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Ginger-supplemented diet ameliorates ammonium nitrate-induced oxidative stress in rats

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The present study was designed to evaluate the capacity of ginger to repair the oxidative stress induced by ammonium nitrate. 50 male rats were divided into 5 groups; they underwent an oral treatment of ammonium nitrate and/or ginger (N mg/kg body weight + G% in diet) during 30 days. Group I served as control (C); group II (G) received a diet with 2% of ginger; group III (N) received a toxic dose of ammonium nitrate and normal diet; group IV (NG) received a toxic dose of ammonium nitrate and a diet containing 2% ginger and group V ($N^{+}G$) received a highly toxic dose of ammonium nitrate and an experimental diet containing 2% ginger. The treatment by ammonium nitrate was found to elicit a rise in blood biochemical parameters, a disorder in hematological parameters and significant decrease in the tissue glutathione level. Feeding ginger supplemented diets to ammonium nitrate treated rats restored all the parameters studied compared to the controls. These findings suggest that ginger treatment exerts a protective effect on metabolic disorders by decreasing oxidative stress.

Key words: Ammonium nitrate, toxicity, ginger, oxidative stress, rats.

INTRODUCTION

The massive use of fertilizers lead to an increase in agricultural outputs, but caused a greater pollution of continental waters and farmlands (Koller, 2009). Ammonium nitrate is one of the most commercially important ammonium compounds in terms of usage. It finds extensive use in the area of nitrogen fertilizers (Testud, 2004), explosives (Presles et al., 2009) and in the manufacturing of meat products (Honikel, 2008). It is a crystalline, hygroscopic and odorless substance which tends to coagulate in lumps, and very soluble in water. Excessive nitrate concentrations in drinking water may have serious implications for public health; it can enhance the proliferation of phytoplankton, contributing to the phenomenon of eutrophication in aquatic ecosystems (Camargo and Álvaro, 2006). Once in the organism, the conversion of nitrate in the mouth is particularly important. The dorsal surface of the tongue symbiotically harbors a specialized flora of anaerobic nitrate reducing bacteria, which can rapidly reduce nitrate to nitrite (Duncan et al., 1995). Nitrite will be converted to varieties of nitrogen compounds in the stomach. The main products are NO and N-nitroso compounds (Mitsui and Kondo, 2002).

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Abbreviations: GPx, Glutathione peroxidases; **SOD,** superoxide dismutases; **CAT,** catalase; **EDTA,** ethylenediaminetetra acetate; **CBC,** complete blood count; **ALP,** alkaline phosphatase; **ALAT,** alanine aminotransferase; **ASAT,** aspartate aminotransferase, **LDH,** lactate dehydrogenase; **GSH,** glutathione; **metHb,** methemoglobin; **MCV,** mean corpuscular volume; **IDDM,** insulin-dependent diabetes mellitus.

Recent research indicates that there is a close correlation between NO generation and nitrite content in plasma; and thereby the nitrite is suggested to be the storage form of NO (Bryan, 2006). Nitric oxide (NO) has been shown to be involved in many important biological events. However, the presence of the unpaired electron gives NO paramagnetic properties, prevents its dimerization and increases its reactivity with a variety of atoms and free radicals leading to oxidative stress. One important reaction of NO is its interaction with oxyhemoglobin to form methemoglobin (metHb) (Mansouri and Lurie, 1993); it can also react with secondary amines to produce N-nitroso compounds susceptible to be carcinogenic (Volkmer et al., 2005). Moreover, NO interact with superoxide anion (O_2^-) to form peroxynitrite ($ONOO^-$); which is a potent oxidant which can nitrosate proteins and nucleic acids, and can cause lipid peroxidation (Squadrito and Pryor, 1998). Additionally, the protonated form of $ONOO^-$ may be decomposed to form highly reactive (OH•) and nitrogen dioxide (Beckman et al., 1990).

Organisms have several mechanisms to counteract damage by free radicals (Koechlin-Ramonatxo, 2006). One important line of defence is an enzyme system, including glutathione peroxidases (GPx), superoxide dismutases (SOD) and catalase (CAT), which decrease concentrations of the harmful oxidants in the tissues. The second line of defence against free radical damage is the presence of antioxidants, which are stable molecules enough to donate an electron to scavenging free radical and neutralize it. Some antioxidants, including glutathione, ubiquinol and uric acid, are produced during normal metabolism in the cells (Yu, 1994). Other antioxidants are found in the diet, the best known are vitamin E, vitamin C and carotenoids. Many natural substances, such as phenolic or polyphenolic compounds, display antioxidant properties, and thus, important for health. Spices are recognized as sources of natural antioxidants which can protect against the oxidative stress and play an important role in the prevention of numerous pathologies (Gião et al., 2010).

