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The Effectiveness of Black Mulberry Bark (*Morus Nigra L.*) Ethanol Extract in Reducing Blood Glucose Levels in Male Mice (*Mus Musculus*) Induced by Aloxan

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KEYWORDS
Diabetes Mellitus, Black mulberry bark (Morus nigra L.), Test of the effectiveness of reducing blood sugar levels.

ABSTRACT
Diabetes Mellitus (DM) is a disorder of carbohydrate metabolism characterized by disruption of the body's ability to produce insulin. The bark of the black mulberry (*Morus nigra* L.) stem has a high chemical content of anthocyanins, phenolics, and flavonoids and has been known to possess many beneficial properties. The purpose of this study was to determine the effectiveness of the ethanol extract of black mulberry (*Morus nigra* L.) stem bark in reducing blood glucose levels in male mice (*Mus musculus*) induced by alloxan. This research is an experimental study conducted on a laboratory scale. The *simplicia* of the black mulberry stem was macerated to produce a dry extract. Antidiabetic testing was carried out by dividing 24 mice into six groups: a healthy control group, a positive control group (glibenclamide), a negative control group (0.5% Na CMC), a 10% b/v dose extract group, a 15% b/v dose extract group, and a 20% b/v dose extract group, all induced with alloxan at a dose of 130 mg/kg BW. Based on the results, the ethanol extract of black mulberry stem bark at a dose of 20% b/v had the highest effect in reducing blood glucose levels, with a percentage decrease of 33.51%, compared to doses of 10% and 15% b/v, which showed decreases of 5.51% and 2.36%, respectively.

INTRODUCTION

Diabetes mellitus is a disorder of carbohydrate metabolism characterized by a disruption of the body's ability to produce or respond to insulin, so that it is unable to maintain normal sugar (glucose) levels in the blood (Abbasi et al., 2013). According to the World Health Organization (WHO), diabetes is a chronic metabolic disease characterized by an increase in blood glucose levels that, over time, causes serious damage to the heart, blood vessels, eyes, kidneys, and nerves (De Freitas et al., 2016; Dipiro et al., 2016). Riskesdas 2018 shows that the prevalence of non-communicable diseases has increased when compared to Riskesdas 2013, such as cancer, stroke, chronic kidney disease, hypertension, and diabetes mellitus. The prevalence of DM disease in Indonesia based on doctor's diagnosis in the population aged ≥15 years increased from 6.9% in 2010–2013 to 8.5% in 2018. The increase in the prevalence of DM disease is related to unhealthy lifestyles, including smoking, consumption of alcoholic beverages, and physical inactivity (Riskesdas, 2018).

Diabetes mellitus requires long-term treatment and expensive costs, so it is necessary to find anti-diabetic drugs that are relatively cheap and affordable for the public (Andallu et al., 2014). As an alternative, it is necessary to conduct research on traditional medicines that have a hypoglycemic effect. In 1980, WHO recommended that research be carried out on plants that have the effect of lowering blood sugar levels because the use of modern drugs is not safe. Herbal medicines have several advantages, such as fewer side effects, good tolerance in patients, and relatively less expense for long-term use (Arshad et al., 2014; Baity, 2015;).

Various studies on the use of plants for the treatment of various diseases have been widely reported, including the black mulberry plant known by its scientific name as *Morus nigra* L., of the Moraceae family (Ahlawat et al., 2016; Khoirunnisa et al., 2025; Lim & Choi, 2019).

The black mulberry plant is generally used only for its leaves as silkworm feed, but over time this plant has many other benefits, especially in treating various diseases. According to Baity (2015), black mulberry leaves can be used to treat diabetes mellitus, hypertension, hypercholesterolemia, and disorders of the gastrointestinal tract. Black mulberry contains major compounds such as flavonoids, polyphenols, alkaloids, terpenoids, and steroids (Andallu, et al., 2014). Each part of this plant has different properties, including to overcome diabetes, anemia, and hypotension (Ozgen, et al., 2016) and to help maintain healthy skin, namely as an antibacterial, antioxidant, and anti-inflammatory (Minhas, et al., 2016). The bark part of the trunk can be used as a laxative, vermifuge, and antidiabetic. The root bark has anthelmintic and cathartic properties (Thabti et al., 2014). In addition, the stem bark also has antioxidant activity at a dosage of around 25–135 µg/mL (Mazimba, 2011).

