

Hepatoprotective Effect of Extract Ethanol *Gynura procumbens* on Liver Injury Induced by Toxic Dose of Paracetamol

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ARTICLE INFO

Article History:
Received: April, 2021
Revise: June, 2022
Accepted: August, 2022

Keywords:
hepatoprotective, *Gynura procumbens*, paracetamol, liver

ABSTRACT

Gynura procumbens is a plant that contains flavonoid compounds as antioxidants and has a hepatoprotective effect. A hepatoprotector is a compound that can protect the liver from liver damage. One way to determine liver function is to measure the enzyme activity of Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT). This study aimed to determine the hepatoprotective effect of the extract ethanol of *Gynura procumbens* on the liver induced by paracetamol toxic dose. This study used 20 white male rats divided into five groups: positive control group, negative control (NaCMC 1%), and paracetamol dose of 2.400mg/kg bw). EDSNY 100 mg/kg bw, 200 mg/kg bw, 300 mg/kg bw + 2.400 mg/kg paracetamol. The rat blood samples were taken through the lateral vein, and then the SGOT and SGPT were measured before, after 4 days, and on the 6th day after paracetamol induction. The results showed that the ethanol extract of *Gynura procumbens* significantly reduced the levels of SGPT in mice at a dose of 300 mg/kg bw. However, the ethanol extract of *Gynura procumbens* leaves could not reduce SGOT levels in rats. From the histopathological results, it was evident that both low, medium, and high doses had not been able to significantly improve liver damage induced by toxic doses of paracetamol. However, the ethanol extract of *Gynura procumbens* leaves appeared to reduce the number of areas of necrosis and degeneration of hepatocytes compared to the negative control group. Based on the study's results, the ethanol extract of *Gynura procumbens* leaves at a dose of 300 mg/kg bw was able to protect against an increase in SGPT. Still, it hadn't shown that it was the best way to protect the liver from damage caused by too much paracetamol.

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INTRODUCTION

Today, traditional treatments utilizing phytopharmaceutical methods are one of the world's concerns. Darminto *et al.* (2011) say that in Thailand and the Philippines, phytopharmaceuticals have been used to kill bacteria, fungi, algae, viruses, weeds, and pests. One disease classified as excessive in Indonesia is heparin disease (Ministry of Health) (2010). The use of drugs of a hepatotoxic nature is one of the causes. Liver disease is caused by drugs called Drug-Induced Hepatitis (DIH). In 2013, the Indonesian Liver Researchers Association (PPHI) found that drugs cause 20–40% of fulminant liver disease, and those drug reactions to heparin cause 50% of severe hepatitis cases. DIH can be caused by using drugs such as aspirin, artemisinin, rifampicin, paracetamol, and other drugs that are metabolized in heparin with prolonged use at excessive doses. These drugs will be metabolized by heparin into an active metabolite. If endogenous antioxidants are lower than the active metabolites of drugs, then the active metabolites of prescriptions can become free radicals that damage cells (Larson, 2007).

Paracetamol is one of the analgesics and antipyretic drugs that can cause liver injury (Larsen and Wendon, 2014). Paracetamol metabolized in the liver forms the metabolite *n*-acetyl-*p*-benzoquinone imine (NAPQI) which is reactive and covalently interacts with liver macromolecules on the part of cysteine, resulting in oxidative stress (Brune *et al.*, 2015; Vakiloddin *et al.*, 2015). These reactions were then alleged to cause liver injury (James *et al.*, 2003). Gowda *et al.* (2009) say that damage to liver function can be measured by an increase in serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT). SGPT is an excellent indicator of liver injury.

Gynura procumbens is a medicinal plant of the Asteraceae family. Alkaloids, flavonoids, anthraquinones, saponins, glycosides, and essential oils are all chemicals in this leaf that are good for people (Kaewseejan, 2012). *Gynura procumbens* is used to treat a number of diseases,

such as high blood pressure, diabetes, high cholesterol, fever, benign tumors, decreased kidney function, exposure to snake venom or caterpillar venom, and dysentery (Dalimartha, 2006).

