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Research Article

Toxicological Assessment of *Parkia Speciosa* Pod Ethanol Extract: The Acute and Sub-Chronic Oral Toxicity Test on Experimental Animal

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ABSTRACT

Parkia speciosa has been used traditionally for the treatment of diabetes, headache, and kidney failure in several countries such as Malaysia and Indonesia. This study tested the toxicity of its pod ethanol extract through acute and sub-chronic toxicity tests on experimental animal. Wistar rats were given orally a single dose of *Parkia speciosa* ethanol extract up to 5000 mg/kg for the acute toxicity test. Toxic symptoms and mortality were observed daily for 7 days. On the other hand, the rats were given the extract doses of 100, 200, 400, and 800 mg/kg/day for 28 days orally for sub-chronic toxicity test. A control group was given 10% Na CMC suspension. On the 29th day, blood of the rats was drawn and the rats were then sacrificed. The toxicity effects were observed through the several parameters namely, body weight, hematology, serum biochemistry, relative organ weight, and histopathology. The acute toxicity analysis showed no mortality and toxic symptoms until the dose of 5000 mg/kg so that the extract was categorized as low toxicity with LD_{50} value of > 5000 mg. In addition, the sub-chronic toxicity analysis showed insignificant alteration in body weight, serum biochemistry, and hematology ($p > 0.05$). However, white blood cells count and neutrophils levels of the female group were significantly different between test group and control group ($p < 0.05$). Furthermore, mild necrosis occurred at the 800 mg/kg group from histopathology analysis. In conclusion, the *Parkia speciosa* pod ethanol extract is relatively safe for oral administered with caution at high doses usage.

Keywords: *Parkia speciosa,* toxicity test, acute, sub-chronic.

INTRODUCTION

Parkia speciosa Has (Fabaceae) is a medicinal plant that is found and widely distributed in Indonesia, Malaysia, the Philippines and Thailand [1]. In Indonesia, it is known as Petai. Traditionally *P. speciosa* seeds are commonly used to treat diabetes [2], kidney failure [3], and headaches [4]. Many studies on the chemical content and pharmacological potencyl of various parts of the *Parkia speciosa* plant have been carried out. The main content of this plant are flavonoid and phenolic compounds [1, 5]. Additionally this plant also contains alkaloid and saponin [6, 7]. The pharmacological activities have been reported from this plant as an antibacterial [8], antiulcer 9, 10], antitumor and antihypertension [11], antioxidant and antiangiogenic [1, 12, 13], antidiabetic [14] anti-inflammatory [15], and immunomodulator [16].

The development of a drug candidate towards herbal medicine should ideally be based on scientific evidence of the chemical content, pharmacological activity, toxicity, and quality standards of the ingredients. Although many studies have been conducted to prove the pharmacological activity of *Parkia speciosa,* but there is no study proving a toxicological effect of this plant [1, 17]. Therefore, this study aims to assess toxicological effects of the *Parkia speciosa* pod ethanol extract on a single and multiple doses for a period of 28 days.

MATERIALS AND METHODS

Plant material

The plant was identified by Indonesian Institute of Science with No. 218/IPH.06/HM/I/2018. The voucher specimen was deposited in the herbarium of Biology Department, Faculty of Mathematics and Natural Sciences, Sriwijaya University.

Ethanol extract preparation

The pods were separated from the seeds. The pods were then dried at 40° C for 2 days. These dried samples were powdered by using an electrical mill. *Parkia* pod powder (1 kg) macerated for 2 days with 96% ethanol (3 L). The maceration process was carried out three times. The liquid extract was concentrated with a rotary evaporator (Buchi®R-100) at a temperature of 70°C until a thick extract reached a constant weight. The *Parkia* pods ethanol extract (PEE) was stored at a temperature of 4°C before being used for experiment.

Animal

The animals in this experiment were male and female wistar rats (150-200 g). All procedures for experimental animals were approved by the Ethics Committee of the Faculty of Medicine, Sriwijaya University decree no: 068/kepkrsmhfkunsri/2020. The rats were housed at 22°C and 12 hours of light / 12 hours of darkness. Food and water were provided according to *ad libitum* standards. Before the experiment, rats were acclimatized under laboratory conditions for 7 days.

Acute toxicity test

The acute toxicity test was carried out on OECD 423 [18]. In a preliminary test, the experimental rats were divided into two groups: the experimental group and the control group, one rat for each group. The initial dose administered was 300 mg/kg BW. If mortality or toxic symptoms did occur in the test animal, we continued by increasing the dose up to 5000 mg/kg BW. In the main test, 3 rats were utilized for each group. The control group was given distilled water and the test group was given 5000 mg/kg PEE orally. Observations were routinely carried out in the first 30 minutes to 4 hours after the test subjects were administered, continued periodically every 2 hours for 24 hours, then every day for 7 days. The observations included the mortality and the toxic symptoms shown by the animals [18, 19].

