Research Article

Hesperidin protects against diethylnitrosamine-induced nephrotoxicity through modulation of oxidative stress and inflammation

Rasha R Ahmed[1], Ayman M Mahmoud[2], Mohamed B Ashour[2], Amira M Kamel[2]

[1]Cell Biology and Histology Division, Zoology Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt.
[2]Physiology Division, Zoology Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt.

Correspondence to: Ayman M Mahmoud, E-mail: ayman.mahmoud@science.bsu.edu.eg

Received July 29, 2015. Accepted August 9, 2015

Abstract

Background: Kidney forms the main controlling organ in sustaining homeostasis and, thus, is vulnerable to toxicity by xenobiotics. **Aims and Objective:** To evaluate the possible protective effects of the citrus flavonoid, hesperidin (HES), against diethylnitrosamine (DEN)-induced nephrotoxicity in rats. **Materials and Methods:** Rats received a single intraperitoneal dose of DEN (200 mg/kg body weight). Two-weeks after DEN administration, rats received 0.5 g/L phenobarbital in drinking water for 12 weeks. HES (50, 100, and 200 mg/kg body weight) were orally administered from the first day of experiment. **Result:** DEN administration induced nephrotoxicity evidenced or DEN-induced nephrotoxicity was evidenced by the histological alterations and significant increase in serum creatinine ($P < 0.001$), urea ($P < 0.01$), and uric acid ($P < 0.001$) levels. DEN-intoxicated rats exhibited a significant ($P < 0.001$) increase in renal lipid peroxidation levels and reduced glutathione content and activity of superoxide dismutase, glutathione peroxidase, and glutathione-S-transferase. Concomitant supplementation with all the doses of HES markedly prevented DEN-induced biochemical and histopathological alterations. **Conclusion:** The study findings provide evidence that HES could protect against DEN-induced renal injury through abolishment of inflammation and oxidative stress and potentiation of the antioxidant defense system.

KEY WORDS: Flavonoids; Renal Injury; Oxidative Stress; Inflammation; Antioxidants

Introduction

Diethylnitrosamine (DEN), a potent hepatocarcinogen, is produced from the metabolism of some drugs and found in tobacco smoke, processed meats, soybean, cheese, and wide variety of foods.[1] The cytochrome P450-dependent monooxidase systems biotransforms DEN, as reported earlier. The lethal effects of DEN are initiated by this metabolic activation.[2] During metabolism, DEN induces oxidative stress, resulting in cytotoxicity, mutagenicity, and carcinogenicity.[3,4] Oxidative stress has been reported to play a key role in the pathogenesis of drug-induced renal damage, and reactive oxygen species (ROS) have been implicated in the mechanisms that lead to tubular necrosis.[5] Hence, the use of antioxidants could offer protective effects against drug-induced renal damage.

A growing number of epidemiologic studies consistently reveals a protective effect of polyphenol-rich foods against many diseases.[6,7] The results of multiple studies conducted in animal models[8–14] and in humans[6,7] have provided an evidence about the therapeutic effects of polyphenols. Flavonoids are nonnutritive dietary polyphenolic components widely distributed in plants[12] and possess a wide range of

Access this article online	
Website: http://www.njppp.com	Quick Response Code:
DOI: 10.5455/njppp.2015.5.2907201567	

National Journal of Physiology, Pharmacy and Pharmacology Online 2015. © 2015 Ayman M Mahmoud. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), allowing third parties to copy and redistribute the material in any medium or format and to remix, transform, and build upon the material for any purpose, even commercially, provided the original work is properly cited and states its license.

biological effects.[15] Hesperidin (HES), one of the most important flavonoids, is the predominant flavonoid in citrus fruits.[16] The peel and membranous parts of sweet orange and lemon have the highest HES.[17] HES exhibits numerous biological and pharmacological effects including antioxidant, anti-inflammatory, antidiabetic, hepatoprotective, and anti-carcinogenic properties.[10,13,14,18] To the best of our knowledge, reports evaluating the protective effects of HES against DEN-induced nephrotoxicity are scarce. Therefore, this study was designed to demonstrate the efficacy of HES in the modulation of oxidative stress, inflammation, and cell damage associated with DEN-induced nephrotoxicity in rats.