MATERIALS AND METHODS

This research is an experimental study using a post-test only control group design. Twenty rats were split into five groups. Group 1 was used as a control, and Group 2 was used as a negative control. Groups 3, 4, and 5 were treated with ethanol extract of life-saving leaves at doses of 100 mg/kg BB, 200 mg/kg BB, and 300 mg/kg BB, respectively.

Tools and Materials

The tools used are rotary evaporator maceration, surgical equipment, paraffin block, water bath, glass object, glass deck, preparation needle, chaff, pellets, sonde, analytical balance, microtome, paraffin mold, pot, tweezers, pipette, measuring flask, oven, filter paper, Humalyzer, minor surgery, test tube, aluminum foil. The ingredients used were life-saving leaves, white rat strains of Wistar, paracetamol, NaCMC, NaCl 0.9%, aqua des, chloroform, 80% alcohol, 96% alcohol, 10% formalin, xylol, paraffin liquid, HE dyes (hematoxylin Eosin), SGOT SGPT reagents, and rat liver organs given the treatment.

Extract Making

The dried *Gynura procumbens* was weighed up to 500 grams, put in a container, and extracted with 70% ethanol using the maceration method. In the mesorization process, the sample is first washed with 70% ethanol until wholly submerged for 15 minutes. After this, they were cleaned with 70% ethanol at room temperature for 3 x 24 hours, which is sufficient again, while occasionally stirring.

After separating the macerate by filtration and remaseration with the same type and amount of solvent, a clearer last macerate is obtained. The liquid extract is collected and evaporated until a viscous ethanol extract is obtained (Larsen and

Wendon, 2014). The thick piece that is left is put into a vial or porcelain dish and weighed against the weight of the extract.

SGOT and SGPT Measurements

Measurements are conducted by the photometric method by mixing serum samples with reagents. Blood serum and SGOT/SGPT reagents are mixed at room temperature (15–30°C). Then 100 µl of blood serum was collected, then 1000 µl of SGOT/SGPT reagents were added and incubated at 37°C. After 60 seconds, the measured absorbance is read and recorded. The mixture is then returned to room temperature and set at 37°C for 60 seconds. The next absorbance is measured in the 1st, 2nd, and 3rd minutes. The measured absorbance is then calculated to obtain the SGOT and SGPT levels.

The treatment of experimental animals

The control group was fed from the first to the 6th day. Blood draws were carried out on the first day, the 4th day, and the 6th day to measure the levels of SGOT and SGPT. Then the rats were heated, and termination was carried out for heparin and subsequently in histopathology. In the negative control group, NaCMC suspension was 1%. Ethanol extract of life-saving leaves (EDSNY) doses of 100, 200, and 300 mg/kg bb, namely the extract, was given on the first day to day 3, and on day 4, blood was taken to measure SGOT and SGPT levels. On day 5, paracetamol was induced at a dose of 2,400 mg/kg on day 5, and on day 6, it was re-measured. SGOT and SGPT levels were then heated and terminated to take heparin and then for histopathology.

Data Analysis

SGOT and SGPT data were tested comparatively using an ANOVA statistical test using post-hoc Tukey HSD.

RESULTS AND DISCUSSION

In this study, white rats were given paracetamol to cause liver damage so that SGOT, SGPT, and

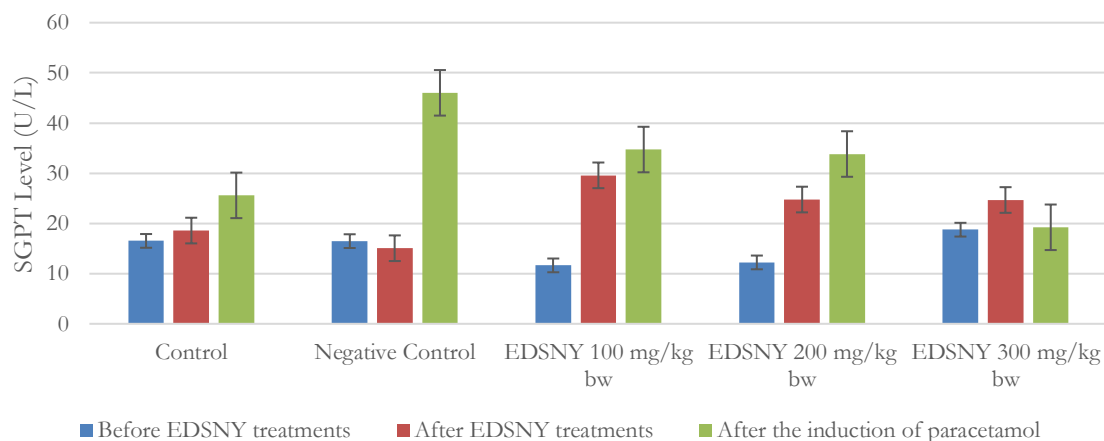
histopathology parameters could be used to see how the ethanol extract of life-saving leaves protected the liver.

