Research Article

Tannic Acid Protects against Cadmium-Induced Renal Damages of Male Albino Rats

Sanatan Mishra[1,2], Debosree Ghosh[1#], Mousumi Dutta[1,2], Aindrila Chattopadhyay[2], Debasish Bandyopadhyay[1*#]
[1]Department of Physiology, University of Calcutta, University College of Science and Technology, 92, APC Road, Kolkata, India.
[2]Department of Physiology, Vidyasagar College, 39, Shankar Ghosh Lane, Kolkata, India.
#Principal Investigator, (CPEPA), University of Calcutta, University College of Science and Technology, 92 APC Road, Kolkata, India.
#Presently, Department of Physiology, Ramananda College, Bishnupur, Nadia, West Bengal, India.
*Corresponding author's E-mail: debasish63@gmail.com

ABSTRACT

Heavy metals are known to accumulate in various vital organs including heart, liver, kidneys etc. and induce generation of free radicals inside animal body. Thus heavy metals cause oxidative damage in tissues like liver, heart and kidneys. The purpose of this study was to investigate the role of tannic acid, a plant polyphenol, against cadmium chloride-induced oxidative stress mediated nephro-toxicity in male rats. The rats of control group were treated orally with normal saline (0.09% NaCl). The rats of second, third, fourth and fifth groups were treated with 0.44mg/ kg body weight cadmium chloride, 12.5 mg/kg bw tanic acid plus 0.44mg/kg body weight cadmium chloride, 25 mg/kg bw tannic acid plus 0.44mg/kg body weight cadmium chloride and 50 mg/kg bw tannic acid plus 0.44mg/kg body weight cadmium chloride, respectively for 14 days. The results indicated that cadmium chloride caused a significant alteration in the level of lipid peroxidation (LPO), protein carbonyl content (PCO), reduced and oxidised glutathione content (GSH and GSSG), total sulfhydryl content (TSH), cytosolic and mitochondrial superoxide dismutase activities (Cu-Zn SOD and Mn SOD), activities of catalase, xanthine oxidase and xanthine dehydrogenase (XO ad XDH) and in the activities of Kreb's cycle as well as respiratory chain enzymes. Pre-treatment of rats with tannic acid at increasing doses ameliorates the changes in a dose-dependent manner and tannic acid at 25 mg/kg bw caused the most significant protection against cadmium induced changes. In addition, the results of histological studies showed that cadmium chloride caused significant damages in the renal tissue morphology of rats and this cadmium induced deteriorative changes were found to be protected in a dose-dependent manner when the rats were pre-treated with tannic acid in increasing doses along with cadmium chloride. The results of the current studies raise the possibility using tannic acid as a potent reno-protective agent, in future days, against cadmium-induced damages.

Keywords: Cadmium, kidneys, oxidative stress, tannic acid

INTRODUCTION

Heavy metals are known to accumulate in various vital organs including heart, liver, kidneys etc. and induce generation of free radicals inside animal body[1,2]. Thus heavy metals cause oxidative damage in tissues like liver, heart and kidneys[2,3]. Cadmium, a toxic environmental pollutant is released into the environment from industries and automobile exhausts etc.[3,4]. The toxicity of cadmium-induced oxidative damages has been recognised as a potent mediator of tissue damages[5]. Cadmium like other toxic heavy metals is associated with several damaging health effects[6]. Heavy metals generate highly reactive oxygen species (ROS), such as superoxide anion free radicals (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH) and lipid peroxides which are known to damage various cellular components including proteins, membrane lipids and nucleic acids[7,8]. Kidney cortex and medulla has been recognised as the second largest site for accumulation of heavy metals like cadmium[9,10]. Cadmium has also been reported to accumulate in tissues and induce generation of free radicals in the tissue[5].

Tannic acid (TA) is a widely distributed plant polyphenol, present in several dietary plants including vegetables and fruits[4]. TA is a naturally occurring antioxidant[11]. TA has been reported to be an antagonist of the mutagenic potency of aromatic hydrocarbons like benzo(a)pyrene[12]. Tannic acid also has medicinal uses including its use in treating burns, diarrhoea etc[13]. Herein, we provide evidence that TA has the capability to provide protection against cadmium-induced renal damages in experimental rats and antioxidant mechanism(s) may perhaps be responsible for such protection.

MATERIALS AND METHODS

All the chemicals used were of analytical grade unless otherwise specified. Thio-barbituric Acid (Spectro Chem., India), Folin and Ciocalteu Phenol reagent, (SRL, India), Ethylene Diamine Tera-acetic acid [EDTA] (Qualigens Fine Chemicals, Glaxo India Ltd), TRIS-HCl tris (hydroxymethyl) amino methane (SRL, India), Tannic Acid (Merk India), Cadmium Chloride (SRL, India) Trichloro acetic acid (TCA) (SRL, India), Dithio bis nitro benzoic acid (DTNB) (SRL, India), GSH (SRL, India), Na_2PO_4 (SRL, India), NaOH (SRL, India), $CuSO_4$ (Merck, India), Na-K-Tartarate (SD Finechem. Ltd, India), Triton x, hydrogen peroxide (H_2O_2) (SRL, India).

Experimental Animals

Male albino rats of Wister strain, weighing 160-180 g were handled as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of environment and forests,

Government of India. All the experimental protocols had the approval of the Institutional Animal Ethics Committee (IAEC) of the Department of Physiology, University of Calcutta (approval no IAEC-III/Proposal /DB-01/ 2013 dated 22.03.2013).

During experiment, the animal room was maintained at a temperature of 25±1°C, humidity 50±10% and a 12-h light/dark cycle and the rats were allowed to access standard diet containing 18% protein (casein) and water *ad libitum* for 7 days (quarantine period). The 18% protein diet was considered as an adequate dietary protein level, which was used on earlier occasions[14].

The animals were divided into five groups in such a way that the average body weights of animals in each group remained similar. Each group contained six animals. The groups were as follows:

GROUP I

Control group

GROUP II

Cadmium chloride treated group: 0.44mg/ kg body weight (BW) cadmium chloride (administered subcutaneously).

GROUP III

Tannic acid (12.5mg/Kg BW) + Cadmium chloride (tannic acid administered orally and cadmium chloride administered subcutaneously).

GROUP IV

Tannic acid (25mg/Kg BW) + Cadmium chloride (tannic acid administered orally and cadmium chloride administered subcutaneously).

