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# The Hepatoprotective Effect of *Moringa oleifera* Leaves on Male Wistar Rat Induced Streptozotocin-Nicotinamide

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## ABSTRACT

Diabetes mellitus is a disease characterized by uncontrolled increases in blood sugar levels, which can cause complications in the liver. Disorder liver disease can be seen from the increasing SGOT, SGPT activity, and the number of necrosis cells in the liver. Moringa leaves contain the flavonoid quercetin with antidiabetic and antioxidant activity. This research aimed to determine the effect of administering the ethyl acetate fraction of Moringa leaves on SGOT activity, SGPT, and liver histopathology in rat induced by streptozotocin and nicotinamide. Moringa leaves macerated with 80% ethanol and fractionated with ethyl acetate were used. The research subjects were 30 rats divided into six groups. The rats were induced with streptozotocin at a dose of 65 mg/kgBW and nicotinamide 100 mg/kgBW for five days, then treated for ten days. Serum SGOT and SGPT activity were measured using a Microlab 300 Semi-Automated at a wavelength of 340 nm. Histopathological observations were carried out when the rats were dislocated, their livers were taken, and then preparations were made using Hematoxylin Eosin (HE) staining. Data were analyzed using the SPSS One Way ANOVA method, followed by the Tukey test with a confidence level of 95%. The results of the study showed that administration of Ethyl Acetate Fraction of *Moringa oleifera* (EA-MO) could reduce the levels of SGOT, SGPT, and the number of liver necrosis cells in rats induced by streptozotocin and nicotinamide.

**Keywords:** Histopatology; Liver; *Moringa oleifera*; SGOT; SGPT

## INTRODUCTION

Diabetes mellitus (DM) is a disease that involves the endocrine hormones of the pancreas, including insulin and glucagon. Lack of insulin causes the lipolysis of spare fat and the release of free fatty acids, the primary energy source for the entire body, except the brain. Diabetes mellitus (DM) is divided into four categories: type-1 DM, type-2 DM, gestational DM, and other types of DM caused by other factors (Faida & Santik, 2020).

Diabetes mellitus is caused by oxidative stress, which causes lipid peroxidation, resulting in liver cell damage (Hardiningtyas et al., 2014). Damaged liver cells can be caused by Type 2 diabetes disease (Goud et al., 2015). Damage and impaired liver function in Type 2 diabetes disease are characterized by increased levels of transaminase enzymes specific to liver damage, namely SGPT (ALT) and SGOT (AST) levels in the bloodstream (Sharma, 2015).

Type 2 diabetes mellitus in test animals can be induced by Streptozotocin at a dose of 50 mg/kgBW. The addition of nicotinamide induction at 120 mg/kg BW can increase SGOT-SGPT activity with the working mechanism of free radical formation, which causes free radical damage and oxidative stress from entering the aminotransferase enzyme—liver cells to blood vessels (Firdous & Singh, 2016). The mechanism of streptozotocin is forming free radicals and causing oxidative stress which result in damage to liver cells. Damage to liver cells can take the form of degeneration and necrosis. Cell necrosis is characterized by the cell nucleus shrinking and becoming dark in color until there is no euchromatin (pyknosis), the cell nucleus ruptures (karyorrhexis), and the cell nucleus disappears (karyolysis) (Irham & Widyaningsih, 2017).

Significant oxidative damage by free radicals can be prevented using antioxidants found in Moringa leaves (Priyanto et al., 2023). Giving the ethyl acetate fraction of Moringa leaves can decrease SGPT and SGOT levels (Wulan et al., 2019). Administration of Moringa leaf extract functions as a hepatoprotective so that oxidative stress can be inhibited and liver damage can be repaired repair liver damage by accelerating hepatocyte regeneration (Syahrin et al., 2016).

