EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 11
Issue: 8
182-193
Year: 2024

FLUORIDE-INDUCED CARDIOTOXICITY IN RATS: HISTOPATHOLOGICAL AND BIOCHEMICAL FINDINGS

A. Shashi* and Shiwani Dogra

Department of Zoology and Environmental Sciences, Punjabi University, Patiala-147002, India.

***Corresponding Author: A. Shashi**
Department of Zoology and Environmental Sciences, Punjabi University, Patiala-147002, India.

Article Received on 07/06/2024 **Article Revised on 27/06/2024** **Article Accepted on 17/07/2024**

ABSTRACT
Chronic fluoride exposure is a widespread environmental concern associated with various health implications beyond its well-known effects on dental and skeletal systems. Eighteen Wistar albino rats were divided into three groups having six in each. The control group was given 1 ml deionized water/kg b.w./day for 40 days. The groups II and III were administered with sodium fluoride (NaF) orally by oral gavage at doses of 300 mg/kg and 600 mg/kg body weight daily for the same period. The animals were sacrificed, cardiac tissue was taken out and processed for histopathological changes and biochemical analysis. Histopathological examination using light microscopy revealed nuclear dissolution, swelling, and degeneration progressing to pyknosis and karyorrhexis, Zenker's degeneration, vascular dilation, myofiber fragmentation, and loss of cross-striations were observed in rats treated with 300 mg NaF. Treatment with 600 mg NaF exacerbated these effects, showing fibrillolysis, fibrous necrosis, interstitial edema, and extensive cellular infiltration. Biochemical results showed a significant ($P < 0.0001$) increase in triglycerides, total cholesterol, low-density lipoproteins, very low-density lipoproteins, and phospholipids, along with a marked decrease in high-density lipoproteins levels in fluoride-exposed rats. Pearson's bivariate correlation and simple linear regression analysis exhibited positive relationship between the levels of fluoride and triglycerides, total cholesterol, low-density lipoproteins, very low-density lipoproteins and phospholipids. On contrary, negative relationship existed between the levels of fluoride and HDL, indicating fluoride-induced dyslipidemia.

KEYWORDS: Fluoride, High-density lipoproteins, Histopathology, Low-density lipoproteins, Phospholipids, Triglycerides, Total cholesterol, Very low-density lipoproteins.

INTRODUCTION
Fluoride, a ubiquitous environmental contaminant, poses significant health risks through chronic exposure. One of the most visible manifestations of excessive fluoride intake is fluorosis, characterized by dental mottling in mild cases and severe skeletal deformities in more extreme instances.[1] Beyond its well-documented effects on dental and skeletal health, fluoride toxicity extends to various organ systems, including the brain[2], skeletal muscles[3], kidneys[4], reproductive organs[5], endocrine glands[6], liver and spleen.[7,8]

Histopathological studies in animals have shown significant pathological changes in cardiac tissue due to chronic sodium fluoride exposure, including myocardial cell necrosis and vacuolation.[9] Previous studies reported structural abnormalities in experimental animals exposed to fluoride.[10,11] These changes are indicative of the cardiotoxic potential of fluoride, leading to cardiovascular diseases. This study aims to elucidate the effects of fluoride on Lipid profile and histopathological changes in the cardiac tissue of albino rats.

The detrimental impact of fluoride on soft tissues has drawn increasing attention, particularly its disruption of enzymatic functions leading to oxidative stress and the generation of free radicals.[12] Among these tissues, the cardiovascular system stands out due to its sensitivity to lipid disturbances, which are pivotal in the development of myocardial diseases. Lipids play essential roles in cellular structure, signaling, and energy storage, and their deregulation is intricately linked to conditions such as atherosclerosis and coronary artery disease.[13] Fluoride exposure has been shown to induce hyperlipidemia and alter lipid profiles over time.[14] Elevated levels of blood lipids, including triglycerides (TG), total cholesterol (TC), low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), phospholipids and reduced high-density lipoproteins (HDL), are recognized markers of cardiovascular risk. Moreover, ability of fluoride to impair thyroid function and induce hypothyroidism

further exacerbates lipid metabolism abnormalities, potentially amplifying cardiovascular risks.[15]

MATERIALS AND METHODS

Experimental protocol

A total of eighteen young Wistar albino rats weighing between 150-200 g were kept in polypropylene cages with stainless steel grill tops and provided standard rat pellet diet (Hindustan Lever Limited, India) and *ad libitum* access to water. Following a two-week acclimatization period, rats were randomly assigned into three groups of six animals each. Groups II and III received daily oral gavage of 300 mg and 600 mg sodium fluoride (NaF) per kg body weight, respectively, for 40 days, while the control group received 1 mL of double deionized water/kg b.w./day for the same time period. After the experimental period, rats were fasted overnight, weighed and excised. The heart tissue was isolated, washed with normal saline, weighed and processed further for histopathological and biochemical analysis.

Histopathological analysis

Cardiac tissue was fixed in Bouin's fluid for 24 hours, dehydrated in 95% alcohol for 45 minutes, treated with tertiary butyl alcohol for 6 hours, cleared in amyl acetate overnight, and embedded in paraffin wax. Serial sections of 7 µm were cut and then stained with haematoxylin and eosin.[16] Histopathological alterations were examined under a research binocular microscope (Leica microsystem) and microphotographed.

Biochemical estimation

Sample preparation: The cardiac tissue was quickly homogenized in 0.1 M phosphate buffer (pH 7.4) using a homogenizer. The homogenate was then centrifuged at 10,000 rpm for 10 minutes, and the resulting supernatants were used for biochemical analysis.

Chemicals and Reagents

Diagnostic kits of Erba Mannheim/Germany were used for the estimation of levels of triglycerides, total cholesterol, low-density lipoproteins, and high-density lipoproteins. The levels of very-low density lipoproteins were estimated using the formula given by Friedewald *et al.*[17] and the phospholipids levels were assessed using a diagnostics kit from DiaSys Diagnostic Systems, by following the instructions provided in the protocols.

Biochemical Assays

The level of fluoride in the cardiac tissue was measured by a potentiometric method by using the ion selective electrode.[18]

The levels of TG, TC, LDL, HDL, VLDL, phospholipids in control and experimental rats were estimated using commercially available reagent kits by following the datasheet instructions.

Statistical analysis

Statistical analyses were conducted using SPSS 16.0 statistical software (IBM). Data are expressed as Mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was employed to assess the significance between experimental groups, followed by post hoc Bonferroni multiple comparison test. Statistical significance was defined as $P< 0.05$. Pearson's bivariate correlation analysis and simple linear regression test was used to examine the relationships between two variables.

Ethical Aspects

All experimental procedures were conducted in accordance with the guidelines approved by the Institutional Animal Ethical Committee of Punjabi University, Patiala (Animal Maintenance and Registration No. 107/GO/ReBi/S/99/CPCSEA/2017-42).

RESULTS

Histopathological findings

The histopathological examination of cardiac tissue of control rat revealed syncytical arrangement of muscle fibres. These fibers were separated by narrow inter-muscular spaces (Fig. 1). Cardiomyocytes had close alignment with typical features and structural integrity and were introduced to fusiform in an orderly manner (Fig. 2). The myofilaments of cardiomyocytes were aligned neatly, with distinct horizontal stripes. Myocardial fibers were uniformly sized, and the cytoplasm was uniformly distributed. A normal blood vessel was also visible (Fig. 3). At higher magnification, the myocardium appeared normal, with a centrally placed nucleus, cross-striated myofibres, and a typical branching pattern (Fig. 4).

In comparison to the control group, marked histopathological changes were observed in sodium fluoride treated rats. The cardiac tissue of rats treated with 300 mg NaF/kg b.w./day for 40 days revealed that the nuclei were dissolving and experiencing mild to moderate swelling. The nucleus showed signs of degeneration with changes in shape and size, ultimately progressing to a state of pyknosis and karyorrhexis (Fig. 5). Zenker's myocardial degeneration was observed along with vascular dilation and congestion. There was fragmentation and separation of myofibers and loss of cross striations (Fig. 6). In fluoridated rats, there were areas in the tissue where the muscle fibers were disorganized, fragmented, and showed signs of cell death (myonecrosis). These areas were also infiltrated by inflammatory cells, mostly lymphocytes, indicating a response to the damage (Fig. 7). The cellular changes such as rupturing of muscle fibers, widened intercellular space and extensive inter-myofibrillar haemorrhage were noted (Fig. 8). The mild degeneration and congestion of myocardial fibers along with cloudy swellings and formation of vacuoles were prominent (Fig. 9). The myocardial fibers appeared to be clumped and distortion of endocardial layer was seen (Fig. 10).

