Renoprotective effect of *Mangifera indica* polysaccharides and silymarin against cyclophosphamide toxicity in rats

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KEYWORDS

*Mangifera indica* L.; Polysaccharides; Oxidative stress; Cyclophosphamide; Silymarin; Nephrotoxicity

Abstract

The present study aims to evaluate the possible protective role of polysaccharides extracted from the Egyptian mango *Mangifera indica* L. (MPS) and silymarin against cyclophosphamide (CP) nephrotoxicity in male albino rats. Male rats were randomly divided into, control group (administered distilled water orally for 10 days) and MPS (500, 1000 mg/kg, p.o.) and/or silymarin (150 mg/kg, p.o.) treated groups for 10 days. In the last 5 days of treatment rats were administered CP (150 mg/kg, i.p). The MPS revealed significant prophylactic effect against kidney injury induced by CP as demonstrated by enhancement of the kidney function via decreasing serum creatinine, urea and uric acid. Treatment of rats with MPS extract and/or silymarin significantly increased the level of reduced glutathione (GSH) and superoxide dismutase (SOD) activity while decreased the level of total malondialdehyde (MDA) and glutathione-S-transferase (GST). Also, histopathological examinations confirmed the protective efficacy of MPS and/or silymarin against CP nephrotoxicity. In conclusion, the obtained results of the present study support the protective antioxidant role of MPS and/or silymarin against CP-induced kidney disorder in rats.

Introduction

The kidney is a primary target for numerous toxic xenobiotics including drugs, environmental chemicals and metals. Acute kidney injury, induced by drugs and other stimuli, is a significant clinical problem, and accounts for the cessation of development of many promising drug candidates Shelton et al. (2013). Drug-induced nephrotoxicity is an extremely common condition and is responsible for a variety of pathological effects on the kidneys Dhodi et al. (2014). Cyclophosphamide (CP) is a potent anticancer agent. CP is effective against a wide spectrum of malignancies, such as leukemia, lymphoma, breast, lung, prostate, and ovarian cancers Khan et al. (2004). The usage of CP is severely limited by its physiological
side effects, such as hepatotoxicity, nephrotoxicity, urotoxicity, cardiotoxicity and myelosuppression Motawi et al. (2010), Lameire et al. (2011) and Newton (2012). However, its clinical use is restricted because of its marked organ toxicity associated with increased oxidative stress and inflammation Nafees et al. (2015). Oxidative stress is reported to play important roles in CP-induced renal damage Abraham and Rabi (2011). Renal damage is one of the dose-limiting side effects of CP Abraham and Rabi (2011). It has been demonstrated that increased generation of reactive oxygen species (ROS) by CP in kidney tissues plays a critical role in the pathogenesis of CP-induced kidney damage Stankiewicz and Skrzylewska (2003) and Abraham and Rabi (2009). So, free radical scavengers and antioxidants can be used in the treatment Sabolic (2006). Cyclophosphamide is now being used in combination with various detoxifying and protective agents with the purpose of reducing or eliminating its adverse toxic effects Neboh and Ufelle (2015) and Nurrochmad et al. (2015).

Silymarin, a bioflavonoid, is the main constituent of Silybumarianum (milk thistle). Chemically, silymarin is a flavonolignan that consists of a mixture of mainly three flavonoids, silybin, silydianin and silychristin Kiruthiga et al. (2007). Silymarin has been reported to possess several pharmacological activities including antioxidant and anti-inflammatory/immunomodulatory Manna et al. (1999), antibacterial Crocenzil and Roma (2006), hepatoprotective, antibacterial, antiallergic, antimutagenic, antiviral, antithrombotic and vasodilatory actions Wellington and Jarvis (2001). Many studies reported that silymarin reduced CP-induced ROS generation Jnaneshvar et al. (2012). Silymarin was shown to have positive effects on preventing or decreasing severity of cyclophosphamide nephrotoxicity Eser et al. (2012). So it is the positive control used in this study.

