EFFECT OF MORINGAOLEIFERA LEAVES EXTRACT AGAINST ACETAMINOPHEN INDUCED HEPATOTOXICITY IN ALBINO RATS.

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ABSTRACT

This study was carried out to evaluate the the effect of moringaoleifera leaves Extract against liver injury induced by APAP in rats. The experimental animals were divided into six groups of male albino rats. Aqueous extract of Moringaoleifera (AMO) and hydroalcoholic extract of Moringaoleifera (HMO) were prepared and pre-administered orally to rats at dose 500 mg/kg body weight; p.o for a period of 28 days prior to a single oral dose of APAP 3 g/kg body weight; p.o. The results showed that APAP induced a significant rise in the activities of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and the level of malondialdehyde (MDA) besides reduction in the activities of glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT). The activities of AST, ALT, ALP and the level of MDA were significantly decreased while the activities of GR, SOD and CAT were significantly increased in rats pre-administered with HMO and AMO prior to APAP treatment compared to the group treated with APAP alone. Rats pre-administered with HMO prior to APAP treatment have provided more significant protection in the activities of liver enzymes and antioxidants enzymes than rats pre-administered with AMO prior to APAP treatment. Pre-administered of HMO and AMO significantly prevented the hepatocellular changes associated with APAP treatment. The results from the present study suggested that the two extracts of moringaoleifera leaves (HAMO and AMO) possess a remarkable hepatoprotective and antioxidant activity against APAP induced hepatotoxicity.

INTRODUCTION

The liver is the most important organ in the body. It plays a bivalent role in regulating various physiological processes (Ahsan et al., 2009). The role played by this organ in the removal of substances from the portal circulation makes it susceptible to first and persistent attack by offending foreign compounds, culminating in liver dysfunction (Bodakhe and Ram, 2007). Liver diseases have become one of the major causes of morbidity and mortality all over the world. Among them, drug induced liver injury is one of the most common causative factor that poses a major clinical and regulatory challenge (Russmann et al., 2009).

Acetaminophen (APAP) is a widely used medication for the treatment of pain and fever in children and pregnant women. Initiation of (APAP) toxicities is believed to be promoted by oxidative stress of liver during the event of overdosage. Acetaminophen is an effective and widely used antipyretic-analgesic drug with excellent safety record when taken at therapeutic doses (Larson, 2007). Despite extensive efforts to conduct studies on the processes of APAP induced toxicity, the exact mechanisms are still incompletely understood. Most evidence suggested the depletion of glutathione and the formation of reactive metabolites is somehow triggers the cascade events of hepatotoxicity. Overdose of APAP results in the generation of free radicals following the depletion of glutathione (Jaeschke and Bajt, 2006).
Moringa oleifera L (Moringaceae) known commonly as Ben oil tree or drumstick tree in English language, ‘Okweoyibo’ in Igbo, ‘Gawara’ or ‘Habiwal’ in Hausa and ‘Adagbamaloye’ or ‘Ewe Igbale’ in Yoruba grows rapidly in most regions and climatic conditions of Nigeria. *M. oleifera* is an important food commodity which has had enormous attention as the ‘natural nutrition of the tropics’ (Anwar et al., 2007). A number of medicinal properties have been ascribed to various parts of this tree. Most parts of this plant: root, bark, gum, leaves, fruit (pods) flowers, seed and seed oil have been used in folk medicine in Africa and South Asia (Fahey, 2005). It has been used for the treatment of inflammation, infectious diseases, cardiovascular, gastrointestinal, hematological and hepatorenal disorders (Siddhuraju and Becker, 2003). Most of plants uses for medicinal purposes have been correlated to their possession of antioxidant activity (Sofidiya et al., 2006). Plants and their products are potential sources of phytochemicals that have been found to counteract free radicals due to their antioxidant activity (Khalafalla et al., 2010). The antioxidant activity of *Moringa oleifera* extracts is due to the presence of various bioactive compounds such as chlorogenic acid, rutin, quercetin-glucoside, and kaempferol-rhamnoglucoside (Atawodi et al., 2010). The leaves are highly essential nutritious when compared to other parts, being a source of protein, β-carotene, B, C and E, riboflavin, nicotinic acid, folic acid, pyridoxine, amino acids, minerals and various phenolic compounds (Khalafalla et al., 2010). Also Moyoe et al. (2012) suggested that *M. oleifera* could be a potential source of polyphenolic compounds with strong antioxidant potential. *M. oleifera* leaves protected the tissues against oxidative stress in rats. This profound protective effect of *M. oleifera* may explain its extensive use in daily life and possible health benefits (Vermaet et al., 2009). The present study aimed to evaluate the hepatoprotective of ethanol and aqueous extract of the leaves of *Moringa oleifera* L against Acetaminophen (APAP) induced hepatotoxicity in rats.

