World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 7.409

BIOCHEMICAL MARKERS OF MYOCARDIAL INJURY AND ULTRASTRUCTURAL CARDIOMYOPATHY FOLLOWING FLUORIDE ADMINISTRATION IN RATS

***Shashi A. and Shiwani Dogra**

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

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

Article Received on 08/06/2024 **Article Revised on 28/06/2024** **Article Accepted on 18/07/2024**

ABSTRACT

Background and Objective: Fluoride uptake in coronary arteries is associated with an increased cardiovascular risk of sudden death. It interferes with numerous enzyme systems resulting in elevation of key risk factors for cardiovascular disease. However, there are few studies about the effects of fluorosis on cardiovascular system. The study aims to investigate the effect of fluoride on cardiac biomarkers and ultrastructural changes. **Materials and Methods:** A total of eighteen Wistar albino rats were divided into three groups of six rats each. The control group received 1 ml deionized water/kg b.w./day and groups II and III were given 300 and 600 mg NaF/kg b.w./day respectively for 40 days. At the end of experimental fluoride, all animals were sacrificed and heart tissue was isolated and processed for biochemical analysis and ultrastructural changes. **Results:** There was significant ($P< 0.0001$) accumulation of fluoride in cardiac tissue of fluorotic rats. The level of cardiac troponin-T and the activities of biomarker enzymes viz; creatine kinase-MB, lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase were significantly elevated ($P< 0.0001$) in the fluoride administered rats as compared to control. Correlation analysis exhibited a positive relationship between levels of fluoride and cardiac troponin-T as well as activities of functional biomarker enzymes. Transmission electron microscopic examination of cardiac tissue showed nuclear condensation, basophilic degeneration, cytomorphosis, wide spaces in sarcoplasm, cytoplasmic matrix edema, thinning and lysis of myocardial myofibrils, swollen and disorganized mitochondria in fluoridated rats. The elongated nuclei, intact nuclear membrane, and a striated appearance of myocardial fibers were noted in control rat. **Conclusion:** The present study revealed that fluoride induces cardiotoxicity through biochemical disruptions and direct ultrastructural damage to cardiac tissue. The observed increase in cardiac biomarkers correlates with the ultrastructural abnormalities, affirming the detrimental impact of fluoride at both biochemical and cellular levels during experimental fluorosis.

KEYWORDS: Cardiac troponin-T, Creatine kinase-MB, Fluorosis, Lactate dehydrogenase, Transaminases, Ultrastructural pathology, Wistar rat.

INTRODUCTION

Fluorosis poses a significant threat to human and animal health and is a major public safety concern in many nations including India. Subchronic fluoride exposure has the potential to cause pathological damage to the heart, although the mechanism of action requires further exploration.[1] Studies have shown that fluoride has severe detrimental effects on cardiac function in myocardial injury.[2] The cardiovascular system is susceptible to be disrupted by a high concentration of fluoride. However, very few studies have ever addressed the mechanism of action of fluorosis in cardiovascular system.

Cardiac biomarkers are critical tools in the diagnosis and management of cardiovascular diseases. Circulating cardiac troponin T (cTnT) is a sensitive biomarker for diagnosing and predicting acute coronary syndromes, with elevated levels linked to heart failure severity.[3] Creatine kinase (CK) is found in various tissues and cells that convert creatine into phosphocreatine and ADP. High CK levels indicate tissue damage in conditions like heart attack and muscular dystrophy.[4] Lactate dehydrogenase, a vital enzyme in anaerobic metabolism, is found in various organs and serves as a checkpoint for gluconeogenesis and DNA metabolism.[5] It is frequently used to detect myocardial infarction, tissue damage, and malignant tumors.[6] Alanine and aspartate aminotransferase, also known as transaminases, are intracellular enzymes that catalyze amino acid transamination to ketonic acid[7], and are often used as early indicators of liver and cardiovascular disease.[8]

Fluoride toxicity increases activities of biomarker enzymes due to damage to myocardial cell membrane.[9]

Cardiomyocytes contract due to an organized cytoskeleton with myofibrils which are contractile fiber bundles composed of sarcomeres.[10] Myofibril ends are connected to the sarcolemma, and the force produced by contracting myofibrils is transmitted through intercalated discs, the specialized cell-cell junctions.[11] Transmission electron microscopy is used in studies on cardiovascular diseases like heart failure and myocardial infarction, which influence mitochondrial function including apoptosis and mitophagy.[12] Hence, the present study elucidated the effects of sodium fluoride on ultrastructure and functional biomarkers of cardiac tissue in fluorosis.

MATERIALS AND METHODS

Experimental design: Eighteen Wistar albino rats weighing 150-200 g were housed in polypropylene cages and fed a commercial rat pellet diet and water was given *ad libitum*. After acclimatization, they were divided into three groups of 6 rats each. The control group received deionized water/kg b.w./day, while groups II and III received 300 and 600 mg NaF/kg b.w./day by oral gavage for 40 days.

Chemicals and reagent kits: All of the reagents and chemicals used in the study were certified analytical grade. ELISA kit by Elabscience for cardiac troponin-T, Tulip diagnostics kit for creatine kinase-MB, Reckon diagnostics kit for lactate dehydrogenase and Erba Diagnostics kit for the estimation of aspartate aminotransferase and alanine aminotransferase were used.

Transmission electron microscopic examination: Small sections of cardiac tissue were fixed overnight at in a glutaraldehyde solution of 2.5% phosphate buffer (0.1M, pH 7.4) and washed 3-4 times in 0.1M phosphate buffer.[13] Tissues were post-fixed in 1% osmium tetraoxide for an hour and then dehydrated with acetone. Ultrathin sections were cut with an ultramicrotome (Leica Ultracut UC7, Austria), uranyl acetate and lead citrate were used to stain ultra-thin sections placed on copper grids and then were studied under transmission electron microscope (Tecnai G2 20 Fei Corporation, The Netherlands) at All India Institute of Medical Sciences, New Delhi, India.

BIOCHEMICAL ANALYSIS

Sample Preparation: The cardiac tissue was homogenized with 0.1 M phosphate buffer (pH 7.4) using a glass Teflon homogenizer before being centrifuged at 10,000 rpm for 20 minutes to estimate the concentration of tissue fluoride and cardiac biomarkers.

Estimation of fluoride: The level of fluoride in the cardiac tissue of experimental and control rats was measured via a potentiometric method using the ion selective electrode.[14]

Estimation of cardiac biomarkers: The level of cTnT and activity of CK-MB, LDH, AST and ALT in control and fluoridated rats were estimated using commercially available reagent kits by following the methods given in respective protocols.