Ginger (*Zingiber officinale*, Roscoe, Zingiberaceae) is one of the most commonly used spices around the world, especially in the South-Eastern Asian countries (Chrubasik et al., 2005). The constituents of ginger are numerous and vary depending on the place of origin and whether the rhizomes are fresh or dry. Many chemical investigations on this plant led to the identification of a large number of compounds, like the gingerols, shogaols, and the gingerdiones (Schwertner and Rios, 2007). Ginger has been demonstrated to have various pharma-cological activities (Ali et al., 2008) such as antiemetic, antiulcer, anti-inflammatory, antioxidant, glucose and lipid lowering, cardiovascular as well as anti-cancer activities. Therefore, the present study was undertaken to investigate the potential role of ginger in reducing the toxic effect and oxidative stress induced by ammonium nitrate in experimental rats.

MATERIALS AND METHODS

Preparation of ammonium nitrate solution

Pure ammonium nitrate (Fluka Macedonia) was dissolved in mineral water, and induced by oral voice (*per os*); the volume of each dose was adjusted to deliver 400 and 600 mg/kg of rat body weight.

Preparation of ginger powder

The rhizomes of fresh ginger was purchased from the local market (imported from China), peeled, washed, coarsely minced, air dried and pulverized with a blender to fine powder. It was preserved in airtight containers at room temperature until the formulation of experimental diets. Ginger powder was added (w/w) to already pulverized feed and thoroughly mixed so as to give a diet containing 2% ginger.

Animals, diet and treatment

Fifty (50) male rats with an average body weight of 200 g were provided by the Algiers Pasteur Institute, Algeria. The animals were housed in clean polypropylene cages, and kept under standard laboratory conditions of light/dark cycle (12/12 h) and controlled temperature room (25 ± 2°C). The rats were given a nutritionally adequate diet which was prepared according to Upreti et al. (1989) and water *ad libitum* throughout the experimental period. After acclimatization, the rats were randomly allocated into five groups of ten animals each and the groups were treated as follows: the 1st group (C) served as control and received mineral water and normal diet (0 + 0); the 2nd group (G) feed with 2% ginger diet (0 + 2%); the 3th group (N) received by gavage 400 mg/kg of ammonium nitrate (400 mg/kg + 0); the 4th group (NG) received 400 mg/kg of ammonium nitrate and feed with 2% ginger diet (400 mg/kg + 2%); and the 5th group (N^+G) received 600 mg/kg of ammonium nitrate and feed with 2% ginger diet (600 mg/kg + 2%).

Experimental methods

After 30 days of treatment, animals were sacrificed by decapitation and blood samples were collected into two tubes: one contained ethylenediaminetetra acetate (EDTA) as anticoagulant for metHb and complete blood count (CBC) test, while the other was kept dry and centrifuged at 5000 rpm for 15 min to obtain serum, which is used for various biochemical investigations. Some organs were retrieved, weighed and preserved at -20°C for the determination of reduced glutathione levels.

Analytical methods

Methemoglobin assay was carried out according to Evelyne and Malloy (1938) method. Hemathological parameters were measured by an automated ERMA INC Analyzer (full automatic blood cell counter model PCE-210N). The serum glucose concentration was measured by portable glucometer (Accu-Chek Active). Uric acid, urea, creatinite, cholesterol, triglycerides, total lipids, alkaline phosphatase (ALP), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), lactate dehydrogenase (LDH), total and direct bilirubin were measured spectrophotometrically in serum with kits purchased from Spinreact (Spain). The concentration of tissue reduced glutathione (GSH) was measured, as described by Weckbecker and Cory (1988). The proportioning of proteins was carried out by the method of Bradford (1976).

Statistical analysis

All data were represented as means ± SD of 10 rats. Data were analyzed by Minitab software (version 13.31) using the Student's t-test to assess differences compared to the control group.

