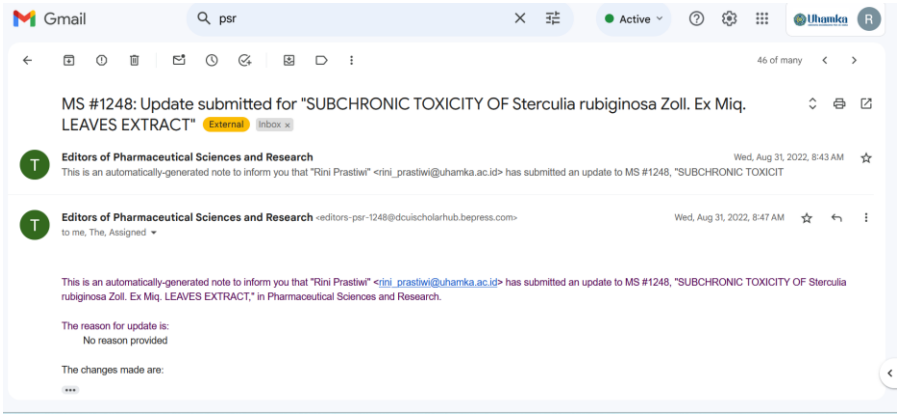
BUKTI KORESPONDENSI
JURNAL NASIONAL SINTA 2
DIGUNAKAN DALAM PERSYARATAN

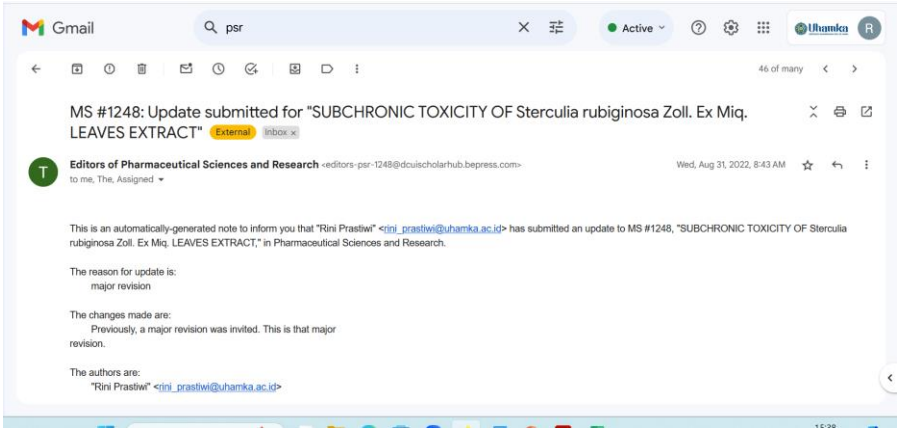
Judul artikel	**Subchronic Toxicity Study of Sterculia rubiginosa Zoll. Ex Miq. Leaves Extract**
Jurnal	Pharmaceutical and Sciences Research, (SINTA 2), volume 10, No.3, 2023
Link Jurnal	: https://scholarhub.ui.ac.id/cgi/viewcontent.cgi?article=1248&context=psr
Penulis	Jumlah Penulis 6/penulis ke-1 / Koresponden Ya Sebagai syarat pengajuan kepangkatan

No.	Perihal	Tanggal
1.	Bukti konfirmasi submit artikel dan artikel yang disbumit	31 Agustus 2022
2.	Bukti konfirmasi review dan hasil review pertama Mayor revisi	31 Agustus 2022
3.	Bukti konfirmasi submit revisi pertama, respon kepada reviewer, dan artikel yang diresubmit	1 September 2022
4.	Bukti konfirmasi review dan hasil review kedua	9 November 2022
5.	Bukti konfirmasi submit revisi kedua, respon kepada reviewer, dan artikel yang diresubmit	24 Januari 2023
6.	Bukti konfirmasi artikel accepted	10 Januari 2023
7.	Bukti konfirmasi artikel published online	29 Desember 2023

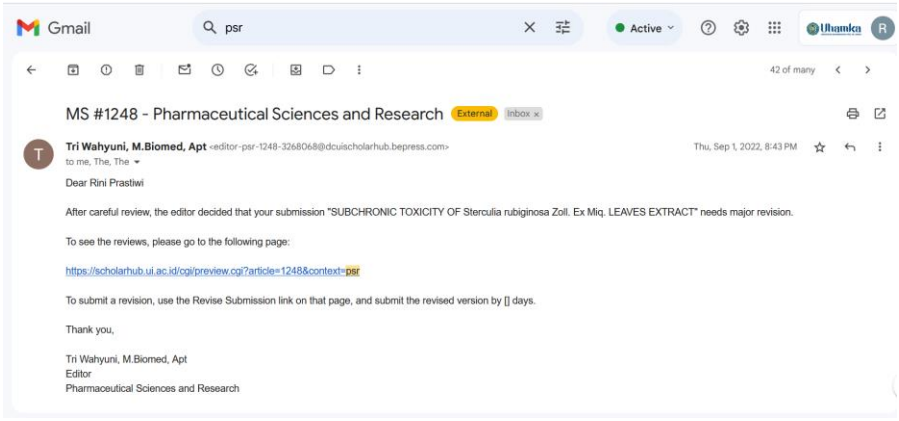
Bukti 1



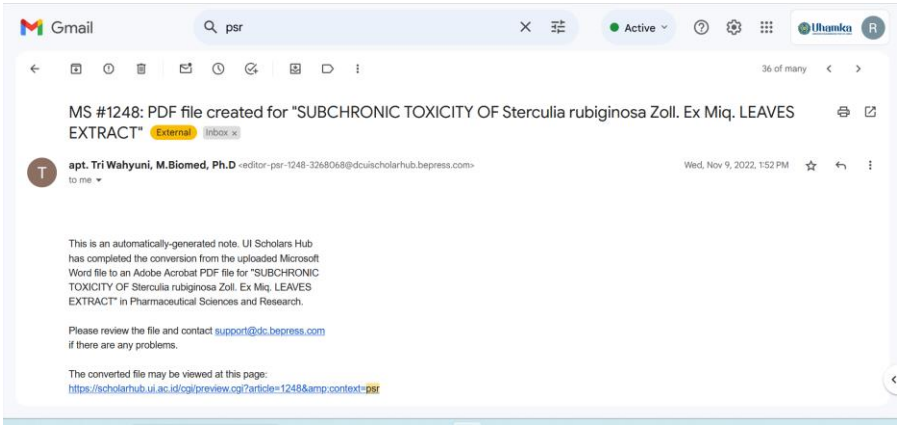
Bukti 2



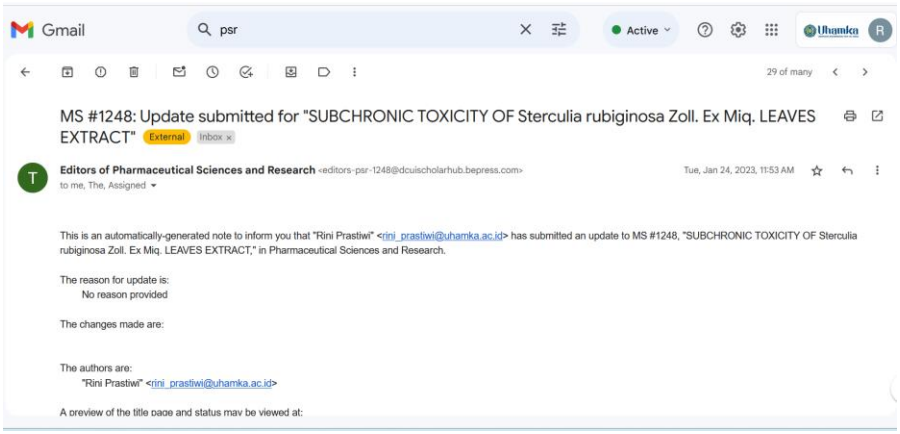
Bukti 3



Bukti 4



Bukti 5



Gmail psr Active Uhamka R

29 of many

MS #1248: Update submitted for "SUBCHRONIC TOXICITY OF Sterculia rubiginosa Zoll. Ex Miq. LEAVES EXTRACT" External Inbox ×

Editors of Pharmaceutical Sciences and Research <editors-psr-1248@dcuischolarhub.bepress.com> Tue, Jan 24, 2023, 11:53 AM

to me, The, Assigned

This is an automatically-generated note to inform you that "Rini Prastiwi" <rini_prastiwi@uhamka.ac.id> has submitted an update to MS #1248, "SUBCHRONIC TOXICITY OF Sterculia rubiginosa Zoll. Ex Miq. LEAVES EXTRACT," in Pharmaceutical Sciences and Research.

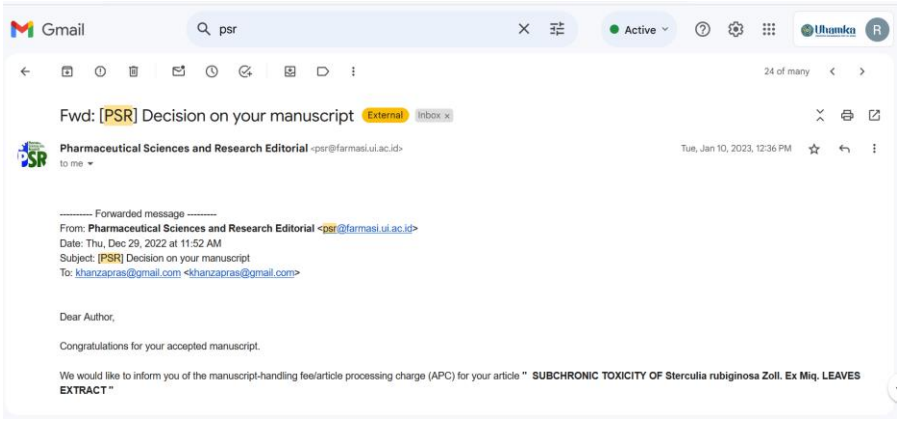
The reason for update is:
No reason provided

The changes made are:

The authors are:
"Rini Prastiwi" <rini_prastiwi@uhamka.ac.id>

A preview of the title page and status may be viewed at:

Bukti 6



Gmail psr Active Uhamka R

24 of many

Fwd: [PSR] Decision on your manuscript External Inbox ×

Pharmaceutical Sciences and Research Editorial <psr@farmasi.ui.ac.id> Tue, Jan 10, 2023, 12:36 PM

to me

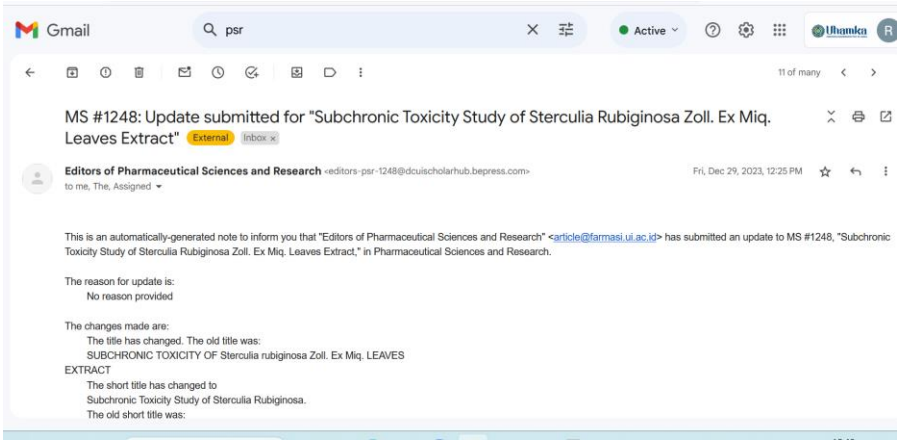
---------- Forwarded message ---------
From: **Pharmaceutical Sciences and Research Editorial** <psr@farmasi.ui.ac.id>
Date: Thu, Dec 29, 2022 at 11:52 AM
Subject: [PSR] Decision on your manuscript
To: khanzapras@gmail.com <khanzapras@gmail.com>

Dear Author,

Congratulations for your accepted manuscript.

We would like to inform you of the manuscript-handling fee/article processing charge (APC) for your article " **SUBCHRONIC TOXICITY OF Sterculia rubiginosa Zoll. Ex Miq. LEAVES EXTRACT** "

Bukti 7



Gmail psr Active

11 of many

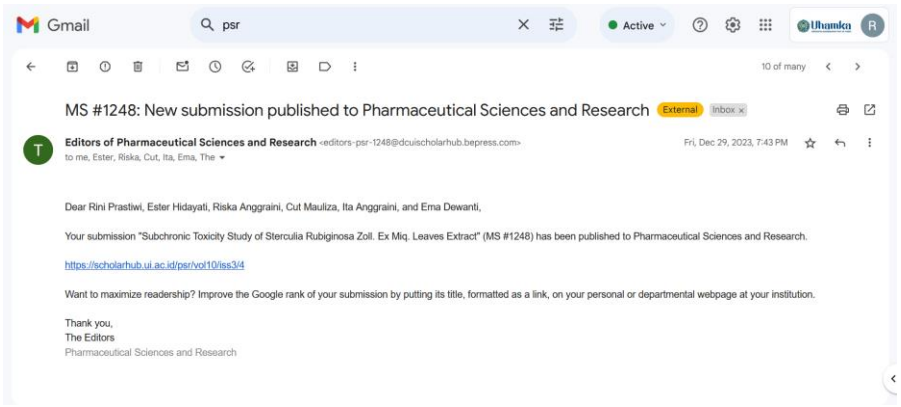
MS #1248: Update submitted for "Subchronic Toxicity Study of Sterculia Rubiginosa Zoll. Ex Miq. Leaves Extract" External Inbox ×

Editors of Pharmaceutical Sciences and Research <editors-psr-1248@dcuischolarhub.bepress.com> Fri, Dec 29, 2023, 12:25 PM
to me, The, Assigned

This is an automatically-generated note to inform you that "Editors of Pharmaceutical Sciences and Research" <article@farmasi.ui.ac.id> has submitted an update to MS #1248, "Subchronic Toxicity Study of Sterculia Rubiginosa Zoll. Ex Miq. Leaves Extract," in Pharmaceutical Sciences and Research.

The reason for update is:
No reason provided

The changes made are:
The title has changed. The old title was:
SUBCHRONIC TOXICITY OF Sterculia rubiginosa Zoll. Ex Miq. LEAVES
EXTRACT
The short title has changed to
Subchronic Toxicity Study of Sterculia Rubiginosa.
The old short title was:



Gmail psr Active

10 of many

MS #1248: New submission published to Pharmaceutical Sciences and Research External Inbox ×

Editors of Pharmaceutical Sciences and Research <editors-psr-1248@dcuischolarhub.bepress.com> Fri, Dec 29, 2023, 7:43 PM
to me, Ester, Riska, Cut, Ita, Ema, The

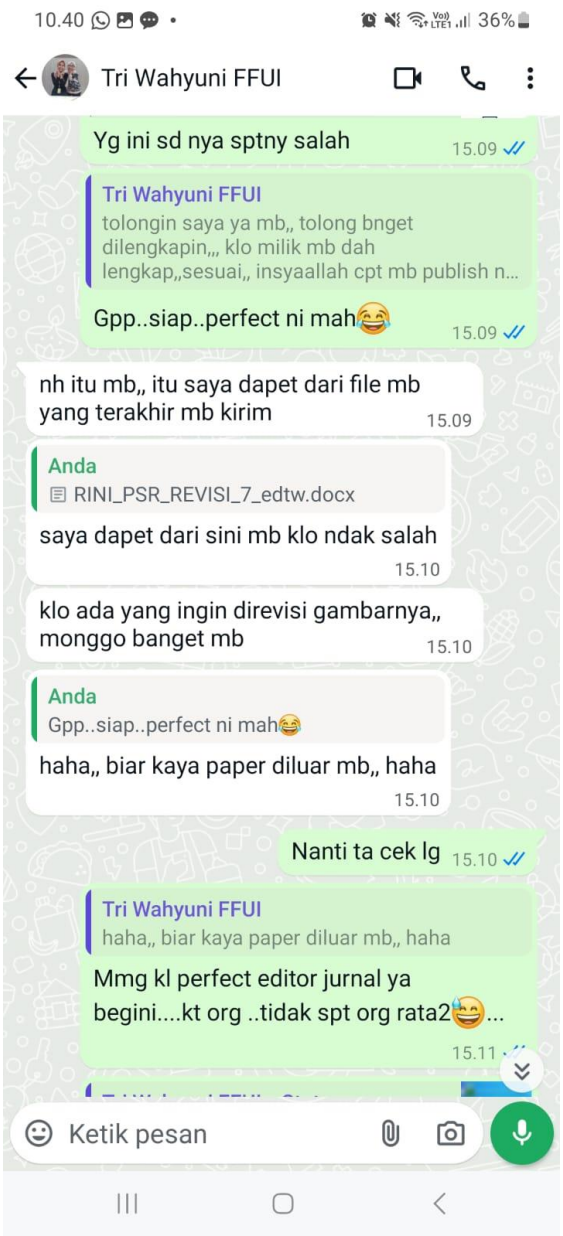
Dear Rini Prastiwi, Ester Hidayati, Riska Anggraini, Cut Mauliza, Ita Anggraini, and Ema Dewanti,

Your submission "Subchronic Toxicity Study of Sterculia Rubiginosa Zoll. Ex Miq. Leaves Extract" (MS #1248) has been published to Pharmaceutical Sciences and Research.

https://scholarhub.ui.ac.id/psr/vol10/iss3/4

Want to maximize readership? Improve the Google rank of your submission by putting its title, formatted as a link, on your personal or departmental webpage at your institution.

Thank you,
The Editors
Pharmaceutical Sciences and Research


10.40
36%
Tri Wahyuni FFUI
Yg ini sd nya sptny salah
15.09
Tri Wahyuni FFUI
tolongin saya ya mb,, tolong bnget dilengkapin,,, klo milik mb dah lengkap,,sesuai,, insyaallah cpt mb publish n...
Gpp..siap..perfect ni mah😂
15.09
nh itu mb,, itu saya dapet dari file mb yang terakhir mb kirim
15.09
Anda
RINI_PSR_REVISI_7_edtw.docx
saya dapet dari sini mb klo ndak salah
15.10
klo ada yang ingin direvisi gambarnya,, monggo banget mb
15.10
Anda
Gpp..siap..perfect ni mah😂
haha,, biar kaya paper diluar mb,, haha
15.10
Nanti ta cek lg
15.10
Tri Wahyuni FFUI
haha,, biar kaya paper diluar mb,, haha
Mmg kl perfect editor jurnal ya begini....kt org ..tidak spt org rata2😅...
15.11
Ketik pesan

10.40 • 36%

Tri Wahyuni FFUI

insyaallah yang desember 13.48

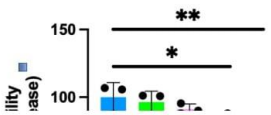
Oh begitu..baik trmksh Bu Tri 🙏 13.48

mb rin 13.53

RINI_PSR_REVISI_7_edtw_230816.docx
6,1 MB • DOCX

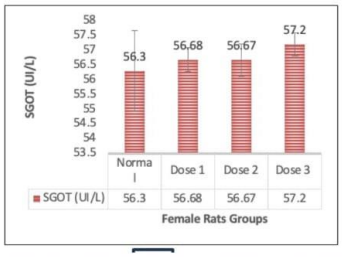
mohon ditambahin dong mb,, yang kmr aku maksud 13.54

mb rini kok blm ditambhin ya 13.54

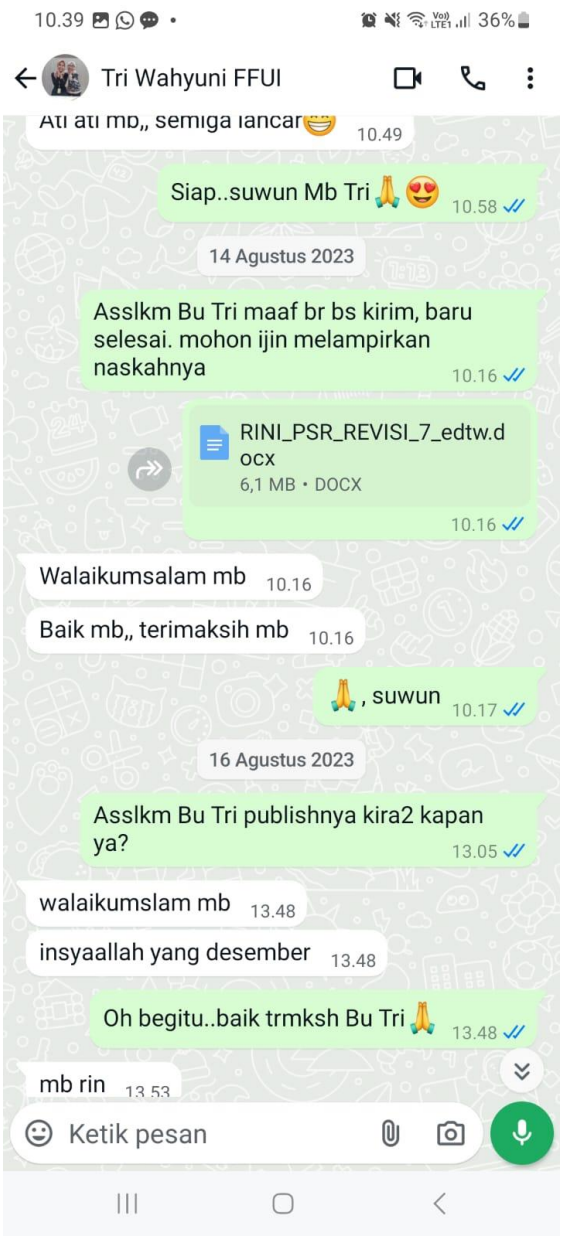


yang kaya gini mb mksdku 13.55

kan mb rini klaim pake analisis datanya spss,,,kan nganalisis tuh 13.55



Ketik pesan


10.39
36%
Tri Wahyuni FFUI
Ati ati mb,, semiga lancar
10.49
Siap..suwun Mb Tri
10.58
14 Agustus 2023
Asslkm Bu Tri maaf br bs kirim, baru selesai. mohon ijin melampirkan naskahnya
10.16
RINI_PSR_REVISI_7_edtw.docx
6,1 MB • DOCX
10.16
Walaikumsalam mb
10.16
Baik mb,, terimaksih mb
10.16
, suwun
10.17
16 Agustus 2023
Asslkm Bu Tri publishnya kira2 kapan ya?
13.05
walaikumslam mb
13.48
insyaallah yang desember
13.48
Oh begitu..baik trmksh Bu Tri
13.48
mb rin
13.53
Ketik pesan

