Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Advances in Applied Science Research, 2011, 2 (2): 69-78

Pelagia Research Library

ISSN: 0976-8610
CODEN (USA): AASRFC

Protective influence of dietary nutrients on antioxidant defense system in the blood of rats treated with cadmium

Deepti gaurav*, Shabad Preet and K. K. Dua

Department of Zoology, Dayalbagh Educational Institute (Deemed University), Dayalbagh, Agra, India

---

ABSTRACT

The present study was planned to elucidate the role of dietary nutrients alone and their combination in the modification of toxicity of cadmium with emphasis on vital organ dysfunction. The animals were randomly divided into seven groups where the control group (Group I) receiving physiological saline (p.o.) and group II was administered with cadmium chloride (0.1mg/kg) only group III, IV, V, VI and VII receiving 2.5 mg/kg dietary nutrients i.e. N-acetyl cystiene, methionine, melatonin, Vit-B_1 and their combination. All dietary nutrients were administered orally for 21 days with a concomitant subcutaneous (s.c.) cadmium treatment. It was shown that exposure to cadmium induced a significant increase in urea, creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum whereas decrease in haematocrit value (Htc), haemoglobin concentration (Hb) and blood glutathione (GSH) in blood of rats. Administering of dietary nutrients alone or their combination with cadmium chloride resulted increase in GSH level in blood and statistically significant decrease in AST, ALT, urea and creatinine level in serum as well as decreased Cd accumulation in liver and kidney in relation to animals receiving cadmium chloride alone.

Keywards: Blood, Serum, Dietary nutrients, Glutathione, Free radicals.
Abbreviations: Alanine aminotransferase (ALT); Aspartate aminotransferase (AST); Haematocrit value (Htc); Haemoglobin concentration (Hb); Blood glutathione (GSH); N-acetyl cysteine (NAC); Methionine (MT); Thiamine (Vit-B_1); Zinc (Zn); Cadmium (Cd); S-adenosylmethionine (SAM)

INTRODUCTION

Cadmium is a common contaminant of hazardous waste site and is released from sources such as fossil fuel combustion and municipal waste incineration and as a component of cigarette smoke. After the intake and resorption, cadmium enters the blood where it binds to the erythrocyte membrane and protein of two molecular mass forming metallothionein [1]. Binding of cadmium

to red blood cells (RBCs) causes their destruction and increased haemolysis and haematological alters. It was induced decreased absorption of intestinal iron and anemia appears. Cadmium derives its toxicological properties from its chemical similarity to zinc an essential micronutrient for plants, animals and humans. It is known that various chelator substances and antioxidant substances like metallothionein, vitamin E, selenium, neominophagen, and melatonin reduce cadmium toxicity and to lessen oxidative stress [2-5].

Zinc (Zn) is an important antioxidant, decreasing ROS production [6]. Some studies have reported the ability of Zn to interact with essential elements such as Cu and Fe, decreasing their content in tissues and retarding the oxidative processes. Numerous studies have shown that Zn supply may reduce Cd absorption and accumulation, and also prevent or reduce the adverse actions of Cd [7], whereas Zn deficiency can intensify Cd accumulation and toxicity [8].

Methionine (MT) is an essential amino acid involved in protein synthesis [9]. It is one of the main sources of glutathione but is also metabolized to S-adenosylmethionine (SAM) that mediates most biochemical methylation reactions [10]. The body also needs plenty of methionine to produce two other sulfur-containing amino acids, cysteine and taurine, which help the body to eliminate toxins, build strong, healthy tissues, and promote cardiovascular health.

N-acetyl cystiene (NAC) can serve as a cysteine donar for GSH synthesis .Glutathione is an antioxidant and can also form complexes with cadmium to alter cadmium distribution and excretion [2]. However, NAC did not cause further elevation in GSH levels over that produced by cadmium administration.