RESULTS

Physiological study

Exposure of rats to ammonium nitrate did not produce any overt sign of toxicity/mortality. We find that hepatosomatic index in (N) group increased significantly ($p < 0.05$) indicating hepatomegaly, as well as the spleenosomatic index in (N^+G) group was significantly decreased (Figure 1).

Hematological study

Table 1 shows the result of hematological parameters in control and ginger-diet supplemented rats. These results reveal that the treatment by ammonium nitrate (N) caused significant changes ($p < 0.05$) in hematocrit, red and white blood cells number; hemoglobin (Hb), metHb level and mean corpuscular volume (MCV). However, ginger supplementation in treated rats prevented alterations in these parameters except hematocrit and metHb.

Renal study

Significant increase ($p < 0.01$) in serum urea, creatinine and uric acid were observed in the ammonium nitrate treated rats compared with control group. Feeding of rats with ginger-supplemented diet maintains these parameters in normal values, which reflect a re-establishment of the renal function (Table 2).

Biochemical study

As presented in Table 3, oral administration of 400 mg/kg of ammonium nitrate to rats caused a significant increase in the concentration of glucose, total and direct bilirubin, cholesterol, triglycerides, total lipids, ALAT, ASAT, LDH and ALP in comparison with control group. Meanwhile, ginger supplementation in NG group restored these biochemical parameters to near control levels. The N^+G group, which was given 600 mg/kg ammonium nitrate and 2% of ginger, showed a very significant alteration in all biochemical markers.

Toxicological study

High nitrate intake caused an impressive oxidative stress which occurs mainly through a significant reduction of GSH levels in all removed organs. On the other hand, ginger supplementation showed a remarkable antioxidant activity. We recorded an increase of GSH levels in all organs and became equivalent to those of the control rats (Figure 2).

DISCUSSION

A large number of xenobiotics have been identified to have potential to generate free radicals in biological systems (Kehrer, 1993). Free radicals have become an attractive means to explain the toxicity of numerous xenobiotics. It is now known that both nitrates and nitrites are the precursor of NO leading to $ONOO^-$ production in particular if oxidative stress is present in biological areas. $ONOO^-$ reacts with and damages many important biological molecules including thiols, lipids, proteins, and nucleic acids by a number of mechanisms (Raat et al., 2009). However, it is clear that dietary intake of naturally occurring antioxidants may be an effective means to develop prevention strategies in biochemical alterations and diseases risk factors associated with free radicals formation.

The most important effect of nitrate in the cellular elements of the blood is its hemolytic action inducing microcytosis (reduction of MCV) (Lukyanenko et al., 2004). Increased formation of MetHb is the most emphasized adverse effect caused by nitrate pollution after its subsequent reduction to nitrite which is ten times more toxic because of its oxidative properties (Sadeq, 2008; Rodrıguez-Estival, 2010).

Nitrites are absorbed into the blood stream, from where they can reach other tissues. It leads to the oxidation of the ferrous iron (Fe^{2+}) to the ferric (Fe^{3+}) valence state, converting the hemoglobin to methemoglobin with a resultant inability to deliver oxygen to tissues, causing hypoxia and cyanosis (Jaffe, 1981). Nitrites can also, be a source of nitric oxide (NO) and other reactive oxygen as well as nitrogen species such as hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$) and superoxide anion (O_2^-), which disturb the balance between pro-oxidants and antioxidants in favor of the former, resulting in oxidative stress (Halliwell and Gutteridge, 1984). Inducing a peroxidation of the unsaturated fatty acids of phospholipids. Thus, it appears like an osmotic brittleness of the erythrocyte membrane as well as a disturbance of membrane transport which leads to the hemolysis (Ozturk et al., 2003).

Therefore, ginger is considered to be effective in reducing the rate of methemoglobin and restoring the values of hematological parameters in combination groups, demonstrating its antioxidant properties. Polyphenols, the largest compound family in ginger roots, possess ideal structure for free radical scavenging activity; and some of them have been shown to be more effective antioxidants *in vitro* than tocopherols and ascorbate (Bolkhina et al., 2010). Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors, and from the ability of the polyphenol-derived radical to stabilize and delocalize the unpaired electron (chain-breaking function), as well as their ability to chelate transition metal

