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Molecular Mechanism of Changes in Gene Expression of Cytosolic Cu/Zn SOD, Lipid Peroxidation and Hepatic Function Impairments during Experimental Fluorosis

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: Long term intake of high amount of fluoride leads to fluorosis causing metabolic, functional and structural damages affecting many tissues and organs including dental and skeletal manifestations. The liver is the most susceptible organ to fluoride toxicity because of its active and major role in digestion and detoxification.
Aim: The present study aims to elucidate the effects of sodium fluoride on hepatic function biomarkers, lipid peroxidation and gene expression of Cu/Zn SOD in albino rats.
Materials and Methods: Wistar albino rats were randomly assigned to three groups. The control rats were given 1 ml deionized water orally for 40 days. Groups II and III were administered 300 and 600 mg NaF/kg b.w. /day for the same period. Animals were sacrificed under anaesthesia, liver tissue was excised and used for biochemical and molecular analysis. The level of fluoride and lipid peroxidation (MDA) as well as reduced glutathione (GSH) content was determined. The activities of cytosolic copper/zinc superoxide dismutase (Cu/Zn SOD), aminotransferases (ALT and AST), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) in the hepatic tissue were

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determined. The analysis of gene expression of Cu/Zn SOD in the liver was determined using Real-time PCR.

Results: The results revealed significantly (*P<0.0001*) higher concentration of fluoride and MDA in the liver of rats exposed to fluoride when compared to control. The GSH content reduced significantly (*P<0.0001*) in fluorotic rats. The activities of hepatic function biomarkers viz; ALT, AST, LDH and ALP elevated significantly (*P<0.0001*) compared to the control. An elevation of 108.60% (ALP), 121.45% (AST), 33.77% (LDH) and least 24.40% (ALT) was found in rats treated with 300 mg/L fluoride and maximum elevation of 226.20% (ALP), 211.52% (AST), 57.75% (LDH) and least 56.79% (ALT) was registered in rats exposed to 600 mg/L fluoride in drinking water. The activity of cytosolic Cu/Zn SOD decreased significantly (*P<0.0001*) in fluorotic rats. Pearson's bivariate correlation and simple linear regression analysis exhibited strong positive correlation between level of hepatic tissue fluoride and activities of ALT (Pearson r= 0.85), AST (Pearson r= 0.94), LDH (Pearson r= 0.89), ALP (Pearson r= 0.86) and in MDA (Pearson r=0.984) while negative correlations existed between levels of fluoride and GSH (Pearson r=-0.93) as well as activity of Cu/Zn SOD (Pearson r= -0.99). The gene expression of cytosolic Cu/Zn SOD was significantly (*P<0.0001*) reduced in fluorotic rats.

Conclusion: The present study revealed that fluoride declined the antioxidant activity of hepatic Cu/Zn SOD at biochemical as well as molecular level which leads to oxidative stress and tissue damage. This further affirms by increased activities of hepatic function biomarkers in correlation with high fluoride level during experimental fluorosis.

Keywords: Fluorosis; gene expression; hepatic function biomarkers; lipid peroxidation.

ABBREVIATIONS

ALT : Alanine transaminase
AST : Aspartate transaminase
ALP : Alkaline phosphatase
B.W. : Body Weight
Cu/Zn SOD : Copper/Zinc superoxide dismutase
DNA : Deoxyribonucleic acid
Fig : Figure
LDH : Lactate dehydrogenase
MDA : Malondialdehyde
µl : Microliter
RNA : Ribonucleic acid
GSH : Reduced glutathione
NaF : Sodium fluoride

1. INTRODUCTION

Prolonged exposure to fluoride can affect the physiological activities of various enzymes in tissues and organs [1]. Excessive ingestion of fluoride over a prolonged period caused fluorosis [2] which occurs both acutely and chronically in humans and animals [3]. Dental and skeletal fluorosis is the main consequences of prolonged exposure of high fluoride concentrations [4, 5].

Being a major detoxifying organ, liver plays many vital roles in the body of humans and animals such as by regulating and metabolising the harmful toxins and also helpful to throw away these harmful toxins from the digestive tract through hepatic detoxification systems [6, 7]. The liver is a most susceptible organ to fluoride toxicity because of its active and major role in digestion and detoxification [8]. It is the central organ for nutrient metabolism and plays a key role in the coordination of nutrient fluxes to support body homeostasis. The liver represents the main detoxifying tissue by processing, neutralizing, and eliminating toxins from the digestive tract through hepatocyte-mediated enzymatic detoxification systems [9]. It is indirectly involved in the process of haematopoiesis by destructing abnormal blood cells [10]. Fluoride has been known to decelerate or trigger a wide range of enzymes including transaminases or phosphatases [11]. Familiar biomarkers of liver function, transaminases (alanine aminotransferase and aspartate aminotransferase) and phosphatases recognized to be significantly raised in adults and young ones with fluorosis which suggests liver damage and dysfunction [12, 13]. These biomarkers are common indicators of hepatic damage. High consumption of fluoride can damage the liver profile and ultimately rise in the levels of these metabolic enzymes [14]. Alkaline phosphatase is an important biomarker of bone disease and also plays an important role to detect various hepatobiliary diseases.

The imbalance between the formation and removal of free radicals causes oxidative stress, which is one of the accepted mechanisms of fluoride toxicity. Free radicals such as reactive

oxygen species (ROS) and reactive nitrogen species (RNS) accumulate when the body's antioxidant capacity can no longer protect the cell from oxidative damage, causing lipid peroxidation (LPO), protein oxidation, and DNA damage [12]. Considering all of these factors, the present study aims to elucidate the effects of experimental fluorosis on the hepatic functions, lipid peroxidation and gene expression of Cu/Zn SOD in rats.

2. MATERIALS AND METHODS

2.1 Animals

The experimental study was performed on Wistar albino rats weighing 150-200 g were housed in propylene cages with spotless grill tops. They were fed on standard commercial rat pellet diet (Hindustan lever limited, Mumbai, India) and water was given *ad libitum.*

Rats were separated into three groups and each group contained six rats. The animals of the group I received 1ml of deionized water orally for 40 days and served as control. The animals of groups II and III were given 300 and 600 mg NaF/kg b.w./day for the same period. At the end of the experimental period, all groups of rats were fasted overnight and sacrificed under anaesthesia.

2.2 Chemicals/kits

Sodium fluoride was purchased from Loba Chemie Pvt. Ltd., India. Commercially available test kits for estimation of aminotransferases (ALT and AST) (Erba diagnostics, Mannheim, Germany) as well as lactate dehydrogenase and alkaline phosphatase (Reckon diagnostics Pvt. Ltd., India) were used in the study.

2.3 Determination of Fluoride Level

The extraction of hepatic fluoride was done by the method of Inkielewicz and Krechniaka [15] and estimation [16] was done on selective ion electrode (ELIT 8221).

2.4 Homogenate Preparation and Enzyme Assays

The hepatic tissue was excised, washed and perfused with normal saline to remove excess blood, homogenized in 0.1M phosphate buffer (pH 7.4) and then centrifuged at 3000 rpm for 15 minutes at 4 ◦ C. The supernatants were taken out and used for further biochemical analysis. Some part of liver samples were kept in RNA later™ (Ambion, USA) for RNA isolation and stored at -80 ◦ c for molecular analysis. The activities of ALT (EC: 2.6.1.2), AST (EC: 2.6.1.1), LDH (EC: 1.1.1.27) and ALP (EC: 3.1.3.1) were determined on UV-Vis spectrophotometer using commercially available kits (Erba diagnostics, Mannheim, Germany and Reckon diagnostics Pvt. Ltd., India) according to manufacturer's protocol.

2.5 Lipid Peroxidation Quantification

The level of MDA in the hepatic tissue was determined by thiobarbituric acid method at the absorbance wavelength of 532 nm [17]. The level of GSH was measured on UV-Vis spectrophotometer by the method of Moron [18].

2.6 Cytosolic Cu/Zn Superoxide Dismutase Activity

The activity of cytosolic Cu/Zn SOD (EC: 1.15.1.1) in the hepatic tissue was determined according to the recommendations proposed by Ewing and Janero [19].

