Quest Journals
Journal of Research in Pharmaceutical Science
Volume 7 ~ Issue 11 (2021) pp: 21-27
ISSN(Online) : 2347-2995
www.questjournals.org

Research Paper

Hepatoprotective Potentials of Green Chireta (*Andrographis paniculata*) and Moringa Leaves (*Moringa oleifera*) Extracts against Streptozotocin-Induced Hepatotoxicity in Rats

Nila Rusdi Rahayu[1,2], Moch Saiful Bachri [1], Laela Hayu Nurani [1]
[1]*Faculty of Pharmacy, Universitas Ahmad Dahlan*
Jl. Prof. Dr. Soepomo, S.H, Janturan, Yogyakarta, Indonesia
[2]*Pharmacy Department, Institut Medika Drg. Suherman*
Jl. Raya Industri Pasir Gombong, Jababeka Cikarang, Bekasi, Indonesia
Corresponding author: Nila Rusdi Rahayu

ABSTRACT
The liver is the main organ where metabolism takes place, and streptozotocin (STZ) is a known cause of damage to liver cells (hepatocytes). The ethanol extracts of moringa leaf (EEML) and green chireta (EEGC) possess hepatoprotective properties because they can reduce lipid peroxidase enzyme activities and increase antioxidant enzymes by neutralizing free radicals and elevating glutathione levels. This study was intended to determine the potential combination of EEML and EEGC as hepatoprotective agents in rats with streptozotocin-induced hepatoxicity. Twenty-four test rats were clustered into six groups receiving different treatments for four weeks, namely normal control (food and water), negative control (CMC-Na solution), positive control (gliclazide at 5 mg/kg BW), treatment group I (EEML+EEGC each at 150 mg/kg BW), treatment group II (EEML at 200 mg/kg BW and EEGC at 100 mg/kg BW), treatment group III (EEML at 100 mg/kg BW and EEGC at 200 mg/kg BW). Blood samples were drawn on Days 0 and 28, and at the end of the treatment, the test rats were sacrificed, and their liver tissues were collected for histopathology by hematoxylin-eosin staining. The hepatoprotective agents administered to treatment group III lowered the SGOT, SGPT, and ALP levels (to 90.88±7.69, 37.12±5.65, and 19±1.58, respectively), which significantly differed from the normal and negative control groups ($P>0.05$) but not from treatment groups I and II ($P<0.05$). All treatments affect the repair process of the damaged histopathologic structure of the liver. Although the combination of EEML and EEGC can reduce the activities of SGOT, SGPT, and ALP, the resultant improvements cannot fully reverse the damage and return the conditions to normal.

KEYWORDS*: Hepatoprotective agent, moringa, green chireta, streptozotocin*

Received 03 November, 2021; Revised: 15 November, 2021; Accepted 17 November, 2021

I. INTRODUCTION

The liver is the main place where metabolism takes place, and free radicals are known to induce damages to liver cells (Kumala, 2017). Streptozotocin (STZ) is a free radical compound that can bind to DNA in the mitochondria, causing necrosis. Necrotic hepatocytes will release the enzymes GOT, GPT, and ALP into the bloodstream, elevating the SGOT, SGPT, and ALP levels. After being metabolized in the liver, STZ will become a reactive compound that most likely causes damages to the liver (Sari *et al*., 2010).

Liver enzymes that can indicate liver damage include aminotransferase (transaminase) and alkaline phosphatase (ALP). The aminotransferase enzyme groups are serum alanine aminotransferase (Serum Glutamic-Pyruvic Transaminase or SGPT) and serum aspartate aminotransferase (Serum Glutamic-Oxaloacetic Transaminase or SGOT). These enzymes function as specific indicators of hepatocyte damage. Alkaline phosphatase (ALP) is a group of enzymes that hydrolyze phosphate esters in an alkaline atmosphere. The highest levels of ALP in the body are found in cells that undergo rapid division, such as intestinal epithelium,

cells of hepatic proximal tubule tissues, and placenta. Increased levels of these enzymes reflect the extent of hepatocyte injury (Sari, 2011).

