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Chemical Constituents of Crude Extracts of *Moringa oleifera* (Lam) Leaf and Biochemical Response of Weanling Wistar Rats Administered Crude Extract

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Abstract
Among moringa species, *Moringa oleifera* is the most common because of its nutritious and numerous medicinal uses. The aim of the study was to investigate the chemical constituents of crude extracts of *Moringa oleifera* leaf and biochemical response of weanling Wistar rats administered crude extract. Twenty, six weeks old seedlings were planted and nurtured till they matured. Thus, fresh *Moringa oleifera* leaves were harvested at random every two weeks and air-dried for 14 days. The dried leaf meal was subjected to nutritional evaluation, determination of proximate and mineral composition as well as phytochemical screening. However, the effect of different ages of harvest was examined on the nutritional evaluation of *Moringa oleifera* leaf. Four-week old Wistar rats were assigned to seven dietary treatments of ten rats each in a Completely Randomised Design (CRD) for each group of alkaloids, saponin and tannin extracts in comparison with crude moringa extract. The rats were fed *ad libitum* for 21 days. Effect of age on proximate, mineral and phytochemical composition of *Moringa oleifera* leaves was not significantly ($p>0.05$) different across the treatments. The higher the concentration of Moringa extract and alkaloids, the lower the body weight gain. Higher doses of alkaloids had more depressive effect ($p<0.05$) on the weight change of the experimental animals when compared with higher doses of Moringa extract. Rats exposed to both Moringa extract and crude alkaloids had significantly ($p<0.05$) lower body weight change when compared with those on control group.

Keywords: Alkaloid, extract, Moringa oleifera, saponin, tannin, toxicology

Introduction

Plants provide food, shelter and are most time used for curing various human and livestock ailments. Modern-day animal nutrition focuses more on the utilization of plant nutrients for feed cost reduction, easy digestibility and absorption of nutrients. As far back as 150 A.D., the ancient kings, queens and warriors of India, Greek, Roman and Egypt have used herbs for treatments of various diseases and revitalization drinks for relieving stress and pain incurred by warriors during world war (Aftab and Sial, 1999; Kar *et al*., 2004). There is this general belief that herbal plants are safe and economical. Leaf meal had been reported by many researchers to contain high content of minerals, vitamins and phytochemicals, but with constraints of high fibre content, presence of anti-nutritional compounds and deficiency in some amino acids. Makkar and Becker (1997) had reported an increasing use of *Moringa oleifera* also known as Drumstick, Horseradish as a good source of protein in livestock feed. Among moringa species, *Moringa oleifera* is the most common because of its nutritious and numerous medicinal uses (Nikkon *et al.*, 2003). A few others species like *Moringa stenopetala, Moringa peregrine* and *Moringa concanensis* have been discovered of having equal potential with *Moringa oleifera*. Most traditional healers do not put into consideration the toxicological effect of these medicinal plants because they believe that the plants are harmless and therefore, there is no need to actually take record of any negative effects on their patients over a long period of time. It is, therefore, very difficult for these traditional healers to detect or monitor delayed effects and rare adverse effects arising from long-term use. There is scanty literature on moringa genus with regards to toxicological studies; however, the few reports on *Moringa oleifera* and *Moringa stenopetala* available are not exhaustive. The study was therefore carried out to investigate the chemical constituents of crude extracts of *Moringa oleifera* (Lam) leaf and biochemical response of weanling Wistar rats administered crude extract.

Materials and Methods

Location of the study

The feeding trial was conducted at the Department of Animal Science, University of Ibadan, Nigeria. It is

located on latitude 7°20′N, 3°50′E, 200m above sea level.

Cultivation of Moringa plant
Twenty, six weeks old seedlings were purchased from National Cereal Research Institute Ibadan, Nigeria and transported to Abeokuta, Nigeria where they were planted. The seedlings were planted during May, 2013 by adopting a spacing of 2m by 2m and were rain-fed. The seedlings were rain-fed. Leaves were harvested at 12, 14, 16, 18 and 20 weeks of age.

Preparation of dry Moringa leaf meal
Fresh leaves of *Moringa oleifera* were harvested at random every two weeks and the leaves were air-dried for 14 days when a constant weight was attained. The selected dried leaves were ground into powder with a motorized electric blender. It was allowed to cool down and kept in a sealed container to prevent absorption of moisture.

