**Ruta graveolens** and its active constituent rutin protect against diethylnitrosamine-induced nephrotoxicity through modulation of oxidative stress

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**ABSTRACT**

The current study was designed to evaluate the possible protective effects of *Ruta graveolens* (Rue) and its active phenolic constituent rutin against diethylnitrosamine (DEN)-induced nephrotoxicity in rats. A single dose of DEN (200 mg/kg body weight) was intraperitoneally injected. Two-weeks after DEN administration, rats received 0.05 % phenobarbital in drinking water for 12 weeks. *Ruta graveolens* (50 mg/kg) and rutin (50 mg/kg) were orally administered from the first day of experiment. DEN administration induced kidney injury evidenced by histological alterations as well as significant increase in serum urea (P<0.01), creatinine (P<0.001) and uric acid (P<0.001), and renal lipid peroxidation levels. On the other hand, renal glutathione content and activity of superoxide dismutase, glutathione peroxidase and glutathione-s-transferase were significantly declined. Concomitant supplementation with either *R. graveolens* extract or rutin markedly alleviated the altered biochemical and histopathological features. In conclusion, the current findings provide evidence that *R. graveolens* and its active phenolic component rutin could protect against DEN-induced renal damage through abolishment of oxidative stress and potentiation of the antioxidant defense system.

**INTRODUCTION**

Drug-induced nephrotoxicity is a serious kidney problem and is responsible for a variety of pathological effects on the kidneys (Sarang and Ameeta, 2001). Oxidative stress has been implicated in the pathogenesis of drug-induced renal damage, and reactive oxygen species (ROS) have been suggested to be the central key in the mechanisms that lead to tubular necrosis (Lopez-Novoa et al., 2011). Diethylnitrosamine (DEN), a hepatocarcinogen, is produced from the metabolism of some drugs and also found in processed meats, tobacco smoke, soybean, cheese and wide variety of foods (Verna et al., 1996). During metabolism, DEN has been reported to induce oxidative stress, leading to cytotoxicity, mutagenicity and carcinogenicity (Pradeep et al., 2007; Farombi et al., 2009). Pradeep et al., (2010) stated that DEN is biotransformed by cytochrome P450 dependent monooxidase systems and its metabolic activation is responsible for the onset of the toxic effects. Due to the key role of oxidative stress in DEN-induced toxicity, the use of antioxidants could offer protection against its deleterious effects.

Medicinal plants play a central role in managing human diseases and numerous drugs have been developed from natural sources (Balunas and Kinghorn, 2005). *Ruta graveolens* L. (Family: *Rutaceae*), commonly known as rue or sadab, is an ancient medicinal plant and currently used for treatment of multiple disorders, including eye problems, aching pain, dermatitis and rheumatism (Miguel, 2003). In addition, *R. graveolens* has been extensively used in treatment of vitiligo, psoriasis, leucoderma, multiple sclerosis, cutaneous lymphomas, and recently reported to possess anticancer, anti-inflammatory, hepatoprotective and antidiabetic activity (Pathak et al., 2003; Preethi et al., 2006; Ahmed et al., 2010; Mahmoud et al., 2014). It has also been used for gastric disorders, stiff neck, dizziness and headache (Conway and Sclocumb, 1979); however, its protective effects against DEN-induced nephrotoxicity were not reported.

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Phytochemical investigations of *R. graveolens* have demonstrated the presence of fats, oils, flavonoids, furoquinolone, alkaloids, terpenoids, glycosides, essential oils, steroids, sterols, tannins, coumarins, saponin, cardioglycosides, carbohydrates, amino acids, protein and others (Inna et al., 2004; Khare, 2007; Ahmed et al., 2010; Rajeshwari et al., 2011). Flavonoids are non-nutritive dietary components widely distributed in plants (Mahmoud, 2012) and possess a wide range of biological effects (Zhao et al., 2007). The flavonoid rutin is extensively found in many plants and it is considered the active constituent of *R. graveolens*. It is also called rutoside, quercitin-3-rutinoside and sophorin. This flavonoid has been reported to exert a broad range of biological activities, including antimicrobial (Arima et al., 2002), antiviral (Middleton et al., 2000), neuroprotective (Gupta et al., 2003), anti-inflammatory (Mahmoud and Soliman, 2013), hepatoprotective (Mahmoud, 2012) and antidiabetic (Ahmed et al., 2010; Mahmoud and Soliman, 2013).

To the best of our knowledge, reports evaluating the protective effects of *R. graveolens* and its major constituent rutin against DEN-induced nephrotoxicity are scarce. Therefore, the intention of the present study was to demonstrate the efficacy of rue ethanolic extract and rutin in the modulation of oxidative stress and cell damage associated with DEN-induced nephrotoxicity in Wistar rats.

