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EFFECT OF DAHLIA TUBER INULIN EXTRACT ON FATTY LIVER IN TYPE 2 DIABETES MELLITUS RATS

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ABSTRACT

Several studies show a correlation between intestinal microbiota disturbances and metabolic disorders, including type 2 diabetes mellitus (T2DM). Fatty liver is one of the complications that occurs due to T2DM. Fatty liver is characterized by the accumulation of lipids in the liver in response to increased triglyceride synthesis in hepatocytes and is thought to be associated with disturbances in the gut microbiota so that changes in the gut microbiota can be a potential target for T2DM treatment. It is known that inulin, a prebiotic, can improve intestinal microbiota disorders. This study aims to analyze the effect of administering inulin from dahlia tubers on fatty liver in T2DM rats.The research design is an experimental study with a post-test-only control group design conducted from June to November 2023. The study used twenty male Rattus norvegicus Wistar strain divided into five groups: a control group, a T2DM-induced group, T2DM groups given inulin extract at doses of 0.5 mg/gBW, 1.0 mg/gBW, and 1.5 mg/gBW. The results showed that the T2DM group had a higher significant percentage of fatty liver ($p < 0.01$) than the control group. Compared to the T2DM group, there was a decrease in the percentage of fatty liver in the T2DM groups given inulin at all doses ($p < 0.05$). This study concluded that inulin administration can reduce fatty liver in T2DM rats.

Keywords : Dahlia tubers., fatty liver., inulin., type 2 diabetes mellitus,

INTRODUCTION

Unhealthy lifestyles, such as lack of physical activity, unbalanced eating patterns, habits of consuming high fat, high sugar, and low fiber are one of the risk factors for type 2 diabetes mellitus (T2DM). This disease is a metabolic disease caused by impaired insulin secretion, insulin resistance, or both, which causes an increase in blood glucose and develops into diabetes.[1,2]

The International Diabetes Federation (IDF) reports that in 2021, there will be 573 million adults (aged 20-79 years) worldwide who suffer from diabetes mellitus. This number represents 10.5% of the world's population in this age group. This figureis expected to increase to 643 million (11.3%) in 2030 and to 783 million (12.2%) in 2045. Meanwhile,in Indonesia, from the same source, it is stated that19.5 million adults are suffering from diabetes mellitus, and it is expected to increase by 47% by 2045. T2DM accounts for approximately 90-95% of all diabetes cases.[3]

Fatty liver is one of the complications that occurs due to T2DM. Fatty liver is characterized by fat deposits in hepatocytes exceeding 5% of the weight of the liver. As many as 50% to 70% of diabetes mellitus sufferers experience fatty liver.[4] The mechanism of fatty liver is very complex and heterogeneous, including the interaction between diet and microbiota gut, genetic factors, and de novo lipogenesis. Current concepts point to insulin resistance as the primary metabolic defect that triggers fatty liver. Insulin resistance causes an increase in blood glucose and free fatty acids, ultimately increasing triglyceride synthesis in the liver. This results in lipid accumulation in hepatocytes, leading to hepatic steatosis.[6]

Currently, functional foods are considered in the management of type 2 diabetes and its complications. Supplementation of prebiotic fibers such as inulin has been investigated as a treatment strategy for obesity and metabolic disorders. Inulin is a carbohydrate from the fructan group, a water-soluble dietary fiber with a low glycemic index. The physicochemical characteristics of inulin make it a functional substance. For example, in the case of inulin used in food as a texture modifier and fat substitute due to its gel formation and viscous properties, this depends on the degree of polymerization. Inulin extract from dahlia tubers has a higher degree of polymerization than other sources[7], so this type of inulin has better bioavailability. The β (2-1) bond of the fructose molecule in inulin prevents inulin from being digested by digestive enzymes in the small intestine and can selectively stimulate the growth and activity of beneficial bacteria in the large intestine. That way, inulin has a calorific value, which is low, and is prebiotic, so it is good for people living with diabetes.[2]

Several studies show a relationship between T2DM and gut microbiota, which also significantly contributes to metabolic disorders, including T2DM. So improving gut microbiota could be a potential target for managing T2DM. Based on the description above, researchers are interested in analyzing the effect of administering inulin from dahlia tubers on fatty liver in type 2 diabetes mellitus rats.