The light microscopy of the cardiac tissue of rats treated with 600 mg NaF/kg b.w./day for 40 days revealed isolated fibrillolysis, early signs of fibrous necrosis, and dissolution of nuclei. Additionally, interstitial spaces showed the presence of a fibrinous, edematous fluid, numerous haemorrhage sites, and infiltration by various cells including histiocytes, lymphocytes, and granulocytes (Fig. 11). The experimental rats exhibited significant vacuolar/fatty degeneration in the cardiac tissue, with dilated blood vessels displaying hyperplasia, leading to detachment from the cardiac muscle (Fig. 12). There was presence of histolytic cells. Spotty necrosis, disarray and collapse of myofibrils and hyaline degeneration were also noted (Fig. 13). The arrangement of myofilaments began to loosen and disordered large gaps between myocardial fibers were prominent (Fig. 14). Cardiac muscle cells experienced denaturation and there was extensive haemorrhage and rupture between the cardiomyocytes (Fig. 15). In the myocardium, there were acute fibrous necrosis, sarcoplasmic vacuolization, small haemorrhages and fibrillolysis observed (Fig. 16).

Lipid Profile

1. Triglycerides

The mean level of TG in cardiac tissue of rats exposed to fluoride significantly increased ($F = 175.835$, $P < 0.0001$) by +20.229% and +46.761% after 40 days of treatment (Fig. 17). Post-hoc Bonferroni test confirmed significant ($P < 0.0001$) elevation in level of TG within and between fluoride-treated groups (95% CI = -18.373 to -13.487).

2. Total Cholesterol

The mean level of TC in cardiac tissue of group II (300 mg NaF/kg b.w./day) and group III (600 mg NaF/kg b.w./day) significantly increased ($F = 680.150$, $P < 0.0001$) by +81.061% and +142.481%, respectively, compared to control after 40 days of fluoride exposure (Fig. 18). Post-hoc Bonferroni test revealed significant ($P < 0.0001$) increase in level of TC within and between fluoride-treated groups (95% CI = -44.183 to -24.618).

3. Low-density Lipoproteins (LDL)

The mean level of LDL in cardiac tissue of rats significantly increased ($F = 639.568$, $P < 0.0001$) compared to controls after 40 days of fluoride administration (Fig. 19). There was +85.232% and +162.837% increase observed in fluoride-administered groups. Post-hoc Bonferroni test exhibited significant ($P < 0.0001$) elevation in level of LDL within and between fluoride-treated groups (95% CI = -29.619 to -19.847).

4. High-density Lipoproteins (HDL)

The mean level of HDL in cardiac tissue of rats significantly decreased ($F = 73.175$, $P < 0.0001$) by -17.963% and -39.180% compared to controls (Fig. 20). Post-hoc Bonferroni test indicated significant ($P < 0.0001$) decrease in level of HDL within and between fluoride-treated groups (95% CI = 2.847 to 7.392).

5. Very Low-density Lipoproteins (VLDL)

The mean level of VLDL in cardiac tissue of rats significantly increased ($F = 175.811$, $P < 0.0001$) after 40 days of fluoride exposure (Fig. 21). There was an incline of +20.232% in group II and +46.760% in group III. Post-hoc Bonferroni test indicated significant ($P < 0.0001$) increase in level of VLDL within and between fluoridated groups (95% CI = -3.675 to -2.697).

6. Phospholipids

The mean level of phospholipids in cardiac tissue of fluoridated rats showed significant accumulation ($F = 55.213$, $P < 0.0001$) compared to control rats, indicating an increase of +17.068% in the 300 mg/kg NaF group and +32.501% in the 600 mg/kg NaF group, respectively (Fig. 22). Post-hoc Bonferroni test after ANOVA revealed significant ($P < 0.0001$) elevation in the level of phospholipids within and between fluoride-treated groups (95% CI = -20.258 to -5.660).

Correlation Analysis

Pearson's correlation and simple linear regression analysis showed a significant positive relationship ($P < 0.0001$) between the levels of fluoride and triglycerides ($R^2 = 0.886$, Pearson r = 0.941; $Y = 63.410 + 800.738X$; Fig. 23); total cholesterol ($R^2 = 0.963$, Pearson r = 0.981; $Y = 40.057+1775.524X$; Fig. 24); low-density lipoproteins ($R^2 = 0.960$, Pearson r = 0.980; $Y = 23.828 + 1271.769X$; Fig. 25); very low-density lipoproteins ($R^2 = 0.886$, Pearson r = 0.941; $Y = 12.682 + 160.150X$; Fig. 26). phospholipids ($R^2 = 0.845$, Pearson r = 0.919; $Y = 76.428 + 662.430X$; Fig. 28), whereas there was significant ($P < 0.0001$) negative relationship between the levels of fluoride and high-density lipoproteins ($R^2 = 0.917$, Pearson r = -0.958; $Y = 26.452 - 268.466X$; Fig. 27) following the 40 day fluoride exposure.

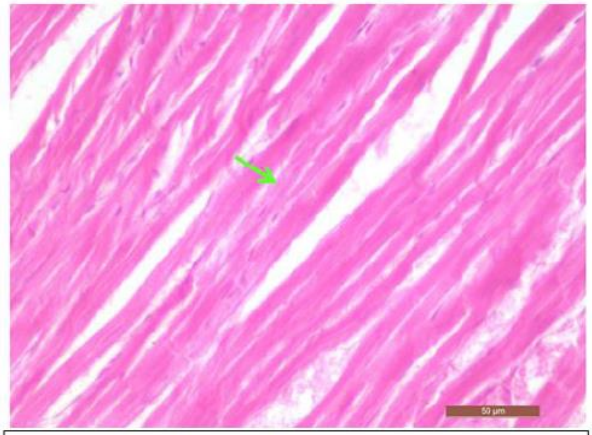

Fig. 1: T.S. of cardiac tissue of control rat showing the normal syncytial arrangement of myocardial fibers (↑). Haematoxylin and EosinX400

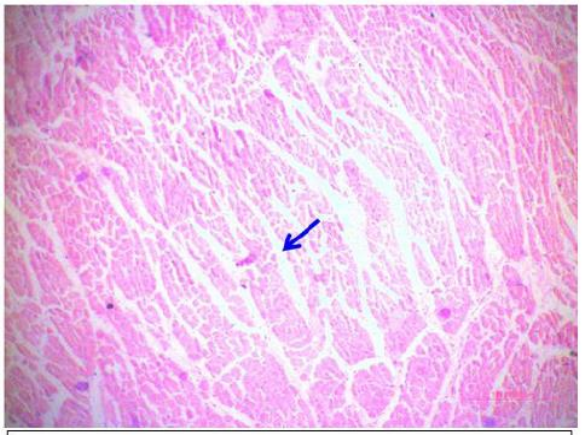

Fig. 2: T.S. of cardiac tissue of control rat showing fusiform order and the intercellular spaces (↑). Haematoxylin and EosinX100

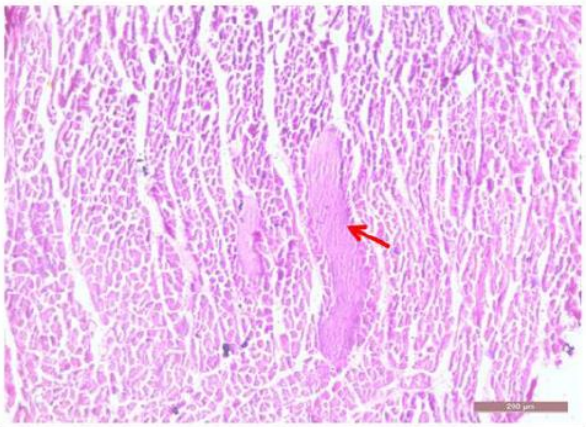

Fig. 3: T.S. of cardiac tissue of control rat showing regular and uniform pattern of cardiac cells arrangement. Narrow intercellular spaces and a blood vessel (↑) were also seen. Haematoxylin and EosinX100

