Activity of Ethanol Extract of Kalangkala seed (*Litseaangulata* B.) as Nephroprotector and Hepatoprotector in Alloxan-induced Diabetic Rats

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KEYWORDS
Litseaangulata B; Kalangkala; Nephroprotective; Hepatoprotective; Alloxan

ABSTRACT
Kalangkala (Litsea angulata B) is a traditional medicine that contains phenolic compounds and flavonoids and has antioxidant activity. Phenolic and flavonoids are reported to protect liver and kidney function and maintain insulin levels in the body. The purpose of this study was to determine the effect of administration of ethanol extract of kalangkala seeds (EEBK) as nephroprotective and hepatoprotective agents in rats (Rattus norvegicus L) induced by alloxan. Kalangkala seed was extracted by ethanol and evaporated to get the concentrated extract. The 30 male Wistar rats were divided into 6 groups including normal group, negative group, metformin positive group and treated groups which treated by EEBK 100, 200 and 400 mg/kgBW. Alloxan was injected intraperitoneally, metformin and EEBK were given orally for 21 days. The blood of rats was taken through the orbital sinus on day 21. The results showed the total flavonoid content of EEBK was 6.27 mgQE/g. EEBK doses of 100, 200 and 400 mg/kg BW as well as metformin can reduce ($p<0.05$) creatinine levels, ureum and SGPT activity but only EEBK doses of 200 and 400 mg/kgBW ($p<0.05$) can reduce SGOT activity. The administration of EEBK doses of 100. 200 and 400 mg / kgBW was able to improve the histopathological picture of the liver. It can be concluded that EEBK has the potential as a nephroprotective and hepatoprotective in diabetic rats (Rattusnorvegicus L) induced by alloxan

INTRODUCTION

Hyperglycemia resulting from abnormalities in insulin production, action, or both characterizes diabetes mellitus (DM), a metabolic illness (American Association of Diabetes, 2018). Diabetes mellitus disrupts glucose homeostasis and the main peripheral tissues involved in the glucose cycle in the body which will affect organs, especially the liver, adipose tissue and skeletal muscle (Manavi et al., 2021). High blood sugar levels that are over acceptable limits in DM patients might harm and destroy the kidneys' capillary blood vessels. Because of this, nephrons are unable to properly filter the body's unclean blood because they are unable to take in enough oxygen and clean blood. This can interfere with the body's general metabolism since there will be a buildup of fluid and salt that cannot be filtered by the kidneys (Sari & Hisyam, 2014). Hyperglycemia in diabetes patients is caused by a deficiency in insulin production, insulin action, or a combination of the two. Hyperglycemia damages several organs, including the kidneys, eyes, heart, vascular system, neurological system, and liver (Mohan, 2013).

The use of plants for treatment has long been recognized by the community. Efforts to develop plants for treatment need to be done considering that plants are easily obtained and cheap. In addition, the side effects caused are very minimal compared to using chemical drugs. The use of plants for treatment needs to be supported by research data so that its scientific efficacy is not in doubt and can be accounted for. This will certainly encourage people to use plants or plants as medicine (Lestari, 2016). One of the herbal plants used as medicine is *Litseaangulata* B. known by the regional names kalangkala (Kalimantan), engkala or pengolaban (Malaysia) which belongs to the Lauraceae tribe. About 22 species of *Litsea* are distributed on the island of Borneo. Kalangkala is a seasonal fruit that bears fruit during the rainy season (September-March). Local people use this plant for traditional medicine (Kutoi et al., 2012).

In previous research, in Tumbang Samba, Central Kalimantan, it was stated that based on the recognition of local residents regarding kalangkala fruit seeds, they used it as an antidiabetic by roasting it and drinking the boiled water directly (Fujianti, 2021). Kalangkala fruit seeds in the area of

origin, Kalimantan, are used by some people for traditional medicine as an antidiabetic drug. The antioxidant activity is allegedly due to the phenolic and flavonoid concentrations seen in kalangkala fruit seeds (*Litseaangulata* B) (Hassan et al., 2013).