GROUP V

Tannic acid (50mg/Kg BW) + Cadmium chloride (tannic acid administered orally and cadmium chloride administered subcutaneously).

Preparation of Cadmium Chloride (CdCl₂) Solution

Twenty two mg of cadmium chloride was dissolved in 50 ml of double distilled water[15,16].

Preparation of Aqueous Solution of Tannic Acid (TA)

A definite amount of tannic acid was dissolved in double distilled water to give a particular concentration of 12.5mg/ml, 25mg/ml and 50mg/ml. These solutions were used to carry out our experiments.

Animal Treatment

After acclimatization to laboratory environment, the animals of cadmium chloride treated group were administered subcutaneously cadmium chloride at a dose of 0.44 mg/kg BW for a period of 15 days. The rats of the control group received the vehicle (0.9% NaCl) only. Another three groups (III, IV and V) of rats were pre-treated with tannic acid (oral administration) at a dose of

12.5mg/kg, 25mg/kg and 50mg/kg BW, respectively, before administration of cadmium chloride, subcutaneously, at a dose 0.44 mg/kg body weight.

Animal Sacrifice and Collection of Blood and Tissue Samples

At the end of the treatment period, the animals of each group were kept fasted over night. The body weight of each group of animals were checked and recorded. Each animal was anaesthetized using ether, sacrificed through cervical dislocation and blood was collected from hepatic vein and was allowed to clot for serum to separate out and then centrifuged at 2500 rpm for 15 minutes. Serum was collected with auto pipette in individual microfuge tube and stored at –20°C[17]. Kidneys were collected separately from each animal, washed in ice cold saline, and blotted dry, immediately weighed, some portion kept for tissue morphological studies and isolation of mitochondria and the rest stored at -20° C until analysis.

Isolation of Mitochondria from Kidney Tissue

The mitochondria from kidney tissue were isolated according to the procedure as adopted by Dutta[18]. In brief, immediately after sacrifice, some portion of the kidney tissue was cleaned and cut into small pieces. Five hundred mg of the tissue was placed in 10ml of sucrose buffer [0.25(M) sucrose, 0.001(M) EDTA, 0.05(M) Tris-HCl (pH 7.8)] at 25°C for 5min. The tissue was then homogenized in cold for 1 minute at low speed by using a Potter Elvenjem glass homogenizer (Belco Glass Inc., Vineland, NJ, USA). The homogenate was centrifuged at 1500rpm for 10 minutes at 4°C. The supernatant was poured through several layers of cheese cloth and kept in ice. This filtered supernatant was centrifuged at 4000rpm for 5minutes at 4°C. The supernatant, thus, obtained, was further centrifuged at 14000rpm for 20 minutes at 4°C. The final supernatant was discarded and the pellet was re-suspended in sucrose buffer and stored at -20°C until further analysis. However, most of the enzymatic assays were carried out with freshly prepared mitochondria.

Measurement of Lipid Peroxidation Level

A portion of the kidney tissue was homogenized (5%) in ice-cold 0.9% saline (pH 7.0) using Potter Elvehjem glass homogenizer (Belco Glass Inc., Vineland, NJ, USA) for 30 seconds and lipid peroxides in the homogenate were determined as thiobarbituric acid reactive substances (TBARS) according to the method of Buege and Aust[19] with some modification (Mishra)[20]. Briefly, the homogenate was mixed with thiobarbituric acid–trichloro acetic acid (TBA–TCA) reagent with thorough shaking and heated for 20 min at 80°C. The samples were then cooled to room temperature. The absorbance of the pink chromogen present in the clear supernatant after centrifugation at 1200 g for 10 min at room temperature was measured at 532 nm using a UV–vis spectrophotometer (Bio-Rad, Smartspec Plus, Hercules, CA, USA). Values were expressed as nmoles of TBARS/mg of tissue protein.

International Journal of Pharmaceutical Sciences Review and Research
Available online at www.globalresearchonline.net
274

Measurement of Tissue Protein Carbonyl (PCO) Content

The PCO content was estimated by DNPH assay[21] with some modifications[22]. The tissue homogenates were centrifuged at 10,000g for 10 min at 4°C. After centrifugation, 0.5 ml of supernatant was taken in each tube and 0.5 ml DNPH in 2.0 M HCl was added to the tubes. The tubes were vortexed every 10 min in the dark for 1 hour. Proteins were then precipitated with 30% TCA and centrifuged at 4000g for 10 min. The pellet was washed three times with 1.0 ml of ethanol: ethyl acetate (1:1, v/v). The final pellet was dissolved in 1.0 ml of 6.0 M guanidine HCl in 20 mM potassium dihydrogen phosphate (pH 2.3). The absorbance was determined spectrophotometrically at 370 nm. The PCO content was calculated using a molar absorption coefficient of 2.2×10^4 M^{-1} cm^{-1}. The values were expressed as nmoles/mg of tissue protein.

Measurement of Reduced Glutathione (GSH), Oxidised Glutathione (GSSG) Content, GSSG:GSH Ratio and Total Sulfhydryl Group (TSH) Content

The level of GSH in the kidney tissue was estimated by its reaction with DTNB (Ellman's reagent) following the method of Sedlak and Lindsey (1968)[21] with some modifications[23]. A portion of the kidney tissue was homogenized (10%) separately in 2 mM ice cold ethylene diamine tetra acetic acid (EDTA). The homogenate was mixed with Tris–HCl buffer, pH 9.0, followed by DTNB for color development. The absorbance was measured at 412 nm using a UV–VIS spectrophotometer (BIORAD, Smartspec Plus). Values were expressed as nmoles/mg of tissue protein.

The GSSG content was measured by the method of Sedlak and Lindsay, 1968[21]. The reaction mixture contained 0.1 mM sodium phosphate buffer, EDTA, NADPH and 0.14 units per ml glutathione reductase. The absorbance was measured at 340 nm using a UV-VIS spectrophotometer to determine the GSSG content. The values were expressed as n moles GSSG/mg of tissue protein. GSSG: GSH ratio was calculated.

Total sulfhydryl group content was measured following the method as described by Sedlak and Lindsay, 1968[21] with some modifications[24]. The values were expressed as nmoles TSH/mg of tissue protein.

