PROTECTIVE EFFECT OF MORINGA PEREGRINA LEAVES EXTRACT ON ACETAMINOPHEN - INDUCED LIVER TOXICITY IN ALBINO RATS.

Samy Abdelfatah Abdel Azim, Mohamed Taha Abdelrahem, Mostafa Mohamed Said, Alshaimaa khattab

National Organization for Drug Control and Research Cairo, Egypt

Abstract

**Background:** Acetaminophen is a common antipyretic drug but at overdose can cause severe hepatotoxicity that may further develop into liver failure and hepatic centrilobular necrosis in experimental animals and humans. This study was undertaken to assess the ameliorative role of *Moringa peregrina* leaves extract against acetaminophen toxicity in rats.

**Materials and methods:** Induction of hepatotoxicity was done by chronic oral administration of acetaminophen (750 mg/kg bwt) for 4 weeks. To study the possible hepatoprotective effect, *Moringa peregrina* leaves extract (200 mg/kg bwt) or Silymarin (50 mg/kg bwt) was administered orally, for 4 weeks, along with acetaminophen.

**Results:** Acetaminophen significantly increased serum liver enzymes and caused oxidative stress, evidenced by significantly increased tissue malondialdehyde, glutathione peroxidase, hepatic DNA fragmentation, and significant decrease of glutathione and antioxidant enzymes in liver, blood and brain. On the other hand, administration of *Moringa peregrina* leaves extract reversed acetaminophen-related toxic effects through: powerful malondialdehyde suppression, glutathione peroxidase normalization and stimulation of the cellular antioxidants synthesis represented by significant increase of glutathione, catalase and superoxide dismutase in liver, blood and brain, besides, DNA fragmentation was significantly decreased in the liver tissue.

**Conclusion:** acetaminophen induced oxidative damage can be improved by *Moringa peregrina* leaves extract-treatment, due to its antioxidant potential.

**Key words:** Moringaceae; NAPQI; Oxidative stress; Antioxidant; DNA fragmentation

**Abbreviations:** ALP: Alkaline phosphatase, ALT: Alanine aminotransferase, APAP: Acetaminophen, AST: Aspartate aminotransferase, bp: base pair, bwt: body weight, CAT: Catalase, CYP450: cytochrome P450, DPPH: 1, 1-diphenyl-2-picrylhydrazyl, FR: free radicals, GGT: Gamma glutamyl transferase, GPx: Glutathione peroxidase, GSH: Reduced glutathione, LPO: lipid peroxidation, MDA: Malondialdehyde, MPL: Moringa peregrina leaves, P.o: per oral, SIL: Silymarin, SOD: Superoxide dismutase.

Introduction

Drug-induced liver injury has become a leading cause of severe liver disease in the world and is initiated by direct hepatotoxic effects of a drug, or a reactive metabolite of a drug (Russman et al., 2009). Acetaminophen (APAP), a widely used over-the-counter (OTC) analgesic and antipyretic, is one of the best known experimental models of hepatotoxicity (Lebda et al., 2013). It is safe in therapeutic doses, but causes fatal hepatic necrosis and hepatic failure in overdose (Bassem and vermeulen, 2001).

It was found that induction of cytochrome P450 (CYP450) isoforms (CYP2E1, CYP3A4, and CYP1A2), depletion of intracellular glutathione (GSH) and oxidative stress are the major mechanisms involved in the pathogenesis of APAP induced liver injury. These actions are mainly ascribed to the formation of N-acetyl-P-benzoquinone imine (NAPQI), a highly toxic, reactive metabolite formed during cytochrome P450 catalyzed oxidation of APAP (Bassem and vermeulen, 2001).

Plants are a rich source of natural bioactive phytochemicals, such as antioxidants that can delay or inhibit the oxidation of lipids and other molecules, by inhibiting the initiation or propagation of oxidative chain reactions (Ali et al., 2011). The combination of medicinal plants with each others also may impact their medicinal values in some cases (Roy et al., 2012).

*Moringa peregrina* (Forssk.) Fiori, [family: moringaceae] is a wild tree that grows in the eastern desert mountains in Egypt (Batanouny et al., 1999). *Moringa peregrina* leaves (MPL) extract was found to contain large number of phytoconstituents (El-Alfy et al., 2011). Scientifically, it has been proven that the leaves possess various pharmacologic effects, including hypoglycemic (El-Alfy et al., 2011), analgesic, anti-inflammatory and hypolipidimic
Azim et al., Afr J Tradit Complement Altern Med., (2017) 14 (2): 206-216
doi:10.21010/ajtcam.v14i2.22

activities (El-Batran et al., 2005). An in vitro study had demonstrated that MPL possessed antioxidant activity (Dehshahri et al., 2012).

To our knowledge, no attempt has been made to study the hepatoprotective potential of MPL. Various reports had shown that antioxidant activity played significant role in the mechanisms of hepatoprotective activity (Muriel, 2009). Based on these data, the present study aimed to trace the antioxidant and hepatoprotective effects of the MPL extract on APAP induced chronic hepatotoxicity in rats in comparison with silymarin as a reference hepatoprotective agent.

Methods
Collection of plant material

The fresh MPL were collected from Al-Orman garden, ministry of agriculture, Giza, Egypt. The plant specimen deposited in orman herbarium under code No. 64M. The plant was identified and authenticated by botanists in the orman herbarium in Al-Orman garden. A voucher specimen was kept in the herbarium of the Applied research center of medicinal plant No.3-6-2009, Moringa peregrine (Moringaceae). The leaves were dried under shade for 7 days at room temperature, separated, and pulverized by mechanical grinder to form coarse powder.

