

Subchronic Toxicity Study of *Sterculia rubiginosa* Zoll. Ex Miq. Leaves Extract

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ABSTRACT

Ki hampelas (*Sterculia rubiginosa* Zoll. Ex Miq.) is traditionally used as an antiasthma. It also reported has antioxidant and nephroprotective activity. This study was conducted to evaluate the subchronic toxicity of the leaf extract of ki hampelas. The extract was orally administered to male and female Sprague-Dawley rats at doses of 50, 200, and 400 mg/kg bodyweight (BW) per day for 28 days. The rats were divided into four groups, consist of consist of normal group (Na CMC 0.5%), dose 1 (50 mg/kg BW), dose 2 (200 mg/kg BW), and dose 3 (400 mg/kg BW) of extract. The extract was administered every day for 28 days. Subchronic toxicity in the male and female rats resulted in no death or treatment-related signs at the highest dose. All the animals survived the duration of the study, with no significant changes in biochemical parameters and there was a change in the liver and kidney histopathology results. There was no significant difference between the SGOT, SGPT, urea, and creatinine levels in the dose groups with extracts and the normal group ($p > 0.05$). However, based on the histological results of the liver and kidneys it was found a significant difference among the groups. This study showed that the leaf extract of ki hampelas is relatively non-toxic according to the normal biochemical parameters results and has no treatment-related signs. There was a change in the liver and kidney histopathology results, but no death was found.

Keywords: subchronic toxicity; histopathology; toxicity; *Sterculia rubiginosa* Zoll. Ex Miq.

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INTRODUCTION

Plants of the genus *Sterculia* have various pharmacological activities and can also be used as additives in the manufacture of pharmaceutical preparations. Some of them such as *Sterculia diversifolia* is used as an immunomodulator and anticancer (Fazle et al., 2017). *Sterculia foetida* is useful as an antimicrobial, antioxidant, and apoptosis inducer (Jafri et al., 2019), and also as an antidiabetic (Swarnalatha et al. 2019). *Sterculia villosa* has sedative (Hossain et al., 2016), antioxidant, and antifibrinolytic effects (Uddin et al., 2015). *Sterculia tragacanth* has analgesic and anti-inflammatory activity (Mogbojuri et al., 2016), and is also an enzyme inhibitor (Bibi et al., 2019).

Sterculia has many benefits. *Sterculia urens* Roxb. is used as a thickener, food emulsifier, laxative, and artificial adhesive while roots of *Firmiana simplex* (L.) W.Wight. was used in Chinese medicine to treat rheumatic disorders, asthma, fractures, and tumors, and the seeds have been used for diarrhea and stomach disorders (Upson, 2012). The stems, wood, leaves, fruit, and roots of *Sterculia* species have been traditionally used in various countries to treat various diseases, including digestive disorders, diabetes, respiratory disorder, and skin disorder. It is also found that *Sterculia*

activity is antimicrobial, anti-inflammatory, antioxidant, and anticancer (Saleh, 2016). Several species of the genus *Sterculia* are used for their stem bark and are also cultivated as ornamental plants.

Ki hampelas (*Sterculia rubiginosa* Zoll. Ex Miq.) is one of the plants that can be used in traditional medicine, as an anti-asthma. This plant is now widely used in tropical and subtropical areas, especially in Sumatra. Ki hampelas activity as a nephroprotective agent and antioxidant was reported by Prastiwi (Prastiwi et al., 2020). This plant contains several chemical elements, the main secondary metabolites are phenolic compounds. While other compounds contained are flavonoids, tannins, alkaloids, terpenoids, steroids, and glycosides (Prastiwi et al, 2018). In the acute toxicity test at doses of 50 mg/kg, 1000 mg/kg, and 2000 mg/kg, it was found there was no difference in levels of SGOT, SGPT, urea, creatinine, and histopathology in the liver and kidneys (Prastiwi et al., 2021).

It is important to use herbs that have efficacy, safety, and quality. To ensure its safety, toxicity tests can be carried out (Priyanto, 2009; Priyanto, 2015). For this reason, it is necessary to study sub-chronic toxicity to determine the effect of long-term administration of 70% ethanol extract of ki hampelas.

Table 1. Phytochemical results of ki hampelas extract

Compound	Reagent	Result
Alkaloids	Bouchardat	+
	Mayer	-
	Dragendorff	+
Flavonoids	Shinoda	+
Tannins	Gelatin test	+
Phenol	FeCl ₃	+
Saponins	Foam Reaction	-
Triterpenoids & Steroids	Lieberman-Bouchard	+

METHODS

Material

Plant material

Plants were obtained from the Bogor Botanical Gardens, Indonesia. Determination was carried out at LIPI, Bogor. The leaves were washed, dried under shade, and coarsely powdered.

