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Is AST? A cardiogenic marker for fluoride induced toxicity in animal model

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Abstract: The aim of the study was to investigate the effect of sodium fluoride on serum lipid profile and AST (aspartate aminotransferase) enzymes and the possible protective role of selenium against fluoride toxicity on serum AST levels and lipid profile. 18 male wistar albino rats were divided into 3 groups control, experimental and interventional groups. Both experimental and interventional group were given 200 ppm fluoride water, whereas the control group received RO filter for drinking. Interventional group received 5mg/kg body weight/day selenium as a single dose orally by using oral gavages for 20days at the end of experimental protocol. Chronic exposure of fluoride resulted in an increase in serum triglyceride and VLDL levels in both the experimental and interventional group. The levels of HDL decreased in experimental and interventional groups while no change was observed in serum cholesterol and LDL levels when compared to control group. There was an increase of serum AST levels in experimental and interventional group. Upon supplementation of selenium for 20 days, resulted in decreased triglyceride, VLDL levels, increased HDL levels and Decreased AST levels in interventional group when compared to experimental group which was statistically significant with control and experimental group. Reversals of these effects were probably due to selenium which is an antioxidant and ameliorative in nature. Supplementation of selenium in fluoride endemic zones can prevent cardiovascular diseases. Screen of AST levels along with lipid profile may probably help in diagnosis of cardiovascular diseases in people residing at fluoride endemic zones.

Key Words: Fluoride, Selenium, AST, Cholesterol, Triglycerides, HDL, LDL and VLDL

INTRODUCTION

Fluoride in water is known for both useful and harmful effects on human body. Sodium fluoride was first fluoride compound used in the fluoridation of drinking water and in the treatment of dental caries. Chronic exposure to fluoride induces skeletal and dental fluorosis which causes damage to major tissues of the body such as cardiac tissue (Sinha *et al.,* 2008). Fluoride accumulation in soft tissues causes oxidative stress by inhibition of different oxidative enzymes and increases generation of free radicals (Nabavi *et al.,* 2012c). Determental effects of high fluoride intake are observed in soft tissues (Monsour and Krauger, 1985). Fluoride-induced oxidative stress plays an important role in progression of a variety of cardiac disorders such as cardiac ischemia (Sinha *et al.,* 2008). Therefore, oxidant and antioxidant balance is an important mechanism in mitigation of the oxidative stress in cardiac tissues. Sinha *et al.,* (2008) have shown that fluoride consumption causes myocardium injuries and dysfunction. Nabavi *et al.,* (2012c) have reported that fluoride increases oxidative stress through abnormal biochemical parameters in different tissues of rats. The free radical-induced oxidative stress is well known as an important mechanism of fluoride intoxication (Nabavi *et al.,* 2012c).

Reactive oxygen species have an important role in cardiac failures. Knowing that reactive oxygen species play a crucial role in fluoride-induced cardiotoxicity and oxidative stress, studies have been carried out on the cardioprotective action of antioxidants against fluoride-induced toxicity in cardiac tissues (Sinha *et al.,* 2008). Previous authors reported that natural antioxidants mitigated the oxidative injuries of fluoride in the cardiac tissues of rat (Nabavi *et al.,* 2012b).

In current research lipid profile was assessed to accumulate the evidence that the fluoride is lipid peroxidative and by assessing the levels of serum AST, to find out the oxidative changes in cardiac tissue which leads to cardiac damage in animal model.

MATERIAL AND METHODS

Animals

The study was performed on 18 Male wistar albino rats which were procured from BRULAC, Saveetha University, Chennai after obtaining the permission from Animal Ethical Committee (Approval No. SU/BRULAC/RD/010/2013).

Chemicals

Sodium fluoride and Selenium dioxide were procured from Ranbaxy laboratories, India. Biochemical kits were procured from Kamineni life sciences.