Statistical Analysis: Standard statistical procedures were followed and the data collected during the experiment was subjected to analysis of variance (ANOVA) and was followed by post hoc Bonferroni multiple comparison test, using SPSS 16.0 statistical software (IBM). The significance was set at $P< 0.05$ levels. The relationship between two variables was examined by using Pearson's bivariate correlation metric analysis and simple linear regression test.

Ethical Aspects: The experimental protocols were approved by the Institutional Animal Ethics Committee at Punjabi University, Patiala (Animal Maintenance and Registration No. 107/GO/ReBi/S/99/ CPCSEA /2017-42), and the animals were cared for in accordance with guidelines and committees.

RESULTS

Fluoride Accumulation: The mean levels of fluoride in cardiac tissue were significantly elevated in experimental rats in a dose-dependent manner ($F = 482.267$, $P < 0.0001$, Fig. 1).

Cardiac functional biomarkers: Cardiac troponin-T is the ultimate marker for myocardial necrosis. The mean level of cTnT significantly ($F= 459.580$, $P< 0.0001$) increased in the fluoridated rats in comparison to control. The incline was +215.342% and +454.046% in rats exposed to fluoride (Fig. 2).

The mean activity of cardiac biomarkers such as creatine kinase-MB ($F= 1880.896$, +100.429% & +173.781%, Fig. 3); lactate dehyrogenase ($F= 2065.456$, +102.564% & +221.457%, Fig. 4) aspartate aminotransferase ($F= 690.900$, +90.156% & +218.348%, Fig. 5) and alanine aminotransferase ($F= 5313.121$, +194.851% and +395.421%, Fig. 6) were found to be increased significantly ($P< 0.0001$) in both the studied groups of rats (300 and 600 mg NaF/kg bw/day respectively) for 40 days when compared to control group.

Post-hoc Bonferroni multiple comparison tests following ANOVA indicated a significant ($P < 0.0001$) rise in the levels of cardiac tissue fluoride (mean difference= -0.024 to 0.014, 95% CI = -0.027 to -0.010); cardiac troponin-T (mean difference= -14.850 to -16.461, 95% CI= -17.633 to -13.678); activities of creatine kinase-MB (mean difference= -216.923 to -154.737, 95% CI= -233.322 to -138.338); lactate dehydrogenase (mean difference= -106.907 to -123.927, 95% CI= -116.590to -114.244); aspartate aminotransferase (mean difference= -29.664 to -42.179, 95% CI= -34.896 to -36.947) and alanine aminotransferase (mean difference= -33.494 to -34.476,

95% CI= -35.270 to -32.700) both between and within the fluoride-treated groups.

Correlation analysis: Pearson's bivariate correlation and simple linear regression witnessed a positive relationship between levels of fluoride and cardiac troponin-T (R^2= 0.925; Pearson r = 0.962; Fig. 7A) as well as activities of creatine kinase-MB (R^2= 0.972; Pearson r = 0.986; Fig. 7B); lactate dehydrogenase (R^2= 0.948; Pearson r = 0.974; Fig. 7C); aspartate aminotransferase (R^2= 0.928; Pearson r = 0.963; Fig. 7D) and alanine aminotransferase (R^2= 0.960; Pearson r = 0.980; Fig. 7E).

Effect of fluoride on the ultrastructure of cardiomyocytes in rats

The transmission electron microscopic examination of cardiac tissue of control rat revealed elongated nuclei with intact nuclear membrane and prominent nucleolus. The euchromatin nuclei were present along with scattered patches of heterochromatin near the cell membrane (Fig. 8). The cardiomyocytes had long parallel myofibrillar arrangements and preserved sarcomeric structure. The myofibrils were tightly packed together, but there were only a few small inter-myofibrillar spaces in some areas (Fig. 9). There was characteristic striated appearance of cardiac muscle consisting of bundles of alternating thick myosin (A-bands) and thin actin (I-bands) filaments, extend the length of the cell. The Z-lines separate each sarcomere, and the mitochondria constitute rows sandwiched between the myofibrils (Fig. 10). Inter-myofibrillar mitochondria with dense matrix and closely packed cristae were prominent (Fig. 11).

Electron microscopic examination of cardiomyocytes showed irregularities in the nuclear membrane along with nuclear pores, with an increase in condensed chromatin (Fig. 12). The fluoridated cardiac tissue depicted cytomorphosis accompanied by dissolved and disorganized mitochondria with broken ridges. Extensive cellular degeneration and marked loss of myofibrils was replaced by wide spaces of sarcoplasm (Fig. 13). The fluoride treatment caused marked irregularities of focal distortion and thinning of myocardial myofibrils (Fig. 14). The myofibrils were disorganized and lacked integrity. The altered myofibrils had normal Z bands. The lysis of cardiac muscle fibers and swelling of mitochondria were visible (Fig. 15). The mitochondrial damage, in the form of severe cristae arrangement disruption, matrix substance loss, the presence of intra-mitochondrial vacuoles, and an area of limiting membrane disruption was prominent (Fig. 16). The less electron dense mitochondria exhibited cristolysis. There was variation in mitochondrial shape and an abnormal cristal architecture was noted (Fig. 17).

The ultrastructural alterations in cardiac tissue of rats exposed to 600 mg sodium fluoride/kg b.w./day revealed that the nucleus had lost its normal shape and had a torturous appearance along with nuclear pores. The sarcoplasmic reticulum was nearly absent in these severely degenerated cells. Cellular sequestration and cellular debris were observed in the enlarged extracellular space as well as swollen mitochondria were prominent (Fig. 18). There were visible light and dark zones within a myocyte due to basophilic degeneration. Disintegration of the sarcoplasmic reticulum was also evident (Fig. 19). The cardiomyocytes contained cytoplasmic vacuoles, particularly in close proximity to the dilated blood vessel in the inter-myofibrillar spaces (Fig. 20). The most noticeable ultrastructural change was dilation of sarcotubules and loosening of myofibrils in the center of cells. The damaged cardiomyocyte exhibited cytoplasmic matrix edema, myofibril separation and disruption. The loss of myofibrils was accompanied by abnormal formation of Z band material that extends to the cell periphery (Fig. 21). The cardiomyocyte displayed thinning of the myofibrils, which appeared attenuated and separated from one another. Fluoride-treated groups also showed loss of band integrity, irregularity and distorted intercalated discs. The hypercomplex nuclear folding was noted (Fig. 22). Fluoride caused disruption of myocardial fibers, extracellular matrix lysis and the formation of intracytoplasmic vacuoles of steatosis i.e. lipid droplet deposition. Fluoride-induced mitochondriopathy was also detected as the pronounced variation in shape of mitochondria and abnormal architecture of cristae (Figure. 23).