The leaves of *Moringa oleifera* Lam, or locally known as horseradish or moringa tree, contains quercetin with proven antioxidant activities that can neutralize free radicals, preventing damages to the cell membrane or called necrosis (Sulistyorini *et al*., 2015). Therefore, quercetin can act as hydrogen atom donors to decelerate autoxidation that inhibits the formation of lipid radicals, i.e., by donating hydrogen atoms to lipid radicals to stabilize them and avert further damage (Gibson, 2015). The ethanol extract of moringa leaf (EEML) can reduce reactive free radicals and, therefore, minimize oxidative damage in rats injected with streptozotocin (Sulistyorini *et al*., 2015). Previous phytochemical screening has discovered secondary metabolites present in moringa leaf extract, such as flavonoids, terpenoids, saponins, and tannins (Anyanwu *et al*., 2015). Quercetin is a flavonoid that has the most potential for antioxidants in EEML, followed by chlorogenic acid and moringinine (Ali *et al*., 2015). It works through the mechanism of reducing lipid peroxidation (MDA) and increasing the activity of antioxidant enzymes (Sulistyorini *et al*., 2015). In Kumala (2017), the histopathological picture of the liver of white rats that have been given EEML at 1,000 mg/200g BW shows an improvement to the damages induced by the injection of toxic doses of paracetamol.

The ethanol extract of green chireta (EEGC) is known to contain lactone diterpene (Okhuarobo *et al*., 2014), which has bioactive compounds like andrographolide, neoandrographolide, deoxyandrographolide, 14-deoxy-11,12 didehydroandrographolide, and isoandrographolide (Chao & Lin, 2010). In a previous study, green chireta extract has been found to prevent liver damage by increasing the activity of antioxidant enzymes and the amount of glutathione and decreasing the activity of lipid peroxidase enzyme (Sari *et al*., 2015). This study was also conducted to scrutinize the synergistic effect of hepatoprotective components, that is, EEML and EEGC on the hepatic condition of rats injected with STZ, mainly because each extract is expected to have hepatoprotective properties. Here, synergism occurs if one component increases the effect of the others.

II. MATERIALS AND METHOD

Materials

The research equipment included a macerator (Iwaki Pyrex), filter cloth, filter paper, Buchner funnel (Iwaki), vacuum pump, rotary evaporator, analytical balance, measuring flask (Iwaki Pyrex), peritoneal injection syringe, oral injection, test tube (Iwaki Pyrex), and glass chamber (Iwaki Pyrex). The research materials were dried powdered moringa and green chireta leaves. They were obtained in Tasikmadu, Karanganyar, Jawa Tengah, 70% ethanol, 0.5% CMC-Na solution, streptozotocin, gliclazide (Diamicron MR 60 mg), silica gel GF-245 (Sigma Aldrich), standards of tannins, flavonoids, and andrographolides, Citric Acid (Merck, Germany), and Na Citrate (Merck, Germany).

Methods

Moringa Leaf Extraction

A total of 2.5 kg of fresh old moringa leaves were weighed, cleaned, washed, dried, ground, and sieved with mesh no. 20 until a dry powder was obtained. Then, 1.316 kg of the dry powder was macerated with 70% ethanol (1:5) for three days, then filtered. The residue was re-macerated with 70% ethanol solution three times, and the result was evaporated in a vacuum rotary evaporator until there were no drips of ethanol vapor and then heated in a water bath at 50^{o}C until a thick ethanol extract (EEML) was formed.

Green Chireta Extraction

A total of 2.3 kg of green chireta was washed, dried in an oven at 50^{o}C, ground, and sieved using mesh No.12. Then, the dry powder was weighed (1.25 kg) and extracted by maceration for three days using 70% ethanol solvent (ratio of powder and solvent was 1:5). After three days of soaking, the process continued to filtering using a filter paper to obtain the filtrate. This filtrate was then evaporated in a vacuum rotary evaporator at 40°C until there were no drips of the solvent and then heated in a water bath at 50^{o}C until the extract (EEGC) was formed.