The kidney is an organ It plays a significant part in detoxifying several toxins. The ureum level is the best estimate that parameters kidney function (Mahfudh & Fajrin, 2019). Increased free radicals in diabetes will also cause Liver cell injury. Increased SGOT and SGPT levels in the blood are one of the indicators of liver cell injury (A. Adetutu & A.O. Owoade, 2013). Research on nephroprotective and hepatoprotective activities of *Litseaangulata* has never been done before, so this study aims to test the nephroprotector and hepatoprotector activities of kalangkala seeds induced by alloxan in male Wistar white rats.

MATERIAL AND METHOD

Material

Kalangkala seeds (*Litseaangulata* B) obtained from Barabai city, Hulu Sungai Tengah regency, South Kalimantan, which have been determined at Lambung Mangkurat University, metformin, alloxan, 96% ethanol, Na-CMC.

Extract Preparation

5 kg of kalangkala fruit seeds were washed, cut, and dried using an oven at 400C, then pulverized. Simplisia powder as much as 2 kg was extracted using 6000 mL of 96% ethanol for 24 hours, then filtered and re-remacerated twiceThe filtrate is evaporated using a rotary evaporator at 600 degrees Celsius until a thick extract is produced (Ramadhan et al., 2020).

Measurement Of Total Flavonoid Content

Determination of use flavonoid content a series solution of quercetin standard levels, wavelength optimization, determination of absorbance of flavonoid isolates and calibration of measurement results. Five series of standard solutions were made and added $AlCl_3$(Chang et al., 2002). The quantitative test performed was to measure the total flavonoid content of kalangkala seed extract with quercetin standard. The process carried out is to make a standard quercetin solution with concentrations of 40, 60, 80, 100 and 120 ppm. Determination of maximum wavelength and determination of operating time.

Preparation of ethanol extract of Kalangkala seed suspension

The treatment dose of ethanol extract of Kalangkala seed (EEBK) were 100, 200 and 400 mg/kgBW. The solvent used is 1% Na-CMC solution. For a dose of 100 mg/kgBW, 20 mg of extract was weighed and then dissolved into 2 mL of 1% Na-CMC. For a dose of 200 mg/kgBW weighed as much as 40 mg of extract after it was dissolved with 2 mL Na-CMC 1% and for a dose of 400 mg/kgBW weighed 80 mg of extract dissolved in 2 mL Na-CMC 1%.

Preparation of alloxan suspension

Stock solution was made by weighing alloxan as much as 150 mg dissolved in NaCl 0.9% to 1 mL. The dose of alloxan used 150mg/kg BW According to past study (Balfas et al., 2018). The concentration of alloxan was prepared in 150 mg/mL in 0,9% of NaCl solution.

Making the suspension of metformin

The adult human dosage of metformin is 500 mg per day; if translated to rats weighing 200 grams at 0.018, the dose of metformin for rats is 9 mg/kg BW. Metformin tablet powder was weighed at 360 mg and suspended in 0.5% Na CMC for 100 mL.

Animal testing procedures

This research has received approval from Research Ethics at Ahmad Dahlan University Commission using the number: 012307116. Thirty male white rats that have been weighed first, then acclimatized for one week in the laboratory (Mahfudh et al., 2021)and provided with regular food and water to drink (Tandi & Wulandari, 2017). The test animals used were white male rats that had been

adequately housed using plastic group cages and given standard feed and drinking water every day for 21 days.

Grouping of test animals

A total of 30 male white rats matched the inclusion requirements and were randomly divided into six groups of five rats each. Table 1 shows how the test animals were grouped.

