Effects of Aqueous Extract of *Moringa oleifera* on Phenylhydrazine-induced Liver Toxicity in Wistar Rats

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**ABSTRACT:** *Moringa oleifera* has been shown to have a variety of medicinal benefits, including hepatoprotective effects. Phenylhydrazine has been used to cause hepatotoxicity in experimental models. In this study, an aqueous extract of *Moringa oleifera* was used to evaluate the ameliorating properties of phenylhydrazine-induced liver damage by assessing liver enzymes and histochachinery employing twenty-five (25) mature Wistar rats were divided into five (5) groups. Group A received 1 ml distilled water, Group B received 50 mg/kg phenylhydrazine twice in 48 hours, Group C received 500 mg/kg body weight of *Moringa oleifera* aqueous extract for four (4) weeks, Group D received 50 mg/kg phenylhydrazine twice in 48 hours and 500 mg/kg body weight of *Moringa oleifera* aqueous extract for four (4) weeks, and Group E received 50 mg/kg of phenylhydrazine twice in forty-eight (48) hours and 70 mg/kg body weight of Silymarin for four (4) weeks. After the administration of phenylhydrazine, significant increases (P<0.05) in the mean concentrations of liver enzymes (ALP, AST, ALT) and total protein were observed, whereas treatment with *Moringa oleifera* resulted in a reversal of those parameters to values comparable to the control and the standard drug - Silymarin.

Overall, the results showed that *Moringa oleifera* aqueous extract had considerable hepatoprotective capability against phenylhydrazine-induced hepatotoxicity.

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Medicinal plants have long been recognized as being critical to the health of individuals and societies (Bruneton, 1993; Nku-Ekpang et al., 2021). Herbal medicines derived from plants are becoming more important in basic health care in many poor nations, and their worldwide commerce has expanded (WHO, 1996). *Moringa oleifera* is one of these plants. *Moringa oleifera* Lam. is a tropical and subtropical tree that grows widely. Commercial cultivation occurs in India, Africa, South and Central America, Mexico, Hawaii, and across Asia and Southeast Asia. It is known as the drumstick tree because of the shape of its immature seed pods, the horse-radish tree because of the flavor of ground root preparations, and the ben oil tree because of the oils generated from its seeds. Immature seed pods are consumed in some locations, while the leaves are commonly utilized as a basic meal because of their high nutritional value (Razis et al., 2014; Mbikay, 2012; Thurber and Fahey, 2009). The tree can reach a height of 10 to 12 m, with a spreading, open crown of drooping, brittle branches, feathery foliage with tripinnate leaves, and thick, corky, deeply fissured white bark. It is primarily appreciated for its edible fruits, leaves, flowers, roots, and seed oil, and it is widely utilized in traditional medicine across its native and introduced areas (Francis and Iogier, 1991; Jahn et al., 1986). Leaves of *Moringa oleifera* have been shown to have a favourable nutritional balance, containing vitamins, minerals, amino acids, and fatty acids (Moyo et al., 2011; Teixeira et al., 2014).

The leaves are up to 45 cm long, bipinnate or tripinnate, and alternately and spirally arranged on the branches. Pinnae and pinnules are opposed; leaflets

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MATERIALS AND METHOD

Plant material: Moringa Oleifera leaves were taken from a farm in Edo state, Nigeria's Ovia North East Local Government Area. A Plant Taxonomist from the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, identified and validated the leaves, which were then air-dried for seven (7) days before being crushed into powder and weighed with an electrical weighing scale. Standard extraction procedures were used (Eze and Akonoafua, 2020).

Experimental animals: The experiment employed twenty-five (25) mature Wistar rats weighing between 190 and 220 g. The rats were obtained from the Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Edo State, and acclimatized for two weeks prior to the start of the study. During this time, the animals were given unlimited access to conventional animal feed (Top feed growers mash) and clean water. Each animal procedure was carried out in accordance with established procedures and recommendations for the proper management and exploitation of laboratory animals employed for research (Buzek and Chastel, 2010).

Induction of hepatotoxicity: Hepatotoxicity was generated in the experimental rats using previously described procedures of Henneh et al (2021), in which the animals were given 50 mg/kg of phenylhydrazine orally twice in 48 hours.

Experimental design: The experimental design of the study is carefully presented in table 1.