Materials and Methods

Chemicals

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

Animals and Treatments

Thirty male Wistar rats, weighing 120–140 g, obtained from the animal house of the National Research Centre (El-Giza, Egypt), were included in this investigation. The animals were housed in plastic well-aerated cages (six rats/cage) at normal atmospheric temperature (25 ± 1°C) and normal 12-h light/dark cycle. Rats were provided with 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 five groups ($N = 6$) and were subjected to the following treatments:

- Group 1 (control): Rats were injected with a single dose of saline (0.9%) and orally administered the vehicle 1% carboxymethylcellulose (CMC).
- Group 2 (DEN): Rats were given a single intraperitoneal injection of DEN (200 mg/kg body weight) dissolved in saline[19] 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[19] for 12 weeks.
- Group 3 (DEN + 50 mg HES): DEN/PB-treated animals received 50 mg/kg hesperidin dissolved in 1% CMC by gavage daily throughout the experimental period.[10]
- Group 4 (DEN + 100 mg HES): DEN/PB-treated animals received 100 mg/kg hesperidin by gavage daily throughout the experimental period.[20]
- Group 5 (DEN + 200 mg HES): DEN/PB-treated animals received 200 mg/kg hesperidin by gavage daily throughout the experimental period.[20]

The doses of HES were balanced consistently as indicated by any change in body weight to keep up the comparable dosage for every kilogram of body weight over the entire period of study. By the end of the experiment, animals were killed, and blood samples were collected, left to coagulate, and centrifuged at 3000 rpm for 15 min to separate the serum. Kidney samples were immediately excised and perfused with ice-cold saline. Frozen samples (10% wt/vol) 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 urea, 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,[21] Young,[22] and Fossati et al.,[23] respectively.

Determination of serum tumor necrosis factor(TNF)-α: Serum levels of TNF-α were determined by specific ELISA kits purchased from R&D Systems (USA), according to the manufacturer's instructions. The concentration of TNF-α was determined spectrophotometrically at 450 nm. Standard plot was constructed by using standard cytokine, and the concentrations for unknown samples were calculated from the standard plot.

Determination of oxidative stress and antioxidant system parameters: Lipid peroxidation, assayed as malondialdehyde (MDA), was determined in kidney homogenates according to the method of Preuss et al.[24] Reduced glutathione (GSH) content was assayed according to the method of Beutler et al.[25] Activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) were measured according to the methods of Marklund and Marklund,[26] Matkovics et al.,[27] and Mannervik and Gutenberg,[28] 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 4-μm 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). Results were expressed as mean ± standard error (SEM), and all the 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

Data summarized in Table 1 show the effect of DEN and HES on renal function markers. The administration of DEN produced marked impairment of kidney function as demonstrated by the

Table 1: Serum creatinine, urea, and uric acid levels in control, DEN, and DEN rats treated with hesperidin.

	Creatinine (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)
Control	0.66 ± 0.10	27.59 ± 2.81	1.80 ± 0.11
DEN	1.07 ± 0.06***	79.36 ± 6.44***	4.67 ± 0.59***
DEN + 50 mg HES	0.77 ± 0.04$^{\#}$	43.38 ± 5.32$^{\#\#\#}$	2.32 ± 0.28$^{\#\#\#}$
DEN + 100 mg HES	0.82 ± 0.04$^{\#}$	39.05 ± 1.96$^{\#\#\#}$	2.26 ± 0.21$^{\#\#\#}$
DEN + 200 mg HES	0.81 ± 0.02$^{\#}$	27.74 ± 3.29$^{\#\#\#}$	2.29 ± 0.14$^{\#\#\#}$

***$P < 0.001$ vs. control, and $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ vs. DEN. Data are expressed as mean ± SEM.

significant ($P < 0.001$) increase in serum urea, creatinine, and uric acid levels. Oral administration of 50, 100, or 200 mg HES significantly decreased the elevated levels of serum urea ($P < 0.001$), creatinine ($P < 0.05$), and uric acid ($P < 0.001$) when compared with the DEN control group.

Figure 1 provides the description of serum level of TNF-α in different treatment groups. Treatment with DEN significantly ($P < 0.001$) increased the serum levels of the proinflammatory cytokine TNF-α. Coadministration of HES produced a significant decrease in the serum levels of TNF-α when compared with the DEN-administered rats.

Histopathological examination revealed normal histology of kidney in the control group [Figure 2A]. Treatment with DEN caused renal damage evident by the histological changes including adenoma, dysplastic renal tubules with karyomegalic nuclei, atrophy of glomerular tuft, inflammatory cells infiltration, protein cast in the lumen of renal tubules, and vacuolation of renal tubules [Figure 2B,C]. On the other hand, treatment of the DEN-administered rats with the 50 [Figure 2D], 100 [Figure 2E], or 200 mg [Figure 2F] dose of HES protected against the DEN-induced histological alterations. The histopathological alterations are summarized in Table 2.