MATERIALS AND METHODS

This research is a continuation of previous research. The results of the inulin extract from this research were used in this research. This research has been declared to have passed ethical review by the Medical and Health Research Ethics Unit, Faculty of Medicine, Riau University with numberB/046/UN19.5.1.1.8/UEPKK/2023.

This experimental research used a post-test only control group design. The experimental animals used were male Wistar rats, *Rattus novergicus*, aged 8-12 weeks and weighing 180-240 grams. There were 20 experimental animals used based on the Resource Equation Method formula, which was divided into five groups, namely group I (control rats; standard diet and potable water), group II (T2DM), group III (T2DM + 0.5 mg/gBW inulin), group IV (T2DM + 1.0 mg/gBW inulin), and group V (DMT2 + 1.5 mg/gBW inulin).

Induction of type 2 DM in this study was carried out by administration of streptozotocin (60 mg/kg BW) intraperitoneally, followed 15 minutes later by the administration of nicotinamide (120 mg/kg) intraperitoneally. After 72 hours, the blood glucose levels of the rats were measured, blood glucose levels above 250 mg/dL were diagnosed with diabetes[9]. Next, inulin was given according to the determined dose orally using a gastric probe for 21 days to T2DM rats.

Liver organs were harvested after the rats were first anesthetized using ether. The organs were then placed in a 10% formalin buffer. The organs were then placed in 10% formalin buffer. Then, preparations were made using hematoxylin eosin (HE). The preparations were examined using a light microscope, and assessments were carried out at 400x magnification in 5 fields of view. An anatomical pathologist carried out the evaluation. The percentage of fat cells are calculated by the formula (microvesicular steatosis + macrovesicular steatosis / normal cells + microvesicular steatosis + macrovesicular steatosis) x 100%.[10] The percentages for each preparation were added up, and the average was calculated. The results obtained were then categorized based on Brunt grading to assess steatosis, with grading as follows: grade 0 (<5%, normal); grade 1 (5–33%, mild); grade 2 (33–66%, moderate) and grade 3 (>66%, severe).[11]

The data obtained were analyzed with SPSS 29.0 software. This research uses non-parametric statistical tests. Kruskal Wallis then continued with the Mann-Whitney test to determine the difference between groups.

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RESULTS

Based on the results of descriptive tests carried out, it was found that the highest mean percentage of fatty liver was in the DMT2 (II) group, namely 16.45% ± 17.72 which was included in the grade 1 category (mild steatosis). Meanwhile, the lowest mean fatty liver was in the control group (I), 0.30% ± 0.25, included in the grade 0 (normal) category. Test carried out non-parametric Kruskal Wallis and obtained significance results <0.05. These results indicate that the administration of dahlia tuber inulin extract significantly affects T2DM-induced fatty liver in rats. The results of descriptive and comparative analysis of differences using Kruskal Wallis are presented in Table 1.

Table 1. Mean percentage of fatty liver in various groups

Groups	Mean ±SD	Med (min-max)	p
I	0,30 ±0,25	0,30 (0,00-0,58)	0,001
II	16,45 ±17,72	3,99 (3,85-41,52)	
III	3,42 ±2,53	2,92 (1,28-7,75)	
IV	2,27 ±0,60	2,46 (1,42-2,80)	
V	1,96 ±0,80	2,44 (0,97-2,69)	

Next, the data was subjected to the Mann Whitney test aims to analyze differences between groups. The Mann-Whitney test results showed that the T2DM group (II) had a higher fat percentage and was statistically significant (p<0.05) compared to the control group (I). In the T2DM group, administration of dahlia tuber inulin extract (III-V). The percentage of fat loss liver was lower compared to the T2DM (II) group, which showed a statistically significant difference.

