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Oxidative Stress of Diethylnitrosamine on the Functions of Kidney in Male Rats and Effective Role of Rutin and/or Selenium

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ABSTRACT

Diethylnitrosamine (DENA), one of the most important environmental carcinogen, causes generation of reactive oxygen species resulting in oxidative stress. The present study was designed to examine the ability of rutin (Ru) and selenium (Se), naturally occurring antioxidants, to attenuate DENA-induced nephrotoxicity in adult albino rats. Administration of DENA at a dose level of 200 mg/kg b.wt. to male rats for three times a final week significantly elevated the levels of serum creatinine, uric acid, urea, cortisol, potassium (K), and lactate dehydrogenase(LDH) activity which indicate injury to the kidney function. Also, the tumor necrosis factor- alpha TNF-α increased in serum. On other hand, DENA decreased renal glutathione (GSH) content, glutathione-S-transferase (GST) and superoxide dismutase (SOD). While, DENA induced lipid peroxidation as indicated by markedly increased of malondialdehyde (MDA). DENA intoxication also induced marked alterations in most of the renal tubules including cell depletion and tubular atrophy. Ru 30 mg/kg b.wt. and Se 0.5 mg/kg b.wt orally for 21 days administered 1h before DENA ameliorated the biochemical toxicity induced by DENA, in the kidney. This was evidenced by a significant reduction in serum creatinine, uric acid, urea, cortisol and LDH activity, K and a significant restoration in Na, GSH, GST and SOD. These results indicate that Ru and Se have a protective effect against DENA-induced damage to kidney. This reflects the beneficial role of Ru and Se in treatment of renovascular hypertention and congestive kidney failure.

Keywords: Diethylnitrosamine, Ru, Selenium, kidney failure, oxidative stress.

INTRODUCTION

The kidney is highly susceptible to toxicants for two reasons; a high volume of blood flowing through it and filtration of large amounts of toxins which can concentrate in the kidney tubules. It can result in systemic toxicity causing: decreased ability to excrete body wastes, inability to maintain body fluid and electrolyte balance and decreased synthesis of essential hormones [45].

Nitrosamine, one of the most important environmental carcinogen, has been suggested to cause the generation of reactive oxygen species (ROS) resulting in oxidative stress, alteration the antioxidant defense system in tissues and cellular injury. Thus it may be one of the factors in the etiology of cancer, causing a wide range of tumors in all animal species and inducing cancer in a variety of rodent organs, such as the kidney and liver [9,50].

Nitrosamines are used in the manufacture of some cosmetics, pesticides, and in most rubber products. It is present in latex products and in many foods. In foods, nitrosamines are produced from nitrites and secondary amines, which often occur in the form of proteins. Under acidic conditions the nitrite forms nitrous acid (HNO_2), which is protonated and splits, into the nitrosonium cation $N{\equiv}O^+$ and water: $H_2NO_2^+ = H_2O + NO^+$. The nitrosonium cation then reacts with an amine to produce nitrosamine. Nitrosamines can also be found in tobacco smoke. Dimethylnitrosamine (DENA), also known as N-Nitrosodimethylamine, is a semi-volatile organic chemical that is highly toxic and is a suspected human carcinogen. At high doses, it is a potent hepatotoxin that can cause fibrosis of the liver in rats and produced liver tumours in rats, approximately 90% of nitrosamine compounds were deemed to be carcinogenic [20,48,41].

Selenium (Se) is an essential dietary trace element which plays an important role in a number of biological processes for humans and many other forms of life. Deficiency of this element induces some pathological conditions, such as cancer, coronary heart disease, and liver necrosis [33,7]. Se taken in the form of selenite, selenate, selenocysteine and selenomethionine gets the most absorbed in the duodenum. After absorption, increased levels of Se have been recorded in the blood plasma proteins and from there it can be distributed into the tissues where it is incorporated in newly synthesized seleno-proteins. A marked uptake of Se by erythrocytes was also found [44]. Se is an essential component of several enzymes such as glutathione peroxidase (GSH-Px), thioredoxin reductase and selenoprotein

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P, which contain Se as selenocysteine. It is also well known that Se is essential for cell culture when a serum-free medium is used and has some protective role from the toxic actions of Cadmium and other heavy metals [33,7,44].

