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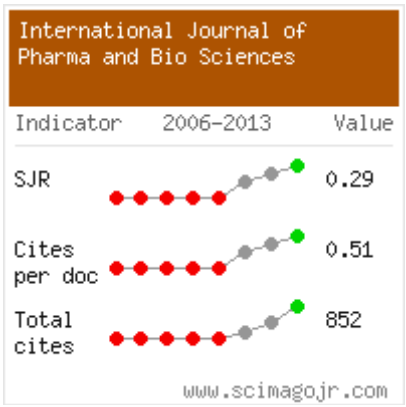

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Int J Pharm Bio Sci 2015 July; 6(3): (B) 1335 - 1341

Research Article **Allied Science**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

AMELIORATIVE EFFECT OF SELENIUM AS ANTIOXIDANT ON FLUORIDE TOXICITY INDUCED OXIDATIVE DEGENERATIVE CHANGES ON CARDIAC TISSUE

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ABSTRACT

Fluorosis is an endemic disease caused due to the chronic intake of fluoride, which alters body physiology, especially antioxidant status and lipid metabolism. There are reports which suggests that degenerative changes occur in cardiovascular system due to fluoride ingestion, may be caused due to oxidative stress. Selenium is a component of the antioxidant enzymes glutathione peroxidase and thioredoxin reductase has an antioxidant property. Hence, the present study was designed to evaluate the antioxidant effect of selenium and its ameliorative action on cardiac tissue damage of fluorotic rats. Eighteen male adult Wister rats were divided into 3 groups, 6 rats in each group. One group served as control and the other two groups as experimental control & experimental group. Both the experimental groups were given 200ppm of fluoride drinking water for 100days and only experiment group received supplement of selenium (5mg/kg body weight) which was given orally as single dose daily for another 20days and both the groups were continued to receive fluorinated drinking water during the extended period also. The control group rats received ordinary drinking water throughout the experimental period. At the end of the experimental period, the rats were sacrificed as per ethical guidelines and the cardiac tissues were harvested for histological & biochemical studies. One ml of blood was collected from retro-orbital plexuses before animals were sacrificed. Fluoride treatment significantly decreased the body weight, increased the oxidative stress, decreased glutathione levels, increased malondialdehyde levels in cardiac tissue homogenate and increased level of serum AST was observed in fluoride ingested rats. The histological scrutiny of the cardiac tissue of these rats showed degenerative changes. The result of experimental group which received oral supplementation of selenium for 20days showed the opposite of only fluoride ingested experimental control group. Here, the MDA & serum AST levels were decreased whereas GSH level was increased. These biochemical changes were supported by histological observation of the experimental rats where, the cytoarchitecture of cardiac tissues showed near normal picture. The result may suggest that dietary supplementation of selenium to animals and humans living in fluoride endemic zones may probably reduce the risk of cardiac tissue damage.

KEYWORDS: Antioxidant, AST, Fluorosis, Oxidative Stress, MDA, GSH. Selenium

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INTRODUCTION

Fluorosis related health problems have become global concern in developing countries. Chronic exposure of fluoride bring about several metabolic alterations by interacting with various cellular process and affects the antioxidant system in body [1, 2]. It is also well known that fluoride is hyper lipidemic agent, as it increased the plasma and tissue lipids in laboratory animals.[3, 4]. Its accumulation in soft tissues causes' injury by enhancing free radical production and disturbed antioxidant level leads to oxidative stress[5]. Studies indicated a slow and altered development of metabolic, functional and structural damages to the myocardium of fluoride exposed rats[6]. Okushi found higher incidences of myocardial damage followed by changes in electrocardiogram and cardiac dilation in inhabitants of fluoride endemic zone, where fluoride levels were 6-13ppm in drinking water[7]. Histopathological studies by Pribilla[8] showed fibrous necrosis, dissolution of nuclei, infiltration of histocytes, lymphocytes, granulocytes in the myocardium of patients with acute slilcofluoride intoxication. Generation of free radicals, lipid peroxidation and altered antioxidant defense system are considered to play important role in toxic effects of fluoride[9,10]. Administration antioxidant ascorbic acid to sodium fluoride treated mice revealed significant recovery from fluoride toxicity[11]. Selenium is an essential constituent of a number of enzymes, some of which have antioxidant functions. Deficiency of the element in animals makes them susceptible to injury by certain types of oxidative stress[12]. In this study we assess the antioxidant property of selenium on cardiac tissue on exposure of chronic fluoride toxicity.

