International Journal of Science Academic Research
Vol. 03, Issue 06, pp.3978-3981, June, 2022
Available online at http://www.scienceijsar.com

ISSN: 2582-6425

Research Article

THE EFFECT OF *ANNONA MURICATA* LEAVES ON THE CHANGES OF HISTOPATHOLOGY OF HEPAR SCRATCHING SWISS WEBSTER STRUCTURED BY ALLOXANT

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Received 18th April 2022; **Accepted** 24th May 2022; **Published online** 27th June 2022

Abstract

Background: Hyperglycemia which triggers an increase in oxidative stress can cause inflammation of the liver tissue. This study aims to look at the effect of the ethanol extract of Annona muricata leaves which has a hepato-protective function on liver histopathological changes in mice that have diabetes mellitus. **Methods:** This study used 25 Swiss Webster mice which were randomly divided into 5 groups: the Negative Control (KN), Positive Control (KP) group, the treatment group dose 150 mg, 300 mg, 600 mg. Histopathological features will be assessed using the NASH CRN score which will then be processed using SPSS version 24 using the Chi-Square test. While blood glucose levels will be tested using the Kruskal-Wallis test with the post hoc Mann Whitney UTest. **Results:** The results of the ethanol extract of Annona murica leaves in the 5 dose groups showed significant differences in lobular inflammation (p = 0,000) and steatosis (p = 0.001). In this studyalso found a significant relationship between steatosis variables with lobular inflammation (p = 0.535). **Conclusion:** There is a significant relationship to the ethanol extract of Annona muricata leaves on changes in steatosis and lobular liver inflammation in mice.

Keywords: Alloxant, Annona muricata, Glucose, Lobular inflammation, Steatosis, Mice.

INTRODUCTION

According to WHO data, the number of people with diabetes has almost doubled since 1980, increasing from 4.7% to 8.5% in 2014[1].Diabetes mellitus can cause serious complications in different organ systems in the body, such as the kidneys, eyes, heart, liver, and other organs[2]. Insulin resistance as the main cause of hyperglycemia and hyperinsulinemia is becoming the main cause of liver damage in diabetic patients[3,4]. The liver as an insulin-sensitive organ, can be affected by the effects of oxidative stress induced by hyperglycemia, which can cause inflammation in liver tissue[4,5]. Both oxidative stress and inflammatory response have roles as agents. Destroyer in aggravating the pathological condition of diabetes mellitus[6,7]. In addition, there is often excessive accumulation of liver fat in some patients with diabetes mellitus which can result in fatty liver (fatty liver disease) liver. Hepatitis steatosis can further progress to fibrosis and cirrhosis[7,8]. The condition of fatty liver in patients with diabetes mellitus can be seen by examination of Anatomic Pathology. Histopathological features of hepatitis steatosis include accumulation of fat cells, lobular inflammation, ballooning hepatocytes, peri cellular/sinusoidal fibrosis[2,3,4]. One of the alternatives chosen in the management and treatment of chronic diseases is medicinal plants. Increasing reports about traditional medicine from various parts of the world support this trend. The management of diabetes mellitus, especially non-insulin dependent (NIDDM) has been widely practiced in the use of medicinal plants throughout the world. Annona muricata Linn, also NCown as soursop, graviola, and guanabana, deserves further investigation regarding its beneficial effects on diabetes mellitus because of its function as an antioxidant, and other functions that can lower total cholesterol, increase HDL cholesterol, and provide a hypoglycemic effect[9,10,11,12].This study aims to look at the effect of the ethanol extract of Annona muricata leaves which has a hepato-protective function on liver histopathological changes in mice that have diabetes mellitus.

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METHODS

The design used in this study is a true experimental one. In this study, 25 male Swiss Webster mice were used which were put into 5 groups with different interventions. The ethics of this research have received information that it has passed the ethical review with the number KET-432/UN2.F1/ETIK/ PPM.00.02/2019. This research was combined in a study entitled "Molecular, Cellular, and Histopathological Studies of the Effect of Annona muricata Extract on the Pancreas, Liver and Testis of Diabetes Mellitus Mice.