Preparation of Plant Extract

The fresh dried powdered MPL was subjected to 70% ethanol extraction whereby 1 kg of powder leaves was macerated in 20 L of ethanol in the ratio of 1: 20 (w/v) for 72 hours. The supernatant was filtered sequentially using cloth filter, cotton wool, and Whatman filter paper number 1. The solvent was then evaporated under reduced pressure (204 mbar) and controlled temperature (40°C) using a vacuum rotary evaporator (Buchi Rotavapor R205, Switzerland). The whole processes were repeated twice for the remaining residue and then lyophilized using (lyophilizer.UM5sm, Nederland) to obtain powdered extract ( Mahmood et al., 2014).

High-performance liquid chromatography (HPLC)-Assay of Flavonoids and phenolic compounds in MPL extract

Flavonoid and phenolic compounds were HPLC-assayed according to the method of Mattila et al. (2000), using HPLC Agilent (series 1200) equipped with autosampling injector, solvent degasser, ultraviolet (UV) detector set at 330 nm and 280 nm for flavonoids and phenolic acids, respectively, and quarter HP pump (series 1050). The column temperature was maintained at 35°C. Separation was carried out with methanol and acetonitrile (2:1) as a mobile phase at a flow rate of 1ml/min. Flavonoids and phenolic compounds standards (Sigma Co.) were dissolved in the mobile phase and injected into HPLC. Retention time and peak area were used to calculate flavonoids and phenolic compounds concentration through data analysis of HEWLLLET Packard software.

1,1Diphenyl -2-picrylhydrazel free radical scavenging activity (DPPH assay).

The antioxidant activity of MPL extract was determined according to the procedure described by Blois (2002), through preparing DPPH solution (0.3 mM in methanol), serial concentrations of MPL extract (5-250 μg/ml in methanol), and a standard ascorbic acid solution, as a positive control. Extracts (2.5 mL) were, then, mixed with DPPH (1.0 mL) and left in dark for 30 minutes, allowing the reaction to take place. The decrease in absorbance of the resulting solution was monitored at 517 nm.

Animals

Sixty adult female albino rats weighing 160 ± 10 g were used. Animals were provided from the animal house of National Organization for Drug Control and Research (NODCAR) Giza, Egypt. They were housed in cages at room temperature of 25°C ±1 with a 12 hr light- dark cycle. Animals fed on standard rat chow diet and water ad libitium and acclimatized to the environment for a week prior to the experiment. Experiment was carried out in accordance with the protocols approved by the local experimental ethics committee.

Experimental design

Experiment lasted for 4 weeks. A total of 60 rats were divided randomly into 5 groups (12 rats each) as follows:

Group1 (N): negative control, received water. Group2 (APAP): positive control, received APAP (750 mg/kg bwt). Group3 (MPL+APAP): received MPL extract (200mg/kg bwt) along with APAP (750 mg/kg bwt). Group4 (SIL+APAP): received Silymarin (SIL) (50mg/kg bwt) along with APAP (750 mg/kg bwt). Group5 (MPL+SIL+APAP): received MPL extract (200mg/kg bwt) and SIL (50mg/kg bwt) along with APAP (750 mg/kg bwt).
Azim et al., Afr J Tradit Complement Altern Med., (2017) 14 (2): 206-216

doi:10.21010/ajtcam.v14i2.22

bwt). In the present study, the choice of the MPL extract dose, 200 mg/kg, was based on a previous report of the acute toxicity study (El-batran et al., 2005).

The animals were fasted for 24 hours prior to the experiment under standard laboratory conditions, but, allowed to freely access distilled water (dH₂O) ad libitum. After 24 hours, each group received the respective dose of test solution orally once daily for 28 consecutive days. The APAP was orally administered one hour after the extract administration. The biochemical parameters were determined 24 hours after the last dose.

Preparation of blood Samples

At the end of the experiment, all animals from each group were fasted overnight and blood samples were withdrawn from retro-orbital plexus vein of each rat according to the procedure described by Schermer (1967). 0.2 ml whole blood was added to 1.8 ml distilled water for determination of reduced glutathione (GSH). 0.1 ml of blood was haemolysed in 250 ml distilled water for estimation of catalase (CAT). The rest of the blood samples were centrifuged at 3000 rpm for 15 minutes.

After centrifugation, the supernatant was separated and plasma samples were kept at -20°C until used for measuring the activities of ALT, AST, ALP, GGT, total protein, albumin and TBARS. Whereas, the red cells, in the residue, were washed twice with cold saline, then, 1ml of bi-distilled water was added and the erythrocytes were re-suspended by agitation and lysed for 2 hours at 4°C. 0.8 ml chloroform/ethanol mixture (3:5 v/v) and 0.3 ml of bi-distilled water were added to 1.8 ml of homolysate to precipitate and centrifuged at 3000 rpm for 10 min. The chloroform/ethanol supernatant was used for superoxide dismutase (SOD) estimation.

Preparation of liver and brain samples

The rats were sacrificed by cervical dislocation and the livers and brains were rapidly removed. Liver and brain were divided into four parts. The first part: was homogenized in phosphate buffer, pH 7.4, at a concentration of 1:10 w/v, using homogenizer (stuart-SHM2-U.K). The homogenate was then centrifuged at 3000 r.p.m for 10 minutes, and the supernatant obtained was used for the determination of hepatic CAT and glutathione peroxidase (GSHPX) in liver and brain. The second part: was homogenized in cold 5% sulfosalicylic acid solution (1:20 w/v) then centrifuged at 3000 r.p.m for 10 minutes, and the supernatant was used for estimating liver and brain glutathione. The third part: was homogenized in ice-cold tris HCl buffer (0.01M), pH 7.5, centrifuged at 15000 r.p.m for 15 minutes, and the supernatant was used for measuring liver and brain superoxide dismutase (SOD) estimation. The fourth part: was homogenized in cold KCl (1.15 %) to make a 10% homogenate, centrifuged at 3000 r.p.m for 10 minutes, and the supernatant was used for measuring liver and brain malondialdehyde (MDA).