MATERIALS AND METHODS

After obtaining the IAEC (Approval No. SU/BRULAC/RD/010/2013) eighteen wistar male albino rats were obtained from BRULAC, Saveetha University, Chennai and were maintained in the BRULAC, Saveetha University, Chennai. They were housed in polypropylene cages with stainless steel grill tops, fed with standard pellet diet ad libitum. The average weight of the rats was 130grams and was randomly divided into 3groups as control, the experimental control and experimental group with equal number of rats. The control group of rats were provided with ordinary drinking water whereas the both the experimental groups of rats were received 200ppm of fluoride for 100days. After 100th day onwards while the other groups continues to receive the same experimental protocol, the experimental group was given supplement of selenium (5mg/kg body weight) daily by oral gavages for another 20days. At the end of the experiment, the groups were scarified under light ether anesthesia. Blood samples were drawn from retro orbital plexuses, rats were perfused transcardially with 0.9% saline and the heart was removed for analysis. Blood samples were centrifuged at 3000rpm for 15 minutes to separate the serum. Cardiac tissue was homogenized 1:40 w/v in 0.1M phosphate buffer, pH 7.4, contain 1mM of EDTA. The cardiac tissue homogenate was used for the assay of MDA, GSH. AST levels was assayed in serum adopted by the standard procedures.[13,14]. The histological slides were prepared and stained by sectioning the cardiac tissue using standard histological procedure.

Statistical analysis

Graphpad prism 6 was used for statistical analysis. Mean and standard error was calculated using one way analysis of variance (ANOVA) with Tukey's multiple comparisons test Level of significance was assumed at $p<0.05$.

RESULTS

The chronic fluoride intake significantly reduced the body weight and also caused degenerative changes in cardiac tissue. The experimental control rat's myocardial tissue showed disorganization and vacuolar degeneration of cells, dissolution of nuclei and vacuolar degeneration of lamina interna of artery (Fig 1, 2 &3). The interstitial edema, fibrosis and

necrosis of myocardial fibers were seen in the histological preparation of experimental control group. The biochemical analysis showed enhanced level of serum AST ($p<0.0001$) and cardiac tissue MDA ($p<0.0001$) but decreased GSH ($p<0.0001$) in experimental control group when compared to the control group. All these parameters in experimental group showed significant shift towards control. Though, between control and experimental group all the parameters significantly varied with experimental control ($p<0.0001$). The result showed that supplementation of selenium reverse the degenerative effect of chronic fluoride intoxication. (Table1)

Table 1

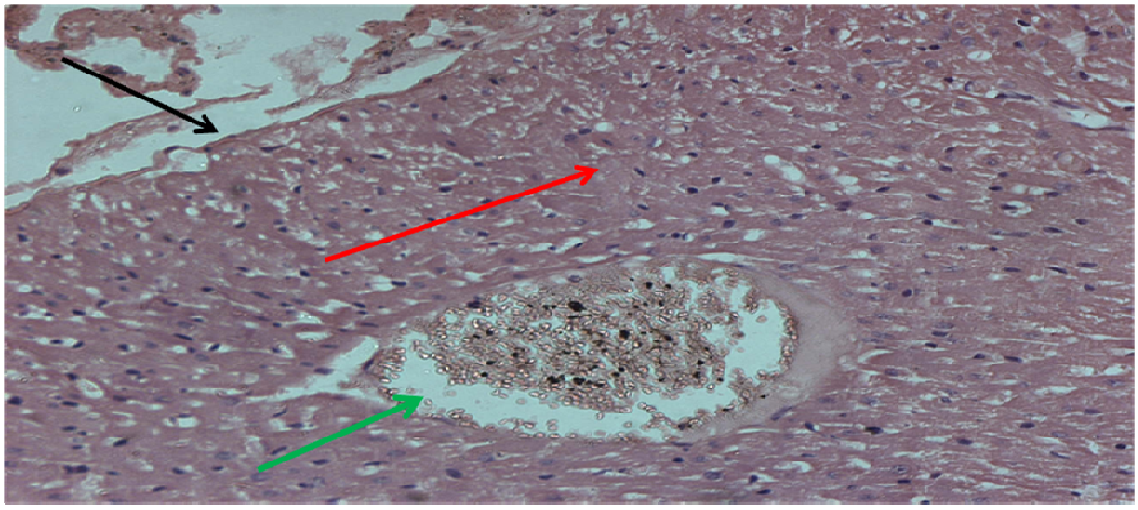
MDA, GSH and serum AST level in Control & Experimental rats: (mean ±SE)

