Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 5, 2014

Original Article

ADDITIVE PROTECTIVE EFFECTS OF SELENIUM AND VITAMIN E AGAINST ARSENIC INDUCED LIPIDEMIC AND CARDIOTOXIC EFFECTS IN MICE

SURAJIT BHATTACHARJEE AND SUDIPTA PAL*

Nutritional Biochemistry Laboratory, Department of Human physiology Tripura University (A Central University) Suryamaninagar, Tripura 799022, India.
Email: sudiptapal@tripurauniv.in

Received: 25 Mar 2014 Revised and Accepted: 28 Apr 2014

ABSTRACT

Objectives: Alteration in lipid profiles and increased levels of oxidative stress have been implicated in the cardiotoxicity induced by arsenic. Selenium and vitamin E against metal-induced cytotoxicity have been showed protective effects and well documented. However, the effectiveness of them to reduce arsenic-induced cardiotoxicity in has not yet been evaluated. So, the present work was undertaken to investigate the cardio protective effects of them against arsenic-induced cardiotoxicity.

Methods: In the present study, intraperitoneal dose of arsenic at 7.2mg/kg b.w./day for 30 days was used in Swiss albino mice as cardiotoxicant. Selenium at a dose of 10 µg/k.g./day and vitamin E at a dose of 20 mg/k.g./day for last 14 days of arsenic treatment were used separately and in combination (orally). They were studied in response to control and antioxidant control groups.

Results: Sub-acute arsenic exposure resulted in decrease in antioxidant enzyme activities like superoxide dismutase, catalase, glutathione reductase, glutathione S-tranferase, glutathione peroxidase activities and elevated transaminase activities, cholesterol, triglycerides, low density lipoprotein and uric acid level in serum. Additionally, arsenic treatment increased protein carbonyl content, free hydroxyl radical and nitric acid formation in cardiac tissue. Other enzyme activities such as xanthine oxidase, nicotinamide adenine dinucleotide phosphate-oxidase, γ-glutamyl transpeptidase in cardiac tissue were also increased by arsenic.

Conclusion: Appreciable beneficial effects of selenium and vitamin E co-administration against arsenic-induced changes in biochemical and histopathological parameters indicate that supplementation of vitamin E along with micronutrient selenium has anti-oxidative as well as anti-lipidemic effects against arsenic-induced cardiotoxicity.

Keywords: Arsenic, Oxidative stress, Cardiac pathology, Selenium, Vitamin E.

INTRODUCTION

Arsenic is available worldwide as both organic and inorganic forms. It is used as an important semiconductor material, taxonomic sample and wood preservation [1]. Therapeutic efficacy of arsenic trioxide (As_2O_3) against acute promyelocytic leukemia is well established [2]. Unfortunately, the clinical usefulness of As_2O_3 has been limited by its cytotoxic effects for long treatment purpose. Cardio-toxic effects of arsenic trioxide, including QT prolongation and sudden cardiac death, have been reported earlier [3]. Arsenic causes oxidative damage in almost whole organ systems of human body by inducing ROS generation [4]. Arsenic is methylated to form monomethylated arsenic acid (MMA) and dimethylated arsenic acid (DMA) in liver. The degree of severity of arsenic toxicity depends on the detoxifying ability and varies on arsenic methylation capability of tissues [5, 6]. Arsenic is thought to be one of the causative factors of cardiovascular diseases [7]. Arsenic exposure via drinking water is associated with hypertension, peripheral vascular disease, cardiomyopathy and ischemic heart disease [8]. Long term arsenic exposure plays a key role in the pathogenesis of myocardial tissue leading to various cardiovascular complications and myocardial injury [9]. Fragmentation of DNA, reactive oxygen species (ROS) generation, changes in cardiac ion channels and apoptosis in the myocardial tissue are the possible mechanisms of arsenic-induced cardiotoxicity [10]. Selenium, an essential trace element for animals has been recognized as an antioxidant [11]. Selenium pre-treatment protected brain against restraint stress-induced oxidative damage in hippocampus, striatum and frontal cortex [12]. Protective effects of selenium against aluminum-induced liver injury were established earlier [13]. Selenium also exhibited protective effects against cadmium-induced testicular toxicity in cocks [14] and oxidative injury in trophoblast cells [15]. Vitamin E (Vit E) is the collective name for a group of lipo-soluble compounds derived from tocol and tocotrienol, alpha tocopherol being the one with greatest biological activity. The characteristic of "biological antioxidant" of Vit E derives from its molecular structure as its capability of fixing ROS types O_2^-, O_2^{2-}, and OH^- [16, 17]. Vitamin E showed protection against bleomycin hydrochloride induced pulmonary fibrosis in rats [18]. Alpha-tocopherol showed prevention against cisplatin-induced nephrotoxicity [19]. In addition, alpha-tocopherol showed protection on vascular complication in spinal cord reperfusion injury in rats [20] as well as hypercholesterolemia-induced age-related diseases [21].

Inadequate information regarding mechanism of arsenic-induced cardiotoxicity draws attention to see whether the cardio-toxic effects of arsenic are mediated via alteration of lipid profile or any other mechanism like oxidative stress mediated tissue damage may also be involved. In addition, the effects of combined supplementation of selenium and vitamin E will be elucidated whether these two antioxidants have any additive beneficial effects against arsenic-induced cardiotoxicity.

MATERIALS AND METHODS

Materials

Sodium arsenite ($NaAsO_2$), BSA, 5,5-Dithio-bis-2-nitrobenzoic acid, NADPH, GSH, TCA, nitro BT, EDTA, selenium, vitamin E, vitamin C, 2-thiobarbituric acid and other chemicals used in the study are of analytical grade and purchased from the Sigma Aldrich, MERCK, CDH and SRL. Biochemical Kits such as cholesterol, triglycerides, HDL, uric acid etc. were purchased from Coral clinical systems.

Experimental Design

42 Swiss albino mice weighing 30-40gm were purchased from CPCSEA registered animal supplier. They were then divided into six groups of equal average body weight and equal number (N=7) and kept in separate cages. Animals were acclimatized under laboratory conditions for two weeks before starting the experiment. All animals

received humane care as per CPCSEA guidelines. Animal ethical committee of Tripura University approved the protocols for the experiments before experimentation.

Treatment schedule of the study

The treatment schedule of the mice' for the present study is given below:-

Group A (Control): 0.9% NaCl/day (i.p.) was given to this group for 30 days
Group B (As-treated): 7.2mg of $NaAsO_2$ /kg/day (i.p.) was administered to this group for a period of 30 days
Group C (Selenium-supplemented): The animals of this group were treated with arsenic at the same dose and duration mentioned above, followed by co administration with selenium at a dose of 10 µg/k.g./day for last 14 days of arsenic treatment.
Group D (Vitamin E supplemented): This group was treated with sodium arsenite at the above mentioned dose and duration, followed by co administration with vitamin E at a dose of 20mg/k.g./day for last 14 days of arsenic treatment.
Group E (Se+ vitamin E supplemented): The animals of this group were treated with arsenic at the dose and duration mentioned earlier, followed by co administration of selenium with vitamin E at their respective doses mentioned above for last 14 days of arsenic treatment
Group F (Positive control vitamin C): Vitamin C at a dose of 100 mg /kg/day was administered in arsenic-treated mice during last 14 days of treatment schedule.