10.40 • 36%

Tri Wahyuni FFUI

28 Agustus 2023

Asslkm 11.03

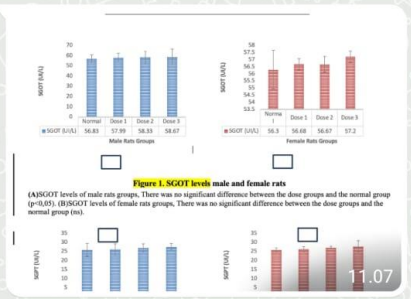
ijin mengirimkan revisian 11.03

RINI_PSR_REVISI_8.docx
6,1 MB • DOCX
11.03

Walaikumsalam mb 11.03

Ok mb 11.03

11.04



mb rin,, izin tanya, tampilan di mb rini spt

Ketik pesan


10.39
36%
Tri Wahyuni FFUI
dengan normal?
10.36
Wah mwsti nambahi lagi ya ditulis ya
10.42
Mb dengan gambar diatas apakah bnar tidak ada perbedaan?
10.43
Ok
10.45
Tri Wahyuni FFUI
apakah ini tidak ada perbedaan bermakna mb?antara dosis 3 dengan normal?
Ada perbedaan
10.46
Narasinya salah ya
10.46
Anda
Ada perbedaan
Di gambar harusnya di kasih info mb „ misal ** atau *
10.46
*
10.46
Tri Wahyuni FFUI
Di gambar harusnya di kasih info mb „ misal ** atau *
Siap
10.46
Jd nunjukin hal yg berbeda gt
10.46
Ketik pesan

10.39 • 36%

Tri Wahyuni FFUI

21.43

12 Agustus 2023

mb rini,,,, 10.29

punten ,, haha 10.30

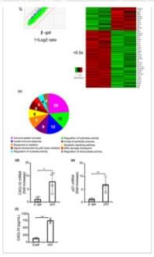


FIGURE 1 Open in figure viewer | PowerPoint

p53 induced CXCL10 expression in cardiomyocytes. DNA microarray was performed using p53-overexpressing NRCMs. (a) Scatter plot shows the genes colored blue with >2-fold upregulate in p53-overexpressing NRCMs compared with β-gal. (b) Heat map shows genes colored red with >2-fold change and green with <0.5-fold change between β-gal and p53. (c) Gene ontology analysis. (d, e) The expression of (d) CXCL10 and (e) p21 transcripts was measured by qPCR ($n = 4$). Experiments were performed three times with similar results. (f) The protein expression of CXCL10 in culture media of p53-overexpressing NRCMs was measured by ELISA ($n = 3$). *$p < 0.05$, **$p < 0.01$. by Student's t-test.

yang figure legend begini mb mksd saya 10.31

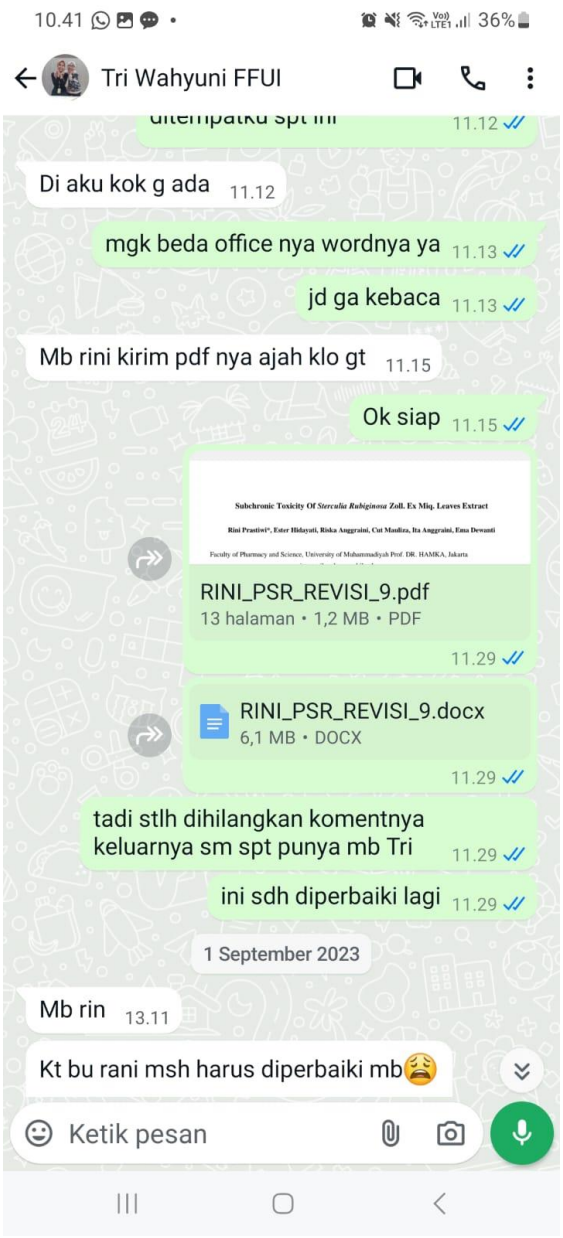
g ada kah mb? 10.31

ya udah klo gt mb klo memang g dikasih keterangan 10.33

nah mksd saya kmr nanya figure legend itu terkait dengan kan bagian method itu ada statistical analysis,, nah karena tidak ada perbedaan bermaksa klo dilihat dari gambar,, jadi itu ada di figure legend 10.35

Ketik pesan


10.39
36%
Tri Wahyuni FFUI
sy pi
10 Agustus 2023
laptop
13.21
Maaf ya bu
13.21
Klo ibu bisa saya telp ya
13.22
Makasih Bu Tri...bantuannya
Smg lancar penelitiannya di jepang, dimudahkan dan diberikan hasil memuaskan..aamiin YRA
13.30
Maaf ya mb rin ngerepotin
13.31
Sy yg ngrepotin malahan
13.32
mb rini,, punten
14.25
mb ini dah siap published,, namun,,,,,
14.25
RINI_PSR_REVISI_6_edtw.docx
6,0 MB • DOCX
14.26
mohon direvisi segera untuk tanda kuning dari aku ya mb,,, tdr dari 1. figure legend setiap figure hampir semua blm ada,, 2 references ikutin cara di psr ya mb,,penulisan secara APA
14.27
itu manuscrip aku ambil dari data mb rini
14.28
Ketik pesan


10.41
36%
Tri Wahyuni FFUI
11.12
Di aku kok g ada 11.12
mgk beda office nya wordnya ya 11.13
jd ga kebaca 11.13
Mb rini kirim pdf nya ajah klo gt 11.15
Ok siap 11.15
Subchronic Toxicity Of Sterculia Rubiginosa Zoll. Ex Miq. Leaves Extract
Rini Prastiwi*, Ester Hidayati, Riska Anggraini, Cut Mauliza, Ita Anggraini, Ema Dewanti
Faculty of Pharmacy and Science, University of Muhammadiyah Prof. DR. HAMKA, Jakarta
RINI_PSR_REVISI_9.pdf
13 halaman • 1,2 MB • PDF
11.29
RINI_PSR_REVISI_9.docx
6,1 MB • DOCX
11.29
tadi stlh dihilangkan komentnya keluarnya sm spt punya mb Tri 11.29
ini sdh diperbaiki lagi 11.29
1 September 2023
Mb rin 13.11
Kt bu rani msh harus diperbaiki mb
Ketik pesan

Bukti artikel yang direvisi

SUBCHRONIC TOXICITY OF *Sterculia rubiginosa* Zoll. Ex Miq. LEAVES EXTRACT

Rini Prastiwi[1]*, Ester Hidayati[1], Riska Anggraini[1], Cut Mauliza[1], Ita Anggraini[1], Ema Dewanti[1]

Faculty of Pharmacy and Science, University of Muhammadiyah Prof. DR. HAMKA, Jakartawrite
email and no. mobile phone

(rini_prastiwi@uhamka.ac.id)

ABSTRACT

Background*:* Ki Hampelas (*Sterculia rubiginosa* Zoll. Ex Miq) is traditionally used as an antiasthma. It alsohas antioxidant and nephroprotective activity.

Objective: This study was conducted to evaluate the subchronic toxicity of the leaf extract of Ki Hampelas. **Materials and Methods**: The extract was orally administered to male and female Sprague-Dawley rats atdoses of 50, 200, and 400 mg/Kg bodyweight/ day for 28 days. The rats were divided into four groups, consist of a the normal group (Na CMC 0.5%) with 50, 200, and 400 mg/kg BW of doses. the rats wadministered the extract every day for 28 days.

Results: Subchronic toxicity in the male and female rats resulted in no death or treatment-related signs at high doses. All the animals survived the duration of the study, with no significant changes in biochemichal parameters, organweight, or histological findings. There was no significant difference between the SGOT, SGPT, urea, andcreatinine levels in the dose groups with extracts and the normal group ($p > 0.05$). In addition, based on the histological results of the liver and kidneys it also found a significant difference among the groups.

Conclusion: This study establishes that the leaves extract of Ki Hampelas is non toxic in rats following oral administration.

Keywords: *Ki Hampelas, Subchronic, Histopathology, Toxicity, Sterculia rubiginosa* Zoll. Ex Miq.

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, while 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.

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, $FeCl_3$, amyl alcohol, H_2SO_4, Liebermann- Burchard reagent, Mayer reagent, ketamine, anhydrous acetic acid, ketamine, gelatine, SGOT kit reagent (Human), SGPT reagent kit (Human), Urea kit reagent (Human), Creatinine reagent kit (Human), rats, animalfeed, Na CMC (Brataco), aqua distillate, Spectrophotometer (Elitech).

Extraction

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

Characteristic extract

The characteristics extract were determined by organoleptic examination, calculation of yield, determinationof water content, and determination of ash content. The procedure was carried out according to Depkes 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

The groups were divided into 4 (eight), 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 giventhe 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 respectivereagents and read using a Clinical Spectrophotometer (BPOM, 2014). Liver and kidney histology preparations were made, and histology preparations were made by the Histology Laboratory, Universitas Indonesia (Mescher 2015).

This study was approved by the Ethics Committee of UHAMKA with No. 02/20.03/0358.

Statistical analysis

Experimental data were recorded using excel and statistically analyzed by SPSS 19 according to the sex of the animals and the test stage. The data on SGOT, SGPT, creatinine, urea, and histopathological organs wereanalyzed for normality

and homogeneity, then proceeded to the ANOVA test by SPSS 19. This test was carriedout to determine the difference between each group, whether it is significantly different or not. Numerical datalike 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.

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 ofmetabolites 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 andevaporated 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 secondarymetabolite 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 stigera* 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 (Djojosumarto, 2008).

The results of giving 70% ethanol extract of ki hampelas leaves which were administered test animals at a doseof 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 animalsdid not experience stress or toxic symptoms caused by the administration of ki hampelas leaf extract.

Repeated administration of the extract for 28 days, it caused an increase in the levels of SGOT andSGPT 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 andSGPT obtained showed $p > 0.05$, which indicates that there is no significant difference in each treatment group.The AST/ALT De Ritis ratio 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 thecytoplasmic contents come out causing ALT (SGPT) to increase higher than AST (SGOT) with an AST/ALT the ratio of <0.8 which indicates mild damage. In chronic or severe inflammation and damage, liver cell damagereaches 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 each group was in a group I which was 2.20, group II was 2.18, group III was 2.17, then group IV was 2.13. 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 UI/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 UI/L.

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 freeradicals, as well as recovery for dead or damaged liver cells (Birt *et al*. 2001). However, if there is an increasein the activity of the SGOT and SGPT enzymes, it can indicate the occurrence.

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 the subchronic toxicity test on liver histopathological preparations with Hematoxylin-Eosin staining, it can be seen 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 changesin the glomerulus in the acute toxicity test showed a significant difference between the normal group and thedose 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 asignificant difference between the 200 mg/kg BW and 400 mg/kg BW dose groups and the normal group, butno death was found and no tubular *casts* were found.

Figure 6, shows that there is a significant difference in the number of pyknotic nuclei between the normalgroup and the test group with $p<0.05$. Based on these results, it was found that there was an increase in necrosisat each increase in dose, the characteristic of necrosis that was seen was the pyknotic 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 leafextract 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 betweenthe glomerulus and Bowman's capsule, changes that occur in the glomerulus are edema which is characterizedby 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 wascalculated 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 $p>0.05$.

In the parameter of the percentage of closed proximal tubules, the percentage of proximal tubule damageis indicated by the number of closed proximal tubules in one field of view compared to the number of all proximal tubules (Shreevastva, 2017). Based on the results of the subchronic toxicity test, the results of statistical tests showed that there was a significant difference between all normal control groups and the test dose group, namely $p<0.05$, as shown in (Figure 8).

CONCLUSION

It can be concluded that the administration of 70% ethanol extract of ki hampelas leaves in the subchronic toxicity test did not cause death and had no toxic effect on the test animals. Based on the results of the examination of levels of SGOT/SGPT and creatinine/Ureum, it showed that there was no significant differencebetween the dose treatment group and the normal group ($p > 0.05$). On the other hand, based on the results ofhistology of the liver and kidneys, it was found there was a significant difference but did not cause death.

ACKNOWLEDGMENT

The author would like to thank the Lemlit UHAMKA for the Research Foundation through the PPI Grant.

References

Aikpe, J. F. A. *et al.* (2020).Antitussive properties of aqueous extracts of *Sterculia setigera* Delile (Sterculiaceae) and mixture of Aframomum melegueta K . Schum (Zingiberaceae) - Citrus aurantifolia(Christm . and panzer) Swingle (Rutaceae). *Journal of Pharmacognosy and Phytochemistry*. 9(2). 1624–1627. doi: 10.22271/phyto.2020.v9.i2aa.11085

Bibi, N. *et al.* (2019). Chemical profiling , antioxidant , enzyme inhibitory and molecular modelling studies on the leaves and stem bark extracts of three African medicinal plants. *Journal of Pharmaceutical and Biomedical Analysis*,174. 19–33.doi : 10.1016/j.jpba.2019.05.041

Birt, D.F., Hendrich, S. & Wang, W. (2001). Dietary agents in cancer prevention: flavonoids andisoflavonoids. *Pharmacology & Therapeutics*. 90(2). 157–77. doi: 10.1016/s0163-7258(01)00137-1

Depkes RI. (2008). Indonesian Herbal Pharmacopoeia Edition 1. *Ministry of Health RI*. Jakarta. 76-80

Depkes RI. (2000). Guidelines for the Implementation of Traditional Medicine Clinical Trials. *Directorate General*

of POM, Directorate of Drug and Food Control. 67-68.

Cahyaningsih, AD, Azizahwati, Dadang (2011). Efek Nefroprotektif Infus Daun Sukun (*Artocarpus altilis* (Park.) Fsb.) Pada Tikus Jantan yang Diinduksi Karbon Tetraklorida. *Majalah Ilmu Kefarmasian*. 8(2).15-18. doi : https://doi.org/10.7454/psr.v8i2.3473

BPOM RI. (2014). Guidelines for Invivo Non-Clinical Toxicity Test. BPOM RI. *BPOM Jakarta*. 34.

Djojosumarto, Panut. (2008). Pesticides and their Applications. Jakarta: *Agromedia Pustaka*. 45-48

Fazle Rabbi, Amir Zada, Achyut Adhikari, Almas Jabeen, A.N. and I.U. (2017). Sterculia diversifolia bears anti-cancer and immunomodulatory activities. *Bangladesh Journal of Pharmacology*, 12. 52–55. doi: 10.3329/bjp.v12i1.29516

Hanani, E. (2015). Phytochemical Analysis. Jakarta: *EGC Medical Book Publisher*. 86, 87

Hossain, F. et *al*. (2016). In vivo sedative activity of methanolic extract of *Sterculia villos*a Roxb leaves. *BMC Complementary and Alternative Medicine*, 16. 10–13. doi : http://dx.doi.org/10.1186/s12906-016-1374-8

Jafri, A. *et al*. (2019). Phytochemical screening of Sterculia foetida seed extract for anti-oxidant, anti- microbial activity , and detection of apoptosis through reactive oxygen species (ROS) generation , mitochondrial membrane potential (MMP) decrease, and nuclear fragmentation in human osteosarcoma cells. *Journal of Histotechnology*, 42(2).1–12. doi: 10.1080/01478885.2019.1592832

Lu, C Frank. (2010). Basic Toxicology. Second edition. Jakarta : *University of Indonesia P*ress.

Prastiwi, R., Elya, B., Sauriasari, R., Hanafi, M., Dewanti, E. 2018. Pharmacognosy, Phytochemical Study and Antioxidant Activity of sterculia rubiginosa Zoll. Ex Miq. leaves. *Pharmacognosy Journal*, Vol 10: 571-575. doi: 10.5530/pj.2018.3.93

Prastiwi, R., Dewanti, E., Nurul, I., Aqilla, N., Salsabila, S.Ladeska, V. 2020. The Nephroprotective And Antioxidant Activity of Sterculia rubiginosa Zoll. Ex Miq. leaves. *Pharmacognosy Journ*al Vol 12, Issue 4, July-Aug, 2020. doi: 10.5530/pj.2020.12.121

Prastiwi, R. *et al*. (2021) , The Antioxidant Activity of Sterculia stipulata Korth Woods and Leaves by FRAPMethod, *Pharmacognosy Journal* 12(2), pp. 236–239. doi: 10.5530/pj.2020.12.36

Prastiwi Rini, Ema Dewanti, Cut Mauliza, Ester Hidayati, Ita Anggraini, Riska Anggraini, Vera Ladeska. (2021). The Acute Toxicity of Ki Hampelas Leaves (Sterculia rubiginosa Zoll. Ex Miq). 13(2). 570-576. doi: 10.5530/pj.2021.13.71

Priyanto. 2009. Toxicology: mechanism, anidotum therapy, and risk assessment. Depok: leskonfi. 67-68 Priyanto. 2015.

Toxicology, Mechanisms, Antidote Therapy, and Risk Assessment. *Lenskofi. Jakarta*. pp. 1, 177, 180, 182.

Rosida, A. 2016. Pemeriksaan laboratorium penyakit hati. Berkala Kedokteran. Vol.12. No.1.123-131

Saleh et al.(2016).M.Phytochemistry, biological activities and economical uses of the genus *Sterculia* and therelated genera: A review. Asian Pacific Journal of Tropical Disease, 6(6):492–501. doi: http://dx.doi.org/10.1016/S2222-1808(16)61075-7

Shreevastva, NK, Pandeya, A., & Mishra, DK 2017 (2017). A study of AST : ALT ratio in alcoholic and nonalcoholic liver diseases. 4929, 1047–1050.https://doi.org/10.21276/sjmps.2017.3.10.5

Swarnalatha, K. *et al*. (2019) Phytochemical Screening , Anti-Diabetic And Anti-Oxidant Activities Of

Kigelia africana (LAM .) and *Sterculia foetida* L. *Rasayan J. Chem*, 12(2).907–914. doi: :10.31788/RJC.2019.1225168

Mogbojuri, O.M., Adedapo, A.A. & Abatan, M.O. (2016) Phytochemical screening , safety evaluation , anti-inflammatory and analgesic studies of the leaf extracts of Sterculia tragacantha. *J Complement IntegrMed*, 2–7. doi: 10.1515/jcim-2015-0114

Mescher, AL (2011). Basic Histology of Junqueira Texts and Atlas. Jakarta: *EGC*. page. 2-4

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	Liberman-Bouchard	+

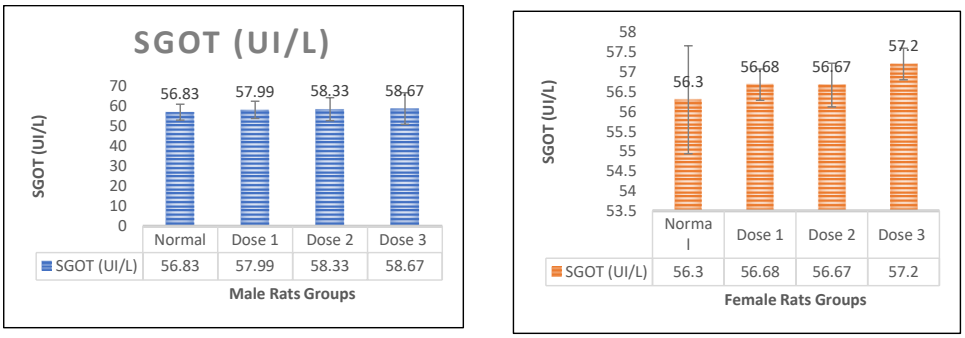


Figure 1. SGOT levels

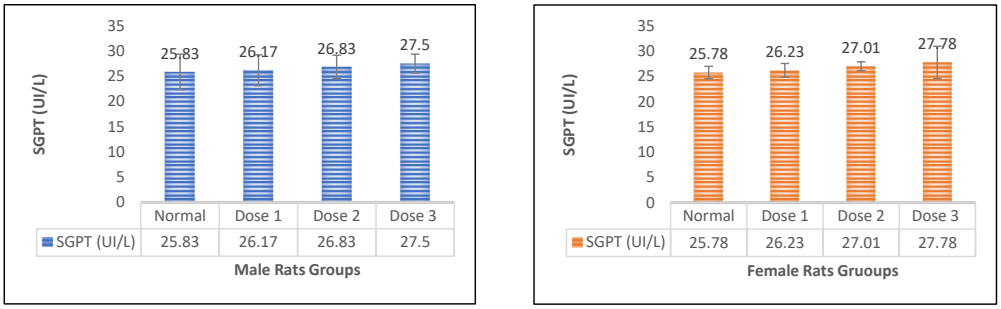
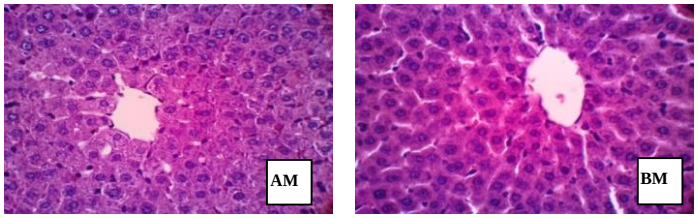
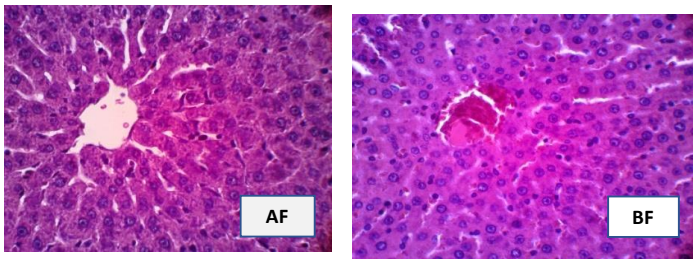