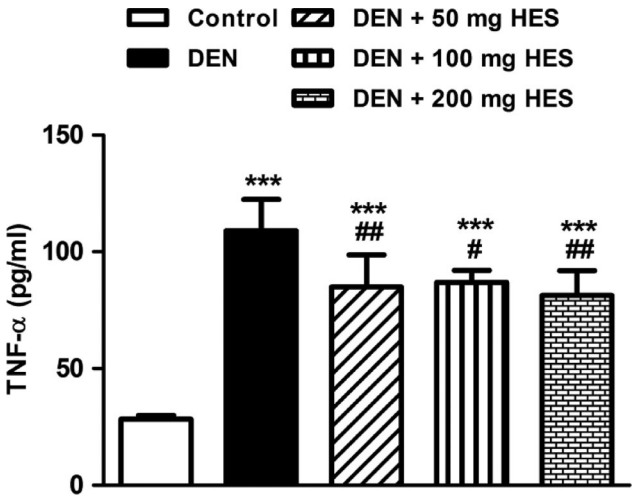

Figure 1: Serum TNF-α levels in control, DEN, and DEN rats treated with hesperidin. Data are expressed as mean ± SEM. ***$P < 0.001$ vs. control, and $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. DEN.

Concerning renal lipid peroxidation, DEN-administered rats exhibited significant ($P < 0.001$) elevation in the renal lipid peroxidation marker MDA when compared with the control group of rats [Figure 3]. Oral supplementation of the 50- and 100-mg doses of HES to the DEN-treated rats significantly ($P < 0.001$) decreased renal MDA content. More or less similar, the higher dose of HES (200 mg) significantly ($P < 0.001$) prevented the DEN-induced lipid peroxidation in the kidney when compared with the DEN control rats. In addition, the 200 mg HES dose significantly decreased the renal lipid peroxidation when compared with the control ($P < 0.05$) and the lower HES dose ($P < 0.01$), as represented in Figure 3.

In contrast, GSH content of the DEN-administered rats showed significant ($P < 0.001$) decrease when compared with the corresponding control group. Oral supplementation with all the three doses of HES significantly ($P < 0.01$) ameliorated renal GSH content, as depicted in Figure 4. Similarly, the activity of SOD showed a significant ($P < 0.01$) decline in the kidney of DEN-intoxicated rats when compared with the control group. The low and high doses of HES significantly ($P < 0.001$) ameliorated the activity of renal SOD. However, nonsignificant differences exist, and the 100 mg dose of HES produced a less potent ($P < 0.05$) ameliorative effect on the SOD activity [Figure 5].

The activity of GPx and GST in kidney of the DEN-administered rats showed a significant ($P < 0.001$) decrease when compared with the control group of rats, as represented in Figures 6 and 7, respectively. The 50-mg HES dose significantly improved the activity of GPx ($P < 0.05$) and GST ($P < 0.001$) when compared with the DEN group. Both the 100- and 200-mg doses of HES markedly ($P < 0.001$) alleviated the activity of GPx and GST.

Table 2: Histopathological lesions in kidney sections of control, DEN, and DEN rats treated with hesperidin.

Histopathological lesions	DEN	DEN + 50 mg HES	DEN + 100 mg HES	DEN + 200 mg HES
Adenoma	+++	–	–	–
Karyomegalic nuclei	++	–	–	–
Atrophy of glomerular tuft	++	–	–	–
Inflammatory cells infiltration	++	–	–	–
Protein cast in the lumen of renal tubules	++	–	+	+
Vacuolation of renal tubules	–	–	+	–