Animal Sacrifice

Animals were sacrificed by cervical dislocation under light ether anesthesia.

Collection of serum

Blood was collected from the hepatic vein of all experimental animals and kept for clotting. Serum was collected and kept at -20°C until biochemical analysis.

Collection of heart tissue

Hearts from the experimental animals were quickly excised and washed in ice-cold saline blotted dry. One heart from each group was kept for histological study and other six animals from each group were used for biochemical analysis.

Preparation of Tissue Homogenate

A 5% tissue homogenate of cardiac tissue was prepared in 0.1 M phosphate buffer (pH 7.4) using all glass homogenizer and kept at -20°C until biochemical analysis was performed.

Biochemical Studies

Assessment of serum markers

Creatine kinase (CK) activity

Creatine kinase (CK) activity of serum was assayed by the method of Jacobus and Lehninger [22]. In the presence of creatinine phosphate CK helps to form ATP and creatinine. The production rate of NADPH by the serum samples were assayed by spectrophotometrically at 340nm. The temperature was maintained 37°c for this assay.

Serum LDH activity determination

The LDH activity was measured by the consumption of by the rate of the consumption of pyruvate and DNPH according to the method of Bergmeyer [23]. In this process potassium buffer at 7.4 pH was using 20 µl of serum sample. The change in optical density at 340nm due to oxidation of DNPH is measured.

Serum glutamic-pyruvic transaminase and glutamic-oxaloacetic transaminase activity

The serum GPT and GOT activities were determined by the method of Reitman & Frankel [24]. For the determination of serum GPT and GOT activities, glutamic-pyruvic substrate and glutamic-oxaloacetate substrate was prepared separately in 0.1M PB at 7.4 pH and used as substrates for them respectively. 2,4,-DNPH was used as colouring reagent in alkaline medium for both cases. 0.4 (N) NaOH was used in these experiments as the alkaline medium provider. After the formation of colour the reading was taken spectrophotometrically at 520nm.

Serum γ -glutamyl transpeptidase determination

Serum γ -glutamyl transpeptidase will be studied according to Szasz [25]. In this assay 1 ml of ammediol-HCl buffer (0.05M & pH 8.6) contain substrate γ-glutamyl-p-nitroanilide monohydrate was taken in a cuvette and 0.1 ml of serum is added to the cuvette. The change in absorbance was noted at 405 nm at regular time intervals. The calculation was done by using the extinction coefficient of γ-glutamyl-p-nitroanilide which is $9900 M^{-1} cm^{-1}$.

Other serum parameters determination by using standard kits

The other serum markers such as cholesterol, triglycerides, HDL and uric acid related to cardiac dysfunction was also evaluated by using standard kits. The LDL cholesterol was determined by using Freidewald's formula,

LDL cholesterol = {total cholesterol – (triglycerides/5) – HDL cholesterol}

Measurement of primary parameter of oxidative stress and endogenous free hydroxyl radical

Protein carbonyl content

Protein carbonyl content of cardiac tissue will be estimated according to the method of Stadtman & Levine [26]. The samples was treated with an equal volume of 10mM 2, 4-DNPH in 2.5 M HCl and incubated for 30 minutes at room temperature by vortexing in every 15 minutes. After that samples are then treated with 20% TCA and allowed to keep in ice. After centrifugation, the supernatant was discarded.

The pallets were then washed two times with ethanol/ethyl acetate (1:1 v/v). The final precipitate was dissolved in 6% SDS and allowed for centrifugation again. The optical density was then recorded at 370nm. The results were expressed as nmol of DNPH-incorporated/mg protein based on the molar extinction coefficient of 22,000/M/cm.

Assay of primary parameters of oxidative stress

Reduced glutathione (GSH) content

The GSH content in heart was estimated by the method of Ellman [27] as modified according to Davila *et al.* [28]. In this assay equal volume of 5% heart tissue homogenate was treated with 20% TCA containing 1 mM EDTA to allow precipitation of proteins. The centrifugate was then treated with freshly prepared Ellman's reagent (DTNB in 1% sodium citrate). The reading was taken in a spectrophotometer at 412 nm. Tissue glutathione level was calculated from the standard curve generated using aliquots of solution having known concentration of GSH.

Tissue lipid peroxidation (LPO) level

Lipid peroxidation (LPO) was measured using the thiobarbituric acid (TBA) assay according to the method described by Buege and Aust [29]. One mill molar EDTA was used in the reaction mixture to chelate iron and reduce its interference in the peroxidation reaction of unsaturated fatty acids. The reaction mixture was then heated at 80°C for colour development. The optical density was read at 533 nm. The molar extinction co-efficient, 1.56×10^5 cm^2/mmol of malondialdehyde was used to calculate the malondialdehyde production.

Free ·OH radical generation determination

2 hours before the sacrifice of animals each group was injected intra-peritonially with 30% dimethylsulfoxide at a dose of 4 ml/kg/ body weight. Tissue homogenates were prepared by adding 10(N) H_2SO_4 and allowed for centrifugation at 3000 rpm for 10 minutes for the determination of free ·OH radical generation [30]. The supernatant is then mixed well with tolune-butanol (3:1) and kept in standing

position until the formation of an organic layer. The aqueous layer is then taken and allowed to mix with saturated butanol. The upper butanol phase was then allowed to react with 0.5M sodium acetate buffer (pH 5.0). After centrifugation the aqueous phase was allowed for the colour development with freshly 30 mM fast blue BB salt and kept in dark. After the formation of yellowish colour of the samples they were measured spectrophotometrically at 425 nm.

Determination of tissue NADPH oxidase activity

Tissue NADPH oxidase activity was measured by the method of Chen *et al* [31]. 0.05M Tris-HCl containing 0.25M sucrose is prepared at 7.4pH and used for this experiment. 100µl of the supernatant with Tris-HCl and 1.06mM NADPH oxidase solution were mixed in a cuvette. Then the cuvette is allowed to read OD the change absorbance for 5 mins. The enzyme activity was calculated by using molar extinction coefficient of NADPH $6.22X10^3$/M/cm.

Xanthine oxidase activity

Xanthine oxidase activity will be assayed according to the method of Bergmeyer *et al.* [32]. In this method 100 µl supernatant is added in 50 mM Potassium Phosphate Buffer, pH 7.5 at 25^0c with 0.15 mM Xanthine solution and mixed well. This mixture is then use to determine the changes in optical density at 290 nm at a regular interval. A blank solution is also run in the same way. Calculation is done by determining the δ-OD/min and by the use of mill molar extinction coefficient of uric acid which is 12.2.

