Naringenin in Combination with Vitamins C and E Potentially Protects Oxidative Stress-Mediated Hepatic Injury in Cadmium-Intoxicated Rats

Selvaraj Milton Prabu1, Kalist Shagirtha2 and Jayapaul Renugadevi1

1Department of Zoology, and 2Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India

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Summary  Cadmium (Cd)-induced oxidative stress and hepatic injury is one of the major outcomes of chronic Cd toxicity, which can be ameliorated by numerous antioxidants. The present study was undertaken to find the therapeutic efficacy of naringenin (NGN) plus vitamins C and E on Cd-induced oxidative hepatotoxicity in Wistar rats. It has been noticed that Cd intoxication significantly elevates the levels of serum hepatic marker enzymes such as alanine amino transferase, aspartate amino transferase, alkaline phosphatase, lactate dehydrogenase, y glutamyl transferase, total bilirubin, and hepatic thiobarbituric acid reactive substances, lipid hydroperoxides, conjugated dienes and protein carbonyls. In addition, Cd also decreases the activities of hepatic enzymatic antioxidants superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase, glutathione reductase and glucose-6-phosphate dehydrogenase and the levels of non-enzymatic antioxidants total sulphhydryl groups, reduced glutathione, vitamins C and E and histopathological changes in liver. Treatment with NGN and vitamins C and E in combination more significantly improved the altered biochemical and histopathological changes in the liver of Cd-intoxicated rats than the NGN or vitamins C and E treatment alone. Conclusion: The present data suggest that combined administration of NGN with vitamins C and E proved to be more beneficial in the treatment of Cd-hepatotoxicity than NGN treatment alone.

Key Words  Cd, liver, oxidative stress, naringenin, vitamins (C and E)

Humans are exposed to cadmium (Cd), a ubiquitous metal with no known biological function, mainly through occupation, environmental contamination and cigarette smoking (1). Cd-induced toxicity is influenced by a number of factors such as the route of administration, the dosage, the chemical form of the metal, the duration of exposure and the age of experimental animals (2). Cd is implicated in many industrial uses, such as electroplating, preparation of paints, dyestuffs, and the metallurgical and mining industries. It is used as a cathode material in nickel-Cd batteries (3). The deleterious effects of Cd reported to date include oxidative stress, generation of reactive oxygen species (ROS), depletion of glutathione, increased lipid peroxidation, alteration of antioxidant enzymes, modulation of apoptosis, pleiosis hepatitis and inhibition of DNA repair enzymes (4–6). As a result, these alterations are manifested in almost all the organs such as liver, kidney, testes, lungs and bones. It also increases the risk of peripheral arterial disease (7).

Cd has been shown to accumulate in the liver after acute and chronic exposure. Cd produces liver injury through the generation of ROS and lipid peroxidation, which in turn depresses the hepatic functions (8). Although, Cd has no redox properties like other transition metals, it produces oxidative stress indirectly by depleting SH-group containing compounds. Cd-induced peroxidation and its deleterious effects on cellular membrane have already been well documented (9). Cd-induced membrane damage promotes the release of intracellular Fe2+ that further augments oxidative damage in biological systems (2). In addition, Cd exposure inhibits the electron transport chain in mitochondria and thus provokes the generation of abnormal levels of ROS (10).

Flavonoids are one of the most numerous and widespread groups of naturally occurring antioxidants that can inhibit lipid oxidation in a biological membrane. They are found in fruits, vegetables, nuts, seeds, leaves, flowers and barks of plants (11). Scavenging of free radicals and chelation of metal ions (Cu2+ and Fe2+) seems to play a crucial role in the antioxidant activity of flavonoids. They usually contain one or more aromatic hydroxyl groups in their moiety which is responsible for the antioxidant activity of the flavonoids (12). Naringenin (NGN, 4,5,7-trihydroxy flavone) (Fig. 1) is a plant bioflavonoid richly found in citrus and grape fruits. NGN has already been pharmacologically evaluated as a potential antioxidant (13), cardioprotective (14), antifibrogenic (15), nephroprotective (16) and hepatoprotective (17). NGN modulates cytochrome P450-dependent monoxygenase, the primary enzyme involved in