Thiamine (Vit-B_1) as a protective agent against short term metal intoxication and it decreased mortality and metal accumulation in different tissue. Thiamin is involved in numerous body functions, including: nervous system and muscle functioning, flow of electrolytes in and out of nerve and muscle cells (through ion channels), multiple enzyme processes (via the coenzyme thiamin pyrophosphate); carbohydrate metabolism and production of hydrochloric acid (which is necessary for proper digestion).

The aim of the present study was to evaluate effects of the individual and combined administration of dietary nutrients i.e. NAC, MT, Vit-B_1 and Zn on erythrocytes GSH, serum ALT, AST, creatinine, and urea after high dose of $CdCl_2$ in male rats. However, no attention has been paid so far to explore its haematoprotective activity in animals and human beings. In continuation of our work exploring protective activities of dietary nutrients on sub chronic serum and haematological alterations.

MATERIALS AND METHODS

Chemicals

Zinc chloride, cadmium chloride anhydrous sodium chloride, melatonin, N-acetyl cystiene methionine and Vit-B_1 from sigma chemical co. (USA). All other chemicals and reagents used were of A.R. grade.

Animals and treatments

Male Wistar albino rats weighing 200±10 g were obtained from the Defense Research and Development Establishment (DRDE) animal facility, Gwalior (India). The animals were maintained in individual stainless steel cages under controlled conditions (23±1°C, 12-h light–dark cycle, relative humidity of 50±10%) and had access to a standard rodent laboratory diet (g) and drinking water. Animals were orally administered dose of dietary nutrients using canula. Monitoring of individual body weights of animals was regularly done (Reg No. 37/99/CPCSEA, dated 11th Mar 1999, renewed 2011). As follows:

Groups I normal saline, orally (negative control)
Groups II 0.1mg/kg $CdCl_2$, sc (positive control)
Groups III 0.1mg/kg $CdCl_2$, sc + Vit-B_1 (2.5mg/kg, orally)
Groups IV 0.1mg/kg $CdCl_2$, sc + methionine (2.5mg/kg, orally)
Groups V 0.1mg/kg $CdCl_2$, sc + N-acetyl cysteine (2.5mg/kg, orally)
Groups VI 0.1mg/kg $CdCl_2$, sc + Zn (2.5mg/kg, orally).
Groups VII 0.1mg/kg $CdCl_2$, sc + Combination (Methionine+ Zn+ Vit-B_1+ NAC, 2.5mg/kg, orally).

The animals were fasted for 12 hrs and then dissected under light chloroform, anesthesia. Blood was collected directly from cardiac puncher. Some fraction of the whole blood was taken in vials with anticoagulant (5 % EDTA). Serum was separated from rest of the blood. For separation of serum, blood samples were kept at room temperature for 30 minutes to permit clot retraction before centrifugation at 2500xg for 10 min. Freshly removed organs like liver and kidney were washed from extraneous material using chilled normal saline solution and blotted dry, weighed and enclosed in parafilm aluminum foil and stored at -20ºC for the estimation of enzymes and metals.

Blood hemoglobin concentration was determined by standard cyanmethemoglobin procedure [11] whereas, PCV was studies by haematocrit method [12]. Analysis of blood GSH concentration was performed by modified method of Ellman [13].

Alanine aminotransferase, aspartate aminotransferase, urea and creatinine were measured using commercially available kit (Ranbaxy India Ltd.).

The concentration of cadmium, copper and zinc in liver and kidney was measured by standard method of Parker [14].

The data are presented as mean ± S.E.M. value. Number of animals per group stated in the table or figure legends. One way analysis of variance (ANOVA) followed by Student-Newman- Keuls test was used to analyze mean differences between experimental groups for each parameter separately after ascertaining the homogeneity of variance between treatment groups by Bartlett`s test. Composite treatments were compared using one-way analysis of variances (ANOVA) and considered significantly different where probability values were found to be equal to or less than 0.05. All ANOVA tests, as well as mean and standard error of mean calculations, were performed using Graph Pad Prism (Graph Pad Software, Inc., San Diego, USA).

