



E-ISSN: 2788-8428
P-ISSN: 2788-8436
ZEL 2024; 4(1): 28-33
Received: 13-11-2023
Accepted: 15-12-2023

Mathias Akoji Godwin
Department of Chemistry,
Kogi State University
Anyigba, Nigeria

Charles David N
Department of Biochemistry
Olusegun Agagu University of
Science and Technology,
Okitipupa, Nigeria

Okoliko Micheal Victor
Department of Biochemistry
Kogi State University
University Anyigba, Nigeria

Ekwoba Lucky
Department of Chemistry,
Kogi State University
Anyigba, Nigeria

Nwobodo Chiamaka Frances
Department of Applied
Biology Biotechnology, Enugu
State University of Science
and Technology, Nigeria

Ozokwo Mary
Department of Biology,
Ahmadu Bello University
Zaria, Nigeria

Olorunfemi Oyewole Babalola
Department of Biochemistry
Olusegun Agagu University of
Science and Technology,
Okitipupa, Nigeria

Omeje Helen E
Professor, Department of
Psychiatry, University of
Social Welfare and
Rehabilitation Sciences USWR
Razi Psychiatric Hospital
Tehran, Iran

Correspondence Author,
Mathias Akoji Godwin
Department of Chemistry,
Kogi State University
Anyigba, Nigeria

Zoological and Entomological Letters

Effects of defatted *Moringa oleifera* seed on skeletal muscle of protein energy malnourished rats

Mathias Akoji Godwin, Charles David N, Okoliko Micheal Victor, Ekwoba Lucky, Nwobodo Chiamaka Frances, Ozokwo Mary, Olorunfemi Oyewole Babalola and Omeje Helen E

Abstract

Background: Malnutrition in form of protein energy malnourishment, is a range of pathological conditions arising from coincident lack of protein and/or energy in varying proportions which can result in muscle wasting and degeneration.

Aim: The study is aimed to investigate the effects of defatted *Moringa oleifera* seed on skeletal muscle of Protein Energy Malnourished rats and to assess its effect on enzyme markers: AST, ALT, Total protein, Albumin, Ca²⁺ ATPase and Na⁺/K⁺ ATPase.

Methods: 16 white albino rats of waster strain (*Rattus norvegicus*) were initially divided into 'A' and 'B' groups; Group A rats served as positive control and was administered normal growers' feed while Group 'B' rats were administered with low protein-based diet to induce muscle wasting for 21 days. After the period of malnourishment, group B was further divided into 3 groups: C, D, E. Group C was sacrificed which served as negative control while group D and E were fed with recovery diet containing 20% *M. oleifera* based diet and 20% fish meal-based diet respectively for another 21 days.

Results: Upon the introduction of recovery diet, the results show that there was an increase in the activities of ALT, AST, Na⁺/K⁺ and Ca²⁺ ATPases as well as the total protein and albumin when compared with the control.

Conclusion: *M. oleifera* leaf-based diet proves to be a sustainable replacement for food protein in the diet.

Keywords: Protein energy malnutrition, *Moringa oleifera*, enzyme markers

1. Introduction

We know that calcium ion maintains the cellular balance between protein synthesis and protein degradation in the muscle cells. Generally, altered protein metabolism in the muscle cells leads to muscle degeneration as well as various disease conditions including micro-nutrient deficiency, over-weight, infections, under-nutrition etc. (Olayinka and Clement, 2017) [22] which can greatly affect calcium ion levels, ATPase activity, oxidation/reduction state of the cell and cause oxidative stress. Calcium adenosine triphosphatase (Ca²⁺ - ATPase) is important for maintaining the overall health of the muscle cells, however inhibition of Ca²⁺ - ATPase prevent the pumping of calcium ion, resulting in muscle wasting and degeneration of muscle tissue (Olayinka and Clement, 2017) [22]. In addition to calcium homeostasis in the muscle cells, Na⁺/K⁺-ATPase have been known to maintain the resting potential, and regulate cellular volume of the cell. It also functions as a signal transducer/integrator to regulate MAPK pathway, and reactive oxygen species in cells (Kasas *et al.*, 2022) [13].