Based on this, it is very important to test the effectiveness of the ethanol extract of black mulberry stem bark (*Morus nigra* L.) as a treatment to help lower blood sugar levels (*diabetes mellitus*). The purpose of this study is to determine the effectiveness of the ethanol extract of black mulberry stem bark (*Morus nigra* L.) in reducing blood glucose levels in male mice (*Mus musculus*) induced by alloxan. This study is expected to provide scientific information and supporting data for future studies related to the ethanol extract of black mulberry stem bark (*Morus nigra* L.) as a safe and effective antidiabetic derived from nature, so that it can be used as a consideration in the formulation of pharmaceutical preparations.

RESEARCH METHOD

The research is an experimental research on a laboratory scale. This research was conducted in November – February 2021 at the Biology - Pharmacology Laboratory of the Makassar College of Pharmaceutical Sciences and the Laboratory of Siloam Hospital Makassar.

The tools used in this study are stirring rods, bisturi, chocolate bottles, spray bottles, porcelain cups, mouse pacifiers, 50 mL and 100 mL beaker cups, 50 mL measuring cups, scissors, glucometer, watch glass, test animal cage, mortar, cannula, newspaper, parchment paper, microscope, tweezers, drip pipettes, 10 mL plastic pots, horn spoons, a set of maceration tools, a set of surgical instruments, probe spet, 1 ml spoit, 10 ml spoit, analytical scale, animal scale.

The materials used in this study include: aluminum foil, aloxan monohydrate, aquadest, aqua pro injection (as a solvent of aloxan monohydrate), godam thread, 70% ethanol, formalin, glibenklamide, filter cloth, black mulberry stem skin (*Morus nigra* L.), cotton, Na-CMC 270.5%, mice *(Mus musculus),* mouse feed, mading nails, silica gel, autocheck brand sugar strip.

Research variables

Free variable : Black mulberry stem bark extract (*Morus nigra* L.)
Bound variable: Blood sugar levels in mice (*Mus musculus*).

Antidiabetic Activity Test

1. Preparation of Experimental Animals

The test animals were adapted to the ± environment for 1 week before being given treatment. All animals were tried to be kept in the same way and previously all mice were fasted for 8-12 hours. Before the treatment is given, all animals are first weighed to calculate the dosage setting.

2. Manufacture of Colloidal Solution Na CMC 0.5 % (w/v)

A total of 0.5 grams of Na CMC is gradually put into a lump containing 50 mL of hot aquadest at (temperature 700C) while being eroded until a colloidal solution is formed, then

the volume is sufficient to 100 mL with aquadest, then the finished solution is put into a brown bottle container.

3. Manufacture of Glibenclamamide suspension

Weighing 10 tablets of glibenclamide (5 mg), the average weight of each tablet is calculated and then put into a lump and grinded until homogeneous. Weighted to 0.2 mg of glibenclamamide and suspended with a sufficient 0.5% b/v colloidal solution of Na CMC and then sufficient volume up to 5 ml.

4. Antidiabetic Activity Test

The test animals used in the study were 24 mice (*Mus musculus*). The test animals are acclimatized Previously with a research environment for 1 week. Then mice are divided into 6 groups each consisting of 4 heads. Grouping is done randomly. Before the treatment of mice, they were fasted for 8 hours by only being given a drink, then the weight of each mouse was weighed and the initial blood glucose level was measured. After that, the mice were induced with 130 mg/kg of allaxan BB intraperitonally (Nugroho, 2004) and 3 days later the blood glucose levels of mice (*Mus musculus) were re-measured.*

The test animals of Mice (*Mus musculus*) were used as many as 24 animals which were divided into 6 groups.where each group consisted of 4 mice, namely: the first group as a healthy control was only given a 0.5% Na-CMC solution without aloxant induction. The second group is the negative control, which is a 0.5% Na–CMC solution. The third group as a positive control is glycenchlamide suspension. The fourth, fifth, and sixth groups as test groups were mice of black mulberry stem bark ethanol extract with doses of 10% b/v, 15% b/v and 20% b/v. On the tenth day after blood collection was carried out for blood sugar level measurement, then all test animals were sacrificed to be isolated from the pancreatic organ for histopathological examination.