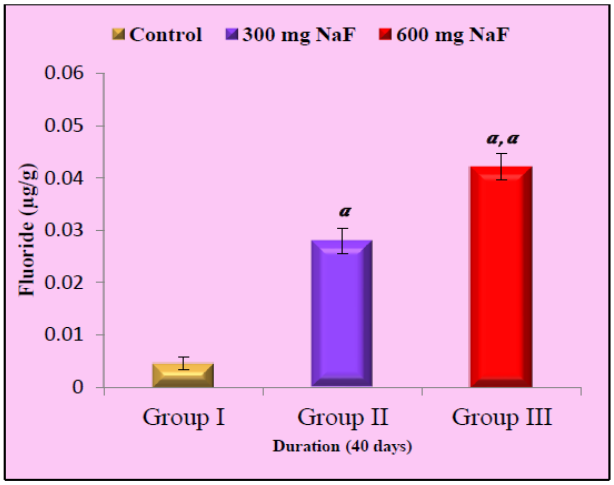


Fig. 1: Mean level of fluoride (µg/g) in cardiac tissue of control and fluoridated rats. Values are expressed as Mean ± SD from six rats in each group. [a]P< 0.0001 Groups II-IIIcompared with Group I; [a,a]P< 0.0001 Group II compared with Group III. Statistical significance was recorded by one way ANOVA followed by post-hoc Bonferroni multiple comparison test.

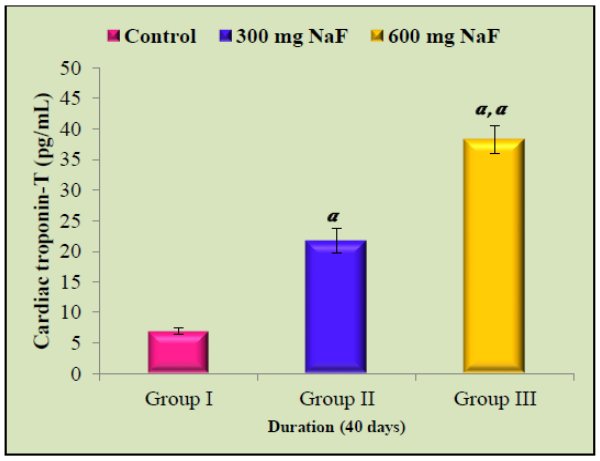


Fig. 2: Mean level of cardiac troponin-T (cTnT) (pg/mL) in control and fluoride treated rats. Values are expressed as Mean ± SD from six rats in each group. aP< 0.0001 Groups II-III compared with Group I; a,a P< 0.0001 Group II compared with Group III. Statistical significance was recorded by one way ANOVA followed by post-hoc Bonferroni multiple comparison test.

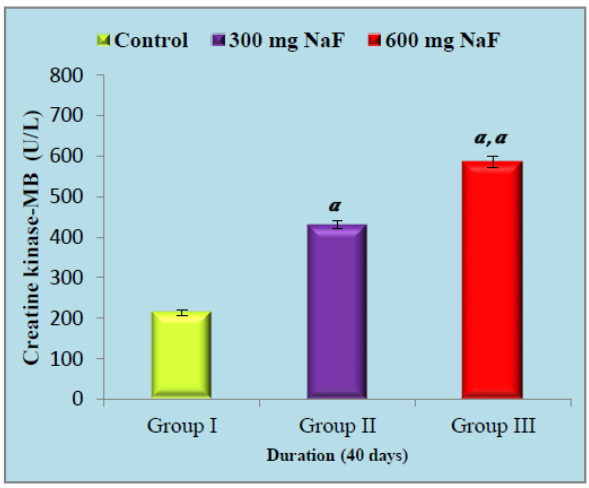


Fig. 3: Mean activity of creatine kinase-MB (CK-MB) (U/L) in control and fluoride treated rats. Values are expressed as Mean ± SD from six rats in each group. aP< 0.0001 Groups II-III compared with Group I; a,a P< 0.0001 Group II compared with Group III. Statistical significance was recorded by one way ANOVA followed by post-hoc Bonferroni multiple comparison test.

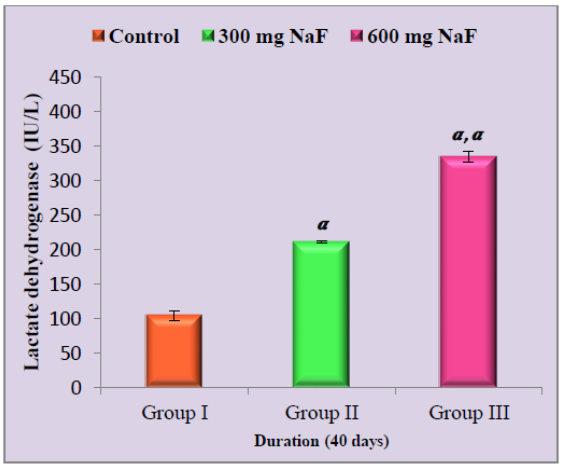


Fig. 4: Mean activity of lactate dehydrogenase (LDH) (IU/L) in control and fluoride treated rats. Values are expressed as Mean ± SD from six rats in each group. ^{a}P<0.0001 Groups II-III compared with Group I; ^{a,a} P< 0.0001 Group II compared with Group III. Statistical significance was recorded by one way ANOVA followed by post-hoc Bonferroni multiple comparison test.

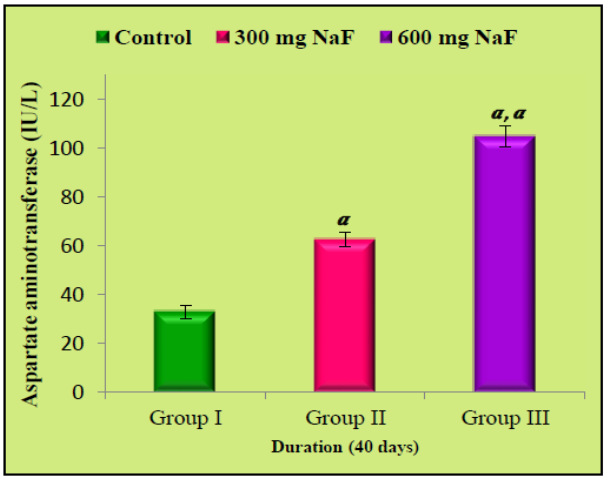


Fig. 5: Mean activity of aspartate aminotransferase (AST) (IU/L) in control and fluoride treated rats. Values are expressed as Mean ± SD from six rats in each group. ^{a}P< 0.0001 Groups II-III compared with Group I; ^{a,a} P<0.0001 Group II compared with Group III. Statistical significance was recorded by one way ANOVA followed by post-hoc Bonferroni multiple comparison test.