RESULTS

Data presented in table 1 exhibits a decrease in the levels of Hb, Htc and GSH by 92%, 67% and 63% in Cd intoxicated rats as compared to rats receiving saline on day 21. Hb concentration does not appears to be affected by dietary nutrients supplementation as a function of time except methionine where the Hb level was significantly ($p<0.01$) higher. A significant decline was observed in the level of GSH induced by Cd was reversed by dietary nutrients supplementation with more pronounced recovery by NAC treated group. However combination of dietary nutrients was found to be most effective in recovering Cd induced decrease in haematological variable. Indeed the levels of Hb, Htc and GSH in treated rats approached the normal value in control rats.

Cd administration resulted in significant increase in AST, ALT, URE and CRE activities in serum by 276%, 276%, 129% and 124% on day 21. All dietary nutrients when administered individually had beneficial effect on altered level of aminotransferase as indicated by a decrease in serum AST, ALT, URE and CRE. Moreover, combined treatment (methionine+NAC+Zn+Vit-B_1) was notably effective in recovery of hepatic (AST and ALT) and renal (URE and CRE) markers which is evident in table 2.

Table 3 shows cadmium concentration in blood, liver and kidney of Cd exposed animals after 21 days and the ability of dietary nutrients either alone or in combination to reduce its concentration. It is evident from the results that a significant increase has occurred (blood, 2.0 fold; liver, 2.5 fold and kidney, 2.7 fold) in Cd exposed animals as compared to the control group. Administration of Vit-B_1 showed non-significant decrease in Cd concentration in the tissues while combined administration of dietary nutrients with Cd provided the best effects in reducing Cd concentration from these tissues than any other treatment group.

Additionally, concentration of copper and zinc was determined in blood, liver and kidney by AAS spectrophotometer. A marked decrease was observed in the level of Cu (blood, 48 %; liver, 70 % and kidney, 89 %) in rats exposed to Cd as compared to control rats (Table 4). Exposure to dietary nutrients produced non significant elevation in the blood and kidney except that there was significant increase noted in Cu concentration of liver in animals individually supplemented with NAC and Vit-B_1 and combinational group as compared to normal group.

Concentration of Zn in blood, liver and kidney of rats following 21 days Cd exposure is shown in table 5. Exposure to Cd resulted in significant depletion of Zn levels (blood, 40 %; liver, 46 % and kidney 71 %) while the supplementation of dietary nutrients had marginal effect in reversing Zn concentration. However, combined treatment with NAC, Zn, Met and Vit-B_1 offered a protective effect against Cd induced toxicity.

DISCUSSION

Cadmium is toxic metal that is widely used in different industries. It promotes an early oxidative stress and afterward contributes to the development of serious pathological conditions because of its long retention in some tissues [15]. The damaging action of cadmium on the soft tissues during its exposure has been extensively studied [16]. The hypothesis that the supplementation of

dietary nutrients either individual or in combination offers beneficial effects in reversing Cd induced oxidative stress was examined in the present study.

The present study reveals that rats exposed to Cd alone showed significant reduction in haematological variables such as Hb, Htc and GSH. The decrease in hematological parameters (Hb, GSH and Htc) is in agreement with the work of Karmakar [17] who showed that cadmium chloride also caused changes in the blood indices of rats. The results obtained in present study show that treatment with Cd induced anemia in rats. It is well known that the presence of cadmium in the rats decreased the level of iron in the blood [18] which was responsible for declined Hb concentration. The reduction in Hb content may be due to increased rate of either destruction or reduction in the rate of formation of erythrocytes. In addition, the reduction in the blood parameters may be attributed to hyperactivity of bone marrow leading to production of red blood cells with impaired integrity that are unstable in the circulation.

