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Haematological and *in-vivo* study of *moringa oleifera* seed

Abstract

The purpose of the study was to determine the hematological and nutritional evaluation of Moringa seed. Dietary Samples investigated consisted of Basal dietary, a nitrogen free diet100%(1) Control dietary (2) Basal80%: Pressure cooked *Moringa olifera* seed10%: soybean10%(3) Basal80%: Oven Roasted Moringa olifera seed10: soybean10%(4) Basal80%: Raw *Moringa olifera* seed10%: Soybean10%(5).

Fifty (50) albino rats were obtained from faculty of Health Science animal breeding centre, Obafemi Awolowo University, Ile-Ife, Nigeria. They were weighed and grouped into five groups of ten each and fed with (Figure 1) five dietary samples for 28days. A commercial product (Milk based) manufactured by Nestle Plc, was purchased from a local supermarket, Ile-Ife, Nigeria and was used as standard control. The growth rate, (non protein diet) for sample dietary 1 declined by 0.7%. Protein samples dietary growth rate increased for samples dietary 2(39.1%), 3(23.9%), 4(23.8%), and 5(17%) respectively. Protein efficiency ratio (PER) Net protein ratio (NPR) Protein retention efficiency (PRE=NPRX16) measured for samples dietary 2, 3, 4, 5 were comparable to the control. The average nitrogen retained in various organs of experimental animals, such as liver, kidney and muscle of the diets 2, 3, 4 5 were favorably comparable to the control respectively. Hematological study of the blood serum analysis of the test rats showed significant ($p<0.05$) healthy conditions of the packed cell volume, hemoglobin, red blood cells, white blood cells and serum protein were comparable to the control rats. In conclusion *Moringa oleifera* dietary samples were good potential to be an alternative source to boost human and animal blood, protein, essential amino acids supplements. It is cheap, easily available, could improve human health, meet nutritional requirement for infants, reach at affordable price and available for the rural dweller populace.

Keywords: *moringa olifera* seed, hematological study, experimental animals, EDTA

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Introduction

Moringa oleifera is a vegetable plant protein with bio-active multipurpose; it is originated from the sub-Himalayan areas of India, Pakistan, Bangladesh, Afghanistan and Tropics. The various parts of *Moringa oleifera* such as leaves, bark, flowers, fruit, seeds, and root are medicinal beneficiary Gunjul et al.[1] *Moringa oleifera* is an important food source in some parts of the world because it can be grown cheaply and easily, and the leaves retain lots of vitamins and minerals when dried. Chemical analysis of *Moringa oleifera* seed consists of oil%, protein%, fiber%, moisture% and ash% contents were 34.80%, 31.65%, 7.54%, 8.90% and 6.53% respectively, thus some people use it as a nutritional supplement or tonic.[2] In India and Africa *Moringa oleifera* is used in feeding programs to fight malnutrition. The immature green pods (drumsticks) are prepared similarly nutritive values to green beans, while the seeds are removed from more mature pods and cooked like peas or roasted like nuts.[3] The leaves are cooked and used like spinach, and they are also dried and powdered for use as a condiment. The seed cake remaining after oil extraction is used as a fertilizer and also to purify well water and to remove salt from seawater.[3] *Moringa oleifera* is medically therapy for prevention of "tired blood" (anemia); arthritis and other joint pain (rheumatism); asthma; cancer; constipation; diabetes; diarrhea; epilepsy; stomach pain; stomach and intestinal ulcers; intestinal spasms; headache; heart problems; high blood pressure; kidney stones; fluid retention; thyroid disorders; and bacterial, fungal, viral, and parasitic infections.[4]

Other medical application of *Moringa oleifera* is to reduce swelling, increase sex drive prevent still birth pregnancy, boost the immune system, and increase breast milk production. *Moringa oleifera* is sometimes applied directly to the skin as a germ-killer or drying agent (astringent). It is also used tropically for treating several infection (abscesses), athlete's foot, dandruff, gum disease (gingivitis), snakebites, warts, and wounds.[4] Socially oil from *Moringa oleifera* seeds is used in foods, perfume, and hair care products, and as a machine lubricant.[4] Hence, the study aimed to determine the hematological analysis and the nutritional evaluation of *Moringa oleifera* seed diets Figures 2 & Figure 3 from locally available, easily accessible plant protein such as *Moringa oleifera* seed soy bean and maize and (carbohydrate).

