Possible Protective role of Calcium against Fluoride Induced Cardio Toxicities in Adult Male Albino Rats

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Abstract: Fluoride contamination in drinking water due to natural and anthropogenic activities has been recognized as one of the major problems worldwide imposing a serious threat to human health. Excessive exposure to fluoride appears to be serious and causes metabolic, functional and structural damages in many organs especially in the heart. Calcium is a chelating agent for fluoride and can reduce its toxicity. This study was **conducted** to investigate some of the toxic effects of sodium fluoride (NaF) on albino rats heart and the role of Ca administration on these toxic changes. Thirty adults' male albino rats were used in this study. The animals were divided into five groups (6 animals each); **Group I (-ve control),** given standard diet and tape water, **Group II:** (+ve control) received 0.1 mL distilled water/ day by oral gavage. **Group III:** received calcium chloride at a dose 20 mg/kg b.wt./day. **Group IV:** received sodium fluoride treatment (NaF) at a dose 20 mg/kg b.wt./day. **Group V:** animals given sodium fluoride (NaF) at a dose 20 mg/kg/day and calcium chloride given 4 hours after NaF treatment at a dose of 20 mg/kg/day. The duration of the study was 28 days. The rats then were anesthetized and blood was collected for estimation of troponin T, Lactate dehydrogenase (LDH) and creatine phosphokinase (CPK). The hearts were saved for light and electron microscopic examinations. **The results** revealed that NaF induced cardio toxicity which reflected in significant increase in troponin T, LDH and CPK. Light microscopic examination of the heart of NaF treated rats showed marked distortion of the myocardial structure including rupture of the muscle fibers, widening of the intercellular space and massive inter-myofibrillar hemorrhage, appearance of cytoplasmic vacuoles, peripheral displacement of its nuclei. There was also dilatation and congestion of the blood capillaries and lymphocytic infiltration in the inter-myofibillar spaces. Electron microscopic examination revealed, irregularities of the nuclear membrane with increase of the heterochromatic patches. The cytoplasm showed swelling of the mitochondria, rupture of the cell membrane of some cardiomyocytes and extravasations of cell organelles in the intercellular space. There was also thinning of the myofibrils that appeared attenuated with marked discontinuity of the intercalated discs. The cytoplasm also contained many vacuoles. In rats treated with NaF and Ca significant decrease in troponin T, LDH and CPK were observed compared to those treated with NaF only. The myofibrils retained its arrangement with few fibers are still thinned with rounded and flat nuclei and areas of cellular distortion. There were also areas of lymphocytic infiltration. The nuclei showed enfolding of its nuclear membrane, thick patches of heterochromatin and numerous nucleoli. It was concluded that NaF is a cardio-toxic agent and these toxicities could be minimized by concomitant use of Ca. It was recommended that exposure to F should be regulated and Ca supplement is better to be received to minimize its side effects.

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1. Introduction:

Fluoride is an essential oligo-element, beneficial for growth, reduction of bone fractures and promoting the development of bones and teeth. Moderate fluoride (0.5–1.5 mg/L) in drinking water is beneficial. However, excessive intake of fluoride is toxic **(Viswanathan *et al.*, 2010 & Cai *et al.*, 2012)**. Fluoride has two sources, it can appear "naturally" in water or it can appear as a toxic waste. Fluoride disperses to the environment through water, food, drugs, cosmetics and several engineering process such as semiconductor manufacturing, coal power plants, glass and ceramic production, uranium refinement, electroplating, rubber and fertilizer manufacturing. The earliest problems associated with toxic wastes involved those manufacturing aluminum with the resulting wastes usually being sodium fluoride **(Islam & Patel, 2011**). Discharges of fluorinated municipal waters can also cause significant increases (about five times the natural background level) in the fluoride concentration of recipient rivers. Therefore, fluoride must be considered as a serious pollutant since its concentration in many aquatic ecosystems is significantly increasing as a consequence of man's activities **(Gonzalo & Camargo, 2012)**. Fluoride is found in many common household products, including toothpaste, vitamins, dietary supplements (e.g. sodium fluoride), glass-etching or chrome-cleaning agents, insecticides and rodenticides **(Islam & Patel, 2011**).

The systemic fluoride absorption from water through the gastrointestinal tract into bloodstream is nearly 100% by the process of simple diffusion **(Viswanathan *et al.*, 2010).** After ingestion peak plasma fluoride concentrations are reached within 30–

60 min, and the levels return to normal within 3–6 hrs. The decline in the concentration is mainly due to calcified tissue uptake and renal excretion **(Sato *et al.*, 2011).**

Excess intake of fluoride leads to various diseases such as osteoporosis, arthritis, cancer, infertility, brain damage, Alzheimer syndrome, and thyroid disorder. Fluorosis is a common symptom of high fluoride ingestion manifested by mottling of teeth in mild cases and embrittlement of bones and neurological damage in severe cases. Fluoride may interfere with DNA synthesis. carbohydrates, lipids, proteins, vitamins and mineral metabolism **(Bhatnagar *et al.*, 2011).** Estimations of human lethal fluoride doses showed a wide range of values, from 16 to 64 mg/kg in adults and 3 to 16 mg/kg in children **(Barbier *et al.*, 2010)**. Heart and its constituting cardiomyocytes are the target for fluorosis **(Cieek *et al.*, 2005)**. It's accumulation in heart causes injury by reducing the potential of removal of free radicals which critically injure the biological cell membranes. Highly free radicals production or disturbed antioxidant level leads to oxidative stress which is known to be a key factor in a variety of many cardiac diseases such as heart failure and ischemic heart disease **(Mahaboob and Suhithe, 2011)**.

There has been an extensive research in the field of finding suitable preventive and therapeutic measures against fluoride toxicity. The available information reveals no satisfactory approach that prevents the toxic effects of fluoride **(Chouhan *et al.*, 2012**).