5. Organ Harvesting Procedure

After being treated for 1 week, the test animals were anesthetized by putting them in a cotton jar that had been given ether. Each test animal was dissected using a scalpel and its pancreatic organs were taken. After that, the organs are put into a container containing 10% formalin (BNF) and the volume of the organ is measured.

6. The process of making histological preparations

The preparation consists of 4 stages, namely fixation, dehydration, embedding, and staining. The fixation stage is carried out by cutting the organ, inserted into a 10% formalin neutral buffer (BNF) for 3x24 hours, then cut again with a thinner size. The organ pieces are continued to the dehydration stage, namely by immersion using multi-stage ethanol (70%, 80%, 96%, absolute I, absolute II). Then ethanol was removed with xylol I, II, and III each for 40 minutes of infiltration 8 using liquid paraffin was carried out at 60°C for 4 times each for 30 minutes. Before printing, the mold is washed with a mixture of 96% ethanol, xylol, and water. Printing is carried out by pouring hot paraffin in a mold block by half with a Tissue Tec tool. Pieces of the organ are inserted into it slowly so that they do not touch the base of the mold and then covered again with liquid paraffin. After freezing, the paraffin internal organs are cut with a microtomy tool 4-5 mμ thick. The obtained pieces are put in warm water (40°C) to melt the paraffin, then placed in the glass of the object. The pieces were dried in an incubator oven at 56°C for one night (Subowo, 2009) At the stage of staining Haematoxylin Eosin (HE) was carried out after parafinition, namely the preparation was soaked using xylol I and xylol II for 2 minutes each, rehydrated with absolute ethanol for 2 minutes, then with 95% and 80% ethanol for 1 minute respectively, and washed with running water. Then the preparation was soaked in Mayer's Haemotoxylin dye for 8 minutes, washed with running water, put into LiCI for 30 seconds, and washed again with running water. Then the preparation slices are given eosin dye for 2-3 minutes, then washed. After that, the organ slices were dipped in 95% ethanol and absolute I 10 times each and continued with absolute ethanol II for 2 minutes, xylol I for 1

minute and xylol II for 2 minutes. Then it is placed on the preparation glass. After the histological preparation is formed, then analysis and observation of the changes that occur in organ cells are carried out using a light microscope and the results of the observations are photographed. The purpose of histopathological examination is to determine the possibility of changes in the structure of pancreatic tissue in test animals caused by the administration of ethanol extract of black mulberry stem bark and aloxan. The brief procedure of histopathological examination is as follows.

Examination and tissue imaging are carried out in a pathology laboratory. The reading of pancreatic histopathological preparations for the presence of a picture of cell damage, a picture of the vacola or the presence of an inflammatory reaction due to the induction of aloxan is carried out by a pathologist.

7. Data analysis

Data in the form of blood glucose level observation results were tested by statistically analyzed with the One-way ANOVA test and Post Hoc LSD analysis.

The results of the examination of pancreatic histopathological preparations are qualitatively analyzed by experts presented descriptively.

