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Original Research Article

Histological observation of the effects of *Moringa oleifera* leaf extract on lead poisoning in the skeletal muscle of wistar rat models

Joshua O. Owolabi[1]*, Philip O. Ogunnaike[1] and Nnenna L Nwobi[2]

[1]Department of Anatomy, Ben Carson Sr. School of Medicine, Babcock University, Nigeria.

[2]Department of Chemical Pathology, Ben Carson Sr. School of Medicine, Babcock University, Nigeria

***Corresponding Author E-mail: Olaowolabi001@yahoo.com; Tel.: +2348064884305**

Abstract

Lead poisoning is no doubt a global health challenge, considering the numerous ways that people are at risk of exposure and the extensive negative effects the metal could produce on several body tissues. While investigations to observe the effects using diverse methods of study are important, so also is the need to find possible remedies, especially when they are of natural products. This investigation observed the effects of lead treatment on the skeletal muscle and the possible positive effects of Moringa against lead poisoning in the tissues. Moringa has been reported to have produced positive anti-lead effects to various degrees in certain other tissues. Twenty four Wistar rats (n=24) were divided into four groups labelled: Group A- control group fed *ad libitum*; Group B- treated with moringa leaf extract only to observe possible extract effects solely; Group C- treated with lead to observe sole effects of lead and Group D- treated with lead and moringa leaf extract concurrently to observe the effects of moringa treatment against lead poisoning within the tissue. Lead dosage wherever applicable was 50mg/kg body weight and moringa extracts was 200mg/kg body weight. All administration was done through the oro-gastric route. The experiment lasted 28 days. Animals were sacrificed and the thigh muscles were excised fixed and processed using the routine Haematoxylin and Eosin staining technique. Photomicrographs obtained were subjected to critical qualitative histological and complimentary histopathological analyses. Histological observations show that lead as used did not produce extensive tissue disruption in skeletal muscles but enough assault on the myocytes to produce mild morphological difference relative to unaffected tissues. Moringa extract effect on myocytes could have been stimulatory, resulting in mild hyperplasia- an effect that could counter the negative effects of lead on the myocytes or increase muscle volume on its own.

Keywords: Anti-toxicity, Lead, Moringa, Muscle, Rat

INTRODUCTION

Lead poisoning is no doubt a global health challenge, considering the numerous ways that people are at risk of exposure and the extensive deleterious effects the heavy metal could produce on several body tissues. It is a common environmental poison that affects neuromuscular junction and potentially might cause

muscle weakness (Hasan *et al.,* 2003). While investigations to observe the effects using diverse methods of study are important, so also is the need to find possible remedies, especially when they are of natural products. This investigation observed the effects of lead treatment on the skeletal muscle the possible positive effects of Moringa against lead poisoning in the tissues. Moringa has been reported to have produced positive anti-lead effects to various degrees in certain other tissues.

Though lead is present in the environment in various forms, and could be ingested in various ways; the United States current reference range for acceptable blood lead concentrations in healthy individuals without excessive exposure to environmental lead sources is less than 5 µg/dL for children (ACCLPP, 2012); less than 25 µg/dL for adults;and the biological exposure index for lead-exposed workers is being palced at 30 µg/dL. High level of lead poisoning has been associated with muscle weakness (MedLine Plus, 2014; ATSDR 1989); as well as slowed growth- a case that also has to do with muscular development, albeit possibly through secondary mechanisms. Lead poising has also been reportedly linked with muscle pain (Rempel, 1989).

Mekonnen (1999) reported a possible antispasmodic property effect of crude moringa oleifera leaf extract on the uterine smooth muscle; while Williams *et al.* (2014) reported a mildly positive anti-lead toxicity effect of moringa leaf extract on the cardiac muscle. It has also been reported that the aqueous, ethanol and ether extracts of *Moringa oleifera* leaves have compounds that cause reduction in heart rate and relaxation of cardiac muscle (Ntulume, 2010). No doubt, not many specific literatures are available on the effects of moringa oleifera parts extracts on various muscle types. There are however good reason to investigate the possible use of moringa leaf extracts in ameliorating or preventing the deleterious effects of lead poisoning on the skeletal muscle. Very important among existing reports are the reported high concentrations of antixoidnats (Njoku and Adikwu, 1997; Siddhurajuand and Becker, 2003; Fahey, 2005) proteins (Fuglie, 1999) and minerals (Babu, 2000) among several other vital phytochemicals (Fahey**,** 2005**)** in the plants leaf and other parts preparations.