Hansen *et al.*(2011) have stated that fluoride has a strong affinity for calcium and forms calcium fluoride with a solubility product as low as 3.9×10^{-11}. Moreover **Das *et al.*(2006)** reported that calcium is a chelating agent for fluoride that results insoluble calcium-fluoride generation and thereby reduces fluoride toxicity

This study was conducted to investigate some of the toxic effects of sodium fluoride (NaF) on the rat heart and the role of Ca co administration on these toxic changes. This was done through estimation of troponin T, Lactate dehydrogenase (LDH) and creatine phosphokinase (CPK). The hearts were examined by light and electron microscopes.

2.Material and Methods

Chemicals:

Sodium fluoride was obtained from Sigma-Aldrich Chemical Company, (St. Louis, MO), USA and Calcium chloride was obtained from Egyptian International Pharmaceuticals Industries (E.P.I.C.O.).

Animals:

Thirty adults' male albino rats were used in this study. Male rats were chosen because females might have possible cardiac protection by estrogen (**Rodriguez-Hernandez *et al.*, 2008),** along with increased levels of telomerase activity, which could increase tissue regeneration capacity in females **(Iing *et al.*, 2006**).

The animals were obtained from the animal house, Faculty of Medicine, Zagazig University. The rats weighting between 150-190 gm. The animals were housed in stainless steel cages and maintained under standard laboratory conditions. They were left for two weeks to acclimatize to laboratory conditions with free access to food and water.

Experimental design:

These animals were randomly divided intro five groups (6 animals each) as follow:

Group I (-ve control), given standard diet and tape water, to investigate the basic parameters.

Group II: (+ve control) received 0.1 mL distilled water/ day by oral gavage for 28 days.

Group III: received calcium chloride at a dose of 20 mg/kg/day dissolved in 0.1 mL distilled water for 28 days **Das *et al.*, 2006).**

Group IV: received NaF at a dose 20 mg/kg/day dissolved in 0.1 mL distilled water for 28 days **(Das *et al.*, 2006).**

Group V: received NaF at a dose 20 mg/kg/day dissolved in 0.1 mL distilled water and calcium chloride given 4 hours after NaF treatment **(Das *et al.*, 2006)** at a dose of 20 mg/kg/day dissolved in 0.1 mL distilled water for 28 days.

One day after the last treatment, the rats then were anesthetized and blood was collected for estimation of troponin T, LDH and CPK. The hearts were saved for light and electron microscopic examinations.

Estimation of serum Troponin T:

Troponin T level was measured by an electrochemiluminescence immunoassay on the Elecsys 2010 (Roche Diagnostics, Mannheim, Germany). The recommended lower limit of 0.03 ng/ml was used to define positive troponin T levels **(Baum *et al.*, 1997)**

Estimation of LDH and CPK:

Lactate dehydrogenase and creatine phosphokinase levels were measured by ultraviolet photometric assay (Roche Diagnostic Ltd., lewes, United Kingdom) **(Woo *et al.*, 1992 & Calbreath, 1992).**

Light microscopic examination:

Small pieces of heart were fixed separately in 10% buffered neutral formalin for 24 hrs. Tissues were processed and embedded in paraffin blocks. Paraffin sections were cut with 5µ thickness. Stained by routine hematoxylin and eosin stain (**Bancroft and Steven, 1996).** The ready sections examined under a Zeiss light microscope.

Electron microscopic examination:

The specimens were fixed in 2.5-4% gluteraldehyde in 0.1 M cocodylate buffer (pH 7.4). Then the specimens were kept in gluteraldehyde for 24-48 hours at 4°C. The specimens were cut into small pieces

and washed with distilled water and postfixed in 1% osmium tetraoxide with 15 mg/ml of potassium ferrocyanide for 1-2 hours at 4°C. Then the specimens were cut using an utlramicrotome (JEOL-JUM-7) and stained with uranyl acetate and lead (**Hayat, 1989**). The specimens were finally examined and photographed using a JEOL, JEM 1010 electron microscope (Jeol Ltd, Tokyo, Japan) in the Electron Microscopy Research Laboratory of Histology and Cell Biology Department, Faculty of Medicine, Zagazig University.

Statistical analysis

Results were expressed as the mean ± SD. Comparison between different groups was carried out by one way analysis of variance test (ANOVA). Percentage values were analyzed by chi square test. The level of significance was set at $p<0.05$.

3.Results

Results of the biochemical studies:

Groups I, II & III (control groups):

Regarding groups I, II & III no significant difference was found among them in the mean values of the levels of troponin T, LDH and CPK (Table 1).

Group IV (NaF group):

In rats treated with NaF, the results of biochemical changes revealed that NaF induced significant increases in the mean values of serum troponin T (5.27 ± 1.9 ng/ ml), LDH (847± 56 µ mol/L) and of serum CPK (602.4± 43 U/L) levels as compared with those of the control group (0.32± 0.18 ng /ml), (235± 29.6 µ mol/L) & (183.9±14 U/L)for troponin T, LDH & CPK respectively with $p<0.05$ (Table 1).

Group V (Combined NaF and calcium chloride):

In rats treated with calcium chloride 4 hours after NaF treatment, the results of biochemical changes showed a significant decrease in the levels of troponin T (0.86± 0.1 ng/ml), LDH (302.2± 32.5 µ mol/L) and CPK (240.3 ± 31 U/L) as compared with the NaF-treated rats ($p<0.05$), and a non- significant change compared to the control group with $P>0.05$ (Table 1).

Results of light and electron microscopic examination:

Groups I, II & III (control groups):

Light microscopic examination of myocardium of the control adult male albino rats (groups I, II & III) showed that the cardiac muscle cells (cardiomyocytes) had a syncytial arrangement of fibers with central oval vesicular nuclei. The muscle fibers are separated by narrow inter-muscular spaces (Fig. 1).

