EVALUATION OF *MONODORA TENUIFOLIA* SEED OIL

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Abstract
The seed oil of Monodora tenuifolia Benth which has previously been characterized was analyzed for fatty acid composition and fed to albino rats to determine its suitability as edible oil. Analyses of the oil for fatty acids revealed that the oil is highly unsaturated (76.22 %) and it contains linoleic and linolenic acids which are two of the essential fatty acids. The main fatty acids of the oil, however, are linoleic acid: 39.37 %; oleic acid: 34.40 % and palmitic acid: 12.56 %. The high level of polyunsaturated fatty acids (40.97 %) in the oil is nutritional significant. The effect on physical appearance, weight gain, organ weight, tissue, plasma cholesterol and triacyglycerol levels was determined in rats fed a diet containing 5 % M. tenuifolia oil. These were compared with those of rats fed with control diets 0 % M. tenuifolia and 5 % groundnut oil. Weekly monitoring of the rats showed good physical appearance and steady weight increase. No visible lesion was observed in all but one of the heart of the test rats during the histopathology examination of the heart, liver, kidney, spleen and lung of the rats and all the parameters analyzed for in the blood samples fall within normal range. The haematological analysis of the blood sample of the rats indicated that they were not anaemic and no mortality was recorded. Monodora tenuifolia seed oil could find application as edible oil/ potential industrial raw material.

Key words: *Monodora tenuifolia*; characterization; fatty acid; histopathology; toxicology

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1 INTRODUCTION

Monodora tenuifolia, a small tree, belongs to the family Annonaceae, (Irvine, 1961). It is generally referred to as African nutmeg while in Nigeria it is referred to as abo-lakoshin by the Yorubas, ehuru ofia by the Igbos and uyenghen by the Edos, (Quattrocchj, 2000). It is widespread (Keay, 1989) and is used as ornamental plant in different locations in University of Ibadan, Nigeria from where it was gathered for this study. Burkill (Burkill, 1985) and Irvine (Irvine, 1961) listed the various uses of its morphological parts in traditional medicine for the treatment of headache (stem bark), dermatitis (root), tooth aches (roots), body itching in children (leaves decoction) and dysentery (bark and root). Its fruits; very rich in flavor; is edible and is a source of edible oil in Nigeria, (Burkill, 1985). *Monodora tenuifolia* contains about 2.5-3 % total alkaloids and has antimicrobial activity, (Adeoye et al., 1986). The presence of volatile oil from its roots (Oguntimehin et al., 1989) and fruits (Adesomoju et al., 1990) has been confirmed. Esuoso, Bayer and Kutubuddin (Esuoso,2000) found triterpene alcohol in the seed and also looked into the phytochemical potency of the seed oil.

Considerable interest has grown in determining the potential toxicity of some uncommon edible oils. Many edible oils are currently referred to as uncommon, non– traditional, minor or unconventional due to inadequate human experience and knowledge about their edible quality, assimilation and freedom from toxicity. *Monodora tenuifolia* seed oil has a share in this even though it has received attention from researchers. The chemical composition of *Monodora tenuifolia* has been reported in literature by Ajayi and Aghanu, (Ajay and Aghanu, 2011). This study assessed the suitability of *Monodora tenuifolia* seed oil as a potential heart friendly and healthy seed oil. It was therefore fed to albino rats in order to ascertain its safety to man. This will contribute to the search of more sources of edible oil since there is need of vegetable oil in a balanced human diet.

2 MATERIALS AND METHODS

2.1. Fruit collection and extraction of the seed oil

Fruits and seeds of *Monodora tenuifolia* were picked from various locations in University of Ibadan. The hard husks of the seed were manually broken to remove the seed coat to give the kernels of *Monodora tenuifolia* which were ground using a domestic grinder to give the grits. The oil was extracted with a soxhlet extractor using normal hexane (40–60 ^{o}C). The oil obtained, after distilling off the hexane, was stored in a labelled flask.

2.2 Fatty acid analysis

Fatty acid analysis of the seed oil was carried out at the Mass Spectrometry Laboratory, University of Sao Paulo, Ribeirao Preto, Brazil. The methyl ester of the raw oil was prepared according to Idouraine, Kohlhepp and Weber (Idouraine et al., 1996) with some slight modification. Oil-solvent mixture was evaporated to dryness under nitrogen and then trans esterified with H_2SO_4 in the presence of methanol for 2h at 7 ^{o}C. To the resulting fatty acid methyl ester was added 40 ml of water after which the organics were extracted with petroleum ether (40-60 ^{o}C) and then dried under nitrogen. The fatty acid methyl esters were redissolved in hexane and analyzed in a Gas Chromatograph Hewlett 5890 Packard Series11. Heptadecanoic acid was used as the internal standard.

