Artikel Fitrya IJPR 2021

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Submission date: 10-Apr-2021 09:34PM (UTC+0700)
Submission ID: 1555386041
File name: Fitrya_manuscript.doc (1.43M)
Word count: 4996
Character count: 26055

Toxicological Assessment Of *Parkia Speciosa* Pods Ethanol Extract: The Acute And Sub-Chronic Oral Toxicity Test On Experimental Animal

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.

Key word : *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 potential 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 that prove the toxicological effect of this plant (1,17). Therefore, this study aims to determine the toxicological assessment of the *Parkia speciosa* pods ethanol extract on a single dose and multiple dose for a period of 28 days.

Materials and Methods

Plant material

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

Ethanol crude extract preparation

The pods were separated from the seeds. The pods then oven-dried at 40 ^{o}C for 2 days. The dry sample was powdered by using an electric mill. *Parkia* pods 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 with constant weight was obtained. The *Parkia* pods ethanol extract (PEE) was stored at a temperature of 4°C before being used for experiment.

Animal

The animal utilized in this study were male and female wistar rats (150-200 g). All procedures for animal experimental were approved by the Ethics Committee of the Faculty of Medicine, Sriwijaya University decree no: 068/kepkrsmhfkunsri/2020. The rats were housed at 22^{o}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 rat 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. If there was no mortality or the appearance of toxic symptoms in the animal, the test would be 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 analisys

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, reticulocyte. The routine hematology analysis was performed by using the hematology analyzer (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 was analyzed by using Clinical Chemistry Analyzer BioSystem A15 (England). The analysis of biochemical parameters including 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 then weighed. Relative organ weights were calculated by using formula: Relative Organ Weight (%) = organ weight / rat body weight x 100 (22). Then the organs of liver, kidney and spleen were 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%.

Result

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].

Sub chronic toxicity analysis

Body weight changes and toxic symptoms

There were no mortality in experimental rats were administered extract of Parkia 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].

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.

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 data are needed to estimate the toxicity of a substance and determine the LD_{50} value (18). The extract showed a fairly wide safety range because there were no mortality up to 5000 mg/kg BW. Therefore, the LD_{50} value of extract is set above 5000 mg/kg BW. Based on the WHO classification, *Parkia* pods 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 profile of a substance, sub chronic analysis are required (21,24).

Results of observations during the 28-day administration of the test substance, there were no mortality nor toxic symptoms which indicate the ethanol extract of Parkia pods did not affect the function of the central nervous, gastrointestinal, and autonomic nervous of the rats. Salivation, diarrhea, and urination associated with the toxic symptoms on the autonomic nerves. Meanwhile, toxic symptoms on the neuromuscular system were indicated by decreased or increased activity and tremors. Moreover, gasping and constipation or diarrhea correlated with toxic signs on respiratory system and gastrointestinal system, respectively (23).

Body weight is an important initial indicator in toxicity analysis (25). The decrease or increase in rat body weight is an indication of important physiological changes, such as hormonal changes or failure to absorb dietary components such as protein, amino acids, and others (26). The chemicals contained in the plant can affect the metabolism and catabolism of the body resulting in weight loss, thus the decrease in weight is an early indication of the toxic effects of a substance (21,27). The increase in body weight of the experimental rats in this study was an indicate that the *Parkia* pods ethanol extracts did not influence normal metabolism, hormones and animal growth.

Hematology parameters analysis aims to determine the extent to which the extract influence the rat's blood (28). Chemical content of plants can influence the spine so that it can interfere with blood cell production (21). Any substance that affects the bone marrow can interfere with the synthesis of blood cells (21). Hematology parameters analysis is important in assessing the toxic effects of the test substances because hematopoietic system is the most sensitive target for the toxic compound. Variations of these parameters can indicate toxicity related to various diseases and conditions, 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). Although the WBC counts of female group significantly different from the control group but only the 800 mg/kg group was not included in the normal range (7-14x$10^3/\mu$L) (31). Based on this research, the increase in the value of RBC, WBC, neutrophils and monocytes after 28 days indicate that the extract is not toxic, because it does not affect the red blood cells circulation, haematopoiesis and leucopoiesis, or otherwise which may cause anemia (32). This increases also corroborate that the *Parkia* pods ethanol extracts have the ability to stimulate 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 occurred 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 platelets value indicated that the extract did not show thrombotic activity (21).