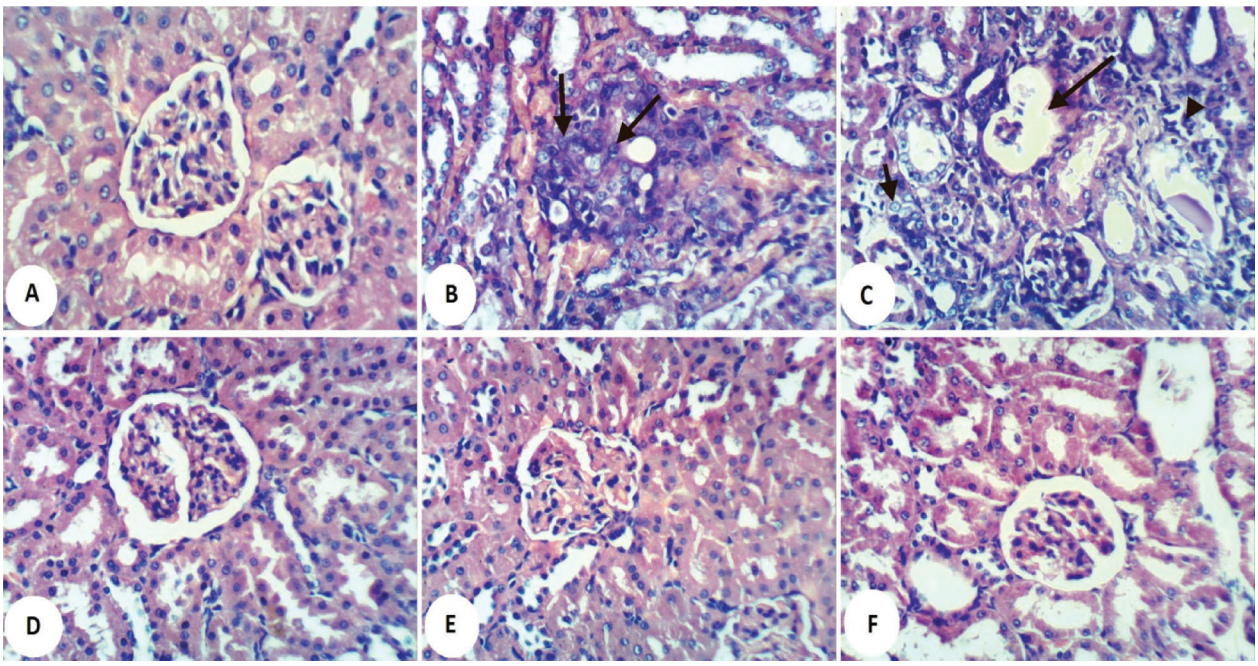

Figure 2: Photomicrographs of H&E-stained kidney sections of control (A) showing normal histological structure; DEN (B and C) showing several lesions including dysplastic renal tubules with karyomegalic nuclei, atrophy of glomerular tuft, and inflammatory cells infiltration; DEN + 50 mg HES (D), DEN + 100 mg HES (E), and DEN + 200 mg HES (D) showing nearly normal renal tubules and renal corpuscles. (× 400).

Discussion

This study showed that the administration of DEN induced renal damage, as evidenced by the increased levels of serum creatinine, urea, and uric acid. Serum creatinine level has been reported to reveal glomerular function and its increase is an indicator of renal failure.[29,30] These findings are in agreement with the studies of Rezaie et al.[31] and Pashmforoosh et al.,[32] who demonstrated increased serum creatinine and urea levels in the DEN-administered rats. Renal injury induced by DEN was further confirmed by the observed histological alterations, including adenoma, dysplastic renal tubules with karyomegalic

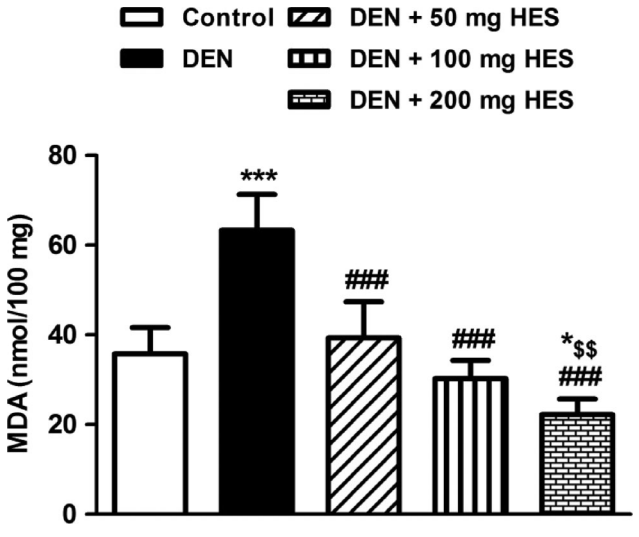

Figure 3: Lipid peroxidation in kidneys of control, DEN, and DEN rats treated with hesperidin. Data are expressed as mean ± SEM. $^{*}P < 0.05$ and $^{***}P < 0.001$ vs. control, $^{\#\#\#}P < 0.001$ vs. DEN, and $^{\$\$}P < 0.01$ vs. DEN + 50 mg HES. MDA, malondialdehyde.

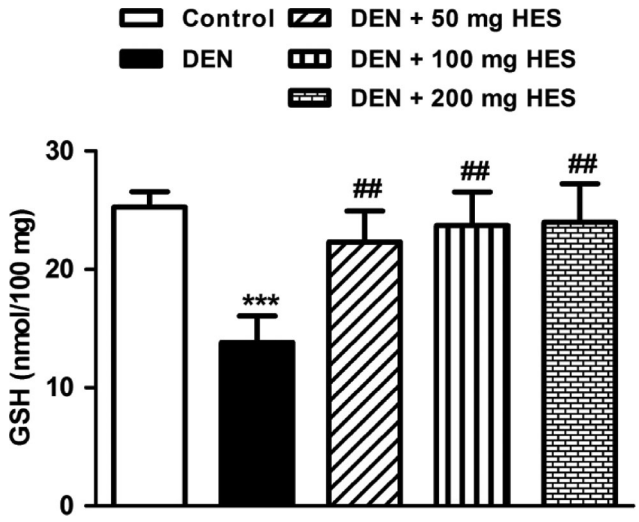

Figure 4: Reduced glutathione (GSH) content in kidneys of control, DEN, and DEN rats treated with hesperidin. Data are expressed as mean ± SEM. $^{***}P < 0.001$ vs. control and $^{\#\#}P < 0.01$ vs. DEN.

