Short-term toxicological evaluation of *Cucumeropsis mannii* seed oil in albino rat

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Abstract: Seed oil of *Cucumeropsis mannii* was extracted and characterized. Acid value, peroxide value, saponification value, iodine value and refractive index had the average values of 1.45 ± 0.07mgKOH/100g, 2.80 ± 0.00Meq/kg, 143.2 ± 0.21mgKOH/100g, 73.6.5 ± 0.21mgI_2/100g and 1.4132 ± 0.00 respectively. 5% of *C. mannii* seed oil was incorporated into the ingredient for compounding a balanced rat feed and served as the experimental feed while 5% of groundnut oil replaced 5% of *C. mannii* oil in the control feed. These feeds were analysed for chemical composition and some mineral content. The moisture content, crude protein, crude fat, crude fibre, ash content and carbohydrate obtained for the experimental diet were 12.96 ± 0.09%, 25.17 ± 0.22%, 6.51 ± 0.03%, 4.37 ± 0.06%, 10.72 ± 0.09% and 44.63 ± 0.25% respectively while those for the control group include 13.08 ± 0.09%, 25.25 ± 0.25%, 7.22 ± 0.09%, 4.68 ± 0.04%, 40.31 ± 0.02% and 44.14 ± 0.29% respectively. The experimental feed had higher concentration of calcium, (8.190%), magnesium (0.388%) and iron (0.104%) than that of control which is 2.040% Ca, 0.300% Mg and 0.075% Fe. Haematological and biochemical examination of rat blood was also carried out to observe the effect of *C. mannii* and groundnut oils on them. The results showed that there was no significant difference between the two groups for all the blood indices. There were no pathological changes in all the organs of the rats from both groups at histology level. *C. mannii* oil appeared not to have adverse effect on the rats; it actually supported their gradual growth.

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1. Introduction

Evidence has shown that consumption of diet high in saturated fatty acids contributes significantly to the risk of developing cardiovascular diseases and some form of cancers and atherosclerotic heart diseases in humans (Romero-Coral *et al.,* 2006). A healthy diet is low in saturated fatty acids and high in mono and poly unsaturated fatty acids and renders the valuable potentials of reducing the risk of cardiovascular diseases (Laidlaw and Holub, 2003**).** It has been confirmed that most diets in developing countries especially African countries are high in carbohydrates and remarkably low in high quality protein and essential fatty acids (Krivanek *et al.*, 2007). Most developing countries depend on starch based foods as the main basic foods for the supply of both energy and protein. Oils and fats are substances of vegetable or animal origin. The most important feature of fats and oils is that they have a caloric energy content more than twice as high as the other food stuffs such as protein, and carbohydrates and they are source of essential fatty acids. Also, they act as lubricant during mixing of ingredients and as medium for heat transfer carrier for fat soluble vitamins.

Cucumeropsis mannii is cultivated widely in West African countries especially in Cameroon (Fomekong *et al.,* 2008). It is a species of melon native to tropical Africa where it is grown for food and oil source. The plant, usually grown during the rainy season (March-September), produces climbing vine up to 4m long and bears small yellow male and female flowers with petals under a centimeter in length. The fruits are egg shaped or an elongated oval shape, up to about 19cm long and 8cm wide, cream in colour with green streaks (Loukou *et al*., 2007). It usually takes 3-4 months to mature for harvesting; it is an annual crop mostly grown as a subsidiary crop which is inter planted with early maize and yam in some savannah belt of Nigeria (Mabalaha *et al.*, 2007). When the fruits are matured, the seeds of *C. mannii* are usually harvested from the fruits and dried, then manually shelled to collect the kernels. The kernels are ground and used for soup or vegetable towards obtaining a balanced diet (Fokou *et al***.,** 2004) and serve as an ingredient for seasoning baked meats, and fish. Anhwange *et al*. (2010), reported the oil yield and physicochemical characteristics of *C. mannii* seed oil. The aim of this study is to evaluate the seed oil of *C. mannii* for nutritional purposes. This is part of our efforts to bring into focus the many lesser known seed oils for nutritional and industrial purposes (Ajayi *et al*., 2004; 2007; 2013).