RESULTS AND DISCUSSION

Samples of black mulberry stems (*Morus nigra* L.) were taken from Soppeng Regency. The sample was then processed in the form of simplicia and obtained a dry weight of 800 grams. The obtained simplicia is then extracted using the maceration method. Extraction by the maceration method has the advantage of ensuring that the extracted active substances will not be damaged (Pratiwi, 2010). During the soaking process of material, there will be a breakdown of the cell wall and cell membrane caused by the difference in pressure between the outside of the cell and the inside of the cell so that secondary metabolites in the cytoplasm will break down and dissolve in the organic solvent used (Novitasari and Putri, 2016). The maceration stage is by soaking simplicia with filtering liquid. The filter fluid used is ethanol
70%. 70% ethanol filtering liquid can attract relatively polar compounds such as phenol compounds, flavanoids, saponins, and other polar compounds contained in the sample, 70% ethanol is used on the grounds that it is more selective, non-toxic, neutral, good absorption, non-toxic, can prevent the growth of molds and germs, as well as the heat required for less concentration (Diniatik, et al., 2016). The results of the black mulberry stem bark extract obtained were in the form of a dry extract with a weight of 23.96 grams so that the soaking value obtained
as many as 4.79 %.

The dried extracts obtained are then phytochemical screened to determine the content of the group of compounds found in the sample.

Table 1. Results of Phytochemical Screening Test of Ethanol Extract Trunk Bark Black mulberry.

No	Compound Groups		Reaction Results	Information
1	Alkaloids	Mayer	No white posits formed	(-)
		Wagner	No red-orange deposits are formed	(-)
		Dragendroff	Brown deposits are not formed	(-)
2	Flavonoids		Red solution	(+)
3	Saponins		The solution is brown, foamed	(+)
4	Tannins		Blackish-green solution	(+)
5	Triterpenoids		Orange color formed	(+)

Source: Processed from research

Description : (+) = Contains compounds
(-) = Contains no compounds

Based on the test results obtained in this study, it shows that ethanol extract from black mulberry stem bark is positive and contains flavanoid compounds, saponins, tannins, and triterpenoids. According to research conducted by Mazimba (2011), it is stated that the bark of the trunk and wood of black mulberry contains compounds such as flavanoids, triterpenoids, and saponins. One of the compounds contained in this plant is flavanoids where the compound has antidiabetic activity through its function as an antioxidant. Antioxidants are able to bind free radicals so that they can reduce oxidative stress. Reduced oxidative stress can reduce insulin resistance and prevent the development of dysfunction and cell damage β pancreas. The presence of tannins is also thought to stimulate glucose uptake and regulate the activity of enzymes involved in carbohydrate metabolism pathways and act like insulin by influencing *the mechanism of insulin* signaling.

After the identification test was carried out, the effectiveness of ethanol extract of black mulberry stem bark was then tested against the blood glucose of aloxan-induced mice. Aloxan is a diabetogenic compound that is cytotoxic to pancreatic islet cells through the formation of free radicals and oxidative stress. Induction of aloxan in test animals can result in damage to pancreatic tissue resulting in a decrease in insulin production by pancreatic islet cells.

In this study, mice were used to try mice, which have similarities with humans in terms of physiology, anatomy, nutrition, pathology, metabolism, and are commonly used in research. The test animals were then grouped into 6 groups, namely the healthy/normal control group that was not induced by aloxan only given to drink to find out the normal blood sugar level of the test animals. The positive control group was induced by the administration of glibenclamide. Glibenklamide is one of the sulfonylurea drugs with a mechanism that is able to stimulate β cells from the langerhans island, so that insulin secretion is increased. The negative control group was induced by aloxan and Na CMC 0.5% which aimed to determine the normal decrease in sugar levels. Meanwhile, the extract treatment group with doses of 10%, 15%, and 20% was induced aloxan with different concentration variations (Cimbiz et al., 2011; Ferraz et al., 2024).

Before being given the treatment of test animals, acclimatization is carried out to adjust to the surrounding environmental conditions. Then the mice were fasted ± 8 hours by not being fed but still given a drink before the blood draw. The blood collection process is by cutting off the tip of the tail of the mouse that has previously been cleaned with an alcoholic cotton swab. Then the initial glucose level measurement was carried out to find out the initial glucose level before being given treatment. Furthermore, aloxan induction with a dose of 130mg/kgBB was carried out intraperitoneally to increase blood glucose levels in mice and blood glucose levels were measured 48 hours after induction. After the induction of the aloxan, it is followed by the administration of treatment which is carried out every day for 7 days. The administration of the extract was carried out by dissolving the extract into 0.5% Na CMC. It was then administered orally to mice and observed the effect of decreasing glucose levels from each dose.