2.3 Animal and experimental diets

15 albino rats weighing between 110 and 145 g were obtained from the Animal House, Physiology Department, University of Ibadan, Ibadan, Nigeria. The animals were divided into three groups (n=5) and were housed in cages in an animal house (with twelve hour of light and dark cycles in ambient conditions) for a period of 8 weeks. They were fed on adaptation diet *ad-libitum* for three weeks. After acclimatization, group A were fed *ad-libitum* with 5% incorporation of *Monodora tenuifolia* oil (MTO) in their feed, group B were fed with 5% incorporation of groundnut oil (GO) in their feed while group C, the control group were fed with the normal rat feed (Ladokun Feeds Limited, Ibadan, Nigeria). Feed intake was noted daily while body weight was recorded weekly together with physical appearance, (Ajayi et al., 2004; Ajayi et al., 2004; Ajayi et al., 2008).

2.4 Collection of samples from animals

Three milliliters of blood was collected from each rat by cardiac puncture into heparinized vials and stored at 10 ^{o}C for haematological analysis the same day that they were sacrificed. The haematological analysis was carried out the same day. Similarly, another 3 ml of blood sample was collected into an EDTA bottle from which plasma was harvested by centrifugation at 300 rpm for 5 min at room temperature and stored at 20 ^{o}C until needed for analysis. The abdominal wall of each rat was dissected through the linear alba and peritoneum using the scalpel blade. The liver, heart, kidney and spleen were carefully examined for gross lesions and weighed after removal of blood by blotting on a filter paper.

2.5 Haematological examination and analysis of sample

The packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cell (RBC) and white blood cell (WBC), differential WBC counts, mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were determined and calculated respectively using the standard technique as described by Jain, (Jain, 1986).

2.6 Plasma and tissue cholesterol and tissue triacyglycerol determination

The plasma concentrations of total cholesterol were estimated according to the method of Searcy and Berquist while the cholesterol level in the heart homogenates was measured according to Gottfried, (Gottfried, 1973).

2.7 Tissue pathology

A sample of the heart, liver, kidney and spleen for each animal in the various groups was fixed in 10 % phosphate-buffered formalin and put through series of dehydration in graded concentration of xylene. They were embedded in paraffin wax, sectioned at 5 µ and transferred to clean glass slides. The thin sections were stained with haemotoxylin and eosin (H and E) dyes for examination under the

light microscope for histological changes in the tissues due to the consumption of *M. tenuifolia* seed oil following the methods of Jain, (Jain, 1986).

2.8 Statistical analysis

Data are expressed as the means and standard errors of five separate contents, except for fatty acid. They were statistically analysed by 2-way analysis of variance (ANOVA) and means were compared by Duncan's multiple range test at 5 % level of significance, (Duncan, 1955).

3. RESULTS AND DISCUSSION

3.1 Fatty acids

The fatty acid composition of *Monodora tenuifolia* seed oil was analysed by gas chromatography. Table 1 shows the major fatty acids present in the *M. tenuifolia* seed oil which are palmitic acid (12.56 %), stearic acid (10.23 %), oleic acid (34.40 %), linoleic acid (39.37%) and linolenic acid (1.43 %). It is reported that linolenic acid helps to relieve flaky or rough skin and maintains smooth moist skin (Gottfried, 1973). *M. tenuifolia* oil contains high percentage of unsaturated fatty acid which is about 76.22 %. The palmitoleic acid content is similar to the one reported for *Bauhinia purpurea* (Ramadan et al., 2006) while the oleic acid content compares with that of low land African *Cucurbita pepo* (Younis and Ghirmay, 2000). This high percent of unsaturates compared favourably with that of *Jatropha curcas* fatty acid composition (Nayak and Patel, 2010). A striking feature of *Monodora tenuifolia* seed oil was the relatively high level of polyunsaturated fatty acids (40.97 %). Trienes [(γ -linolenic acid, GLA, C18:3n-6) and (α-linolenic acid, ALA, C18:3n-3)] as well as EPA (C20:5) were also found in relatively lower amounts. A great deal of interest has been placed on the few oils that contain PUFA, especially GLA. Moreover, interest in the PUFA as health-promoting nutrients has expanded dramatically in recent years (Ramadan et al., 2006). A rapid growing literature illustrates the benefits of PUFA, in alleviating cardiovascular, inflammatory conditions, heart disease, athrosclerosis, autoimmune disorders, diabetes and other diseases (Finsley and Shahidi, 2001).

