HISTOPATHOLOGICAL AND IN-VIVO STUDY OF *MORINGA OLEIFERA* SEED

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Abstract
The purpose of the study was to determine the histopathological and In-vivo study of Moringa Oleifera Seed of moringa oleifera seed dietary.
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 five dietary samples for 28 days. Dietary samples investigated consisted of basal dietary, a nitrogen free diet100% (1) control dietary (2) basal80%: pressure cooked moringa seed10%: soybean10% (3) basal80%: oven roasted moringa seed10: soybean10%(4) basal80%: raw moringa seed10%: Soybean10% (5). The result revealed that histopathological study including spleen, heart, lungs, kidney, liver were healthy and chemical analysis such as pH, titratable acidity%, brix% and vitamin Cmg/100g were comparable with control samples, They were ranged from 6.05-6.60, 0.01-0.04%,5-10% and 22.5-28.5 Cmg/100g. Proximate composition includes protein%, moisture%, fat%, ash%, crude fiber%, CHO% and caloric values. They were ranged from 10.20-10.30%, 4.25-4.34%, 2.68-3.80%, 2.30-2.64%, 0.68-0.88%, 79.07-88.85%, 382-389 Kcal% respectively.
Histopathological and In-vivo study of formulated moringa samples dietary1-5 showed to improve health and promote growth. The tissues showed no infarcted zone, no oedema, no inflammatory cells and separation of cardiac muscle fibres, no cellular disintegration, and no toxicity symptom hence fit for human consumption.

Key words: Histopathological study, *moringa oleifera* seed, In-vivo study

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1. INTRODUCTION

Moringa oleifera is native to Asian and African continents, is a vegetable protein with multiple bio-chemical and has several medicinal values, indispensable and could improving health (Anwar *et al*., 2007).
Thirteen of moringa species are well documented, it contains about fifteen multi-vitamins, and hence it is a cheap way of acquiring multi-vitamins at doorstep (Farooq anwar and Umer rashid 2007). Chemical composition of moringa seeds consisted of oil content amount to 34.80% moisture 8.90% fiber 7.54% ash 6.53 % protein 31.65%(Anwar *et al*., 2007).. Moringa *oleifera* seed has been reported to be a potential anticancer and endowed with several bioactive compounds with antitumor activity. Some workers have highlighted that combination of nitrile, mustard oil glycosides and thiocarbamate constituent of moringa *oleifera* leaves confirmed to be lowering blood pressure and diuretic activity (Morton, 199, Anwar *et al*., 2007). This compound is capable of reducing cholesterol level from the serum of high fat diet fed rats (Ghasi *et al*., 2000).
Moreover,other bioactive component such as phytoconstituent, (that is, b-sitosterol) is linked with cholesterol lowering. The moringa leaf reported to be hypolipidimic, help to lowering body weight, reduce heart weight serum triglycerides level and serum cholesterol level (Ghasi *et al 2000*). High protein and low fibre contents of moringa food products could be succor for those that are suffering from malnutrition (Aberra, *et al 2013)*.
The iron content of the *moringa oleifera* leaves is high, and they are reportedly prescribed for anemia patents and also act as protective effect on cardiovascular. The *moringa oleifera* seeds could be used as a supplement to scurvy patients; this is attributed to high content of vitamin in it. It is also acts as water purified for

families and rural communities (Ndabigengeser, *et al*1998).
Hence, the purpose of study is to determine histopathological and In-vivo study of *Moringa oleifera* seed to ensure safety, health therapy for human consumption.

2. MATERIALS AND METHOD

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

Materials

Mature unshelled moringa oleifera seeds was collected for Obafemi Awolowo University Agriculture farm, Ile-Ife.

Chemical Analysis

The proximate analyses were carried out according to A.O.A.C. methods and carbohydrate content was determined by difference and energy by Atwater factors, (AOAC, 2000, Adeniyi *et al* 2014).

Animal Bioassay

Fifty (50) weaning albino rats were obtained from College of Heath Science animal breeding centre, Obafemi Awolowo University, Ile-Ife, Nigeria. The rats were weighed and randomly distributed to metabolic cages.
The average weights and ages were ranged from 83-83.74g and their ages were 4 to 6 old weeks respectively. The albino rats were accommodated in metabolic cages fixed with a feeding plate and sizable plastic bottle to supply food and water *ad libitum.*
The animals were acclimatised to the new environment by feeding them for seven days on pellets specially prescribed 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, 1-5, had almost similar weights at the beginning of the experiment 83.70g, 83.74g 83.45g, 83g and 83.20g respectively. Groups (1-5) were placed on experimental diets for 28 days. 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 histopathological analysis (Ibironke,*et al* 2012, 2014a).