Determination of tissue Nitric oxide (NO) production

Nitric oxide level in heart was measured by the method of Raso *et al.,* [33]. The 5% tissue homogenate was prepared in 0.25 M sucrose solution and centrifuged at 6000 rpm at 4^0C for 10 minutes. Equal volume of 0.25 M sucrose solution, 1% sulfanilamide and 0.1% napthylethylene diamine hydrochloride were then added to the supernatant and mixed well. After 20 minutes of the total reaction, the optimal density was read at 550 nm in a spectrophotometer.

Determination of antioxidant enzyme activities

GPX activity

GPX activity of tissues will be assayed by the method as used by Maiti and Chatterjee [34]. 5% tissue homogenate was prepared in 0.1M phosphate buffer (pH 7.4). Supernatant is taken for this assay as sample. 50 µl of sample is added with glutathione reductase in presence of 10mM GSH and phosphate buffer. It is then incubated for 10 minutes at 37^0c. Then 1.5mM NADPH is added to that and the optical density was recoded at 340 nm. After then H_2O_2 is added to the cuvette and OD value was recoded at 340 nm again. The enzyme activity was expressed as nmole NADPH oxidized/min/mg protein.

Glutathione S-transferase activity

Glutathione S-transferase activity will be assayed by the method of Warholm *et al.,* [35]. 0.01M (pH 6.5) phosphate buffer containing 1 mM EDTA, 20 mM GSH and 20 mM CDNB were added to the supernatant in a cuvette and the increase in absorbance was noted at 340 nm. The activity of GST was expressed as micromole of GSH-CDNB conjugate formed/min /mg of protein.

Superoxide dismutase (SOD) activity of tissues

The assay of SOD activity is based on the SOD-mediated increase in the rate of auto oxidation of hematoxylin in aqueous alkaline solution, which yields a chromophore with maximum absorbance at 560 nm [36]. The enzyme activity was expressed as units per minute per mg of protein.

Cardiac catalase activity

Cardiac catalase activity will be measured by the method of Aebi [37]. A 5% tissue homogenate was prepared with 0.1M phosphate buffer (pH 7.4) with 1% triton X-100. The catalase activity was measured by calculating the rate of degradation of H_2O_2, the substrate of the enzyme. The enzyme activity was expressed as µmole H_2O_2 utilized per minute per mg of protein.

Glutathione Reductase (GR) activity of cardiac tissue

The 10,000 g supernatant of 5% tissue homogenate was used for estimation of GR activity by the method of Carlberg and Mannervik [38]. The assay system consisted of phosphate buffer (0.2M, pH 7.0, containing 2mM EDTA), 20 mM GSSG, 2 mM NADPH and supernatant.

The enzyme activity was quantitated at 25^oC by measuring the disappearance of NADPH at 340 nm. The enzyme activity was expressed as nmole of NADPH oxidized per minute per mg of protein.

Tissue protein content

Protein content was estimated by the method of Lowry *et al* [39] using bovine serum albumin as the standard protein.

Statistical Analysis

The values were expressed as mean ± SEM. The data were statistically analyzed by one way ANOVA followed by multiple comparison T test to determine the significance of differences between the two related groups. The $p < 0.05$ was considered statistically significant.

Histopathological Studies

Before doing histopathology, the size, shape and weight of hearts from each of the groups were noted. Then tissues were preserved in 4% buffered formal solution for 24 hours. After proper dehydration in graded alcohol, clearing, impregnation and embedding, tissue sections were prepared by rotary microtome.

These sections obtained from the respective groups were stained using hematoxylin-eosin and examined under high power microscope (40x) and photomicrographs were taken.

Table 1: Change in Cholesterol, Triglycerides, HDL, LDL, GPT and GOT in serum of mice due to exposure of arsenic with selenium, vitamin E and their combination.

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	GPT (U/dl)	GOT (U/dl)
Control (6)	158.77±5.45	212.62±16.4	71.34±2.27	44.90±7.03	35.60±1.07	26.48±1.56
As (6)	288.52±9.28 p^{a***}	399.61±15.30 p^{a***}	39.25±1.54 p^{a***}	169.36±7.53 p^{a***}	68.83±2.36 p^{a***}	64.73±2.8 p^{a***}
As +Se (6)	203.25±14.16 p^{a**} p^{b***}	223.67±13.76 $p^{a\#}$ p^{b***}	60.20±1.62 p^{a**} p^{b**}	98.31±13.39 $p^{a**}p^{b**}$	45.45±1.53 $p^{a**}p^{b***}$	47.7±1.63 $p^{a**}p^{b**}$
As +Vit. E (6)	233.98±5.96 p^{a**} p^{b**}	166.67±10.73 p^{a*} p^{b***}	56.46±1.84 p^{a**} p^{b**}	144.19±5.76 $p^{a***}p^{b*}$	47±1.08 $p^{a**}p^{b***}$	44.25±2.79 $p^{a**}p^{b***}$
As +Se +Vit. E (6)	190.80±5.84 p^{a*} p^{b***}	158.97±22.24 p^{a*} p^{b***}	71.03±1.97 $p^{a\#}$ p^{b***}	87.98±6.41 $p^{a**}p^{b***}$	39.08±1.41 $p^{a\#}p^{b***}$	32.78±1.99 $p^{a\#}p^{b***}$
As +Vit. C (6)	177.81±7.11 $p^{a\#}$ p^{b***}	222.2±12.6 $p^{a\#}p^{b***}$	66.67±2.71 $p^{a\#}$ p^{b***}	66.69±5.84 $p^{a*}p^{b***}$	39.35±1.93 $p^{a\#}p^{b***}$	32.23±2.55 $p^{a\#}p^{b***}$

Values are Means±S.E.M. p^a compared with control group. p^b compared with arsenic-treated group. *** indicates $p<0.001$, ** indicates $p<0.01$, * indicates $p<0.05$ and # indicates $p>0.05$.

RESULTS

Table 1 represents that arsenic treatment at the present dose and duration caused significant increase in the serum cholesterol and triglyceride levels by 81.73% and 87.95%. Selenium and vitamin E alone partially counteracted arsenic-induced changes in serum cholesterol. The counteraction was 29.5% and 18.9% respectively, whereas their combined supplementation restored the elevated cholesterol level towards the control by 33.9%. Vitamin C as a positive control antioxidant also showed appreciable counteractive effects against arsenic-induced alteration of serum cholesterol. Triglyceride level in serum also increased markedly following arsenic treatment. Selenium alone almost completely restored the triglyceride level in serum, and vitamin E reduced that even below the control value. Their combined supplementation did not exhibit additional beneficial effect though restored triglyceride level appreciably.

Serum HDL level decreased markedly (44.98%) ($p<0.001$) after arsenic exposure, whereas LDL level increased by four fold ($p<0.001$) in arsenic-treated mice. HDL level was counteracted partially by selenium and vitamin E supplementation alone, while their combination exhibited better effect to counteract HDL level almost completely to the respective control value. Selenium supplementation individually counteracted LDL level in serum in a better efficacy than vitamin E. Synergistic beneficial effect of selenium and vitamin E was found in restoration of serum LDL level. The restoration was found to be 48%. Vitamin C also restored both the parameters significantly.

