Histological Study on the Possible Protective Role of Moringa Oleifera Leaves Extract on Paracetamol Induced Liver Damage in Adult Male Albino Rats

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ABSTRACT

Introduction: Moringa oleifera is a tropical tree whose leaves are rich in antioxidant compounds. Paracetamol hepatotoxicity is a significant public health concern.

Aim: The aim of this study was to investigate the possible protective role of Moringa oleifera leaves (MOL) aqueous extract on paracetamol induced liver injury in adult male albino rats.

Materials and Methods: Forty adult male albino rats were used in the current study and were divided into four equal groups.: Group I served as control; Group II were given MOL for 14 days; Group III were given water for 7 days followed by paracetamol for another seven days; Group IV were given MOL for 14 days and Paracetamol on the seventh day for 7 days. All medications were given by nasogastric tube. Specimens of liver were excised and processed for histological and histochemical studies. Blood samples were collected for biochemical study. Statistical analysis of the results was done.

Results: MOL administration in group II didn’t affect hepatic architecture or liver function tests. There was a significant increase in glutathione peroxidase and catalase activity and no significant difference in malondialdehyde level as compared to control. Paracetamol administration led to vacuolization of hepatocytes, cellular infiltrations and congestion in central and portal veins, with apparent increase in number of cholangiocytes. There were also significant decrease in the area percentage of PAS positive granules and significant decrease in area percentage of collagen fibers as compared to control. By electron microscope, distorted mitochondria, decrease of rough endoplasmic reticulum and increase of smooth endoplasmic reticulum & collagen fibrils were observed. Administration of Moringa before paracetamol ameliorated damaging effects of paracetamol. Minimal cellular infiltrations and apparent increase in number of cholangiocytes were still observed.

Conclusion: MOL extracts could protect the liver against paracetamol effects through inhibition of lipid peroxidation and enhancement of antioxidant enzymes.

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Key Words: Histopathology, liver, moringa oleifera, paracetamol.

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INTRODUCTION

Liver plays an important role in biotransformation of drugs and toxins, so the drug induced hepatotoxicity should be a major concern in drug development and chronic drug therapy[3]. More than 900 drugs have been found to cause liver injury and this is the most common reason for a drug withdrawal from the market[2].

Natural medications from plants are considered as effective and safe alternative treatments for hepatotoxicity[3]. Plants have thus been investigated for hepatoprotective and antioxidant effects against hepatotoxin induced liver damage[8]. Previous studies have shown that hepatoprotective effects are usually due to the presence of phytochemicals rich in natural antioxidants[12].

Moringa oleifera is an evergreen tree[9] whose edible parts have been used for homeopathic aspects since ancient times[9]. Although being native to Africa[7], such a use is not known by the public[9]. It was known along the Nile valley by the name of ‘Shagara al Rauwaq’, which means ‘tree for purifying’[9]. Many organizations are promoting for its uses as an untapped opportunity[9].

Recently Moringa oleifera is coming to the forefront as a result of scientific evidence that Moringa is an important source of naturally occurring phytochemicals[11]. Phytochemical analyses have shown that its leaves are rich in potassium, calcium, phosphorous, iron, vitamins A and D, essential amino acids, as well as known antioxidants such as β-carotene, vitamin C, and flavonoids[12].

Paracetamol was first introduced as an analgesic in 1893, yet was not popular until the 1960s, after knowing that phenacetin, a frequently used analgesic in those days, was nephrotoxic in chronic abusers[14]. Nowadays, paracetamol has become more popular due to the increased concern about aspirin mediated gastrointestinal bleeding and Rye’s syndrome. According to the US Food and Drug
Administration, each week approximately 50 million adults in the United States take paracetamol containing products. Today, paracetamol toxicity accounts for 50% of all cases of acute liver failure in the United States and Great Britain. Annually, it accounts for a high percentage of the admissions to the poison control centers and also for the deaths. The United States spend about $87 million dollars annually in treatment of paracetamol induced hepatotoxicity. Paracetamol over dose is the most common cause of drug induced hepatotoxicity worldwide and its side effects are multiplied when given to chronic liver patients.

Previous studies showed that Moringa oleifera extract could protect the liver tissue against the induced hepatotoxicity by enhancing the antioxidant defense system. The anti hepatotoxic nature of Moringa oleifera leaves aqueous extract against paracetamol induced liver injury in rats has not been fully investigated, therefore the target of the present work is to study the hepatoprotective role of Moringa oleifera leaves extract on paracetamol induced liver injury.

**AIM**

This study aimed to investigate the possible protective role of Moringa oleifera leaves aqueous extract on paracetamol induced liver injury in adult male albino rats.

**MATERIALS AND METHODS**

**Animals**

Forty adult male albino rats (4-5 months) with an average weight ranged from 200 to 250 grams were housed in wire mesh cages and were freely allowed to a chow diet and water ad libitum. The experiment was conducted in the scientific research center at Ain Shams University. Animals were kept for seven days before the beginning of the experiment for acclimatization. All animal procedures were carried out according to the recommendation of the institutional animal ethics committee at the faculty of Medicine, Ain Shams University. The animals were divided into four equal groups, ten rats each.