Based on the results of the study, data were obtained on each group, namely in the positive control group, the negative control group, and the extract treatment group with doses of 10%, 15%, and 20% in the following table:

Table 2. Data on the Effectiveness Test Results of Mulberry Stem Ethanol Extract Black

Treatment	Replication	Fasting Blood Glucose Level (mg/dL)			
		Beginning	After Induction	After Treatment	%Changes
Control (+) (Glibenclamide)	1	92	152	93	38,82
	2	78	111	76	31,53
	3	95	165	86	47,88
	Average ± SD	**88.33 ± 9,07**	**142.7 ± 28,18**	**85 ± 8.54**	**39,41**
Control (-) (NaCMC)	1	96	106	122	-15,09
	2	73	154	160	-3,89
	3	75	133	139	-4,51
	Average ± SD	**81.33 ± 12,74**	**131 ± 24,06**	**140.33 ± 19,03**	**-7,83**
Group I	1	70	151	111	26,49
	2	88	105	123	-17,14
	3	85	139	129	7,19
	Average ± SD	**81 ± 9.64**	**131.7 ± 23,86**	**121 ± 9,17**	**5,51**
Group II	1	73	142	91	35,92
	2	90	144	95	34,02
	3	81	105	171	-62,85
	Average ± SD	**81.33 ± 8,50**	**130.3 ± 21,96**	**9 ± 45,07**	**2,36**
Group III	1	71	161	96	40,37
	2	77	170	86	49,41
	3	94	279	249	10,75
	Average ± SD	**80.66 ± 11,93**	**203.33 ± 65,68**	**143.66 ± 91,36**	**33,51**

Source: Processed from research data

Information:
Control (+) : Glibenclamamide
Control (-) : Na CMC
Group I: Black mulberry stem bark extract concentration 10% Group II: Black mulberry stem bark extract concentration 15% Group III: Black mulberry stem bark extract concentration 20%

Based on table 3, normal, positive control and negative control comparisons were used to determine whether or not the administration of black mulberry stem bark extract had an effect on the reduction of blood glucose levels. Based on the results of blood glucose level measurement data, it showed that in the positive control group with the administration of glibenclamamide drugs resulted in a decrease in blood glucose levels in the range of 85 ± 8.54 mg/dL with a % change of 39.41%. The mechanism of action of glibenclamamide is to stimulate insulin production by inhibiting the attachment of sulfonylurea receptors in the β cells of the pancreas and finally there is a tension in the opening of calcium channels so that it causes an increase in intracellular calcium β (Akash, 2013). In the treatment group with a concentration of 10% it was in the range of 121 ± 9.17 mg/dL with a % change of 5.51%, in the concentration of 15% it was in the range of 119 ± 45.07 mg/dL with a % change of 2.36%, and in group III with a concentration of 20% it was in the range of 143.66 ± 91.36 mg/gL with a % change of 33.51%.

Based on research by Da Silva Júnior *et* al, 2017 on *Morus nigra* L extract, there is a reduction in blood sugar levels due to the lack of oxidative stress by malondialdehyde, and an increase in the antioxidant levels of glutathione in the liver of animals due to the content of flavonoids, isoquercetin, and kaempferritrin. In addition, according to Sharma *et al,* who investigated the antidiabetic potential of 400 mg/kg extract *of Morus rubra* L resulted in remarkable glycemic control, evidenced by a significant decrease in hemoglobin glycosylation with an increase in plasma insulin and C-peptide levels. Not only that, this extract is also antioxidant because there is a significant increase in the activity of superoxide dismutase and catalase enzymes, as well as a reduction in lipid peroxide and glutathione peroxide in animal liver erythrocytes (Sharma *et al,* 2010)

For the negative control group with the administration of the Na solution. CMC 0.5% did not decrease blood glucose levels, was in the range of 140.33 ± 19.03 mg/dL with a % change of -7.83%. This indicates that in addition to the negative control group, there is a real difference with other treatment groups in lowering blood glucose levels. This is because there are no active substances contained in Na-CMC that can lower blood glucose levels .