SGPT Levels

The results of SGPT levels before, after treatment, and after paracetamol induction can be seen in table 1 and Figure 1. Blood is taken on the first day before treatment, the fourth day after the extract is given, and the sixth day after paracetamol is given. Average SGPT levels were seen, and an increase occurred in negative controls after the induction of toxic doses of paracetamol. This means that paracetamol has an influence on SGPT enzyme levels, which is an indication of liver damage. Meanwhile, the treatment group was given life-saving leaf ethanol extract of 300 mg/kg bb and had an average SGPT of 18.75 U/L, 24.67 U/L, and 19.23 U/L. This means that the dose of life-saving leaf ethanol extract of 300 mg/kg bb was able to prevent an increase in SGPT levels after paracetamol induction, where the value was much lower than the administration of life-saving leaf ethanol extract doses of 100 mg/kg bb and 200 mg/kg bb. The results of the ANOVA statistical test stated that there was a significant influence ($P < 0.05$) of the treatment on the SGPT levels of experimental rats. Based on the Tukey HSD Test, paracetamol induction pct administration in animals that were only given 1% NaCMC (placebo) caused a significant increase in SGPT levels. This can indicate liver cell damage by administering toxic doses of paracetamol (2,400 mg/kg). Table 1 shows that the administration of ethanol extract at a dose of 300 mg/kg bw was significantly lower than the negative control. The average level of SGPT in the negative control group was 46.02 U/L, while the group given treatment (after paracetamol induction) was the EDSNY group, 300 mg/kg bb of 19.23 U/L. This means that an increase in SGPT levels caused by too much paracetamol can be stopped by giving EDSNY 300 mg/kg bb.

Table 1. Average SGPT levels before the extract treatments, after the extract treatments, and induction of paracetamol

Rat Group	Average SGPT (U/L) \pm SEM		
	Before EDSNY treatments	After EDSNY treatments	After the induction of paracetamol
Control	16.52 \pm 1.83	18.58 \pm 1.98	25.59 \pm 1.72
Negative Control	16.47 \pm 1.48	15.06 \pm 3.04	46.02 \pm 4.49
EDSNY 100 mg/kg bb	11.64 \pm 0.12	29.58 \pm 0.42	34.72 \pm 5.93
EDSNY 200 mg/kg bb	12.23 \pm 1.35	24.76 \pm 1.41	33.83 \pm 2.04
EDSNY 300 mg/kg bb	18.75 \pm 0.91	24.67 \pm 2.40	19.23 \pm 2.24

