

The Effect of Moringa Leaf Extract on Hyperglycemic Rat Liver Function

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Abstract. One of the management of diabetics is to maintain stable glucose levels. Often diabetes treatment combines chemical drugs with medicinal plants. People have consumed a lot of Moringa leaves which are believed to be able to maintain body condition. One of the benefits of Moringa leaves with phytochemical components in it is as a hepatoprotector. The aim of this study was to analyze the role of Moringa leaf extract on liver function parameters of hyperglycemic rats. There were 4 groups, normal control (K0), hyperglycemic rats with induction of alloxan 125 mg/kg bw and divided into three groups treated with moringa leaf extract at a dose of 0 mg (K1), 200 mg (T1) and 400 mg/kg bw (T2) for 21 days. The variables measured were aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, total cholesterol. Data were analyzed with Anova $P < 0.05$ and Least Significance Different (LSD) follow-up test. The results showed that the levels of AST and ALT were highest in the K1 group which then decreased significantly in the group that received Moringa extract (T1 and T2). Statistically there was a significant difference ($p < 0.05$) between the control and treatment groups. Cholesterol levels in the control group were significantly lower than the diabetes and treatment groups, but there was no significant difference between the treatment groups. The conclusion is that Moringa leaf extract has an effect on reducing liver enzyme levels and cholesterol in hyperglycemic rats. This research adds to the study of the hepatoprotective potential of Moringa leaf extract in hyperglycemic rats.

Key words: cholesterol; hyperglycemia; liver enzymes; Moringa extract

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INTRODUCTION

Moringa (*Moringa oleifera*) is known as the “miracle tree” because it has various pharmacological properties with significant nutritional value and has been scientifically evaluated for various medicinal applications. The leaves of this wonder plant are a source of protein, -carotene, vitamins (A, B, C, E, riboflavin), nicotinic acid, folic acid, pyridoxine, amino acids, minerals, various phenolic compounds (Mallya *et al.*, 2017; Bhattacharya *et al.*, 2018). As a source of protein, Moringa leaf powder is used as an addition to Tambaqui fish feed (*Colossoma macropomum*) which accelerates growth and can be used as a supplement to replace soybean flour (Safrida *et al.*, 2020).

Moringa has natural antioxidants with high concentrations such as vitamins A, C, E and phenolics also contain 46 antioxidants that help cells to neutralize free radicals (Arise *et al.*, 2019). Moringa leaves have been shown to have hepatoprotective activity and lower plasma lipids in rats fed a high-fat diet. It also has a cardioprotective

role in isoproterenol-induced myocardial infarction by influencing the activity of several enzymes associated with oxidation (Almatrafi *et al.*, 2017).

Many studies show that Moringa extract can act as an anti-inflammatory, isothiocyanic components, phenolic acids, flavonoids, and terpenoids have antihyperglycemic activity (Wardhani, 2020). The antioxidant and hepatoprotective activities of Moringa leaves are related to free radical scavenging activity by the presence of total phenolics and flavonoids in the extract (Singh *et al.*, 2014). The biological and pharmacological activities of Moringa can help reduce cell death and proliferation, such as its antiproliferative effect on human cancer cells (Omodanisi *et al.*, 2017).

Previous studies have shown that the content of bioactive substances in Moringa leaves, such as phenolic compounds and flavonoids, can reduce plasma lipid profiles which may be caused by upregulation of low-density lipoprotein receptor expression, inhibition of hepatic lipid synthesis, lipoprotein secretion (Bhandari *et al.*, 2011). The

results of the study by Fakurazi *et al.* (2012) confirmed that a high-fat diet causes histopathological changes in the form of hepatocellular damage. Moringa extract given to hyperlipidemic rats was able to temporarily reduce enzyme levels and prevent liver damage.

Hyperglycemia is a characteristic of diabetes mellitus, a disorder or abnormality in metabolism. In diabetes, hyperglycemia generates free radicals through increased glycolysis and glucose auto-oxidation (Chikezie *et al.*, 2015), mediated by the byproducts of non-enzymatic glycolysis (AGEs) and the polyol pathway (Lucchesi *et al.*, 2013; Kumar, 2018). The goals of diabetes management include maintaining stable blood glucose levels and the use of therapeutic agents that have few side effects due to long-term diabetes treatment. An alternative is to use plants in the treatment and management of diabetes to reduce the severe side effects of antidiabetic drugs.

