RESEARCH ARTICLE

# EFFECT OF KELOR LEAVES (*MORINGA OLEIFERA*) IN LIVER FUNCTION OF STREPTOZOTOCIN INDUCED DIABETIC *SPRAGUE DAWLEY* STRAIN MALE RATS

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## ABSTRACT

**Background:** Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia. Prolonged hyperglycemia can damage various organs of the body including the liver. *Moringa oleifera* leaves extract contain flavonoids which can decrease blood glucose levels and act as a hepatoprotector. To evaluate its hepatoprotective activity, liver function marker s*erum glutamic oxaloacetic transaminase* (SGOT) and *serum glutamic pyruvate transminase* (SGPT ) was used. In this study, we aimed to determine the effect of *Moringa oleifera* leaves extract on the liver function test of male *Sprague dawley* rats that have diabetes mellitus due to streptozotocin induction.

**Methods:** This was experimental study conducted from February 2019 to July 2019 at clinical pathology and animal house laboratory, Faculty of Medicine UIN Syarif Hidayatullah, Jakarta. This study conducted in 25 male *Sprague dawley* rats divided into 5 groups, those were negative controls group (non-DM), positive controls group (DM rats without giving *Moringa oleifera* leaves extract), and 3 treatment groups which given oral *Moringa oleifera* leaves extract at doses of 200 mg/kgBW, 400 mg/kgBW, and 600 mg/kgBW, respectively, for 14 days. Intra-peritoneal induction of streprozotocin was done at a dose of 40 mg/kgBW. Samples were collected on day 26th to measure SGOT and SGPT using ELISA method. Blood glucose levels were assayed on day 6th and 26thusing rapid glucose meter.

**Results:** There was no significant decrease in SGOT (p = 0.069) and SGPT (p = 0.345) levels in the administration of *Moringa oleifera* leaves extract to male *Sprague dawley* rats with diabetes mellitus induced by streptozotocin at a dose of 200 mg/KgBW and 400 mg/KgBW. At a dose of 600 mg/kgBW SGOT levels were higher than the positive control.

**Conclusions:** *Moringa oleifera* leaves extract did not significantly decrease SGOT and SGPT.

**Key words :** *Moringa oleifera*, SGOT,SGPT, *sprague dawley*, streptozotocin.

## INTRODUCTION

Diabetes Mellitus (DM) is a group of metabolic diseases characterized by high blood glucose levels caused by insulin abnormalities.[1] The etiology of diabetes mellitus is insulin resistance in the muscles and liver, and damage to pancreatic β cells. Streptozotocin (STZ) is toxic to pancreatic β cells which can result in insulin deficiency. Diabetes mellitus is a predisposing factor for non-alcoholic fatty liver, non-alcoholic steatohepatosis, and advanced liver fibrosis.[2]

Condition of non alcoholic fatty liver can be detected using several serological methods including *serum glutamic oxalocetic transaminase* (SGOT) and *serum glutamic pyruvate transminase* (SGPT).[3] Results of the study at Gondar University found that 33,3% of patients with type 2 diabetes mellitus had abnormal liver function tests and 23,3% experienced an increase in SGOT.[4]

*Moringa oleifera* leaves extract contain β-sitosterol, phenolic acid, and flavonoids which are anti-inflammatory, antioxidant, hepatoprotector and has effect in decreasing blood glucose and triglyceride levels.[5,6] Another study reported that *Sprague dawley* rats who have streptozotocin induced diabetes mellitus and given *Moringa oleifera* leaves extract for 8 weeks had a significant decrease in fasting blood glucose levels.[7] Administration of *Moringa oleifera* leaves extract at a dose of 250 mg/kgBW for 42 days in diabetic rat showed to have an effect in decreasing liver enzymes and lipid profile parameters.[8] Toppo et al reported that giving *Moringa oleifera* leaves extract at a dose of 500 mg/kgBW for 28 days showed protection against cadmium-

induced hepatotoxicity of rats.[9]

Therefore, this study aims to investigate the effect of *Moringa oleifera* leaves extract on the SGOT and SGPT of streptozotocin-induced diabetic *Sprague dawley* rats.

## METHODS

### Study design and sample

The design of this study is experimental. The study was conducted at clinical pathology and animal house laboratory, Faculty of Medicine UIN Syarif Hidayatullah, Jakarta from February 2019 to July 2019 with a total sample of 25 male *Sprague dawley* rats according to inclusion and exclusion criteria. The number of samples in this study using Mead formula.