Experimental Protocol

18 Male adult wistar rats were randomly divided into 6 cages, 3 animals in each cage and cages are randomly divided into 3 groups control, experimental and interventional. Duration of the studies was 120 days. Control group of animals were fed with normal pelleted diet and RO filter water and both the experimental and interventional group were given fluoride water for drinking purpose and normal pelleted diet. Interventional group received selenium (in form of selenium dioxide) 5mg/ kg body weight dissolved in water for 20 days at the end of experimental protocol as an intervention.

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Experiment protocol - Control, Experimental and Interventional Groups

Group	No of animals	Exposure of Fluoride - Drinking
Control	6	RO filter water
Experimental	6	200ppm Fluoride
Interventional	6	200ppm Fluoride + Selenium for 20 days (end of experimental protocol)

All the animals were maintain at BRULAC and exposed to 12 hours dark and light cycle and hygienic conditions were maintained to get proper results. Care was taken during the preparation stock solution and proper dilutions to achieve good results. As the experimental protocol was finished animals were euthanized under isoflurane anesthesia and blood was collected by bleeding retro orobital plexuses and centrifuged it to get clear supernatant serum and stored at -20^{0}C for further estimations.

Assay of serum biochemical parameters

Lipid profile and AST (aspartate amino transferase): Measurement of lipid profile: by standard kit methods procured from Kamineni Life Sciences. Measurement of Serum AST: AST activity in serum was assayed using the coupled –enzyme method (IFCC 1976).

Statistical analysis

Data were collected, tabulated as Mean ±SE (Standard Error of Mean) for all the groups, each group was considered as single experimental unit. Data were analyzed by (GraphPad prism 6 software for statistical purpose, Version 6.07 using ANOVA (F-Test) and significant of means among the groups were done by using Dunnet's test with a P value < 0.05.

RESULTS

Effect of sodium fluoride on lipid profile

Chronic intake of fluoride on lipid profile was assessed by estimation of serum total cholesterol, triglyceride, HDL, LDL and VLDL the results showed that among the lipid profile, the cholesterol and LDL level did not change in experimental and interventional group. Triglycerides and VLDL levels are significantly increased in experimental fluoride group then that of control group, while in interventional group the levels are declined then that of experimental fluoride group which was statistically significant with Dunnett's multiple comparison test ($p<0.05$). Decreased HDL levels were observed in the fluoride group when compared to control and interventional group which is statistically significant with Dunnett's multiple comparison test ($p<0.05$). Table 1, Figure 1

Effect of Sodium fluoride on AST

AST is one of the specific nonfunctional enzyme of cardiac tissue damage. Exposure of chronic sodium fluoride toxicity, it was observed that there was an increase in serum AST levels in experimental fluoride group and interventional group then that of control group which is statistically significant with Dunnet's multiple comparison test that $P<0.05$, but it was observed that the levels of AST was shown decreased trend when compared to experimental fluoride group after the supplementation of selenium in interventional group. Table 2, Figure 2

Table 1: Serum Lipid Profile in Control, Experimental and Interventional Groups

		Mean ±SE	F	P
Control				
1.	Cholesterol	81±6.9		
2.	Triglycerides	107±14**	37.26	<0.0001
3.	HDL	12±0.63*		
4.	LDL	47±5.3		
5.	VLDL	21±2.7**		
Experimental				
1.	Cholesterol	78±7.5		
2.	Triglycerides	150±13	37.26	<0.0001
3.	HDL	12±1*		
4.	DL	36±4.5		
5.	VLDL	30±2.6**		
Interventional				
1.	Cholesterol	68±5.7		
2.	Triglycerides	74±8.9**	37.26	<0.0001
3.	HDL	15±0.36*		
4.	LDL	38±5.7		
5.	VLDL	15±1.8**		

Mean±SE, P<0.05 with AOVA with Dunnett's multiple comparison

Table 2: Serum AST (Aspartate Amino Transferase) in Control, Experimental and Interventional groups

	Mean ±SE	F	P
Control			
AST IU/l	32±1.9***	360.9	<0.0001
Experimental			
AST IU/l	142±3.3***	360.9	<0.0001
Interventional			
AST IU/l	106±2***	360.9	<0.0001

