ORIGINAL ARTICLE

Effect of Turmeric Powder and Extract on the Level of Triglyceride, Total Cholesterol and Liver Histopathological Appearance in Alloxan-induced Wistar Rats

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ABSTRACT

Introduction: Dyslipidemia and hyperglycemia can cause damage to the body, such as liver damage. Curcumin in turmeric is known to have potential anti-hypercholesterolemia and hepatoprotective effects. The aim of this study was to determine the effect of turmeric extract and powder on liver histopathological appearance, total cholesterol, and triglyceride in alloxan-induced Wistar rats. **Methods:** An experimental study using twenty male Wistar rats, divided into 4 groups. C1 was the normal control group, C2 was induced by intraperitoneal injection of alloxan with a single dose (160 mg/kg BB). T1 and T2 were induced by alloxan and orally administered by turmeric extract and powder (200mg/kg BW) for 21 days. The indicator of the examination was the percentage of liver damage from histopathological assessment and the lipid profile levels. **Results:** The steatosis, ballooning degeneration, inflammation degree, and NASH category result of C2 and T2 showed significant differences (p<0,05). The result of C2 and T1 in steatosis, inflammation degree, and NASH category showed a significant difference (p<0,05), but in ballooning degeneration, there was no difference. The cholesterol level on C2 (78,7±4,73mg/dL) was significantly higher than T2 (57,8±11,24 mg/dL; p=0,028) and T1 (55,6±5,98 mg/dL; p=0,009). Triglyceride level on C2 (134,6±36,49 mg/dL) was significantly higher than T2 (60,78±14,34 mg/dL; p=0,028) and T1 (103,1±54,77 mg/dL; p=0,716). **Conclusion:** Turmeric extract and powder has an effect in decreasing the level of total cholesterol, serum triglyceride, and can reduce the liver damage in histopathology appearance of Alloxan induced Wistar rats.

Keywords: Turmeric, NAFLD, Cholesterol, Triglycerides

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INTRODUCTION

Diabetes mellitus is a disease with an increase in blood glucose level (hyperglycemia), caused by a sufficient production of insulin or insulin resistance (1). Hyperglycemia can cause liver damage, wherein the liver is a collection of insulin-sensitive tissue (2). From the study of Mohammed et al, it has been shown that diabetes mellitus can cause fatty liver, resulting from the accumulation of fat cells in the liver (2). NAFLD is classified into non alcoholic steatohepatitis (NASH) and nonalcoholic fatty liver (NAFL). NAFL is a benign condition, whereas NASH is a progressive disease that can progress to cirrhosis liver and hepatocellular carcinoma. In some cases, NAFL can progress to NASH (3). NAFLD activity score uses the cumulative score of steatosis, ballooning degeneration, and inflammation to grade NAFLD (4).

Diabetes mellitus can initiate liver damage, coronary heart disease, atherosclerosis, or even stroke, due to dyslipidemia risk factors or disruption of lipid metabolism that can cause an increase of total cholesterol and triglyceride (TG) level (5). Dyslipidemia in diabetes consists of an elevated serum concentrations of TG-rich lipoproteins, low concentrations of cholesterol-rich high-density lipoprotein (HDL)2-C, and a high prevalence of small dense low-density lipoprotein (LDL). A central lipoprotein abnormality is an increase in large TG-rich very-low-density lipoprotein (VLDL) and other lipoprotein abnormalities

that are metabolically linked to increased TRLs (6). In previous research Harikrasnha et al, patients with NAFLD have other clinical characteristics that qualify them for the diagnosis of metabolic syndrome (88% of those with steatohepatitis had metabolic syndrome). A common operative definition of the metabolic syndrome is the presence of any three of the five abnormalities: abdominal obesity (increased waist size or waist ratio), impaired glucose tolerance, elevated triglycerides, low concentrations of cholesterol-rich high-density lipoprotein (HDL)2-C, and elevated blood. (3).