Table 1. Proximate[a] and fatty acid[b] compositions of *Monodora tenuifolia* seed oil

Proximate composition (%)	Mean±SD
Moisture content	16.20±1.85
Ash content	2.85±0.40
Crude protein	33.91±1.42
Carbohydrate	5.20±1.58
Oil yield	32.09±1.58
Fatty acid	(%)
$C_{8:0}$	0.02
$C_{10:0}$	0.02
$C_{11:0}$	0.10
$C_{12:0}$	0.03
$C_{14:0}$	0.03
$C_{16:0}$	12.56
$C_{16:1}$	0.13
$C_{18:0}$	10.23
$C_{18:1\ n-9}$	34.40
$C_{18:2\ n-6}$	39.37
$C_{18:3\ n-3}$	0.13
$C_{18:3\ n-6}$	1.30
$C_{20:1}$	0.72
$C_{20:3\ n-3}$	0.06
$C_{20:5\ n-3}$	0.11
Total saturates	22.99
Total unsaturates	76.22
Unknown	0.79
U/S[c]	3.32

[a] Ajayi and Aghanu (2011)
[b] Present work
[c] U/S = (Unsaturates)/(Saturates)

3.2 Intake of feed and changes in body weight

The feed intake and resultant body weight changes of test and control rats are shown in Tables 2. Weights of group B rats is higher but is not far from those of rats of groups C when compared to group A. Note that the initial weight of group A rats was not close to groups B and C. Generally, rats in groups B and C consumed more feed than their counterparts in group A. Despite the seed oil incorporated into group A's feed, the rats in this groups were not as big as those in groups A and B as noted from their initial body weight.

Table 2. Feed consumed and weight increase of control and test rats per week (g)

Parameter	Feed			Weight*		
Week	MTO	GO	CONT	MTO	GO	CONT
0	-	-	-	115.0±7.07	128.0±15.65	139.8±8.56
1	120	465	667	124.8±7.56	137.8±13.22	145.6±7.02
2	672	980	1000	137.2±7.40	154.0±17.24	154.0±6.52
3	760	977	1040	115.0±8.66	178.0±34.25	188.0±10.95
4	895	975	1115	128.0±11.51	199.0±15.97	206.0±17.82
5	980	570	1205	148.0±19.24	212.0±10.95	224.0±26.08
6	1026	1238	1121	150.4±18.93	248.3±54.88	233.6±25.11
7	1084	1261	1230	161.4±16.73	277.5±33.04	242.0±33.47
8	1040	996	1400	142.0±8.37	262.5±20.62	234.0±39.75

MTO= *Monodora tenuifolia* oil group; GO= Groundnut oil group; CONT= Control group
*Values are expressed as mean±SD

3.3 Physical appearances of the rats

Eyes of rats were looking normal initially but during the first and second weeks, groups A rats registered weak eyes while groups B and C had bright eyes. By the third week the eyes of the rats in group A had improved and just like other groups; they continued to be normal throughout the experimental period. The mouth of the animals used in their respective groups appeared normal throughout. All rats maintained fine and smooth hair all through the experimental period. No offensive odour was perceived in all the groups throughout the experimental period.

3.4 Organ weights

The kidney weight for group A rats (0.46±0.06 g) was significantly different from those of groups B and C. This difference in the kidney weight of group A rats emanated from the initial body weight differences in the rats. Liver weights of the rats in the three groups varied significantly from each other (Table 3).

Table 3. Weight of organs[a] (g) of control and test rats

Tissue[b]	MTO	GO	CONTROL
Kidney	0.46±0.06[c]	0.71±0.13[d]	0.76±0.09[d]
Liver	6.43±1.35[c]	11.22±0.59[d]	9.43±0.73[e]
Lungs	1.22±0.19[c]	1.57±0.09[d]	2.02±0.22[d]
Heart	0.41±0.02[c]	0.76±0.09[d]	0.69±0.09[d]
Spleen	0.59±0.16[c]	1.24±0.05[d]	0.80±0.20[c]

[a]Means with the same letter are not significantly different at p<0.0
[b]Values are expressed as mean±SD

Liver weights from 12.8±0.60 g to 16.0±0.98 g were obtained for *Vicia faba,* canola soybean, virgin olive and sesame oils (Macarulla et al., 2001; Baba and Ghossoub, 2000; Agbedana). Lungs and heart weights of the rats were found to follow the trend of that of the kidney. The spleen of group B rats (1.24±0.05 g) was significantly different from those of groups A and C respectively.