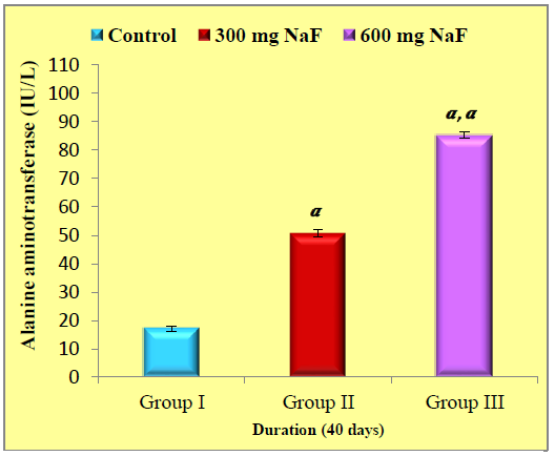
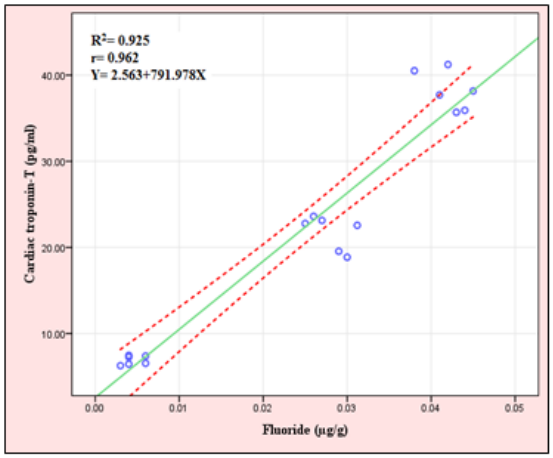
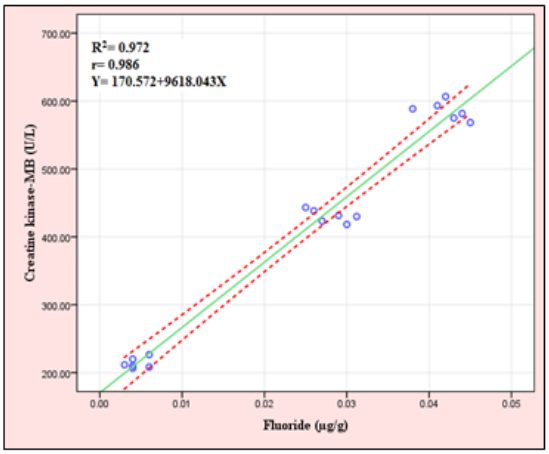


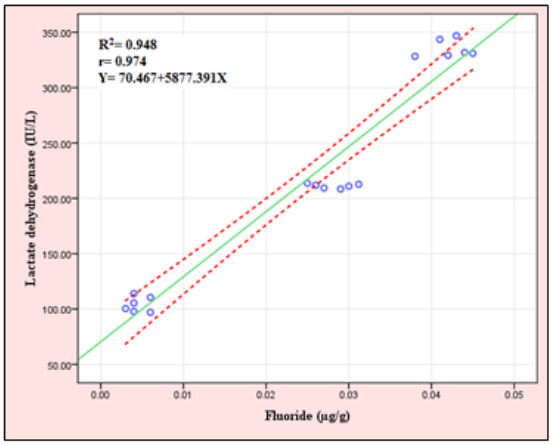
Fig. 6: Mean activity of alanine aminotransferase (ALT) (IU/L) in control and fluoride treated rats. Values are expressed as Mean ± SD from six rats in each group. ^{a}P<0.0001 Groups II-III compared with Group I; ^{a,a} P< 0.0001 Group II compared with Group III. Statistical significance was recorded by one way ANOVA followed by post-hoc Bonferroni multiple comparison test.



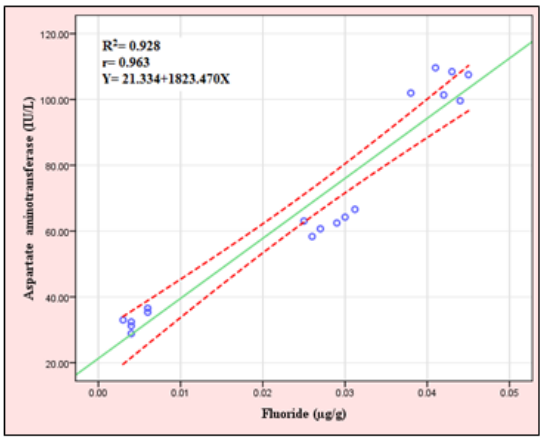
A



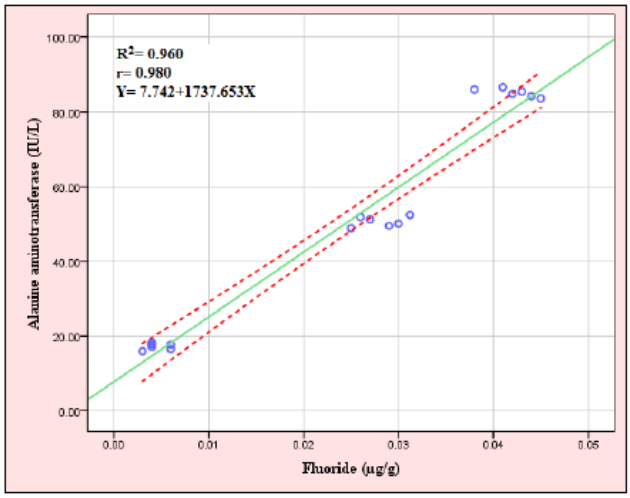
B



C



D



E

Fig. 7: Scattterplot showing Pearson's bivariate correlation and simple linear regression between levels of cardiac tissue fluoride (µg/g) and A. Cardiac troponin-T; activities of cardiac biomarker enzymes: B. Creatine kinase-MB, C. Lactate dehydrogenase, D. Aspartate aminotransferase, E. Alanine aminotransferase in test rats after 40 days of fluoride exposure.

