Original Research Article

Protective role of *Moringa oleifera* leaf-based diet on protein-energy malnutrition induced skeletal muscle degeneration

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**ABSTRACT**

**Background:** Sequel to the diverse diseases resulting in muscle mass degeneration and its key role in the prognosis of the diseases. For instance, currently, only resistance exercise can be used to promote recovery of mass/strength following disuse atrophy. But in contrast, many patients are unable or unwilling to exercise at a sufficient intensity to promote muscle growth. It would therefore be of great advantage to develop natural compounds that counteract the negative effects of skeletal muscle degenerative diseases especially in the form of therapeutic feed). The present study was aimed at elucidating the mechanism by which *M. oleifera* ameliorates skeletal muscle degeneration caused by malnutrition.

**Methods:** The experimental animals were malnourished with low protein iso-caloric diets for four weeks after which they were treated with 25% *M. oleifera* leaf – based diet, vitamin E supplemented feed and soy bean based-diet for another four weeks.

**Results:** There was a significant reduction in the activity of calcium ATPase in the skeletal muscle of animals induced with skeletal muscle degeneration. However, this activity was significantly increased following treatments with the most significant increase observed in the skeletal muscle of animals treated with *M. Oleifera* leaf based diet followed by those fed with vitamin E supplemented diet.

**Conclusions:** In summary, the study revealed that the mechanism by which *M. oleifera* leaf corrects muscle degeneration caused by PEM might be by increasing calcium ATPase activity and/or its synthesis and by preventing oxidative stress due to its antioxidant properties.

**Keywords:** Calcium ATPase, Mechanism, Iso-caloric diet, *M. oleifera*, Muscle atrophy

**INTRODUCTION**

Muscle degeneration occurs following alterations in normal balance between protein synthesis and degradation which results in down-regulation of protein synthesis pathways, and an activation of protein degradation. An important ion that maintains this strict balance in the cell is calcium ion. Diseases and conditions which cause decrease in muscle mass include HIV/AIDS, cancer, infections and tuberculosis. Most of these diseases negatively affect calcium homeostasis, ATPase activity and redox state of cells causing oxidative stress.

Inhibition of calcium adenosine triphosphatase (Ca^{2+}-ATPase) prevents pumping of calcium in the muscle cell which would otherwise be used for maintaining overall health of the muscle, thus resulting in a wasting and degeneration of (muscle) tissue.

There is a very large trans-membrane electrochemical gradient of calcium ions driving its entry into cells, yet it is very important for cells to maintain low concentrations of calcium ions for proper cell signalling. Thus, it is necessary for cells to employ ion pumps to remove...
excess calcium ions. Calcium ATPase is a form of P-ATPase that transfer calcium after muscle contraction. The enzyme exits in two forms namely, plasma membrane calcium ATPase (PMCA) and sarcoplasmic endoplasmic reticulum calcium ATPase (SERCA). They are located on various membrane types and serve to translocate calcium ions across these membranes against very steep concentration gradients. PMCA resides in the sarcoplasmic reticulum (SR) within muscle cells where it transfers Ca²⁺ from the cytosol of the cell to the lumen of the SR at the expense of ATP hydrolysis during muscle relaxation.

Malnutrition is a medical condition caused by improper or insufficient diet. It is a category of diseases that includes under-nutrition, obesity and overweight, and micronutrient deficiency among others. It is frequently used to mean just under nutrition from either inadequate calories or inadequate specific dietary components for whatever reason. The term “severe malnutrition” is however often used to refer specifically to protein-energy malnutrition (PEM) which is often associated with micronutrient deficiency.