Sub chronic Toxicity Test

Oral extract administration

The sub chronic toxicity test was based on OECD 407 [20]. Experimental rats were randomly divided into 5 groups: the normal control group was given 10% Na CMC, the experimental groups (I-IV) were given an extract dose of 100, 200, 400 and 800 mg/kg of body weight. Each group consisted of 5 male rats and 5 female rats. The extract was given to the test groups orally once a day for 28 days. Standard food and drink were given throughout the experiment.

Body weight changes and Toxic symptoms evaluation

The toxic effect of the extract was evaluated through observations of rat mortality and toxic symptoms such as tremor, salivation, diarrhea, urination, gasping and stress. Toxic symptoms and rat mortality were observed daily. The body weight of the rats was measured every week.

Haematology analysis

Blood was analyzed on day 29th. Blood was drawn via the retro orbitalis and collected in 2 different tubes, tubes containing EDTA were used for routine hematology analysis. The second tube without anticoagulants, it was used for biochemical analysis [21]. The parameter for hematology analysis including hemoglobin, erythrocytes, leukocytes, neutrophils segment, lymphocytes, monocytes, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), platelet, and reticulocyte. Analyses of hematology parameters were performed by using Sysmex® KX-21N, Japan. For the biochemical parameters analyses, the blood was centrifuged at 2000 rpm for 15 minutes and stored at 4°C [22]. Biochemical serum parameters were analyzed by using Clinical Chemistry Analyzer Bio System A15 (England). The analyzed biochemical parameters included AST, ALT, total protein, cholesterol, triglycerides, glucose, urea, and creatinine.

Histopathology analysis

On the 29th day the rat were sacrificed by cervical dislocation and then dissected. Observations were made on the macroscopic organs of the liver, heart, kidney, and spleen. The organs were removed, washed in normal saline, and they were then weighed. Relative organ weights were calculated by using formula: Relative Organ Weight (%) = organ weight / rat body weight x 100 [22]. The organs of liver, kidney and spleen were then placed into bottles containing 10% formalin for histopathology analysis [21].

Data Analysis

Data are presented as mean ± standard deviation (SD) and statistically analyzed by using One Way Analysis of Variance (ANOVA) followed by post hoc Tukey's to compare with the control group with a confidence level of 95%.

RESULTS

Acute toxicity analysis

The preliminary and the main test showed that no death or toxic symptoms were found in the experimental rats administered up to a dose 5000 mg/kg of extract. The body weight of the experimental group did not significantly different from the control group ($p > 0.05$). The results of the acute toxicity test are shown in [Table 1].

Table 1. Effect of the extract on acute toxicity test (n=3)

Dose (mg/kg BW)	Mortality (%)	Toxic Symptom
0	0.00	none
300	0.00	none
2000	0.00	none
5000	0.00	none

Sub chronic toxicity analysis

Body weight changes and toxic symptoms

There were no mortality in experimental rats were administered extract of Parkia pod and no symptoms were found which indicate the toxicity of the extracts such as walking backwards, salivation, diarrhea, constipation, tremors, stress or gasping. There is no significant difference in body weight of experimental group with the control group ($p> 0.05$). The results of the body weight observation are shown in [Table 2].

Table 2. Effect of the extract on the body weight of rats after 4 weeks of administration (n=5)

Groups	Dose (mg/kg BW)	Weekly weight of rats (g)				
		0	1	2	3	4
Male	Control	136.05 ± 2.38	135.9 ± 1.02	158.41 ± 0.97	171.18 ± 0.86	182.57 ± 1.03
	100	129.87 ± 1.00	140.86 ± 0.27	166.57 ± 0.97	169.71 ± 0.91	173.28 ± 0.97
	200	135.57 ± 0.10	144.6 ± 0.93	162.91 ± 0.55	170.71 ± 0.95	174.55 ± 1.49
	400	133.78 ± 2.92	148.9 ± 2.80	159.76 ± 2.99	176.16 ± 3.86	185.66 ± 1.66
	800	138.39 ± 0.63	141.99 ± 0.83	165.18 ± 0.86	178.94 ± 0.98	188.46 ± 0.58
Female	Control	131.19 ± 2.72	144.39 ± 1.16	150.44 ± 1.36	162.1 ± 1.54	168.25 ± 1.02
	100	136.83 ± 2.85	147.04 ± 2.18	156.43 ± 1.02	166.32 ± 2.42	168.31 ± 1.80
	200	133.43 ± 2.86	137.9 ± 1.02	154.25 ± 1.35	162.10 ± 1.80	173.2 ± 2.92
	400	134.27 ± 3.12	140.56 ± 2.07	150.65 ± 2.44	164.92 ± 2.59	172.86 ± 3.74
	800	135.66 ± 2.33	141.82 ± 3.34	153.23 ± 8.01	164.23 ± 4.51	170.47 ± 3.94