Malnutrition is simply imbalances, deficiencies, or excesses intake of nutrients and energy. The term includes all the disease condition mentioned earlier and protein energy malnutrition (PEM). PEM refers to a form of malnutrition which is defined as a range of pathological conditions arising from coincident lack of protein and/or energy in varying proportions. The condition varies in forms ranging from mild through moderate to severe degrees. (Jee *et al.*, 2021) [12]. Kwashiorkor (protein malnutrition predominant), Marasmus (deficiency in calorie intake) and Marasmic Kwashiorkor (marked protein deficiency and marked calorie insufficiency signs present, sometimes referred to as the most severe form of malnutrition) are the common types of PEM (Ogunniyi, 2022) [21].

Moringa oleifera (family: Moringaceae), also known as the "Miracle tree", is a worldwide distributed medicinal plant and characterized by a series of pharmacological and health-promoting properties (Meireles *et al.*, 2020) [17]. *M. oleifera* has multiple uses because all parts of the tree are edible.

Interestingly, the consumption of this plant has been reported to contribute significantly to the intake of some essential nutrients and health-promoting phytochemicals in humans (Singh *et al.*, 2018) [28]. Accordingly, *M. oleifera* is described as a great remedy to fight malnutrition (Sujatha and Patel, 2017) [30] because of its easiness in the cultivation and distribution of phytochemicals in each part of the plant, including leaves, flowers, pods, and seeds (Mushtaq *et al.*, 2021) [18]. In this regard, the most abundant constituents are vitamins (Glover-Amengor *et al.*, 2017) [10], polyphenols and carotenoids (Zhu *et al.*, 2020) [31], phytosterols and tocopherols (Saini *et al.*, 2016) [26], glucosinolates (Lopez-Rodriguez *et al.*, 2020) [16], folic acid (Saini *et al.*, 2016) [26], polyunsaturated fatty acids (Aly *et al.*, 2016) [3], and minerals (Meireles *et al.*, 2020) [17]. According to the literature, more than 20 different pharmacological activities have been reported for this plant from both *in vivo* and *in vitro* studies (Saucedo-Pompa *et al.*, 2018) [27]. The proximate analysis of the plant revealed high percentage

yield of carbohydrate and protein which is suitable for foods fortification and their use as nutritional supplements is highly promising (Bamishaiye *et al.*, 2011) [4]. Hence, this study is aimed to investigate the effects of defatted *Moringa oleifera* seed on skeletal muscle of protein energy malnourished rats.

2. Materials and Methods

Plant materials

Moringa oleifera seed was bought from Kogi State University farm Anyigba. The seeds were removed from the seed coat and dried at 60°C and pulverized. A known weight of the pulverized seeds was measured into a savette paper wrapped by the use of a white thread; it was thereafter kept in a washed and dried conical flask. N-Hexane was added to the conical flask and shaken vigorously and left to stand for about three hours to defat properly. The defatted seed was untied and spread on a new savette paper for proper drying at room temperature.

Table 1: Components of the Control and Test Diets

| Diet Components | Protein energy malnutrition diet | Fish meal-based recovery diet | Defatted <i>Moringa oleifera</i> seed-based recovery diet |
|--|----------------------------------|-------------------------------|---|
| Defatted grounded <i>Moringa oleifera</i> seed | 40 g | - | 100 g |
| Grounded fish | - | 100 g | - |
| Corn chaff | 530 g | 470 g | 470 g |
| Vitaflash amino WSP (vitamins-amino acids) | 30 g | 30 g | 30 g |
| Vegetable oil | 50 g | 50 g | 50 g |
| Sucrose | 350 g | 350 g | 350 g |