Hematocrit is another haematological variable directly related to Hb content and variations in Hb content are directly manifested in PCV value. There may be three possible causes for the decrease of the Htc during the stress: increase on the volume of the plasma, loss of water in the erythrocyte and haemolysis of erythrocytes in the blood stream. The response to stress is characterized by hormone change (catecholamine and corticosteroid) that induced alteration on the haematological parameter. Shukla [19] and Hamada [20] have also confirmed the similar reason for decreasing packed cell volume in rats.

The results of our experiments showed that, in animals exposed to Cd, the GSH was significantly decreased as compared to control rats. The reduction in activity of GSH might be due to its consumption in the scavenging free radicals generated by Cd [21 & 22]. Also, GSH may be consumed in the detoxification of Cd. In fact, it has been reported that the sulfhydryl group of cysteine moiety of glutathione has a high affinity for metals such as Cd, forming thermo-dynamically stable mercaptides complexes which are inert and excreted *via* the bile [23].

The activity of AST and ALT enzymes in blood serum may also be used as stress indicator. The significant changes in the activities of these enzymes in blood serum indicate tissue impairment caused by stress [24]. In the present study there were significant changes in AST and ALT activities in serum of rats exposed to Cd compared to the control group. The increase in concentration of AST and ALT in blood serum indicates impairment of parenchymatous organ such as liver. Additionally, elevated serum levels of these variables may be due to hepatocellular necrosis which causes increase in the permeability of the cell membrane resulting the release of transaminases in the blood stream. The increase in plasma AST and ALT activities indicates an active transamination of amino acids and involvement of keto acids that are probably fed into tricarboxylic acid cycle (TCA) for oxidation. The increase in the liver AST and ALT activities may be due to liver dysfunction and disturbance in the synthesis of these enzymes. Therefore, the increase in the activities of AST and ALT in plasma indicating the hepatotoxic effect of cadmium chloride and is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream [25]. Administration of dietary nutrients was beneficial in reversing the levels of ALT and AST very close to the normal. These results are in agreement with the findings of Konar [26] who noticed protective effects of various combinations of melatonin, Vit-E and Se in Cd exposed rats. The application of dietary nutrients and their combination restored the control

value in different serum contents tested during different time of exposure. It may testify the possibility of dietary nutrients arresting cadmium free radicals, consequently decreasing the damage caused by this metal.

Previous studies have demonstrated that Cd intoxication may also result in renal tubule damage specifically glomerular filtration impairment [27]. This may account for the increase of urea and creatinine concentration in the animals receiving cadmium chloride in the present study. The damaging effects of cadmium on kidneys have also been described by other authors [28 & 29].

Present results coincide with earlier reports that a significant increase in serum urea and creatinine or decrease in potassium levels [30 & 31] might indicate a nephrotoxic condition in Cd-treated rats and may be due to kidney damage caused by the enhanced generation of ROS.

In the present study, activity of creatinine was increased significantly in the kidney of Cd exposed rats. This may be due to the damage of large number of nephrons. Only renal dysfunction changes the results, however, the serum creatinine level will not rise until at least half of the kidney's nephron are destroyed or damaged. Because creatinine rise and fall more slowly than urea levels, CRE levels are often preferred to monitor renal function on a long term exposure [32].

Our findings show that 0.1 mg/kg $CdCl_2$ administration for 21 days significantly increased levels of serum AST, ALT, URE and CRE. These findings are similar to the findings put forth by the previous researchers [33 & 34]. Several researchers reported that various dietary nutrients like Vit-E [35], Se [36], NAC [37] and melatonin [26] were effective against haematological toxicity caused by cadmium chloride.

Our results indicated a significant increase in the toxic metal level in the liver, kidney and blood with higher amount in the kidney which was evident from the data showing maximum accumulation of cadmium after 21 days (Table 3). Our observations are in agreement with the findings of previous workers [38 & 39].

