

Full Length Research Paper

Ameliorative effects of *Embilica officinalis* and *Rosmarinus officinalis* on cadmium-induced oxidative stress in Wistar rats

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The study was designed to assess the antioxidative potential of three dietary antioxidants, vitamin C, Rosemary (*Rosmarinus officinalis*) leaf extract (10% carnosic acid) and Amla (*Embilica officinalis*) fruit extract (aqueous) on cadmium-induced oxidative stress and the related histopathology of the liver and kidney of Wistar rats. Cadmium was administered as cadmium chloride (15 mg/kg body weight/day) for 4 weeks with five groups co-treated with the antioxidants, vitamin C (100 mg/kg/day) Rosemary leaf extract (15 and 30 mg/kg/day) and amla fruit extract (100 and 200 mg/kg/day). The results showed that cadmium induced a significant increase in both kidney and liver malondialdehyd (MDA) levels coupled with an inhibition of the superoxide dismutase (SOD) and catalase (CAT) activity. Co-treatment of cadmium and the antioxidants did alleviate the oxidative stress which was observed as a significant reduction in the MDA levels and an increase in the SOD activity both in the kidney and liver. CAT activity was only increased by vitamin C and Rosemary (15 mg/kg) co-treatment in the kidney. Furthermore, cadmium induced cellular disorganization of the kidney and liver was restored with the co-treatment of the antioxidants being more pronounced with the plant extracts. Rosemary leaf extract was efficacious at both the doses while the amla fruit extract had the most ameliorative effect at a higher dose (200 mg/kg).

Key words: Cadmium, *Rosmarinus officinalis*, *Embilica officinalis*, vitamin C, antioxidants, oxidative stress, histopathology.

INTRODUCTION

Cadmium (Cd) is a toxic heavy metal ubiquitously present in the environment. Due to its non-corrosive nature it has a myriad of uses including electroplating or galvanizing. The major anthropogenic sources which make up for 90% of Cd in the environment include smelting operations, phosphate fertilizers, pigments, cigarette smoke, automobiles etc. which lead to its bioaccumulation in

humans and animals. Cadmium has been reported to exert deleterious effects in organisms at low levels of exposure (Cope et al., 1994) which are nephro-toxic, cyto-toxic, geno-toxic, immune-toxic and carcinogenic (ASTDR, 1999; Lippmann, 2000; Rissode-Faverney et al., 2001). The literature is replete with the reports on metal induced oxidative stress that has been recently

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implicated in the pathogenesis of metal toxicities. It is established that metals can generate reactive oxygen species (ROS) which in turn overwhelms the cell's innate antioxidant defenses leading to oxidative stress. Studies have demonstrated that Cd stimulates free radical production, resulting in oxidative deterioration of lipids, proteins and DNA which eventually leads to membrane damage, protein dysfunction and DNA damage. These conditions can further culminate into pathological conditions both in humans and animals (Waisberg et al., 2003) including diabetes, cardiovascular diseases, cancer etc. Antioxidants are compounds that mop up the free radicals and prevent this cellular damage (Sen et al., 2010). They can be classified as exogenous and endogenous. The exogenous antioxidants coming from plants are the polyphenols-flavonoids, flavonols and proanthocyanides. These phyto-constituents have been a subject of interest and research in the past decade as they are able to terminate free radical reactions preventing and ameliorating the cells from metal induced oxidative damage.

The efficacy of a wide variety of phyto-compounds as antioxidants has been reported. Flavonoids like quercetin in grape seed extract (Bu et al., 2011; Renugadevi and Prabhu, 2009), hydro alcoholic leaf extract of Holy basil, *Ocimum sanctum* (Ramesh and Satakopan, 2010) and cabbage extract (Onwuka et al., 2010) have been reported to alleviate the cadmium induced oxidative stress in mammals. Garlic extract has also been reported to have an ameliorative effect on cadmium induced oxidative stress in fish (Banerjee et al., 2003; Kumar et al., 2009). Leaves of a culinary herb, *Murraya koenigii* have been reported to prevent cadmium induced lipid peroxidation and tissue accumulation in broilers (Bharavi et al., 2010).

Emblica officinalis Gaertn, commonly known as amla or the Indian gooseberry, is a member of the small genus, *Emblica* (Euphorbiaceae). It grows in tropical and subtropical parts of China, India, and Indonesia. Amla has been mentioned in the texts of Ayurveda, the Indian traditional medicine, owing to its pharmacological properties (Scartezzini and Speroni, 2000). The fruit is consumed raw, cooked or pickled and is an important dietary source of vitamin C, minerals and amino acids. The fruit extract is also rich in poly phenols like tannins which contribute to its pharmacological potential which has been widely investigated. The fruit extract has been found to inhibit mutagenicity induced by metals such as cadmium (Scartezzini and Speroni, 2000). It has also been reported to have potent antidiabetic (Sabu and Kuttan, 2002; Rao et al., 2005), hepato-protective (Jose and Kuttan, 2000) and antibacterial (Saeed and Tariq, 2007) properties owing to its antioxidative nature.

Rosemary (*Rosmarinus officinalis*, Labiatae) is a native to the Mediterranean and is a widely used culinary herb. It is commonly used as a spice and flavoring agent in food processing (Saito et al., 2004) and cosmetics (Al-Sereiti et al., 1999; Etter, 2004; Aguilar et al., 2008; Panda,

2009; Ibarra et al., 2010).

Rosemary leaf extract has also been proposed by the European Community Reference Laboratory (CRL) as a feed additive (Dossier no. FAD -2004-0003) in the class of antioxidants. The antioxidative activity of Rosemary is attributed to the phenolic diterpenes, carnosol and carnosic acid. Rosemary leaf extract has been demonstrated to possess strong antioxidant activity, predominantly due to the carnosic acid which is present in the alcoholic extract (10%). A recent study by Ozogul et al. (2010) showed the potential of Rosemary extract as a natural preservative agent in fish preservation owing to its antioxidant properties. Furthermore, the efficacy of Rosemary extracts as antioxidants to combat oxidative stress has been clearly demonstrated in previous studies on animal models (Posadas et al., 2010; Ahmed and Abdella, 2010; Ibarra et al., 2011).

With this premise, the present study was designed to study the ameliorative effect of the phyto-constituents present in, *E. officinalis* fruit extract and *R. officinalis* leaf extract on cadmium induced oxidative stress in male Wistar rats.

MATERIALS AND METHODS

Chemicals

All chemicals and Rosemary leaf extract were obtained commercially and were of analytical grade, Cadmium chloride ($\text{CdCl}_2 \cdot \text{H}_2\text{O}$, 98%) and ascorbic acid (Loba Chemie), India also. Rosemary leaf extract (10% carnosic acid) was procured from Hunan Geneham Biomedical Technology Ltd. (China). ELISA kits for the estimation of enzyme activity (CAT, SOD) and lipid peroxidation, measured as TBARS were used (Cayman Chemicals, USA).

Preparation of aqueous amla (*Emblica officinalis*) fruit extract

Shade dried amla fruit were obtained from the local market in Riyadh. 100 g of dried amla fruits were ground in an electrical grinder and dissolved in 500 ml distilled water (Gohil et al., 2010). The mixture was left for 24 h with a magnetic stirrer at room temperature. The next day the mixture was strained out in a fine sieve and the crude extract was air evaporated for three days. The concentrated fruit extract was then orally administered to the rats in different treatment groups (100 and 200 mg/kg body weight) using a syringe.