Table 3. Effect of the extract on hematological parameters after 28 days of administration (n=5)

Parameters	Sex	Control	Group 100 mg/kg BW	Group 200 mg/kg BW	Group 400 mg/kg BW	Group 800 mg/kg BW
Haemoglobin (g/dL)	Male	14.50 ± 2.43	12.13 ± 0.42	14.27 ± 1.88	14.63 ± 1.32	15.00 ± 1.20
	Female	11.50 ± 0.17	12.87 ± 1.71	13.57 ± 1.53	13.17 ± 0.99	13.40 ± 0.90
RBC (10^6/mL)	Male	8.33 ± 1.50	6.50 ± 0.30	7.63 ± 0.95	8.60 ± 0.61	8.91 ± 0.89
	Female	6.40 ± 0.26	7.03 ± 0.76	7.77 ± 0.75	7.40 ± 0.70	7.60 ± 0.50
WBC (10^3/mL)	Male	10.17 ± 3.37	11.47 ± 1.21	12.53 ± 1.91	13.57 ± 2.28	17.96 ± 3.26
	Female	4.30 ± 0.89	10.60 ± 3.80	11.90 ± 2.46*	13.47 ± 0.84*	18.90 ± 3.30*
Neutrophils (%)	Male	12.00 ± 3.00	13.67 ± 3.78	18.33 ± 7.77	20.00 ± 7.21	30.33 ± 13.65
	Female	14.67 ± 5.86	9.67 ± 5.69	26.67 ± 7.64*	27.33 ± 5.77*	21.50 ± 1.50*
Lymphocytes (%)	Male	61.67 ± 9.71	58.33 ± 6.5	73.00 ± 2.12	72.00 ± 4.36	84.50 ± 1.50 *
	Female	69.00 ± 3.60	70.67 ± 13.8	65.00 ± 6.24	60.00 ± 9.54	89.50 ± 1.50*
Monocytes (%)	Male	5.60 ± 1.40	5.75 ± 0.66	4.67 ± 1.51	5.83 ± 1.89	4.75 ± 0.75
	Female	5.70 ± 0.260	5.63 ± 1.38	5.40 ± 0.53	5.17 ± 1.04	4.50 ± 2.500
Haematocrit (%)	Male	45.33 ± 8.14	37.67 ± 2.89	46.33 ± 5.51	46.67 ± 3.21	50.00 ± 3.00
	Female	35.00 ± 0.00	40.33 ± 5.13	42.33 ± 4.62	41.67 ± 3.78	44.50 ± 3.50
MCV (mm^3)	Male	55.00 ± 1.73	58.00 ± 2.00*	60.67 ± 3.05*	54.00 ± 1.00	57.00 ± 2.00
	Female	55.33 ± 2.08	57.67 ± 1.53	54.33 ± 1.15	56.67 ± 0.58	58.00 ± 1.00
MCH (pg)	Male	17.67 ± 0.58	19.00 ± 0.00*	18.67 ± 1.15	17.33 ± 0.58	17.00 ± 0.00
	Female	18.00 ± 1.00	18.33 ± 0.58	17.67 ± 0.58	18.33 ± 0.58	18.00 ± 0.00
MCHC (g/L)	Male	318.33 ± 5.51	321.67 ± 10.69	309.00 ± 5.29	315.33 ± 5.13	300.5 ± 6.50*
	Female	327.33 ± 5.69	319.67 ± 1.15*	320.67 ± 3.51*	316.67 ± 8.08*	303.00 ± 3.00*
Reticulocytes (%)	Male	16.67 ± 3.51	15.67 ± 3.21	21.67 ± 5.43	20.00 ± 3.46	14.00 ± 2.00
	Female	14.33 ± 2.08	17.67 ± 6.66	18.67 ± 1.53	24.67 ± 6.66	27.00 ± 2.00
Platelets (103/mL)	Male	600.00 ± 22.89	275.67 ± 10.73	438.67 ± 22.79	659.67 ± 28.73	667.50 ± 25.50
	Female	244.67 ± 21.03	469.33 ± 26.17	573.84 ± 13.09	679.67 ± 20.10	529.00 ± 25.00

*Value expressed as mean ± SD, value with superscript * significantly different from control group ($p<0.05$)*

Hematology and Biochemical Serum Analysis

The results of hematology and biochemical rat serum analysis after 28 days of oral administration are shown in [Table 3] and [Table 4]. Overall, the hematology parameters of the experimental group did not significantly different with the control group ($p > 0.05$) except for the parameters of white blood cells count, neutrophils level and the MCHC level of female experimental group ($p < 0.05$). Likewise the biochemical serum parameters, only the urea of the female experimental group was significantly different from the control group ($p < 0.05$).