Table 1. Test Animal Groupings

Group	Alloxan Injection	Treatment
Normal	-	Normal rats (only fed and watered)
Negative	√	Alloxan injection 150 mg/kgBW
Positive	√	Alloxan injection 150mg/kgBW+ metformin 45 mg/kgBW
EEBK 100 mg/kgBW	√	Alloxan injection 150mg/kgBW + EEBK 100mg/kgBW
EEBK 200 mg/kgBW	√	Alloxan injection 150mg/kgBW + EEBK 200mg/kgBW
EEBK 400 mg/kgBW	√	Alloxan injection 150mg/kgBW + EEBK 400 mg/kgBW

Blood sampling

Blood samples from the orbital sinus were taken using a microhematocrit (±2 mL), then transferred to an Eppendorf tube. To extract serum from other blood components, the blood was centrifuged for 15-20 minutes at 3000 rpm(Mahfudh & Ikarini, 2018).

Examination of creatinine and blood ureum levels

The examination of creatinine and urea levels was carried out using DiaSys® Creatinine FS and Urea FS reagent kits. Levels were determined using UV Vis spectrophotometry.

Measurement of SGOT and SGPT levels

Analyzing SGOT and SGPT levels was carried out using DiaSys® reagent kit, and Levels were determined using UV-Vis spectrophotometry.

Histopathology preparation and observation

Observation of the anatomical structure of rat liver cells was performed with hematoxylin and eosin (HE) staining to see cell nuclei, sinusoids and blood vessels. Rats were sacrificed and liver organs were taken for histopathology preparation and observation. Observations are based on the degree of damage or necrosis in hepatocytes, as supporting evidence in addition to the results of liver function tests through measurement of SGOT and SGPT levels (Widarti & Nurqaidah, 2019). Preparation using paraffin method and observation using hematoxilin-eosin dye.

Analytical statistics

With the use of SPSS (Statistical Product and Service Solution) analysis, the study data was examined. The normality test was carried out utilizing the Shapiro-Wilk test to assess whether or not the data was distributed normally. The One-Way Anova approach was used if the information was consistent and frequently disseminated. The Anova test was followed by the Thukey test to determine whether or not there was a significant difference between each treatment group (Bolton, S and Bon, 2010). Data on the examination of liver histopathology images will be analyzed descriptively by observing the photos obtained.

RESULTS

Measurement of total flavonoid content

Quercetine was utilized as a reference solution to calculate the sample's total flavonoid content. Since quercetin is a flavonoid belonging to the flavol group and is widely distributed in plants, it is used as a standard (Azizah et al., 2014). Almost all plant species contain quercetin, a polyphenolic flavonoid molecule that possesses potent antioxidant properties. Quercetin is a naturally occurring substance (Eddy, 2010). The measurement of flavonoid was run on a wavelength of 435 nm and presented in Table 2.

Table 2. The total amount of flavonoids in the Kalangkala seed ethanol extract

Replication	Total flavonoidcontent (mgQE/g extract)	Mean (mgQE/g)	SD (mgQE/g)
1	5.76		
2	7.09	6.27	0.70
3	6.03		

Measurement of creatinine and ureum levels

Creatinine and ureum levels of white rats between groups were tested using the UV-Vis spectrophotometric method on 30 blood serum samples that had been previously centrifuged. From the data that has been obtained, the average creatinine and ureum levels in experimental animals can be seen in table 3.

Table 3. The creatinine and ureum levels of alloxan induced rat treated with Kalangkala seed extract

Group	Creatinin(mg/dL)	Urea (mg/dL)
Normal	0.58 ±0.15 [(a)]	18.46 ± 2.77 [(a)]
Negative	1.87 ± 0.69[(b)]	124.36 ± 8.01[(b)]
Positive (metformin)	0.44 ± 0.20 [(a)]	15.38 ± 3.85 [(a)]
EEBK100 mg/kgBW	0.67 ± 0.13 [(a)]	20.51 ± 2.22[(a)]
EEBK 200 mg/kgBW	0.62 ± 0.08 [(a)]	17.95 ± 2.22 [(a)]
EEBK 400 mg/kgBW	0.44 ± 0.08 [(a)]	16.67 ± 2.22 [(a)]

Description:[(a)]notably distinct from the negative group.
[(b)] notably distinct from the positive group.