Flavonoid affect basic cell function such as growth, differentiation and apoptosis. Also, they were showed to be potent antioxidant because of their radical-scavenging activity; ability to complex heavy metal ion and to antagonize a broad spectrum of enzymes such as tyrosine protein kinase [30].

Rutin a quercetin-3-rutinosid or vitamin-P is considered as one of flavonoid glycosides, which is found in onions, apples, tea and red wine. It is well known to exhibit multiple pharmacological activities including antibacterial, antitumor, anti-inflammatory, anti-diarrheal, antiulcer, anti-mutagenic, vasodilator and immunomodulator. Furthermore, rutin showed an inhibitory effect against membrane lipid peroxidation and has renal protective effects via its antioxidant activities which suggest its protective role in oxidative stress-mediated diseases [32]. However, until our knowledge, the protective effect of rutin and Se against DENA-induced nephrotoxicity has not been investigated.

The current study was designed with major two goals; (1) to investigate DENA-induced nephrotoxicity using albino rats as an animal model; (2) to evaluate the potential beneficial effects of rutin and/or selenium supplementation to improve nephrotoxicity.

Materials and Methods

Chemicals:

Diethylnitrosamine (DENA), Selenium(Se) and Rutin (Ru) were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA) and given by i.p at dose levels of 200 mg/kg b.w. DENA [29], orally 0.5 mg/kg b.w. of Se [16] and 30 mg/kg b.w. of Ru [1], respectively. All other chemicals and solvent used were of highest available commercial grade.

Experimental animals:

Fifty male Sprague–Dawely rats, each weighing 160 ± 10 g, were obtained from the Breading Unit of the Egyptian Organization for Biological and Vaccine production, A.R.E. The animals were housed in stainless steel cages after grouping in batches of five under standard animal house conditions of relative humidity (55 ± 5%), temperature (25 ± 2 ˚C) and a 12 hr light/12 hr dark cycle. Rats were allowed free access to standard commercial feed and tap water and were acclimatized to laboratory conditions for a period of one week before the onset of experimentation.

Experimental protocol:

Animals were allocated to five groups each of ten rats as follows:

Group I: (Control) pre- treated with dimethylsulfoxide (DMSO) 1 ml/kg b.w. once daily for 21 days and treatment continued with saline (1 ml/kg b.w.) three times a final week of the experimental period of 21 days.

Group II: (DENA group) pre-treated with DMSO (1 ml/kg b.w.) once daily for 21 days and treatment continued with DENA (200 mg/kg b.w.) three times a final week of the experimental period of 21 days.

Group III: (Se and DENA group) pre-treated with Se in a dose of 0.5 mg in 1 ml DMSO / kg b.w. */o.s.* for 21 days and treatment continued with DENA (200 mg /kg b.w.) three times a final week of the experimental period of 21 days.

Group IV: (Ru and DENA group) pre-treated with rutin (30 mg/kg b.w.) for 21 days and treatment continued with DENA (200 mg/kg b.w.) for 3 times a final experimental week.

Group V: (Ru, Se and DENA group) pre-treated with rutin and Se for 21 days and treatment continued with DENA (200 mg/kg b.w.) for three times a final experimental week.

At the end of the experimental period, the tested animal groups were sacrificed after 24 hrs of the last dose of different administrations and their blood were collected, by carotid bleeding, in centrifuge tubes and serum was obtained from the blood after centrifugation at 3000 rpm for 10 min. Kidneys were removed and washed with ice cold saline, freed from surrounding fats, blotted with a piece of filter paper, weighed and homogenized in ice cold 0.15M KCl. Serum and kidney samples were stored at -20°C until analysis studies.

