

Acute and Subchronic Toxicity Assessment of 70% Ethanol Extract of Gendarusa Leaves In Vivo

by Retno Widyowati

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Acute and Subchronic Toxicity Assessment of 70% Ethanol Extract of Gendarusa Leaves *In Vivo*

Luthfiatu Kanina¹, Mabluhatus Solehah¹, Hani Plumeriastuti², Retno Widyowati³, Bambang Prajogo Eko Wardoyo^{3*}

¹Master Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

²Department of Veterinary Pathology, Faculty of Veterinary, Universitas Airlangga, Surabaya, Indonesia

³Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

*Corresponding author: prajogo_ew@ff.unair.ac.id

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Abstract

Background: *Justicia gendarussa* Burm. f., has been used traditionally in Indonesia for antifertility. Nowadays, a capsule containing 70% ethanol extract of *J. gendarussa* leaves has been studied for safety. **Objective:** This study aimed to determine the acute and sub-chronic toxicity of 70% ethanol extract of *J. gendarussa* leaves *in vivo*. **Methods:** In the acute toxicity study, a single dose of 2,000 mg/Kg BW was orally administered to mice ($n = 10$), which were monitored for 24 days. For the subchronic toxicity study, rats were randomly divided into four groups ($n = 10$). The control group received distilled water, while the treatment groups received a repeated dose of 40, 200, and 1,000 mg/Kg BW orally for 90 days. Blood samples were collected for hematological and biochemical evaluations. Gross pathology and histopathology of liver and kidneys were assessed. **Results:** No mortality and non-observed adverse effect level (NOAEL) were observed in the acute toxicity study. The hematological analysis did not show significant differences in the subchronic toxicity study. The SPGT, SGOT, and creatinine values showed no change in groups 2 and 4, but the level of SGPT increased in groups 3. The increasing level of BUN was observed in all treated groups. Abnormalities or histopathological changes were observed in the liver and kidney in groups 3 and 4. **Conclusions:** Using 70% ethanol extract of *J. gendarussa* leaves at a therapeutic dose is safer, but it needs attention at a higher dose.

Keywords: acute toxicity, *Justicia gendarussa* Burm. f., sub-chronic toxicity

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INTRODUCTION

Justicia gendarussa Burm.f. (JG), commonly known as Gendarusa, can be found in Indonesia, Malaysia, Sri Lanka, and India (Heyne, 1987). In Papua, Indonesia, JG is said to be a traditional male antifertility medicine (Moeso & Agus, 1985). *In vitro* and *in vivo* antifertility studies of JG n-butanol fractions reveal a competitive and reversible suppression of the spermatozoa hyaluronidase enzyme (Prajogo *et al.*, 2009). The presence of flavonoid molecules, particularly C-glycosyl flavone groups with an apigenin base structure, is responsible for the hyaluronidase enzyme's inhibitory activity. Apigenin and vitexin, a glycoside of apigenin, have anti-inflammatory and anticancer properties in JG (Prajogo *et al.*, 2008). As nonhormonal male contraception, JG natural medicine can be turned into a phytopharmaceutical product (Prajogo *et al.*, 2008).

According to an FDA (Food and Drug Administration) report in the Poisonous Plant Database (Plant List), the JG plant is one of the potentially poisonous plants (Solehah, 2018). Based on previous research, the acute toxicity test using the water phase and ethanol phase of 60% JG leaves in rats shows relatively harmless results (Ekaputri, 2015). In addition, the subacute toxicity test in rabbits does not affect the liver and kidney function parameters. The water and ethanol phases of 60% JG leave also do not have a teratogenic effect on rats. Even the water phase does not have a carcinogenic effect, but it does not provide histopathological changes in rats' testes, liver, kidneys, intestines, and lungs. However, giving the water phase for 52 days can cause changes in liver histopathology and erosion of the small intestine mucosa but does not show differences in kidney histopathology. Therefore, before being used as a medicine, further research is needed regarding the subchronic toxicity of this plant using ethanolic solvent (Ekaputri, 2015).

MATERIALS AND METHODS

This study was designed through the Animal Care and Use Committee (ACUC), Faculty of Veterinary Universitas Airlangga, with the reference number of 715-KE.

