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Research Article

Evaluation of Toxic Effect of *Phaleria macrocarpa* (Scheff.) Boerl Leaf Extract on Hematological Parameters

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A B S T R A C T

Objectives: This current study was conducted to evaluate the toxic effect of *P. macrocarpa* leaf extract (*Phaleria macrocarpa* (Scheff.) Boerl) on hematology parameters in rats.
Design: The toxicity of ethanol extract of *P. macrocarpa* leaf was evaluated by OECD guidelines. The extract at doses of 100, 500, 1000 mg/kg body weight (bw), control satellite and satellite group dose 1000 mg/kg bw were orally administered to the test animal for 90 days. Hematological parameters were observed for 90 days and 118 days for treatment and satellite group, respectively.
Interventions: The variable that was intervened in this study was the doses of *P. macrocarpa* extract.
Main outcome measure: The main results in this study were the toxic effect of *P. macrocarpa* leaf on hematology parameters.
Results: The etanol extract of *P. macrocarpa* did not cause any changes in hematological parameters, these include red blood cells (RBC), hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet, white blood cells (WBC), neutrophils, lymphocytes, monocytes, eosinophils, and basophils levels as compared to normal control (P>0.05)
Conclusion: The ethanol extract of *P. macrocarpa* leaf did not cause any toxic effect on hematological parameters for long-term use.

Keywords:, *P. macrocarpa* leaf, hematology parameters, toxic effect, long term use

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INTRODUCTION

Phaleria macrocarpa, commonly known as God's crown, Mahkota dewa or Pau is an Indonesian plant of family Thymelaceae that grows in tropical areas of Papua island. It is a complete tree, including stem, leaves, flowers and fruits. Its height ranges from 1 m to 18 m with 1 m long straight root exuding sap, brownish green bark and white wood. It grows 10-1,200 m above sea level with a productive age that ranges from 10 to 20 years. The leaves are green and tapering with length and width ranging from 7 cm to 10 cm and 3-5 cm respectively. The flowers make a compound of 2-4, with color from green to maroon. Pit is round, white and poisonous, and fruit is of eclipse shape with a diameter of 3 cm. Fruits are green when un-ripened and become red on ripening[1]. The leaves contain alkaloids, saponins, and polyphenols (lignans); the skin fruit contains alkaloids, saponins, and flavonoids; while the fruit contains alkaloids, tannins, flavonoids, phenols, saponins, lignans, essential oils, and sterols. Flavonoids are the most compounds found in *P. macrocarpa*[2].

Leaves of *P. macrocarpa* are found to have antibacterial activity[3,4]. leaves of *P. macrocarpa* are found to possess flavanoids and phenolics, which make it a potent antioxidant[5]. The leaves and fruit of P. macrocarpa have been used to counter a number of disease including vascular problems and high blood pressure[1,6]. Extracts of *P. macrocarpa* are reported for a number of pharmacological activities, including anti-tumor, anti-hyperglycemia, anti-inflammation, anti-diarrhoeal, vasodilator, anti-oxidant, anti-viral, anti-bacterial and anti-fungal effect. Its stem is used to treat bone cancer; egg shells of seeds are used in treating breast cancer, cervix cancer, lung diseases, liver and heart diseases while leaves contain constituents that

reat impotence, blood diseases, allergies, diabetes mellitus and tumors[7,8,9].

However, the safety assessment of this plant has not been done completely. Therefore, the evalution of toxic effect, especially in long term duration should be done to determine the range of safe dose as well as toxic effect that may appear in high dose or in long term treatment. Blood tests are needed as a consideration for drug use, determination of dosage, monitoring of unwanted drug reactions and assessing drug toxicity. Several hematological parameters are routinely used clinically, these include the erythrocyte sedimentation rate, hemoglobin concentration, hematocrit, bleeding and clotting times, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean cell volume, and determination of blood groups. Abnormal range of hematological parameters indicates health problems such as anemia, pregnancy, dehydration, overhydration, infectious disease, cancer, thyroid disease, and autoimmune conditions[10]. This current study was performed to provide information of the untoxic dose *P. macrocarpa* leaf for the clinical use, especially on hematological parameters.

MATERIAL AND METHODS

Material

The materials used in this study include plant materials and chemicals. Plant material used was the *Phaleria macrocarpa* (Scheff.) Boerl leaf. The chemicals used were 0,9% NaCl, CMC Na 0,5%, ethanol 95%, and distilled water.