Experimental animals

Two months old male Wistar rats weighing 300 to 345 g were used. The animals were fed with standard laboratory chow and had free access to water under well ventilated conditions of 12 h day and 12 h dark cycles. The animals were acclimated to laboratory conditions prior to the experiment. The animals were divided into seven groups each comprising five rats. The experimental period was four weeks and the groups were as follows:

Group 1 was used as control.

Group 2 exposed to cadmium (15 mg Cd/kg body weight/day as CdCl_2 ; Ognjanović et al., 2008).

Group 3 exposed to cadmium (15 mg Cd/kg body weight/day as

CdCl₂ + Vitamin C (100 mg/kg body weight/day; Dawud et al., 2011).

Group 4 exposed to cadmium (15 mg Cd/kg body weight/day as CdCl₂ + Rosemary leaf extract (15mg/kg body weight/day).

Group 5 exposed to cadmium (15 mg Cd/kg body weight/day as CdCl₂ + Rosemary leaf extract (30 mg/kg body weight/day).

Group 6 exposed to cadmium (15 mg Cd/kg body weight/day as CdCl₂ + Amla fruit extract (100 mg/kg body weight/day).

Group 7 exposed to cadmium (15 mg Cd/kg body weight/day as CdCl₂ + Amla fruit extract (200 mg/kg body weight/day; Reddy et al., 2010).

Cadmium chloride, vitamin C, amla fruit extract powder were dissolved in normal saline while the Rosemary leaf extract (organic) was dissolved in 0.02% ethanol. All chemicals and plant extracts were administered orally through a syringe. The oral administration of the vitamin C, Rosemary leaf extract and amla fruit extract was done 30 min before the cadmium chloride administration in groups 3, 4, 5, 6 and 7.

After the exposure period the animals were sacrificed from each group and the liver and kidneys were excised out for enzyme assays and histological investigation. For enzyme assays, the organs were preserved at -80°C till further analysis.

Histological analysis

Samples of fragments of fresh organs of both testis and liver were fixed in buffered 10% formalin solution. Thereafter the tissues were dehydrated through a graded series of ethanol (from 70 to 100% alcohol in subsequent steps). Xylene was used as a clearing agent. Tissues were embedded in paraffin (58.6°C). Sections of paraffin blocks were cut by a rotary microtome (5 µm). Sections were stained with hematoxylin and eosin, and were examined and photographed using a photomicroscope (Nikon, Japan).

Biochemical analysis

Tissue homogenates for liver and kidney from each experimental group were prepared in accordance with the protocol provided with the enzyme assay kits (Cayman Chemicals, USA). Cayman's TBARS assay kit was used to measure lipid peroxidation in terms of MDA (malondialdehyde). For this assay 25 mg of tissue was weighed and 250 µl RIPA buffer was added with 50 µl EDTA. The tissue was then sonicated over ice. After this the contents were centrifuged at 1,600 xg for 10 min at 4°C. The supernatant was stored in ice and used for the assay.

Cayman's superoxide dismutase assay kit was used to measure the SOD activity. For this, 0.5 g of tissue was homogenized in 3 to 6 ml of cold 20 mM HEPES buffer, pH 7.2 containing 1 mM EGTA, 210 mM mannitol and 70 mM sucrose. The contents were then centrifuged at 1,500 xg for 5 min at 4°C. The supernatant was stored on ice and used for the assay.

Cayman's catalase assay kit was used to measure the CAT activity. For this, 0.5 g of tissue was homogenized on ice in 2 to 5 ml of cold PBS (phosphate buffered saline). Thereafter the contents were centrifuged at 10,000 xg for 15 min at 4°C. The supernatant was stored on ice and used for the assay.

Statistical analysis

Differences between obtained values (mean±SEM, n = 5) were carried out by one-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison test. A p value of 0.05 or less was taken as a criterion for a statistically significant difference.

RESULTS

Biochemical analysis

Our results indicate that lipid peroxidation in terms of thiobarbituric reactive substances (TBARS) concentration significantly increased both in the kidney and the liver of rats exposed to Cd in comparison to the control group (p< 0.0001). Co-treatment with the antioxidants reduced the TBARS levels in the kidney being significant in group 4 (Cd + 15 mg Rosemary), group 5 (Cd + 30 mg Rosemary) and group 7 (Cd + 200 mg Amla) (p< 0.0001), in comparison to the Cd exposed group (Figure 1). The TBARS levels in the liver, however showed a significant decrease in all Cd exposed groups co-treated with antioxidants (p< 0.0001) in comparison to the group exposed to Cd alone (Figure 2). In comparison to the control group, SOD activity was significantly (p<0.05) inhibited both in the kidney and liver in the Cd exposed group. In comparison to group 2 (Cd alone), all Cd exposed groups co-treated with antioxidants did not show any statistical significant change in the SOD activity in the kidney (Figure 3). However, the SOD activity in the liver showed significant increase in group 4 (Cd + 15 mg Rosemary), group 5 (Cd + 30 mg Rosemary), group 6 (Cd + 100 mg Amla) and group 7 (Cd + 200 mg Amla) (p< 0.0001), in comparison to group 2 (Cd alone) (Figure 4).

CAT activity was significantly reduced (p<0.03) only in the kidney (Figure 3) of the rats exposed to Cd alone in comparison to the control group. In comparison to the group exposed to Cd alone (group 2), among the co-treated groups there was a significant increase (p<0.01) in CAT activity in the kidney of rats from group 3 (Cd + Vit C) and group 5 (Cd + 30 mg Rosemary) (p<0.04) (Figure 5). Contrariwise, there was no significant change observed in the CAT activity in the liver of rats from all experimental groups exposed to Cd (Figure 6).

Histopathological effects on the liver and kidney

Liver

The sections of the control liver showed normal hepatic architecture with a central vein and hepatic lobule. A higher magnification showed well defined plates of binucleate hepatocytes. (Figure 7A, B). Sections of liver of rats from Group 2 exposed to Cd alone showed disorganized hepatic structure with dilated central vein, hepatocytes with pycnotic nuclei and Kupffer cells (Figure 7C, D). Liver sections from cadmium exposed rats being treated with Vit C (Group 3) showed a slightly better hepatic organization with more organized hepatic strands than Group 2 (Figure 7E, F). The hepatic architecture in sections from rats of Group 4 being treated with a lower dose of Rosemary leaf extract (15 mg/kg) was more or