Materials

The 70% ethanol extract of gendarusa leaves was obtained from a series of processes in all parts of the *Justicia gendarussa* Burm. F leaves from Gempol, Pasuruan that was identified by Indah Yulia Ningsih with number herbarium 01. Na citrate, 70% ethanol, CMC Na, aquadest, acetonitrile, and methanol were

used as chemical materials. Male Balb/C mice were used as experimental animals weighing 15 - 30 grams and aged 6 - 8 weeks, and male Wistar rats weighing between 150 - 300 grams and 6 - 8 weeks old were used as experimental animals. Variation in body weight did not exceed 20% of the average body weight.

Preparation of dried leaves

JG leaves that were nine months old were harvested. Fresh leaves were sorted to separate them from impurities or foreign materials such as soil, gravel, grass, unnecessary plant parts, etc. After sorting, they were washed and then dried in the oven at a temperature of 50°C. Subsequently, the leaves were made into powder by grinding.

Preparation of alkaloid free dried leaves

The JG dried leaves were acidified with a solution of citric acid with a pH of 3. Acidification was carried out for 3 x 24 hours (with stirring) and then washed with aquadest until the pH was neutral. Then, it was dried at 50°C until the drying shrinkage reached 10%.

Preparation of 70% ethanolic extract

Alkaloid free dried leaves were extracted with 70% ethanol for 12 hours without heating by maceration technique. This process was repeated three times and the filtrate obtained from each process was collected. The filtrate was then concentrated until reaching a total solid of $\geq 90\%$.

Preparation of animal models

The animal models used in the acute and sub-chronic toxicity test evaluations were male mice and rats. Before treatment, both were adapted to the environment for one week. They were kept in the same way and had the same diet. Then, they were weighed to calculate the dosage setting. The animal models were randomized in such a way that the distribution of body weight was evenly distributed across groups with variations in body weight not exceeding 20% of the mean of body weight. Their body weights were measured before, during, and after treatment. Monitoring of weight gain was done twice a week.

Preparation of CMC Na compound

The administration of each dose was in the form of an extract suspended in 0.5% CMC Na. The negative control was given 0.5% CMC Na. Preparation of 0.5% CMC Na was by weighing 0.5 grams of CMC Na, sprinkled over 20 times of hot water, allowed to expand (± 15 minutes), and crushed until it formed mucilage. Then, it was transferred to a bottle calibrated and added water to 100 ml. Afterwards it was given to the control group orally.

Acute toxicity test

Dose of acute toxicity test

The dosing method used for the acute toxicity test was the fixed-dose method, namely the preliminary, main, and limit tests. In the preliminary test, the fixed doses selected were 5, 50, 300, and 2,000 mg/Kg BW as doses expected as toxic effects. The limit dose of this experiment was 2,000 mg/Kg BW. Previous research on LD₅₀ oral administration of 60% ethanol fraction and water fraction of JC leaves in mice was 17.82630 g/Kg BW and 15.63389 g/Kg BW (Ekaputri, 2015) the primary test was carried out at a dose rate of 2000 mg/Kg BW.

Provision of test preparations and volume of administration

Two groups of mice were prepared, in which each group consisted of 10 mice. Each experimental animal was given JC extract for the treatment group according to its respective weight. Before the treatment, the mice were fasting for 3 - 4 hours; after that, drinking water could be given. The administration was administered orally once and then observed for 24 hours and continued for 14 days. Control group was given 0.5% CMC Na suspension. Treatment was given JC extract, where one mouse was for the preliminary test and the other four were for the main test. The volume of fluid given was 2 mL/100 g BW.

Observation of animal behavior

The test animals were observed individually for at least the first 30 minutes after administration of the test preparation, periodically every four hours for 24 hours, and once a day for 14 days. The development and remission of toxicity symptoms were thoroughly recorded in separate records for each animal (particularly if there was a propensity for delayed toxic indications). If the animal had poisoning symptoms on a regular basis, additional observations were made. Skin, hair, eyes, mucous membranes, the respiratory system, the autonomic nervous system, central nervous system, somatomotor activity, and behavior were all observed. Furthermore, the following circumstances were observed: shaking, convulsions, salivation, diarrhea, weakness, drowsiness, and coma. The following items were observed during the observation period: animal behaviors, such as walking backwards and walking on the stomach, and animal body weight would be tracked at the time of the test preparation and for at least a week afterward.