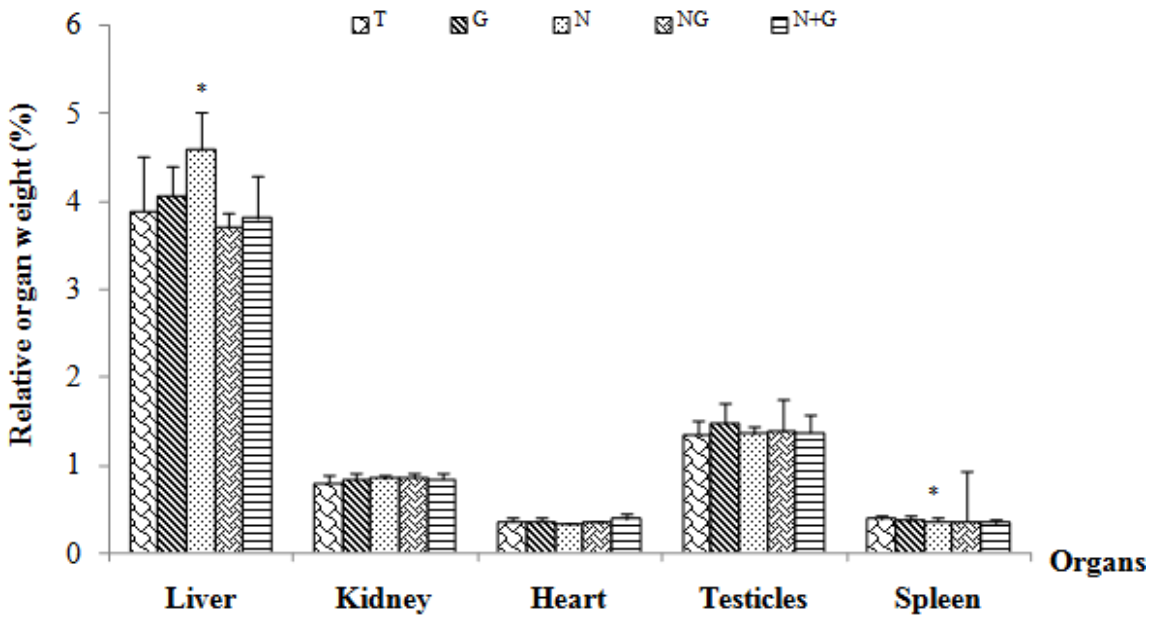

Figure 1. Organosomatic indexes (relative organ weight) in control (C) and treated rats with ammonium nitrate and ginger (G, N, NG and N^+G) after 30 days treatment. Each value represents the mean ± SD of 10 rats. *, Significantly different from control at $P < 0.05$.

Table 1. Concentrations of hematological parameters in control (C) and treated rats with ammonium nitrate and ginger (G, N, NG and N^+G) after 30 days treatment, (each value represents the mean ± SD of 10 rats).

Parameter	Experimental batch				
	C (0 + 0)	G (0 + 2%)	N (400 mg/kg + 0)	NG (400 mg/kg + 2%)	N^+G (600 mg/kg + 2%)
WBC ($x10^3$/ul)	9.07 ± 1.26	8.54 ± 0.80	7.68 ± 1.22*	8.96 ± 1.41	9.66 ± 1.26
LY ($x10^3$/ul)	5.98 ± 0.84	5.72 ± 0.90	5.32 ± 0.74	6.08 ± 0.61	6.54 ± 0.92
MO ($x10^3$/ul)	1.21 ±0.27	1.22 ± 0.28	1.05 ± 0.16	1.14 ± 0.17	1.24 ± 0.24
RBC ($x10^6$/ul)	8.92 ± 0.67	7.97 ± 1.34	7.79 ± 0.57*	8.89 ±0.55	8.55 ± 0.63
Hgb (g/dl)	13.54 ± 0.85	12.52 ±1.29	12.12 ±1.47*	13.80 ± 1.03	12.88 ± 1.27
HCT (%)	51.21 ± 3.37	47.54 ± 5.69	39.66 ± 4.89***	45.02 ± 4.77**	42.11 ± 3.12***
MCV (fl)	54 ± 3.79	50.26 ± 4.46	49.53 ± 2.39*	50.49 ± 2.68	50.30 ± 3.20
MCHC (g/dl)	32.33 ± 2.90	33.03 ± 3.47	31.80 ± 2.65	32.17 ± 2.56	31.89 ± 3.22
PLT ($x10^3$/ul)	303.6 ± 80.1	248.4 ± 37.2	235.2 ± 90.7	290.6 ± 67.8	282.1 ± 55
MetHb (%)	1.69 ± 0.82	1.75 ± 0.60	10.51 ± 1.50***	3.65 ± 1.11**	4.62 ± 1.02***

WBC, White blood cells; LY, lymphocytes; MO, monocytes; RBC, red boold cells; Hgb, hemoglobin; HCT, hematocrit; MVC, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet; MetHb, methemoglobin; *, **, and ***, significantly different from control at $P < 0.05$; $P < 0.01$; $P < 0.001$, respectively.