Making of EEML and EEGC Suspensions

The combination/suspension of EEML and EEGC was prepared by weighing the extracts (as much as needed) and suspending them in 0.5% CMC-Na solution. For test rats weighing 200 g, the extract suspension given amounted to 1.0 ml.

Production of Citrate Buffer (0.1 M, pH 4.5)

Citrate buffer solution (0.1 M, pH 4.5) was made by carefully weighing 0.516 g of citric acid, dissolving it in 30 ml of aquadest, and placing the mixture in a measuring flask. Also, Na citrate was weighed carefully (0.576 g), dissolved in 20 mL of aquadest, and poured into the previous measuring flask that contained citric acid solution.

This liquid mixture was then homogenized and measured with a pH meter, with a target of pH 4.5 (Ajizah, 2017).

Making of STZ solution

STZ was dissolved in 0.1 M citrate buffer (pH 4.5) to produce adequate total volume for intraperitoneal injections (i.e., 14 mL). Assuming that the injected volume was 0.2 ml for a test rat that weighed 150 g and received STZ at a dose of 45 mg/kg BW, the required weight of STZ to be dissolved in the citrate buffer solution (pH 4.5) would be 0.47 g.

Treatments given to the test rats

The test animals used were male Wistar rats originating from Solo, Central Java, aged 7-8 weeks with a body weight of 180-250 grams, kept in the air-conditioned (AC) test animal room in UAD, and were given food pellets and drinking distilled water and has passed the research code of ethics permit at the Ethics Committee No 011801014 UAD.

A total of 24 test rats were divided into six groups, each consisting of four rats. The six test groups were normal control (only given food and drink), negative control (injected with STZ at 45 mg/kg BW i.p. and 0.5% CMC-Na p.o.), positive control (STZ i.p. and gliclazide suspension p.o. at 5 mg/kg BW), treatment group I (STZ i.p. and EEML+EEGC (1:1) suspension p.o. at 150 mg/kg BW), treatment group II (STZ i.p. and a suspension (2:1) of EEML at 200 mg/kg BW and EEGC at 100 mg/kg BW p.o.), and treatment group III (STZ i.p. and a suspension (1:2) of EEML at 100 mg/kg BW and EEGC at 200 mg/kg BW p.o.). Intraperitonial injection of 45 mg / kg of streptozotocin was carried out on day 0. The treatment was carried out for 4 weeks. Blood sampling was carried out on days 0 and 28 and after treatment the rats were sacrificed and their hepatic tissue was taken for histopathologic staining with hematoxylin-eosin.

Data Analysis

The SGOT, SGPT, and ALP levels were analyzed in the SPSS 16 program using analysis of One Way Anova.

III. RESULTS AND DISCUSSION

Effects of EEML and EEGC on streptozotocin-induced changes in the serum hepatic enzymes Increased liver serum enzymes (SGOT, SGPT, and ALP) are indicators of liver damage. These hepatic marker enzymes were analyzed to evaluate the hepatoprotective effects of EEML and EEGC in rats receiving STZ, and the results are presented in Table 1.

An improvement in SGOT activity was observed from the mean difference (Δ) between Day 28 and Day 0. In the negative control group, the treatment significantly increased SGOT activity ($p<0.05$) more than the normal control group, meaning that, to some extent, it induces liver damage. Meanwhile, based on the SGOT levels in the treatment group I (EEML+EEGC, 1:1), there was no protective activity against liver damage. It is likely to occur because the combined doses of EEML and EEGC in treatment groups I (1:1) and II (2:1) were too small. The SGOT data in treatment group III (EEML+EEGC, 1:2) were significantly different ($p<0.05$) from the negative control group (STZ). In other words, the combination of EEML and EEGC (1:2) can protect against liver damage, as characterized by a more significant decrease in SGOT levels in the treatment group III than the negative control (STZ). Also, compared to the normal control group, the Wistar rats injected with STZ (negative control) showed a substantial increase in SGOT levels. Overall, the combined administration of EEML and EEGC at the ratios of 1:1, 2:1, and 1:2 can lower SGOT levels in Wistar rats with STZ-induced liver damage. In this context, administrations at different doses can produce varying effects on the reduction of the SGOT level, although not significant ($p>0.05$).