Subchronic toxicity test

Dose of sub-chronic test

three dose groups and one control group were used. The doses chosen for subchronic toxicity tests were based on acute toxicity data and consideration of doses that had pharmacological effects. The highest dose of the test substance caused toxic effects but did not cause death or severe toxicity symptoms, medium doses caused milder toxic symptoms, while the lowest dose did not cause toxic symptoms (NOAEL).

Provision of test preparations and volume of administration

Four groups of rats were prepared, in which each group consisted of 10 rats. Each experimental animal was given JC extract according to their respective dosages for all groups except the control group. The administration was carried out orally (with a maximum volume of 1 - 2 mL of test preparation/100 g animal body weight) every three days a week for 90 days. During treatment, the toxic and clinical symptoms were observed every day. Meanwhile, weight monitoring was carried out twice a week. Animals were weighed daily to determine the volume of test preparation to be administered. After 90 days of treatment, the blood of each rat was taken intracardially. Then, the SGOT, SGPT, BUN, and creatinine levels were measured and complete blood profile testing by kinetic enzymatic methods based on IFCC (International Federation of Clinical Chemistry) (Sardini, 2007). In addition, the liver, kidneys, small intestine, lungs, lymph, and heart were also taken from rats for microscopic observation. From the SGOT, SGPT, BUN, and creatinine levels and microscopic observation of the liver, kidney, small intestine, lung, lymph, and heart, could be analyzed for the presence of organ damage or not. The following was each group of treatments: [I] Control group (group 1): it was given 0.5% CMC Na suspension. [II] Group 2: it was given JC extract at a dose of 40 mg/Kg BW. [III] Group 3: it was given JC extract at a dose of 200 mg/Kg BW. [IV] Group 4: it was given JC extract at a dose of 1000 mg/Kg BW. From the acute toxicity, it was known that LD₅₀ score was up to 2,000 mg/Kg BW (NOAEL) or based on OECD included in the fifth category.

Analysis of Enzyme SGOT, SGPT, BUN, and creatinin

All data obtained from the biochemical parameters were analyzed with ANOVA (One Way) at the 95% degree of confidence to determine whether there were significant differences between treatment groups.

Table 1. Body weight of mice in acute toxicity test

	Initial experiment	Body weight of mice (g)	
		7th day	24th day
Control group	28 ± 1	29 ± 1	31 ± 2
Test group dose (2000 mg/Kg BW)	28 ± 1	31 ± 1	32 ± 1

Table 2. Results of biochemical examination

	SGOT	SGPT	BUN	Creatinine
Group 1	131.9 ± 40.9	58.5 ± 18.9	20.7 ± 3.7	0.6 ± 0.2
Group 2	144.9 ± 35.5	65.4 ± 11.1	26.0 ± 2.4	0.6 ± 0.1
Group 3	173.4 ± 69.2	84.0 ± 40.9	24.2 ± 2.7	0.5 ± 0.2
Group 4	159.4 ± 34.4	61.5 ± 19.5	29.5 ± 2.5	0.6 ± 0.6

Note: average ± standard deviation

Blood draw

Blood was taken using a sterile syringe and always kept from being exposed to water (to avoid hemolysis). After the animals were anesthetized with ketamine solution, blood was taken from the heart slowly using a 3 m syringe, one syringe for one animal, and then put into a blood tube for further testing.

Execution of experimental animals

The experimental animals were executed by "dislocating the neck bones" to remove the liver, kidneys, small intestine, lungs, lymph, and heart. Furthermore, surgery was carried out to remove these organs. Then, the organs obtained were fixed with a buffer solution of 10% formalin to make histopathological preparations.

Histopathological preparations

Procedures for making histopathological preparations were based on BPOM regulations (Solehah, 2018).

Examination of histopathological preparations

Light microscopy was used to observe the rat organ preparations microscopically. At first, 100 times magnification was used and then a 400 magnification was used. The method of assessment was in the form of scoring (Sukardja, 1998).

Analysis of histopathological preparation data

The data on changes in the histopathological image of the rat organs that had been given a score were processed by ranking then analyzed using the Kruskal Wallis test as non-parametric statistical tests.