Pharmacotherapy using natural substances can be currently regarded as a very promising future alternative to conventional therapy Wang et al. (2013). Plants have been the major source of therapeutic agents for curing the human diseases Zahid et al. (2013). Mango (Mangifera indica L.) is one of the most important and popular tropical fruits, mainly due to its attractive flavor Chen et al. (2012). M. indica L. is an important member of the family Anacardiaceae and belongs to genus Mangifera order sapindales Ross (1999). In recent years, some bioactive polysaccharides isolated from natural sources have attracted much attention in the field of biochemistry and pharmacology Wang et al. (2013) and Xue et al. (2015). Some polysaccharides isolated from natural sources show various important biological activities, such as antitumor, immunomodulatory, and anti-inflammatory effects, which are strongly affected by their chemical structures and chain conformations Liu et al. (2015). It has been well documented that polysaccharides increase immunity through production of interleukins and antibodies Yang et al. (2008) and therefore could be explored as novel prospective antioxidants Sun et al. (2010) and Chen et al. (2012). Polysaccharides can be currently regarded as a very promising future alternative to conventional therapy in kidney diseases Chiu et al. (2014) and Lu et al. (2014). When multiple antioxidants are used in combination, they protect against vulnerability to other agents and synergistically potentiate their antioxidant properties Aleisa et al. (2013). So, the present study was designed to evaluate the efficacy of polysaccharides extracted from the mango pulp (MPS) and/or silymarin against renal injury induced by cyclophosphamide in male albino rats.

Materials and methods

Chemicals and reagents

Methanol 95%, ethanol, absolute acetone, and cyclophosphamide (Endoxan) were purchased from Bexter Oncology GmbH (Germany), silymarin was purchased from SEDICO Pharmaceutical Co. (6 October City, Egypt). All other chemicals were of analytical grade. Kits for all biochemical parameters were purchased from Biodiagnostic Company (El-Dokki, Giza, Egypt).

Collection of Mangifera indica L.

Mature green mango (M. indica L.) cv. Fagrkelan fruits were freshly harvested from Egypt fields through a local Dealer in September 2012, M. indica washed thoroughly with running tap water, seeds and peels are removed from mango.

Extraction of polysaccharides from Mangifera indica L.

Polysaccharides extracted from mango pulp by the technique described by Devaki et al. (2009) with some modifications. Briefly, 1670 g of mango pulp was boiled in 16.7 L of distilled water at 100 °C for 3 h. The slurry was separated by gauze and then, was filtered. The filtrate was dialyzed against tap water for 48 h, and then concentrated to about 350 ml under reduced pressure using rotary evaporator. Then, the concentrated filtrate was precipitated using four volumes of 95% ethanol to one volume of the extract (about 1400 ml 95% ethanol). The extract was washed with absolute acetone, filtrated and then dried in a vacuum desiccator over anhydrous calcium chloride. About 65 g of polysaccharides extract is obtained and used for animals’ experiments.

Animals

The experimental animals used in this study were adult male albino Wistar rats (Rattus norvegicus) weighing (150–160 ± 5 g). The animals were obtained from the National Research Center (NRC, Dokki, Giza). Animals were grouped and housed in polyacrylic cages (Five animals per cage). Animals were given food and water ad libitum. Rats were maintained in a friendly environment with a 12 h/12 h light–dark cycle at room temperature (22–25 °C). Rats were acclimatized to laboratory conditions for 7 days before commencement of the experiment. The protocol was approved by the Cairo University, Faculty of Science Institutional Animal Care and Use Committee (IACUC, Egypt) (CUFS/S/PHY/28/14), and all the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

Toxicity study (OECD 420)

Adult male albino rats (R. norvegicus) weighing (150–160 ± 5 g) were used for acute toxicity studies. The rats were divided into control and test groups containing five animals each. The rats were administered orally with polysaccharides extract of mango at dose levels of 5000 mg/kg (high dose)
and 2000 mg/kg (low dose). Normal control rats received the same amount of vehicle (distilled water) only. Rats were observed carefully for 24 h after extract administration and then for the next 14 days. At the end of this experimental period, the rats were observed for signs of toxicity, morphological behavior, and mortality. Acute toxicity was evaluated based on the number of deaths (if any). Acute toxicity was calculated as per Organization for Economic Co-operation and Development (OECD) guidelines 420 (Fixed dose method) (Van den Heuvel et al., 1990; Whitehead and Curnow, 1992).

Experimental design

Thirty-five rats were randomly divided into seven groups (5 animals/group) as follows: **Group 1:** Served as a control group. **Group 2:** Rats were administered distilled water (2 ml) orally for 10 days. **Group 3:** Rats treated orally with MPS (dissolved in distilled water) in a dosage of 500 mg/kg body weight for 10 days. **Group 4:** Rats treated orally with MPS in a dosage of 1000 mg/kg body weight for 10 days. **Group 5:** Rats treated orally with silymarin (dissolved in distilled water) in a dosage of 150 mg/kg body weight for 10 days. **Group 6:** Rats treated orally with MPS in a dosage of 500 mg/kg body weight and silymarin 150 mg/kg body weight at the same time for 10 days. **Group 7:** Rats treated orally with MPS in a dosage of 1000 mg/kg body weight and silymarin 150 mg/kg body weight at the same time for 10 days (see Fig. 1). Rats of all groups except group one treated intraperitoneally with CP in a dosage of 150 mg/kg body weight in the last 5 days (Jnaneshwari et al., 2012).

