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EFFECTS OF DEFATTED *MORINGA OLEIFERA* SEED ON SKELETAL MUSCLE OF PROTEIN ENERGY MALNOURISHED RATS

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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 results 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, Ca^{2+} ATPase and Na^{+}/K^{+} ATPase. **Methods:** 16 white albino rats of waster strain (*Rattus novergicus*) 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 Ca^{2+} 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.

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*. [22] which can greatly affect calcium ion levels, ATPase activity, oxidation/reduction state of the cell and cause oxidative stress. Calcium adenosine triphosphatase (Ca^{2+} - ATPase) is important for maintaining the overall health of the muscle cells, however inhibition of Ca^{2+} - ATPase prevent the pumping of calcium ion, resulting in muscle

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wasting and degeneration of muscle tissue [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 [13].

Malnutrition is the outcome of an unbalanced, inadequate, or excessive intake of nutrients and energy. The phrase covers both protein energy malnutrition (PEM) and all of the previously listed disease symptoms. PEM is a kind of malnutrition that is described as a set of clinical symptoms brought on by simultaneous protein and/or energy deprivation in varied ratios. The severity of the ailment ranges from moderate to severe, depending on the form [12].

The three most prominent types of PEM are Kwashiorkor (protein malnutrition predominate), Marasmus, and Marasmic Kwashiorkor (major protein deficiency and strong calorie insufficiency signs present; occasionally referred to as the most severe form of malnutrition) [21].

The "Miracle tree," *Moringa oleifera* (family: Moringaceae), is a commonly used medicinal plant that possesses a number of pharmacological and health-improving qualities [17]. Due to the fact that every part of the tree is edible, *M. oleifera* has a range of uses. It's noteworthy to mention that consumption of this plant has been demonstrated to drastically boost the intake of certain critical minerals and phytochemicals that are known to promote human health [28].

Due to its ease of growing and the dispersion of phytochemicals in all of the plant's components, including the leaves, flowers, pods, and seeds [18-30] identify *M. oleifera* as a wonderful remedy to combat malnutrition. According to studies by [3, 10, 16, 17, 26] and [31] vitamins, polyphenols, carotenoids, phytosterols, and tocopherols are the most prevalent ingredients. From both *in-vivo* and *in-vitro* research, the literature reveals that this plant has more than 20 different pharmacological effects [27].

The plant's proximate analysis demonstrated a high percentage production of protein and carbs that are appropriate for food fortification and have significant potential as nutritional supplements [4]. Therefore, the goal of this study is to examine into the effects of defatted *Moringa oleifera* seed on the skeletal muscle of protein-energy deficient rats.

MATERIALS AND METHODS:

Plant Materials: *Moringa oleifera* seed was bought from Kogi State University farm Anyigba. The seeds were taken from the seed coat and dried at 600C then crushed.

A known weight of the pulverized seeds was measured into a savette paper wrapped by the use of a white thread; it was thereafter maintained in a washed and dried conical flask. N-Hexane was added to the conical flask and shaken violently and permitted to stand for roughly three hours to defat adequately. The defatted seed was untied and put on a new savette paper for optimum 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 *Moringa oleifera* seed based recovery diet
Defatted grounded *Moringa oleifera* seed	40g	-	100g
Grounded fish	-	100g	-
Corn chaff	530g	470g	470g
Vitaflash Amino WSP (vitamins-amino acids)	30g	30g	30g
Vegetable oil	50g	50g	50g
Sucrose	350g	350g	350g

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

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 57-120g) 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 21days of feeding the animals with the recovery diets, the rats were decapitated by cervical dislocation and were sacrifice.

Preparation of Tissue Homogenate: The rodents were killed by the dislocation of the cervical spine, and blood was obtained using a jugular puncture. For serum and hematological analysis, blood specimens were put into plain bottles and some into EDTA coated sample vials (to limit clotting). (250 mM sucrose, 10 mMtris, pH 7.4) was administered to quickly extract skeletal muscle from the hind limbs [2].