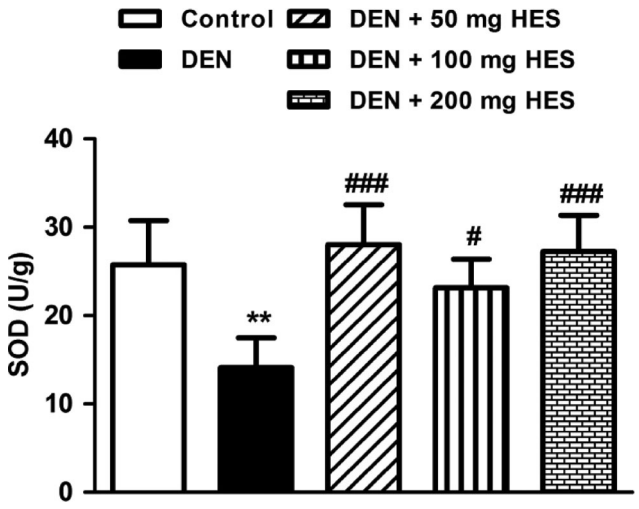

Figure 5: Superoxide dismutase (SOD) activity in kidneys of control, DEN, and DEN rats treated with hesperidin. Data are expressed as mean ± SEM. $^{**}P < 0.01$ vs. control, and $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ vs. DEN.

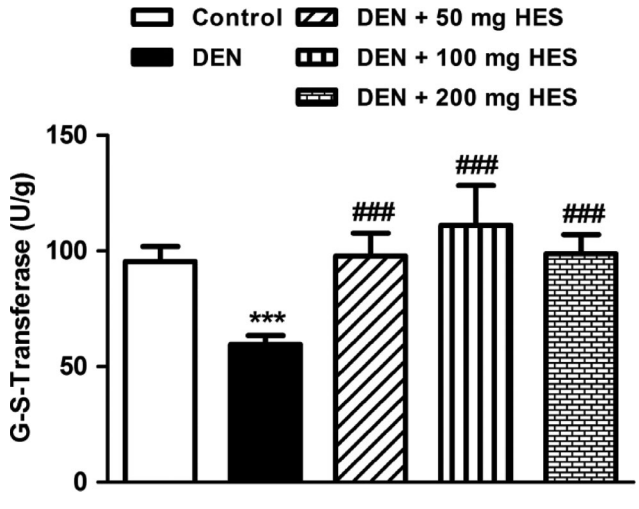

Figure 7: Glutathione-S-transferase (GST) activity in kidneys of control, DEN, and DEN rats treated with hesperidin. Data are expressed as mean ± SEM. $^{***}P < 0.001$ vs. control and $^{\#\#\#}P < 0.001$ vs. DEN.

nuclei, atrophy of glomerular tuft, and inflammatory cells infiltration. Concurrent administration of HES, in a dose-dependent manner, significantly decreased the serum levels of creatinine, urea, and uric acid and markedly prevented the DEN-induced renal histological alterations. The nephroprotective effects of HES in DEN-administered rats have not been previously demonstrated. Recently, HES has been demonstrated to protect rats against gentamicin-induced nephrotoxicity.[33]

The proinflammatory cytokine TNF-α showed a significant increase in the serum of DEN-administered rats when compared with the normal control group. The contribution of the immune system to the drug-induced nephrotoxicity has been well recognized.[34] Numerous studies demonstrated that several nephrotoxicants could induce an inflammatory response, leading to organ injury.[35] The toxicant-induced generation of inflammatory mediators promotes migration and infiltration of leukocytes and aggravates the primary injury induced by the toxicant.[36] Alleviation of the altered serum TNF-α following HES administration might be attributed to its anti-inflammatory properties. We have previously reported the potent anti-inflammatory effect of HES in diabetic[10] and cyclophosphamide-intoxicated rats.[14]

