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Effect of Fluoride Induced Toxicity on Cardiac Tissue: Possible Role of Oxidative Stress in Degenerative Changes of Cardiac Tissue.

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ABSTRACT

Fluorosis is an endemic disease caused due to ingestion of excess of fluoride in the form of drinking water and ingestion of vegetables sprayed on with pesticides etc. The current study was undertaken to determine degenerative changes takes place in cardiac tissue following ingestion of 200ppm of fluoride water for 100 days by studying histo-pathological changes and AST, MDA and GSH levels in cardiac tissue. In general, the fluoride ingested experimental group showed significantly less gain in their body weight during the experimental period when compared with the control. The histopathological observation showed that chronic fluoride ingestion causes severe cardiac tissue damage and the increased levels of AST (a non specific enzyme elevated in cardiac tissue damage) observed in experimental group supports the histopathological findings. Further, the fluoride treatment significantly increased the lipid peroxidation as determined by increase in MDA level and significantly decreased the level of GSH, an antioxidant enzyme in fluoride consumed experimental animal when compared with the control indicate that the degenerative damages that take place in the cardiac tissue might be possible through oxidative stress. Thus, biochemical changes were supported by histological observations and suggest that chronic exposure of fluoride may damage cardiac tissues may be by generating oxygen free radicals and depressing body's oxidative defense.The results of the study can be undertaken as an index of cardio toxicity in albino rats exposed to water fluorination and results may help people living in fluoride endemic zones to identify different screening tests to detect the damage of cardio vascular system.

Keywords: fluorosis, oxidative stress, MDA, GSH, AST, Lipid peroxidation

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INTRODUCTION

Fluorosis caused by excess ingestion fluoride in the form of drinking water, pesticides sprayed on vegetables, industrials waste products contaminating ground water resources is growing concern of the world. Daily intakes of fluoride can vary significantly according to the various sources of exposure. Values ranging from 0.46 to 3.6–5.4 mg/day have been reported in several studies [1]. In areas where water is fluoridated this can be expected to be a significant source of fluoride, however fluoride is also naturally present in huge range of foods, in a wide range of concentrations[2]. The lethal dose for most adult humans is estimated at 5 to 10 g (which is equivalent to 32 to 64 mg/kg elemental fluoride/kg body weight) [3-5].

Fluoride drinking water is known to cause both beneficial and toxic effects on health [6]. Cicek et al (2005) reported that when consumed in excess besides inducing skeletal and dental fluorosis, it also known to cause damage major organs of the body, including heart. They further indicted a slow and altered development of metabolic functions and structural changes in myocardium, as consequences of fluoride exposure for long duration [7]. Okushi et al found higher incidences of myocardial damage followed by changes in electro cardiogram and cardiac dilation in inhabitants of fluoride endemic zones, where fluoride levels were 6-13ppm. They also reported cloudy swelling's infiltration with round cells, thickening of adventitia, diffuse hemorrhage, vacuolar and colloidal degeneration of myocardium of rabbits fed with 10 to 100mg of sodium fluoride (NaF) for 132 days. Electrocardiographic studies by Takmore et al [8] showed direct relationship between increased myocardial damage and mottled teeth enamel. Pribilla et al [9] noticed fibrous necrosis, dissolution of nuclei, fibrinolysis, interstitial edema, minute hemorrhages, histocytes infiltration of lymphocytes and granulocytes in myocardium of patients with acute silicofluoride intoxication. In soft tissues fluoride interferes with numerous enzymes, finally leading to the production of free radicals [10].

Fluoride is known to induce oxidative stress and alter the antioxidant status in blood and tissues of humans and experiment animals. In the red blood cells of fluorotic humans, lipid peroxidation was increased and blood levels of antioxidants namely, superoxide dismutase and glutathione peroxidase decreased [11,12]. Further one study revealed that increased malonaldehyde with decreased levels of glutathione and glutathione peroxidase in blood of rats which received 10 and 30ppm fluoride in drinking water for 8months[13]. Some other reports are also stated that fluoride treatment for 5 and 10mg NaF/Kg body weight for 30 days caused increased malonaldehyde, and decreased superoxide dismutase, catalase, glutathione peroxidase , glutathione and ascorbic acid in the tissues of mice [14, 15]. In brain and muscle of mice subjected to fluoride toxicity (20mg NaF/Kg) for 14 days, levels of antioxidants enzymes decreased while activity of prooxidant enzymes xanthine oxidase was increased[16].