***Vitaflash Amino WSP (vitamins-amino acids) composition per 1000 gram:** vitamin A, 10000000i.u; vitamin D3, 2000000i.u; vitamin E, 15000 mg; vitamin K3, 2500 mg; vitamin B1,1000 mg; vitamin B2, 2000 mg; vitamin B6, 2000 mg; vitamin B12, 10000 mcg; Folic acid,300 mg; Ca-d-pantothenate, 7500 mg; Nicotinic acid, 20000 mg; choline chloride, 15000 mg; vitamin C, 40000 mg; DL-methionine, 50000 mg; L-lysine, 50,000 mg; Amino acids, 52,000 mg

Experimental animals

Sixteen (16) female albino rats of waster strain (*Rattus Novergicus*) weighing between 57-120g were used for the study. The rats were obtained from the animal house of the department of Biochemistry, Kogi State University, Anyigba, Kogi State. All the rats were fed with growers' marsh and clean water for a week in the animal house of Biochemistry, Kogi State University, Anyigba for them to acclimatize prior to experimentation. They were kept in properly ventilated cages.

Animal grouping

Sixteen albino rats (weighing 57-120 g) were initially grouped into two groups of 'A' (Made up of the four {4} positive control fed with growers' feed) and 'B' (made up of the twelve rats fed with the Protein Energy Malnutrition diet). At the end of the twenty-one (21) days of malnourishment four rats under 'B' were randomly selected and sacrificed with subsequent removal of their skeletal muscles to serve as the negative control (Group C). The remaining eight rats under 'B' were grouped into 'D' and 'E' made up of four {4} rats each. Group A (Positive control): fed with normal growers' feed. Group C (Negative control): fed only with Protein Energy

Malnutrition diet.

Group D: fed with 20% *M. oleifera* seed-based recovery diet.

Group E: fed with 20% fish meal-based recovery diet.

At the end of the 21 days of feeding the animals with the recovery diets, the rats were decapitated by cervical dislocation and were sacrifice.

Preparation of tissue homogenate

The rats were sacrificed by cervical dislocation and blood was collected by jugular puncture. Blood samples were collected into plain and some into EDTA coated sample bottles (to prevent clotting) for serum and hematological analysis respectively. Skeletal muscle from the hind limbs was quickly extracted into iced cold solutions of 250 mM sucrose buffer (250 mM sucrose, 10 mMTris, pH 7.4) (Akanji *et al.*, 1989) [2].

Serum was thereafter prepared by centrifuging the blood samples at 3000 rpm for 5 minutes (Ogbu and Okechukwu, 2001) [20]. The skeletal muscle was homogenized in an iced cold mortar and pestle using the buffer as the homogenizing medium. The suspension of tissue homogenate was stored in aliquot units in Eppendoff tubes and stored in the freezer. The homogenate was kept frozen overnight to ensure maximum release of the enzymes (Ngha *et al.*, 1989) [19] and thereafter used for enzyme assay.

Biochemical assay

The protein concentration in the tissue homogenates was determined using Biuret method described by Gornall *et al.* (1949) [11] using bovine serum albumin as the standard protein. Serum albumin concentration was quantified by the method described by Doumas *et al.*, (1971) [8]. Activity of aspartate transaminase (AST), alanine transaminase (ALT),

Na⁺/K⁺ ATPase, in skeletal muscle tissue homogenate were determined following the method reported by Reitman and Frankel (1957) [25]. Ca²⁺ -ATPase was assayed in the skeletal muscle tissue homogenate after the forth and eight weeks using the procedure described by Bewaji (2004) [5].

Analysis

All data are expressed as the mean of four (4) replicates. Statistical evaluation of mean was performed by MS excel.

3. Results

3.1 Animal morphology

The weekly mean weight of the animals showed progressive decrease during malnourishment except in the control group. The reverse was case when treatments with recovery diets commenced. Group A (control) animals grew well, had smooth body fur, an oblong face and tails covered with fur;

no loss of fur was observed in any part. In contrast, the malnourished animals (Group B) experienced loss of appetite which could have led to the observed loss of body fur, developed moon face, circumference of the head remaining the same, scaly tails, bulged eyes, muscle wasting. There was a gradual improvement in the aforementioned morphological changes as the treatment progressed.