Mean±SE, P<0.05 with ANOVA with Dunnett's multiple comparison

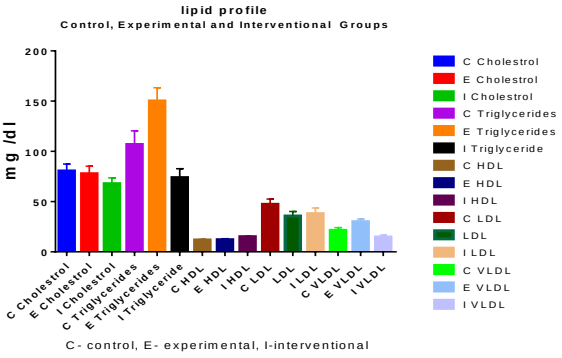

Figure 1: Serum lipid profile in control, experimental and intervention groups

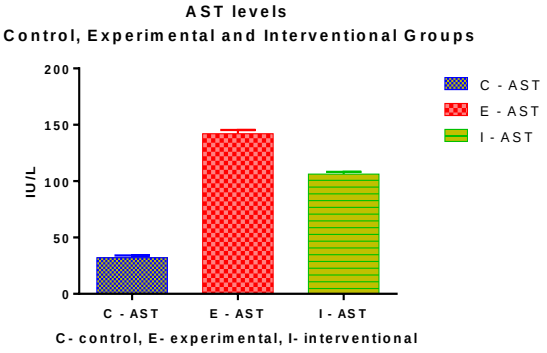

Figure 2: Serum AST levels in control, experimental and interventional groups

DISCUSSION

The current study was an attempt to evaluate the toxic effects of sodium fluoride on lipid profile and AST (Aspartate Aminotransferase) and possible protective role of natural antioxidant selenium and its ameliorative role.

Supplementation of sodium fluoride 200 ppm for prolonged duration, resulted in change of lipid profile, the results were in agreement with Czerny *et al.,* (2000) they stated that these changes are due abnormal activity of lipases, it appears that these enzymes are inhibited by fluoride. Levels of cholesterol and LDL were not affected both by fluoride intoxication and after the administration of antioxidant selenium. These finding were in agreement with report of Grucka-Mamczar *et al.,* (1997) where the authors stated that the rats which received 30 ppm of fluoride water for 3 months, there was no change in serum cholesterol levels. Further in current study, the triglyceride levels and VLDL levels were increased during supplementation of fluoride and reverted back, decreased on selenium supplementation for 20 days in an interventional group. There was decrease in HDL levels in experimental fluoride group then that of control and interventional groups. This proves that fluoride is atherogenic, upon the supplementation of selenium there was increase of HDL levels. These results are in accordance with Bennis *et al.,* (1993). Changes in HDL levels are probably because of ameliorative role of antioxidant selenium. Table 1, Figure 1

In the present study serum AST levels were increased in both the groups experimental and interventional group when compared to that of control group. AST is most often measured in clinical practice to accesses the damage of cardiac tissue. Clinical observations and experimental studies made it clear that subtle membrane changes are sufficient to allow passage of intracellular enzymes to extracellular spaces (Friedel *et al.,* 1979). Upon cellular damage permeability of the cell membrane increases and cytosolic enzymes such as AST, Alt, LDH and ALP spills into sinusoids from there escapes into blood elevating the enzyme levels. Lipid peroxidation represent most frequent reaction's resulting from free radical attack on biological structures. The damage to cellular membrane occurs due to generation of free radical (Stohs 1995).

The results serum AST showed that the enzymes levels have increased 3 folds in experimental and interventional groups ($P<0.05$) when compared to control group. Upon supplementation of selenium to interventional group the levels of AST were in line of decrease. Table 2, Figure 2

CONCLUSION

Timely evaluating the levels of Lipid profile and AST one can assess the oxidative damage to cardiac tissue and preventive measures can be taken to avoid the further damage of cardiac tissue. Supplementation of Selenium protects oxidative damage of cardiac tissue. Evaluation of serum lipid profile and AST may probably help the people leaving in fluoride endemic zones to evaluate the cardiac risk.

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