Biochemical blood analysis

Hepatic injury was assessed through measuring plasma levels of ALT, AST (Retiman et al., 1957), ALP (Belfield et al., 1971), GGT (Szasz, 1969), total protein (TP) (Tietz, 1994) and Albumin (Doumas et al., 1971). The aforementioned parameters were determined spectrophotometrically using commercial kits (Biodiagnostic co., Egypt) and UV- visible spectrophotometer (thermo. Nicolet evolution100- England).

Estimation of biomarkers of lipid peroxidation, oxidative stress and antioxidant enzymes in plasma and erythrocytes

Plasma lipid peroxidation level was measured as the concentration of MDA, using the method described by Buege and Aust (1978). Whereas, GSH concentration, Cu/Zn-SOD and CAT activities were determined in erythrocytes using the methods of Beutler et al. (1963), Marklund and Marklund (1974) and Aebi (1984), respectively.

Estimation of oxidative stress biomarkers in liver and brain

Liver and brain tissue homogenates were used for spectrophotometric determination of the following parameters: 1) MDA level, as an index of lipid peroxidation, was measured according to the colorimetric method of Uchiyama and Mihtara (1978). 2) GSH concentration was measured according to Beutler et al. (1963). 3) The activities of SOD and 4) CAT were determined using kinetic colorimetric methods of Marklund and Marklund (1974) and Aebi (1984), respectively. 5) Glutathione peroxidase (GPx) activity was measured using the method described by Rotruck et al. (1973). 6) Total protein, needed for tissue parameters calculation, was determined through the method of Lowry et al. (1951).
Analysis of total genomic DNA, using DNA fragmentation assay

DNA fragmentation assay was performed using the salting out extraction technique (Aljanabi and Martinez, 1997), followed by agarose gel electrophoresis (Hassab El-Nabi, 2004). The DNA bands intensities were assessed using Biogene software (Biogene software, France).

Statistical analysis

Results of biochemical assays were presented as mean ± S.E. and analysed statistically using one-way analysis of variance (SPSS 17.0) according to Snedecor and Cochran (1981), followed by Tukey’s post-hoc test for multiple comparisons. Results were considered to be significantly different when p < 0.05.

Results

HPLC-Assay of Flavonoids and phenolic compounds in MPL extract

As presented in Table 1 and Fig 1, the flavonoids identified in MPL that showed the highest concentrations were rutin: 487.3 mg/100 g dry extract, naringin: 45.4 mg/100 g dry extract and quercetin: 14.32 mg/100 g dry extract, while, the lowest flavonoid concentration identified in MPL, was that of 7-OH flavone: 0.31 mg/100 g dry extract.

Whereas, Table 2 and Fig 1 show that the highest concentrations of phenolic compounds identified in MPL, were those of 3-OH-tyrosol: 1763.7, protocatechein: 444.4 and epicatechein: 413.1 (mg/ 100 g dry extract). Table 2 and Fig 1 also reveal that the lowest phenolic compound concentration identified in MLE was that of P-coumaric acid: 1.96 mg/100 g dry extract.

![HPLC chromatograms of MPL](image)

**Figure 1:** High-performance liquid chromatography (HPLC) chromatograms of MPL, at 280 nm (A) and 330 nm (B).

| Flavonoids     | Concentration (mg/100g) | Flavonoids     | Concentration (mg/100g) |
|----------------|-------------------------|----------------|-------------------------|
| Vitexin        | 16.52                   | Narengenin     | 1.1                     |
| Naringin       | 45.43                   | Kampferol      | 1.82                    |
| Rutin          | 487.3                   | Hisperetin     | 2.27                    |
| Rosmarinic     | 3.67                    | Apegenin       | 5.43                    |
| Quercetrin     | 6.96                    | 7-OH flavone   | 0.31                    |
| Quercetin      | 14.32                   |                |                         |

**Table 1:** Concentrations of Flavonoids in MPL extract.
Table 2: Concentrations of phenolic compounds in MPL extract:

| Phenolic compound     | Concentration (mg/100g) | Phenolic compound     | Concentration (mg/100g) |
|-----------------------|-------------------------|-----------------------|-------------------------|
| Gallic                | 15.1                    | Chicoric              | 96.9                    |
| Pyrogallol            | 243.14                  | Ferulic               | 52.1                    |
| 3-OH-Tyrosol          | 1763.74                 | Iso-ferulic           | 29.8                    |
| 4-Amino-benzoic       | 23.1                    | e-vanillic            | 485.25                  |
| Protocatechuic        | 444.43                  | Reversetrol           | 19.5                    |
| Chlorogenic           | 93.42                   | Ellagic               | 60.82                   |
| Catechol              | 165.65                  | Alpha-coumaric        | 12.1                    |
| Epichatecin           | 413.1                   | Benzoic               | 71.57                   |
| Salicylic             | 157.65                  | 3,4,5-methoxy-cinnamic| 10.5                   |
| Caffeine              | 64.33                   | Coumarin              | 8.24                    |
| P-OH-benzoic          | 51.72                   | P-coumaric            | 1.96                    |
| Caffeic               | 51.44                   | Cinnamic              | 6.02                    |
| Vanillic              | 49.22                   |                       |                         |

**DPPH assay:**

Scavenging of DPPH represents the free radicals reducing activity of antioxidants based on a one-electron reduction which was determined by the decrease of its absorbance at 520 nm. MPL extract exhibited significant antioxidant activity in the DPPH assay in a concentration-dependent manner. The leaf extract showed IC₅₀ value of 7.1 ± 0.31 μg/ml compared with ascorbic acid, which was used as the positive control with IC₅₀ value of 4.6 ± 0.22 μg/ml (Fig. 2).