**Figure 1.** The average levels of SGPT before and after the extract was given and after paracetamol was given.

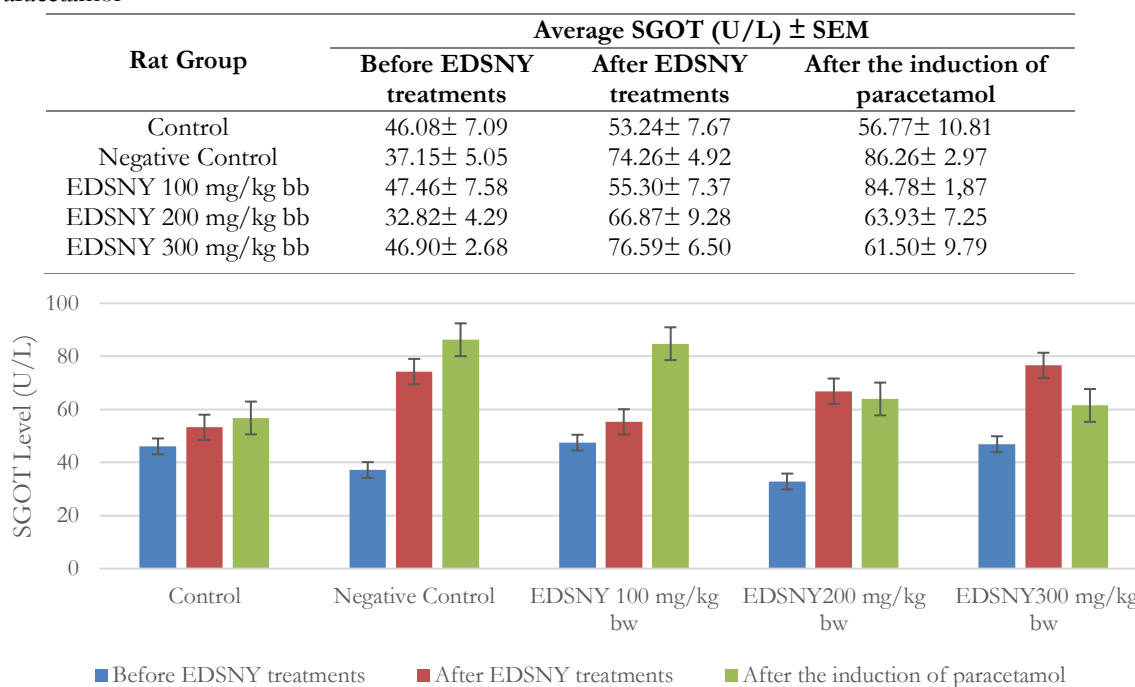
Description: The symbol a stands for p 0.05 against EDSNY 100 mg/kg bb and 200 mg/kg bb before the extract was given. The symbol b stands for p 0.05 against the negative control (NaCMC1%), and the symbol c stands for p 0.05 against the negative control after induction.

SGOT levels

The results of SGOT levels before administration, after treatment, and after paracetamol induction can be seen in table 2 and Figure 2. Based on the SGOT level data, the control group showed normal SGOT levels of between 46.08-56.77 IU/L until the last day of the study. This is because the group of animals is not induced with paracetamol, so there is no liver damage in this group. However, after paracetamol induction, the negative control group showed the highest value compared to the extract treatment group ($p < 0.05$). This suggests that high doses of paracetamol may have caused the most liver damage in the group.

In the group of animals given ethanol extract from life-saving leaves, it was seen that the average level of SGOT was greater than that of

the standard group. This shows the occurrence of liver toxicity due to the induction of paracetamol. Still, the average levels of these two parameters are much smaller than the negative control group (placebo), which was not given ethanol extract of life-saving leaves, only given 1% NaCMC, and toxic doses of paracetamol (2,400 mg/kg). These are statistically significant different. Average SGOT levels of 37.15 \pm 5.05 U/L, 74.26 \pm 4.92, and 86.26 \pm 2.97 U/L in the negative control group or those only given paracetamol showed higher than normal levels. Based on the Anova One Way statistical test, the significance value showed $p < 0.05$ (significant difference), meaning that there were substantial and noticeably different SGOT levels in each group before the administration of the extract/treatment.

Table 2. Average SGOT levels before the extract treatments, after the extract treatments, and after the induction of paracetamol**Figure 2.** Diagram of the average SGOT levels before and after administration of the extract and after induction of paracetamol. The significant value ($p > 0.05$) was not significantly different, meaning there was no significant or noticeable difference in SGOT levels in each group.

Analysis of liver histopathology

The treatment group, namely the group given life-saving leaf extract and toxic doses of paracetamol, showed that there were histopathological changes in hepar in the presence of degeneration, sinusoid dilatation, necrosis, and inflammation but not as much as the negative control group, which was only given 1% NaCMC and toxic doses of paracetamol. It also shows that giving poisonous substances like poisonous doses of paracetamol can cause less damage to the liver when combined with life-saving leaf ethanol extract than when paracetamol is given alone (Table 3).