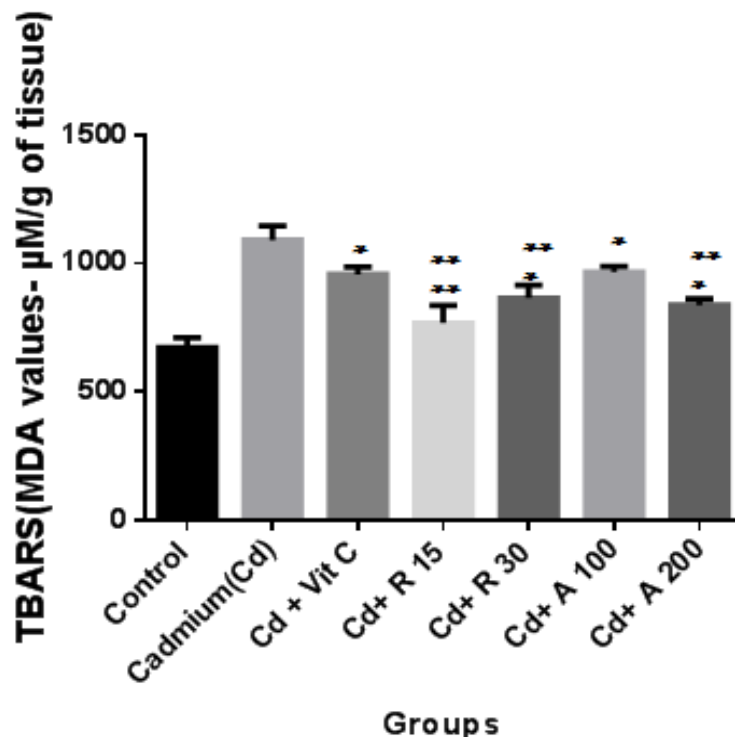


Figure 1. Malondialdehyde levels in kidney of controls and rats exposed to cadmium (Cd) and co-treated with Rosemary leaf extract (Cd+R15, Cd+R30) and Amla fruit extract (Cd+A100, Cd+A200). Data are presented as the mean \pm SEM (n=5). Significantly different from Cadmium exposed group (Cd); * P < 0.05.

less normal showing hepatic strands of hepatocytes with fewer Kupffer cells in comparison to Groups 2 and 3 (Figure 7G, H). A co-treatment of 30 mg/kg of Rosemary leaf extract did not restore the hepatic disorganization caused by the cadmium exposure which was shown by swollen hepatocytes with pyknotic nuclei, blood sinusoids with hemorrhage. However the structural disarray observed was less than Group 2, but more than Groups 3, 4 and 7 (Figure 7I, J). Sections of liver from rats exposed to Cd and treated with 100 mg/kg did not show much restoration in the cellular structure with disorganized hepatic strands (Figure 7K, L). However, a treatment of 200 mg/kg of amla fruit extract (Group 7) restored the normal hepatic architecture with hepatic strands radiating from central vein (CV) with binucleated hepatocytes (Figure 7M, N). The observations made show that the high dose of amla fruit extract (200 mg/kg b.wt) had the most marked ameliorative effect on the cadmium induced histopathology, among all the co-treatments used in the study.

Kidney

Sections of kidney of rats from the control group (Group1) Showed normal structural details with glomerulus with

surrounding renal tubules, proximal convoluted tubule and distal convoluted tubule (Figure 8A, B). Kidney sections from Cd exposed rats (Group 2) showed structural disarrangement with diffused hemorrhage, infiltration by lymphocytes and collapsed tubular lumina (Figure 8C, D, E). Treatment with Vit C in Group 3 showed a slight restoration in the structural organization in terms of less hyper-cellularity of glomerulus and normal renal tubules with lumina, in comparison to Group 2 (Figure 8F, G, H, I). Sections of kidney from Group 4, Cd exposed rats treated with a lower dose of Rosemary leaf extract (15 mg/kg) retained the normal appearance of glomerulus and structure of renal tubules with a decreased number of destructive tubules (Figure 8J, K). At a higher dose of 30 mg/kg the Rosemary (Group 5) treatment did show restoration in the structural arrangement in the kidney sections when compared to Group 2; however its effect was less pronounced than observed in Group 4 (Figure 8L, M). Further, a treatment with the fruit extract of amla was dose dependent as the higher dose (200 mg/kg) showed a noticeable restoration in the renal architecture in Group 7 (Figure 8P, Q) which was evident with the normal cellularity observed in contrast to the hypercellularity observed in Group 6 exposed to a lower dose (100 mg/kg) of the fruit extract, which did not show any distinctive improvement in the Cd

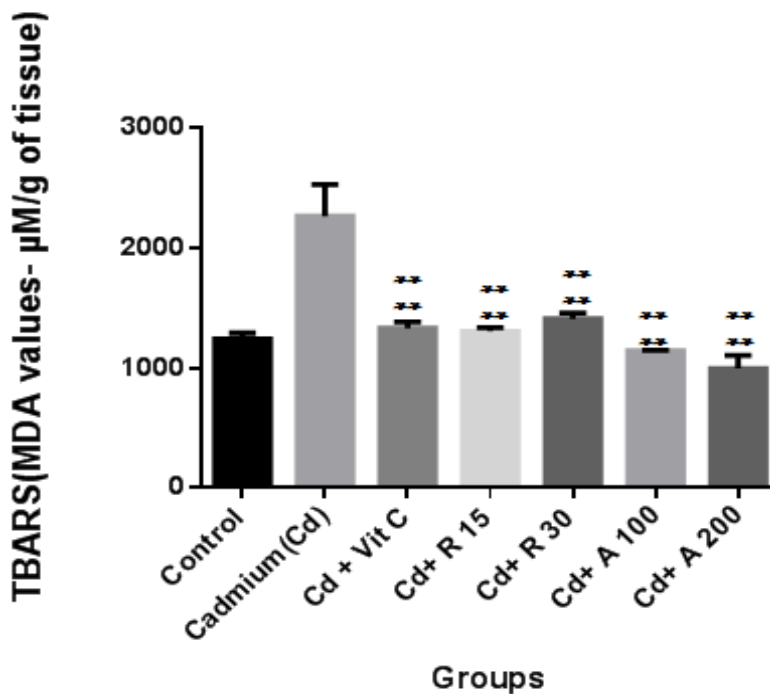


Figure 2. Malondialdehyde levels in liver of controls and rats exposed to cadmium (Cd) and co-treated with Rosemary leaf extract (Cd+R15, Cd+R30) and Amla fruit extract (Cd+A100, Cd+A200). Data are presented as the mean \pm SEM (n=5). Significantly different from Cadmium exposed group (Cd); * P < 0.05.

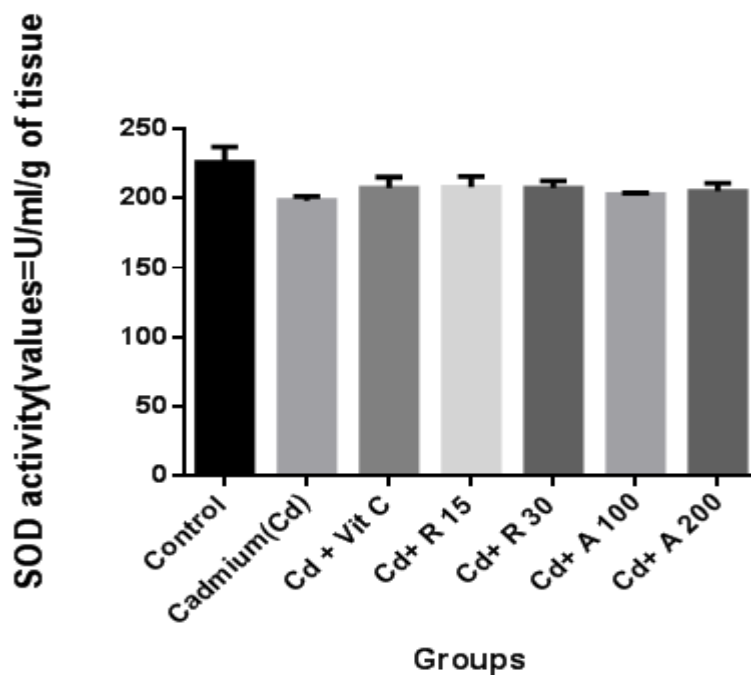


Figure 3. SOD activity in kidney of controls and rats exposed to cadmium (Cd) and co-treated with Rosemary leaf extract (Cd+R15, Cd+R30) and Amla fruit extract (Cd+A100, Cd+A200). Data are presented as the mean \pm SEM (n=5). Significantly different from Cadmium exposed group (Cd); * P < 0.05.