Histopathology analysis

The organs that were collected from the experimental animal including spleen, heart, lungs, kidney, liver and small intestine were fixed in 10% formyl saline for 24hr dehydrated in ascending concentration (50%, 70%, 90%, then twice in 100%) for interval of 1hr to enable it to be embedded in paraffin.
The tissues was sectioned to 6-micron thin films using a rotary microtome and stained with Hematoxylin and Eosin. Then they were examined with a zeiss EM light microscope (Kuku *et al 2013).*

Statistical Analysis

Statistical analysis of the data was carried out using the one-way Analysis of Variance (ANOVA) technique (SPSS 17.0 for windows), and the differences were separated using Duncan's Multiple Range Test (DMRT) at a level considered to be significant at $p < 0.05$.

Ethical consideration

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

***Moringa oleifera* seeds with seeds without seed coating**

3. RESULTS AND DISCUSSION

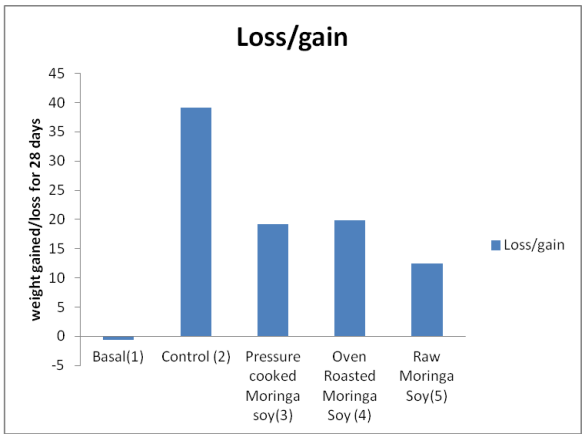


Figure 1: Flow chart of the experimental animals' growth response for 28 days

Figure 1 reflects the flow chart of the experimental animals' growth response for 28 days. Control dietary, (2) ranked the best growth performance, followed by oven roasted moringa, (4) pressure cooked (3), raw moringa (5) and basal dietary (1). Growth performance of dietary 2 - 5 may be as a result of quality and quantity protein present in the dietary regime. Moringa samples dietary were comparable to control sample but basal dietary declined growth, which may be due to the fact that diet is nitrogen free hence, lack quality protein. This is quite identical with previous finding. (Ibironke,*et al* 2014b).

Table 1 shows the chemical parameters of dietary samples consist of pH ranged from 6.05-6.60, titratable acidity 0.01-0.04%, Vitamin Cmg/100 ranged from 22.5-28.5, Brix% ranged from 5-10%.

Table 1 show Physico- Chemical Parameters of Dietary Samples

Dietary Samples	pH	Titratable acidity%	Vitamin C	Brix%
1	6.14^{b}±02	0.01^{a}±03	26.5^{c}±02	5^{a}± 01
2	6.05^{a}±03	0.03^{c}±02	25.5^{b}±01	10^{b}± 03
3	6.45^{c}±01	0.02^{b}±03	28.5^{d}±03	10^{b}± 02
4	6.64^{e}±04	0.04^{d}±03	26.5^{c}±01	10^{b}± 01
5	6.60^{d}±01	0.02^{b}±04	22.5^{a}±01	10^{b}± 04

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 seed10%: soybean10%: (3) Basal80%: Oven Roasted moringa seed10%: soybean10% (4) Basal80%: Raw Moringa seed10%:Soybean10% (5).

The sugar level was moderate, could be natural beneficiary, nutritional supplement to help lower blood glucose levels that could increase blood circulation (Anwar, 2007). It has also been found to help reduce blood pressure levels and a succor to a diabetic patient, this was confirmed earlier (Anwar, 2007). The pH fall within acceptable level of range from 6.0- 8.0 for drinking water as specified by World Health Organization. Also protein content of *Moringa oleifera* seeds solublised easily to basic amino acids that are necessary to improve health (Anwar, 2007, Mangale, *et al* 2012, Ibironke,*et al* 2014c).