Metabolic disorders in diabetes can affect many organs, such as the liver, which plays a key role in the regulation of carbohydrate, lipid, and protein metabolism. Elevated levels of AST, ALT, and -glutamyltransferase (GGT) commonly observed in diabetes are the most specific markers of liver injury (Music *et al.*, 2015). A recent report demonstrated a significant association of elevated ALT and AST with insulin resistance, type 2 diabetes, and metabolic syndrome (Idris *et al.*, 2013)

Insulin resistance triggers lipolysis which causes free fatty acids to accumulate in hepatocytes and causes oxidative stress in mitochondria (Regnell & Lernmark, 2011; Loria *et al.*, 2013). These combined actions ultimately lead to inflammation and cellular necrosis (Lucchesi *et al.*, 2013). Severe liver damage can be demonstrated by elevated levels of damage biomarkers, such as ALT and AST. Both enzymes have physiological and clinical significance. ALT levels in the liver have much higher concentrations than elsewhere, therefore increased ALT activity specifically reflects liver damage, which is a normal occurrence in diabetes possibly due to leakage of the

enzyme into the bloodstream (Woldekidan *et al.*, 2021). Various forms of cell death, both apoptosis and hepatocellular necrosis, have the potential to cause an increase in cell membrane permeability resulting in the release of transaminases in the bloodstream (Mallya *et al.*, 2017; Omodanisi *et al.*, 2017).

This study aimed to analyze the role of Moringa leaf extract on liver function parameters, such as AST, ALT, and cholesterol levels in alloxan-induced hyperglycemic rats. The results of the study in the form of liver function parameters added to the study of the potential of Moringa leaf extract as a hepatoprotector.

METHODS

Animals

Experimental animals were acclimatized for 1 week with food and drink ad libitum, placed in groups in plastic cages. Rat were kept in 12:12 bright dark lighting. The sample consisted of 20 male Wistar rats weighing 150-200 grams which were divided into 2 groups, the control group (K0) and the hyperglycemic group given Moringa leaf extract 0 mg (K1), 200 mg (T1) and 400 mg/kg body weight. (T2). Hyperglycemic rats were induced by alloxan 125 mg/kg with blood glucose levels > 120 mg/dl. Treatment for 21 days with the observed variables in the form of ALT and AST levels. Research using experimental animals has obtained approval from the Health Research Ethics Commission, Faculty of Sports Science, State University of Semarang No: 276/KEPK/EC/2021.

Extraction of Moringa leaf

Fresh Moringa leaves were cut into pieces, dried at room temperature, smoothed using a blender and then sieved to obtain Moringa leaf powder. The



Figure 1. Moringa leaf extract application on hyperglycemic albino rats

Table 1. Mean \pm SD ALT, AST, and Cholesterol levels in Rats after 21 days

No	Group	ALT (U/L)	AST (U/L)	Cholesterol (mg/dl)
1.	K0	136.9 \pm 22.2 ^a	87.6 \pm 36.7 ^{bd}	39.1 \pm 3.43 ^{bcd}
2.	K1	163.2 \pm 69.4 ^c	262.1 \pm 79.5 ^{acd}	86.5 \pm 4.58 ^{acd}
3.	T1	101.2 \pm 26.3 ^b	122.7 \pm 23.0 ^b	63.9 \pm 5.43 ^{ab}
4.	T2	137.8 \pm 37.6 ^a	161.7 \pm 29.2 ^{ab}	60.7 \pm 13.22 ^{ab}

^{abcd} = different letters indicate significant differences

powder was macerated and soaked using 96% ethanol as solvent. The results of the maceration were tightly closed and stored at room temperature while stirring every day until the macerate was at the bottom. The macerate was filtered with a flannel cloth and the resulting solution was put in the oven to obtain a powder.

Variable Measurement

Blood was taken from the retroorbital vein and centrifuged at 5000 rpm for 15 minutes to obtain serum as a measurement of liver function indicators. Variables including AST, ALT and cholesterol levels were measured by Colorimetric Assay Kit Catalog #: K753, K752 and K623 (BioVision).

Statistics analysis

Data in the form of AST, ALT and cholesterol levels obtained were expressed as Mean \pm Standard Deviation. Statistical test using ANOVA with $p < 0.05$ and continued with the smallest significant difference test (LSD).

RESULTS AND DISCUSSION

The research variables observed were liver function biomarkers in the form of AST and ALT serum levels in normal rats and hyperglycemic rats induced by alloxan 125 mg/kg body weight. Treatment rats were given Moringa extract with a successive dose of 0 mg, 200 mg, and 400 mg/kg for 21 days. The results showed that the variables measured in the positive control group (K1) had the highest levels and then had a downward trend with the administration of Moringa leaf extract (T1 and T2). Although the levels of AST, and ALT in the normal group were still the lowest compared to other groups.