Although acute toxicity of fluoride on hard tissue is well known, information of extent and nature of degenerative changes and also causative factor induced these effects in chronic fluoride exposure on heart tissue is still scanty.

MATERIALS AND METHODS

Sixteen male albino rats (Wister strain of one month old) were procured from BRULAC, Saveetha University, Chennai after obtaining the animal Ethical Clearance (IAEC- No SU/BRULAC/RD/010/2013 Dated 11/07/2013). For the experiment, the rats were randomly divided into to two groups; as Control (Group 1) of 6 rats and Experimental (Group 2) with 10 rats. The rats were maintained in metabolic cages and were exposed to 12H light and dark cycle at a room temperature of 25 ± 1^{0}c and provided with normal laboratory diet.

The control group rats were fed with normal laboratory diet and water for drinking adlibitum. The experimental animals along with normal diet instead of ordinary drinking water, 200ppm of fluoride water was given for drinking. The experimental procedure was continued for 100 days to study the chronic fluoride effect.

At end of 100 days, after collecting 1ml of blood from the retro orbital plexus by sclera puncture, rats were scarified by following standard anesthetic procedure and the cardiac tissues were isolated and stored in 10% formalin for biochemical & histological studies. Histology of the tissue was done by using hemotoxcilin and eosin stains and the tissue homogenized in phosphate buffer used for biochemical investigations of MDA, GSH. The serum obtained from the blood collected was used for the estimation of AST.

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The tissue homogenate was prepared by homogenized the weighed cardiac tissue in chilled potassium chloride (1.15%). The homogenates were centrifuged at 6,000 g for 15 min at 4°C. The supernatant was used for biochemical analysis.

The MDA (Malonaldehyde) estimation was done by the method of Ohkawa et al (1979) and the value was expressed as µmoles/gm tissue.

Assay of the GSH (Glutathione) was assayed by a modification of the method Beutler et al (1963) mg of GSH/gm Tissue AST (Aspartate transaminase) activity in the serum was assayed using the coupled-enzyme method (IFCC1976) and the values as expressed as IU/L.

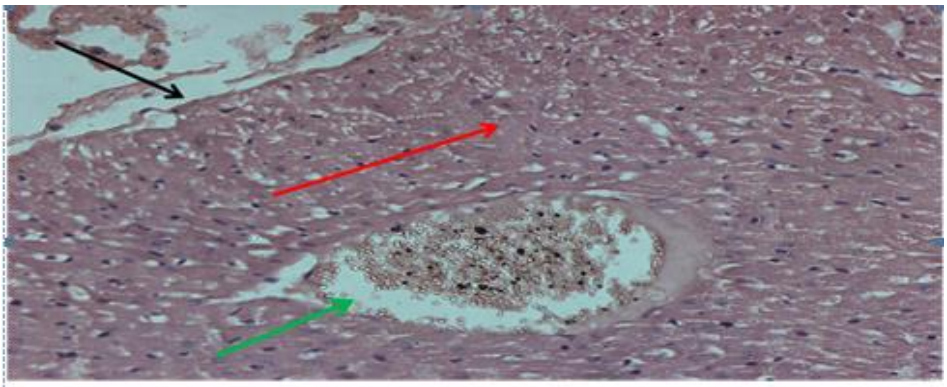
Statistical analysis

Graphpad prism 6 was used for statistical analysis. Mean and standard error was calculated using unpaired t test two tailed and F test to compare variances. Level of significance was assumed at $p<0.05$.

