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Effect of Ethanol Extract of Sugar Apple (*Annona squamosa* L.) Stem Bark on Rat SGPT and SGOT

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Abstract: This study aims to compare hepatoprotector potency of the ethanol extract of sugar apple (*A. squamosa)* stem bark (EESSB) against the enzyme activity of SGOT and SGPT in rats induced by toxic doses of paracetamol. The method used in this study is Completely Randomized (CRD) consisting of 6 treatment groups and 3 replications. The parameters that are being observed in this study are phytochemical, antioxidant, clinical symptoms, macroscopic, SGOT and SGPT rats. Data were analyzed using Analysis of Variance (ANOVA) with a significant level of 5% with the Duncan test as a follow-up test. The clinical and macroscopic symptoms of rat liver including changes in color, surface structure and consistency as well as relative organ weight. Data were analyzed using ANOVA significant level 5% and Duncan's test as a follow-up test. Extracts contains alkaloid, steroid and phenolic compounds. The results showed that there was a significant difference (P <0.05) in the levels of SGOT and SGPT after giving ethanol extract of stem bark *A.squamosa* (EESSB). Based on the results of the study, it was concluded that all EESSB doses had the potential to reduce the levels of AST and ALT in paracetamol induced rats. The results showed that there was a significant difference (P <0.05) in the macroscopic changes in the liver based on changes in color and degree of liver damage. Relative organ weight had no significant effect (P> 0.05). The ethanol extract of *A. squamosa* (EESSB) stem bark has the potential to be a hepatoprotector, however a dose of 150 mg/kg BW is a more effective dose as a hepatoprotector against paracetamol.

Keywords: Ethanol; Extract; SGOT;SGPT; Stem Bark; Sugar Apple

Introduction

Paracetamol is widely available but it is significantly more toxic than other painkillers available without a prescription (Freo et al., 2021; Hawton et al., 2019). Where data are available, we estimate that acetaminophen is involved in 6% of poisonings, 56% of severe acute liver injury and acute liver failure, and 7% of drug-induced liver injury. These estimates are limited by the lack of available data for many countries, especially in Asia, South America and Africa. The harmful effects of acetaminophen can be reduced through better identification of high-risk overdoses and better treatment regimens. Massive overdoses and cases involving modified-release Paracetamol pose a high risk and may be the target of legislative changes (Chidiac et al., 2023). Dosage reduction is recommended in patients with liver disease or malnutrition. Genotyping can improve efficacy and safety. As part of the current trend to minimize the analgesic effects of opioids, it is often included in multimodal, non-opioid or non-opioid therapies. Paracetamol is recommended by guidelines as a first-line or second-line medication for the treatment of acute and chronic pain, particularly in patients with

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limited treatment options and in the elderly (Freo et al., 2021).

Acetaminophen poisoning, whether accidental or intentional, is a persistent global problem that continues to lead to cases of hepatotoxicity, acute liver failure, and even irreversible liver damage requiring liver transplantation. Due to the increasing prevalence of combined drug use in the form of analgesics and antihistamines, Paracetamol remains an important cause of acute hepatotoxicity, as evidenced by the fact that Paracetamol contributes to more than half of cases of liver failure. acute liver disease in the United States. This is of particular concern because when co-administered with other medicinal products, the increase in Paracetamol serum concentrations may be delayed beyond the 4-hour mark after ingestion which is currently used to identify patients requiring treatment. medical. The present study explores the outcome of acetaminophen-associated DILI cases and its relationship to liver transplantation and other treatment modalities (Rotundo & Pyrsopoulos, 2020).

Abnormalities in the liver can be seen from increased levels of liver enzymes in serum, including increased serum levels of glutamate oxaloacetate transaminase (SGOT) and serum levels of glutamate pyruvate transaminase (SGPT), which are aminotransferase enzymes. Both enzymes are active in serum and can be used as an indication of symptoms of liver dysfunction. Cell damage or cell degeneration determines the high number of enzymes released from the damaged liver (Engelking, 2011). The higher the increase in the levels of SGOT and SGPT enzymes indicates the higher the damage to liver cells. Damage to the cell membrane causes the enzymes SGPT and SGOT to come out of the damaged cytoplasm, and their numbers increase in the blood so that they can be used as indicators of liver damage (Sukohar, 2019). Transaminase enzymes include the enzyme alanine transaminase (ALT) or serum glutamate pyruvatetransferase (SGPT) and aspartate transaminase (AST) or serum glutamate oxaloacetate transferase (SGOT). Activity measurement Serum SGPT and SGOT can be obtained indicates liver cell abnormalities certain, although not a test Liver function is actually a measurement the activity of this enzyme is still recognized as a test liver function (Rosida, 2016).