E-mail: smprabu73@gmail.com
the metabolism of many xenobiotics (18). Considering the relationship between Cd exposure and oxidative stress, attention has been focused on compounds having antioxidant, metal chelating and free radical scavenging properties which are beneficial to ameliorate Cd-induced oxidative stress. Structure activity relationship (SAR) studies revealed that NGN possess higher antioxidant, free radical scavenging and metal chelating properties than the other flavonoids (19).

Vitamins C and E are used as effective free radical scavengers in various toxicity studies (20, 21). Vitamin E belongs to the family of lipid-soluble vitamins and acts as an antioxidant in cells, interrupting the propagation of lipid peroxidation in the plasma membrane, and thus preserves the membrane integrity, inhibiting free radical formation and effectively minimizing the lipid peroxidation in biological systems (22). Vitamin C is hydrophilic and a most important free radical scavenger in extracellular fluids, trapping free radicals in the aqueous phase and protecting biomembranes from peroxidative damage (23). In our laboratory, the hepatoprotective efficacy of NGN (17) and vitamins C and E (21) in Cd-intoxicated rats has been reported. As combined therapeutic modalities are gaining promising results in the clinical scenario, in this present study we investigated the hepato protective efficacy of NGN plus vitamins C and E on Cd-induced oxidative hepatotoxicity in Wistar rats.

MATERIALS AND METHODS

Animals. Adult male albino rats (Rattus norvegicus) of the Wistar strain (180–200 g) were used in the present experiment. They were procured from the Central Animal House, Rajah Muthiah Medical College & Hospital, Annamalai University, Annamalainagar and housed in plastic cages (12-h light/12-h dark cycle, 50% humidity and 25 ± 3 °C). The animals were fed with a standard pellet diet procured from M/s. Pranav Agro Industries Ltd., Bangalore, India and water ad libitum. This study was approved (vide No. 160, 2007) by the Institutional Animal Ethics Committee of Annamalai University and the study was conducted in accordance with the “Guidance for the Care and Usage of Laboratory Animals.”

Chemicals. NGN was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Cd was purchased from Pfizer, India. Vitamin C (L-ascorbic acid) and E (DL-α-tocopheryl acetate) were supplied by Merck (Germany). Commercial kits used to estimate serum hepatic markers were procured from Sigma Diagnostics (I) Pvt. Ltd. (Baroda, India). All other chemicals and biochemicals were of analytical grade obtained from local firms.

Treatments. In the present experiment, a total of 36 rats were used and they were randomly divided into 6 groups (n = 6). All the substances were administered via gavage in the morning between 09.00 and 10.00 h to non-fasted rats. NGN was suspended in 0.5% carboxy methyl cellulose (CMC). Vitamins C and E were dissolved in water and corn oil, respectively. CdCl₂ as a source of Cd was dissolved in normal physiological saline.

Group 1: Control rats received only the vehicles every day for 28 d.

Group 2: Rats orally received NGN alone (50 mg/kg BW) for 28 d.

Group 3: Rats orally received NGN (50 mg/kg BW) in combination with vitamins C (50 mg/kg BW) and E (50 mg/kg BW) every day for 28 d.

Group 4: Rats orally received Cd (5 mg/kg BW) every day for 28 d.

Group 5: Rats orally received NGN (50 mg/kg BW) and Cd (5 mg/kg BW) every day for 28 d.

Group 6: Rats orally received Cd (5 mg/kg BW) and NGN (50 mg/kg BW) in combination with vitamins C (50 mg/kg BW) and E (50 mg/kg BW) every day for 28 d. Antioxidants were given 1 h prior to the administration of Cd.