Histopathological observations on the liver steatosis showed more microvesicular and macrovesicular steatosis in

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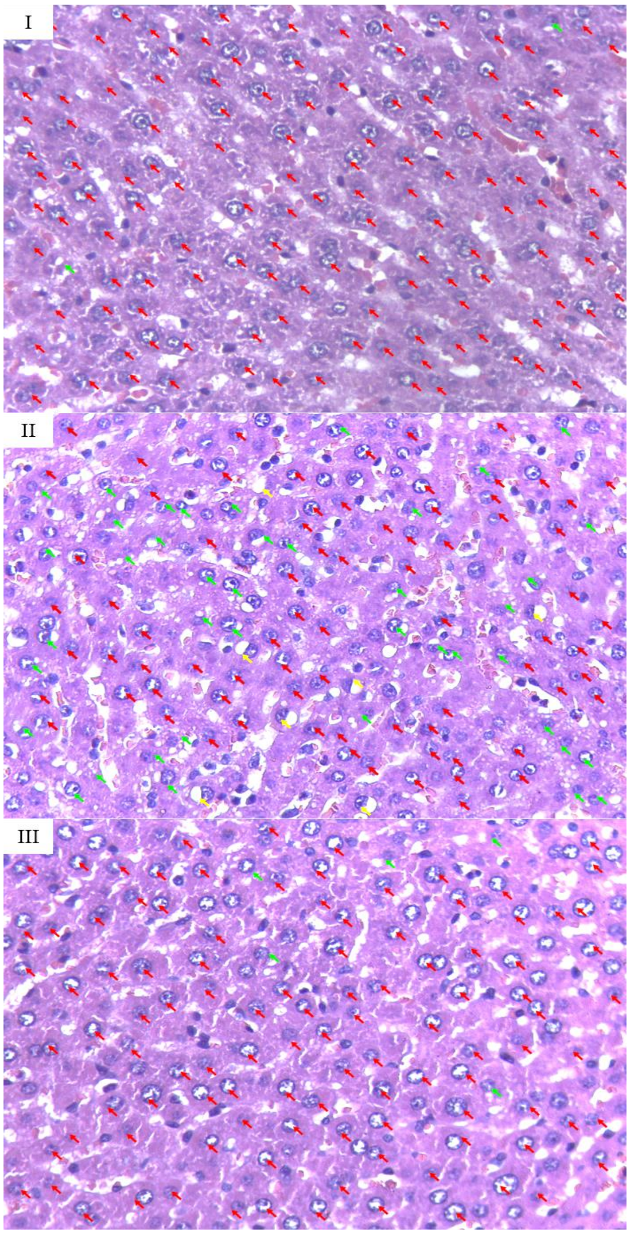
the T2DM group (II) compared to the control group (I). Microvesicular steatosis is characterized by the cytoplasm of hepatocytes containing tiny lipid center of the cell. Meanwhile, macrovesicular steatosis is characterized by one large fat droplets or tiny, well-defined fat droplets occupying the hepatocyte cytoplasm and pushing the nucleus to the edge.[12] Meanwhile, the T2DM group given inulin (III-V) showed a similar reduction in steatosis control group preparations. The histopathological preparations of fatty liver can be seen in Figure 1.



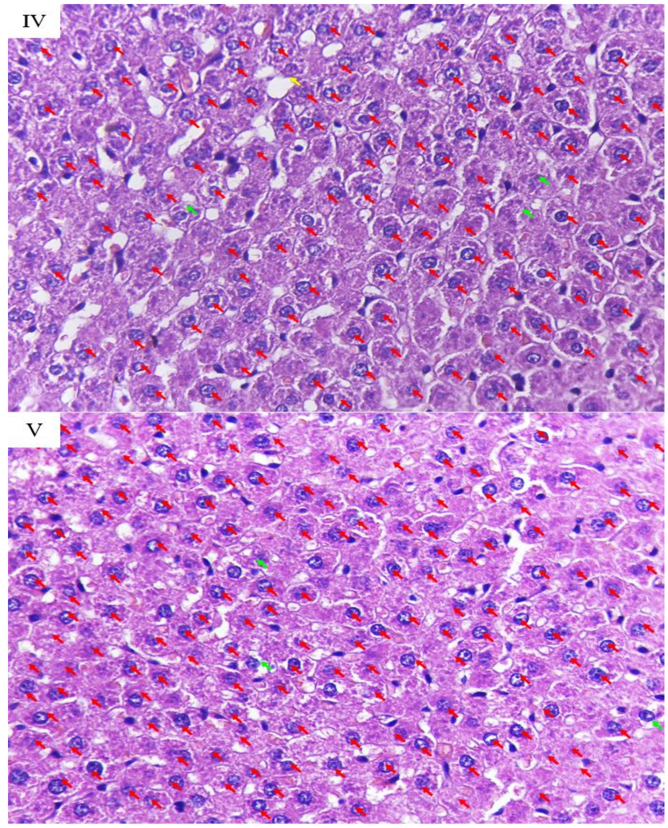


Figure 1. Histopathology of fatty liver of mice from all treatment groups (HE, 400x). Normal cells (red arrow), microvesicular cells (green arrow), macrovesicular cells (yellow arrow).