In fact, it has been reported that, after its absorption, Cd is taken up by the hepatocytes, and then from the liver it circulates in blood bound to metallothionein (MT). The Cd–metallothionein complex (CdMT), because of its small molecular size, gets easily filtered through the glomerular membrane and taken up by renal tubular cells. MT is then catabolized releasing Cd ions in the cytoplasm where they induce synthesis of new MT molecules. This, in turn, binds and retains Cd in the kidney for a long period of time [40]. In Zn co-treated animals, although we have found a significant decrease in renal and hepatic Cd levels. We noticed an improvement in the Cd-induced damage in the liver, but above all, a complete prevention of the renal structural changes was observed. Our findings are in agreement with the work of Jacquiller [41] who reported that the effect of co-treatment with Zn during Cd administration completely prevented the changes in the renal function produced by the toxic metal in the rat, even though they did not find any significant difference in the renal Cd content. Previous studies [42] concluded that Zn protection is perhaps due to redistribution of Cd in the organism since Zn is able to induce synthesis of MT in the liver and kidney. In recent studies, Zn has been demonstrated to play an active role in preventing oxidative stress, apoptosis and necrosis induced by Cd [8].

In addition to Cd, Cu and Zn levels were also determined as they are essential elements for the maintenance of life and health. In the rats treated with Cd there was a significant decrease in the levels of these trace elements as compared to control. This may be due to interference of cadmium on absorption and transport of these trace elements, which would have resulted in the depletion of these metals in this group of rats. Cd may inhibit zinc activities at many stages, interfering with absorption, distribution and transport of zinc into cells or into several intracellular structures [43 & 44]. Co-administration of dietary nutrients either individually or in combination normalized the levels of these trace elements in blood and tissue as compared to Cd intoxicated rats [45].

Table 1: Cd induced changes in haemoglobin, blood glutathione and haematocrit and their response to treatment with dietary nutrients alone or in combination in blood of albino rats, during three weeks of exposure.

Groups	Treatments	Haemoglobin (mg/dl)	Blood glutathione (GSH) (mg/ml)	Haematocrit (%)
I	Control	14.62±0.41	3.29±0.04	37.4±2.18
II	Cd alone	13.54±0.18^{x}	2.08±0.28^{x}	25.0±0.95^{x}
III	Cd+NAC	14.07±0.40^{a}	2.78±0.30^{a}	37.0±1.87^{a}
IV	Cd+Met	13.99±0.54^{a}	2.53±0.31^{a}	35.0±1.03^{b}
V	Cd+Zn	13.99±0.55^{a}	5.53±0.08^{b}	33.4±1.40^{a}
VI	Cd+Vit-B_1	14.31±0.28^{a}	3.82±0.17^{b}	34.0±2.97^{a}
VII	Cd+Combination	15.87±0.39^{a}	6.30±0.19^{b}	37.8±2.01^{b}

Results are expressed as mean ±S.E.M. (n=8). $^{x}p<0.05$ *compared to control,* $^{a}p<0.05$, $^{b}p<0.01$ *compared to cadmium treated rats.*

Table 2: Cd induced changes in activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine and their response to treatment with dietary nutrients alone or in combination in serum of albino rats, during three weeks of exposure.

Groups	Treatments	Aspartate aminotransferase (AST) (IU/L)	Alanine aminotransferase (ALT) (IU/L)	Urea (mg/dl)	Creatinine mg/dl
I	Control	29.2±0.86	123±4.89	38.0±1.14	2.5±0.19
II	Cd alone	80.6±1.21	340±31.5^{x}	59.7±2.13^{x}	3.1±0.22^{x}
III	Cd+NAC	29.4±0.67^{b}	98.0±5.58^{b}	44.3±0.88^{b}	2.4±0.36^{b}
IV	Cd+Met	34.0±0.42	114±6.67^{b}	32.4±0.51^{b}	1.8±0.18^{b}
V	Cd+Zn	34.2±0.71	106±3.68^{b}	37.2±1.72^{b}	0.8±0.03^{b}
VI	Cd+Vit-B_1	34.2±1.17	99.7±0.68^{b}	34.0±2.63^{b}	0.9±0.03^{b}
VII	Cd+Combination	25.0±0.93^{b}	97.3±0.64^{b}	28.2±1.26^{b}	0.8±0.02^{b}

Results are expressed as mean ±S.E.M. (n=8). $^{x}p<0.05$, *compared to control,* $^{b}p<0.01$ *compared to cadmium treated rats*.