Nutritional evaluation
One hundred grams of dried powdered samples were soaked in 500 ml of distilled water for 12 hrs to prepare aqueous extract of the leaves. Methanolic and ethanoic extracts of the leaves from different ages were prepared using the same method for the aqueous extract. The three extracted solvents were compared in terms of rate of recovery to determine which solvent gave the highest quantity of extract. This was done by weighing the quantity of extract and the residue. The extract from methanol solvent was used for the determination of proximate, elemental and phytochemical quantity.

Determination of proximate and mineral composition
Proximate analyses were carried out on the methanol leaf extract according to the procedure of Association of Official Analytical Chemist (AOAC, 1990). All determinations were done in triplicates and the values reported in percentages. Atomic absorption spectrophotometer (aas-buck 205) was used to determine magnesium, calcium, potassium, sodium, iron, iodine, phosphorus and chloride (AOAC, 1990). All determinants were done in triplicates.

Phytochemical screening
Phytochemical screening procedures of Odebiyi and Sofowora (1979) on plant analysis were adopted. Bioactive compounds analysed were, alkaloids, flavonoids, tannins, phenols, cyanides and saponins. All determinants were done in triplicates.

Extraction and isolation of Moringa alkaloids
Seven hundred grams (700 g) of moringa leaf meal was taken and extracted with 4 litres of 100% methanol (x3) at room temperature for 72 hours each time. Extracts were pooled together and concentrated to dryness in vacuo on a rotary evaporator yielding 90 g. Fifty gram (50 g) of the crude extract was suspended in distilled water and partitioned with n-hexane, ethyl acetate and butanol successively. The organic fractions and the aqueous mother liquor were concentrated to dryness in vacuo on a rotary evaporator. The n-butanol fraction was dissolved in water (200 ml), acidified with 5% HCl and partitioned with dichloromethane (4*400ml), concentrated and coded F1. The aqueous acidic medium was basified with ammonia, and the liberated bases were extracted with di-chloromethane. The organic solvent was removed in vacuo to give an extract coded F2 which tested positive with Draggendorffs' reagent for Alkaloids. The crude Alkaloids isolated was kept in the refrigerator for proper preservation.

Preparation of crude extract solution
One gramme of crude alkaloid extract was dissolved in 10 mL of distilled water to produce homogenous solution from which three different volumes; 1 mL, 1.5 mL, 2 mL were administered orally. Similarly, three volumes of the moringa extract were also administered orally. All rats were fed with the same basal diet, which also served as control. The same procedure was followed for saponin and tannin experiments.

Experimental animals and management
For each group of the studies (alkaloids, saponins and tannins), seventy weanling 4-weeks old Wistar rats were assigned to seven dietary treatments of ten rats each in a completely randomised design. The rats were fed *ad libitum* for 21 days. The rats were given the extracts orally each morning after which feed and water were served two hours later.

Experimental design
The design of the study was a Completely Randomized Design.

Statistical analysis
Data obtained were subjected to one-way analysis of variance (ANOVA) according to Steel and Torrie (1980) and analyzed using statistical analysis software (SAS, 2012). Significant means were separated using Duncan's Multiple Range test at $p<0.05$ (Duncan, 1955).

Results and Discussion
Results
Gross composition of basal diet fed to the experimental animals is shown in Table 1. Effect of age of growth on proximate composition of *Moringa oleifera* leaves and on growth on mineral compositions of *Moringa oleifera* leaves are shown in Tables 2 and 3 respectively. Tables 4 and 5 show the quantitative phytochemical constituent of *Moringa oleifera* leaves at different ages of growth and the effect of moringa alkaloid, saponin and tannin extract on growth performance of rats. Effect of age on proximate, mineral and phytochemical composition of *Moringa oleifera* leaves was not significantly different across the treatments. Comparison of moringa extract with crude moringa alkaloids on growth performance of Wistar rats (Table 5) shows that feed intake was significantly ($p<0.05$) higher in rats exposed to 1 and 1.5mg/ml moringa alkaloids when compared with those on control and 1and 1.5 mg/ml moringa extract. Rats exposed to 2 mg/ml moringa alkaloids had significantly

($p<0.05$) lower feed intake when compared with those fed 1 and 1.5 mg/ml alkaloids, 2 mg/ml moringa extract and control. The higher the concentration of moringa extract and alkaloids, the lower the body weight gain. Higher doses of alkaloids had more depressive effect on the weight change of the experimental animals when compared with higher doses of moringa extract. Rats exposed to both moringa extract and crude alkaloids had significantly ($p<0.05$) lower body weight change when compared with those on control group. Rats on 1 and 1.5 and 2 mg/ml crude moringa alkaloids had highest feed conversion ratio than the animals in other groups. The higher the concentration of moringa extract and crude moringa alkaloids, the higher the feed conversion ratio.