- **Group I, Control group** in which rats received 0.5ml of water by nasogastric tube for 14 days.
- **Group II, Moringa Oleifera group**: rats were given Moringa oleifera leaves aqueous extract (500 mg/kg body weight) by nasogastric tube for 14 days.
- **Group III, Paracetamol group**: rats received water by nasogastric tube for 7 days then received paracetamol (400mg/kg body weight) that was given by nasogastric tube on the seventh day for another seven days.
- **Group IV, Moringa Oleifera and paracetamol group**: rats were given Moringa oleifera leaves aqueous extract (500 mg/kg body weight) by nasogastric tube for 14 days and Paracetamol (400 mg/kg body weight) by nasogastric tube on the seventh day for seven days.

**Preparation of plant extract**

The plant materials are harvested in Cairo - Alexandria desert road, Egypt and purchased from Pure Life co. Egypt. The plant aqueous extract was prepared by mixing 1 gm of dried and powdered leaves of Moringa oleifera with 10 ml boiling water for 5 minutes. The mixture was then filtered twice through 2 µm pore sterile filter paper into a sterile tube. The aqueous extract stock solution (100mg/ml) was freshly prepared for each set of experiment and stored at 4ºc for up to 5 days.

**The drug**

Paracetamol was purchased as Cetal 100mg/ml and given orally by nasogastric tube. Dose of 400 mg/kg body weight, equivalent to 40mg/100gm body weight (which is equivalent to the maximum therapeutic dose in human), was prepared to be given to group III later.

On day 15 the rats were fasted, then anaesthetized, blood was collected via cardiac puncture then the animals were sacrificed by cervical dislocation. The Liver specimens were excised and processed to obtain paraffin blocks. Sections of 5µm thickness were cut and stained with the following:

1. Hematoxylin and Eosin (H&E) stain
2. Masson's trichrome stain
3. Periodic Acid Schiff's reaction (PAS).

Also, small pieces of liver tissue were rapidly fixed and then processed for Transmission electron microscopic study (TEM). Specimens were examined and photographed with JEOL-1010 Transmission electron microscope made in Japan at EM unit at the Regional Center of Mycology and Biotechnology, Azhar University, Cairo, Egypt.

**Morphometric study**

Mean area % of glycogen granules in hepatocytes and mean area % of the green stained collagen fibers in Masson’s trichrome stained sections were measured. This was performed in five non-overlapping fields from five different sections of ten different rats in each group using image analyzer Leica QwinV.3 Program connected to a camera (DM2500, Leica microsystems, Wetzlar, Germany) at the Histology Department, Faculty of Medicine, Ain Shams University.

**Biochemical study**

At time of sacrifice blood samples were collected to measure Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP). They were assessed in the lab of biochemistry department at Ain Shams university using diagnostic kits purchased from Roche Diagnostic Ltd (Germany).
**Oxidative state of liver tissue was investigated**

Four to five mm slices of liver tissue were rapidly stored at -80°C to be processed and handled to prepare tissue homogenate.

Liver was weighed and homogenized in ice cold 0.15 M Tris-KCL (0.15M KCL+10mM Tris-HCl, PH7.4). The homogenate was then centrifuged at 600 g for 10 min. Supernatant was collected and centrifuged again at 10,000 g for 30 min[22].

Tissue Malondialdehyde, tissue Glutathione peroxidase (GSHPx), and tissue Catalase were estimated as follows:

MDA level was measured by thiobarbituric acid test[23]. Activity of Glutathione Peroxidase enzyme (GSHPx) level was estimated by commercial kinetic method kits (The Glutathione Peroxidase UV method Activity Assay kit) purchased from Bio-Diagnostic, Egypt according to the instructions of the manufacturer. Activity of Catalase level was estimated by commercial kinetic method kits (Catalase Assay, Colorimetric method) purchased from Bio-Diagnostic, Egypt according to the instructions of the manufacturer.

**Statistical analysis**

All data of morphometric and biochemical analysis were collected, revised and then subjected to statistical analysis using one-way Analysis of Variance (ANOVA) method to test the mean differences observed among the four different groups. The test was performed by SPSS package (version 23 for Windows). P. value less than 0.05 was considered significant.

**RESULTS**

**Histological results**

H&E stained liver section of rats from Group I showed that liver parenchyma consists of branching and anastomosing cords of hepatocytes radiating from the central vein. Blood sinusoids separating hepatic cords were lined by flat endothelial cells and Kupffer cells. The polygonal hepatocytes had acidophilic cytoplasm with basophilic bodies and single, central, round euchromatic nucleus of variable size with prominent nucleoli. Some cells appeared binucleated (Figure 1). Branches of hepatic artery, portal vein and bile ducts could be seen at the portal tracts (Figure 2). Examination of liver sections of rats from group II showed that they were nearly similar to those of group I.

In group III, Paracetamol led to mononuclear cellular infiltrations in some areas within the hepatic lobules. Most of the hepatocytes surrounding the congested central vein appeared with vacuolated cytoplasm. Some vacuolated hepatocytes appeared with rounded vesicular nuclei (Figure 3), while others showed shrunken, irregular and darkly stained nuclei. Blood sinusoids were hardly visible between the ballooned hepatocytes (Figure 4). Cellular infiltrations close to congested and dilated portal vein branch were also detected (Figure 5). Portal area showed an apparent increase in the number of the lining cells of bile ductules as compared to that of the control group. The hepatocytes around portal area were vacuolated (Figure 6).