![Figure 2: Percentage of DPPH inhibition against different concentrations of MPL extract and Ascorbic acid. Values are mean ± standard error of triplicate determinations.](image)

**Effects of MPL Extract on serum hepatic marker enzymes**

The activities of serum hepatic marker enzymes, ALT, AST, ALP and GGT were significantly elevated in APAP treated rats, compared to normal rats. (Table 3). The decrease in total protein and albumin were non-significant when compared to N group. Meanwhile, oral administration of MPL, SIL and MPL+SIL together with APAP significantly decreased the increased activities of serum hepatic marker enzymes in comparison with the normal control group, where treatment with the mixture (MPL+SIL) showed no more pronounced effects, compared to the groups administered one extract only with APAP.
Azim et al., Afr J Tradit Complement Altern Med., (2017) 14 (2): 206-216

doi:10.21010/ajtcam.v14i2.22

Table 3: Effects of MPL and SIL on hepatic ALT, AST, ALP and GGT levels in APAP induced hepatotoxicity in experimental rats.

| Parameter | Groups | N     | APAP     | MPL + APAP | SIL + APAP | MPL+SIL+APAP |
|-----------|--------|-------|----------|------------|------------|--------------|
| ALT (U/L) |        | 25.3 ± 1.1(b) | 71.8 ± 2.7(ad) | 37.8 ± 1.3(ab) | 33.3 ± 1.7(ab) | 30.8 ± 1.2(b) |
| AST (U/L) |        | 67.6 ± 3.7(b)  | 128 ± 6.4(a)   | 73.6 ± 3.0(b)  | 72.8 ± 3.8(b)  | 71.3 ± 2.7(b)  |
| ALP (U/L) |        | 117.7 ± 5.1(bo) | 156.5 ± 1.8(b) | 127.4 ± 3.8(b) | 123.6 ± 5.3(b) | 120.2 ± 4.9(bf) |
| GGT (U/L) |        | 8.3 ± 0.3(b)   | 12.8 ± 0.83(a) | 6.7 ± 0.33(b)  | 7.7 ± 0.33(b)  | 7.2 ± 0.26(b)  |
| TP (mg/dl) |       | 8.1 ± 0.4      | 6.5 ± 0.32     | 7.9 ± 0.56     | 7.8 ± 0.32     | 8.2 ± 0.56     |
| Albumin (mg/dl) |      | 3.6 ± 0.16     | 3.3 ± 0.16     | 3.5 ± 0.16     | 3.3 ± 0.19     | 3.2 ± 0.22     |

Values are expressed as means ±SEM of 12 rats per group; p<0.05 (using one-way ANOVA followed by Tukey’s HSD post-hoc test); a significant difference from normal control group; b significant difference from APAP treated group.

Effect of MPL Extract on Oxidative Stress Markers in APAP Induced Toxicity

Tables 4-6 showed that APAP intoxication resulted in a state of oxidative stress in the liver, revealed by the results of the biochemical tests. Administration of APAP to experimental animals caused an increase in lipid peroxidation, and hence, in MDA production in blood, liver and brain compared to the negative control group. Groups treated with either MPL or SIL showed significant reduction of the elevated MDA levels. On the contrary, reduced glutathione (GSH), was severely diminished in liver, brain and blood of animals given APAP. Administration of MPL extract or SIL, either alone or in combination, along with APAP, succeeded in replenishing GSH stores in blood, liver and brain. Meanwhile, the activities of SOD, CAT and GPx were significantly lowered in APAP intoxicated rats. After administration of MPL extract or SIL, there was a powerful enhancement in the activities of CAT and SOD, as well as a complete normalization in GPx activity.

Table 4: Effect of MPL and SIL on blood GSH, CAT, SOD and MDA in APAP induced hepatotoxicity in experimental rats.

| Group Parameters | N     | APAP     | MPL + APAP | SIL + APAP | MPL+SIL+APAP |
|------------------|-------|----------|------------|------------|--------------|
| GSH (mg/dl)      | 40.4 ± 2.80(b) | 30.8 ± 1.65(a) | 42.9 ± 1.02(b) | 42.6 ± 1.39(b) | 44.5 ± 2.35(b) |
| CAT (U/ml)       | 97.3 ± 5.6(b)  | 69.9 ± 4.2(a)  | 97.98 ± 6.5(b) | 102.7 ± 4.8(b) | 93.8 ± 3.2(b)  |
| SOD (U/mg Hb)    | 23.7 ± 1.1(b)  | 14.0 ± 1.2(a)  | 22.8 ± 0.9(b)  | 23.5 ± 0.9(b)  | 25.1 ± 1.6(b)  |
| MDA (nM/ml)      | 8.64 ± 0.42(b) | 13.34 ± 0.79(a) | 9.83 ± 0.69(b) | 10.56 ± 0.56(b) | 9.24 ± 0.27(b) |

Values are expressed as means ±SEM of 12 rats per group; p<0.05 (using one-way ANOVA followed by Tukey’s HSD post-hoc test); a significant difference from normal control group; b significant difference from APAP treated group.