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Although limited pre-clinical and clinical studies regarding anti-diabetic *Moringa oleifera* have been carried out, research on *Moringa oleifera* related to diabetes complications is still limited. Research on liver damage parameters in test animals with DM can serve as an important basis for future studies directed at developing the management of diabetes complications, especially on liver function (Mthiyane et al., 2022).

Based on this background, it is necessary to conduct research to examine the activity of the ethyl acetate fraction in Moringa leaves, which can reduce SGOT, SGPT activity, and liver histopathological features in mice induced by streptozotocin and nicotinamide.

## MATERIALS AND METHODS

### Materials

The materials used in this research were Moringa leaves (*Moringa oleifera*) obtained from the Gunung Kidul area of Yogyakarta, 80% ethanol, aquabidestilata, n-hexane (Brataco Chemical, Yogyakarta), CMC-Na (Brataco Chemical, Yogyakarta), Natrium 0.9% chloride, methanol p.a, 10% formalin buffer, 80% ethanol, citrate buffer, distilled water, n-hexane, ethyl acetate, quercetin (Sigma Aldrich), Gallic acid (Sigma Aldrich), $NaOH_2$ (Merck), NaOH (Merck), $AlCl_3$ (Merck), xylol, alcohol with varying concentrations (100%, 95%, 80%, 70%), technical paraffin, tymol, Mayer hematoxylin solution, Eosin solution, Streptozotocin (Nacalai), nicotinamide obtained from the Laboratory Gajah Mada University Natural Ingredients Pharmacy, SGOT and SGPT (Dyasis) Reagents obtained from the Clinical Pathology Laboratory of Gajah Mada University.

### Methods

#### Preparation of *Moringa oleifera* Extraction

Extraction of *Moringa oleifera* leaf powder was carried out by macerating 1.5 kg of the powder in 10.5 L of 80% ethanol for five days, filtering the mixture using a vacuum filter, and then macerating the residue again using 3 L of 80% alcohol for 24 hours. The resulting macerate was concentrated using a vacuum rotary evaporator at 60ºC and a water bath at 60ºC until a thick extract was obtained (Sulistyawati et al., 2017).

#### Preparation of Ethyl Acetate Fraction of *Moringa oleifera* (EA-MO)

The thick ethanol extract was dissolved in distilled water (1:2). The water-soluble extract was dissolved in an N-hexane solution (1:1). The fraction was fractionated three times. The water-soluble fraction from adding n-hexane was dissolved with ethyl acetate (1:2). Separation was carried out using a separating funnel, taking the ethyl acetate fraction located at the top and the insoluble fraction at the bottom. The insoluble ethyl acetate fraction was fractionated again using new ethyl acetate with a ratio of water-soluble fraction: new ethyl acetate (1:2 and 1:1.5). After that, the soluble ethyl acetate fraction was left in an acid cupboard for ± 3 days so that the ethyl acetate evaporated completely, and the ethyl acetate fraction of Moringa leaf extract was ready to be induced into test animals (Erika et al., 2014).

#### Total Flavonid Content of Ethyl Acetate Fraction of *Moringa oleifera* (EA-MO)

The quantitative analysis of the total flavonoid content followed Ramadhani et al.'s (2022) method with a few modifications. Standard measurements were conducted with quercetin of 5; 7.5; 10, 12.5, 15, 17.5, and 20 ppm. Then, the ethyl acetate fraction of *Moringa oleifera* (EA-MO) was carefully weighed, ±250 mg each, put into a 100.0 mL measuring flask, added with methanol p.a. up to the limit mark, and homogenized. A 10 mL flask was filled to capacity with 1.0 mL of sample, 1.0 mL of $AlC_3$, 3.0 mL of distilled water, and 5.0 mL of metanol pa. After 15 minutes, the absorbance of the solution was measured three times at a wavelength of 424.5 nm using a UV/VIS spectrophotometer.