While, examination of liver sections of rats of Group IV showed that the structure of liver was comparable to that of the control group. Branching and anastomosing cords of polygonal hepatocytes with vesicular nuclei were seen radiating from the central vein. Few cells showed small dark nuclei. The cytoplasm of the hepatocytes was hardly vacuolated in comparison to paracetamol group. The blood sinusoids could be clearly seen between the hepatocytes. They were lined by endothelial cells and Kupffer cells (Figure 7). Portal area showed minimal cellular infiltration. However, apparent increase in number of cells lining bile ductules within the portal area and the nearby bile canaliculi at the periphery of the hepatic lobules was noticed in some sections (Figure 8).

On examination of Liver sections stained with PAS, most of the hepatocytes in group I (Figure 9) and group II had PAS positive glycogen granules in their cytoplasm. While in group III, patchy distribution of PAS negative hepatocytes was noticed with an apparent decrease in the PAS positive glycogen granules, compared with group I (Figure 10). In group IV, the PAS positive glycogen granules in the hepatocytes were almost similar to that of the control group (Figure 11).

Liver sections stained with Masson's trichrome stain revealed minimal amount of green stained collagen fibers surrounding the central vein and portal areas and in between hepatocytes in group I (Figures 12 a&b) and group II. While, in group III apparent increase in collagen fibers was noticed around the central vein and the portal area with appearance of collagen fibers running between and surrounding the hepatocytes (Figures 13 a&b). In group IV, the collagen fibers content in the portal tracts and around the central vein was almost similar to that in control group (Figures 14 a&b).

Electron microscopic examination of the control group revealed that hepatocytes have central rounded euchromatic nuclei surrounded by nuclear membrane. The cytoplasm showed numerous mitochondria variable in shape, with cristae. The mitochondria appeared associated with rough endoplasmic reticulum (rER). Rosettes of glycogen granules scattered in cytoplasm were also seen (Figure 15). Bile canaliculi in between adjacent hepatocytes were obvious, with short microvilli from the hepatocyte surface being projecting into their lumina. Close to the canaliculi junctional complexes were seen attaching hepatocytes to each other (Figure 16). Hepatic stellate cells (HSCs, Ito cells, stellate fat storing cells) lying in between hepatocytes were detected. HSC had elongated nucleus and multiple lipid droplets in its cytoplasm (Figure 17). The ultra structure of the hepatocytes in group II was nearly similar to that of the control group.
In Group III, electron microscopic examination showed that some of the hepatocytes had vacuolated cytoplasm and shrunken, irregular nucleus with more peripheral heterochromatin. Small lipid droplets of variable size with well-defined edges were seen in the cytoplasm. Apparent decrease in the mitochondria and rough endoplasmic reticulum was noticed as compared to that of the control group (Figure 18). On the other hand, some hepatocytes showed elongated distorted mitochondria and numerous profiles of smooth endoplasmic reticulum (sER) as compared to that of the control group (Figure 19). Other sections showed adjacent hepatocytes with lipid droplets of variable size with well-defined edges scattered in the cytoplasm. Collagen fibrils in between the hepatocytes were noticed (Figure 20). Microvilli projecting into the lumen of the bile canaliculi were apparently shorter and lacking in some sites when compared to that of the control group. Close to the canaliculi junctional complexes were seen attaching hepatocytes to each other. Elongated mitochondria were evident (Figure 21). HSC in association with collagen fibrils were frequently seen. They appeared elongated and their cytoplasm was nearly devoid of lipid droplets as compared to that of the control group (Figure 22).

Electron microscopic examination of the liver in Group IV, showed hepatocytes containing euchromatic nuclei. Their cytoplasm contained many mitochondria with apparent cristae. Rough endoplasmic reticulum and glycogen granules were also seen (Figure 23). Bile canaliculi showed microvilli projecting into their lumina. Cell membranes of the adjacent hepatocytes were attached to each other by junctional complexes close to the canaliculus (Figure 24). Hepatic stellate cells have euchromatic nuclei and multiple lipid droplets in their cytoplasm. No collagen fibrils were noticed in between hepatocytes (Figure 25).

**Statistical results**

**Morphometric parameters**

Area percentage of glycogen granules was significantly decreased in group III as compared to that of the other groups. There was no significance difference of area percentage of glycogen granules in group I, group II or group IV (Table 1).

Area percentage of collagen fibers was significantly increased in group III as compared to that of the other groups. There was no significance difference of area percentage of collagen fibers in the control group (Group I), group II or group IV (Table 2).

**Biochemical parameters**

Serum ALT, AST and ALP Levels were significantly elevated after paracetamol administration, in group III as compared to that of the control group. In group IV, ALT, AST and ALP levels were significantly reduced as compared to that of group III. While there was no significant difference in the level of serum ALT, AST and ALP of the control group, group II and group IV (Table 3).

Tissue MDA was significantly increased in group III as compared to that of the other groups. There was no significance difference of the tissue MDA level in group I, group II or group IV (Table 4).