3.2 Effects of diets on liver function indices (AST, ALT, serum albumin, Protein)

As shown in figure 1, the serum concentration of AST, ALT, serum albumin, and protein was reduced in experimental rats after feeding with low protein iso-caloric diets. This serum concentration of these markers greatly increased after feeding the malnourished animals with the formulated treatment feeds.

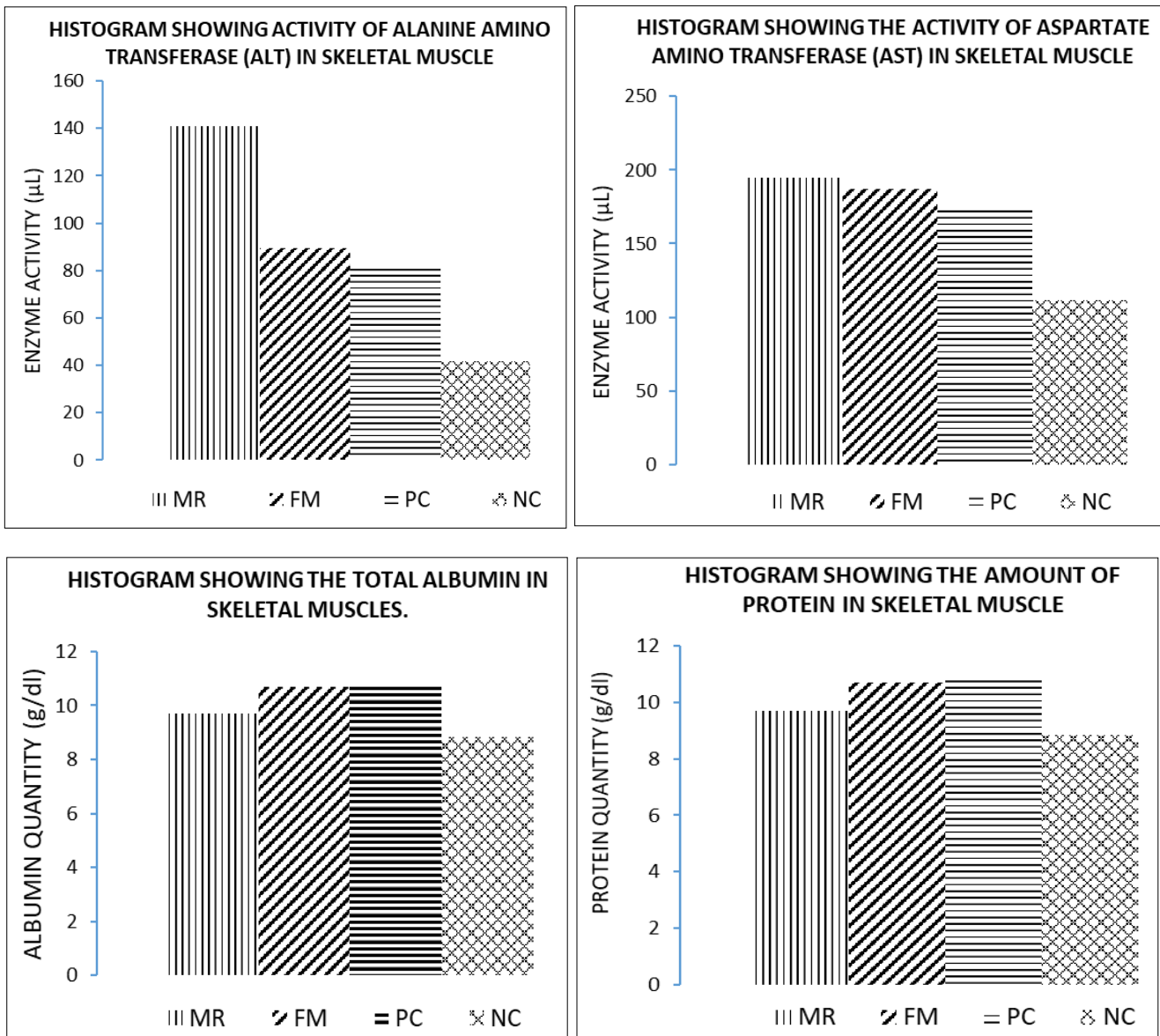


Fig 1: The effect of administration of defatted *Moringa oleifera* seed, fishmeal feed and normal grower’s feed on the AST, ALT, serum albumin, and protein level in skeletal muscle of albino rats. MR= *Moringa oleifera* rich meal; FM= fish meal; PC= positive control; NC= negative control

