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Changes in Electrophoretic Protein Profiles of Skeletal Muscle of Rats Exposed to Fluoride

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Abstract:

Aim: *In this study, we aimed to determine the changes of electrophoretic protein profiles of skeletal muscle of rat during 40 days fluoride toxicity. Changes in muscle proteins of control and test rats have been examined using sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Densitometric Analysis.*

Study design: *Wistar albino rats were maintained under controlled conditions of 12 h light-dark cycle, room temperature of 20-25 ^{0} C and relative humidity of 40-50 %. The animals were divided into three groups (Group I, II and III) of six animals each. The first group served control group and was administered 1 ml double distilled water /kg bw/day. Rats of the second group were intoxicated with 300 mg/kg NaF and the third group received 600 mg/kg NaF for 40 days by oral gavage.*

Methodology: *At the end of the experimental period, rats of all groups were sacrificed under ether anaesthesia. Skeletal muscle tissue were excised and weighed. Aliquots were used to determine the total protein content. The relationship of fluoride with proteins was assessed by correlation and linear regression analysis. To elucidate the possible mechanism for skeletal muscle damage due to fluoride, the skeletal muscle homogenates were subjected to SDS-PAGE and Densitometric analysis.*

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Results: *The level of muscle total protein declined significantly (F=7.457, P<0.01) in fluoride treated groups as compared to control. Pearson's bivariate correlation analysis revealed a significant (r=-0.671, P<0.01) negative relationship between level of fluoride and total proteins in skeletal muscle of test rats after 40 days of fluoride treatment. Further regression analysis (Y=23.2 -18.23 X, R^2= 0.45) showed that as the level of fluoride increases in skeletal muscle of rats, protein content also declined. SDS-PAGE and densitometric analysis revealed that intensity and the number of some protein bands were reduced while the bands of actin didn't change significantly during fluoride intoxication . Group III exhibited the greatest protein changes, as evidenced by the reduction in the number of protein bands to 4 as compared to Group I and II and disappearance of Myosin heavy chain (MHC) band with molecular weight of 200 kDa .*

Conclusion: *The results of polyacrylamide gel electrophoresis (SDS-PAGE) and densitometric analysis demonstrated that fluoride disturbed the banding pattern of skeletal muscle proteins.*

Key words: Contractile proteins; Densitometry; Fluorosis; Rats; SDS-PAGE electrophoresis; Skeletal muscle ; Sodium fluoride.

1. INRODUCTION

Environmental contamination due to fluoride and its subsequent deleterious health effects, in overt or subtle forms in human and animals, merits serious attention globally. Fluorosis is a major health problem in both man and animals all over the world [1, 2, 3].

Skeletal muscle accounts for 40% of mammalian body weight and atleast 25% of whole body protein turnover occurs in this tissue. Discordant changes in the muscle protein synthesis and/or protein degradation lead to either protein loss or accumulation [4].

Skeletal muscle plays a major role in whole body protein metabolism, and the changes in the rates of synthesis and degradation of proteins are likely to lead to characteristic

changes in the amounts of different proteins in muscle under various physiological and pathological conditions.

Myofibrillar proteins, present in muscle fibres of skeletal muscles are potential indicators of energy metabolism in living muscle, and can influence the quality skeletal muscle tissue [5].The major myofibrillar proteins are myosin, actin, tropomyosin, and troponins [6].

Low molecular myofibrillar proteins (troponin) hydrolyzed by specific proteinases are of great interest because these proteins are hampered by the fragments produced from bigger molecule proteins such as Myosin heavy chain [7].

Numerous studies have shown that fluorosis may induce nucleotide damage, inhibition of protein synthesis and alters mitochondrial functions resulting in energy deficient state of cell [8, 9]. Toxic effects of any environmental pollutant depend on the dose and duration of its exposure as well as the susceptibility of the tissue to that particular toxicant [10, 11].

Despite the importance of skeletal muscle to whole body physiology, the mechanisms that govern protein accretion or loss in this tissue are not fully understood. It is, therefore, clear that understanding the mechanisms that contribute to tissue protein imbalance provides a bases of devising novel therapeutic strategies to reverse the pathological manifestations seen in the myopathic conditions. However, investigations have largely focused on single proteins such as actin and myosin or chemically defined groups of proteins such as the mixed contractile protein fraction of skeletal muscle. Such a limited approach imposes constraints on our understanding of myopathies since numerous proteins and pathways may be disturbed, as exemplified by the complex etiological mechanisms inherent in the pathogenesis of fluoride toxicity.