**MATERIALS AND METHODS**

**Chemicals**

Rutin, diethylaminoethyl (DEN), phenobarbital (PB), glutathione (GSH), pyrogallol, thiobarbituric acid (TBA) and 5,5'-dithiobis- (2-nitrobenzoic acid) (DTNB) were purchased from Sigma (USA). All other chemicals were of analytical grade and obtained from standard commercial supplies.

**Collection of plant and extract preparation**

*R. graveolens* (sadab) was obtained from the Experimental Station of Medical Plants (ESMP), Faculty of Pharmacy, Cairo University, Egypt. The plant leaves were cleaned, air dried and ground with an electric grinder. The extract was prepared by maceration in 80% aqueous ethanol for 24 h at room temperature. After filtration, the filtrate was concentrated under vacuum in a rotary evaporator. The residue obtained was stored frozen till use.

**Animals and treatments**

Twenty-four male Wistar rats weighing 130-150 g, obtained from the animal house of the National Research Centre (El-Giza, Egypt) were included in the present investigation. The animals were housed in plastic well-aerated cages (6 rats/cage) at normal atmospheric temperature (25 ± 5°C) and normal 12 h light/dark cycle. Rats had free access to water and were supplied daily with laboratory standard diet of known composition *ad libitum*. All animal procedures were undertaken with the approval of Institutional Animal Ethics Committee of Beni-Suef University (Egypt). Rats were divided to four groups (N = 6) and were subjected to the following treatments:

Group 1 (Control): animals were injected with a single dose of saline (0.9%) and orally administered the vehicle 1% carboxymethylcellulose (CMC)

Group 2 (DEN): animals were given a single intraperitoneal injection of DEN (200 mg/kg b.wt) (Banakar et al., 2004) and given 1% CMC by gavage daily throughout the experimental period. Two-weeks after DEN administration, rats received 0.5 g/L Phenobarbital in drinking water (Banakar et al., 2004) for 12 weeks.

Group 3 (DEN + *R. graveolens*): DEN/PB-treated animals received 50 mg/kg *R. graveolens* dissolved in 1% CMC by gavage daily throughout the experimental period (Ratheesh and Helen, 2007).

Group 4 (DEN + Rutin): DEN/PB-treated animals received 50 mg/kg rutin by gavage daily throughout the experimental period (Mahmoud, 2012).

The doses of *R. graveolens* and rutin were balanced consistently as indicated by any change in body weight to keep up comparable dosage for every kg body weight over the entire period of study. By the end of the experiment, animals were sacrificed and blood samples were collected, left to coagulate and centrifuged at 3000 rpm for 15 min to separate serum. Kidney samples were immediately excised and perfused with ice-cold saline. Frozen samples (10% w/v) were homogenized in chilled saline and the homogenates were centrifuged at 3000 rpm for 10 min. The clear homogenates were collected and used for subsequent assays.

**Biochemical assays**

**Determination of serum creatinine and uric acid**

Serum urea, creatinine and uric acid levels were assayed using reagent kits purchased from Biosystems (Spain), following the methods of Kaplan (1984), Young (1995) and Fossati et al. (1980), respectively.

**Determination of oxidative stress and antioxidant system parameters**

Lipid peroxidation, assayed as malondialdehyde (MDA), was determined in kidney homogenates according to the methods of Preuss et al. (1998). Reduced glutathione (GSH) content was assayed according to the method of Beutler et al. (1963). Activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione-s-transferase (GST) were measured according to the methods of Marklund and Marklund (1964), Matkovics et al. (1998) and Mannervik and Gutenberg (1981), respectively.

**Histopathological study**

The kidney samples were flushed with cold saline and then fixed in 10% buffered formalin for at least 24 h. The specimens were then dehydrated in ascending series of ethanol, cleared in xylene and embedded in paraffin wax. Blocks were prepared and 4μm thick sections were cut by a sledge microtome.
The paraffin embedded sections were deparaffinized, washed and stained with hematoxylin and eosin (H&E). The stained slides were examined under light microscope.

**Statistical analysis**

Statistical analysis was performed using GraphPad Prism 5 software (GraphPad Software, San Diego, CA, USA). Results were expressed as mean ± standard error (SEM) and all statistical comparisons were made by means of the one-way ANOVA test followed by Tukey’s test post hoc analysis. A P value <0.05 was considered significant.

**RESULTS**

DEN-administration resulted in marked impairment of kidney function as demonstrated by the significant increase in serum urea (P<0.01), creatinine (P<0.001) and uric acid (P<0.001) levels (Table 1). Oral administration of either *R. graveolens* or rutin to DEN-treated rats significantly decreased the elevated levels of serum urea, creatinine and uric acid when compared with the DEN control group. However non-significant differences exist, rutin seemed to be more effective in ameliorating serum urea, creatinine and uric acid than *R. graveolens*.