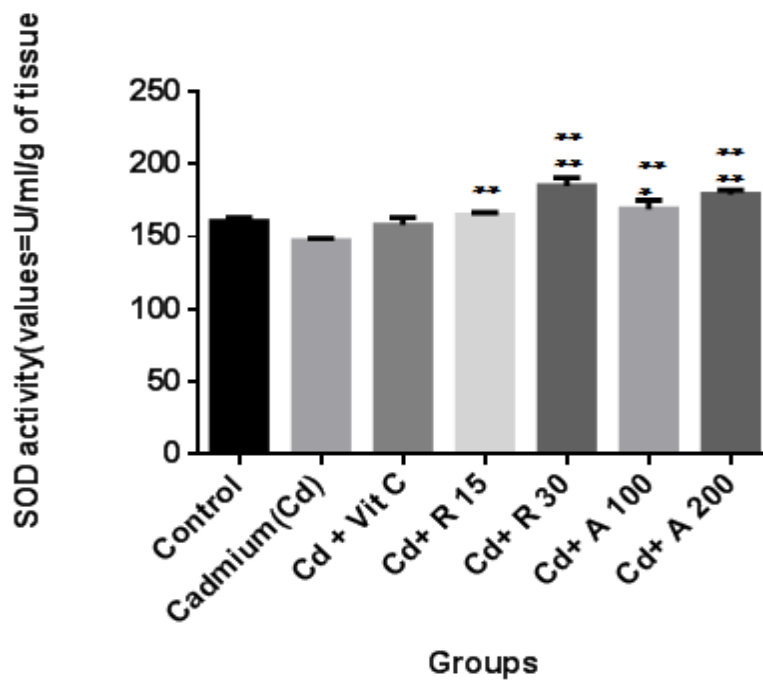


Figure 4. SOD activity in liver of controls and rats exposed to cadmium (Cd) and co-treated with Rosemary leaf extract (Cd+R15, Cd+R30) and Amla fruit extract (Cd+A100, Cd+A200). Data are presented as the mean \pm SEM (n=5). Significantly different from Cadmium exposed group (Cd); * P < 0.05.

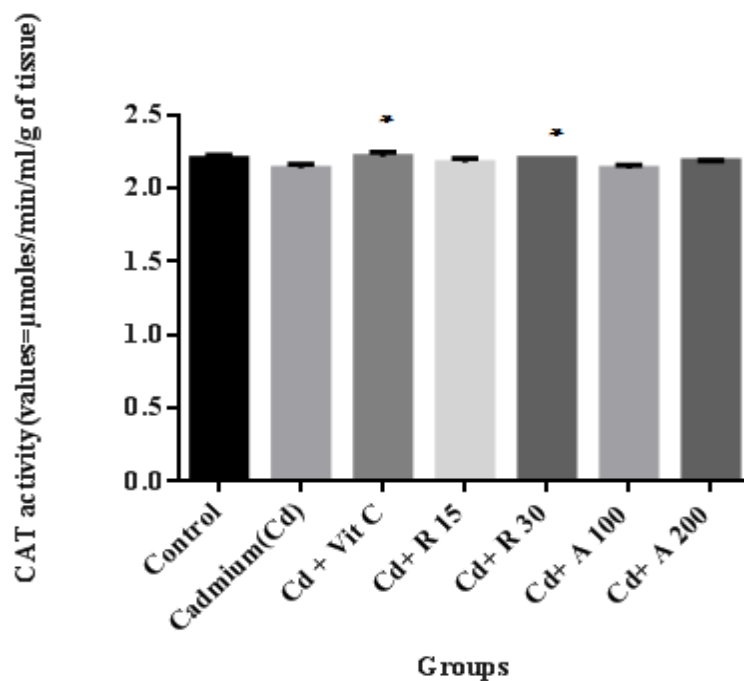


Figure 5. CAT activity in kidney of controls and rats exposed to cadmium (Cd) and co-treated with Rosemary leaf extract (Cd+R15, Cd+R30) and Amla fruit extract (Cd+A100, Cd+A200). Data are presented as the mean \pm SEM (n=5). Significantly different from Cadmium exposed group (Cd); * P < 0.05.

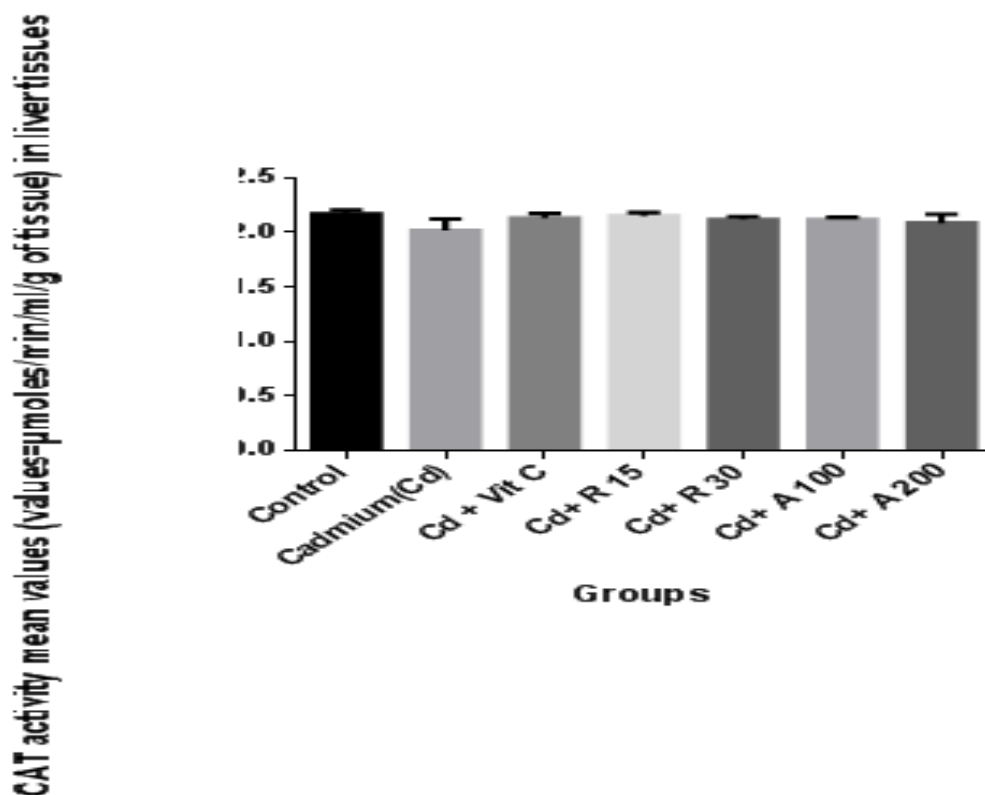


Figure 6. CAT activity in liver of controls and rats exposed to cadmium (Cd) and co-treated with Rosemary leaf extract (Cd+R15, Cd+R30) and Amla fruit extract (Cd+A100, Cd+A200). Data are presented as the mean \pm SEM (n=5). Significantly different from Cadmium exposed group (Cd), * $P < 0.05$.

induced cellular damage (Figure 8N, O).