After that, serum was created by centrifuging the blood samples at 3000 rpm for 5 minutes [20]. The buffer was utilised as the homogenizing medium to mix the skeletal muscle in an ice-cold mortar and pestle. Aliquots of the tissue homogenate suspension were stored in Eppendoff tubes and kept in the freezer. To achieve the maximal potential release of the enzymes, the homogenate was kept frozen overnight [19]. The homogenate was then utilised for enzyme testing.

Biochemical Test: Using bovine serum albumin as the reference protein, the protein concentration in the tissue homogenates was assessed using the Biuret method, which was first described by [11].

The method provided by [8] was used to test the concentration of serum albumin. The method described by [25] was used to test the activities of aspartate transaminase (AST), alanine transaminase (ALT), and Na+/K+ ATPase in skeletal muscle tissue homogenate. Using the approach recommended by Bewaji (2004), Ca2+ -ATPase was assessed in the skeletal muscle tissue homogenate after four and eight weeks.

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

RESULTS:

Animal Morphology: With the exception of the control group, the animals' weekly mean weight gradually decreased during malnutrition. When recovery diet treatments began, the situation was the opposite. The animals in Group A (control) developed well, with smoother skin fur, an oblong face, and tail covered in fur; no fur loss was noted in any place.

In contrast, the starved animals (Group B) demonstrated decrease of appetite, which may have contributed to the body fur loss, moon face development, unchanged head circumference, scaly tails, enlarged eyes, and muscle atrophy that were seen. The morphological modifications mentioned above improved over the course of the treatment.

Effects of Diets on Liver Function Indices (AST, ALT, Serum Albumin, Protein): As shown in **Fig. 1**, the blood concentration of AST, ALT, serum albumin, and protein was lowered in experimental rats after feeding with low protein iso-caloric diets. This serum concentration of these markers considerably increased after feeding the malnourished animals with the designed treatment meals.