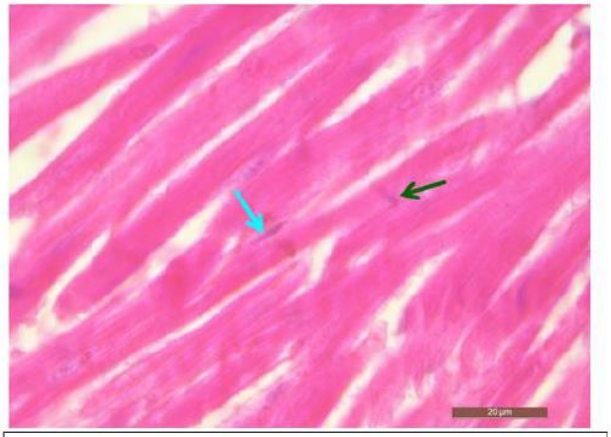

Fig. 4: T.S. of cardiac tissue of control rat showing cardiac muscle fibers with centrally placed nuclei (↑). Intercalated discs were also visible (↑). Haematoxylin and EosinX1000 (under oil immersion)

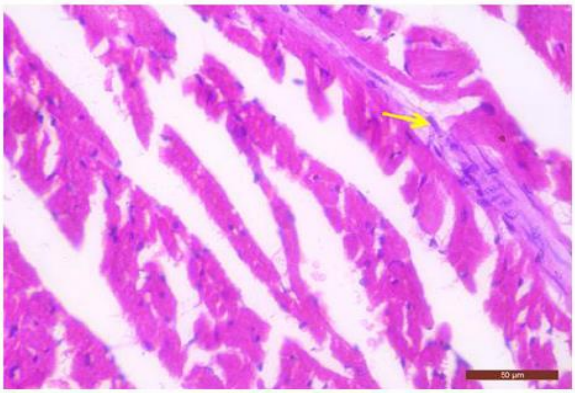

Fig. 5: T.S. of cardiac tissue of rat treated with 300 mg NaF/kg b.w./day for 40 days showing dissolution of nuclei (↑) and nuclear pyknosis, karyolysis, and chromatin margination in cardiac myocytes. Haematoxylin and EosinX400

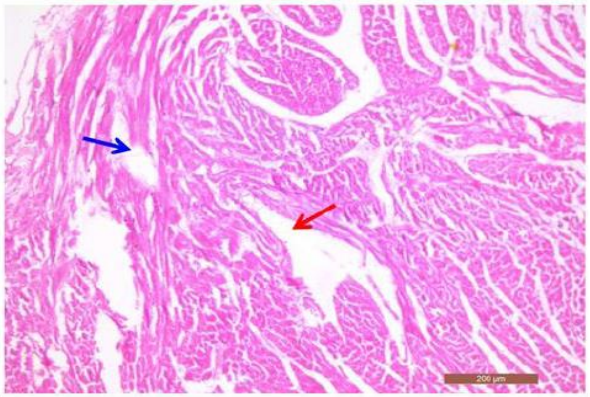

Fig. 6: T.S. of cardiac tissue of rat treated with 300 mg NaF/kg b.w./day for 40 days showing Zenker's degeneration (↑) with widely separated myocardial fibers (↑) exhibiting wavy orientation. Haematoxylin and EosinX100

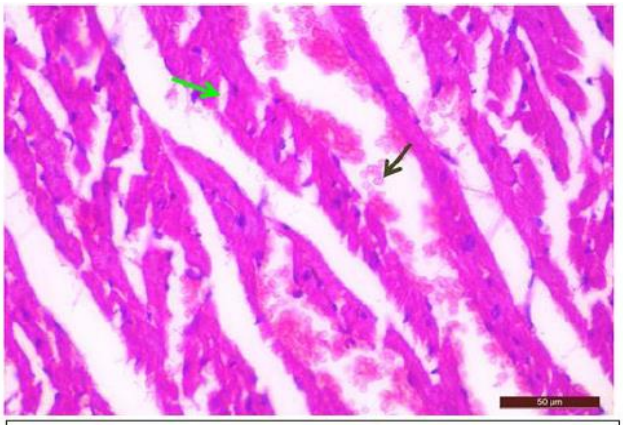

Fig. 7: T.S. of cardiac tissue of rat treated with 300 mg NaF/kg b.w./day for 40 days showing presence of numerous cytoplasmic vacuoles (↑) and lymphocytic infiltration (↑). Haematoxylin and EosinX400

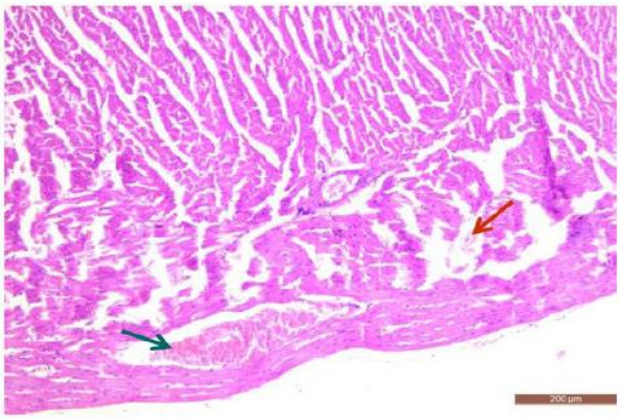

Fig. 8: T.S. of cardiac tissue of rat treated with 300 mg NaF/kg b.w./day for 40 days showing dilated blood vessel (↑) and hemorrhage (↑). Haematoxylin and EosinX100

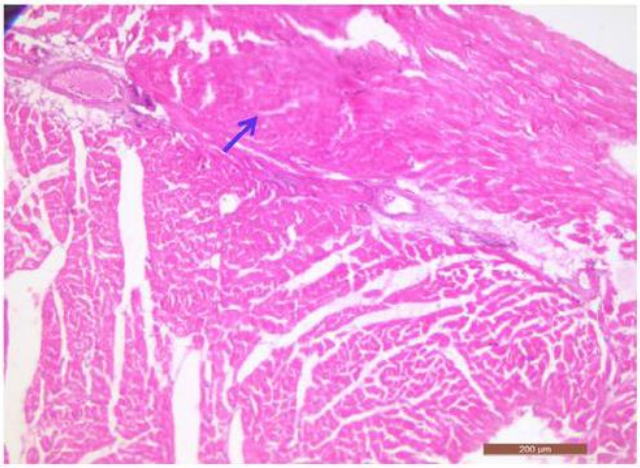

Fig. 9: T.S. of cardiac tissue of rat treated with 300 mg NaF/kg b.w./day for 40 days showing cloudy swellings (↑) and congestion of myocardial fibers. Haematoxylin and EosinX100

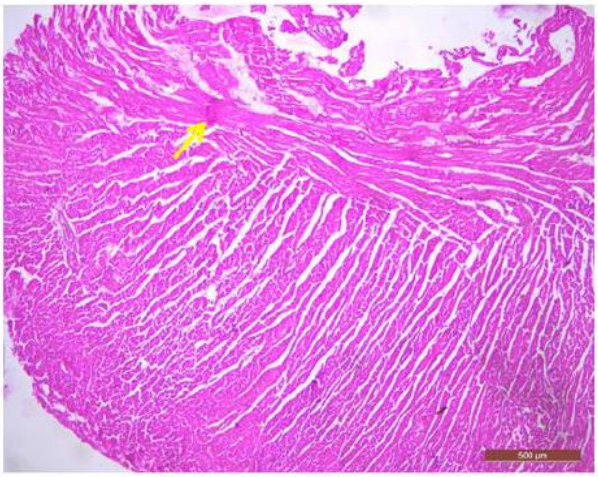

Fig. 10: T.S. of cardiac tissue of rat treated with 300 mg NaF/kg b.w./day for 40 days showing clumped fibers (↑). Haematoxylin and EosinX40

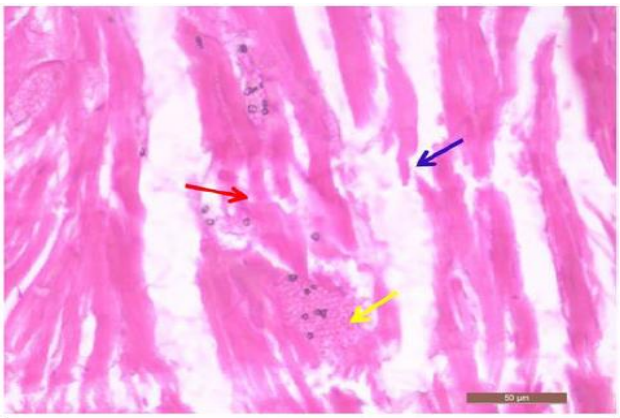

Fig. 11: T.S. of cardiac tissue of rat treated with 600 mg NaF/kg b.w./day for 40 days showing interstitial edema (↑), fibrillolysis (↑) and fibrous necrosis (↑). Haematoxylin and EosinX400