*Moringa oleifera* is an angiosperm belonging to the family Moringaceae. Its English names include horseradish tree, drumstick or ben oil seed tree and locally known as ‘Zogalegandi’ in Hausa, ‘Ewe igbale’ in Yoruba and ‘Okweoyibo’ in Igbɔ. It is a fast growing, drought resistant tree native to the southern foot hills of the Himalayas in north western India. However, it is now cultivated in all regions of the world. Several biological properties ascribed to various parts of this plant have been reviewed in the past. These include its use as an antioxidant, anticarcinogenic, antiulcer, antibacterial, and antifungal. Phytochemical analyses of its leaves have been particularly rich in potassium, calcium, phosphorous, iron, vitamins A and D, essential amino acids as well as antioxidants such as β-carotene, vitamin C, and flavonoids. Studies on proximate and phytonutrient analysis of the leaf by Bamishaiye et al, 2011 also showed that it has high percentage of carbohydrate and protein and compared favourably with other high protein/ carbohydrates food crops. It is however a potential leaf source of food that is suitable for fortification of foods and their use as nutritional supplements is highly promising. Moreover, its fruit pod and leaves have been used to combat malnutrition, especially among infants and nursing mothers for enhancing milk production. Dietary consumption of its part is therein promoted as a strategy of personal health preservation and self-medication in various diseases.

In view of the aforementioned effects of muscle degeneration on calcium homeostasis in disease conditions, it would therefore be of great advantage to develop natural compounds that counteract the negative effects of skeletal muscle degenerative diseases without adversely affecting calcium levels or the redox state of the muscle cells. Although *M. oleifera* has been used effectively in cases of malnutrition, this study evaluates its mechanism of ameliorating skeletal muscle degeneration caused by malnutrition. This might be a useful tool in formulating drugs for the management and treatment of skeletal muscle degeneration resulting from different diseases of global concern.

**METHODS**

**Chemicals and reagents**

Adenosine triphosphate (ATP) is a product of Sigma Aldrich Chemical Company Poole England. All other chemicals and reagents used were of analytical grade.

**Feed materials**

Dried blended *M. oleifera* leaf was purchased from Faculty of Agriculture university of Ilorin. DL-methionine, vitamin- mineral mix, corn chaff, sucrose, yellow corn, soy bean, and soy oil were purchased from Ilorin. These were formulated as presented in Table 1.

**Table 1: Components of the control and test diets.**

| Diet composition | Control diet (g/kg) (25%) | Test diet (4% soy bean) (g/kg) | Test diet (4% Moringa leave) (g/Kg) |
|------------------|---------------------------|-----------------------------|----------------------------------|
| Soy bean         | 250                       | 40                          | ---                              |
| *Moringa leaves* | ---                       | ---                         | 40                               |
| Corn starch      | 516                       | 100                         | 100                              |
| Soy bean oil     | 40                        | 40                          | 40                               |
| Cellulose        | 40                        | 400                         | 400                              |
| Sucrose          | 100                       | 366                         | 366                              |
| DL-methionine    | 4                         | 4                           | 4                                |

*Vitamin/ Mineral mix: Vitamin A 4,000,000 i.u; Vitamin D3, 800,000 i.u; Tocopherols, 400 i.u; Vitamin K3 800mg, Folacin, 200 mg; Thiamine, 600mg; Riboflavin 1,800 mg; Niacin, 6000 mg; Calcium pantothenate, 4 mg; Biotin, 8 mg; Manganese, 30,000 mg. Zn, 20,000 mg; Iron, 8,000 mg; Choline chloride 80,000 mg; Copper, 2,000 mg; Iodine, 480 mg; Cobalt, 80 mg; Selenium, 40 mg; BHT, 25,000 mg Anti- caking agent 6000 mg.

**Experimental animals**

Forty female weanling albino rats (*Rattus norvegicus*) with mean weight of 65± 0.26 g were used for this research. They were procured from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin. All the animals were fed with commercially prepared feed and clean water *ad libitum* for one week to acclimatize.
**Induction of muscle degeneration**

Skeletal muscle degeneration was induced by feeding the animals with low protein (4%) iso-caloric diet *ad libitum* for four weeks (a slight modification of the method used by Nadia et al.16)

**Animal grouping**

The animals were randomly divided into four groups of ten animals each. Control animals constituted Group 1. Group 2 and Group 3 animals were fed low protein isocaloric diet (4% soy meal- based diet) while Group 3 feed was supplemented with vitamin E (40 mg/Kg body weight). Group 4 animals were also fed low protein iso-caloric diet (constituted with 4% *M. oleifera* leaf- based diet).