The mean difference (Δ) in the SGPT activities of the negative control group (STZ) between Day 28 and Day 0 was higher than that of the normal control group. Such an increase in SGPT activity confirms that the administration of STZ can indeed cause liver damage. Based on the SGPT levels, treatment groups I (EEML+EEGC, 1:1) and II (EEML+EEGC, 2:1) had low SGPT levels on Day 28, but the mean difference was not significant ($p> 0.05$) compared to the negative control group (STZ). In other terms, the administration of EEML and EEGC at both ratios can only reduce SGPT activity without protecting the liver from STZ-induced damages. It is likely to occur because the doses in both groups were categorically small. Table 1 shows that in treatment group III (EEML+EEGC, 1:2), the SGPT activities decreased significantly ($p<0.05$) when compared to the negative control group (STZ), meaning that this treatment gives a protective effect against liver damage. Also, the SGPT levels of the Wistar rats in the three treatment groups (EEML+EEGC) lowered more substantially than that of the negative control group, which signifies the ability of the EEML+EEGC combination to reduce SGPT activities depending on the administered doses.

These treatment groups (EEML+EEGC at 1:1, 2:1, and 1:2) could reduce ALP activities more significantly ($p<0.05$) than the negative control group and, accordingly, produce better protective effects against

liver damage. Observation on Day 28 found that the ALP activity of the negative control (STZ) group increased dramatically ($p < 0.05$) than that of the normal group, which means that the injection of STZ induces damages to the liver. On the contrary, when compared to the negative control group, the treatment groups were able to reduce the ALP levels ($p > 0.05$) significantly. In conclusion, the combination of EEML and EEGC successfully produce protective activities against liver damage.

Effects of EEML and EEGC on streptozotocin-induced liver damage

Observations on the histopathologic slides of the liver in both normal and positive control groups showed no histopathologic changes, meaning that despite the different treatment received by the latter, it still creates a normal liver condition similar to the former. Necrosis was present in the negative control group, as apparent from the lots of inflammations or black spots scattered and concentrated around and near the Kierman triangle. STZ can injure liver because it is a hepatoxic agent that can damage the mitochondria and inhibit the formation of energy in hepatocytes.

Table 1. Effects of EEML+EEGC combination on SGOT, SGPT, and ALP levels of rats injected with STZ (in mean±SD).

Groups	SGOT (U/L)			SGPT (U/L)			ALP (U/L0		
	Day 0	Day 28	mean Δ	Day 0	Day 28	mean Δ	Day 0	Day 28	mean Δ
Normal	76.98±7.22	86.52±7.11	9.54	50.94±1.87*	44.69±2.79*	-6.25	7.13±0.73*	21.28±0.99*	14.5
Negative Control	138.16±8.83	126.05±17.33	-12.11	48.92±1.67	46.2±3.0	-2.72	8.47±0.37	12.11±1.77	3.64
EEML+EEGC (1:1)	104.83±10.66 *	105.68±13.81	0.85	54.01±6.04*	58.01±11.00	4	8.28±0.71*	22.50±1.35	14.22
EEML+EEGC (2:1)	90.82±3.84*	101.62±13.27	10.8	58.19±5.11*	46.21±5.11	-11. 98	5.42±0.29*	21.01±1.71*	15.59
EEML+EEGC (1:2)	83.87±3.69*	90.88±7.69*	7.01	55.14±2.34*	52.39±4.78	-2.75	8.15±0.12*	19.78±1.58*	11.63
Gliclazide 5 mg/kg BW	132.39±5.59	104.36±13.78	-28.3	50.32±4.72*	39.62±8.87*	-10.7	3.60±0.45*	19.00±1.58*	15.4