Table 2. Concentrations of renal parameters in control (C) and treated rats with ammonium nitrate and ginger (G, N, NG and N^+G) after 30 days treatment, (each value represents the mean ± SD of 10 rats).

Parameter	Experimental batch				
	C (0 + 0)	G (0 + 2%)	N (400 mg/kg + 0)	NG (400 mg/kg + 2%)	N^+G (600 mg/kg + 2%)
Uric acid (mg/dl)	4.90 ± 0.85	3.70 ± 0.87*	6.80 ± 1.23**	5.63 ± 1.07	6.36 ± 1.25*
Urea (mg/dl)	27.08 ± 4.51	26.71 ± 3.69	46.41 ± 11.20**	35.19 ± 9.94	39.28 ± 10.50**
Creatinine (mg/dl)	1.02 ± 0.25	1.29 ± 0.29	2.06 ± 0.58**	1.34 ± 0.35	1.67 ± 0.39**

* and **, Significantly different from control at $P < 0.05$; $P < 0.01$, respectively.

Table 3. Concentrations of biochemical parameters in control (C) and treated rats with ammonium nitrate and ginger (G, N, NG and $N^{+}G$) after 30 days treatment.

Parameter	Experimental batch				
	C (0 + 0)	G (0 + 2%)	N (400 mg/kg + 0)	NG (400 mg/kg + 2%)	$N^{+}G$ (600 mg/kg + 2%)
Glucose (g/l)	1.15 ± 0.15	1.10 ± 0.14	1.45 ± 0.27*	1.17 ± 0.13	1.19± 0.10
Cholesterol (mg/dl)	96.69 ± 14.40	76.86 ± 15.40*	150.71 ± 27.20***	121.70 ± 25.40*	130.56 ± 25.50**
Triglyceride (mg/dl)	132.25 ± 22.20	87.83 ± 16.20 **	199.81 ± 33***	160.63 ± 24.90*	169.36 ± 25.40**
Total lipids (mg/dl)	326.95 ± 84.50	281.94 ± 80.50	720.85 ± 98.60***	426.79 ± 90.60*	506.56 ± 92.80**
ASAT (UI/l)	30.07 ± 9.32	37.26 ± 9.55	60.50 ± 10.30***	42.33 ± 9.82*	46.39 ± 10.10**
ALAT (UI/l)	26.43 ± 6.45	32.89 ± 6.54	45.47 ± 8.20***	35.58 ± 6.09*	41.05 ± 7.38**
PAL (UI/l)	130.90 ± 14.60	145.25 ± 13.20	243.83 ± 23.70***	159.63 ± 20.60*	169.75 ± 20.90**
LDH (UI/l)	165.08 ± 32.80	177.93 ± 31.90	296.18 ± 36.20***	229.60 ± 34.50**	274.76 ± 35.40***
Total bilirubin (mg/dl)	1.13 ± 0.28	0.92 ± 0.27	1.74 ± 0.31**	1.25 ± 0.30	1.44 ± 0.31*
Direct bilirubin (mg/dl)	0.56 ± 0.20	0.61 ± 0.22	1.01 ± 0.24**	0.76 ± 0.21	0.86 ± 0.22***

* and **, Significantly different from control at $P < 0.05$; $P < 0.01$, respectively. Each value represents the mean ± SD of 10 rats.