Contents of myofibrillar proteins in muscle tissue have been the subject of extensive research efforts aimed at understanding a number of biological phenomenon. The most

frequently used method is an SDS-polyacrylamide gel electrophoresis system which separates proteins according to their molecular size. Hence, the present study was designed to explore the use of SDS-PAGE and Densitometric analysis for separating skeletal muscle proteins and to elucidate the possible mechanism for skeletal muscle damage due to fluoride toxicity.

2. MATERIALS AND METHODS

2.1 Chemicals

Sodium fluoride was purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals used in the present study were of analytical grade. Kits used in the present study were the products of Erba Diagnostics (Manheim/ Germany).

2.2 Animals and Experimental design

This study was performed on thirty Wistar albino rats of 150–200 g body weight. Rats were housed in polypropylene cages. Rats were allowed free access to standard rat chow and water. The animals were maintained under controlled conditions of 12 h light-dark cycle, room temperature of 20- 25^{o} C, relative humidity of 40-50 %. After 2 weeks of acclimatization to the laboratory conditions, rats were randomly divided into 3 experimental groups (6 rats in each) as follows: The first group served control group and was administered 1 ml double distilled water /kg bw/day. Rats of the second group were intoxicated with 300 mg/kg NaF and the third group received 600 mg/kg NaF for 40 days by oral gavage. At the end of the experimental duration rats of all groups were sacrificed under ether anaesthesia. Skeletal muscle tissue were excised and weighed. Aliquots were used to determine the protein content.

2.3 Ethical Aspects

The conduct and procedures involving animal experiments were approved by the Instituitional Animal Ethics Committe, Punjabi University, Patiala, India.

2.4 Laboratory Analysis

2.4.1 Protein estimation

Protein concentration of skeletal muscles of control and fluoride treated rats was determined by the method of Lowry *et al.* [12]. Bovine serum albumin was used as the standard, and absorbance was recorded at 660 nm.

2.4.2 SDS-PAGE Electrophoresis

The electrophoresis was performed using slab gels of 100mm X 150mm X 1mm of 10% polyacrylamide containing 1% SDS and 10% glycerol by the method of Laemmli [13]. Experiments were run at 20mA/gel until the tracking dye entered the separating gel, and next the current was raised to 30mA/gel, maintained throughout the electrophoresis. To visualize protein bands , the gel was stained with Coomassie brilliant blue.

2.4.3 Molecular weight estimation and Densitometric analysis

Gels were scanned and the bands were analysed using Image J software, to provide values under each peak. The proteins in the bands were identified by estimating their molecular weights (MW) using MW standard markers, and compairing these values to the MW of proteins which have been described in the literature [14].

2.5 Statistical Analysis

The protein quantities of control and fluoride treated skeletal muscle tissue were subjected to variance analysis (One-way ANOVA) using SPSS-20 windows software, and the mean

values of important variance sources were compared with Post-Hoc Tukey's HSD multiple comparision test at P=0.01 significance level.

3. RESULTS

3.1 Total Proteins

The mean level of total proteins in skeletal muscle of test rats showed a significant (F=7.457, P<0.01) decline after 40 days of fluoride toxicity (Fig.1.). Most prominent decrease was registered in 600 ppm NaF group (-52.37%).

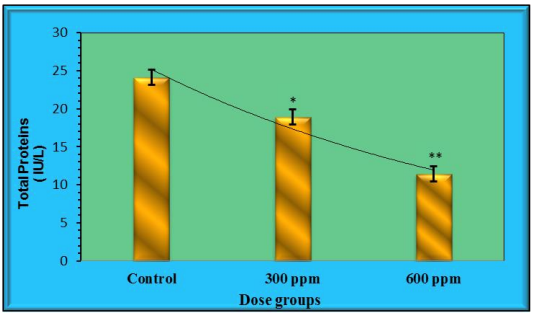


Fig. 1. Mean total proteins (IU/L) levels in skeletal muscle of control and fluoride treated rats.

Values are expressed as Mean ± SD.

* p<0.05 NaF treated group compared with control

** p<0.01 NaF treated group compared with control

Post- Hoc Tukey's multiple HSD comparison test after ANOVA (F=7.457, P<0.01) revealed significant decrease in the level of total proteins in skeletal muscle (95%CI=-6.083 to 3.3063) with mean difference of 5.1983 to -7.4800 among all fluoride treated groups as well as compared to control.

