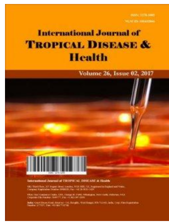

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SDS-PAGE and Densitometric Analysis of Myofibrillary Proteins: Actin and Myosin in Skeletal Muscle of Fluoride Intoxicated Rats

A. Shashi[1*] and Nidhi Rana[1]

[1]*Department of Zoology and Environmental Sciences, Punjabi University, Patiala, Punjab, India.*

Authors' contributions

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

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ABSTRACT

Aim: The present study was undertaken to evaluate the contractile proteins; Actin and Myosin in the skeletal muscle of rats exposed to three different concentrations of fluoride during 40 days of 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. The percentage decrease in both Actin and Myosin proteins was observed for all treated groups.

Study Design: 8 weeks old, Wistar albino rats of both sexes were used for the experiments. The rats were divided into three groups (Group I, Group II and Group III) of six animals each. The first group served as control group and was administered 1 ml deionized water /kg bw/ day. Sodium fluoride was given to Group II and Group III at the dose rate of 300 mg NaF/ kg bw/day and 600 mg NaF / kg bw /day orally for 40 days. Animals were housed in polypropylene cages lined with husk and kept in light/dark condition (12h light/ 12 h dark) .The animals had free access to water and were supplied with standard pellet diet.

Methodology: At the end of the experimental period, rats were fasted overnight and anaesthetized

**Corresponding author: Email: shashiuniindia@yahoo.co.in;*

using ether anaesthesia. Skeletal muscle tissue were dissected out in ice-cold saline. The tissues were weighed and 10% tissue homogenate was prepared with the 0.1 M Tris-HCl buffer, pH 7.5 using a ultrasonic homogenizer. After centrifugation at 2000 rpm for 10 minutes, the clear supernatant was used for the analysis of contractile proteins.

Results: SDS-PAGE and Densitometric analysis revealed that intensities of Actin and Myosin protein bands were reduced due to fluoride intoxication. The intensity of Myosin in Control group was found to be 2.57, 2.00 in Group II and 0.41 in Group III. The intensity of Actin in Control group was 3.041, 2.11 in Group II and 0.30 in Group III. The 600 mg NaF fluoride treated group exhibited more prominent decrease that the intensity of Myosin was decreased to 0.41 and Actin was decreased to 0.30. In the present study the percentage decrease of both Actin and Myosin proteins showed that Actin was 46% decreased in 300 ppm group and 58% decreased in 600 ppm group. Similarly, Myosin was 41% decreased in 300 ppm group and 87% decreased in 600 ppm group.

Conclusion: The results of polyacrylamide gel electrophoresis (SDS-PAGE) and densitometric analysis demonstrated that fluoride disturbed the contractile pattern of skeletal muscle proteins. The results suggested that skeletal muscle damage, induced by fluoride, is probably due to its proteolytic action on myofibrils, which are responsible for the maintainence of the cellular architecture.

Keywords: Actin; contractile proteins; densitometry; fluorosis; myosin; rats; SDS-PAGE electrophoresis; skeletal muscle; sodium fluoride.

1. INTRODUCTION

Fluorosis is a serious public health problem in many parts of the world. Drinking water is the major source of daily intake of fluoride. Effulent containing high concentration of fluoride is discharged from industries and forms the major source of external contamination to water bodies. Due to its strong electronegativity, fluoride is attracted to positively charged calcium in teeth and bones which causes dental fluorosis. With prolonged exposure to higher fluoride concentrations, dental fluorosis progresses to skeletal fluorosis [1].

Skeletal muscle fibres are multinucleated cells, composed of large number of parallel running myofibrils. The myofibrils, in turn, consist of overlapping and parallel thin actin and myosin filaments [2].

Muscles serve as a source of amino acids that can be used for energy production by various organs. However, excessive protein degradation in skeletal muscle, is highly detrimental for the economy of human body and can lead to death [3].

Muscle is the largest repository of proteins and amino acids in the body. The functional capacity of muscle depends on both the quality and quantity of muscle proteins. Both the quality and quantity of muscle protein are likely to be maintained through a continuous remodeling process involving protein synthesis and breakdown. A decline in the synthesis rate of specific protein is an indication of a diminished the modelling process with possible functional consequences [4].