The induction of alloxan increase the creatinine and ureum level significantly ($p<0.05$) compare to normal group which is only given food and drink treatment. The ureum and creatinine level of alloxan induced rats were found to exeed the normal values. The normal values for blood creatinine and ureum levels for normal male white rats are 0.20-0.80 mg/dL and 15.00-21.00 mg/dL (L. Pratiwi et al., 2016). This indicates that alloxan causes damage to kidney function. High creatinine levels indicate impaired glomerular filtration rate in the kidney.

Data analysis of the positive group (metformin treatment) did not differ significantly ($P> 0.05$) to the EEBK group 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW or with the normal group in reducing creatinine levels. This show that metformin as antidiabetic could decrease the blood glucose level and followed by a decrease in creatinine and urea levels due to the cessation of oxidative stress and lipid peroxidation processes that damage glomerular endothelial cells (Perdanawati et al., 2022). Administration of EEBK doses of 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW had a significant effect ($P < 0.05$) on ureum levels compared to the negative group. But statistical analysis between the three EEBK groups were not significantly different ($P> 0.05$).

Measurement of SGOT And SGPT levels

The analysis of SGOT and SGPT activity diabetic white rats treated with EEBK are presented in table 4.

Table 4. The SGOT and SGPT activities of rats treated with ethanol extract of Kalangkala seed after they were stimulated with alloxan

Group	SGOT (U/L)	SGPT (U/L)
Normal	66.86 ± 4.94 [(a)]	29.24 ± 1.08 [(a)]
Negative	164.99 ± 64.94 [(b)]	133.71 ± 11.36 [(b)]
Positive (metformin)	52.84 ± 4.94 [(a)]	18.43 ± 3.65 [(a)]
EEBK100 mg/kgBW	74.41 ± 3.23	28.04 ± 1.87 [(a)]

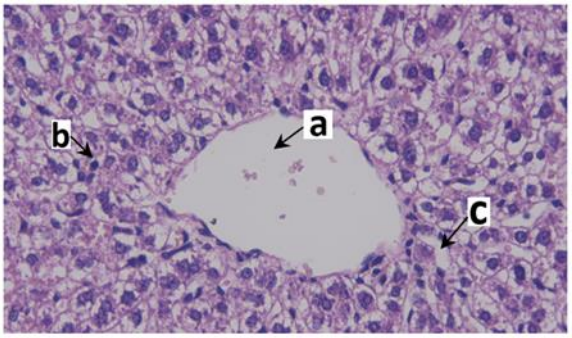
EEBK 200 mg/kgBW	55.00 ± 3.24 [(a)]	26.96 ± 1.87 [(a)]
EEBK 400 mg/kgBW	55.00 ± 3.24 [(a)]	22.65 ± 3.24 [(a)]

Description:[(a)]notably distinct from the negative group.
[(b)] notably distinct from the positive group.

Table 4 shows that negative group showed an increase in the value of SGOT and SGPT levels, it proves that the induction of diabetes mellitus by alloxan can lead to hyperglycemia and disturbances in hepatic cells. Disturbances in hepatic cells are characterized by increased levels of SGOT above the normal value of 17.5-30.2 U/L (Gad, 2016) and SGPT above the normal value of 10-80 U/L (Evans, 2009). Treatmen of EEBK at doses of 100 mg/kgBW, 200mg/kgBW, and 400mg /kgBW can reduce SGOT and SGPT levels in rats to normal levels. This show the capability of EEBK in hepatoprotective against diabetogenic agent.