Chemical and reagent

Chemicals used are 70% ethanol, concentrated HCl, Mg, FeCl₃, amyl alcohol, H₂SO₄, Liebermann- Burchard reagent, Mayer reagent, ketamine, anhydrous acetic acid, ketamine, gelatine, SGOT kit reagent (Human®, Germany), SGPT reagent kit (Human®, Germany), Urea kit reagent (Human®, Germany), Creatinine reagent kit (Human®, Germany), rats, animal feed, Na CMC (Brataco), aqua distillate, Spectrophotometer (Microlab 300, Netherlands).

Extraction

The powder (200 g) was macerated at room temperature with 70% ethanol. Maceration was carried out for 3 days with stirring several times a day. The filtrate was evaporated under a vacuum at 45°C by rotary evaporator. Maceration was carried out for 3 days with stirring several times a day. The filtrate was filtered and separated. The pulp was macerated again until the color of the solvent changed to clear. After that, the macerate was concentrated using a vacuum rotary evaporator at a temperature of 50°C until the extract was thick, then subjected to a water bath at the same temperature (Depkes RI, 2008).

Characteristic Extract

The extract characteristic was determined by organoleptic examination, calculation of yield, determination of water content, and determination of ash content. The procedure was carried out according to BPOM RI (2000).

Phytochemical Screening

The phytochemical screening consists of an examination of alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, and steroids, which can be seen in Table 1. The procedure was carried out according to Hanani (2015).

Subchronic Toxicity

This study was approved by the Ethics Committee of UHAMKA with No. 02/20.03/0358. The groups were divided into 4 (four), the male and female rats were administered extract groups with doses of 50 mg/kg BW, 200 mg/kg BW, 400 mg/kg BW, and normal groups of 5% Na CMC. Every group consists of 6 rats. Animals were acclimatized for 7 days so that they animals adjust to their new environment. Every day the group was given the extract of 5% Na.CMC for 28 days. The blood was drawn through the orbital sinus and the serum was separated. After that, the levels of SGOT/SGPT and creatinine/ureum were measured using their respective reagents and read using a Clinical Spectrophotometer (BPOM, 2014). Liver and kidney histology preparations were made by the Histology Laboratory, Universitas Indonesia (Mescher, 2015).

Statistical Analysis

Experimental data were recorded using excel and statistically analyzed by SPSS 19 and GraphPad Prism 10.0 version for mac software according to the sex of the animals and the test stage. The data on SGOT, SGPT, creatinine, urea, and histopathological organs were analyzed for normality and homogeneity, then proceeded to the ANOVA test by SPSS 19. This test was carried out to determine the difference between each group, whether it is significantly different or not. Numerical data like BW etc were analyzed using one-way analysis of variance (ANOVA) test and continued with multiple comparisons of Tukey Test. P values lower than 0.05 were considered significant.

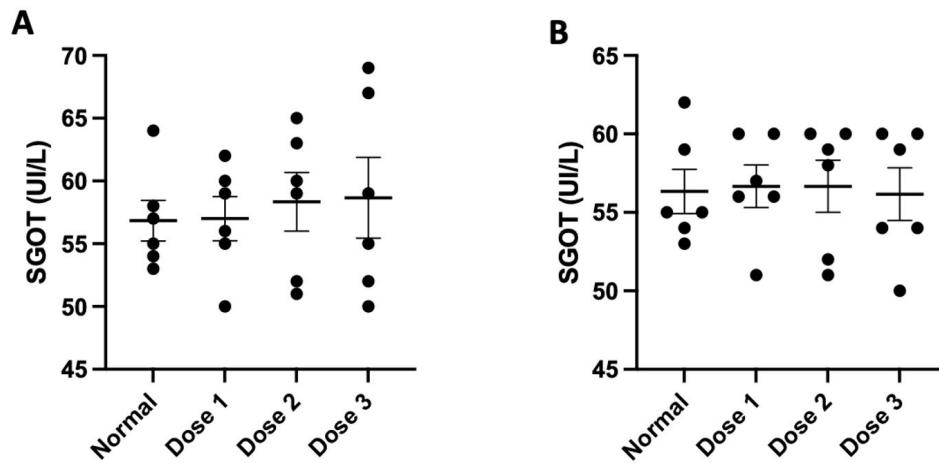


Figure 1. SGOT levels in male and female rats

(A) SGOT levels of male rats groups. (B) SGOT levels of female rats groups. There was no significant difference between the dose groups and the normal group