RESULTS AND DISCUSSION

Acute toxicity

The administration of 70% ethanol extract of JG leaves at a dose of 2,000 mg/Kg BW of the mice was found not to cause death. There were no toxic symptoms, such as standing hair, yellow eyes, and abnormal behaviors (not staying in one place and not biting certain body parts). From the acute toxicity, it was known that the LD₅₀ score was up to 2,000 mg/Kg BW
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(NOAEL). Table 1 describes the observation of the body weight of mice in the acute toxicity test.

Subchronic toxicity

In the subchronic toxicity test for 90 days of the experimental animals with 70% ethanol extract of JG leaves, it was found no mortality, behavioral observations, and signs of clinical toxicity, such as not causing changes in behavior (walking backward, biting certain body parts, yellowing eyes, and standing hair).

Biochemical data from the results of subchronic toxicity tests such as SGOT, SGPT, BUN, and creatinine be seen in Table 2. The p-value of the SGOT level (0.170) was greater than 0.05, indicating that there was no significant difference between treatment groups. On the other hand, the SGPT level p-value (0.039) was less than 0.05, showing a difference between treatment groups (group 2, 3, and 4). The 200 mg/Kg BW dosage group (group 3) was substantially different from the control group according to the post hoc test results. BUN level as kidney function measurement had a p-value of 0.000, which was less than 0.05, indicating a difference between treatment groups. The post hoc test revealed that the 200 mg/kg BW dosage group differed significantly from the control group (Table 3). Then hematology data from the results of subchronic toxicity test be seen in Table 4. There was no significant difference between treatment groups because the p-value for creatinine (0.176) was greater than 0.05. In general, the test groups' p-values of leukocytes and hematocrit were less than 0.05, which indicated the difference between treatment groups (Table 5). The difference in leukocytes was described in group 3 with p-value of 0.044, while the level of hematocrit was observed in group 4 (p = 0.039).

The histopathological changes were carried out using scoring referred to Arsad *et al.* (2014) and the scoring data of hepar and kidney are shown in Table 6. On the microscopic examination of the liver and kidneys, a dose of 40 mg/Kg BW (group 2) JC extract did not affect all histopathological parameters of the

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liver and kidneys, while a dose of 200 mg/Kg BW (group 3) and 1,000 mg/Kg BW (group 4), microscopic

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changes in the liver and kidneys of all histopathological parameters were shown at Figure 1 and 2.

Table 3. Results of biochemical statistical data processing of rat blood with SPSS

Parameter	Difference between treatment groups (p)	Difference between treatment groups(p)		
		1 & 2	1 & 3	1 & 4
SGOT	0.170	-	-	-
SGPT	0.039	0.330	0.012	0.232
BUN	0.000	0.002	0.083	0.000
Creatinine	0.176	-	-	-

Note : Control group (group 1): it was given 0.5% CMC Na suspension. Treatment group [I] Group 2: it was given JC extract at a dose of 40 mg/Kg BW. [II] Group 3: it was given JC extract at a dose of 200 mg/kg BW. [III] Group 4: it was given JC extract at a dose of o1000 mg/Kg BW

Table 4. Results of hematology examination

Parameter	Group 1	Group 2	Group 3	Group 4
HB	14.0 ± 1.1	14.1 ± 0.8	13.9 ± 0.8	13.5 ± 0.8
Leukocyte	17.8 ± 4.3	16.9 ± 2.1	17.4 ± 6.0	16.2 ± 2.9
Thrombocyte	1139 ± 162.5	1082.6 ± 151.7	948.8 ± 99.8	942.4 ± 212.2
Eosinophil	4.7 ± 1.2	6.8 ± 2.0	4.6 ± 1.2	5.3 ± 1.5
Basophil	0.7 ± 0.5	0.2 ± 0.4	0.2 ± 0.4	0.8 ± 0.6
Neutrophil	17.1 ± 7.3	14.2 ± 3.3	10.7 ± 4.3	16.0 ± 4.9
Lymphocyte	70.3 ± 6.6	70.4 ± 3.7	77.3 ± 5.1	71.5 ± 5.0
Monocyte	7.2 ± 3.5	8.4 ± 3.5	7.2 ± 1.4	6.4 ± 1.9
LED	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
Erythrocyte	8.6 ± 0.7	8.5 ± 0.42	8.3 ± 0.6	8.2 ± 0.4
Hematocyte	47.8 ± 3.8	45.7 ± 2.9	44.1 ± 3.0	43.4 ± 3.3
MCV	53.7 ± 2.5	52.5 ± 0.9	53.3 ± 2.1	53.2 ± 2.0
MCH	16.4 ± 0.8	16.7 ± 0.4	17.0 ± 0.6	16.4 ± 0.5
MCHC	30.8 ± 0.6	31.9 ± 0.6	31.7 ± 0.6	31.0 ± 0.6
G	54.5 ± 15.9	37.0 ± 17.8	65.5 ± 23.0	38.0 ± 19.9