Relative organ weight

The results of the relative organ weight observations are shown in [Table 5]. There was no significant difference in the relative organ weight of rats except for the spleen organ in the male groups at doses of 100, 400 and 800 mg/kg. Macroscopic observation found no alteration in the morphology and color of the organ compared to the control group.

Table 4. Effect of the extract on biochemical parameters after 28-day administration (n=5)

Parameters	Sex	Groups				
		Control	100 mg/kg BW	200 mg/kg BW	400 mg/kg BW	800 mg/kg BW
AST (IU/L)	Male	262.11 ± 7.58	197.33 ± 11.71	197.93 ± 2.19	187.34 ± 3.99	205.92 ± 4.78
	Female	211.93 ± 8.38	181.44 ± 4.29	194.94 ± 6.33	172.34 ± 7.20	229.72 ± 5.65
ALT (IU/L)	Male	76.18 ± 3.26	86.77 ± 8.15	71.37 ± 14.39	74.78 ± 3.85	89.17 ± 5.53
	Female	101.76 ± 3.76	54.88 ± 3.90	60.78 ± 4.50	62.98 ± 4.53	97.97 ± 9.08
Total Protein (g/L)	Male	82.00 ± 8.72	77.33 ± 7.37	76.00 ± 1.73	75.00 ± 5.29	79.00 ± 3.00
	Female	90.67 ± 7.77	88.67 ± 7.50	83. 67 ± 5.51	84.33 ± 7.50	79.00 ± 3.00
Cholesterol (mg/dL)	Male	60.09 ± 5.14	62.66 ± 7.31	53.14 ± 4.34	50.82 ± 5.46	59.15 ± 6.96
	Female	56.23 ± 1.94	54.56 ± 2.79	59. 96 ± 9.10	62.02 ± 4.40	57.15 ± 4.64
Triglyceride (mg/dL)	Male	106.58 ± 2.55	113.820 ± 7.53	104.22 ± 6.74	108.94 ± 3.23	134.63 ± 9.14
	Female	72.04 ± 4.49	88.82 ± 4.19	118.39 ± 5.77	91.23 ± 3.34	137.55 ± 5.75
Glucose (mmol)	Male	5.94 ± 0.88	6.24 ± 0.47	5.83 ± 0.76	6.38 ± 1.28	5.90 ± 0.20
	Female	5.85 ± 0.51	5.61 ± 0.12	5.74 ± 0.45	5.41 ± 0.22	5.80 ± 0.10
Urea (mg/dL)	Male	43.63 ± 4.98	45.07 ± 3.14	41.4 ± 5.77	35.90 ± 0.90	38.26 ± 0.60
	Female	71.03 ± 6.77	46.57 ± 2.93*	44.17 ± 7.65*	45.90 ± 12.35*	36.47 ± 0.36*
Creatinine (mg/dL)	Male	0.47 ± 0.06	0.33 ± 0.06	0.43 ± 0.06	0.33 ± 0.06	0.41 ± 0.00
	Female	0.53 ± 0.06	0.50 ± 0.00	0.40 ± 0.10	0.43 ± 0.06	0.46 ± 0.00

*Value expressed as mean ± SD, value with superscript * significantly different from control group ($p < 0.05$)*
AST = aspartate aminotransferase, ALT = alanine aminotransferase

Table 5. Relative organ weight of rats after 28-day extract administration (n=5)

Group	Liver		Heart		Kidney		Spleen	
	Male	Female	Male	Female	Male	Female	Male	Female
Control	3.18 ± 0.53	3.30 ± 0.66	0.44 ± 0.16	0.39 ± 0.05	0.82 ±0.02	0.75 ± 0.11	0.31 ± 0.03	0.37 ± 0.08
100	3.10 ± 0.36	3.16 ± 0.72	0.40 ± 0.01	0.37 ± 0.02	0.84 ± 0.04	0.73 ± 0.06	0.38 ± 0.03*	0.34 ± 0.13
200	3.13 ± 0.82	3.40 ± 1.20	0.41 ± 0.07	0.38 ± 0.06	0.86 ± 0.18	0.81 ± 0.13	0.32 ± 0.22	0.35 ± 0.24
400	3.17 ± 0.21	3.28 ± 0.09	0.37 ± 0.01	0.45 ± 0.03	0.81 ± 0.07	0.77 ± 0.08	0.38 ± 0.06*	0.36 ± 0.11
800	3.27 ± 0.40	3.38 ± 0.16	0.44 ± 0.02	0.40 ± 0.01	0.86 ± 0.06	0.80 ± 0.05	0.37 ± 0.03*	0.38 ± 0.08