Table 1: Serum urea, creatinine and uric acid levels in control, DEN and DEN + Rutin treated with *R. graveolens* and rutin.

<table>
<thead>
<tr>
<th></th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
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<tbody>
<tr>
<td>Control</td>
<td>29.05 ± 3.39</td>
<td>0.59 ± 0.108</td>
<td>0.45 ± 0.027</td>
</tr>
<tr>
<td>DEN</td>
<td>71.53 ± 9.28</td>
<td>1.062 ± 0.056</td>
<td>1.168 ± 0.149</td>
</tr>
<tr>
<td>DEN + <em>R. graveolens</em></td>
<td>31.41 ± 5.59</td>
<td>0.747 ± 0.027</td>
<td>0.601 ± 0.043</td>
</tr>
<tr>
<td>DEN + Rutin</td>
<td>38.21 ± 1.91</td>
<td>0.73 ± 0.044</td>
<td>0.515 ± 0.081***</td>
</tr>
</tbody>
</table>

Data are expressed as M ± SEM. **P<0.01 and ***P<0.001 vs control, and #P<0.05, ##P<0.01 and ###P<0.001 vs DEN.

Histopathological examination of the kidney sections of control rats revealed normal histological structure (Fig. 1A). On the other hand, DEN administration produced histological changes and several lesions including dysplastic renal tubules with karyomegalic nuclei, atrophy of glomerular tuft and inflammatory cells infiltration (Fig. 1B). The histopathological alterations are summarized in Table 2. Treatment of the DEN-administered rats with *R. graveolens* extract (Fig. 1C) as well as rutin (Fig. 1D) produced marked improvement in the kidney histological structure and prevented the DEN-induced alterations.

Table 2: Histopathological lesions in kidney sections of control, DEN and DEN + Rutin rats treated with *R. graveolens* and rutin.

<table>
<thead>
<tr>
<th>Histopathological lesions</th>
<th>DEN</th>
<th>DEN + <em>R. graveolens</em></th>
<th>DEN + Rutin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoma</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Karyomegalic nuclei</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Atrophy of glomerular tuft</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Inflammatory cells infiltration</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Protein cast in the lumen of renal tubules</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vacuolation of renal tubules</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</table>

DEN-administered rats exhibited significant (P<0.001) elevation in the renal lipid peroxidation marker MDA when compared with the control group of rats, as represented in Figure 2. Oral supplementation of either *R. graveolens* or rutin to the DEN-treated rats significantly (P<0.001) decreased MDA content.

**Fig. 1:** Photomicrographs of H&E stained kidney sections of control (A) showing normal histological structure, DEN (B) showing several lesions including dysplastic renal tubules with karyomegalic nuclei, atrophy of glomerular tuft and inflammatory cells infiltration, DEN + *R. graveolens* (C) and DEN + Rutin (D) showing nearly normal renal tubules (i) and renal corpuscles. (X400).

**Fig. 2:** Lipid peroxidation in kidneys of control, DEN and DEN + Rutin rats treated with *R. graveolens* and rutin. Data are expressed as M ± SEM. **P<0.01 vs control and ###P<0.001 vs DEN.

**Fig. 3:** Reduced glutathione (GSH) content in kidneys of control, DEN and DEN rats treated with *R. graveolens* and rutin. Data are expressed as M ± SEM. **P<0.01 vs control, ***P<0.001 vs control, and $P<0.05$ vs DEN + *R. graveolens*. 

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In contrast, GSH content showed significant (P<0.001) decrease in renal homogenate of DEN-administered rats when compared with the corresponding control group (Figure 3). Supplementation with either R. graveolens or rutin significantly (P<0.001) ameliorated renal GSH content with more potent effect offered by R. graveolens.

Similarly, DEN-administered rats exhibited significant decline in the activity of SOD (P<0.05), GPx (P<0.001) and GST (P<0.001) when compared with the normal control rats. R. graveolens markedly ameliorated the activity of SOD (P<0.001), GPx (P<0.01) and GST (P<0.001). Rutin as well produced significant amelioration in the activity of SOD (P<0.01), GPx (P<0.05) and GST (P<0.05) as represented in Figures 4, 5 and 6, respectively.

**DISCUSSION**

DEN was suggested to be important environmental carcinogen, leading to generation of ROS and eventually resulting in oxidative stress and cellular injury (Bartech et al., 1989). Being metabolized by cytochrome P450, DEN generates highly reactive free radicals and, initiates lipid peroxidation of the cell and endoplasmic reticulum membranes (Archer, 1989; Vitaglione et al., 2004). These resulting radicals have the ability to cause oxidative damage in DNA, proteins and lipids (Vitaglione et al., 2004). Therefore, the use of antioxidants could protect against DEN-induced oxidative stress and nephrotoxicity. We designed the current study to test the hypothesis that R. graveolens and its main constituent rutin, through their antioxidant efficacy, could protect against DEN-induced renal damage.