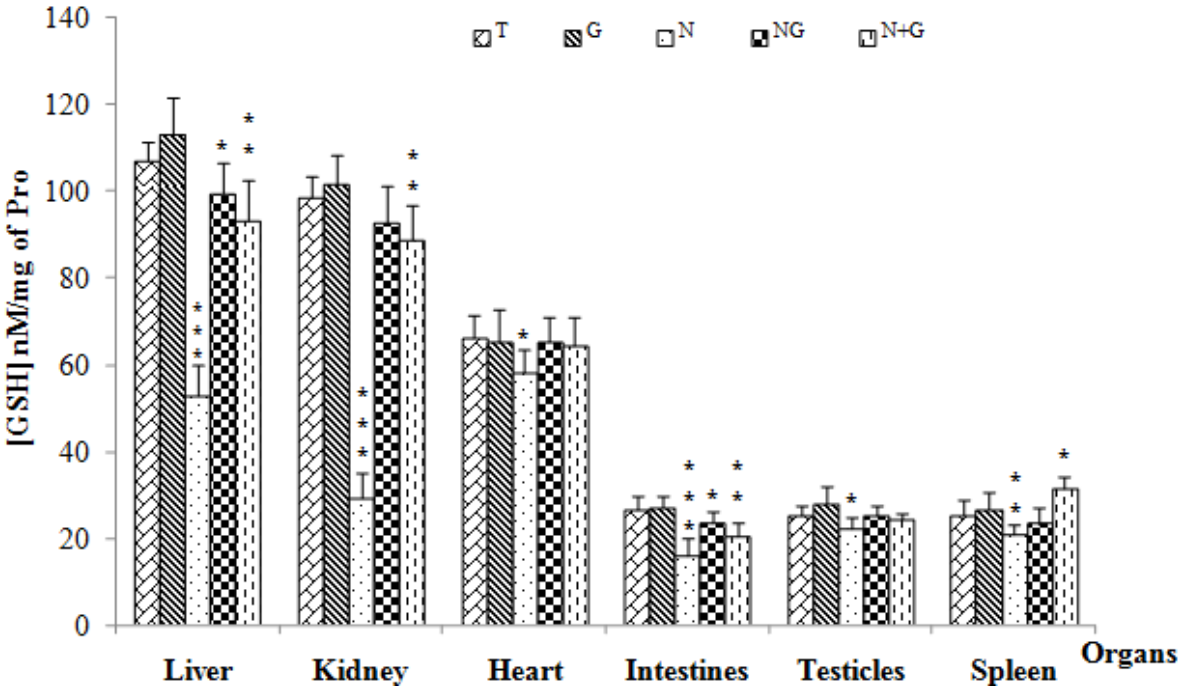

Figure 2. Glutathione levels in control (C) and treated rats with ammonium nitrate and ginger (G, N, NG and $N^{+}G$) after 30 days treatment (each value represents the mean ± SD of 10 rats); *, **, and ***, significantly different from control at $P < 0.05$; $P < 0.01$; $P < 0.001$, respectively.

ions (termination of the Fenton reaction) (Rice-Evans et al., 1997). Another mechanism underlying the antioxidative properties of phenolics is the ability of flavonoids to alter peroxidation kinetics by modification of the lipid packing order and to decrease fluidity of the membranes. These changes could sterically hinder diffusion of free radicals and restrict lipids peroxidative reactions (Arora et al., 2000).

The results obtained in the present study showed an increase in blood glucose levels in ammonium nitrate treated rats (N). Nitrate has been suspected to have a diabetogenic effect in children. Thus, several studies conducted in Europe have attempted to determine if a relationship exists between drinking contaminated water with nitrate and childhood-onset Type 1 insulin-dependent diabetes mellitus (IDDM) (Parslow et al., 1997; Moltchanova et al., 2004). Peroxynitrite acts as a terminal mediator of cellular injury in various pathophysiologic conditions. Typical cytotoxic reaction pathways triggered by peroxynitrite include lipid peroxidation, DNA breakage

and base modification, activation of the nuclear enzyme poly (ADP-ribose) polymerase, as well as tyrosine nitration (Beckman et al., 1990). Tyrosine nitration has been demonstrated in a variety of pathophysiologic conditions, including diabetes mellitus. In the pancreatic islets of spontaneous autoimmune diabetic mice, a significant increase in tyrosine nitration was found and the degree of beta-cell destruction showed a good correlation with the frequency of nitrotyrosine-positive beta cells. It was therefore proposed that the intra-islet formation of peroxynitrite plays an active pathogenic role in the pathogenesis of diabetes mellitus (Ischiropoulos et al., 1992). However, the administration of ginger to ammonium nitrate treated rats reduced blood glucose levels, in accordance with earlier reports (Al-Amin et al., 2006; Shahidul-Islam and Choi, 2008). *Zingiber officinale* may have beneficial effects on diabetes that hold the hope of a new generation of antidiabetic drugs.