3.5 Haematological parameters

An average PCV (%) in the rat blood varied from 26.00±13.86 to 40.25±3.77 while the RBC (10^6/µl) also varied on the average of 4.93±1.45 to 7.06±0.72. Haemoglobin concentration in group B rats (8.47±4.74 mg/dl) was slightly different from those of groups A and C (12.06±1.66 and 12.95±1.00 respectively). Percentage MCHC did not record differences in all the groups. White blood cell count in groups A and C rats were similar but group B recorded higher value of 13850.00±1011.19 (10^3/µl). This showed that there was a significant difference among the groups. Percentage lymphocyte (72.00±8.60) for group A rats was different while those of groups B and C were similar just like their percentage neutrophilis and eosinophilis. Percentage monocyte varied significantly among the groups. Absolute values of lymphocyte, neutrophilis, eosinophilis and monocytes reflected their percentage values. Platelets of rats in groups A, B and C were not significantly different. A close look at Table 4 revealed that group A rats' Hb (mg/dl) and MCV(fl) was significantly different from those of groups B and C but PCV (%) and RBC (10^6/µl) of group A was significantly different

Table 4. Result[a] of haematological analysis of control and test rats

Parameter[b]	MTO	GO	CONTROL
PCV (%)	37.40±5.03^{cd}	26.00±13.86^{d}	40.25±3.77^{c}
RBC (10^6/µl)	6.37±0.89^{cd}	4.927±1.46^{d}	7.05±7.05^{c}
Hb (mg/dl)	12.06±1.66^{cd}	8.47±4.74^{c}	12.95±1.0^{c}
MCV (fl)	58.40±1.66^{cd}	49.67±16.17^{c}	16.95±2.38^{c}
MCHC (%)	31.80±0.45^{c}	31.67±1.53^{c}	33.25±0.50^{c}
WBC (10^3/µl)	11590.00±1628.04^{c}	13850.00±1011.19^{c}	11900.00±2670.52^{c}
Lymphocyte (%)	72.00±8.60^{c}	64.33±6.66^{c}	62.25±8.14^{c}
Neutrophilis (%)	24.20±9.63^{c}	32.33±0.58^{c}	32.00±7.62^{c}
Eosinophilis (%)	2.20±1.9^{c}	1.33±0.58^{c}	1.25±0.50^{c}
Monocytes (%)	1.80±0.84^{c}	2.00±0.00^{c}	1.50±1.00^{c}
Platelets	109800.00±12814.05^{c}	97333.33±12858.20^{c}	107500.00±9036.96^{c}

[a]Values are expressed as mean±SD
[b]Values in the same row with different superscripts are significantly different at P< 0.05

from group C alone. Other haematological parameters analysed showed no significant difference among the three groups.

3.6 Plasma and tissue cholesterol and tissue triacyglycerol

The result of the total organ cholesterol and total triacyglycerol of the hearts of the rats from both the test and control groups is presented in Fig. 1.

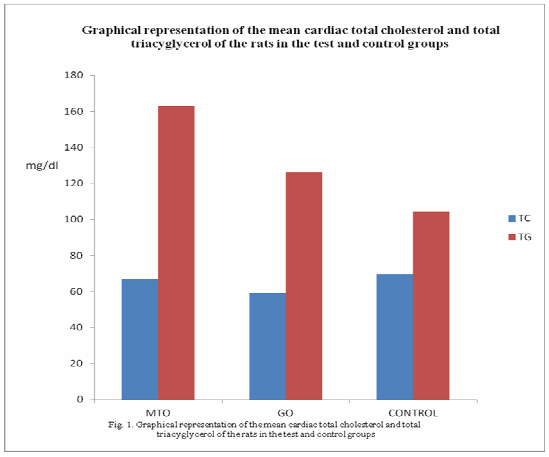

Fig. 1. Graphical representation of the mean cardiac total cholesterol and total triacyglycerol of the rats in the test and control groups

There were significant differences ($p < 0.05$) in the cholesterol levels in the hearts of the rats in the test and control groups. Abnormal lipid deposition in the heart tissue of rats with puromycin amino nucleoside induced nephrosis has been reported by Agbedana, Yamato, Moriwake, Suda, Takaash and Higashino (Agbedana et al, 1993). There was no previous report suggesting specific excessive cardiac lipid deposition in rats consuming *M. tenuifolia* seed oil, but excessive cardiac lipid deposition was reported in rats fed on certain athregenic diets (Hung et al., 1976). The triglyceride level of the organs of the rats in the test groups was significantly different from those of the control. The result of the mean plasma low density lipoprotein total cholesterol (LDLTC) and total triglyceride (LDLTG) and high density lipoprotein total cholesterol (HDLTC) are presented in Fig. 2.