#### Experimental Animals and Design

This research has received approval from the Research Ethics Committee of Ahmad Dahlan University number 021703005. It used test animals which were divided into six treatment groups and each group consisted of 5 rats. Group I, the normal control, was given standard feed and drinking water ad libitium. Group II, the negative control, was given streptozotocin orally at a dose of 65 mg/kgBW + nicotinamide 100 mg/kgBW. Group III, the positive control, was given streptozotocin at a dose of 65 mg/kgBW + nicotinamide 100 mg/kgBW orally plus metformin at a dose of 45 mg/kgBW once a day. Groups IV-VI were given streptozotocin at a dose of 65 mg/kgBW+nicotinamide 100 mg/kgBW, and ethyl acetate fraction of Moringa leaves at successive doses of 12.5 mg/kgBW, 25 mg/kgBW, 50 mg/kgBW orally. Streptozotocin and nicotinamide were given on day one. The groups were observed until diabetes occured for five days until diabetes occurred for five days with the rats' blood sugar level ≥ 200 mg/dL, which can be included in the positive diabetes category included in the positive diabetes category (Rani et al., 2019).

**Measurement of SGOT and SGPT Activities**

SGOT and SGPT activity measurements were performed on 10 days after treatment. The sample used was male Wistar rat serum taken through the orbital sinus of the eye. SGOT and SGPT activity were measured using a modified Microlab 300 Semi-Automated at a wavelength of 340 nm recommended by the IFCC (International Federation of Clinical Chemistry and Laboratory Medicine).

**Liver Histopthology Profile of Male Rats Wistar**

Liver histopathological preparations in male Wistar rats were conducted using the Hematoxylin Eosin (HE) staining method. Liver histopathology images were read in 5 fields of view on one slice using a microscope with 400x magnification. The results of liver histopathology images were taken using a digital camera to observe lipid degeneration and count the number of necrosis cells (hepatocytes cells) (Roosdiana et al., 2019).

**Statictical analysis**

SGOT and SGPT activity data were analyzed statistically using a one-way variance analysis (ANOVA) followed by the Tukey test. The results of statistical significance analysis can be seen in the differences between groups at the significance level ($p<0.05$). The study used SPSS 25.0 for Windows, while the liver histopathological observations were analyzed descriptively.

## RESULTS

**Determination of Total Flavonoid Content of Ethyl Acetate Fraction of *Moringa oleifera* (EA-MO)**

The total flavonoid content of the Ethyl Acetate Fraction of *Moringa oleifera* (EA-MO) was determined using the $AlCl_3$ method, which is based on the complexation reaction between aluminum ions and flavonoid compounds in an alkaline state so that a red chelate is formed. The total flavonoid content can be determined from the linear regression equation obtained from the standard curve of the quercetin common solution, which can be seen in Figure 1.

As Figure 1 indicates, the linear regression equation is obtained, namely $y = 0.0495x - 0.0046$ with an $R^2$ value of 0.9998 so that the total flavonoid content in Ethyl Acetate Fraction of *Moringa oleifera* (EA-MO) can be determined from the absorbance value obtained. The result of total flavonoid content can be seen in Table I.

**Observation of SGOT and SGPT activity**

SGOT and SGPT activity in Wistar rat serum were measured on the 10th day after treatment using a Microlab 300 Semi-Automated at a wavelength of 340 nm in each group. The result of testing the effect from testing the effect of Moringa leaf ethyl acetate fraction (EA-MO) on SGOT and SGPT activity can be seen in Table II.

**Observation of Liver Histopathology**

The histopathological examination of Wistar rats' liver stained with Hematoxylin Eosin (HE) was examined under a light microscope with 400x magnification, and reading was carried out in 5 fields of view on one slice. The histopathological appearance of liver cells can be seen in Figure 2.

The results of calculating the number of necrosis cells in all research groups were taken from the total of five fields of view and one slice in each research group, and then the average was taken for each group. The results of calculating the number of liver cells experiencing necrosis can be seen in Table III.