Measurement of the Activities of Cytosolic (Cu-Zn type) and Mitochondrial (Mn-type) Superoxide Dismutase (SOD)

Copper-zinc superoxide dismutase (Cu-Zn SOD) activity was measured by hematoxylin auto-oxidation method of Martin[25] with some modifications[20]. Briefly, the kidney tissues were homogenized (10%) in ice-cold 50 mM phosphate buffer containing 0.1 mM EDTA, pH 7.4. The homogenate was centrifuged at 12,000 g for 15 min and the supernatant, thus obtained, was collected. Inhibition of hematoxylin auto-oxidation by the cell free supernatant was measured at 560 nm using a UV–Vis spectrophotometer. The enzyme activity was expressed as units/mg of tissue protein.

Manganese superoxide dismutase (Mn-SOD) activity was measured by pyrogallol auto-oxidation method[26] with some modifications[27]. A weighed amount of kidney tissue was homogenized (10%) in ice-cold 50 mM Tris–HCl buffer containing 0.1 mM EDTA, pH 7.4 and centrifuged at 2000 rpm for 5 min. The supernatant was carefully collected and centrifuged again at 10,000 rpm in cold for 20 min. The supernatant was discarded and the pellet was suspended in 50 mM Tris–HCl buffer, pH 7.4. One ml of assay mixture contains 50 mM of Tris–HCl buffer (pH 8.2), 30 mM EDTA, 2 mM of pyrogallol and suitable volume of the mitochondrial preparation as the source of enzyme. An increase in absorbance was recorded at 420 nm for 3 min in a UV/VIS spectrophotometer. The enzyme activity was expressed as units/ mg of mitochondrial protein.

Measurement of the Activity of Catalase (CAT)

Catalase was assayed by the method of Beers and Sizer[28]. The kidney tissue was homogenized (5%) in ice-cold 50 mM phosphate buffer, pH 7.0. The homogenate was centrifuged in cold at 12,000 g for 12 min. The supernatant, thus obtained, was then collected and the aliquots of the supernatant serving as the source of enzyme were incubated with 0.01 ml of absolute ethanol at 4°C for 30 min, after which 10% Triton X-100 was added to have a final concentration of 1%. The sample, thus obtained, was used to determine the catalase activity by measuring the breakdown of H_2O_2 spectrophotometrically at 240 nm. The enzyme activity was expressed as μmoles H_2O_2 consumed/mg of tissue protein.

Measurement of Xanthine oxidase (XO) and Xanthine Dehydrogenase (XDH) Activities of Rat Kidney Tissue

The activity of XO in rat kidney tissue was measured by the conversion of xanthine to uric acid following the method of Greenlee and Handler[29]. The kidney tissue was homogenized in cold (10%) in 50 mM phosphate buffer, pH 7.8. The homogenate was then centrifuged at 500 g for 10 minutes. The supernatant, thus obtained, was again centrifuged at 12,000 g for 20 min. The final supernatant was used, as the source of enzyme, for spectrophotometric assay at 295 nm, using 0.1 mM xanthine in 50 mM phosphate buffer, pH 7.8, as the substrate. The enzyme activity was expressed as milli units/mg of tissue protein.

Xanthine dehydrogenase activity was measured by following the reduction of NAD^+ to NADH according to the method of Strittmatter (1965)[30]. The weighed amount of rat kidney tissue was homogenized in cold (10%) in 50 mM phosphate buffer with 1 mM EDTA, pH 7.2. The homogenate was centrifuged in cold at 500g for 10 min. The supernatant, thus obtained, was further centrifuged in cold at 12,000g for 20 min. The final supernatant was used as the source of the enzyme, and the activity of the

International Journal of Pharmaceutical Sciences Review and Research
Available online at www.globalresearchonline.net
275

enzyme was measured spectrophotometrically at 340 nm with 0.3 mM xanthine as the substrate (in 50 mM phosphate buffer, pH 7.5) and 0.7 mM NAD^+ as an electron donor. The enzyme activity was expressed as milli units/mg of tissue protein.

Measurement of the Activities of the Pyruvate Dehydrogenase (PDH) and some of the Kreb's Cycle Enzymes

Pyruvate dehydrogenase activity was measured spectrophotometrically according to the method of Chretien[31] with some modifications[32] following the reduction of NAD^+ to NADH at 340nm using 50mM phosphate buffer, pH-7.4, 0.5mM sodium pyruvate as the substrate, and 0.5mM NAD^+ in addition to mitochondria as the source of the enzyme. The enzyme activity was expressed as Units/mg of mitochondrial protein.

Isocitrate dehydrogenase (ICDH) activity was measured according to the method of Duncan[33] with some modifications[34] by measuring the reduction of NAD+ to NADH at 340nm with the help of a UV-VIS spectrophotometer.

One ml assay volume contained 50mM phosphate buffer, pH-7.4, 0.5 mM isocitrate, 0.1mM $MnSO_4$, 0.1mM NAD+ and suitable aliquot of mitochondria as the source of the enzyme.

The enzyme activity was expressed as units/mg of mitochondrial protein.

Alpha-ketoglutarate dehydrogenase (α-KGDH) activity was measured spectrophotometrically according to the method of Duncan[33] with some modifications[35] by measuring the reduction of 0.35 mM NAD+ to NADH at 340nm using 50 mM phosphate buffer, pH 7.4, as the assay buffer and 0.1mM α-ketoglutarate as the substrate and suitable amount of mitochondria as the source of the enzyme. The enzyme activity was expressed as units/mg of mitochondrial protein.

Succinate Dehydrogenase (SDH) activity was measured spectrophotometrically by following the reduction of potassium ferricyanide (K_3FeCN_6) at 420nm according to the method of Veeger[36].

One ml assay mixture contained 50mM phosphate buffer, pH 7.4, 2% (w/v) BSA, 4 mM succinate, 2.5 mM K_3FeCN_6 and the suitable aliquot of mitochondria as the source of the enzyme.

The enzyme activity was expressed as units/mg of mitochondrial protein.

Histological Studies

A portion of the extirpated rat kidneys were fixed immediately in 10% formalin and embedded in paraffin following routine procedure as used earlier by Dutta[34]. Sections of the kidney tissue (5 µm thick) were prepared.

The tissue sections were stained with hematoxylin–eosin stain and examined under Leica microscope and the images were captured with a digital camera attached to it.

Estimation of Protein

The protein content of the different samples was determined by the method of Lowry[37].