The shape of the histopathological picture of the hepar of rats in the treatment group with the administration of life-saving leaf ethanol extracts in both low, medium, and high doses has not provided a significant improvement in the effect of administering toxic doses of paracetamol but can reduce necrosis,

degeneration, and sinusoid dilatation only slightly in some places compared to the negative control group that was not given the extract. In each group, it was found to have hydropic degeneration and fatty degeneration. Hydropic degeneration is a severe form of damage that looks like a water-filled vacuole in the cytoplasm that does not contain fat or glycogen (Tatukude, 2014).

Table 3. The average value of the heparin change score

Treatment Groups	Mouse Code			
	I	II	III	IV
Control	0	0	0	0
NaCMC 1%	2	2	2	2
EDSNY 100 mg/kg bb	1	2	1	2
EDSNY 200 mg/kg bb	1	2	2	1
EDSNY 300 mg/kg bb	2	1	1	2

Note:

- 0: no heparin-treated or normal cell damage
- 1: degeneration/necrosis in a single location
- 2: some areas of degeneration/necrosis
- 3: general degeneration/necrosis of the premises

The control group, given only standard feed (Figure 3) daily, showed no abnormalities in the visible liver cells. The negative control group given NaCMC 1% and paracetamol 2,400 mg/kg (Figure 4) showed abnormalities, namely, the discovery of fatty degeneration, hydropic degeneration, inflammation, and sinusoid dilatation in almost all places. In the treatment group, they were given an ethanol extract of life-saving leaves at a dose of 100 mg/kg bb, then induced with a paracetamol dose of 2,400 mg/kg (Figure 5), there was visible damage to hepar, namely the appearance of fatty degeneration, hydropic degeneration, inflammation, and

sinusoid dilatation in several places. Still, the severity was a little lower than the negative control. In the treatment group, where rats were given ethanol extract of life-saving leaves at 200 mg/kg bb and then induced with paracetamol doses of 2,400 mg/kg (Figure 6), there were abnormalities in the form of fat degeneration, hydropic degeneration, and sinusoid dilatation only in some places. The group of mice who were given ethanol extract of 300 mg/kg bb leaves and given paracetamol at a dose of 2,400 mg/kg (Figure 7) saw damage to liver cells, namely the discovery of necrosis, degeneration, and sinusoid dilatation in several places.

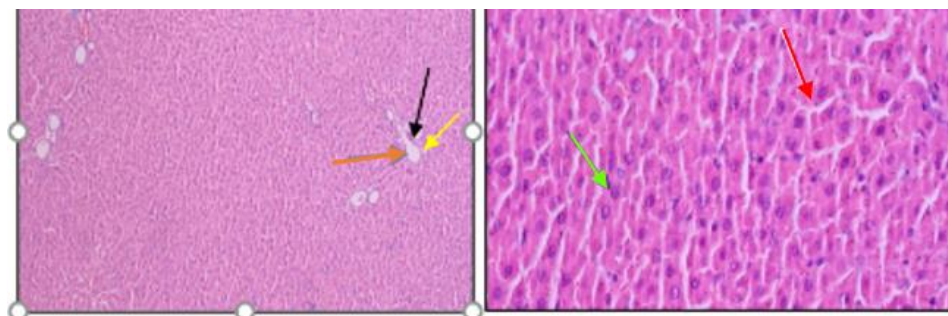


Figure 3. Histological overview of the liver of control group mice given only standard feed. The visible standard liver structure in rats does not appear to have any abnormalities. a. Black arrows indicate bile ducts; orange arrows indicate port veins; yellow arrows indicate hepatic arteries; green arrows indicate normal hepatocyte cells; and red arrows indicate a normal sinusoid with an average degree of damage. Description: (Yellow arrow of hydropic degeneration, red arrow of inflammation, orange arrow of fat degeneration, black arrow of sinusoid dilatation, green arrow of necrosis, blue arrow of hemorrhage)