In conclusion, this study demonstrated that oral supplementation of individual dietary nutrients or their combination protect against Cd induced oxidative damage and ameliorated the negative effects of Cd on antioxidant status with lowering the Cd levels in tissues, thus act by mechanisms different from therapeutic approaches. However, this antioxidative capacity of nutrients became most effective when administered in combination (Met+Zn+Vit-B_1+NAC).

Table 3: Effect of dietary nutrients on cadmium concentration in blood, liver and kidney of albino rats exposed to cadmium, during three weeks of Cd exposure.

Groups	Treatments	Blood (µg/ml)	Liver (µg/g)	Kidney (µg/g)
I	Control	0.92±0.07	0.02±0.55	0.05±0.02
II	Cd alone	2.88±0.13^{x}	2.52±0.42^{x}	2.73±0.42^{x}
III	Cd+NAC	2.32±0.48	1.51±0.09^{b}	2.49±0.41
IV	Cd+Met	1.93±0.15^{b}	1.85±0.24	2.56±0.53
V	Cd+Zn	1.99±0.14^{b}	2.02±0.23	2.13±0.10
VI	Cd+Vit-B_1	2.18±0.51	1.68±0.19	2.14±0.21
VII	Cd+Combination	1.83±0.05^{b}	1.75±0.18	1.65±0.28

Results are expressed as mean ±S.E.M. (n=8). $^{x}p<0.05$ compared to control, $^{a}p<0.05$, $^{b}p<0.01$ compared to cadmium treated rats.

Table 4: Effect of dietary nutrients on copper concentration in blood, liver and kidney of albino rats exposed to cadmium, during three weeks of exposure.

Groups	Treatments	Blood (µg/ml)	Liver (µg/g)	Kidney (µg/g)
I	Control	0.83±0.21	0.44±0.04	0.46±0.073
II	Cd alone	0.40±0.8	0.31±0.04^{x}	0.41±0.28
III	Cd+NAC	0.50±0.07	0.39±0.18^{b}	0.48±0.09
IV	Cd+Met	0.61±0.23	0.48±0.18	0.51±0.09
V	Cd+Zn	0.62±0.07	0.50±0.15	0.47±0.07
VI	Cd+Vit-B_1	0.62±0.08	0.46±0.06^{b}	0.54±0.09
VII	Cd+Combination	0.78±0.15	0.53±0.07^{b}	0.56±0.08

Results are expressed as mean ±S.E.M. (n=8). $^{x}p<0.05$, compared to control, $^{b}p<0.01$ compared to cadmium treated rats.

Table 5: Effect of dietary nutrients on zinc concentration in blood, liver and kidney of albino rats exposed to cadmium, during three weeks of exposure.

Groups	Treatments	Blood (µg/ml)	Liver (µg/g)	Kidney (µg/g)
I	Control	0.82±0.22	3.76±2.02	1.59±0.08
II	Cd alone	0.33±0.01	1.73±0.26	1.13±0.37
III	Cd+NAC	0.63±0.20	1.82±0.25	1.43±0.09
IV	Cd+Met	0.54±0.02^{b}	1.52±0.07	1.26±0.07
V	Cd+Zn	0.55±0.20	1.81±0.19	1.76±0.24
VI	Cd+Vit-B_1	0.67±0.15^{a}	1.68±0.09	1.54±0.19
VII	Cd+Combination	0.77±0.14^{b}	3.79±0.22^{b}	2.07±0.19

Results are expressed as mean ±S.E.M. (n=8). $^{b}p<0.01$, compared to cadmium treated rats.