After 4 weeks of induction, the animals were subdivided into treatment groups. Group 2a and 2b were treated with 25% soy and 25% *M. oleifera* leave based diet respectively. Group 3a and b were treated with 25% soy supplemented with vitamin E and 25% soy alone respectively while group 4 animals were treated with 25% *M. oleifera* leaf- based diet. These animals were treated for another 4 weeks before they were sacrificed for analysis.

**Anthropometric measurements**

Anthropometric measurements taken include; weight, circumference of the head and length of the whole rat. The anthropometric study was done before the animals were induced with skeletal muscle atrophy (by PEM), and during the administration of treatment feeds.

**Collection of blood and preparation of homogenates**

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 haematological 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).

Serum was thereafter prepared by centrifuging the blood samples at 3000 rpm for 5 minutes.17 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 Eppendorf tubes and stored in the freezer. The homogenate was kept frozen overnight to ensure maximum release of the enzymes 18 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, using bovine serum albumin as the standard protein.19 Serum albumin concentration was quantified by the method described by Doumas et al. 20 Ca²⁺-ATPase was assayed in the skeletal muscle tissue homogenate after the forth and eight weeks using the procedure described by Bewaji.21

**Haematological analysis**

The haematological parameters analyzed include, red blood cell count, white blood cell count, blood haemoglobin, full blood count. These were analyzed using automated haematological analyzer.

**Statistical analysis**

Data were analyzed using one-way ANOVA and differences were considered significant when P <0.05. Values presented are mean±SEM. SPSS Version 16.0 software for windows was used to analyse the data.

**RESULTS**

**Animal morphology**

The weekly mean weight of the animals showed progressive decrease except in the control group as shown in Figure 1. The reverse was however the case when treatments commenced as in Figure 2. Group 1 (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 (i.e Groups2, 3 and 4) 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.

**Haematological analysis**

The result of the haematological analysis is presented in Tables 2 and 3. All the haematological parameters assessed had significant reduction during malnutrition in all the test groups. However the indices increased significantly after treatment and could be compared to the control.

**Serum albumin**

The serum albumin concentration was significantly reduced in all the groups compared with the control.
during weeks of malnutrition. However, a significant increase was observed after treatments across all the groups with the highest increase in group 4 animals as shown in Table 4.

**ATPase activity**

The specific activity of Ca$^{2+}$-ATPase of the animals was used to plot Michaelis-Menten curve as depicted in Figure 3 to Figure 8. These curves showed that the calcium ATPase of the malnourished animals had lower activities compared with the control, except for group 4 where which the enzyme activity curve was seen not to have a similar characteristic with Michaelis-Menten curve though with higher activity. However, ATPase activity in all the groups had significant increase in activities after treatment though with varying activities.

![Figure 1: Average weight of malnourished rats per week.](image1)

![Figure 2: Average weight of treated rats per week.](image2)

**Table 2: Haematological indices of rats before treatment.**

|               | Group 1        | Group 2        | Group 3        | Group 4        |
|---------------|----------------|----------------|----------------|----------------|
| **RBC (x10$^6$/µl)** | 7.46 ± 0.01$^a$ | 6.16 ± 0.08$^b$ | 6.22 ± 0.02$^b$ | 6.36 ± 0.07$^b$ |
| **WBC (10$^3$/µl)** | 17.8 ± 0.35$^a$ | 12.3 ± 0.10$^b$ | Xxx            | 12.9 ± 0.05$^b$ |
| **HGB (g/dl)**   | 12.7 ± 0.15$^a$ | 10.1 ± 0.05$^b$ | 11.2 ± 0.15$^c$ | 11.5 ± 0.10$^c$ |
| **PCV (%)**      | 48.4 ± 0.20$^a$ | 35.0 ± 0.25$^b$ | 39.0 ± 0.30$^c$ | 38.1 ± 0.40$^d$ |