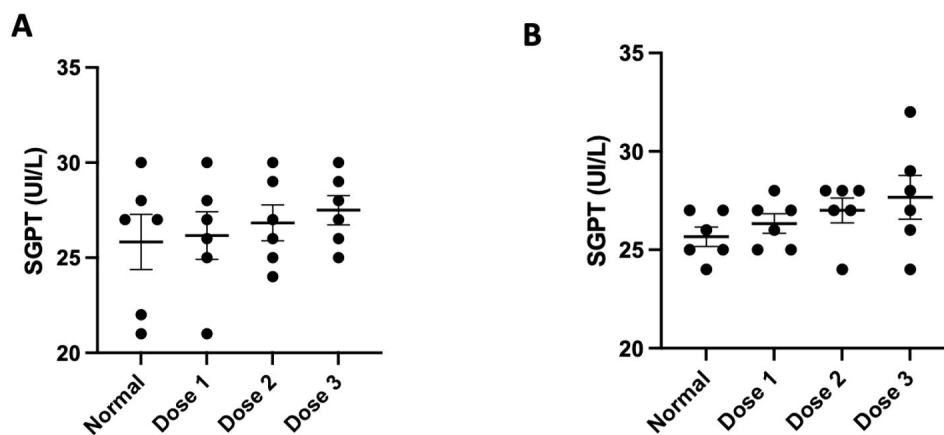


Figure 2. SGPT levels in male and female rat

(A) SGPT levels of male rats groups. (B) SGPT levels of female rats groups. There was no significant difference between the dose groups and the normal group

RESULTS AND DISCUSSION

Characterization of the Leaves Extract of Ki Hampelas

Results obtained a yield of 13.19%. These results indicate the presence of 13.19 grams of metabolites in 100 grams of simplicia powder. The result of water content was 7.95%. This shows that the water content in the extract is less than 10%, which means the extract is according to the requirements for water content in the extract. Therefore, the extract can be stored for a long time and is maintained from contamination by microorganisms.

The measurement of ash content aimed to provide an overview of the internal and external mineral content

from the initial process until the formation of a thick extract. The principle of determining the ash content is that the material is heated at a temperature where organic compounds and their derivatives are destroyed and evaporated so that only mineral and inorganic elements remain (Depkes RI 2000). The measurement results obtained were 8.54%.

Phytochemical Screening Results

Phytochemical screening of 70% ethanolic extract of ki hampelas leaf was carried out to determine secondary metabolite compounds contained in ki hampelas leaf extract. The results of phytochemical screening can be seen in Table 1.

The results of the compound content in Hampelas ki extract are the same as the content of other plants belonging to the genus *Sterculia*, including *Sterculia stigeri* Delile (Aikpe, J.F. A et al., 2020), *Sterculia foetida* L. (Swarnalatha et al., 2019), *Sterculia stipulata* Korth (Prastiwi et al., 2020), *Sterculia rubiginosa* Zoll.x. Miq. (Prastiwi et al., 2020), *Sterculia tragacantha* (Bibi et al., 2019).

Subchronic Toxicity Test Results of Ki Hampelas Leaf Extract in Rats

A subchronic toxicity test is a test to determine the adverse effects arising from repeated daily doses of drugs, chemicals, or exposure to these substances which lasts about 10% of their life span. However, some researchers used a shorter period, for example, the administration of substances for 14 and 28 days (Djojsumarto, 2008).

The results of giving 70% ethanol extract of ki hampelas leaves which were administered test animals at a dose of 50 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW for 28 days showed no death, or changes in behavior such as weakness, seizures, excessive diarrhea. There was no shedding, there was no change in the color of the stool or urine, and an active attitude, which is a normal attitude. It can be interpreted that the test animals did not experience stress or toxic symptoms caused by the administration of ki hampelas leaf extract.

Repeated administration of the extract for 28 days caused an increase in the levels of SGOT and SGPT between the normal group and the group that was given the test preparation, as can be seen in (Figures 1 & 2). However, the increase is still within the normal range. The results of the analysis on the levels of SGOT and SGPT obtained showed $p > 0.05$, which indicates that there is no significant difference in each treatment group.