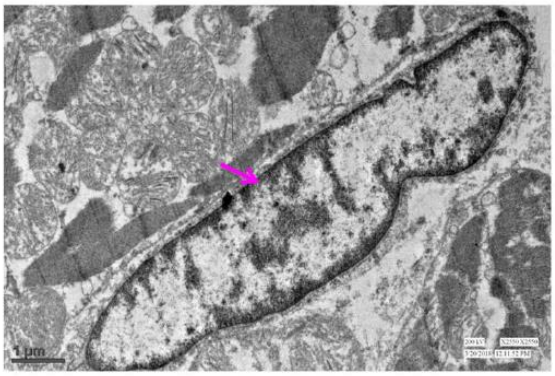


Fig. 8: Transmission electron micrograph of cardiac tissue of control rat showing elongated nucleus of cardiomyocyte exhibiting sparse and a thin layer of dense chromatin ( ) in the inner boundary of nuclear membrane. X2550

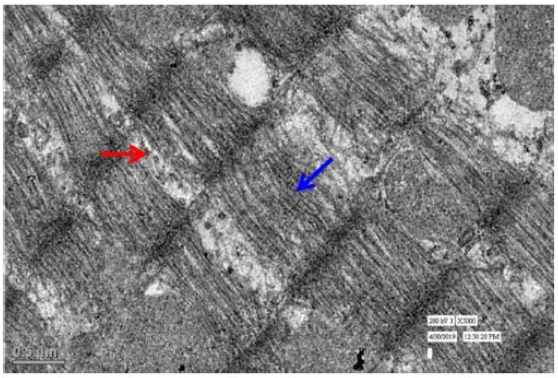


Fig. 9: Transmission electron micrograph of cardiac tissue of control rat showing normal arrangement of myofibrils ( ) and sarcoplasmic reticulum ( ). X5000

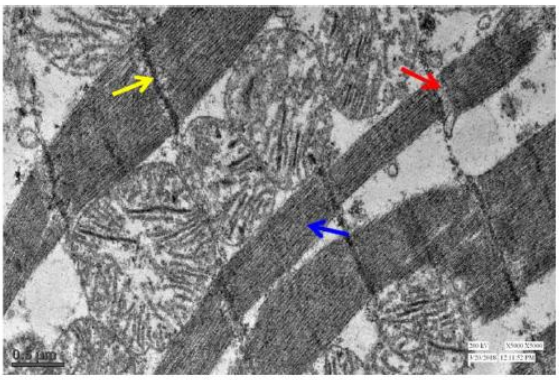


Fig. 10: Transmission electron micrograph of cardiac tissue of control rat showing alternating dark A ( ) and light I bands ( ) with regular Z lines ( ). Non altered mitochondria with their usual shape were also seen. X5000

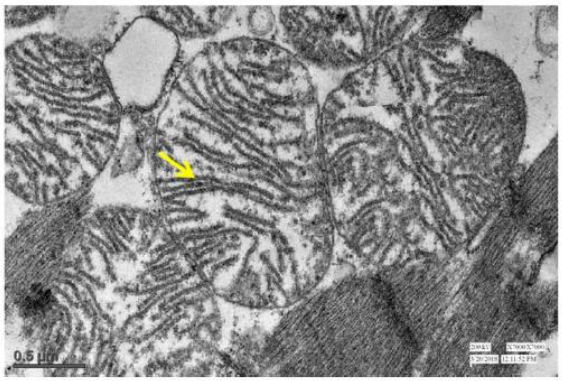


Fig. 11: Transmission electron micrograph of cardiac tissue of control rat showing long and closely packed cristae ( ). X7000

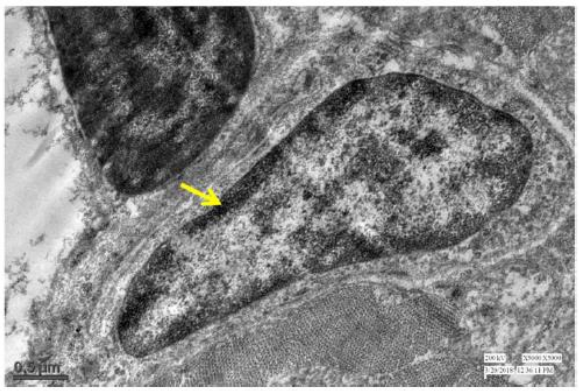


Fig. 12: Transmission electron micrograph of cardiac tissue of rat treated with 300 mg NaF/kg b.w./day showing elongated nucleus of cardiomyocyte exhibiting large condensation of chromatin constituting a thick layer ( ) in inner boundary of nuclear membrane. X5000

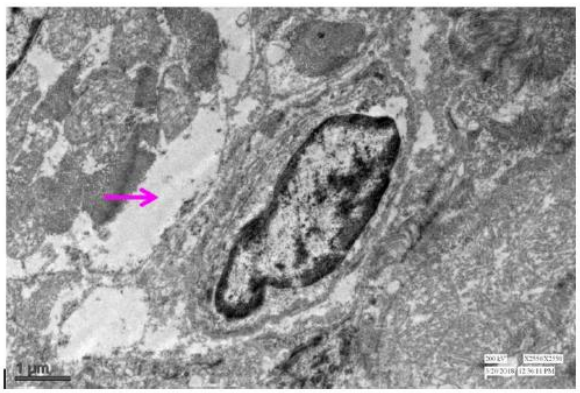


Fig. 13: Transmission electron micrograph of cardiac tissue of rat treated with 300 mg NaF/kg b.w./day showing wide spaces in sarcoplasm ( ) of cardiac myocytes. X2550

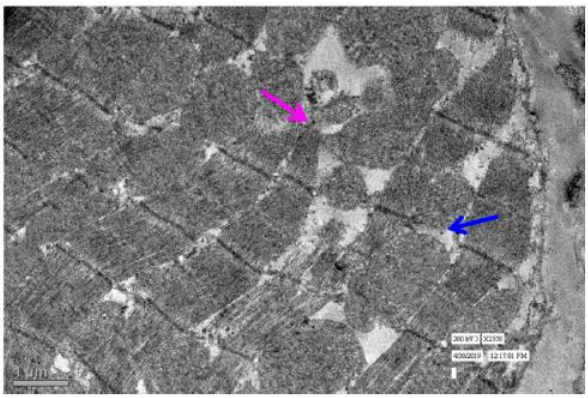


Fig. 14: Transmission electron micrograph of cardiac tissue of rat treated with 300 mg NaF/kg b.w./day showing focal distortion of myofibrils ( ) and disarrangement of mitochondria ( ). X5000

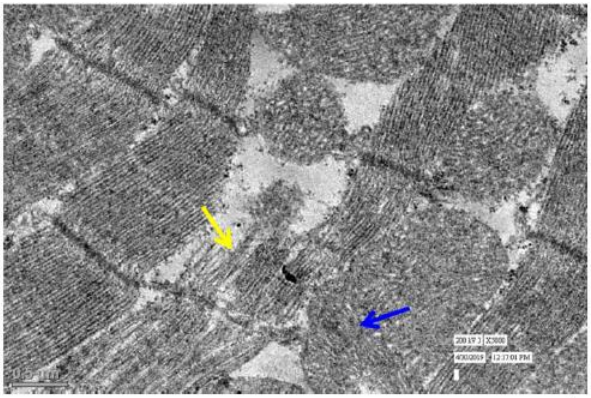


Fig. 15: Transmission electron micrograph of cardiac tissue of rat treated with 300 mg NaF/kg b.w./day showing moderate lysis of myofibrils ( ) and swelling of mitochondria ( ). X5000