$^a$Data with different superscript across the same row are significantly different at p < 0.05

RBC = Red blood cells, WBC = White blood cells, HGB = haemoglobin, PVC = Pack cell volume

Group 1= control animals, Group 2= malnourished with 4% soy, Group 3= malnourished with 4% soy + vit. E, Group 4 = malnourished with M. oleifera.
Table 3: Haematological indices of rats after treatment.

|                  | RBC (x10^6/µl) | WBC (10^3/µl) | HGB (g/dl) | PCV (%) |
|------------------|-----------------|---------------|------------|---------|
| Group 1          | 6.75 ± 0.03 a   | 14.75 ± 0.38 a| 11.60 ± 0.10 a| 40.20 ± 0.10 a |
| Group 2a         | 7.39 ± 0.05 b   | 21.40 ± 0.20 b| 12.70 ± 0.15 a| 51.80 ± 0.10 c |
| Group 2b         | 6.58 ± 0.01 b   | 14.30 ± 0.15 a| 11.30 ± 0.10 a| 39.30 ± 0.35 a |
| Group 3a         | 7.45 ± 0.03 a   | 21.30 ± 0.40 b| 12.00 ± 0.15 a| 47.50 ± 0.10 bc |
| Group 3b         | 7.03 ± 0.01 b   | 18.70 ± 0.50 ab| 12.30 ± 0.10 a| 48.65 ± 0.33 c |
| Group 4          | 6.66 ± 0.06 a   | 14.50 ± 3.20 | 11.60 ± 0.95 a| 45.10 ± 5.20bc |

*Data with different superscript along the same column are significantly different at p < 0.05.
RBC = Red blood cells, WBC = White blood cells, HGB= haemoglobin, PVC = Pack cell volume
Group 1= control animals, Group 2= malnourished with 4% soy, Group 3= malnourished with 4% soy + vit. E, Group 4= malnourished with 4% M. Oleifera.

Table 4: Effects of muscle degeneration on blood serum concentration of rats.

| Serum albumin conc (g/l) | Group 1 | Group 2a | Group 2b | Group 3a | Group 3b | Group 4 |
|-------------------------|---------|----------|----------|----------|----------|---------|
| Before treatment        | 3.690±0.05 a | 1.407±0.00 b | 1.407±0.00 b | 0.352±0.00 c | 0.352±0.00 c | -1.583±0.01 d |
| After treatment         | 14.39±0.05 a | 15.37±0.01 c | 19.62±0.04 e | 18.64±0.07 d | 13.08±0.06 b | 23.06±0.13 f |

*Data with different superscript across the same row are significantly different at p < 0.05
Group 1= control animals, Group 2= malnourished with 4% soy, Group 3= malnourished with 4% soy + vit. E, Group 4= malnourished with 4% M. oleifera.

Figure 3: Ca^{2+} ATPase activity in the skeletal muscle of malnourished rats.

Figure 4: Ca^{2+} ATPase activity in the skeletal muscle of group 2a rats.
Group 1= control animals, Group 2= malnourished with 3% soy, Group 2b= treated with 25% *M. oleifera*.

**Figure 5:** Ca$^{2+}$ ATPase activity in the skeletal muscle of group 2b rats.

Group 1= control animals, Group 3= malnourished with 3% soy + vitamin E, Group 3a= treated with 25% soy.

**Figure 6:** Ca$^{2+}$ ATPase activity in the skeletal muscle of group 3a rats.

Group 1= control animals, Group 3= malnourished with 3% soy + vitamin E, Group 3b= treated with 25% soy.