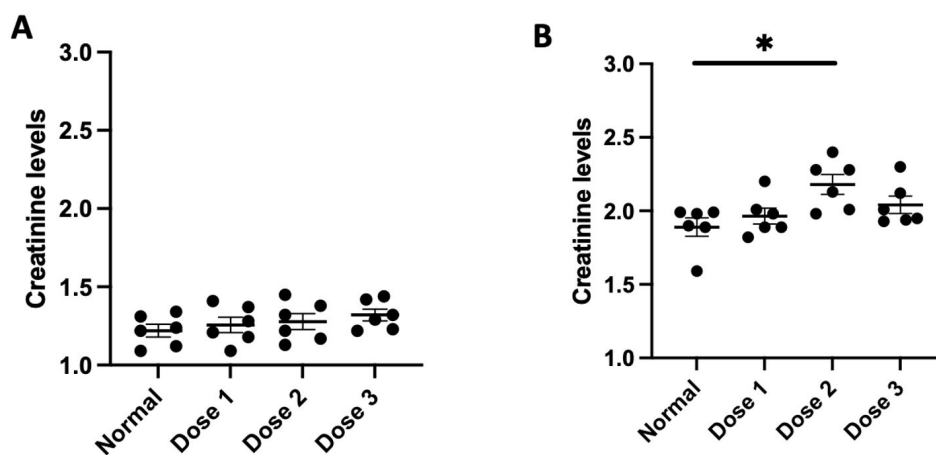


Figure 3. Creatinine levels in male and female rats

(A) The creatinine levels of male rats groups. (B) The creatinine levels of female rats groups. There was significant difference between the dose groups and the normal group, $*p < 0.05$

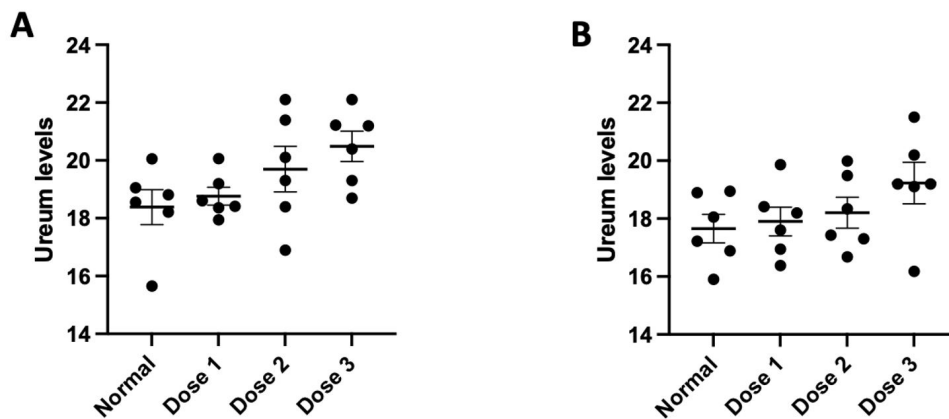


Figure 4. Ureum levels in male and female rats

(A) The ureum levels of male rats groups. (B) The ureum levels of female rats groups. There was no significant difference between the dose groups and the normal group

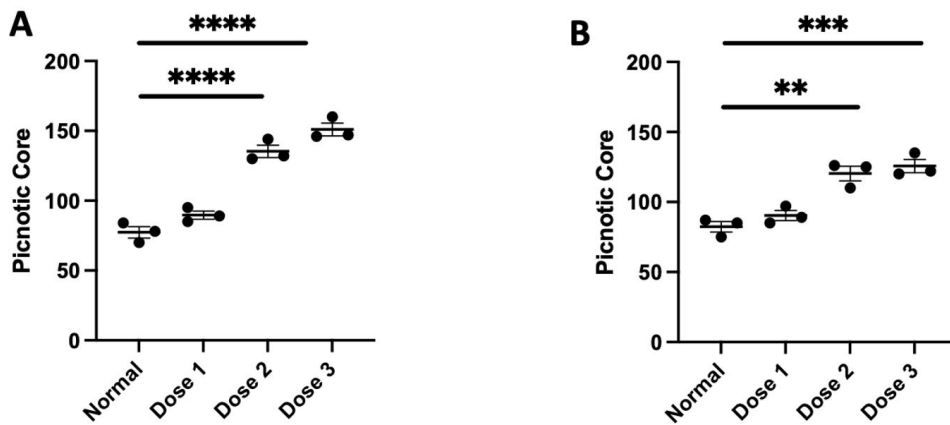


Figure 5. Picnotic core in male and female rats

(A) The picnotic core of male rats groups, There were significant difference between the dose 2 and dose 3 with the normal group ****p < 0.0001. (B) The picnotic core of female rats group, There were significant difference between the dose 2 and dose 3 with the normal group, **p < 0.01, ***p < 0.001