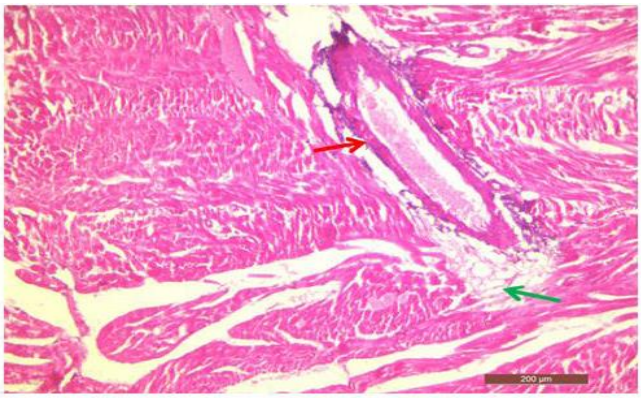

Fig. 12: T.S. of cardiac tissue of rat treated with 600 mg NaF/kg b.w./day for 40 days showing thickening of coronary artery (↑). Vacuolar degeneration (↑) of myocardium was also seen. Haematoxylin and EosinX100

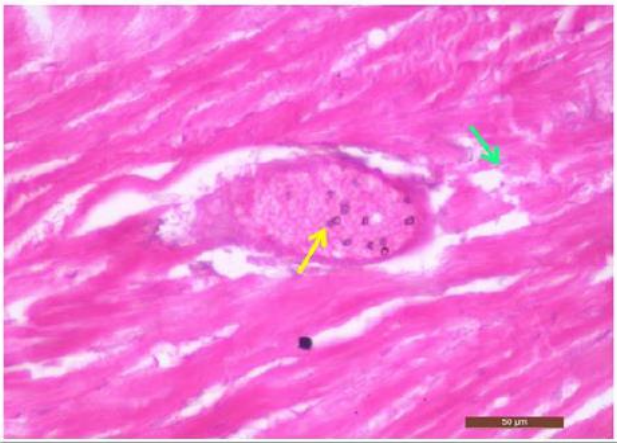

Fig. 13: T.S. of cardiac tissue of rat treated with 600 mg NaF/kg b.w./day for 40 days showing interstitial inflammatory cells ( ) and hyaline degeneration ( ). Haematoxylin and EosinX400

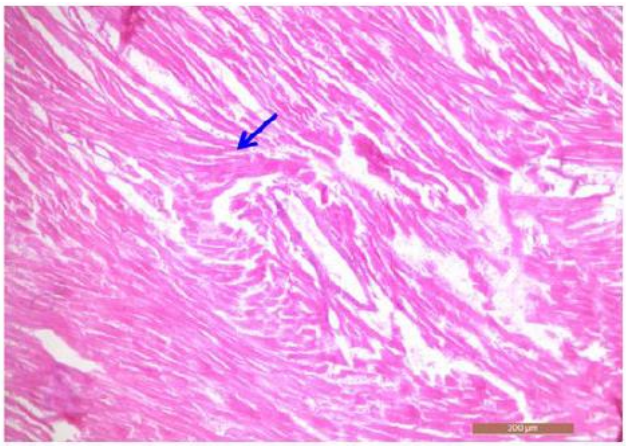

Fig. 14: T.S. of cardiac tissue of rat treated with 600 mg NaF/kg b.w./day for 40 days showing disarrayed pattern of cardiac myofibers ( ) and loosely arranged myofilaments. Haematoxylin and EosinX100

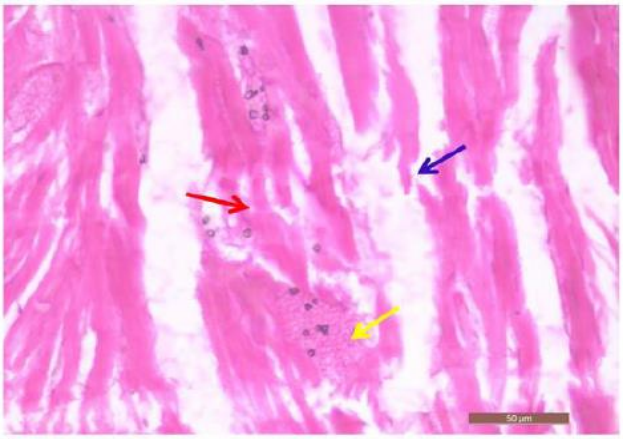

Fig. 15: T.S. of cardiac tissue of rat treated with 600 mg NaF/kg b.w./day for 40 days showing interstitial edema ( ), fibrillolysis ( ) and fibrous necrosis ( ). Haematoxylin and EosinX400

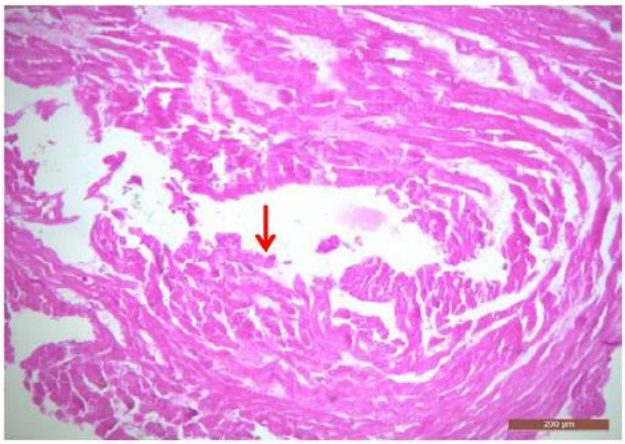

Fig. 16: T.S. of cardiac tissue of rat treated with 600 mg NaF/kg b.w./day for 40 days showing acute fibrous necrosis and damage to parenchyma ( ). Haematoxylin and EosinX100

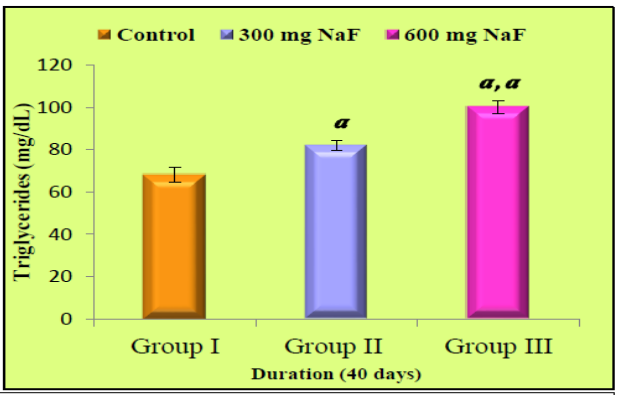

Fig. 17: Mean level of triglycerides (mg/dL) in control and fluoridated rats. *P< 0.0001 Groups II-III compared with Group I; ** P< 0.0001 Group II compared with Group III. One way ANOVA followed by post-hoc Bonferroni multiple comparison test.

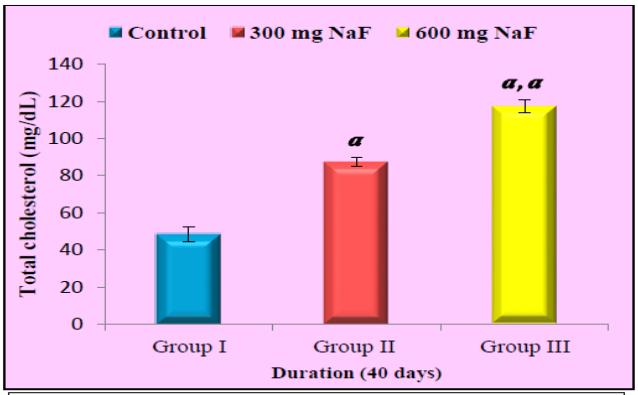

Fig. 18: Mean level of total cholesterol (mg/dL) in control and fluoride treated rats. *P< 0.0001 Groups II-III compared with Group I; ** P< 0.0001 Group II compared with Group III. One way ANOVA followed by post-hoc Bonferroni multiple comparison test.