Liver histopathology observation

Liver histopathology observation aims to determine the effect of EEBK in improving liver function. Observation of the anatomical structure of rat liver cells was performed with hematoxylin and eosin (HE) staining to see cell nuclei, sinusoids and blood vessels. HE is one of the main tissue stains used in histology. HE is a combination of two histological stains: hematoxylin and eosin, the use of two types of reagents (Hematoxyline-Eosin) in this method, will facilitate the observation of pathological changes by coloring organelles and cell nuclei separately. The cytoplasm (organelles) will be colored pink by the presence of eosin and the cell nucleus will be colored purplish blue by the presence of hematoxyline (Mescher, 2012). Based on the results of observations with HE staining, it appears that the transversely sliced liver has a display that can be observed clearly. The clearest image can be observed at 400 times magnification. The results of observations of the anatomical structure of cells, sinusoids and blood vessels can be seen in Figure 1.



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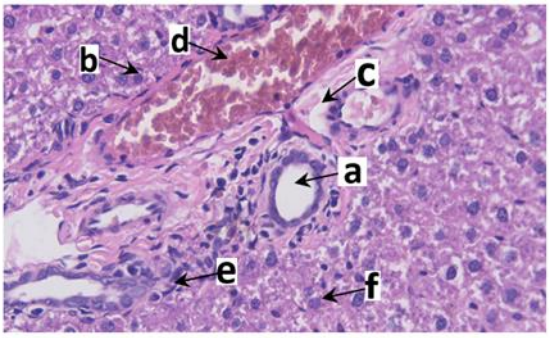

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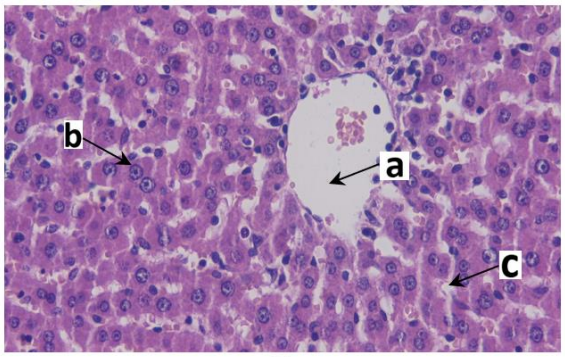

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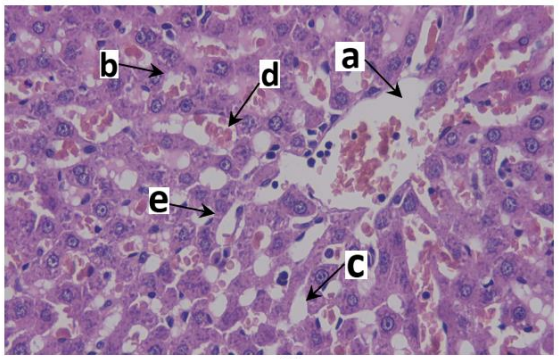

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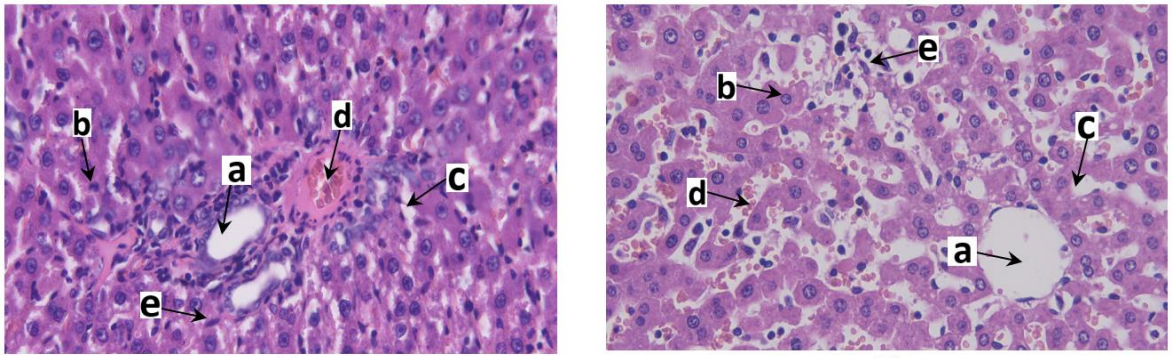