**MATERIALS AND METHODS**

**Materials:**

**Chemicals:**

Acetaminophen was purchased from Sigma Chemical Co. (St Louis, MO, USA) and solvents were purchased from Merck (Darmstadt, Germany). Kits used for the estimation of analyzed parameters were purchased from Biosystem, Spain and Biodiagnostic, France.

**Plant material:**

*Moringa oleifera* (MO) leaves powder was obtained from National Research Center, Dokki, Cairo, Egypt.

**Corn starch, corn oil, cellulose and sucrose:**

Corn stachard corn oil were purchased from the local market while sucrose and cellulose were purchased from Sigma Chemical Co. (St Louis, MO, USA).

**Mineral salts and vitamins:**

Mineral salts and vitamins used for the preparation of the mineral salt and vitamin mixtures were obtained from Sigma Chemical Co. (St Louis, MO, USA) and prepared according to AIN-1993 (Reeves et al., 1993).
Animals:

Adult male albino rats weighing (120 ±5g) were obtained from Faculty of Veterinary Medicine, Cairo University.

Methods:

Plant materials and extraction:

The dried leaves powder was stored in polyethylene bags in refrigerator at 4°C until further use. 10 g of leaves powder was extracted using 100 ml boiling distilled water or 80% hydroalcoholic solvent (80% ethanol: 20% distilled water) with constant shaking at room temperature for overnight. The two extracts were filtered and the residue were then resuspended in boiling distilled water or 80% hydroalcoholic solvent for another 24 hours and refiltered. The two filtrates were then concentrated using a rotary evaporator (RV 10 digital, Germany) under reduced pressure at 40 ⁰C and then lyophilized using a freeze dryer (Labconco, Model Lyph. Lock 6). The two dark green mass were obtained and stored at -20 ⁰C until further use. The two crude extracts, were resuspended in distilled water before administration to the animals.

APAP induction of liver injury:

Liver injury was induced by an oral administration of 3 g/kg APAP in 40% sucrose buffer, 1 ml/kg body weight, on day 28 (Fakurazi et al., 2008).

Experimental animals and study design:

The animals were housed in separated cage and maintained under control laboratory conditions at 25°C ± 2°C on a 12h light: 12h dark cycle for a week with free access to standard food and water ad libitum. The animals were handled accordance to the rules and regulations by the animal's house of Regional Center for Food and Feed, Agriculture Research Center, Giza, Egypt. Standard food consists of corn starch 62.5% casein 14%, sucrose 10%, cellulose 5% corn oil 4%, salt mixture 3.5% and vitamin mixture 1%. Treatment consisted of pretreatment phase of MO in distilled water followed by the second phase in which the animals were given 3 g/kg APAP in 40% sucrose buffer on day 28. During the experimental period (four weeks), the body weight of animals was recorded. The animals were divided into six groups each consisting of 5 rats as follows:

Group A served as normal control group.

Group B served as hepatotoxic control group which animals received a single dose of APAP (3 g/kg body weight; p.o) on day 28.

Group C animals were pre-administered with AMO extract (500 mg/kg body weight; p.o) for 28 days

Group D animals were pre-administered with HAMO extract (500 mg/kg body weight; p.o) for 28 days

Group E animals were pre-administered with AMO (500 mg/kg body weight; p.o) for 28 days before being intoxicated with APAP.

Group F animals were pre-administered with HAMO extract (500 mg/kg body weight; p.o) for 28 days before being intoxicated with APAP.