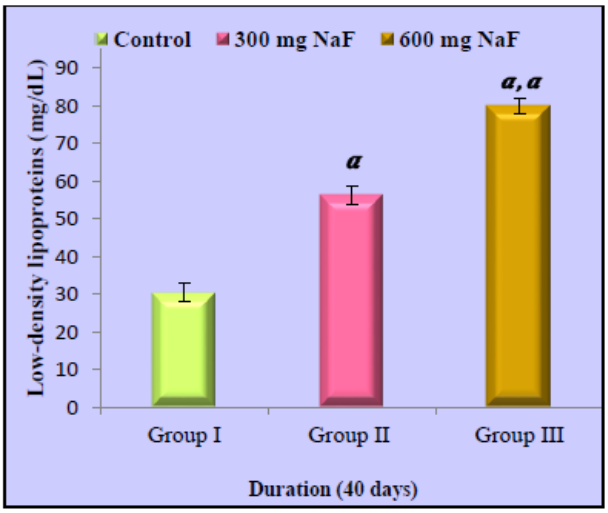

Fig. 19: Mean level of low-density lipoproteins (mg/dL) in control and fluoride-administered rats. *P< 0.0001 Groups II-III compared with Group I; ** P< 0.0001 Group II compared with Group III. One way ANOVA followed by post-hoc Bonferroni multiple comparison test.

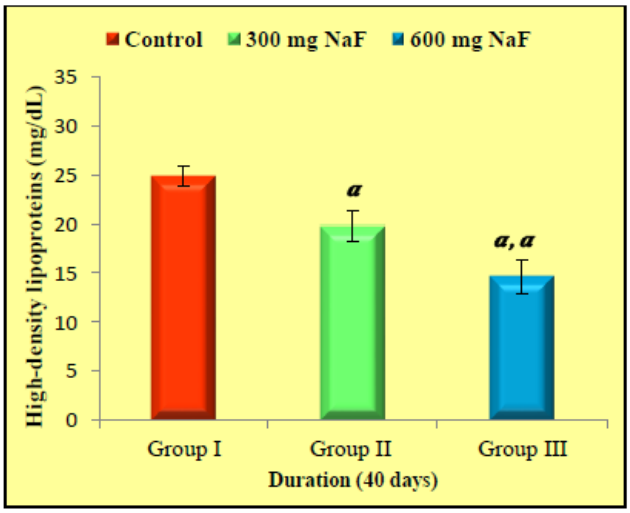

Fig. 20: Mean level of high-density lipoproteins (mg/dL) in control and fluoride-induced rats. *P< 0.0001 Groups II-III compared with Group I; ** P< 0.0001 Group II compared with Group III. One way ANOVA followed by post-hoc Bonferroni multiple comparison test.

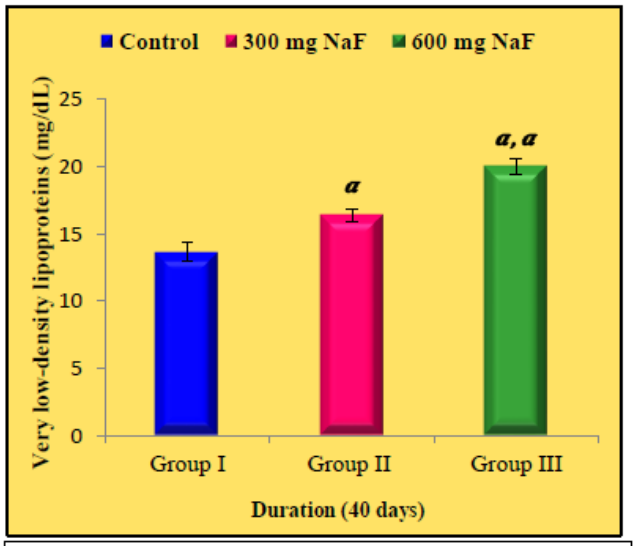

Fig. 21: Mean level of very low-density lipoproteins (mg/dL) in control and fluoride-administered rats. *P< 0.0001 Groups II-III compared with Group I; ** P< 0.0001 Group II compared with Group III. One way ANOVA followed by post-hoc Bonferroni multiple comparison test.

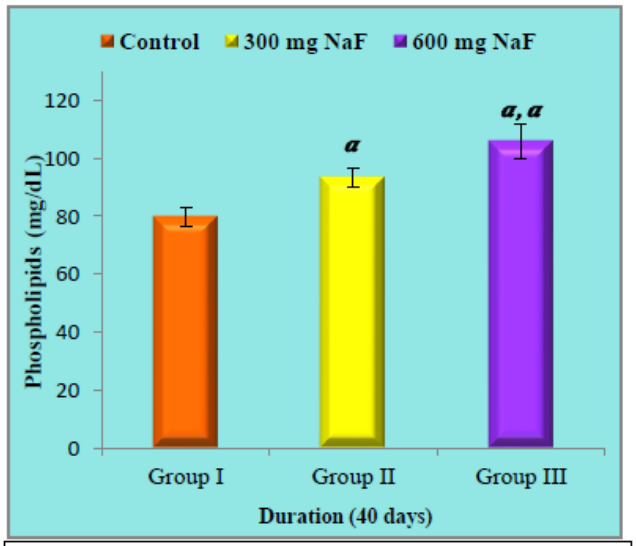

Fig. 22: Mean level of phospholipids (mg/dL) in control and fluoride-administered rats. *P< 0.0001 Groups II-III compared with Group I; ** P< 0.0001 Group II compared with Group III. One way ANOVA followed by post-hoc Bonferroni multiple comparison test.

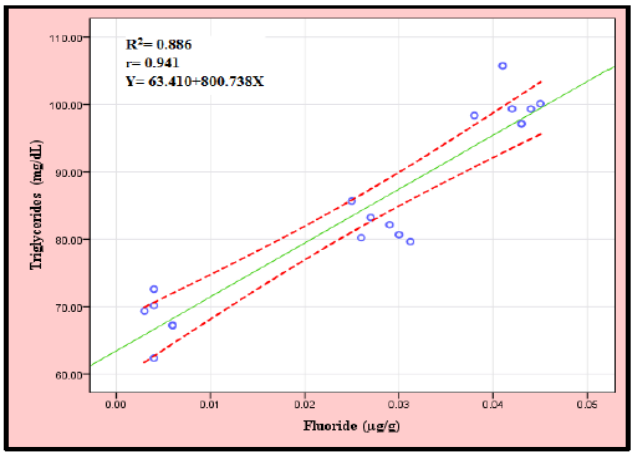

Fig. 23: Scattterplot showing Pearson's bivariate correlation and simple linear regression between levels of cardiac tissue fluoride (µg/g) and triglycerides (mg/dL) in cardiac tissue of rats after 40 days of fluoride treatment.

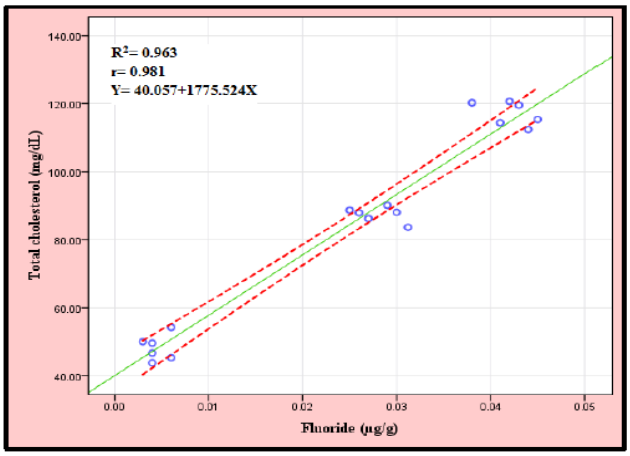

Fig. 24: Scattterplot showing Pearson's bivariate correlation and simple linear regression between levels of cardiac tissue fluoride (µg/g) and total cholesterol (mg/dL) in cardiac tissue of rats following 40 days of fluoride treatment.