*Value expressed as mean ± SD, value with superscript * significantly different from control group ($p < 0.05$)*

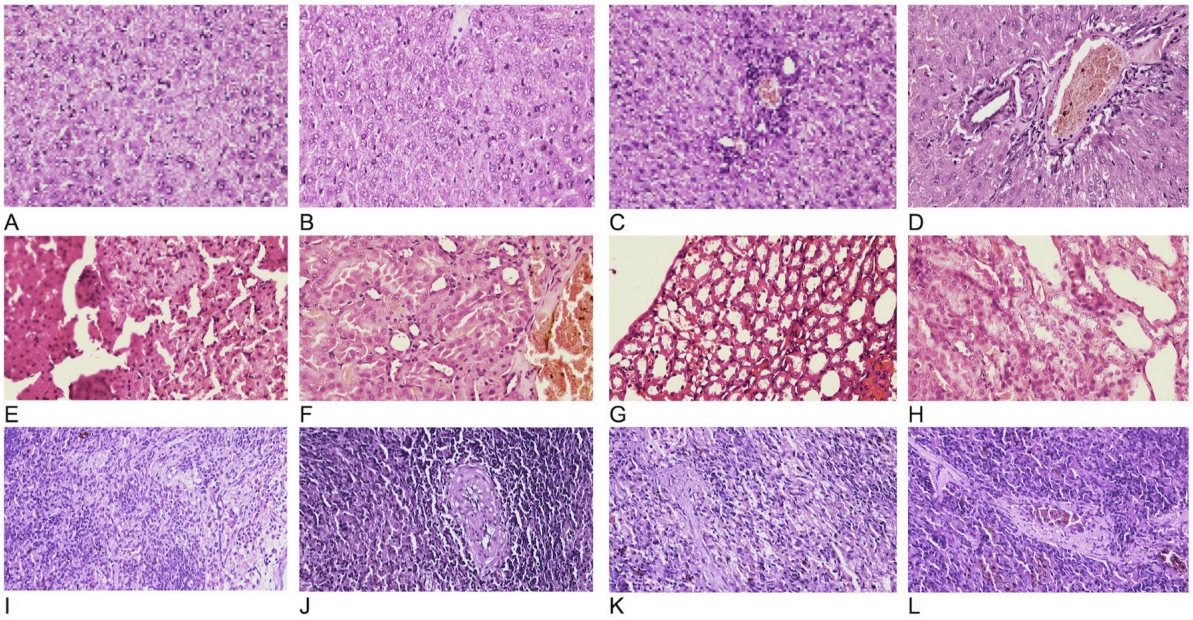

Figure 1. Microphotograph (HE 400x) after 28 days administration of *Parkia speciosa* pod extract: Liver (A-D), Kidney (E-H) and Spleen (I-L) of female and male from the highest dose group (800 mg/kg BW) and the control group respectively.

Histopathology Analysis

Histopathology observations were made on the liver, kidney, and spleen organs. No change was found in the lymph and kidney microphotograph. However, in the liver microphotograph, mild necrosis was found, especially in the male and female of the highest dose groups [Figure 1].

DISCUSSION

The oral acute toxicity are needed to estimate the toxicity of a substance and to determine the LD_{50} value [18]. The extract showed a wide range of safety because there was no mortality at doses up to 5000 mg/kg BW. Therefore, the LD_{50} extract was set more than 5000 mg/kg BW. Based on the WHO classification, *Parkia* pod ethanol extract is included in the slightly toxic category (Class 5) [23]. However, acute toxicity analysis cannot show the cumulative toxic effects of a substance. Therefore, to evaluate the safety of a substance, sub chronic analysis are required [21, 24].

There were no mortalities or toxic symptoms from observation results during the 28-day administration of the test substance. It indicates the ethanol extract of the Parkia pod not affecting the function of the central nervous, gastrointestinal, and autonomic nervous of the rats. Salivation, diarrhea, and urination were associated with the toxic symptoms on the autonomic nerves. Meanwhile, toxic symptoms on the neuromuscular system were indicated by a decrease or an increase of rat activities and occurring tremors. Moreover, gasping was correlated with toxic signs on respiratory system. While constipation or diarrhea was correlated with toxic signs on gastrointestinal system [23].