2. Materials and Methods

C. mannii seeds used for this study were purchased from Bodija market in Ibadan, Nigeria. The seeds were decorticated and the whole seeds were air dried. These seeds were reduced in size and subjected to solvent extraction using soxhlet extractor and n-hexane as the solvent for eight hours to achieve maximum yield (AOAC, 2008).

The moisture, crude protein, crude fat, total carbohydrates, crude fibres were determined using AOAC methods (AOAC, 1995). Characterization was carried out to access the quality of the oil extracts using standard methods. Assessment was carried out for the following parameters; specific gravity, refractive index, iodine value, saponification value and colour (Ajayi *et al.*, 2007). Fe, Mg, Ca were determined after wet digestion of the seed with a mixture of nitric, sulphuric and hydrochloric acid. These nutritionally significant metals were determined by atomic absorption spectrophotometer (Perkin-Elmer, Model 2380, USA) as outlined by AOAC (1996).

Fourteen weanling albino wistar rats (weighing between 50g and 80g) were obtained from the experimental Animal House of the Department of Veterinary Anatomy, University of Ibadan, Nigeria. These rats were divided into two groups of seven rats each for the experimental and the control group. The animals were fed for eight weeks.

Feed was formulated to meet the entire nutrient requirement for young albino rats. The feed was prepared according to the formula described by Toyomizu *et al.* (2003) with little modification (Table 1). Rats in the control group were fed with compounded feed containing 5% crude groundnut oil while their counterpart in the experimental group were fed with compounded feed containing 5% crude *C. mannii* seed oil. These rats had unlimited access to water and feed. The daily feed intake and weekly body weight gain of individual rats were recorded for the period of eight weeks of the experiment. Physical appearance of all the rats was closely observed.

At the end of the feeding period of eight weeks, the rats were fasted over night after which they were sacrificed. About 3ml of blood sample was collected from each rat in the two groups through the eye using capillary tubes into EDTA tubes on the day they were sacrificed. One portion of the blood was subjected to haemotological examination while the other portion was centrifuged to separate plasma from red blood cells after which total cholesterol, triglycerides, high density lipoprotein (HDL) and low density lipoprotein (LDL) were analyzed for. Tissues of rats collected include brain, heart, liver, kidney, spleen and small intestine. These organs were quickly weighed and preserved in formalin after which they were fixed for further studies.

White and red blood cells were determined using Neubaurer haemocytometer. Packed cell volume (PCV) was determined by microhaematocrit centrifuge. Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) were determined according to the method of Jain (1986). Total protein (TP), Albumin (ALB) and Globulin (GLB) were also analyzed for.

The method of estimating the plasma concentration of total cholesterol was according to Fredrickson *et al.* (1986). Cholesterol in the heart homogenate was measured using the method outlined by Gottfried (1973) as detailed in our previous study (Ajayi *et al.*, 2013).

Means were analyzed using a one-way analysis of variance (ANOVA) and complemented with Student's t-test. Differences with values of $P < 0.05$ were considered statistically significant (Mahajan, 1997).

3. Results

Table 1. Composition of the feed

Composition (kg)	Experimental	Control
Maize	4.000	4.000
Soybean	1.820	1.820
Dicalcium phosphate	0.330	0.330
Salt	0.079	0.079
Groundnut cake	0.945	0.945
Corn bran	0.700	0.700
Palm kernel cake	0.700	0.700
Wheat	0.200	0.700
Limestone	0.226	0.226
C. mannii oil	0.000	0.500
Groundnut oil	0.500	0.000