Figure 2. SGPT levels



CM DM



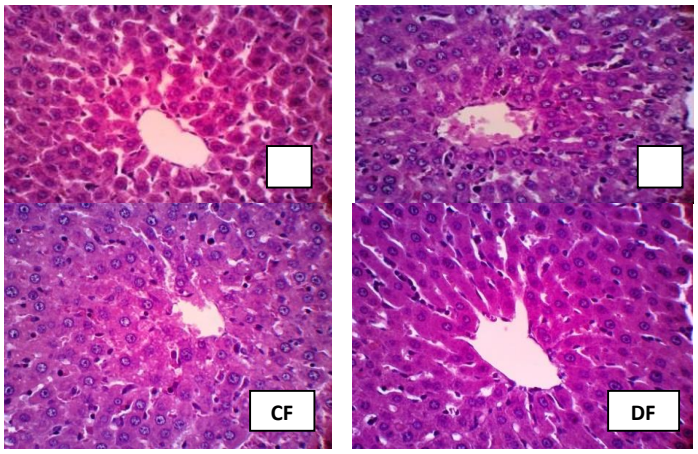
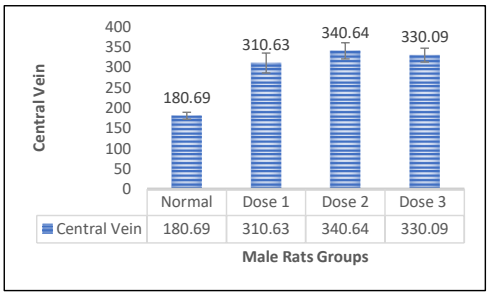


Figure 3. Histopathology of the liver

Information: Transverse incision histology of rat liver organ with hemotoxin-eosin staining at 40x10magnification. M: Male, F: Female, (AM) 0.5% NaCMC, (BM) 50 mg/kgBW, (CM 200 mg/kgBW, and (DM) 400 mg/.kgBW. (AF) 0.5% NaCMC, (BF) 50 mg/kgBW, (CF) 200 mg/kgBW, and (DF) 400 mg/.kgBW



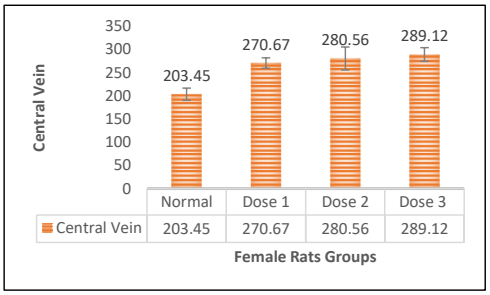
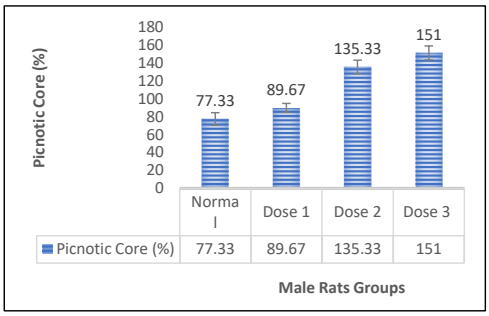


Figure 4. Diameter of the Central Vein



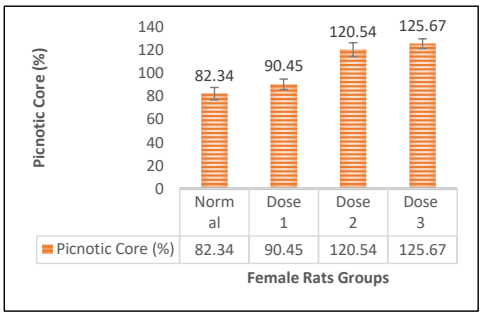
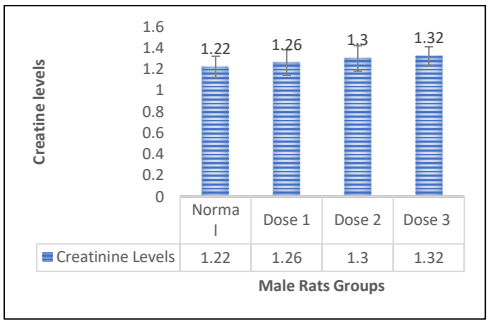


Figure 5. Picnotic Core



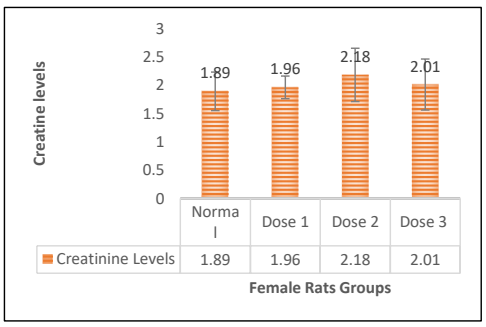


Figure 6. Creatinine Levels

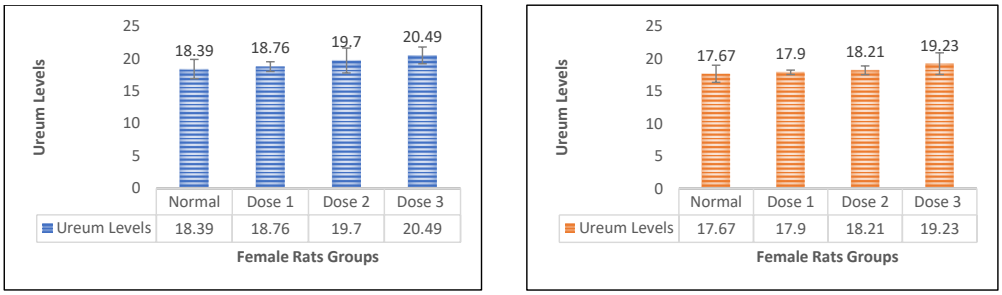


Figure 7. Ureum levels

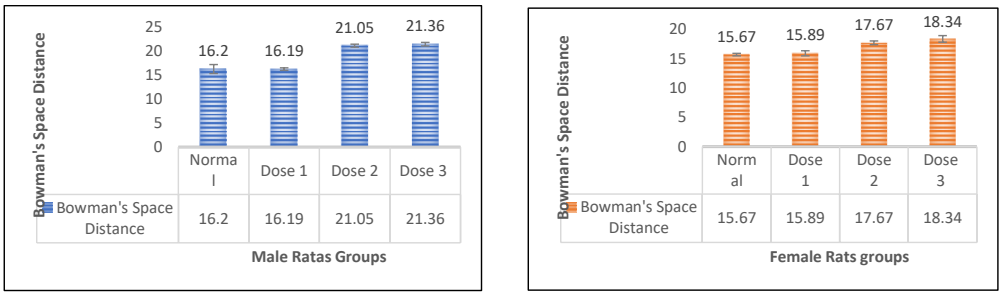


Figure 8. Bowman's Space Distance

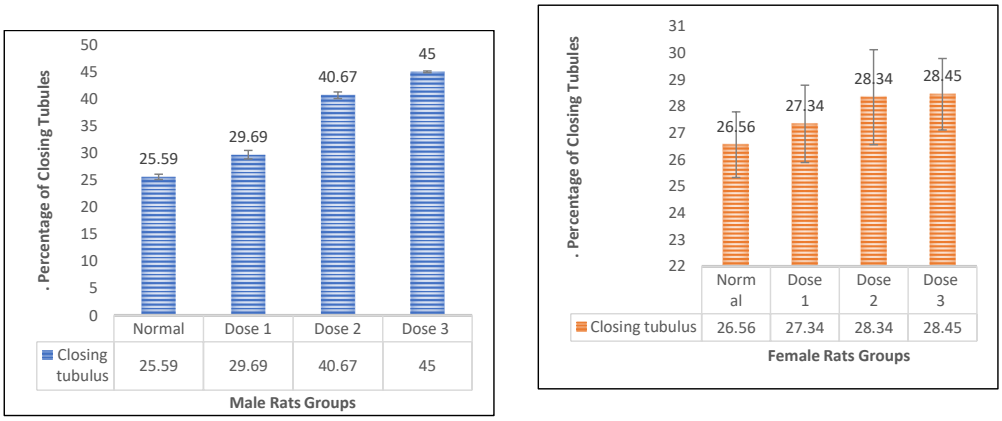


Figure 9. Percentage of Closing Tubules

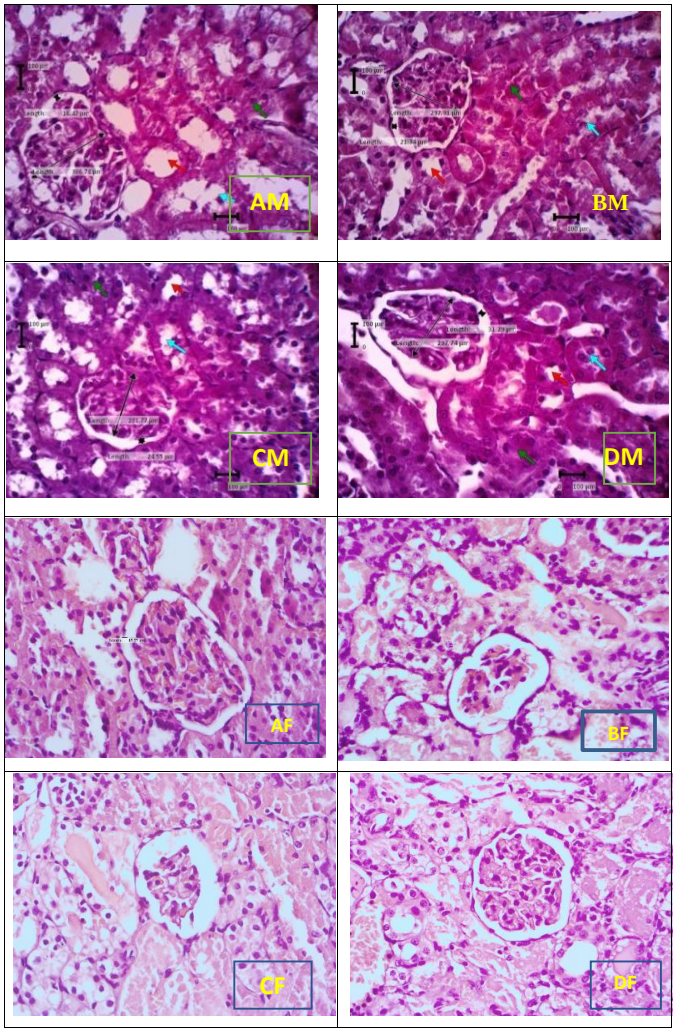


Figure 10. Kidney Histopathology

Information: Transverse incision histology of rat Kidney organ with hemotoxin-eosin staining at 40x10magnification. M: male, F: Female (AM) 0.5% CMC, (BM) 50 mg/kgBW, (CM) 200 mg/kgBW, and (DM) 400 mg/.kgBW, (AF) 0.5% CMC, (BF) 50 mg/kgBW, (CF) 200 mg/kgBW, and (DF) 400 mg/.kgBW.

	Komisi Etik Penelitian Kesehatan **Universitas Muhammadiyah Prof. DR. HAMKA** **(KEPK – UHAMKA) Jakarta** http://www.lef.afit.uhamka.ac.id	**POB-KE.B/008/01.0** Berlaku mulai: 19 Mei 2017 FL/B.06-008/01.0

SURAT PERSETUJUAN ETIK

PERSETUJUAN ETIK
ETHICAL APPROVAL

No : 02/20.03/0358

Bismillaahirrohmaanirrohiim
Assalamu'alaikum warohmatullohi wabarokatuh

Yang bertanda tangan di bawah ini, Ketua Komisi Etik Penelitian Kesehatan Universitas Muhammadiyah Prof. DR. HAMKA (KEPK-UHAMKA), setelah dilaksanakan pembahasan dan penilaian oleh reviewer yang bersertifikat, memutuskan bahwa protokol penelitian/skripsi/tesis dengan judul :

"UJI TOKSISITAS EKSTRAK ETANOL 70% DAUN KI HAMPELAS (*Sterculia rubiginosa*) PADA TIKUS PUTIH"

Atas nama

Peneliti utama	: Rini Prastiwi, M.Si., Apt.
Peneliti lain	: Ema Dewanti, M.Si., Cut Mauliza, Ester Hidayati, Ita Anggraini, Riska Anggraini
Program Studi	: S1 FARMASI
Institusi	: UNIVERSITAS MUHAMMADIYAH PROF. DR. HAMKA JAKARTA

dapat disetujui pelaksanaannya. Persetujuan ini berlaku sejak tanggal ditetapkan sampai dengan batas waktu pelaksanaan penelitian seperti tertera dalam protokol.

Pada akhir penelitian, laporan pelaksanaan penelitian harus diserahkan kepada KEPK-UHAMKA dalam bentuk *soft copy* ke email kepk@uhamka.ac.id. Jika terdapat perubahan protokol dan/atau perpanjangan penelitian, maka peneliti harus mengajukan kembali permohonan kajian etik penelitian (amandemen protokol).

Wassalamu'alaikum warohmatullohi wabarokatuh

Jakarta, 02 Maret 2020
Ketua Komisi Etik Penelitian Kesehatan
UHAMKA

(Dr. Emma Rachmawati, Dra., M.Kes)

Subchronic Toxicity Of *Sterculia Rubiginosa* Zoll. Ex Miq. Leaves Extract

Rini Prastiwi*, Ester Hidayati, Riska Anggraini, Cut Mauliza, Ita Anggraini, Ema Dewanti

Faculty of Pharmacy and Science, University of Muhammadiyah Prof. DR. HAMKA, Jakartawrite
email and no. mobile phone

(rini_prastiwi@uhamka.ac.id)

ABSTRACT

Ki Hampelas (*Sterculia rubiginosa* Zoll. Ex Miq) is traditionally used as an antiasthma. It alsohas 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 atdoses of 50, 200, and 400 mg/Kg bodyweight/ day for 28 days. The rats were divided into four groups, consist of a the normal group (Na CMC 0.5%) with 50, 200, and 400 mg/kg BW of doses. the rats administered the extract every day for 28 days. Subchronic toxicity in the male and female rats resulted in no death or treatment-related signs at high doses.All the animals survived the duration of the study, with no significant changes in biochemichal parameters, organweight, or histological findings. There was no significant difference between the SGOT, SGPT, urea, andcreatinine levels in the dose groups with extracts and the normal group ($p > 0.05$). In addition, based on the histological results of the liver and kidneys it also found a significant difference among the groups. This study establishes that the leaves extract of Ki Hampelas is non toxic in rats following oraladministration.

Keywords: *Ki Hampelas, Subchronic, Histopathology, Toxicity, Sterculia rubiginosa* Zoll. Ex Miq.

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, while 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.

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, $FeCl_3$, amyl alcohol, H_2SO_4, Liebermann- Burchard reagent, Mayer reagent, ketamine, anhydrous acetic acid, ketamine, gelatine, SGOT kit reagent (Human), SGPT reagent kit (Human), Urea kit reagent (Human), Creatinine reagent kit (Human), rats, animalfeed, Na CMC (Brataco), aqua distillate, Spectrophotometer (Elitech).

Extraction

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

Characteristic extract

The characteristics extract were determined by organoleptic examination, calculation of yield, determinationof water content, and determination of ash content. The procedure was carried out according to Depkes 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

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 giventhe 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 respectivereagents and read using a Clinical Spectrophotometer (BPOM, 2014). Liver and kidney histology preparations were made, and histology preparations were made by the Histology Laboratory, Universitas Indonesia (Mescher 2015).
This study was approved by the Ethics Committee of UHAMKA with No. 02/20.03/0358.

Statistical analysis

Experimental data were recorded using excel and statistically analyzed by SPSS 19 according to the sex of the animals and the test stage. The data on SGOT, SGPT, creatinine, urea, and histopathological organs wereanalyzed for normality and homogeneity, then proceeded to the ANOVA test by SPSS 19. This test was carriedout to determine the difference between each group, whether it is significantly different or not. Numerical datalike 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.

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 ofmetabolites 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 andevaporated 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 secondarymetabolite 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 stigera* 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 (Djojosumarto, 2008).

The results of giving 70% ethanol extract of ki hampelas leaves which were administered test animals at a doseof 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 animalsdid not experience stress or toxic symptoms caused by the administration of ki hampelas leaf extract.

Repeated administration of the extract for 28 days, it caused an increase in the levels of SGOT andSGPT 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 andSGPT obtained showed $p > 0.05$, which indicates that there is no significant difference in each treatment group.The AST/ALT De Ritis ratio 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 thecytoplasmic contents come out causing ALT (SGPT) to increase higher than AST (SGOT) with an AST/ALT the ratio of <0.8 which indicates mild damage. In chronic or severe inflammation and damage, liver cell damagereaches 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 each group was in a group I which was 2.20, group II was 2.18, group III was 2.17, then group IV was 2.13. 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 UI/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 UI/L.

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 freeradicals, as well as recovery for dead or damaged liver cells (Birt *et al*. 2001). However, if there is an increasein the activity of the SGOT and SGPT enzymes, it can indicate the occurrence.

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 the subchronic toxicity test on liver histopathological preparations with Hematoxylin-Eosin staining, it can be seen 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 changesin the glomerulus in the acute toxicity test showed a significant difference between the normal group and thedose 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 asignificant difference between the 200 mg/kg BW and 400 mg/kg BW dose groups and the normal group, butno death was found and no tubular *casts* were found.

Figure 6, shows that there is a significant difference in the number of pyknotic nuclei between the normalgroup and the test group with $p<0.05$. Based on these results, it was found that there was an increase in necrosisat each increase in dose, the characteristic of necrosis that was seen was the pyknotic 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 leafextract 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 betweenthe glomerulus and Bowman's capsule, changes that occur in the glomerulus are edema which is characterizedby 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 wascalculated 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 $p>0.05$.

In the parameter of the percentage of closed proximal tubules, the percentage of proximal tubule damageis indicated by the number of closed proximal tubules in one field of view compared to the number of all proximal tubules (Shreevastva, 2017). Based on the results of the subchronic toxicity test, the results of statistical tests showed that there was a significant difference between all normal control groups and the test dose group, namely $p<0.05$, as shown in (Figure 8).

CONCLUSION

It can be concluded that the administration of 70% ethanol extract of ki hampelas leaves in the subchronic toxicity test did not cause death and had no toxic effect on the test animals. Based on the results of the examination of levels of SGOT/SGPT and creatinine/Ureum, it showed that there was no significant differencebetween the dose treatment group and the normal group ($p > 0.05$). On the other hand, based on the results ofhistology of the liver and kidneys, it was found there was a significant difference but did not cause death.

ACKNOWLEDGMENT

The author would like to thank the Lemlit UHAMKA for the Research Foundation through the PPI Grant.

References

Aikpe, J. F. A. *et al.* (2020).Antitussive properties of aqueous extracts of *Sterculia setigera* Delile (Sterculiaceae) and mixture of Aframomum melegueta K . Schum (Zingiberaceae) - Citrus aurantifolia(Christm . and panzer) Swingle (Rutaceae). *Journal of Pharmacognosy and Phytochemistry*. 9(2). 1624–1627. doi: 10.22271/phyto.2020.v9.i2aa.11085

Bibi, N. *et al.* (2019). Chemical profiling , antioxidant , enzyme inhibitory and molecular modelling studies on the leaves and stem bark extracts of three African medicinal plants. *Journal of Pharmaceutical and Biomedical Analysis*,174. 19–33.doi : 10.1016/j.jpba.2019.05.041

Birt, D.F., Hendrich, S. & Wang, W. (2001). Dietary agents in cancer prevention: flavonoids andisoflavonoids. *Pharmacology & Therapeutics*. 90(2). 157–77. doi: 10.1016/s0163-7258(01)00137-1

Depkes RI. (2008). Indonesian Herbal Pharmacopoeia Edition 1. *Ministry of Health RI*. Jakarta. 76-80

Depkes RI. (2000). Guidelines for the Implementation of Traditional Medicine Clinical Trials. *Directorate General of POM, Directorate of Drug and Food Control*. 67-68.

Cahyaningsih, AD, Azizahwati, Dadang (2011). Efek Nefroprotektif Infus Daun Sukun (*Artocarpus altilis* (Park.) Fsb.) Pada Tikus Jantan yang Diinduksi Karbon Tetraklorida. *Majalah Ilmu Kefarmasian*. 8(2).15-18. doi : https://doi.org/10.7454/psr.v8i2.3473

BPOM RI. (2014). Guidelines for Invivo Non-Clinical Toxicity Test. BPOM RI. *BPOM Jakarta*. 34.

Djojosumarto, Panut. (2008). Pesticides and their Applications. Jakarta: *Agromedia Pustaka*. 45-48

Fazle Rabbi, Amir Zada, Achyut Adhikari, Almas Jabeen, A.N. and I.U. (2017). Sterculia diversifolia bears anti-cancer and immunomodulatory activities. *Bangladesh Journal of Pharmacology*, 12. 52–55. doi: 10.3329/bjp.v12i1.29516

Hanani, E. (2015). Phytochemical Analysis. Jakarta: *EGC Medical Book Publisher*. 86, 87

Hossain, F. et *al*. (2016). In vivo sedative activity of methanolic extract of *Sterculia villos*a Roxb leaves. *BMC Complementary and Alternative Medicine*, 16. 10–13. doi : http://dx.doi.org/10.1186/s12906-016-1374-8

Jafri, A. *et al*. (2019). Phytochemical screening of Sterculia foetida seed extract for anti-oxidant, anti- microbial activity , and detection of apoptosis through reactive oxygen species (ROS) generation , mitochondrial membrane potential (MMP) decrease, and nuclear fragmentation in human osteosarcoma cells. *Journal of Histotechnology*, 42(2).1–12. doi: 10.1080/01478885.2019.1592832

Lu, C Frank. (2010). Basic Toxicology. Second edition. Jakarta : *University of Indonesia Pr*ess.

Prastiwi, R., Elya, B., Sauriasari, R., Hanafi, M., Dewanti, E. 2018. Pharmacognosy, Phytochemical Study and Antioxidant Activity of sterculia rubiginosa Zoll. Ex Miq. leaves. *Pharmacognosy Journal*, Vol 10: 571-575. doi: 10.5530/pj.2018.3.93

Prastiwi, R., Dewanti, E., Nurul, I., Aqilla, N., Salsabila, S.Ladeska, V. 2020. The Nephroprotective And Antioxidant Activity of Sterculia rubiginosa Zoll. Ex Miq. leaves. *Pharmacognosy Journ*al Vol 12, Issue 4, July-Aug, 2020. doi: 10.5530/pj.2020.12.121

Prastiwi, R. *et al*. (2021) , The Antioxidant Activity of Sterculia stipulata Korth Woods and Leaves by FRAPMethod, *Pharmacognosy Journal* 12(2), pp. 236–239. doi: 10.5530/pj.2020.12.36

Prastiwi Rini, Ema Dewanti, Cut Mauliza, Ester Hidayati, Ita Anggraini, Riska Anggraini, Vera Ladeska. (2021). The Acute Toxicity of Ki Hampelas Leaves (Sterculia rubiginosa Zoll. Ex Miq). 13(2). 570-576. doi: 10.5530/pj.2021.13.71

Priyanto. 2009. Toxicology: mechanism, anidotum therapy, and risk assessment. Depok: leskonfi. 67-68 Priyanto. 2015.