Electron microscopic examination of myocardium of the control groups showed that the cardiac muscle cells had long parallel arrays of myofibrils and oval euchromatic nuclei with prominent nucleoli. The mitochondria are present in rows between myofibrils. The myofibrils were closely adherent to each other, however few and small inter-myofibrillar spaces are found in some areas (Fig. 2). The myofibrils revealed alternating dark and light bands and regular Z lines. the cardiomyocytes contain elongated nuclei with the long axis of the cell. The nuclei contain euchromatin with scattered patches of heterochromatin close to the cell membrane (Figs. 2, 3). The cell membranes at the ends of the cardiomyocytes showed many areas of gap junctions ; intercalated discs (Figs. 4).

Group IV (NaF group):

Light microscopic examination of the cardiomyocytes of the NaF-treated rats showed marked distortion of its structure. The cellular changes included rupture of the muscle fibers, widening of the intercellular space and massive inter-myofibrillar hemorrhage (Fig. 5). The cytoplasm of the cells showed appearance of numerous cytoplasmic vacuoles and marked displacement of its nuclei toward the periphery of the cell. There was also marked dilatation and congestion of the blood capillaries and marked lymphocytic infiltration in the inter-myofibillar spaces (Fig.6). All these histopathological changes showed significant increase compared to the control groups with $p<0.05$ (Table 2).

Electron microscopic examination of the cardiomyocytes showed marked irregularities of its nuclear membrane with increase of the heterochromatic patches. The cytoplasm showed marked swelling of the mitochondria that are arranged parallel with the long axis of the cell (Fig. 7). There is also rupture of the cell membrane of some cardiomyocytes with extravasations of the cell organelles in the intercellular space that contain also bundles of collagen fibers (Fig. 8). The cardiomyocytes showed marked thinning of the myofibrils that appeared attenuated and separated from each other. Large rounded, swollen mitochondria with marked discontinuity of the intercalated discs was also seen (fig. 9, 11). The cytoplasm of the cells contained large vacuoles especially in close relation to the intervening blood capillaries and fibroblasts in the inter-myofibrillar spaces (Fig. 10).

Group V (Combined NaF and calcium chloride):

Light microscopic examination of the myocardium showed that the myofibrils retained their syncytial arrangement with few small intercellular spaces among them. Few muscle fibers are still showed thinning of their myofibrils with rounded and flat nuclei. There is also few areas of lymphocytic infiltration (figs.12 & 13). Statistical analysis of these lesions showed non significant changes compared to the control groups with $p>0.05$ (Table 2).

Electron microscopic examination showed that the myocardial cells retained their normal architecture; however some of them showed areas of cellular distortion. The muscle bundles are separated by small inter-myofibrillar space. The cytoplasm contained large rounded mitochondria and nuclei. The

nuclei of some cells showed enfolding of its nuclear membrane with thick patches of heterochromatin and numerous nucleoli however, other cells showed normally formed nucleus and smooth nuclear membrane with diffuse euchromatin (Figs. 14, 15).

Table (1): Statistical analysis of the levels of serum Troponin T, LDH and CPK to investigate the possible protective role of (calcium chloride 20mg/kg/day) orally for 28 days against cardiotoxicity of NaF 20mg/kg/day orally in adult male albino rats by ANOVA test.

Enzymes / Groups	TroponinT (ng/ml)	LDH (µ mol/L)	CPK(unit/L)
	Mean ± SD	Mean ± SD	Mean ± SD
Group I	0.32 ± 0.18	235 ± 29.6	183.9 ± 14
Group II	0.47 ± 0.4	299.3 ± 23.2	201.6 ± 20.5
Group III	0.55 ± 0.05	220.1 ± 21	247.9 ± 19.6
Group IV	5.27 ± 1.9 [a]	847 ± 56 [a]	602.4 ± 43 [a]
Group V	0.86 ± 0.1	302.2 ±32.5	240.3 ± 31

Number of animals in each group = 6.
[a] Significantly different from control (group I) (p<0.05).

Table (2): Statistical comparison of histopathological changes of the heart to investigate the possible protective role of (calcium chloride 20mg/kg/day) orally for 28 days against cardiotoxicity of NaF 20mg/kg/day orally for 28 days in adult male albino rats by by Chi square test.

Group / Pathology	Group I		Group II		Group III		Group IV		Group V	
	No	%	No	%	No	%	No	%	No	%
Wide intercellular space	1	16.7	0.0	0.0	1	16.7	6	100[a]	1	16.7
Interstitial hemorrhage	0	0.0	0.0	0.0	0	0.0	5	83.5[a]	1	16.7
Cytoplasmic vacuolization	0.0	0.0	0.0	0.0	0.0	0.0	5	83.5[a]	0.0	0.0
Vascular dilatation & congestion	1	16.7	1	16.7	1	16.7	5	83.5[a]	1	16.7
Inflammatory cellular infiltration	0.0	0.0	0.0	0.0	0.0	0.0	3	50 [a]	1	16.7
Rupture of muscle fibers	0.0	0.0	0.0	0.0	1	16.7	4	66.8 [a]	1	16.7

N.B: number of rats in each group=6.
[a] Significantly different from control (group I) (p<0.05).