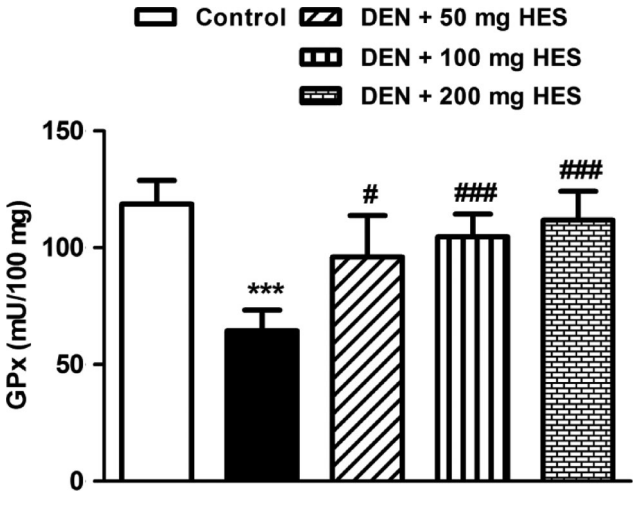

Figure 6: Glutathione peroxidase (GPx) activity in kidneys of control, DEN, and DEN rats treated with hesperidin. Data are expressed as mean ± SEM. $^{***}P < 0.001$ vs. control, and $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ vs. DEN.

DEN was suggested to induce generation of ROS and eventually resulting in oxidative stress and cellular injury.[37] ROS have the ability to cause oxidative damage in DNA, proteins, and lipids.[38] The kidneys are susceptible to the injury caused by ROS because of the plenty of long chain polyunsaturated fatty acids found in the composition of renal lipids.[39] In this study, DEN-induced rats exhibited a significant increase in levels of MDA, indicating a serious damage to kidney tissue. Nakae et al.[40] reported that DEN could intercalate with the membrane lipids and form ROS, which increase lipid peroxidation. Increased lipid peroxidation leads to the alteration of the membrane functions through decreasing its fluidity and changing the activity of its bounding enzymes and their receptors.[41] Concurrent treatment with HES markedly ameliorated the elevated levels of MDA in a dose-dependent manner. This observation could be attributed to the potent free radical-scavenging activity of HES, which we have confirmed previously.[10,14]

On the other hand, DEN-administered rats exhibited significant decrease in renal GSH content when compared with the control group. GSH, a potent antioxidant, protects the cellular constituents against the damage induced by ROS[42] through its ability to form S-conjugates with the products of lipid peroxidation.[43] Hence, GSH depletion leads to lowered

cellular defenses against free radical-induced cellular injury.[44] More or less similar, reduction in activity of the antioxidant enzymes SOD, GPx, and GST was observed in the kidneys of the DEN-administered rats. SOD and GPx play a significant role in maintaining the body's defense mechanism against the deleterious effects of ROS,[45–47] and GST is an extra key detoxifying enzyme.[48] The recorded reduction in GSH and the enzymatic antioxidants may be attributed directly to the excessive production of ROS in the DEN-induced rats. Oral administration of all HES doses markedly alleviated renal GSH content and the activity of the antioxidant enzymes. Therefore, we assume that the nephroprotective mechanism of HES against the DEN-induced oxidative stress is partially mediated by preventing GSH decline and potentiation of the enzymatic antioxidant defenses. These findings provide evidence on the radical scavenging and antioxidant activity of HES documented in our previous studies.[10,14]

Conclusion

Data of the current study indicate that HES, in a dose-dependent manner, exerts protection against the DEN-induced renal toxicity in albino rats. Their renoprotective effects could be attributed to attenuation of the proinflammatory cytokine production and inhibition of the lipid peroxidative system through prevention of GSH depletion and enhancement of the enzymatic antioxidants.