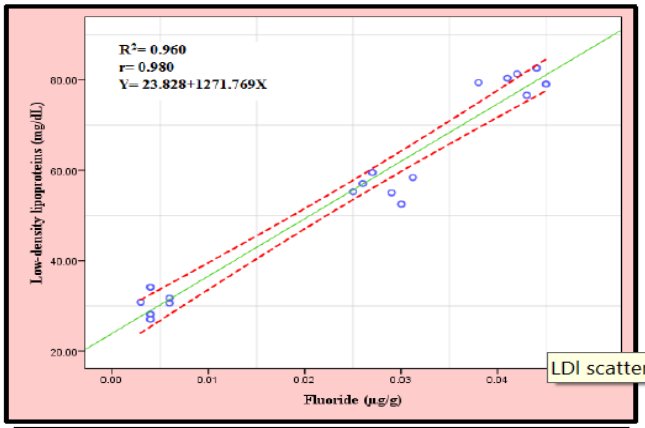

Fig. 25: Scattterplot showing Pearson's bivariate correlation and simple linear regression between levels of cardiac tissue fluoride (µg/g) and low-density lipoproteins (mg/dL) in cardiac tissue of rats after 40 days of fluoride exposure.

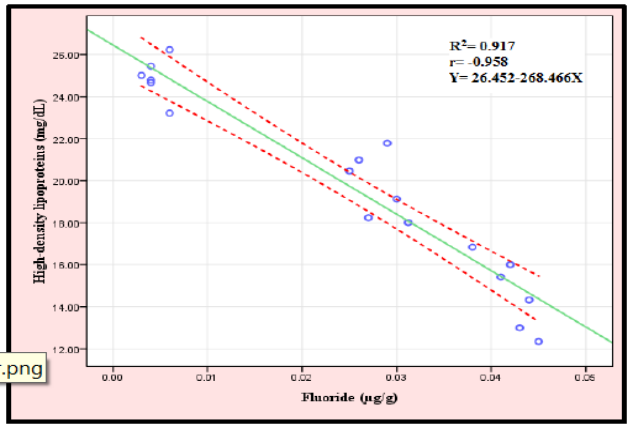

Fig. 26: Scattterplot showing Pearson's bivariate correlation and simple linear regression between levels of cardiac tissue fluoride (µg/g) and high-density lipoproteins (mg/dL) in cardiac tissue of rats after 40 days of fluoride treatment.

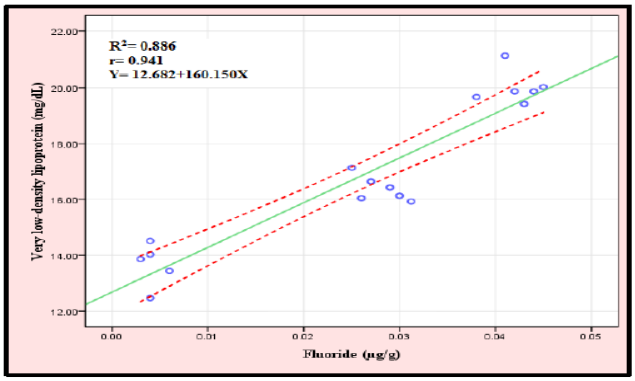

Fig. 27: Scattterplot showing Pearson's bivariate correlation and simple linear regression between levels of cardiac tissue fluoride (µg/g) and very low-density lipoproteins (mg/dL) in cardiac tissue of rats following 40 days of fluoride treatment.

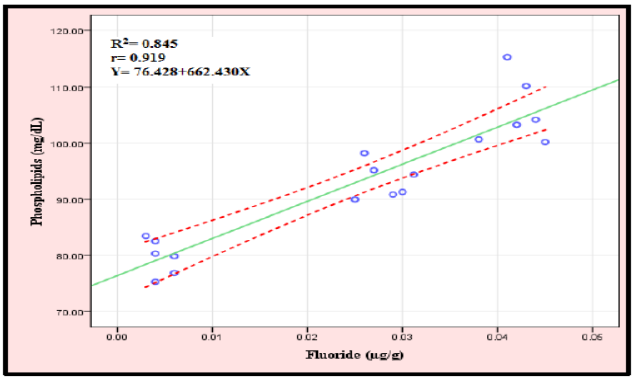

Fig. 28: Scattterplot showing Pearson's bivariate correlation and simple linear regression between levels of cardiac tissue fluoride (µg/g) and phospholipids (mg/dL) in cardiac tissue of rats after 40 days of fluoride administration.

DISCUSSION

In the current investigation, the histopathological findings of rats subjected to 300 and 600 mg/kg of NaF b.w./day respectively for 40 days unveiled significant pathological alterations i.e. myocardial cell necrosis, fibrillolysis, extensive cytoplasmic vacuole formation, swollen myocardial fibers, nucleus dissolution, clumped fibers, and interstitial inflammatory infiltrate. These findings are in accordance with the study of Okushi[19] who documented several pathological alterations such as cloudy swelling, infiltration with round cells, thickening of adventitia, diffuse hemorrhages, and vacuolar and colloid degeneration of the myocardium in rabbits exposed to sodium fluoride. The present study highlights the interference of fluoride with myocardial metabolism, a condition witnessed by Iwase[20] who illustrated through histochemical analysis that fluoride induced degenerative alterations followed by changes in the localization of glycogen in the myocardium of rabbits receiving 10-30 mg NaF/kg/ b.w./day for 15-169 days. Cloudy swelling, vacuolar degeneration, hemorrhages, and round-cell infiltration in the cardiac tissue of rabbits under the effect of fluoride was noted.[21]

In fluorotic patients, Muller and Bock[22] demonstrated myocardial edema accompanied by the infiltration of erythrocytes and leukocytes, as well as acute right dilatation of the heart and general venous hyperemia. Another study reported degenerative changes, notably fragmentation of muscle fibers, in the heart of a fluorotic patient, where delicate sarcolemma was replaced by a fibrous structure.[23] The study done by Pribilla[24] revealed fibrous necrosis, nuclear dissolution, fibrillolysis, interstitial edema, minute hemorrhages, and infiltration of histiocytes, lymphocytes, and granulocytes in the myocardium of fluorotic patients.

In rabbits subjected to fluoride exposure, Shashi and Thapar[25] displayed significant pathological changes in the myocardium. These changes included extensive interstitial edema, fibrous necrosis, and cloudy swellings. Particularly in the group receiving the highest dose of 50 mg of NaF/kg b.w./day, there was marked presence of myocardial fiber disintegration, round cell infiltration, and acute hemorrhages. Similar changes were reported by Cicek *et al.*[26] who observed significant histological alterations, including myocardial cell necrosis, cytoplasmic vacuole development, dissolution of nucleus, inflated and clumped myocardial fibers, fibrillolysis, interstitial oedema, tiny hemorrhagic regions as well as hyperaemic blood vessels in cardiac tissues of rat treated with 50 and 100 mg/L NaF.

The present investigation supports the findings of Stawiarska-Pięta *et al.*[27], who found that long-term exposure to fluorine compounds causes morphological changes and impaired function. They also reported pathological changes in the heart of the rabbits supplemented with 3 mg NaF/kg b.w./day in their drinking water. Varol *et al.*[28] postulated that the production of reactive oxygen species in chronic fluorosis could cause myocardial cell damage and death.

Kaithwas *et al.*[29] documented focal areas of myocardial degeneration with sparse infiltration and vacuolation in rats treated with doxorubicin. Cardiac myofibers were found to be in disarrayed pattern in the present study. This concurs with the work of Thent *et al.*[30] who presumed that this is due to the degeneration of the structural protein in mitochondria of the cytoplasm in diabetic rats.

During the present study, the myocytes were separated from each other by wide intracellular spaces and some myocardial fibers exhibited vacuolated sarcoplasm, along with localized areas of hemorrhage and congested blood capillaries. These observations align with the findings of Basha and Sujitha[31], who reported similar histopathological changes, including edema, plasmic vacuolization, small hemorrhages, and fibrous necrosis in the cardiac tissue of fluoridated rats. Other experimental studies also showed similar results in the cardiac tissue of rats.[32,33,34] In the present study, the cardiac tissue of fluoridated rats exhibited mild mononuclear cell infiltration between cardiac muscle fibers, focal sarcolytic changes, fibroblast proliferation, small pockets of hemorrhages, and thickened blood vessels. These alterations could be due to reduced antioxidant enzyme activity or membrane lipid peroxidation induced by fluoride.[35]

During present investigation, there was loss of cross-striations, fragmentation and Zenker's necrosis in the cardiac tissue of fluoride treated rats. Some studies have documented myocardial cell damage following fluoride intoxication in both in vivo and in vitro experiments.[36,37]

Dyslipidemia is one of the major risk factors of coronary artery disease.[38] Fluoride alters the lipid profile, which is an important risk factor for the occurrence of cardiovascular disease. In the present investigation, the fluoride-exposed treated group exhibited significantly ($P< 0.0001$) elevated levels of total cholesterol, triglycerides, low-density lipoprotein cholesterol, very low-density lipoprotein cholesterol and phospholipids, meanwhile, the level of high-density lipoproteins was significantly ($P< 0.0001$) declined.