Statistical Evaluation

Each measurement was repeated at least three times for each rats. Data are presented as means ± SE. Significance of mean values of different parameters between the treatments groups were analyzed using one way analysis of variances (ANOVA) after ascertaining the homogeneity of variances between the treatments. Pairwise comparisons were done by calculating the least significance. Statistical tests were performed using Microcal Origin version 7.0 for Windows.

RESULTS

Level of Lipid Peroxidation and Protein Carbonyl Content

Figure 1(A-B) shows that treatment of rats with cadmium chloride increased the level of lipid peroxidation (LPO) and protein carbonyl content significantly compared to that of control (152.38 % and 133.00% respectively, p<0.001). When the rats were pre-treated with TA, the level of LPO and PCO were found to be protected from being increased in a dose-dependent manner (53.49% and 54.61% respectively, compared to cadmium treated group, p<0.001). It was found that there was maximum protection at the dose of 25 mg/kg bw of TA.

Reduced Glutathione (GSH), Oxidised Glutathione (GSSG) and Total Sulphydryl (TSH) Content

Figure 2 (A-C) shows that treatment of rats with cadmium chloride decreased the content of reduced glutathione (GSH) and increased the content of oxidised glutathione (GSSG) and decreased total sulphydryl content (TSH) significantly compared to that of control (57.12 %, 102.77% and 20% respectively, p<0.001). When the rats were pre-treated with TA, the level of GSH, GSSG and TSH were found to be protected from being altered compared to cadmium chloride treated group, also, in a dose-response manner. Here also, the maximum protection was found to be at the dose of 25 mg/kg bw of TA (95.38%, 46.57% and 20.41% respectively compared to the cadmium chloride-treated group, p<0.001).

Activities of Cu-Zn Superoxide Dismutase (Cu-Zn SOD), Mn-Superoxide dismutase (Mn-SOD) and Catalase (CAT) Enzymes

Figure 3 (A-C) shows that cadmium chloride treatment of rats caused a marked alteration in the activities of Cu-Zn SOD, Mn-SOD and Catalase in the kidney tissue compared to that of control rats (46.29%, 43.40% and 34.37% respectively, p<0.001). When the rats were pre-treated with TA, the activities of Cu-Zn SOD, Mn-SOD and Catalase were found to be protected from being altered, in a dose-dependent manner. Tannic acid, at the dose of

25mg/kg bw exhibited maximum protection against cadmium chloride-induced alterations (72.97%, 61.16% and 24.60% respectively compared to the cadmium chloride-treated group, p<0.001).

Activities of Xanthine Oxidase (XO) and Xanthine Dehydrogenase (XDH) Enzymes

Figure 4 (A-B) shows that treatment of rats with cadmium chloride increased the activities of XO and XDH in the kidney tissue of rats compared to that of control animals (133.90% and 55.41%, p<0.001). When the rats were pre-treated with TA, the activities of XO and XDH were found to be protected from being increased, also, in a dose-dependent manner. Here also, TA offered maximum protection against cadmium chloride-induced alterations at the dose of 25 mg/kg bw (51.38% and 34.04% respectively compared to the cadmium chloride-treated group, p<0.001).

Activities of Pyruvate Dehydrogenase (PDH), Isocitrate Dehydrogenase (ICDH), Alpha-keto Glutarate Dehydrogenase (alpha-KGDH) and Succinate Dehydrogenase (SDH) Enzymes

Figure 5 (A-D) shows that treatment of rats with cadmium chloride significantly decreased the activities of PDH, ICDH, alpha-KGDH and SDH in the kidney tissue of rats compared to that of control rats (80.29%, 59.74%, 47.68% and 77.82%, respectively, p<0.001). When the rats were pre-treated with TA, the activities of PDH, ICDH, alpha-KGDH and SDH were found to be protected from being decreased, in a dose-dependent manner. The TA, at the dose of 25 mg/kg bw, offered maximum protection against cadmium chloride-induced alteration in the activities of PDH and some of the Kreb's cycle enzymes as mentioned above (380.54%, 70.79%, 310.90% and 122.22% respectively compared to the cadmium treated group, p<0.001).
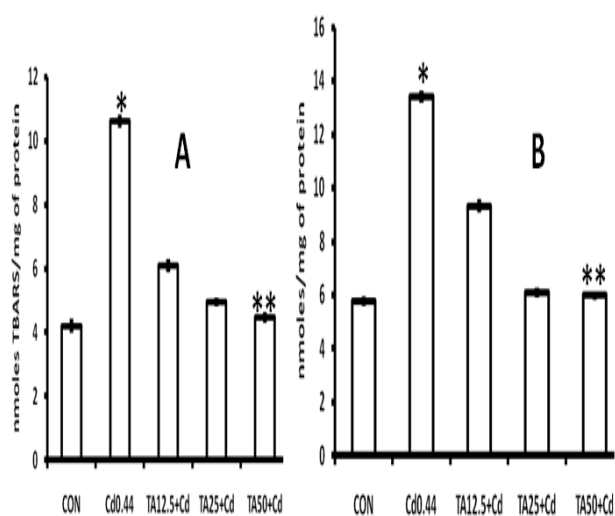

Figure 1: Protective effect of tannic acid against cadmium chloride-induced alteration in the level of lipid peroxidation (A) and protein carbonylation (B) of kidney tissue of rat. Values are expressed as Mean ± SE of 6

animals in each group. Cd = cadmium chloride treated group, dose 0.44 mg/kg bw; TA12.5+Cd 0.44= group pre-treated with tannic acid (administered orally) at the dose of 12.5 mg/kg bw followed by cadmium chloride (administered subcutaneously) 0.44mg/kg bw, respectively; TA25+Cd 0.44= group pre-treated with tannic acid (administered orally) at the dose of 25mg/kg bw followed by cadmium chloride (administered sub-cutaneously) with 0.44 mg/kg bw' respectively; TA50+Cd 0.44= group pre-treated with tannic acid (administered orally) at the dose of 50mg/kg bw followed by cadmium chloride (administered sub-cutaneously) with 0.44 mg/kg bw, respectively;

*P < 0.001 compared to control values using ANOVA.