Sample Preparation and Extraction

The leaves of *Phaleria macrocarpa* (Scheff.) Boerl were collected from Medan Sumatera Utara, Indonesia. The plant identification was confirmed by *Herbarium Medanense* (MEDA) Universitas Sumatera Utara. Fresh samples were washed, dried and powdered in a grinder and stores in an airtight jar. An amount of 500 g the dried leaf of *P. macrocarpa* were exctracted with maceration method using 5 L ethanol until discoloration. Then the ethanol macerate was evaporated at ± 40 ° C in a rotary vacuum evaporator and thickened by heating in a water bath at ± 40 ° C. The yield of ethanol extract of *P. macrocarpa* leaf was 130, 6 g.

Animals

All procedure were evaluated by *Animal Research Ethics Committees* (AREC) Faculty of Mathematics and Natural Science, Biological Departement, University of Sumatera Utara. Sixty Animals used were male and female wistar rats weighing 150-200g, 6-8 weeks old (30 males and 30 females). Before the experiment begins, the animals were acclimatized in the experimental room for 7-14 days with room temperature and conditions 12 hours of light and 12 hours of darkness. The rats were fed on a standard pellet diet and provided access to water ad libitum.

Treatment

The toxicity evaluation was followed OECD guidelines[11]. Animals were divided into 6 groups of females and males, each consisting of 5 rats:

I : Na-CMC suspension 0.5%w/v
II : *P. macrocarpa* leaf extract 100 mg/kg bw
III : *P. macrocarpa* leaf extract 500 mg/kg bw
IV : *P. macrocarpa* leaf extract 1000 mg/kg bw
V : Control Satellite Na-CMC 0.5% w/v
VI : Dose Satellite 1000 mg/kg bw

The treatment were administered orally at a single dose to the test animal for 90 days. The toxic symptoms, mortality, body weight were observed weekly for 90 days and for the satellite groups, the observation were continued until 118 days to evaluate the recovery process from toxic effect. Observation of toxic symptoms and clinical symptoms in the form of physical behaviors such as diarrhea, salivation, weakness, strange movements such as walking back and using the abdomen were performed by placing test animals on a flat field, Blood for haematological were collected by cardiac arteries. The plasma was used to evaluate haematological parameters.

Statistical analysis

Data were analyzed using SPSS 25.0 with Kolmogorov-Smirnov normality test, one-way ANOVA and Kruskall (Kruskal) Wallis to identify the differences between test groups with significance ($p>0.05$).

RESULT AND DISCUSSION

Phytochemical screening

Table: 1. Phytochemical Constituent of *P. macrocarpa* leaf

No	Secondary metabolite	Constituents
1	Alkaloids	+
2	Flavonoids	+
3	Glycosides	+
4	Saponins	+
5	Tannins	+
6	Steroids/triterpenoids	+

Notes : (+) positive : contains of phytochemicals compound

Phytochemical screening on *P. macrocarpa* leaf showed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, and steroids/triterpenoids.

Hematological parameters

Table 2 and 3 shows that there was no significant difference ($p> 0.05$) between the hematological parameter

values of each group, hence it can be concluded that the ethanol extract of *P. macrocarpa* leaf did not affect the hematology value of the animal test.

Table: 2. Effect of of *P. macrocarpa* leaf extract on hematological parameters of male rats (Mean ± SEM)