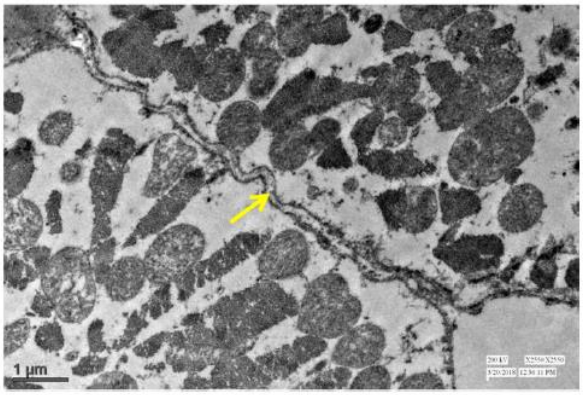


Fig. 16: Transmission electron micrograph of cardiac tissue of rat treated with 300 mg NaF/kg b.w./day showing disruption of limiting membrane ( ) and loss of matrix substance. X2550

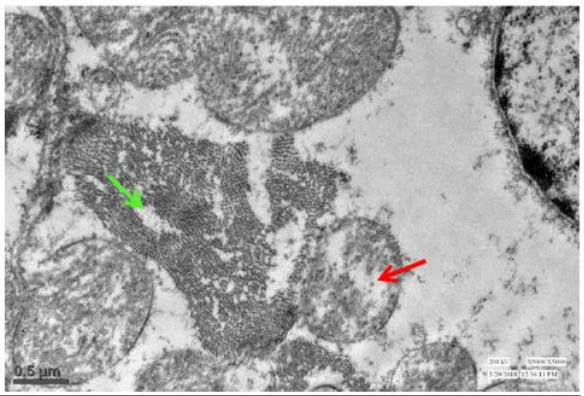


Fig. 17: Transmission electron micrograph of cardiac tissue of rat treated with 300 mg NaF/kg b.w./day showing fragmented myofibrils ( ) and altered mitochondria with loss of cristae ( ). X5000

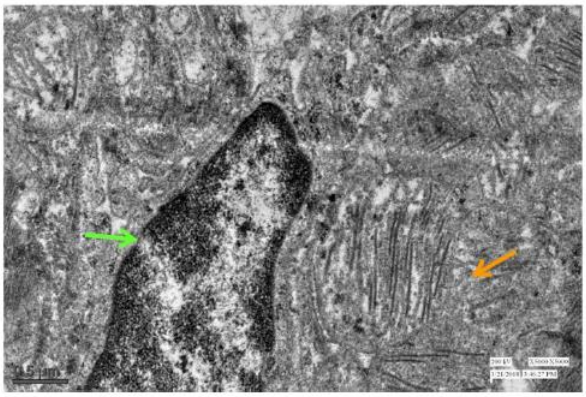


Fig. 18: Transmission electron micrograph of cardiac tissue of rat treated with 600 mg NaF/kg b.w./day showing nuclear pores in the nucleus ( ) and mitochondria appeared swollen ( ) within the cytoplasm. X5000

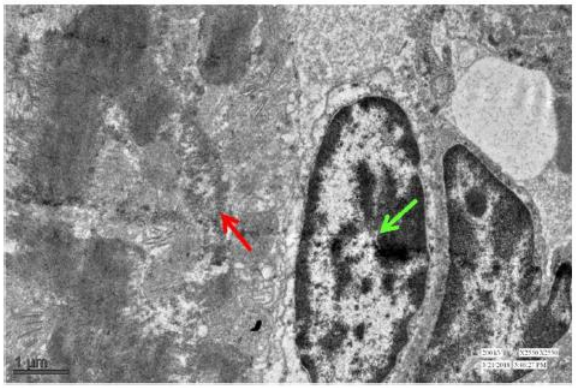


Fig. 19: Transmission electron micrograph of cardiac tissue of rat treated with 600 mg NaF/kg b.w./day showing basophilic degeneration with light and dark zones within a nucleus ( ) along with disruption of sarcoplasmic reticulum ( ). X2550

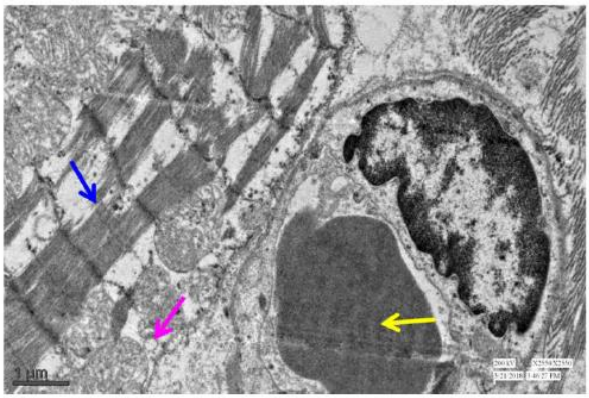


Fig. 20: Transmission electron micrograph of cardiac tissue of rat treated with 600 mg NaF/kg b.w./day showing cytoplasmic vacuoles ( ), necrotic myofibrils ( ) and dilation of blood vessel ( ). X2550

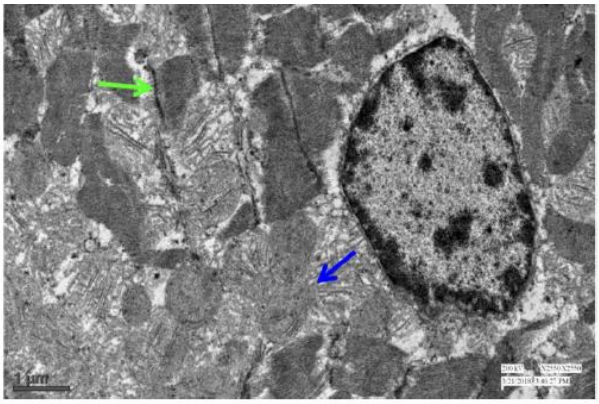


Fig. 21: Transmission electron micrograph of cardiac tissue of rat treated with 600 mg NaF/kg b.w./day showing damaged myocyte with edema of cytoplasmic matrix ( ), loosening of myofibrils ( ) and dilation of sarcotubules. X2550