The contractile proteins are an important cellular component critical to maintaining cell shape mass and other cellular function. Myosin heavy chains contributes to 20 to 25% of overall muscle protein synthesis in humans, while Actin display both lower and higher protein turnover compare to mixed muscle proteins [5].

Myosin is a major structural component of skeletal muscle and is considered to be the molecular motor that converts free energy derived from the hydrolysis of ATP into mechanical work. The approximate expression of myosin in skeletal muscle is critical to motor cell function [6].

The study of the molecular size and intensity of the fibrous proteins of skeletal muscle has an interest in its own right, but in addition it has a particular importance in that these proteins are believed to provide the ultimate molecular ‘building blocks’ for the contract and system of the living muscle [7].

The aim of the present study was to evaluate the contractile proteins; Actin and Myosin in the skeletal muscle of rats exposed to three different concentrations of fluoride during 40 days of 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.

2. MATERIALS AND METHODS

8 weeks old, Wistar albino rats of both sexes were used for the experiments. Animals were housed in polypropylene cages lined with husk and kept in light/ dark condition (12h light/ 12 h dark). The animals had free access to water and were supplied with standard pellet diet. Animal handling and experimental procedures were approved by the Institutional Animal Ethics Committee, Punjabi University, Patiala, India.

2.1 Experimental Design

In the present study, fluoride was administered orally as sodium fluoride (NaF, 99% pure, Loba chemie, Mumbai, India). Control (group 1) received 1 ml deionized water only, experimental rats were sub-divided into two groups (2 and 3). In the experiment, a total of 18 rats were used. The rats were randomly divided into three groups of 6 animals each.

Group 1 : Control recieved 1 ml double distilled water for 40 days
Group 2 : Rats administered with sodium fluoride (300 mg NaF / kg bw/day) for 40 days
Group 3 : Rats administered with sodium fluoride (600 mg NaF / kg bw/day) for 40 days

At the end of the experimental period, rats were fasted overnight and anaesthetized using ether anaesthesia .Gastrocnemius muscle tissue were dissected out in ice-cold saline. The tissues were weighed and 10% tissue homogenate was prepared with the 0.1 M Tris-HCl buffer, pH 7.5 using a ultrasonic homogenizer. After centrifugation at 2000 rpm for 10 minutes, the clear supernatant was used for biochemical assay. The samples were processed for Electrophoresis and analysed.

2.2 Estimation of Total Proteins

The concentration of total protein in the tissue was assayed by the method of Lowery et al. [8].

2.3 Analysis of Skeletal Muscle Proteins Using SDS-PAGE Electrophoresis

The electrophoresis was performed using slab gels of 100 mm X 150 mm X 1 mm of 10% polyacrylamide containing 1% SDS and 10% glycerol. Experiments were run at 20 mA/gel until the tracking dye entered the separating gel, and next the current was raised to 30 mA/gel, maintained throughout the electrophoresis. The amount of total protein loaded to each gel lane was 30 µL. To visualize protein bands, the gel was stained with Coomassie brilliant blue G250 for polypeptides Further the protein bands were confirmed by determining the molecular weight in gel documentation system. The molecular weight were thus compared with literature available,thereby confirming the samples to be as Myosin and Actin. The samples were analysed using Image J software and the densitometric analysis was further done. The molecular weight ladder was also used and marked as ‘M’.

2.4 Analysis of Muscle Protein Bands by Densitometry

To estimate molecular weights or to quantify protein in an individual bands, densitometry of photo prints or gel scans was performed using Image J software. Image J software confirms presence of same proteins on the basis of molecular weight on comparing bands of protein ladder and bands obtained after running samples. As far as analysis of bands through software Image J is concerned, it is an open source image processing program designed for scientific multidimensional images.

3. RESULTS

3.1 SDS-PAGE Analysis

SDS-PAGE patteren of contractile proteins from of skeletal muscle from control and fluoride treated samples are presented in Fig. 1.

When tissue sample was fully purified for actin and myosin on SDS-PAGE then the bands were analyzed using Image J software..In first lane, there was a protein ladder that ranges molecular weight of actin and myosin.

The pattern reveals multiple bands in the molecular weight range between 200 KDa and 12 KDa. The band appearing at over the marker of 200 KDa is Myosin heavy chain and at 45 kDa is actin.