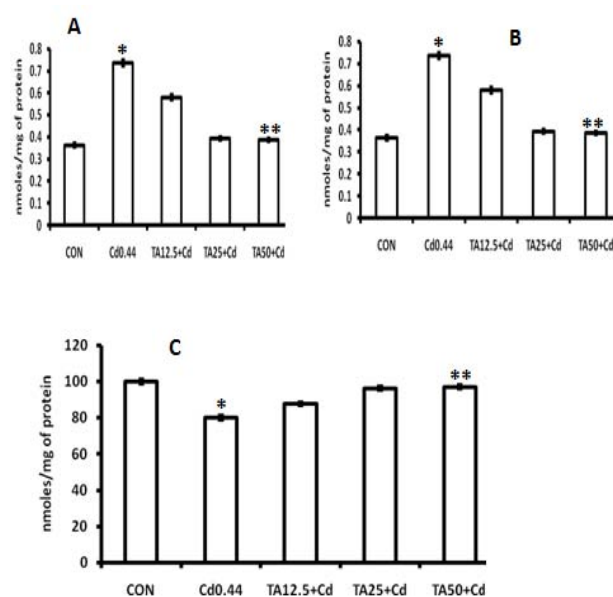**P < 0.001 compared to cadmium chloride-treated group using ANOVA.

Figure 2: Protective effect of tannic acid against cadmium-induced alteration in the contents of reduced (A) and oxidised (B) glutathione (GSH and GSSG) and total sulphydryl (C) in kidney tissue of rats. Values are expressed as Mean ± SE of 6 animals in each group.

Cd = cadmium chloride treated group, dose 0.44 mg/kg bw; TA12.5+Cd 0.44= group pre-treated with tannic acid (administered orally) at the dose of 12.5 mg/kg bw followed by cadmium chloride (administered subcutaneously) 0.44mg/kg bw, respectively; TA25+Cd 0.44= group pre-treated with tannic acid (administered orally) at the dose of 25mg/kg bw followed by cadmium chloride (administered subcutaneously) with 0.44 mg/kg bw' respectively; TA50+Cd 0.44= group pre-treated with tannic acid (administered orally) at the dose of 50mg/kg bw followed by cadmium chloride (administered subcutaneously) with 0.44 mg/kg bw, respectively;

*P < 0.001 compared to control values using ANOVA.

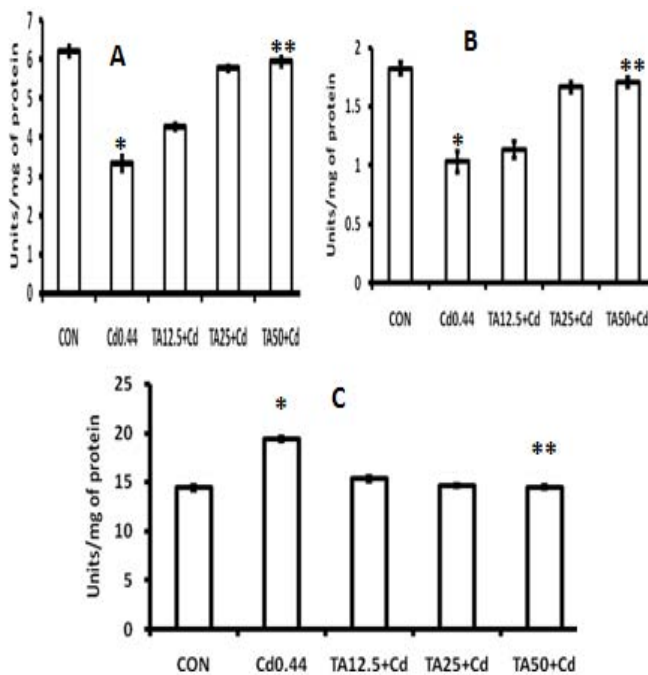**P < 0.001 compared to cadmium chloride-treated group using ANOVA.

Figure 3: Protective effect of tannic acid against cadmium-induced alteration in the activities of Cu-Zn superoxide dismutase (Cu-Zn SOD) (A), Mn-Superoxide dismutase (Mn-SOD) (B) and Catalase (CAT) (C) in kidney tissue of rats. Values are expressed as Mean ± SE of 6 animals in each group. Cd = cadmium chloride treated group, dose 0.44 mg/kg bw; TA12.5+Cd 0.44= group pre-treated with tannic acid (administered orally) at the dose of 12.5 mg/kg bw followed by cadmium chloride (administered subcutaneously) 0.44mg/kg bw, respectively; TA25+Cd 0.44= group pre-treated with tannic acid (administered orally) at the dose of 25mg/kg bw followed by cadmium chloride (administered subcutaneously) with 0.44 mg/kg bw' respectively; TA50+Cd 0.44= group pre-treated with tannic acid (administered orally) at the dose of 50mg/kg bw followed by cadmium chloride (administered subcutaneously) with 0.44 mg/kg bw, respectively; *P < 0.001 compared to control values using ANOVA. **P < 0.001 compared to cadmium chloride-treated group using ANOVA.
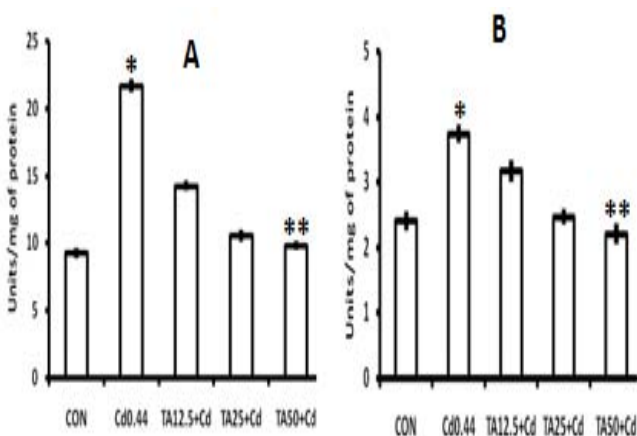