At the present schedule of treatment arsenic significantly increase the serum GPT and GOT activities (table 1). GOT and GPT activities increased by 48.28% ($p<0.001$), and 144.45% ($p<0.001$) respectively following arsenic treatment. Combined supplementation of selenium and vitamin E exhibited better response than their individual effects in restoration of those transaminase enzyme activities.

Table 2 represents that arsenic treatment at the present dose and duration caused a remarkable decrease in cardiac GSH content and an increase in NO generation in cardiac tissue. The decrease was found to be 69.01% ($p<0.001$) in case of GSH and the increase was 105.17% ($p<0.001$) in case of NO production. Supplementation of vitamin E alone restored depleted GSH content near to the normal, whereas selenium restored the depleted GSH completely. Counteractive effect of vitamin E against depleted GSH content was enhanced by selenium. Similarly combined supplementation of vitamin E and selenium exhibited additive beneficial effects against arsenic-induced NO generation. Vitamin C, a well-known antioxidant, has been included in the current study as a positive control, which also protected cardiac tissues from arsenic-induced perturbation of GSH content and NO generation.

Table 2: Changes in GSH, NO content, LPO level, protein carbonyl content, NADPH Oxidase activity and ·OH radical in cardiac tissue due to exposure of arsenic with selenium, vitamin E and their combination.

Groups	GSH (µmol/mg protein)	NO (µM/mg protin)	LPO (nmol/mg protein)	PCC (nmol/mg prtein)	NADPH(O) activity (nM/mg protein)	·OH radical (nM/mg tissue)
Control (6)	29.11±1.71	1.16±0.04	1.85±0.07	9.80±0.42	630.68±47.19	4.45±0.28
As (6)	12.12±0.74 p^{a***}	2.38±0.05 p^{a***}	7.39±0.16 p^{a***}	20.72±0.32 p^{a***}	1897.62±113.74 p^{a***}	13.08±0.47 p^{a***}
As +Se (6)	24.83±0.57 p^{a*} p^{b***}	1.61±0.04 p^{a***} p^{b***}	4.94±0.14 p^{a***} p^{b***}	15.50±0.24 $p^{a***}p^{b***}$	734.84±108.95 $p^{a\#}$ p^{b***}	5.56±0.34 $p^{a\#}$ p^{b***}
As +Vit. E (6)	34.96±1.12 p^{a**} p^{b***}	1.74±0.03 p^{a***} p^{b***}	2.57±0.16 p^{a**} p^{b**}	12.86±0.28 p^{a**} p^{b***}	780.18±65.58 $p^{a\#}$ p^{b***}	6.55±0.30 p^{a*} p^{b***}
As +Se +Vit. E (6)	35.22±0.96 p^{a**} p^{b***}	1.42±0.02 p^{a**} p^{b***}	1.78±0.09 $p^{a\#}$ p^{b***}	10.73±0.31 $p^{a\#}$ p^{b***}	610.90±47.45 $p^{a\#}$ p^{b***}	4.46±0.41 $p^{a\#}$ p^{b***}
As +Vit. C (6)	29.92±0.91 $p^{a\#}$ p^{b***}	1.61±0.03 p^{a***} p^{b***}	1.94±0.11 $p^{a\#}$ p^{b***}	11.17±0.63 p^{a*} p^{b***}	687.28±28.09 $p^{a\#}$ p^{b***}	4.60±0.27 $p^{a\#}$ p^{b***}

Values are Means±S.E.M. p^a compared with control group. p^b compared with arsenic-treated group. *** indicates $p<0.001$, ** indicates $p<0.01$, * indicates $p<0.05$ and # indicates $p>0.05$.

The LPO level (table 2) was increased in cardiac tissue significantly (p^a <0.001) after arsenic exposure. Vitamin E showed better protective effect in restoration of LPO level than selenium. The restoration was found to be 33.15% ($p^a<0.001$) and 65.22% ($p^a<0.01$) in selenium and vitamin E supplemented groups respectively.

Conjoint administration of selenium and vitamin E exhibited 75.91% restoration ($p^a>0.05$) of cardiac LPO level, which indicated additive beneficial effects of selenium and vitamin E in scavenging harmful lipid peroxides. Vitamin C also showed appreciable effect in restoration of cardiac LPO level.

Arsenic treatment significantly increased protein carbonyl content in cardiac tissue ($p<0.001$) (Table 2). Selenium, vitamin E and their combination counteracted the changes in protein carbonyl content by 25.19% ($p^a<0.001$), 37.92% ($p^a<0.01$) and 48.22% ($p^a>0.05$) respectively. This observation further indicates better protective effect of combined supplementation of selenium and vitamin E against arsenic-induced cardiotoxicity. Vitamin C restores the protein carbonyl content significantly (p^b <0.001) by 46.09% than the control group.

The NADPH oxidase enzyme activity in cardiac tissue increased significantly ($p<0.001$) due to arsenic toxicity. Conjoint administration of vitamin E and selenium restored the enzyme activity in a better efficacy than their individual supplementation.

The ·OH radical generation in cardiac tissue was increased significantly ($p^a<0.001$) by 193.93% due to arsenic intoxication (Table 2). Supplementation of selenium eliminated OH radical much better than vitamin E, whereas their combined supplementation restored the free OH radical generation similar to that of control value.

Table 3 represents that both the catalase and SOD activities were significantly decreased following arsenic treatment. The decrease was found to be 65.77% ($p<0.001$) and 42.61% ($p<0.001$), respectively, whereas the activity of xanthine oxidase (XO) increased significantly by 42.04% (p^a <0.001) in arsenic-treated mice.

The results further reveal that selenium or vitamin E partially counteracted arsenic-induced alteration of catalase and XO activities, whereas their conjoint administration shows synergistic beneficial effects against those altered parameters. The glutathione reductase (GR), glutathione S-transferase (GST) and glutathione peroxidase (GPX) enzyme activities are shown in table 3.

All of the enzyme activities in cardiac tissue of arsenic intoxicated mice were significantly decreased ($p^a<0.001$) by 61.12%, 51.83% and 67.95, respectively. Treatment with selenium, vitamin E alone counteracted partially the altered enzyme activities, whereas their combined supplementation exhibited appreciable beneficial effects to restore the suppressed activities of those enzymes to their respective control values.

Table 3: Effects of selenium and vitamin E on arsenic-induced alteration of CAT, SOD, GR, GST, GPX and XO activities in cardiac tissue.