(5) (6)

Figure 1: Histopathologic picture of the liver in groups (1) Normal, (2) Negative, (3) Positive (metformin), (4) EEBK 100 mg/kgBW, (5) EEBK 200 mg/kgBW, (6) EEBK 400 mg/kgBW. Description: (a) vein centralis. (b) hepatocytes, (c) sinusoids, (d) inflammatory cell infiltration, (e) extravasation, (f) necrosis. Magnification 400x, HE staining.

The histological analysis of the liver revealed that the normal group's hepatocyte cell, sinusoidal, and blood vessel structures were normal due to the group's sole nutritional and hydration regimen. Treatment with EEBK 100 mg/kgBW showed that hepatocyte cells partially began to return to normal, most were still swollen, still obtained dilatation of blood vessels and sinuses, infiltration of inflammatory cells and extravasation of erythrocytes between hepatocytes. The increasing of dose treatment (EEBK 200 mg/kgBW) showed hepatocyte cells returned to normal, some blood vessels and sinuses were dilated, and inflammatory cell infiltration. EEBK 400 mg/kgBW group hepatocyte cells returned to normal, still obtained dilatation of blood vessels and sinuses, infiltration of inflammatory cells and extravasation of erythrocytes between hepatocytes. EEBK showed regenerative improvement in biochemical parameters and a decrease in serum creatinine, ureum, SGOT and SGPT. This reveals that EEBK has hepatoprotective activity. The capacity of flavonoids to both form complexes with free radicals and transfer an electron to a free radical compound accounts for their antioxidant qualities.

DISCUSSION

Natural substances called flavonoids have the ability to function as antioxidants, preventing free radicals from destroying the immune system and oxidizing lipids and proteins, which is how degenerative illnesses begin (Rais, 2015). Numerous investigations have verified the overall flavonoid composition of kalangkala plants. Within the research (Rizki et al., 2023) determined the total flavonoid content in the leaves of kalangkala plants, the results obtained were 0.395%. The study's conclusions (Amalia et al., 2022) stated the results of the total flavonoid content of kalangkala leaves was 0.7 mg QE/g. Other researchers (Astuti et al., 2023) obtained data from the determination of total flavonoid levels in kalangkala leaves of 8.367 mg QE/g. The study's conclusions obtained the entire amount of flavonoids in EEBK of 6.27±0,7 mg QE/g extract.

Analysis of kidney function parameters is carried out by measuring creatinine and ureum levels because creatinine and ureum levels in a blood serum are one of the examination parameters in kidney function. Greater levels of creatinine in the blood than normal values suggest decreased renal function. Creatinine is eliminated by the kidneys via a mix of filtration and secretion; its concentration in plasma is generally constant (Corwin, 2001). Creatinine levels are more accurate than ureum levels in measuring kidney function. This is because creatinine is constantly produced by the muscles and then filtered almost completely by the glomerulus. In addition, ureum is the result of protein metabolism derived from food, while creatinine is the result of the breakdown of keratin phosphate which is directly related to body muscle mass so it is more accurate to describe kidney dysfunction (Khan et al., 2012). Ureum is the largest nitrogen product formed in the liver and

excreted through the kidneys, ureum is the end product of protein metabolism and must be removed from the body (Price and Wilson, 2006).