Body weight is an important initial indicator in toxicity analysis [25]. A change in rat body weight is an indication of important physiological changes, such as hormonal changes or failure to absorb dietary components [26]. Chemical substances contained in the plant can affect the metabolism and catabolism of the body resulting in weight loss. The decreases in body weight is thus an early indication of the toxic effects of a substance [21, 27]. In contrast, the increase in body weight in this study indicated that the *Parkia* pod ethanol extracts did not influence normal metabolism, hormones, and animal growth.

Hematology parameters were analyzed to understand extract effects on the rat's blood [28]. Chemical content of plants can influence the spine so that it can disturb blood cell production [21]. Any substance affecting the bone marrow caused an abnormal synthesis of blood cells [21]. Hematology parameters play important rules for assessing the toxic effects on the test substances. In addition hematopoietic system is the most sensitive target for a toxic compound. Variations of these parameters can indicate toxicity related to various diseases, such as anemia, leukemia, inflammation and infection reaction [29, 30]. Leukocyte (WBC) is the first line of defense of the haemopoietic system to fight infection while erythrocyte (RBC) is important for erythropoiesis and morphology of red blood cells [29]. The WBC counts of female group were significantly different from those of the control group. However, only the 800 mg/kg BW group was not included in the normal range (7-14 x 10^3/µL) [31]. Based on this study, increases in RBC, WBC, neutrophils, and monocytes after 28 days administration indicated that the extract was not toxic. It means that the extract did not affect the red blood cells circulation, haematopoiesis and leucopoiesis [32]. This finding also support the study of the *Parkia* pod ethanol extracts stimulating body's immune system [16]. Even though there were differences in MCV, MCH and MCHC values, these values were still within the normal range [31] and only occurring in several groups. In general there was no significant difference in MCV, MCHC and MCH, indicating that the extract did not affect cell circulation in experimental rats [30]. Platelets play an important role in homeostasis. These cellular fragments are required to prevent vascular leakage through the formation of platelet plugs during injury. No alteration in a platelet value indicated that the extract did not show a thrombotic activity [21].

The liver and kidney are two organs that play an important role in the detoxification process [33]. The assessment of both organs is a vital index to evaluate the toxicity of drug and plant extract which is possible to change renal and hepatic functions due to extracts administration [29, 34]. Although AST and ALT of test groups were not significantly different from those of the control group, they tend to decrease in the test groups. The decrease in AST and ALT levels indicated an ability of the extract to improve liver function [33, 35]. However, the results of histopathology analysis showed hepatocytes and mild necrosis presented in the liver, especially in the 800 mg/kg BW dose test group. This means that the extract caused a liver damage at high doses. Glucose level in the blood of experimental rats not changing indicated that the extract did not cause hyperglycemia or hypoglycemia. Some studies reported an anti diabetic activity from various parts of the *Parkia speciosa* plant [1, 2, 14].

In addition, several serum biochemical parameters of the experimental group slightly increased or decreased compared to those of the control group although they were insignificant. Total protein levels of the test groups tend to be lower than those of the control group, while the triglyceride levels tend to be higher than the control group, especially on 800 mg/kg BW dose. A decrease in total protein and an increase in triglyceride levels were related to reduction of a liver synthetic function or possibly due to an impaired hepatocellular function [27, 36].

Urea and creatinine are important parameters in the evaluation of kidney function [33]. There was no significant difference between creatinine and urea levels in the male groups indicating that a renal function of the experimental rats was normal. Significantly different and lower levels of urea of the female test groups compared to those of the control groups indicated that the renal function was not affected by the extract. This finding is supported by the results of renal histopathology analysis showing a normal microphotograph. Decreases in the creatinine and urea levels indicate that the extract can improve a kidney function. The results can strengthen a rationally traditional use of *Parkia speciosa* for treatments of a kidney failure [3].

A calculated relative organ weight is more indicative than an absolute organ weight to indicate a toxic effect [37]. A relative organ weight has reflected pathological changes in damaged organs [38]. There was no significant difference in relative organ weight on liver, heart, and kidney organs. In addition, morphological alteration was not found. However, microphotography observations found hepatocyte cells indicating mild necrosis in the liver organs with highest dose administered.

CONCLUSION

The main finding of this study revealed that the Parkia pod ethanol extract has relatively low toxic with the value of LD_{50} more than 5000 mg/kg BW. Repeated administration generally did not alter hematology and biochemical parameter profiles as well as the morphology of the main organ. However, the long-term administration with high doses is not recommended due to its effect causing mild liver necrosis.

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Conflicts of Interest:
The authors report no conflict of interest.