Table 2: Chemical Composition of Dietary Samples

Dietary Samples	Protein%	Moisture%	Fat%	Ash%	Crude fiber%	CHO%	Caloric value kcal%
1	-	4.25^{a}±01	3.80^{e}±03	2.30^{a}±02	0.80^{c}±01	88.85^{e}±01	389^{d}±03
2	10.20^{a}±03	4.33^{c}±03	3.20^{c}±03	2.34^{c}±01	0.86^{d}±01	79.07^{b}±01	385^{c}±04
3	10.20^{a}±03	4.34^{d}±01	2.78^{b}±03	2.32^{b}±02	0.76^{b}±02	79.60^{d}±01	384^{b}±02
4	10.30^{b}±02	4.26^{b}±03	3.30^{d}±02	2.35^{d}±03	0.88^{d}±03	78.91^{a}±02	386^{d}±01
5	10.20^{a}±01	4.34^{d}±02	2.68^{a}±01	2.64^{d}±03	0.68^{a}±04	79.46^{c}±02	382^{a}±02

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 seed10%: soybean10%: (3) Basal80%: Oven Roasted moringa seed10%: soybean10%: (4) Basal80%: Raw Moringa seed10%: Soybaen10% (5).

Table 3: Functional Properties of Dietary Samples

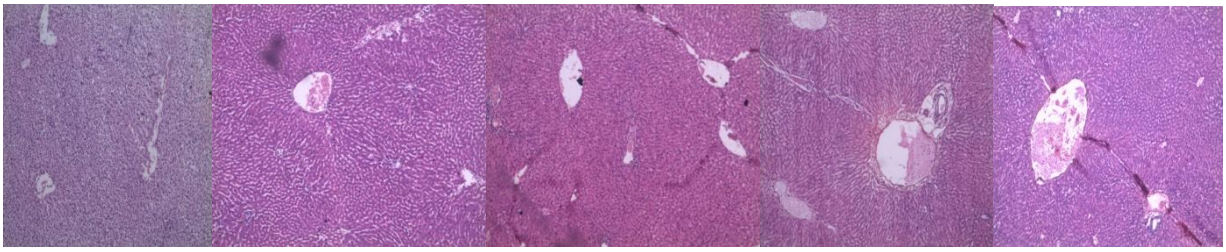
Dietary Samples	Bulk Density g/ml	Water Absorption Capacity g/g	Swelling Power%	Gelation Power%
1	0.79^{e}±03	0.89^{b}±02	22.22^{a}±01	12.50^{a}±03
2	0.57^{a}±02	2.17^{c}±03	27.77^{b}±03	14.30^{b}±04
3	0.66^{b}±01	0.72^{a}±01	32.25^{c}±01	15.34^{c}±02
4	0.67^{c}±04	0.88^{b}±03	37.03^{e}±03	15.45^{d}±03
5	0.72^{d}±04	0.98^{d}±02	34.48^{d}±02	15.55^{e}±01

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 seed10%: soybean10%: (3) Basal80%: Oven Roasted moringa seed10%: soybean10% (4) Basal80%: Raw Moringa seed10%: Soybean10% (5).

Table 2 further stressed the proximate composition of dietary samples which include protein%, moisture% Fat%, Ash%, Crude fiber%, CHO% and Caloric value kcal%. They were ranged from 10.20-10.30, 4.25-4.34, 2.68-3.80, 2.30-2.64, 0.68-0.88, 79.07-88.85, 382-389 kcal% respectively. The dietary were nutritional adequate to prepare a *moringa oleifera* meals and could meet the estimated daily nutrient requirements for elderly and infants, these confirmed the previous literature (Ibironke, *et al*, 2014d).

Table 3 shows functional properties of dietary samples ranged from bulk densityg/ml, Water Absorption Capacity g/g, Swelling Power%, Swelling Power% ranged from 0.57-0.72, 0.89-2.165,22.22-37.03 and 12.50-15.55 respectively (Oloyede, *et al* 2011). Samples dietary had higher values than control sample; bulk density is a reflection of the load the sample can carry if allowed to rest directly on one another. High bulk density of powdered food is desirable for packing, since it allows more weight to be contained in a limited volume. Water absorption capacity absorbed more water than sample dietary conformed to other studies. This may be as a result of production of liquid gruel due to breakdown of starch by the enzymes (Adeniyi *et al* 2014) Swelling capacity dietary samples were better than control sample dietary, this may be due to presence of enzymes call amylase released and breakdown the starch into dextrin-maltose which does not swell so well when cooked into gruel. But nitrogen free diet had poor swelling capacity. Gelation power of moringa samples dietary form gel at high concentrations are ideal for weaning foods because they would require a lot of dilution in an attempt to improve digestibility in relation to volume (Adeniyi *et al* 2014).