The liver and kidney are two organs that play an important role in the detoxification process (33). The assessment of both organ is vital index to evaluate drug and plant extracts toxicity on the possibility of changes in renal and hepatic function due to extracts administration (29,34). Although it was not significantly different from the control group,

there was a tendency of the decrease of AST and ALT level in test group compared to the control group. The decrease of AST and ALT levels indicate the 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 liver damage at high doses. No alteration in glucose level in the blood experimental rats indicate that the extract did not cause hyperglycemia or hypoglycemia. Some study reporting an anti diabetic activity from various parts of *Parkia* plant (1,2,14).

In addition, several serum biochemical parameters showed a slight increase or decrease in the experimental group compared to the control group although not significant. Total Protein levels tend to be lower than the control group, 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 are signs of reduced liver synthetic function or possibly due to 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 group indicating that the renal function of the experimental rats was normal. As for the female group, although significantly different, the urea levels were lower than the control group, this indicates that renal function was not affected. This is supported by the results of renal histopathology analysis of which showed normal microphotograph. The decreased levels of creatinine and urea compared to the control group indicate that the extract improve kidney function. The results of this research can strengthen the rationale of the traditionally use of *Parkia* seeds to treat kidney failure (3).

In this study, the calculated relative organ weight tap a is more indicative than the absolute organ weight (37). Relative organ weight values have been shown to reflect pathological changes in damaged organs (38). There was no significant difference in relative organ weight on liver, heart and kidney organs and no morphological alteration were found. However, microphotograph observations found hepatocyte cells indicating mild necrosis in the liver organs of the group with highest dose administered.

Conclusion

Based on the results of the research, it was found that the Parkia pods ethanol extract has relatively low toxic with the value of $LD_{50} > 5000$ mg/kg BW. Repeated administration generally did not alter hematology and biochemical parameter profiles as well as the

morphology of the main organ but the long-term administration of high doses is not recommended because it can cause mild liver necrosis.

Acknowledgments

Author would like to thank you to the Research and Community Service Institution of Sriwijaya University for research funding through DIPA of Public service Agency of Universitas Sriwijaya 2020. [0685/UN9/SK.BUK.KP/2020]

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Table 1 : Effect of *Parkia speciosa* pods ethanol 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

Table 2. Effect of *Parkia speciosa* pods ethanol extract on the body weight of rats after 4 weeks 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 ± 0,1	144,6 ± 0,93	162,91 ± 0,55	170,71 ± 0,95	174,55 ± 1,49
	400	133,78 ± 2,92	148,9 ± 2,8	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 ± 1,8
	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 Parkia speciosa pods ethanol extract on hematological parameters after 28 days administration (n=5)

Parameters	Sex	Groups				
		Control	100 mg/kg BW	200 mg/kg BW	400 mg/kg BW	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±1,2
	Female	11,50 ± 0,17	12,87 ± 1,71	13,57 ± 1,53	13,17 ± 0,99	13,4±0,9
RBC (10^6/µL)	Male	8,33 ± 1,5	6,50 ± 0,3	7,63 ± 0,95	8,6 ± 0,61	8,91 ± 0,89
	Female	6,40 ± 0,26	7,03 ± 0,76	7,77 ± 0,75	7,40 ± 0,7	7,6 ± 0,5
WBC (10^3/µL)	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 ± 3,8	11,90 ± 2,46*	13,47 ± 0,84*	18,9 ± 3,3*
Neutrophils (%)	Male	12,0±3,0	13,67 ± 3,78	18,33 ± 7,77	20 ± 7,21	30,33 ± 13,65
	Female	14,67 ± 5,86	9,67 ± 5,69	26,67 ± 7,64*	27,33 ± 5,77*	21,5 ± 1,5*
Lymphocytes (%)	Male	61,67±9,71	58,33±6,5	73±2,12	72±4,36	84,5±1,5 *
	Female	69±3,6	70,67±13,8	65±6,24	60±9,54	89,5±1,5*
Monocytes (%)	Male	5,6 ± 1,4	5,75 ± 0,66	4,67 ± 1,51	5,83 ± 1,89	4,75 ± 0,75
	Female	5,7 ± 0,26	5,63 ± 1,38	5,4 ± 0,53	5,17 ± 1,04	4,5 ± 2,5
Haematocrit (%)	Male	45,33 ± 8,14	37,67 ± 2,89	46,33 ± 5,51	46,67 ± 3,21	50 ± 3
	Female	35 ± 0	40,33 ± 5,13	42,33 ± 4,62	41,67 ± 3,78	44,5 ± 3,5
MCV (µm3)	Male	55 ± 1,73	58 ± 2*	60,67 ± 3,05*	54 ± 1	57 ± 2
	Female	55,33 ± 2,08	57,67 ± 1,53	54,33 ± 1,15	56,67 ± 0,58	58 ± 1
MCH (pg)	Male	17,67 ± 0,58	19 ± 0*	18,67 ± 1,15	17,33 ± 0,58	17 ± 0
	Female	18 ± 1	18,33 ± 0,58	17,67 ± 0,58	18,33 ± 0,58	18 ± 0