The use of paracetamol in the long term can trigger the production of free radicals which are toxic to liver cells. Liver damage caused by free radicals can be prevented and cured with antioxidant compounds. Sugar apple (*Annona squamosa* L.) plants from the Annonaceae tribe contain antioxidants that can be used as hepatoprotectors. Based on these antioxidant activities, it is necessary to conduct research on the effect of giving ethanol extracts of sugar apple plants on the liver function of rats (*Rattus novergicus* L.) induced hepatotoxicity by exposure to paracetamol through testing the activity of SGOT and SGPT enzymes. Paracetamol is the first choice for the community for the treatment of fever and pain as an antipyretic and analgesic. Paracetamol is also sold freely in the market, so people can consume it without having to use a prescription from a doctor. Public knowledge about the dangers of drug toxicity is still lacking, especially when used in excessive doses (Sailellah, 2016). Paracetamol drug when used in large quantities and for a long period of time can cause damage to the liver.

This study used types of sugar apple (*Annona squamosa* L.) stem bark which may be used as herbal medicines or liver protectors (hepatoprotectors). Therefore, this study examined the hepatoprotective potential of the ethanol extract of the stem bark through a study of phytochemicals, possible clinical symptoms, and analysis of changes in the structure of the liver organ macroscopically.

This study aims to compare the hepatoprotective potential of the ethanol extracts sugar apple (*Annona squamosa* L.) stem bark on the activity of SGOT and SGPT enzymes in rats induced by paracetamol. The aim of this research was to detect the phytochemical content of the ethanol extract of the stem bark of sugar apple (*Annona squamosa* L.) stem bark. To detect macroscopic changes in liver structure and clinical symptoms after administration of ethanol extract of sugar apple (*Annona squamosa* L.) stem bark.

Method

This study used an experimental method with a completely randomized design (CRD) consisting of 9 treatments and 3 replications, namely: normal control: Rats given distilled water for 14 days, negative control: Rats induced by paracetamol 1350 mg/kgBB at 7th day, positive control: Rats given Hepa-Q 11.34 mg/kgBW for 14 days + paracetamol 1350 mg/kg on the 7th day. Treatment 1: Rats given *A. squamosa* 150 ethanol extract mg/kgBB for 14 days + paracetamol 1350 mg/kgBB on the 7th day. Treatment 2: Rats given *A. squamosa* 300 ethanol extract mg/kgBB for 14 days + paracetamol 1350 mg/kgBB on the 7th day. Treatment 3: Rats given *A. squamosa* 600 ethanol extract mg/kgBB for 14 days + paracetamol 1350 mg/kgBB on the 7th day. Determination of the treatment dose of A. squamosa ethanol extracts based on the range of doses in previous studies (Zakiah et al., 2017).

Sample Extraction

The dried bark samples were weighed as much as 1000 g, put into the maceration container. Extraction was carried out by maceration using absolute ethanol for 3x 24 hours. The filtered filtrate was then concentrated using a rotary evaporator at 30 – 40°C. The extract obtained was then placed in a closed bottle and stored in the refrigerator at 15°C. The extract is made in the form of a suspension in the form of a liquid extract dissolved in distilled water to facilitate administration to experimental animals.

Maintenance of Experimental Animals

This study used 27 male Wistar rats aged 3 months with an average body weight of 267.5185 ± 14.835 g. The rats used were obtained from the Experimental Animal Laboratory, Faculty of Veterinary Medicine, Syiah Kuala University. Mice were acclimatized for 7 days in cages measuring 50 cm x 40 cm x 30 cm with a lid in the form of wire mesh. The cage is lined with husks as high as 3 cm and cleaned every 3 days. Maintenance in a cage at the Experimental Animal Laboratory, Faculty of Veterinary Medicine, Unsyiah. Rats were given food in the form of standard pellets and drinks were given ad libitum. Reference of Ethical Approval for using animals' number: 203/KEPH/III/2023.

Alkaloid Test

Bark samples of *A. squamosa* (10 g) which had been dried, air-dried, crushed, and mashed were then moistened with concentrated ammonia for 2 hours. The sample is then macerated with 5 mL CH_2Cl_2 and then crushed or shaken to speed up the extraction process. The filtrate was filtered and concentrated until the volume became 1 mL. The filtrate was added with 5% HCl (5 mL), shaken vigorously, allowed to stand for a while until the HCl and CH2Cl2 solutions separated. The HCl layer was taken and divided into three test tubes and then 3 drops of Dragendorf, Meyer, and Wagner reagent were added to each. A positive alkaloid result is indicated by the formation of a white precipitate on Mayer's reagent, a red precipitate on Dragendorf's reagent and a brown precipitate on Wagner's reagent.

Test Steroids, Terpenoids and Saponins

Samples of *A. squamosa* (10 g) were finely ground and then extracted using hot methanol. The filtrate obtained was evaporated using a rotary evaporator and then extracted with ethyl acetate to form soluble and insoluble fractions. The dissolved fraction was tested with the Liebermann-Burchard reagent and then the precipitate formed at the bottom of the tube was observed. A green or blue precipitate indicates the presence of steroids while a purple or red precipitate indicates the presence of terpenoids. The insoluble fraction was added to water and then shaken. The foam formed was stable for approximately 30 minutes indicating the presence of saponins.

Flavonoid Test

Samples of *A. squamosa* (10 g) were extracted with methanol. The filtrate obtained was concentrated with a rotary evaporator, then the methanol extract was extracted with n-hexane. The resulting residue was extracted with 10 mL of 80% ethanol then added 0.5 g magnesium and 0.5 M HCl. The positive extract contained flavonoids if a pink or purple color was formed.