Acknowledgments

The authors are thankful to U.G.C., New Delhi for providing financial assistance and Department of Zoology, Dayalbagh Educational Institute for providing necessary facilities for this work.

REFERENCES

[1] Bauman, J.W.; Liu, J; Klaassen, C.D., *Fundamental Applied Toxicology*. **1993,** 21, 15-22.
[2] Rana, S.V.S; Verma, S., *Biological Trace Element Research.* **1996,** 51,161–168.
[3] Sarkar, S; Yadav, P; Bhatnagar, D.J., *Biological Trace Element Medicine*. **1997**, 11, 8–13.
[4] Longoni, B; Salgo, M.G; Pryor, W.A; Marchiafava, P.L., *Life Science*. **1998**, 62, 853–859.
[5] Casalino, E; Sblano, C; Calzaretti, G; Landriscina, C., *Toxicology*. **2006**, 217, 240–245
[6] Fernandez, E; Gustafson, A; Anderson, M; Hellman, B; Dencker, L., *Toxicological Science*. **2003,** 10, 85–99.
[7] Brzoska, M; Moniuszko-Jakoniuk, J., *Food Chemical Toxicology*. **2000,** 39, 967–80.
[8] Jemai, H; Messaoudi, I; Chaouch, A; Kerkeni, A., *Journal Trace Element Medicine Biology*. **2007,** 21, 269–273.
[9] Poirson-Bichat, F; Lopez, R; Bras, Goncalves, R.A; Miccoli, L; Bourgeois, Y; Demerseman, P; Poisson, M; Dutrillaux, B; Poupon, M.F., *Life Science*. **1997,** 60, 919-931.
[10] Griffith, W.O., *Methods Enzymology*. **1987,** 143, 366.
[11] Clegg, J.W; King, E.J., *B M J*. **1968,** 11, 329-330.
[12] Garby, L; Vuille, J.C., *Scandinavian Journal of Clinical and Laboratory Investigation*. **1961**, 13, 642-645.
[13] Ellman, G.L., Tissue sulphydral groups. *Archieve of Biochemical and Biophysics*. **1959,** 82, 70-77
[14] Parker, M.M; Humoller, F.L; Mahler, D.J., *Clinical Chemistry*. **1967,** 13, 40-48.
[15] Bagchi, D; Bagchi, M; Stohs, S.J; Ray, S.D; Kuszynski, C.A; Pruess, H.G. *Toxicology*. **2000,** 148, 187-197.
[16] Jihen, E.H; Imed, M; Fatima; H; Abdelhamid, K., *Food and Chemical Toxicology*. **2008,** 46, 3522–3527.
[17] Karmakar, R; Bhattacharya, R; Chatterjee, M., *Biometals*. **2000,** 13, 231-239.
[18] Kostic, M.M; Ognjanovic, B; Dimitrijevic, S; Zikic, R.V; Stajn, A; Rosic, G.l; Zivkovic, R.V., *European Journal of Haematology*. **1993a,** 51, 86-92.
[19] Shukla, A; Shukla, G.S; Srimal, R.C., *Human & Experimental Toxicology*. **1996,** 15, 400-405.
[20] Hamada, T; Tanimoto, A; Arima, N; Ide, Y; Sasaguri, T; Shimajiri, S; Murata, Y.K.Y; Sasaguri, Y., *Sangyo Ika Daigaku Zasshi*. **1998,** 20, 11-19.
[21] Bagchi, D; Bagchi, M; Hassoun, E.A; Stohs, S.J; *Biological Trace Element Research*. **1996,** 52, 143-154.
[22] Nigam, D; Shukla, G.S; Agarwal, A.K., *Toxicology Letters*. **1999,** 106, 151–157.
[23] Mohanpuria, P; Rana, N.K; Yadav, S.K., *Environmental Toxicology*. **2007,** 22, 368–374.
[24] Sarkar, S; Yadav, P; Bhatnagar, D., *BioMetals*. **1998,** 11, 153–157.
[25] Shrama, M.K; Kumar, M; Kumar, A., *Journal of Experimental Biology*. **2002**, 40, 1079-1082.
[26] Konar, V; Kara, H; Yilmaz, M; Dayangac, A; Karatas, F., *Biological Trace Element Research*. **2007,** 118, 131-137.
[27] Shibutani, M; Mitsumori, K; Satoh, S; Hiratsuka, H; Satoh, M; Sumiyoshi, M; Nishijima, M; Katsuki, Y; Suzuki, J; Nakagawa, J; Akagi, T; Imazawa, T; Ando, M., *Journal of Toxicology Science*. **2001,** 26, 337–358.
[28] Jarup, L., *Nephrology Dialysis Transplantation*. **2002,** 17, 35–39.
[29] Gaurav, D; Preet, S; Dua, K.K., *Annals of Biological Research.* **2010,** 1, 121-127