RESULTS

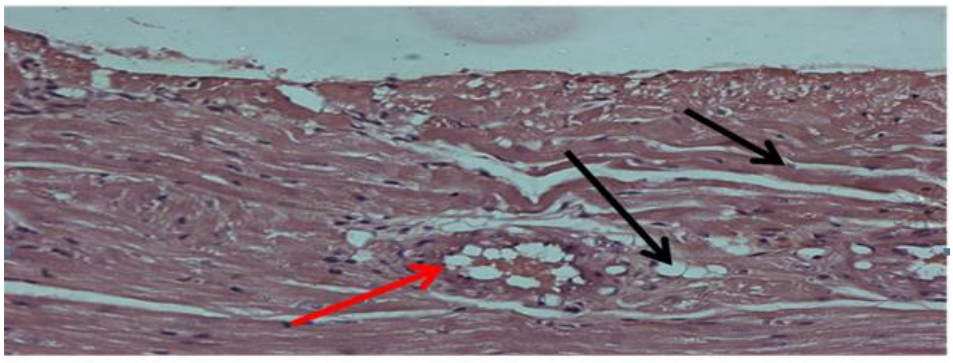
The histopathological & biochemical results of the control & experimental groups were analyzed and the outcome is given below.

Histopathological finding of cardiac tissue of control animal had shown normal cytological architecture (Fig.1) where as the experimental group shown the following degenerative changes in their cyto-architecture of cardiac tissue (Fig. 2).



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

Figure 1: Normal histology of cardiac muscle in control albino rats



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

Figure 2: Histopathological changes in fluoride treated albino rats

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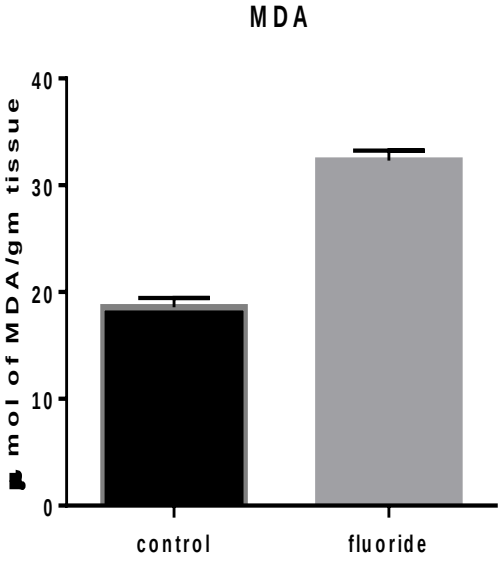
The experimental rats showed prominent vacuolar degeneration in the cardiac tissue (Fig.2). In those rats, the cardiac tissue had severe dilated bundles of muscles which were disorganized and the intercalated discs become prominent by swollen up. The cells of cardiac muscle shown mild to moderate swelling of nucleus, which are various degree of degeneration with altered shape and size and become pyknotic and karyorhesis.

The blood vessels here become dilated and showed hyperplasia hence detached from cardiac muscle.

The result biochemical analysis of rats showed that the MDA level increased (P<0.0001) and the GSH level decreased (p<0.001) in the experimental group than that of the control. The result of AST showed that the enzyme AST level increased 3 folds in experimental groups (p<0.0001) compared to control. . (Table 1, Figure 3,4,5).