After feeding for 28 d, the rats were fasted overnight and the blood samples were collected into tubes without adding anticoagulant by puncturing the heart. Animals were sacrificed by cervical decapitation under ketamine anesthesia. The collected blood samples were centrifuged (1,000 × g for 10 min at 4 °C) for the separation of serum to analyze various hepatic markers.

The liver was dissected out, weighed and washed using chilled physiological saline solution. Tissue was minced and homogenized (10%, w/v) in an appropriate buffer (pH 7.4) and centrifuged (3,000 × g for 10 min). The resulting clear supernatant was used for various enzymatic and non-enzymatic biochemical assays. All rats from each group were sacrificed and used for serum and tissue biochemical assays.

Biochemical assays. The activities of serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and total bilirubin (TB) were assayed spectrophotometrically according to the standard procedures given in the diagnostic kits (Sigma Diagnostics (I) Pvt. Ltd.). γ glutamyl transferase (GGT) activity was determined by the method of Rosalki et al. (24) using γ-glutamyl-p-nitroanilide as substrate. Lipid peroxidation in the liver was estimated calorimetrically by measuring thiobarbituric acid reactive substances (TBARS) and hydroperoxides as described by Niehiaus and Samuelsson (25) and Jiang et al. (26), respectively. As a hallmark of protein oxidation, total protein carbonyl content was determined in the liver by a spectrophotometric method described by Levine et al. (27) and expressed as nmol/mg protein. Reduced glutathione (GSH) was determined by the method of Moron et al. (28) based on the reaction with Ellman’s reagent (19.8 mg dithionitrosobenzoic
Serum Markers

Acid in 100 mL of 0.1% sodium citrate. Total sulfhydryl groups (TSH) in the liver homogenate were measured after the reaction with dithionitrobis benzoic acid using the method of Ellman (29). Vitamin C and vitamin E concentrations were measured by the methods of Omaye et al. (30) and Desai (31), respectively. Superoxide dismutase (SOD) activity was determined by the method of Kakkar et al. (32) in which the inhibition of the formation of NADPH-phenazine methosulphate nitroblue tetrazolium formazon was measured spectrophotometrically at 560 nm. Catalase (CAT) activity was assayed colorimetrically as described by Sinha (33) using dichromate-acetic acid reagent. Glutathione peroxidase activity (GPx) was assayed by a method based on the reaction between glutathione remaining after the action of GPx and 5,5'-dithiobis-2-nitrobenzoic acid to form a complex that absorbs maximally at 412 nm (34). Glutathione S-transferase (GST) activity was determined spectrophotometrically by using dichloro-2,4-dinitrobenzene as the substrate by the method of Habig et al. (35). Glutathione reductase (GR), which utilizes NADPH to convert metabolized glutathione (GSSG) to the reduced form, was assayed by the method of Horn and Burns (36). The estimation of glucose-6-phosphate

Fig. 2. Effects of NGN in combination with and without vitamins C and E on levels of serum markers of liver damage in rats intoxicated with and without Cd. Values are mean±SD for 6 rats in each group. a–d Values are not sharing a common superscript letter (a, b, c and d) differ significantly at p<0.05 (DMRT).
Serum hepatic marker enzymes and bilirubin

Statistical analysis. All the data were expressed as mean±SD of the experiments (n=6). The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS Version 9.0 (SPSS, Cary, NC, USA) and the individual comparisons were obtained by Duncan’s multiple range test (DMRT). Values were considered statistically significant when p>0.05.

RESULTS

Serum hepatic marker enzymes and bilirubin

Table 1 depicts the effects of Cd, NGN, vitamins C and E significant (p<0.05) increase the levels of serum hepatic functional markers such as AST, ALT, ALP, LDH, GGT and total bilirubin when compared with control rats.