2.7 RNA Isolation

RNA was isolated using Trizol kit (G-Biosciences, USA, 2018). The isolated RNAs were checked using RNA gel electrophoresis and Nanodrop spectrophotometer (USA).

2.8 cDNA Synthesis

cDNA was synthesized using the Reverse Aid™ first strand cDNA synthesis kit (Thermo scientific, USA, 2018) according to the manufacturer's protocol.

2.9 Primers

The list of primers used is presented in Table 1.

2.10 Gene Expression Analysis

The gene expression profiles were measured using the SYBR green (Thermoscientic, USA, 2018) based qPCR method. Briefly, 4µl of total RNA was reverse transcribed into cDNA using 2µl of oligo $(dT)_{18}$ and Reverse Aid™ first strand cDNA synthesis kit in a final volume of 20 µl. From this reaction product, 4 µl was used to

serve as a template strand for PCR in a mixture with 2 µl each of Cu/Zn SOD forward and reverse primers amplified with β-actin taken as housekeeping gene. Primers were selected to bind specifically to the target gene [Table 1]; [20]. PCR reactions were performed in ABI prism SDS 7000. Co-amplification of primers with β-actin was incorporated to make sure that equal amounts of RNA were reverse transcribed and amplified in each reaction tube. The reaction conditions were as follows: initial denaturation at 95°C for 10 minutes was carried out. Further, denaturation at 95°C for 15 seconds, annealing at 60°C for 30 seconds, extension at 72°C for 30 seconds with a final extension at 72°C for 10 minutes. An overall of 40 cycles was performed. Gene expression was calculated according to the $2^{-\Delta\Delta Ct}$ formula [21]. The dissociation curve was also analyzed. 1.0% agarose gel was used to resolve the amplified products, stained with ethidium bromide and visualized in Gel Documentation System and photographed.

2.11 Statistical Analysis

The data was analysed using the statistical package SPSS (version 20.0 SPSS Inc., Chicago, IL, USA, 2018). Data were expressed as Mean ± SD. One-Way ANOVA was used to analyze the statistical differences between control and treatment groups. Multiple comparisons among all treatment groups were carried out by Post hoc Tukey's HSD test. To examine the correlation between fluoride and enzymatic activities, Pearson's bivariate correlation and simple linear regression analysis was conducted. P-value of less than 0.05 was considered as statistically significant.

Table 1. The primers used in qPCR analysis of gene expression

Gene symbols	Accession Number	Primer	Sequence (5'-3')	Product size
β-actin	NM_031144.3	Forward Reverse	cctgcttgctgatccaca ctgaccgagcgtggctac	505 bp
Cu/Zn SOD	NM_017050.1	Forward Reverse	gcagaaggcaagcggtgaac tagcaggacagcagatgagt	447 bp

3. RESULTS

3.1 Fluoride Level

The level of hepatic fluoride in test rats of groups II and III increased significantly (*P<0.0001*) in comparison with control group (Fig. 1) after 40 days of exposure.

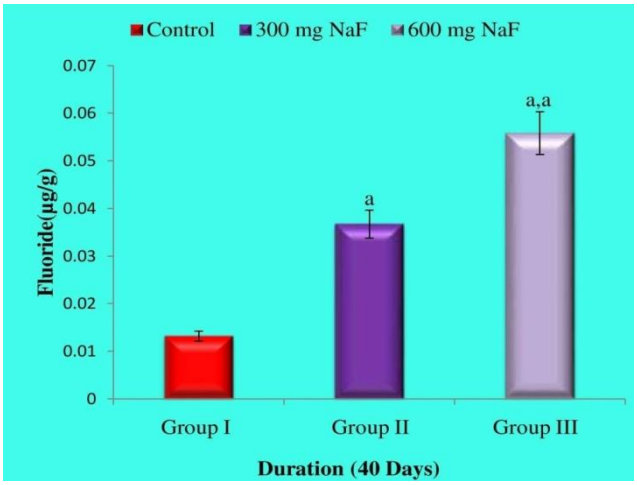

Fig. 1. Fluoride levels in the liver of rats after 40 days of exposure. The Values are represented as Mean ± SD (n=6).

[a]P<0.0001 Groups II and III vs Group I, [a,a]P<0.0001 Group II vs Group III.

n= Number of animals; SD= statistical difference; a,aa= distinct letters in the graph indicate statistical significant difference at P<0.0001

3.2 Aminotransferases

3.2.1 Alanine aminotransferase

The activity of ALT in groups II (300 mg/L NaF) and III (600 mg/L NaF) were significantly (*P<0.0001*) higher than the control group with respectively increasing +24.40% and +56.79% (Fig. 2).

Post hoc Tukey's HSD multiple comparison test after ANOVA revealed significant increase (*P<0.0001*) in the activity of ALT between and within groups (95% CI= -97.465 to 110.47995) in rats exposed to fluoride.

Pearson's bivariate correlation and simple linear regression analysis displayed a strong positive relationship between level of hepatic tissue fluoride and activity of ALT (Pearson r= 0.85; Fig. 3) in rats treated with fluoride. This indicated that with increase in the concentration of fluoride in hepatic tissue, activity of alanine aminotrans-ferase also increased due to hepatic cell damage.

3.2.2 Aspartate aminotransferase

The activity of AST in groups II and III were significantly elevated than the control group with respectively increasing +121.45% (*P<0.0001*) in group II received 300mg/L NaF and +211.52% in group III received 600 mg/L NaF (*P<0.0001*) (Fig. 4).

Post hoc Tukey's HSD multiple comparison test after ANOVA displayed a significant increase (*P<0.0001)* in the activity of AST between and within groups (95% CI= -164.319 to 131.561) after 40 days of fluoride treatment.

Pearson's bivariate correlation and simple linear regression analysis exhibited a strong positive relationship between level of hepatic tissue fluoride and activity of AST (Pearson r= 0.94; Fig. 5) in rats with fluoride intoxication. The activity of aspartate aminotransferase was more pronounced with increased concentration of fluoride in liver.

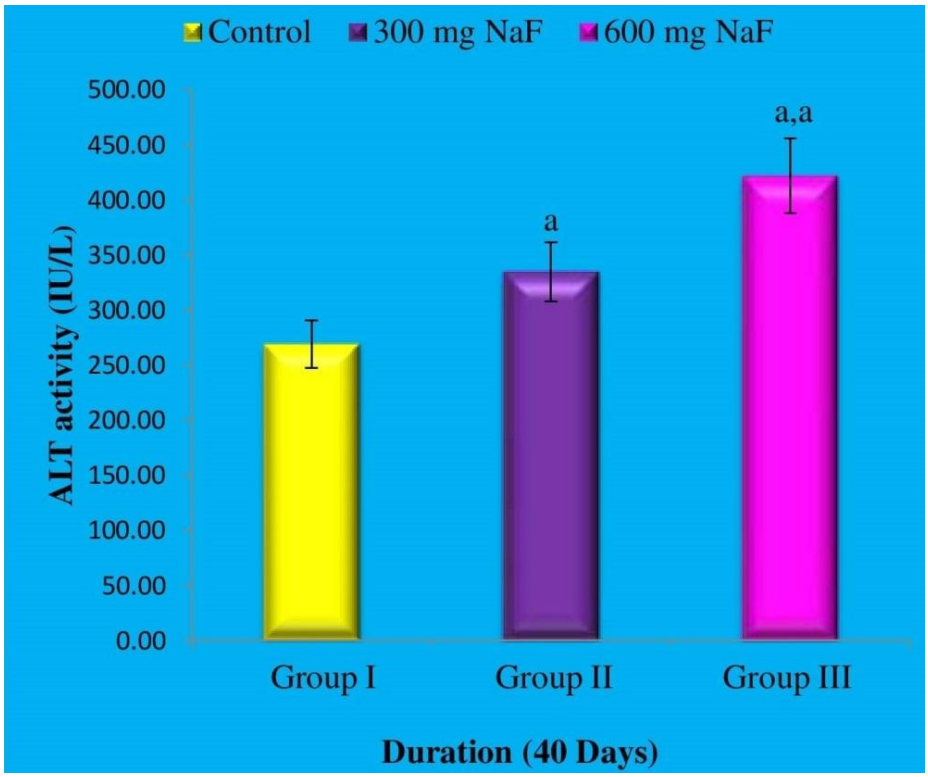

Fig. 2. Effect of fluoride on the mean activity of ALT in the hepatic tissue of control and fluoridated rats. The values represent Mean±SD (n=6).