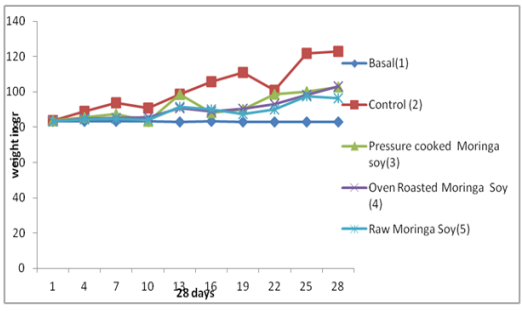

Figure 1 Shows the Growth response of animal fed with *Moringa olifera* dietary samples for 28days.

Figure 2 *Moringa oleifera* seeds with seed coating.

Figure 3 *Moringa oleifera* seeds without seed coating.

Materials and methods

Maize, soybean and a commercial product (Milk based) manufactured by Nestle plc was purchased from a local supermarket, Ile-Ife, Nigeria.

Seed collection and processing

Mature unshelled *Moringa oleifera* seed was collected for Obafemi Awolowo University Agriculture farm, Ile-Ife. The maize was soaked for one day, wet milled, made into dough and allowed to ferment for 48 hours, after which it was dried and milled into fine flour. Soy bean was also made into flour by first soaking for 48hr, blanched at 100^0C, dehulled, oven dried at 80^0C, and milled into fine flour. Sample dietary was prepared *Moringa oleifera* seed was cleaned, was at cooked at 100^0C dried and milled into fine flour, Pressure cooked Moringa seed10%: soybean10%(3) *Moringa oleifera* seed was cleaned sorted, oven dried at 80^0C, milled into fine flour Basal80%: Oven Roasted *Moringa oleifera* seed10: soybean10%(4), *Moringa oleifera* seed was sorted, cleaned, sun dried, milled into fine flour, Raw Moringa *oleifera* seed10%: Soybean10%(5).The flours were packed into air-tight polyethylene bags and stored in the freezer.[5–7]

Biological Assay

FIfty (50) weaning albino rats were obtained from College of Heath Science animal breeding centre, Obafemi Awolowo University, Ile-Ife, Nigeria. The albino rats were weighed and randomly allocated to metabolic cages. The weights and ages ranged from 83g-83.74g and 4 to 6 old weeks respectively. The albino rats were accommodated in metabolic cages fixed with a feeding plate and a small plastic bottle to supply food and water *ad libitum*. The animals were acclimatized to the new environment by feeding them for seven days on pellets specially prepared for animals. The animals were then re-weighed and grouped into five of ten per group in such a way that the weights were similar. For example groups, 1, 2, 3, 4 and 5 had almost similar weights at the beginning of the 83.70, 83.74 83.45, 83 and 83.20 respectively. Groups (1-5) were placed on experimental diets for 28days. They were given a noted weighed quantity of each experimental diet, in a feeding dish and water was supplied *ad libitum* via a plastic bottle attached to the cage. Daily consumption of samples was carefully recorded and the weights were noted. Weight gain/loss of the experimental animals was taken every three days. Prior to the end of the experiment, which was twenty-eight days, the experimental animals were sacrificed. The organs collected from the animal including spleen, heart lungs kidney liver and small intestine were fixed immediately in 10% formyl saline for nitrogen determination.[8–12]