References

1. Verna L, Whysner J, Williams GM. N-nitrosodiethylamine mechanistic data and risk assessment: bioactivation, DNA-adduct formation, mutagenicity, and tumor initiation. Pharmacol Ther. 1996;71:57–81.
2. Pradeep K, Raj Mohan CV, Gobianand K, Karthikeyan S. Protective effect of *Cassia fistula* Linn. on diethylnitrosamine induced hepatocellular damage and oxidative stress in ethanol pretreated rats. Biol Res. 2010;43:113–25.
3. Pradeep K, Mohan CV, Gobianand K, Karthikeyan S. Silymarin modulates the oxidant-antioxidant imbalance during diethylnitrosamine induced oxidative stress in rats. Eur J Pharmacol. 2007;560:110–6.
4. Farombi EO, Shrotriya S, Surh YJ. Kolaviron inhibits dimethyl nitrosamine-induced liver injury by suppressing COX-2 and iNOS expression via NF-kappaB and AP-1. Life Sci. 2009;84:149–55.
5. Lopez-Novoa JM, Quiros Y, Vicente L, Morales AI, Lopez-Hernandez FJ. New insights into the mechanism of aminoglycoside nephrotoxicity: an integrative point of view. Kidney Int. 2011;79:33–45.
6. Buijsse B, Weikert C, Drogan D, Bergmann M, Boeing H. Chocolate consumption in relation to blood pressure and risk of cardiovascular disease in German adults. Eur Heart J. 2010;31:1616–23.
7. Catalgol B, Batirel S, Taga Y, Ozer NK. Resveratrol: French paradox revisited. Front Pharmacol. 2012;3:141.
8. Auclair S, Milenkovic D, Besson C, Chauvet S, Gueux E, Morand C, et al. Catechin reduces atherosclerotic lesion development in apo E-deficient mice: a transcriptomic study. Atherosclerosis. 2009;204:e21–7.
9. Mahmoud AM, Soliman AS. Rutin attenuates hyperlipidemia and cardiac oxidative stress in diabetic rats. Egypt J Med Sci. 2013;34:287–302.
10. Mahmoud AM, Ahmed OM, Ashour MB, Abdel-Moneim A. Hesperidin and naringin attenuate hyperglycemia-mediated oxidative stress and proinflammatory cytokine production in high fat fed/strptozotocin-induced type 2 diabetic rats. J Diabetes Complications. 2012;26:483–90.
11. Mahmoud AM, Ahmed OM, Abdel-Moneim A, Ashour MB. Upregulation of PPARγ mediates the antidiabetic effects of citrus flavonoids in high fat diet fed-streptozotocin induced type 2 diabetic rats. Int J Bioassays. 2013;2:756–61.
12. Mahmoud AM. Influence of rutin on biochemical alterations in hyperammonemia in rats. Exp Toxicol Pathol. 2012;64:783–9.
13. Mahmoud AM. Hematological alterations in diabetic rats; role of adipocytokines and effect of citrus flavonoids. EXCLI J. 2013;12:647–57.
14. Mahmoud AM. Hesperidin protects against cyclophosphamide-induced hepatotoxicity by upregulation of PPARγ and abrogation of oxidative stress and inffammation. Can J Physiol Pharmacol. 2014;92:717–24.
15. Zhao M, Yang B, Wang J, Liu Y, Yu L, Jiang Y. Immunomodulatory and anticancer activities of flavonoids extracted from litchi (*Litchi chinensis* Sonn) pericarp. Int Immunopharmacol. 2007;7:162–6.
16. Barthe GA, Jourdan PS, Mclntosh CA, Mansell RL. Radioimmunoassay for the quantitative determination of hesperidin and analysis of its distribution in *Citrus sinensis*. Phytochemistry. 1988;27:249–54.
17. Leuzzi U, Caristi C, Panzera V, Licandro G. Flavonoids in pigmented orange juice and second-pressure extracts. J Agric Food Chem. 2000;48:5501–6.
18. Chen MC, Ye YI, Guang JI, Liu JW. Hesperidin upregulates heme oxygenase-1 to attenuate hydrogen peroxide-induced cell damage in hepatic L02 cells. J Agric Food Chem. 2010;58:3330–5.
19. Banakar MC, Paramasivan SK, Chattopadhyay MB, Datta S, Chakraborty P, Chatterjee M, et al. 1alpha, 25-dihydroxyvitamin D3 prevents DNA damage and restores antioxidant enzymes in rat hepatocarcinogenesis induced by diethylnitrosamine and promoted by phenobarbital. World J Gastroenterol. 2004;10:1268–75.
20. Shi X, Liao S, Mi H, Guo C, Qi D, Li F, Zhang C, Yang Z. Hesperidin prevents retinal and plasma abnormalities in streptozotocin-induced diabetic rats. Molecules. 2012;17:12868–81.
21. Kaplan A. Urea. In: Kaplan A (Ed.). *Clinical Chemistry*. St Louis/Toronto/Princeton: The CV Mosby, Co., 1984. pp. 1257–60, 437, 418.
22. Young DS. *Effects of Drugs on Clinical Laboratory Tests*, 4th edn. Washington, DC: AACC Press, 1995.
23. Fossati P, Prencipe L, Berti G. Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clin Chem. 1980;26:227–31.
24. Preuss HG, Jarrell ST, Scheckenbach R, Lieberman S, Anderson RA. Comparative effect of chromium vanadium and gymnema sylvestre on sugar-induced blood pressure elevation in SHR. J Am Coll Nutr. 1998;17:116–23.
25. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Mid. 1963;61:882–8.
26. Marklund SL, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974;47:469–74.
27. Matkovics B, Szabo L, Varga IS. Determination of enzyme activities in lipid peroxidation and glutathione pathways (in Hungarian). Lab Diagn. 1998;15:248–9.