3.3 Calcium ATPase activity

As shown in figure 2, the calcium ATPase activity of the malnourished rats had a rise in activities compared with the

positive control However, ATPase activity in the *Moringa oleifera* treated group had great increase in activity after treatment

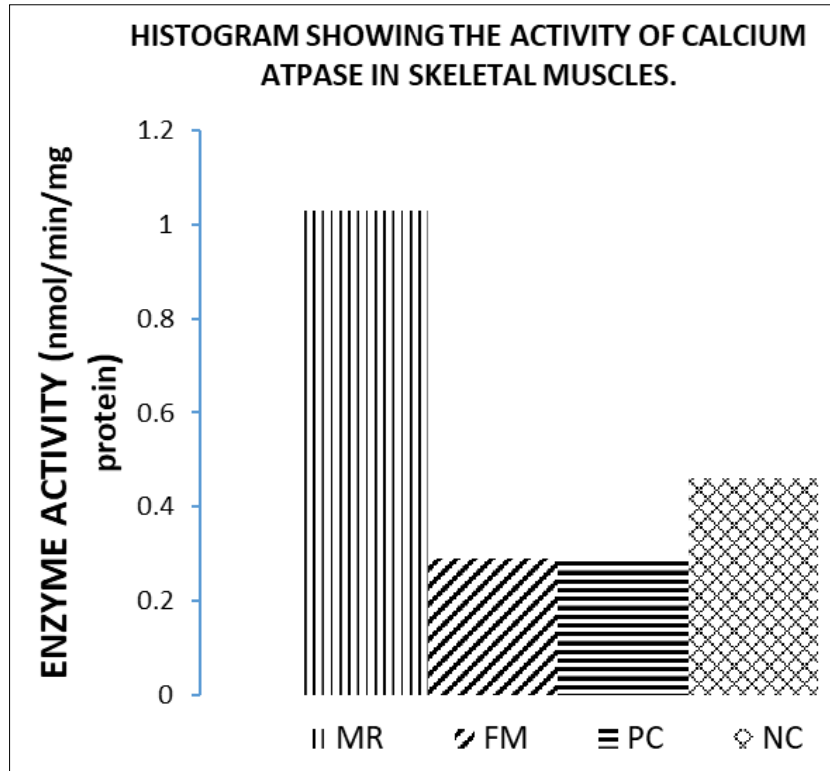


Fig 2: The effect of administration of defatted *Moringa oleifera* seed, fishmeal feed and normal grower’s feed on calcium ATPase activity in skeletal muscle of albino rats. MR= *Moringa oleifera* rich meal; FM= fish meal; PC= positive control; NC= negative control

3.4 Na⁺/K⁺ ATPase activity

As shown in figure 3, the Na⁺/K⁺ ATPase activity was reduced in experimental rats after feeding with low protein

iso-caloric diets. This serum concentration of Na⁺/K⁺ ATPase increased after feeding the malnourished animals with the formulated treatment feeds.