The level of Tissue GSHPx and catalase were significantly increased in group II as compared to that of the control group. Paracetamol administration in group III produced a significant decrease in the level of tissue GSHPx and catalase as compared to that of the other groups. Group IV showed non significant difference in the level of GSHPx and catalase as compared to that of the control group (Table 5).
Fig. 3: Showing swollen hepatocytes with vacuolated cytoplasm and rounded vesicular nuclei (green arrow ↑). Some nuclei are darkly stained (*). Other hepatocytes appear with acidophilic cytoplasm and vesicular nucleus (green arrow head▲). Cellular infiltration (i) is seen in close proximity to the congested central vein (CV). Group III, H&E x 400

Fig. 4: Swollen hepatocytes with highly vacuolated cytoplasm(*) and shrunken, irregular and darkly stained nuclei are noticed. Blood sinusoids are hardly visible between the ballooned hepatocytes. Group III, H&E x 400

Fig. 5: Swollen hepatocytes with highly vacuolated cytoplasm (*) and shrunken, irregular and darkly stained nuclei are seen. Note the congested and dilated portal vein branch (PV) with nearby cellular infiltrations (↑). Group III, H&E x 400

Fig. 6: Apparent increase in the number of lining cells (black arrow ↑) of bile ductule (B) as compared to that of the control group is detected. Portal vein (PV) branch is dilated and congested. Note cellular infiltration (green arrow head▲) within the portal area. Vacuolated hepatocytes around portal area also seen (green arrow ↑). Group III H&E x 400

Fig. 7: Hepatocytes appeared polygonal with acidophilic cytoplasm and vesicular nuclei (↑) nearly similar to the control. Binucleated cells are seen (green arrow↑). The blood sinusoids are lined by endothelial cells (e) and Kupffer cells (k). Group IV, H&E x 400

Fig. 8: Shows portal area with minimal cellular infiltration (↑). Notice the apparent increase in number of cells lining bile ductule and nearby bile canaliculi (green arrow↑). Note hepatic artery branch (HA), Portal vein branch (PV) and bile ductules (B). Group IV, H&E x 400
Fig. 9: PAS positive granules are prominent within the cytoplasm of most of hepatocytes. Group I, PAS x 400

Fig. 10: Hepatocytes appeared with a decrease in the PAS positive glycogen granules (↑) as compared to that of the control. Group III, PAS x 400

Fig. 11: PAS positive glycogen granules appeared within the cytoplasm of most of hepatocytes similar to the control. Group IV, PAS x 400

Fig. 12: (a&b) Showing minimal amount of green stained collagen fibers surrounding the central vein (CV) [a], within perisinusoidal spaces (↑) [a&b] and in the portal tract (PT) [b]. Group I, Masson’s trichrome x 400

Continued
Fig. 13: (a&b). Apparent increase in green stained collagen fibers around the central vein (CV) [a] and the portal area [b] with appearance of collagen fibers (↑) running between and surrounding the hepatocytes [a&b]. Note the congested branches of Portal vein (PV) and hepatic artery (HA) [b]. Group III, Masson’s trichrome x 400

Fig. 14: (a&b). Minimal amount of collagen fibers are seen around the central vein (CV) [a], portal tract (PT) [b] and within perisinusoidal spaces (↑) [a&b]. Group IV, Masson’s trichrome x 400

Fig. 15: An electron micrograph showing part of a hepatocyte containing euchromatic nucleus (N). Mitochondria (m) variable in shape with apparent crista are seen in association with rough endoplasmic reticulum (rER). Rosettes of glycogen granules (red arrow↑) scattered in cytoplasm are also conspicuous. The cell membrane between two adjacent hepatocytes is noticed (red arrow head▲). Group I, TEM x 8000

Fig. 16: An electron micrograph showing bile canaliculus in between adjacent hepatocytes (red arrow head ▲), with short microvilli from the hepatocytes surface projecting into its lumen. Junctional complexes (red arrow↑) appeared attaching hepatocytes to each other close to the bile canaliculus. Notice the mitochondria (m), rough endoplasmic reticulum (rER) and rosettes of glycogen granules (yellow arrow↑) within the hepatocytes. Group I, TEM x 25000
Fig. 17: An electron micrograph showing hepatic stellate cell (HSC) with elongated nucleus (N) and multiple lipid droplets (↑) is seen within the cytoplasm Group I, TEM x 12000.

Fig. 18: An electron micrograph showing hepatocyte with vacuolated cytoplasm (red arrow↑) and shrunken, irregular nucleus with more peripheral heterochromatin (N). Small lipid droplets of variable size with well-defined edges are scattered in the cytoplasm (*). Apparent decrease in mitochondria (m) and rough endoplasmic reticulum is noticed as compared to the control. Group III, TEM x 8000.

Fig. 19: An electron micrograph showing elongated distorted mitochondria (m) are evident in hepatocyte cytoplasm. Numerous profiles of smooth endoplasmic reticulum (sER) and few rER (red arrow↑) are also noticed as compared the control group. Group III, TEM x 12000.