Toxicology, Mechanisms, Antidote Therapy, and Risk Assessment. *Lenskofi. Jakarta*. pp. 1, 177, 180, 182.

Rosida, A. 2016. Pemeriksaan laboratorium penyakit hati. Berkala Kedokteran. Vol.12. No.1.123-131

Saleh et al.(2016).M.Phytochemistry, biological activities and economical uses of the genus *Sterculia* and therelated genera: A review. Asian Pacific Journal of Tropical Disease, 6(6):492–501. doi: http://dx.doi.org/10.1016/S2222-1808(16)61075-7

Shreevastva, NK, Pandeya, A., & Mishra, DK 2017 (2017). A study of AST : ALT ratio in alcoholic and nonalcoholic liver diseases. 4929, 1047–1050.https://doi.org/10.21276/sjmps.2017.3.10.5

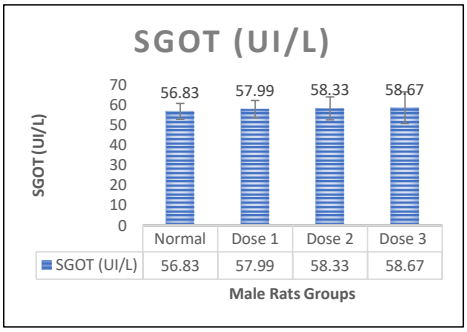
Swarnalatha, K. *et al*. (2019) Phytochemical Screening , Anti-Diabetic And Anti-Oxidant Activities Of *Kigelia africana* (LAM .) and *Sterculia foetida* L. *Rasayan J. Chem*, 12(2).907–914. doi: :10.31788/RJC.2019.1225168

Mogbojuri, O.M., Adedapo, A.A. & Abatan, M.O. (2016) Phytochemical screening , safety evaluation , anti-inflammatory and analgesic studies of the leaf extracts of Sterculia tragacantha. *J Complement IntegrMed*, 2–7. doi: 10.1515/jcim-2015-0114

Mescher, AL (2011). Basic Histology of Junqueira Texts and Atlas. Jakarta: *EGC*. page. 2-4

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	Liberman-Bouchard	+



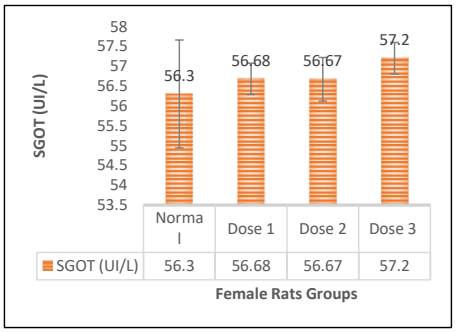
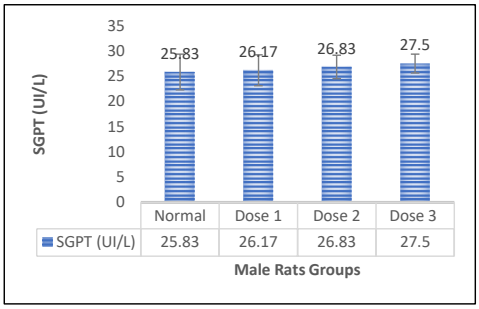


Figure 1. SGOT levels



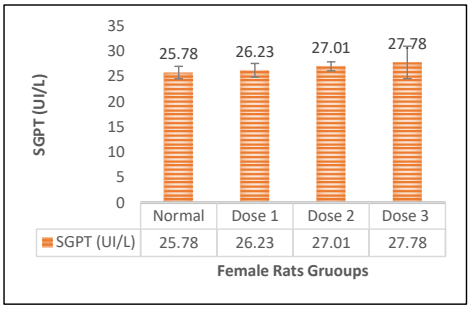
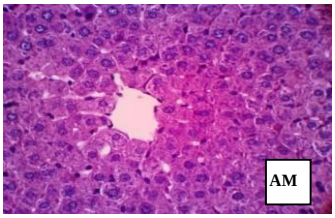
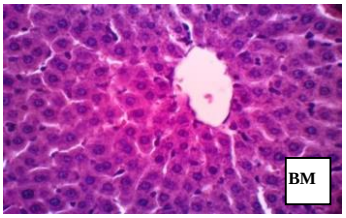


Figure 2. SGPT levels





CM **DM**

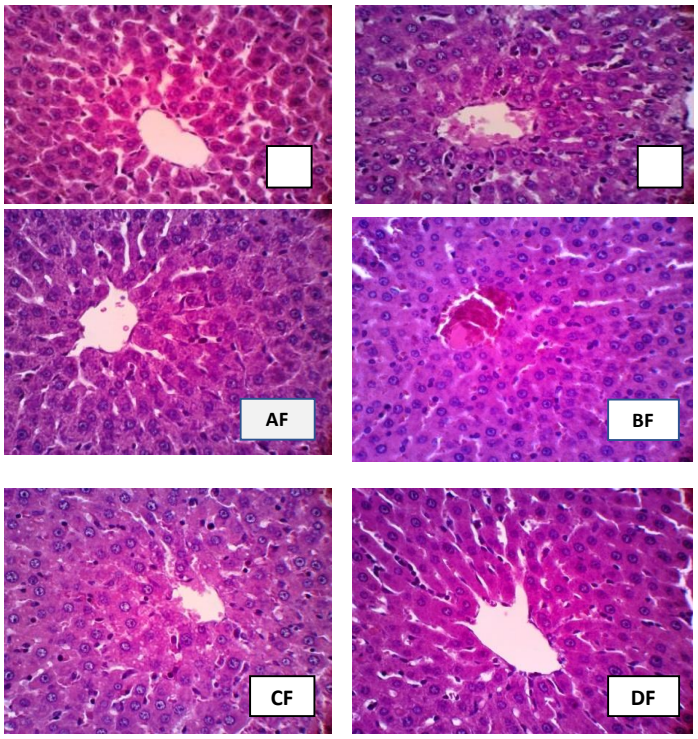
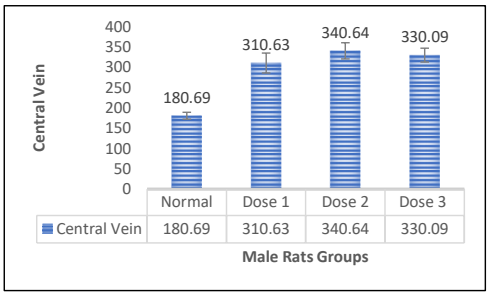


Figure 3. Histopathology of the liver

Information: Transverse incision histology of rat liver organ with hemotoxin-eosin staining at 40x10magnification. M: Male, F: Female, (AM) 0.5% NaCMC, (BM) 50 mg/kgBW, (CM 200 mg/kgBW, and (DM) 400 mg/.kgBW. (AF) 0.5% NaCMC, (BF) 50 mg/kgBW, (CF) 200 mg/kgBW, and (DF) 400 mg/.kgBW



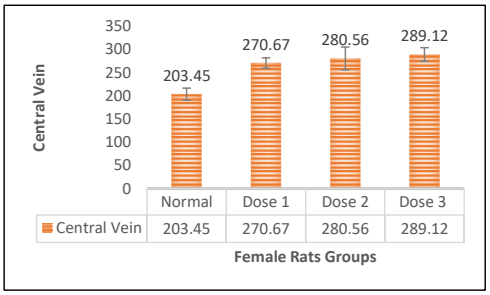
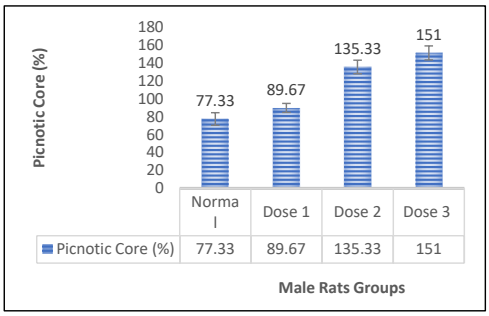


Figure 4. Diameter of the Central Vein



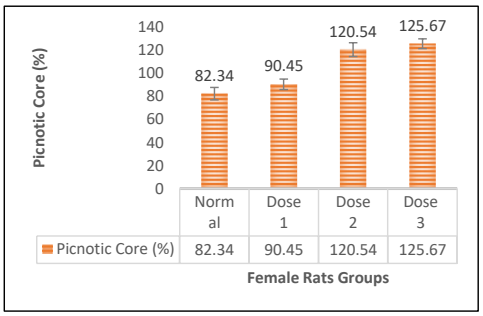
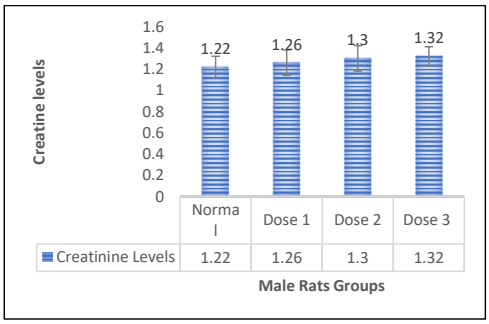


Figure 5. Picnotic Core



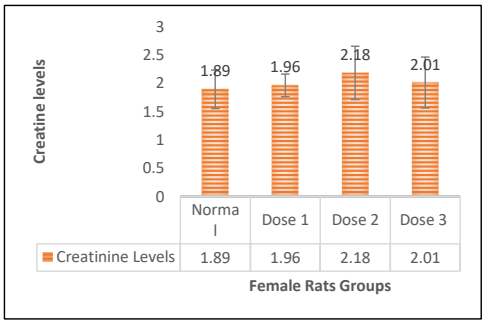
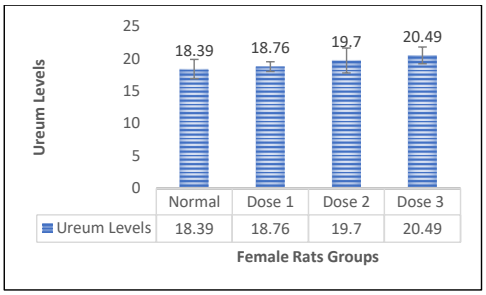


Figure 6. Creatinine Levels



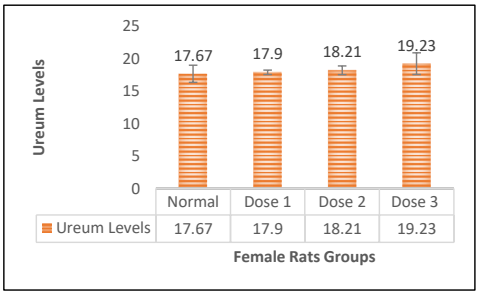
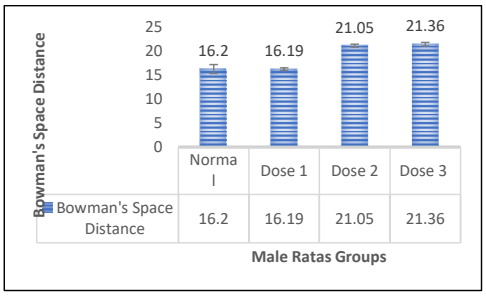


Figure 7. Ureum levels



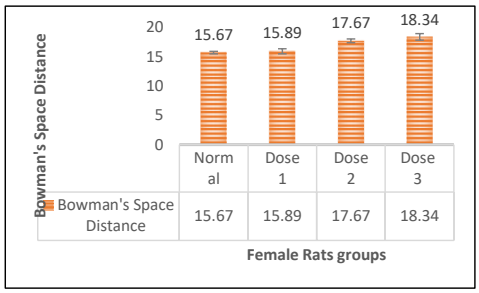
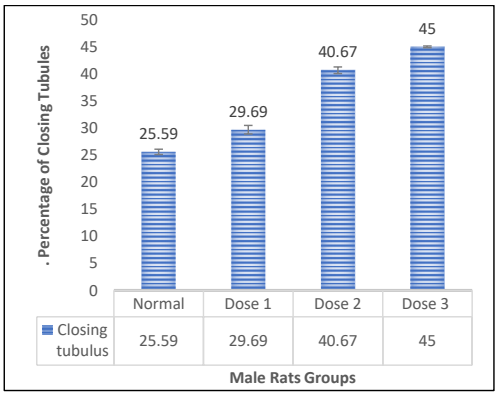


Figure 8. Bowman's Space Distance



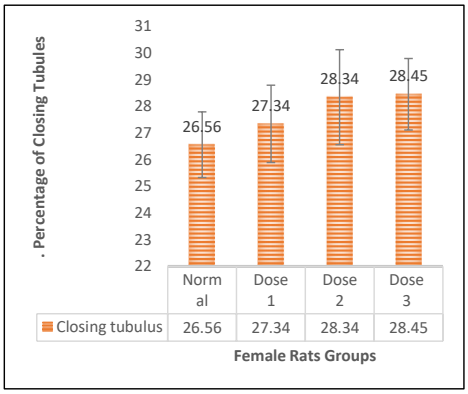


Figure 9. Percentage of Closing Tubules

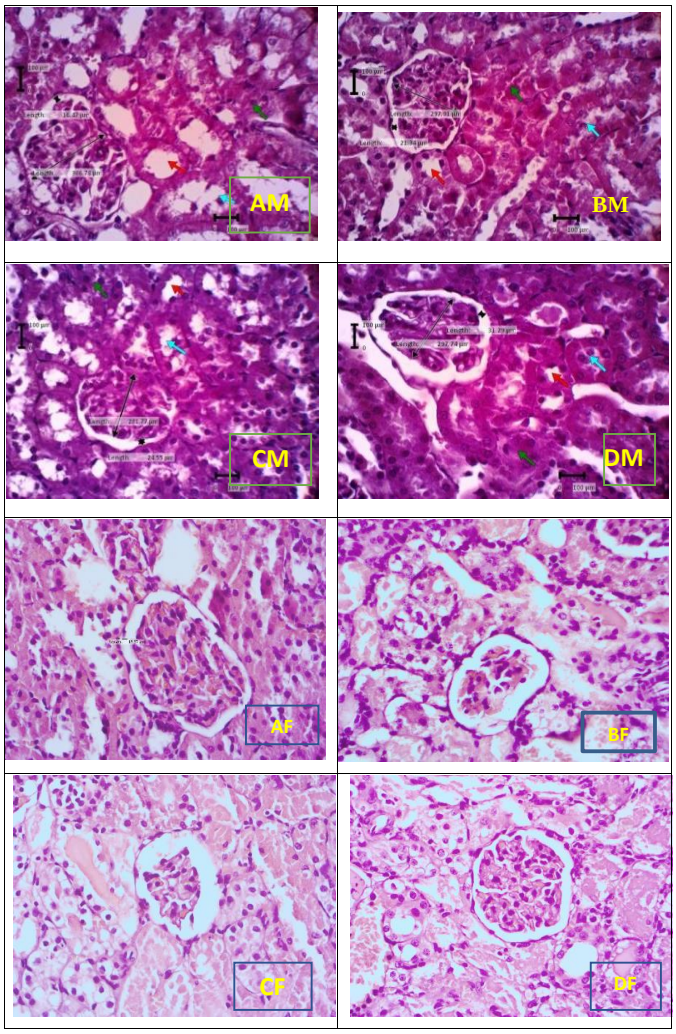


Figure 10. Kidney Histopathology

Information: Transverse incision histology of rat Kidney organ with hemotoxin-eosin staining at 40x10magnification. M: male, F: Female (AM) 0.5% CMC, (BM) 50 mg/kgBW, (CM) 200 mg/kgBW, and (DM) 400 mg/.kgBW, (AF) 0.5% CMC, (BF) 50 mg/kgBW, (CF) 200 mg/kgBW, and (DF) 400 mg/.kgBW.

Subchronic Toxicity Of *Sterculia Rubiginosa* ZZoll. EEx MMiq. Leaves Extract

Rini Prastiwi[1]*, Ester Hidayati[1], Riska Anggraini[1], Cut Mauliza[1], Ita Anggraini[1], Ema Dewanti[1]

Faculty of Pharmacy and Science, University of Muhammadiyah Prof. DR. HAMKA, Jakartawrite
email and no. mobile phone

Formatted: Font: 14 pt

Formatted: Font: 14 pt, Italic

Formatted: Font: 14 pt

(rini_prastiwi@uhamka.ac.id)

ABSTRACT

~~Background:~~ Ki Hampelas (*Sterculia rubiginosa* Zoll. Ex Miq) is traditionally used as an antiasthma. It alsohas antioxidant and nephroprotective activity.

~~Objective:~~ This study was conducted to evaluate the subchronic toxicity of the leaf extract of Ki Hampelas.

~~Materials and Methods:~~ The extract was orally administered to male and female Sprague-Dawley rats at doses of 50, 200, and 400 mg/Kg bodyweight/ day for 28 days. The rats were divided into four groups, consist of a the normal group (Na CMC 0.5%) with 50, 200, and 400 mg/kg BW of doses. the rats administered the extract every day for 28 days.

~~Results:~~ Subchronic toxicity in the male and female rats resulted in no death or treatment-related signs at high doses. All the animals survived the duration of the study, with no significant changes in biochemichal parameters, organweight, or histological findings. There was no significant difference between the SGOT, SGPT, urea, andcreatinine levels in the dose groups with extracts and the normal group ($p > 0.05$). In addition, based on the histological results of the liver and kidneys it also found a significant difference among the groups.

~~Conclusion:~~ This study establishes that the leaves extract of Ki Hampelas is non toxic in rats following oral administration.

Keywords: *Ki Hampelas, Subchronic, Histopathology, Toxicity, Sterculia rubiginosa* Zoll. Ex Miq.

Formatted: Normal, Right: 0 cm, Space Before: 6.85 pt

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).

Formatted: Font: Italic

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, while 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.

Formatted: Font: Italic

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).

Formatted: Font: Italic

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.

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, $FeCl_3$, amyl alcohol, H_2SO_4, Liebermann- Burchard reagent, Mayer reagent, ketamine, anhydrous acetic acid, ketamine, gelatine, SGOT kit reagent (Human), SGPT reagent kit (Human), Urea kit reagent (Human), Creatinine reagent kit (Human), rats, animalfeed, Na CMC (Brataco), aqua distillate, Spectrophotometer (Elitech).

Extraction

The powder (200 g) was macerated at room temperature with 70% ethanol. Maceration was carried outfor 3 days

with stirring several times a day. The filtrate was evaporated under a vacuum at 45 ºC by rotary evaporatorMaceration was carried out for 3 days with stirring several times a day. The filtrate was filtered andseparated. The pulp was macerated again until the color of the solvent changed to clear. After that, the maceratewas concentrated using a vacuum rotary evaporator at a temperature of 50 ºC until the extract was thick, then subjected to a water bath at the same temperature (Depkes, 2008).

Characteristic extract

The characteristics extract were determined by organoleptic examination, calculation of yield, determinationof water content, and determination of ash content. The procedure was carried out according to Depkes 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

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 giventhe 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 respectivereagents and read using a Clinical Spectrophotometer (BPOM, 2014). Liver and kidney histology preparations were made, and histology preparations were made by the Histology Laboratory, Universitas Indonesia (Mescher 2015).
This study was approved by the Ethics Committee of UHAMKA with No. 02/20.03/0358.

Statistical analysis

Experimental data were recorded using excel and statistically analyzed by SPSS 19 according to the sex of the animals and the test stage. The data on SGOT, SGPT, creatinine, urea, and histopathological organs wereanalyzed for normality and homogeneity, then proceeded to the ANOVA test by SPSS 19. This test was carriedout to determine the difference between each group, whether it is significantly different or not. Numerical datalike 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.

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 ofmetabolites 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 andevaporated 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 secondarymetabolite 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 stigera* 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 (Djojosumarto, 2008).

The results of giving 70% ethanol extract of ki hampelas leaves which were administered test animals at a doseof 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 animalsdid not experience stress or toxic symptoms caused by the administration of ki hampelas leaf extract.

Repeated administration of the extract for 28 days, it caused an increase in the levels of SGOT andSGPT 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 andSGPT obtained showed $p > 0.05$, which indicates that there is no significant difference in each treatment group.The AST/ALT De Ritis ratio 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 thecytoplasmic contents come out causing ALT (SGPT) to increase higher than AST (SGOT) with an AST/ALT the ratio of <0.8 which indicates mild damage. In chronic or severe inflammation and damage, liver cell damagereaches 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 each group was in a group I which was 2.20, group II was 2.18, group III was 2.17, then group IV was 2.13. 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 UI/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 UI/L.

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 freeradicals, as well as recovery for dead or damaged liver cells (Birt *et al*. 2001). However, if there is an increasein the activity of the SGOT and SGPT enzymes, it can indicate the occurrence.

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 the subchronic toxicity test on liver histopathological preparations with Hematoxylin-Eosin staining, it can be seen 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 changesin the glomerulus in the acute toxicity test showed a significant difference between the normal group and thedose 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 asignificant difference between the 200 mg/kg BW and 400 mg/kg BW dose groups and the normal group, butno death was found and no tubular *casts* were found.

Figure 6, shows that there is a significant difference in the number of pyknotic nuclei between the normalgroup and the test group with $p<0.05$. Based on these results, it was found that there was an increase in necrosisat each increase in dose, the characteristic of necrosis that was seen was the pyknotic 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 leafextract 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 betweenthe glomerulus and Bowman's capsule, changes that occur in the glomerulus are edema which is characterizedby 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 wascalculated 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 $p>0.05$.

In the parameter of the percentage of closed proximal tubules, the percentage of proximal tubule damageis indicated by the number of closed proximal tubules in one field of view compared to the number of all proximal tubules (Shreevastva, 2017). Based on the results of the subchronic toxicity test, the results of statistical tests showed that there

was a significant difference between all normal control groups and the test dose group, namely $p<0.05$, as shown in (Figure 8).

CONCLUSION

It can be concluded that the administration of 70% ethanol extract of ki hampelas leaves in the subchronic toxicity test did not cause death and had no toxic effect on the test animals. Based on the results of the examination of levels of SGOT/SGPT and creatinine/Ureum, it showed that there was no significant differencebetween the dose treatment group and the normal group ($p > 0.05$). On the other hand, based on the results ofhistology of the liver and kidneys, it was found there was a significant difference but did not cause death.