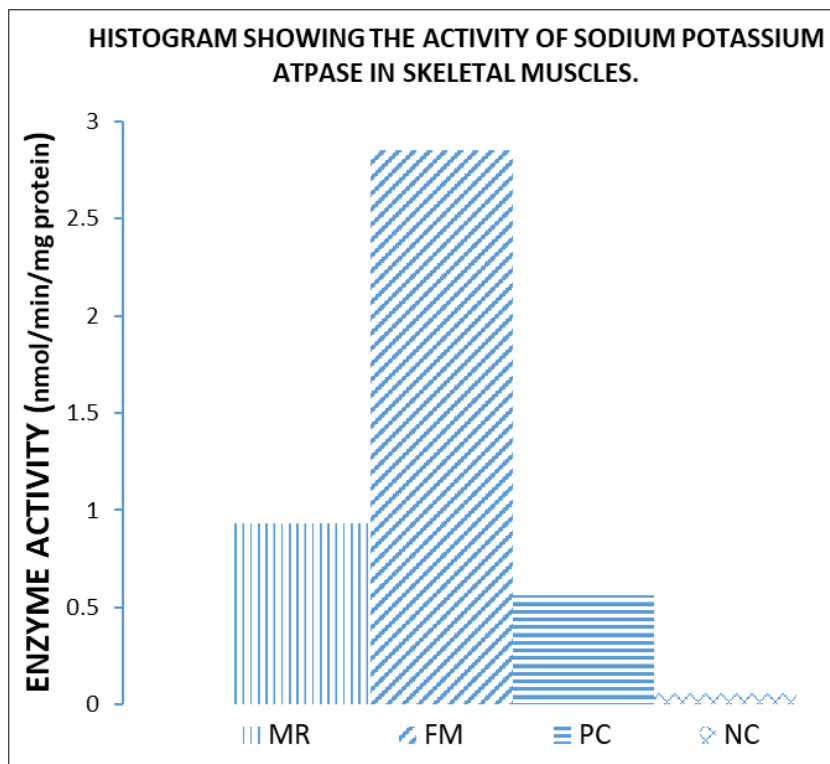


Fig 3: The effect of administration of defatted *Moringa oleifera* seed, fishmeal feed and normal grower’s feed on Na⁺/K⁺ ATPase activity in skeletal muscle of albino rats. MR= *Moringa oleifera* rich meal; FM= fish meal; PC= positive control; NC= negative control.

4. Discussions and Conclusion

Malnutrition, particularly Protein-Energy Malnutrition (PEM), can lead to various morphological changes such as

edema, diarrhea, weight loss, hair loss, retinopathy, vulnerability to infections, and reduced total protein levels (Raphael *et al.*, 2020) [24]. The present research aligns with

these findings, showing that improved nutrition can reverse these adverse morphological changes observed in test animals to some extent, though with varying capacity.

Patients with protein deficiency (Kwashiorkor) are known to have low hematological indices, including AST, ALT, serum albumin, and protein levels (Adesola, 1986; Bolarinwa *et al.*, 1991) ^[1, 6]. However, feeding animals with a diet based on *Moringa oleifera* leaves, which are rich in β -carotene, protein, vitamin C, calcium, and potassium (Sreeja *et al.*, 2021) ^[29], indicates its potential as a sustainable substitute for food protein in rat feed. Moreover, the inclusion of *M. oleifera* leaf-based diet in this study restored liver markers, such as serum albumin levels and AST/ALT activities, suggesting improved liver function.

Following skeletal muscle degeneration induction, the activity of Ca^{2+} ATPase in the skeletal muscle of all groups decreased. However, animals fed with the *M. oleifera* leaf-based diet exhibited better resistance to the effects of malnutrition on enzyme activity compared to other groups. The reduction in Ca^{2+} ATPase activity during malnutrition might result from decreased enzyme synthesis or inactivation, leading to unhealthy high concentrations of Ca^{2+} within the cell and eventual cell death (Carafoli, 1991) ^[7]. Conversely, the significant increase in Ca^{2+} ATPase activity observed in the *M. oleifera* leaf-based diet group could be due to enzyme activation or increased synthesis during treatment, suggesting the reversibility of the effect of malnourishment on this enzyme.

The Na^+/K^+ -ATPase plays a crucial role in maintaining the concentration of ions across the plasma membrane, contributing to the resting membrane potential in cells. Studies have shown that its levels are low in certain metabolic conditions (Dufayet *et al.*, 1998; Radosinska and Vrbjar, 2021) ^[9, 23], which aligns with the findings of this study. Notably, animals fed with *M. oleifera* leaf-based diet and fish meal diet exhibited a significant increase in serum Na^+/K^+ -ATPase activity after treatment, possibly indicating enzyme activation or increased synthesis during the treatment, suggesting the reversible nature of the effect of malnutrition on Na^+/K^+ -ATPase.