Hematology parameters (Mean ± SD)	Samples					
	Control	Dose 100 mg/kg bw	Dose 500 mg/kg bw	Dose 1000 mg/kg bw	Control Satellite	Satellite dose 1000 mg/kg bw
WBC	7.67 ± 0.77	8.04 ± 1.30	8.65 ± 0.77	9.12 ± 0.53	7.44 ± 0.58	7.82 ± 1.27
RBC	8.69 ± 1.09	9.05 ± 0.82	8.17 ± 1.00	7.94 ± 1.11	8.20 ± 1.12	7.15 ± 0.68
Platelets	899.00 ± 162.26	924.20 ± 182.26	868.60 ± 75.12	960.25 ± 181.82	900.25 ± 121.36	1009.25 ± 147.38
Hemoglobin	16.00 ± 1.11	15.10 ± 1.06	14.80 ± 0.78	14.67 ± 1.62	15.07 ± 1.31	15.57 ± 0.61
Hematocrit	50.57 ± 1.37	45.64 ± 5.26	50.48 ± 3.57	51.70 ± 4.61	48.17 ± 1.87	51.45 ± 3.01
MCH	17.37 ± 0.66	17.84 ± 0.98	18.40 ± 0.82	17.55 ± 1.40	17.27 ± 0.75	17.52 ± 0.61
MCV	55.40 ± 0.70	53.70 ± 2.52	56.08 ± 1.17	53.52 ± 2.81	54.87 ± 0.87	53.70 ± 1.13
MCHC	33.20 ± 1.01	34.10 ± 2.24	34.62 ± 1.82	36.05 ± 1.85	32.67 ± 1.48	34.02 ± 2.14
Eosinophils	3.25 ± 0.68	3.30 ± 0.52	3.40 ± 0.51	3.25 ± 0.77	3.47 ± 0.67	2.97 ± 0.38
Monocytes	1.92 ± 0.27	1.96 ± 0.86	2.40 ± 0.63	3.07 ± 0.51	3.22 ± 0.71	3.02 ± 0.61
Basophils	0.42 ± 0.11	0.36 ± 0.07	0.42 ± 0.08	0.41 ± 0.10	0.37 ± 0.03	0.41 ± 0.06
Lymphocytes	66.02 ± 17.40	66.06 ± 13.66	68.58 ± 12.88	69.87 ± 11.53	65.10 ± 3.69	64.15 ± 4.82
Neutrophils	27.92 ± 6.52	27.92 ± 4.84	31.70 ± 5.65	34.02 ± 7.93	29.80 ± 3.89	34.25 ± 5.85

*P<0.05 significant to respective control

Table: 3. Effect of of *P. macrocarpa* leaf extract on hematological parameters of female rats (Mean ± SEM)

Hematology parameters (Mean ± SD)	Samples					
	Control	Dose 100 mg/kg bw	Dose 500 mg/kg bw	Dose 1000 mg/kg bw	control Satellite	Satellite dose 1000 mg/kg bw
WBC	6.96 ± 0.86	6.89 ± 1.07	7.93 ± 1.04	7.94 ± 0.96	8.39 ± 0.84	7.05 ± 1.01
RBC	7.98 ± 0.80	8.17 ± 0.60	7.61 ± 0.59	7.29 ± 0.88	7.85 ± 0.61	6.91 ± 0.88
Platelets	786.60 ± 110.17	832.80 ± 203.52	894.20 ± 176.65	829.40 ± 207.69	885.00 ± 104.39	871.50 ± 151.39
Hemoglobin	15.72 ± 1.01	15.68 ± 1.08	15.96 ± 1.03	15.60 ± 1.01	15.40 ± 1.14	15.77 ± 0.69
Hematocrit	44.10 ± 2.75	43.74 ± 3.41	45.20 ± 3.17	45.24 ± 2.93	43.65 ± 2.65	43.42 ± 2.76
MCH	17.94 ± 0.48	18.42 ± 0.52	18.20 ± 0.65	18.46 ± 0.97	18.50 ± 0.66	18.67 ± 0.94
MCV	53.82 ± 2.17	55.30 ± 1.50	57.18 ± 0.92	56.60 ± 0.93	52.85 ± 2.05	55.95 ± 1.21
MCHC	34.58 ± 1.08	35.68 ± 1.74	35.48 ± 1.94	36.08 ± 1.00	34.42 ± 0.84	35.90 ± 1.06
Eosinophils	3.28 ± 0.69	3.22 ± 0.60	3.66 ± 0.58	3.68 ± 0.49	3.00 ± 0.62	3.22 ± 0.22
Monocytes	2.04 ± 0.91	2.54 ± 0.57	2.32 ± 0.76	2.56 ± 0.66	2.02 ± 0.73	2.15 ± 0.38
Basophils	0.33 ± 0.07	0.37 ± 0.07	0.40 ± 0.09	0.39 ± 0.08	0.34 ± 0.05	0.35 ± 0.04
Lymphocytes	53.16 ± 4.54	58.44 ± 9.67	66.98 ± 13.22	69.74 ± 9.67	65.10 ± 6.67	66.17 ± 8.58
Neutrophils	27.34 ± 3.69	26.76 ± 4.56	28.98 ± 5.28	32.08 ± 2.52	27.92 ± 2.32	31.20 ± 2.11

*P<0.05 significant to respective control

CONCLUSION

The ethanol extract of *P. macrocarpa* leaf did not induce toxic effect on hematological parameters at dose of 100, 500 and 1000 mg/kg bw in long term treatment. However, further studies are required to determine its toxic effect on organs.

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