The protein profile assessed by SDS-PAGE showed no difference in protein patterns in control rat at 40 days (Fig. 2A.). Similarly, there were no changes observed in actin and myosin bands intensity either.

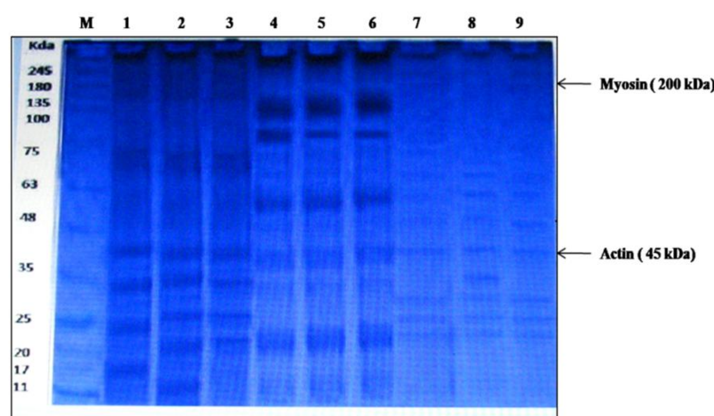


Fig. 1. SDS-PAGE polypeptide pattern profile of the skeletal muscle of rat on 10% gel. Lane 1,2,3 (the samples without fluoride treatment), Lane 4,5,6 (samples treated with 300mg NaF/kg bw/day), Lane 7,8,9 (samples treated with 600mg NaF/kg bw/day) after 40 days. M-Marker

When the changes in contractile proteins were investigated for the rats treated with 300 m g NaF and 600 m g NaF respectively, for 40 days, it was noted that the intensity of protein bands started to decrease while some protein bands completely disappeared in 600 mg NaF treated group.

In addition there was an evident decrease in the intensity of actin and myosin bands in 300 mg fluoride treated samples (Fig. 2B.).

The intensity of myosin protein bands over 200 kDa molecular weight started to decrease in 600 mg NaF group and degraded (Fig. 2C)

3.2 Densitometry Results

The comparative electrophoretic and densitometric analysis revealed that intensity of Myosin in control group was found to be 2.57 and Actin was 3.041 (Fig. 3.).

The results at 300 ppm fluoride treatment showed that the intensity of Myosin was decreased to 2.00 in 300 ppm and Actin was decreased to 2.11 (Fig. 4.).

3.3 Percentage Decrease

In the present study the percentage decrease of both Actin and Myosin proteins showed that Actin was 46% decreased in 300 ppm group and 58% decreased in 600 ppm group. Similarly, Myosin was 41% decreased in 300 ppm group and 87% decreased in 600 ppm group.

The 600 ppm fluoride treated group exhibited more prominent decrease that the intensity of Myosin was decreased to 0.41 and Actin was decreased to 0.30 (Fig. 5.).

4. DISCUSSION

The present study has revealed the electrophoretic pattern and densitometric analysis exhibited by two important contractile proteins, actin and myosin due to fluoride intoxication.

Protein estimation is a reliable parameter and is recommended for broad usage. Since proteins are involved in the architecture and physiology of the cell, they appear to occupy a key role in cell metabolism [9].

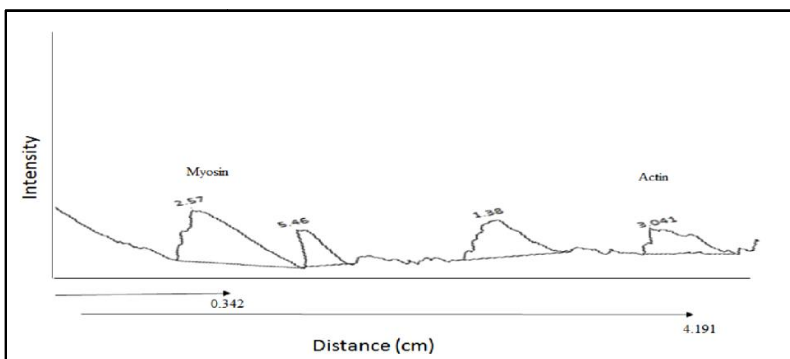
In the present investigation, rat exposed to fluoride concentrations for 40 days showed decrease in the level of proteins. The present work agrees with the previous studies which also reported that total protein content was decreased due to fluoride toxicity and it may be due to the breakdown of proteins in the fabrication of some amount of energy for organism [10-12].