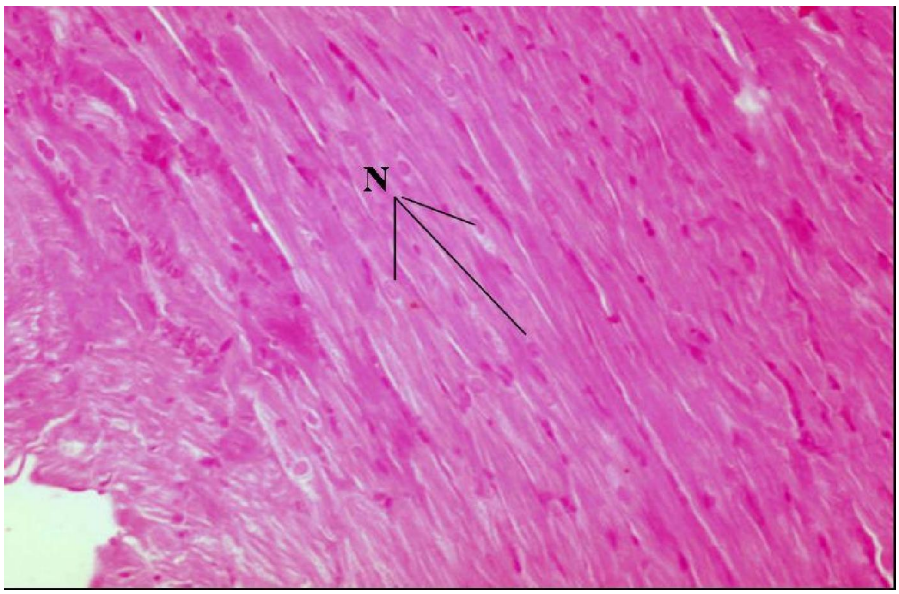

Fig. (1): A photomicrograph of rat heart from group I (control group) showing the normal syncytial arrangement of muscle fibers with central oval vesicular nuclei (N). The muscle fibers are separated by very narrow intermuscular spaces (H&E .X 400)

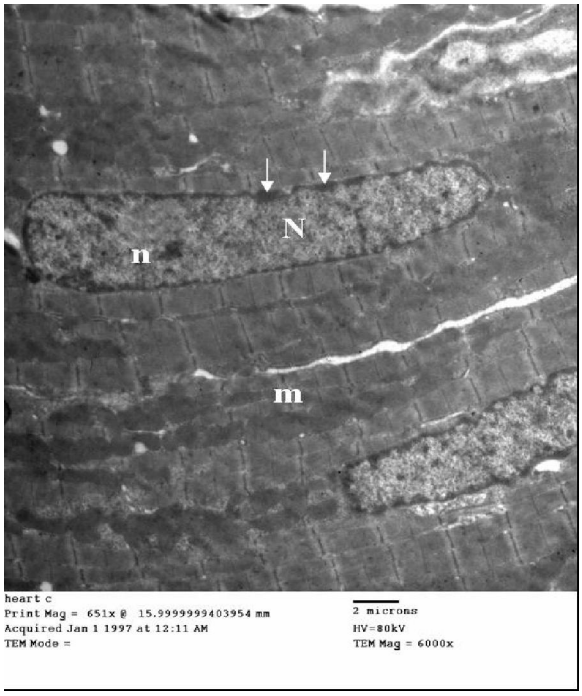

Fig. (2): An electromicrograph of rat heart from group I (control group) showing cardiac muscle cells had long parallel arrays of myofibrils with an oval euchromatic nucleus (N) with patches of heterochromatin close to cell membrane (arrow) and prominent neucleolus (n). Rows of mitochondria (m) between myofibrils are seen (X 6000).

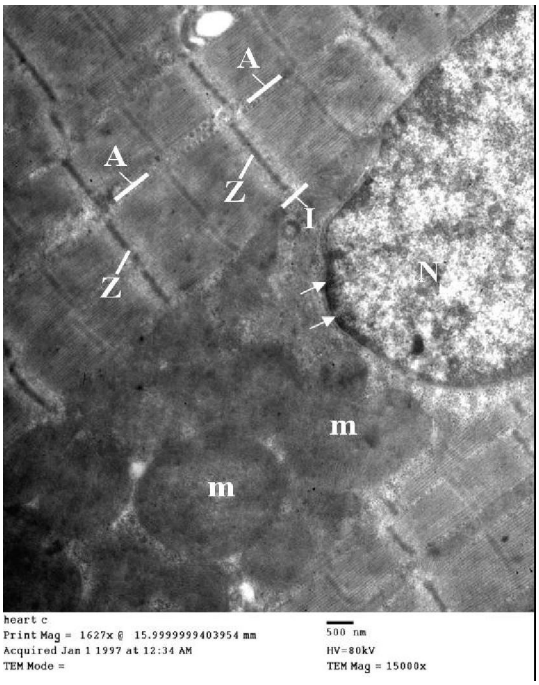

Fig. (3): An electromicrograph of rat heart from group I (control group) showing alternating dark (A) and light (I) bands with regular Z lines in the middle of (I) bands. The nucleus (N), patches of heterochromatin (arrow) and mitochondria (m) are seen.(X 15000)

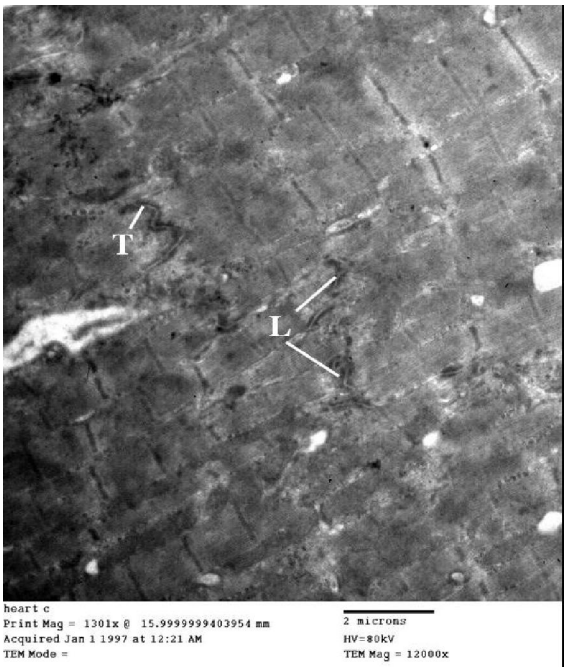

Fig. (4): An electromicrograph of rat heart from group I (control group)showing intercalated discs that formed of transverse portion (T) and longitudinal portion (L). (X 12000)