ACKNOWLEDGMENT

The author would like to thank the Lemlit UHAMKA for the Research Foundation through the PPI Grant.

REFERENCES~~References~~

Aikpe, J. F. A. Baï Huguette Akakpo, Fidel Assogba,~~*et al.*~~ (2020).Antitussive properties of aqueous extracts of *Sterculia setigera* Delile (Sterculiaceae) and mixture of Aframomum melegueta K . Schum (Zingiberaceae) - Citrus aurantifolia (Christm . and panzer) Swingle (Rutaceae). *Journal of Pharmacognosy and Phytochemistry*. 9(2). 1624–1627. doi: 10.22271/phyto.2020.v9.i2aa.11085

Bibi, N. , Euglio jose L., Kuoadio B. M.F. Mahomoodally., A. Mollica., K.I. Sinan., A. Stefanucci., A. Riaguas., M.L.F. Cordova., G.Zengin

~~*et al.*~~ (2019). Chemical profiling , antioxidant , enzyme inhibitory and molecular modelling studies on the leaves and stem bark extracts of three African medicinal plants. *Journal of Pharmaceutical and Biomedical Analysis*,174. 19–33.doi : 10.1016/j.jpba.2019.05.041

Birt, D.F., Hendrich, S. ,~~&~~ Wang, W. (2001). Dietary agents in cancer prevention: flavonoids andisoflavonoids. *Pharmacology & Therapeutics*. 90(2). 157–77. doi: 10.1016/s0163-7258(01)00137-1

Depkes RI. (2008). Indonesian Herbal Pharmacopoeia Edition 1. *Ministry of Health RI*. Jakarta. 76-80

Depkes RI. (2000). Guidelines for the Implementation of Traditional Medicine Clinical Trials. *Directorate General of POM, Directorate of Drug and Food Control*. 67-68.

Cahyaningsih, AD, Azizahwati &~~,~~ Dadang (2011). Efek Nefroprotektif Infus Daun Sukun (*Artocarpus altilis* (Park.) Fsb.) Pada Tikus Jantan yang Diinduksi Karbon Tetraklorida. *Majalah Ilmu Kefarmasian*. 8(2).15-18. doi : https://doi.org/10.7454/psr.v8i2.3473

BPOM RI. (2014). Guidelines for Invivo Non-Clinical Toxicity Test. BPOM RI. *BPOM Jakarta*. 34.

Djojosumarto &~~,~~ Panut. (2008). Pesticides and their Applications. Jakarta: *Agromedia Pustaka*. 45-48

Fazle Rabbi, Amir Zada , Achyut Adhikari,-Almas Jabeen, A.N. and I.U.-(2017). Sterculia diversifolia bears anti-cancer and immunomodulatory activities. *Bangladesh Journal of Pharmacology*, 12. 52–55. doi: 10.3329/bjp.v12i1.29516

Hanani, E. (2015). Phytochemical Analysis. Jakarta: *EGC Medical Book Publisher*. 86, 87

Hossain, F. Bashudeb T., Mohammad N.R, Tasnim R., T.S. Nipun, S.M. Naim Uddin, S.M.M. Hossen. ~~*et al.*~~ (2016). In vivo sedative activity of methanolic extract of *Sterculia villos*a Roxb leaves. *BMC Complementary and Alternative Medicine*, 16. 10–13. doi : http://dx.doi.org/10.1186/s12906-016- 1374-8

Jafri, A.Shabana B., & Juhi R. ~~*et al.*~~ (2019). Phytochemical screening of Sterculia foetida seed extract for anti-oxidant, anti- microbial activity , and detection of apoptosis through reactive oxygen species (ROS) generation , mitochondrial membrane potential (MMP) decrease, and nuclear fragmentation in human osteosarcoma cells. *Journal of Histotechnology*, 42(2).1–12. doi: 10.1080/01478885.2019.1592832

Lu, C Frank. (2010). Basic Toxicology. Second edition. Jakarta : *University of Indonesia Pr*ess.

Prastiwi, R., Elya, B., Sauriasari, R., Hanafi, M., Dewanti, E. (2018). Pharmacognosy, Phytochemical Study and Antioxidant Activity of sterculia rubiginosa Zoll. Ex Miq. leaves. *Pharmacognosy Journal*, Vol 10: 571-575. doi: 10.5530/pj.2018.3.93

Prastiwi, R., Dewanti, E., Nurul, I., Aqilla, N., Salsabila, S.Ladeska, V.(2020). The Nephroprotective And Antioxidant Activity of Sterculia rubiginosa Zoll. Ex Miq. leaves. *Pharmacognosy Journ*al Vol 12, Issue 4, July-Aug, 2020. doi: 10.5530/pj.2020.12.121

Prastiwi, R. Elya B., Hanafi M. , Desmiaty Y., Sauriasari R., ~~*et al.*~~ (2021) , The Antioxidant Activity of Sterculia stipulata Korth Woods and Leaves by FRAP Method, *Pharmacognosy Journal* 12(2), pp. 236–239. doi: 10.5530/pj.2020.12.36

Prastiwi Rini, Ema Dewanti, Cut Mauliza, Ester Hidayati, Ita Anggraini, Riska Anggraini, Vera Ladeska. (2021). The Acute Toxicity of Ki Hampelas Leaves (*Sterculia rubiginosa* Zoll. Ex Miq). 13(2). 570-576. doi: 10.5530/pj.2021.13.71

Priyanto. 2009. Toxicology: mechanism, anidotum therapy, and risk assessment. Depok: leskonfi. 67-68 Priyanto. 2015. Toxicology, Mechanisms, Antidote Therapy, and Risk Assessment. *Lenskofi. Jakarta*. pp. 1, 177, 180, 182.

Rosida, A. 2016. Pemeriksaan laboratorium penyakit hati. Berkala Kedokteran. Vol.12. No.1.123-131

~~Sale~~Saleh M., El-Sherei1M.M., Ragheb A.Y.,Kassem M.E., Marzouk M.M., Mosharrafa S.A., Abdel N.,

.(2016).M.Phytochemistry, biological activities and economical uses of the genus *Sterculia* and therelated genera: A review. *Asian Pacific Journal of Tropical Disease*, 6(6):492–501. doi: http://dx.doi.org/10.1016/S2222-1808(16)61075-7

Shreevastva, NK, Pandeya, A., & Mishra, DK 2017 (2017). A study of AST : ALT ratio in alcoholic and nonalcoholic liver diseases. 4929, 1047–1050.https://doi.org/10.21276/sjmps.2017.3.10.5

~~Swarnalatha, K. *et al.*~~Swarnalatha K., CH. Venkata Kishore Babu, & B. Hari Babu (2019) Phytochemical Screening , Anti-Diabetic And Anti-Oxidant Activities Of

Kigelia africana (LAM .) and *Sterculia foetida* L. *Rasayan J. Chem*, 12(2).907–914. doi: :10.31788/RJC.2019.1225168

Mogbojuri, O.M., Adedapo, A.A. & Abatan, M.O. (2016) Phytochemical screening , safety evaluation , anti-inflammatory and analgesic studies of the leaf extracts of Sterculia tragacantha. *J Complement IntegrMed*, 2–7. doi: 10.1515/jcim-2015-0114

Formatted: Font: Not Italic

Formatted: Font: Italic

Formatted: Indent: First line: 0 cm

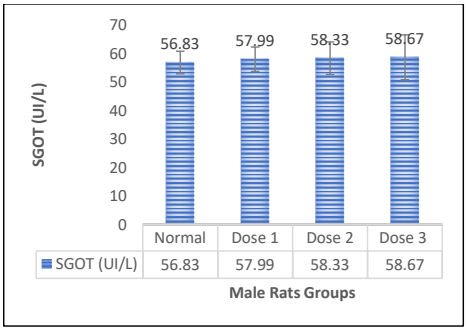
Formatted: Font: Italic

Formatted: Left

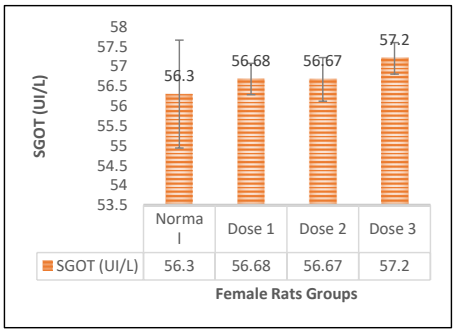
Mescher, AL (2011). Basic Histology of Junqueira Texts and Atlas. Jakarta: *EGC*. page. 2-4

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	Liberman-Bouchard	+



A

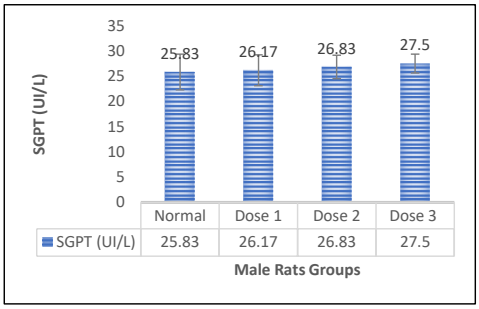


B

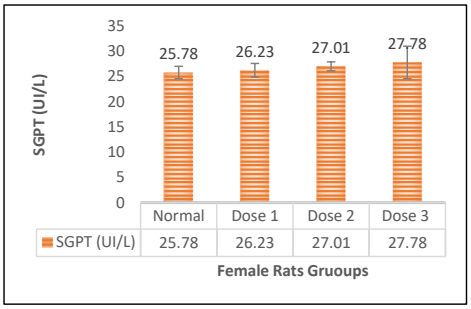
Figure 1. SGOT levels male and female rats

(A)SGOT levels of male rats groups, There was no significant difference between the dose groups and the normal group ($p<0,05$). (B)SGOT levels of female rats groups, There was no significant difference between the dose groups and the normal group ($p<0,05$).

Formatted: Centered
Formatted: Centered
Formatted: Highlight
Formatted: Centered
Formatted: Font: Not Bold
Formatted: Font: Not Bold
Formatted: Font: Not Bold



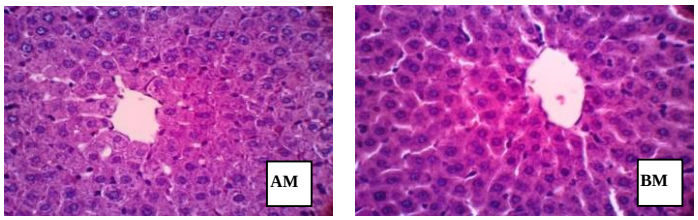
A



B

Figure 2. SGPT levels male and female rat

(A)SGPT levels of male rats groups, There was no significant difference between the dose groups and the normal group (p<0,05). (B)SGPT levels of female rats groups, There was no significant difference between the dose groups and the normal group (p<0,05).



Formatted: Body Text, Space Before: 0 pt
Formatted: Centered
Formatted: Centered
Formatted: Highlight
Formatted: Font: Not Bold
Formatted: Heading 1, Indent: Left: 1.48 cm, Space Before: 9.9 pt
Formatted: Font: Not Bold
Formatted: Font: Not Bold

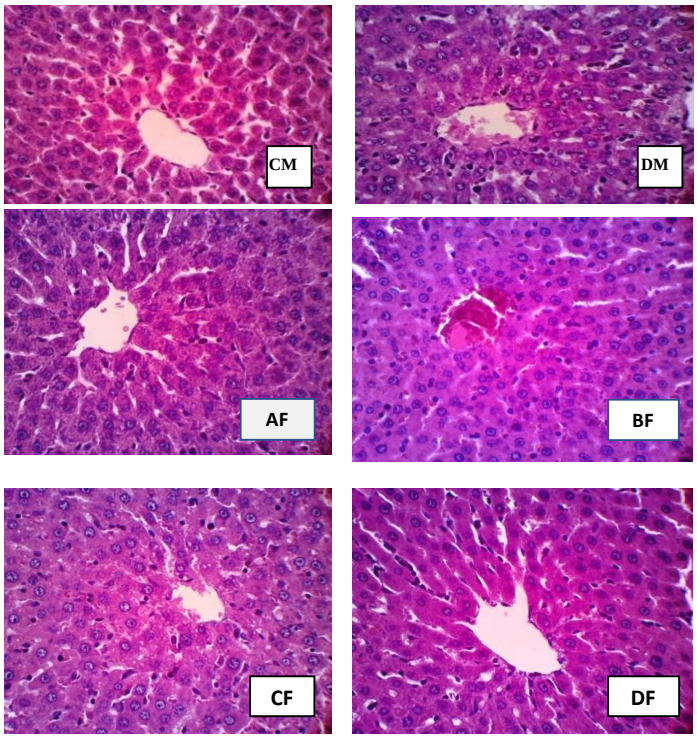
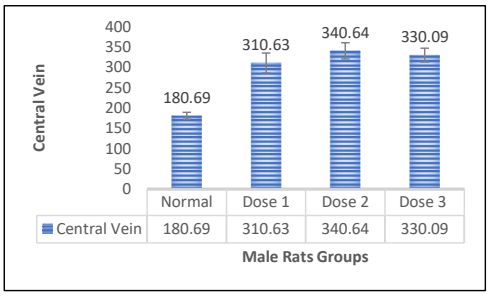


Figure 3. Histopathology of the liver male and female rats

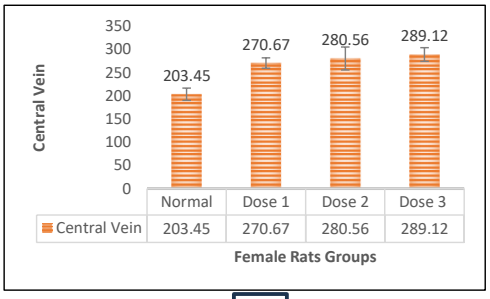
~~**Information:**~~Transverse incision histology of rat liver organ with hemotoxin-eosin staining at 40x10magnification. M: Male, F: Female, (AM) 0.5% NaCMC, (BM) 50 mg/kgBW, (CM 200 mg/kgBW, and (DM) 400 mg/.kgBW. (AF) 0.5% NaCMC, (BF) 50 mg/kgBW, (CF) 200 mg/kgBW, and (DF) 400 mg/.kgBW

Formatted: Centered

Formatted: Justified, Line spacing: single



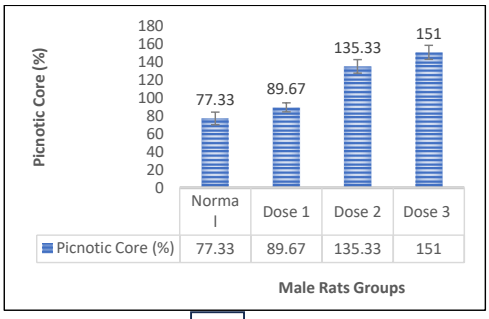
A



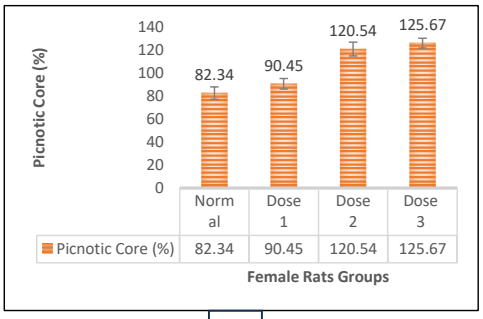
B

Figure 4. Diameter of the c~~C~~entral v~~V~~ein male and female rats

(A) Diameter of the central vein of male rats groups, There was a significant difference between the dose groups and the normal group (p<0,05). (B) Diameter of the central vein of female rats groups, There was a significant difference between the dose groups and the normal group (p<0,05).



A

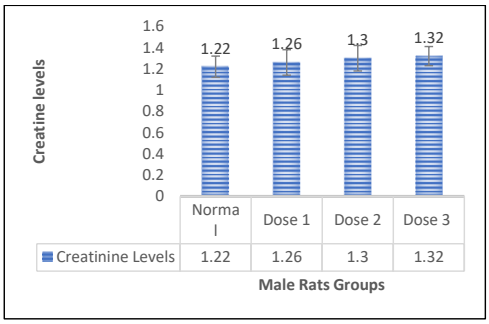


B

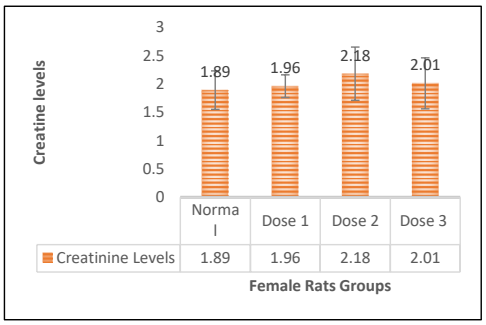
Figure 5. Picnotic core male anda female rats ~~Core~~

(A) The picnotic core of male rats groups, There was a significant difference between the dose 2 and dose 3 with the normal group (p<0,05). And there no significant difference between dose 1 anda normal group (p<0,05). (B) The picnotic core of female rats group, There was a significant difference between the dose 2 and dose 3 with the normal group (p<0,05). And there was no significant difference between dose 1 anda normal group (p<0,05).

Formatted: Centered
Formatted: Centered
Formatted: Highlight
Formatted: Font: 11 pt, No underline
Formatted: Font: 11 pt, Not Bold, No underline
Formatted: Font: 11 pt, No underline
Formatted: Font: 11 pt, No underline
Formatted: Normal, Space Before: 0 pt
Formatted: Font color: Text 1
Formatted: Centered
Formatted: Centered
Formatted: Highlight
Formatted: Centered
Formatted: Font: Not Bold
Formatted: Font: Not Bold



A



B

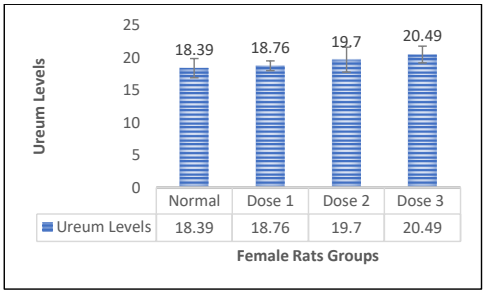
Figure 6. Creatinine ~~l~~ Levels male and female rats

Formatted: Centered

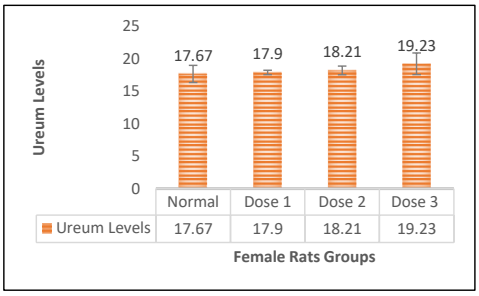
Formatted: Centered

Formatted: Highlight

(A) The creatinine levels of male rats groups, There was no significant difference between the dose groups and the normal group (p<0,05). (B) The creatinine levels of female rats groups, There was no significant difference between the dose groups and the normal group (p<0,05).



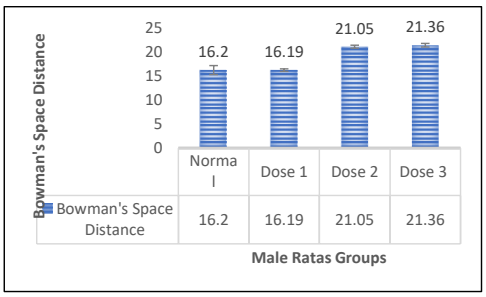
A



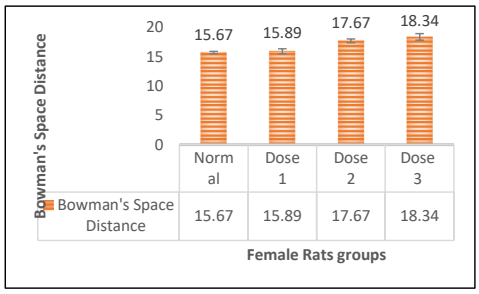
B

Figure 7. Ureum levels male and female rats

(A) The ureum levels of male rats groups, There was no significant difference between the dose groups and the normal group (p<0,05). (B) The ureum levels of female rats groups, There was no significant difference between the dose groups and the normal group (p<0,05).



A

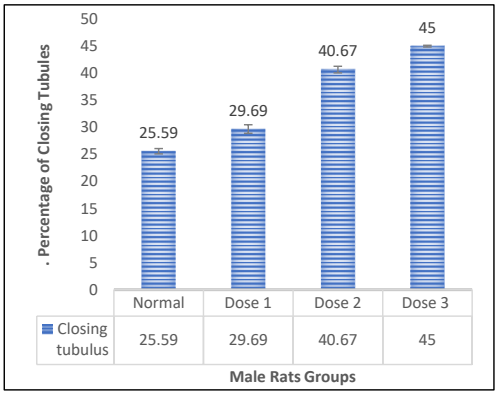


B

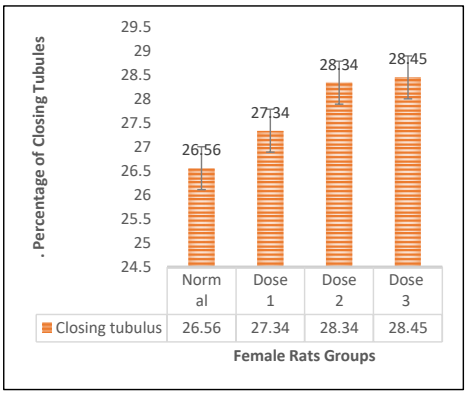
Figure 8. Bowman's sSpace dDistance male and female rats

(A) The Bowman's space of male rats groups, There was a significant difference between the dose 2 and dose 3 with the normal group (p<0,05). And there no significant difference between dose 1 anda normal group (p<0,05). (B) The picnotic core of female rats group, There was a significant difference between the dose 2 and dose 3 with the normal group (p<0,05). And there was no significant difference between dose 1 and normal group (p<0,05).