Note : average ± standard deviation

Table 5. Results of hematology statistical data processing of rat hematology with SPSS

Parameter	Difference between treatment Group (p)	Difference between treatment groups(p)		
		1 & 2	1 & 3	1 & 4
Hemoglobin	0.444	-	-	-
Leukocyte	0.004	1.000	0.044	1.000
Thrombocyte	0.080	-	-	-
Erythrocyte	0.601	-	-	-
Hematocyte	0.033	0.295	0.121	0.039

Note: Control group (group 1): it was given 0.5% CMC Na suspension. Treatment group [I] Group 2: it was given JC extract at a dose of 40 mg/Kg BW. [II] Group 3: it was given JC extract at a dose of 200 mg/Kg BW. [III] Group 4: it was given JC extract at a dose of o1000 mg/Kg BW.

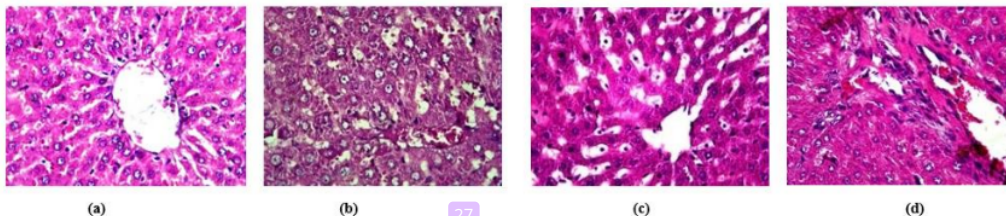
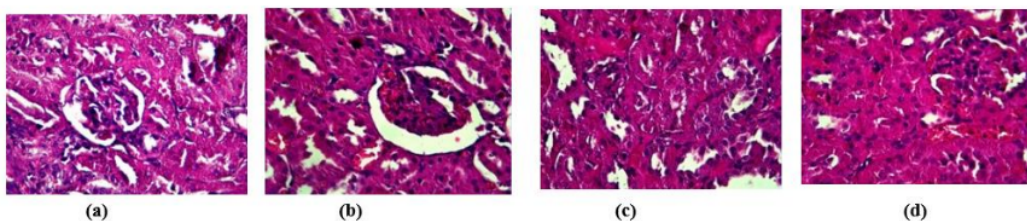


Figure 1. Tissue section of hepar (a) group 1, (b) group 2, (c) group 3, (d) group 4



Note: This picture represented certain of hepar and kidney tissue

Figure 2. Tissue Section of kidney (a) group 1, (b) group 2, (c) group 3, (d) group 4

Table 6. Scoring degree of change in the organ of rats

Organ	Observed change of each group (average)	Group			
		K-(group 1)	P1 (group 2)	P2 (group 3)	P3 (group 4)
Hepar	Activated kupper cells	0.60	0.78	1.02	1.50
	Sinusinoiddillatation	0.40	0.52	0.87	1.76
	Cytoplasm Vacuole	1.20	1.00	2.54	2.84
	Karyolysis	1.10	1.02	2.20	2.24
	Karyopicnotis	1.00	0.96	1.74	1.86
	Average	0.86	0.86	1.67	2.04
Kidney	Granular Cast	0.10	0.08	1.06	1.16
	Cellular Cast	0.10	0.10	1.00	1.16
	Protein Cast	0.20	0.18	0.88	0.50
	Pyknotic cells	0.60	0.62	0.90	1.26
	Hydropic degeneration	0.40	0.40	1.60	1.72
	Average	0.28	0.27	1.08	1.16