Alloxan which is chemically known as 5,5-dihydroxyl pyrimidine- 2,4,6-trione is an organic compound, an urea derivative, a carcinogen, and cytotoxic glucose analog. Alloxan is one of the common diabetogenic agents that often used to assess the antidiabetic potential of both pure compounds and plant extracts in diabetes studies. Alloxan has been successfully induced in a variety of animal species; rabbits, mice, rats, monkeys, cats and dogs, by a mechanism which basically involves partial degradation of the beta cells of pancreatic islets and subsequent compromise in the quality and quantity of insulin produced by these cells. Alloxan can inhibit glucose-stimulated insulin secretion and trigger selective necrosis of pancreatic beta cells by inducing the formation of reactive oxygen species(ROS) (7).

Herbal medicines play an important role in the management of diabetes mellitus, especially in developing countries where resources are limited. Curcumin in turmeric is known to have potential anti-hypercholesterolemia and hepatoprotective effects. In previous research Olatunde et al, it has been proven that turmeric contained high antioxidant activity, the hepatoprotective effect, anti-hyperlipidemia, and anti-hypercholesterol effects (8). From the study of Maithili et al, it has been shown that curcumin can be used as an antihyperglycemic, as well as inhibits the development of further complications (9).

The objective of this study is to determine the effects of turmeric extract and powder on liver histopathological appearance, total cholesterol, and triglyceride in alloxan-induced Wistar rats. Extracts and powder forms are expected to have the same efficacy, and can be applied more effectively

MATERIALS AND METHODS

Samples and Experimental Design

This study was done at Animal Laboratory of Universitas Negeri Semarang, Indonesia. It was an experimental research using 20 Wistar rats with the inclusion criteria were male, 150-300 grams weighed, two-three months of aged, healthy, active, and had no anatomical abnormality. The samples were divided into four groups. C1 was the normal control group, C2 was induced by intraperitoneal injection of alloxan with a single dose (160 mg/kg BB). T1 were induced by alloxan and orally administered by turmeric extract (200mg/kg BW) for 21 days and T2 were induced by alloxan and orally administered by turmeric powder (200mg/kg BW) for 21 days (8).

Turmeric Powder Making

Turmeric rhizome was cut and peeled with a knife and cleaned. Then, it was blanched for ten minutes using a steaming pot under 60^{o}C. The turmeric was then sliced in a 3 mm diameter with a knife. Then, it was dried using an oven under 50^{o}C for 24 hours. The last process was grinding the turmeric with a blender, and then sifted using an 80 mesh sieve (10).

Turmeric Extract Making

Turmeric rhizomes were weighed ±500 grams, then washed, drained, and dried with 50^{o}C oven to obtain dry weight. Dry weights were weighed and powdered (milled and sieved). Dry powder weighed ±50 mg then put into filter paper and incorporated soxhlet flask. Furthermore, soxhletation process was done with 96% alcohol solvent (±500 mL). Turmeric rhizome extract obtained in the form of thick (paste). To facilitate the administration of white mice, the extract is diluted with aquadest. Termination and preparation of the sample for histopathological analysis (11).

Termination and preparation of sample for histopathological analysis

On the twenty-first day, the rats were terminated using chloroform steam. The liver was carefully dissected, trimmed from the fats and connective tissues to remove blood. The liver was fixated in 10% formal saline. The tissue processing was conducted manually in two days. On the first day, the tissues dehydrated in alcohol content. On the second day, the tissues were chemically cleaned using xylene, then infiltrated and planted in paraffin. Then, paraffin block tissue was cut using rotary microtome and perpendicular oriented towards the liver's length axis. The obtained serial part with 3 µm thickness was placed in a clean preparation glass covered in albumin. The slide was colored with Haematoxylin Eosin (HE). The microscopic analysis was conducted using a Leica DM 750 microscope of LZ software serial (12).

The Measurement of Total Cholesterol, and Triglyceride

The observation on the total cholesterol and triglyceride levels of the rats was conducted at the end of the research of each tested animal. The observation was conducted at the Medical Laboratory with Cholesterol Oxidase-PAP (CHOD-PAP) and GPO-PAP methods in mg/dl unit.

Liver histopathology assessment

The histopathology assessment were blindly made by two anatomical pathologist from the Faculty of Medicine, Diponegoro University, Indonesia. NAFLD assessment

used a scoring system that is based on North American Steatohepatitis Clinical Research Network. This system assesses three main things, which are steatosis, lobular inflammation, and liver cell's ballooning degeneration, but also merged them all together into a score of 'NAFLD Activity Score' (NAS) (13).