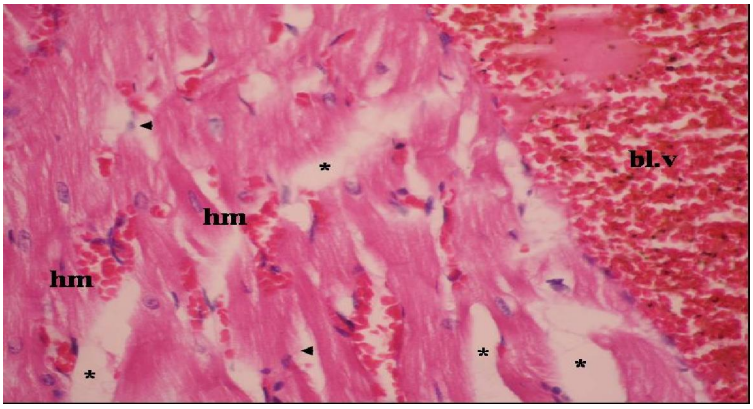

Fig. (5): A photomicrograph of rat heart from group treated with Naf 20mg/kg/day for 28 days (group IV) showing rupture of myocytes (star), widening of intercellular space (arrow head) and hemorrhage (hm). Notice the congested blood vessels (bl.v). (X 400)

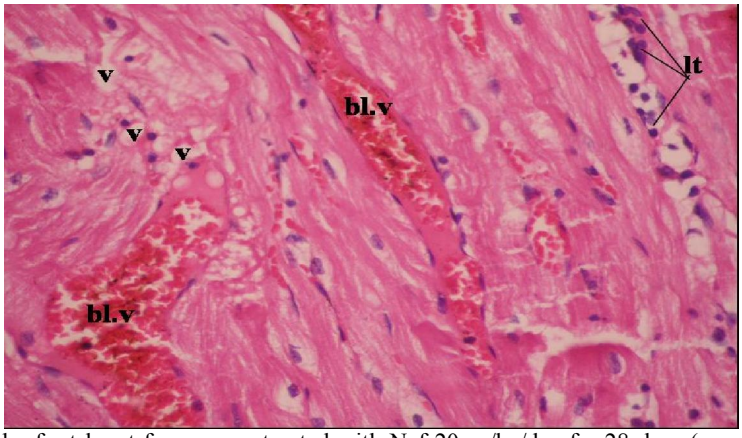

Fig. (6): A photomicrograph of rat heart from group treated with Naf 20mg/kg/day for 28 days (group IV) showing presence of numerous cytoplamsic vacuoles (v), dilated congested blood vessels (bl.v) and lymphocytic infiltration (lt). (X 400)

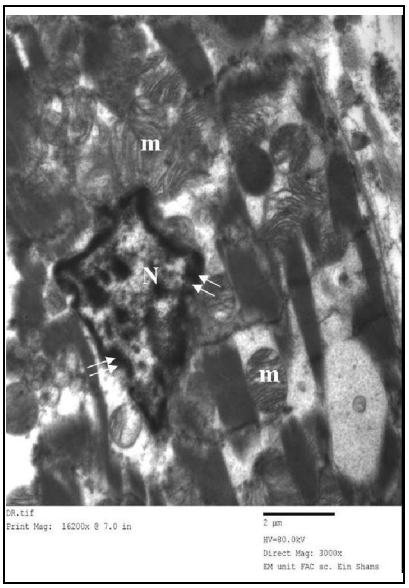

Fig. (7): An electromicrograph of rat heart from group treated with Naf 20mg/kg/day for 28 days (group IV) showing nucleus (N) with marked infoldings of nuclear membrane and increase of heterochromatin patches (double arrow). The mitochondria (m) appeared swollen within the cytoplasm. (X 30000)

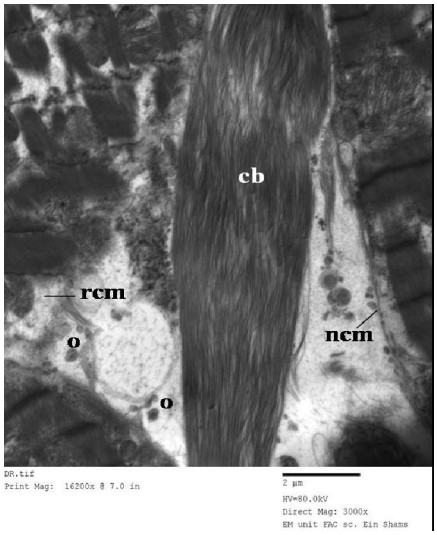

Fig. (8): An electromicrograph of group II showing some of cells with normal cell membrane (ncm) and the other showing rupture of cell membrane (rcm) with extravasation of cell organelles in the intercellular space (o). Notice the collagen bundle (cb) in the intercellular space. (X 3000)

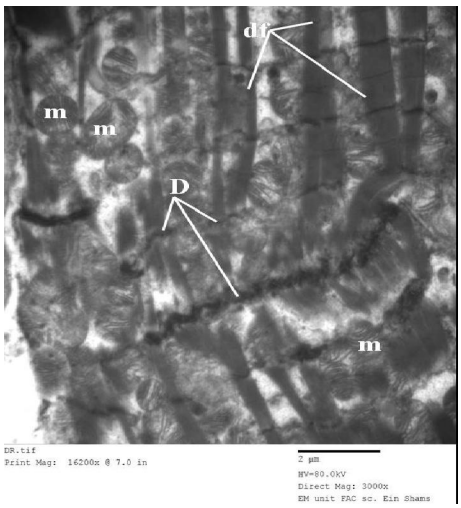

Fig. (9): An electromicrograph of rat heart from group treated with Naf 20mg/kg/day for 28 days (group IV) showing thin, attenuated myofibrils (df) with distorted intercalated discs (D). Notice the change in size and disarrangement of mitochondria (m). (X 3000)