Administration of NGN in combination with vitamins C and E significantly (p<0.05) decreased the activities of serum hepatic biochemical markers when compared to Cd and Cd along with NGN treated rats. The levels of AST, ALT, ALP, LDH, GGT and total bilirubin were found to be near normal levels in rats administered with NGN in combination with vitamins C and E alone.

Body weight gain, food intake, water intake and organ-body weight ratio

Table 2 shows the serum hepatic functional markers of control and experimental rats. Oral administration of Cd significantly (p<0.05) increased the levels of serum hepatic functional markers such as AST, ALT, ALP, LDH, GGT and total bilirubin when compared with control rats.

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Body weight gain, food intake, water intake and organ-body weight ratio

Table 1. Effects of NGN in combination with and without vitamins C and E on body weight, body weight gain, food intake and liver-body weight ratio in rats intoxicated with and without Cd.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>NGN</th>
<th>NGN+vitamins C and E</th>
<th>Cd</th>
<th>Cd+NGN</th>
<th>Cd+NGN+vitamins C and E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Final (g)</td>
<td>232±11.79</td>
<td>231±10.78</td>
<td>229±11.42</td>
<td>201±10.57</td>
<td>216±8.69</td>
</tr>
<tr>
<td>Body weight gain (%)</td>
<td>20.31</td>
<td>21.64</td>
<td>21.16</td>
<td>7.48</td>
<td>13.08</td>
<td>18.61</td>
</tr>
<tr>
<td>Food intake (g/100 g bw/d)</td>
<td>12.07±0.91a</td>
<td>12.01±0.84a</td>
<td>11.93±0.81a</td>
<td>8.17±0.69b</td>
<td>8.74±0.61bc</td>
<td>10.58±0.83d</td>
</tr>
<tr>
<td>Water intake (ml/rat/d)</td>
<td>18.20±2.40a</td>
<td>18.41±2.20a</td>
<td>17.40±2.0ad</td>
<td>12.20±1.20b</td>
<td>12.50±1.40bc</td>
<td>16.20±1.50d</td>
</tr>
<tr>
<td>Organ-body weight ratio (%)</td>
<td>Liver 2.94±0.38a</td>
<td>2.97±0.34a</td>
<td>3.02±0.42a</td>
<td>3.87±0.49b</td>
<td>3.64±0.38bc</td>
<td>3.24±0.36d</td>
</tr>
</tbody>
</table>

Values are expressed mean±SD for six rats in each group.

Table 2. Effects of NGN in combination with and without vitamins C and E on levels of hepatic TBARS, lipid hydroperoxide and protein carbonyls in rats intoxicated with and without Cd.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>NGN</th>
<th>NGN+vitamins C and E</th>
<th>Cd</th>
<th>Cd+NGN</th>
<th>Cd+NGN+vitamins C and E</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS</td>
<td>0.87±0.07a</td>
<td>0.84±0.06c</td>
<td>0.76±0.06c</td>
<td>1.34±0.11b</td>
<td>1.02±0.09c</td>
<td>0.91±0.06d</td>
</tr>
<tr>
<td>Hydroperoxides</td>
<td>0.92±0.07a</td>
<td>0.87±0.06a</td>
<td>0.81±0.06c</td>
<td>1.31±0.12b</td>
<td>1.09±0.09c</td>
<td>0.98±0.07d</td>
</tr>
<tr>
<td>Protein carbonyls</td>
<td>1.78±0.18a</td>
<td>1.73±0.16a</td>
<td>1.69±0.16c</td>
<td>4.27±0.37b</td>
<td>2.43±0.24c</td>
<td>2.02±0.20d</td>
</tr>
</tbody>
</table>

Values are expressed mean±SD for 6 rats in each group.