Twenty-four hours after APAP administration, all rats were anesthetized with diethyl ether and blood samples were collected from the retro-orbital vein using a glass capillary tube. Animals were then sacrificed by cervical decapitation and liver from each group was excised, washed in ice-cold saline to remove blood and duly weighed.
Biochemical assay:

The serum and plasma were separated and collected into sterilized tubes and stored at -20°C for biochemical parameters. Serum was separated by centrifuging (Hettich, Universal 16, German) at 3000 rpm for 20 min at 4°C. Activities of serum enzymes AST and ALT were assayed according to the methods of Gella et al., (1985) while ALP was determined according to the method of Rosalki et al., (1993). Plasma was prepared by collect blood using an anticoagulant heparin and separated by centrifuging at 4000 rpm for 10 min at 4°C for antioxidant studies. The activities of GR, SOD and CAT were measured according to the method reported by Goldberg and Spooner (1983), Nishikimi et al. (1972) and Aebi (1984), respectively. The level of lipid peroxidation was determined as malondialdehyde (MDA) according to the method reported by Onkawa, (1979).

Histopathological studies:

Liver from each group was fixed in 10% buffered formalin and embedded in the paraffin. Microtome sections of 3-4 μm thickness were prepared according to the standard procedure and stained with haematoxylin and eosin. Sections were then examined for pathological findings of such as centrilobular necrosis, fatty and lymphocytes infiltration by the light microscope (Banchroft et al., 1996).

Statistical analysis:

Statistical analysis of the obtained data was done using the least significant difference test (LSD) at the 5% level of probability as outlined by Snedecor and Cochran (1980). Using the Duncan test institute program used a computer in the statistical analysis.

RESULTS AND DISCUSSION

Body and liver weights:

The body weight and relative liver weight of each group are found in Table (1). There are no significant differences in the initial body weights, final body weights, body weight gains and relative liver weights among the six groups of animals. These results are in line with the findings of Brown et al. (2012) who found that the body weights were similar between all treatment groups at 4 h after APAP administration. Also these results are in disagreement with Adedapo et al. (2009) who demonstrated that the aqueous extract of the leaves of Moringa oleifera did not cause any significant change in the weight of liver. Also similar trend was observed by Oinam et al. (2012) who also did not showed any significant difference in weight gain, final weight and mean weight of liver between rats in different treatments after 8 weeks of supplementation with Moringa oleifera.
Table (1) Effect of MOL extracts on body weight and relative liver weight of APAP-induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Body weight (g)</th>
<th>Relative liver weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Group A (Normal control)</td>
<td>124.8±0.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>156.0±0.71&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group B (APAP)</td>
<td>124.0±0.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>155.4±1.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group C (AMO)</td>
<td>124.0±0.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>155.6±1.78&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group D (HAMO)</td>
<td>124.0±0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>156.6±0.93&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group E (AMO + APAP)</td>
<td>124.2±0.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>155.2±1.66&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group F (HAMO + APAP)</td>
<td>124.0±0.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>155.2±0.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

LSD P < 0.05  
2.25  3.58  3.66  0.91

Values are mean ± SE of 5 rats. Within the same column, various superscript letters indicate significant differences (Duncan, P < 0.05).

**Determination of liver enzymes activity:**

Liver function tests help in the diagnosis of any abnormal/normal condition of liver. Leakage of cellular enzymes into serum indicates the sign of hepatic tissue damage. The serum activities of liver enzymes ALT, AST and ALP are shown in Table (2). Rats treated with 3 g/kg APAP has significant elevation of ALT, AST and ALP activities (58, 54 and 347 U/l, respectively) when compared to those groups pre-administered with HAMO and AMO extracts prior to APAP treatment. Our results are consistent with Fakurazi et al. (2008) who suggested that rats treated with a single high dose of APAP (3 g/kg body weight; p.o) have significantly elevated the serum transaminases and ALP activities. Meanwhile Hamza (2010) found that treatment with Moringa oleifera has completely blocked the CCl<sub>4</sub> induced elevation of serum ALT and AST activities. Another study by Nadro et al. (2005) has also shown that MO leaves extract has prevented the release of these enzymes from hepatocytes into the bloodstream when induced with high level of ethanol administration in rats.