Methods of analysis:

Serum urea was estimated by using the method of Pathson and Nauth [46] and creatinine was determined according to the method of Rock *et al.* [51]. Uric acid and cortisol were determined by method of the Barham and Trinder [10] and Coolidge [15]. Lactate dehydrogenase (LDH) activity was determined in serum by the method described by Mc Queen [39]. Sodium (Na) and potassium (K) were measured by using the method of Kokko and Tannen, [31] The tumor necrosis factor- alpha. TNF-α in serum were measured by sandwich enzyme-linked immunosorbent assay. Renal GSH was assayed by the method of Hissin and Hilf [25]. Glutathione-S-transferase GST was assayed by the method of William *et al.* [58]. The activity of renal superoxide dismutase SOD was determined by assessing the inhibition of pyrogallol autooxidation [37]. Malondialdehyde MDA in the kidney was determined using the method of Mihara and Uchiyama [40].

Statistical analysis:

Statistical analyses were done using InStat version 2.0 (GraphPad, ISI Software, Philadelphia, PA, USA, 1993) computer program. The results were expressed as mean ± SE. Multiple comparisons were done using one-way ANOVA followed by Tukey-Kramer as a post-ANOVA test.

Results:

The effect of diethylnitrosamine (DENA):

As shown in Table 1&2 the present data recorded that DENA induced significant increase (P<0.001) in the levels of serum creatinine, uric acid and urea as well as cortisol level. Also, the level of serum potassium (K), TNF-α and LDH activity were significant elevated (P<0.001) in DENA group II compared to control group I, which indicate injury to the kidney function.

Regarding the effect of DENA in renal tissues (Table 3), the obtained results showed a significant reduction in renal glutathione (GSH) content and superoxide dismutase (SOD) (P<0.001) as well as glutathione-S-transferase (GST) activity and sodium level (Na) in DENA group II compared to control group I. On other hand DENA induced lipid peroxidation as indicated by markedly increased of MDA (P<0.001) in the treated rats with DENA group II compared to control group I.

The effect of Ru and Se:

The administration of Ru and Se plus DENA exhibited significant enhancement in all previous parameters (Tables1 &2). The results recorded a significant decrease (P<0.001) in the levels of serum creatinine, uric acid and urea as well as cortisol level. Also, the level of serum potassium (K), TNF-α and LDH activity were reduced significantly (P<0.001) in group V as compared to the control group I.

The data in Table 2&3 showed that the treatment with Ru and Se elevated significantly these low concentrations (P<0.001). Renal GSH content and SOD (P<0.001) as well as GST activity and Na level increased and decreased in renal MDA after Ru and Se administration compared to the values in the control group I. Also, these changes were statistically significant as compared to the values obtained after injection with DENA alone group II.

Table 1: The effect of Selenium (Se) & Rutin (Ru) on Diethylnitrosamine (DENA) - induced changes on serum urea, creatinine, uric acid & cortisol levels.

Groups	Parameters			
	Urea mg/dl	creatinine mg/dl	Uric acid mg/dl	Cortisol U/dl
Control	34.25 ± 0.09	0.78 ± 0.006	20.55±0.19	6.73 ±0.41
GII (DENA)	59.26 ± 0.07a**	1.65 ±0.01a**	35.99 ±0.14a**	14.47±0.13a**
GIII (Se+ DENA)	57.52 ± 0.01ab**	1.05 ±0.01a*b**	29.05 ±0.22ab**	11.8 ±0.16ab**
GIV (Ru + DENA)	50.87 ±0.6abc**	1.07 ±0.05a*b**	26.71 ±0.13a bc**	10.22 ±0.69abc**
GV (Se+Ru + DENA)	43.16 ± 0.08abc**	0.84 ± 0.006b**c*	23.35 ±0.12abc**	9.11 ±0.05abc**

Data are expressed as mean ± S.E. (n = 8 in each group).
a: Significant change at p< 0.05 with respect to control group.
b: Significant change at p< 0.05 with respect to group II.
c : Significant change at p< 0.05 with respect to group III.
*Highly significant change at p < 0.01.
**Very highly significant change at p < 0.001.

Table 2: The effect of Selenium (Se) & Rutin (Ru) on Diethylnitrosamine (DENA) - induced changes on serum TNF, LDH, K & Na levels.