Formatted: Font: Not Bold
Formatted: Font: Not Bold
Formatted: Centered
Formatted: Centered
Formatted: Highlight
Formatted: Centered
Formatted: Font: Not Bold
Formatted: Font: Not Bold
Formatted: Font: Not Bold
Formatted: Centered
Formatted: Centered
Formatted: Highlight
Formatted: Centered
Formatted: Font: Not Bold
Formatted: Font: Not Bold



A



B

Figure 9. Percentage of cClosing tTubules male and female rats

(A) The percentage of closing tubulus of male rats groups, There was a significant difference between the dose groups with the normal group ($p<0,05$). (B) The percentage of closing tubulus of female rats group, There was a significant difference between the dose 2 and dose 3 with the normal group ($p<0,05$). And there was no significant difference between dose 1 and normal group ($p<0,05$)

Formatted: Centered
Formatted: Centered
Formatted: Highlight
Formatted: Font: Not Bold
Formatted: Justified
Formatted: Font: Not Bold
Formatted: Font: Not Bold

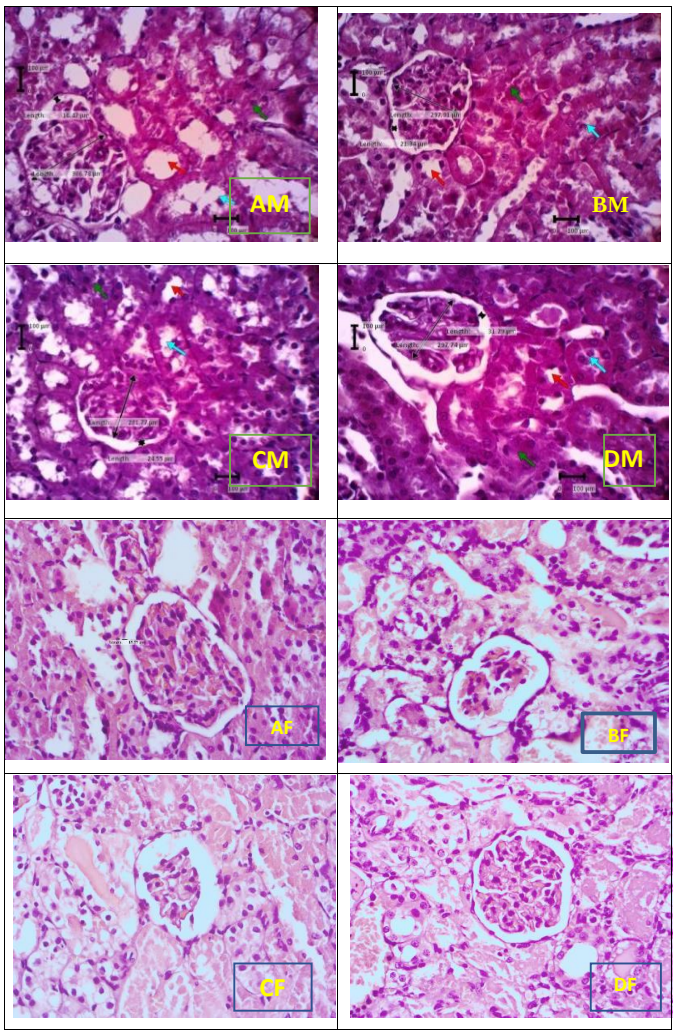


Figure 10. Kidney hHistopathology male and female rats

Information: Transverse incision histology of rat Kidney organ with hemotoxin-eosin staining at 40x10magnification. M: male, F: Female (AM) 0.5% CMC, (BM) 50 mg/kgBW, (CM) 200 mg/kgBW, and (DM) 400 mg/.kgBW, (AF) 0.5% CMC, (BF) 50 mg/kgBW, (CF) 200 mg/kgBW, and (DF) 400 mg/.kgBW.

	Komisi Etik Penelitian Kesehatan **Universitas Muhammadiyah Prof. DR. HAMKA** **(KEPK – UHAMKA) Jakarta** http://www.lrf.mfit.uhamka.ac.id	**POB-KE.B/008/01.0** Berlaku mulai: 19 Mei 2017 FL/B.06-008/01.0

SURAT PERSETUJUAN ETIK

PERSETUJUAN ETIK
ETHICAL APPROVAL

No : 02/20.03/0358

Bismillaahirrohmaanirrohiim
Assalamu'alaikum warohmatullohi wabarokatuh

Yang bertanda tangan di bawah ini, Ketua Komisi Etik Penelitian Kesehatan Universitas Muhammadiyah Prof. DR. HAMKA (KEPK-UHAMKA), setelah dilaksanakan pembahasan dan penilaian oleh reviewer yang bersertifikat, memutuskan bahwa protokol penelitian/skripsi/tesis dengan judul :

"UJI TOKSISITAS EKSTRAK ETANOL 70% DAUN KI HAMPELAS (*Sterculia rubiginosa*) PADA TIKUS PUTIH"

Atas nama
Peneliti utama : Rini Prastiwi, M.Si., Apt.
Peneliti lain : Ema Dewanti, M.Si.,
Cut Mauliza,
Ester Hidayati,
Ita Anggraini,
Riska Anggraini
Program Studi : S1 FARMASI
Institusi : UNIVERSITAS MUHAMMADIYAH PROF. DR. HAMKA JAKARTA

dapat disetujui pelaksanaannya. Persetujuan ini berlaku sejak tanggal ditetapkan sampai dengan batas waktu pelaksanaan penelitian seperti tertera dalam protokol.

Pada akhir penelitian, laporan pelaksanaan penelitian harus diserahkan kepada KEPK-UHAMKA dalam bentuk *soft copy* ke email kepk@uhamka.ac.id. Jika terdapat perubahan protokol dan/atau perpanjangan penelitian, maka peneliti harus mengajukan kembali permohonan kajian etik penelitian (amandemen protokol).

Wassalamu'alaikum warohmatullohi wabarokatuh

Jakarta, 02 Maret 2020
Ketua Komisi Etik Penelitian Kesehatan
UHAMKA

(Dr. Emma Rachmawati, Dra., M.Kes)

Formatted: Indent: Left: 0 cm

Subchronic Toxicity Of *Sterculia Rubiginosa* Zoll. Ex Miq. Leaves Extract

Rini Prastiwi*, Ester Hidayati, Riska Anggraini, Cut Mauliza, Ita Anggraini, Ema Dewanti

Faculty of Pharmacy and Science, University of Muhammadiyah Prof. DR. HAMKA, Jakartawrite
email and no. mobile phone

(rini_prastiwi@uhamka.ac.id)

ABSTRACT

Ki Hampelas (*Sterculia rubiginosa* Zoll. Ex Miq) is traditionally used as an antiasthma. It alsohas 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 atdoses of 50, 200, and 400 mg/Kg bodyweight/ day for 28 days. The rats were divided into four groups, consist of a the normal group (Na CMC 0.5%) with 50, 200, and 400 mg/kg BW of doses. the rats administered the extract every day for 28 days. Subchronic toxicity in the male and female rats resulted in no death or treatment-related signs at high doses.All the animals survived the duration of the study, with no significant changes in biochemichal parameters, organweight, or histological findings. There was no significant difference between the SGOT, SGPT, urea, andcreatinine levels in the dose groups with extracts and the normal group ($p > 0.05$). In addition, based on the histological results of the liver and kidneys it also found a significant difference among the groups. This study establishes that the leaves extract of Ki Hampelas is non toxic in rats following oraladministration.

Keywords: *Ki Hampelas, Subchronic, Histopathology, Toxicity, Sterculia rubiginosa* Zoll. Ex Miq.

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, while 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.

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, $FeCl_3$, amyl alcohol, H_2SO_4, Liebermann- Burchard reagent, Mayer reagent, ketamine, anhydrous acetic acid, ketamine, gelatine, SGOT kit reagent (Human), SGPT reagent kit (Human), Urea kit reagent (Human), Creatinine reagent kit (Human), rats, animalfeed, Na CMC (Brataco), aqua distillate, Spectrophotometer (Elitech).

Extraction

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

Characteristic extract

The characteristics extract were determined by organoleptic examination, calculation of yield, determinationof water content, and determination of ash content. The procedure was carried out according to Depkes 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

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 giventhe 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 respectivereagents and read using a Clinical Spectrophotometer (BPOM, 2014). Liver and kidney histology preparations were made, and histology preparations were made by the Histology Laboratory, Universitas Indonesia (Mescher 2015).
This study was approved by the Ethics Committee of UHAMKA with No. 02/20.03/0358.

Statistical analysis

Experimental data were recorded using excel and statistically analyzed by SPSS 19 according to the sex of the animals and the test stage. The data on SGOT, SGPT, creatinine, urea, and histopathological organs wereanalyzed for normality and homogeneity, then proceeded to the ANOVA test by SPSS 19. This test was carriedout to determine the difference between each group, whether it is significantly different or not. Numerical datalike 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.

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 ofmetabolites 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 andevaporated 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 secondarymetabolite 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 stigera* 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 (Djojosumarto, 2008).

The results of giving 70% ethanol extract of ki hampelas leaves which were administered test animals at a doseof 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 animalsdid not experience stress or toxic symptoms caused by the administration of ki hampelas leaf extract.

Repeated administration of the extract for 28 days, it caused an increase in the levels of SGOT andSGPT 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 andSGPT obtained showed $p > 0.05$, which indicates that there is no significant difference in each treatment group.The AST/ALT De Ritis ratio 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 thecytoplasmic contents come out causing ALT (SGPT) to increase higher than AST (SGOT) with an AST/ALT the ratio of <0.8 which indicates mild damage. In chronic or severe inflammation and damage, liver cell damagereaches 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 each group was in a group I which was 2.20, group II was 2.18, group III was 2.17, then group IV was 2.13. 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 UI/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 UI/L.

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 freeradicals, as well as recovery for dead or damaged liver cells (Birt *et al*. 2001). However, if there is an increasein the activity of the SGOT and SGPT enzymes, it can indicate the occurrence.

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 the subchronic toxicity test on liver histopathological preparations with Hematoxylin-Eosin staining, it can be seen 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 changesin the glomerulus in the acute toxicity test showed a significant difference between the normal group and thedose 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 asignificant difference between the 200 mg/kg BW and 400 mg/kg BW dose groups and the normal group, butno death was found and no tubular *casts* were found.

Figure 6, shows that there is a significant difference in the number of pyknotic nuclei between the normalgroup and the test group with $p<0.05$. Based on these results, it was found that there was an increase in necrosisat each increase in dose, the characteristic of necrosis that was seen was the pyknotic 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 leafextract 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 betweenthe glomerulus and Bowman's capsule, changes that occur in the glomerulus are edema which is characterizedby 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 wascalculated 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 $p>0.05$.

In the parameter of the percentage of closed proximal tubules, the percentage of proximal tubule damageis indicated by the number of closed proximal tubules in one field of view compared to the number of all proximal tubules (Shreevastva, 2017). Based on the results of the subchronic toxicity test, the results of statistical tests showed that there was a significant difference between all normal control groups and the test dose group, namely $p<0.05$, as shown in (Figure 8).

CONCLUSION

It can be concluded that the administration of 70% ethanol extract of ki hampelas leaves in the subchronic toxicity test did not cause death and had no toxic effect on the test animals. Based on the results of the examination of levels of SGOT/SGPT and creatinine/Ureum, it showed that there was no significant differencebetween the dose treatment group and the normal group ($p > 0.05$). On the other hand, based on the results ofhistology of the liver and kidneys, it was found there was a significant difference but did not cause death.

ACKNOWLEDGMENT

The author would like to thank the Lemlit UHAMKA for the Research Foundation through the PPI Grant.

REFERENCES

Aikpe, J. F. A. Baï Huguette Akakpo, Fidel Assogba,. (2020).Antitussive properties of aqueous extracts of *Sterculia setigera* Delile (Sterculiaceae) and mixture of Aframomum melegueta K . Schum (Zingiberaceae) - Citrus aurantifolia(Christm . and panzer) Swingle (Rutaceae). *Journal of Pharmacognosy and Phytochemistry*. 9(2). 1624–1627. doi: 10.22271/phyto.2020.v9.i2aa.11085

Bibi, N. , Euglio jose L., Kuoadio B. M.F. Mahomoodally., A. Mollica., K.I. Sinan., A. Stefanucci., A. Riaguas., M.L.F. Cordova., G.Zengin

(2019). Chemical profiling , antioxidant , enzyme inhibitory and molecular modelling studies on the leaves and stem bark extracts of three African medicinal plants. *Journal of Pharmaceutical andBiomedical Analysis*,174. 19–33.doi : 10.1016/j.jpba.2019.05.041

Birt, D.F., Hendrich, S. , Wang, W. (2001). Dietary agents in cancer prevention: flavonoids andisoflavonoids. *Pharmacology & Therapeutics*. 90(2). 157–77. doi: 10.1016/s0163-7258(01)00137-1

Depkes RI. (2008). Indonesian Herbal Pharmacopoeia Edition 1. *Ministry of Health RI*. Jakarta. 76-80

Depkes RI. (2000). Guidelines for the Implementation of Traditional Medicine Clinical Trials. *Directorate General of POM, Directorate of Drug and Food Control*. 67-68.

Cahyaningsih, AD, Azizahwati & Dadang (2011). Efek Nefroprotektif Infus Daun Sukun (*Artocarpus altilis* (Park.) Fsb.) Pada Tikus Jantan yang Diinduksi Karbon Tetraklorida. *Majalah Ilmu Kefarmasian*. 8(2).15-18. doi : https://doi.org/10.7454/psr.v8i2.3473

BPOM RI. (2014). Guidelines for Invivo Non-Clinical Toxicity Test. BPOM RI. *BPOM Jakarta*. 34.

Djojosumarto & Panut. (2008). Pesticides and their Applications. Jakarta: *Agromedia Pustaka*. 45-48

Fazle Rabbi, Amir Zada , Achyut Adhikari,Almas Jabeen, A.N. and I.U.(2017). Sterculia diversifolia bears anti-cancer

and immunomodulatory activities. *Bangladesh Journal of Pharmacology*, 12. 52–55. doi: 10.3329/bjp.v12i1.29516

Hanani, E. (2015). Phytochemical Analysis. Jakarta: *EGC Medical Book Publisher*. 86, 87

Hossain, F. Bashudeb T., Mohammad N.R, Tasnim R., T.S. Nipun, S.M. Naim Uddin, S.M.M. Hossen. (2016). In vivo sedative activity of methanolic extract of *Sterculia villos*a Roxb leaves. *BMC Complementary and Alternative Medicine*, 16. 10–13. doi : http://dx.doi.org/10.1186/s12906-016- 1374-8

Jafri, A.Shabana B., & Juhi R. (2019). Phytochemical screening of Sterculia foetida seed extract for anti-oxidant, anti-microbial activity , and detection of apoptosis through reactive oxygen species (ROS) generation , mitochondrial membrane potential (MMP) decrease, and nuclear fragmentation in human osteosarcoma cells. *Journal of Histotechnology*, 42(2).1–12. doi: 10.1080/01478885.2019.1592832

Lu, C Frank. (2010). Basic Toxicology. Second edition. Jakarta : *University of Indonesia Pr*ess.

Prastiwi, R., Elya, B., Sauriasari, R., Hanafi, M., Dewanti, E. (2018). Pharmacognosy, Phytochemical Study and Antioxidant Activity of sterculia rubiginosa Zoll. Ex Miq. leaves. *Pharmacognosy Journal*, Vol 10: 571-575. doi: 10.5530/pj.2018.3.93

Prastiwi, R., Dewanti, E., Nurul, I., Aqilla, N., Salsabila, S.Ladeska, V.(2020). The Nephroprotective And Antioxidant Activity of Sterculia rubiginosa Zoll. Ex Miq. leaves. *Pharmacognosy Journ*al Vol 12, Issue 4, July-Aug, 2020. doi: 10.5530/pj.2020.12.121

Prastiwi, R. Elya B., Hanafi M. , Desmiaty Y., Sauriasari R., (2021) , The Antioxidant Activity of Sterculia stipulata Korth Woods and Leaves by FRAP Method, *Pharmacognosy Journal* 12(2), pp. 236–239. doi: 10.5530/pj.2020.12.36

Prastiwi Rini, Ema Dewanti, Cut Mauliza, Ester Hidayati, Ita Anggraini, Riska Anggraini, Vera Ladeska. (2021). The Acute Toxicity of Ki Hampelas Leaves (*Sterculia rubiginosa* Zoll. Ex Miq). 13(2). 570-576. doi: 10.5530/pj.2021.13.71

Priyanto. 2009. Toxicology: mechanism, anidotum therapy, and risk assessment. Depok: leskonfi. 67-68 Priyanto. 2015. Toxicology, Mechanisms, Antidote Therapy, and Risk Assessment. *Lenskofi. Jakarta*. pp. 1, 177, 180, 182.

Rosida, A. 2016. Pemeriksaan laboratorium penyakit hati. Berkala Kedokteran. Vol.12. No.1.123-131

Saleh M., El-Sherei1M.M., Ragheb A.Y.,Kassem M.E., Marzouk M.M., Mosharrafa S.A., Abdel N., .(2016).M.Phytochemistry, biological activities and economical uses of the genus *Sterculia* and therelated genera: A review. *Asian Pacific Journal of Tropical Disease*, 6(6):492–501. doi: http://dx.doi.org/10.1016/S2222-1808(16)61075-7

Shreevastva, NK, Pandeya, A., & Mishra, DK 2017 (2017). A study of AST : ALT ratio in alcoholic and nonalcoholic liver diseases. 4929, 1047–1050.https://doi.org/10.21276/sjmps.2017.3.10.5

Swarnalatha K., CH. Venkata Kishore Babu, & B. Hari Babu (2019) Phytochemical Screening , Anti-Diabetic And Anti-Oxidant Activities Of *Kigelia africana* (LAM .) and *Sterculia foetida* L. *Rasayan J. Chem*, 12(2).907–914. doi: :10.31788/RJC.2019.1225168

Mogbojuri, O.M., Adedapo, A.A. & Abatan, M.O. (2016) Phytochemical screening , safety evaluation , anti-

inflammatory and analgesic studies of the leaf extracts of Sterculia tragacantha. *J Complement IntegrMed*, 2–7. doi: 10.1515/jcim-2015-0114

Mescher, AL (2011). Basic Histology of Junqueira Texts and Atlas. Jakarta: *EGC*. page. 2-4

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	Liberman-Bouchard	+

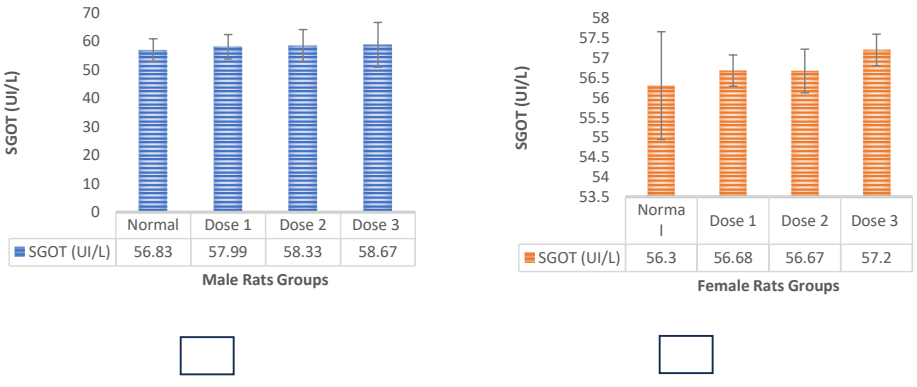


Figure 1. SGOT levels male and female rats

(A)SGOT levels of male rats groups, There was no significant difference between the dose groups and the normal group (p<0,05). (B)SGOT levels of female rats groups, There was no significant difference between the dose groups and the normal group (ns).

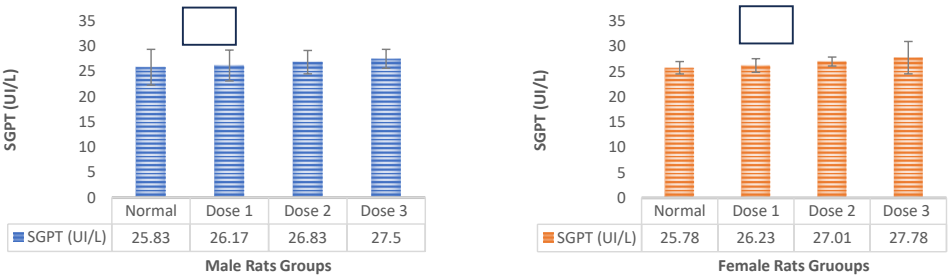
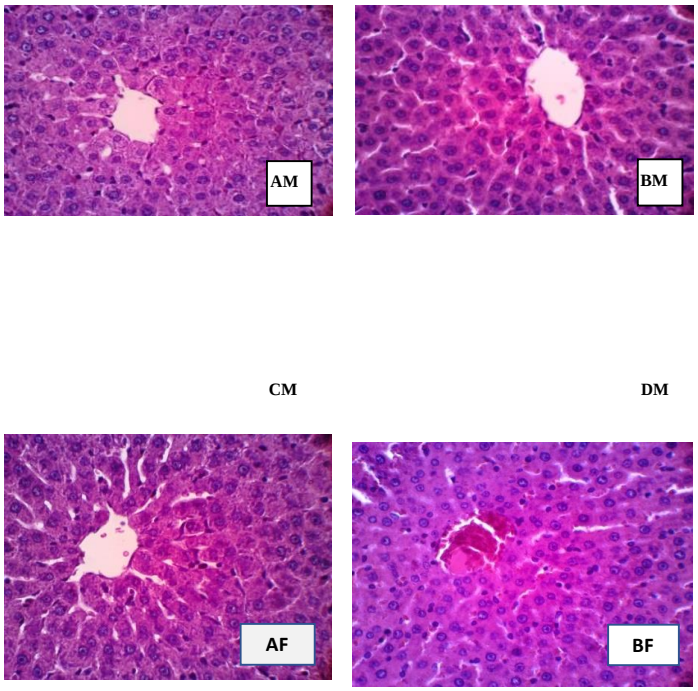


Figure 2. SGPT levels male and female rat(A)SGPT levels of male rats groups, There was no significant difference between the dose groups and the normal group (*p<0,05). (B)SGPT levels of female rats groups, There was no significant difference between the dose groups and the normal group (ns).