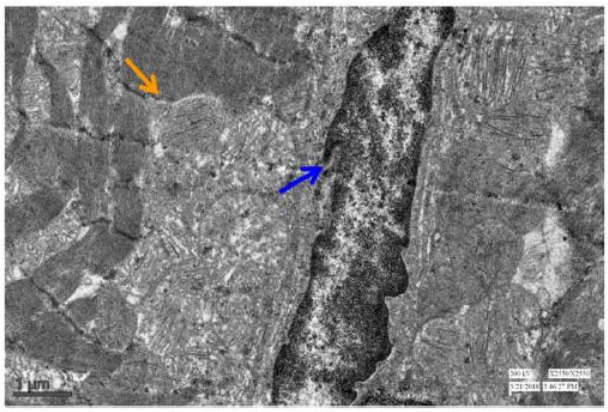


Fig. 22: Transmission electron micrograph of cardiac tissue of rat treated with 600 mg NaF/kg b.w./day showing nucleus with irregular borders ( ) and thin and attenuated myofibrils ( ) with distorted intercalated discs. X2550

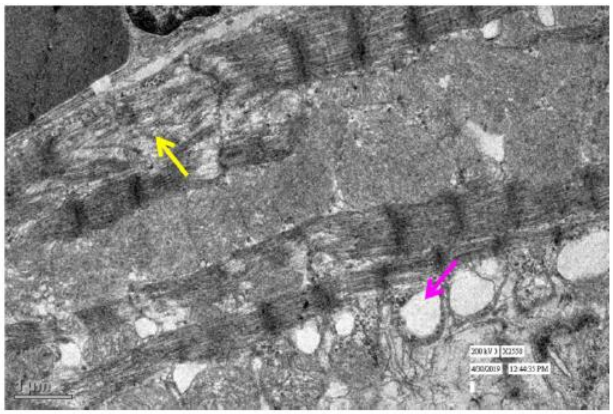


Fig. 23: Transmission electron micrograph of cardiac tissue of rat treated with 600 mg NaF/kg b.w./day showing disruption of myocardial fibers ( ) along with intracytoplasmic vacuoles of steatosis ( ). X2550

DISCUSSION

Cardiac biomarkers such as cTnT, CK-MB, LDH, AST, and ALT can help identify cardiac disease, and changes in these enzymes have been linked to myocardial injury.[15] Assessing the degree and intensity of increase in these cardiac markers is the most reliable way to diagnose the severity of heart damage. In the current investigation, cardiac troponin T, a myocardial tissue-specific protein that is a highly sensitive and precise indicator of myocardial injury[16], was shown to be elevated in fluoride-administered rats. Miltonprabu and Thangapandiyan[17] found similar results, claiming elevated cTnT levels might be due to cardiotoxic effects of fluoride. Creatine kinase has two subunits that are present in three unique molecular types: CK-MM, CK-MB, and CK-BB. CK-MB is a myocardium-specific enzyme that measures the intensity and type of myocyte damage.[18] The current study revealed that rats treated with fluoride had an increase in their CK-MB activity. This incline is due to fluoride-induced free radical formation which causes membrane peroxidation and disruption of cardiac myocytes, resulting in the synthesis of an excessive amount of CK-MB.[19] LDH is an enzyme found in the cytoplasm of all cells in the body. The present investigation demonstrated an increase in LDH activity, which is consistent with the findings of Umarani *et al.*[20], who claimed that fluoride increases LDH activity in rats subjected to sodium fluoride via increasing membrane permeability. Transaminases (AST and ALT) are heart-specific indicators that facilitate aminotransfer catalysis processes and are used to assess cardiac tissue damage. In this study, a similar trend of increased transaminase activity was seen in the fluorotic groups. These findings are in line with the results reported by Abdel-Baky *et al.*[21], who observed that rats given 10 mg/kg of sodium fluoride for four weeks had significantly increased AST and ALT activity.

Transmission electron microscopic examination of cardiac tissue of control rat revealed unaltered mitochondria with tightly packed cristae, as well as long and parallel arrangement of myofibrils consisting of dark A and light I bands. These observations are consistent with the findings of Chongwan and Daijei[22], who observed mitochondria with long and tightly packed cristae and a syncytial arrangement of cardiac muscle fibers with cross striations of both dark and light bands and intercalated discs at the ends. In the current study, congested blood vessels and deteriorated muscle fibers in the myocardium were seen in the fluoride treated groups. These findings are in line to alterations observed in chronic myocardial infarction.[23] Fluoride-induced ultrastructural changes, such as an invaginated nucleus, and enlarged mitochondria with a distorted matrix and absence of cristae, are important indications of apoptosis and necrosis. Myofibril lysis, interrupted Z lines, as well as dilated, fragmented sarcoplasmic reticulum has also been reported previously.[24] The present study demonstrated that the accumulation of fluoride ions within cardiomyocytes causes oxidative stress, culminating in the production of reactive oxygen species, which further contribute to mitochondrial damage and, eventually, cell death. There was rupturing of myocardial fiber and mitochondrial disintegration, providing significant evidence for fluoride-induced heart failure, infarction, and ischemia.[25] A remarkably high level of mitochondrial damage in fluorotic rats was witnessed, including membrane disruption and cristolysis. These findings are in agreement with the study of Yan *et al.*[26] in the H9c2 cardiac cell line, which demonstrated fluoride-induced mitochondrial membrane damage, increased membrane permeability, and apoptotic pathway activation. The current investigation also revealed fluoride-induced mitochondriopathy, which is consistent with the findings of Quadri *et al.*[27], who evaluated the effects of sodium fluoride on heart tissue using transmission electron microscopy.

CONCLUSION

In conclusion, NaF exposure to rats elicited elevation in cardiac biomarkers and alterations in ultrastructure. These changes might be due to fluoride diffusion across the sarcolemma, which affects structural integrity. The present investigation clearly depicts the cardiotoxic impact of fluoride by indicating changes in cardiac biomarkers which further aligns with the ultrastructural damage at the cellular level.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

FUNDING FOR THE PROJECT

The research did not receive any specific grant from funding agencies in the public, commercial or non-profit sectors.