Shashi[39] observed elevated levels of triglycerides (TG) in rats administered at doses of 5, 10, 20 and 50 mg NaF/kg b.w./day, these findings are in agreement with the current study. Triglycerides underwent hydrolysis by a hormone-sensitive lipase to form free fatty acids and glycerol. Fluorides are known as strong inhibitors of various enzyme systems like lipase, bone phosphatase, esterases. Lipase, a hydrolyzing enzyme is particularly susceptible to the inhibitory action of fluoride in amounts as low as 1 part in 5 million. This type of inhibition of lipase may result in an increased level of triglycerides during chronic fluoride intoxication.[40,41] Afolabi *et*

al.[42] observed that male rats treated with 100 mg/L of fluoride in drinking water for seven weeks had significant hypertriglyceridemia. This was due to enhanced fatty acid supply and impaired removal of very low-density lipoprotein from the plasma resulting to high levels of triglycerides.[43]

In the current study, the fluoridated rats exhibited a highly significant (P< 0.0001) increase in the levels of total cholesterol (TC). These findings are in concordance with the study done by Rupal and Narasimhacharya (2010) who reported hypercholesterolemia in fluoride exposed animals, indicating significant (P< 0. 001) increases in cholesterol biosynthesis. Fluoride-induced hypercholesterolemia is thought to involve the disruption of lipid metabolism in the liver. This disruption results in accumulation of cholesterol and other lipids, which in turn raises the levels of serum cholesterol. These findings are further supported by Abdel-Wahab[44] who noted that sodium fluoride administration raised the levels of total lipids and cholesterol. Shashi and Bhardwaj[45] have reported rise in serum cholesterol levels among fluorotic patients.

The present study revealed a highly significant (P< 0. 0001) increase in the level of low-density lipoprotein (LDL) in the cardiac tissue of fluoridated rats. This is in concordance with the study of Abdel-Baky and Abdel-Rahman[46], who reported a significant increase in the level of serum LDL in sodium fluoride treated animals compared to control. The increase in LDL is particularly concerning as it leads to the development of atherosclerosis and cardiovascular diseases. The observed rise in myocardial cholesterol levels in the fluorotic rats is attributed to increased uptake of LDL by myocardial membranes from the bloodstream.

The current investigation revealed a highly significant (P< 0. 0001) reduction in the level of high-density lipoprotein (HDL) in the fluoridated animals. HDL is known for its protective role in cardiovascular health by facilitating the removal of cholesterol from tissues and efflux of cholesterol from tissues to liver fro excretion. The decrease in the level of HDL is in line with the study conducted by Afolabi *et al.*[42], who observed decreased levels of HDL in male rats administered with 50 mg/L and 100 mg/L of fluoride in drinking water. This decline in HDL further emphasizes the potential of fluoride to contribute to atherogenesis and cardiovascular diseases.

The study also reported a significant elevation (P< 0.0001) in the levels of very low density lipoprotein (VLDL) in the fluorotic rats. VLDL is a precursor to LDL and is involved in the transport of triglycerides and cholesterol to the peripheral tissues. The effect of fluoride on VLDL may be linked to its effect on lipid metabolism. Machoy and Wieczorek[47] proposed that fluoride administration leads to alterations in the activity of enzymes involved in lipid transformation, including triglyceride lipase and nonspecific esterase. These changes lead to raised VLDL levels and other lipid abnormalities.

Present experiments showed a significant (P<0.0001) elevation of phospholipids during chronic fluoride intoxication in cardiac tissue which might result from a defect in the lipoprotein metabolism involving either the failure of the lipid to couple the protein moiety or the release of the lipoprotein from the liver into the plasma.[40] Arpita and Bidyut[48] reported incline in the level of phospholipids after the treatment of fluoride at doses of 5, 10, 15, 20 ppm for 60 days. In contrary, some studies documented decreased levels of phospholipids after the fluoride exposure.[49]

CONCLUSION

The present study aims to explore the impact of chronic fluoride exposure on lipid profile and cardiac tissue integrity in Wistar rats. These findings depict the potential of fluoride to induce dyslipidemia and provoke adverse histopathological changes in cardiac tissues, highlighting the importance of assessing fluoride toxicity in relation to cardiovascular health.

REFERENCES

1. Bhatnagar A, Kumar E, Sillanpää M. Fluoride removal from water by adsorption-A review. Chem Eng J., 2011; 171(3): 811-840. https://doi.org/10.1016/j.cej.2011.05.028
2. Shashi A, Kumar J. Neurotoxicity induced by fluoride in rat cerebral cortex. Int J Curr Microbiol App Sci., 2016; 5(10): 938-951. http://dx.doi.org/10.20546/ijcmas.2016.510.101
3. Shashi A, Rana N. SDS-PAGE and densitometric analysis of myofibrillary proteins: actin and myosin in skeletal muscle of fluoride intoxicated rats. Int J Trop Dis Health, 2017; 27(2): 01-08. https://doi.org/10.9734/IJTDH/2017/36186
4. Shashi A, Kaur J. Protective and curative effects of Boerhaavia diffusa L. against fluoride induced renal oxidative stress and antioxidant enzymes in rats. Arch Anat Physiol, 2017; 2(1): 036-041. https://dx.doi.org/10.17352/aap.000009
5. Shashi A, Khan I. Alleviation of fluoride induced alterations in antioxidant status and fecundity of spermatozoa of rats by Boerhaavia diffusa L. Int J Pharm Pharm Sci, 2017; 9(10): 93-104. https://dx.doi.org/10.22159/ijpps.2017v9i10.20695
6. Shashi A, Thakur R. Therapeutic effect of Boerhaavia diffusa L. on scanning electron microscopy of rat exocrine pancreas induced by fluoride. Eur Acad Res, 2020; 7(11): 5762-5775.
7. Shashi A, Thakur S. Molecular mechanism of changes in gene expression of cytosolic Cu/Zn SOD, lipid peroxidation and hepatic function impairments during experimental fluorosis. J Pharm Res Int, 2021; 33(46B): 247-265. https://dx.doi.org/10.9734/jpri/2021/v33i46B32939
8. Shashi A, Thakur S. Gene expression and alterations of antioxidant enzymes in spleen of rats exposed to