## DISCUSSION

Based on Table I, Ethyl Acetate Fraction of *Moringa oleifera* (EA-MO) shows a total flavonoid content of 8.408 ± 0.067 mg QE/g sample because ethyl acetate is a semi-polar solvent that can dissolve flavonoid compounds in antioxidant intermediates which act as lipophilic antioxidants and antioxidants hydrophilic (Santoni et al., 2023). To measure flavonoid levels, the sample reacts with $AlCl_3$ in an acidic environment and causes a colometry reaction. Adding $AlCl_3$ creates a complex reaction between flavonoids and $AlCl_3$, which can change the wavelength towards the visible and produce a yellow color (Lindawati & Ma'ruf, 2020).

Table II shows a higher SGOT activity in the negative Group SGOT activity in the negative group compared to the normal group. There was no significant decrease in the EA-MO 50 mg/kgBW group compared to the negative group, but there was a decrease in SGOT activity in the Metformin 45 mg/kgBW group and the EA-MO 12.5 mg/kgBW and EA-MO 25 mg/kgBW groups compared to the negative group. There was a decrease in SGPT activity in the Metformin 45 mg/kgBW group, EA-MO 12.5 mg/kgBW group, and EA-MO 25 mg/kgBW group compared to the negative group.

Normally, the liver also has cells that experience necrosis as shown in Figure 2. Eventually, the dead cells can be removed by the body through defensive mechanisms (Iorga et al., 2017). The results of the study showed that the number of necrotic cells was still significantly greater in the negative control group compared to the normal group.

Based on the data, it can be seen that the SGOT and SGPT activity of Wistar rats induced with