Statistical analysis

The test used for histopathological appearance was Chi-Square comparative and Fisher's Exact tets. The bivariate analysis used for total cholesterol and triglyceride was One Way ANOVA and Kruskal-Wallis. Statistical analyses were done using SPSS version 23.0.

Ethical Clearance

The ethical clearance was obtained from the Health Research Ethics Committee of the Faculty of Medicine, Diponegoro University (No. 41/EC/H/FK-RSDK/V/2018).

RESULTS

Blood Glucose Level

The blood glucose levels of each group that measured before the start of the study showed that it was in the normal range, which was 84-95 mg/dl. There was an increase in blood glucose levels in C2, T1, T2 after being induced by alloxan. T1 and T2 showed a decrease in blood glucose levels after the 7th day.

Steatosis Assessment

As shown in Fig. 1A, the statistic test result between C1 and C2 showed p=0,008 which could be concluded that there was a significant difference. The result between C2 and T1 showed p=0,014 and C2 with T1 showed p=0,014, displaying that there were significant differences. As shown in Fig. 2 score 0, showed that no steatosis was found or the discovered steatosis was <5% that was considered as physiological steatosis. Score 1, showed the image of a cell with 5-33% steatosis. Score 2, showed the image of a cell with 34-66% steatosis. Score 3, showed the image of cells with >66% steatosis or almost all cells had steatosis.

Ballooning Degeneration Assessment (Liver Cell Inflammation)

As shown in Fig. 1B the statistic test result between C1 and C2 showed p=0,008 which could be concluded that there was a significant difference. An insignificant difference was found on C2 and T1 with p=0,165, while on C2 and T2 was found a significant difference with p=0,045. As shown in Fig. 3 score 0, showed the image of liver cells that did not experience ballooning degeneration. Score 1, showed that there was a little image of liver cells which experienced ballooning degeneration. Score 2, showed that there were many liver cells that experienced ballooning degeneration.

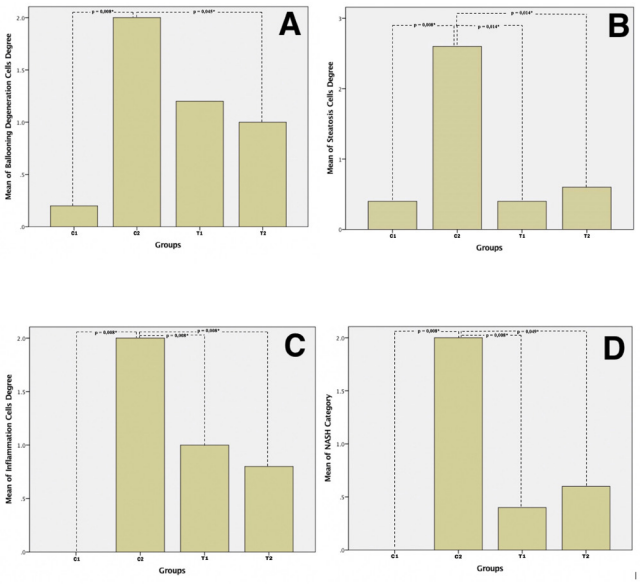

Fig. 1 : Comparative Analysis of Steatosis, Ballooning Degeneration, Lobular Inflammation, and NASH Category in Liver Histopathology Assessment. C1 (Normal Control Group); C2 (Alloxan-Induced Group); T1 (Turmeric Extract Group); P2 (Turmeric Powder Group).