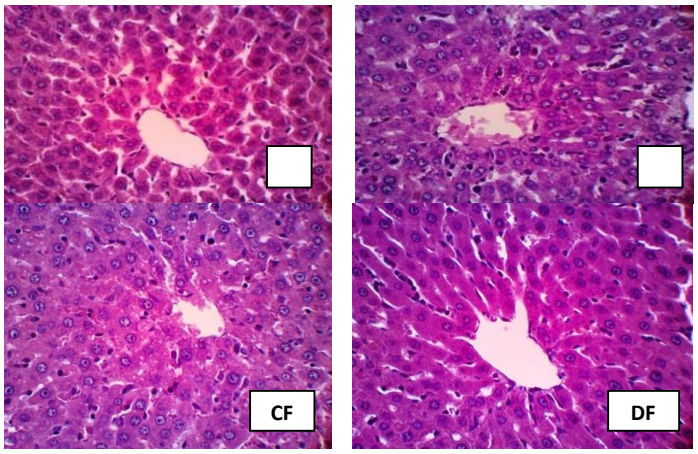
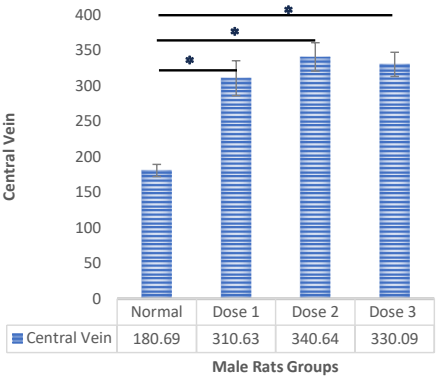
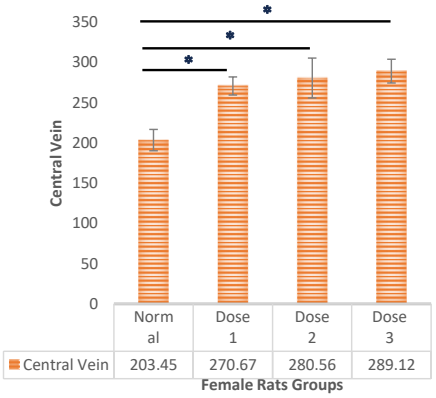


Figure 3. Histopathology of the liver male and female rats

Transverse incision histology of rat liver organ with hemotoxin-eosin staining at 40x10magnification. M: Male, F: Female, (AM) 0.5% NaCMC, (BM) 50 mg/kgBW, (CM 200 mg/kgBW, and (DM) 400 mg/.kgBW. (AF) 0.5% NaCMC, (BF) 50 mg/kgBW, (CF) 200 mg/kgBW, and (DF) 400 mg/.kgBW

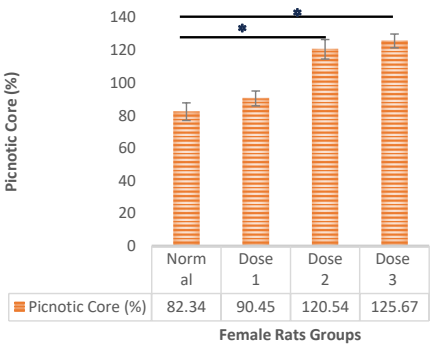

400
350
300
250
200
150
100
50
0
Central Vein
Normal
Dose 1
Dose 2
Dose 3
Central Vein
180.69
310.63
340.64
330.09
Male Rats Groups


350
300
250
200
150
100
50
0
Central Vein
Norm al
Dose 1
Dose 2
Dose 3
Central Vein
203.45
270.67
280.56
289.12
Female Rats Groups

B

Figure 4. Diameter of the central vein male and female rats

(A) Diameter of the central vein of male rats groups, There was a significant difference between the dose groups and the normal group (p<0,05). (B) Diameter of the central vein of female rats groups, There was a significant difference between the dose groups and the normal group (p<0,05).



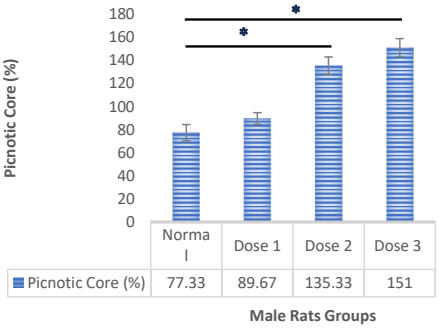
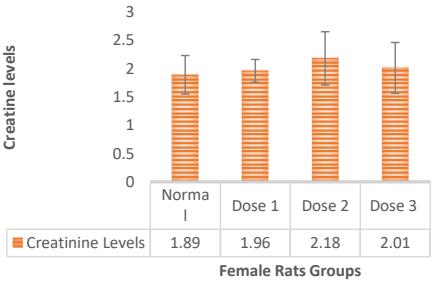
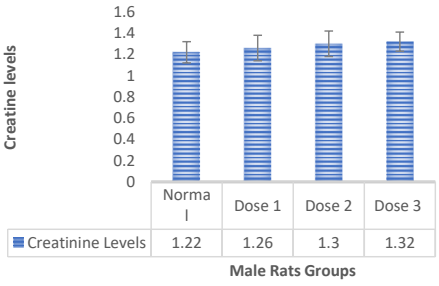


Figure 5. Picnotic core male anda female rats

(A) The picnotic core of male rats groups, There was a significant difference between the dose 2 and dose 3 with the normal group (p<0,05). And there no significant difference between dose 1 anda normal group (p<0,05). (B) The picnotic core of female rats group, There was a significant difference between the dose 2 and dose 3 with the normal group (p<0,05). And there was no significant difference between dose 1 anda normal group (p<0,05).

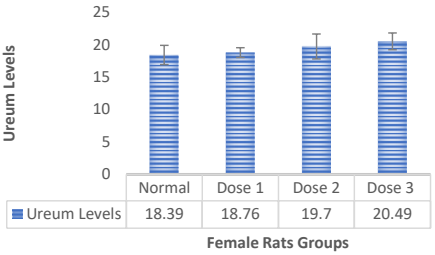




A

Figure 6. Creatinine levels male and female rats

(A) The creatinine levels of male rats groups, There was no significant difference between the dose groups and the normal group (*p<0,05). (B) The creatinine levels of female rats groups, There was no significant difference between the dose groups and the normal group (ns).



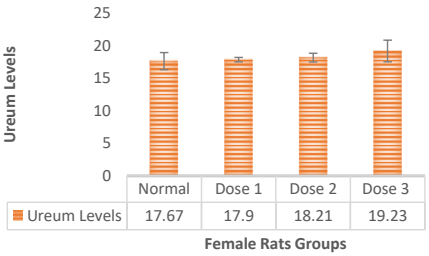
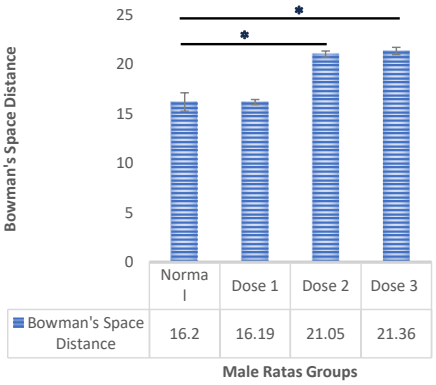


Figure 7. Ureum levels male and female rats

(A) The ureum levels of male rats groups, There was no significant difference between the dose groups and the normal group (*p<0,05). (B) The ureum levels of female rats groups, There was no significant difference between the dose groups and the normal group (ns).



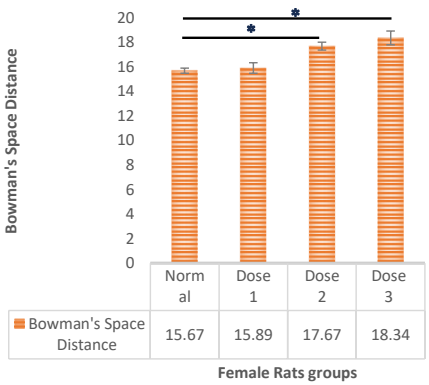


Figure 8. Bowman's space distance male and female rats

(A) The Bowman's space of male rats groups, There was a significant difference between the dose 2 and dose 3 with the normal group (p<0,05). And there no significant difference between dose 1 anda normal group (p<0,05). (B) The picnotic core of female rats group, There was a significant difference between the dose 2 and dose 3 with the normal group (p<0,05). And there was no significant difference between dose 1 and normal group (p<0,05).

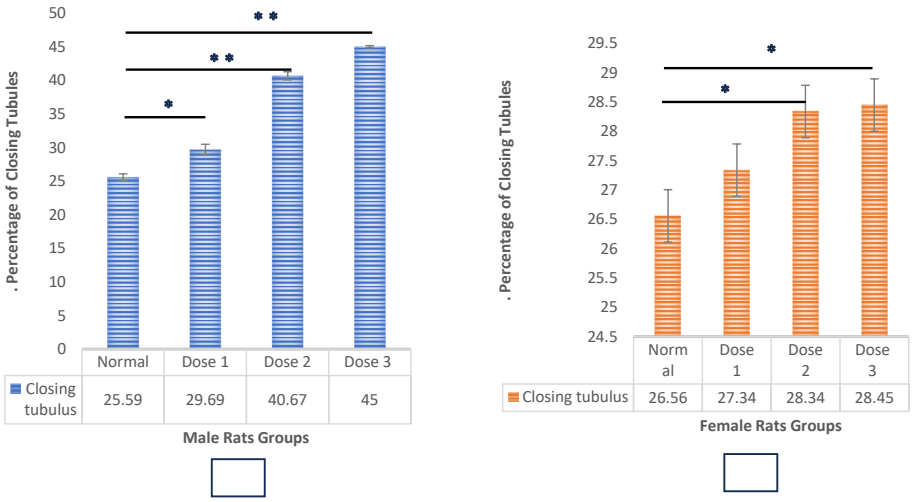


Figure 9. Percentage of closing tubules male and female rats

(A) The percentage of closing tubulus of male rats groups, There was a significant difference between the dose groups with the normal group ($p<0,05$). (B) The percentage of closing tubulus of female rats group, There was a significant difference between the dose 2 and dose 3 with the normal group ($p<0,05$). And there was no significant difference between dose 1 and normal group ($p<0,05$)

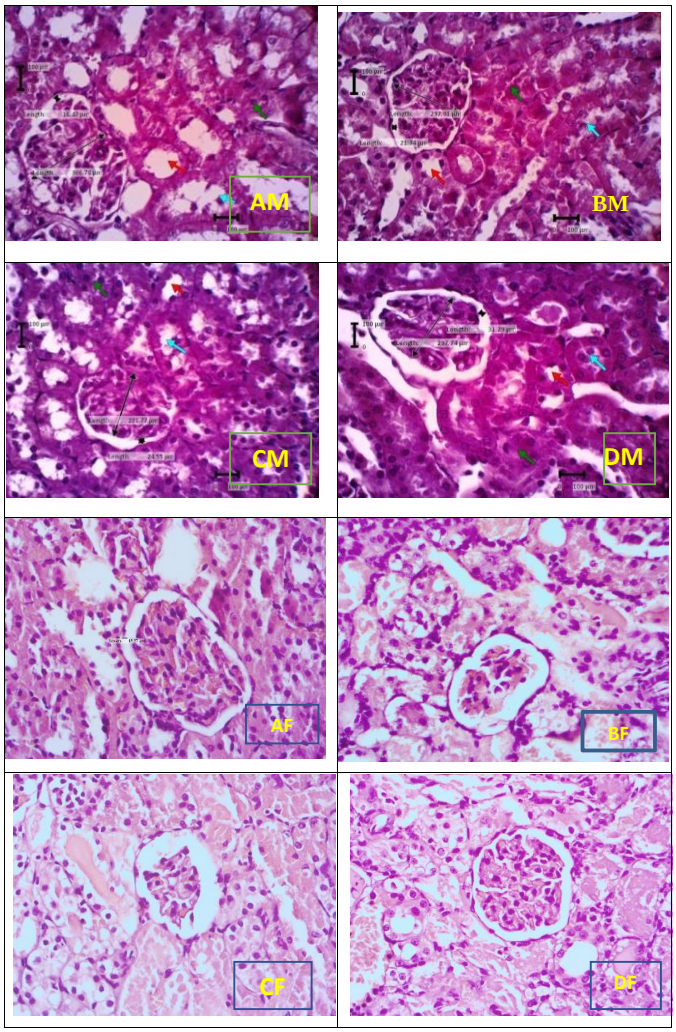


Figure 10. Kidney histopathology male and female rats

Information: Transverse incision histology of rat Kidney organ with hemotoxin-eosin staining at 40x10magnification. M: male, F: Female (AM) 0.5% CMC, (BM) 50 mg/kgBW, (CM) 200 mg/kgBW, and (DM) 400 mg/.kgBW, (AF) 0.5% CMC, (BF) 50 mg/kgBW, (CF) 200 mg/kgBW, and (DF) 400 mg/.kgBW.

Subchronic Toxicity Of *Sterculia Rubiginosa* ZZoll. EEx MMiq. Leaves Extract

Formatted: Font: 14 pt

Formatted: Font: 14 pt, Italic

Formatted: Font: 14 pt

Rini Prastiwi[1]*, Ester Hidayati[1], Riska Anggraini[1], Cut Mauliza[1], Ita Anggraini[1], Ema Dewanti[1]

Faculty of Pharmacy and Science, University of Muhammadiyah Prof. DR. HAMKA, Jakartawrite
email and no. mobile phone

(rini_prastiwi@uhamka.ac.id)

ABSTRACT

~~Background:~~ Ki Hampelas (*Sterculia rubiginosa* Zoll. Ex Miq) is traditionally used as an antiasthma. It alsohas antioxidant and nephroprotective activity.

~~Objective:~~ This study was conducted to evaluate the subchronic toxicity of the leaf extract of Ki Hampelas. **~~Materials and Methods:~~** The extract was orally administered to male and female Sprague-Dawley rats atdoses of 50, 200, and 400 mg/Kg bodyweight/ day for 28 days. The rats were divided into four groups, consist of a the normal group (Na CMC 0.5%) with 50, 200, and 400 mg/kg BW of doses. the rats administered the extract every day for 28 days.

~~Results:~~ Subchronic toxicity in the male and female rats resulted in no death or treatment-related signs at high doses. All the animals survived the duration of the study, with no significant changes in biochemichal parameters, organweight, or histological findings. There was no significant difference between the SGOT, SGPT, urea, andcreatinine levels in the dose groups with extracts and the normal group ($p > 0.05$). In addition, based on the histological results of the liver and kidneys it also found a significant difference among the groups.

~~Conclusion:~~ This study establishes that the leaves extract of Ki Hampelas is non toxic in rats following oraladministration.

Keywords: *Ki Hampelas, Subchronic, Histopathology, Toxicity, Sterculia rubiginosa* Zoll. Ex Miq.

Formatted: Normal, Right: 0 cm, Space Before: 6.85 pt

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).

Formatted: Font: Italic

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, while 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

Formatted: Font: Italic

(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).

Formatted: Font: Italic

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.

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, $FeCl_3$, amyl alcohol, H_2SO_4, Liebermann- Burchard reagent, Mayer reagent, ketamine, anhydrous acetic acid, ketamine, gelatine, SGOT kit reagent (Human), SGPT reagent kit (Human), Urea kit reagent (Human), Creatinine reagent kit (Human), rats, animalfeed, Na CMC (Brataco), aqua distillate, Spectrophotometer (Elitech).

Extraction

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

Characteristic extract

The characteristics extract were determined by organoleptic examination, calculation of yield, determinationof water content, and determination of ash content. The procedure was carried out according to Depkes 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

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 giventhe 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 respectivereagents and read using a Clinical Spectrophotometer (BPOM, 2014). Liver and kidney histology preparations were made, and histology preparations were made by the Histology Laboratory, Universitas Indonesia (Mescher 2015).

This study was approved by the Ethics Committee of UHAMKA with No. 02/20.03/0358.

Statistical analysis

Experimental data were recorded using excel and statistically analyzed by SPSS 19 according to the sex of the animals and the test stage. The data on SGOT, SGPT, creatinine, urea, and histopathological organs wereanalyzed for normality and homogeneity, then proceeded to the ANOVA test by SPSS 19. This test was carriedout to determine the difference between each group, whether it is significantly different or not. Numerical datalike 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.

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 ofmetabolites 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 andevaporated 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 secondarymetabolite 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 stigera* 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 (Djojosumarto, 2008).

The results of giving 70% ethanol extract of ki hampelas leaves which were administered test animals at a doseof 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 animalsdid not experience stress or toxic symptoms caused by the administration of ki hampelas leaf extract.

Repeated administration of the extract for 28 days, it caused an increase in the levels of SGOT andSGPT 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 andSGPT obtained showed $p > 0.05$, which indicates that there is no significant difference in each treatment group.The AST/ALT De Ritis ratio 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 thecytoplasmic contents come out causing ALT (SGPT) to increase higher than AST (SGOT) with an AST/ALT the ratio of <0.8 which indicates mild damage. In chronic or severe inflammation and damage, liver cell damagereaches 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 each group was in a group I which was 2.20, group II was 2.18, group III was 2.17, then group IV was 2.13. 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 UI/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 UI/L.

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 freeradicals, as well as recovery for dead or damaged liver cells (Birt *et al*. 2001). However, if there is an increasein the activity of the SGOT and SGPT enzymes, it can indicate the occurrence.

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 the subchronic toxicity test on liver histopathological preparations with Hematoxylin-Eosin staining, it can be seen 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 changesin the glomerulus in the acute toxicity test showed a significant difference between the normal group and thedose 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 asignificant difference between the 200 mg/kg BW and 400 mg/kg BW dose groups and the normal group, butno death was found and no tubular *casts* were found.

Figure 6, shows that there is a significant difference in the number of pyknotic nuclei between the normalgroup and the test group with $p<0.05$. Based on these results, it was found that there was an increase in necrosisat each increase in dose, the characteristic of necrosis that was seen was the pyknotic 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 leafextract 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 betweenthe glomerulus and Bowman's capsule, changes that occur in the glomerulus are edema which is characterizedby 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 wascalculated 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 $p>0.05$.

In the parameter of the percentage of closed proximal tubules, the percentage of proximal tubule damageis indicated by the number of closed proximal tubules in one field of view compared to the number of all proximal tubules (Shreevastva, 2017). Based on the results of the subchronic toxicity test, the results of statistical tests showed that there was a significant difference between all normal control groups and the test dose group, namely $p<0.05$, as shown in (Figure 8).

CONCLUSION

It can be concluded that the administration of 70% ethanol extract of ki hampelas leaves in the subchronic toxicity test

did not cause death and had no toxic effect on the test animals. Based on the results of the examination of levels of SGOT/SGPT and creatinine/Ureum, it showed that there was no significant differencebetween the dose treatment group and the normal group ($p > 0.05$). On the other hand, based on the results ofhistology of the liver and kidneys, it was found there was a significant difference but did not cause death.

ACKNOWLEDGMENT

The author would like to thank the Lemlit UHAMKA for the Research Foundation through the PPI Grant.

REFERENCES~~References~~

Aikpe, J. F. A. Baï Huguette Akakpo, Fidel Assogba,~~*et al.*~~ (2020).Antitussive properties of aqueous extracts of *Sterculia setigera* Delile (Sterculiaceae) and mixture of Aframomum melegueta K . Schum (Zingiberaceae) - Citrus aurantifolia (Christm . and panzer) Swingle (Rutaceae). *Journal of Pharmacognosy and Phytochemistry*. 9(2). 1624–1627. doi: 10.22271/phyto.2020.v9.i2aa.11085

Bibi, N. , Euglio jose L., Kuoadio B. M.F. Mahomoodally., A. Mollica., K.I. Sinan., A. Stefanucci., A. Riaguas., M.L.F. Cordova., G.Zengin ~~*et al.*~~ (2019). Chemical profiling , antioxidant , enzyme inhibitory and molecular modelling studies on the leaves and stem bark extracts of three African medicinal plants. *Journal of Pharmaceutical and Biomedical Analysis*,174. 19–33.doi : 10.1016/j.jpba.2019.05.041

Birt, D.F., Hendrich, S. ,~~&~~ Wang, W. (2001). Dietary agents in cancer prevention: flavonoids andisoflavonoids. *Pharmacology & Therapeutics*. 90(2). 157–77. doi: 10.1016/s0163-7258(01)00137-1

Depkes RI. (2008). Indonesian Herbal Pharmacopoeia Edition 1. *Ministry of Health RI*. Jakarta. 76-80

Depkes RI. (2000). Guidelines for the Implementation of Traditional Medicine Clinical Trials. *Directorate General of POM*, *Directorate of Drug and Food Control*. 67-68.

Cahyaningsih, AD, Azizahwati &~~,~~ Dadang (2011). Efek Nefroprotektif Infus Daun Sukun (*Artocarpus altilis* (Park.) Fsb.) Pada Tikus Jantan yang Diinduksi Karbon Tetraklorida. *Majalah Ilmu Kefarmasian*. 8(2).15-18. doi : https://doi.org/10.7454/psr.v8i2.3473

BPOM RI. (2014). Guidelines for Invivo Non-Clinical Toxicity Test. BPOM RI. *BPOM Jakarta*. 34.

Djojosumarto &~~,~~ Panut. (2008). Pesticides and their Applications. Jakarta: *Agromedia Pustaka*. 45-48

Fazle Rabbi, Amir Zada , Achyut Adhikari,-Almas Jabeen, A.N. and I.U.-(2017). Sterculia diversifolia bears anti-cancer and immunomodulatory activities. *Bangladesh Journal of Pharmacology*, 12. 52–55. doi: 10.3329/bjp.v12i1.29516

Hanani, E. (2015). Phytochemical Analysis. Jakarta: *EGC Medical Book Publisher*. 86, 87

Hossain, F. Bashudeb T., Mohammad N.R, Tasnim R., T.S. Nipun, S.M. Naim Uddin, S.M.M. Hossen. ~~*et al.*~~ (2016). In vivo sedative activity of methanolic extract of *Sterculia villos*a Roxb leaves. *BMC Complementary and Alternative Medicine*, 16. 10–13. doi : http://dx.doi.org/10.1186/s12906-016- 1374-8

Jafri, A.Shabana B., & Juhi R. ~~*et al.*~~ (2019). Phytochemical screening of Sterculia foetida seed extract for anti-oxidant,

anti- microbial activity , and detection of apoptosis through reactive oxygen species (ROS) generation , mitochondrial membrane potential (MMP) decrease, and nuclear fragmentation in human osteosarcoma cells. *Journal of Histotechnology*, 42(2).1–12. doi: 10.1080/01478885.2019.1592832

Lu, C Frank. (2010). Basic Toxicology. Second edition. Jakarta : *University of Indonesia Pr*ess.

Prastiwi, R., Elya, B., Sauriasari, R., Hanafi, M., Dewanti, E. (2018). Pharmacognosy, Phytochemical Study and Antioxidant Activity of sterculia rubiginosa Zoll. Ex Miq. leaves. *Pharmacognosy Journal*, Vol 10: 571-575. doi: 10.5530/pj.2018.3.93

Prastiwi, R., Dewanti, E., Nurul, I., Aqilla, N., Salsabila, S.Ladeska, V.(2020). The Nephroprotective And Antioxidant Activity of Sterculia rubiginosa Zoll. Ex Miq. leaves. *Pharmacognosy Journ*al Vol 12, Issue 4, July-Aug, 2020. doi: 10.5530/pj.2020.12.121

Prastiwi, R. Elya B., Hanafi M. , Desmiaty Y., Sauriasari R., ~~*et al.*~~ (2021) , The Antioxidant Activity of Sterculia stipulata Korth Woods and Leaves by FRAP Method, *Pharmacognosy Journal* 12(2), pp. 236–239. doi: 10.5530/pj.2020.12.36

Prastiwi Rini, Ema Dewanti, Cut Mauliza, Ester Hidayati, Ita Anggraini, Riska Anggraini, Vera Ladeska. (2021). The Acute Toxicity of Ki Hampelas Leaves (*Sterculia rubiginosa* Zoll. Ex Miq). 13(2). 570-576. doi: 10.5530/pj.2021.13.71

Priyanto. 2009. Toxicology: mechanism, anidotum therapy, and risk assessment. Depok: leskonfi. 67-68 Priyanto. 2015. Toxicology, Mechanisms, Antidote Therapy, and Risk Assessment. *Lenskofi. Jakarta*. pp. 1, 177, 180, 182.