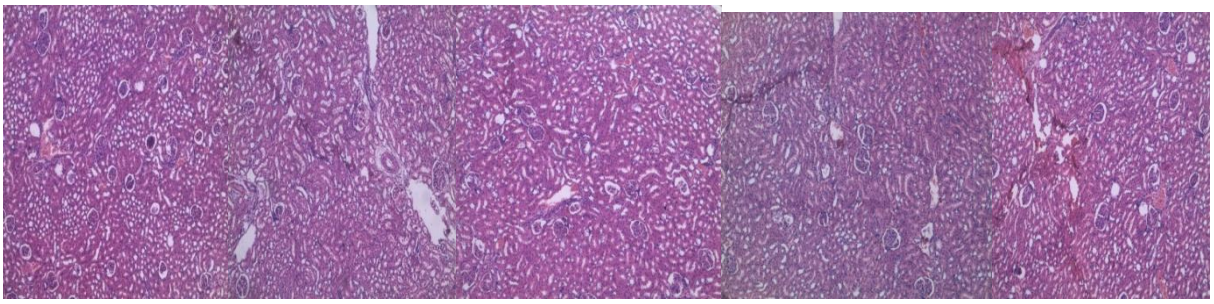
Histopathological Study



1 2 3 4 5

Figure 2a: Photomicrographs showing the transverse sections of livers(x100) for experimental groups 1-5.

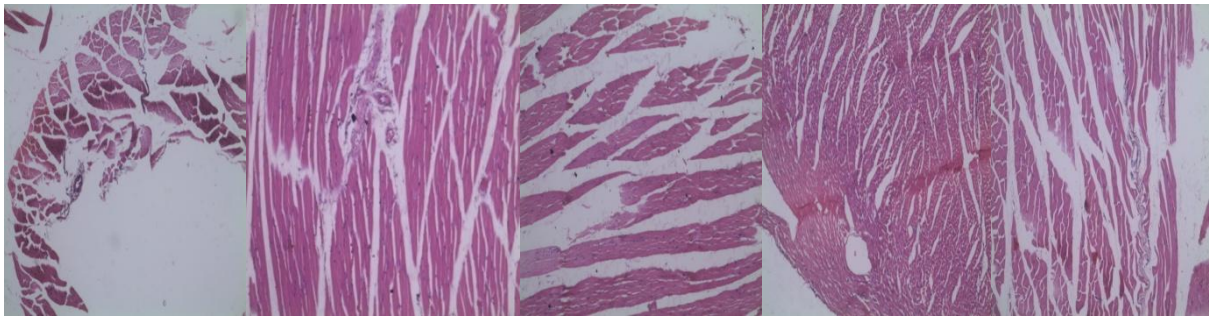
The livers of animal experimental groups 1-5 indicated no infarcted zone, no oedema, no inflammatory cells and separation of cardiac muscle fibres, no cellular disintegration, and no toxicity hence fit for consumption (Gunjul *et al* 2010 Kuku, *et al* 2013 Ibironke,*et al* 2015f, Jibril,2015).



1 2 3 4 5

Figure 2b: Photomicrographs showing the transverse sections of kidneys(x100) for experimental groups1-5.