Automated blood count

Procedures

Blood Count was performed in the Department of Hematology and Immunology, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife using an Abbott Cell-Dyn 1700 automatic analyzer. The blood sample was collected through cardiac puncture, drawing the blood into a test tube containing an anticoagulant (EDTA) to stop it from clotting. The sample is then transported to the laboratory for immediate processing by an automated counter. The blood was well mixed (though not shaken) and placed on a rack in the analyzer. This instrument has flow cells, photometers and apertures that analyze different elements in the blood. The cell counting component counts the numbers and types of different cells within the blood. The results are printed out. This blood counting machine aspirates a very small amount of the specimen through narrow tubing followed by an aperture and a laser flow cell. Laser eye sensors count the number of cells passing through the aperture, and can identify them; this is flow cytometry. The two main sensors used are light detectors and electrical impedance. The instrument measures the type of blood cell by analyzing data about the size and aspects of light as they pass through the cells (called front and side scatter). Other instruments measure different characteristics of the cells to categorize them. Because an automated cell counter samples and counts so many cells like White cells, Red cells, Hemoglobin, Hematocrit, MCV, MCH, MCHC, RDW, Platelets etc., the results are very precise AACC.[13]

Ethical consideration

This study was approved by the Ethical Review Committee of the Obafemi Awolowo University, Osun State, Ile-Ife, Nigeria.

Results and discuss

Table 1 reflects the chemical composition of raw materials of

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the dietary samples in percentage include protein%, moisture%, fat%, ash%, fibre%, CHO% and energy/kcal%, It was found to be nutritional adequate to formulate dietary and meet daily nutritional needs for infants and adults. The data are mean±SD values of three determinations with different superscript in a column are significantly different (P<0.05). Foot note: Basal dietary, a nitrogen free diet100%(1) Control dietary(2) Basal80%: Pressure cooked *Moringa olifera* seed10%: soybean10%:(3) Basal80%: Oven Roasted *Moringa olifera* seed10%: soybean10%(4) Basal80%: Raw *Moringa olifera* seed10%:Soybean10%(5).

Table 2 showed the highlights of haematological study of *Moringa oleifera* samples dietary include WBC10$^{-3/UL}$, LYM%, MON%, GRAN%, LYM#$^{-3/UL,}$ MON#$^{-3/UL,}$ GRAN#$^{-3/UL,}$ RBC$^{-6/UL}$ HGBg/dl, HCT%, MCVfL, MCHpg, MCHC g/dl, RDW-SDfL, RDW-CV%, PLT10-$^{3/UL,}$ MPVfL, PDW%, PCT% and P.LCR%. Hematological study of the blood serum analysis of the experiment rats showed significant (p<0.05) healthy conditions of the packed cell volume, hemoglobin, red blood cells, white blood cells and serum protein were comparable to the control sample dietary experimental rats. This is in agreement with earlier report that *Moringa oleifera* could boost blood and hence an alternative food for anemia patients.[14,15]

The data are mean±SD values of three determinations with different superscript in a column are significantly different (P<0.05). Foot note: Basal dietary, a nitrogen free diet100%(1) Control dietary(2) Basal80%: Pressure cooked *Moringa oleifera* seed10%: soybean10% (3) Basal80%: Oven Roasted *Moringa oleifera* seed10%: soybean10% (4) Basal80%: Raw Moringa seed10%:Soybean10%(5). Table 3 highlights the chemical analysis of the five dietary samples which include protein%, moisture%, fat%, ash%, fibre%, CHO% and energy/Kcal%. They were ranged from 10.22 to10.30%, 4.25 to 4.34%, 2.68 to 3.80%, 2.30 to 2.64%, 0.68 to 0.88%, and 78.91 to 88.85% and 382 to 389Kcal% respectively. Samples dietary protein were formulated and regulated at 10% to enable calculate biological values. Levels of moisture% and fat% were less than 10% this values give an advantage to the samples dietary for example moisture% higher than 10% favour food spoilage and microorganism multiplication. Fat content below 10% could discourage rancidity and increase shelf life, higher CHO may result in higher energy supply to the body. Corroborate other studies.[6,8,12,16,17]