Notes: * significantly different from the control group ($p<0.05$), $p<0.05$ compared to the negative control

When administered at a dose of 45 mg/kg BW, STZ results in reversible degeneration in hepatocytes, leading to liver damage and high levels of ROS in the liver. Moreover, it can induce inflammation in hepatocytes that further release inflammatory mediators, damage hepatocytes, and finally cause necrosis. In the negative control group, the entire liver cells (100%) were necrotic, and this damage is irreversible.

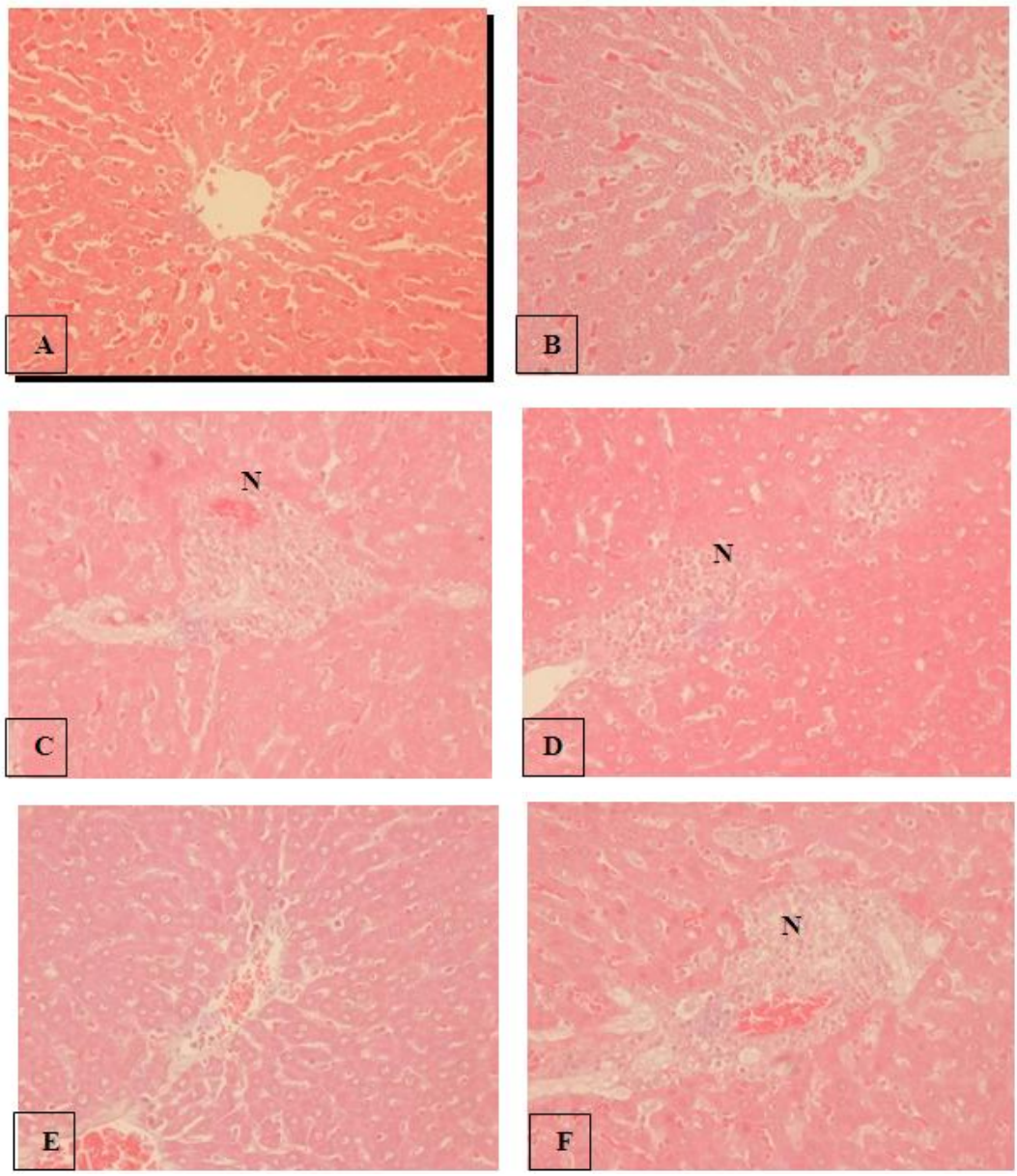

Figure 1. Histopathological profiles of the liver of Wistar rats injected with STZ and treated with EEML and EEGC. No degeneration and necrosis are apparent in the normal (A) and positive control (B), while necrotic cells (N) are visible in the profile of the negative control group (C). There are signs of degeneration and necrosis in treatment groups I (EEML+EEGC, 1:1, picture D) and II (EEML+EEGC, 2:1, F), but not in treatment group III (EEML+EEGC, 1:2, E) (coloring HE, 40X).

Table II. Liver histopathological test results.

Groups	Day-28	
	Rats	Liver observation results
Normal	1	K,N+
	2	-
	3	K
	4	K
Negative Control	1	N+
	2	N+
	3	N+
	4	N+
Glieclazid 5mg/KgBB	1	-
	2	-
	3	-
	4	-
EEML+EEGC (1:1)	1	-
	2	K
	3	K,N+
	4	K
EEML+EEGC (2:1)	1	N+
	2	N+
	3	K
	4	K
EEML+EEGC (1:2)	1	-
	2	DV+
	3	K
	4	K

Table II presented on the treatment groups I (EEML+EEGC, 1:1) and II (EEML+EEGC, 2:1), necrosis was apparent after the induction of liver damage by injecting streptozotocin at a dose of 45 mg/kg BW. Streptozotocin is a toxic compound that can increase ROS, and therefore, by administering the combination of EEML and EEGC (1:1), the resultant liver cell degeneration is preventable. Observations on the histopathological slides of the murine liver in the treatment group III (EEML+EEGC, 1:2) affirmed that this combination gave a better histopathologic