Phenol and Tannin Test

Samples of *A. squamosa* (1 g) were extracted with 20 ml of ethanol, then the extract (1 mL) was added with 2 drops of $FeCl_3$. The result of a green or blue green precipitate indicates the presence of phenol.

Experimental Animal Treatment

Administration of extracts and paracetamol

Test animals were given stem bark extracts of *A. squamosa* (EESSB) with three dose levels, namely EESSB 150 mg/kgBB (P4), EESSB 300 mg/k for 14 days after acclimatization. The extract is administered orally using a gastric tube. Mice in each group except K0 were given paracetamol on the 7th day with a toxic dose of 1350 mg/kgBW with a volume of 1 mL administration.

Measurement of clinical symptoms and toxic indications in treated rats

Observation of clinical symptoms was carried out for 4 hours after treatment (Makiyah et al., 2017). Observations continued for 14 days of treatment until all rats were ready to be tested and observed for organ damage. The signs of toxicity observed were strange reactions, weakness, activity, abnormal tails, death, and aggressive behavior in treated rats. Observation of toxic indications and clinical symptoms was carried out based on the spectrum of toxic effects from organ color. All these spectrum values will later become qualitative data using Adobe Photoshop CC 6.0.

Parameters measured in this study were the phytochemical content of *A. squamosa* L. qualitatively, clinical symptoms qualitatively, and observing the structure of the liver organ macroscopically by observing color changes, degree of liver damage and relative organ weight quantitatively, rat blood SGPT and SGOT levels.

The data obtained from the results of the phytochemical tests of *A. squamosa* L. stem bark and observations of clinical symptoms were analyzed descriptively. Data on the degree of organ damage, the

relative weight of the organ and changes in the color of the liver will be statistically analyzed using Analysis of Variance (ANOVA). Quantitative data in the form of SGPT and SGOT levels were analyzed using One Way ANOVA (Analysis of Variance) and further tested with Duncan. The software used is statistical SPSS 25.

Result and Discussion

Phytochemical

Phytochemical tests of the ethanol extract of the stem bark the ethanol extract of the stem bark of *Annona squamosa* L. (EESSB) were carried out to qualitatively detect the presence or absence of secondary metabolites in plants.

Table 1. Phytochemical test results of the ethanol extract of the stem bark of *Annona squamosa* L. (EESSB).

Secondary Metabolites	Test Results
	A.squamosa
Alkaloids	
Mayer	+
Wagner	+
Dragendorff	+
Steroid	+
Terpenoid	-
Saponin	-
Flavonoids	-
Phenolic	+

Note: + = Contains compounds
- = Does not contain compounds

Based on Table 1 it is known that the ethanol extract of *Annona squamosa* L. stem bark (EESSB) contains alkaloid, steroid and phenolic compounds. The content of alkaloid compounds was detected using three reagents namely Dragendorff, Mayer and Wagner. According to Adedayo et al. (2021) and Macáková et al. (2019) alkaloid compounds have antioxidant activity so that they can stop free radical reactions. The content of steroid and terpenoid compounds was detected using a reagent, namely Liebermann-Burchard (LB). Suryelita et al. (2017) stated that steroids are a class of compounds that are widely used as medicinal ingredients in the medical field.

Putri et al. (2019) stated that phenolic compounds are also antioxidants because they act as hydrogen donors to free radicals, so these free radicals become stable and are not reactive to form new free radicals. Fahrudin et al. (2017) added that phenol compounds have the potential to be hepatoprotectors because they can reduce liver enzyme levels in the blood. According to Putri et al. (2019) phenolic secondary metabolites, flavonoids, triterpenoids, and alkaloids have high antioxidants and can be used to improve liver histology. Flavonoid active compounds can act as antihistamines. According to Harbone (1987) said that compounds such as alkaloids, saponins and tannins can be used in the field of medicine.

The EESSB phytochemical test results were found to not contain terpenoids, saponin and flavonoids. Samples of EESSB stem bark ethanol extracts did not contain saponins. According to Atun (2014), secondary metabolites function to maintain the existence of a plant in its environment.

Clinical Symptoms

Observation of clinical symptoms caused by administration of ethanol extract of stem bark *A. squamosa* L. (EESSB) was carried out qualitatively, namely on changes in behavior caused in rats (*Rattus novergicus*) for 14 days after administration of the extracts given. The observed symptoms can be seen in Table 2.

Table 2. Clinical symptoms in treated animals after administration of skin extracts

Clinical symptoms	Treatments					
	K0	K-	K+	P1	P2	P3
Activity	-	-	-	-	-	-
Weird reaction	-	-	-	-	-	-
Tail looks stiff	-	-	-	-	+	+
Aggressive behavior	-	-	-	-	-	-
Weaknesses	-	+	-	-	-	-
Death	-	-	-	-	-	-
Hair loss	-	-	-	-	+	+

Information:
-: showing no symptoms
+: showing symptoms.