Research Sample Preparation

This study used 31 male Swiss Webster mice aged 12-14 weeks with a body weight of 30-40 grams. which were categorized into 5 groups, namely negative control group (given alloxan 40 mg/kg BW), positive group (given alloxan 40 mg/kg BW and given glibenclamide 0.65 mg/ml/head/day45 for 14 days), group low dose treatment (given alloxan 40 mg/kgBW and given ethanol extract of AM leaves 150 mg/kgBW/day for 14 days), medium dose treatment group (given alloxan 40 mg/kgBW and given AM leaf extract 300 mg/kgBW/day for 14 days), high dose treatment group (given alloxan 40 mg/kgBW and given ethanol extract of AM 600 mg/kgBW/day for 14 days).

Hematoxylin-Eosin stain

The slide preparations were stained with HE staining in the following steps. First, the process of immersion in xylol I, xylol II, xylol III was carried out for 5 minutes each called deparafanization which aims to remove paraffin that has adhered to the preparation.

The preparations were immersed in alcohol, absolute, 90% alcohol, and 70% alcohol for 5 minutes each with the aim of removing xylol carried by the preparation and introducing water into the tissue (rehydration). After the rehydration process, the preparations were stained by dipping them in lithium carbonate, the slides were washed with running water for 10 minutes and immersed in eosin solution for 3 minutes. The next process is dehydration by dipping into alcohol which has an increasing concentration of 70%, 90%, and 100% for five times each. Then after the dehydration process was carried out, the preparations were immersed in a solution of xylol I, xylol II, xylol III for 5 minutes each. Furthermore, the preparation is dripped with one entelan and closed again with a cover glass.

Histopathological Results Assessment

Histopathological features of hepatitis steatosis include accumulation of fat cells, lobular inflammation, ballooning hepatocytes, peri cellular/sinusoidal fibrosis, and triglyceride levels, liver weight. Histopathological parameters were calculated using the NASH CRN (Non Alcoholic Steatohepatitis Clinical Research Network). Readings were made at 400 times magnification. Histopathological readings using an Axiocam ERc 5s camera to a computer.

Data analysis

The interpretation results of the histopathological examination of the liver of mice are presented in the form of "Yes" and "No" statements. Because the data includes categorical data, the statistical test used is a nonparametric test. The data will be analyzed using the Chi-Square test. If the p value <0.05, it indicates the null hypothesis (H0) is rejected and the Alternative Hypothesis (Ha) is accepted. If the p value> 0.05, it shows that H0 is accepted and Ha is rejected. H0 states that the two variables are independent (no association), while Ha states that the two variables are not independent (no association).

RESULTS

The results of the test of ethanol extract of Annona murica leaves on histopathological changes in the liver of mice for a picture of stetatosis, p value = 0.001 which means p <0.05. This indicates that there is a significant relationship between the administration of AM leaf ethanol extract on the appearance of steatosis in the liver. For the picture of lobular inflammation, the value of p = 0.000 which means p <0.05. This indicates that there is a significant relationship between the administration of AM leaf ethanol extract on the lobular inflammation of the liver. The significant relationship shown by the test results cannot be known between which treatment groups have significant differences. This is because the test results show a constant value when compared between treatment groups.

Table 1. Liver NASH CRN Scores of Swiss Mice Webster

	Indikator	Yes	No
NC	Steatosis	100%	0%
	Inflamaasi lobular	0%	100%
PC	Steatosis	80%	20%
	Inflamaasi lobular	0%	100%
LD	Steatosis	100%	0%
	Inflamaasi lobular	100%	0%
MD	Steatosis	100%	0%
	Inflamaasi lobular	100%	0%
HD	Steatosis	100%	0%
	Inflamaasi lobular	100%	0%

Information: NC = Negative Control (given alloxan 40 mg/kgBW), PC = Positive Control (givenAlloxan 40 mg/kgBW and glibenclamide 0.65 mg/ml/head/day), LD=Low dose (given AM extract dose 150 mg/kgBW/day), MD=Medium dose (given AM extract dose 300 mg/kgBW/ days), HD = High Dosage (given a dose of soursop extract 600 mg/kgBW/day).