Note: Control group [K] (group 1): it was given 0.5% CMC Na suspension. Treatment group [P1] Group 2: it was given JC extract at a dose of 40 mg/Kg BW. [P2] Group 3: it was given JC extract at a dose of 200 mg/Kg BW. [P4] Group 4: it was given JC extract at a dose of 1000 mg/Kg BW

Discussion

In the acute toxicity test, there were no hazardous symptoms, and no animals died due to it. According to the findings, the 70% ethanol extract of JC was not harmful, thus, it may be claimed that these changes are not toxicologically important. As a result, this research demonstrates that JC extract does not produce acute toxicity at the dosage examined and with LD50 values more than 2,000 mg/Kg. The limit check methodology is intended to serve as a reference for identifying crude extracts based on the estimated dose stage at which the animal would live (Roopashree et al., 2009). Based on the categorization of acute system toxicity established by the OECD (Kennedy et al., 1986), the crude extract of JC extract was once assigned category 5 popularity (LD50 > 2000 mg/Kg), which was once the lowest toxicity class.

The median lethal dose is the dose needed to kill one or more tested populations in a group after a certain amount of test duration. According to Kennedy et al. (1986), extract for treatment or drug with an oral LD50 greater than 2,000 mg/Kg is considered safe or nearly harmless. Some medicinal plants have been studied and reported to be toxic to both humans and animals

(Kennedy et al., 1986). Therefore, it has to be emphasized that the standard use of any plant for medicinal purposes by no means ensures the protection of such plants. The facts that the acute and subchronic toxicity research on medicinal vegetation or preparations derived from it have to be bought to extend the self-belief in its protection to humans, particularly for the use in the improvement of prescribed pharmaceuticals (Ukwuani et al., 2012). Choosing the best test and dosing regimens showing a large margin of safety is crucial in planning human security. Because no hazardous outcomes were discovered during the acute toxicity investigation, a comparison was conducted to look at the subchronic toxicity of JC extract in rats for up to 90 days to compile the complete toxicology information for this ancient medicinal plant.

The oral subchronic toxicity test is used to detect hazardous effects when repeated doses of the test preparation are given orally to test animals for a portion of their lives, but not more than 10% of their whole lives. Subchronic studies show the negative consequences of continuous or repetitive exposure to plant extracts or chemicals in experimental animals like rats during a portion of their lifespan. They are designed to identify

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no observable adverse impact levels and provide information on target organ toxicity (NRC, 2006). Subchronic evaluation can also aid in selecting dosing regimens for longer-term investigations. As a result, the subchronic toxicity of JC extract was tested in rats for 90 days at dosages of 40, 200, and 1,000 mg/Kg/day. There was no satellite group in this study. For at least 14 days after treatment, an extra satellite group administered with the maximum dose should be considered to observe reversibility, persistence or delayed onset of systemic toxic effects, and recovery from toxic effects. Satellite groups are animal groups that are part of a toxicity study's conception and execution. Moreover, the satellite group will be treated and housed in the same conditions as the animals in the main experimental. Based on subchronic toxicity test findings of JC extract, the data of weight organ, especially kidney and liver were not recorded. The usefulness of weighing organs in toxicity research consists of their sensitivity to predict toxicity, enzyme induction, physiologic perturbations, and acute injury; this function of weighing is regularly a goal organ of toxicity; it correlates nicely with histopathological changes; there is little inter animal variability; historic managed varied statistics are available (Ukwuani *et al.*, 2014). The serum hematology and scientific biochemistry analyses are done to evaluate the possible changes in hepatic and renal features influenced by the extracts. Liver and kidney feature evaluations are essential in the toxicity comparison of drug and plant extracts as they are each fundamental for the survival of an organism (Ukwuani *et al.*, 2014).