In conclusion, this research demonstrates that malnutrition-induced muscle degeneration negatively impacts Ca^{2+} ATPases, Na^+/K^+ -ATPase, AST, ALT, protein levels, and serum albumin in skeletal muscles. However, the adverse effects are most effectively improved by the *M. oleifera* leaf-based diet. The mechanism by which *M. oleifera* leaf corrects muscle degeneration caused by PEM might involve increasing the synthesis and/or activity of the examined markers, thereby preventing oxidative stress and possibly preventing energy depletion in the skeletal muscles. Thus, the *M. oleifera* leaf-based diet proves to be a sustainable replacement for food protein in the diet.

5. References

- Adesola AO. The influence of severe protein deficiency (Kwashiorkor) on gastric acid secretion in Nigerian children. *The British Journal of Surgery*. 1968;55(11):866.
- Akanji MA, Ngaha EA. Effect of repeated administration of berenil and urinary excretion with corresponding tissue pattern in rats; *Pharmaceutical toxicology*. *Pharmaceutical Toxicology*. 1989;64:272-275.
- Aly AA, Maraie RW, Ali HGM. Fatty acids profile and chemical composition of Egyptian *Moringa oleifera* seed oils. *Journal of the American Oil Chemists' Society*. 2016;93(3):397-404.
- Bamishaiye EI, Olayemi FF, Awagu EF, Bamshaiye OM. Proximate and phytochemical composition of *Moringa oleifera* leaves at three stages of maturation. *Advance Journal of Food Science and Technology*. 2011;3(4):233-237.
- Bewaji CO. Enzymes-Determination of the Adenosine Triphosphatase Activities in Rat Brain. *Experimental Biochemistry*. Klobex Academic Publishers; c2004. p. 45-46.
- Bolarinwa AF, Oyebola DDO, Akindeinde GB. Effect of malnutrition on basal and induced gastric acid secretion. *Nigerian Journal of Physiological Sciences*. 1991;5:144-148.
- Carafoli E. Calcium pump of the plasma membrane. *Physiological Reviews*. 1991;71(1):129-153.
- Doumas BT, Watson WA, Biggs HG. Albumin Standards and the Measurement of Serum albumin with Bromocresol Green. *Clinica. Chimica. Acta*. 1971;31:87.
- Dufayet De La Tour D, Raccach D, Jannot MF, Coste T, Rougerie C, Vague P. Erythrocyte Na^+/K^+ ATPase activity and diabetes: relationship with C-peptide level. *Diabetologia*. 1998;41:1080-1084.
- Glover-Amengor M, Aryeetey R, Afari E, Nyarko A. Micronutrient composition and acceptability of *Moringa oleifera* leaf-fortified dishes by children in Ada-East district, Ghana. *Food Science & Nutrition*. 2017;5(2):317-323.
- Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry*. 1949;177:751-766.
- Jee KO. Protein energy malnutrition: An overview. *International Journal of Homoeopathic Sciences*. 2021;5(1):368-373.
- Kasas AE, Farag IM, Darwish HR, Soliman YA, Nagar EE, Ibrahim MA, *et al.* Molecular characterization of alpha subunit 1 of sodium pump (ATP1A1) gene in *Camelus dromedarius*: Its differential tissue expression potentially interprets the role in osmoregulation. *Molecular Biology Reports*. 2022;49(5):3849-3861.
- Sharma M, Sharma SB. Antioxidant evaluation of *Moringa oleifera* leaf extracts. *Int. J. Biol. Sci*. 2020;2(1):22-23. DOI: 10.33545/26649926.2020.v2.i1a.19
- Liu M, Yang H, Mao Y. Magnesium and liver disease. *Annals of Translational Medicine*. 2019;7(20).
- Lopez-Rodriguez NA, Gaytán-Martínez M, de la Luz Reyes-Vega M, Loarca-Pina G. Glucosinolates and isothiocyanates from *Moringa oleifera*: Chemical and biological approaches. *Plant Foods for Human Nutrition*. 2020;75:447-457.
- Meireles D, Gomes J, Lopes L, Hinzmann M, Machado J. A review of properties, nutritional and pharmaceutical applications of *Moringa oleifera*: integrative approach on conventional and traditional Asian medicine. *Advances in Traditional Medicine*. 2020;20:495-515.
- Mushtaq BS, Hussain MB, Omer R, Toor HA, Waheed M, Shariati MA, Sergey P, Heydari M. *Moringa*