The Statistical tests showed that the data were normally distributed (sig. > 0.05), homogeneous (sig. > 0.05) and the administration of Moringa extract had an effect ($P < 0.05$). The difference in the effect of each group was carried out by using the LSD test. ALT levels in the diabetes group (163.2 \pm 69.4) were significantly different from the group receiving 200 mg/kg BW extract (101.2 \pm 26.3), but not significantly different from the control group and those receiving 400 mg/kg extract. AST levels in the

diabetes group were significantly different from all groups, as well as for the control group, it was significantly different from the group receiving 400 mg/kg extract, but there was no difference between groups receiving Moringa leaf extract. Measurement of liver enzyme levels is shown in Table 1.

Liver function biomarkers such as low ALT and AST levels in the control group (136.9 \pm 22.2 and 87.6 \pm 36.7) increased in the diabetic rat group (163.2 \pm 69.4 and 262.1 \pm 79.5). After getting Moringa leaf extract for 21 days, it showed a decreasing trend as shown in Figure 1.

In addition to AST and ALT which are normal enzymes secreted by the liver, the cholesterol synthesized by hepatocytes was also measured. Cholesterol levels in the normal group (K0) were the lowest which would then increase in hyperglycemic rats. Giving Moringa leaf extract (T1 and T2) slowly lowers cholesterol levels according to the dose.

Statistical analysis with the LSD further test showed significant cholesterol levels between K0 and all groups, but there was no difference between the treatment groups (T1 and T2). Cholesterol levels in the control group of 39.1 mg/dl increased significantly to 86.5 mg/dl in diabetic rats (K1) and tended to decrease significantly in the T1 (63.9) and T2 (60.7) groups (Figure 2).

The results showed that diabetes significantly increased levels of ALT, AST, creatinine, urea, triglycerides, cholesterol, and uric acid compared to a control group of normal animals. These results support the statement of Mathur et al. (2016) who showed that liver enzymes have shown higher activity in type 2 diabetic patients than individuals without diabetes. The reasons behind the increase in this enzyme due to insulin resistance include oxidative stress from reactive lipid peroxidation and increased proinflammatory cytokines (Mandal et al., 2018).

Non-diabetic rats had significantly lower levels. This study showed that serum levels of ALT and AST were significantly increased only in the diabetic group (K1) when compared to control (K) and treatment groups (T1 and T2). This is an indication of impaired liver function. Increased liver enzyme activity may reflect hepatocyte or biliary epithelial

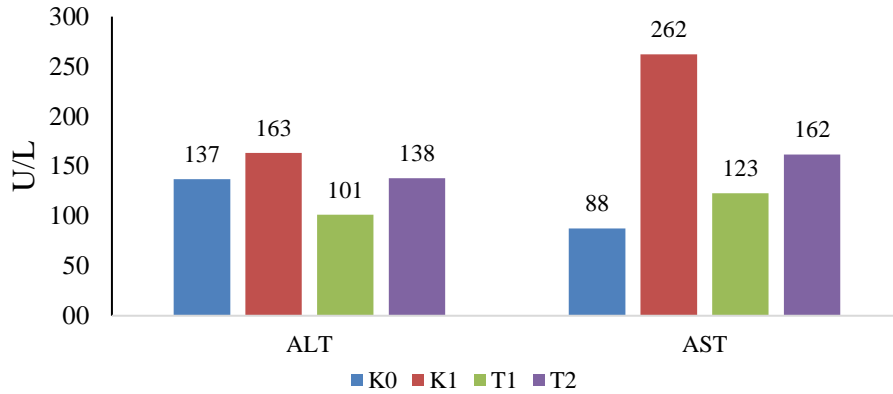


Figure 2. Mean ALT and AST levels in all groups

Note :

K0 = control group

K1 = hyperglycemic group given Moringa leaf extract 0 mg/kg body weight

T1 = hyperglycemic group given Moringa leaf extract 200 mg/kg body weight

T2 = hyperglycemic group given Moringa leaf extract 400 mg/kg body weight

necrosis, compromising the integrity of the hepatocyte membrane. Damaged hepatocyte membranes are likely to cause hepatotoxicity which induced release of enzymes into the circulation leading to an increase in these enzymes in the blood (Singh et al., 2014).

Previous studies evaluated an increase in liver marker enzyme levels in patients with Type 2 DM due to an increase in the effect of glycogen/insulin on liver cells. Increased glycogenolysis and gluconeogenesis from non-carbohydrate precursors into primary metabolic pathways (Balaji et al., 2013). The overload of free fatty acid release due to insulin resistance induces fat mobilization and

results in hepatocyte toxicity (Sunitha et al., 2015).