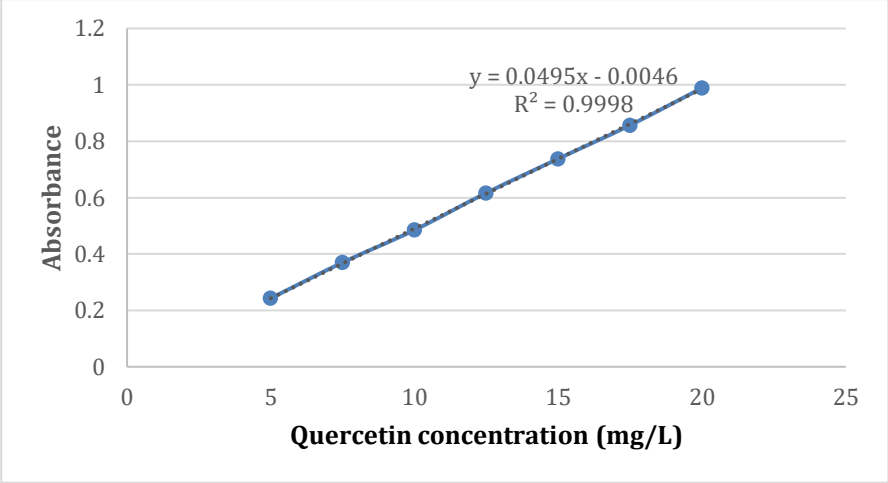


**Figure 1. Standard curve of quercetin standard solution**

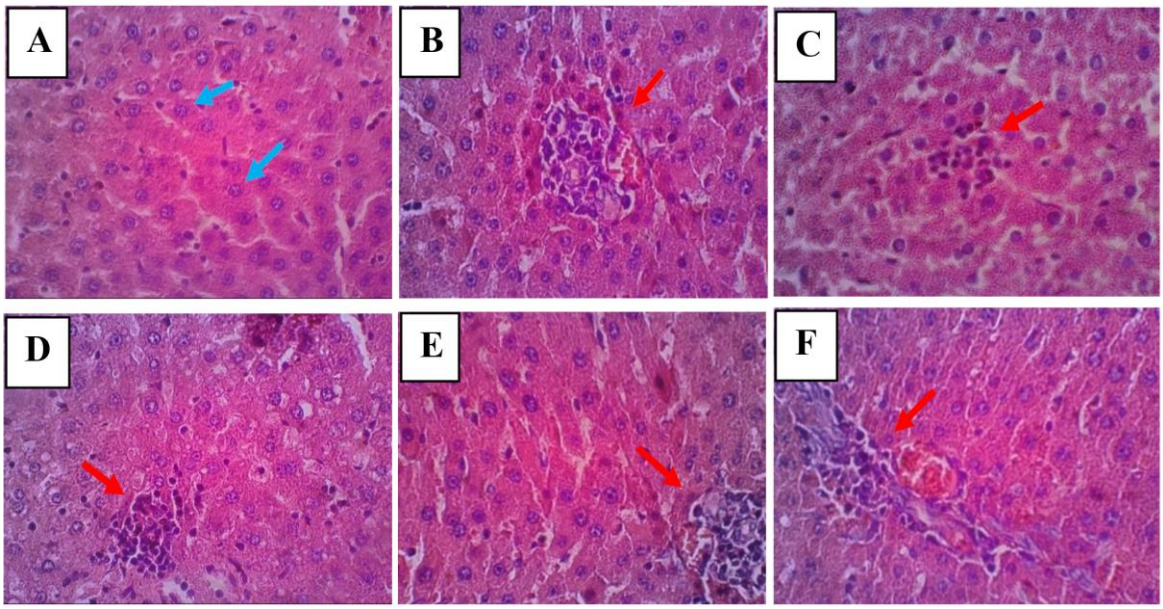


**Figure 2. The liver histopathologic features of male Wistar rats induced by STZ after *Moringa oliefera* ethyle acetate fraction administration for 10 days. A: Normal, B: Negative Control, C: Metformin 45 mg/KgWB, D: EA-MO 12,5 mg/KgWB, E: EA-MO 25 mg/KgWB, F: EA-MO 50 mg/KgWB. Hepatocyte with normal nucleus (blue arrow) and necrosis hepatocyte with picnotic nucleus (red arrow). Hematoxyline eoisin staining with magnification 400x.**

**Table I. The result of total flavonoid content in Ethyl Acetate Fraction of *Moringa oleifera* (EA-MO)**

| Sample Weight (µg/ml) | Absorbance | Rate (mg QE/g) | Average ± SD (mg QE/g) |
|---|---|---|---|
| 250 | 0.412 | 8.422 | |
| 250 | 0.408 | 8.336 | 8.408 ± 0.067 |
| 250 | 0.415 | 8.468 | |

65 mg/kgBW of streptozotocin and 100 mg/kgBW of nicotinamide were higher than those of the normal group which had an average serum SGOT activity of 117.50 U/L and serum SGPT of 100.75 U/L. This happens because streptozotocin and nicotinamide can increase blood glucose levels in mice (Sulistyawati et al., 2017). The STZ-NA mechanism in creating a type 2 diabetes mouse model is related to nitric oxide levels, which result in the death of pancreatic β cells, increasing blood

**Table II. Average SGOT and SGPT activities after 10 days of therapy with Ethyl Acetate Fraction of *Moringa oleifera* (EA-MO)**

| Group | SGOT activity (U/L) | SGPT activity (U/L) |
|---|---|---|
| Normal | 71.00 ± 12.52[c] | 42.25 ± 3.40[c] |
| Negative Control | 117.50 ± 8.58 | 100.75 ± 4.77 |
| Metformin 45 mg/KgBW | 86.75 ± 3.86[ac] | 72.50 ± 4.04[c] |
| EA-MO 12,5 mg/KgBW | 79.00 ± 7.44[a,b] | 60.25 ± 4.03[b,c] |
| EA-MO 25 mg/KgBW | 95.25 ± 15.92[a,b] | 68.50 ± 5.07[b,c] |
| EA-MO 50 mg/KgBW | 112.75 ± 35.67 | 96.75 ± 5.38 |

**Notes:** The results of the data were expressed as mean ± standard deviations (n=5); The Tukey test showed that there was a big difference between the top letter meanings of the 6 groups ($P<0.05$); [a]: not significantly different from the normal group; [b]: not significantly different from the metformin group (positive control); [c]: significantly different from the negative control group

lucose and ROS (Firdaus et al., 2016). This damage can cause the entry of liver cell aminotransferase enzymes into the blood vessels (Goud et al., 2015).