Tissue collection, processing and staining: After four (4) weeks, the rats were anesthetized with chloroform and sacrificed. By making a midline incision in the ventral abdominal wall, each rat was sacrificed. The livers of each rat were instantly extracted. Blood was collected directly from the abdominal aorta to assess serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), alkaline phosphatase (ALP), conjugated bilirubin, and total bilirubin. The liver tissues were fixed in 10% buffered formalin for 24 hours before being stained with haematoxylin and eosin and processed histologically. Tissue staining was accomplished using standard techniques (Drury et al., 1976).

Table 1: Table showing experimental design

<table>
<thead>
<tr>
<th>Groups</th>
<th>DOSAGE</th>
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<tr>
<td>Group A</td>
<td>Received 1 ml distilled water only for four (4) weeks</td>
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<tr>
<td>Group B</td>
<td>Received 50 mg/kg of phenylhydrazine twice in forty-eight (48) hours</td>
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<tr>
<td>Group C</td>
<td>Received 500 mg/kg body weight of Moringa oleifera aqueous extract for four (4) weeks</td>
</tr>
<tr>
<td>Group D</td>
<td>Received 50 mg/kg of phenylhydrazine twice in forty-eight (48) hours and 500 mg/kg body weight of Moringa oleifera aqueous extract for four (4) weeks</td>
</tr>
<tr>
<td>Group E</td>
<td>Received 50 mg/kg of phenylhydrazine twice in forty-eight (48) hours and 70 mg/kg body weight of Silymarin for four (4) weeks</td>
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</table>
Effects of aqueous extract of *Moringa oleifera* on phenylhydrazine-induced toxicity in the liver of exposed Wistar rats were investigated and compared to the control group in the presence of a well-known standard drug, silymarin. The liver function test and histopathology data were used to assess the toxicity of phenylhydrazine and the effectiveness of *Moringa oleifera*. Liver function tests are an efficient modality for detecting hepatic impairment and are a useful screening tool.

**Photomicrography:** A histopathologist examined the H&E stained liver slides using a Leica DM750 research microscope with a connected digital camera (Leica CC50). The tissues were photographed electronically at x400 magnification.

**Statistical analyses:** The IBM SPSS statistics application (Statistical Package for Social Science) Version 25 (SPSS, Inc., Chicago, Illinois, USA) was used to evaluate all of the data and create acceptable statistical values. Using a one-way analysis of variance, the values of the treatment groups were compared to those of the control group (ANOVA). P values less than 0.05 were considered significant. LSD was used as a post-hoc test.

**RESULTS AND DISCUSSION**

Photomicrographs of H&E-stained slides: Each photomicrograph taken at x400 magnification is a representative of each group. Plate 1 shows the liver of the control group showing normal histoarchitecture of the liver. They received 1 ml of water daily. Plate 2 shows the liver of the group that received phenylhydrazine only. Heavy periportal infiltrates of inflammatory cells, vascular ulceration and congestion, and necrosis were observed.

<table>
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<th>Table 2: Table showing results of liver function tests</th>
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<tr>
<td>Control</td>
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<tr>
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</tr>
<tr>
<td>ALP (U/L) 300.67±55.03</td>
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<tr>
<td>ALT (U/L) 85.00±5.00</td>
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<tr>
<td>AST (U/L) 118.33±21.85</td>
</tr>
<tr>
<td>TP (g/dL) 7.27±0.20</td>
</tr>
</tbody>
</table>

*Significantly different from the control group at P<0.05; The results are mean of rats in each group ± SEM

Plate 2 is a photomicrograph of Group 3 (*Moringa oleifera* only) showing normal histoarchitecture of the liver and comparable with the control group. Plate 4 is a photomicrograph of Group 4 (phenylhydrazine + *Moringa oleifera*). Kupffer cell activation and periportal lymphocytosis were seen, while the hepatocytes were normal. Plate 5 (Group 5) also shows Kupffer cell activation, periportal lymphocytosis and normal hepatocytes.

Plate 1: Control liver, composed of A: hepatocytes, B: sinusoids, C: portal vein and D: bile duct (H&E x 400)

Plate 2: Rat given phenylhydrazine only, showing A: heavy periportal infiltrates of inflammatory cells, B: vascular ulceration, C: necrosis and D: vascular congestion (H&E x 400)

Plate 3: Rat given *Moringa oleifera* only, showing A: normal hepatocytes, B: periportal lymphocytosis and C: kupffer cell activation (H&E x 400)
Because the liver conducts so many activities, no one test can offer an accurate measure of the liver's function (Thapa and Walia, 2007). Phenylhydrazine was employed to generate hepatotoxicity in this investigation. While in the body, phenylhydrazine (C6H8N2) is harmful and damages various tissues (Giffin and Allen 1993; Mansuy et al. 1982).