Administration of Moringa extract reduces the increase in enzyme activity and recovery that is able to affect hepatocytes thereby causing accelerated regeneration of parenchymal cells and lysosomes, thereby protecting against lysosomal integrity and cell membrane fragility, and therefore reducing leakage of marker enzymes into the circulation (Otomoso et al., 2015). The reduction in ALT and AST activity as a result of Moringa leaf extract showed an early improvement in the cellular membrane integrity of liver cells which is a clear manifestation of the anti-hepatotoxic effect (Elbakry et al., 2016). Recovery of these enzymes to

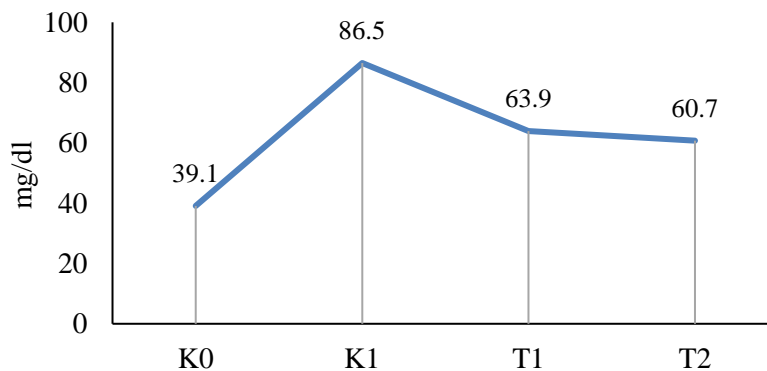


Figure 3. Lowering Cholesterol Levels with Moringa Leaf Extract

Note :

K0 = control group

K1 = hyperglycemic group given Moringa leaf extract 0 mg/kg body weight

T1 = hyperglycemic group given Moringa leaf extract 200 mg/kg body weight

T2 = hyperglycemic group given Moringa leaf extract 400 mg/kg body weight

normal levels indicates the return of normal liver function after administration of Moringa leaf extract. This suggests revival of insulin secretion and regenerative activity of pancreatic islets of Langerhans cells (Woldekidan et al., 2021).

Moringa leaf extract has sufficient effect ability to prevent hepatotoxicity due to DM in part as a result of chemical constituents having hepatoprotective properties. The antioxidant and hepatoprotective activity of *M. oleifera* leaf extract in DM rats was caused by the presence of total phenolic and flavonoid active constituents - sitosterol, quercetin and kaempferol (Singh et al., 2014). Moringa leaf extract has a repair effect on liver damage as one of the complications by reducing serum levels of ALT, AST in the treatment group (Otomoso et al., 2015). Hepatotoxic especially those that follow free radical-mediated mechanisms require materials to overcome liver injury. Flavonoids can control blood glucose by increasing glycolysis and glycogenesis by stimulating glucose utilization so that blood glucose levels decrease. This causes glucose to be available in cells so that it can inhibit the occurrence of gluconeogenesis in the liver (Yuneldi et al., 2018)

The results of the study which proved that there was a decrease in cholesterol levels in the treatment group after being given Moringa leaf extract complemented the results of Reddy et al. (2017). It is suspected that the hypocholesterolemic activity of phenols and statins has been associated with increased bile cholesterol and bile acid concentrations. The decrease in cholesterol levels is also due to the presence of active substances that are hypocholesterolemic. Saponins are active compounds from the steroid or triterpene group that bind to sugars, compounds that have a biological effect on lowering cholesterol (Rame et al., 2020). The flavonoids and saponins present in Moringa leaves are reported to increase HDL and reduce LDL and cholesterol in hypercholesterolemic rats / Decrease cholesterol absorption by inhibiting the solubility of cholesterol micelles (Almatrafi et al., 2017).

Polyphenolic phytochemical extracts were found to counteract the effects of free radicals by a scavenger mechanism (El-Bakry et al., 2016). Liver marker enzymes such as ALT and AST are known as indicators of liver disease and are released from damaged tissue into the bloodstream. The hepatoprotective activity of moringa is thought to prevent leakage of intracellular enzymes from hepatocytes by stabilizing their membranes. From the research of Arise et al. (2019) showed that serum aminotransferase levels tend to be normal in line

with liver cell regeneration.

This study showed that Moringa leaves can reduce liver function parameters which increase in hyperglycemic conditions. The bioactive content in the leaves, such as the flavonoid group, is believed to be involved in maintaining glucose levels in the blood. The antioxidant properties of flavonoids are able to prevent liver damage resulting in increased secretion of liver enzymes into the circulation. The results of the study will contribute to the community in utilizing Moringa leaves to maintain hepatocyte activity or as a hepatoprotector.

CONCLUSION

The proposed conclusion is that Moringa leaf extract plays a role in reducing liver function parameters of hyperglycemic rats, such as levels of AST, ALT, and cholesterol. Suggestion for the next research is to measure oxidative stress parameters to increase the potency of Moringa leaves as an antioxidant agent.

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