Figure 4: Protective effect of tannic acid against cadmium chloride-induced alteration in the activities of xanthine oxidase (A) and xanthine dehydrogenase (B) of kidney

tissue of rats. Values are expressed as Mean ± SE of 6 animals in each group. Cd = cadmium chloride treated group, dose 0.44 mg/kg bw; TA12.5+Cd 0.44= group pre-treated with tannic acid (administered orally) at the dose of 12.5 mg/kg bw followed by cadmium chloride (administered subcutaneously) 0.44mg/kg bw, respectively; TA25+Cd 0.44= group pre-treated with tannic acid (administered orally) at the dose of 25mg/kg bw followed by cadmium chloride (administered subcutaneously) with 0.44 mg/kg bw' respectively; TA50+Cd 0.44= group pre-treated with tannic acid (administered orally) at the dose of 50mg/kg bw followed by cadmium chloride (administered sub-cutaneously) with 0.44 mg/kg bw, respectively; *P < 0.001 compared to control values using ANOVA. **P < 0.001 compared to cadmium chloride-treated group using ANOVA.
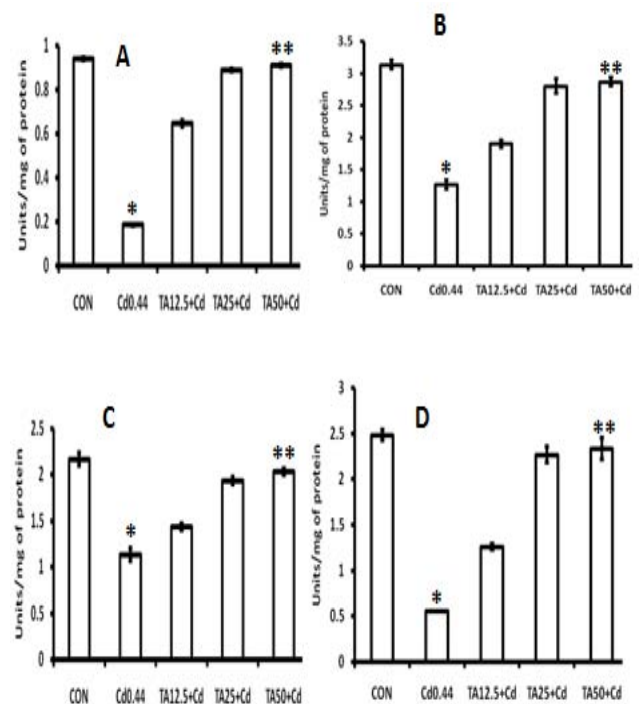

Figure 5: Protective effect of tannic acid against cadmium chloride-induced alteration in the activities of pyruvate dehydrogenase (A), isocitrate dedydrogenase (B), alpha-keto glutarate dehydrogenase (C) and succinate dehydrogenase (D) of kidney tissue of rats. Values are expressed as mean ± SE of 6 animals in each group.

Cd = cadmium chloride treated group, dose 0.44 mg/kg bw; TA12.5+Cd 0.44= group pre-treated with tannic acid (administered orally) at the dose of 12.5 mg/kg bw followed by cadmium chloride (administered subcutaneously) 0.44mg/kg bw, respectively; TA25+Cd 0.44= group pre-treated with tannic acid (administered orally) at the dose of 25mg/kg bw followed by cadmium chloride (administered subcutaneously) with 0.44 mg/kg bw respectively; TA50+Cd 0.44= group pre-treated with tannic acid (administered orally) at the dose of 50mg/kg bw followed by cadmium chloride (administered subcutaneously) with 0.44 mg/kg bw, respectively;*P < 0.001 compared to control values using ANOVA. **P <

0.001 compared to cadmium chloride-treated group using ANOVA.

Histological Studies

Tissue morphological studies showed damage in renal tubules in kidneys of cadmium treated rats. The damage was found to be dose-dependently ameliorated with tannic acid. Here also, the protection by TA was found to be almost complete at the dose of 25 mg/kg bw (Figure 6).
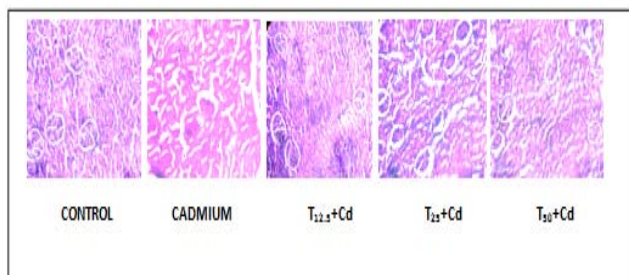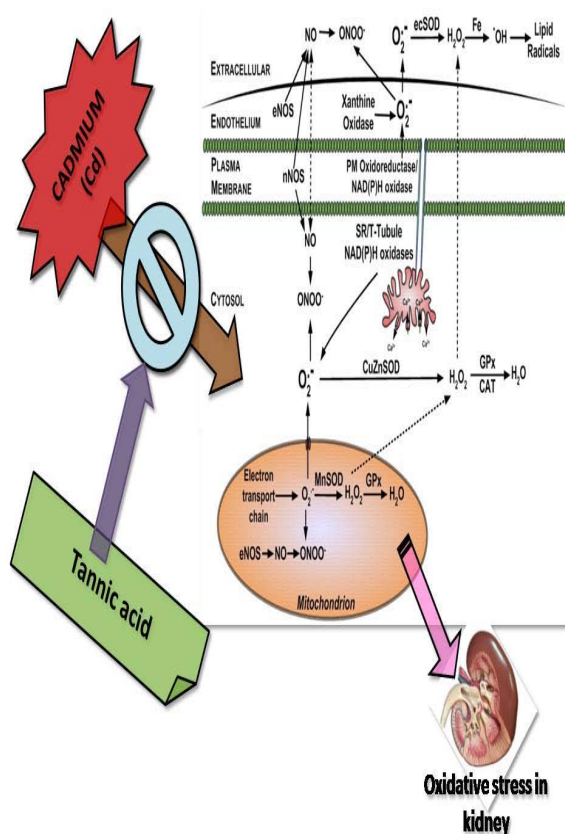

Figure 6: Histopathological studies of renal tissue [200X]

Scheme 1: Mechanism of the protective action(s) of tannic acid against cadmium-induced nephro-toxicity

DISCUSSION

From the results of our study it is seen that cadmium chloride when administered subcutaneously at a dose of 0.44 mg/kg bw to rats caused a significant increase in the level of lipid peroxidation, changes in the content of protein carbonyl, reduced glutathione, oxidised glutathione, total glutathione, activities of cytosolic superoxide dismutase, mitochondrial superoxide dismutase, catalase. Treatment of rats with cadmium chloride also altered the activities of the PDH and some of the Kreb's cycle enzymes and damaged the normal cyto-architechture of renal tissue in male Wistar rats.

Increase in the level of lipid peroxidation is a well established observation for ascertaining oxidative stress in experimental models[2]. Cadmium stimulates generation of free radicals in tissues they reach[3].

Those free radicals generated in presence of oxygen are primarily reactive oxygen species which initiates and propagates a chain oxidation reaction process with the lipid and protein components of the cellular membranes. Proteins are altered and oxidised to protein carbonyls[38]. Lipids get converted to peroxides[39]. Thus, those changes disrupt the normal cellular membrane integrity and cause cellular damages.