Chinoy and Memon [13] pointed out that decreased protein content might also be attributed to the destruction or necrosis of cellular function and consequent impairment in protein synthetic machinery.

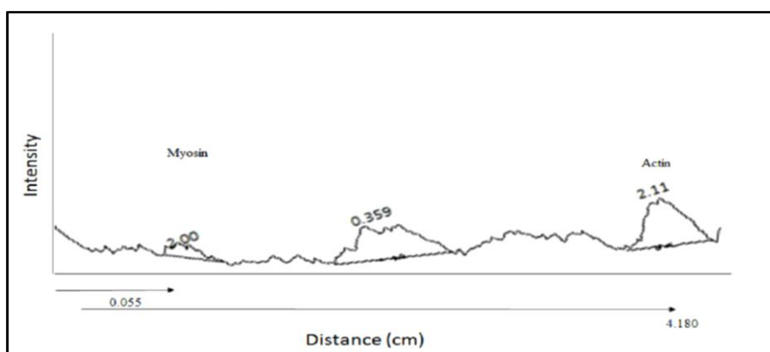
Skeletal muscle plays a major role in whole body metabolism and changes in rates of synthesis and degradation of proteins are likely to lead to

characterstic changes in the quality of different proteins in muscle under various physiological and pathological conditions [14].

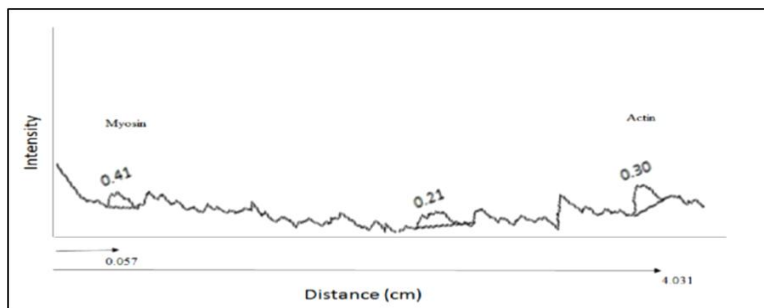
In the present study, results revealed that actin and myosin bands showed decrease in intensity when exposed to 300 and 600 ppm fluoride dosage as compared to control. The intensities of contractile proteins were found to be more decreased at higher dose of 600 ppm than 300ppm. Similar studies also reported the decrease in banding pattern and intensities of contractile proteins when skeletal muscle of different animals were exposed to different toxins [15,16].



(A)



(B)



(C)

Fig. 2. The densitometric analysis of contractile proteins of the skeletal muscle of rat after 40 days of fluoride treatment. (A) Skeletal muscle sample kept as Control, (B) Skeletal muscle sample treated with 300 mg NaF/ kg bw/day, (C) Skeletal muscle sample treated with 600mg NaF/ kg bw/day

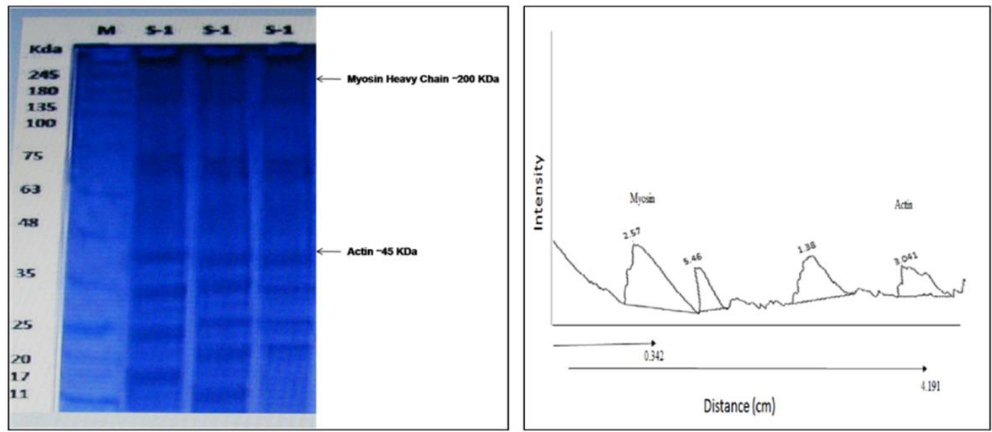


Fig. 3. The electrophoretic and densitometric analysis of contractile proteins of skeletal muscle of control rat after 40 days of fluoride treatment