Experimental procedures

After 10 days of treatment, the animals were fasted overnight (12–14 h). Rats of each group were sacrificed by exsanguinations under sodium pentobarbital anesthesia, and the blood samples were collected from each animal into sterilized centrifuge tubes for serum separation. Kidney was removed and immediately blotted using filter paper to remove traces of blood. The first part was immersed in 10% formal saline solution for fixation preparatory histopathological examinations. The other part of kidney was stored at 4°C for biochemical analysis. Blood samples were centrifuged at 3000 rpm for 15 min. Serum was separated and stored in Eppendorf at −20°C to be used for biochemical analysis. The kidney was homogenized (10% w/v) in ice cold 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 3000 rpm for 15 min at 4°C and the resultant supernatant was used for estimation of oxidative stress markers.

Assessment of serum biochemical parameter

All kits for biochemical analysis were purchased from Biodiagnostic Company. Determination of serum creatinine according to colorimetric method was described by Tietz and Andresen (1986). Urea was determined in serum by modified urease-Berthlot method according to Tietz et al. (1990). Serum uric acid was determined colorimetrically according to Tietz (1990).

Assessment of oxidative stress markers

Oxidative stress markers were detected in the resultant supernatant of kidney homogenate by using Biodiagnostic kits. Lipid peroxide (Malondialdehyde) MDA was determined in tissue according to Ohkawa et al. (1979). Reduced glutathione (GSH) was determined in tissue according to Beutler et al. (1963). Superoxide dismutase (SOD) activity was determined according to Nishikimi et al. (1972). Glutathione-S-transferase (GST) activity was determined according to Habig et al. (1974).

Histopathological analysis

Histological sections (4 μm thick) were prepared from paraffin blocks of kidney tissues fixed in 10% formal saline. Sections were stained with hematoxylin and eosin (H&E) (Mayer, 1903) and then, examined under light microscope under ×400 magnifications.

Statistical analysis

Values were expressed as mean ± SEM. (standard error) to evaluate differences between the groups studied, a one way analysis of variance (ANOVA) with the Duncan post hoc test was used to compare the group means and P < 0.05 was considered statistically significant. SPSS for Windows (version 19.0) was used for the statistical analysis.

Results

Toxicity study

Nevertheless, none of the five rats of the test group died and did not show any sign of toxicity after administration of MPS at higher dosage (5000 mg/kg) in the first 24 h or during the experiment period (14 day) and the activities of serum ALAT and serum ASAT are at normal range compared with the control group. In addition, there is no histopathological change in both liver and kidney. The single lethal dose of MPS that kills half of the animals (LD50) was therefore taken as above 2000 mg/kg. MPS is tested at two dosage levels (500 and 1000 mg/kg) as they represented 1/10th and 1/5th of the proposed LD50.

Improvement of kidney function in M. indica polysaccharides (MPS) extract and/or silymarin treated rats

As shown in (Table 1), serum creatinine, urea and uric acid levels were significantly increased (P < 0.05) following administration of CP compared to the control group. However, treatment with MPS (500, 1000 mg/kg) and/or silymarin significantly showed enhancement in the recorded kidney function markers.
Table 1  Effect of *Mangifera indica* polysaccharides (MPS) extract and/or silymarin on kidney functions parameter in serum of cyclophosphamide (CP) – treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum creatinine (mg/dL)</th>
<th>Serum urea (mg/dL)</th>
<th>Serum uric acid (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>57 ± 2.54a</td>
<td>123.34 ± 5.03bc</td>
<td>3.442 ± 0.108a</td>
</tr>
<tr>
<td>CP Vehicle(saline)</td>
<td>81 ± 1.87d</td>
<td>219.18 ± 11.74d</td>
<td>4.406 ± 0.135b</td>
</tr>
<tr>
<td>MPS (500 mg/kg)</td>
<td>69.2 ± 1.41c</td>
<td>94.16 ± 5.37a</td>
<td>3.3 ± 0.133a</td>
</tr>
<tr>
<td>MPS (1000 mg/kg)</td>
<td>71.5 ± 1.87c</td>
<td>102.52 ± 1.02ab</td>
<td>3.328 ± 0.058a</td>
</tr>
<tr>
<td>Silymarin</td>
<td>63.5 ± 4.71abc</td>
<td>127.5 ± 13.42c</td>
<td>3.19 ± 0.081a</td>
</tr>
<tr>
<td>MPS (500 mg/kg)+ silymarin</td>
<td>68 ± 2.42abc</td>
<td>104.16 ± 5.43abc</td>
<td>3.438 ± 0.132c</td>
</tr>
<tr>
<td>MPS (1000 mg/kg)+ silymarin</td>
<td>60.7 ± 1.72ab</td>
<td>113.34 ± 3.58abc</td>
<td>3.272 ± 0.086a</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM for 5 rats in each group. Values with different superscript letters are significantly different at $P < 0.05$.