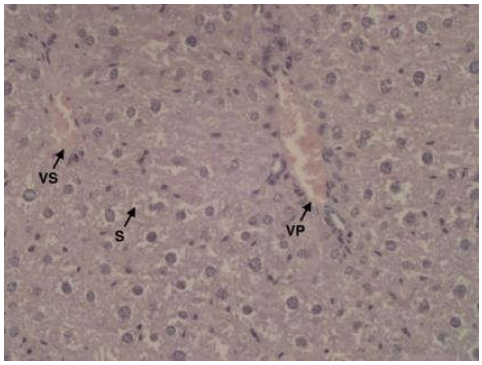
DISCUSSION

Hepatic Histopathology

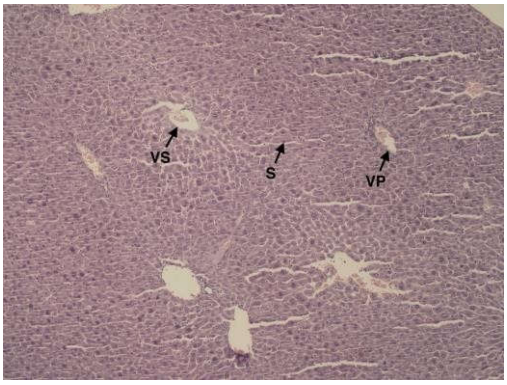
The results of data analysis from the histopathology of mice stated that there was a significant relationship between the administration of AM leaf ethanol extract on the appearance of steatosis and lobular inflammation in the liver. The scoring used is NASH CRN to see the severity of liver damage based on the presence of components of steatosis, lobular inflammation, and balloon cell degeneration. This happened because from the observations on the histopathological picture of the liver, the entire group did not find balloon cell degeneration[13]. Therefore, the researcher simplified the writing of the score to "0=No" and "1=Yes". Previous studies have shown the effect of giving AM leaf extract on histopathological changes in mice with diabetes[14]. This is supported by the presence of flavonoid compounds in AM leaf extract which have antioxidant functions so that they can repair liver damage[15]. However, the histopathological results in the group NC showing only a small amount of fat without lobular inflammation. Meanwhile, in the dose group (DR, DS, DT) it was found that there was fatness accompanied by lobular inflammation. In addition, cytoplasmic vacuolization was also found in the histopathological picture of the dose group.

Researchers suspect that the liver of the test animals may not have experienced the effects of complications from diabetes after alloxan induced. The damage that occurred in the liver of mice in the dose group (DR, DS, DT) was higher than the NC group, presumably due to the hepatotoxic nature of the AM extract leaves. Previous studies have shown that AM leaf extract can cause injury to healthy hepatocyte cells with degeneration and necrosis in healthy liver cells of Rattus norvegicus rats after being given AM leaf extract at a dose of 1000 mg/kgBW/day for 90 days[16]. Drugs in the form of synthesis, herbal or natural can have side effects on certain organs, one of which is the liver which is the site of drug metabolism and detoxificatio[17].

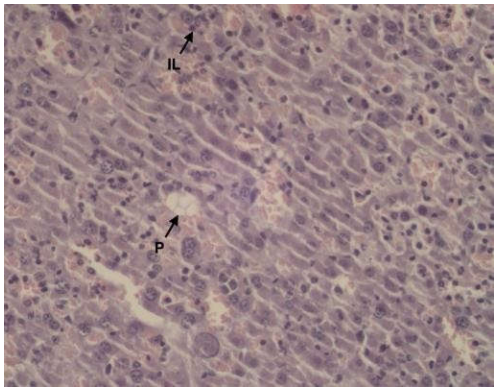
This liver damage is thought to be caused by the content of AM leaf extract, namely acetogenin, which can interfere with mitochondrialactivity[18]. Disrupted mitochondria can lead to decreased ATP production. Physiologically, a decrease in ATP production that occurs in the long term can cause cells to degenerate and die due to necrosis or apoptosis[19,20]. Liver cell damage can be characterized by the presence of clear cell foci of the liver or cytoplasmic vacuolization[21]