Substance	Control	Exp. control	Experimental group
MDA(µmol of MDA/gm tissue)	18.59±0.35	32.32±0.29	22.75±0.18****
GSH(mg of GSH/gm tissue)	144.6±25.40	107.5±0.94	136.1±0.60****
AST (IU/L)	32.08 ± 1.90	142 ± 3.31	106.3 ±1.98 IU/L****

P<0.0001; * significant with experimental control***

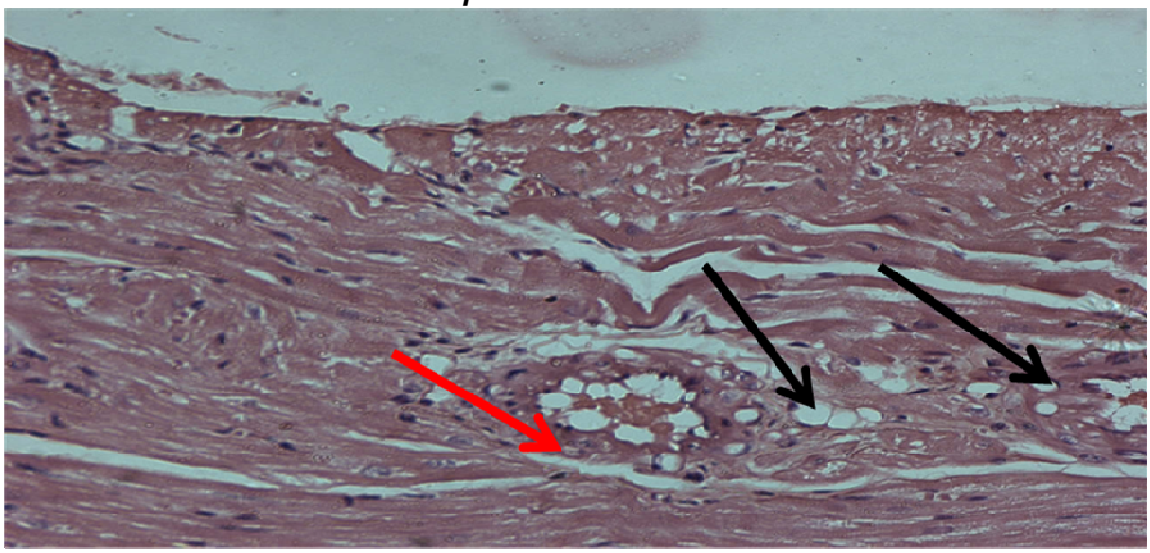
Figure 1

Control



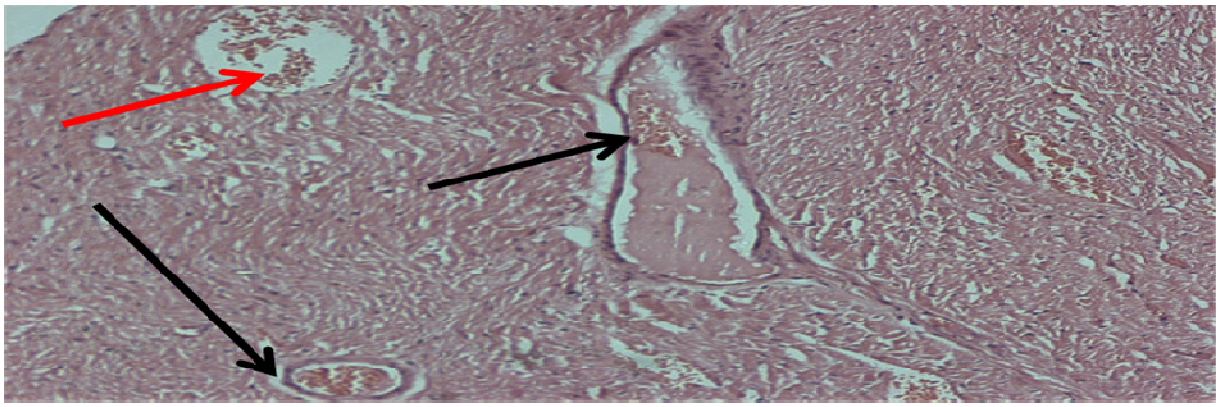
Pericardial layer of heart appeared normal [black arrow]. Myocardial layer is appeared normal [red arrow]. Coronary artery appeared normal [green arrow]