Based on the above data, it can be seen that the difference in blood sugar level reduction in mice between the negative control and the positive control and the extract group shows a significant difference where the negative control with the administration of Na CMC does not affect blood sugar levels (Zheng et al., 2010). While in the treatment group with a variation in concentration able to reduce blood sugar levels in mice, the highest reduction in sugar levels was in the extract treatment group with a concentration of 20% and the lowest decrease in sugar levels was in the treatment group with a concentration of 10%, but in groups I and II although the effect given was relatively low, it still showed a decrease in blood sugar levels in mice (Tangkuman et al., 2017; Tchabo et al., 2015; Thabti et al., 2014).

To find out whether there is a significant difference from each treatment, a statistical data analysis test was carried out using the one-way ANOVA test. Before conducting a parametric test, the requirements for the test must meet the requirements of the normality test, so a data normality test is first carried out. The test results met ($p>0.05$), so it was continued with the ANOVA test.

Based on the results of the ANOVA test, the data obtained in (Appendix 6) showed that each treatment group had statistically different results ($P<0.05$). It states that the dose of ethanol extract of black mulberry bark has efficacy in lowering blood glucose levels. To find out which group was different, a follow-up test was carried out, namely the post hoc test of the LSD method (Appendix 6), The results showed that there was no group that experienced significant differences, all treatment doses had effectiveness in lowering blood glucose levels in mice, but the treatment group with a concentration of 20% had the best effectiveness in lowering blood glucose levels.

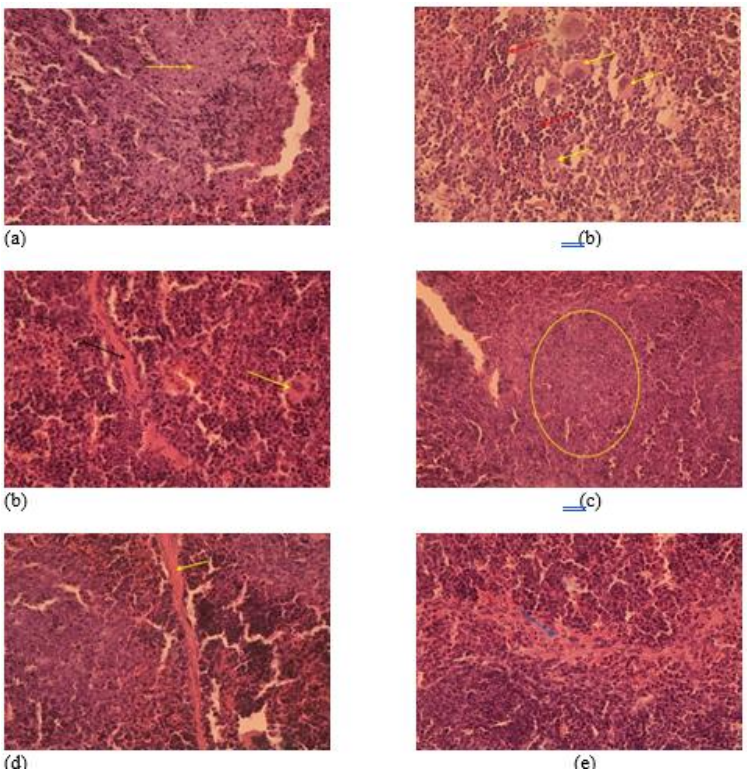

Figure 1. Histopathological picture of pancreatic organs in normal mice and mice in posttreatment diabetic models with hematoxilin eosin (HE) staining at 400X magnification. Source: Histopathological observation result