For moringa extract and crude moringa saponins, feed intake decreased significantly ($p<0.05$) with increase doses of moringa extract and saponins. Rats in both moringa extract and saponin groups followed a similar trend. Rats exposed to 1 mg/ml of moringa extract and saponins compared with the control group for feed intake. Weight change was significantly ($p<0.05$) lower for both moringa extract and saponin groups when compared with the control group. The higher the concentration of crude saponin the lower the body weight changes of the experimental animals. Crude saponin had more depressive effect on body weight change of the experimental animals. Rats on 2 mg/ml saponin had the highest feed conversion ratio when compared with other treatments. The experimental animals on the control group had the least feed conversion ratio. Rats on all groups of moringa extract were statistically similar in feed conversion ratio. However, for saponin group, the higher the concentration of saponin, the higher the feed conversion ratio of the experimental animals. The experimental rats exposed to 1 and 1.5 mg/ml moringa extract and crude tannins compared with those on control group for feed intake, while at 2 mg/ml significantly ($p<0.05$) reduced feed intake of the animals when compared with those on control group. Rats on 2 mg/ml tannins had the least feed intake. The higher the concentration of tannin, the lower the body weight change of the experimental animals, although body weight change was significantly ($p<0.05$) lower for moringa extract and crude saponin groups when compared with the control group. Feed conversion was significantly higher ($p<0.05$) for crude moringa tannin group when compared with the moringa extract and control groups. The least feed conversion ratio was obtained by rats on control group followed by those on 1 mg/ml moringa extract group.

Discussion

The rate of recovery for Ethanol, Methanol and aqueous solvents were examined. Methanol solvent was the highest and the aqueous the least, this might be because organic solvent have been proved to extract better (Ezekwe *et al.,* 2013). The extracts of *M. oleifera* from the three different solvents shown that tannins, alkaloids and saponins were significantly present compared with others. Bukar and Loyeyi (2010) earlier reported that saponins wee more detected in *Moringa oleifera* ethanol leaf extract, though with the presence of alkaloids and tannins as recorded in this study. Proximate analysis results of the *Moringa* leaves showed that the leaves are rich. Generally, vegetables make up of essential components that supply protein, calcium, iron, vitamins and other nutrients (Adenipekun and Oyetunji, 2010). Because vegetable fats and oil are lower in lipids their regular consumption always results in reduce occurrence of (Martins, 2007). In comparison with other plants, the ash content of moringa leaves were lower than that of some leafy vegetables commonly consumed in Nigeria such as: *Talinum triangulare* (20.05%), Acalypha *marginata* (15.68%) but they compared favorably with some other vegetables such as *Occimum graticimum* (8.00%) and *Hibiscus esculentus* (8.00%) (Akindahunsi and Salawu, 2005). Crude protein of Moringa leaves ranges from 22.13-22.82% for the different ages of growth, they compared favourably with; *Amaranthus caudatus* (20.59%), cassava leaves (*Manihot utilisima*) 24.88%, *Piper guineeses* 29.78% and *Talinum triangulare* 31.00% (Akindahunsi and Salawu, 2005). However, Yang *et al*. (2006) recommended that moringa leave meal should be promoted for greater consumption to improve nutrition and strengthen immune functions. Lipid content was very low when compared to Talinum *triangulare* (5.90%), *Baseila alba* (8.71%), *Amaranthus hybridus* (4.80%), *Calchorus africanum* (4.20%) and *Acalypha racemosa* (6.30%) (Akindahunsi and Salawu, 2005).