Groups	Parameters			
	TNF Pg/ml	LDH U/L	K meq/L	Na meq/L
Control	10.69 ± 0.13	275.93 ± 0.41	2.17±0.07	136.67 ± 0.06
GII (DENA)	31.37 ± 0.11a**	312.46 ± 0.31a**	5.19 ±0.07a**	110.30±0.16a**
GIII (Se+ DENA)	29.05 ± 0.18a**	310.65± 0.42a**, n.s.	5.09 ±0.05ab**	110.81 ±0.27n.s.
GIV (Ru + DENA)	25.42 ± 0.11abc**	294.09 ± 0.40ab**	4.27 ± 0.02a bc**	123.44 ± 0.08 a bc**
GV (Se+Ru + DENA)	16.67± 0.19abc**	281.45 ± 0.27b**c**	3.30 ± 0.07abc**	130.18 ± 0.28abc**

Data are expressed as mean ± S.E. (n = 8 in each group).
a: Significant change at p< 0.05 with respect to control group.
b: Significant change at p< 0.05 with respect to group II.
c : Significant change at p< 0.05 with respect to group III.
*Highly significant change at p < 0.01.
**Very highly significant change at p < 0.001.

Table 3: The effect of Selenium (Se) & Rutin (Ru) on Diethylnitrosamine (DENA) - induced changes on renal tissue GSH, GST, SOD and MDA contents.

Groups	Parameters			
	GSH Ug/g wet tissue	GST U/g wet tissue	SOD U/g wet tissue	MDA U/g wet tissue
Control	82.52 ± 0.104	20.79 ± 0.16	62.91 ± 0.061	52.32 ±0.205
GII (DENA)	63.07 ± 0.189a**	14.8 ±0.19a**	51.24 ± 0.139a**	77.02 ±0.123a**
GIII (Se+ DENA)	68.42 ± 0.093ab**	16.22± 0.18ab**	54.30 ±0.132ab**	69.34±0.093ab**
GIV (Ru + DENA)	70.38 ± 0.100abc**	18.39 ± 0.22abc**	56.59 ± 0.092abc**	68.82 ±1.199ab**
GV (Se+Ru + DENA)	79.45 ± 0.130abc**	20.65 ±0.2bc**	60.67 ± 0.062abc**	55.45 ±0.150a*b**c**

Data are expressed as mean ± S.E. (n = 8 in each group).
a: Significant change at p< 0.05 with respect to control group.
b: Significant change at p< 0.05 with respect to group II.
c : Significant change at p< 0.05 with respect to group III.
*Highly significant change at p < 0.01.
**Very highly significant change at p < 0.001.

Discussion:

Acute renal failure is characterized by disorders in some biochemical parameters in DENA treated rats. All rats injected with DENA showed a decrease in glomerular filtration indicated by significantly elevated levels of serum creatinine, uric acid, urea, cortisol, K and increase LDH activity. These results confirm that DENA produces nephrotoxicity as previously reported by Vargas-Olvera, *et al* [56]. These changes reflect the severity of renal function insufficiency which occurred in association with the sudden fall in glomerular filtration rate in acute tubular necrosis because of the majority of administrated DENA enters specifically the proximal tubular epithelial cells, binds to anionic phospholipids in the target cells inducing abnormalities in the function and metabolism of multiple intracellular membranes and organelles then developed injury in the proximal tubular epithelial cells of kidney that caused acute renal failure [2,47]. Increased in LDH in acute injury of cardiac muscle, liver, kidney and lung causing tissue necrosis [3].

Creatinine is excreted by filtration through the glomerulus and by tabular secretion. Reduction in glomerular filtration rate, serum creatinine increased. LDH responsible to heart failure and related to decreased renal blood flow [23]. Urea and creatinine values are commonly evaluated together due to acute renal failure and related blood flow to the kidney, decreased renal excretion and accumulation in circulating blood. Uric acid is created when the body breaks down purine nucleotides; it is an end product of nucleoprotein metabolism and excreted by the kidney. An increase in uric acid evaluate liver damage that result in high cellular turnover release nucleic acids into circulation which are converted to uric acid by the liver [35,52].