Information from Table 2 shows that after administration of EESSB to rats no clinical symptoms were found in the normal control (K0), positive control (K+), and P1 treatments. The clinical symptoms observed in the negative control (K-) treated rats that were given paracetamol at a toxic dose of 1350 mg/kg on the 7th day were symptoms of weakness such as lethargy and sleep. Saubaki & Sudharmono (2019) and McCrae et al. (2018) state that the drug paracetamol given in excessive doses without a doctor's prescription can cause drowsiness and dizziness. Some of the symptoms can be seen in Figure 1.

Figure 1. Various clinical symptoms after treatment with stem bark extract (*A. squamosa* L.) (a) tail stiffness, (b) hair loss and (c) weakness.

Based on Figure 1, various clinical symptoms can be seen in rats after treatment. Figure (a) shows the behavior of the rats in the form of a tail that looks stiff on the red arrow. Clinical symptoms in figure (b) hair loss. These two clinical symptoms occurred in treated rats that were given EESSB at doses of 300 mg/kg BW and 600 mg/kg BW, respectively. The clinical symptoms shown in figure (c) are weakness in rats after treatment. EESSB at doses of 300 mg/kg BW and 600 mg/kg BW found toxic symptoms such as a stiff tail and hair loss. Hair loss is thought to be caused by free radicals from paracetamol. Restuati & Nasution (2019) stated that the physical changes experienced by white rats after administration of carcinogens experienced hair loss in mice, this was allegedly due to the stress that occurred in the test animals. According to Setyowati et al. (2019)hair growth is influenced by flavonoids, saponins and polyphenols which can support hair growth. Hairunnisah et al. (2019) states that the higher the dose given, the greater the toxic symptoms that arise. The indications of these clinical symptoms are thought to be due to excessive analgesic effects due to toxic doses of paracetamol. According to (Im et al., 2012) and Karandikar et al. (2016), the analgesic mechanism of paracetamol is to increase the performance of serotonin in the central nervous system. Pourhamzeh et al. (2022) stated that Serotonin plays a role in maintaining emotional stability such as anxiety, depression, and drowsiness.

Paracetamol that enters the body can increase serotonin levels in the cortex and pons of the brain. Serotonin levels can increase after giving paracetamol doses of 10-50 mg/kg. Rezanty (2020) reported that the toxic dose for paracetamol use was 10-50 g (200-250 mg/kgBB) in a single dose. During 14 days of treatment in test animals did not show death. According to Masykur et al. (2022) changes in the behavior of test animals due to the administration of toxic compounds are also a process of adaptation to test animals that experience stress after treatment.

Organ Color

The results obtained after the liver color data of the test animals were analyzed using Adobe Photoshop CS 6.0 showed a change in the color of the liver in the treatment of the experimental animals (Table 3). Organ color change data was tested using a normality test to determine whether the data obtained was normally distributed or not.

The data that has been normally distributed is then tested for homogeneity to find out whether there are differences in variation between the groups given the treatment. Based on the results of the homogeneity test, a significant value was obtained (P> 0.05). This shows that the data obtained has the same variation so that it is declared homogeneous. The data is then followed by the ANOVA test. Based on the results of the ANOVA test, a significantly smaller value was obtained α (P <0.05). The test results showed that the administration of EESSB had a significant effect on changes in the color of the liver of rats. The data was then further tested using Duncan's test at a level of 0.05. The results of the average value of changes in liver color can be seen in Table 3.

Table 3. The mean value of liver discoloration in various treatments of the administration of ethanol extract of the stem bark of *Annona squamosa* L. (EESSB).

Treatments	Treatment Average change in liver color ($\bar{X}$ ± SD)
K0	32.70 $^{a\,b}$ ± 3.33
K-	39.30 $^{b\,c}$ ± 5.64
K+	35.46 $^{a\,b\,c}$ ± 7.03
P1	37.82 $^{a\,b\,c}$ ± 2.37
P2	41.82 ^{c} ± 3.39
P3	42.15 ^{c} ±3.67

Information:
Different letter superscripts (a, b, and c) indicate there is a significant difference (p<0.05)
K0: Normal control by administering distilled water.
K-: Negative control of paracetamol administration at a dose of 1350 mg/kg BW
K+: Positive control given paracetamol 1350 mg/kg BW & HEPA-Q at a dose of 11.34 mg/kb BW.
P1: Administration of paracetamol 1350 mg/kg BW and EESSB dose of 150 mg/kg BW

P2: Administration of paracetamol 1350 mg/kg BW and EESSB dose of 300 mg/kg BW
P3: Administration of paracetamol 1350 mg/kg BW and EESSB dose of 600 mg/kg BW

Based on Table 3 the administration of EESSB doses influences organ color changes. The higher the value obtained, the paler the color produced in the liver. The discoloration of the liver (Figure 2) to a pale color is thought to be due to free radicals from paracetamol, in the form of N-acetyl-p-benzoquinone (NAPQI), which causes microscopic liver damage. Congestion is the accumulation of blood that occurs in the veins due to slowed or even stopped blood flow. This failure of blood flow can cause the organs to turn pale due to lack of oxygen. K+ treatment was not significantly different from K0. The improvement that occurred in the liver of the K+ treatment was thought to be due to the administration of Hepa-Q supplements which could neutralize and protect liver damage from the accumulation of paracetamol metabolites. Hepa-Q was given to the treated rats at a dose of 11.34 mg/kg BW and given a toxic dose of paracetamol on the 7th day and continued with HEPA-Q again. HEPA-Q is a supplement to help maintain liver function which contains active ingredients in the form of sylimarin (87.5 mg), Curcuma xanthorrizae which contains curcumin (21 mg) (Rousdy & Yanti, 2018), Oleum xanthorrizae (10 mg) and Fructus schisandrae extract 7.5 mg. According to Vargas-Mendoza et al. (2014), silymarin is a natural compound produced by the species Silybum marianum (Milk Thistle) (Khazaei et al., 2022). According to Ranjbar et al. (2020) curcumin as an antioxidant can prevent damage to liver cells.