Phytochemicals which can also be referred to as bioactive compounds are non-nutritive plant chemicals with ability to instill physiological effects on farm animals. The quantitative result revealed that the leaves contain tannins, saponins and alkaloids in appreciable amount. Saponins in *Moringa oleifera* leaves possess both beneficial (cholesterol lowering) and deleterious (cytotoxic; permeabilization of the intestine) properties (Price *et al.,* 1987, Oakenful and Sidhu, 1989). Although some saponins have been shown to be highly toxic under experimental conditions, acute poisoning is relatively rare both in animals and man (Osagie, 1988). The beneficial effect of saponins includes lowering blood cholesterol levels, cancer prevention, bone health and stimulation of the immune system. Both tannins and alkaloids have also been reported to having the ability to prevent some basic animal and human disease (Kasolo *et al*., 2010). In general, the presence of these Phytochemicals could account for the much-touted medicinal properties of these leaves in various disease conditions. The administrative effect of methanol moringa leaf extract on the feed intake is determined by appetite of the animal. The bitter taste of the extract usually affects the feed intake; this is attributed mainly to condensed tannins (Dixon *et al*., 2005). Evers (2008) reported that high rate of saponins caused significant reduction in the absorption of dietary nutrients in the gastrointestinal tract due to *leaky gut*. This might have played a part in reduction of intake of the animals. It had been documented that administration of moringa leaf extract lowered feed intake of rats in an almost dose-dependent manner (Oyewo *et al*., 2013b). The reduction

in feed intake had been linked to slow metabolism of ingested substances in the gastrointestinal tract.

The loss in body weight in a dose-dependent manner by the experimental animals is in line with report of Stanek *et al*. (2015) who that stated that rats were sensitive to the bitter taste of alkaloids. Lupine seeds alkaloid had a negative effect on feed intake, resulting in lower body weight gains of rats. Sobotka *et al.* (2013) on the other hand reported that rats' response to dietary alkaloid was low, but with non-significant decrease in the growth rate. Similar trend was reported by Butler *et al*. (1996). Robbins *et al*. (1996) noted that reduction in feed intake in the first two weeks of feeding may be traced to alkaloid intake. Reddy *et al.* (2012) reported that saponin-rich aqueous leaf extract of *Gymnema sylvestre* reduced weight gain and feed consumption of rats. Nakamura *et al.* (2001) submitted that tannic acid at a dose of only 0.1 g/kg of body weight led to a reduction of live weight gain of rats which suggested to arise from a negative influence of tannic acid on nutrient digestibility and absorption. The most tannic acid-sensitive nutrient is protein (Mueller-Harvey, 2006). It may precipitate by binding to tannins which can lead to the inhibition of enzyme activity and significant reduction of protein and dry matter digestibility (Jansman, 1993). The result obtained on the effect of saponin in the present study was corroborated by the report of Igwilo *et al*. (2013) who noted that soaked *Moringa oleifera* seed did not support growth of albino rats. Similarly, Oyewo *et al.* (2013a) reported weight loss in rats exposed to increasing concentration of the aqueous leaf extract. The trend of result obtained indicates crude Moringa extract and its phytochemicals possess growth depressive effect on the experimental rats.

Conclusion

No significant ($p>0.05$) difference was observed across the treatments for the effect of age on proximate, mineral and phytochemical composition of *Moringa oleifera* leaves. The higher the doses of Moringa extract and alkaloids, the lower the body weight gain. Higher doses of alkaloids had more depressive effect ($p<0.05$) on the weight change of the experimental animals when compared with higher concentrations of Moringa extract. Experimental animals on both Moringa extract and crude alkaloids had significantly ($p<0.05$) lower body weight change when compared with those on control group. Similar studies that investigate the effect of Moringa phytochemicals on challenged animals are therefore recommended.

Table 1: Gross composition of basal diet fed to weanling Wistar rats

Ingredients	Inclusion (g/100g)
Corn starch	58.87
Casein	10.53
Glucose	5.00
Sucrose	7.00
Cellulose	5.00
Vitamin premix*	0.30
Salt	0.30
Di-calcium phosphate	2.00
Lime stone	1.00
Total	100.00

Premix composition: vit A 10,000000iu, Vit D3 2,000000iu, Vit E20,000mg, Vit k3 2,000mg, Vit B1 3,000mg, Vit.b2 5,000mg, Niacin 4,500mg, Vit B6 4,000mg, Vit B12 20mgBiotin 100mg, Choline Chloride 3000000mg, Manganese 50mg, Iron 300000mg, Zinc 120,000mg, Copper 80,000mg, Iodine 15000mg, Cobalt 300mg, Selenium 120mg, Anti-oxidant 120,000mg

Table 2: Effect of ages of growth on proximate composition of *Moringa oleifera* leaves