Rosida, A. 2016. Pemeriksaan laboratorium penyakit hati. Berkala Kedokteran. Vol.12. No.1.123-131

~~Saleh et al~~ Saleh M., El-Sherei1M.M., Ragheb A.Y.,Kassem M.E., Marzouk M.M., Mosharrafa S.A., Abdel N.,

.(2016).M.Phytochemistry, biological activities and economical uses of the genus *Sterculia* and therelated genera: A review. *Asian Pacific Journal of Tropical Disease*, 6(6):492–501. doi: http://dx.doi.org/10.1016/S2222-1808(16)61075-7

Shreevastva, NK, Pandeya, A., & Mishra, DK 2017 (2017). A study of AST : ALT ratio in alcoholic and nonalcoholic liver diseases. 4929, 1047–1050.https://doi.org/10.21276/sjmps.2017.3.10.5

~~Swarnalatha, K. *et al.*~~Swarnalatha K., CH. Venkata Kishore Babu, & B. Hari Babu (2019) Phytochemical Screening , Anti-Diabetic And Anti-Oxidant Activities Of *Kigelia africana* (LAM .) and *Sterculia foetida* L. *Rasayan J. Chem*, 12(2).907–914. doi: :10.31788/RJC.2019.1225168

Mogbojuri, O.M., Adedapo, A.A. & Abatan, M.O. (2016) Phytochemical screening , safety evaluation , anti-inflammatory and analgesic studies of the leaf extracts of Sterculia tragacantha. *J Complement IntegrMed*, 2–7. doi: 10.1515/jcim-2015-0114

Mescher, AL (2011). Basic Histology of Junqueira Texts and Atlas. Jakarta: *EGC*. page. 2-4

Formatted: Font: Not Italic

Formatted: Font: Italic

Formatted: Indent: First line: 0 cm

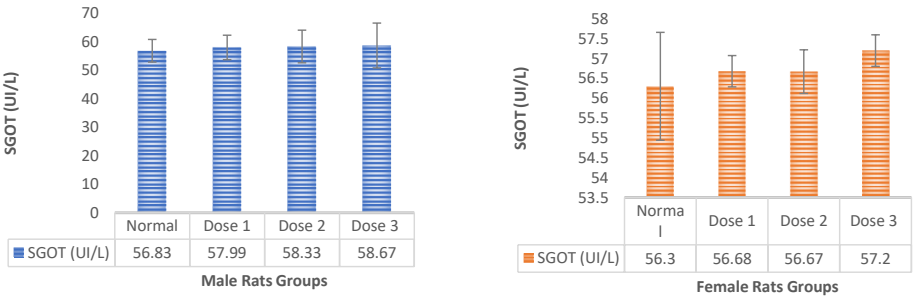
Formatted: Font: Italic

Formatted: Normal, Left, Right: 0 cm, Space Before: 0 pt

Formatted: Left

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	Liberman-Bouchard	+



A B

Figure 1. SGOT levels male and female rats

(A)SGOT levels of male rats groups, There was no significant difference between the dose groups and the normal group ($p<0,05$). (B)SGOT levels of female rats groups, There was no significant difference between the dose groups and the normal group (ns).

Formatted: Centered
Formatted: Centered
Formatted: Font: Not Bold
Formatted: Font: Not Bold
Formatted: Font: Not Bold

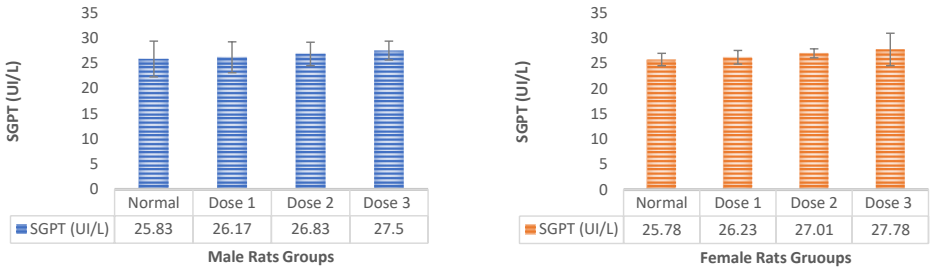
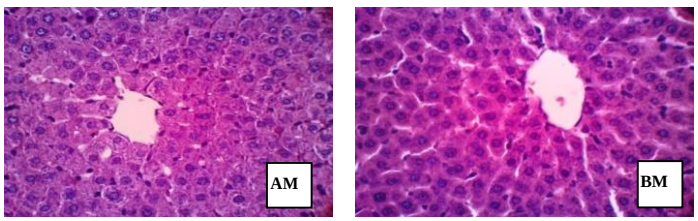


Figure 2. SGPT levels male and female rat

(A)SGPT levels of male rats groups, There was no significant difference between the dose groups and the normal group (*p<0,05). (B)SGPT levels of female rats groups, There was no significant difference between the dose groups and the normal group (ns).



Formatted: Centered
Formatted: Centered
Formatted: Body Text, Centered, Space Before: 0 pt
Formatted: Centered
Formatted: Font: Bold
Formatted: Font: Not Bold
Formatted: Heading 1, Indent: Left: 1.48 cm, Space Before: 9.9 pt
Formatted: Font: Not Bold
Formatted: Font: Not Bold
Formatted: Font: Not Bold
Formatted: Font: Not Bold

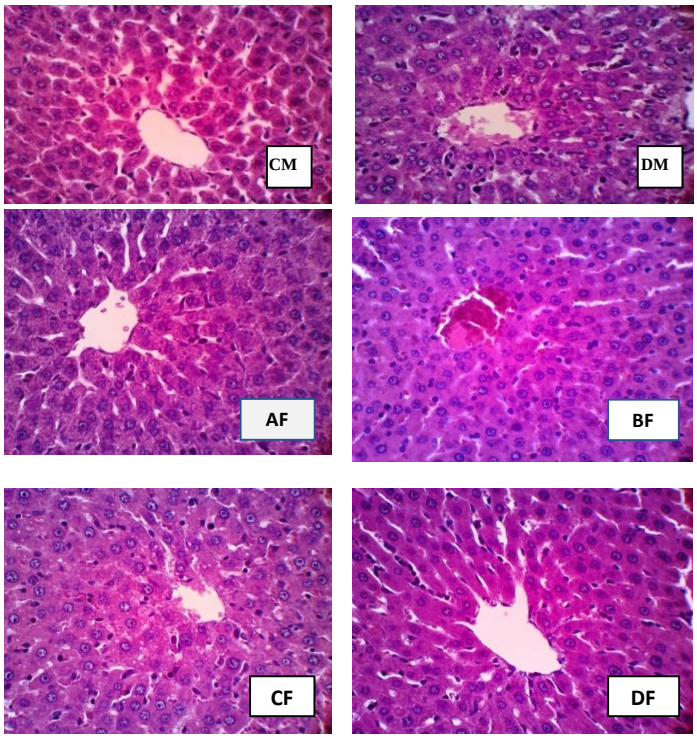
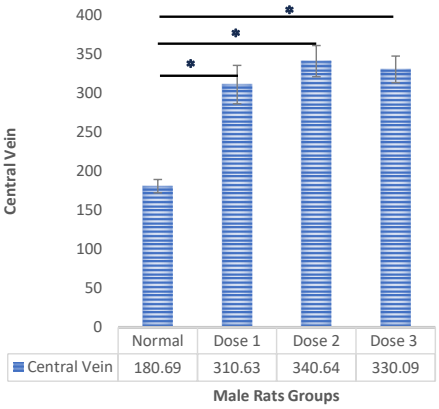


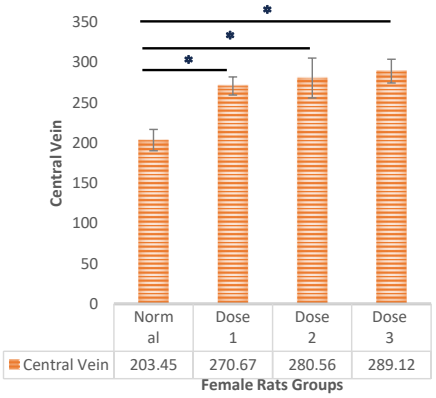
Figure 3. Histopathology of the liver male and female rats

~~**Information:**~~Transverse incision histology of rat liver organ with hemotoxin-eosin staining at 40x10magnification. M: Male, F: Female, (AM) 0.5% NaCMC, (BM) 50 mg/kgBW, (CM 200 mg/kgBW, and (DM) 400 mg/.kgBW. (AF) 0.5% NaCMC, (BF) 50 mg/kgBW, (CF) 200 mg/kgBW, and (DF) 400 mg/.kgBW

Formatted: Centered

Formatted: Justified, Line spacing: single



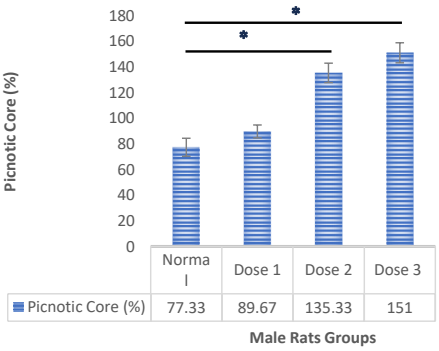


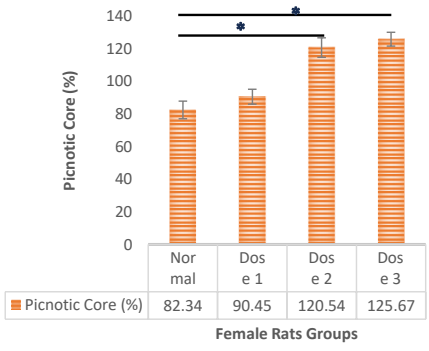
A

B

Figure 4. Diameter of the c~~C~~entral v~~V~~ein male and female rats

(A) Diameter of the central vein of male rats groups, There was a significant difference between the dose groups and the normal group (p<0,05). (B) Diameter of the central vein of female rats groups, There was a significant difference between the dose groups and the normal group (p<0,05).





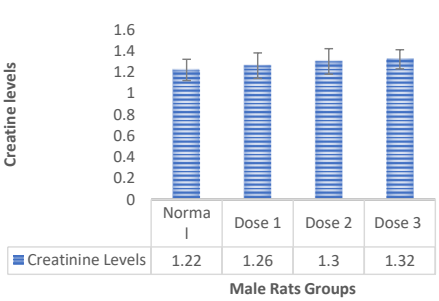
A

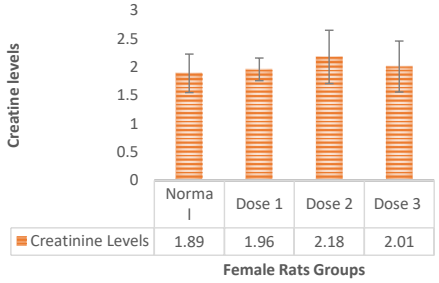
B

Formatted: Centered
Formatted: Centered
Formatted: Font: 11 pt, No underline
Formatted: Font: 11 pt, No underline
Formatted: Normal, Indent: Left: 1.27 cm, Space Before: 0 pt
Formatted: Font color: Text 1
Formatted: Centered
Formatted: Centered

Figure 5. Picnotic core male anda female rats ~~Core~~

(A) The picnotic core of male rats groups, There was a significant difference between the dose 2 and dose 3 with the normal group (p<0,05). And there no significant difference between dose 1 anda normal group (p<0,05). (B) The picnotic core of female rats group, There was a significant difference between the dose 2 and dose 3 with the normal group (p<0,05). And there was no significant difference between dose 1 anda normal group (p<0,05).

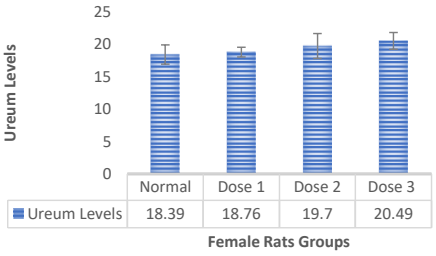


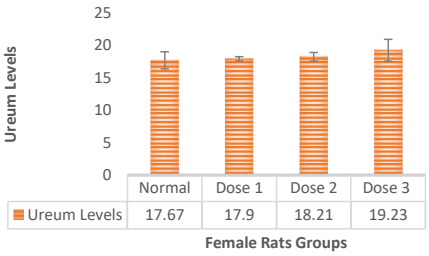


A B

Figure 6. Creatinine l~~L~~evels male and female rats

(A) The creatinine levels of male rats groups, There was no significant difference between the dose groups and the normal group (*p<0,05). (B) The creatinine levels of female rats groups, There was no significant difference between the dose groups and the normal group (ns).





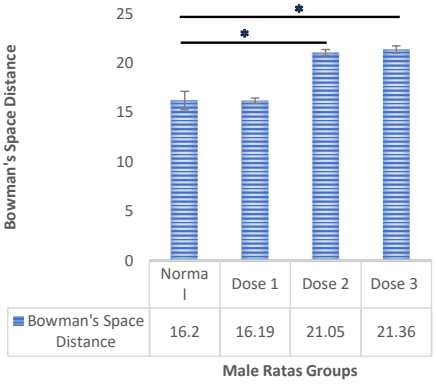
A B

Figure 7. Ureum levels male and female rats

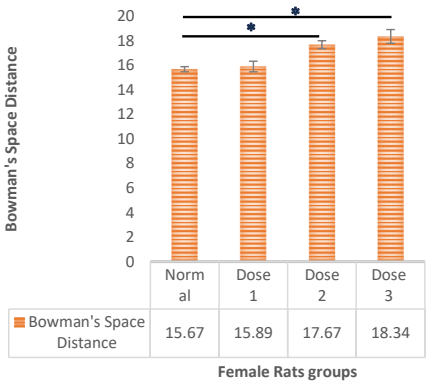
(A) The ureum levels of male rats groups, There was no significant difference between the dose groups and the normal group (*p<0,05). (B) The ureum levels of female rats groups, There was no significant difference between the dose

Formatted: Centered
Formatted: Font: Not Bold
Formatted: Font: Not Bold
Formatted: Centered
Formatted: Centered
Formatted: Font: Not Bold
Formatted: Font: Not Bold
Formatted: Centered
Formatted: Centered
Formatted: Centered
Formatted: Font: Not Bold
Formatted: Font: Not Bold

groups and the normal group (ns).



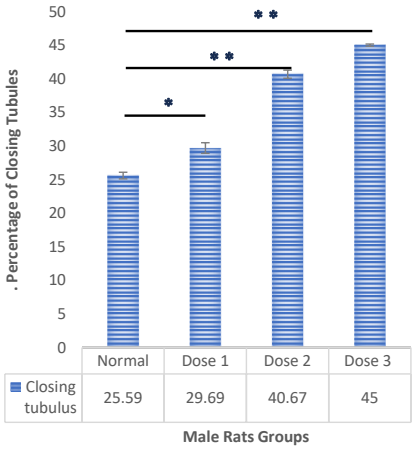
A



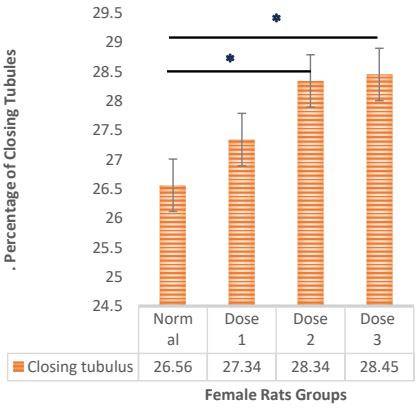
B

Figure 8. Bowman's sSpace dDistance male and female rats

(B) The Bowman's space of male rats groups, There was a significant difference between the dose 2 and dose 3 with the normal group (p<0,05). And there no significant difference between dose 1 anda normal group (p<0,05). (B) The picnotic core of female rats group, There was a significant difference between the dose 2 and dose 3 with the normal group (p<0,05). And there was no significant difference between dose 1 and normal group (p<0,05).



A



B

Formatted: Font: Not Bold

Formatted: Centered

Formatted: Centered

Formatted: Centered

Formatted: Font: Not Bold

Formatted: List Paragraph, Numbered + Level: 1 + Numbering Style: A, B, C, … + Start at: 1 + Alignment: Left + Aligned at: 0.63 cm + Indent at: 1.27 cm, No widow/orphan control, Don't adjust space between Latin and Asian text, Don't adjust space between Asian text and numbers

Formatted: Font: Not Bold

Formatted: Centered

Formatted: Centered

Figure 9. Percentage of cClosing tTubules male and female rats

(A) The percentage of closing tubulus of male rats groups, There was a significant difference between the dose groups with the normal group (p<0,05). (B) The percentage of closing tubulus of female rats group, There was a significant difference between the dose 2 and dose 3 with the normal group (p<0,05). And there was no significant difference between dose 1 and normal group (p<0,05)

Formatted: Font: Not Bold

Formatted: Justified

Formatted: Font: Not Bold

Formatted: Font: Not Bold

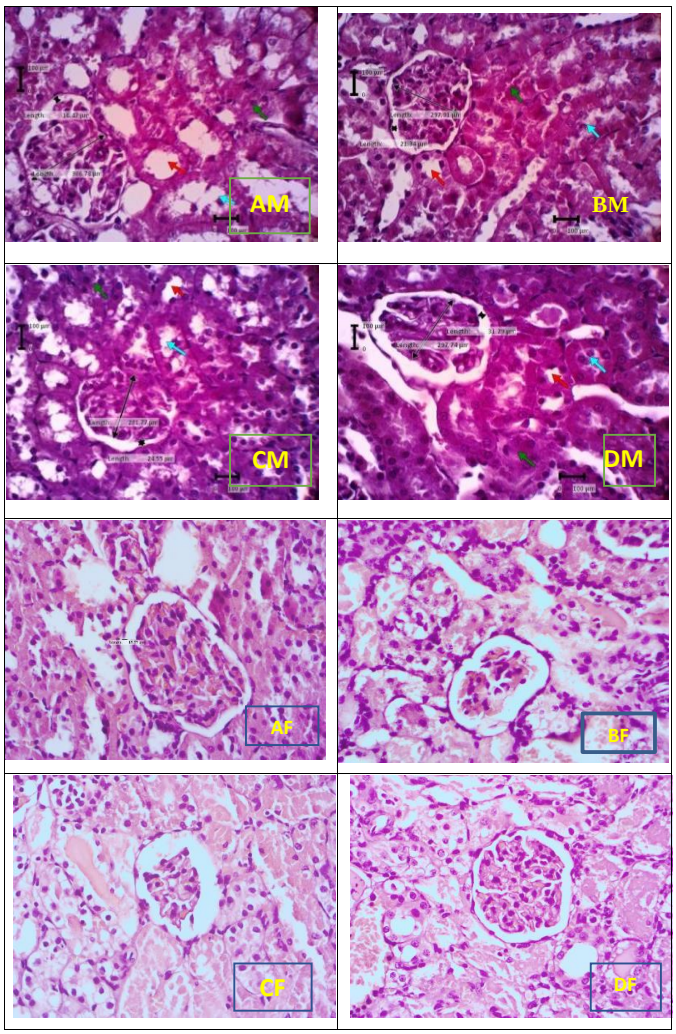


Figure 10. Kidney hHistopathology male and female rats

Information: Transverse incision histology of rat Kidney organ with hemotoxin-eosin staining at 40x10magnification. M: male, F: Female (AM) 0.5% CMC, (BM) 50 mg/kgBW, (CM) 200 mg/kgBW, and (DM) 400 mg/.kgBW, (AF) 0.5% CMC, (BF) 50 mg/kgBW, (CF) 200 mg/kgBW, and (DF) 400 mg/.kgBW.

	Komisi Etik Penelitian Kesehatan **Universitas Muhammadiyah Prof. DR. HAMKA** **(KEPK – UHAMKA) Jakarta** http://www.lrf.uhamka.ac.id	**POB-KE.B/008/01.0** Berlaku mulai: 19 Mei 2017 FL/B.06-008/01.0

SURAT PERSETUJUAN ETIK

PERSETUJUAN ETIK
ETHICAL APPROVAL

No : 02/20.03/0358

Bismillaahirrohmaanirrohiim
Assalamu'alaikum warohmatullohi wabarokatuh

Yang bertanda tangan di bawah ini, Ketua Komisi Etik Penelitian Kesehatan Universitas Muhammadiyah Prof. DR. HAMKA (KEPK-UHAMKA), setelah dilaksanakan pembahasan dan penilaian oleh reviewer yang bersertifikat, memutuskan bahwa protokol penelitian/skripsi/tesis dengan judul :

"UJI TOKSISITAS EKSTRAK ETANOL 70% DAUN KI HAMPELAS (*Sterculia rubiginosa*) PADA TIKUS PUTIH"

Atas nama
Peneliti utama : Rini Prastiwi, M.Si., Apt.
Peneliti lain : Ema Dewanti, M.Si.,
Cut Mauliza,
Ester Hidayati,
Ita Anggraini,
Riska Anggraini
Program Studi : S1 FARMASI
Institusi : UNIVERSITAS MUHAMMADIYAH PROF. DR. HAMKA JAKARTA

dapat disetujui pelaksanaannya. Persetujuan ini berlaku sejak tanggal ditetapkan sampai dengan batas waktu pelaksanaan penelitian seperti tertera dalam protokol.

Pada akhir penelitian, laporan pelaksanaan penelitian harus diserahkan kepada KEPK-UHAMKA dalam bentuk *soft copy* ke email kepk@uhamka.ac.id. Jika terdapat perubahan protokol dan/atau perpanjangan penelitian, maka peneliti harus mengajukan kembali permohonan kajian etik penelitian (amandemen protokol).

Wassalamu'alaikum warohmatullohi wabarokatuh

Jakarta, 02 Maret 2020
Ketua Komisi Etik Penelitian Kesehatan
UHAMKA

(Dr. Emma Rachmawati, Dra., M.Kes)

Formatted: Indent: Left: 0 cm