According to research, phenylhydrazine promotes oxidative stress, free radical generation, lipid peroxidation, oxidative destruction of spectrum cell membranes, and red blood cell lysis (Ferrali et al. 1997; Giffin and Allen 1993). Phenylhydrazine also promotes haemolysis (increases ferrous, ferritin, and erythropoietin levels), liver enlargement, and chronic failure (through hypertrophy of liver cells) (Ferrali et al. 1997; Goldberg and Stern 1975). According to our findings, phenylhydrazine substantially (p 0.05) raised the levels of ALP, AST, ALT and total protein, demonstrating hepatotoxicity. This is consistent with the findings of Zangeneh et al (2019), who conducted a similar research. Despite the hepatotoxic effects of phenylhydrazine, Moringa oleifera therapy has shown a positive chance that M. oleifera extract possesses hepatoprotective properties due to a considerable decrease in serum transaminases and ALP activity, as well as by avoiding the histological alterations found with phenylhydrazine therapy. In another research, Moringa oleifera enhanced liver biochemical markers such as ALT and AST when compared to the acetaminophen-treated group (Fakurazi et al., 2008).

Aminotransferases are a class of enzymes that catalyse the reversible transfer of an a-amino acid’s amino acid group to an oxo acid. These enzymes are not typical plasma components, and their function outside of the organ of origin is unclear (James et al., 2003). The cytoplasm of hepatic parenchymal cells contains the most ALT (Okuda, 1997). AST, on the other hand, is present in the cytosol and mitochondria of hepatocytes, as well as cardiac muscle, skeletal muscle, the pancreas, and the kidney (Shyamal et al., 2006). As a result, measuring ALT is more liver-specific for determining hepatocellular damage (Shyamal et al., 2006). Despite this, AST is still widely used as a laboratory test to measure liver function since it is regarded as a sensitive sign of mitochondrial damage, particularly in the centrilobular areas of the liver (Panteghini, 1990). Meanwhile, ALP is an enzyme family that hydrolyzes phosphate esters at alkaline pH and is frequently employed as a diagnostic for cholestatic liver disease. The liver, however, is not the only source of ALP. Significant levels are also found in the bone, placenta, kidney, and gut (Gaw et al., 1998). The leakage of cellular enzymes into plasma is a classic indicator of hepatic injury or destruction (Ramaiah, 2007; Kumar et al., 2004). Furthermore, the presence or absence of particular enzymes in the circulation can be used to determine the amount and type of liver injury or damage (Kumar et al., 2004). In general, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels are often utilized as hepatotoxicity markers (Yapallewar et al., 2002; Yen et al., 2007). Previous research found that the root and flower of Moringa oleifera reduced acetaminophen-induced ALT, AST, and ALP increase in rats and mice (Ruckmani et al., 1998). Meanwhile, another study found that Moringa oleifera leaves extract substantially reversed the rise of ALT, AST, and ALP levels caused by isoniazid to normal levels (Pari and Kumar, 2002). In a recent research, Nadro et al (2005) found that Moringa oleifera leaves extract inhibited the release of these enzymes from hepatocytes into the circulation in rats when triggered with a high amount of ethanol administration. Our findings are consistent with prior research, which suggests that Moringa oleifera may retain the structural integrity of hepatocytes when exposed to hepatotoxicants, hence reducing enzyme leakage into the plasma.

**Plate 4:** Rat given phenylhydrazine + Moringa oleifera, showing A: normal hepatocytes, B: periportal lymphocytosis and C: Kupffer cell activation (H&E x 400)

**Plate 5:** Rat given phenylhydrazine + Silymarin showing A: normal hepatocytes, B: periportal lymphocytosis and C: Kupffer cell activation (H&E x 400)
plasma. The findings also revealed that the plant extract is just as efficient as silymarin in avoiding a rise in liver enzymes when exposed to a hepatotoxic dosage of acetaminophen. Silymarin is a well-known hepatoprotective medication that has been shown to reverse aberrant changes in these enzymes in drug-induced hepatotoxicity (Pradhan and Girish, 2006). The capacity of silymarin to operate as a radical scavenger, so preserving membrane permeability, is connected with its ability to prevent drug-induced hepatotoxicity (Song et al., 2006). Because of its known hepatoprotective activity, silymarin is utilized as a reference agent for comparing the hepatoprotective effects of plant principles (Dhiman and Chawla, 2005).

**Conclusion:** The aqueous extract of *Moringa oleifera* demonstrates significant hepatoprotective potentials, based on the above findings.

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