Histopathological examination aims to see for the presence of organ damage at the cellular level that is not visible by macroscopic observation. The organ examined is the rat pancreatic organ because the pancreas is the place where insulin is produced. In Figure (a) is a healthy control that is not given treatment only by giving Na CMC it appears in the image that there is Langerhans islet, pale cytoplasm (yellow arrow) (Gundogdu et al., 2011; Hall et al., 2016). This indicates that the island of langerhans is in a normal state or has not been damaged. Based on the results of observations on the histopathology of the pancreatic tissue of mice (*Mus musculus*) with the Hematoxylen-Eosin(HE) staining method, it can be seen that langerhans islands are spread throughout the pancreatic organs, shaped like islands and are often passed by blood capillaries, look paler compared to the surrounding asynary gland cells so that langerhans islands are easy to distinguish, while in animals with DM they will experience morphological changes on Langerhans Island, both in number and size. In figure (b) is a positive control, namely the proliferation of multinucleate cells (yellow arrow) between the asinar cells (red arrow). In the histopathological picture of the pancreas, it can be seen that there is a decrease in the number of island cells, this is in accordance with the statement of Vessal et al., (2001) that beta cells are 60% of the formation of Langerhans island so that the damage to many Langerhans island beta cells will reduce the diameter of the Langerhans island cells. Damage to pancreatic beta cells can be caused by many factors. These factors include genetic factors, infection by germs, nutritional factors, diabetogenic substances and free radicals (oxidative stress). Aloxan compound is one of the diabetogenic substances that is toxic, especially to pancreatic beta cells and when given to experimental animals such as mice (*Mus musculus*) can cause experimental mice to become diabetic (Iqbal et al., 2012; Khalid et al., 2011; Koyuncu et al., 2014). In addition, experimental animals induced by aloxan will experience a decrease in the number of spermatogenic cells and sertoli cells, the mechanism is

to start from an increase in free radicals due to hyperglycemic conditions (Muliasari et al., 2017). Hyperglycemic conditions lead to glucose autooxidation, protein glycation, and activation of polyol metabolic pathways which further accelerate the formation of reactive oxygen compounds. The formation of reactive oxygen compounds can increase the modification of lipids, DNA, and proteins in various tissues. Molecular modifications in these tissues result in an imbalance between protective antioxidants (antioxidant defense) and increased free radical production. It is the beginning of oxidative damage known as oxidative stress (Hojjatpanah et al., 2011).

In Figure (c) is a negative control with the administration of aloxan with the boundary between groups of asinar cells becoming unclear (cytoplasm becomes eosinophilic, yellow arrow). where it can be seen that the islet of Langerhans has been damaged, the presence of hydropic degeneration, edema, fat degeneration, and the discovery of necrosis in the organ proves that the administration of aloxan can damage pancreatic cells, especially beta cells. In figures (d), (e), and (f) are the treatment controls where in figure (d) there is a proliferation of Langerhans islet cells (yellow circle). In Figure (e), there is fibrohyaline connective tissue between the asinar cells and the Langerlans islet (yellow arrow). and in figure (f) there is connective tissue between the asinar cells, containing histiocytes. Pancreatic organ changes in treatment groups I and II showed poorer outcomes than positive controls, while treatment group III gave similar results to positive controls. Damage in treatment groups I, and II was seen to be reduced when compared to the negative control group. Disorders such as edema, degeneration, and necrosis are still found even in small amounts. Meanwhile, in treatment group III, the damage due to allocation was almost entirely recovered, characterized by a dense cell state, close spacing between cells, and slight hydropic degeneration without any edema and necrosis.

CONCLUSION

From the research that has been carried out, it can be concluded that ethanol extract of black mulberry stem bark can provide effectiveness in reducing blood glucose levels in mice induced by aloxan with the best concentration, namely in group III with a dose concentration of 20% compared to the concentration of dose 10 and 15%. However, all dose variations can be said to be able to have a decreasing effect on blood glucose in mice. For further research development, it is recommended to conduct toxicity tests of the extract to ensure long-term safety, as well as to explore more practical and stable formulation preparations. Additionally, further studies using animal models more relevant to human diabetic conditions are needed, along with a deeper investigation of the molecular mechanisms to strengthen the scientific basis for the use of black mulberry stem bark as an alternative therapy for diabetes mellitus.

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