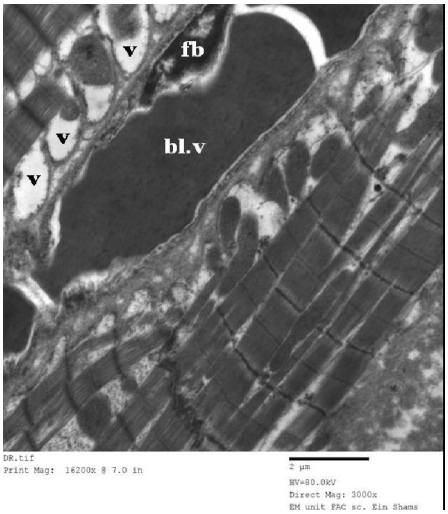

Fig. (10): An electromicrograph of rat heart from group treated with Naf 20mg/kg/day for 28 days (group IV) showing cytoplasmic vacuoles (v). Notice the dilated blood vessels (bl.v) and fibroblast (fb). (X 3000)

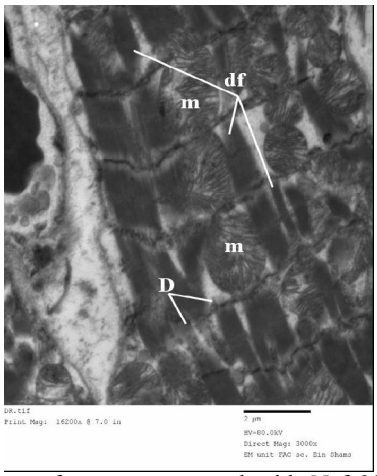

Fig. (11): An electromicrograph of rat heart from group treated with Naf 20mg/kg/day for 28 days (group IV) showing thin, attenuated myofibrils (df) with distorted intercalated discs (D). Notice round swollen mitochondria (m). (X 3000)

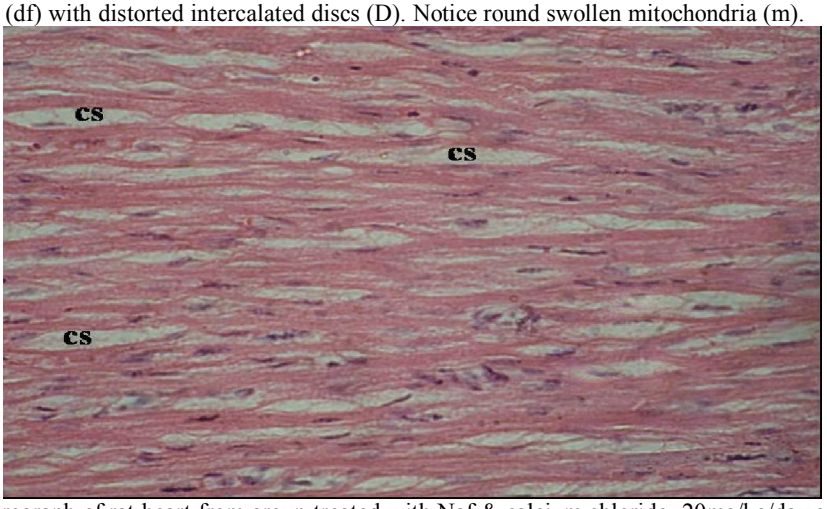

Fig. (12): A photomicrograph of rat heart from group treated with Naf & calcium chloride 20mg/kg/day each for 28 days (group V) showing syncytial arrangement of cardiac muscle fibers with few small intercellular spaces (cs). (X 400)

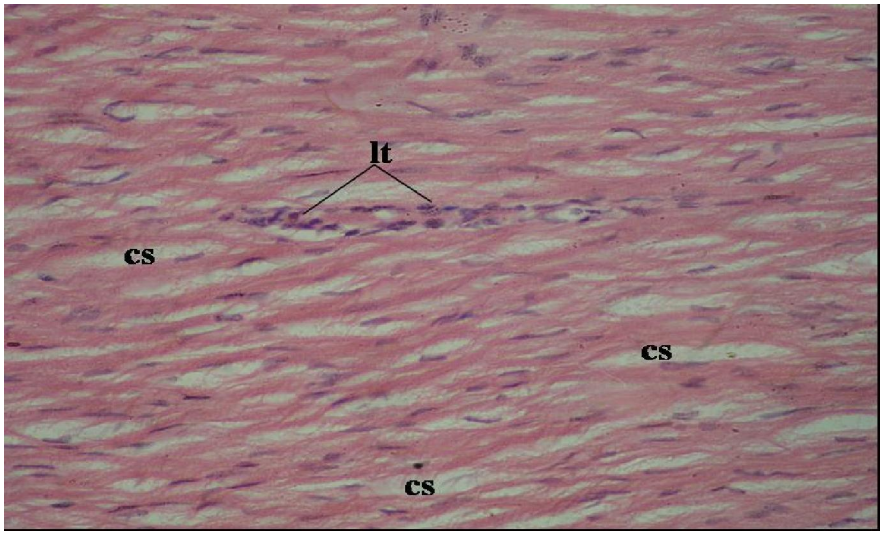

Fig. (13): A photomicrograph of rat heart from group treated with Naf & calcium chloride 20mg/kg/day each for 28 days (group V) showing syncytial arrangement of cardiac muscle fibers with few small intercellular spaces (cs) and lymphocytic infiltration (lt).(X 400)