The high value of the standard deviation in the K+ treatment is due to outlier data. This is thought to be caused by individual sensitivity to toxic substances. According to Nurmawati (2017) states that there are differences in individual responses to changes in the body will be carried out by carrying out homeostasis or body balance as an adaptation. The liver can be damaged by various types of substances that act directly on the organs or indirectly through the central nervous system or blood vessels (Kholmurodovich, 2021).

The normal control treatment (K0) was given dark colored distilled water with a slippery structure and a rubbery consistency. These results are like those reported by Anggraini (2008) regarding the anatomical characteristics of normal rat livers. Figure 2 (a) shows the normal control treatment (K0), with a smooth surface and a rubbery, brownish-red consistency. According to Randall et al. (1986), stated that a normal liver is reddish brown or light brown and if the food is high in fat, it will be yellow (Xiong et al., 2020).

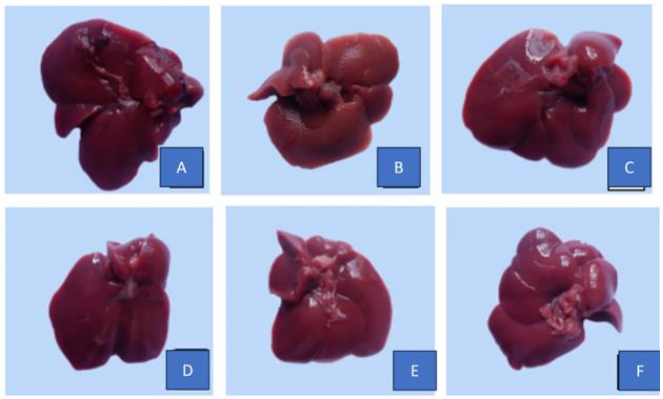

Figure 2. Visualization of organs after EESSB treatment, normal control (a), negative control (b), positive control (c), P1 sugar apple (g), P2 sugar apple (h) and P3 sugar apple (i).

The normal control treatment (K0) was significantly different from the liver of negative control rats (K-) which were given paracetamol at a toxic dose of 1350 mg/kg BW on the 7th day. Visually, the color changes to pale, the rough surface has raised spots (bumps) and the consistency is soft. The results of (Bardos et al., 2018), stated that the livers of mice given paracetamol were pale in color compared to other treatment groups. As for the condition of the liver which is mottled according to Surasa et al. (2014), indicating a condition in which the liver is experiencing fattening due to a deficiency of unsaturated fatty acids resulting in infiltration of fat into the liver cells. the condition of hepatocytes filled with a certain amount of fat is caused by fatty acids that are not properly esterified by mitochondria (Satapathy et al., 2015). The mitochondrial damage is caused by NAPQI which is produced from paracetamol.

Treatments P1 were not significantly different from the positive control (K+) and normal control (K0) treatments, where treatments P1 were given treatment was given EESSB at a dose of 150 mg/kg body weight and given paracetamol on the 7th day then continued with extract administration. The average value of changes in liver color of P1 rats was not much different from the normal control and positive control treatments, this indicates that EESSB can be used as hepatoprotector drugs. According to research by (Zakiah et al., 2017), stated that soursop leaves have high antioxidants which play an important role in capturing free radicals to prevent damage to liver cells induced by paracetamol at toxic doses.

Giving EESSB to P2 and P3 cannot restore organ color like K+. This condition is because the administration of EESSB at all dose levels is thought to have not been able to trigger the binding of NAPQI in the liver so that there is no significant color change. The higher the dose given is thought to cause an overdose of plant secondary metabolites in test animals. Overdosage of secondary metabolites causes too high a buildup of

antioxidants resulting in liver damage. This is supported. That some secondary metabolites can be pro-oxidants, for example the flavonoid and phenolic groups. Excessive levels of flavonoids in the body can be oxidized by peroxidase enzymes to form free radical compounds that can oxidize glutathione in hepatocytes.

Furthermore, data on the degree of damage to the liver organ with observations in the form of changes in color, changes in surface structure and changes in structure consistency are calculated using a scoring value. The score for the degree of liver damage was then tested using the ANOVA test. The results of the ANOVA test obtained a significantly smaller value than the α value (P <0.05), the administration of EESSB had a significant effect on the degree of liver damage in rats. After analyzing the data, it was continued using Duncan's test, the results were obtained as shown in Table 4.

Table 4. The average value of the degree of liver damage in various treatments of the administration of ethanol extract of the stem bark of *Annona squamosa* L. (EESSB).