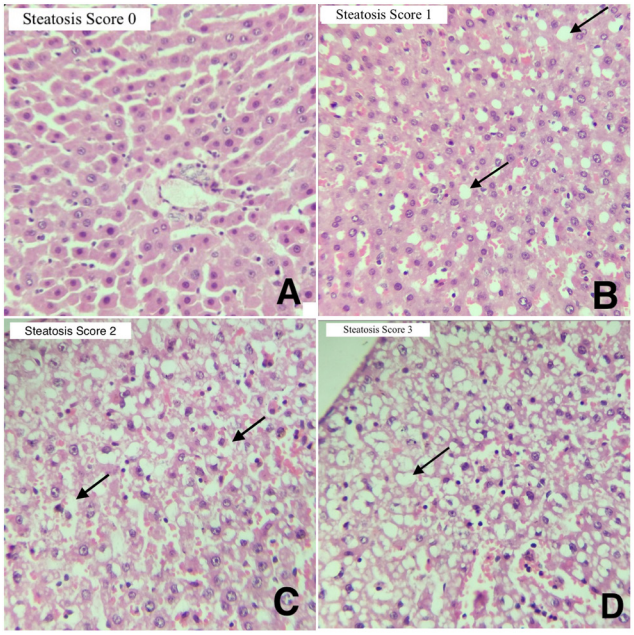

Fig. 2 : Steatosis Score in Liver Histopathology Appearance. (A) Score 0, (B) Score 1, (C) Score 2, (D) Score 3.

Lobular Inflammation Assessment

As shown in Fig.1C the statistic test result between C1 and C2 showed a significant difference with p=0,008 which could be concluded that there was a significant difference. A significant difference was also found between C2 and T1 with p=0,008 and between C2 and

T2 with p=0,008. As shown in Fig. 4 score 0 showed the image of normal liver or no lobular inflammation was found. Score 1, showed that there was lobular inflammation of <2 inflammation focus. Score 2, showed that there were lobular inflammations of 2-4 inflammation focuses.

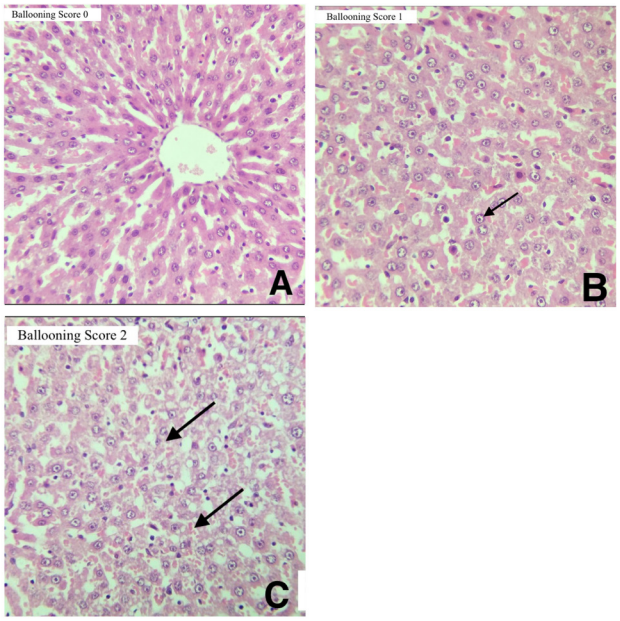

Fig. 3 :Ballooning Degeneration Score in Liver Histopathology Appearance. (A) Score 0, (B) Score 1, (C) Score 2.

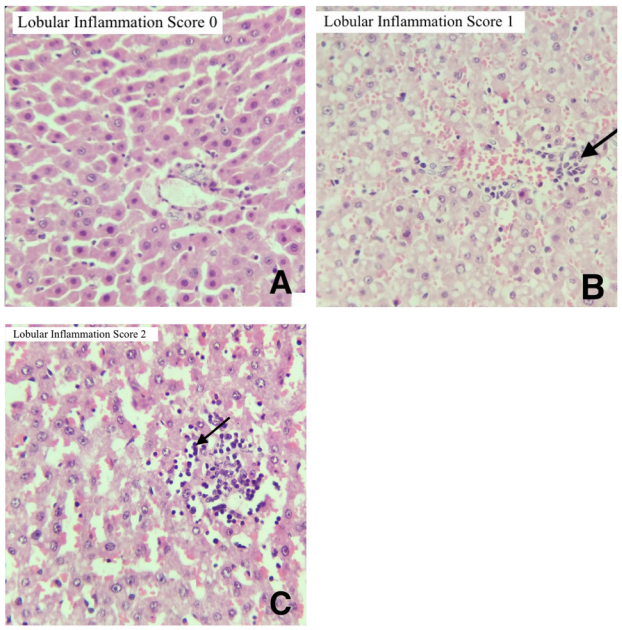

Fig. 4 : Lobular Inflammation Score in Liver Histopathology Appearance. (A) Score 0, (B) Score 1, (C) Score 2.