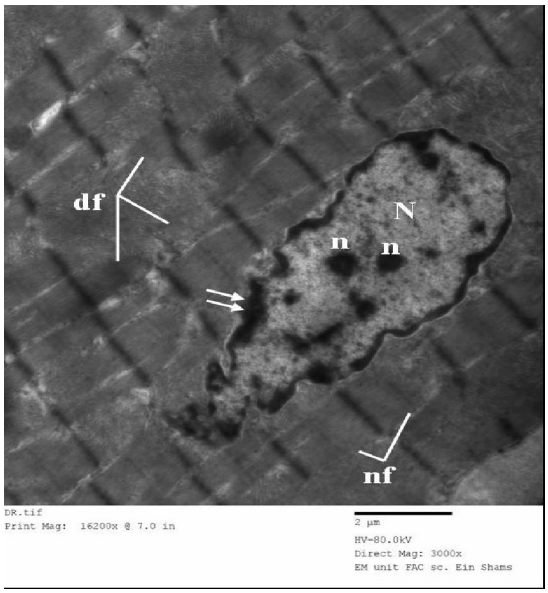

Fig. (14): An electromicrograph of rat heart from group treated with Naf & calcium chloride 20mg/kg/day each for 28 days (group V) showing most of fibralis retained its normal architecture (nf). The others all still distorted (df). The nucleus (N) showing infolding of its nuclear membrane with thick patches of heterochromatin (double arrow) and numerous nuclei (n). (X 3000)

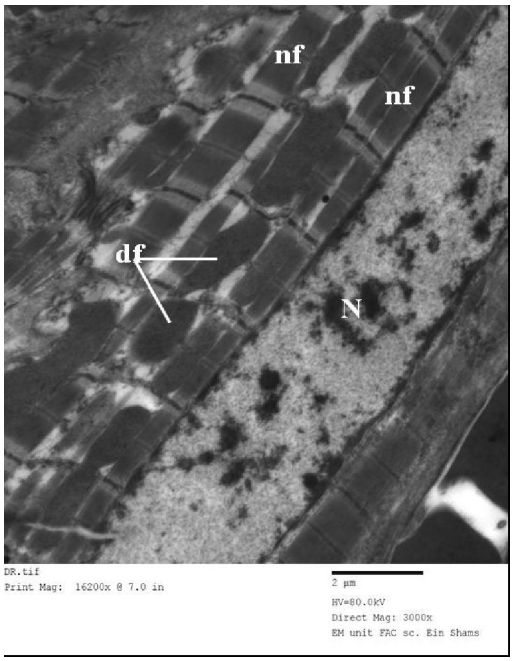

Fig. (15): An electromicrograph of rat heart from group treated with Naf & calcium chloride 20mg/kg/day each for 28 days (group V) showing normal myofibralis (nf) and distorted other (df). The nucleus (N) showing smooth nuclear membrane with diffuse euchromatin. (X 3000) cytoplasmic swelling (S) (X 4000).

4. Discussion

Through the twentieth century, fluoride attracted the interest of toxicologists due to its deleterious effects at high concentrations in human populations and experimental models. **(Barbier *et al.*, 2010)**

The main objective of this study was to investigate some of the cardiotoxic effects of NaF and the possible protective role of calcium against it in adult male albino rats.

In the current study, the serum levels of troponin T, LDH & CPK enzymes were significantly elevated in the NaF treated group as compared to the control groups. The results of the present study are in agree with **Kaczor *et al.*(2005)** and **Hassan & Yousef (2009)** who found that the activities of lactate dehydrogenase (LDH) and creatine kinase (CK) were significantly increased in rats treated with sodium fluoride. They stated that this may be attributed to a generalized increase in membrane permeability and is particularly useful in the diagnosis of muscular dystrophy.

Akhlaghi & Bandy (2010) reported that the release of LDH to the medium is a commonly used indicator of myocardial necrosis in the isolated heart model and has been used clinically as an indicator of myocardial infarction. **Guan *et al.*(2012**) stated that cardiomyocyte damage can be confirmed by assessing LDH and CPK, which are two cardiac-specific enzymes that reflect the degree of myocardial damage.

In the study of **Jiang *et al.*(2011)** they evaluated the correlation between early plasma troponin T levels with later development of congestive heart failure and pulmonary hypertension in rats. They found early plasma troponin T concentrations correlated with left ventricular systolic dysfunction and with histological infarct size. In addition **Kusumoto *et al.*(2012)** mentioned that Circulating cardiac troponin T is a very sensitive and specific biomarker of cardiomyocyte injury, and is used as the diagnostic and prognostic marker of acute coronary syndromes.

In the present study light microscopic examination of myocardium from NaF treated rats showed that myocytes were separated from each other by wide intracellular spaces. Some of myocardial fibers attained vacuolated sarcoplasm with localized area of hemorrhage and congested blood capillaries with extravasated red blood cells. These finding observed also by **Mahaboob and Sujitha, 2011** who reported that NaF causes histological changes in the heart like oedema, plasmic vacuolization small, hemorrhage and fibrous necrosis. Also **Cicek *et al.*(2005)** found Significant histopathological changes in the myocardial tissue of rats treated with 50 and 100 mg/L NaF. These were myocardial cell necrosis, extensive cytoplasmic vacuole formation, nucleus dissolution in myosits, swollen and clumped myocardial fibers, fibrillolysis, interstitial oedema, small hemorrhagic areas and hyperaemic vessels. The result of the current study are in agreement also with **Stawiarska-Pięta *et al.*(2012)** who reported that long term exposure to fluorine compounds induces morphological changes in many organs, leading to an impairment of their function. They reported the occurrence of pathological changes in the heart.

Other studies also suggested that the acute and chronic fluoride exposure could cause myocardial damage in experimental animals, like rats, sheep and rabbits. **(Ghosh *et al.*, 2008)**. **Varol *et al.*(2010a)** assumed that myocardial cell damage and cell death could be induced by the generation of reactive oxygen species in chronic fluorosis.