The levels of TBARS are expressed as mg/g tissue; lipid hydroperoxides, mmol/g tissue; protein carbonyls, nmol/mg protein.
**Hepatic oxidative stress markers**

The changes in the levels of hepatic oxidative stress markers in control and experimental rats are shown in Table 2. The levels of lipid peroxidation products, namely TBARS, LOOH and PC, significantly \( p<0.05 \) increased in Cd treated rats when compared with control. Oral administration of NGN in combination with vitamins C and E significantly \( p<0.05 \) decreased the levels of TBARS, LOOH and PC in the liver tissue of rats when compared to Cd-treated and Cd-along with NGN-treated rats. Administration of NGN in combination with vitamins C and E alone significantly \( p<0.05 \) reduced the levels of TBARS, LOOH and PC when compared with the control group.

**Hepatic non-enzymatic antioxidants**

Table 3 illustrates the alterations in the levels of non-enzymatic antioxidant systems in the liver of control and experimental rats. A significant decrease \( p<0.05 \) in the levels of non-enzymatic antioxidants (GSH, TSH, vitamins C and E) in the liver tissue was observed in rats treated with Cd when compared to control. Administration of NGN in combination with vitamins C and E significantly \( p<0.05 \) increased the levels of non-enzymatic antioxidants to near normalcy when compared with Cd-treated and Cd-along with NGN-treated rats.

**Hepatic enzymatic antioxidants**

The activities of enzymatic antioxidants, namely SOD, CAT, GPx and GST, and glutathione metabolizing enzymes (GR and G6PD) significantly \( p<0.05 \) decreased in the liver tissue of Cd treated rats when compared with control. Administration of NGN in combination with vitamins C and E in Cd-intoxicated rats significantly \( p<0.05 \) increased the activities of these antioxidants and glutathione-metabolizing enzymes (Table 4) when compared with Cd-treated and Cd-along with NGN-treated rats. Rats administrated with NGN in combination with vitamins C and E alone showed a significant increase in the level of enzymatic and non-enzymatic antioxidants when compared with control.

**Histological changes**

Histopathological investigations showed that the administration of Cd produced severe hepatic damage including extensive degeneration of hepatocytes with necrosis, inflammation, vacuolization, inflammatory cell infiltration and fatty degenerative changes (Fig. 3B and C) when compared with control rats (Fig. 3A). The above histopathological abnormalities were effectively attenuated in the liver of rats treated with Cd along with NGN (Fig. 3E) and Cd-along with NGN in combination with vitamins C and E (Fig. 3P). The histoarchitectural patterns of liver were almost normal in rats treated with NGN in combination with vitamins C and E (Fig. 3B).
Generally heavy metals exert their cytotoxic effects by damaging cell membranes. In particular, Cd induces membrane damage that results from strong interaction with lipids and proteins. Cell damage is followed by release of cytoplasmic enzymes into the blood, a phenomenon that provides the basis for clinical diagnosis. In the present study, the increased levels of serum AST, ALT, ALP, LDH and GGT in Cd-intoxicated rats agreed with the findings of Pari and Murugavel (39) who reported that Cd caused alterations in the liver trans-

**DISCUSSION**

Generally, heavy metals exert their cytotoxic effects by damaging cell membranes. In particular, Cd induces membrane damage that results from strong interaction with lipids and proteins. Cell damage is followed by release of cytoplasmic enzymes into the blood, a phenomenon that provides the basis for clinical diagnosis. In the present study, the increased levels of serum AST, ALT, ALP, LDH and GGT in Cd-intoxicated rats agreed with the findings of Pari and Murugavel (39) who reported that Cd caused alterations in the liver trans-