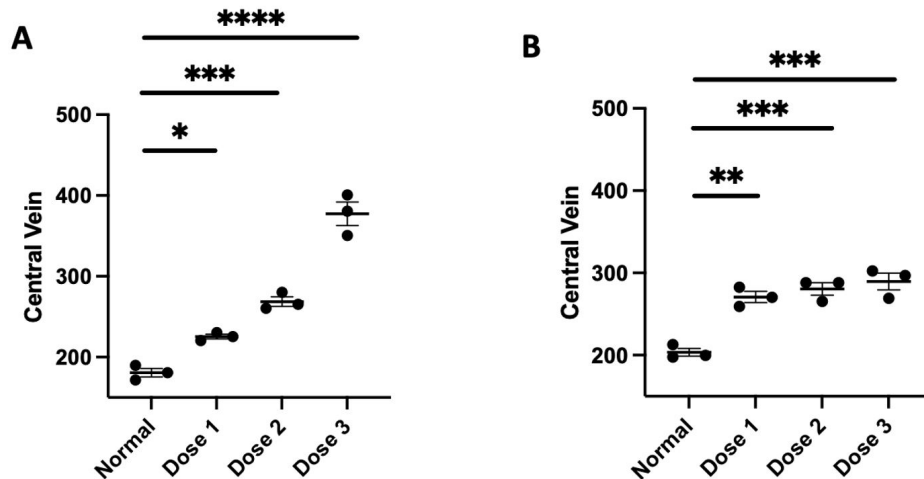


Figure 6. Diameter of the central vein in male and female rats

(A) Diameter of the central vein of male rats groups. There were significant difference between the dose groups and the normal group *p < 0.05, ***p < 0.001 and ****p < 0.0001. (B) Diameter of the central vein of female rats groups, There were significant difference between the dose groups and the normal group **p < 0.01, ***p < 0.001

The De Ritis ratio of AST (SGOT) and ALT (SGPT) can be used to help determine the severity of liver cell damage. In inflammation and early (acute) hepatocellular damage, there will be leakage of cell membranes so that the cytoplasmic contents come out causing ALT to increase higher than AST with an AST/ALT the ratio of <0.8 which indicates mild damage. In chronic or severe inflammation and damage, liver cell damage reaches the mitochondria causing an increase in AST levels higher than ALT so that the AST/ALT ratio is >0.8 which indicates severe or chronic liver damage (Rosida 2016). Based on the value of the ratio (AST: ALT ratio) the subchronic toxicity test of was 2.20 (dose 1 group), 2.18

(dose 2 group), 2.17 (dose 3 group), and 2.13 (dose 4 group). So the value of the arthritis ratio of each group > 0.8 indicates possibility of severe or chronic liver damage. In the results of the SGOT values of female rats, there was no difference between the normal group and the 1, 2, and 3 dose groups. When compared to the male group, the SGOT values were also not too different, between 56-58 IU/l. In the results of the SGPT values of female rats, there was no difference between the normal group and the 1, 2, and 3 dose groups. When compared to the male group, the SGPT values were also not too different, between 25-27 IU/l.