improvement and protection of the liver function than the negative control group. This finding is consistent with Gibson (2015), which states that the administration of moringa leaf extract causes a significant change in the histopathologic profile of rats injected with paracetamol. Quercetin present in EEML can scavenge free radicals because its molecular structure is composed of a phenolic hydroxyl group that it can donate hydrogen atoms and decelerate autoxidation that inhibits the formation of lipid radicals, then stabilize them and prevent further cell damage (Gibson, 2015). Furthermore, quercetin has been reported to exhibit antioxidant activities that help neutralize free radicals and prevent damages to cell membrane, i.e., necrosis (Sulistyorini *et al*., 2015).

Meanwhile, in EEGC, andrographolide plays a crucial part in repairing cells and, therefore, protecting liver from damage by not only increasing the activity of antioxidant enzymes and the amount of glutathione but also decreasing the activity of the lipid peroxidase enzyme (Sari *et al*., 2015). The treatment group III (EEML+EEGC, 1:2) produced the best histopathologic profile of liver improvement because no visible sign of necrosis was found on the slides indicating similar conditions with normal liver cells and proving that this combination can protect the liver of the test rats. Based on the histopathologic pictures of the murine livers in all groups, the combination of EEML and EEGC at the ratio of 1:2 can improve the liver condition of rats with STZ-induced hepatoxicity. Most importantly, it produced histopathologic features that were nearly identical to that of the normal and positive control groups. Meanwhile, the combined administration of EEML and EEGC at the ratio of 1:1 does not significantly reverse the detrimental effects of STZ injection but can still reduce necrosis better than the negative control group

.