- oleifera* in malnutrition: A comprehensive review. *Current Drug Discovery Technologies*. 2021;18(2):235-243.
19. Ngaha EO, Akanji MA, Madusolumuo MA. Studies on correlations between chloroquine-induced tissue damage and serum enzyme changes in the rat. *Experientia*. 1989;45:143-146.
 20. Ogbu SI, Okechukwu EI. The effect of storage temperature prior to separation on plasma and serum potassium. *Journal of Medical Laboratory Science*. 2001;10:1-4.
 21. Ogunniyi KAB. A Study of Protein Energy Malnutrition (PEM) Among Under-Five Children Okuku, Odootin Local Government Area of Osun State, Nigeria. *Adeleke University Journal of Science*. 2022;1(1):258-266.
 22. Olayinka LM, Clement B. Protective role of *Moringa oleifera* leaf-based diet on protein-energy malnutrition induced skeletal muscle degeneration. *International Journal of Scientific Reports*. 2017;3(2):54-62.
 23. Radosinska J, Vrbjar N. Erythrocyte deformability and Na, K-ATPase activity in various pathophysiological situations and their protection by selected nutritional antioxidants in humans. *International Journal of Molecular Sciences*. 2021;22(21):11924.
 24. Raphael JE, Atanu FO, Mohammed SS, Abdulrahman SI. Anti-anaemic effects of *Moringa oleifera* seeds (Lam) in protein energy malnourished rats. *GSC Biological and Pharmaceutical Sciences*. 2020;13(1):197-202.
 25. Reitman S, Frankel S. Colometric methods for aspartate and alanine aminotransferase. *American Journal of Clinical Pathology*. 1957;28:55-60.
 26. Saini RK, Sivanesan I, Keum YS. Phytochemicals of *Moringa oleifera*: a review of their nutritional, therapeutic and industrial significance. *Biotechnology*. 2016;6:203.
 27. Saucedo-Pompa S, Torres-Castillo JA, Castro-López C, Rojas R, Sánchez-Alejo EJ, Ngangyo-Heya M, *et al.* *Moringa* plants: Bioactive compounds and promising applications in food products. *Food Research International*. 2018;111:438-450.
 28. Singh VP, Arulanantham A, Parisipogula V, Arulanantham S, Biswas A. *Moringa oleifera*: Nutrient dense food source and world's most useful plant to ensure nutritional security, good health and eradication of malnutrition. *European Journal of Nutrition and Food Safety*. 2018;8(4):204-214.
 29. Sreeja M, Jayasri P, Keerthi N, Yeshashwini J, Praveen J. *Moringa oleifera*: A review on nutritive importance and its potential use as nutraceutical plant. *Journal of Medicinal Plants*. 2021;9(2):15-7.
 30. Sujatha BK, Patel P. *Moringa oleifera*—nature's gold. *Imperial Journal of Interdisciplinary Research*. 2017;3(5):1175-1179.
 31. Zhu Y, Yin Q, Yang Y. Comprehensive investigation of *Moringa oleifera* from different regions by simultaneous determination of 11 polyphenols using UPLC-ESI-MS/MS. *Molecules*. 2020;25(3):676.
 32. Lambe MO, Bewaji CO. *Moringa oleifera* leaf-based diet combats malnutrition in rats. *Biokemistri*. 2022;33(2).