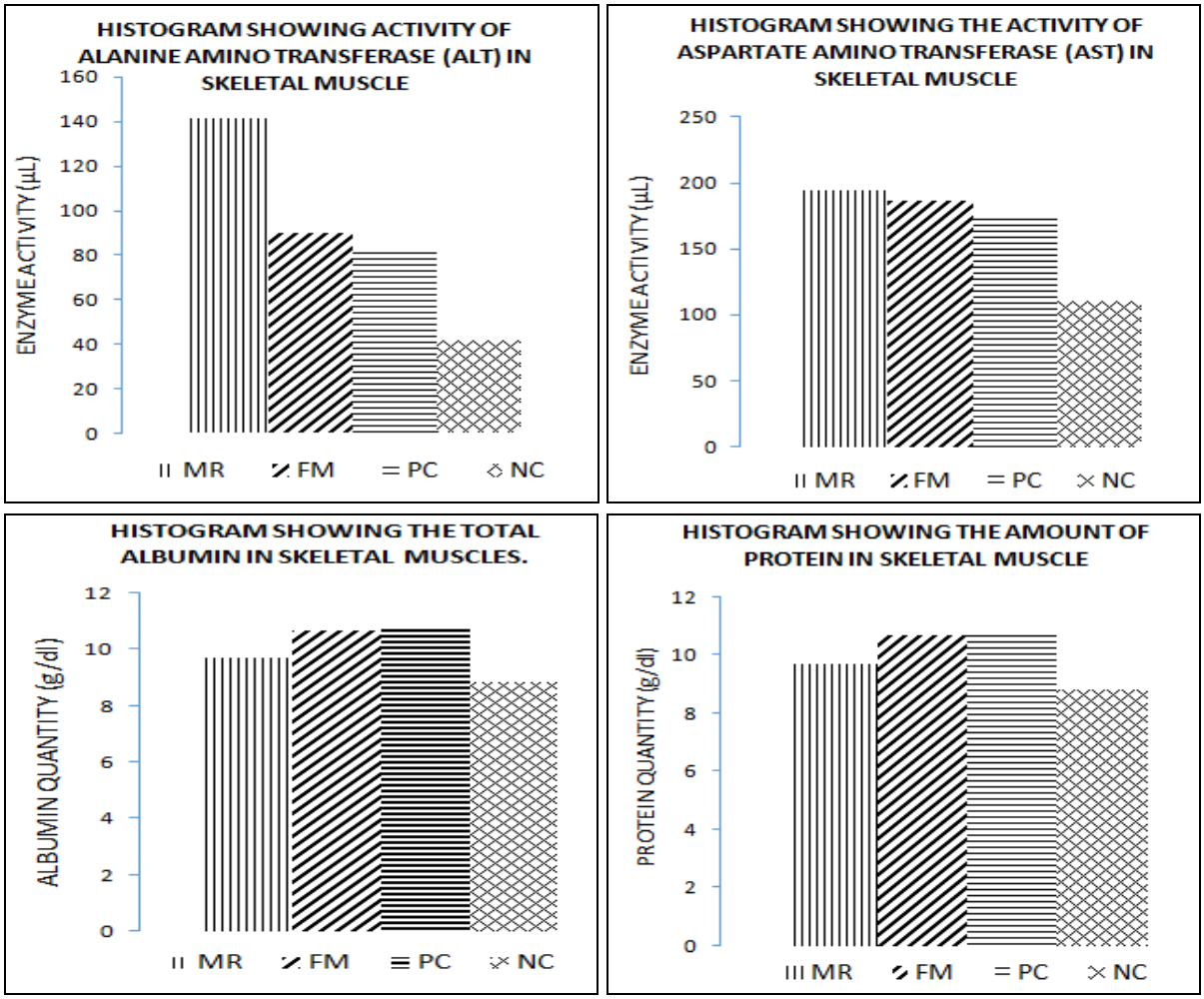

FIG. 1: THE EFFECT OF ADMINISTRATION OF DEFATTED *MORINGA OLEIFERA* SEED, FISHMEAL FEED AND NORMAL GROWER'S FEED ON THE AST, ALT, SERUM ALBUMIN, AND PROTEIN LEVEL IN SKELETAL MUSCLE OF ALBINO RATS. MR= *Moringa oleifera* rich meal; FM= fish meal; PC= positive control; NC= negative control.

Calcium ATPase Activity: As shown in **Fig. 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

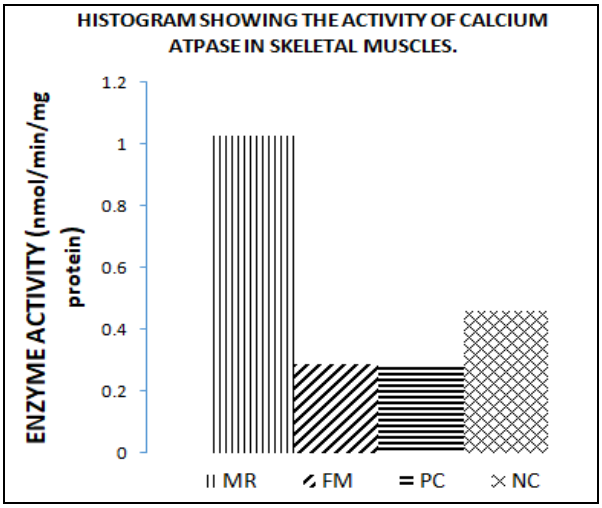

FIG. 2: THE EFFECT OF ADMINISTRATION OF DEFATTED *MORINGA OLEIFERA* SEED, FISHMEAL FEED AND NORMAL GROWER'S FEED ON CALCIUM ATPASE ACTIVITY IN SKELETAL MUSCLE OF ALBINO RATS. MR= *Moringa oleifera* rich meal; FM= fish meal; PC= positive control; NC= negative control.

Na^+/K^+ ATPase Activity: According to **Fig. 3**, the Na+/K+ ATPase activity was decreased in the experimental animals after feeding with low protein iso-caloric diets. This blood concentration of Na+/K+ ATPase rose after feeding the impoverished animals with the planned therapeutic meals.