DISCUSSION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by persistent hyperglycemia that occurs due to impaired insulin secretion, insulin resistance, or both.[1] The establishment of the rats model in this study took advantage of the contradictory effects of two chemicals on β cells in the form of streptozotocin (STZ) which is cytotoxic to β cells, while nicotinamide (NA) is cytoprotective. Therefore, STZ-induced β cell damage can be reduced by giving NA so, increasing the percentage of viable cells, and responding to glucose stimulation to release insulin, creating a state of partial insulin deficiency, similar to that in T2DM.[9]

Fatty liver occurs due to insulin resistance and increased blood glucose levels in diabetes. This condition increases lipid synthesis (lipogenesis) including increased production of fatty acids and triglycerides in the liver. As a result of high-fat metabolism, fat accumulates in liver cells and causes fatty liver or steatosis.[13]

In this study, the T2DM group that was given inulin extract at all doses showed a significant reduction in the percentage of fatty liver ($p<0.05$). This reduction in fatty liver percentage shows that the dose of 0.5 mg/g BW in this study is the minimum against fatty doses to produce a protective effect liver in T2DM mice. The results of this study are in line with research by Monter et al. (2022), which shows that administration of inulin reduces the percentage of liver fat in mice induced by a high-fat diet.[14] Inulin can reduce liver fat content by reducing the expression of the enzyme acetyl-CoA carboxylase and fatty acid synthase activity in the liver, which are involved in lipogenesis. [15,16]

Increased insulin sensitivity was found with inulin administration through reducing systemic inflammatory responses and improving gut microbiota.[17] A meta-analysis showed that inulin can reduce fasting blood glucose [18] lowering lipids in the blood.[19] Reducing blood glucose and blood lipid profiles will reduce triglyceride synthesis in the liver.

Inulin as a prebiotic has shown adequate protection against metabolic disorders. The dysbiosis of the gut microbiota in diabetes plays a role in the severity of hepatic steatosis. Besides food, microbiota dysbiosis can disrupt the integrity of the intestinal mucosa. Compromised intestinal mucosal integrity makes it easier for microbiota products, including LPS and intraluminal bacteria, to translocate to the enterohepatic circulation. LPS activates pro-inflammatory cytokines in the liver, which can worsen fatty liver. Dysbiosis of the gut microbiota also causes abnormalities in Short Chain Fatty Acid (SCFA) components. Apart from playing a role in regulating fat synthesis in the liver, SCFA also maintains the intestinal barrier and has effects anti-inflammatory.[20,21]

Research shows that inulin alleviates gut microbiota dysbiosis by increasing *Lactobacillus*, *Bacteroides*, *Phascolarctobacterium*, and *Lachnoclostridium* are SCFA-producing bacteria.[22] Inulin also reduces body mass index and improves liver steatosis through *Akkermanisa* and *Butyricicoccus* enrichment in on mice obese mice.[15] Then, research induced by a high-fat diet showed that the administration of inulin reduced LPS, TLR-4 and NF- κB levels. Inulin also increased serum levels of glucagon-like peptide-1 (GLP-1) in diabetic mice.[23] Other research showed that the administration of inulin to diabetic rats increased glucose tolerance and reduced levels of TNF-α, IL-6 and LPS by increasing *Bacteroides* in the intestines of diabetic rats.[24]

CONCLUSION

Based on the research results, it was concluded that the inulin extract of dahlia tubers (*dahlia variabilis*) could reduce fatty liver in diabetes mellitus rats.The dose of 0.5 mg/gBW of dahlia tuber inulin extract in this study was the lowest dose that provided a protective effect.

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