DISCUSSION

The toxicity of Cd as a metal pollutant is well recognized. The generation of ROS has been attributed to the Cd induced pathotoxicity that leads to a disruption in the pro-oxidant/antioxidant balance, a condition coined as oxidative stress. A quest for safe phyto-constituents as antioxidants has been the mainstay of recent research in phytotherapy to alleviate the pathologies that are associated with oxidative cellular damage. Studies have indicated that Cd is an inducer of cell oxidative stress, either in a variety of cell culture systems (He et al., 2008) or in *in vivo* models through all routes of exposure (Amara et al., 2008).

The prime manifestation of the oxidative cell damage induced by metal exposure is the increased lipid peroxidation in the cells. Lipid peroxidation has been observed in Cd toxicity. A study by Yiin et al. (2001) demonstrated that the administration of Cd at various doses significantly increased the thiobarbituric acid

reactive-substances (TBARS), a well known indicator of lipid peroxidation, in rat adrenals glands. Our results showed a marked increase in the lipid peroxidation both in the kidney and liver of rats exposed to Cd which is in consensus with the previous reports on Cd induced oxidative stress in rats (Shaikh et al., 1999; Ognjanović et al., 2008) in poultry (Bharavi et al., 2010; Kant et al., 2011) and fish (Kumar et al., 2009). It is well established that Cd does not directly generate free radicals like other heavy metals but does generate non-radical hydrogen peroxide that eventually is a source of free radicals through the Fenton chemistry (Liochev, 1999). Thus the increased levels of MDA (malondialdehyde), an end-product of lipid peroxidation, in the liver and kidney of Cd exposed rats is mainly due to the degeneration of the lipids caused by the hydrogen peroxide formed.

Metal-induced modulation in the endogenous antioxidant enzyme activity has been extensively reported in the literature. The increase in lipid peroxidation due to Cd toxicity has been attributed to alterations in the antioxidant defense system. SOD and CAT are important endogenous antioxidant enzymes that are specifically affected by Cd exposure (Ercal et al.,

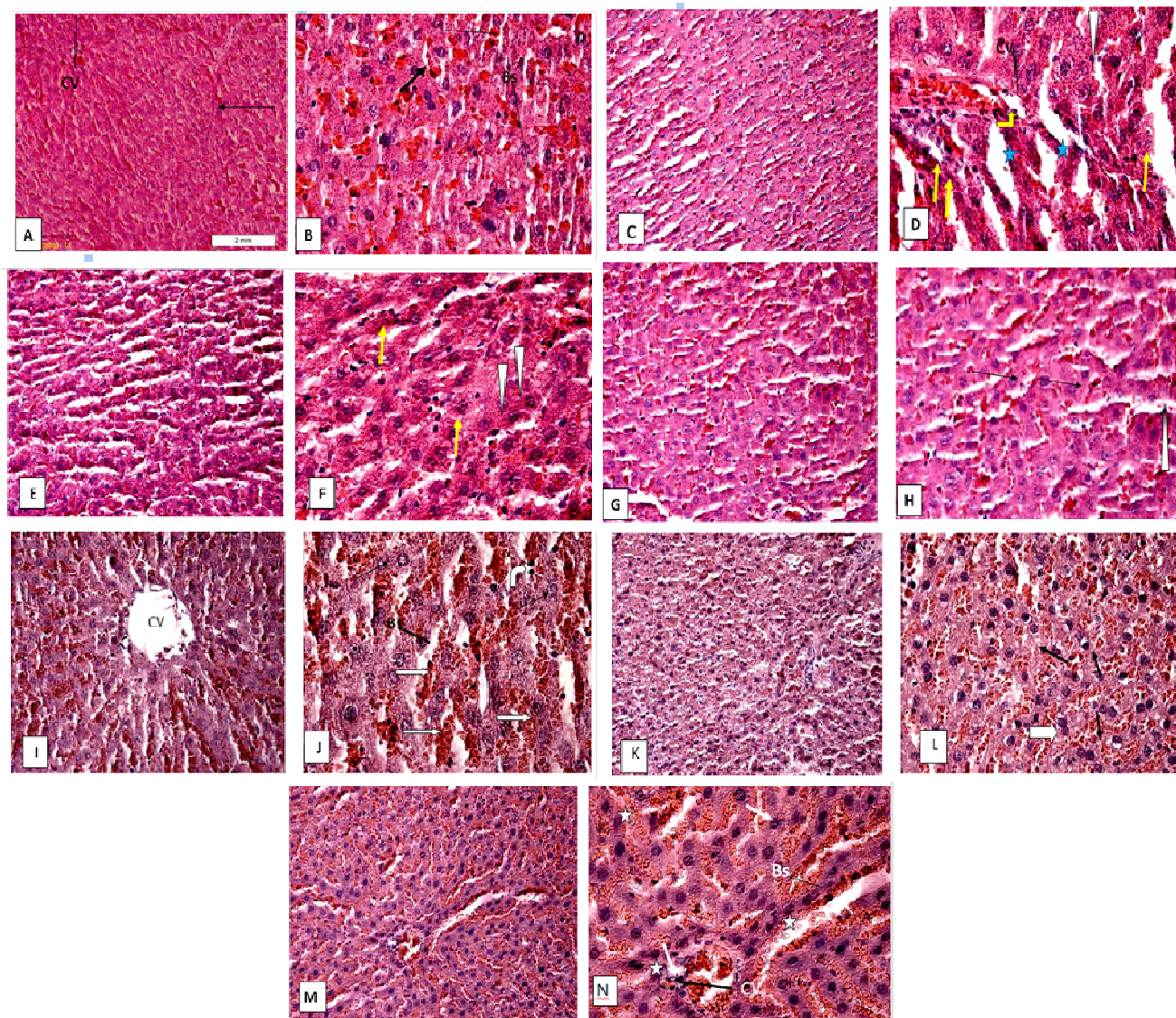
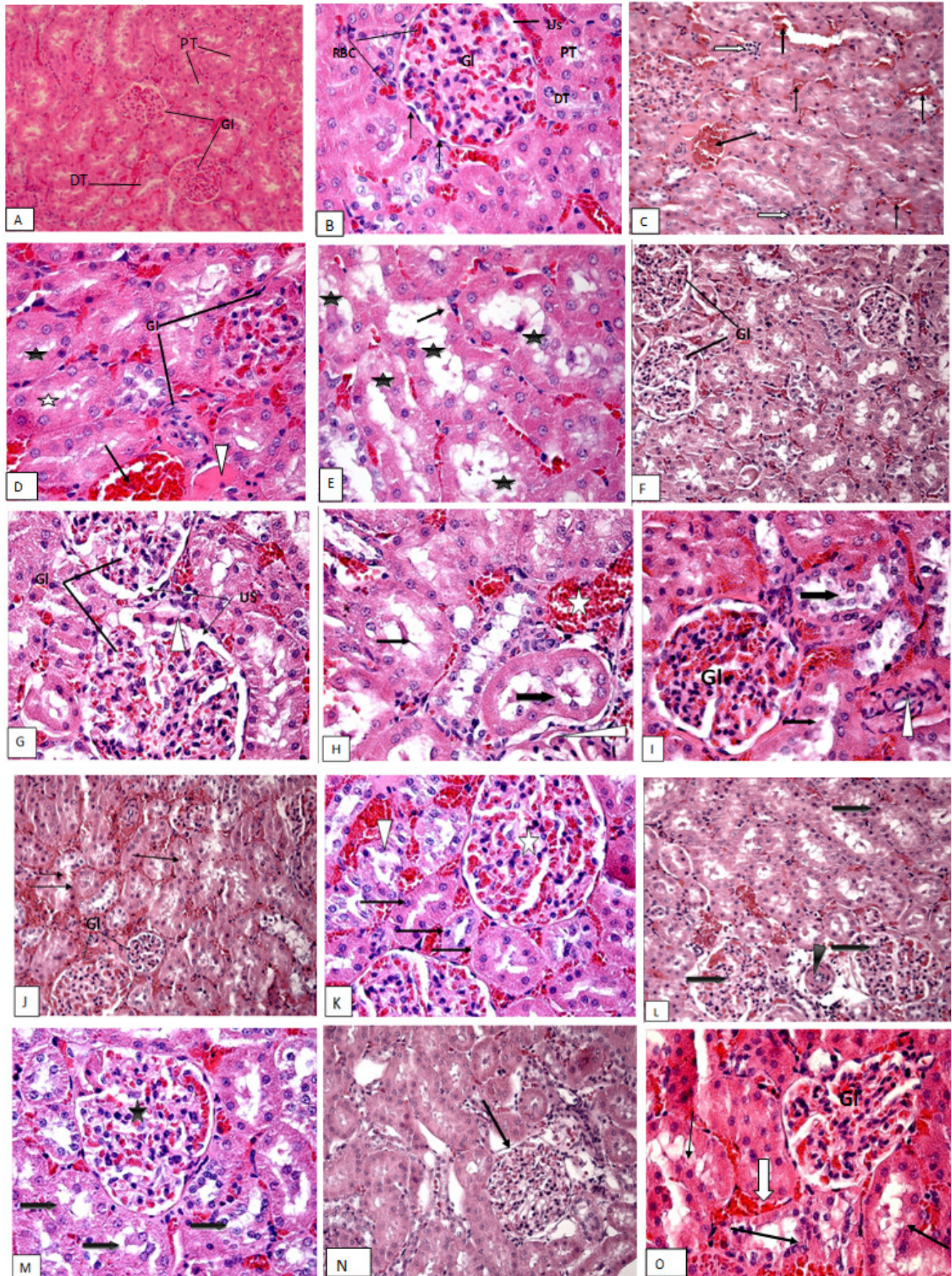