Groups	CAT (µmol/min/mg protein)	SOD (U/min/mg protein)	GR (nmol/min/mg protein)	GST (nmol/min/mg protein)	GPX (nmol/min/mg protein)	XO (µmol/min/mg protein)
Control (6)	51.65±1.73	117.76±1.17	94.46±1.95	2.74±0.18	183.65±4.86	0.540±0.02
As (6)	17.68±0.40 p^{a***}	67.58±0.52 p^{a***}	36.73±1.78 p^{a***}	1.32±0.10 p^{a***}	58.86±1.16 p^{a***}	0.767±0.03 p^{a***}
As +Se (6)	32.11±0.51 p^{a***} p^{b***}	90.64±1.34 p^{a***} p^{b***}	66.35±1.86 p^{a***} p^{b***}	2.27±0.10 $p^{a\#}$ p^{b***}	123.49±3.09 $p^{a**}p^{b***}$	0.346±0.01 p^{a**} p^{b***}
As +Vit. E (6)	33.81±0.52 p^{a***} p^{b***}	96.79±0.83 p^{a***} p^{b***}	79.08±1.24 p^{a**} p^{b***}	2.13±0.20 p^{a*} p^{b***}	138.24±3.89 $p^{a*}p^{b***}$	0.380±0.04 p^{a**} p^{b***}
As +Se +Vit. E (6)	43.62±0.81 p^{a**} p^{b***}	102.16±0.64 p^{a**} p^{b***}	88.88±1.19 $p^{a*}p^{b***}$	2.45±0.10 $p^{a\#}$ p^{b***}	194.92±4.97 $p^{a\#}p^{b***}$	0.360±0.01 p^{a**} p^{b***}
As +Vit. C (6)	44.37±1.36 p^{a*} p^{b***}	98.27±0.68 p^{a***} p^{b***}	82.49±1.56 p^{a**} p^{b***}	2.45±0.13 $p^{a\#}$ p^{b***}	173.79±3.76 $p^{a\#}p^{b***}$	0.330±0.01 p^{a**} p^{b***}

Values are Means±S.E.M. p^a compared with control group. p^b compared with arsenic-treated group. *** indicates p<0.001, ** indicates p<0.01, * indicates p<0.05 and # indicates p>0.05.

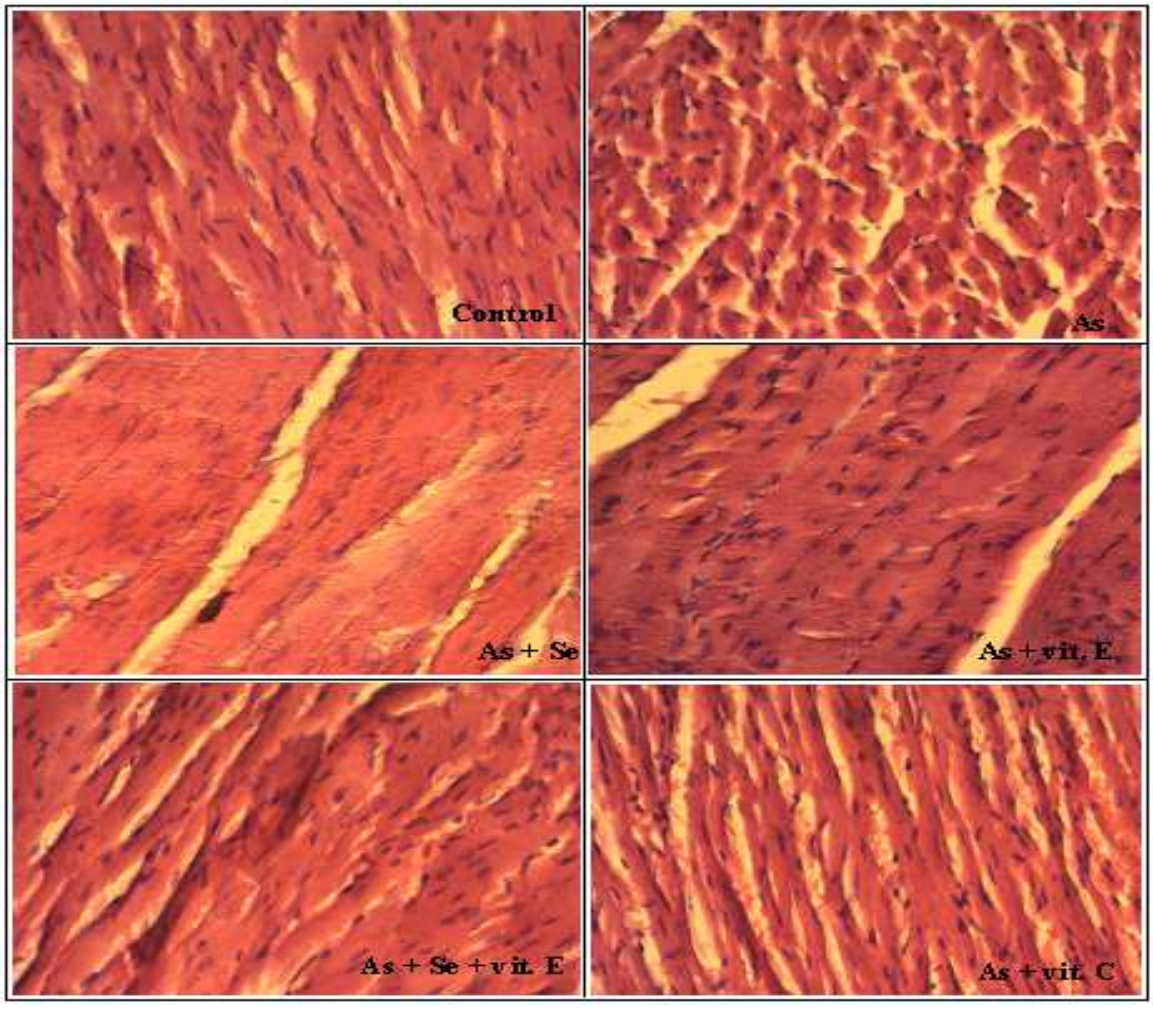

Fig. 5: Effects of selenium, vitamin-E and their combination on histological changes in mice hearts by hematoxylin and eosin staining (x40)

A significant increase (91.9%, p^a<0.001) in serum γ–glutamyltranspeptidase (γ-GT) activity occurred in arsenic intoxicated mice (fig. 1). Conjoint administration of selenium and vitamin E appreciably restored the elevated γ-GT activity to its respective control value.

The serum CK activity increased significantly (p^a<0.001) by 71.82% in arsenic treated mice (Figure 2). Combined supplementation of selenium and vitamin E showed 99.9% (p^b<0.001) counteractive response against arsenic-induced altered CK activity. Their synergistic effect was better than their individual one.

Lactate dehydrogenase (LDH) activity (Fig. 3) increased significantly by in arsenic treated mice. Selenium alone restored the elevated LDH activity by 48.9%, whereas vitamin E counteracted it by 40%. Combined supplementation of selenium and vitamin E counteracted the LDH activity by 60.6%, which further indicates additive beneficial effects of selenium and vitamin E against arsenic toxicity.