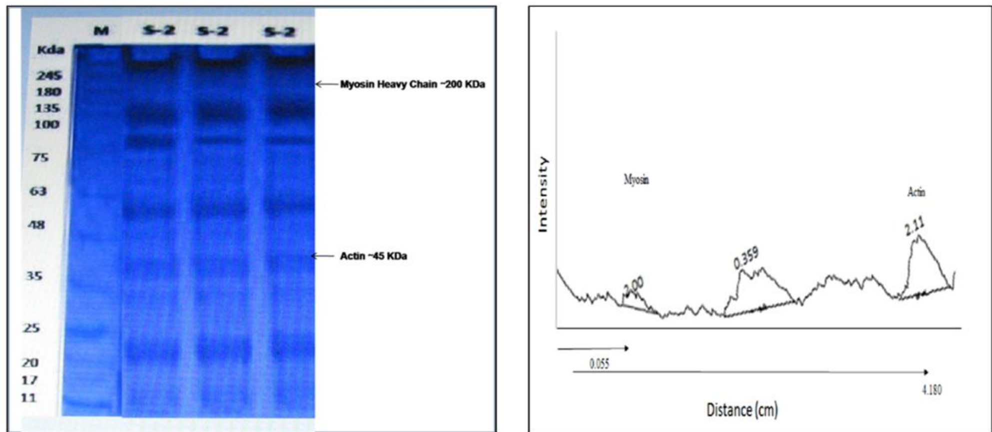


Fig. 4. The electrophoretic and densitometric analysis of contractile proteins of skeletal muscle of rat treated with 300 mg NaF/ kg bw/day after 40 days of fluoride treatment

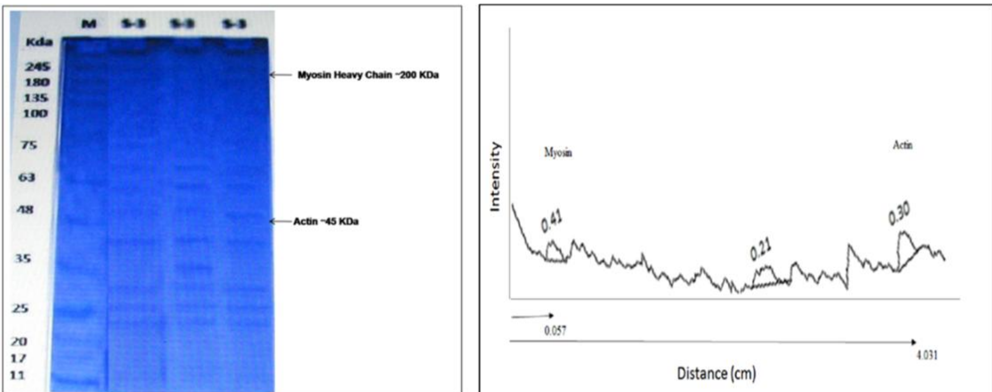


Fig. 5. The electrophoretic and densitometric analysis of contractile proteins of skeletal muscle of rat treated with 600 mg NaF/ kg bw/day after 40 days of fluoride treatment

In order to understand the damage induced by fluoride the skeletal muscle was analysed by transmission and scanning electron microscopy in our previous study [17,18]. After fluoride toxicity skeletal muscle showed conspicuous damaged of muscle fibres. Degradation of actin and myosin filaments which are critical for muscle architecture and functioning was observed. It is important to point out that cell movement, muscular contraction, cytokinesis, membrane trafficking and signal transduction are driven by contractile protein myosin that move undirectionally along actin filaments. Thus, degradation of myosin and actin proteins induced by fluoride could interfered with the muscle cell function.

5. CONCLUSION

The results of polyacrylamide gel electrophoresis (SDS-PAGE) and densitometric analysis demonstrated that fluoride disturbed the contractile pattern of skeletal muscle proteins. The results suggested that skeletal muscle damage, induced by fluoride, is probably due to its proteolytic action on myofibrils, which are responsible for the maintainence of the cellular architecture.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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