Treatment	Degree of liver damage ($\bar{X}$ ± SD)
K0	$1.00^{a} \pm 0.00$
K-	$3.33^{c} \pm 0.57$
K+	$1.00^{a} \pm 0.00$
P1	$1.33^{a\,b} \pm 0.57$
P2	$1.66^{a\,b} \pm 0.57$
P3	$2.00^{b} \pm 0.00$

Information:
Different letter superscripts (a, b, and c) indicate there is a significant difference (p<0.05)
K0: Normal control by administering distilled water.
K-: Negative control of paracetamol administration at a dose of 1350 mg/kg BW
K+: Positive control given paracetamol 1350 mg/kg BW & HEPA-Q at a dose of 11.34 mg/kb BW.
P1: Administration of paracetamol 1350 mg/kg BW and EESSB dose of 150 mg/kg BW
P2: Administration of paracetamol 1350 mg/kg BW and EESSB dose of 300 mg/kg BW
P3: Administration of paracetamol 1350 mg/kg BW and EESSB dose of 600 mg/kg BW

The treatment of test animals that were given EESSB with a dose of P6 could not restore the condition of the liver to the condition of K0. This is because the doses of EESSB used in this study were extracts with a single dose, namely without the presence of mixed ingredients from the composition of various other plants. Toxic reactive metabolites and free radicals from paracetamol can damage liver cell membranes and then continue to liver damage. The damage to hepatocyte cells is characterized by changes in cell structure in the form of swelling of cells which are called degenerative changes. Swelling of the cells can occur due to the accumulation of fluid in the cell vacuoles. The accumulation of toxic substances can cause disturbances in the mitochondrial organelles that produce energy Adenosine Triphosphate (ATP) resulting in disruption of the sodium (Na+) pumping process. Na+ which has the property of attracting water is inside the cell, cannot get out of the cell and causes cell permeability. Fluid that is in the extracellular will enter the intracellular in large quantities resulting in the formation of clear, small, and numerous vacuoles. These vacuoles then unite to form a single larger vacuole that occupies the cytoplasm and replaces the cell nucleus and swelling occurs in certain parts.

The increase in the degree of liver damage was also influenced by the sensitivity of the rat's body response to the administration of toxic substances. Generally, the toxicant will only affect one or a few organs. This is also caused by more sensitivity of an organ, or higher levels of chemicals and their metabolites in the organ.

Relative Organ Weight

Measurement of clinical symptoms in organs was carried out by weighing organs before treatment, during treatment and after treatment. The results of the body weight of the rats tested showed that in the normal control group (K0) the rats' body weight increased. The K0 animal treatment did not show weight loss due to normal rat appetite, whereas in the negative control (K-), positive control (+), P1, P2, P3. The treated rats experienced a decrease in body weight allegedly because after administration of EESSB with various doses in the treated rats it caused the rats to become weak and there might be lesions in the hypothalamic nucleus (as a center of hunger).

Relative organ weight data were statistically analyzed including the normality test, homogeneity test, and ANOVA test. Based on the normality test, it is known that organ weight data are relatively normally distributed (P>0.05), while the homogeneity test results (P<0.05). This shows that the data obtained does not have the same variation so that it is not homogeneous. Meanwhile, the results of the ANOVA test with EESSB had no significant effect (P<0.05) (Appendix 12) on the rat's liver weight, so further tests were not necessary. The results of statistical testing can be seen in Table 5.

Based on the results of data processing statistically the ANOVA test showed that the significant value was greater than the value of α (P> 0.05), so in this study the administration of EESSB to rats did not significantly affect the relative weight of the liver. The mean value of relative organ weight K- is a statistical trend of increasing relative organ weight of the liver, but the change has no significant effect. This is presumably

because the treatment was carried out using the oral route, the test compound will experience an enterohepatic cycle, after absorption occurs in the gastrointestinal tract, the compound will be carried by the portal vein to the liver (Ozougwu, 2017). The liver is an organ that has enormous cell recovery capabilities, in the liver there are cytochrome p-450 enzymes that can metabolize foreign substances in the body, by making some toxicants less toxic and more soluble in water. The results of determining relative organ weights are indicators of toxicity that are significantly sensitive and consistent, but histopathological testing will better explain whether there is damage to liver cells.

Table 5. The mean relative organ weight values in the various treatments of the administration of the ethanol extract of the stem bark of *Annona squamosa* L. (EESSB).