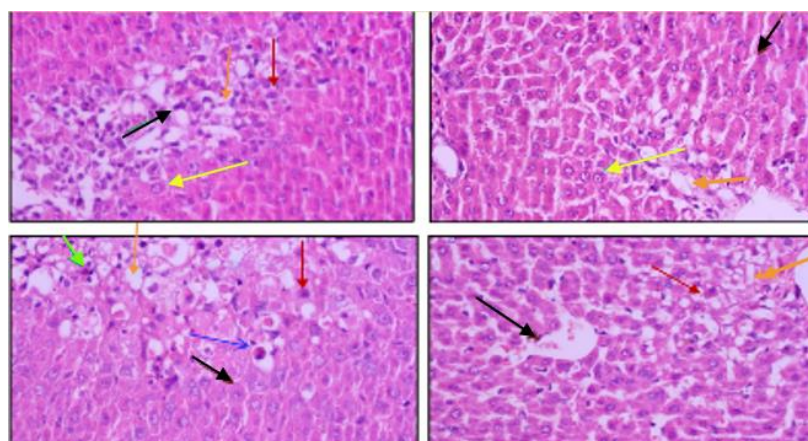


Figure 4. Liver histopathological features of rats infected with NaCMC 1% and infected with toxic dose paracetamol (2,400 mg/kg). Description: (Yellow arrow of hydropic degeneration, red arrow of inflammation, orange arrow of fat degeneration, black arrow of sinusoid dilatation, green arrow of necrosis, blue arrow of hemorrhage)

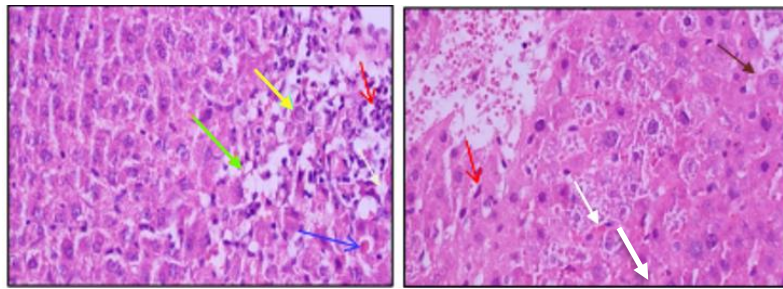


Figure 5. Shows a histopathological image of hepatocyte cells treated with EDSNY100 mg/kg bb and a toxic dose of paracetamol (2,400 mg/kg). Description: The green arrow shows fatty degeneration, the blue arrow shows bleeding, the red arrow shows inflamed cells, and the red arrow shows hydropic degeneration. Yellow arrow, white arrow necrosis, brown arrow sinusoid dilatation.

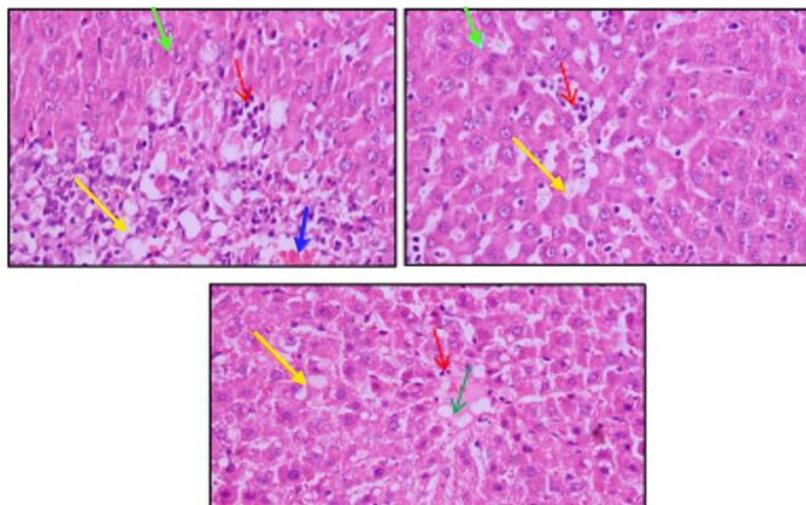


Figure 6. Histopathological picture of EDSNY hepatocyte cells 200 mg/kg bb and a toxic dose of paracetamol (2,400 mg/kg). Description: yellow arrow indicating fat degeneration, hemorrhagic blue arrow, inflammatory red arrow, green arrow easy hydropic degeneration, dark green arrow necrosis.