Figure 1  Schematic diagram shows the experimental design of treatment.

Figure 2  Effect of *Mangifera indica* polysaccharides (MPS) extract and/or silymarin on kidney malondialdehyde (MDA) in serum of cyclophosphamide (CP) – treated rats. Values are given as mean ± SEM for 5 rats in each group. Values with different superscript letters are significantly different at ($P < 0.05$).

Figure 3  Effect of *Mangifera indica* polysaccharides (MPS) extract and/or silymarin on kidney glutathione reduced (GSH) in serum of cyclophosphamide (CP) – treated rats. Values are given as mean ± SEM for 5 rats in each group. Values with different superscript letters are significantly different at ($P < 0.05$).
Improvement of some oxidative parameters in M. indica polysaccharides (MPS) extract and/or silymarin treated rats

Administration of CP significantly ($P < 0.05$) increased the level of malondialdehyde (MDA) and the activity of glutathione-S-transferase (GST) in the kidney tissue compared to the control group (Figs. 2 and 5). Administration of MPS extracts (500 mg/kg body weight) and/or silymarin significantly increased the activities of SOD (Fig. 4). On the other hand treatment with MPS extracts (1000 mg/kg body weight) and/or silymarin failed to increase the SOD activity (Fig. 4).

Kidney histopathological analysis

Representative views of kidney sections are shown in (Fig. 6). As shown in tissue sections stained with hematoxylin and eosin, CP caused dilation and congestion of renal blood vessel, vacuolations of epithelial lining renal tubules and atrophy of glomerular tuft (Fig. 6B–D). It can also be noticed protein cast in the lumen of renal tubules. Oral administrations of MPS extract (500 and 1000 mg/kg body weight) and/or silymarin improved the kidneys architecture compared to the CP group (Fig. 6E–H).

Discussion

Chemotherapeutic drugs cause renal injury at different levels and their effect ranges from an elevation in the level of serum creatinine to renal failure. Among which renal toxicity is a dreadful complication developed in cancer patients upon cyclophosphamide (CP) therapy Sinanoglu et al. (2012) and Rehman et al. (2012). For the therapeutic strategies of renal injury and disease during CP treatment, it is important to find complementary antioxidant compound that is able to block renal injuries. Polysaccharides chemoprevention activity against cyclophosphamide has been indicated in several reports Huang et al. (2013), Zhang et al. (2013), Yu et al. (2014). The present study throws light for the first time on the therapeutic effect of M. indica polysaccharides (MPS) and/or silymarin against nephrotoxicity induced by CP in rats.

During the progression of the renal disease, loss of kidney function is accompanied by failing organ function leading to accumulation of a series of compounds Vanholder et al. (2008). Hepatorenal syndrome is most frequently first diagnosed by a finding of increasing concentrations of serum creatinine or blood urea nitrogen (BUN) Gine’s et al. (2003). The marked increase in the levels of serum urea and creatinine is a marker for the nephrotoxicity and kidney damage Cagler et al. (2002). In consistent with the finding of Cagler et al. (2002) and Rehman et al. (2012), the results of the present investigation showed significant increase in the serum urea and creatinine following CP administration. Creatinine is an endogenous substance produced by muscle from creatine and creatine phosphate, with a rate of production proportionate to the muscle mass Kurniawan et al. (2013). The measurement of serum creatinine proves useful in diagnosing renal failure and diseases where, directly proportional relationship exists between creatinine levels and renal function Sharma (2011). This increase in serum creatinine may be due to the hepatic damage which evolved into a stage with features of hepatorenal syndrome Arroyo and Jimenez (2000) including reduction in creatinine clearance and low glomerular filtration rate Briglia and Anania (2002).

Figure 5
Effect of Mangifera indica polysaccharides (MPS) extract and/or silymarin on kidney glutathione-S-transferase (GST) in serum of cyclophosphamide (CP) – treated rats. Values are given as mean ± SEM for 5 rats in each group. Values with different superscript letters are significantly different at ($P < 0.05$).