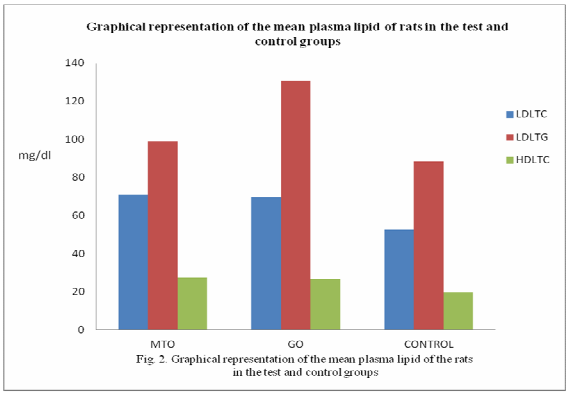

Fig. 2. Graphical representation of the mean plasma lipid of the rats in the test and control groups

The cholesterol levels in the plasma of the rats in both the test and control groups were below the recommended limit despite the fact that the plasma cholesterol of rats from the test groups was significantly higher ($p < 0.05$) than those from the control groups. These results are comparable to that of Kritchevsky who reported that the level of dietary fat was perceived as the governing factor in human cholesterolemia. It has been demonstrated that there is a strong relationship between the percentage of dietary fat and cholesterolemia in

a number of populations and data are already accumulating to show that the type of fat (saturated or unsaturated) played an important role in human or animal cholesterolemia (Kritchevsky et al., 1995; Kritchevsky et al., 1982). Results of the present study show a good indication that the consumption of MTO seed can likely be used to lower cholesterol levels in blood. This is because of the high unsaturated fatty acid content of the test oil, since it has been reported by many authors that oils containing unsaturated fatty acids, especially linoleic and oleic acids, can be used to lower plasma cholesterol (Ahmed and Young, 1982; Melgarejo and Gee, 1994). Kaplan and Pesce reported that diets high in plant foods such as fruits and vegetables are associated with a lower occurrence of coronary heart disease, (Kaplan, 1989). The oil from *M. tenuifoila* seeds, being of vegetable origin, is thus likely to lower the occurrence of coronary heart diseases, if consumed. The value of the plasma cholesterol: 52.61 mg/dl (MTO) to 70.86 mg/dl (control group) is within the 73.0–100.0 mg/dl reported in literature by Dong, Barban, Gazzaz, Venaventura and Holcomb (Dong, 1990). According to Magne, hyperlidaemia may be diagnosed if the total plasma cholesterol concentration is greater than 275 mg/dl. It is generally accepted that, in the absence of other risk factors of coronary disease, it is desirable that plasma cholesterol concentrations be maintained below 275 mg/dl. In this study, the plasma cholesterol for all the rats in the test groups is less than 275 mg/dl, which suggests that the MTO, if consumed, cannot raise the plasma cholesterol of the individual consuming the oil to above the specified limit (Magne, 1971).

3.7 Histopathology

No major lesions were found in the heart of all the rats in the three groups except one of the rats in the MTO group that has hemorrhage; some in the GO group had broken blood vessels while there was a slight haemorrhage in one of the rats of the control group. There was no visible lesion except for the case of a swollen hepatocyte in the liver of one of the rats fed with GO but there was multifaecal necrosis with multiple foci of infiltrating cells in the central area and congestion of sinusoids in one of the ones fed with MTO and swollen hepatocytes in two others. The rats fed with control feed did not show any visible lesion except for the case of cellular infiltration in one of them which may be due to infection.

4. CONCLUSION

The seed oil of *M. tenuifolia* did not have any deleterious effect on the physical appearance, body weight gain, organ weights and haemotological parameters of the test rats. However, some lesions were found in the heart and liver of one or two of the rats fed with the oil. *M. tenuifolia* seed oil consist of high percentage of unsaturated fatty acid including a relatively high level of polyunsaturated fatty acids which makes it a healthy low-fat food and is nutritionally significant. It contains essential fatty acid; essential fatty acid nourishes skin, hair and nails. *M. tenuifolia* oil could probably serve as edible oil, especially when it is refined, and it has a great potential for oleochemical application.

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