The reduction in the activities of ALT, AST and ALP enzymes in group that was pre-administered with HAMO extract prior to APAP treatment were more significantly pronounced (45, 40 and 326 U/l, respectively) compared to the group that was pre-administered with AMO extract prior to APAP treatment (50, 45 and 335 U/l, respectively). The observed results could be due to different degree of polarity of the solvents used for the extraction of polyphenolic compounds. Similar observation was demonstrated by Verma et al. (2009) who reported the leaves of M. oleifera, have higher amount of phenols (gallic, chlorogenic, ellagic and ferulic acid) and flavonoids (kaempferol, quercetin and rutin), which are protected the tissues against CCl<sub>4</sub> induced oxidative stress in rats.

Meanwhile, no significant differences were observed in the liver enzymes activities between the rats administered with HAMO (33, 36 and 312 U/l, respectively) and AMO extracts (33, 36 and 311 U/l, respectively) when compared to normal control group (33, 36 and 313 U/l, respectively).
Table (2) Effect of MOL extracts on liver enzymes activities of APAP-induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Biochemical parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALT (U/l)</td>
</tr>
<tr>
<td>Group A (Normal control)</td>
<td>33±1.14d</td>
</tr>
<tr>
<td>Group B (APAP)</td>
<td>58±1.16a</td>
</tr>
<tr>
<td>Group C (AMO)</td>
<td>33±0.81d</td>
</tr>
<tr>
<td>Group D (HAMO)</td>
<td>33±0.68d</td>
</tr>
<tr>
<td>Group E (AMO + APAP)</td>
<td>50±0.86b</td>
</tr>
<tr>
<td>Group F (HAMO + APAP)</td>
<td>45±1.30c</td>
</tr>
<tr>
<td></td>
<td>2.97</td>
</tr>
</tbody>
</table>

Values are mean ± SE of 5 rats. Within the same column, various superscript letters indicate significant differences (Duncan, P <0.05).

**Determination of antioxidant enzymes activities and levels of MDA:**

Antioxidant enzymes including GR, SOD and CAT are important enzymes for preventing liver damage by oxidative stress. Antioxidant enzyme activity (GR, SOD and CAT) and the levels of MDA in each group are presented in Table (3). Twenty-four hours after APAP administration, rats treated with a single high dose of APAP has caused remarkable reduced in the activities of antioxidant enzymes (GR, SOD and CAT (20.0, 334 and 29.6 U/ml, respectively) besides elevating the levels of MDA (29.6 nmol/ml) when compared to those groups pretreated with HAMO and AMO extracts prior to APAP treatment. Das and Kanodia (2012) reported that the treatment with ethanolic extract of *Moringa oleifera* leaves inhibits the decrease of CAT and SOD level and increase of MDA in rat treated with acetic acid.

Pre-treatment of rats with HAMO extract for 28 days prior to APAP administration caused more significant depletion in the MDA level (18.0 nmol/ml), while improving the activities of antioxidant enzymes (GR, SOD and CAT (25.0, 344 and 37.6 U/ml, respectively) than group that was pretreated with AMO extract prior to APAP administration, at 21.8 nmol/ml for MDA level and 23.0, 339 and 34.6 U/ml for GR, SOD and CAT activities, respectively. This may be due to high phenolic and flavonoid content of methanolic extract of *M. oleifera* as found by Siddhuraju and Becker, (2003). Our findings are in accordance with the results of Verma et al. (2009) who found that co-treatment of rats with polyphenolic fraction of *M. oleifera* leaves at a dose of 50 mg/kg and 100 mg/kg/day, for 14 days significantly inhibited the toxicity produced by CCl4 administration as seen from the decreased lipid peroxides (LPO) and increased in SOD and CAT activities and glutathione (GSH) levels near to normal levels.