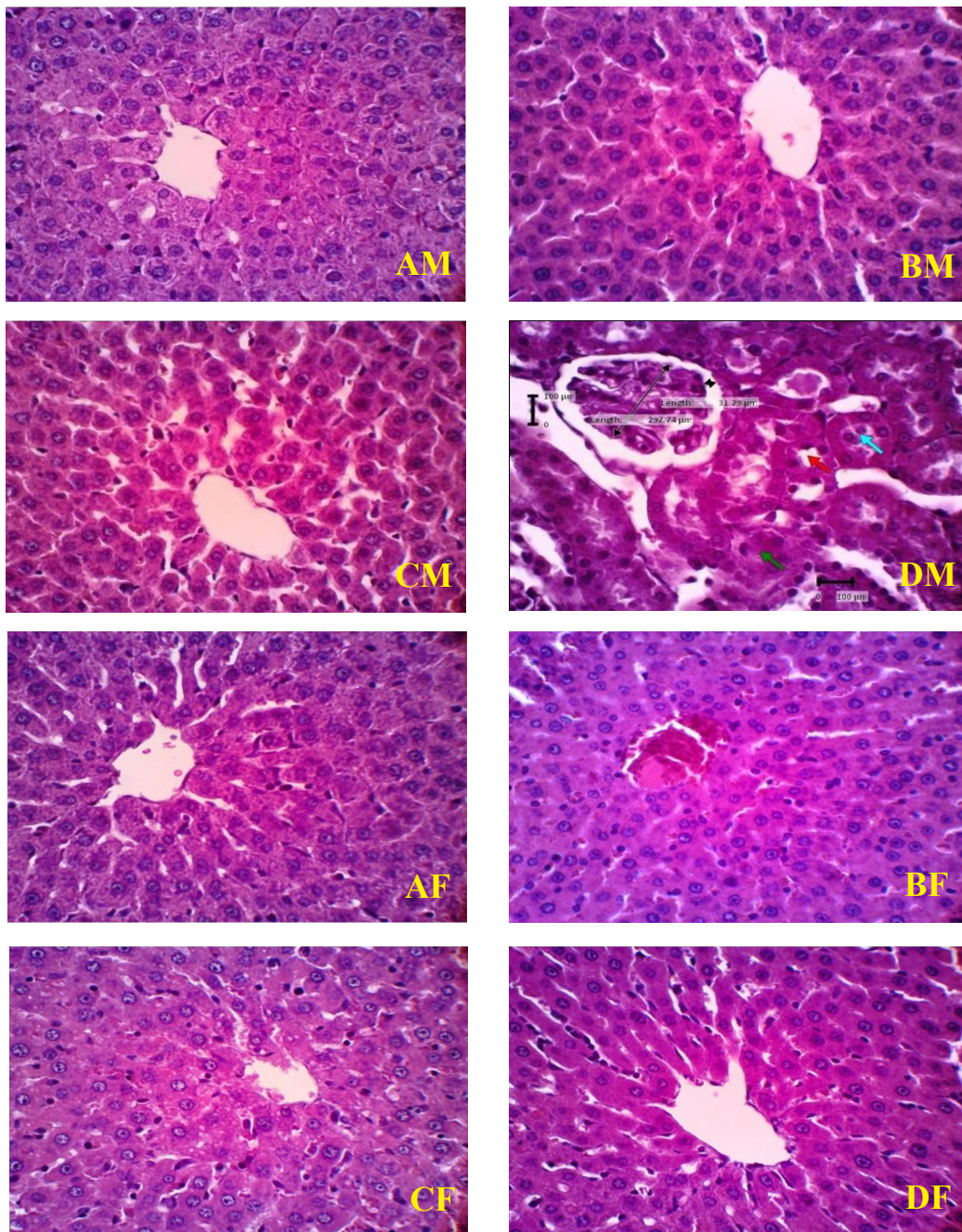


Figure 7. Histopathology of the liver male and female rats

Transverse incision histology of rat liver organ with hematoxylin-eosin staining at 40x10 magnification. M: Male, F: Female, (AM) 0.5% NaCMC, (BM) 50 mg/kg BW, (CM) 200 mg/kg BW, and (DM) 400 mg/kg BW. (AF) 0.5% NaCMC, (BF) 50 mg/kg BW, (CF) 200 mg/kg BW, and (DF) 400 mg/kg BW

However, liver damage is only clinically significant if there is an increase in SGOT levels between three and ten times the normal range. In the administration of ki hampelas leaves extract, the subchronic toxicity test for orally and repeatedly for 28 days experienced a significant change with increasing levels of SGOT and SGPT in white rats when compared to the acute toxicity test. The 70% ethanol extract of ki hampelas leaves showed the presence of alkaloids, flavonoids, phenols,

tannins, triterpenoids, and steroids. (Prastiwi et al. 2018). The flavonoid content contained in the ethanol extract of ki hampelas leaves has the potential as an antioxidant so that it can reduce the levels of SGOT and SGPT. Flavonoids can be used as free radicals, as well as recovery for dead or damaged liver cells (Birt et al. 2001). However, if there is an increase in the activity of the SGOT and SGPT enzymes, it can indicate the occurrence.

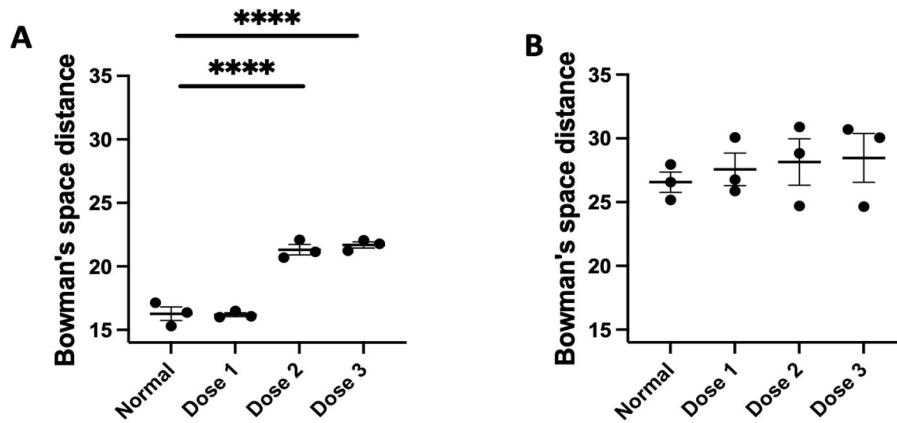


Figure 8. Bowman's space distance in male and female rats
 (A) The Bowman's space of male rats groups, There were significant difference between the dose 2 and dose 3 with the normal group ****p < 0.0001. (B) The picnotic core of female rats group. There was no significant difference between the dose groups and the normal group