The sample were male *Sprague dawley* rats aged 2-3 months and weighing 150-200 mg. The exclusion criteria for this study were rats that died during the study and blood glucose levels <200 mg / dL after being induced by STZ. *Moringa oleifera* leaves extract obtained from the Indonesian Institute of Sciences (LIPI) Serpong from 5 kg of fresh *Moringa oleifera* leaves were dried and extracted by maceration method to obtain 105 grams of extract. The solvent used was 70% ethanol.

The rats were divided into 5 groups (K1,K2,K3,K4,and K5) and each group consisting of 5 rats. K1 was the negative control that was not induced by STZ. Diabetes mellitus was induced in K2-K5 by giving 40 mg/kgBW streptozotocin. In positive control group (K2), *Moringa oleifera* leaves extract was not administered. In group K3, K4, and K5 *Moringa oleifera* leaves extract were administered at the dose of 200 mg/kgBW, 400 mg/kgBW, and 600 mg/kgBW, respectively (Table 1).

**Table 1. Treatment group**

| Treatment group | STZ injection | *Moringa oleifera* |
|---|---|---|
| K1 (negative control) | No | No |
| K2 (positive control) | 40 mg/kgBW | No |
| K3 | 40 mg/kgBW | 200 mg/kgBW |
| K4 | 40 mg/kgBW | 400 mg/kgBW |
| K5 | 40 mg/kgBW | 600 mg/kgBW |

*Sprague dawley* rats were adapted for 5 days in the animal house laboratory, during the adaptation period they were fed M512 and drank ad libitum every day. Cages and bedding are cleaned every 3 days until the termination and excision process. The positive control treatment group, the K3, K4, and K5 treatment groups were fasted for 10 hours and then blood glucose levels were assasyed and injected Streptozotocin 40 mg/KgBW intraperitoneally on the 6th day. Blood glucose was assayed on the 10th day. Treatment groups K3, K4 and K5 were given Moringa oleifera leaves extract at a dose of 200 mg/KgBW, 400 mg/KgBW, and 600 mg/KgBW on the 11th day. Every day, all rats in the treatment group were weighed.

### Blood sampling and assays

Blood glucose levels were assayed on the 6th day before being induced by STZ, on the 11th day in the positive control group and treatment group to ensure that the rats had diabetes mellitus, and on the 26th day after being given treatment and before necropsy. Blood glucose level were assayed using the Glucocheck.

The rats were terminated on the 26th day by inhalation anesthesia using ether, then necropsy was performed. Three mililiters blood from inferior vena cava was drawn using *vacutainer clott activator*. The tube was left for 30 minutes until it was clotted then centrifuged at 5000 rpm for 10 minutes to separate the serum. Blood serum were stored at -20°C and until SGOT and SGPT assayed using ELISA method.

The normality test on the distribution of blood glucose level data was done by Shapiro-Wilk test, the analysis continued with paired t-test. The normality test and homogenity test of SGOT and SGPT data in all groups were analysed with Shapiro-Wilk and Levene and then proceed with the One Way ANOVA parametric test.

### ETHICAL APPROVAL

The Study was approved by ethics committee of the Faculty of Medicine with protocol number B-007/F12/KEPK/TL00/9/2019.

## RESULTS

A total of 24 *Sprague dawley* rats were involved in this study. One *Sprague dawley* rats in experimental group with a dose of 200 mg/kgBW were found dead on day 26th. This is probably due to environmental factors that make it easily infected or stressed.

### Blood glucose level

Based on Table 1, the negative control had normal blood glucose levels while the experimental group at doses of 200

mg/kgBW, 400 mg/kgBW, and 600 mg/kgBW had high blood glucose levels after being induced by STZ. There was no significant increase in blood glucose level in the positive control group and the negative control group. The decreasing of in blood glucose level was observed in all experimental groups, furthermore, a significant decrease in blood glucose levels was found at a dose of 400 mg/KgBW ($p < 0.05$).

**Table 1. Blood glucose level (mg/dL)**

| Group | Before treatment *Moringa oleifera* (mean ± SD) | After treatment *Moringa oleifera* (mean ± SD) | *p*-value |
|---|---|---|---|
| Negative control/non DM | 100.2 ± 15.3 | 115.6 ± 1.8 | 0.078 |
| Positif control/DM without *Moringa oleifera* leaves extract | 428.0 ± 142.8 | 442.2 ± 79.3 | 0.893 |
| DM + *Moringa oleifera* leaves extract 200 mg/kgBW | 444.6 ± 108.3 | 201.25 ± 251.0 | 0.144 |
| DM + *Moringa oleifera* leaves extract 400 mg/kgBW | 399.8 ± 100.6 | 201.4 ± 163.6 | 0.018 |
| DM + *Moringa oleifera* leaves extract 600 mg/kgBW | 464.0 ± 78.3 | 298.4 ± 169.4 | 0.165 |