[a]P<0.0001 Groups II and III vs Group I, [a,a]P<0.0001 Group II vs Group III

n= Number of animals; SD= statistical difference; a,aa= distinct letters in the graph indicate statistical significant difference at P<0.0001

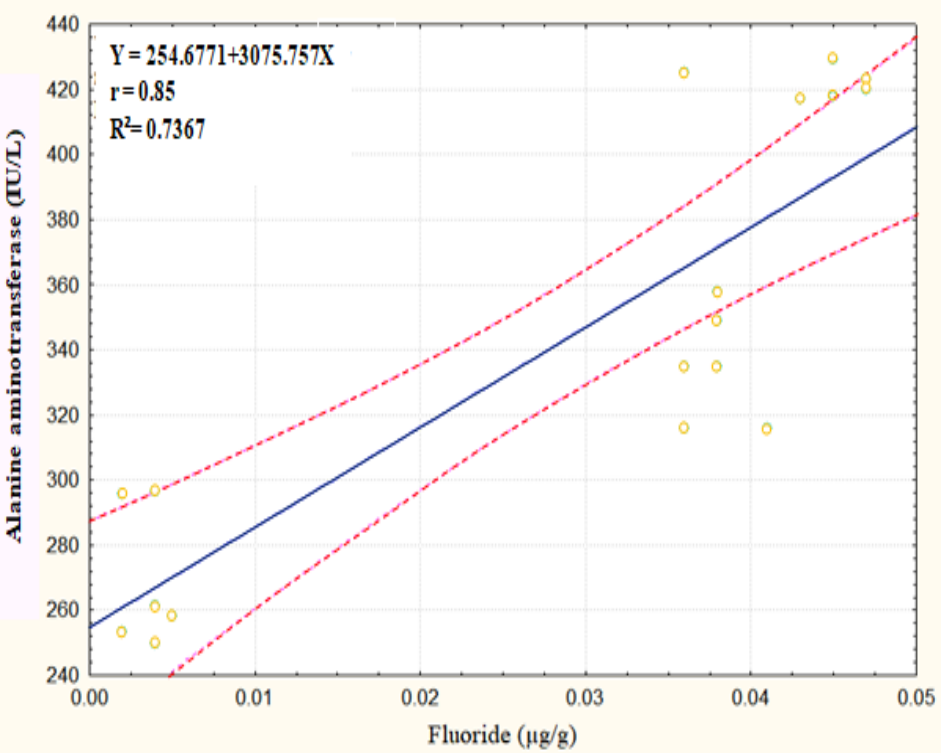

Fig. 3. Pearson's bivariate correlation and simple linear regression between level of fluoride and activity of alanine aminotransferase (IU/L) in the hepatic tissue of fluoridated rats.

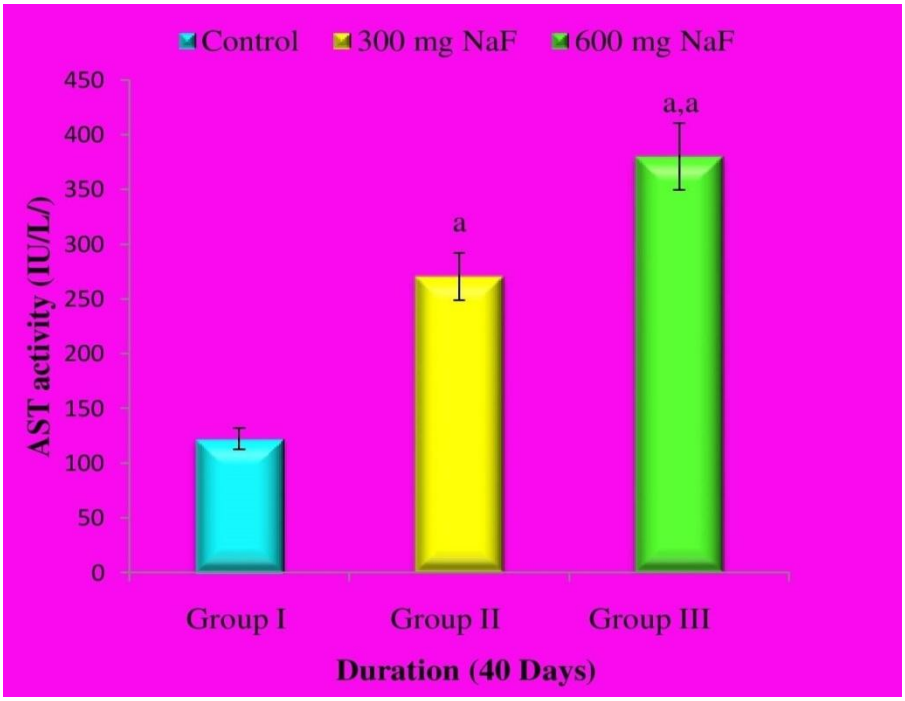

Fig. 4. Effect of fluoride on the mean activity of AST in the hepatic tissue of control and fluoridated rats. The values represent Mean±SD (n=6).

[a]P<0.0001 Groups II and III vs Group I, [a,a]P<0.0001 Group II vs Group III

n= Number of animals; SD= statistical difference; a,aa= distinct letters in the graph indicate statistical significant difference at P<0.0001

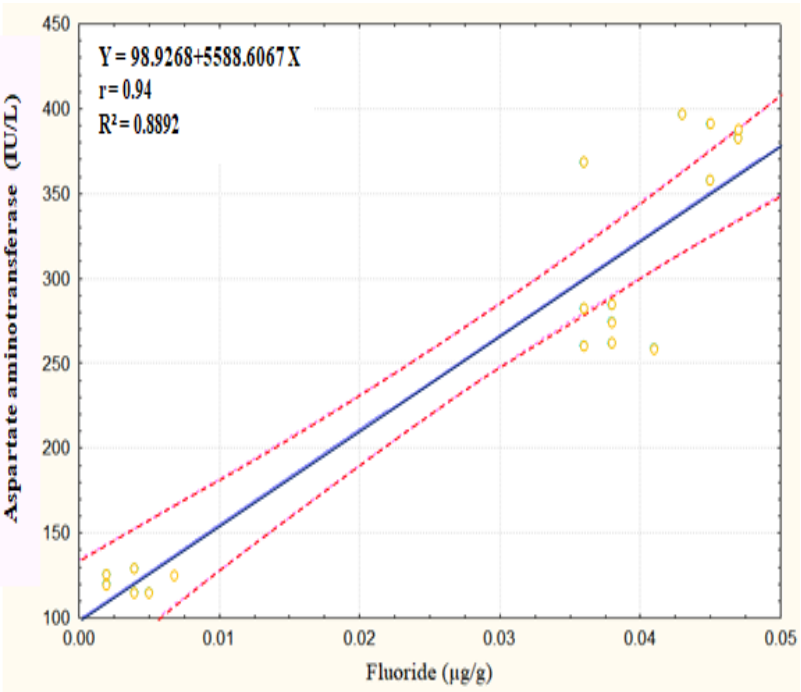

Fig. 5. Pearson's bivariate correlation and simple linear regression between level of fluoride and activity of aspartate aminotransferase (IU/L) in the hepatic tissue of rats exposed to fluoride