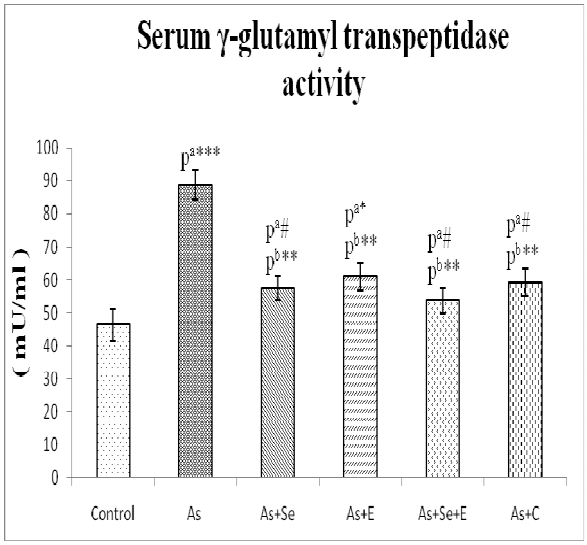

Fig. 1: Effects of co-administration of selenium and vitamin E in restoration of serum γ-glutamyl transpeptidase activity in arsenic-exposed animals

Values are Means±S.E.M., p^a compared with control group., p^b compared with arsenic-treated group. ,*** indicates p<0.001, ** indicates p<0.01, * indicates p<0.05 and # indicates p>0.05.

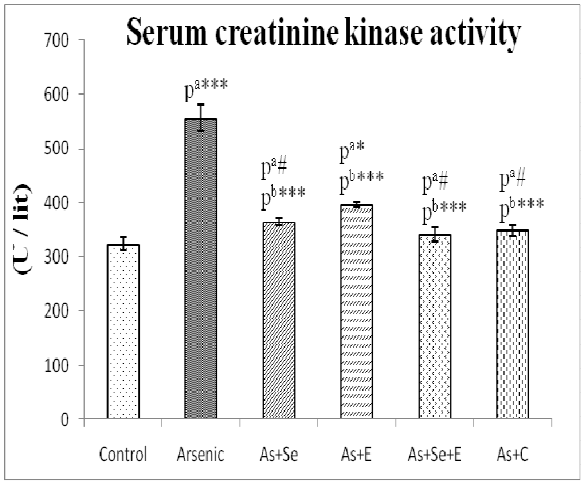

Fig. 2: Effects of co-administration of selenium and vitamin E in restoration of serum creatinine kinase activity in arsenic-exposed animals

Values are Means±S.E.M. p^a compared with control group. p^b compared with arsenic-treated group.*** indicates p<0.001, ** indicates p<0.01, * indicates p<0.05 and # indicates p>0.05.

Uric acid level in serum was elevated significantly by 30.02% (p<0.001) in arsenic-treated group of animals (Fig. 4). Selenium, vitamin E and their combined exposure prevented arsenic-induced alteration of serum uric acid level. The restoration was found to be 23.61%, 35.97% and 23.6%, respectively. Here combined administration shows almost similar counteractive effect as selenium.

Figure 5 illustrates the histopathological changes in cardiac tissue of experimental animals. In normal tissue of heart the nuclei are long cylindrical in shape and are well organised. Arsenic treatment caused severe disorganization of normal cardiac tissue architecture and also transformed the nuclei into globular shape as compared to control.

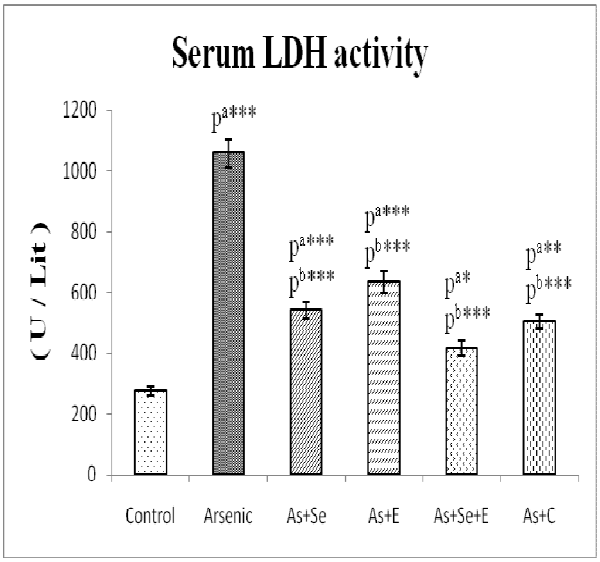

Fig. 3: Effects of co-administration of selenium and vitamin E in restoration of serum LDH activity in arsenic-exposed animals

Values are Means±S.E.M. p^a compared with control group. p^b compared with arsenic-treated group. *** indicates p<0.001, ** indicates p<0.01, * indicates p<0.05 and # indicates p>0.05.

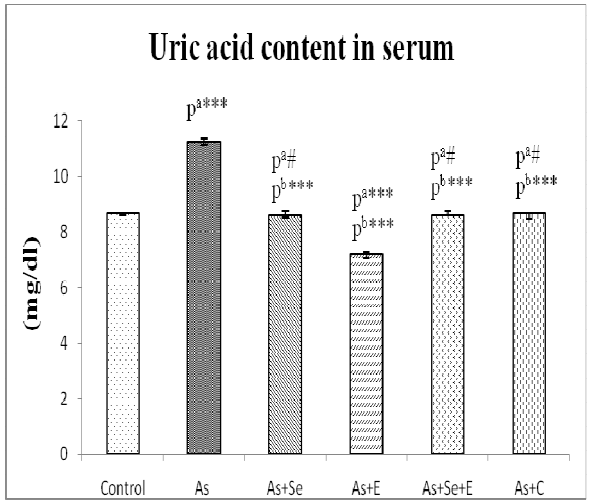

Fig. 4: Effects of co-administration of selenium and vitamin E in restoration of serum uric acid content in arsenic-exposed animals

Values are Means±S.E.M. p^a compared with control group. p^b compared with arsenic-treated group. *** indicates p<0.001, ** indicates p<0.01, * indicates p<0.05 and # indicates p>0.05.

Supplementation with selenium and vitamin E or their combination appreciably restored all the changes of cardiac tissue similar to that of control mice.

DISCUSSION

Synergistic beneficial effects of selenium and vitamin E against arsenic-induced oxidative insult in cardiac tissue and altered lipid profile were evaluated. Arsenic caused significant elevation of lipid markers of cardiac damage indicated by increased level of cholesterol, triglyceride and LDL, whereas decreased the HDL level in serum. Restoration of lipid parameters near to normal by vitamin E and selenium indicated that these micronutrients may have suppressing effects on cholesterol biosynthesis and also may increase the uptake of LDL from blood by liver. Abnormal lipid

metabolism may be associated with cardiovascular diseases [40]. Arsenic causes overt endothelial cell injury, cell proliferation and changes in monolayer binding of labeled low-density lipoprotein and permeability of albumin [41]. Epidemiologic evidences showed incidence of cardiovascular diseases, stroke and peripheral arterial disease in arsenic exposed areas of Taiwan [42].