Table 5: Effect of MPL and SIL on hepatic GSH, CAT, SOD, MDA and GPx in APAP induced hepatotoxicity in experimental rats.

| Parameter | Groups | N     | APAP     | MPL + APAP | SIL + APAP | MPL+SIL+APAP |
|-----------|--------|-------|----------|------------|------------|--------------|
| GSH (µg/mg pt) | 29.88 ± 1.71(b) | 13.96 ± 0.98(a) | 28.87 ± 1.75(b) | 27.29 ± 2.17(b) | 30.0 ± 2.34(b) |
| CAT (U/ mg pt) | 64.7 ± 1.92(b)  | 38.3 ± 1.35(a)  | 59.5 ± 2.98(b)  | 58.7 ± 2.68(b)  | 60.9 ± 2.11(b)  |
| SOD (U/mg pt)  | 14.54 ± 0.68(b) | 6.40 ± 0.45(a)  | 14.10 ± 0.63(b) | 13.22 ± 0.78(b) | 14.2 ± 0.59(b)  |
| MDA (nM/ mg pt) | 1.35 ± 0.11(b)  | 2.71 ± 0.17(a)  | 1.40 ± 0.07(b)  | 1.51 ± 0.09(b)  | 1.43 ± 0.08(b)  |
| GPx (µg of glutathione utilized/min/mg pt) | 21.9 ± 1.12(b) | 29.5 ± 1.91(a) | 23.6 ± 1.0(b) | 22.9 ± 1.32(b) | 21.2 ± 1.21(b) |

Values are expressed as means ±SEM of 12 rats per group; p<0.05 (using one-way ANOVA followed by Tukey’s HSD post-hoc test); a significant difference from normal control group; b significant difference from APAP treated group.
Table 6: Effect of MPL and SIL on brain GSH, SOD, MDA and GPx in APAP induced hepatotoxicity in experimental rats.

| Groups                             | Parameter          | N          | APAP       | MPL+ APAP   | SIL+ APAP   | MPL+SIL+APAP |
|------------------------------------|--------------------|------------|------------|-------------|-------------|--------------|
|                                    | GSH (µg/mg pt)     | 6.45 ± 0.44(b) | 3.72 ± 0.16(ab) | 6.36 ± 0.21(b) | 6.10 ± 0.45(b) | 6.87 ± 0.22(b) |
|                                    | SOD (U/ mg pt)     | 5.2 ± 0.4  | 3.8 ± 0.2  | 5.9 ± 0.2(b) | 5.4 ± 0.5   | 6.1 ± 0.5(b)  |
|                                    | MDA (nM / mg pt)   | 1.38 ± 0.05(b) | 2.07 ± 0.10(ab) | 1.60 ± 0.04(b) | 1.65 ± 0.06(b) | 1.50 ± 0.03(b) |
|                                    | GPx (µg of glutathione utilized/min/mg pt) | 5.77 ± 0.49(b) | 7.94 ± 0.69(ab) | 6.78 ± 0.27 | 6.90 ± 0.35 | 6.51 ± 0.30 |

Values are expressed as means ±SEM of 12 rats per group; p<0.05 (using one-way ANOVA followed by Tuckey's HSD post-hoc test); a significant difference from normal control group; b significant difference from APAP treated group.

Analysis of total genomic DNA

Analysis of total genomic DNA, using DNA fragmentation assay

Table (7) and Fig 3 and 4 show the effects of different treatments on the total genomic DNA damage induced by APAP in albino rats' liver, after 4 wks of treatment. Where, severe damage was observed in the DNA of the APAP treated rats livers, appeared in the second lane, L2. As indicated, the massive DNA release in L2 was followed by migration in the form of smear shape indicating necrosis.

This pattern of migration of the released DNA (necrosis) disappeared in all the lanes representing the other treatments, L3: APAP+MPL; L4: APAP+SIL; L5: and APAP+SIL+MPL, indicating the presence of protective effects, attributed to these treatments.

The power of protection against DNA damage was in the following descending order: L3> L5> L4. Where, only a small portion of the DNA in L3 was released and migrated only to 2000 bps, showing the highest level of protection for MPL treatment amongst the other treatments. In L5, less protection was shown by mixture treatment, where, most of the DNA remained intact at 4000 bps, however the remaining little portion was fragmented into a few apoptotic bands at 2000, 1000 and 800. Whereas, silymarin treatment exerted the least level of protection, where, it only protected a smaller portion of DNA, from being released and migrated into several apoptotic bands, at 2000, 1000, 800 and 600 bps.

Figure 3: Digital photograph of DNA electrophoresis of liver tissues shows the protective effects of different treatments against APAP toxicity, where, L1: negative control (N); L2: APAP; L3: APAP+MPL; L4: APAP+SIL; L5: APAP+SIL+MPL and M: DNA marker.
Figure 4: The analytical peaks for DNA lanes which resemble the same lanes in Fig. 2 using Biogene software.