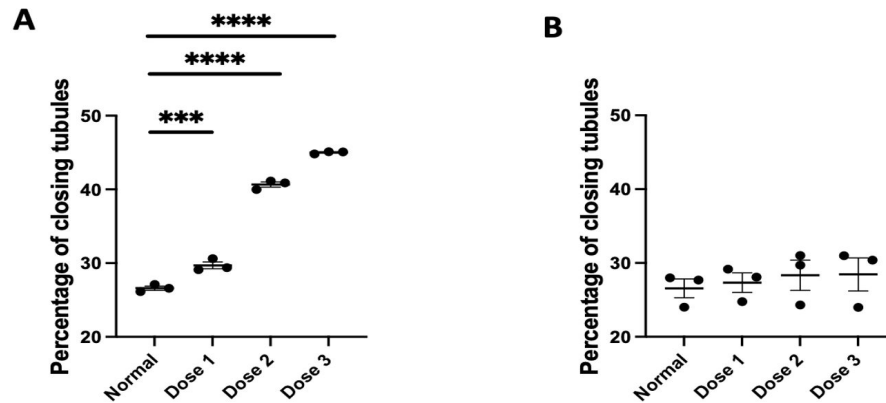


Figure 9. Percentage of closing tubules in male and female rats
 (A) The percentage of closing tubulus of male rats groups, There were significant difference between the dose groups with the normal group ***p < 0.001 and ****p < 0.0001. (B) The percentage of closing tubulus of female rats group. There was no significant difference between the dose groups and the normal group

Observation of liver and kidney histopathological preparations was carried out to determine the effect of ki hampelas leaf extract on changes in the structure of the liver and kidneys. . The results of this study are different from previous research by Prastiwi (2021) which gave results that there was no difference in the histological liver and kidney.

The results of the subchronic toxicity test on liver histopathological preparations with Hematoxylin-Eosin staining showed that an increase in extract dose associated with an increase in the size of the central vein in the extract group that each increase in dose showed a difference in the size of the central vein diameter and the number of pycnotic nuclei as shown in Figure 5 (p<0.05).

Based on the statistical analysis of the subchronic toxicity test, the ratio of the kidney to the body weight of rats showed no difference between the normal group and the test group. The number of closed tubules and changes in the glomerulus in the acute toxicity test showed a significant difference between the normal group and the dose group of 1000 mg/kg BW and 2000 mg/kg BW, but no death and no tubular casts were found in all test groups. In the subchronic toxicity test, the number of closed tubules and changes in the glomerulus showed a significant difference between the 200 mg/kg BW and 400 mg/kg BW dose groups and the normal group, but no death was found and no tubular casts were found.