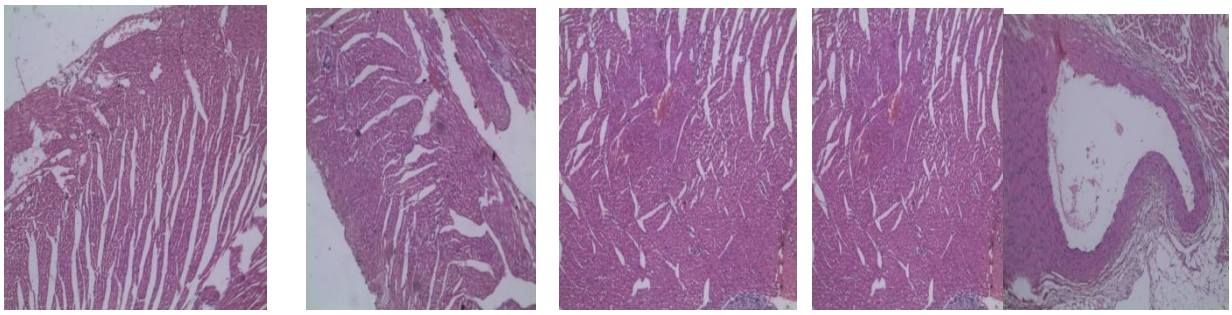
The kidneys of animal experimental groups 1-5 indicated no infarcted zone, no oedema, no inflammatory cells and separation of cardiac muscle fibres, no cellular disintegration no toxicity hence fit for consumption (Gunjul *et al* 2010 Kuku, *et al* 2013 Ibironke,*et al* 2015f, Jibril,2015).



1 2 3 4 5

Figure 2c: Photomicrographs showing the transverse sections of muscles(x100) for experimental groups 1-5.

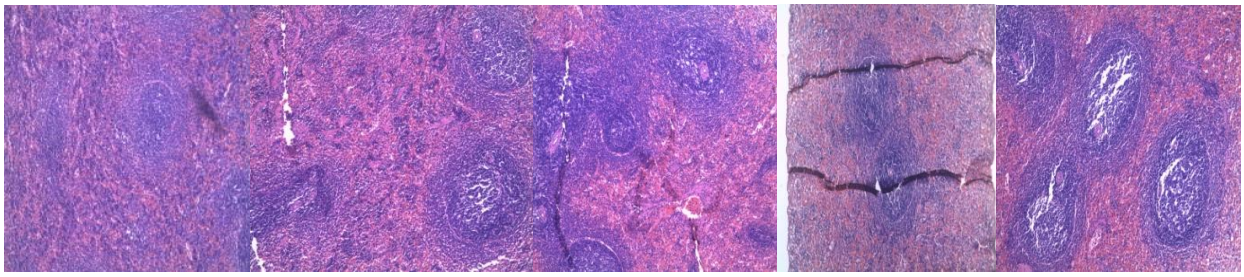
The muscle of animal experimental groups 1-5 indicated no infarcted zone, no oedema, no inflammatory cells and separation of cardiac muscle fibres, no cellular disintegration, no toxicity hence fit for consumption (Gunjul *et al* 2010 Kuku, *et al* 2013 Ibironke,*et al* 2015f, Jibril,2015).



1 2 3 4 5

Figure 2d: Photomicrographs showing the transverse sections of hearts(x100) for experimental animal groups 1-5.

The hearts of animal experimental groups 1-5 indicated no infarcted zone, no oedema, no inflammatory cells and separation of cardiac muscle fibres, no cellular disintegration no toxicity hence fit for consumption (Gunjul *et al* 2010 Kuku, *et al* 2013 Ibironke,*et al* 2015f, Jibril,2015).



1 2 3 4 5

Figure 2e: Photomicrographs showing the transverse sections of spleen(x100) for experimental animal groups1-5.

The spleens of animal experimental groups 1-5 indicated no infarcted zone, no oedema, no inflammatory cells and separation of cardiac muscle fibres, no cellular disintegration, no toxicity symptom hence fit for consumption no infarcted zone, without oedema, no inflammatory cells and separation of cardiac muscle fibres, no cellular disintegration, no damage to tissue and comparable to control (Gunjul *et al* 2010 Kuku, *et al* 2013 Ibironke,*et al* 2015f, Jibril,2015).

4. CONCLUSIONS

Moringa seed samples dietary have proved that moringa food is functional and consumable. Histopatological parameters of experimental animal groups fed on moringa samples dietary1-5 showed no infarcted zone, no oedema, no inflammatory cells and separation of cardiac muscle fibres, no cellular disintegration, hence no toxicity symptom and fit for human consumption. Proximate analysis and chemical is nutritional adequate to supplement protein to meet nutritional daily need, that the body requires. It is highly digestible, optimum in water uptake, desirable for packing; suitable for adult and mothers at home to quantify diets (1g/ml) during preparation.

5. ACKNOWLEDGEMENTS

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