The data are mean±SD values of three determinations with different superscript in a column are significantly different (P<0.05). Foot note: Basal dietary, a nitrogen free diet100% (1) Control dietary (2) Basal80%: Pressure cooked *Moringa olifera* seed10%: soybean10%:(3) Basal80%: Oven Roasted *Moringa olifera* seed10%: soybean10%(4) Basal80%: Raw *Moringa olifera* seed10%:Soybean10%(5). Table 4 depicts the weight of various tissues of experimental animals in gram such as Liver (g), Muscle (g), Kidney (g), Heart (g) and Spleen (g). They were ranged from 3.54 to 4.54, 0.46 to 0.78, 0.62 to 0.90, 0.32 to 0.52 and 0.46 to 0.62 respectively. Samples dietary 2-5 were higher compared with sample dietary 1 because sample dietary 1 lack adequate nutrient to promote inner tissues growth.[1,6,11,17]

The data are mean±SD values of three determinations with different superscript in a column are significantly different (P<0.05). Foot note: Basal dietary, a nitrogen free diet100% (1) Control dietary (2) Basal80%: Pressure cooked *Moringa olifera* seed10%: soybean10%: (3) Basal80%: Oven Roasted *Moringa olifera* seed10%: soybean10% (4) Basal80%: Raw *Moringa olifera* seed10%:Soybean10% (5). Table 5 shows the Bioassay of experimental animals include biological value (BV %), True digestibility (TD %), Food efficiency ratio (FER), and Protein retention efficiency (PRE) Protein efficiency ratio (PER), net protein retention (NPR), net protein utilization (NPU) values They were 95.66 to 98.68, 72.45 to 80.55, 3.0 to3.7, 0.28 to 0.37, 2.9 to 3.6 and 46.4 to 57.6 comparable to control samples and similar to biological value of an egg, the combination of two proteins in the formulation of sample dietary had reported previously to complement each other to form a complete amino acid profile but diet 1 lack biological value being a single plant protein, nitrogen free diet contain sulphur amino acid such as methionine but limited in lysine and tryptophan respectively.[6,9,17]

The data are mean±SD values of three determinations with different superscript in a column are significantly different (P<0.05). Foot note: Basal dietary, a nitrogen free diet100% (1) Control dietary (2) Basal80%: Pressure cooked *Moringa olifera* seed10%: soybean10%:(3) Basal80%: Oven Roasted *Moringa olifera* seed10%: soybean10% (4) Basal80%: Raw *Moringa olifera* seed10%:Soybean10%(5). Table 6 shows the total nitrogen content in tissue of experimental animal mg/100g, the nitrogen content which includes Liver (mg/100g), Muscle (mg/100g) and Kidney (mg/100g). They were ranged from 35.37 to 50.27, 36.03 to 50.53, 35.03 and 50.20 respectively. The tissue store enough nitrogen that could promote growth and carry out other daily activities, however sample dietary 1 had lower nitrogen compared to group 2 to 5, this might be because group 1 lack quality protein, single plant protein can not complete amino acid profile this is in agreement with other findings.[6,8,17]

Table 1 Proximate Composition of raw materials procured for dietary samples %

Sample code	Protein%	Moisture%	Fat%	Ash%	Fibre%	CHO%	Energy/kcal%
Moringa oleifera seed	31.62±02	2.56±01	34.80±03	6.53±03	1.54±01	22.95±01	531±01
Maize	9.88±03	2.67±04	0.45±01	2.88±02	1.68±02	82.44±03	373±03
Soy Bean	38.66±01	2.58±01	20.56±02	2.45±01	1.88±01	33.87±02	681±01