The study found that alloxan is not only diabetogenic but also kidney damage. Alloxan generates free radicals that damage the DNA of pancreatic β cells, resulting in decreased insulin production and secretion and hyperglycemia(Rohilla & Ali, 2012). Test animals with diabetes brought on by alloxan exhibit severe polyuria, hyperglycemia, and a gradual loss of kidney function as ROS levels rise (Radenkovic et al., 2016). Damage that occurs in the kidneys results in decreased renal filtration function so that serum creatinine levels will increase (Rodrigues et al., 2014) and an increase in ROS can cause glomerulosclerosis so that ureum levels increase (Almaghrabi, 2015). Increased blood sugar causes oxidative stress, in these conditions the production of NO increases, causing the release of vasoconstrictive mediators that will affect kidney function, namely a decrease in glomerular filtration rate which will result in increased creatinine and ureum levels. This damage can occur as a result of being around harmful compounds like alloxan which is a diabetagonizing agent that can destroy pancreatic β cells (Rohilla & Ali, 2012). High levels of creatinine and ureum in the blood indicate damage to the kidneys (Cheon et al., 2016).

The ethanol extract of Kalangkala seed contains flavonoid compounds (Ramadhan et al., 2020), which have the potential as antioxidants to increase glomerular filtration rate and reduce creatinine and ureum levels (Perdanawati et al., 2022). Flavonoid compounds are able to donate their hydrogen electrons to ROS so that ROS become stable molecules (Kumar & Pandey, 2013). The mechanism of the hypoglycemic effect of flavonoids is to reduce glucose absorption by inhibiting GLUT2 (Song et al., 2002). Flavonoid compounds increase antioxidant enzyme activity and reduce free radicals to prevent further oxidative stress (Triastuti et al., 2009).

The current study used SGPT and SGOT for parameters of liver function. SGPT and SGOT are intracellular enzymes located in the heart, liver and muscle tissue (Price & Wilson, 2005). The intracellular release of enzymes into the blood is due to necrosis of hepatic cells or acute hepatic damage causing their activity to increase (Wibowo et al., 2008). SGOT is an enzyme derived from the liver and serves to produce protein by converting aspartate into α-ketoglutarate to produce oxaloacetate and glutamate. SGOT is found in the heart and liver in large amounts. Moderate levels of SGOT can also be found in the pancreas, skeletal muscle, and kidney (Sing & Sharma, 2014). SGPT is an enzyme that plays a role in amino acid metabolism and gluconeogenesis. SGPT helps produce glutamate and pyruvate from the conversion of alanine to α-ketoglutarate. SGPT is considered much more specific for assessing liver damage, as the presence of large amounts of SGPT enzyme can be found in the liver. SGPT is also found in the heart, kidney and skeletal muscle in small amounts which is effective for the diagnosis of hepatocellular destruction (Sing & Sharma, 2014).

Uncontrolled lipolysis, glycogenolysis, and gluconegoenesis are caused by hyperglycemia situations in order to keep glucose levels in cells (Mohamed et al., 2016). The process increases cellular metabolic processes, causing an increase in the end result of metabolism as an example of ROS (Reactive Oxygen Species). The more ROS produced will induce hepatocyte apoptosis and the release of pro-inflammatory cytokines that will cause an increase in adhesion molecules and leukocyte infiltration. The combination of these things results in more extensive tissue damage. Hepatocyte apoptosis causes enzymes contained in cells to fill the extracellular space and can be detected in the blood.

The positive control group had the lowest average SGOT and SGPT level values (metformin). Metformin in the liver will reduce gluconeogenesis in the liver by reducing ATP production so that the formation of ROS can be reduced. In the intestine metformin reduces glucose absorption so that blood glucose levels do not increase. In addition, metformin will increase the use of glucose through the process of microbial modification in the gastrointestinal tract (Liu et al., 2014).