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REFERENCES

1. Kamisah Y, Othman F, Qodriyah H, Jaarin K. Parkia speciosa Hassk.: A potential phytomedicine. Evidence-based Complement Altern Med. 2013; 2013: 1-9.
2. Azliza MA, Ong HC, Vikineswary S, Noorlidah A, Haron NW. Ethno-medicinal resources used by the Temuan in Ulu Kuang village. Stud Ethno-Medicine. 2012; 6(1): 17–22.
3. Samuel AJSJ, Kalusalingam A, Chellappan DK, Gopinath R, Radhamani S, Husain HA, et al. Ethnomedical survey of plants used by the Orang Asli in Kampung Bawong, Perak, West Malaysia. J Ethnobiol Ethnomed. 2010; 6(5): 1-6.
4. Milow P, Ghazali NH, Mohammad NS, Ong HC. Characterization of plant resource at Kampung Parit Tok Ngah, Perak, Malaysia. Sci Res Essays. 2011; 6(13): 2606–2618.
5. Ko HJ, Ang LH, Ng LT. Antioxidant activities and polyphenolic constituents of bitter bean Parkia speciosa. Int J Food Prop. 2014; 17(9): 1977–1986.
6. Gmelin R, Susilo R, Fenwick GR. Cyclic polysulphides from Parkia speciosa. Phytochemistry. 1981; 20(11): 2521–2523.
7. Ghasemzadeh A, Jaafar HZE, Bukhori MFM, Rahmat MH, Rahmat A. Assessment and comparison of phytochemical constituents and biological activities of bitter bean (Parkia speciosa Hassk.) collected from different locations in Malaysia. Chem Cent J. 2018; 12(1): 1–9.
8. Uyub AM, Azlan A, Fariza SS, Nwachukwu IN. In-vitro antibacterial activity and cytotoxicity of selected medicinal plant extracts from Penang Island Malaysia on some pathogenic bacteria. Ethnobot Res Appl. 2010; 8: 95–106.
9. Al-Batran R, Al-Bayaty F, Al-Obaidi MMJ, Abdualkader AM, Hadi HA, Ali HM, et al. In Vivo Antioxidant and Antiulcer Activity of Parkia speciosa Ethanolic Leaf Extract against Ethanol-Induced Gastric Ulcer in Rats. PLoS One. 2013; 8(5): e64751.
10. Maria MS, Devarakonda S, Kumar AT, Balakrishnan N. Anti Ulcer Activity of Ethanol Extract of Parkia Speciosa againts indomethacin induced peptic ulcer in Albino rats. Int J Pharm Sci Res. 2015; 6(2): 895–902.
11. Siow LH, Gan CY. Extraction of antioxidative and antihypertensive bioactive peptides from Parkia speciosa seeds. Food Chem. 2013; 141(4): 3435–3442.
12. Tangkanakul P, Trakoontivakorn G. Extracts of Thai Indigenous Vegetables as Rancid Inhibitor in a Model System. Kasetsart J. 2005; 39(2): 274–283.
13. Aisha AFA, Abu-Salah KM, Alrokayan SA, Ismail Z, Abdul Majid AMS. Evaluation of antiangiogenic and antoxidant properties of Parkia speciosa Hassk extracts. Pak J Pharm Sci. 2012; 25(1): 7–14.
14. Jin CB, Noor H. The hypoglycemic effect of aqueous seed extract of Parkia speciosa on rats. J Trap Med Plants. 2014; 9(May): 16–20.
15. Mustafa N h., Ugusman A, Jalil J, Kamisah Y. Anti-inflammatory property of Parkia speciosa empty