Figure 2
Experimental Control

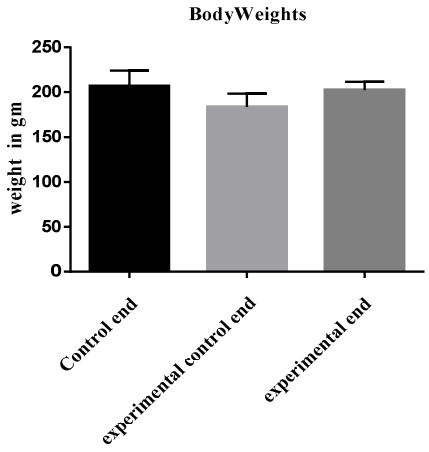


Thickening of coronary artery and vacuolar degeneration of intimae layer of coronary artery [red arrow]
Mild vacuolar Fatty degeneration of myocardium noticed [black arrow]

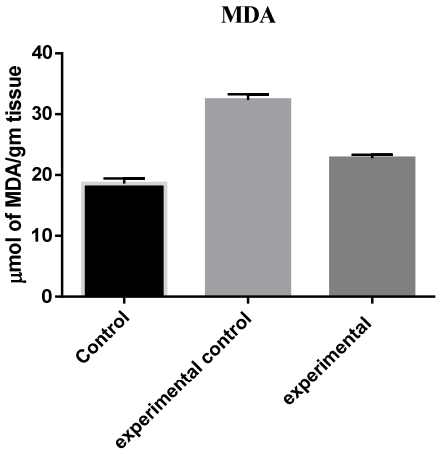
Figure 3
Experimental (Selenium)



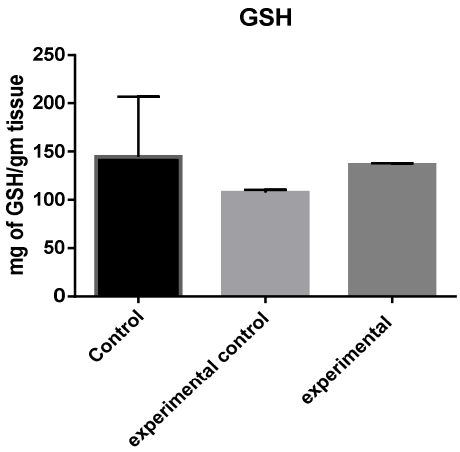
Coronary artery appeared normal black arrow, Coronary vein appeared normal red arrow