Table (7): Protective effect of different treatments on total genomic DNA damage in albino rats

| Groups          | N  | APAP | MPL+APAP | SIL+ APAP | MPL+SIL+APAP |
|-----------------|----|------|----------|-----------|--------------|
| Intact DNA (4000 bp) | 2900 | 2180 | 5065     | 1820      | 4160         |
| 2000 bp         | -  | -    | 902      | 3405      | 1265         |
| 1000 bp         | -  | -    | -        | 400       | 950          |
| 800 bp          | -  | -    | -        | 1750      | 1050         |
| 600 bp          | -  | 6830 | -        | 870       | -            |

Discussion

The present study examined the in vitro antioxidant potential of MPL extract, using DPPH assay. It aimed, also, to point out the in vivo antioxidant and free radical scavenging activities of MPL against APAP toxicity in rats. The obtained results of DPPH assay revealed that MPL extract possesses a high free radical scavenging activity compared to vitamin C. The antioxidant potential of MPL extract can be attributed to the presence of considerable amounts of phenolic compounds and flavonoids, identified in the extract. Where, phenolics, generally, act as free radical scavengers (Dai J and Mumper, 2010) and flavonoids have been reported to exert antioxidant, anti-inflammatory and hepatoprotective activities (Mahmood et al., 2014).

In the present study, the deleterious effect of the toxic APAP dose on liver function is emphasized by significant elevation in plasma AST, ALT, ALP and GGT activities in APAP rats, as compared to N group. The activities of ALT, AST, ALP and GGT used as biochemical markers for evaluation of early hepatic injury.

These enzymes are normally located in the cytoplasm, mitochondria or microsomes. Consequently, these enzymes are released into the circulation after cellular leakage and loss of functional integrity of cell membrane, in liver, due to this hepatotoxicity (Lahkar and Thakuria, 2012). Furthermore, alterations in the permeability of cell membrane, increased synthesis or decreased catabolism of aminotransferases may explain this elevation (Nuduka, 1999). A possible explanation by which serum ALP level was elevated may be related to the increase in its synthesis by cells lining bile canaliculi in response to cholestasis and increased biliary pressure. The aforementioned increase of liver parameters in APAP-rats is also supported by preceding studies that agreed with our consequences (Lebda et al., 2013).

Based on all of the abovementioned outcomes, our experiment provided an evidence for the hepatoprotective effect of MPL, as revealed by its potential in significantly attenuating the increased activities of serum hepatic enzymes, after administration of MPL, alone or in combination with silymarin, in comparison with APAP group. The decreased enzymes level, after treatment with MPL, SIL or mixture of both, indicates that these treatments might preserve the structural integrity of the hepatocellular membrane against APAP.

Despite the insignificant effect of APAP on total protein and albumin in the present study, there are conflicting results reported by Lotkova et al. (2009) who demonstrated a significant decrease in albumin synthesis by 30% in APAP treated group in comparison with controls.

The observations of the current study clearly demonstrated that, at the end of the experimental period, APAP overdose induced imbalance between ROS production and antioxidant defense which resulted into oxidative stress in the liver.
the liver and lipid peroxidation (LPO) as shown by the increased MDA content and GPx enzyme activity, and the decrease in GSH, SOD and CAT in liver, blood and brain.

Previous mechanistic studies indicated that the metabolic activation of APAP to the reactive metabolite NAPQI (Lu, 2009), which can directly react with GSH a non-enzymatic antioxidant thiol, exhibits dramatic depletion of cellular content of GSH. At APAP overdose, significant decrease in GSH causes the endogenous reactive oxygen species to bind to cellular macromolecules leading to initiation of LPO processes, membrane breakdown, and cell death indicating that oxidative stress has a prime role in the mechanism of APAP toxicity (Mohammed and Safwat, 2013).

Likely, SOD is known to be the primary defense system against oxidative stress. CAT is one of the most important intracellular enzymes in the detoxification of the oxidant hydrogen peroxide. The reduction in the activities of CAT and SOD enzymes in APAP rats was attributed to the increased utilization of these enzymes in scavenging and neutralizing the free radicals and lipid peroxides (Singh et al., 2011). The aforementioned data are in harmony with those obtained by El-Banna et al. (2013) and Soares et al. (2013).

According to the current results, administration of MPL, solely or combined with silymarin, maintained the activities of antioxidant enzymes within normal levels and increased level of reduced glutathione. These actions are mainly ascribed to the high content of phenolic compounds and flavonoids (rutin, quercetin and naringin), present in MPL, which possess antioxidant abilities (El-Alfy et al., 2011). Flavonoids has been proposed to exert antioxidant effects through enhancement of endogenous antioxidant SOD and CAT (Cetin et al., 2008). Additionally, quercetin has been reported to inhibit cytochrome P450 enzymes so decreases the formation of toxic reactive metabolite NAPQI (Sandhar et al., 2011). Moreover, quercetin mediates induction of GSH synthesis through activation of gene expression of most of the key proteins implicated in the GSH synthesis (Nájera et al., 2013). These results showed that MPL extract was able to preserve the amount of GSH, which is important to counteract the APAP toxic effects.

In the present study, another evidence for the implication of APAP in the induction of oxidative stress was emerged by the elevation in MDA level, a toxic product of lipid peroxidation. It has been proven that hydrogen peroxide and superoxide anion are generated during biotransformation of APAP in the CYP450 system and from mitochondria during APAP intoxication (Hinson et al., 2002). The MPL extract was able to maintain normal levels of lipid peroxidation (MDA) in blood and both tissues. The phenomenon can, in principle at least, be attributed to the free-radical scavenging ability of the MPL extract. Also it has been shown that apigenin and rutin inhibit many enzymes including, cyclooxygenase, lipoxygenase, and phospholipase which play significant role in generation of ROS (Tunon et al., 2009).

Furthermore, our investigation demonstrated that GPx was significantly increased in APAP group. On one hand, there is accumulating evidence in harmony with our data, established by Soares et al. (2013) and Shull et al. (1991), who postulated that exposure of the epithelial cells to H₂O₂ causes a significant induction in mRNA of GPx, while, insufficient amounts of intracellular GSH, substrate of GPx, might explain why the level of lipid peroxidation in the liver was not affected by GPx overexpression, as well (Lubos et al., 2011).