Two major tubular abnormalities could be involved in the decrease in glomerular function in DENA treated rats: obstruction and back leak of glomerular filtrate. The alterations in glomerular function in DENA treated rats may also be secondary to ROS (reactive oxygen species) which induce mesangial cells contraction, altering the filtration surface area and modifying the ultrafiltration coefficient factors that decrease the glomerular filtration rate [11,47]. In pharmacokinetics studies, it has been found that DENA is degraded and contact with renal tubular epithelium and is converted to active electrophilic species following α or β hydroxylation, resulting in the formation of unstable hydroxyalkyl compounds that are subsequently converted to alkyl carbonium ions [47].

Serum sodium and potassium were disturbed significantly ($p<0.001$) in DENA treated rats as compared with control animals. Lower value of serum sodium indicates inability of kidney to conserve sodium and chloride. Also excessive loss through renal excretion, renal absorption is blocked and decreased the ability of renal tubules to reabsorb sodium. Haemodilution may be involved in the fall of sodium value via excess water intake and or increased production of endogenous water. In turn, the reversed increases of potassium observed may be due to reduced excretion of potassium aggravated by leakage of intracellular potassium into blood stream as a result of DENA induced lesions in renal tubular epithelium. These results are in harmony with Heibashy and Abdel Moneim [22] and Mazen, [38].

The content of GSH, GST and SOD in rat kidney tissues were significantly ($p<0.001$) reduced after DENA injection as compared to control group (Table 3). This result is confirmed by other studies, which have pointed to reduction of GSH, GST and SOD levels after DENA injection. An explanation to GSH depletion after DENA treatment might be due to NADPH depletion or increased consumption of GSH in non-enzymatic removal of oxygen-radicals [21]. In addition, oxidation of GSH to GSSG by the oxidant stress, with efflux of oxidized glutathione GSSG being the major factor responsible for maintenance of the redox ratio. GSSG is of great biological importance, since it allows fine-tuning for

the cellular redox environment under normal conditions and upon the onset of stress, provides the basis for GSH stress signaling and the conversion of GSSG to GSH is mediated through inhibition of the enzyme glutathione reductase [55,38]. GSH has a very important role in protecting against oxygen free radical damage by providing reducing equivalents for several enzymes; GSH is also a scavenger of hydroxyl radicals and singlet oxygen [17]. Antioxidants can improve the reduction of TNF-α and LDH levels and increase GSH and SOD. The antioxidants also inhibit the lipid peroxidation as observed by the reduced thiobarbituric acid reactive substances level and reduced superoxide anions and free radicals generated in the case of toxicity resulting from oxidative stress by generating cellular antioxidant enzymes, such as GST and superoxide dismutase in the different tissues [43]. TNF-α is produced mainly by macrophages but also by a broad variety of other cell types including lymphoid cells, mast cells, endothelial cells, fibroblasts, and neuronal cells [57. TNF-α induces cell death via apoptosis and necrosis pathways, so inhibiting TNF-α production decreases tissue injury [18].

In this study a significant decrease ($p<0.001$) was observed in serum creatinine, uric acid, urea, Cortisol, LDH and MDA in Se treated group when compared to DENA treated groups and these results are in agreement with Leblond, *et al* [34], Mohamed, *et al* [42], Fatmi *et al* [19] and Kasaikina *et al* [28]. The biological importance of selenium is multifactorial. First, it forms the prosthetic group of some critical selenocysteine-containing enzymes, such as glutathione peroxidase, iodothyronine 5,-deiodinase, and thioredoxin reductase. Second, sodium selenite is protective against a number of toxicants, Third, selenium excessive intake cause toxic potential. Sodium selenite as an exogenous source of selenium is used for endogenous selenoprotein synthesis to scavenge the free radicals, so it involves antioxidant defense mechanisms because Se acts on H2O2 and reduced GSH converting them into H2O and oxidized GSSG [35,44]. Al-Bader, *et al* [4] and Begum, *et al* [11], reported that SOD act as a cellular defense element against potentially harmful effects of superoxide ions by catalyzing the dismutation of these ions [11].