REFERENCES

1. Hou, L., Dong, H., Zhang, E., Lu, H., Zhang, Y., Zhao, H. and Xing, M. A new insight into fluoride induces cardiotoxicity in chickens: Involving the regulation of PERK/IRE1/ATF6 pathway and heat shock proteins. Toxicology, 2024; 501: 153688. https://doi.org/10.1016/j.tox.2023.153688.
2. Basha, M.P. and Sujitha, N.S. Chronic fluoride toxicity and myocardial damage: Antioxidant offered protection in second generation rats. Toxicology International, 2011; 18(2): 99-104. https://doi.org/10.4103/0971-6580.84260.
3. Kusumoto, A., Miyata, M., Kubozono, T., Ikeda, Y., Shinsato, T., Kuwahata, S., Fujita, S., Takasaki, K., Yuasa, T., Hamasaki, S. and Tei, C. Highly sensitive cardiac troponin T in heart failure: Comparison with echocardiographic parameters and natriuretic peptides. J. Cardiol., 2012; 59(2): 202–208. https://doi.org/10.1016/j.jjcc.2011.11.012.
4. Rashid, S., Malik, A., Khurshid, R., Faryal, U. and Qazi, S. 2019. The diagnostic value of biochemical cardiac markers in acute myocardial infarction. In IntechOpeneBooks. https://doi.org/10.5772/intechopen.76150.
5. Farhana, A. and Lappin, S.L. 2023. Biochemistry, Lactate dehydrogenase. In StatPearls. StatPearls Publishing.
6. Agarkov, N.M., Markelov, M.Y., Markelova, E.A., Lutai, Y.A. and Volkov, P.S. The information content and predictive value of cardiac markers in myocardial infarction in the elderly. Adv. Gerontol., 2020; 33(1): 82–86.
7. Kim, S.Y., Lee, J.P., Shin, W.R., Oh, I.H., Ahn, J.Y. and Kim, Y.H. Cardiac biomarkers and detection methods for myocardial infarction. Mol. Cell. Toxicol., 2022; 18(4): 443–455. https://doi.org/10.1007/s13273-022-00287-1.
8. Jasiewicz, M., Siedlaczek, M., Kasprzak, M., Gorog, D.A., Jilma, B., Siller-Matula, J., Obońska, K., Dobosiewicz, R., Pstrągowski, K. and Kubica, J. Elevated serum transaminases in patients with acute coronary syndromes: Do we need a revision of exclusion criteria for clinical trials? Cardiol. J., 2023; 30(5): 747–752. https://doi.org/10.5603/CJ.a2021.0081.
9. Yildrim, S., Ekin, S., Huyut, Z., Oto, G., Comba, A., Uyar, H., Sengul, E. and Çinar, A. 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: 274-286.
10. Gautel, M. The sarcomeric cytoskeleton: Who picks up the strain? CurrOpin. Cell Biol., 2011; 23(1): 39–46. https://doi.org/10.1016/j.ceb.2010.12.001.
11. Sheikh, F., Ross, R.S. and Chen, J. Cell-cell connection to cardiac disease. Trends Cardiovasc. Med., 2009; 19(6): 182–190. https://doi.org/10.1016/j.tcm.2009.12.001.
12. Mielańczyk, Ł., Matysiak, N., Klymenko, O. and Wojnicz, R. 2015. The Transmission Electron

Microscope- Theory and Applications. InTech. https://doi.org/10.5772/60680
13. Karnovsky, M.J. A formaldehyde-glutarldehyde fixative of high osmolarity for use in electron microscopy. J. Cell Biol., 1965; 27: 137-138.
14. Harwood, J.E. 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.
15. Howard-Alpe, G.M., Sear, J.W. and Foex, P. Methods of detecting atherosclerosis in non-cardiac surgical patients; the role of biochemical markers. Br. J. Anaesth., 2006; 97(6): 758–769. https://doi.org/10.1093/bja/ael303.
16. Gupta, S., Singh, K.N., Bapat, V., Mishra, V., Agarwal, D.K. and Gupta, P. Diagnosis of acute myocardial infarction: CK-MB versus cTn-T in Indian patients. Indian J. Clin. Biochem., 2008; 23(1): 89–91. https://doi.org/10.1007/s12291-008-0021-7.
17. Miltonprabu, S. and 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.
18. Al-Hadi, H.A. and Fox, K.A. Cardiac markers in the early diagnosis and management of patients with acute coronary syndrome. Sultan Qaboos Univ. Med. J., 2009; 9(3): 231–246.
19. Oyagbemi, A.A., Omobowale, T.O., Asenuga, E.R., Adejumobi, A.O., Ajibade, T.O., Ige, T.M., Ogunpolu, B.S., Adedapo, A.A. and Yakubu, M.A. Sodium fluoride induces hypertension and cardiac complications through generation of reactive oxygen species and activation of nuclear factor kappa beta. Environ. Toxicol., 2016; 32(4): 1089-1101. https://doi.org/ 10.1002/tox.22306.
20. Umarani, V., Muvvala, S., Ramesh, A., Lakshmi, B.V. and 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.
21. Abdel-Baky, E.S. and Abdel-Rahman, O.N. 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.
22. Chongwan, Z. and Daijei, H. Ultrastructural findings in liver, kidneys, thyroid gland and cardiac muscle of rabbits following sodium fluoride administration. Fluoride, 1988; 21(1): 32-38.
23. Qu, X., Fang, W., Ye, J., Koh, A.S., Xu, Y., Guan, S., Li, R. and Shen, Y. Acute and chronic myocardial infarction in a pig model: Utility of multi-slice cardiac computed tomography in assessing myocardial viability and infarct parameters. Eur. J. Radiol., 2012; 81(4): 431-437. https://doi.org/10.1016/j.ejrad.2011.03.062.
24. Panneerselvam, L., Raghunath, A. and Perumal, E. Acute fluoride poisoning alters myocardial cytoskeletal and AMPK signaling proteins in rats. Int. J. Cardiol., 2017; 229: 96-101. https://doi.org/10.1016/j.ijcard.2016.11.221.
25. Wang, H.W., Liu, J., Zhao, J., Lin, L., Zhao, W.P., Tan, P.P., Tian, W.S. and Zhou, B.H. Ca2+ metabolic disorder and abnormal expression of cardiac troponin involved in fluoride-induced cardiomyocyte damage. Chemosphere, 2018; 201: 564–570. https://doi.org/10.1016/j.chemosphere.2018.03.053.
26. Yan, X., Wang, L., Yang, X., Qiu, Y., Tian, X., Lv, Y., Tian, F., Song, G. and Wang, T. Fluoride induces apoptosis in H9c2 cardiomyocytes via the mitochondrial pathway. Chemosphere, 2017; 182: 159-165. https://doi.org/10.1016/j.chemosphere.2017.05.002
27. Quadri, J.A., Sarwar, S., Pinky, Kar, P., Singh, S., Mallick, S.R., Arava, S., Nag, T.C., Roy, T.S. and Shariff, A. Fluoride induced tissue hypercalcemia, IL-17 mediated inflammation and apoptosis lead to cardiomyopathy: Ultrastructural and biochemical findings. Toxicol., 2018; 406-407: 44-57. https://doi.org/10.1016/j.tox.2018.05.012.