Table 2. Proximate composition of the diets of experimental and control rats

Parameter (%)	Experimental	Control
Moisture content	12.96 ± 0.09^{r}	13.08 ± 0.09^{r}
Crude protein	25.17 ± 0.22^{r}	25.25 ± 0.25^{r}
Crude fat	6.51 ± 0.03^{r}	7.22 ± 0.09^{r}
Crude fibre	4.37 ± 0.06^{r}	4.68 ± 0.04^{r}
Ash content	10.72 ± 0.09^{r}	10.31 ± 0.02^{r}
Total carbohydrate	44.63 ± 0.25^{r}	44.14 ± 0.29^{r}

Means with the same alphabets are not significantly different at $p < 0.05$

Table 3. Physicochemical properties of *Cucumeropsis mannii* seed oil

Parameter	Range	Mean ± SD
Acid value (mgKOH/g oil	1.4 - 1.5	1.45±0.07
Saponification (mgKOH/g oil	142.5	143.20±0.21
Iodine value (mgI_2/100g)	73.5 - 73.8	73.65±0.21
Peroxide value (mEq/kg oil)	2.8	2.80±0.00
Refractive index (25^0C)	1.4129 -1.4134	1.4132±0.00

Table 4. Percentage mineral element composition of diets

Mineral	Experimental	Control
Calcium	8.190	2.040
Magnesium	0.388	0.300
Iron	0.104	0.075
Lead	0.005	0.001

Table 5. Organ weights of rats (g)

Organ	Experimental	Control
Brain	1.26 ± 0.08^r	1.39 ± 0.21^r
Spleen	0.56 ± 0.14^r	0.70 ± 0.17^r
Kidney	0.81 ± 0.04^r	1.06 ± 0.05^s
Liver	4.47 ± 0.51^r	5.63 ± 0.98^s
Lungs	0.97 ± 0.29^r	1.24 ± 0.29^r
Heart	0.47 ± 0.08^r	0.60 ± 0.10^s
Intestine	1.29 ± 0.31^r	1.63 ± 0.56^r

Means with different alphabets are significantly different at $p < 0.05$

Table 6. Haematological and biochemical indices of rat blood

Parameter	Experimental	Control
PCV (%)	42.00 ± 3.27^r	42.57 ± 1.90^r
Hb (mg/dl)	13.57 ± 0.80^r	13.64 ± 0.61^r
RBC (10^6/µl)	7.11 ± 0.53^r	7.08 ± 0.36^r
WBC (10^3/µl)	4657.14 ± 1259.44^r	5236.57 ± 1311.37^r
Platelets	81142.86 ± 21667.40^r	94714.29 ± 17726.76^r
Lymphocyte	66.57 ± 10.01^r	61.71 ± 13.38^r
Neutrophyl	29.00 ± 10.74^r	33.57 ± 11.76^r
Monocyte	2.29 ± 0.76^r	3.00 ± 0.82^r
Eosinophyl	2.29 ± 0.95^r	1.71 ± 1.60^r
Total protein	8.09 ± 0.43^r	7.96 ± 0.42^r
Albumin	4.74 ± 0.36^r	4.76 ± 0.37^r
Globulin	3.34 ± 0.09^r	3.20 ± 0.20^r
A/G ratio	1.39 ± 0.11^r	1.47 ± 0.08^r