Organs get damaged and thus their functions are also hampered. Glutathione is oxidised in the process of scavenging the reactive oxygen species[40]. Hence in situation of oxidative onslaught, reduced glutathione and oxidized glutathione contents are altered. We have observed an increase in oxidised glutathione content which seems normal and expected in condition of cadmium induced oxidative stress. Pre-treatment of rats with TA was found to protect the biomarkers of oxidative stress from being altered.

Reactive oxygen species formed in the presence of cadmium could be responsible for its toxic effects in various organs. The nephro-toxic actions of cadmium may be mediated by cadmium–metallothioneins (MT) complex release from damaged kidney cells filtered through the glomerulus into the urinary space, where it is endo-cytosed by proximal tubular cells and degraded by lysosomes, resulting in the release of cadmium. The released cadmium may stimulate the production of MT in the proximal tubular cells thereby directly damaging the integrity of microvilli and intracellular vesicles, indirectly inhibit the transporter activity through changes in membrane fluidity due to oxidative stress, increase the LPO by binding with membrane phospholipid and target various intra-cellular proteins and membrane transporters at the cytoplasmic side by binding to their reactive SH groups[35]. The TA was found to be potent enough in protecting cadmium-induced renal damage. Considering the relationship between cadmium exposure and oxidative stress, it is reasonable that the administration of some anti-oxidant should be an important therapeutic approach against metal induced oxidative stress which is an emerging area of research.

Mitochondria are the seat of generation of ROS. In this study, there has been considerable decrease in the activities of PDH and some of the TCA cycle enzymes, such as ICDH, Alpha KGDH and SDH following cadmium treatment of rats. However, when the rats were pre-treated with TA, the activities of PDH and TCA cycle enzymes were found to be almost completely protected.

This indicates that TA is able to protect the mitochondria from the damaging effects of ROS, perhaps by either scavenging the ROS or by preventing ROS generation.

The histo-pathological examination of the rat kidney tissue in cadmium treated rats showed tubular necrosis, inflammatory cell infiltration, tubular degeneration, haemorrhage, swelling of tubules and vacuolization and damages of podocytes. This could be due to either formation or accumulation of free radicals as well as increased LPO by free cadmium ions in the renal tissues of cadmium treated rats.

The current study reveals that TA may protect the renal tissues against cadmium-induced damages possibly through its antioxidant activity. The probable mechanism of action of tannic acid against cadmium induced deteriorative oxidative damages in rat kidneys is represented schematically in scheme 1.

REFERENCES

1. Ghosh D, Firdaus SB, Mitra E, Dey M, Chattopadhyay A, Pattari S K, Dutta S, Jana K and Bandyopadhyay D, Hepatoprotective activity of aqueous leaf extract of *Murraya koenigii* against lead-induced hepatotoxicity in male Wistar rat, Int J Pharm Pharm Sci, 5, 2013, 285-295.

2. Ghosh D, Mitra E, Dey M, Firdaus SB, Ghosh AK, Mukherjee D, Chattopadhyay A, Pattari SK, Dutta S And Bandyopadhyay D, Melatonin protects against lead-induced oxidative stress in rat liver and kidney, Asian J Pharma Clin Res, 6, 2013, 137-145.

3. Mitra E, Ghosh D, Ghosh AK, Basu A, Chattopadhyay A, Pattari S K, Datta S, Bandyopadhyay D, Aqueous tulsi leaf (*ocimum sanctum*) extract possesses antioxidant properties and protects against cadmium-induced oxidative stress in rat heart, Int J Pharm Pharm Sci, 6, 2014, 500-513.

4. Khalid G. Al-Fartosi, Tannic acid (TA) protects against cadmium acetate induce toxicity in female rats (Role of tannic acid as an antioxidant), J Thi-Qar Sci, 2, 2010, 216-226.

5. Mitra E, Ghosh AK, Ghosh D, Mukherjee D, Chattopadhyay A, Datta S, Pattari S K, Bandyopadhyay D, Protective effect of aqueous leaf (Murraya koenigii) extracts against cadmium-induced oxidative stress, Food Chem Toxicol, 50, 2012, 1340–1353.

6. Gutteridge J.M, Lipid peroxidation and antioxidants as biomarkers of tissue damage, Clin Chem, 41, 1995, 1819-828.

7. Gurer H, Ercal N, Can antioxidants be beneficial in the treatment of lead poisoning? Free Radic Biol Med, 29, 2000, 927-945.

8. Halliwell B, Gutteridge JMC, Protection against oxidants in biological systems: the superoxide theory of oxygen toxicity, Free Radic Biol Med, 1989, 86-123.

9. Mudipalli P, Lead hepatotoxicity and potential health effects. Ind J Med Res, 126, 2007, 518-527.

10. Ghosh D, Mitra E, Dey M, Firdaus SB, Ghosh AK, Mukherjee D, Chattopadhyay A, Pattari SB, Dutta S, Bandyopadhyay D, Melatonin protects against lead-induced oxidative stress in rat liver and kidney, Asian J Pharma Clin Res, 6, 2013, 137-145.

11. Block G, Patterson D, Subar A, Fruit, vegetables and cancer prevention: a review of the epidemiological evidence, Nutr Cancer, 18, 1992, 1-29.

12. Daniel EM, Stoner GD, The effect of ellagic acid and 3-cis retinoic acid on N-nitrobenzymethylamine induced esophageal tumorigenesis in rats, Cancer Lett, 56, 1991, 117-124.

13. Hirono I, Naturally occurring carcinogens of plant origin, 1987, 161-162.

14. Dutta M, Ghosh D, Ghosh AK, Rudra S, Bose G, Dey M, Bandyopadhyay A, Pattari SK, Mallick S, Chattopadhyay A, Bandyopadhyay D. High fat diet aggravates arsenic induced oxidative stress in rat heart and liver, Food Chem Toxicol, 66, 2014, 262-277.

15. El-Sokkary GH, Kamel ES, Reiter RJ. Prophylactic effect of melatonin in reducing lead-induced neurotoxicity in the rat. Cell Mol Biol Lett, 8, 2003, 461-470.