[30] Atessahin, A; Yilmaz, S; Karahan, I; Ceribasi, A.O; Karaoglu, A., *Toxicology*. **2005,** 212, 116–123.
[31] Gaurav, D; Preet, S; Dua, K.K., *Archives of Applied Science Research.* **2010,** 2, 390-397
[32] Obidah, W; Saad, U.A; Wurochekke, A.U., *African Journal of Biochemical Research.* **2009,** 3, 229-231.
[33] Chakraborty, P; Hossain, S.K.U; Murmu, N; Das, J.K; Pal, S; Bhattacharya, S; *Journal of Cancer Molecules.* **2009,** 4, 183-189.
[34] Kumar, P; Singh, K; Kumar, A., *Annals of Biological Research.* **2010**, 1, 27-35.
[35] Nemmiche, S; Chabane-Sari, D; Guiraud, P., *Chemico-Biological Interactions*. **2007,** 170, 221–230.
[36] Alhazza, I.M., *Research Journal of Environmental Science*. **2008,** 2, 305-309.
[37] Tandon, S.K; Singh, S; Prasad, S; Khandekar, K; Dwivedi, V.K; Chatterjee, M; Mathur, N., *Toxicology Letters*. **2003,** 145, 211–217.
[38] Kim, C.Y; Lee, M.J; Lee, S.M; Lee, C; Kim, J.S., *Tohoku Jouranl of Experimental Medicine*. **1998,** 186, 205-213.
[39] Ognjanovic, B.I; Markovic, S.D; Pavlovic, S.Z; Zikic, R.V; Stajn, A.S; Saicic, Z.S., *Physiological Research*. **2008,** 57, 403-411.
[40] Nordberg, G.F; Jin, T; Nordberg, M., *Environmental Health Perspectives.* **1994,** 102, 191–194.
[41] Jacquillet, G; Barbier, O; Cougnon, M; Tauc, M; Namorado, M.C; Martin, D; Reyes, J.L; Poujeol, P., *American Journal of Physiology- Renal Physiology*. **2006,** 290, 127–137.
[42] Bonner, F.W; King, L.J; Parke, D.V., *Toxicology*. **1981,** 19, 247–254.
[43] Nath, R; Gill, K.D., Effect of cadmium on lipid peroxidation and antioxidant defense enzymes in various regions of adult rat brain exposed to ethanol. *In: health and disease* (Ahish Publishing House, New Delhi) **1993**.
[44] Bin, Q.H; Garfinkal, D., *Medicinal Hypothesis*. **1994,** 42, 380-384.
[45] Mitsumori, K; Shibutani, M; Sato, S; Onodera, H; Nakagawa, J; Hayashi, Y; Ando, M., *Achieves of Toxicology.* **1998,** 72, 545–552.