fluoride. J Trace Elem Med Biol, 2022; 72: 126966. https://doi.org/10.1016/j.jtemb.2022.126966
9. Labib H, Badr AM, Abdelgwad M, Abd El Galil TI. KEAP1/NrF2 pathway in sodium fluoride-induced cardiac toxicity and the prophylactic role of vitamin C versus platelet-rich plasma. Folia Morphol, 2022; 81(3): 663-678. https://dx.doi.org/ 10.5603/FM.a2021.0053
10. Wang T, Yan X, Dong N, Hao X, Xing Y, Tian X, Feng J, Xie J, Lv Y, Wei C, Gao Y, Qiu Y. Comparative transcriptomics reveals the role of the toll-like receptor signaling pathway in fluoride-induce cardiotoxicity. J Agric Food Chem, 2019; 67(17): 5033-5042. https://doi.org/10.1021/acs.jafc.9b00312
11. Zhao S, Guo J, Xue H, Meng J, Xie D, Liu X, Yu Q, Zhong H, Jiang P. Systematic impacts of fluoride exposure on the metabolomics of rats. Ecotoxicol Environ Saf, 2022; 242: 113888. https://doi.org/10.1016/j.ecoenv.2022.113888
12. Nabavi SF, Nabavi SM, Mirzaeia M, Moghaddam AH. Protective effect of quercetin against sodium fluoride induced oxidative stress in rat's heart. Food Funct, 2012; 3(4): 437-441. https://doi.org/10.1039/c2fo10264a
13. Algridi MA, Azab AE. Antidyslipidemic effect of fenugreek seeds powder against sodium fluoride-induced dyslipidemia in male rabbits. J Biotech Bioprocess, 2021; 2(3): 1-8. https://doi.org/10.31579/2766-2314/028
14. Vasant RA, Narasimhacharya AVRL. Alleviatory effects of Emblica officinalis G. as a food supplement in fluoride induced hyperlipemia and oxidative stress. Int J Pharm Pharm Sci., 2012; 4(1): 404-408.
15. Melila M, Rajendran R, Lumo AK, Arumugam G, Kpemissi M, Sadikou A, Lazar G, Amouzou K. Cardiovascular dysfunction and oxidative stress following human contamination by fluoride along with environmental xenobiotics (Cd & Pb) in the phosphate treatment area of Togo, West Africa. J Trace Elem Med Biol., 2019; 56: 13-20. https://doi.org/10.1016/j.jtemb.2019.07.002
16. Drury AR, Wallington EA. Carleton's histological techniques 5th edn. Oxford University Press. London, 1980; 520.
17. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of preparative centrifuge. Clin Chem, 1972; 18(6): 499-502. https://doi.org/10.1093/clinchem/18.6.499
18. Harwood JE. The use of an ion-selective electrode for routine fluoride analyses on water samples. Water Res., 1969; 3(4): 273-280. https://doi.org/10.1016/0043-1354(69)90024-4
19. Okushi I. Experimental studies on the effects of sodium fluoride upon the heart muscle of rabbits. Shikoku Acta Medica, 1954; 5: 238-245.
20. Iwase T. Studies on the glycogen and phosphorylase variations in myocardium, skeletal muscle, liver in experimental fluorosis. Part I Influence of fluorine on glycogen. Shikoku Acta Medicina, 1958; 12: 616-623.
21. Takamori T. The heart changes of growing albino rats fed on varied contents of fluorine. The Toxicology of Fluorine Symposium, 1962; 125-129.
22. Müller W, Bock KD. Acute poisoning with hydrosilicofluoric acid. Medizinische Klinik, 1958; 53(13): 502-503.
23. Fassake E. Acute fluoride poisoning. Gordonoff T, editor. In: The toxicology of fluorine symposium, 1962 Oct 15-17; Bern, Switzerland. Basel/Stuttgart: Schabe, 1964; 130-135.
24. Pribilla O. Four cases of acute silicofluoride intoxication clinical and pathological findings. Fluoride, 1968; 1(2): 103-109.
25. Shashi A, Thapar SP. Histopathology of myocardial damage in experimental fluorosis in rabbits. Fluoride, 2001; 34(1): 43-50.
26. Cicek E, Aydin G, Akdogan M, Okutan H. Effects of chronic ingestion of sodium fluoride on myocardium in a second generation of rats. Hum Exp Toxicol, 2005; 24(2): 79-87. https://doi.org/10.1191/0960327105ht505oa
27. Stawiarska-Pięta B, Birkner E, Stojko R, Grucka-Mamczar E, Szaflarska-Stojko E, Maj A, Birkner B, Wyszyńskaa M. Influence of fluoride on rabbit organ morphology in atheromatosis. Fluoride, 2007; 40(2): 116-127.
28. Varol E, Akcay S, Ersoy IH, Koroglu BK, Varol S. Impact of chronic fluorosis on left ventricular diastolic and global functions. Sci Total Environ, 2010; 408(11): 2295–2298. https://doi.org/10.1016/j.scitotenv.2010.02.011
29. Kaithwas G, Dubey K, Pillai KK. Effect of *Aloe vera* (*Aloe barbadensis* Miller) gel on doxorubicin-induced myocardial oxidative stress and calcium overload in albino rats. Indian J Exp Biol., 2011; 49(4): 260-268.
30. Thent ZC, Lin TS, Das S, Zakaria Z. Histological changes in the heart and the proximal aorta in experimental diabetic rats fed with *Piper sarmentsoum*. Afr J Tradit Complement Altern Med., 2012: 9(3): 396–404.
31. Basha MP, Sujitha NS. Chronic fluoride toxicity and myocardial damage: Antioxidant offered protection in second generation rats. Toxicol Int., 2011; 18(2): 99-104. https://doi.org/10.4103/0971-6580.84260
32. Cheng R, Nie Q, Sun H, Zhang Y, Wu L, Ma Y, Yan X. Fluoride-induced oxidative stress in rat myocardium through the Bax/Bcl-2 signalling pathway. Fluoride, 2013; 46(4): 198-203.
33. Thangapandiyan S, Miltonprabu S. Protective effect of Epigallocatechin gallate on sodium fluoride induced changes in histology of selected organs in rats. J Toxicol, 2013; 3(1): 01-07.
34. Umarani V, Muvvala S, Ramesh A, Lakshmi BV, Sravanthi N. Rutin potentially attenuates fluoride-induced oxidative stress-mediated cardiotoxicity, blood toxicity and dyslipidemia in rats. Toxicol

Mech Methods, 2015; 25(2): 143-149. https://doi.org/10.3109/15376516.2014.1003359
35. Navathej A, Sujatha K, Srilatha C. Effect of Aloe vera whole leaf extract on fluoride induced pathomorphological changes in soft tissues in growing rats. J Vet Sci Technol, 2015; S(13): 01-06. https://doi.org/10.4172/2157-7579.1000S13-003
36. Yildirim S, Ekin S, Huyut Z, Oto G, Comba A, Uyar H, Sengul E, Cinar DA. Effect of chronic exposure to sodium fluoride and 7,12 dimethylbenz [a] anthracene on some blood parameters and hepatic, renal, and cardiac histopathology in rats. Fluoride, 2018; 51(3): 278-290.
37. Zhao S, Guo J, Xue H, Meng J, Xie D, Liu X, Yu Q, Zhong H, Jiang P. Systematic impacts of fluoride exposure on the metabolomics of rats. Ecotoxicol Environ Saf., 2022; 242: 113888. https://doi.org/10.1016/j.ecoenv.2022.113888
38. Limbu YR, Rai S, Ono K, Kurokawa M, Yanagida J-I, Rai G, Gurung N, Rai C. Lipid profile of adult Nepal Med Coll J, 2008; 10(1): 4-7.
39. Shashi A. Biochemical effects of fluoride on lipid metabolism in the reproductive organs of male rabbits. Fluoride, 1992; 25: 149–54.
40. Shashi A, Singh JP, Thapar SP. Effect of fluoride in excess on lipid constituents of respiratory organs in albino rabbits. Fluoride, 1989; 22(1): 33-39.
41. Grucka-Mamczar E, Birkner E, Kasperczyk S, Kasperczyk A, Chlubek D, Samujlo D. Lipid balance in rats with fluoride induced hyperglycemia. Fluoride, 2004; 37: 195-200.
42. Afolabi OK, Oyewo EB, Adekunle AS, Adedosu OT, Adedeji AL. Oxidative indices correlate with dyslipidemia and pro-inflammatory cytokine levels in fluoride-exposed rats. Arh Hig Rada Toksikol, 2013; 64: 521-529. https://doi.org/10.2478/10004-1254-64-2013-2351
43. Miltonprabu S, Thangapandiyan S. Epigallocatechin gallate potentially attenuates fluoride induced oxidative stress mediated cardiotoxicity and dyslipidemia in rats. J Trace Elem Med Biol., 2015; 29: 321-335. https://doi.org/10.1016/j.jtemb.2014.08.015
44. Abdel-Wahab WM. Protective effect of thymoquinone on sodium fluoride-induced hepatotoxicity and oxidative stress in rats. J Basic Appl Zool, 2013; 66(5): 263-270. https://doi.org/10.1016/j.jobaz.2013.04.002
45. Shashi A, Bhardwaj M. Dose effect relationship between high fluoride intake and biomarkers of lipid metabolism in endemic fluorosis. Biomed Prev Nutr, 2013; 3: 121-127. https://doi.org/10.1016/j.bionut.2012.10.006
46. Abdel-Baky ES, Abdel-Rahman ON. Cardioprotective effects of the garlic (Allium sativum) in sodium fluoride-treated rats. J Basic Appl Zool, 2020; 81: 01-07. https://doi.org/10.1186/s41936-020-0140-0
47. Machoy-Mokrzynska A, Put A, Ceglecka M, Mysliwiec Z. Influence of essential phospholipids (EPL) on selected biochemical parameters of lipid metabolism in rats chronically exposed to ammonium fluoride vapours. Fluoride, 19954; 7(4): 201-204.
48. Arpita S, Bidyut B. Effect of fluoride toxicity on some clinical, biochemical and physiological aspects of albino rats. Int J Res Chem Environ, 2012; 2(1): 160-165.
49. Wang Y, Xiao K, Liu J, Dallner G, Guan Z. Effect of long term fluoride exposure on lipid composition in rat liver. Toxicol, 2000; 146(2-3): 161-169. https://doi.org/10.1016/S0300-483X(00)00167-0