MATERIAL AND METHODS

Twenty four Wistar rats (n=24) were divided into four groups labelled: Group A- control group fed *ad libitum*; Group B- treated with moringa leaf extract only to observe possible extract effects solely; Group C- treated with lead to observe sole effects of lead and Group D- treated with lead and moringa leaf extract concurrently to observe the effects of moringa treatment against lead poisoning within the tissue. Lead preparation was made using lead acetate salt dissolved in water. Ethanolic *Moringa oleifera* leaf extract was also prepared from air dried Moringa leaves.

Lead dosage wherever applicable was 50mg/kg body weight and moringa extracts was 200mg/kg body weight. All administration was done through the oro-gastric route. This was done with the aid of suitable orogastric canula. The experiment lasted 28 days, after which animals were sacrificed by cervical dislocation and the skeletal muscle of the animal's thigh regions excised and fixed; then processed using the routine Haematoxylin and Eosin staining techniques (Sheehan and Hrapchak, 1980; Luna, 1992). Photomicrographs of the tissues obtained using the Accuscope Phomicrographic Set, suitably at magnifications X160 and X640. These were properly labelled and subjected to critical qualitative histological and complimentary histopathological analysis.

RESULTS AND DISCUSSION

Group A animals were considered normal and suitably serving as the control. Photomicrographs show that the entire histo-architecture appears normal, hence healthy. The fascicles (MF, Figure A1) are observable as well as the myomesium (Blue arrows, Figure A1 and A2) that demarcates them. Muscle cells- myocytes are observable, elongated as they are cross-sectioned majorly in their longitudinal axis, being peripheral to the muscle cells. The myofibril rich myomesium is also observable (Figure A2).

Group B animals were treated to observe possible effects of moringa leaf extract. All important histo-architectural features are clearly observable- the fascicles are clearly defined (Figure B1) and separated by connective tissue perimysium. The myocytes are also present and observable (Figure B2). These observations are characteristic of normal muscle histology, especially relative to the control in Figure A (1 and 2). Therefore, moringa treatment of this group in this investigation did not produce observable histological disruption or anomaly.

While it is impossible to rule out possible deleterious effects of lead treatment on the skeletal muscles of the Group C animals (administered lead only); it is clearly not a case of massive or extensive disruption. Histo-architecture is still clearly defined. Cells are still observable and the fascicles individually and in relation to adjacent ones are not showing marked signs of disruption or displacement. It is however important to note that the relatively reduced prominence of muscle cells- in number and morphology are pointers to the negative effects of lead on the cells. Thus, while the tissue does not suffer extensive disruption, the individual cells could have suffered chemical assault, that may reduce their mechanical and physiological efficiency. This could be an additional factor responsible for the reported muscle weakness effects of lead poisoning (Hasan *et al.,* 2003).