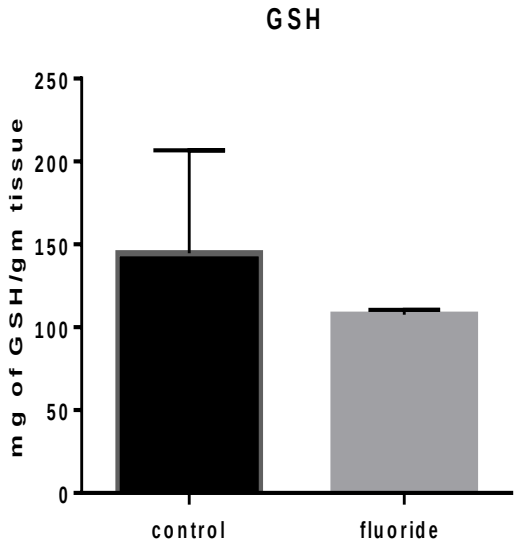


Figure: 3 Malonaldeyde levels(MDA) **Figure: 4 Reduced Glutathione levels(GSH)**

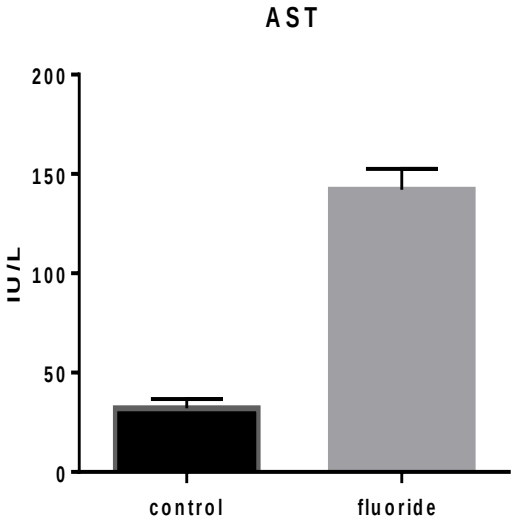


Figure : 5 Aspartate amino transferase (AST)

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Table: 1 MDA, GSH and AST level in Control & Experimental rats: (mean ±SE)

parameters	Control	Experimental
MDA (µmoles/gm tissue)	18.59±0.35	32.32±0.29****
GSH (mg/gm Tissue)	144.6±25.40	107.5±0.94****
AST IU/L (IU/L)	32.08 ± 1.90	142 ± 3.31 ****

P<0.0001; **** significant

DISCUSSION

The present study was devised to explore the damaging effect of chronic fluoride toxicity on cardiac tissue and the mode of degenerative changes. The experiment was carried out to verify assumption that oxidative stress is the main cause for the degenerative changes take place in tissues following chronic exposure to fluoride toxicity. In this work, to find out oxidative stress role in fluoride induced toxicity, the cardiac tissue MDA level was measured. MDA, as an end product of lipid peroxidation, level of which proportionate with the extent of oxygen free radicals induce degenerative effect. Further, GSH as an antioxidant, level of estimation, give some direction about the state of body's defense against the oxidative stress due to fluoride intoxication. Again, AST level was measured in this study to identify the degree of degenerative changes take place in cardiac tissue during fluorosis.

Various studies reported that the AST levels increases in various form of diseases and the enzymes such as ALT, AST, LDH, ALP and GGT, CK, CKMB, ACP, amylases and lipases are most often measured clinically to measure to access damage of tissue. During degenerative changes permeability of mitochondria increases which account increased cellular enzymes. In cardiac tissues, the enzymes thus liberated are LDH, LDH_1, CK, CKMB, and AST. Out of this, the AST is specific for heart and used as a marker in cardiac tissue damage. In this study, the fluoride toxicity increased AST enzyme level in the experimental rats indicating that the degenerative changes take place in cardiac tissue due to high ingestion of fluoride. This biochemical result was supported by the histological observation of the study. Here, the cyto-architecture of cardiac tissue exposed to chronic fluoride showed degenerative changes such as severe dilated bundles of muscles which were disorganized and the intercalated discs become prominent by swollen up. The cells of cardiac muscle showed mild to moderate swelling of nucleus, which are various degree of degenerative changes.

Generally an increase in MDA levels indicating increased oxidative stress, and decreased GSH, GSH-Px, catalases and SOD, indicting decreased ability of the tissue to handle free radicals, and are considered to be the makers of increases oxidative stress in tissues. In the present study, the rats exposed to chronic fluorosis showed marked increase in MDA level and decrease in GSH in the cardiac tissue. Various studies on oxidative stress on chronic fluoride toxicity on other organs in experimental animals have revealed decreased GSH and GSH-Px in tissues and blood. Zhi-zhong et al (1989) reported that decrease of GSH and GSH-Px in erythrocytes was observed in rats exposed to 30ppm of fluoride drinking water for 8month. Similarly various other reports are available stating decreased levels of GSH, GSH-Px, catalases and SOD in tissues in animals exposed chronic fluoride. Thus, the result of the present study also supports the findings of the above reports and further suggests that fluoride toxicity induce damage in cardiac tissue also mediated through oxidative stress.

CONCLUSION

The present study proved that chronic exposure of the fluoride causes degenerative changes particularly in cardiac tissue. Further, this work proved that these degenerative changes are effected through oxidative stress induced free radical generation and weakening of antioxidant system leads to damage of the tissues.

Thus, the knowledge of the study indicates that screening of MDA, GSH and AST levels may help the fluoride endemic zones population to access the early detection of cardiovascular damage and to take necessary steps to prevent oxidative damage caused to cardiovascular system by fluorosis.

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