MCHC (g/L)	Male	318,33 ± 5,51	321,67 ± 10,69	309 ± 5,29	315,33 ± 5,13	300,5 ± 6,5*
	Female	327,33 ± 5,69	319,67 ± 1,15*	320,67 ± 3,51*	316,67 ± 8,08*	303 ± 3*
Reticulocytes (%)	Male	16,67 ± 3,51	15,67 ± 3,21	21,67 ± 5,43	20 ± 3,46	14 ± 2
	Female	14,33 ± 2,08	17,67 ± 6,66	18,67 ± 1,53	24,67 ± 6,66	27 ± 2
Platelets (10^3/µL)	Male	600 ± 22,89	275,67 ± 10,73	438,67 ± 22,79	659,67 ± 28,73	667,5 ± 25,5
	Female	244,67 ± 21,03	469,33 ± 26,17	573,84±13,09	679,67 ± 20,1	529 ± 25

*Value expressed as mean ± SD, value with superscript * significantly different from control group (p<0,05)*

RBC = Red Blood Cells, WBC = White Blood Cells, MCV=Mean cell volume, MCH= Mean corpuscular haemoglobin, MCHC= Mean corpuscular haemoglobin concentration

Table 4 : Effect of *Parkia speciosa* pods ethanol extract on biochemical parameters after 28 days 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±3,9	60,78 ± 4,50	62,98±4,53	97,97 ± 9,08
Total Protein (g/L)	Male	82 ± 8,72	77,33 ± 7,37	76 ± 1,73	75 ± 5,29	79 ± 3
	Female	90,67 ± 7,77	88,67 ± 7,5	83, 67 ± 5,51	84,33 ± 7,5	79 ± 3
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	113,82±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	5,9±0,2
	Female	5,85 ± 0,51	5,61 ± 0,12	5,74 ± 0,45	5,41 ± 0,22	5,80± 0,1
Urea (mg/dL)	Male	43,63 ± 4,98	45,07 ± 3,14	41,4 ± 5,77	35,90 ± 0,90	38,26 ± 0,6
	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	0,40 ± 0,1	0,43 ± 0,06	0,46 ± 0,0

*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 days of *Parkia speciosa* pods ethanol 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 ± 1,2	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 ± 0,4	3,38 ± 0,16	0,44 ± 0,02	0,40 ± 0,01	0,86 ± 0,06	0,8 ± 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* pods extract: Liver (A-D), Kidney (E-H) and Spleen (I-L) of female and male the highest dose group (800 mg/kg BW) and the control group respectively.

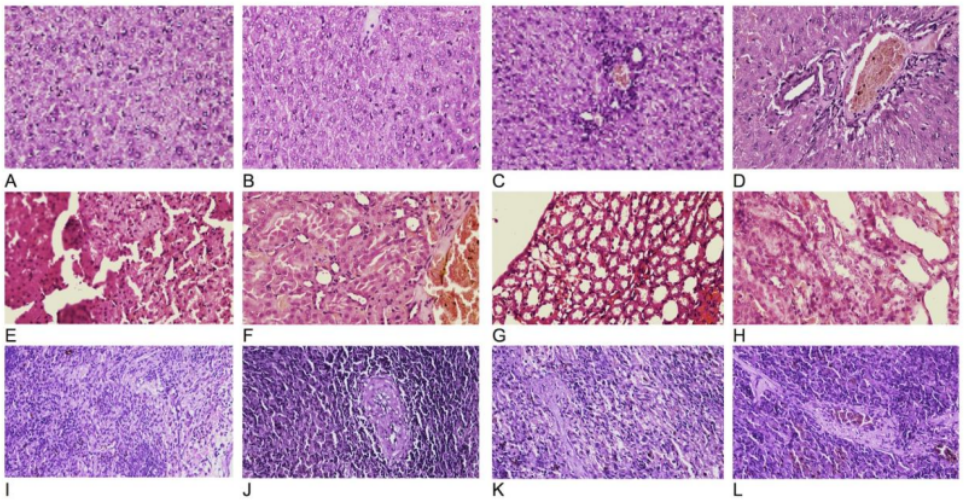

Figure 1.

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