In this study, a positive group was induced by streptozotocin and nicotinamide and then given metformin treatment. Metformin was used because it can reduce blood sugar levels in Wistar rats (Sulistyawati et al., 2017). Metformin can inhibit ROS and DNA damage (Algire et al., 2012). The data shows a decrease in SGOT and SGPT activity in the positive group compared to the negative group, with an average SGOT activity of 86.75 U/L and SGPT of 72.5 U/L. The statistical test results obtained a significance value of 0.000 ($p<0.05$), meaning that Metformin could significantly reduce SGOT and SGPT activity compared to the negative group.

The Ethyl Acetate Fraction of *Moringa oleifera* (EA-MO) at doses of 12.5 mg/kgBW, 25 mg/kgBW, and 50 mg/kgBW can reduce blood glucose levels. This decrease was due to the quercetin content which serves as an antioxidant in Moringa leaves for blood sugar (Sulistyawati et al., 2017). The dose of the EA-MO could reduce SGOT and SGPT activity 12.5 mg/kgBW and 25 mg/kgBW could significantly reduce SGOT and SGPT activity compared to the negative group. This follows previous research, which states that quercetin can reduce SGOT and SGPT levels in rat mice induced by streptozotocin (Kılıçarslan & Dönmez, 2016).

The administration of streptozotocin to test animals can cause liver damage, as indicated by an increase in SGOT and SGPT activity in the blood. SGPT can be used as a more sensitive indicator of liver damage than SGOT. This is because the primary source of SGPT activity lies in hepatocyte cells, while SGOT activity is found in other tissues such as the heart, skeletal muscle, kidney, and brain (Bhakuni et al., 2016).

Streptozotocin is a compound that can cause test animals to develop diabetes (Szkudelski, 2012). Diabetic conditions caused by streptozotocin, which is an alkylating agent, result in damage to the liver. Streptozotocin metabolism liberates nitrite and increases ROS (Goud et al., 2015). Oxidative stress conditions cause lipid peroxidation, damaging liver cells (Hardiningtyas et al., 2014). Damage to liver cells can be observed on histopathological examination. The histopathological appearance of liver cells can be seen in Figure 2.

The histopathological examination of the liver in normal controls showed no histopathological changes, so the liver was still in a normal condition. Meanwhile, in the negative control, Metformin and EA-MO treatment with each dose variation showed changes on microscopic examination, namely necrosis in hepatocytes. Damage to liver cells is characterized by a degeneration process, namely cell swelling. Cell swelling can be reversible until it can return to normal. The next stage of damage to liver cells is necrosis, where the damage is irreversible in that the cells will experience death (Hasana et al., 2019). Necrosis is the death of cells or tissue in living organisms. Cells that experience death are characterized by the cell nucleus shrinking until it become dark and there is no euchromatin (pyknosis), the cell nucleus ruptures (karyorrhexis), and the cell nucleus disappears (karyolysis) (Irham & Widyaningsih, 2017).