**Figure 7:** Ca$^{2+}$ ATPase activity in the skeletal muscle of group 3b rats.
Malnutrition (PEM) results into a lot of morphological changes which includes; muscle wasting (especially in the thigh and buttocks), alopecia (loss of fur), oedema, anaemia, infections and the subject becomes apathetic and lethargic among others, hence the morphological changes observed in the test animals. However, morphological changes occurring as a result of malnutrition are often reversed by improved nutrition. The result of this study showed that all the adverse morphological changes observed in the test animals was improved with the administration of the improved diets though with different capacity. Animals fed only 40% soy-based diet had the lowest improvement when compared with those fed vitamin E supplemented diet and those fed M. oleifera-based diet. The leaves of M. oleifera is a source of both macro- and micronutrients such as β-carotene, protein, vitamin C, calcium, and potassium and its use as an antioxidant is documented. Higher growth pattern observed in group 3 and group 4 may have been because of their antioxidant properties they have in common added to other nutritional qualities. However, the highest improvement was observed in M. oleifera-based diet fed animals.

One of the common complications of protein-energy malnutrition is anaemia. Animals fed on protein calorie malnourished diets had been reported to have significant reduction in haemoglobin concentrations. It has also been well documented that kwashiorkor and marasmus (protein energy malnutrition) patients had low levels of haematological indices. Similarly, in this report, there was reduction in the haematological parameters observed during malnutrition. However, after treatment, all the parameters were significantly increased in all animal groups. This is also in accordance with the report of researchers which have shown that several protein-rich foods increase Hb concentrations in human and animal studies.

In 1991, Bolarinwa et al reported a significant reduction in the levels of total protein in protein-calorie malnourished rats. Similarly, a correlation had earlier been drawn between total protein levels and severity of protein energy malnutrition. Serum albumin test being a liver function test, decrease in its concentration following the malnutrition suggests impaired absorption of protein in the intestine or even liver damage. Impaired intestinal absorption of protein may provide the liver with inadequate supply of amino acids to synthesize serum proteins (such as albumin), leading to a drop in serum protein level. Also, liver damage may impair the synthesis of serum proteins in the liver thereby leading to low serum levels. The reduction in the serum albumin concentration during malnutrition could be a sign of low protein.

Kinetic analysis of enzymes permits scientists to reconstruct the number and order of the individual steps by which enzymes transform substrates into products. Kinetic data combined with detailed information about an enzyme’s structure and its catalytic mechanisms, provide some of the most powerful clues to the enzyme’s biological function(s) and may suggest ways to modify it for therapeutic purpose. After the induction of skeletal muscle degeneration, Ca^{2+} ATPase activities in the skeletal muscle of all the Groups except Group 4 were significantly reduced. This might imply that M. oleifera leaf-based diet fed animals were able to resist the effects of malnutrition on the enzyme activity. The significant reduction in the activity might be as a result of a reduction in the synthesis or inactivation of the enzyme during the malnourished condition. Since the main function of the Ca^{2+} ATPase is to pump out Ca^{2+} from the muscle cell during Ca^{2+} ATPase deactivation.
cell thereby keeping the concentration of Ca\(^{2+}\) low, impaired or reduced activity of the enzyme will result in an unhealthy high concentration of Ca\(^{2+}\) within the cell which might finally result in cell death.\(^6\)

The significant increase in the activity of Ca\(^{2+}\) ATPase across all the groups after treatment could be as a result of activation or increase synthesis of the enzyme during treatment and however implies that the effect of malnourishment on Ca\(^{2+}\) ATPase is reversible. There was slight increase in calcium ATPase activity in groups fed 40% soy-based diet but that of \textit{M. Oleifera} leaf-based diet was significantly higher than other groups. This could as well imply that one of the corrective mechanisms of muscle degeneration by \textit{M. oleifera} is by activating the Ca\(^{2+}\) ATPases in the skeletal muscle thereby reinstalling Ca\(^{2+}\) homeostasis. A pioneering study has demonstrated association between an immobilized rodent skeletal muscle with increased level of oxidative stress, which could partially be arrested by vitamin E supplementation. However, subsequent investigation provided the definite mechanistic link between acute atrophy and oxidative stress.\(^33,34\) Vitamin E supplemented diet also resulted into a much significant increase in activity similar to those of \textit{M. Oleifera} leaf-based diet, as this might imply some relationship between the two feeds probably because of their antioxidant properties, perhaps calcium ATPase activity and oxidative stress are both key factors affected in muscle atrophy.\(^35\)