Means with the same alphabets are not significantly different at $p < 0.0$

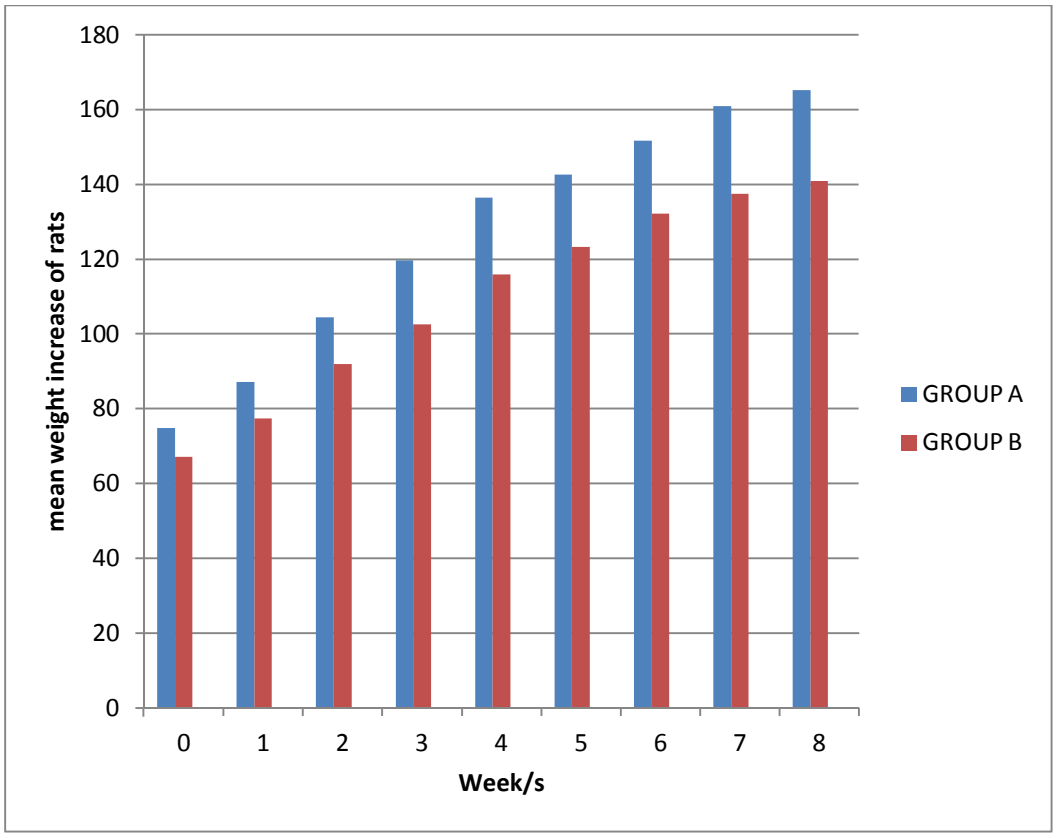

Figure 1. Graph of body mean weight

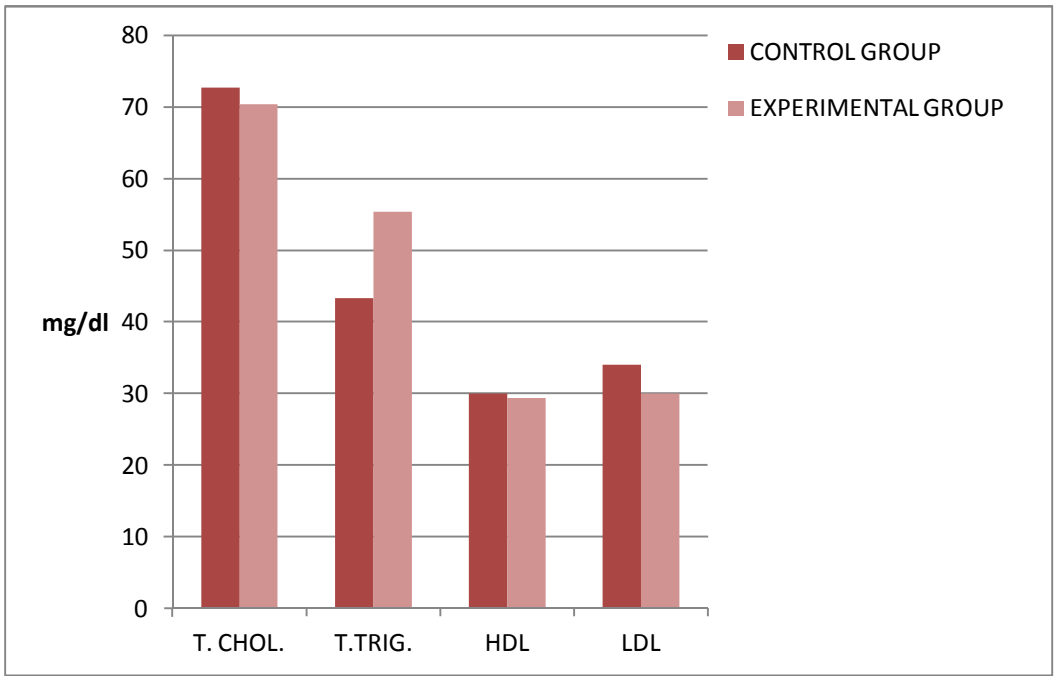

Figure 2. Mean cardiac total cholesterol, total triglyceride, HDL and LDL of rats

4. Discussions

Table 2 shows the result of proximate analysis of compounded feed for both experimental and control groups. There were no significant differences in all the proximate parameters of the two diets. The two diets are rich in carbohydrate (44%) and protein (25%) and low in fat (6-7%). Fat content of *C. manii* diet compares well with that of fenugreek seed meal (Ahmed *et al*., 2009). The values obtained for crude protein in both feeds are higher than the value reported for defatted fluted pumpkin (Agatemor, 2007).

The physicochemical properties of *C. mannii* seed oil are shown on Table 3. The result for acid value, peroxide value, saponification value, iodine value and refractive index were 1.45±0.07mgKOH/100g, 2.80±0.00meq/kg, 143.20±0.21mgKOH/g, 73.65±0.21mgI_2/100g and 1.4132±0.00 (25°C) respectively. These values are lower than the values reported in literature by Anhwange *et al*. (2010) and Essien *et al*. (2012). The very low peroxide value obtained indicates that the oil is not likely to be liable to oxidative rancidity at room temperature (Odoemelam, 2005 and Anyasor *et al*., 2009). Its acid value ranging from 1.4 to 1.5 mgKOH/g oil is within the allowable limit for edible oil. The saponification number is close to that of *G. mangostana* seed oil (Ajayi *et al*., 2007).

Presented on Table 4 is the results of the mineral composition of the compounded diets for experimental and control groups. The experimental diet had higher concentration of calcium (8.19%) in comparison to the control one (2.04%). Both diets contain low concentration of magnesium and iron which are needed for normal physiological functioning of the body.