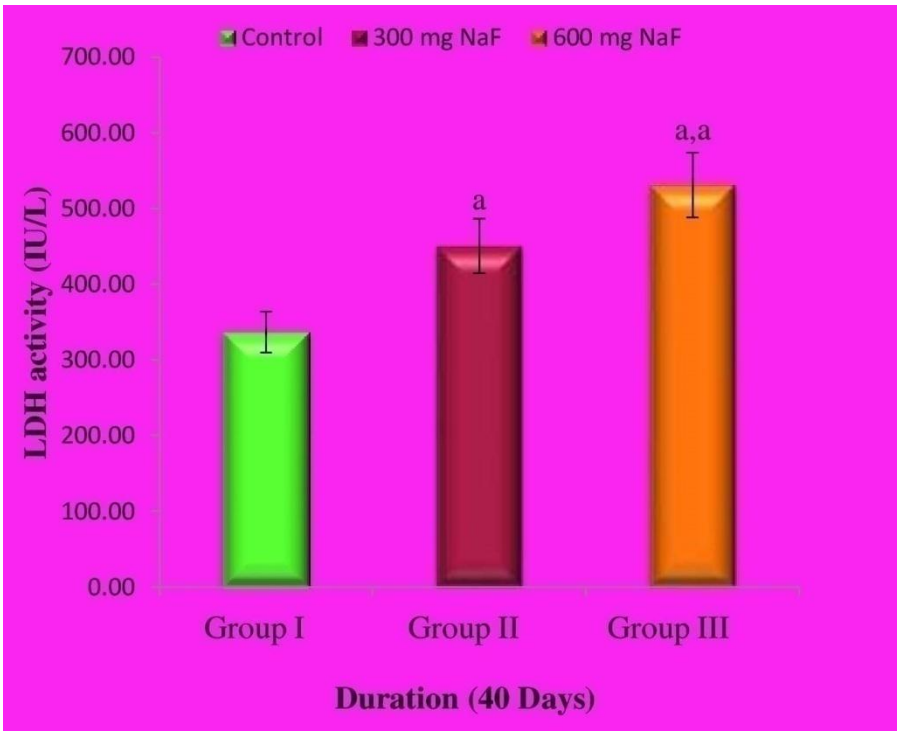

Fig. 6. Effect of fluoride on the mean activity of LDH in the hepatic tissue of control and fluoridated rats. The values represent Mean±SD (n=6).

[a]P<0.0001 Groups II and III vs Group I, [a,a]P<0.0001 Group II vs Group III.

n= Number of animals; SD= statistical difference; a,aa= distinct letters in the graph indicate statistical significant difference at P<0.0001

3.3 Lactate Dehydrogenase

The activity of LDH in groups II (300 mg/L NaF) and III (600 mg/L NaF) were higher than the control group with respectively increasing +33.77% (*P<0.0001*) and +57.75% (*P<0.0001*) (Fig. 6).

Post hoc Tukey's HSD multiple comparison test after ANOVA determined a significant (*P<0.0001*) increase in the activity of LDH between and within groups (95% CI= -142.668 to 142.222) in fluoride exposed rats.

Pearson's bivariate correlation and simple linear regression analysis showed a strong positive relationship between level of hepatic tissue fluoride and activity of LDH (Pearson r= 0.89; Fig. 7) in fluoridated rats.

3.4 Alkaline Phosphatase

The activity of ALP in groups II and III were elevated significantly (*P<0.0001*) than the control group with respectively increasing +108.60% and +226.20% (Fig. 8).

Post hoc Tukey's HSD multiple comparison test after ANOVA displayed a significant (*P<0.0001)* increase in the activity of ALP between and within groups (95% CI= -19.5728 to 23.0814) in fluoridated rats.

Pearson's bivariate correlation and simple linear regression analysis revealed a strong positive relationship between level of hepatic tissue fluoride and activity of ALP (Pearson r= 0.86; Fig. 9) in rats exposed to fluoride.

3.5 Lipid Peroxidation

The level of MDA in groups II and III were significantly higher than the control group with respectively increasing +142.97% in group II received 300 mg/L NaF (*P<0.0001*) and +352.22% in group III received 600 mg/L NaF (*P<0.0001*) (Table 2; Fig. 10).

Post hoc Tukey's HSD multiple comparison test after ANOVA revealed a significant increase (*P<0.0001*) in the level of MDA between and within groups (95% CI= -1.2572 to 1.8388) after 40 days of fluoride exposure.

Pearson's bivariate correlation and simple linear regression analysis displayed a strong positive relationship between levels of hepatic tissue fluoride and MDA (Pearson r= 0.98; Fig. 11) in rats exposed to fluoride which indicated that as level of fluoride increases, lipid peroxidation due to oxidative stress was also increased.

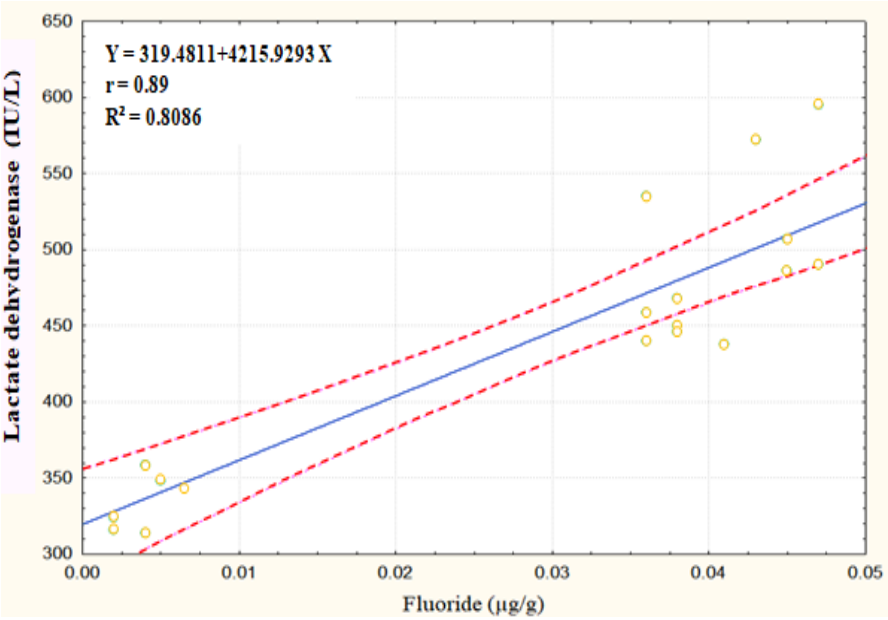

Fig. 7. Pearson's bivariate correlation and simple linear regression between level of fluoride and activity of lactate dehydrogenase (IU/L) in the hepatic tissue of fluoridated rats

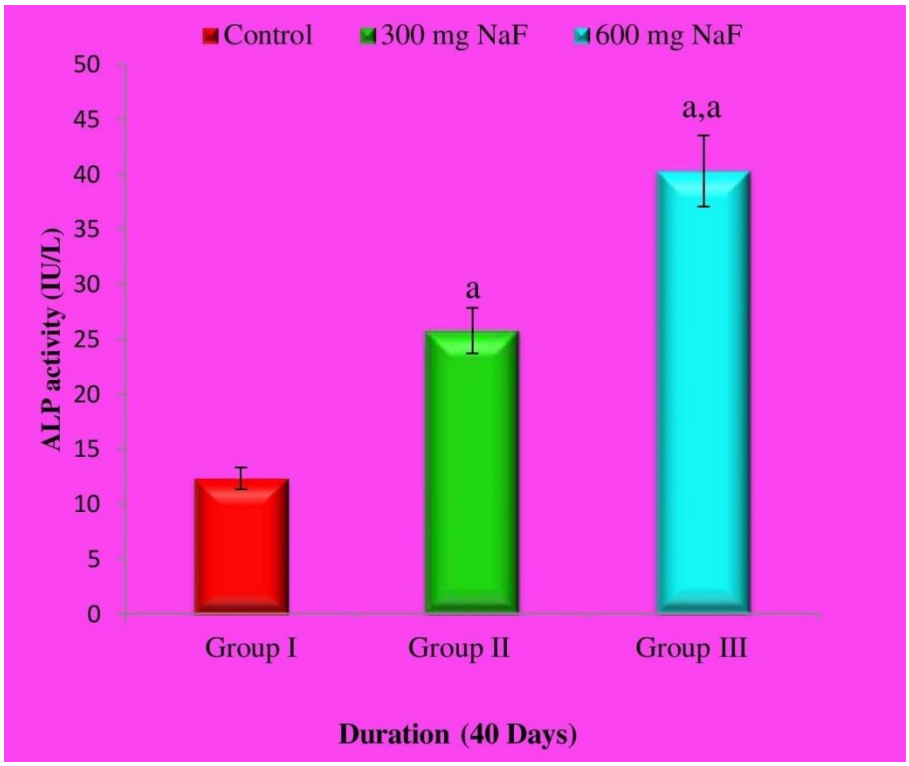

Fig. 8. Effect of fluoride on the mean activity of ALP in the hepatic tissue of control and fluoridated rats. The values represent Mean±SD (n=6).