Figure 7. Effects of Cadmium and co-treatments with antioxidants, vitamin C, Rosemary leaf extract and Amla fruit extract on the histology of the liver of Wistar rats. **A)** Section of liver of rat from the control group, showing normal liver architecture. Notice, central vein (CV), hepatic lobule (arrow). **B)** Higher magnification of control liver, showing arrangement of plates of hepatocytes, one cell thick alternating with blood sinusoids (Bs). Notice, binucleated hepatocytes (arrows). **C)** Section of liver of rat exposed to cadmium, showing disorganized hepatic architecture. **D)** Higher magnification of liver of rat from the previous group, showing dilated central vein (CV) and blood sinusoids (stars), pyknotic nuclei (arrows) of autolytic and degenerate hepatocytes with intense pink cytoplasm (arrow head), accumulation of cells around central vein (curved arrow) which could be Kupffer cells. **E)** Section of liver of cadmium exposed rat treated with vitamin C (Group 3), showing dilated blood sinusoids than control and a slight organized hepatic strands. **F)** Higher magnification of the sections from the previous group, showing euchromatic nuclei and prominent nucleoli (arrow heads) of hepatocytes, Kupffer cell (arrows) are less than Group 2 but greater than Group 1. **G)** Section of liver of Cd exposed rat co-treated with Rosemary leaf extract (15 mg/kg) (Group 4), showing more or less normal architecture of liver in comparison to Group 2. **H)** Higher magnification of liver from the previous group, showing hepatic strands of hepatocytes with euchromatic nuclei (arrows) and blood sinusoids (arrow head). Notice, Kupffer cells are less than Group 3. **I)** Section of liver Cd exposed rat co-treated with Rosemary leaf extract (30 mg/kg) (Group 4), showing central vein (CV) with hypertrophied, degenerate hepatic strands and hemorrhage. **J)** Higher magnification of liver from the previous group, showing swollen hepatocytes with intense pink cytoplasm (black arrows), Pyknotic nuclei (curved arrow), blood sinusoids with hemorrhage (arrows) which is less than Group 2, but more than Groups 3, 4 and 5. **K)** Cross section of liver of cadmium exposed rats co-treated with + Amla fruit extract (100 mg/kg) (Group 6), showing disorganized hepatic strands. **L)** Higher magnification of a section of liver from the previous group, showing pyknotic nucleus (arrows); blood cell infiltrations (thick arrow). **M)** T.S of liver of Cd-exposed rat co-treated with 200 mg/kg of Amla fruit extract (Group 7), showing that liver almost retained its normal architecture in comparison to all co-treated groups. **N)** Higher magnification of the liver from the previous group, showing normal liver architecture with hepatic strands radiating from central vein (CV) with binucleated hepatocytes (arrow), blood sinusoids (Bs). Notice, hemorrhage (stars) but less than Group 2.