Fig. 20: An electron micrograph showing lipid droplets of variable size with well-defined edges scattered in the cytoplasm (*). Notice the collagen fibrils (red arrow↑) in between the hepatocytes. Group III, TEM x 12000.
Fig. 21: An electron micrograph showing bile canaliculus with short microvilli projecting into its lumen and lacking in some sites when compared to that of the control group (red arrow head ▲). Junctional complexes are seen attaching hepatocytes to each other (red arrow↑). Distorted elongated mitochondria are noticed (m). Group III, TEM x 25000

Fig. 22: An electron micrograph showing elongated Hepatic stellate cell (HSC) in between vacuolated hepatocytes (*) with cytoplasm nearly devoid of lipid droplets as compared to that of the control. Collagen fibrils are seen in between hepatocytes (red arrow↑). Group III, TEM x 12000

Fig. 23: An electron micrograph showing part of a hepatocyte containing euchromatic nucleus (N). Mitochondria (m), Rough endoplasmic reticulum (rER) and glycogen granules are also seen (red arrow↑). Group IV, TEM x 8000

Fig. 24: An electron micrograph showing bile canaliculus with microvilli projecting into its lumen (red arrow head ▲). Cell membranes of the adjacent hepatocytes are attached to each other by junctional complexes close to the canaliculus (red arrow↑). Mitochondria (m), rough endoplasmic reticulum (rER) and rosettes of glycogen (yellow arrow↑) are seen. Group IV, TEM x 23000
Table 1: Showing changes in area percentage of glycogen granules represented by mean + standard deviation in different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area percentage of glycogen granules (%)</td>
<td>30.056+</td>
<td>31.694+</td>
<td>8.012+</td>
<td>25.918+</td>
</tr>
<tr>
<td></td>
<td>9.55398*</td>
<td>8.73946*</td>
<td>5.34855**</td>
<td>7.7991**</td>
</tr>
</tbody>
</table>

* Significant difference from group I.
† Significant difference from group II.
■ Significant difference from group III.
▲ Significant difference from group IV.
O Non-significant difference from group I.

Table 2: Showing changes in area percentage of collagen fibers represented by mean + standard deviation in different groups

<table>
<thead>
<tr>
<th>Group</th>
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<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
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</thead>
<tbody>
<tr>
<td>Area percentage of glycogen granules (%)</td>
<td>13.33+</td>
<td>13.019+</td>
<td>18.162+</td>
<td>14.281+</td>
</tr>
<tr>
<td></td>
<td>2.33877*</td>
<td>2.21434*</td>
<td>5.80943**</td>
<td>3.43168*</td>
</tr>
</tbody>
</table>

* Significant difference from group I.
† Significant difference from group II.
■ Significant difference from group III.
▲ Significant difference from group IV.
O Non-significant difference from group I.

Table 3: Showing changes in biochemical measurement (ALT, AST and ALP) represented by mean + standard deviation in different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU/L)</td>
<td>28.4+8.127*</td>
<td>27.1+3.665*</td>
<td>67.9+9.689**</td>
<td>28.9+9.871*</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>23.2+9.199*</td>
<td>20.8+5.412*</td>
<td>82.0+18.3*</td>
<td>24.0+8.014*</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>68.3+15.6*</td>
<td>65.6+14.976*</td>
<td>265+91.308**</td>
<td>67.2+15.259*</td>
</tr>
</tbody>
</table>

* Significant difference from group I.
† Significant difference from group II.
■ Significant difference from group III.
▲ Significant difference from group IV.
O Non-significant difference from group I.

Table 4: Showing changes in biochemical measurement of tissue MDA represented by mean + standard deviation in different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue MDA (umol/g)</td>
<td>4.629+1.2937*</td>
<td>4.427+1.62077*</td>
<td>10.819+1.52343**</td>
<td>4.435+1.42364*</td>
</tr>
</tbody>
</table>

* Significant difference from group I.
† Significant difference from group II.
■ Significant difference from group III.
▲ Significant difference from group IV.
O Non-significant difference from group I.

Table 5: Showing changes in biochemical measurement of Tissue GSHPx and catalase represented by mean + standard deviation in different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue GSHPx (U/g)</td>
<td>672.113+143.13622*</td>
<td>816.667+392.02487**</td>
<td>191.193+51.87027**</td>
<td>656.447+149.0886**</td>
</tr>
<tr>
<td>Tissue Catalase (umol/g)</td>
<td>3.539+0.43778*</td>
<td>4.732+1.44193**</td>
<td>1.151+0.38339**</td>
<td>3.49+0.77236*</td>
</tr>
</tbody>
</table>

* Significant difference from group I.
† Significant difference from group II.
■ Significant difference from group III.
▲ Significant difference from group IV.
O Non-significant difference from group I.
In this study, orally given aqueous extract of Moringa leaves was chosen; as it is easily prepared and readily administered[20] so, it can be easily prepared at home by any one. Moreover, toxicity studies have shown that it has no significant adverse effects in rats and rabbits[20]. In the present study, rats treated with Moringa oleifera Leaf extract (Group II) showed hepatic architecture nearly similar to that of the control. No hepatocyte injury was observed. This was also confirmed by the electron microscope examination, where the ultra structure of the liver tissue was comparable to that of the control. This was in consistence with some authors[23] who reported that rats received Moringa oleifera alone showed normal cellular architecture by light microscopy with no remarkable changes in comparison with the control. Any marked necrosis of the liver cells leads to a significant rise of Liver transaminases in the blood and a substance that doesn’t affect their level is considered non-toxic to the hepatocytes[20]. So the blood liver function tests in the current study were done and it was found that there was no significant difference between the control rats and the rats treated with Moringa oleifera leaf extract (Group II).