On the other hand, there are conflicting results concerning this theory reported by lebda et al. (2013) who demonstrated that APAP administration was accompanied by reduced GPx activity. Their hypothesis was that GPx could be consumed during the process of protection against chemically-induced oxidative destruction of lipids and proteins.

In the current study, the DNA analysis, through gel electrophoresis, showed severe DNA damage to the liver tissues of APAP overdose treated rats; this result is in accordance with that of Gujral et al. (2002). The DNA damage observed in this study might be due to the oxidative stress state. Oxidative stress is known to cause severe DNA damage to various tissues.

In this study, administration of MPL, silymarin or mixture significantly reduced the liver DNA damage. This protective effect against DNA damage was almost attributed to the antioxidant effects of these treatments.

Finally, in the current study, the use of a mixture of MPL and silymarin was to detect whether it would have a synergistic effect as a hepatoprotective agent. However, we reported a synergistic effect for this mixture only in case of increasing the decreased GSH and SOD levels, and in case of decreasing the increased ALT and ALP levels in APAP treated rats. This weak synergism that has been shown to the mixture might be due to the similarity of the active constituents present in both MPL and silymarin, to which antioxidant and hepatoprotective effects are attributed. It worth nothing saying that similarity in structure gives similarity in activity. Furthermore, there was no summative effect in most of the parameters, and this was consistent with the preliminary studies, preceding this study, where we used a high dose of MPL comparing it to a low dose. Both doses gave nearly the same effect with no significant difference. This result encouraged the use of the low dose in the present study.

In conclusion, the present data highlights that MPL is considered to be one of the antioxidants-rich plants which had a significant ameliorative effect on liver function and oxidative stress biomarkers in comparison with silymarin as a reference agent in APAP-induced hepatotoxicity in rats.

References

1. Aebi, H. (1984). Catalase in-vitro. In: Packer L (ed.). Methods of enzymology. Vol. 105. San Diego: Academic Press Inc., p. 121-6.
Azim et al., Afr J Tradit Complement Altern Med. (2017) 14 (2): 206-216
doi:10.21010/ajtcam.v14i2.22

Ali, S.I.; Said, M.M. and Hassan, E.K. (2011). Prophylactic and curative effects of purslane on bile duct ligation-induced hepatic fibrosis in albino rats. J. Hepatol., 10 (3): 340-346.

Aljanabi, S.M. and Martinez, I. (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res., 25:4692-4693.

Batanouny, K.H.; shabana, M., Aboutabl, E. and Soliman, F. (1999). Wild Medicinal Plants in Egypt. Palm press, Cairo, Egypt., p.151.

Belfield, A. and Goldberg, D.M. (1971). Revised assay for serum phenyl phosphatase activity using 4-aminoantipyrine. Enzyme, 12: 561-573.

Bessem, J.G.M. and Vermeulen, N.P.E. (2001). Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. Crit. Rev. Toxicol., 31(1):55-138.

Beutler, E.; Duran, O. and Mikus, B. (1963). Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61(6): 882-888.

Blois, M.S. (2002). Antioxidant determinations by the use of a stable free radical. Nature., 26: 1199-1200.

Buege, J.A. and Aust, S.D. (1978). Microsomal lipid peroxidation. Methods Enzymol., 12: 302-310.

Cetin, A.; Kaynar, L., Kocyigit, I., Hacioglu, S.K., Saraymten, R., Ozturk, A., Sari, I. and Sagdic, O. (2008). Role of grape seed extract on methotrexate induced oxidative stress in rat liver. Am. J. Clin. Med., 36: 861-872.

Choi, Y.; Kang, J., Han, J., Park, Y., Lee, Y., Choi, J., Kang, Y.H. (2003). Polyphenolic Flavonoids Differ in Their Antiapoptotic Efficacy in Hydrogen Peroxide – Treated Human Vascular Endothelial Cells. J. nutr. , 133: 985-991.

Dai, J. and Mumper, J.R. (2010). Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. Molecules, 15: 7313-7352.

Dehshahri, S.H.; Wink, M., Afsarypuor, S., Asghari, G. and Mohagheghzadeh, A. (2012). Antioxidant activity of methanolic leaf extract of Moringa peregrina (Forssk.) Fiori. J. Pharmac. Sci., 7: 111-118.

Doumas, B.T.; Watson, W.A. and Biggs, H.G. (1971). Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chim. Acta., 7: 109-115.

El-Alfy, T.S.; Ezzat, S.M., Hegazy, A.K., Amer, A.M. and Kamel, G.M. (2011). Isolation of biologically active constituents from Moringa peregrina (Forssk.) Fiori. (family: Moringaceae) growing in Egypt. Pharmacog. Mag., 7(26):109-115.

El-Banna, H.; Soliman, M. and Al-wabel, N. (2013). Hepatoprotective Effects of Thymus and Salvia Essential oils on Paracetamol-Induced Toxicity in Rats. J. Phys. Pharm. Adv., 3(2): 41-47.

El-Batran, S.A.; Abdel-Salam, O.M., Abdelshfeek, K.A., Nazif, N.M., Ismail, S.I. and Hammouda, F.M. (2005). Phytochemical and pharmacological investigation on Moringa peregrina (Forssk) Fiori. Nat. Prod. Sci., 11: 199-206.