Rutin, a glycoside of the flavonoid quercetin, exerts its antioxidant effects by scavenging free superoxide and hydroxyl radicals on one hand and by inhibiting xanthine oxidase activity and lipid peroxidation on the other [6]. In the current study, rutin significantly enhanced the content of GSH, GST and the activity of SOD enzyme but reduced the elevated levels of MDA, LDH and TNF-α. These results indicate the ability of rutin to transfer electrons and free radicals in addition to activation of antioxidants enzymes and decreases oxidative stress through its antioxidant properties [6]. Also, it has been reported that rutin has effectively reversed the biochemical, behavioral, and neurochemical changes in rat treated with haloperidol and improved the antioxidants enzymes system in human hepatoma cell line (Hep G2) by inhibition MDA levels and increasing CAT activity and therefore preventing or delay oxidative damage and its adverse effects [24,5,36,38].

The present study demonstrates that the administration of rutin and/or Se exerts a renal protective effect in a rat model of nephrotoxicity provoked by DENA. Co-administration of rutin and Se to the nephrotoxic rats significantly decreased the DENA-induced nephrotoxicity demonstrated by prevention of the increase in renal enzyme activities and preservation of GSH and antioxidant enzymes levels in kidneys. This could be due to the ability of these antioxidants substance to transfer electrons free radicals, chelate metals, catalayse and activate antioxidant enzymes [26,38]. Moreover, the maximum ameliorating effects of two antioxidants pronounced according to the synergistic effects which improve the physical and pharmakinetics properties of them. These improvements depend on the time of administration**.** Protection of cells from DENA-induced oxidative processes caused by ROS and free radicals is in the form of both enzymatic and non-enzymatic defense mechanisms present in the cell. Antioxidants include the enzymes SOD, catalase, (GPx) and esterases, molecules such as (GSH) and trace metals such as Se. Trace elements such as Se have been known to have beneficial effects on DENA-induced oxidative stress. Se is an important co-factor of antioxidant enzymes such as GPx and thioredoxin. Se a component of several enzymes such as iodothyronine 5-deiodinase, thioredoxin reductase and glutathione peroxidase, plays an important role in living organisms [27,13,54,14,44].Thus, combining Se with rutin may effectively prevent DENA-induced nephrotoxicity.

Rutin and Se may have a selective influence on gene transcription and RNA production, which explains their regulation of the biosynthesis of specific proteins, can regulate endogenous pattern of antioxidant defense enzyme expression in the different rat tissues. Furthermore, DENA induced elevation in creatinine, uric acid, urea, Cortisol and LDH were significantly attenuated by rutin and Se combination with an extent that was much better than administration of each alone. MDA is well known to induce cytotoxicity and their action on kidney has been implicated in the pathogenesis of various diseases. Several studies reported that rutin or Se have a potent ability to damage free radicals produced through biological processes in many extracellular and intracellular reactions. Therefore, this study suggest that the combined renal cytoprotective effects of both rutin and Se are due to their free radical scavenging capability which seems to be increased by both factors combination and significantly decreased MDA and elevate GSH

kidney levels as compared to their altered levels in DENA group suggesting that the antioxidant properties of rutin may be attributed to its protective effects on lipid peroxidation [8]. Also, Rutin and Se combination enhance the production of ROS, increase the antioxidant enzyme levels significantly inhibited the rise in serum TNF-α level and these results were in harmony with the finding reported by Ramesh, & Reeves, [49]. Also, Bell & Gochenaur, (2006) reported that antioxidant activity, free radical scavenging property, antiinflammatory effect, vasodilatory action could be reduce the toxic renal damage due to cisplatin. Thus, the toxic effects and degree of renal failure induced by DENA were decreased by rutin and/or Se.

In conclusion, renal enzymes decreased by the antioxidant activities and anti-inflammatory effects of rutin and/or Se. This study clearly showed the potential antioxidant usefulness of rutin and Se combination better protective effect against nephrotoxicity induced by DENA in rats than administration of each alone.

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