In addition, no significant differences were observed in the antioxidant enzymes activities (GR, SOD and CAT) between the rats pretreated with HAMO (29.0, 360 and 43.0 U/ml, respectively) and AMO extracts (28.0, 360 and 43.2 U/ml, respectively) when compared to normal control group (29.0, 357 and 43.2 U/ml, respectively). Also similar trends were observed in the MDA levels of rats pretreated with HAMO and AMO (14.2 and 14.0 nmol/ml, respectively).
Table (3): Effect of MOL extracts on antioxidants enzymes activities and levels of MDA in APAP-induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Biochemical parameters</th>
<th>GR (U/ml)</th>
<th>SOD (U/ml)</th>
<th>CAT (U/ml)</th>
<th>MDA (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (Normal control)</td>
<td></td>
<td>29.0±0.40^a</td>
<td>357±4.54^a</td>
<td>43.2±0.37^a</td>
<td>14.0±0.45^d</td>
</tr>
<tr>
<td>Group B (APAP)</td>
<td></td>
<td>20.0±0.51^a</td>
<td>334±1.36^c</td>
<td>29.6±0.87^a</td>
<td>29.6±0.81^a</td>
</tr>
<tr>
<td>Group C (AMO)</td>
<td></td>
<td>28.0±0.98^a</td>
<td>360±1.89^a</td>
<td>43.2±0.58^a</td>
<td>14.0±0.71^a</td>
</tr>
<tr>
<td>Group D (HAMO)</td>
<td></td>
<td>29.0±0.55^a</td>
<td>360±1.36^a</td>
<td>43.0±0.55^a</td>
<td>14.2±0.37^a</td>
</tr>
<tr>
<td>Group E (AMO + APAP)</td>
<td></td>
<td>23.0±0.68^c</td>
<td>339±0.51^bc</td>
<td>34.6±0.51^c</td>
<td>21.8±0.86^b</td>
</tr>
<tr>
<td>Group F (HAMO + APAP)</td>
<td></td>
<td>25.0±0.66^b</td>
<td>344±1.21^b</td>
<td>37.6±0.51^b</td>
<td>18.0±0.71^c</td>
</tr>
<tr>
<td>LSD P &lt; 0.05</td>
<td></td>
<td>1.91</td>
<td>6.48</td>
<td>1.71</td>
<td>1.97</td>
</tr>
</tbody>
</table>

Values are mean ± SE of 5 rats. Within the same column, various superscript letters indicate significant differences (Duncan, P <0.05).

Effect of MOL extracts on liver histopathology of APAP-induced hepatotoxicity in rats

Fig. (1), showed the histopathological examination of liver sections of untreated control rats (group A) revealed normal histological structure of hepatic lobule (a). Also liver sections of rats from group C and D which pre-administered with AMO and HAMO extracts respectively showed normal histological structure of hepatic lobules (c and d). Conversely, examined liver sections of rats from group B which treated with 3g/kg APAP sacrificed after 24 hours showed coagulation necrosis and apoptosis of hepatocytes associated with inflammatory cells infiltration (b). Whereas, liver sections of rats from group E which pre-administered with AMO extract prior to treatment 3g/kg APAP sacrificed after 24 hours demonstrated hydropic degeneration of hepatocytes and kupffer cells activation (e). Meanwhile, liver sections of rats from group F which pre-administered with HAMO extract prior to treatment 3g/kg APAP sacrificed after 24 hours showed slight activation of kupffer cells (f). A similar observation has been reported by Ahmad et al. (2012) who showed that oral administration of APAP induced renal damage by inducing apoptotic hepatocytes, centrlobular necrosis and inflammation in renal tubular cells. On the other hand these results are in agreement with Uma et al. (2010) who found that pre-treatment of MO significantly prevented the hepatocellular changes associated with paracetamol (PCM) intoxication. Meanwhile, the hydroalcoholic extract of MO alone did not induce any hepatocellular damage and similar to those animals in the control groups.
Fig.(1). Photomicrographs of liver histopathology after pre-administration with AMO and HAMO extracts in APAP-induced hepatotoxicity in rats (hematoxylin and eosin, 400×). a: Normal control; b: APAP hepatotoxin control (3 g/kg b. w); c: AMO (500 mg/kg b.w); d: HAMO (500 mg/kg b.w); e: AMO (500 mg/kg b.w) + APAP (3 g/kg b. w); f: HAMO (500 mg/kg b.w) + APAP (3 g/kg b. w).
CONCLUSIONS:

The present investigation showed that a single high dose of APAP administration (3 g/kg body weight) has significantly elevated the activities of AST, ALT, ALP enzymes and the level of MDA besides reducing the activities of antioxidant enzymes GR, SOD and CAT. According to these results, we suggested that the aqueous and ethanol extracts of M. oleifera leaves were found to have significantly prevented the changes seen in the liver enzymes activities, MDA level, antioxidant enzymes activities and protected the liver tissues against APAP induced hepatotoxicity in rats. The antioxidant potential may be attributed to the presence of polyphenolic compounds. The protective effect of M. oleifera may explain its extensive use in life and possible health benefits.

REFERENCES