The present study showed that the administration of DEN has induced renal damage and this was evident by the increased levels of serum toxicity markers like urea, creatinine and uric acid of DEN-induced rats. It has been reported that serum creatinine level relates to glomerular function and its rise is an indicator of renal failure (Adejuwon and Adokiye, 2008; Nenad et al., 2008). These findings are in agreement with the studies of Rezaie et al. (2013) and Pashmforoosh et al. (2015) who demonstrated increased serum urea and creatinine levels in DEN-administered rats. Also, Lily and James (2008) stated that the elevation of uric acid in blood (hyperuricemia) is considered a sensitive marker of inflammation taking place at various sites of the body. In addition, renal injury induced by DEN was confirmed by the observed histological alterations, including adenoma, dysplastic renal tubules with karyomegalic nuclei, atrophy of glomerular tuft and inflammatory cells infiltration. Concurrent administration of either R. graveolens or rutin markedly decreased serum levels of urea, creatinine and uric acid, and potentially prevented the DEN-induced histological alterations in the kidney. The nephron-protective effects of R. graveolens and rutin have been demonstrated in few studies. Shaheen (2013) reported the renoprotective effects of rutin in DEN-induced animals. We recently reported that both R. graveolens and rutin were able to decrease serum urea, creatinine and uric acid levels in ammonium chloride-induced hyperammonememic rats (Mahmoud, 2012; Mahmoud et al., 2014).

The abundance of long chain polyunsaturated fatty acids in the composition of renal lipids makes the kidney vulnerable to damage caused by ROS (Ozbek, 2012). The metabolism of nitrosamines has been suggested to generate ROS (Kaul et al., 1993). The produced ROS induce cellular injury, DNA fragmentation, protein damage and lipid peroxidation, and alter the antioxidant defense system (Bansal et al., 2005; Mittal et al., 2006; Nencini et al., 2007). In our study it was noticed that the homogenate of kidney of the DEN-induced rats exhibited a significant increase in levels of MDA, indicating a serious damage to kidney tissue. The elevated MDA levels could be explained by the study of Nakae et al. (1997) who reported that DEN intercalate with membrane lipids and form free radicals which increase lipid
peroxidation. Subsequently, the membrane function is altered by decreasing its fluidity and changing the activity of its bounding enzymes and their receptors (Arulselvan and Subramanian, 2007). Daily treatment with rutin and *R. graveolens* markedly ameliorated the elevated levels of MDA suggesting evidence that either treatment possess a potent free radical scavenging activity. We have confirmed the radical scavenging activity of rutin and *R. graveolens* in hyperammonemic (Mahmoud, 2012; Mahmoud et al., 2014) and diabetic rats (Mahmoud and Soliman, 2013).

In contrast, DEN-administered rats exhibited significant decrease in renal GSH content. GSH is a potent antioxidant which protects the cellular constituents against the damage induced by the free radicals (Franco et al., 2007), through the formation of S-conjugates with products of lipid peroxidation (Laurent et al., 2000). Therefore, GSH decline leads to lowered cellular defense against free radical induced cellular injury resulting in cell death (Srivastava and Shivanandappa, 2010). Similarly, reduction in the activity of the antioxidant enzymes SOD, GPx and GST was observed in kidneys of DEN-administered rats. SOD and GPx play a significant role in maintaining the body’s defense mechanism against the deleterious effects of ROS (Chandra et al. 2000; Swamy et al., 2010; Wei et al. 2011), and GST is an extra key detoxifying enzyme (Mate’s et al., 2010). The noticed reduction in the activity of the antioxidant enzymes may be attributed directly to the excessive production of ROS in DEN-induced rats. On the other hand, oral administration of either *R. graveolens* or rutin markedly alleviated renal GSH content as well as activity of the antioxidant enzymes. Therefore, we assume that the nephroprotective mechanism of *R. graveolens* and rutin against DEN-induced oxidative stress is partially mediated by preventing GSH decline and potentiation of the enzymatic antioxidant defenses. These findings provide an evidence on the antioxidant and radical scavenging activity of *R. graveolens* and its flavonoid rutin documented in our previous studies (Ahmed et al., 2010; Mahmoud, 2012; Mahmoud and Soliman, 2013; Mahmoud et al., 2014).

**CONCLUSION**

The present findings indicate that *R. graveolens* and its active phenolic component rutin exert protection against DEN-induced renal toxicity in albino rats. Their renoprotective effects could be attributed to the inhibition of lipid peroxidative system through prevention of GSH decline and enhancement of the enzymatic antioxidants.

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