Rats received Moringa extract in the current study, showed significant increase in GSHPx and catalase activity and no significant difference of MDA level as compared to that of the control. This was in accordance with some investigators[34]. Other authors reported that treatment with Moringa oleifera or with phytochemicals isolated from Moringa oleifera have shown elevation of a variety of antioxidant enzymes[20]. It was found that tocopherols, phenolics and carotenoids are natural antioxidant components found in Moringa oleifera[23].

The light microscopic examination of liver sections of rats treated by paracetamol (Group III), in the current study, showed significant increase in GSHPx and catalase activity and no significant difference of MDA level as compared to that of the control. This was in accordance with some investigators[34]. Other authors reported that treatment with Moringa oleifera or with phytochemicals isolated from Moringa oleifera have shown elevation of a variety of antioxidant enzymes[20]. It was found that tocopherols, phenolics and carotenoids are natural antioxidant components found in Moringa oleifera[23].

In the present study, rats treated with Moringa oleifera Leaf extract (Group II) showed hepatic architecture nearly similar to that of the control. No hepatocyte injury was observed. This was also confirmed by the electron microscope examination, where the ultra structure of the liver tissue was comparable to that of the control. This was in consistence with some authors[23] who reported that rats received Moringa oleifera alone showed normal cellular architecture by light microscopy with no remarkable changes in comparison with the control. Any marked necrosis of the liver cells leads to a significant rise of Liver transaminases in the blood and a substance that doesn’t affect their level is considered non-toxic to the hepatocytes[20]. So the blood liver function tests in the current study were done and it was found that there was no significant difference between the control rats and the rats treated with Moringa oleifera leaf extract (Group II).

In this study, orally given aqueous extract of Moringa leaves was chosen; as it is easily prepared and readily administered[20] so, it can be easily prepared at home by any one. Moreover, toxicity studies have shown that it has no significant adverse effects in rats and rabbits[20]. In the present study, rats treated with Moringa oleifera Leaf extract (Group II) showed hepatic architecture nearly similar to that of the control. No hepatocyte injury was observed. This was also confirmed by the electron microscope examination, where the ultra structure of the liver tissue was comparable to that of the control. This was in consistence with some authors[23] who reported that rats received Moringa oleifera alone showed normal cellular architecture by light microscopy with no remarkable changes in comparison with the control. Any marked necrosis of the liver cells leads to a significant rise of Liver transaminases in the blood and a substance that doesn’t affect their level is considered non-toxic to the hepatocytes[20]. So the blood liver function tests in the current study were done and it was found that there was no significant difference between the control rats and the rats treated with Moringa oleifera leaf extract (Group II).

Mitochondrial damage causes activation of Kupffer cells which in turn release cytokines and chemokines that recruit neutrophils and monocytes and cause cellular injury. The direct action of cytokines and chemokines, rather than inflammatory cells, is thought to cause cellular injury by altering intracellular events within hepatocytes[35]. Paracetamol administration leads to an increase in pro-inflammatory cytokines; as tumor necrosis factor (TNF)-α or interleukin (IL)-1β[36]. The appearance of congestion was attributed to the induction of morphologic changes and to alterations in the relationship between hepatocytes and sinusoidal lining cells as a result of paracetamol toxicity[34]. Cellular infiltrations (lymphocytes and neutrophils) were also reported in another work[36]. Infiltration of inflammatory cells into perivascular spaces and portal triad was observed in paracetamol treated rats[37].

An apparent increase in the number of the lining cells of bile ductules was also noticed in paracetamol treated group of this study. This was reported in a previous work[38]. It was hypothesized that when liver is exposed to injurious stimulus, cholangiocytes hyperplasia may occur. Three types of cholangiocytes proliferation were suggested; Type I “Typical”, Type II “Atypical” and Type III “Oval cell”.

In type I, proliferation arises from older ducts, confined to portal areas and functional characteristics of cholangiocytes are preserved. Proliferating cholangiocytes exhibit stem cell markers. This type of proliferation can be associated with sprout of new side branches. This theory could be the case in our study which still needs further investigation. On the other hand, type II which is more common with chronic injury, results from retro-differentiation (trans-differentiation) of hepatocytes into cholangiocytes. Proliferating cholangiocytes are characterized by being not confined to portal areas, irregular, with no lumen and exhibit functional and cellular properties of both hepatocytes and cholangiocytes. While, Type III results from proliferation of non parenchymal germ cells (Oval cells) giving disorganized ducts that are randomly spread into hepatic lobules leading to distorted hepatic tissues.
architecture. Oval cells can differentiate into hepatocytes, cholangiocytes and exocrine portion of pancreatic cells.