Treatment	Mean relative organ weight ($\bar{X}$ ± SD)
K0	9.58 ± 0.21
K-	11.79 ± 0.49
K+	10.36 ± 0.11
P1	11.09 ±0.22
P2	10.93 ± 0.87
P3	11.09 ± 1.90

Note: Different letter superscripts (a and b) indicate there is a significant difference (p <0.05)
K0: Normal control by administering distilled water.
K-: Negative control of paracetamol administration at a dose of 1350 mg/kg BW
K+: Positive control given paracetamol 1350 mg/kg BW & HEPA-Q at a dose of 11.34 mg/kb BW.
P1: Administration of paracetamol 1350 mg/kg BW and EESSB dose of 150 mg/kg BW
P2: Administration of paracetamol 1350 mg/kg BW and EESSB dose of 300 mg/kg BW
P3: Administration of paracetamol 1350 mg/kg BW and EESSB dose of 600 mg/kg BW

SGOT and SGPT

SGOT and SGPT levels were measured on the 15th day after treatment with the ethanol extract of Annona squamosa L. stem bark ethanol extract (EESSB) for 14 days. The results of calculations of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) levels in rats given the ethanol extract of stem bark of *A. squamosa* L. (EESSB) showed that the doses of EESSB affected SGOT levels and SGPT levels of rats. Data on SGOT and SGPT levels obtained were then statistically tested including the normality test, homogeneity test, Analysis of Variance (ANOVA) test, and Duncan's advanced test. The average levels of SGOT and SGPT in test rats and Duncan's test results in the form of notations can be seen in the Figure 3.

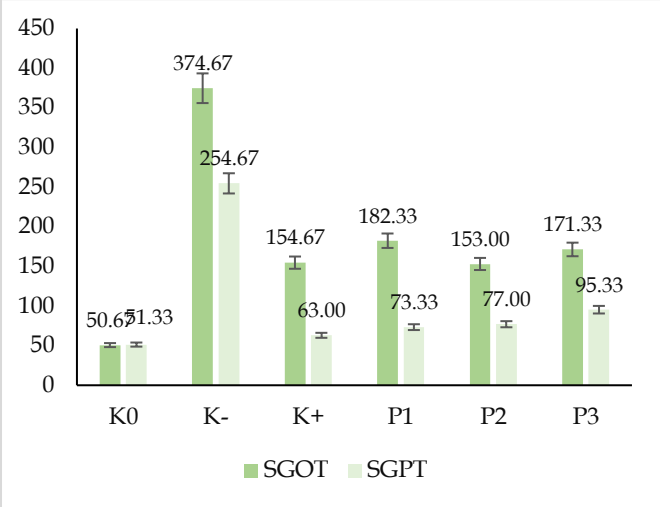

Figure 3. Average SGOT and SGPT levels of rats after administration of paracetamol and continued with administration of various doses of *A squamosa* L. (EESSB) stem bark extracts.

Information :
Different letter superscripts (a, b and c) indicate a significant difference (P < 0.05).
K0 = Normal control (aquadest)
K- = negative control (paracetamol 1350 mg/kg/bb and distilled water)
K+ = positive control (paracetamol 1350 mg/kg/bb and Hepa-Q 11.34 mg/kg/bb)
P1 = Treatment of giving paracetamol 1350 mg/kg/bb and EESSB dose of 150 mg/kg/bb
P2 = Treatment of giving paracetamol 1350 mg/kg/bb and EESSB dose of 300 mg/kg/bb
P3 = Treatment of giving paracetamol 1350 mg/kg/bb and EESSB dose of 600 mg/kg/bb

Figure 3 showed that the treatment of EESSB influenced the results of rat SGOT and SGPT levels. SGOT and SGPT levels of normal control rats (K0) were not significantly different from SGOT and SGPT levels of rats treated with P1, P2, P3. SGOT and SGPT levels in the normal control treatment (K0) were 50.67 (UI/L) and 51.33 (IU/L). This is in line with the study of Sujono, *et al.* (2015) which stated that the normal SGOT level in male rats is (45.7-80.8 IU/L) and the normal SGPT value in male rats is 42.9-67.4 (IU/L).

The results of SGOT and SGPT from the K- treatment which was treated with toxic doses of paracetamol showed higher levels of SGOT and SGPT compared to the other treatments. This indicates that paracetamol of 1350 mg/kgBW given on the 7th day can induce impaired liver function which is indicated by an increase in SGOT and SGPT in rats. This is in line with Sukohar's study (2019) which used paracetamol at a dose of 1.8 g/kg BW for 7 days which resulted in an increase in SGOT and SGPT levels in Sprague Dawley rats. Likewise with a study conducted by Indahsari (2017),

administration of paracetamol at a dose of 200 mg/200g BW for 7 days resulted in a lot of inflammation in the histopathological picture of the liver and there was also a large amount of necrosis. According to Putri et al. (2018) if paracetamol is consumed at a dose of more than 7 g/kg/day in adults it will increase the formation of N-acetyl-p-benzoquinone (NAPQI). If the amount of paracetamol consumed far exceeds the therapeutic dose, the reserves of glucuronic acid and sulfuric acid in the liver will be depleted, then an excess of reactive metabolites N-acetyl-p-benzoquinone (NAPQI) will be formed. As a result of the accumulation of NAPQI metabolites, there will be an oxidative stress process that can damage mitochondria and inhibit hepatocyte cell energy formation, resulting in liver damage. N-acetyl-p-benzoquinone (NAPQI) is a minor metabolite of paracetamol which is very active and toxic to the liver and kidneys. Under normal circumstances this reactive product will quickly bind to glutathione in the liver, so that it becomes a non-toxic material. However, if paracetamol is used in an overdose or continuously it will cause NAPQI to continue to increase and is not proportional to the glutathione levels, then NAPQI will bind to form macromolecules with liver cells which will result in liver cell necrosis.