Active substances that have been shown to have antidiabetic effects are flavonoids. Flavonoids have three mechanisms as antioxidants, namely: 1) reducing ROS production, 2) scavenging mechanism, and 3) as protection (Patel et al., 2012). To reduce ROS production, flavonoids will suppress the process of suppressing glycogenolysis and gluconeogenesis which can indirectly reduce the formation of ROS or by inhibiting enzymes in the process of ROS formation

such as NADH oxidase. The scavenging mechanism carried out by flavonoids is by donating hydrogen atoms so that free radicals are more stable and cannot have an oxidizing effect. In pancreatic beta cells, flavonoids will repair cell damage so that beta cells can secrete insulin. Flavonoids can also increase the sensitivity of insulin receptors so that the use of insulin in the body will be more optimal and can reduce blood glucose levels (D. B. Pratiwi et al., 2020).

Within the cohort of rats administered just with alloxan injections (negative group) there were changes in hepatocyte cells that partially appeared swollen, blood vessels and sinuses were partially dilated, and inflammatory cell infiltrates in the sinuses, periporta and hepatocytes. These changes may be caused by alloxan which is diabetogenic for pancreatic and liver β-cells, sulfhydryl groups, alterations in the transfer of electrolyte membranes, chelation action, and enzyme and metabolic modifications(Nwosu et al., 2011), increased lipoperoxidation (Soto et al., 1994), decreased antioxidant activity (Soto et al., 1998) and oxidative stress (Eldahshan & Abdel-Daim, 2015). Alloxan can cause various types of toxic effects on various organelles in liver cells, such as fatty liver (steatosis), necrosis, cholestasis, and cirrhosis. Alloxan is a chemical commonly used for research animals to induce diabetes, resulting in hyperglycemic animals (Irdalisa et al., 2021). These conditions over an extended length of time lead to changes in the histological picture of the liver so that a picture of fibrosis, accumulation and intracellular degeneration, regeneration, necrosis (Utomo et al., 2012). In experimental animals, alloxan induction can result in hyperglycemia, and at certain dosages, it can harm liver tissue (Etuk, 2010). Research by (Lucchesi et al., 2013)claims that the mechanism of alloxan-induced chronic liver disease results in diabetes mellitus and oxidative stress in the liver. In comparison to rats, mice in the induced group had much lower plasma insulin levels and higher levels of blood glucose, urine glucose, and glycosylated hemoglobin. Another study assessing the long-term consequences of diabetic rat liver caused by alloxan resulted in morphological alterations and liver ultrastructure that closely mirrored human illness, including liver fibrosis, steatosis, and steatohepatitis (Lucchesi et al., 2015).

Dilatation of hepatic sinusoids is indicative of hepatic sinusoid injury. In the rat liver, sinusoid dilatancy is believed to be the result of hepatocyte necrosis (Hayati & Sunaryo, 2014). Necrosis causes hepatocytes to change shape, which affects how the hepatocytes are arranged in the lobules. The sinusoids that surround the hepatocytes enlarge as a result. High blood toxin concentrations that go through the sinusoids and into the central vein can also result in sinusoid dilatation(Madihah, 2017). It is easy for sinusoids to come into touch with hepatocyte-derived toxicants. The sinusoid wallcomprises of cells called endothelium. Sinusoids and hepatocytes are only limited by subendothelial gaps that contain microvilli from hepatocytes. This makes it easier for the surface of sinusoids and hepatocytes to come into contact, which promotes the interchange of substances, including toxicants(Surasa et al., 2014).

In the group given metformin that was able to repair hepatocyte cells so that it began to return to normal, but still obtained dilatation of blood vessels and sinuses, infiltration of inflammatory cells in the liver of rats. By inhibiting caspase-3 activity, promoting the creation of Glucagon Like Peptide-1 (GLP-1), and encouraging the release of endocrine L cells in the colon that are involved in controlling cell proliferation, metformin has an antiapoptotic impact. Metformin can improve organ function by stimulating cell regeneration and reducing excessive cell death through several pathways (Katzung, 2012)-(Detaille et al., 2005).

CONCLUSION

The ethanol extract of Kalangkala seed have nephroprotective and hepatoprotective activity against alloxan induced rat. This finding suggested the use of Kalangkala seed extract for diabetic patients and protect the kidney and liver damage.

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