Significant increase was obtained in serum cholesterol, triglyceride and total lipids levels in rats treated by ammonium nitrate. This hyperlipidemia finds several explanations. The hyperglycemia, dysthyroidism and renal failure observed in these rats can cause dyslipidemia, which can be deteriorated according to the intensity of the imbalance (Krauss and Siri, 2004; Pearce, 2004; Baumelou et al., 2005). Furthemore, possible relation between nitrate intake and effects on the thyroid have also been reported. Experimental study has shown that inorganic nitrate is a goitrogenic agent at short term inducing a hypertrophy of the epithelial cells of the thyroid gland follicles (Gatseva and Argirova, 2008). On the other hand, ginger diet supplemented rats witnessed reduced levels of cholestrol, triglyceride and total lipids. These data were consistent with the previous study (Schwertner and Rios, 2007). The lipid lowering effect of ginger could have possibly resulted from several phenomena, such as attenuation of cellular cholesterol biosynthesis, which was associated with increased activity of the LDL receptor leading to the enhancement of the removal of LDL from plasma (Ness et al., 1996), inhibition of intestinal absorbtion of dietary fat by inhibiting its hydrolysis (Han et al., 2005), reducing lipid peroxidation (Liu et al., 2003), increasing pancreatic lipase and amylase activity (Patel and Srinivavasan, 2000), increasing intestinal peristalsis (Hashimoto et al., 2002), and/or increasing cholesterol conversion to bile acids (Srinivasan and Sambaiah, 1991).

Creatinine, urea and uric acid are wastes produced by protein metabolism and eliminated by the kidneys, generally used as indicators of renal function. When kidneys failure occurs, rates of these parameters increase; this was observed in rats of the N group proving the nephrotoxic effect of ammonium nitrate (Boukerche et al., 2007). In the present study, the levels of creatinine, uric acid and urea were decreased significantly in group with ginger supplementation. The results agree with the earlier published data (Al-Qattan et al., 2008). Also, Ajith et al. (2007) reported that ginger ameliorated cisplatin-induced nerphrotoxicity and this protection is mediated either by preventing the cispaltin-induced decline of renal antioxidant defense system or by direct free radical scavenging activity of ginger.

Oxidative stress generated by external toxic agents may have a negative impact on many tissues, including liver. The obtained result of hepatosomatic index in this study shows that a higher intake of ammonium nitrare can cause hepatomegaly and in order to assess the extent of the injury, determination of some biochemical parameters were performed. The activities of ASAT, ALAT, PAL and LDH were significantly elevated in N group compared to control group. Also, the increased levels of bilirubin in the serum indicate impaired excretory and synthetic functions of the liver (Ogur et al., 2005; Rodriguez-Estival et al., 2010). The obtained results in this study show the effecttiveness of ginger in the prevention of ammonium nitrate-induced hepatotoxicity as indicated by normalization of the hepatic enzymes and bilirubin (El-sharaky et al., 2009).

Glutathione serves as a sensitive marker of oxidative stress and it plays an important role in maintaining the integrity of the cell. GSH is involved in several detoxification-reactions in the organism and it is one of the most prominent non-enzymatic antioxidants (Meister and Anderson, 1983). It was therefore interesting to study the GSH level in the liver, kidneys, testes, intestines, spleen and heart. In this study, the GSH level decreased in all organs of ammonium nitrate treated-group. Significant depletion in tissue GSH levels enhanced cellular damage caused by oxidative stress and suggests its increased utilisation against reactive oxygen species (Tachi et al., 2001). However, ginger treatment in combination groups reversed the GSH to normal levels; which also implies that ginger has an antioxidant property (Ahmed et al., 2008; Shanmugam et al., 2011). Gingerol was found to exert inhibitory effect on xanthine oxidase responsible for generation of ROS, such as superoxide anion (Chang et al., 1994). Other studies revealed that [6]-gingerol, the major pungent constituent of ginger, inhibits nitric oxide production and prevents oxidation and nitration reactions induced by peroxynitrite (Shimodo et al. 2010).

Conclusion

In conclusion, our study shows that ginger exerts a protective effect against oxidative stress induced by ammonium nitrate via increasing the antioxidant defence (GSH) and restoring the levels of biochemical and hematological parameters to their normal values. Further study on antioxidant enzyme activities and MDA levels in tissues is necessary to get a better idea on the antioxidant properties of ginger.

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