16. Ghosh D, Firdaus SB, Mitra E, Dey M, Chattopadhyay A, Pattari SK, Dutta S, Jana K and Bandyopadhyay D. Aqueous leaf extract of *Murraya koenigii* protects against lead-induced cardio toxicity in male Wistar rats. Int J Pharmacol, 4, 2013, 119-132.

17. Mishra S, Ghosh D, Dutta M, Chattopadhyay A, Bandyopadhyay D. Melatonin protects against lead-induced oxidative stress in stomach, duodenum and spleen of male Wistar rats, J Pharm Res, 1, 2013, 997-1004.

18. Dutta M, Ghosh AK, Mohan V, Shukla P, Rangari V, Chattopadhyay A, Das T, Bhowmick D, Bandyopadhyay D. Antioxidant mechanism(s) of protective effects of Fenugreek 4-hydroxyisoleucine and trigonelline enriched fraction (TF4H (28%)) Sugaheal® against copper-ascorbate induced injury to goat cardiac mitochondria *in vitro*. J Pharm Res, 8, 2014, 798-811.

19. Buege JA, Aust SG. Microsomal Lipid Peroxidation, Methods Enzymol, 52, 1978, 302–310.

20. Mishra S, Dutta M, Mondal S K, Dey M, Paul S, Chattopadhyay A, Bandyopadhyay D. Aqueous bark extract of *Terminalia arjuna* protects against adrenaline-induced hepatic damage in male albino rats through antioxidant mechanism(s): a dose response study, J Pharm Res, 8, 2014, 1264-1273.

International Journal of Pharmaceutical Sciences Review and Research
Available online at www.globalresearchonline.net
280

21. Sedlak J, Lindsay RH. Estimation of total, protein-bound, nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem, 25, 1968, 192–205.

22. Dutta M, Ghosh AK, Basu A, Bandyopadhyay D, Chattopadhyay A. Protective effect of aqueous bark extract of *Terminalia arjuna* against copper-ascorbate induced oxidative stress *in vitro* in goat heart mitochondria. Int J Pharm Pharma Sci, 5, 439-447.

23. Dutta M, Ghosh AK, Mishra P, Jain G, Rangari V, Chattopadhyay A, Das T, Bhowmick D, Bandyopadhyay D. Andrographolide, One of the Major Components of *Andrographis paniculata* Protects against Copper-Ascorbate Induced Oxidative Damages to Goat Cardiac Mitochondria *In-Vitro.* Int J Pharma Sci Rev Res, 28, 2014, 237-247.

24. Dutta M, Chattopadhyay A, Ghosh A K, Chowdhury U R, Bhowmick D, Guha B, Das T, Bandyopadhyay B. Benzoic acid, one of the major components of aqueous bark extract of *Terminalia arjuna* protects against Copper-Ascorbate induced oxidative stress in human placental mitochondria through antioxidant mechanism(s): an *in vitro* study, J Pharm Res, 9, 2015, 64-88.

25. Martin JP, Daily M, Sugarman E, Negative and Positive assays of superoxide dismutase based on hematoxyline autooxidation, Arch Biochem Biophysi, 255, 1987, 329-326.

26. Marklund S, Marklund G, Involvement of the superoxide anion radical in the autoxidation of pyragallol and a convenient assay for superoxide dismutase, Eur J Biochem, 47, 1974, 469-474.

27. Dutta M, Ghosh AK, Mishra P, Jain G, Rangari V, Chattopadhyay A, Das T, Bhowmick D, Bandyopadhyay D, Protective effects of piperine against copper-ascorbate induced toxic injury to goat cardiac mitochondria *in vitro*. Food Funct, 5, 2014, 2252–2267.

28. Beers RF Jr, Sizer IW, A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase, J Biol Chem, 195, 1952, 133-140.

29. Greenlee L, Handler P, Xanthine oxidase. IV, Influence of pH on substrate specificity, J Biol Chem, 239, 1964, 1090–1095.

30. Strittmatter C, Studies on avian xanthine dehydrogenases: properties and patterns of appearance during development, J Biol Chem, 240, 1965, 2557-2564.

31. Chretien D, Pourrier M, Bourgeron T, Séné M, Rötig A, Munnich A, Rustin P, An improved spectrophotometric assay of pyruvate dehydrogenase in lactate dehydrogenase contaminated mitochondrial preparations from human skeletal muscles, Clin Chim Acta, 240, 1995, 129-136.

32. Dutta M, Ghosh AK, Rangari V, Jain G, Khobragade SM, Chattopadhyay A, Bhowmick D, Das T, Bandyopadhyay D, Silymarin protects against copper-ascorbate induced injury to goat cardiac mitochondria *in vitro*: involvement of antioxidant mechanism(s), Int J Pharm Pharm Sci, 6, 2014, 422-429.

33. Duncan M, Fraenkel DG, Alpha-ketoglutarate dehydrogenase mutant of Rhizobium meliloti, J Bacteriol, 137, 1989, 415-419.

34. Dutta M, Chattopadhyay A, Bose G, Ghosh A, Banerjee A, Ghosh A, Mishra S, Pattari S K, Das T and Bandyopadhyay D, Aqueous bark extract of *Terminalia arjuna* protects against high fat diet aggravated arsenic-induced, *J Pharm Res,* 8, 2014, 1285-1302.

35. Dutta M, Ghosh AK, Mohan V, Thakurdesai P, Chattopadhyay A, Das T, Bhowmick D, Bandyopadhyay D, Trigonelline [99%] protects against copper-ascorbate induced oxidative damage to mitochondria: an *in vitro* study, J Pharm Res, 8, 2014, 1694-1718.

36. Veeger C, DerVartanian DV, Zeylemaker WP, Succinate dehydrogenase, Method Enzymol, 13, 1969, 81-90.

37. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with Folin phenol reagent, J Biol Chem, 193, 1970, 265–275.

38. Romero-Puertas MC, Palma JM, Gómez M, Del Río L A and Sandalio LM, Cadmium causes the oxidative modification of proteins in pea plants, Plant Cell Environ, 25, 2002, 677–686.

39. Inoue M, Inter-organ metabolism of glutathione as the defense mechanism against oxidative stress. In: Sakamoto Y, Higashi T, Taniguchi N, eds. Glutathione centennial. New York: Academic Press, 1989, 381–394.

40. Geetha RK, Vasudevan DM, Inhibition of lipid peroxidation by botanical extracts of *Osmium sanctum*: *in vivo* and *in vitro* studies. Life Sci, 76, 2004, 21-28.