Parameters (g/100g)	Week 12	Week 14	Week 16	Week 18	Week 20
Moisture	8.96±0.52	8.81±0.39	8.56±0.14	7.93±0.49	7.82±0.60
Crude protein	22.82±0.40	22.66±0.24	22.36±0.07	22.13±0.29	22.11±0.31
Ether extract	2.38±0.08	2.42±0.04	2.48±0.07	2.48±0.02	2.53±0.07
Ash	5.59±0.66	6.16±0.09	6.26±0.01	6.36±0.11	6.51±0.26
Crude fibre	7.16±0.70	7.90±0.16	8.39±0.33	8.41±0.35	8.45±0.39
NFE	53.09±0.70	52.05±0.34	51.95±0.44	52.69±0.30	52.58±0.19

NFE = nitrogen free extract

Table 3: Effect of ages of growth on mineral compositions of *Moringa oleifera* leaves

Parameters (mg/g)	Week 12	Week 14	Week 16	Week 18	Week 20
Calcium	741.67±26.33	761.67±6.33	766.67±1.33	770.00±2.00	800.00±32.00
Iron	17.32±0.51	17.28±0.47	16.63±0.18	16.44±0.37	16.39±0.42
Magnesium	128.33±7.45	130.55±5.23	136.67±0.89	141.66±5.88	141.67±5.89
Sodium	611.67±15.22	625.56±1.33	627.78±0.89	632.22±5.33	637.22±10.33
Chlorine	305.56±27.77	328.335. ±00	340.00±6.67	345.67±12.34	346.111±2.78
Potassium	238.33±16.89	250.00±5.22	253.33±1.89	264.44±9.22	270.00±14.78
Iodine	0.28±0.05	0.30±0.03	0.30±0.03	0.36±0.03	0.40±0.07
Phosphate	658.11±36.38	694.89±0.00	697.17±2.28	705.56±10.67	705.11±10.22

Table 4: Quantitative phytochemical constituent of *Moringa oleifera* leaves at different ages of growth

Parameters (mg/g)	Week 12	Week 14	Week 16	Week 18	Week 20
Alkaloids	235.56±8.66	242.78±1.44	244.22±0.00	247.78±3.56	250.00±5.78
Saponins	153.27±4.28	154.22±3.33	159.16±1.61	159.55±2.00	161.33±3.78
Tannins	132.22±17.56	146.11±3.67	150.56±0.78	150.56±0.78	169.44±19.99

Table 5: Effect of moringa alkaloid, saponin and tannin extract on growth performance of rats

Parameters (g)	Control	Moringa extract			Crude Moringa alkaloids			SEM
		1 mg/mL	1.5 mg/mL	2 mg/mL	1 mg/mL	1.5 mg/mL	2 mg/mL	
Feed intake	32.20^{b}	27.48^{bc}	24.25^{c}	37.25^{a}	36.23^{a}	36.23^{a}	23.60^{c}	1.44
Weight change	39.08^{a}	24.00^{b}	16.75^{c}	16.00^{c}	25.25^{b}	13.00^{d}	8.20^{e}	1.06
FCR	0.82^{d}	1.27^{c}	1.64^{b}	1.52^{b}	1.48^{cb}	2.79^{a}	2.88^{a}	0.08
Parameters (g)	**Control**	**Moringa extract**			**Crude Moringa saponins**			**SEM**
		1 mg/mL	1.5 mg/mL	2 mg/mL	1 mg/mL	1.5 mg/mL	2 mg/mL	
Feed intake	32.20^{a}	30.40^{a}	27.48^{b}	24.25^{c}	31.00^{a}	27.48^{b}	24.73^{c}	1.44
Weight change	39.08^{a}	24.00^{b}	16.75^{c}	16.00^{c}	16.25^{c}	10.00^{d}	8.45^{e}	1.11
FCR	0.82^{e}	1.27^{d}	1.64^{cd}	1.52^{cd}	1.91^{c}	2.75^{b}	2.93^{a}	0.12
Parameters (g)	**Control**	**Moringa extract**			**Crude Moringa tannins**			**SEM**
		1 mg/mL	1.5 mg/mL	2 mg/mL	1 mg/mL	1.5 mg/mL	2 mg/mL	
Feed intake	30.88^{a}	31.00^{a}	27.89^{ab}	25.00^{b}	32.25^{a}	31.60^{a}	21.38^{c}	1.49
Weight change	38.00^{a}	24.00^{b}	17.66^{bc}	17.01^{c}	17.25^{bc}	16.75^{c}	11.50^{d}	0.97
FCR	0.81^{d}	1.29^{c}	1.58^{b}	1.47^{b}	1.87^{a}	1.87^{a}	1.86^{a}	0.10

$^{a, b, c, d}$ Means with different superscripts along the same row are significantly (P<0.05) different. SEM = Standard Error Mean

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