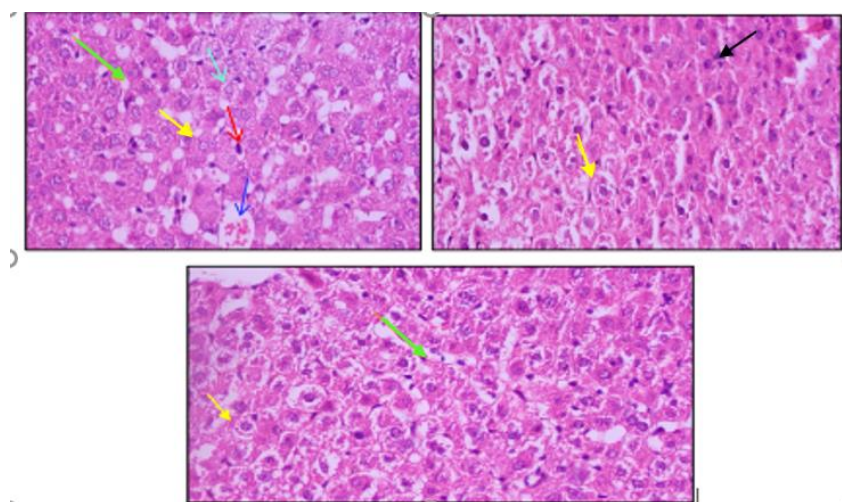


Figure 7. Histopathological description of hepatocyte cells in the rat group with EDSNY dose treatment, namely 300 mg/kg bb and toxic dose paracetamol (2,400 mg/kg). Description: blue arrow indicating hemorrhage, green arrow easy fatty degeneration, inflamed red arrow, yellow arrow sinusoid dilatation, normal hepatocyte black arrow.

In each experimental animal, both negative control and treatment caused fatty degeneration. Fatty degeneration in cell structures is characterized by morphological changes and a decrease in the liver organs' functioning due to fat accumulation in the cytoplasm of cells in the liver organs. Microscopically visible, small clear-colored fat spots. Toxins inhibit lipid transfer from cells to the bloodstream, which results in fatty in hepar (Sherlock, 2004). From the results of hetopathology, rat liver damage was the highest in the negative control group, namely the opposing group. This follows the theory that paracetamol damages hepatocytes by producing its metabolite in the form of N-acetylbenzoquinone imine (NAPQI), which produces superoxide ions which are both reactive. NAPQI binds covalently to macromolecules of hepatocyte proteins while superoxide ions turn into hydroxyl radicals that will bind lipids as well as hepatocyte membrane proteins. This mechanism will lead to the occurrence of enzyme system dysfunction and lipid and protein oxidation of hepatocyte membranes that will damage the hepatocyte membrane and continue with hepatocyte damage (Sastrowardoyo, 2004). This is also in accordance with the research that Heirmayani conducted in 2007 on the effect of paracetamol administration on the liver and kidneys of rats. The results of his study showed that paracetamol caused damage comparable to the induced dose (Heirmayani, 2007). In the treatment group, which was given 100 mg/kg bb, 200 mg/kg bb, 300 mg/kg bb, and 2,400 mg/kg paracetamol (all of which are toxic doses), the level of damage to hepatocyte cells were almost the same, but a little lower than in the negative control group.

CONCLUSION

The ethanol extract of life-saving leaves (*Gynura procumbens*) was able to reduce SGPT levels, especially at a dose of 300 mg /kg bb, but has not been able to mitigate SGOT levels in rats experiencing paracetamol inexpiration

significantly. The administration of life-saving leaf ethanol extracts in both low, medium, and high doses has not provided a meaningful improvement to the damage to the liver structure due to the administration of toxic doses of paracetamol. But when the extract was given, the number of areas with necrosis and degeneration was less than in the negative control group.

ACKNOWLEDGEMENTS

A word of gratitude is conveyed to the supervisor who has guided us during the research process and to the head of the Pharmacognosy–Phytochemical laboratory, The Biopharmaceuticals laboratory, Faculty of Pharmacy, Hasanuddin University Makassar; and the Clinical Pharmacy Laboratory, Faculty of Pharmacy, Hasanuddin University Makassar, have provided the infrastructure for the implementation of this research.

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