[a]P<0.0001 Groups II and III vs Group I, [a,a]P<0.0001 Group II vs Group III.

n= Number of animals; SD= statistical difference; a,aa= distinct letters in the graph indicate statistical significant difference at P<0.0001

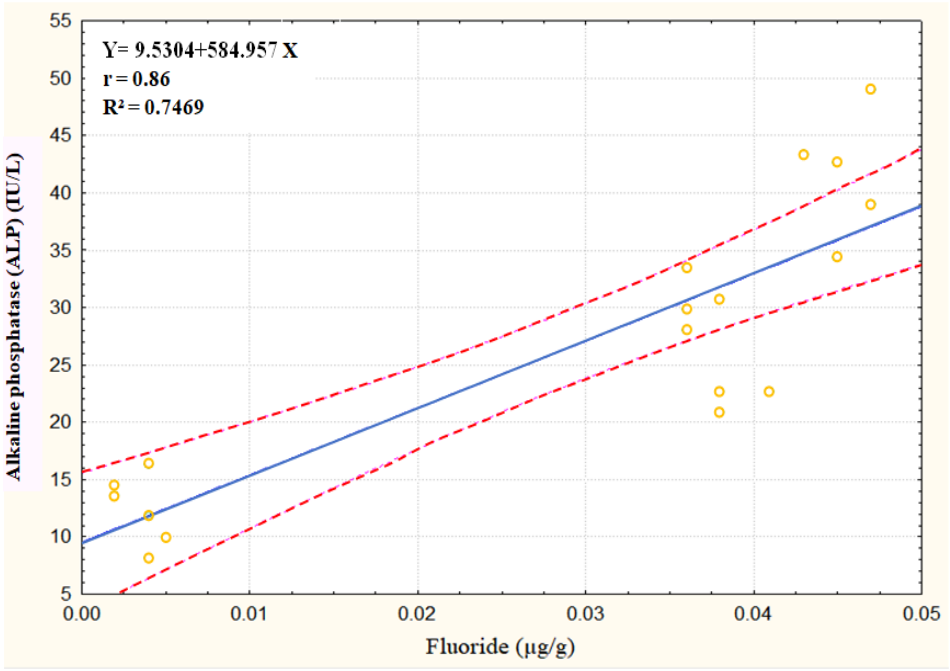

Fig. 9. Pearson's bivariate correlation and simple linear regression between level of fluoride and activity of alkaline phosphatase (IU/L) in the hepatic tissue of fluoride exposed rats

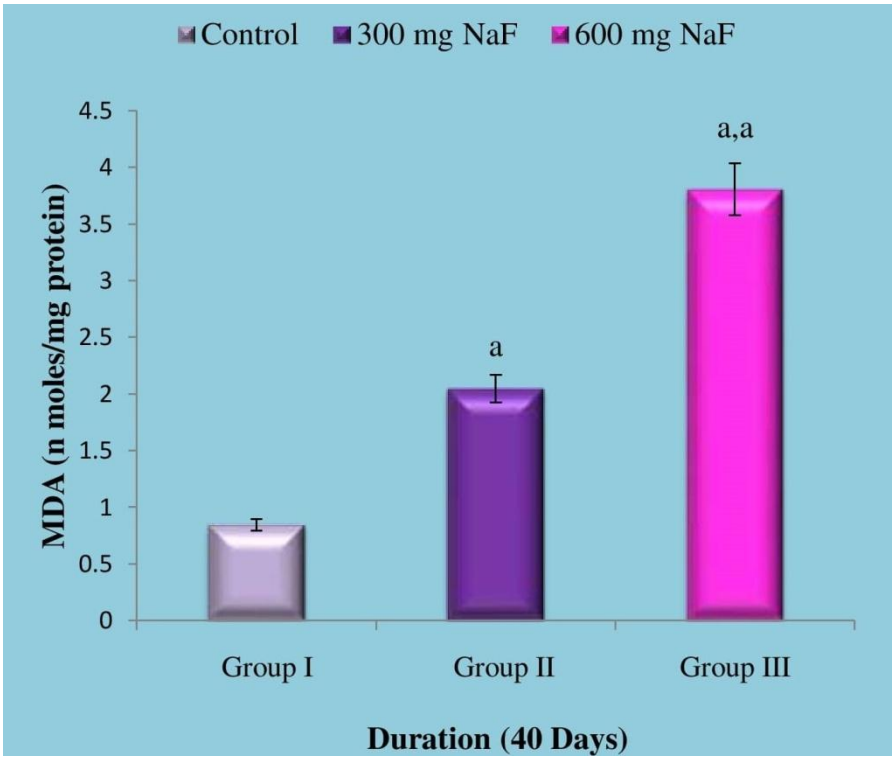

Fig. 10. Effect of fluoride on the mean level of MDA in the liver of rat after 40 days of exposure. The values represent Mean±SD (n=6).

[a]P<0.0001. Groups II and III vs Group I, [a,a]P<0.0001 Group II vs Group III.
n= Number of animals; SD= statistical difference; a,aa= distinct letters in the graph indicate statistical significant difference at P<0.0001

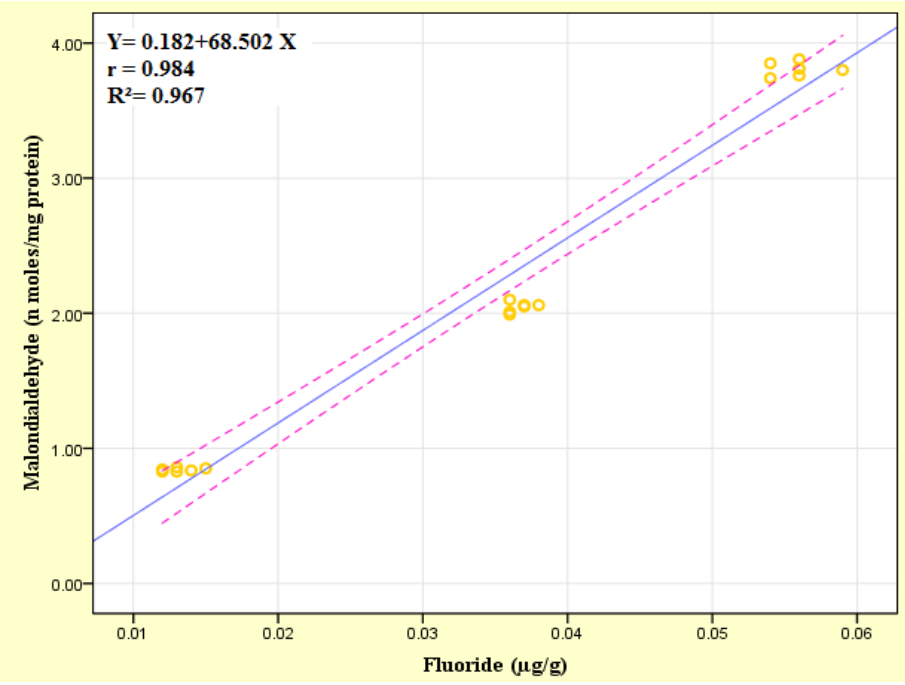

Fig. 11. Pearson's bivariate correlation and simple linear regression between levels of fluoride and malondialdehyde (n moles/mg protein) in the liver of rats after 40 days of fluoride exposure

3.6 Reduced Glutathione

The GSH content in groups II and III were lowered than the control group with respectively decreasing -61.28% (*P<0.0001*) and -70.88% (*P<0.0001*) (Table 2; Fig. 12).

Post hoc Tukey's HSD multiple comparison test after ANOVA revealed a significant derease (*P<0.0001*) in GSH content between and within groups (95% CI= 0.1733 to 0.3717) after 40 days of fluoride treatment.