Elevation of CK, LDH and transaminase enzyme activities in arsenic exposed mice is clear indication of cardiac muscle damage. These enzymes are leak out to blood during low oxygen or glucose supply to myocardium causing myocardial infarction [43]. The oxidation of hypoxanthine/xanthine to uric acid is catalyzed by XO which is associated with the generation of superoxide radical ($O_2^{\cdot-}$) [44]. Uric acid is associated with risk of cardiovascular mortality [45]. Selenium in combination of vitamin E ameliorated arsenic-induced altered enzyme profile and reversed their levels almost near to their normal range. Oxidative stress plays major role in cardio-toxicity by generation of lipid peroxidation, protein carbonylation and altered antioxidant enzyme activities. Myocardial tissues are susceptible to free radical damage due to suppressed activities of antioxidant enzymes like SOD, catalase and GPx in the heart [43, 46]. Overproduction of superoxide anions may be the causative factor of arsenic-induced inhibited SOD and catalase activities [47, 48]. Decreased content of GSH might be a causative factor for decreased activities of GST and GPx which utilize GSH to remove free radicals from myocardium [49]. Decrease in GSH content may be due to suppressed activity of GR. Ddecreased GSH content in myocardial tissue due to arsenic indicates GSH-linked oxidative damages [50]. Total thiol in cardiac tissue was significantly decreased, whereas the protein carbonyl content increased due to arsenic toxicity. Increase in γ-GT enzyme activity in arsenic-treated mice indicates GSH catabolism and plasma membrane damage. Treatment with selenium and vitamin E prevented arsenic-induced reduction in antioxidant power and protect the cell from oxidative insult.

NADPH oxidase plays a central role in generation of cardiovascular disorder by ROS [51]. Increased NADPH in arsenic-treated animals may lead to atherosclerosis in conjugation with increased cholesterol to form foam cells. Additionally, arsenic intoxicated cells enhance NO level and subject to toxicity by generating reactive nitrogen species [10]. These changes were inhibited significantly by the concomitant administration of selenium and vitamin E.

The histopathological study shows abnormal ultra structural changes in arsenic-intoxicated cardiac tissue. A marked disorganization of normal radiating pattern of myocardial cells associated with changes in shape of nuclei was found. Combined supplementation of selenium and vitamin E maintain the normal ultra structure of the heart.

In previous studies it was also established that arsenic causes oxidative stress which leads to myocardial damage [49, 52]. Both the selenium and vitamin E showed protective effects on oxidative damage of erythrocytes [53], reproductive toxicity [54] and antioxidant defense in streptozotocin-induced diabetic rats [55].

CONCLUSION

It is thus concluded that arsenic causes myocardial damage by changing lipid profiles, generating ROS and lowering antioxidant enzymes. Combination therapy of vitamin E with selenium is found to be more effective in restoration of arsenic-induced changes of the above studied parameters as well as ultra-structural organization of the cardiac tissue.

ACKNOWLEDGEMENT

We express our sincere thanks to University Grant Commission, New Delhi, Government of India for funding this project [UGC-MRP-F.No.39-682/2010 (SR) dated 14/01/2011] and help us with financial support for carrying out research in the field of arsenic toxicity.

CONFLICT OF INTEREST

We declared that we have no conflict of interest.

REFERENCES

1. Grund SC, Hanusch K, Uwe HW. Arsenic and Arsenic Compounds, Ullmann's Encyclopedia of Industrial Chemistry; Weinheim: Wiley-VCH; 2005.
2. Miller WH, Schipper HM, Lee JS, Singer J, Waxman S. Mechanisms of action of arsenic trioxide. Cancer Res 2002; 62: 3893-3903.
3. Vizzardi E, Zanini G, Antonioli E, D'Aloia A, Raddino R, Cas LD. QT prolongation: a case of arsenical pericardial and pleural effusion. Cardiovasc Toxicol 2008; 8: 41-44.
4. Ratnaike RN. Acute and chronic arsenic toxicity. Postgrad Med J 2003; 79: 391-396.
5. Wang H, Xi S, Liu Z, Yang Y, Zheng Q, Wang F, Xu Y, Wang Y, Zheng Y, Sun G. Arsenic methylation metabolism and liver injury of acute promyelocytic leukemia patients undergoing arsenictrioxide treatment. Environ Toxicol 2013; 28: 267-275.
6. Tseng CH. Arsenic methylation, urinary arsenic metabolites and human diseases: Current perspective. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 2007; 25: 1-22.
7. Sun HL, Chu WF, Dong DL, Liu Y, Bai YL, Wang XH, Zhou J, Yang BF. Choline-modulated arsenic trioxide-induced prolongation of cardiac repolarization in Guinea pig. Basic Clin Pharmacol Toxicol 2006; 98: 381-388.
8. Rossman T. Arsenic. In: Rom W and Markowitz S eds. Environmental and occupational medicine, 4th ed. Hagerstown, MD: Lippincott Williams & Wilkins; 2007. p. 1006-1017.
9. Tseng CH, Chong CK, Tseng CP, Hsueh YM, Chiou HY, Tseng CC, Chen CJ. Long-term arsenic exposure and ischemic heart disease in arseniasis-hyperendemic villages in Taiwan. Toxicol Lett 2003; 137: 15-21.
10. Shi H, Shi X, Liu K. Oxidative mechanism of arsenic toxicity and carcinogenesis. Mol Cell Biochem 2004; 255: 67-78.
11. Battin EE, Perron NR, Brumaghim JL. The central role of metal coordination in selenium antioxidant activity. Inorg Chem 2006; 45: 499-501.
12. Atif F, Yousuf S, Agrawal SK. Restraint stress-induced oxidative damage and its amelioration with selenium. Eur J Pharmacol *2008;* 600: 59-63.
13. Viezeliene D, Jansen E, Rodovicius H, Kasauskas A, Ivanov L. Protective effect of selenium on aluminium-induced oxidative stress in mouse liver in vivo. Environ Toxicol Pharmacol 2011; 31: 302-306.
14. Li JL, Gao R, Li S, Wang JT, Tang ZX, Xu SW. Testicular toxicity induced by dietary cadmium in cocks and ameliorative effect by selenium. Biometals 2010; 23: 695-705.
15. Watson M, van Leer L, Vanderlelie JJ, Perkins AV. Selenium supplementation protects trophoblast cells from oxidative stress. Placenta 2012; 33: 1012-1019.
16. Lexis LA, Fassett RG, Coombes JS. Alpha-tocopherol and alpha-lipoic acid enhance the erythrocyte antioxidant defence in cyclosporine A-treated rats. Basic Clin Pharmacol Toxicol 2006; 98: 68-73.
17. de Arriba G, de Hornedo JP, Rubio SR, Fernandez MC, Martínez SB, Camarero MM, Cid TP. Vitamin E protects against the mitochondrial damage caused by cyclosporin A in LLC-PK1 cells. Toxicol Appl Pharmacol 2009; 239: 241–250.
18. Deger Y, Yur F, Ertekin A, Mert N, Dede S, Mert H. Protective effect of alpha tocopherol on oxidative stress in experimental pulmonary fibrosis in rats. Cell Biochem Funct 2007; 25: 633-637.
19. Maliakel DM, Kagiya TV, Nair CK. Prevention of cisplatin-induced nephrotoxicity by glucosides of ascorbic acid and alpha-tocopherol. Exp Toxicol Pathol 2008; 60: 521-527.
20. Morsy MD, Mostafa OA, Hassan WN. A potential protective effect of alpha-tocopherol on vascular complication in spinal cord reperfusion injury in rats. J Biomed Sci 2010; 55: 1-9.
21. Catalgol B, Ozer NK. Protective effects of vitamin E against hypercholesterolemia-induced age-related diseases. Genes Nutr 2012; 7: 91-98.
22. Jacobus WE, Lehninger AL. Creatine kinase of rat heart mitochondrea. J BiolChem 1973; 248: 4803-4810.