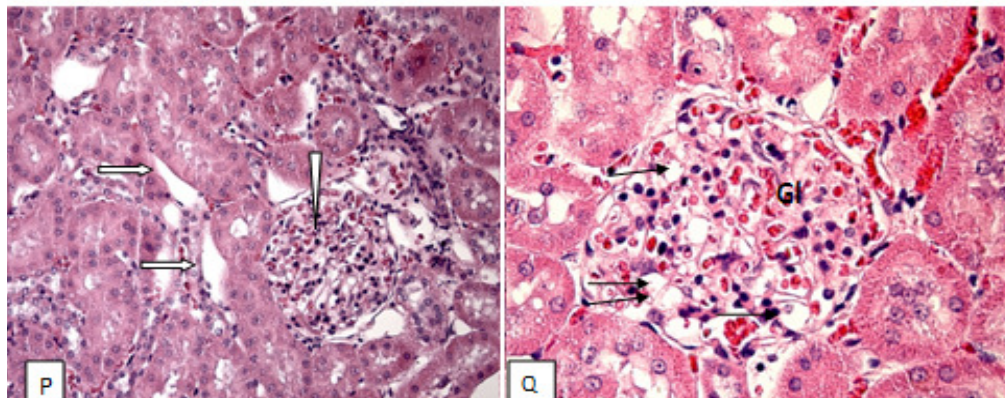


Figure 8. Effects of Cadmium and co-treatments with antioxidants, vitamin C, Rosemary leaf extract and Amla fruit extract on the histology of the kidney of Wistar rats. **A)** T.S Control (Group 1) showing normal glomerulus (G) with surrounding normal renal tubules, proximal convoluted tubules (PT), distal convoluted tubule (DT). **B)** Higher magnification of T.S of control kidney .Notice, glomerulus (G) with its cells (visceral, endothelial and mesangial cells); parietal epithelium (arrows): urinary space (Us); Proximal convoluted (PT)with basically located euchromatic prominent nuclei and nucleoli , distal convoluted tubule (DT) with apical prominent nuclei; Red Blood cells (RBCs) **C)** T.S Treated Kidney of rats exposed to cadmium (Group 2), showing diffuse hemorrhage (arrows) and interstitium infiltrated by lymphocytes (white arrows), hypercellularity of the glomerulus (G), collapsed lumina of tubules. **D)** Higher magnification of the previous section showing cellular crescent of glomeruli (G) due to proliferation of parietal cells , intense hemorrhage (arrow), detached brush border of proximal convoluted tubules (stars), infiltration by acellular material replacing normal structure (arrow head). **E)** Higher magnification of a section of kidney of the rat exposed to cadmium, showing evident acute tubular injury (star), tubular cells are flattened and many proximal cells lack brush border staining, lumina are relatively dilated. One tubule contains cells and debris in lumen and is incompletely lined by epithelium (arrow) (that is, tubulitis). **F)** T.S of kidney of Cd exposed rat co-treated with vitamin C (Group 3) showing a slight improvement and less hypercellularity of glomerulus (G), compared with figure 8d, normal renal tubules with lumina. **G)** Higher magnification of the kidney section from the previous group showing a slight improvement of glomerulus (G) with a prominent urinary space (US), slight migration of parietal cells (arrow head). **H)** Another section from Group3, showing a slight improvement with distinct hemorrhage (star), renal tubules with fragmented cells and nuclei within its lumen (arrows), interstitial fibrosis (arrow head) compared with figure 8d. **I)** Another section with high magnification from Group 3, showing a hemorrhage within pyknotic hypercellularity glomerulus (G), movement of nuclei (arrows) towards the lumen in renal tubules (marked tubulitis) , intertubular accumulation of fibroblast (arrow head). **J)** T.S of kidney of Cd exposed rat co-treated with Rosemary leaf extract (15 mg/kg) (Group 4), showing normal appearance of glomerulus (G) and improvement of renal tubules when compared to figure 8d. (80% improvement). **K)** Higher magnification of the previous section showing normal cellularity of glomerulus (star) and normal appearance of renal tubules (arrows) with its euchromatic nuclei , prominent nucleoli and open lumen. Notice very small number of destructive tubule (arrow head). **L)** T.S of kidney of Cd exposed rat co-treated with Rosemary leaf extract (30 mg/kg) (Group 5), showing a slight improvement than GII but less than GV .Notice, hypercellularity of glomerulus, pyknosis of cellular glomerulus (arrows) and renal tubule , interstitial fibrosis (arrow head). **M)** Higher magnification of the previous treatment, showing mild tubulitis of renal tubules (arrows), and hypercellularity of glomerulus (star). **N)** Cross sections of renal cortex of kidney of cadmium exposed rats co-treated with + Amla fruit extract (100 mg/kg) (Group 6), showing a hypercellularity of renal corpuscle (arrow) and disrupted renal tubules. **O)** Higher magnification of the previous section showing renal cortex with blood cell infiltration in the interstitial spaces (thick arrow), hypercellularity and pyknotic nuclei of glomerular cells (G); open lumen containing cellular debris (arrows). **P)** T.S of kidney of Cd-exposed rat co-treated with 200 mg/kg of Amla fruit extract (Group 7), showing marked improvement than all co-treated groups. Notice, more or less normal cellularity of glomerulus, renal tubules with its euchromatic nuclei and prominent nuclei. **Q)** Higher magnification of the kidney from the previous group, showing patent lumen of glomerular endothelium (arrows) and normal cellularity of glomerulus (G) in comparison to all treated groups.

2001). A measurement of the activities of these enzymes has been validated as a non-invasive method to assess the level of oxidative cellular damage caused by Cd exposure. However, the effect of the Cd exposure on the

alteration of the SOD and CAT activity exhibits variability in different studies which is attributed to the difference in dose, time and route of exposure in the experimental design. Long-term exposure to Cd increases lipid

peroxidation and causes inhibition of SOD activity indicating oxidative damage in liver, kidney and testes (Patra et al., 1999). In consensus to this, our results clearly showed a significant reduction in the SOD activity in the kidney and liver of the Cd exposed group in comparison to the control group. Ognjanović et al. (2008) reported a similar decrease in the SOD activity in liver and kidney of rats exposed to Cd (15 mg Cd/kg b.m./day as CdCl₂ for 4 weeks), decreased activities of SOD and CAT were also reported by Hassan and Awad (2007) in erythrocytes of rats given an oral dose of 5 mg CdCl₂/kg b.wt. for 30 days which is further supported through a study by Uchida et al. (2004). The decreased SOD activity may be due to inactivation of SOD by either cadmium induced lipid peroxidation or the antagonistic effect of cadmium with copper and zinc, which are important cofactors for the SOD activity (Gasiewicz and Smith, 1978). The CAT activity in our study was significantly decreased only in the kidney of the Cd exposed rats while there was no detectable significant change observed in the liver. Contrary to our results, Ognjanović et al. (2008) reported a decrease in CAT activity only in the liver and not in the kidney of rats exposed to Cd (15 mg Cd/kg b.wt./day as CdCl₂) for 4 weeks. However, a study on the effect of sub lethal concentration of cadmium chloride (12 mg/l) on CAT activity in brain, gill, kidney and liver of freshwater fish *Heteropneustes fossilis* at different exposure periods for 5, 10, 20 and 45 days, showed that in the brain, gill, and kidney the CAT activity was significantly inhibited. But in liver, non-significant increase was noted up to 20 days, but significant increase was noted only after 45 days exposure period (Radhakrishnan, 2009). This variability could be attributed to a differential accumulation of the ROS in the different tissues depending on the duration of exposure periods. Our study showed marked effect of the Cd exposure in terms of the oxidative stress caused in the kidney and the liver which resulted and is positively correlated with the histopathological damages caused in the tissues. In comparison to the control group, the Cd exposed group showed a clear disorganization of the hepatocellular structure with the presence of Kupffer cells and hepatocytes with pycnotic nuclei. The kidney of the rats exposed to Cd also showed structural disarray with collapsed tubular lamina. This is consistent with the previous reports on histopathological effects of cadmium exposure (Habeebu et al., 2000; Jose and Kuttan, 1988; Obianime and Roberts, 2009) on the liver, kidney and testis of rats.