28. Mannervik B, Guthenberg C. Glutathione transferase (human placenta). Methods Enzymol. 1981;77:231–5.
29. Adejuwon AA, Adokiye SB. Protective effect of the aqueous leaf and seed extract of *Phyllanthus amarus* on gentamicin and acetaminophen-induced nephrotoxic rats. J Ethnopharmacol. 2008;118:318–23.
30. Stojiljkovic N, Mihailovic D, Veljkovic S, Stoiljkovic M, Jovanovic I. Glomerular basement membrane alterations induced by gentamicin administration in rats. Exp Toxicol Pathol. 2008;60:69–75.
31. Rezaie A, Fazlara A, Haghi-Karamolah M, Zadeh HN, Pashmforosh M. Effects of *Echinacea purpurea* on hepatic and renal toxicity induced by diethylnitrosamine in rats. Jundishapur J Nat Pharm Prod. 2013;8:60–4.
32. Pashmforoosh M, Rezaie A, Haghi-Karamallah M, Fazlara A, Shahriari A, Najafzadeh H. Effects of caffeine on renal toxicity induced by diethylnitrosamine. Zahedan J Res Med Sci. 2015;17:7–9.
33. Jain DP, Somani RS. Antioxidant potential of hesperidin protects gentamicin induced nephrotoxicity in experimental rats. Austin J Pharmacol Ther. 2015;3:1071.
34. Mahmoud AM, Galaly SR, Ahmed OM. Thymoquinone and curcumin attenuate gentamicin-induced renal oxidative stress, inflammation and apoptosis in rats. EXCLI J. 2014;13:98–110.
35. Araujo LP, Truzzi RR, Mendes GE, Luz MA, Burdmann EA, Oliani SM. Annexin A1 protein attenuates cyclosporine-induced renal hemodynamics changes and macrophage infiltration in rats. Inflamm Res. 2012;61:189–96.
36. Akcay A, Nguyen Q, Edelstein CL. Mediators of inflammation in acute kidney injury. Mediators Inflamm. 2009;2009:137072.
37. Bartech H, Heathen E, Melville C. Carcinogenic nitrosamines: free radical aspects of their action. Free Radic Boil Med. 1989;7:637–44.
38. Vitaglione P, Morisco F, Caporaso N, Fogliano V. Dietary antioxidant compounds and liver health. Crit Rev Food Sci Nutr. 2004;44:575–86.
39. Ozbek E. Induction of oxidative stress in kidney. Int J Nephrol. 2012;2012:465897.
40. Nakae D, Kobayashi Y, Akai H, Nabuaki A. Involvement of 8-hydroxyguanine formation in the inhibition of rat liver carcinogenesis by low dose levels of N-nitrosodiethylamine. Cancer Res. 1997;57:1281–7.
41. Arulselvan P, Subramanian SP. Beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultrastructural changes of pancreatic β-cells in experimental diabetes in rats. Chem Biol Interact. 2007;165:155–64.
42. Franco R, Schonveld OJ, Papa A, Panayiotidis MI. The central role of glutathione in the pathophysiology of human diseases. Arch Physiol Biochem. 2007;113:234–58.
43. Laurent A, Perdu-Durand E, Alary J, DE Brauwer L, Cravedi JP. Metabolism of 4-hydroxynonenal, a cytotoxic product of lipid peroxidation in rat precision-cut liver slices. Toxicol Lett. 2000;114:203–14.
44. Srivastava A, Shivanandappa T. Hepatoprotective effect of the root extract of *Decalepis hamiltonii* against carbon tetrachloride-induced oxidative stress in rats. Food Chem. 2010;118:411–7.
45. Swamy AHMV, Kulkarni RV, Thippeswamy AHM, Koti BC, Gore A. Evaluation of hepatoprotective activity of *Cissus quadrangularis* stem extract against isoniaized-induced liver damage in rats. Indian J Pharmacol. 2010;42:397–400.
46. Chandra J, Samali A, Orrenius S. Triggering and modulation of apoptosis by oxidative stress. Free Radic Biol Med. 2000;29:323–33.
47. Wei XJ, Hu TJ, Chen JR, Wei YY. Inhibitory effect of carboxymethylpachymaran on cyclophosphamide-induced oxidative stress in mice. Int J Biol Macromol. 2011;49:801–5.
48. Matés JM, Segura JA, Alonso FJ, Márquez J. Roles of dioxins and heavy metals in cancer and neurological diseases using ROS-mediated mechanisms. Free Radic Biol Med. 2010;49:1328–41.

How to cite this article: Ahmed RR, Mahmoud AM, Ashour MB, Kamel AM. Hesperidin protects against diethylnitrosamine-induced nephrotoxicity through modulation of oxidative stress and inflammation. Natl J Physiol Pharm Pharmacol 2015;5:391-397.

Source of Support: Nil, **Conflict of Interest:** None declared.