*Serum oxaloacetic glutamic transaminase* (SGOT) and *serum glutamic pyruvate transminase* (SGPT)

The highest level of SGOT was observed in 600 mg/kgBW experimental group and the lowest was in 200 mg/kgBW experimental group (Table 2). Lower level of SGOT was found in 200 mg/kgBW and 400 mg/kgBW experimental groups compared to the positive control, on the contrary, higher level was found in the 600 mg/kgBW experimental group.

**Table 2. *Serum oxalocetic glutamic transaminase* (SGOT) and *serum glutamic pyruvate transminase* (SGPT) activities**

| Group | SGOT (IU/L) (mean ± SD) | SGPT (IU/L) (mean ± SD) |
|---|---|---|
| Negative control/non DM | 85,2 ± 21,0 | 58,3 ± 8,9 |
| Positif control/DM without *Moringa oleifera* leaves extract | 118.4 ± 27.7 | 80.5 ± 30.1 |
| DM + *Moringa oleifera* leaves extract 200 mg/kgBW | 91.2 ± 24.5 | 52.9 ± 20.4 |
| DM + *Moringa oleifera* leaves extract 400 mg/kgBW | 115.9 ± 27.2 | 58.1 ± 16.8 |
| DM + *Moringa oleifera* leaves extract 600 mg/kgBW | 125.8 ± 20.4 | 90.3 ± 41.6 |

SGOT *Anova* $p > 0.05$
SGPT *Kruskal-Wallis* $p > 0.05$

Lower level for SGOT and SGPT was found in 200 mg/kgBW and 400 mg/kgBW compared to the positive control and negative control. Higher level of SGPT was observed in 600 mg/kgBW experimental group compared with positive and negative control.

## DISCUSSION

*Sprague dawley* rats that have been induced by STZ were confirmed to have diabetes mellitus. This was in accordance with the theory that STZ able to damage pancreatic β cells thus insulin is not synthesized. Mostafavinia et al explained that STZ at a dose of 40 mg/kgBW can induce type 1 DM.[10] Phenolic acid and flavonoids in *Moringa oleifera* leaves extract can restore the integrity of the pancreatic β cell membrane and increase insulin sensitivity in peripheral tissues. Kaempferol, a substance in *Moringa oleifera* leaves extract reported to stimulate glucose uptake in soleus muscle through phosphatidyl inositol-kinase (PI3K) and protein kinase C (PKC) pathway. Quercetin in *Moringa oleifera* leaves extract can inhibit glucose and fructose transport by GLUT 2 in the brain and stimulates expression and translocation of GLUT 4 in skeletal muscles, two mechanisms that can decreased blood glucose level.[11]

The flavonoids in *Moringa oleifera* leaves extract have a hepatoprotective effect by reducing the expression of diacylglycerol acyltransferase (DGAT). Diacylglycerol acyltransferase is an enzyme that helps the formation of triglycerides in the liver, thus lowering its expression can

prevent liver steatosis.[12] An anti-inflammatory effect of flavonoids was based on its capability to decrease activity of nuclear factor kappa-beta (NF-kβ) resulted in resolution of damaged liver cells.[13] The decreasing of hepatic enzyme markers and lipid profile parameters was observed after administration of *Moringa oleifera* leaves extract at a dose of 250 mg/kgBW for 42 days in diabetic rats.[6]

Administration of *Moringa oleifera* leaves extract at a dose of 50 mg/kgBW for 16 weeks to arsenic-induced toxicity rats was shown to inhibit SGOT and SGPT increment in blood serum.[14] This result is in contrast to the previous studies probably due to differences in the duration of administration of *Moringa oleifera* leaves extract and diabetes mellitus inducers.

The experimental group *Moringa oleifera* leaves extract treated with 600 mg/kgBW had higher SGOT and SGPT levels than the positive control. This is probably due to environmental factors that cause infection and stress in rats and possibility of hepatotoxicity that might occurred in higher dose of *Moringa oleifera* leaves extract.

## CONCLUSION

There was no significant decrease in SGOT and SGPT levels in the administration of *Moringa oleifera* leaves extract to male *Sprague dawley* rats with diabetes mellitus induced by streptozotocin at doses of 200 mg/KgBW and 400 mg/KgBW for 14 days.

**Conflicts of interest**
No conflict of interest regarding this publication

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