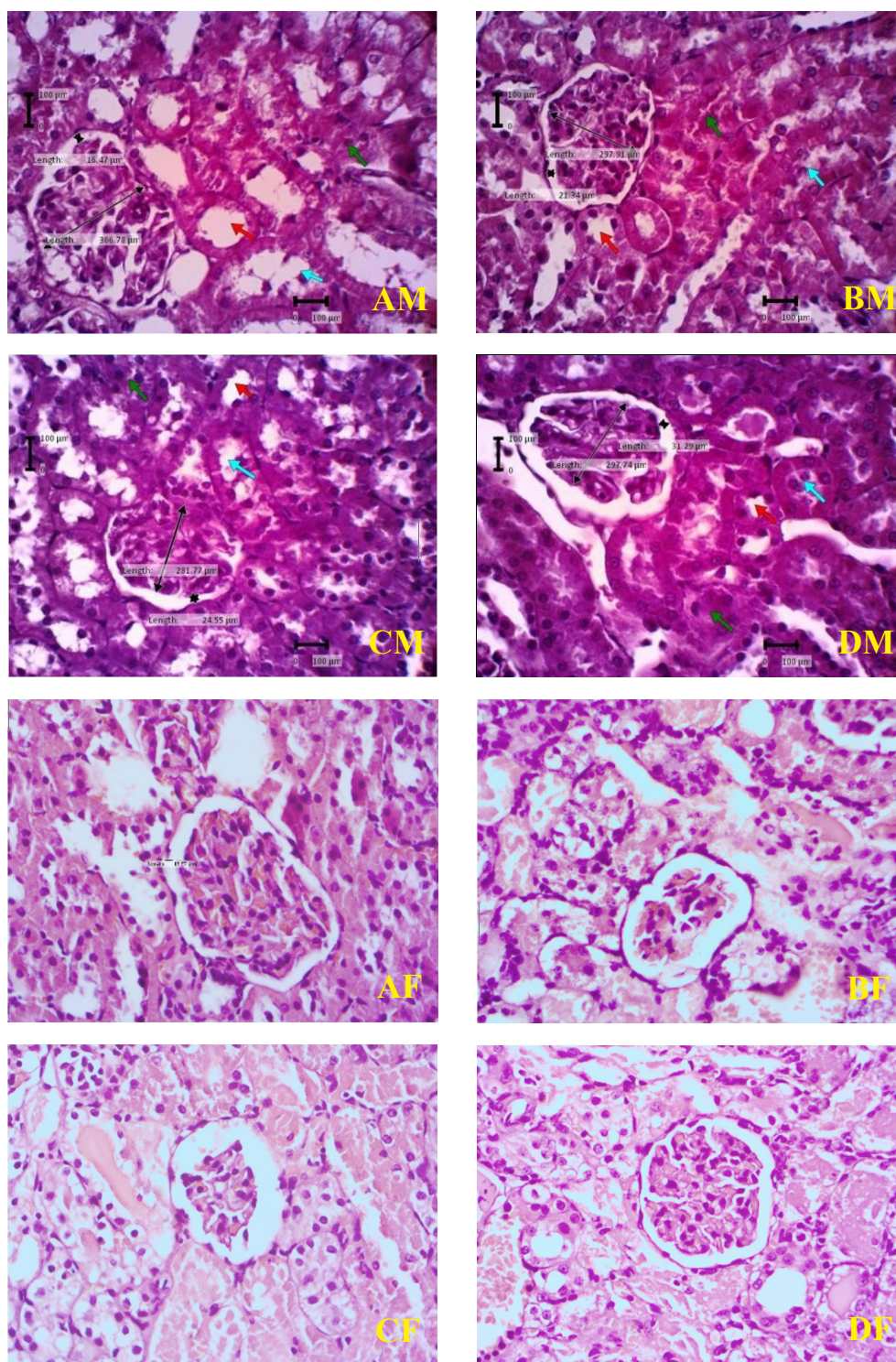


Figure 10. Kidney histopathology male and female rats

Information: Transverse incision histology of rat kidney organ with hemotoxin-eosin staining at 40x10 magnification. M: male, F: Female (AM) 0.5% CMC, (BM) 50 mg/kg BW, (CM) 200 mg/kg BW, and (DM) 400 mg/kg BW, (AF) 0.5% CMC, (BF) 50 mg/kg BW, (CF) 200 mg/kg BW, and (DF) 400 mg/kg BW

Figure 6 shows that there was a significant difference in the number of picnotic nuclei between the normal group and the test group with $p < 0.05$. Based on these results, it was found that there was an increase in necrosis at each increase in dose, the characteristic of necrosis that was seen was the picnotic nucleus, namely the nucleus condensed so that it looked smaller in size and had a more concentrated color with Hematoxylin-Eosin staining (Lu, C Frank, 2010).

Creatinine and urea levels of rats showed that repeated administration of the extract with different doses had a slight increase in both creatinine and urea, but was still within normal limits. The results showed that the levels of creatinine and urea (Figures 3 & 4) it was found that the subchronic toxicity of ki Hampelas leaf extract had no significant effect on creatinine and urea levels ($p > 0.05$), this indicates that the ethanol extract of ki hampelas leaves did not cause a toxic effect.

On the observation of renal histopathology with parameters of the distance between the glomerulus and Bowman's capsule and the percentage of proximal tubules that close. In the parameter of the distance between the glomerulus and Bowman's capsule, changes that occur in the glomerulus are edema which is characterized by the presence of protein deposits in the mesangium up to Bowman's space or the occurrence of atrophy (shrinkage) in the glomerulus which is marked by the increasing space between the glomerulus and Bowman. Therefore, in this study, observations were made by measuring the distance of the Bowman's space which was calculated from the furthest distance from the edge of Bowman's capsule to the edge of the glomerulus (Cahyaningsih, 2011). In the subchronic toxicity test, statistical test results showed that there was no significant difference between the normal test group and the 50 mg/kg BW dose group.

In the parameter of the percentage of closed proximal tubules, the percentage of proximal tubule damage is indicated by the number of closed proximal tubules in one field of view compared to the number of all proximal tubules (Shreevastva, 2017). The results of statistical tests showed that there was a significant difference between all normal control groups and the test dose group, as shown in Figure 8 and Figure 9.

CONCLUSION

This study showed that the leaves extract of Ki hampelas (*Sterculia rubiginosa* Zolll. Ex Miq.) is relatively non toxic according to the normal biochemical parameters results and no treatment-related signs. There was a change in the liver and kidney histopathology results, but no death was found.

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