23. Bergmeyer HU. Methods of Enzymatic analysis. VerlagChemie, GmbH, Weinheim /Bergstr, New York; 1963. p.736.
24. Reitman S, Frankel S. Determination of serum glutamic oxaloactic and glutamic pyruvic transaminase. Am J ClinPathol 1957; 28: 56-60.
25. Szasz G. A kinetic photometric method of serum γ - glutamyltranspeptidase. Clin Chem 1969; 15: 124-136.
26. Stadtman ER, LevineRL. Protein oxidation. Ann N Y AcadSci 2000; 899: 191-208.
27. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82: 70–77.
28. Davila JC, Davis PJ, Acosta D. Changes in glutathione cellular energy as potential mechanisms of papaverine-induced hepatptotoxicity in vitro. Toxicol Appl Pharmacol 1991; 108: 28-36.
29. Buege A, Aust SD. Microsomaal lipid peroxidation. Methods Enzymol 1978; 52: 302-310.
30. Babbs CF, Steiner MG. Detection and quantitation of hydroxyl radical using dimethyl sulfoxide as molecular probe. Methods Enzymol 1990; 186: 137-47.
31. Chen YC, Lin SY, Lin JK. Involvement of reactive oxygen species and caspase 3 activation in arsenic-induced apoptosis. J CellPhysiol 1998; 177: 324-333.
32. Bergmeyer HU, Gawehn K, Grassl M. Methods of Enzymatic Analysis. Academic Press Inc 1974; 1: 521-522.
33. Raso GM, Meli R, Gualillo O. Prolactin induction of nitric oxide synthase in rat C6 glioma cells. J Neurochem 1999; 73: 2272-277.
34. Maiti S, Chatterjee AK. Differential response of cellular antioxidant mechanism of liver and kidney to arsenic exposure and its relation to dietary protein deficiency. Environ Toxicol Pharmacol 2000; 8: 227-235.
35. Warholm M, Guthenberg C, von Bahr C, Mannervik B. Glutathione transferases from human liver. Methods Enzymol 1985; 113: 499-502.
36. Martin JP, Dailey M, Sugarman E. Negetive and positive assay of superoxide dismutase based on hematoxylin autooxidation. Arch Biochem Biophys 1987; 255: 329-36.
37. Aebi H. Catalase in vitro. Methods Enzymol 1984; 105: 121-126.
38. Carlberg I, Mannervavik B. Glutathione reductase. Methods Enzymol 1985; 113: 484-485.
39. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin's phenol reagent. J Biol Chem 1951; 193: 265–275.
40. Katz AM, Messineo FC. Lipid-membrane interactions and the pathogenesis of ischemic damage in the myocardium. Circ Res 1981; 48: 1-16.
41. Hoorn CM. Environmental toxicants and endothelial function. Crisp Data Base National Institute of Health; 1995.
42. Navas-Acien A, Sharrett AR, Silbergeld EK, Schwartz BS, Nachman KE, Burke TA, Guallar E. Arsenic exposure and cardiovascular disease: a systematic review of the epidemiologic evidence. Am J Epidemiol 2005; 162: 1037-1049.
43. Shaik AH, Rasool SN, Vikram Kumar Reddy A, Abdul Kareem M, Saayi Krushna G, Lakshmi Devi K. Cardioprotective effect of HPLC standardized ethanolic extract of Terminalia pallida fruits against isoproterenol-induced myocardial infarction in albino rats. J Ethnopharmacol 2012; 141: 33-40.
44. Singh K, Ahluwalia P. Effect of monosodium glutamate on lipid peroxidation and certain antioxidant enzymes in cardiac tissue of alcoholic adult male mice. J Cardiovasc Dis Res 2012; 3: 12-18.
45. Fang J, Alderman MH. Serum uric acid and cardiovascular mortility the NHANES I epidemiologic follow up study, 1971-1992. National Health and Nutrition Examination Survey. JAMA 2000; 283: 2404-2410.
46. Singh G, Singh AT, Abraham A, Bhat B, Mukherjee A, Verma R, Agarwal SK, Jha S, Mukherjee R, Burman AC. Protective effects of Terminalia arjuna against Doxorubicin-induced cardiotoxicity. J Ethnopharmacol 2008; 117: 123-129.
47. Yamanaka K, Hasegawa A, Sawamura R, Okada S. Cellular response to oxidative damage in lung induced by the administration of dimethylarsinic acid, a major metabolite of inorganic arsenics, in mice. Toxicol Appl Pharmacol 1991; 108: 205-213.
48. Kono Y, Fridovich I. Superoxide radical inhibits catalase. J Biol Chem 1982; 257: 5751–5754.
49. Manna P, Sinha M, Sil PC. Arsenic-induced oxidative myocardial injury: protective role of arjunolic acid. Arch Toxicol 2008; 82: 137-149.
50. Chen YC, Lin-Shiau SY, Lin JK. Involvement of reactive oxygen species and caspase 3 activation in arsenite-induced apoptosis. J Cell Physiol 1998; 177: 324-333.
51. Konior A, Schramm A, Czesnikiewicz-Guzik M, Guzik TJ. NADPH Oxidases in aVascular Pathology. Antioxid Redox Signal 2013; [Epub ahead of print].
52. Das AK, Sahu R, Dua TK, Bag S, Gangopadhyay M, Sinha MK, Dewanjee S. Arsenic-induced myocardial injury: protective role of *Corchorus olitorius* leaves. Food Chem Toxicol 2010; 48: 1210-1217.
53. Ben Amara I, Soudani N, Hakim A, Bouaziz H, Troudi A, Zeghal KM, Zeghal N. Dimethoate-induced oxidative damage in erythrocytes of female adult rats: possible 1. protective effect of vitamin E and selenium supplemented to diet. Toxicol Ind Health 2012; 28: 222-237.
54. Oda SS, El-Maddawy ZKh. Protective effect of vitamin E and selenium combination on deltamethrin-induced reproductive toxicity in male rats. Exp Toxicol Pathol 2012; 64: 813-819.
55. Ghaffari T, Nouri M, Irannejad E, Rashidi MR. Effect of vitamin e and selenium supplement on paraoxonase-1 activity, oxidized low density lipoprotein and antioxidant defense in diabetic rats. Bioimpacts 2011; 1: 12.