pod extract in human umbilical vein endothelial cells. J Appl Pharm Sci. 2018; 8(1): 152–158.
16. Fitrya F, Amriani A, Novita RP, Elfita, Setiorini D. Immunomodulatory effect of Parkia speciosa Hassk. pods extract on rat induced by Salmonella typhimurium. J Pharm Pharmacogn Res. 2020; 8(5): 457–465.
17. Zaini N, Mustaffa F. Review: Parkia speciosa as Valuable, Miracle of Nature. Asian J Med Heal. 2017; 2(3): 1–9.
18. OECD: Organization for Economic Cooperation and Development. OECD Guidance Document On Acute Oral Toxicity Testing. 2001. p. 8–13.
19. BPOM Republik Indonesia. Peraturan Kepala Badan Pengawas Obat dan Makanan. 2014; 7: 34–37.
20. OECD: Organization for Economic Cooperation and Development. Repeated Dose-28 Day Oral Toxicity Study in Rodents. OECD Guid Test Chem. 2008; 407(October): 1–13.
21. Musila MN, Ngai DN, Mbiri JW, Njagi SM, Mbinda WM, Ngugi MP. Acute and Sub-Chronic Oral Toxicity Study of Methanolic Extract of Caesalpinia volkensii (Harms). J Drug Metab Toxicol. 2017; 08(01): 1–8.
22. Peng KZ, Zhang SY, Zhou HL. Toxicological evaluation of the flavonoid-rich extract from Maydis stigma: Subchronic toxicity and genotoxicity studies in mice. J Ethnopharmacol. 2016; 192: 161–169.
23. Lu FC, Sam K. Lu's Basic Toxicology. Fourth. London and New York: Taylor & Frances; 2003.p. 98–99.
24. Aniagu SO, Nwinyi FC, Akumka DD, Ajoku GA, Dzarma S, Izebe KS, et al. Toxicity studies in rats fed nature cure bitters. African J Biotechnol. 2005; 4(1): 72–78.
25. Rojas-Armas J, Arroyo-Acevedo J, Ortiz-Sánchez M, Palomino-Pacheco M, Castro-Luna A, Ramos-Cevallos N, et al. Acute and repeated 28-day oral dose toxicity studies of Thymus vulgaris L. essential oil in rats. Toxicol Res. 2019; 35(3): 225–232.
26. Lee MY, Shin IS, Seo CS, Kim JH, Han SR, Shin HK. Subchronic oral toxicity studies of the traditional herbal formula Bangpungtongseong-san in Crl: CD (SD) rats. J Ethnopharmacol. 2012; 144(3): 720–725.
27. Yuet Ping K, Darah I, Chen Y, Sreeramanan S, Sasidharan S. Acute and subchronic toxicity study of euphorbia hirta L. methanol extract in rats. Biomed Res Int. 2013; 2013.
28. Zhang Y, Guan E, Zhao X, Wang B, Yin L, Zhang L, et al. A subchronic toxicity study of ethanol root extract of baked Aconitum flavum in rats. Rev Bras Farmacogn. 2016; 26(4): 438–445.
29. Porwal M, Khan NA, Maheshwari KK. Evaluation of acute and subacute oral toxicity induced by ethanolic extract of marsdenia tenacissima leaves in experimental rats. Sci Pharm. 2017; 85(3): 1-11.
30. Sutrisni NNW, Soewandhi SN, Adnyana IK, Sasongko LDN. Acute and subchronic (28-day) oral toxicity studies on the film formulation of k-carrageenan and konjac glucomannan for soft capsule application. Sci Pharm. 2019; 87(9): 1–12.
31. Derelanko MJ, Hollinger MA. Handbook of toxicology: Second edition. Second. Handbook of Toxicology, Second Edition. Florida, USA: CRC Press LLC; 2001. pp. 55-57.
32. Janaki B, Sashidhar RB. Subchronic (90-day) toxicity study in rats fed gum kondagogu (Cochlospermum gossypium). Food Chem Toxicol. 2000; 38(6): 523–534.
33. Koumba Madingou NO, Traore A, Souza A, Boukandou Mounanga MM, Aworet Samseny RR, Ouedraogo S, et al. Preliminary studies of acute and sub-chronic toxicity of the aqueous extract of Guibourtia tessmannii (Harms) J. Leonard stem barks (Caesalpiniaceae) in mice and rats. Asian Pac J Trop Biomed. 2016; 6(6): 506–510.
34. Tagne Fokam MA, Noubissi PA, Kamgang R. Acute and Subchronic Oral Toxicity Studies of an Ethanol/Water Extract of *Euphorbia scordifolia* Jacq (Euphorbiaceae) in Mice and in Rats. Int J Pharmacol Phytochem Ethnomedicine. 2017; 7:18–29.
35. Awodele O, Oreagba IA, Odoma S, Teixeira Da Silva JA, Osunkalu VO. Toxicological evaluation of the aqueous leaf extract of Moringa oleifera Lam. (Moringaceae). J Ethnopharmacol. 2012; 139(2): 330–336.
36. Harizal SN, Mansor SM, Hasnan J, Tharakan JKJ, Abdullah J. Acute toxicity study of the standardized methanolic extract of Mitragyna speciosa Korth in Rodent. J Ethnopharmacol. 2010; 131(2): 404–409.
37. Demma J, Gebre-Mariam T, Asres K, Ergetie W, Engidawork E. Toxicological study on Glinus lotoides: A traditionally used taenicidal herb in Ethiopia. J Ethnopharmacol. 2007; 111(3): 451–457.
38. Li IC, Chen YL, Lee LY, Chen WP, Tsai YT, Chen CC, et al. Evaluation of the toxicological safety of erinacine A-enriched Hericium erinaceus in a 28-day oral feeding study in Sprague-Dawley rats. Food Chem Toxicol. 2014; 70: 61–67.