Pearson's bivariate correlation analysis revealed a significant (r=-0.671, P<0.01) negative relationship between level of fluoride and total proteins in skeletal muscle of test rats after 40 days of fluoride treatment. Further regression analysis (Y=23.2 -18.23 X, R^2= 0.45) showed that as the level of fluoride

increases in skeletal muscle of rats, protein content also declined (Fig.2).

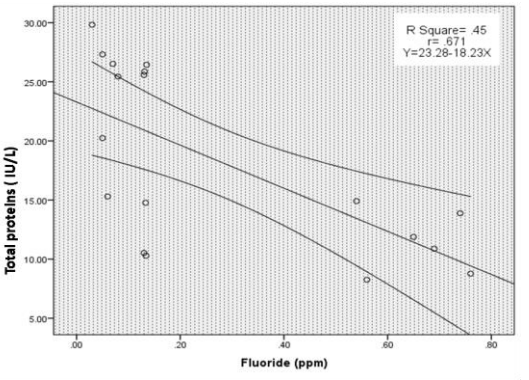


Fig. 2. Correlation and simple regression between tissue fluoride and skeletal muscle total proteins.

3.2. Protein profile in muscle tissue by Densitometry

The electrophoretic analysis of contractile proteins in skeletal muscle of control and fluoride treated rats are presented in Figures (3-5).

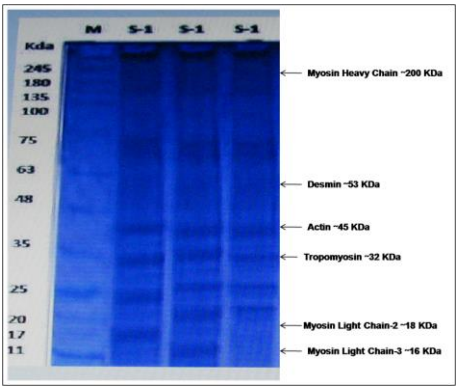


Fig. 3. SDS-PAGE polypeptide profile of total proteins in skeletal muscle of control rats after 40 days. M-Marker, S1- Control (Untreated samples)

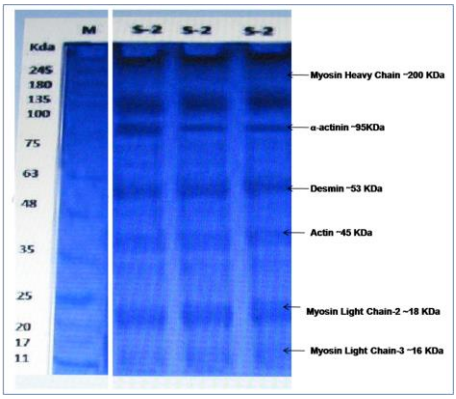


Fig.4. SDS-PAGE polypeptide profile of total proteins in skeletal muscle of control rats and on 40 days exposure to fluoride. M-Marker, S2- 300mg NaF/kgbw /day.

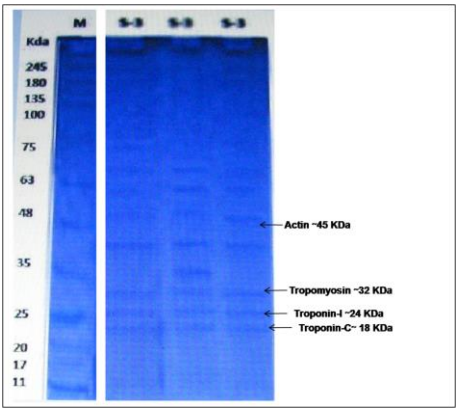
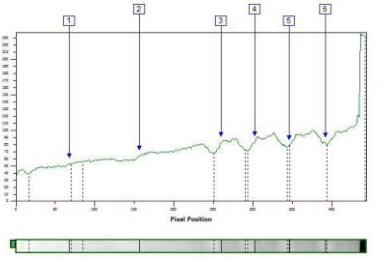


Fig.5. SDS-PAGE polypeptide profile of total proteins in skeletal muscle of control rats and on 40 days exposure to fluoride. M-Marker, S3- 600mg NaF/kgbw /day.

In the skeletal muscle tissue of control group, six polypeptide bands were detected. The molecular weights of these six polypeptides were : 197.088, 61.486, 39.996, 32.573 , 20.238 and 15.155 KDa (Fig. 6).