The hepatotoxic activity of paracetamol occurs through the mechanism of toxic reactive metabolites, namely NAPQI and free radical reactive oxygen species (ROS) which are formed from the parent compound by a mixed-function oxidation system of cytochrome P450 which are abundant in the central vein area. ROS free radicals are molecules that have one or more unpaired electrons in their outer orbit, causing these molecules to be highly reactive and unstable. There are ROS that come from the body (endogenous) and outside (exogenous). Exogenously, ROS can originate from various toxic substances such as paracetamol. Toxic reactive metabolites and ROS free radicals can disrupt the integrity of hepatocyte cell membranes, causing the release of amino transferase enzymes from hepatocytes into the bloodstream so that this is an indicator of liver damage. Excessive free radicals will cause oxidative stress which triggers the process of peroxidation of lipids and can lead to liver damage (Sukohar, 2019).

In addition to being given toxic doses of paracetamol, the K+ treated rats were also given HEPA-Q, a supplement to maintain liver function, while the K-treated rats were only given toxic doses of paracetamol. The treatment of rats given pharmacological drug therapy in the form of Hepa-Q (K+) was not significantly different from the normal control treatment (K0). The test animals in the positive control (K+) did not experience a significant increase in SGOT and SGPT levels even though they were given a toxic dose of paracetamol on the 7th day during the treatment. The levels of SGOT and SGPT in the K+ treatment was 154.67 and 63.00 IU/L. This shows that the administration of Hepa-Q can reduce SGOT and SGPT levels although it cannot completely reduce them to normal levels as in K0. This is thought to be due to the presence of Hepa-Q supplements which can neutralize and protect liver damage caused by the accumulation of paracetamol metabolites. This is what causes a significant decrease in SGOT and SGPT levels in K+ treated rats compared to K-treated rats. K+ rats experienced regeneration of liver function as indicated by a decrease in the levels of these two liver enzymes.

Hepa-Q is a synergistic extract supplement that is used to treat liver dysfunction which contains sylimarin 87.5 mg and 21 mg curcumin which are efficacious for repairing liver damage (Putri et al. 2018). The Monthly Index of Medical Specialties (MIMS) (2020) states that the ingredients contained in Hepa-Q are Silybum marianum extract which contains sylimarin 87.5 mg, Curcuma xanthorrizae extract which contains curcumin 21 mg, Oleum xanthorrizae 10 mg and fructus schisandrae extract 7.5 mg. Mendoza et al. (2014) explained that sylimarin is a natural active compound derived from Sylibum marianum. Based on research conducted by Trappoliere et al. (2009) it is known that sylimarin has biological activities such as antioxidant, immunomodulatory, antifibrosis, antiproliferation and antiviral. Therefore, sylimarin can maintain the integrity of hepatocyte cell membranes and can inhibit the entry of toxic substances. According to the results of research conducted by Panjaitan (2011) it is known that sylimarin is a hepatoprotector drug that has been proven to reduce SGOT and SGPT levels. Yahya (2013) added that sylimarin can significantly reduce SGOT and SGPT levels. In addition, Karimi (2011) and Milic (2013) reported that sylimarin can stabilize ROS and play a role in the process of intracellular glutathione so that it can be used to treat hepatitis, hepatic cirrhosis, and other liver disorders. The content of sylimarin in Hepa-Q is thought to have a free radical scavenging effect on K+ mouse cells. According to Shaker (2010) sylimarin exerts a hepatoprotective effect through several mechanisms, including antioxidant activity and free radical scavenging, increasing cellular glutathione concentrations, stimulating DNA polymeration and stabilizing the hepatocellular membrane.

Conclusion

The EESSB phytochemical test results contained alkaloids, terpenoids, flavonoids and phenolic compounds, while the EESSB phytochemical test results contained alkaloids, steroids, and phenolic compounds.

Observation of clinical symptoms observed for 14 days were strange reactions, weakness, stiff tail, and hair loss. The toxic effects caused after administration EESSB were mildly toxic at the highest dose (600 mg/kg BW), giving EESSB can cause discoloration of the liver and affect the degree of damage to the liver, administration of EESSB had no effect on relative organ weight in rat livers. The ethanol extract of *A. squamosa* L. (EESSB) stem bark has potential as a hepatoprotector, but a dose of 150 mg/kg BW is an effective dose as a hepatoprotector against paracetamol. Administering a toxic dose of paracetamol 1350 mg/kg BW causes damage to the liver in the form of changes in color and surface structure. Annona squamosa L. stem bark has potential as a hepatoprotector. Administration of a dose of 150 mg/kg and a dose of 300 mg/kg of the ethanol extract of the stem bark of *A. squamosa* L. caused SGPT levels in paracetamol-induced rats to be lower than the negative control (K-).

Author Contributions

Conceptualization and supervision, Masykur and Nurdin; methodology, Lukman Hakim; validation and formal analysis, Rosnizar; investigation, resources, data curation and visualization, Widya Sari; writing—original draft preparation, Munira Ulfa and Novi Yana Sari; writing—review and editing and project administration, Ria Ceriana; funding acquisition, Masykur. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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