Antioxidants mitigate the free radical induced cellular damage through various modes which include, suppression of hydroperoxides and hydrogen peroxides, averting the propagation of peroxidative chain reaction or by clearing away the active free radicals (Sen et al., 2010). There have been previous reports showing that antioxidants like vitamins C and E alleviate the cadmium induced toxicity in different animal models (Beytut et al.,

2003; McMurray and Tainer, 2003; Jin et al., 2003; Ognjanović et al., 2003). Supplementation of these natural antioxidants reduces ROS levels, lipid peroxidation, and alters enzymatic and non-enzymatic components of antioxidant defence system. The co-treatment of Cd exposed rats with antioxidants in our study clearly showed a significant alleviation of the lipid peroxidation in comparison to the Cd-exposed group, both in the liver and kidney. However in the liver the effect was significant with all the three antioxidants used, vitamin C, Rosemary leaf extract (15 and 30 mg/kg) and amla fruit extract (100 and 200 mg/kg) while in the kidney the effect was more pronounced with both doses of Rosemary leaf extract (15 and 30 mg/kg) and with the higher dose of amla fruit extract (200 mg/kg). All the antioxidant co-treatments with Cd showed a significant increase in the SOD activity in the liver which was comparable to the control. However, the CAT activity in the kidney was enhanced significantly only in Group 3 (Cd + Vit C) and Group 5 (Cd + 30 mg Rosemary). Overall, the antioxidants used did aid in combating the Cd induced oxidative stress which was also reflected in the histological studies. Our results on the co-treatment of Cd exposed rats with antioxidants show alleviation of the histopathological changes in both liver and kidney. The co-treatment of vitamin C with Cd in group 3 showed a mild restoration both in the hepatic and renal cellular organization in comparison to the group 2 exposed to only Cd. Vit C is one of the most studied and potent non-enzymatic antioxidant (Shiraishi et al., 1993; Kini et al., 2011) as it directly scavenges the superoxide and hydroxyl radicals and breaks down the hydrogen peroxide by the ascorbate peroxidase reaction (Noctor and Foyer, 1998) which clearly explains a decrease in the lipid peroxidation in the liver and kidney of the rats from Group 3 that mirrors the observed restoration in the tissue damage. The phenolic compounds are known to exhibit strong antioxidative properties which is attributed to their chemical structure that facilitates free radical scavenging activity and has been shown to be more efficacious than the tocopherols and ascorbate *in vitro* studies (Blokina et al., 2003). Our results also showed a similar trend, as in comparison to Vit C, the plant extracts (amla fruit and Rosemary leaf) used showed a more marked ameliorative effect on the Cd induced oxidative damage in liver and kidney in terms of the oxidative stress and the associated histopathological alterations observed.

The antioxidative property of amla fruit extract was clearly exhibited in our results; at both doses (100 and 200 mg/kg) it showed a significant ($p < 0.0001$) decrease in the MDA levels in liver and at a higher dose (200 mg/kg) in the kidney. A significant ($p < 0.0001$) increase in the SOD activity was observed in the liver on co-treatment with amla fruit extract at both doses used. The histopathological study revealed that co-treatment with amla (100 and 200 mg/kg) showed restoration in the

cellular damage in the liver which was a dose dependent effect being more noticeable at the higher dose (200 mg/kg). Literature has reported the antioxidative potential of amla fruit which has been attributed to its high vit C content. However, recent studies report the presence of bioactive tannoid principles in the fruit, comprising of emblicanin A, emblicanin B, punigluconin and pedunculagin, which have been shown to exhibit antioxidant activity *in vitro* and *in vivo* and are responsible for preventing the oxidation of the ascorbic acid even in dried fruit (Scartezzini et al., 2006). Therefore, it is the synergistic antioxidative effect of the vit C and polyphenols such as tannins which attribute to the ameliorative effect of the amla fruit extract in our study. Our results are strongly supported by a plethora of evidence from the past literature which report a hepatoprotective and antioxidative (Bhattacharya et al., 1999, 2000; Scartezzini et al., 2006) role of amla fruit extract. Both *in vitro* and *in vivo* studies have reported the antioxidant property of carnosic acid-rich Rosemary leaf extract by oxygen radical absorbance capacity and ferric-reducing/antioxidant power assays (Ibarra et al., 2010). The results of our study do reveal the antioxidant potential of Rosemary leaf extract as the co-treatment of Cd exposed rats showed a significant ($p < 0.0001$) decrease in the lipid peroxidation both in the liver and kidney and the effect was not dose dependent. Both doses (15 and 30 mg/kg b.wt) of Rosemary leaf extract showed a significant increase in the SOD activity in the liver while the CAT activity was increased only in the kidney with the low dose co-treatment. These results corroborate findings in earlier studies that have reported a decrease in lipid peroxidation and decrease in antioxidant enzyme activity in the brain and heart of aged rats following the administration of Rosemary extract (Posadas et al., 2009). Furthermore, in a study by Rasha and Abdella (2010) on Albino mice, a pretreatment of doxorubicin exposed animals with a dose (125 mg/kg) of Rosemary leaf extract (aqueous) significantly reduced level of lipid peroxidation in the liver and kidney. This was co-related to the amelioration in the histological lesions observed in the liver and kidney of doxorubicin exposed rats co-treated with the Rosemary leaf extract. Parallel to these findings, our results on histological studies reveal that the co-treatment with Rosemary leaf extract showed marked restoration in the hepatic architecture and the tubular organization in the kidney of the Cd exposed rats, which was more pronounced at the lower dose (15 mg/kg b.wt). The main bioactive constituents in the Rosemary leaves are the polyphenols, including carnosic acid, carnosol, rosmarinic acid, ursolic acid, etc (Ramirez et al., 2006; Richheimer et al., 1996; Seronans et al., 2000). Among these, carnosic acid, a phenolic diterpene compound and carnosol are the most potent antioxidant constituents contributing about 90% of antioxidant activity. This clearly explains the antioxidative effect of Rosemary leaf extract used in our study.

Our results suggest that the phytonutrient rich plant

extracts (Rosemary leaf and amla fruit extract) were more efficacious than the nutrient derived antioxidant, vit C in alleviating the Cd-induced oxidative stress in terms of the biochemical parameters studied. The same pattern was observed in the restoration of the cellular organization in the liver and kidney tissues. Thus both the Rosemary and amla extracts are rich in phyto compounds that bring about a free radical quenching effect. The plant extracts showed a marked ameliorative effect on oxidative stress that subsequently causes tissue damage.

The key takeaways from our study are that, efficacy of Rosemary leaf extract was dose independent while the amla fruit extract proved to be more effective at the higher dose (200 mg/kg) which was also the best antioxidant co-treatment for the Cd-induced cellular damage in the present study.

Available evidence in the past literature suggests that the pathological and toxicological consequences of oxidative stress are associated with a host of human diseases. The use of phyto nutrients to combat this oxidative damage does offer a novel therapeutic approach to restore the normal body functioning. It is an established fact that phytotherapy is free of side effects, devoid of resistance to microbes and is less phytotoxic (Kumar, 2009). The plant extracts used in our study have a wide culinary use, thus garnering a status of potential dietary supplements in our daily life.

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