In the present study, the paracetamol treated group also showed an apparent decrease in the PAS positive glycogen granules compared to that of the control, which was confirmed by the morphometric analysis. This was supported by preceding studies that agreed with these consequences. Also, significant increase in collagen fibers around the central vein, portal area and intercellular hepatocytes were observed in group III of this study as compared to the control. Additionally, this result was demonstrated by the electron microscopic examination which showed collagen fibrils in between the hepatocytes. Moreover, HSCs in association with collagen fibrils were frequently seen in between hepatocytes and they appeared elongated with no fat droplets in their cytoplasm. This was in accordance with some authors who, described that the presence of some fibrosis was consequent with ballooning degeneration, and that fibrosis is classically found encircling hepatocytes, so called peri-cellular fibrosis. Following liver injury, HSCs undergo activation. Activated HSCs evolves into myofibroblast-like cells without lipid droplets and synthesize a large amount of extracellular matrix components including collagen, proteoglycan and adhesive glycoproteins. Additionally, activated HSCs also synthesize tissue inhibitor of interstitial collagenases. So prevent collagen degradation leading to increase extracellular matrix. HSCs activation was attributed to the release of cytokines and growth factors as a result of Kupffer cells activation by hepatocytes damage. While, others accredited HSCs activation to oxidative stress.

Histopathological findings in this study were verified by the biochemical analysis. The liver function tests showed significant increase in ALT, AST and ALP in paracetamol treated rats as compared to that of the control, similarly as reported by another researchers. The transport function and membrane permeability are impaired by hepatocyte injury as a result of paracetamol toxicity, leading to leakage of enzymes from these cells. In addition, Paracetamol could be achieving its toxic effects by triggering oxidative stress. In the present study it was found that tissue MDA was significantly increased and tissue GSHPx and Catalase were significantly decreased as compared to the control in paracetamol group. This was in consistence with the results of some researchers. They clarified that paracetamol leads to increased generation of reactive oxygen species (ROS). They added that the high level of ROS is known to cause impairment of antioxidant enzyme activities resulting in oxidative stress. Oxidative stress in turn induces various actions including lipid peroxidation, which is characterized by oxidative degradation of membrane phospholipids. Malondialdehyde (MDA) level reflects the degree of lipid peroxidation in tissues especially hepatocytes and elevated MDA product coincides with hepatotoxicity seen with administration of paracetamol. The paracetamol induced liver injury was further explained by another authors. Their theory denoted that cytochrome P4502E (CYP2E1) is the major catalyst involved in the metabolism of drugs, and Paracetamol is mainly metabolized by CYP2E1 to form an electrophilic metabolite, N-acetyl-p-benzoquinone imine (NAPQI), which is primarily inactivated by conjugation with glutathione (GSH) and binding with other proteins to form protein adducts. Accumulation of the intermediate metabolites and the depletion of GSH are key mechanisms of paracetamol hepatotoxicity that directly cause liver damage. Hepatocytes containing adducts are undergoing necrotic changes as evidenced by vacuolization and pyknotic changes in the nuclei. In addition, the only hepatocytes observed to develop necrosis were those containing paracetamol protein adducts. Moreover, paracetamol induced CYP2E1 expression, which increases NAPQI formation associated with paracetamol hepatotoxicity.

In the current study, pre-administration of Moringa leaf extract prior to paracetamol in group IV led to amelioration of the toxic effect of paracetamol on the liver. The hepatocytes appeared less vacuolated and with vesicular nuclei compared to that of the paracetamol treated group (Group III). Minimal cellular infiltration around portal area was observed. Periodic Acid Schiff and Masson’s trichrome stained sections showed nearly similar findings as compared to that of the control. These findings were confirmed by the morphometric analysis and the electron microscope examination as hepatocytes and bile canaliculi ultra structure appeared nearly similar to that of the control. No collagen fibrils were observed associated with HSCs which appeared with lipid droplets in their cytoplasm. These findings were in consistence with that of some authors. They outlined that Moringa oleifera extracts protected the liver against paracetamol induced hepatic toxicity.

Also in this study, group IV showed an apparent increase in the number of cells lining bile ductules in the portal area and nearby bile canaliculi. This finding was in consistence with another investigators who reported mild proliferation of bile ducts in liver sections from rats treated by paracetamol preceded by Moringa oleifera. It was suggested that these proliferating cells might be proliferating cholangiocytes exhibiting stem cell markers (Type I “typical cholangiocytes proliferation”). Cholangiocytes might proliferate in an attempt to recover after exposure to injurious stimulus.

It was suggested that the Moringa leaf extract afforded the hepatoprotection through decreased production of free radicals and by its membrane stabilizing activity. This effect could be explained, at least in part, by the active ingredients present in the extracts. High amount of B-carotene was reported in Moringa oleifera extracts. Moreover, Moringa extract contains all essential amino acids. It was hypothesized that the B-carotene of Moringa oleifera is responsible for the hepatoprotective activity. B-carotene may exhibit a good radical trapping antioxidant activity. Besides carotene, Moringa oleifera has been also reported to contain antioxidants such as vitamin C.
In addition, vitamin C present in Moringa oleifera might be responsible for the antioxidant activity\cite{58}. Moringa leaf extracts were capable of scavenging peroxyl and superoxyl radicals. The major bio-active compounds of phenolics isolated from Moringa leaf extract were flavonoid groups such as quercetin and kaempferol and concluded that Moringa leaves are potential source of natural antioxidants due to their marked antioxidant activity\cite{59}.