Gujral, J.S.; Knight, T.R., Farhood, A., Bajt, M.L. and Jaeschke, H. (2002). Mode of cell death after acetaminophen in mice: apoptosis or oncotic necrosis? Toxicol. Sci., 67: 322-328.

Hassab El-Nabi, S.E. (2004). Molecular and cytogenetic studies on the antimutagenic potential of eugenol in human lymphocytes culture treated with depakine and apetyl drugs. J. Egypt Ger. Soc. Zool., 43(c): 171-196.

Hinson, J.A.; Bucci, T.J., Irwin, L.K., Michael, S.L. and Mayeux, P.R. (2002). Effect of inhibitors of nitric oxide synthase on acetaminophen-induced hepatotoxicity in mice. Nitr. Oxid., 6: 160-167.

Lahkar, M. and Thakuri, B. (2012). Comparative study of the hepatoprotective activities of Vitex Negundo linn. and Moringa Oleifera lam. in paracetamol induced hepatotoxicity in experimental animals. Int. Res. J. Pharm. App. Sci., 2(5): 74-79.

Lebda, M.A.; Taha, N.M., Korshom, M.A. and Mandour, A.E.A. (2013). Ginger (Zingiber officinale) potentiate paracetamol induced chronic hepatotoxicity in Rats. J. Med. Plant Res., 7(42): 3164-3170.

Lotková, H.; Kučera, O., Roušar, T., Endlicher, R., Křiváková, P., Garnol, T. and Červinková, Z. (2009). Effect of S-adenosyl methionine on acetaminophen-induced toxic injury of rat hepatocytes in vitro. Acta. Vet. Brno., 78: 603-613.

Lowry, O.H.; Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.

Lu, S.C. (2009). Regulation of glutathione synthesis. Mol. Aspects Med., 30: 42-59.

Lubos, E.; Loscalzo, J. and Handy, D.E. (2011). Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. Antiox. Redox. Sig., 15(7): 1957-1997.

Mahmood, N.D.; Mamat, S.S., Kamisan, F.H., Yahya, F., KamaroZaman, M.F., Nasir, N., Mohtarrudin, N., Tohid, S.F. and Zakaria, Z.A. (2014). Amelioration of paracetamol-induced hepatotoxicity in rat by the administration of methanol extract of muntingia calabura I. Leaves. Biomed. Res. Int., 2014: 695678.

Marklund, S.L. and Marklund, G. (1974). Involvement of superoxide anion radical in the autoxidation of pyragallol and a convenient assay for superoxide dismutase. Eur. J. Biochem., 47: 469-474.

Mattila, P.; Astola, J. and Kumpulainen, J. (2000). Determination of flavonoids in plant material by HPLC with diode-array and electro-array detection. J. Agric. Food Chem., 48: 5834-5841.

McGill, M.R.; Sharpe, M.R., Williams, C.D., Taha, M., Curry, S.C. and Jaeschke, H. (2012). The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. J. Clin. Invest., 122: 1574-1583.
31. Mohammed, E.T. and Safwat, G.M. (2013). Assessment of the ameliorative role of selenium nanoparticles on the oxidative stress of acetaminophen in some tissues of male albino rats. Beni-suef Univ. J. Bas. Appl. Sci., 2: 80- 85.
32. Muriel, P. (2009). Role of free radicals in liver diseases. Hepatol. Int., 3: 526- 536.
33. Nájera, M.O.; Tinajero, I.S., Páez, L.R., Toledo, S.M. and Sánchez, J.M. (2013). Quercetin improves antioxidant response in diabetes through maintenance of reduced glutathione levels in blood. Afr. J. Pharm. Pharmacol., 7(36): 2531- 2539
34. Nuñuda, N. (1999). Clinical biochemistry for students of pathology, Longman Nigerian Plc., p. 1- 236.
35. Retiman, S. and Frankel, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G. and Hoekstra, W.G. (1973). Selenium: Biochemical role as a component of glutathione peroxidase. Science., 179: 588- 590.
36. Roy, S.D.; Das, S., Shil, D. and Dutta, K.N. (2012). Herbal hepatoprotective agents: a review. World J. Pharma. Res., 1 (2): 87- 99.
37. Russmann, S.; Kullak-Ublick, G.A. and Grattagliano, I. (2009). Current Concepts of Mechanisms in Drug-Induced Hepatotoxicity. Curr. Med. Chem., 16 (23): 3041- 3053.
38. Sandhar, H.K.; Kumar, B., Prasher, S., Tiwari, P., Salhan, M. and Sharma, P. (2011). A review of phytochemistry and pharmacology of flavonoids. Inter. Pharma. Sci., 1(1): 25- 41.
39. Shull, S.; Heintz, N.H., Periasamy, M., Manohar, M., Janssen, Y.M.W., Marsh, J.P. and Mossman, B.T. (1991). Differential regulation of antioxidant enzymes in response to oxidants. J. Biol. Chem., 266: 24398- 403.
40. Szasz, G. A. (1969). kinetic photometric method for serum γ-glutamyl transpeptidase. Clin. Chem., 15112-136.
41. Tietz, N.W. (1994). Total protein in: "Clinical Guide to Laboratory Tests". 2nd. Ed. WB Saunders press, Philadelphia. p. 610- 611.
42. Tunon, M.J.; Garcia-Mediavilla, M.V., Sanchez-Campos, S. and Gonzalez-Gallego, J. (2009). Potential of flavonoids as anti-inflammatory agents: modulation of pro-inflammatory gene expression and signal transduction pathways. Curr. Drug. Metab., 10 (3): 256- 271.
43. Uchiyama, M. and Mihara, M. (1978). Determination of malondialdehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem., 86: 279- 286.