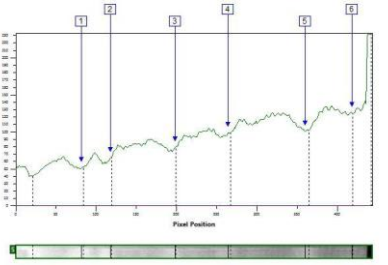
Band Table

Band No	Volume	Vol+BkGnd	Calib Vol(ug)	MW	Rf
1	136104.00	136104.00	-	197.088	0.152
2	235274.00	235274.00	-	61.486	0.348
3	474809.00	474809.00	-	39.996	0.577
4	169648.00	169648.00	-	32.573	0.670
5	160392.00	160392.00	-	20.238	0.817
6	208552.00	208552.00	-	15.155	0.910

Fig.6. Electrophoregram and densitometric scan image of total proteins profile in the skeletal muscle tissue of control rats after 40 days.

The skeletal muscle tissue from 40 days treated rats showed variation in total number of polypeptide bands due to two different fluoride concentrations.

In rats treated with 300 mg NaF/kg bw/day six polypeptide bands were detected . The molecular weights of these six polypeptides were : 157.132, 83.360, 57.691, 38.436, 20.361 and 12.183 KDa (Fig. 7).

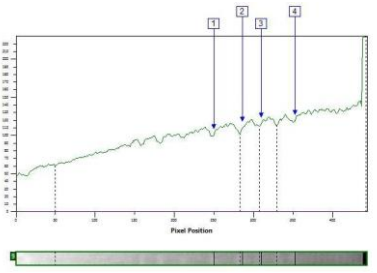


Band Table

Band No	Volume	Vol+BkGnd	CalibVol(ug)	MW	Rf
1	172943.00	172943.00	-	157.132	0.186
2	107744.00	107744.00	-	83.360	0.267
3	324955.00	324955.00	-	57.691	0.448
4	324875.00	324875.00	-	38.436	0.597
5	559636.00	559636.00	-	20.361	0.814
6	337679.00	337679.00	-	12.183	0.946

Fig.7. Electrophoregram and densitometric scan image of total proteins profile in the skeletal muscle tissue of rats after 40 days of exposure to 300ppm fluoride concentration

At 600 mg NaF/kg bw/day concentration, the number of polypeptide bands in skeletal muscle were reduced to four with molecular weights of 40.364, 28.598, 24.613 and 21.012 KDa (Fig. 8).



Band Table

Band No	Volume	Vol+BkGnd	Calib Vol(ug)	MW	Rf
1	868336.00	868336.00	-	40.364	0.572
2	426090.00	426090.00	-	28.598	0.710
3	121842.00	121842.00	-	24.613	0.753
4	599964.00	599964.00	-	21.012	0.803

Fig.8. Electrophoregram and densitometric scan image of total proteins profile in the skeletal muscle tissue of rats after 40 days of exposure to 600 ppm concentration of fluoride

In 300mg NaF dose group, the band intensity and molecular weight of myosin and actin were declined as compared to control. However, two proteins , α-actinin and myosin light chain-3, different to control group were observed (Fig.7).

In 600mg NaF dose group, myofibrillar proteins degraded and important contractile protein like myosin heavy

chain , α-actinin , desmin, disappeared completely (Fig.8). Troponin I and Troponin C were uniquely present in this group.

4. DISCUSSION

The skeletal muscle has been extensively used as a model to understand pathological alterations induced by various toxins [15]. The electrophoretic technique remains a promising tool for identifying the protein profile in response to stressful and sublethal level of groundwater pollutants. Catabolism of proteins and amino acids make a major contribuition of the total energy production in rats [16].

In the present study, the administration of fluoride resulted in a significant decrease (P <0.01) in the attention of total protein as compared to control group. Similar results have been reported in mice treated with fluoride [17] and rats [18].

A direct deleterious action of fluoride on protein metabolism can have a role in its protein depleting action because tissue protein has been reported to be the most sensitive and the earliest affected parameter in fluoride – treated animals .Earlier studies reported a similar reduction in fluoride treated animals and related to inhibition of decarboxylation of branched chain amino acids and simultaneously promoting protein breakdown [19].

Fluoride is well known to affect protein synthesis by suppressing $Na^+ – K^+$ activated ATPase [20] , an enzyme essential for the uptake of amino acids by tissues, causing impairment in polypeptide chain inhibition [21], weak incorporation of amino acids into proteins [22], and abnormal accumulation or inhibition of RNA synthesis [23].