IV. CONCLUSION

The ethanol extracts of moringa leaf (*Moringa oleifera* Lam) and green chireta (*Andrographis paniculata*), when combined at a ratio of 1:2, can prevent an increase in SGOT, SGPT, and ALP levels in Wistar rats injected with streptozotocin at a dose of 45 mg/kg BW. However, when administered at a combination of 1:2, they can improve the histopathologic features of the liver organ in these test animals.

REFERENCES

[1]. Ajizah P.R, 2017 Pemberian ekstrak kering buah okra (*Abelmoschus esculentus*) memperbaiki glukosa darah sewaktu dan indeks apoptosis sel jantung pada tikus Sprague dawley yang mengalami diabetes karena induksi streptozotocin.Universitas Islam Negeri, Jakarta

[2]. Ali, F. T., Hassan, N. S., & Abdrabou, R. R. (2015). Potential Activity of Moringa oleifera Leaf Extract and Some Active Ingredients Against Diabetes in Rats. *International Journal of Scientific & Engineering Research*, *6*(5).

[3]. Anyanwu, A., Salako, O., & Olaide, A. (2015). Effect of the Ethanolic Leaf Extract of Moringa oleifera on Insulin Resistance in Streptozotocin Induced Diabetic Rats. *Journal of Plant Sciences*, *2*(6), 5. https://doi.org/10.11648/j.jps.s.2014020601.12

[4]. Chao, W.W., & Lin, B.F. (2010). Isolation and identification of bioactive compounds in Andrographis paniculata (Chuanxinlian). *Chinese Medicine*. https://doi.org/10.1186/1749-8546-5-17

[5]. Gibson, N. E. (2015). Efek Hepatoprotektor Ekstrak Etanol Lidah Buaya (Aloe Vera Linn.) Terhadap Gambaran Histopatologi Hati Tikus Jantan Putih (Rattus Norvegicus) Galur Wistar Yang Diinduksi Parasetamol. *Jurnal Mahasiswa Fakultas Kedokteran Untan*, *3*(1).

[6]. Kumala I, N. (2017). Gambaran Histopatologi Hepar Tikus Putih (Rattus Novergicus) yang Diinduksi dengan Parasetamol dosis Toksik Pasca Pemberian Ekstrak Etanol Daun Kelor (Moringa Oleifera). *Jurnal Kimia Riset*, *2*(2), 123. https://doi.org/10.20473/jkr.v2i2.6700

[7]. Okhuarobo, A., Ehizogie Falodun, J., Erharuyi, O., Imieje, V., Falodun, A., & Langer, P. (2014). Harnessing the medicinal properties of Andrographis paniculata for diseases and beyond: A review of its phytochemistry and pharmacology. *Asian Pacific Journal of Tropical Disease*. https://doi.org/10.1016/S2222-1808(14)60509-0

[8]. Sari, A. K., Adi, S., & Saichudin. (2010). Efek Latihan Aerobik Interval Dan Continuous Terhadap Kadar SGOT Dan SGPT Pada Tikus Diabetes Induksi Streptozotocin.

[9]. Sari, K. R. P., Sudarsono, & Nugroho, A. E. (2015). Effect of herbal combination of Andrographis paniculata (Burm.f) Ness and Gynura procumbens (Lour.) Merr ethanolic extracts in alloxan-induced hyperglycemic rats. *International Food Research Journal*. Sari, W. (2011). *Care Yourself, Hepatitis*. Jakarta: Penebar Plus+.

[10]. Sulistyorini, R., Sarjadi, Johan, A., & Djamiatun, K. (2015). Pengaruh Ekstrak Etanol Daun Kelor (Moringa oleifera) pada Ekspresi Insulin dan Insulitis Tikus Diabetes Melitus. *Majalah Kedokteran Bandung*, *47*(2). https://doi.org/10.15395/mkb.v47n2.456