The results of calculating the number of necrosis cells in all research groups were taken from the total of five fields of view and one slice in each research group, and then the average was taken for each group. The results of calculating the number of liver cells experiencing necrosis can be seen in Table III. The results of calculating the number of liver necrosis cells in Table III show that there is a large number of liver cells experiencing

**Table III. The result of calculating of liver cell necrosis after 10 days of therapy with Ethyl Acetate Fraction of *Moringa oleifera* (EA-MO) which was observed under a microsope with 400x magnification and reading 5 fields of view in 1 slice**

| Group | Average of liver cell necrosis ± SD |
|---|---|
| Normal | 7.60 ± 1.98[c] |
| Negative Control | 14.80 ± 2.02[b] |
| Metformin 45 mg/KgBW | 9.50 ± 1.27[a,c] |
| EA-MO 12,5 mg/KgBW | 10.10 ± 2.27[a,c] |
| EA-MO 25 mg/KgBW | 10.30 ± 2.02[a,c] |
| EA-MO 50 mg/KgBW | 10.90 ± 1.34[a,c] |

**Notes:** The results of the data were expressed as mean ± standard deviations (n=5); The Tukey test showed that there was a big difference between the top letter meanings of the 6 groups ($P<0.05$); [a]: not significantly different from the normal group; [b]: not significantly different from the metformin group (positive control); [c]: significantly different from the negative control group

necrosis in the negative control compared to the normal group. This also happened in all EA-MO treatment groups, but the 12.5 mg/KgBW dose group showed liver cell necrosis with the smallest value compared to the 25 mg/KgBW and 50 mg/KgBW dose groups.

The Metformin 45 mg/KgBW group experienced the lowest reduction in necrosis cells compared to all groups. This is because using metformin can reduce blood sugar levels in Wistar rats as it inhibits the formulation of ROS and DNA damage (Sulistyawati et al., 2017). If the formation of ROS is inhibited, the liver can regenerate damaged cells so that they will be replaced with new cells (Syahrin et al., 2016).

The administration of Ethyl Acetate Fraction of *Moringa oleifera* (EA-MO) can reduce the levels of SGOT, SGPT, and the number of liver necrosis cells in rats induced by streptozotocin and nicotinamide. The Ethyl Acetate Fraction of *Moringa oleifera* (EA-MO) is known to contain the flavonoid quercetin in Moringa leaves. The total flavonoid concentration in the ethanol extract of MO leaves was 23.05 ± 0.77 mg routine equivalent/g (Wulan et al., 2019). Quercetin can be used as an antioxidant against damage caused by oxidative stress in liver cells due to the induction of Streptozotocin which causes Diabetes Mellitus (Kılıçarslan & Dönmez, 2016).

The quercetin has a specific structure, namely 3-OH and 3',4'-catechol in ring B. The 3-OH structure is a significant factor in fighting ROS because the hydrogen atoms are donated to hydroxyl, peroxyl, and peroxynitrite radicals so that they can stabilize free radicals (Alwaraiq & Abdullah, 2014). Antioxidant mechanisms remove, help activate endogenous anti-oxidation enzymes, chelating metals, reducing α-tocopheryl radicals, inhibiting oxidation processes, reducing oxidative stress caused by nitrites, increasing uric acid levels, and changing the prooxidant properties of low antioxidant molecules (Procházková et al., 2011).

The findings of this research align with other previous studies which indicate that plant extracts have a promising potential for treating diabetes-related problems, particularly liver-related ones. Numerous research works have examined the impact of various plant extracts on the liver function of rats with diabetes. Studies on extracts alone and in combination with various plants demonstrate their effectiveness in mending diabetic liver damage (Hong et al., 2015). These results imply that plant extracts may be able to lessen liver damage brought caused by problems from diabetes and enhance liver function. To exaamine the mechanism of action and pinpoint the precise bioactive substances causing these effects, more further research is required.

## CONCLUSION

The Ethyl Acetate Fraction of *Moringa oleifera* (EA-MO) can reduce SGOT, SGPT levels and liver cell necrosis in rats induced by streptozotocin and nicotinamide. The most effective dose of Ethyl Acetate Fraction of *Moringa oleifera* (EA-MO) in reducing SGOT, SGPT levels, and liver cell necrosis in rats induced by streptozotocin and nicotinamide is 12.5 mg/KgBW.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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