In the present study, SDS polyacrylamide gel electrophoresis was performed for the skeletal muscle exposed to different concentrations of fluoride. When compared to control, the protein subunits of skeletal muscle exposed to fluoride , the bands showed decrease in intensity. The changes

in protein subunit band patterns may be due to change in the turn over (Synthesis/degradation) of various proteins.

In the present study, six distinct bands are accounted in the muscle tissue of rats . Extensive disruption in the number of banding is well documented at 600 ppm fluoride concentration with reduction to three polypeptide fractions during 40 days of exposure. Tripathi and Shukla [24] observed alterations in the cytoplasmic protein pattern of fish *Clarias batrachus* by performing electrophoresis of the cytoplasmic protein fractions of the liver and the skeletal muscle exposed to endosulfan and methyl parathion for 28 days. These authors also found that the disappearance and polymorphism of protein fractions were dependent on the degree of pollution in each water locality. The results are in agreement with the present study in which sodium fluoride retarded the protein syntheisis in direct proportion to the the amount of dosage administered.

In a study on muscle extracts from sea bass (*Dicentrarchus labrax*), Cheret et al.[25] found that 205, 105, 42, 36, 34 KDa protein bands belonged to myosin, α-actinin, actin , glycerlaldehyde-3-phosphate dehydrogeanse and tropomyosin, respectively. Ladart et al. [26] identified 53 KDa protein band as desmin . Samejima and Wolfe [27] reported 36 KDa protein as Tropomyosin in chicken breast muscle.

The present study demonstrated that in control group, 197.088, 61.486, 39.996, 32.573 , 20.238 and 15.155 KDa protein bands were identified as Myosin heavy chain , Desmin, Actin, Tropomyosin, Myosin light chain-2 and Mysoin light chain-3 . In animals treated with 300mg NaF, the 157.132, 83.360, 57.691, 38.436, 20.361 and 12.183 KDa protein bands were found as Myosin heavy chain, α-Actinin , Desmin , Actin, Myosin light chain-2 and Mysoin light chain-3. In animals treated with 600mg NaF , 40.364, 28.598, 24.613 and 21.012 KDa protein bands corresponded to Actin ,Tropomyosin , Troponin I and Troponin C.

Bradbury et al [28] pointed out that the decreased protein content might also be attributed to the destruction or necrosis of cellular function, and consequent impairment in protein synthetic machinery. Changes in protein sub units are regarded as important biomarkers of the metabolic potential of cells, as these play the main role in regulating the activities of cells.

5. CONCLUSION

The results of polyacrylamide gel electrophoresis (SDS-PAGE) and densitometric analysis demonstrated that fluoride disturbed the banding pattern of skeletal muscle proteins . It is also evident that degradation of myosin and other proteins induced by fluoride profoundly interfered with the muscle cell function, causes energy crisis and altered protein metabolism.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shashi A, Sharma N. The pathology of muscle lesions in experimental fluorosis. Int J Sci Innov Discov. 2011; 1(2) : 255-62.
2. Gupta AR, Dey S, Saini M, Swarup D. Down regulation of expression of Type I collagen gene in dentin of fluoride exposed rats. Ind J Anim Res. 2014; 48:601-04.
3. Shashi A, Rana N. 2016. Effect of fluoride on the expression of type I collagen gene (COL1A1) in skeletal muscle of rats. Asian J Biol Life Sci. 2016; 5(1): 62-65.
4. Senthil Elango P and Muthulingam M. Impact of heavy metal chromium on protein and amino acid contents in brain and muscle of freshwater fish *Oreochromis*

mossambicus (PETERS). Int J Curr Res. 2014; 6(1):4841-45.