In the current study, blood liver function tests in rats pretreated with Moringa oleifera leaf extract (Group IV) showed that enzyme levels were nearly similar to the control group. Similar findings were observed in a previous work\cite{22}. The reversal of elevated serum intracellular enzyme levels by Moringa oleifera extract may be attributed to the stabilizing ability of the cell membrane preventing enzymes leakages\cite{60}. Moreover, restoration of MDA level, GSHPx activity and catalase activity as compared to that of the paracetamol treated rats of group III was also noticed in this study. These results goes in parallel with another researchers\cite{56} who outlined that pre-treatment by Moringa Oleifera extracts in paracetamol treated rats reversed the reduction of GSHPx and catalase activity and the elevation in MDA. The role of the plant extract against paracetamol mediated hepatotoxicity could be explained by the ability of the plant to prevent GSHPx and catalase activity impairment. It has effectively scavenged the occurrence of free radicals generated during paracetamol metabolism and subsequently reduced the destructive effects of oxidative stress. Apart from this, phytochemical analysis has revealed the presence of natural antioxidants in Moringa extracts such as phenolics compounds including flavonoid groups such as quercetin and kaempferol\cite{60}. Induction of hepatotoxicity by ROS can be prevented following the administration of an agent with antioxidant capacity\cite{61}. Moringa oleifera remarkably modulated the oxidative stress due to its free radicals scavenging capability\cite{62}.

Consequently, it was concluded that Moringa oleifera extracts could protect the structure and function of the liver against the effects of paracetamol. This hepatoprotective effect is due to inhibition of lipid peroxidation and enhancement of antioxidant enzymes.

**CONFLICTS OF INTEREST**

There are no conflicts of interest

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دراسة هستولوجية عن الدور الوقائي المحتمل لمستخلص أوراق المورينجا أوليفيرا على تلف الكبد الناجم عن الباراسيتامول في ذكور الجرذان البيضاء البالغة

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المورينجا أوليفيرا هي شجرة استوائية عرفت أوراقها بكونها غنية بالمواد المضادة للأكسدة. يعد التسمم الكبدي الناجم عن الباراسيتامول من المحاذير الهامة للصحة العامة.

الهدف من هذا البحث: هو دراسة الدور الوقائي المحتمل للمستخلص المائي لأوراق المورينجا أوليفيرا في اصابة الكبد الناجمة عن الباراسيتامول في الجرذان البيضاء البالغة.

المؤلفون والطرق: تم استخدام أربعين ذكرا من الجرذان البيضاء البالغة في الدراسة الحالية. وتم تقسيمهم إلى أربع مجموعات متساوية: المجموعة الأولى: المجموعة الضابطة (المجموعة المضادة للباراسيتامول)، المجموعة الثانية: تناولت ماء الباراسيتامول لمدة 7 أيام ثم تناولت المستخلص المائي لأوراق المورينجا أوليفيرا المائي لمدة 7 أيام، المجموعة الثالثة: تناولت ماء الباراسيتامول لمدة 14 يوماً، المجموعة الرابعة: تناولت مستخلص أوراق المورينجا أوليفيرا المائي لمدة 14 يوماً.

في نهاية التجربة، تم أخذ عينات الكبد وتجهيزها للدراسات الهستولوجية والهستوكيميائية. كما تم جمع عينات الدم وتعقب الأمور المطلوبة. تم تحليل النتائج باستخدام التحليل الإحصائي.

النتائج: تناول مستخلص أوراق المورينجا أوليفيرا المجموئة الثانية لم يؤثر في بنية الكبد وخلاياه. حيث أن مقاطع الكبد كانت تقريباً مشابهة للمجموعة الضابطة. و قد تم تلخيص ذلك بإنثاثات ووظائف الكبد التي تم تظهر تغير عن المجموعة الضابطة. إضافة إلى ذلك كان هناك زيادة ذو دلالة إحصائية في نشاط الجلوتاتيون بيراسكوديدز والكالسيتليين و لم يكن هناك تغير في مستوى الألانين أثر دهان معاد مقارنة بالمجموعة الضابطة. بينما سفراء الباراسيتامول للمجموعة الثالثة عن ظهور تجفيفات في الخلايا الكبدية. وتم ملاحظة احترار وإرتشاح خلوي حول الوريد البالبي ووريد المركز. و كان هناك زيادة ذو دلالة إحصائية في النسبة المئوية لمساحة الألياف الكولاجين مقابلة بالمجموعة الضابطة بينما نقصت نسبة الموليدن البنفسجية باللوبيسيني الباسبب الأسبوع بالمركبات الكبدية، مع زيادة الإلكترون التحسسية ووجود ميتوكيندريات طويلة ومشوهة ونقص ملحوظ في الشبكة القدالية الكبدية مع زيادة ملحوظة في الشبكة القدالية الكبدية. بالإضافة إلى أن الخلايا النمطي للظهيرة الكبدية مع آلياف الكولاجين ظهرت بكترة بين خلايا الكبد. تناول مستخلص أوراق المورينجا أوليفيرا قبل الباراسيتامول في المجموعة الرابعة أدى إلى تقليل الأثار الناجمة عن الباراسيتامول على الكبد. لقد لوحظ إرتشاح خلوي في الأوعية البالي البباية الكبدية مع زيادة ملحوظة في عدد الخلايا المبطنة للقناطر المرارية.
الاستنتاج: مسخل أوراق المورينجا أوليفيرا له دور وقائي على الكبد ضد تأثيرات الباراسيتامول، و ذلك لقدرته على تثبيط أكسدة الدهون و تحفيز الإنزيمات المضادة للأكسدة.