When reactive oxygen system is out of balance in chronic fluorosis, the free radicals will increase relatively, leading to degradation of cytomembranes, which cause descending fluidity and elevated permeability of the cell membrane, and even collapse of membrane structure **(Stawiarska-Pięta *et al.*, 2009)**. The modifications of membrane lipid contents and fatty acid composition, caused by free radical, have also been observed in brain and liver affected by long-term fluoride exposure. It suggests that the membrane lipid changes occur in multiple organs as a general result of fluorosis **(Feng et al., *et al.*, 2012).**

By electron microscopic examination, the result of the current study showed that the cell membrane of some myocytes show loss of its continuity with leakage of intracellular organells into the interstial space bundle of collagen fibers, numerous congested blood vessels, inflammatory cell and degenerated muscle fibers in myocardium of NaF treated group is noted.

Similar changes had been detected in chronic myocardial infarction **(Qu *et al.*, 2012)**. Many investigations have previously demonstrated that fluoride is a cytotoxic agent inducing apoptosis, and disrupting cell cycle progression in many types of cells. It is well known that collagen is a target of excessive F exposure **(Yan *et al.*, 2011)**.

The pathophysiology of cardiac dysfunction in heart disease is a complex process that involves the interplay and alterations of various extracellular and intracellular proteins and molecules. One of the key families of enzymes involved is the proteases which specialize in cleaving protein peptide bonds. Under pathological conditions these regulatory and control mechanisms are altered leading to marked increases in protease activities **(Willis *et al.*, 2009)**

Mendoza-Schulz *et al.*(2009) mentioned that among the systemic toxic manifestations in humans, the main toxic effect of fluoride on cellular metabolism is its interaction with enzymes. In most cases, fluoride inhibits enzymatic activity, but in other cases, fluoride ions actually stimulate enzymatic activity. The

mechanism depends on the type of enzyme that is affected.

Sodium fluoride exposure has been linked to increase in the risk of coronary artery disease as in a recent study from their clinc **Varol *et al.*(2010b)** have found that elastic properties of ascending aorta are impaired in patients with endemic fluorosis. They have thought that fluorosis had an effect on all cardiovascular system including heart and major vessels arising from the heart. Hence structural changes observed in myocardium of NaF treated group may be due to occurrence of minute segmental infarction.

In present work, electron microscopic examination revealed some mitochondrial changes which were seen as disarrangement and variation in size and shape. This finding was in accordance with **Seyed *et al.*, 2010** who reported that NaF intoxication significantly altered all indices related to the pro-oxidant-antioxidant states of heart. Also they found that superoxide dismutase, catalase and reduced glutathion contents decreased within myocardium and all these antioxidant agents located mainly in mitochondria which help to protect the biological tissue by avoiding the accumulation of hydrogen peroxide. Moreover, **Ingrida *et al.*(2009)** stated that increase the concentration of NaF resulted in reproducible decrease in oxidation rate of rat cardiac mitochondria.

In the present study there was disorganization of the intercalated discs in the myocardium treated with NaF. **Hu *et al.* (2005)** stated that deformed intercalated discs lead to loss of effective contraction force in most of the affected area. Moreover in previous studies, prolonged P–Q interval, sinus bradycardia and increased duration and amplitude of T wave have been reported in electrocardiogram (ECG) of animals with chronic fluorosis (**Kilicalp *et al.*, 2004 and Kant *et al.*, 2009**). In humans, sinus irregularity, sinus bradycardia, low voltage, ST and T wave changes and increased QT and QTc intervals were reported in patients with endemic fluorosis living in endemic fluorosis area **(Varole *et al.*, 2010a)**

All the above mentioned biochemical & histological changes support the hypothesis that fluoride affects the heart muscle fibers.

Since the heart has low cell proliferation capacity, attempts directed to prevent death of myocardial cells are of therapeutic value **(Akhlaghi & Bandy, 2010).** Therefore, the present study aimed to avoid cardiac damage resulting from fluoride exposure through calcium chloride administration.

The results of the present study reveled that calcium chloride ameliorated the cardiotoxic effect of NaF where significant decrease in troponin T, LDH and CPK were observed compared to those treated with NaF only. As regard light and electron microscopic examination of group V, there were almost a similar light and ultrastructural profile of cardiomyocytes to the control group with few number of cardiac myocytes exhibited some changes in the form of mild widening of intercellular spaces and leucocytic infiltration. Few number of cardiac myocytes exhibited distorted myofibrils with evident cross striations and multiple sacroplasmic vacuoles.

The results of the current study are in agree with Previous studies where supplementation of dietary calcium has been shown to reduce the bioavailability of fluoride in rats and rabbits, reduced clinical sign of fluoride poisoning and reduced fluoride accumulation **(Hansen *et al.*, 2011)** Also **Emara *et al.*(2010)** concluded that administration of Ca during withdrawal from NaF showed significant improvement of the chronic fluoride toxicity on male reproductive organs. **Das (2006)** stated that after calcium co-administration, partial recovery was noted in fluoride-treated rats that may be due to interference of oxidative stress imposed by fluoride. Moreover, **Sunil *et al.* (2011)** showed significant improvement in skeletal fluorosis children in the area consuming water containing 4.5 pm of fluoride after ingestion of calcium. Also, **Bhardwaj & Shashi (2012)** stated that as fluoride binds with many calcium cations and causes hypocalcaemia. It is well known that poor nutrition and low calcium intake enhance the deleterious effect of fluoride . The resultant hypocalcemia is responsible for various manifestation of fluorosis.

Conclusion and Recommendation:

From the data of the present study **it is concluded** that short term administration of fluoride to albino rats, induced cardiotoxicity. Furthermore, this effect was ameliorated by the use of calcium. **So**, **it is recommended** to use dietary calcium supplements for amelioration of toxic manifestations in fluoride exposed population. Also, presence of fluoride in the environment and drinking water should be monitored regularly.

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