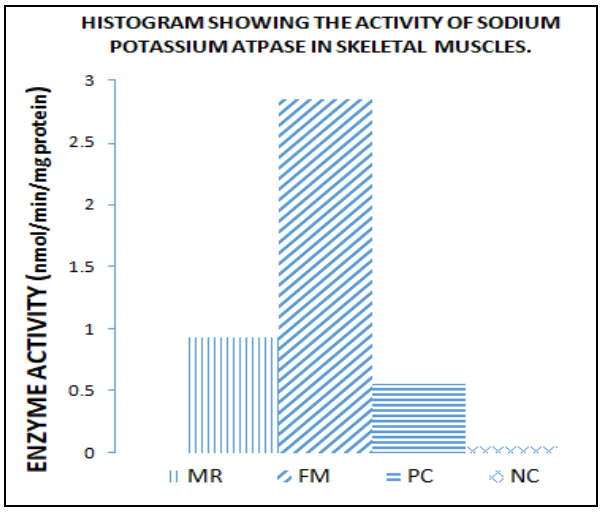

FIG. 3: THE EFFECT OF ADMINISTRATION OF DEFATTED *MORINGA OLEIFERA* SEED, FISHMEAL FEED AND NORMAL GROWER'S FEED ON NA^+/K^+ ATPASE ACTIVITY IN SKELETAL MUSCLE OF ALBINO RATS. MR= *Moringa oleifera* rich meal; FM= fish meal; PC= positive control; NC= negative control.

DISCUSSION AND CONCLUSION: Malnutrition, particularly Protein-Energy Malnutrition (PEM), can lead to different morphological changes such as edema, diarrhea, weight loss, hair loss, blindness, vulnerability to infections, and lower total protein levels [24].

The present research fits with these findings, suggesting that increased diet can restore these detrimental morphological alterations reported in test animals to some extent, though with different capacity. Patients with protein deficiency (Kwashiorkor) are known to have low hematological indicators, including AST, ALT, serum albumin, and protein levels [1, 6].

However, feeding rodents with a diet based on *Moringa oleifera* leaves, which are rich in β-carotene, protein, vitamin C, calcium, and potassium [29], indicates its potential as a sustainable substitute for dietary protein in rat feed. Moreover, the inclusion of *M. oleifera* leaf-based diet in this study restored liver indicators, such as blood albumin levels and AST/ALT activity, suggesting enhanced liver function. Following skeletal muscle degeneration induction, the activity of Ca2+ ATPase in the skeletal muscle of all groups reduced. However, animals fed with the *M. oleifera* leaf-based diet displayed higher resilience to the effects of starvation on enzyme function compared to other groups. The reduction in Ca^{2+} ATPase function during starvation can come from decreased enzyme production or inactivation, leading to hazardous high concentrations of Ca^{2+} within the cell and eventual cell death [7].

Conversely, the considerable increase in Ca2+ ATPase activity found in the *M. oleifera* leaf-based diet group could be attributable to enzyme activation or enhanced synthesis during treatment, showing the reversibility of the effect of malnourishment on this enzyme.

The Na+/K+-ATPase serves a key function in maintaining the concentration of ions across the plasma membrane, contributing to the resting membrane potential in cells. Studies have revealed that its levels are low in specific metabolic situations [9-23], which coincides with the findings of this investigation. 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 indicates that malnutrition-induced muscle degeneration

considerably alters Ca2+ ATPases, Na+/K+-ATPase, AST, ALT, protein levels, and serum albumin in skeletal muscles. However, the adverse consequences are most adequately addressed by the *M. oleifera* leaf-based diet. The process by which *M. oleifera* leaf improves muscle degeneration induced by PEM might comprise improving the production and/or activity of the studied markers, thereby minimizing the impact of oxidative stress and possibly decreasing the loss of energy in the skeletal muscles. Thus, the *M. oleifera* leaf-based diet proves to be a sustainable replacement for dietary protein in the diet.

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CONFLICT OF INTEREST: Nil

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