NC



PC



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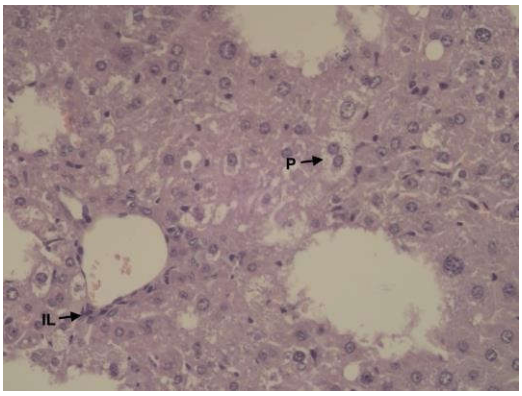
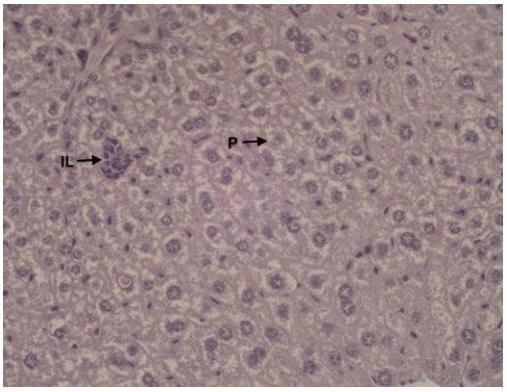


MD



HD

Histopathological appearance of the liver with HE staining at 20xmagnification
NC = Negative Control (given alloxan 40 mg/kgBW), PC = Positive Control (given alloxan 40 mg/kgBW and glibenclamide 0.65 mg/ml/head/day), LD = Low dose (given dose of soursop extract 150 mg/day). kgBW/day), MD=Medium Dose (given dose of soursop extract 300 mg/kgBW/day), HD=High Dose (given dose of soursop extract 600 mg/kgBW/day). Signs in the figure: VS= Central Vein; S= sinusoids; VP= Portal vein; P = Fatty (Steatosis); IL = Lobular Inflammati

Figure 4.1 Histopathological overview of the liver

Diabetes mellitus can cause excessive accumulation of fat cells in the liver. The prominent histopathological change in the liver of patients with DM is the presence of fat accumulation that replaces the liver cytoplasm, NCown as microvesicular steatosis. Steatosis develops and worsens with steatohepatitis which is characterized by the presence of lobular inflammation[4,7].The severity of liver damage itself is influenced by several factors, such as genetic and environmental factors. One example of gene expression that affects the severity of diabetes complications is a member of the cytochrome P450, namely CYP2E1 which is expressed higher in the liver than other organs. Excessive unsaturated fatty acid metabolism by CYP2E1 can produce cytotoxic products that are harmful to the liver.

Therefore, the severity of complications caused by DM conditions in each individual can be different[22,23]. Meanwhile, in the correlation test between the variables of steatosis and lobular inflammation, there was a significant relationship. Liver abnormalities associated with diabetes, one of which is well NCown is NASH, which is an inflammation of the liver due to abnormal fat accumulation in the liver that can be caused by insulin resistance, in the absence of significant alcohol use[24,25]. The histopathological features that can be seen are: the presence of steatosis and lobular inflammation[47,25]. Steatosis is the accumulation of triglycerides in hepatocytes[25]Generally, steatosis is macrovesicular and located in the centrolobulararea[25] While lobular inflammation generally consists of mixed inflammatory cells such as mononuclear and polymorphonuclear leukocytes[24,25].

Conclusion

There was a significant relationship between the administration of AM leaf ethanol extract on changes in the histopathological picture of mice induced by alloxan.

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