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Fig. 3. Representative photographs of liver tissues obtained from Cd-treated and untreated rats with and without NGN or NGN in combination with vitamins C and E. (A) Section of liver from control group showing the normal appearance of central vein and radiating hepatocytes around the central vein (H and E, 40×). (B) Section of liver treated with NGN in combination with vitamins C and E alone showing the normal liver histology (H and E, 40×). (C) Section of liver treated with Cd showing the focal necro-inflammatory changes, portal inflammation, degenerated hepatocytes and inflammatory cell infiltration (H and E, 40×). (D) Section of liver treated with Cd showing the complete rupture of central vein, dilated sinusoids, inflammatory cell infiltration, necrotic changes and derangement of hepatic cords (H and E, 40×). (E) Section of liver treated with Cd and NGN showing a significant improvement in liver histology (H and E, 40×). (F) Section of liver treated with Cd alone with NGN in combination with vitamins C and E showing the normal histological pattern of hepatic parenchyma (H and E, 40×). CV, central vein; DHV, damaged hepatic vein; H, hepatocytes; ICI, inflammatory cell infiltration; S, sinusoids.
aminases in rats. Any damage to the hepatic tissue leads to the release of hepatic marker enzymes into the circulation, thereby showing increased serum level with a concomitant decrease in the liver (40). Administration of NGN in combination with vitamins C and E significantly normalized the activities of hepatic enzymes in the serum of Cd-exposed rats, which indicates the protective influence of NGN and vitamins C and E on alterations in membranes. This may reduce the hepatic dysfunction caused by Cd and prevent the leakage of these hepatic marker enzymes into the blood.

A significant elevation of hepatic LPO level in terms of TBARS and LOOH was observed following Cd exposure. Cd-induced oxidative damage was evidenced by the elevated level of TBARS and LOOH in liver (41). A possible mechanism of Cd-induced lipid peroxidation through displacement of iron from its binding sites led to acceleration of free radical production (2). Cd exposure resulted in a wide variety of cellular responses including oxidative stress. In the present study Cd also increased the level of PC in the liver of rats. Protein carbonyl groups provide a reasonable marker for free radical-induced protein oxidation as a result of oxidative damage to proteins (42).

Administration of NGN in combination with vitamins C and E significantly decreased the levels of LPO and protein oxidation in the liver because of the free radical scavenging effect of NGN (43). It has been proposed that NGN terminates chain radical reactions by forming a flavonoid radical (44). Aluntas et al. (20) have reported that a combination of vitamins C and E reduces the LPO induced by methyldiasthion. Vitamin E neutralizes the lipid peroxidation of unsaturated membrane lipids through its oxygen scavenging effect. In addition, vitamin C may also remove free radicals that are bound to vitamin E, thus serving to regenerate vitamin E (45). Because of these free radical scavenging abilities of both NGN and vitamins C and E, the cell membranes are protected from the peroxidative damage induced by Cd.

In the present study, Cd intoxication significantly depleted the GSH, TSH, vitamins C and E levels in the liver and thus reduced the antioxidant potential of these organs. In Cd-induced oxidative stress, GSH gets converted to oxidized glutathione (GSSG) and this depletion leads to an increased lipid peroxidation (10). Moreover the sulfhydryl group of cysteine moiety of glutathione has high affinity for metals, forming a thermodynamically stable mercaptide complex with Cd (46). These complexes are inert and excreted via the bile. So the decreased GSH level may be due to its consumption in Cd detoxification (47).

Decrease in the levels of vitamins C and E during Cd intoxication leads to increased susceptibility of the tissue to free radical damage. In this context a remarkable depletion of tissue non-enzymatic antioxidants in Cd intoxication might lead to the participation of free radicals in mediating the Cd-induced oxidative cell injury. In the present study a severe depletion in the levels of non-enzymatic antioxidants, viz. GSH, TSH, vitamins C and E, by Cd was in line with previous reports (39). Administration of NGN in combination with vitamins C and E in Cd-treated rats significantly restored the depleted non-enzymatic antioxidants via their synergistic antioxidant activity. NGN and vitamins C and E have been shown to protect the cells against the damaging effects of reactive oxygen species (e.g., superoxide anion radical, hydroxyl radicals, peroxyl radicals) and inactivate free radicals by hydrogen/electron transfer or metal chelation (48).