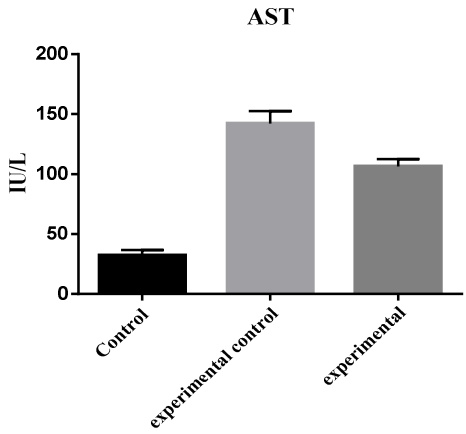
Graph 1 Body Weights



Graph 2 MDA levels



Graph 3 GSH levels



Graph 4 AST levels

DISCUSSION

In the present study sodium fluoride solution (NaF) was used to induce fluoride toxicity. The chronic exposure of fluoride considerably decreases the body weight while selenium supplement prevent the effect of fluorosis on body weight (Graph 1). The result of the body weight change following fluoride ingestion is in agreement with the report of Collins et al. They stated that the decrease of body weight on NaF ingestion primarily could be due to malabsorption which can be reversed by selenium supplementation[15]. Increased generation of ROS and enhanced lipid peroxidation are considered responsible for the toxicity of a wide range of compounds[16]. Various authors have investigated the relation between the fluoride and free radical reactions[17]. Earlier studies have reported increased lipid peroxidation[18,19] or unaltered GSH levels[20] following fluoride toxicity in various tissues. In the current study, in cardiac tissue the activities of GSH content decreased and MDA level increased in fluoride fed animals (Table 1 graph 2,3). There was an increased level of serum AST of fluoride treated rats, the enzyme, whose level increases proportionate to cardiac damage. (Table 1 graph 4) The histopathological evidence also supports the findings. Thus, the result of the present study clearly indicate that the chronic exposure of fluoride cause cardiac tissue damage which was attributed to increased generation of free radicals causing increased lipid peroxidation and decreased body's antioxidant defence. In

this study when the experimental group was supplemented with selenium the entire parameters were reversed in the direction of control group. The histopathological observation and biochemical observation such as MDA, GSH & AST levels tends to move in the direction of control group. (Table 1, Graph 2,3&4) Supplementation of selenium increased oxidative stability of cardiac tissues by increasing the endogenous antioxidants. Altered serum enzyme activities and lipids were observed in fluorosis and their recovery was pronounced on selenium supplementation[21]. It appears that the interference of selenium in balancing the redox state and controlling the activation of kinases and transcription factors that might have contributed to bring amelioration in the level of affected antioxidant status. Selenium plays a critical role in the maintenance of proper functioning and acts as protective agent against the free radical through the expression of selenoproteins as they involve in regulation of redox status under physiological conditions[22]. The role of selenium has been associated with the control of lipid peroxidation, as selenium supplement alleviates lipid peroxidation in fluoride treated animals[23]. Administration of the antioxidants is beneficial in promoting the recovery from fluoride induced toxicity, perhaps by augmentation of glutathione system; its involvement in detoxification process might help to delay the lipid peroxidation rate[24]. In the present study, the selenium administration has increased the body's antioxidant defense system that decreases the tissue damage which is caused due to free radical generation by fluoride toxicity, which supports earlier reports.

CONCLUSION

The present study reveals that the constant exposure of fluoride through drinking water decreases body weight by inducing malabsorption. Further, it states that fluorosis increases generation of free radicals which causes an increased lipid peroxidation results in increased MDA level in the tissues. Again, chronic ingestion of fluoride weaken body's antioxidant defense showed by decreased level of GSH and thereby increases the degenerative changes in the cardiac tissue as the AST level increases in serum. Selenium, an antioxidant when administered after the 100 days of exposure of fluoride, exhibit protective effect over the fluoride toxicity induced degenerative damages. As the experiment is terminated in 20 days, the 100 days degenerative effect of fluoride toxicity could not be reversed fully. Continued selenium administration may be the solution for the fluoride toxicity. This ameliorative effect of selenium could be attributed to its antioxidant property. Thus this study suggests that Selenium administration may probably be an antidote for fluoride toxicity.

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