The body weight of rats and their mean weekly weight increase for both experimental and control groups is given on Tables 5. There were significant differences in body weight between the groups throughout the period of the study with the exception of the first and second weeks. The weight change of the rats in the control group was more remarkable than that of experimental group; the groundnut oil was probably more nutritive than the *C. mannii* oil or there might be some growth inhibitors in the *C. mannii* oil which needs to be removed. The body weight observed in this study followed the pattern of rats fed with 5% inclusion of *G. mangostana* seed oil in their feed (Ajayi *et al*., 2007).

Table 7 shows the organ weights of the experimental and control rats fed for eight weeks. The organs harvested and weighed were heart, liver, kidney, lung, spleen, brain and intestine. There were significant differences between the weight of kidney, liver and heart of both experimental and control rats. This could be as a result of the differences observed in the body weights of these rats (Table 5).

The result of haematological and biochemical indices obtained for rats in the experimental group compared favourably with those obtained for rats in the control group (Table 6). There were no significant differences ($P > 0.05$) in all cases in both groups. Histopathological results revealed that no visible lesions were found in all the organs of the rats in the two groups.

Presented on Figure 4 is the total cholesterol and total triacyglycerol of the hearts of the control and experimental rats. There is a difference in the total triacyglycerol of the rats in the control and experimental groups. Other parameters differed to various extents but within a closer range; the result suggests that *C. mannii* seed oil could be used to lower the occurrence of coronary heart diseases if consumed. Similar report is given by Ajayi *et al*. (2013) for *M. myristica* seed oil.

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