Antioxidant enzymes are considered to be the first line of cellular defense that prevents cellular components from the deleterious effects of oxidative damage. Among them SOD, CAT, GPX and GST mutually function as important enzymes in the elimination of ROS. In the present study, Cd intoxication significantly decreased the activities of antioxidant enzymes in the liver. It is reported that Cd accumulates in the tissues, interacts with metal moieties of SOD (Cu, Zn or Mn) and thereby inhibits its enzyme activity. During oxidative stress, catalase activity decreases. H₂O₂ accumulates and more peroxidation of lipids is favoured. Hence there was decreased activity of catalase, and increased LPO was observed in the liver of Cd-exposed rats. GPX removes the toxic free radicals and H₂O₂ from the living system. Its enzyme activation requires GSH, which is depleted by Cd and thereby reduces GPX activity. Cd inhibits GPX by competing with GSH and also forms a complex with selenium which is present in the active site of the enzyme. GST, the xenobiotic metabolizing enzyme, also decreased in the liver of Cd-exposed rats. G6PD, an important enzyme in pentose phosphate pathway, generates NADPH from NADP⁺. GR, a crucial enzyme is responsible for the reduction of oxidized glutathione (GSSG) to GSH. In the present study the activities of GR and G6PD significantly decreased in the liver tissues of Cd-treated rats. Our findings are in line with previous reports that showed depleted antioxidant activity in Cd-treated rats (49).

Administration of NGN in combination with vitamins C and E significantly increased the activities of these antioxidants and glutathione metabolizing enzymes in the liver tissues of Cd-treated rats. NGN is a polyphenol which is shown to possess an excellent anti-oxidative and antioxidant benefit to facilitate the increased level of antioxidant enzymes in the liver tissues of Cd-intoxicated rats (50). The protective role of vitamins C and E against the toxicity of oxidants may be due to their ability in quenching the hydroxyl radicals. Shaikh et al. (8) reported that oxidative stress appeared to play a major role in chronic Cd-induced hepatotoxicity since the inhibition of components of the antioxidant defense system by Cd could accelerate the toxicity. The administration of NGN in combination with vitamins C and E protected the tissues against Cd-induced oxidative damage via their co-ordinated antioxidant activities.

In the present study, the hepatic histocharchitecture of the Cd-treated rats showed severe necrotic changes, inflammatory cell infiltration, fatty degeneration and
vacuolization. It might be due to the formation of highly reactive radicals and subsequent lipid peroxidation induced by Cd. The accumulated hydroperoxide can cause cytotoxicity, which is associated with the peroxidation of membrane phospholipids by lipid hydroperoxides, the basis for hepatocellular damage. The necrotic conditions coincided with our biochemical observations, which showed the increased level of lipid peroxidation. Administration of NGN in combination with vitamins C and E reduced the histological alterations provoked by Cd quite appreciably. It can be attributed to the antiradical antioxidant and metal-chelating efficacy of these antioxidants which significantly reduced the oxidative stress leading to the reduction of histopathological alterations and restoration of the normal physiological state of an organism.

Taken together our findings indicated that the combined treatment modality exhibited a significant protective role against Cd-induced oxidative hepatic injury. It can be speculated that the chelating property of NGN could enhance the elimination of Cd from the liver tissue via its polyphenol groups and thus reduced the intracellular Cd burden. In addition the antioxidant power of vitamins C and E also accelerated the detoxification of Cd in the liver tissue of rats. Further, the membrane stabilizing and antiliperoxidative effects of these antioxidants may trigger their prophylactic role against Cd-induced oxidative hepatic dysfunction. However, further studies are warranted to find the exact molecular pathways by which these antioxidants exhibit their protective roles against Cd-induced hepatotoxicity in rats.

CONCLUSION

The present study clearly demonstrated NGN along with vitamins C and E in combination significantly inhibits oxidative stress, improves antioxidant status and reduces histopathological changes in the liver of Cd-intoxicated rats. This might be due to the contribution of each antioxidant for the alleviation of Cd-induced oxidative damage.

REFERENCES