Conclusively, this research shows that though malnutrition induced muscle degeneration has adverse effects on Ca\(^{2+}\) ATPases of the skeletal muscle; the effects were most significantly improved by 25% \textit{M. oleifera} leaf- based diet. However, the activity of Ca\(^{2+}\) ATPases in the skeletal muscle of rats malnourished in the presence of \textit{M. oleifera} leaves was not significantly affected. Moreover, the parameters assessed shows that supplementing animal feed with Vitamin E has no significant effect on the changes induced by malnutrition.

Summarily, the mechanism by which \textit{M. oleifera} leaf corrects muscle degeneration caused by PEM might be by increasing Ca\(^{2+}\)-ATPase synthesis and/or activity, preventing oxidative stress, and by possibly preventing energy depletion in the skeletal muscles.

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\textbf{REFERENCES}

1. Ravasco P, Monteiro-Grillo I, Vidal PM, Camilo ME. Nutritional Deterioration In Cancer: The Role of Disease And Diet. Clin Oncol. 2003;15(8):443–50.
2. Baro M, Deubel TF. Persistent Hunger: Perspectives on Vulnerability, Famine, And Food Security In Sub-Saharan Africa. Annual Rev Anthropol. 2006;35:521.
3. Stefanie A, Thomas K, Dietmar P, Eva O, Alexander R, Lisa Z, Ilja R, Marius EM, Christoph G, Stephen P. Skeletal Muscle Depletion and Markers for Cancer Cachexia Are Strong Prognostic Factors in Epithelial Ovarian Cancer. PLoS One. 2015;10(10):1-13.
4. Bababunmi EA. Formulation And Method For Treating Skeletal Muscle Degeneration Caused By Malnutrition And Disease. United States Patent 6887499, 2002.
5. Sandri M. Signalling In Muscle Atrophy And Hypertrophy. Physiol. 2008;23:160-70.
6. Carafoli E. Calcium Pump of the Plasma Membrane. Physiol Rev. 1991;71(1):129–53.
7. Bababunmi EA, Olorunsogo OO, Bewaji CO. Pathological and chemical effectors of the erythrocyte calcium pumping protein: A review. Trop J Hlth Sci. 1994;1:33–47.
8. Laditan AA. Some Clinical Data of Prognostic Importance in Protein Calorie Malnutrition (PCM). Trop Geogr Med. 1976;28:216-9.
9. Jensen TP, Buckby LE. Expression f Plasma Membrane Ca2+ ATPase Family Members and Associated Synaptic Proteins in Acute and Cultured Organotypic Hippocampal Slices from Rat. Developmental Brain Res. 2004;152(2):129-36.
10. Katsilambros N. Clinical Nutrition In Practice. John Wiley & Sons; 2011: 37.
11. Dalziel JM. Useful Plants of West Tropical Africa. London: Crown Agents of Oversea Government and Administration; 1956: 23.
12. Anwar F, Latif S, Ashraf M, Gilani AH. Moringa oleifera: a food plant with multiple medicinal uses. Phytother Res. 2007;21:17-25.
13. Amaglo NK, Bennett RN, Lo Curto RB, Rosa EAS, Lo Turco V, Giuffrid A, et al. Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree Moringa oleifera L., grown in Ghana. Food Chem. 2010;122:1047–51.
14. Bamishaiye EI, Olayemi FF, Awagu EF, Bamshaiye OM. Proximate and Phytochemical Composition of Moringa oleifera Leaves at Three Stages of Maturation. Advance J Food Sci Technol. 2011;3(4):233-7.
15. Siddhuraju P, Becker K. Antioxidant Properties Of Various Solvent Extracts Of Total Phenolic Constituents From Three Different Agroclimatic Origins Of Drumstick Tree (Moringa oleifera Lam.) leaves. J Agricultural Food Chem. 2003;51(8):2144–55.
16. Bennis-Taleb N, Remacle C, Hoet JJ, Reusens B. A Low- Protein Isocaloric Diet During Gestation Affects Brain Development And Alter Permanently Cerebral Cortex Blood Vessels In Rat Offspring. J Nut. 1999;129(8):1613-9.
17. Ogbi SI, Okechukwu EI. The Effect of Storage Temperature Prior To Separation On Plasma and Serum potassium. J Mediterr Sci. 2001;10:1-4.
18. Ngaha EO, Akanji MA, Madusolomo MA. Studies on Correlation Between Chloroquine–Induced Tissue Damage And Serum Changes In Rats. Experimentia. 1989;45:143.

19. Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. J Biol Chem. 1949;177:751-66.

20. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with brom cresol green. Cli Chem acta. 1971;31:87.

21. Bewaji CO. Enzymes-Determination of The Adenosine Triphosphatase Activities in Rat Brain. Klobex Academic Publishers; 2004: 45-46.

22. Grover Z, Looi CE. Protien-Energy Malnutrition. Ped Clin North Am. 2009;56(5):1055-68.

23. Oyagbemi AA, Odetola AA, Azeez OI. Ameliorative effects of Cnidoscolus aconitifolius on anaemia and osmotic fragility induced by protein-energy malnutrition. African J Biotechnol. 2008;7(11):1721-6.

24. Verma AR, Vijayakumar M, Mathela CS, Rao CV. In vitro and in vivo antioxidant properties of different fractions of Moringa oleifera leaves. Food Chem Toxicol. 2009;47:2196–201.

25. Borelli P, Blatt S, Pereira J, deMaurino BB, Tsujita M, deSonja AC, et al. Reduction of Erythroid Precursors in Protein-Energy Malnutrition. Br J Nutr. 2007;97:2.

26. Bolariwaw AF, Ajayi FF, Alak OO, Akande OO. Effect of Malnutrition on Basal and Induced Gastric Acid Secretion. Nig J Physiol Sci. 1991;5:144-8.

27. Adesola AL. The Influence of Severe Protein Deficiency (Kwashiorkor) On Gastric Acid Secretion In Nigerian Children. Br J Surg. 1968;55:866.

28. Coward WA, Whitehead RG. Changes in Haemoglobin Concentrations During The Development Of Kwashiorkor. Br J Nutr. 1972;28:468-9.

29. Mitchell HS. Protein Limitation And Human Growth. J Am Diet Assoc. 1966;44:165-71.

30. Grant GH, Kachmar JF. In Fundamental of Clinical Chemistry. In: Tietz NW, editor. 3rd Edition. Philadelphia: W.B. Saunders Company; 1987: 298-320.

31. Robert MK, Darly GK, Peter MA, Victor RW. Enzyme kinetics. In: Illustrated Biochemistry. 26th edition. USA: Lange medical books/McGraw-Hill; 2003: 60-71.

32. Donald V, Voet Judith G, Pratt Charlotte W. Lipid Metabolism. In: Fundamentals of Biochemistry. Newyork: John Wiley And Sons; 1999: 569.

33. Agten A, Maes K, Smuder A, Powers SK, Decramer M, Gayan-Ramirez G. N-Acetylcysteine protects the rat diaphragm from the decreased contractility associated with controlled mechanical ventilation. Crit Care Med. 2011;39:777–82.

34. Min K, Smuder AJ, Kwon OS, Kavazis AN, Szeto HH, Powers SK. Mitochondrial-targeted antioxidants protect skeletal muscle against immobilization-induced muscle atrophy. J Appl Physiol. 2011;111:1459–66.

35. Mc Ardle A, Jackson MJ. Intracellular Mechanisms Involved In Damage To Skeletal Muscle. Basic Appl Myol. 1994;4(1):43-50.

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