Figure 4
Effect of Mangifera indica polysaccharides (MPS) extract and/or silymarin on kidney superoxide dismutase (SOD) in serum of cyclophosphamide (CP) – treated rats. Values are given as mean ± SEM for 5 rats in each group. Values with different superscript letters are significantly different at ($P < 0.05$).
Uric acid, a product of purine metabolism, is degraded in most mammals by the hepatic enzyme, urate oxidase (urate oxidase), to allantoin, which is mainly excreted by the kidneys (65–75%) and intestines (25–35%) De Oliveira and Burini (2012). The significant increase in uric acid following CP administration in the present study may be due to reduction in glomerular filtration rate and renal urate excretion Vaziri et al. (1995). Hyperuricemia also may result from increased net tubular absorption. After filtration, uric acid undergoes both reabsorption and secretion in the proximal tubule, and this process is

**Figure 6** Photomicrographs of hematoxylin-eosin stained kidney histology of male albino rats (×400 magnifications). (A) Kidney section from control group shows normal histological structure of renal parenchyma with normal structure of glomerulus. (B) Kidney section from CP treated group showing dilation and congestion of renal blood vessel (BV). (C) Kidney section from CP treated group showing vacuolations of epithelial lining renal tubules and atrophy of glomerular tuft. (D) Kidney section from CP treated group showing protein cast (PC) in the lumen of renal tubules. (E), (F), (G) and (H) showing kidney section from the group treated with MPS (500 and 1000 mg/kg) and/or silymarin showing the regeneration of kidney parenchyma.
mediated by a urate/anion exchanger and a voltage sensitive urate channel Leal-Pinto et al. (1999) and Enomoto et al. (2002). In consonance with the finding of Yang et al. (2015), the present study showed a significant decrease in the serum creatinine, urea and uric acid following treatment with MPS (500, 1000 mg/kg) and/or silymarin that may be a contributory self-healing mechanism restoring the kidney structure and function.

Reduced glutathione (GSH) is a major constituent of the detoxification pathways. The amino acid constituent cysteine of GSH and proteins is regarded as being the first line of defense, and neutralizes the hydroxyl radical and plays an important role against inflammation and oxidative stress Circu and Aw (2011). The high electron-donating capacity of the SH group of cysteine provides great reducing power and is used to regulate a complex thiol-exchange system. In agreement with the reports of Atessahin et al. (2005) and Ahyanci et al. (2010), the GSH level in the kidney tissues treated with CP significantly decreased which may be due to the nephrotoxicity and accumulation of free radicals. Treatment with MPS and silymarin shows significant increase in the level of GSH which may be due its antioxidant effect.

Superoxide dismutase (SOD) is the first antioxidant enzyme to deal with oxyradicals by accelerating the dismutation of superoxide to hydrogen peroxide. Catalase (CAT) is a peroxisomal hem protein that catalyzes the removal of hydrogen peroxide formed during the reaction catalyzed by SOD. Thus, SOD and CAT act as mutually supportive antioxidative enzymes that provide protective defense against ROS Cohen et al. (1978). Viewed in conjunction the finding of Jayaraman et al. (2008), the present study shows significant decrease in the level of SOD in the kidney of CP treated rats. The enhanced free radical concentration resulting from the oxidative stress conditions can cause loss of enzymatic activity Sanzgiri et al. (1997). However, treatment with MPS in the present study restored the activity of the studied SOD.

Glutathione-S-transferase (GST) is an enzyme that participates in the detoxification process due to conjugation reaction between GSH and xenobiotics Adang et al. (1990). In conson- 
cence with the results of Yousefipour et al. (2005), the present study indicates a significant increase in GST activity following CP administration. An increase in GST activity might be a physiological response to increased free radical level. Since glutathione is utilized by the body to detoxify xenobiotics and remove free radicals through its direct antioxidant effect, the toxic levels of acrolein stimulate induction of GST activity, thereby depleting GSH and causing direct damage to the vasculature due to free radical accumulation Yousefipour et al. (2005). However, treatment with MPS normalized the GST level through its antioxidant effect that has the ability to scavenge free radicals.

In conclusion, the present study showed that MPS had positive antioxidant effect against CP-induced oxidative stress in the kidney tissues, as it alleviates the alterations in urea, creatinine and uric acid levels as well as the oxidative stress markers in the kidney tissues (MDA, GSH, SOD and GST). In addition, combining MPS with silymarin showed more pronounced effect than each one individually. It is worth to be mentioned that MPS (500 and 1000 mg/kg body weight) and silymarin treatments restore the MDA and GSH nearly to control levels.

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Renoprotective effect of Mangifera indica polysaccharides and silymarin


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