Pearson's bivariate correlation and simple linear regression analysis exhibited a strong negative relationship between levels of hepatic tissue fluoride and GSH (Pearson r= -0.93; Fig. 13) in rats with fluoride intoxication.

3.7 Cytosolic Cu/Zn Superoxide Dismutase

The activity of Cu/Zn SOD in groups II and III were significantly (*P<0.0001*) lowered than the control group with respectively decreasing -39.80% in 300 mg/L NaF and -65.24% in 600 mg/L NaF (Fig. 14).

Post hoc Tukey's HSD multiple comparison test after ANOVA revealed a significant decrease (*P<0.0001*) in the activity of Cu/Zn SOD between and within groups (95% CI= 1.5805 to 2.1528) in rats exposed to fluoride.

Pearson's bivariate correlation and simple linear regression analysis displayed a strong negative relationship between level of hepatic fluoride and activity of Cu/Zn SOD (Pearson r= -0.99; Fig. 15) in rats with fluoride intoxication.

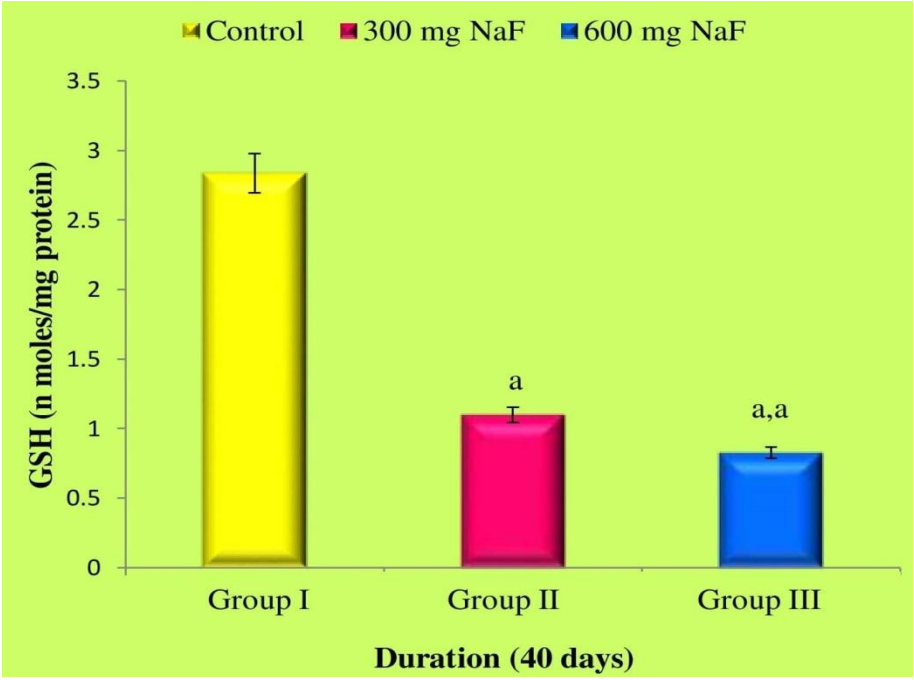

Fig. 12. Effect of fluoride on the mean level of GSH in the liver of rat after 40 days of exposure. The values represent Mean±SD (n=6).

[a]P<0.0001. Groups II and III vs Group I, [a,a]P<0.0001 Group II vs Group III.

n= Number of animals; SD= statistical difference; a,aa= distinct letters in the graph indicate statistical significant difference at P<0.0001

Table 2. Effect of fluoride on hepatic malondialdehyde (MDA) and glutathione (GSH) contents in control and two groups after 40 days of fluoride treatment

Groups	Group I	Group II	Group III
MDA (n moles/mg Protein)	0.841±0.012	2.045±0.039[a]	3.806±0.052[a,a]
GSH (N moles/mg protein)	2.837±0.106	1.098±0.072[a]	0.826±0.008[a,a]

Data are expressed as Mean±SD (n=6). [a]P<0.0001 Groups II and III vs Group I, [a,a]P<0.0001 Group II vs Group III.

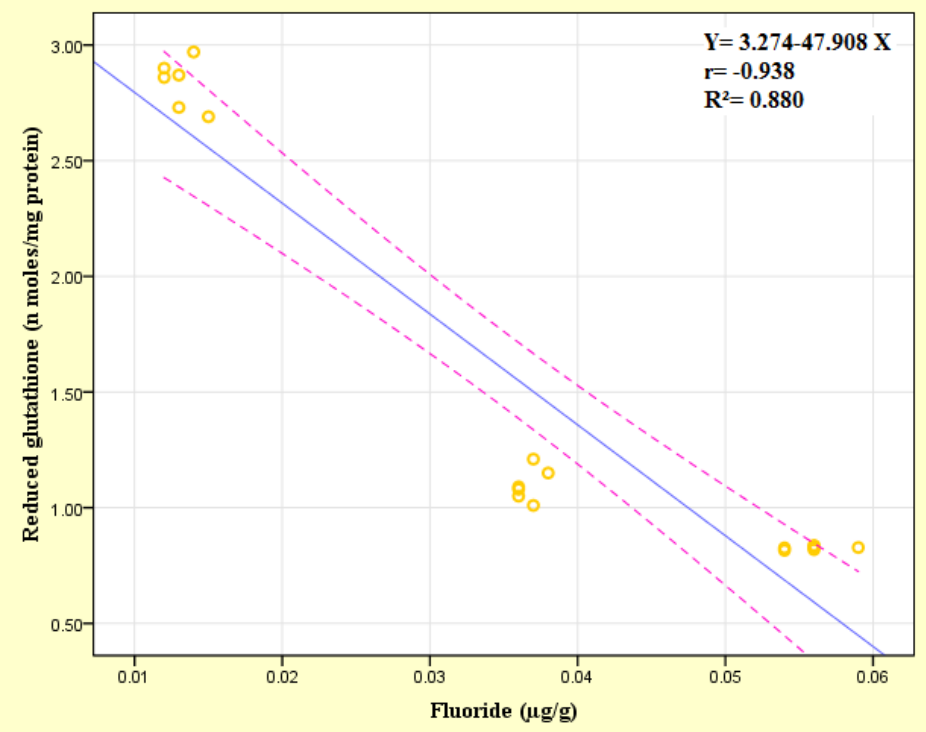

Fig. 13. Pearson's bivariate correlation and simple linear regression between levels of fluoride and reduced glutathione (n moles/mg protein) in the liver of rats after 40 days of fluoride treatment

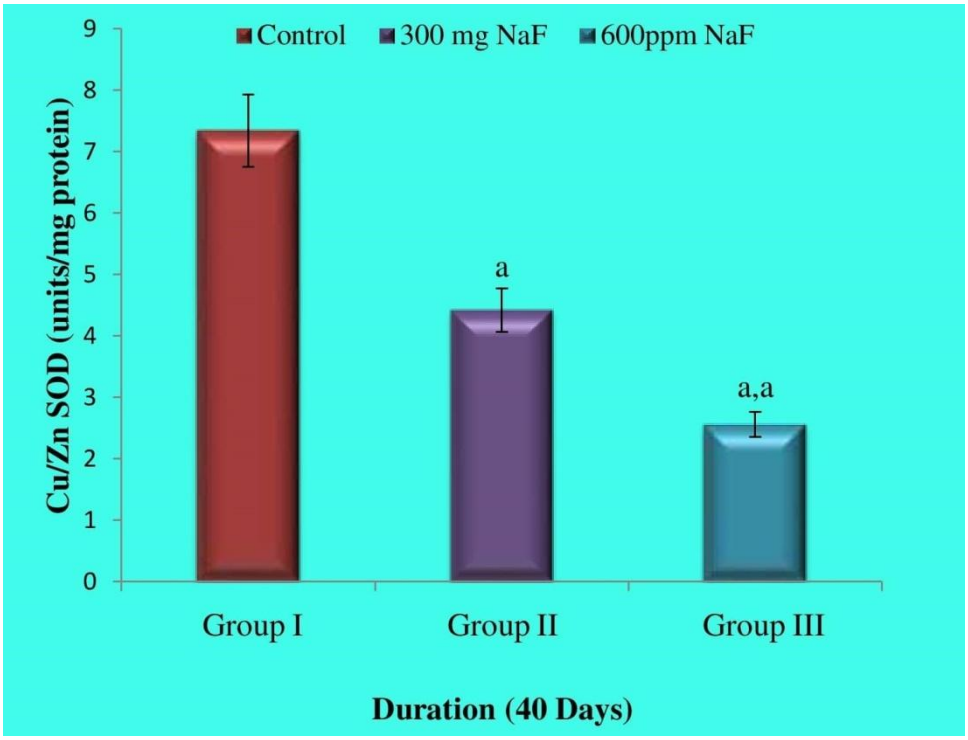

Fig. 14. Effect of fluoride on the mean activity of Cu/Zn SOD in the hepatic tissue of control and fluoridated rats. The values represent Mean±SD (n=6).
[a]P<0.0001 Groups II and III vs Group I, [a,a]P<0.0001 Group II vs Group III