From the study results based on biochemical parameters, it was known that at a dose of 40 mg/KgBW, the ethanol extract of 70% leaves of JC extract did not affect SGOT, SGPT, and Creatinine but did affect BUN. However, the 200 mg/Kg BW dose affected SGPT, and the BUN parameter was affected by both the 40 mg/Kg BW dose and the 1,000 mg/Kg BW dose, but it was not affected by the 200 mg/Kg BW dose. SGPT values were found to be normal in the control group with Doses of 40 mg/kg BW and 1,000 mg/Kg BW, but there was an increase at a dose of 200 mg/Kg BW, probably due to the appearance of SGPT first at a higher dose (dose of 1,000 mg/Kg BW). It can be assumed that there has been an improvement at this high dose at the 91st day of surgery. Meanwhile, at lower doses, the increase in SGPT occurred afterwards so that, on the day of surgery, there was no improvement. Renal dysfunction can be assessed using concurrent measurements of urea, creatinine, and uric acid, and

their normal ranges replicate at the decreased probability of renal problem (Roopashree *et al.*, 2009). In the current study, adjustments in creatinine level in JC extract dealt with corporation confirmed non-significant variations indicating a normal renal function. However, modifications in the BUN stage at 200 mg/Kg BW doses confirmed that there were enormous variations, indicating an abnormal renal function

The amount of the harmful effect of JC extract on an animal's blood can be determined using hematological measures. It can also be used to explain why blood is drawn to specific characteristics of a plant extract or its derivatives (Yakubu *et al.*, 2006). Furthermore, such an assessment is useful to threat comparison since changes in the hematological system have a higher predictive value for human toxicity when statistics from animal studies are translated (Olson *et al.*, 2000). The JC extract does not affect the erythropoiesis, shape, or osmotic fragility of red blood cells, as evidenced by its nonsignificant impact on complete red blood cells, suggestion of corpuscular volume, suggestion of corpuscular Hb, and platelets (Olorunnisola *et al.*, 2012). Leukocytes are the first line of cell defence that respond to infectious agents, tissue injury, or inflammatory processes. Furthermore, in general, the p-values of leukocytes and hematocrit in the test group that are much less than 0.05 indicate the distinction between treatment groups. There is an exchange in hemotogram; however, no mortality facts are shown.

In comparison to the control group, macroscopic examinations of the organs of rats treated with several doses of JC extract show no changes in shade in Figure 1 and Figure 2. Organ hypertrophy is a first-hand indicator of chemical or organic substance toxicity. However, this study examined hypertrophy of organs at 200 mg/Kg BW and 1,000 mg/Kg BW of agencies. Any insult to the parenchymal liver cells causes blood transaminase levels to rise (Slichter, 2004). In addition, when compared to the control group and changes in the BUN stage at 200 mg/Kg BW dosages, there was no significant increase in creatinine in the subchronic administration of JC extract. This study used histological findings of kidney tissue to confirm this finding. These results suggest that JC extract at a 40 mg/kg BW concentration has no effect on liver or kidney characteristics and has no hazardous effects. However, it is necessary to study the changes and improvements in several test parameters at doses of 200 mg/Kg BW and 1,000 mg/Kg BW.

CONCLUSION

Using 70% ethanol extract of *J. gendarussa* leaves at a therapeutic dose is safer, but it needs attention at a higher dose. These results suggest that further research is needed to ensure its safety for a clinical study.

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AUTHOR CONTRIBUTIONS

Conceptualization, B.P.E.W.; Methodology, B.P.E.W.; Validation, B.P.E.W.; Formal Analysis, T.B.; Investigation, L.K.; Resources, M.S.; Data Curation, R.W.; Writing - Original Draft, H.P.; Writing - Review & Editing, R.W., B.P.E.W.; Visualization, L.K.; Supervision, R.W., B.P.E.W.; Funding Acquisition, L.K., B.P.E.W.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