NASH Category

As shown in Fig. 1D the statistic test result between C1 and C2 showed p=0,008 which could be concluded that there was a significant difference. A significant difference was also found between C2 and T1 with p=0,008 and between C2 and T2 with p=0,049.

Cholesterol Level

Based on the data in Table I, the mean cholesterol level was found in C2 (78,7 ±4,73 mg/dL), while the lowest one was in the T1 (55,6±5,98 mg/dL).

Triglyceride Level

Based on the data in Table I, the highest LDL level was found in C2 (134,6 ±36,49 mg/dL), while the lowest one was in C1 (32,3 ± 16,55 mg/dL).

Table I : Blood Cholesterol and Triglyceride Level on All Groups

No	Group	Cholesterol level (mg/dl)		Triglyseride Level (mg/dl)	
		Mean Standard Deviation	Median (min-max)	Mean Standard Deviation	Median (min-max)
1.	Control 1 (C1)	77,6 ± 9,17	72,8 (69,90 – 90,30)	32,3 ± 16,55	35,4 (4,21 – 47,10)
2.	Control 2 (C2)	78,7 ± 4,73	77,3 (75,60 – 87,00)	134,6 ± 36,49	132,6 (83,40 – 174,70)
3.	Treatment (T1)	55,6 ± 5,98	56,9 (45,60 – 61,40)	103,1 ±54,77	86,1 (50,50 – 185,00)
4.	Treatment (T2)	57,8 ± 11,24	57,5 (47,80 – 76,10)	60,78 ± 14,34	58,9 (45,90 – 84,50)

DISCUSSION

The result of this research showed a liver morphology changes in the alloxan-induced rat. It is consistent with the previous research which stated that alloxan induction may result in liver damage (11). The insulin resistance is thought to have a major influence on the onset of Non-Alcoholic Fatty Liver Disease (NAFLD) caused by an increase of lipogenesis and increase of lipolysis due to the high levels of free fatty acids in the liver. Lipogenesis and lipolysis ultimately lead to hepatic steatosis. Increased levels of oxidative stress and inflammation occurs where elevated levels of reactive oxygen species (ROS) and lipid peroxidase will activate stellate cells, causing steatohepatitis and fibrogenesis, which eventually led to the further development of NAFLD to Non-Alcoholic Steato Hepatitis (NASH) and cirrhosis.

This research proved that turmeric can reduce and prevent morphology changes on the liver, as consistent with the previous research which stated that curcumin had been proven to relieve and prevent steatosis and inflammation on the liver. Curcumin-administered

groups experienced less NASH compared to alloxan-induced control groups, showing that the turmeric's curcumin content successfully reduced and prevent NASH from occurring on the tested subjects. An initial study showed that curcumin might play a useful role in inhibiting NASH growth through its anti-inflammation and antioxidant effect (14), as well as its hepatoprotective effect (15). Since the increase of oxidative stress and liver inflammation plays a key role in the growth of NASH, curcumin supplementation is the feasible candidate to be used as the potential therapy for this disease (14), and the other research also stated that curcumin had been proven to prevent the accumulation of oxidative stress on the liver (16).

There was no difference between turmeric extract group and turmeric powder group means that both turmeric extract and powder had similar effects on NASH growth. It can be concluded that both turmeric extract and powder have the same effectiveness towards NASH prevention.

This study also showed that rats from T1 and T2 groups had lower cholesterol levels if compared to control 1 and control 2 groups. This is in accordance with the hypothesis that curcumin's wide pharmacological aspects (anti-inflammation, antioxidant, antimicrobial, and cancer prevention) are effective for many diseases, including neoplastic, neurologic, cardiovascular, and metabolic disorders. Another study also showed that it has a hypolipidemic and hypoglycemic attribute (17). Triglyceride level within this research presented that rats from T1 and T2 groups had lower triglyceride levels compared to rats from the control 2 group. Turmeric powder also displayed hypolipidemic effects by inhibiting liver triglyceride's secretion. Meanwhile, triglyceride is the most efficient fat to store energy. In the development stage, a lot of fats are used for energy needs.

CONCLUSION

Based on this research, it can be concluded that turmeric extract and powder has an effect in decreasing the level of total cholesterol, serum triglyceride, and can reduce the liver damage in histopathology appearance of Alloxan induced Wistar rats.

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