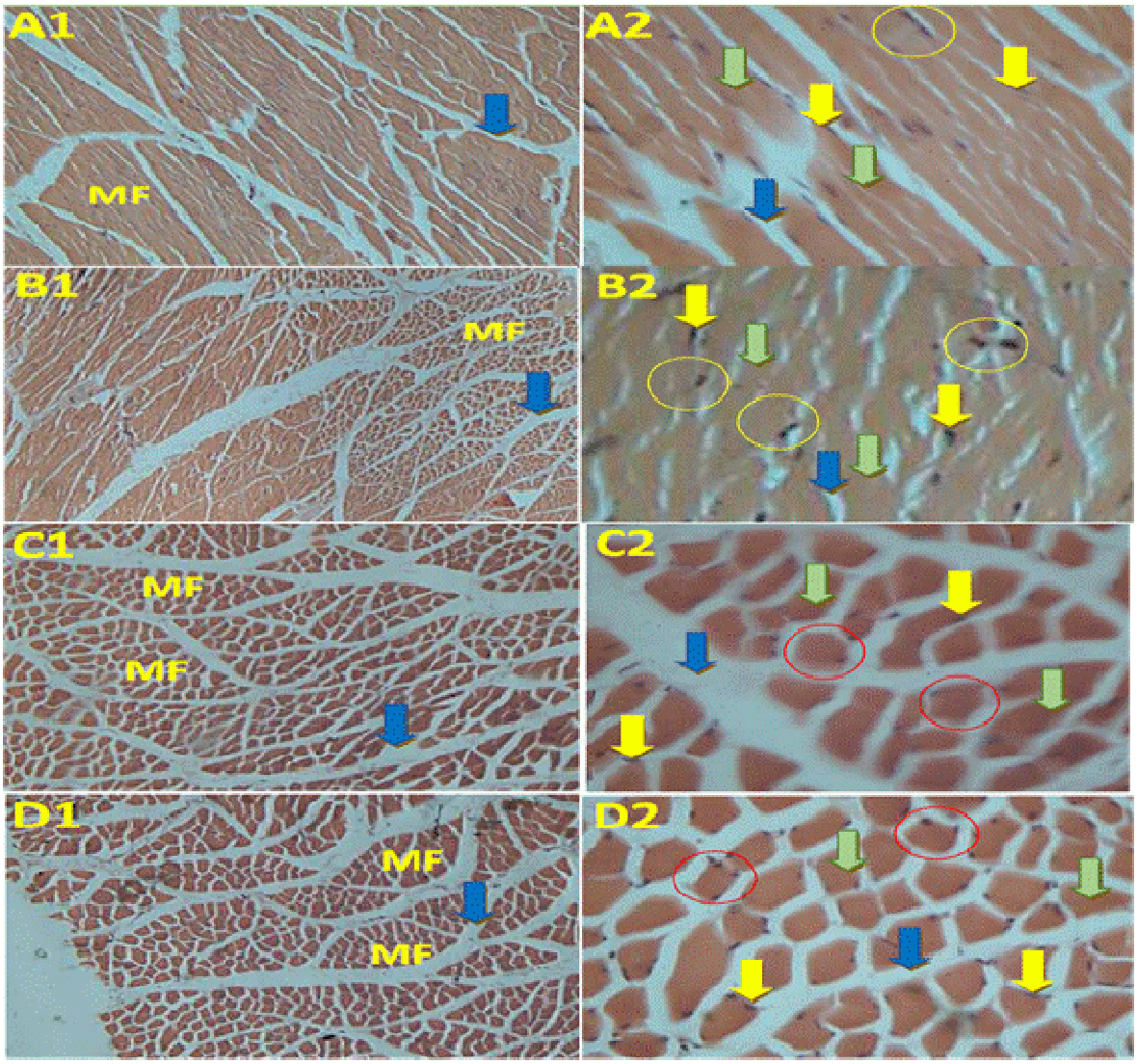

Figure 1A-D. Figures A are Photomicrographs of the skeletal muscle of the Control Group A at X 160 (A1) and X 640 (A2) magnifications: tissues are normal and healthy in terms of histological architecture. Figures B are Photomicrographs of the skeletal muscle of the Group B treated with moringa leaf extract only, at X 160 (B1) and X 640 (B2) magnifications: tissues are also normal and healthy in terms of histological architecture; relative to the Control Group A, frequently occurring adjacent paired cells suggest increased rate of cell division. Figures C are Photomicrographs of the skeletal muscle of the Group C treated with lead only at X160 (C1) and X640 (C2) magnifications: tissues do not exhibit gross histological disruption; myocytes however appear pale and less properly defined. Figure D are Photomicrographs of the skeletal muscle of the Group D treated with lead and moringa leaf extract concurrently at X 160(D1) and X640 (D2) magnifications: myocytes appear prominent and relatively quite abundant, suggesting hyperplasia.

Legend

MF:	MF	Muscle Fascicle
Yellow Arrow:		Myocyte Nucleus
Green Arrow:		Myocytes Sarcoplasm and Fibrils
Blue Arrow:		Connective Tissue Connective Tissue Perimysium
Red Circle:		Muscle Fibre Cross Section Showing Nuclei
Yellow Circle:		Muscle Fibre Cross Section Showing Nuclei

When lead and moringa leaf extract was administered concurrently in Group D; general histo-architecture is observable. Fascicles are well defined. When compared with the histological presentation of the lead-treated Group C; there are more cells per bundle muscle fibre (Red circles in Figure C2 and D2). While this does not justify the presence of structural defect in the lead-treated Group C; it suggests that moringa leaf extract could have

produced a stimulating effect that could have resulted in muscle cell division, a process that could lead on to hyperplasia. It is therefore important to note that if moringa leaf extract treatment produced a stimulatory positive effect on the myocytes in this investigation; it is more of hyperplasia- an increase in the number of cells per unit volume of tissue rather than hypertrophy- an increase in the size or volume of unit cell. Though both are typically physiological, especially hyperplasia which can ether result from chemical substance stimulation or in response to mechanical activities. This mechanism has been reported on the avian specie (Antonio and Gonyea, 1994). More so, when Figure A2 is compared with B2, there are more features or scenarios (encircled in yellow colour) to suggest the hyperplasia effects of moringa- by virtue of increased normal cell division, in Figure B2 (when the animals are administered moringa extract only) than in Figure A2 when the animals are untreated controls. This result again, is in line with our previously reported positive anti-lead toxicity effects of moringa leaf extract in various tissues, especially, cardiac muscle (Williams *et al.,* 2014).

CONCLUSION

Altogether, the histological observations show that lead as used did not produce extensive tissue disruption in skeletal muscles but enough assault on the myocytes to produce mild morphological difference relative to unaffected tissues. Moringa extract effect on myocytes could have been stimulatory, resulting in hyperplasia- an effect that could counter the negative effects of lead on the myocytes or increase muscle volume on its own.

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