Charles River Laboratories, Canada. 2008
27. Sukandar EY, Sheba SH. Acute and Sub-chronic Toxicity Studies of Combination of *Physalis angulata L*. (*Cecendet*) Extract and Methylprednisolone on Animals. Int J Integr Health Sci. 2019: 7: 48-55.
28. Alimuddin A, Murtini S, NurhayatiS. Behaviour, Histopathology and Physiological Responses of Rat Fed Diets Containing Growth Hormone Transgenic Fish Meal. Hayati J Biosci. 2019; 26: 1-6.
29. Liu W, Du JJ, Li ZH, Zhang XY, Zuo HD. Liver injury associated with acute pancreatitis: The current status of clinical evaluation and involved mechanisms. World J Clin Cases. 2021; 9: 10418-29.
30. Sharma A, Hirulkar NB, Wadel P, Das P. Influence of Hyperglycemia on Renal Function Parameter in Patients with Diabetes Mellitus. IJPBA 2011; 2(Suppl 2): 734–739.
31. Schoeffner DJ, Warren DA, Muralidhara S. Bruckner JV, Simmons JE. Organ Weights and Fat Volume in Rats As a Function of Strain and Age. J Toxicol Environ Health. 1999; 56: 449-62.
32. Yan Y, Yong Z, Chunying L, Yushi Z, Yang B, Yalan Y, et al. Toxicity study of ethanol extract from Oroxylumindicum (L.) Vent in rats. J Tradit Chin Med. 2018; 38(5): 714-725
33. Weidemann DK, Weaver VM, Fadrowski JJ. Toxic environmental exposures and kidney health in children. Pediatr Nephrol. 2016;31:2043-2054.
34. Westbrook RH, Dusheiko G, Williamson. Pregnancy and Liver Disease, J Hepatol 2016; 64: 933-45.
35. Mahadevan V. Anatomy of the live. Surgery (Oxford) 2020; 38: 427-431.
36. Jung J, Ryu S, Ki IA, Woo HA, Lee K. Some Biological Consequences of the Inhibition of Na, K-ATPase by Translationally Controlled Tumor Protein (TCTP). Int J Mol Sci. 2018 4;19:1657.
37. Puteri FH, Widjaja J, Cahyani F, Mooduto L, Wahjuningrum DA. The comparative toxicity of xanthones and tannins in mangosteen (*Garcinia mangostana* Linn.) pericarp extract against BHK-21 fibroblast cell culture. Contemp Clin Dent 2019;10:319-23.
38. Arthur FKN, Woode E, Terlabi EO, Larbie C. Evaluation of acute and subchronic toxicity of *Annona Muricata* (Linn.) aqueous extract in animals. Eur J Exp Biol. 2011;1(Suppl 4):115– 124.
39. Alshalani A, Acker JP. Red blood cell membrane water permeability increases with length of *ex vivo* storage. Cryobiology. 2017;76:51-58.
40. Lasrado N, Jia T, Massilamany C, Franco R, Zsolt Illes Z, and Reddy. Mechanisms of sex hormones in autoimmunity: focus on EAE. Biol Sex Differ. 2020; 11:50