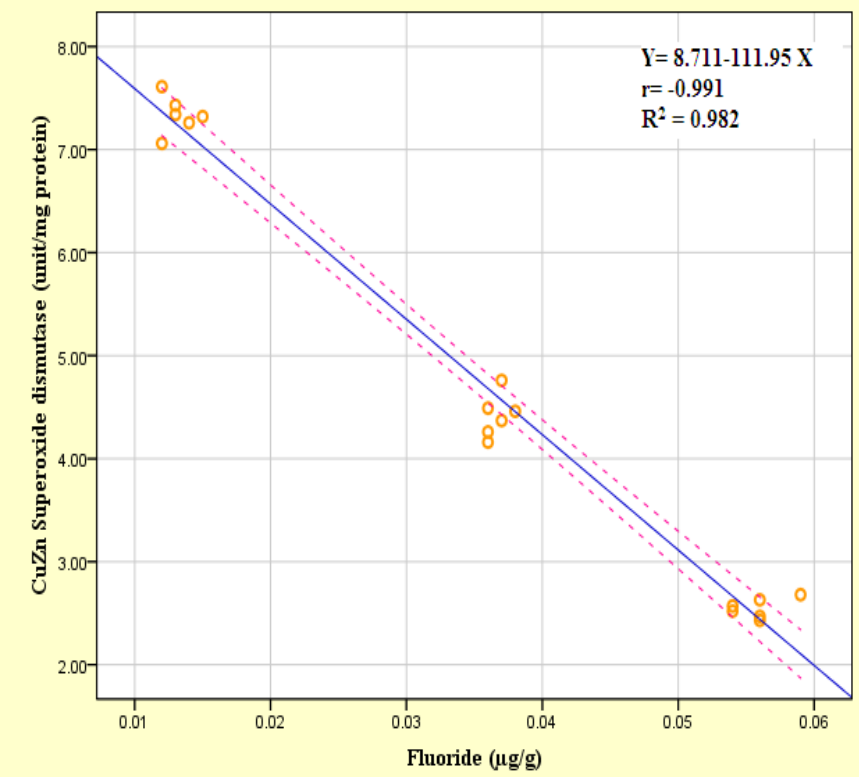

Fig. 15. Pearson's bivariate correlation and simple linear regression between level of fluoride and activity of Cu/Zn superoxide dismutase in the hepatic tissue of fluoride exposed rats.

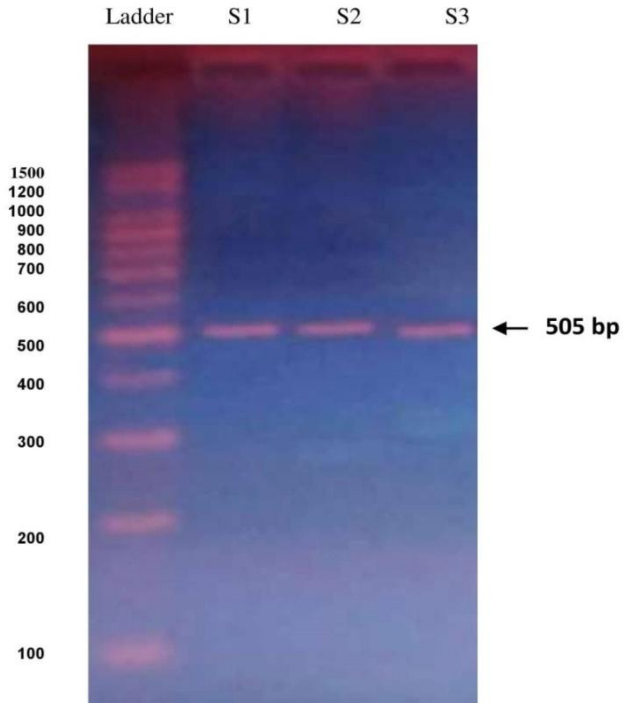

Fig. 16. Gel electrophoresis showing PCR products of β-actin gene stained with ethidium Bromide 100 bp ladder containing molecular weight markers Lane S1- Control, Lane S2- 300 mg NaF, Lane S3- 600 mg NaF

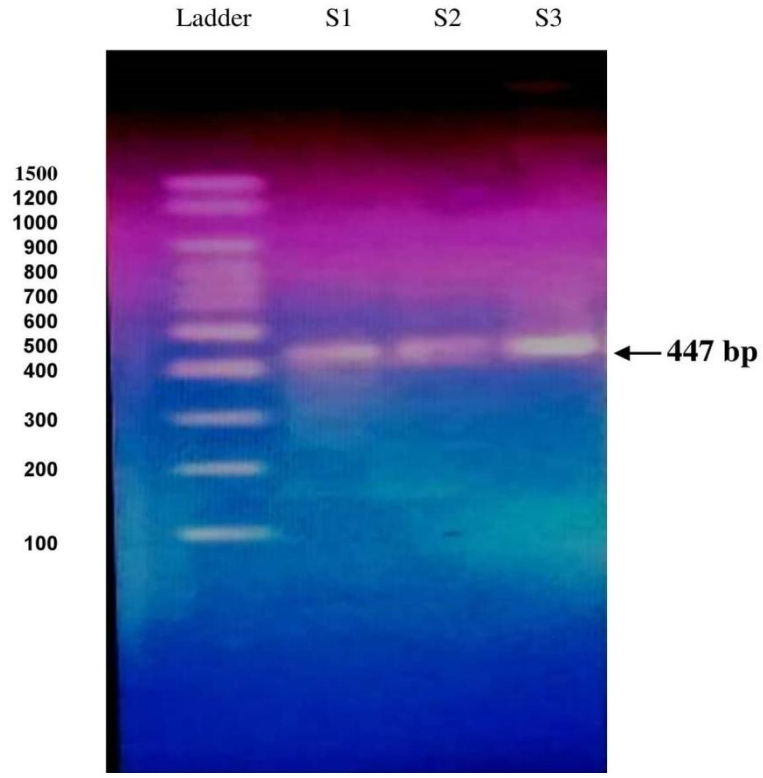

Fig. 17. Gel electrophoresis showing PCR products of Cu/Zn SOD gene stained with ethidium bromide 100 bp ladder containing molecular weight markers Lane S1- Control, Lane S2- 300 mg NaF, Lane S3- 600 mg NaF