5. Saikia PP, Rout PK, Nigam R. Electrophoretic separation of myofibrillar protein isoforms in skeletal muscle of Indian goats.Animal Sci. Reporter. 2014; 8(3): 108-13.
6. Perry SV. Properties of the muscle proteins-A comparative approach. J Exp Biol.1985; 115:31-42.
7. Pangkey H, Hara K, Tachibana K, Cao MJ, Osatomi K, Ishibrara T. Substrate specificity of carp hepatopancreatic cathepsin S for peptides and myofibrillar proteins . Fisheries Sci. 2000; 66: 1138-43.
8. Jengh JH, Hsieh CC, Lan WH, Chang MC, Lin SK, Hahn LJ, Kuo MY. Cytotoxicity of sodium fluoride on human oral mucosal fibroblasts and its mechanisms. Cell Biol Toxicol. 1998; 14:383.
9. Bouaziz H, Ketata S, Jammossi K, Boudwara T, Ayedi F, Ellouze F , Zeghal N. Pesticide biochem. Physiol. 2006; 86:124-30.
10. Shashi A, Singh JP, Thapar SP. Protein degradation in skeletal muscle of rabbit during experimental fluorosis. Fluoride. 1992; 25(3):155-58
11. Sarkar C, Pal S, Das N, Dinda B. ameliorative effects of oleanolic acid on fluoride induced metabolic and oxidative dysfunctions in rat brain; Experimental and biochemical studies. Food Chem Toxicol. 2014; 66: 224-36.
12. Lowry OH, Rosebrough NJ , Farr AL and Rendall RJ . Protein measurement with the folin-phenol reagent. J Biol Chem. 1951 ;193 : 265-75.
13. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature.1970; 227:680-85.

14. Murakami U, Uchida K. Contents of myofibrillar proteins in cardiac, skeletal, and smooth muscles. J Biochem. 1985; 98:187-97.
15. Baldo C, Ferreira MJ, Lopes DS, Izidoro LFM, Gomes AO, Ferro EAV, Hamaguchi A, Homsi-Brandeburgo MI, Rodrigues VM. Action of neuwidase, a mettaloproteinase isolated from *Bothrops neuweidi* venom, on skeletal muscle; an ultrastructural and immunocytochemistry study. The J. Venemous Animals Toxins including Trop Discov. 2010; 16(3):462-69.
16. Nagarjuna A, Rayendra PS, Siraj MS, Ravi Sarkar P, Jacob boss P. Aleration in protein metabolic profiles in gastrocnemius muscle tissue of rats during cypermethrin toxicosis. J Ind Society Toxicol. 2008; 4(1) :17-21.
17. Pillai KS , Mathai AT, Deshmukh PB. Effect of subacute dosage of fluoride on male mice. Toxicol. 1988 ; 8(2) : 155-59.
18. Chinoy NJ, Sharma M, Mathews M .Beneficial effects of ascorbic acid and calcium on reversal of fluoride toxicity in rats. Fluoride. 1993; 26(1): 45-46
19. Opit LJ, Potter H, Charnock JS. The effect of anions on (Na^+ -K^+) - activated ATPase .Biochem Biophys Acta. 1966; 120(1966) : 159-164.
20. Godehaux W , Atwood KC. Structure and function of initiation complexes which accumulate during inhibition of fluoride ion. J Biol Chem. 1976; 251 : 292-301.
21. Chinoy NJ, Narayana MV, Sequeira E, Joshi SM, Barrot JM, Purohit RM, Parikh DJ Ghodsara NB. Studies on effects of soft tissue functions in 36 villages of Mehsana district, North Gujrat. Fluoride. 1992; 25:101-110.
22. Mathew M , Barot VV, Chinoy NJ. Investigations of soft tissue functions in fluorotic individuals of North Gujrat . Fluoride. 1996; 63-71.

23. Holland I. Fluoride inhibition of protein and collagen biosynthesis in rabbit dental pulp in vitro . Acta Pharma et Toxicol. 1979; 45: 96-101.
24. Tripathi G, Shukla SP. Malate and lactate dehydrogenases of a fresh water cat fish: impact of endosulfan. Biomed Environ Sci. 1990; 3:53-64
25. Cheret R, Hernandez-Andres A, Delbarre-Ladart C, De lamballerie M, Verrez-Bagnis V. Proteins and proteolytic activity changes during refrigerated storage in sea bass (*Dicentrarchus labrax L.)* muscle after high-pressure treatment. Eur Food Res Tech. 2006; 222:527-35.
26. Ladart C, Chaplet M, Verrez-Bagnis V, Noel J, Fleurence J. In vitro proteolysis of myofibrillar and sarcoplasmic proteins from white muscle of sea bass (*Dicentrarchus labrax L.)*: effects of cathepsins B,D and L. Food Chem. 2003; 81(4): 517-25.
27. Samejima K, Wolfe FH. Degradation of myofibrillar protein component during post mortem aging of chicken muscle. J Food Sci. 1976; 41:250-54.
28. Bradbury SP, Mc Kim JM, Coasts JR. Physiological responses of rainbow trout, *Salmo gaurdneri* to acute fenvalerate intoxication. Pesticides Biochem Physio.1987; 27:275-88.