Table 2 Haematological study of moringa oleifera seed

Parameter	1	2	3	4	5	Limits	Alerts
WBC10$^{-3/UL}$	6.3	6.6	6.8	11.6	9	2.5-10.5	m
LYM%	73.9	89.2	84.1	73.9	62.2	20-40	H
MON%	68	6.6	9.9	12	13.6	15-Jan	m
GRAN%	14.1	4.3	6	14.1	24.3	50-70	L
LYM#$^{-3/UL}$	8.6	6.6	6.6	8.6	6.6	0.6-4.1	H
MON#$^{-3/UL}$	1.4	0.4	0.7	1.4	1.2	0.1-1.8	m
GRAN$^{\#-3/UL}$	1.6	0.3	0.3	1.6	2.2	2.0-7.8	L
RBC$^{-6/UL}$	6.76	6.62	8.5	6.76	6.64	3.50-6.50	m
HGBg/dl	12	12.7	16.4	12	11.3	16-Nov	m
HCT%	34.2	39.1	48	34.2	31.8	36-48	m
MCVfL	60.6	69.1	56.5	60.6	48	80-99	L
MCHpg	17.7	19.1	19.2	17.7	17	26-32	L
MCHC g/dl	32	32.4	34.1	36	35.5	32-36	m
RDW^{-SDfL}	24.1	35.3	29.7	24.1	24.1	37-54	L
RDW$^{-CV\%}$	13.6	17.4	16.3	13.6	14.2	11.5-14.5	H
PLT10$^{-3/UL}$	440	503	391	511	449	90-400	m
MPVfL	6	6.7	7	9.2	10.7	7.4-10.4	L
PDW%	7	7.9	7.9	10	8.4	17-Oct	L
PCT%	0.47	0.33	0.27	0.1	0.48	0.10-0.28	m
P.LCR%	13.3	18.3	18.3	13	23.7	13-43	L

Table 3 Chemical analysis of five dietary samples

Dietary samples	Protein%	Moisture%	Fat%	Ash%	Fibre%	CHO%	Energy Kcal%
1	-	4.25±04	3.80±01	2.30±01	0.80±04	88.85±02	389±04
2	10.25±01	4.33±01	3.20±02	2.34±03	0.86±01	79.07±01	385±02
3	10.22±02	4.34±03	2.78±01	2.32±04	0.76±04	79.60±03	384±01
4	10.30±03	4.26±01	3.30±03	2.35±01	0.88±01	78.91±02	386±03
5	10.24±01	4.34±02	2.68±03	2.64±03	0.68±02	79.46±01	382±04

Table 4 Weight of various tissues of experimental animals in gram

Dietary samples	Liver(g)	Muscle(g)	Kidney(g)	Heart(g)	Spleen(g)
1	3.44±01	0.44±01	0.62±03	0.30±01	0.36±02
2	4.54±03	0.58±03	0.86±01	0.32±01	0.62±03
3	3.98±02	0.58±04	0.90±02	0.52±02	0.46±01
4	3.50±01	0.46±01	0.84±01	0.38±03	0.46±04
5	3.54±04	0.66±02	0.82±02	0.44±03	0.54±01

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Table 5 Bioassay of experimental animals

Dietary samples	BV%	TD%	PER	FER	NPR	PRE
1	-	-	-	-	-	-
2	98.68±03	80.55±00	3.7±03	0.37±03	3.6±03	57.6±03
3	96.65±01	76.56±01	3.5±02	0.34±01	3.5±01	56.0±02
4	95.66±02	74.55±03	3.4±03	0.39±03	3.2±02	51.2±01
5	95.80±03	72.45±02	3.0±01	0.28±02	2.9±03	46.4±01

Table 6 The total nitrogen content in tissue of experimental animal mg/100g

Dietary samples	Liver(mg/100g)	Muscle(mg/100g)	Kidney(mg/100g)
1	35.37±01	36.03±03	35.03±02
2	50.27±02	50.53±01	50.00±01
3	50.25±03	50..50±02	50.20±04
4	50.26±03	50..53±01	50.00±02
5	48.27±01	43..53±02	48.00±03

Conclusion

In conclusion *Moringa oleifera* dietary has prove to be biochemical active, alternative plant protein source to boost human and animal blood, protein, and essential amino acids supplements. It is highly digestible, could promote growth, improve health, meet nutritional requirement for infants and adult, could be reached at affordable price and available for the rural dweller populace could be applied for health therapy and human consumption, healthy conditions of the packed cell volume, hemoglobin, red blood cells, white blood cells and serum protein were comparable to the control sample dietary experimental rats.

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Conflicts of interest

The author declares no conflict of interest.

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