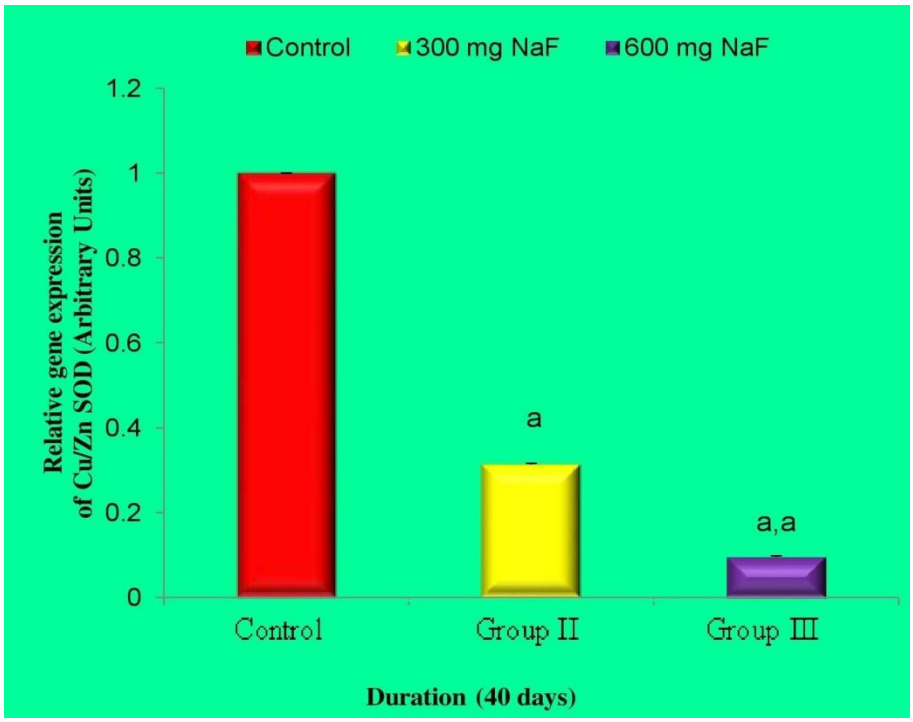

Fig. 18. Effect of fluoride on the hepatic Cu/Zn SOD gene expression after 40 days of fluoride treatment

3.8 Effect of Fluoride on Cytosolic Cu/Zn SOD Relative Gene Expression

The gene expression (Fold change) of Cu/Zn SOD enzyme in the liver of control group was observed to be 1.0. β-actin was taken as housekeeping gene. Gel electrophoresis results further confirm the specificity of used primers by using a single band of appropriate product length of β-actin (505 bp) (Fig. 16).

3.9 Real-Time PCR Analysis of Cu/Zn SOD Gene

Gel electrophoresis showed the specificity of used primers by using a single band of appropriate product length of Cu/Zn SOD (447 bp) (Fig. 17).

Relative gene expression of targeted gene was significantly downregulated than the control group with respectively decreasing -62.30% (*P<0.0001*) and -88.86% (*P<0.0001*) (Fig. 18).

4. DISCUSSION

The present study evaluated the harmful effects of sodium fluoride on the biochemical functions of hepatic biomarkers, lipid peroxidation and transcription of Cu/Zn SOD in albino rats. For the past few years, fluoride not only influenced bones and teeth but also affects the soft tissues. It can rapidly cross the cell membrane and be distributed in bones [22], teeth [4], liver [11] and thus, affecting the health of humans and animals [3].

During present study, significant (*P<0.0001*) increase in the concentration of fluoride as well as activities of aminotransferases, LDH and ALP in the liver of fluorotic rats was observed in comparison with control. A similar increase in the level of plasma fluoride was also observed in mice treated with 10 mg/L and 50 mg/L NaF for 60 days when compared to control group [23].

Liver is an important target organ for fluoride toxicity. It is the main detoxifying organ which takes part in many metabolic functions. Aminotransferases are biomarkers of liver function though they play a crucial role in the biosynthesis of proteins. Drinking fluoride rich water over a prolong period can harm the liver. Increased activities of aminotransferases demonstrated liver damage in fluoridated rats, as reported in past investigations [24, 25]. The significant (*P<0.001*) elevation in the activity of SGPT, SGOT and ALP has also been recorded in rats treated with 6 mg/L NaF for 15 and 30 days [26].

Pearson's bivariate correlation and simple linear regression analysis showed strong positive correlation between the level of fluoride and activities of aminotransferases, LDH and ALP in fluoridated rats. As the level of fluoride increases in the hepatic tissue, the activities of bio-indicators were also increased due to hepatic cell damage leading to leakage of cytoplasmic enzymes.

ALP is the important marker of liver damage an ectoenzyme of the plasma membrane and endoplasmic reticulum and is also used to check the integrity of the plasma membrane. ALP is an external membrane bound enzyme and its increased activity indicates liver injury [27]. LDH is an important biomarker and helpful in the diagnosis of muscular dystrophy. An increased activity of LDH indicates that biological membrane of tissue cells was not intact due to excessive fluoride exposure as lipid peroxidation from oxidative stress disturbs the integrity of cellular membranes leading to the leakage of cytoplasmic enzymes. Study of Wang et al. [28] and Bouasla et al. [29] has also reported the same [28, 29].

Malondialdehyde is the important biomarker of lipid peroxidation in animals exposed to different environmental toxicants [30]. Oxidative stress caused by excessive ROS in the body leads to damage to the membrane structure and peroxidation of membrane lipids has begun in cells, thus MDA is produced as an end product [31]. The present study demonstrated that sodium fluoride exposure leads to oxidative stress in rats and an increased level of MDA and decreased GSH content in the hepatic tissue was observed when compared with control. GSH is an important indicator of oxidative stress caused by sodium fluoride because it is a vital enzyme that protects against reactive oxygen radicals [32].

Significant decrease in the activity of cytosolic Cu/Zn SOD was observed in fluorotic rats in comparison with control. Antioxidant enzymes are important in the conversion of highly reactive oxygen species into less reactive, non-toxic molecules. Several studies have found that sodium fluoride produces reactive oxygen species, which causes oxidative stress and

changes in antioxidant enzyme activity in the livers of mice and rats [33, 34].

In the present study, gene expression of Cu/Zn SOD in groups II and III was downregulated significantly. The expression profile of Cu/Zn SOD and its enzymatic activity was equally reliable. The imbalance between the anti-oxidative function of enzyme and reactive oxygen species, which leads to oxidative stress and participates in hepatic injury, is linked to sodium fluoride's reduction in gene expression as well as activity of antioxidants. Cu/Zn SOD was regarded to be one of the most essential antioxidative enzymes, removing the bulk of free radicals and reactive oxygen species (ROS) [35]. Its direct mechanism of inhibition attributes competitive inhibition of the enzyme by fluorine ions. Fluorine may get attached itself to the active site of copper on enzyme and thus, removing water [36]. Fluoride cytotoxicity is associated with a decrease in the biosynthesis of protein, DNA and RNA which could be an indirect mechanism of its inhibition [37, 38].

The decreased Cu/Zn SOD expression might be explained as fluorine is an active element and it inhibits the absorption of Mg^{2+} ion in the intestine which produced MgF^{+} and MgF_2, which are not easily absorbed [39, 40]. As Mg^{2+} ion is actively participated in cell transcription, a drop in Mg^{2+} availability may result to a decrease in Cu/Zn SOD production and expression level. As a result, elimination of free radicals decreased resulting in oxidative `stress and directly inhibit the nucleic acid and protein synthesis. The effect of fluoride on Cu/Zn SOD production was presumably dose-dependent effect at 300 and 600 mg/kg NaF, and decrease in Cu/Zn SOD activity could be driven by mRNA damage, resulting in decreased Cu/Zn SOD biosynthesis [41].

A significant increase in the activities of aminotransferases in present study might be due to oxidative stress generated by sodium fluoride which could alter the membrane permeability of hepatocytes in rats treated with 300 and 600 mg/L NaF. Fluoride toxicity induced oxidative damage leads to liver dysfunction and deformities in the levels of serum biomarker transaminases [42]. A marked increase in the activity of ALT was attributed to a change in the permeability of the hepatic cell membrane [43]. A similar investigation was conducted by some workers on rats [44]. The previous study has demonstrated a significant positive relationship between serum fluoride level and activities of ALT, AST, ALP and LDH in population of fluoride endemic area [14, 45].

Mitta et al. [46] reported an abnormal increase in the concentrations of aminotransferases and serum ALP level indicate the disruptive functions of the liver in rats given 100 mg NaF for four weeks. These findings have coincided with the results of previous study [47].

5. CONCLUSION

Fluoride causes a decrease in gene expression of cytosolic Cu/Zn SOD and reduced its antioxidant activity leads to decline ability to scavenging free radicals with excessive production of lipid peroxidation which damages the hepatic structure and function.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Institutional Animal Ethics Committee of Punjabi University, Patiala approved all experimental procedures with experimental animals (Animal maintenance and registration number 107/GO/ReBi/S/99/CPCSEA/2017-40).

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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