- Arsad, S. S., Esa, N. M. & Hamzah, H. (2014). Histopathologic Changes in Liver and Kidney Tissue from Male Sprague Dawley Rats Treated with *Rhapidophora decursiva* (Roxb). *Journal of Cytology and Histology*; 2014; 1-6. doi: 10.4172/2157-7099.S4-001.
- Ekaputri, A. A. (2015). Studi Toksisitas secara In Silico Kandungan Senyawa Flavonoid dan Alkaloid pada Daun *Justicia gendarussa* Burm.f. *Skripsi*; Fakultas Farmasi Universitas Airlangga, Surabaya.
- Heyne. (1987). Tumbuhan berguna indonesia, Jilid 3. Jakarta: Yayasan Sarana Wana.
- Kennedy, G. L., Ferenz R. L. & Burgess, B. A. (1986). Estimation of acute Oral Toxicity in Rats by Determination of the Approximatelethal Dose Rather than the LD50. *Journal of Applied Toxicology*; 6; 145-148. doi: 10.1002/jat.2550060302.
- Moeso, M. & Agus, P. (1985). Laporan perjalanan ke Jayapura Sentani (Irian Jaya). Yogyakarta: Fakultas Biologi UGM.
- National Research Council (NRC). (2006). Toxicity Testing for Assessing Environmental Agents. (Interim Report). Washington, DC: National Academies Press.
- Olorunnisola, O. S., Bradley, G. & Afolayan, A. J., (2012). Acute and Sub-Chronic Toxicity Studies of Methanolic Extract of *Tulbaghiaviolacea* Rhizomes in Wistar Rats. *African Journal of Biotechnology*; 11; 14934-14940.
- Olson, H., Betton, G. & Robinson, D. (2000). Concordance of the Toxicity of Pharmaceuticals in Humans and in Animals. *Regulatory Toxicology and Pharmacology*; 32; 56-67. doi: 10.1006/rtp.2000.1399.
- Prajogo, B. E. W., Juliaan, F., Hinting, A., Pramesti, M.P., Anggraeni, M., Radjaram & Musta'ina, A. (2008). Laporan Pelaksanaan Uji Klinik Fase I; Universitas Airlangga dan Badan Koordinasi Keluarga Berencana Nasional. Surabaya: Fakultas Farmasi Universitas Airlangga.
- Prajogo, B. E. W., Juliaan, F., Hinting, A., Pramesti, M. P. Anggraeni, M., Radjaram & Musta'ina, A. (2009). Laporan Pelaksanaan Uji Klinik Fase II; Universitas Airlangga dan Badan Koordinasi Keluarga Berencana Nasional. Surabaya: Fakultas Farmasi Universitas Airlangga.
- Ratih, G. A. M., Imawati, M. F., Nugroho, R. R., Prajogo, B. E. W. & Indrayanto, G. (2019). Phytochemicals of Gandarussa (*Justicia gendarussa*) and Its Preparations. *Natural Product Communications*; 2019; 1-10. doi: 10.1177/1934578X19851406.
- Roopashree, T. S., Raman, D., Rani, R. H. S., & Narendra, C. (2009). Acute Oral Toxicity Studies of Antipsoriatic Herbal Mixture Comprising of Aqueous Extracts of *Calendula officinalis*, *Momordica charantia*, *Cassia tora* and *Azadirachta indica* Seed Oil. *Thai Journal of Pharmaceutical Science*; 33; 74-83.
- Sardini, S. (2007). Penentuan Aktivitas Enzim GOT dan GPT dalam Serum dengan Metode Reaksi Kinetik Enzimatik sesuai IFCC. Jakarta: BATAN.
- Slichter, S. J. (2004). Relationship between Platelet Count and Bleeding Risk in Thrombocytopenic Patients Transfusion. *Medicine Reviews*; 18; 153-167. doi: 10.1016/j.tmr.2004.03.003
- Solehah, M. (2018). Profil Toksisitas Oral Akut dan Subkronis 90 Hari Ekstrak Etanol 70% Daun JC (*Justicia gendarussa* Burm. f.). Tesis; Fakultas Farmasi Universitas Airlangga, Surabaya.
- Sukardja, I. D. G. (1998). Correlation of Clinical and Pathological Diagnosis of Neoplasm. *Modern Pathology for Service and Resesarch on Cancer*; 1; 15-35. doi: 10.1016/j.jvoice.2020.10.003.

Ukwuani, A. N., Abubakar, M. G., Hassan, S. W. & Agaie, B. M. (2012). Toxicological Studies of Hydromethanolic Leaves Extract of *Grewia crenata*. *International Journal of Pharmaceutical Science and Drug Research*; 4; 245–249.

Yakubu, M. T., Akanji, M. A. & Oladiji, A. T. (2007). Haematological evaluation in Male Albino Rats Following Chronic Administration of Aqueous Extract of *Fadogia agrestis* Stem. *Pharmacognosy Magazine*; 3; 34–38.

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