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Short-term Toxicological Evaluation of *Monodora myristica* Seed Oil

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Abstract: *A short-term preliminary toxicological evaluation of Monodora myristica seed oil was carried out in order to determine the suitability of the oil for nutritional purposes. The total unsaturated fatty acids found in M. myristica seed oil was 79.93% while the total saturated fatty acid was 20.07% with oleic and linoleic acid making up the major portion of the unsaturated fatty acids; both accounted for 68.67% of the total fatty acids in the oil. The effects of the oil on physical appearance, feed intake, weight gain, plasma and tissue cholesterol and triacyglycerol levels in rats were determined. There seemed to be no toxicological effects on weanling albino rats when fed with 5% M. myristica seed oil in their diet for 8 weeks. Weekly monitoring of the rats showed good physical appearance and steady weight gain, with no mortality recorded for the study period of eight weeks. Haematological analysis of the test rats showed that the rats were not anaemic. M. myristica seed oil is a promising edible oil.*

Key Words: *albino rats, haematological analysis, M. myristica, seed oil, toxicology*

I. Introduction

Monodora myristica (Gaertn.) Dunal, belonging to the family Annonaceae, is an evergreen and deciduous forest tree, up to 35 cm high by 2 m in girth. It is called African nutmeg or false nutmeg in English while the local name is 'Ariwo'. The seeds, which are embedded in a white sweet-smelling pulp, are the most economically important part of the tree. They are aromatic and are used after grinding into powder as a food flavor resembling that of nutmeg (Burkill 1985). The seed have been reported to contain oil: 22.79 g/100 g dry matter; protein: 20.79 g/100 g dry matter and carbohydrate: 44.29 g/100 g dry matter (Ajayi *et al*. 2004). This result showed that *M. myristica* deserves to be investigated as promising source of new edible oil. There is little doubt that dietary cholesterol influences plasma cholesterol level; epidemiological studies showed that the probability of coronary artery disease decreased linearly as the quantity of the unsaturated fatty acids in foodstuff increased (Younis *et al.* 2000). The percentage of saturated fat and cholesterol in the diet are the major determinants of atherosclerosis and coronary heart disease in different populations. Vegetable oils with a high amount of linoleic acid have ability to reduce serum cholesterol (El- Adawy and Taha 2001).The potential health benefits of various dietary oils in relation to cardiovascular disease and cancer are currently receiving considerable attention (Baba *et al.* 2000). Some workers have stressed the importance of the ratio of polyunsaturated (PUFA) and saturated (SFA) fatty acids in the diet as determinant of plasma cholesterol level (Fuchs *et al*. 1994). SFA causes a rise in plasma cholesterol while PUFA causes low plasma cholesterol; a great deal of interest has been placed on a few oils that contain PUFA (Ramadan *et al*. 2006). Moreover, interest in PUFA as a health-promoting nutrient has expanded dramatically in recent years. A rapidly growing literature illustrates the benefits of PUFA in alleviating cardiovascular, inflammatory conditions, heart disease, atherosclerosis, autoimmune disorders, and diabetes (Finsley and Shahidi 2001; Riemersma 2001, Ramadan *et al.* 2006). 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) plays an important role in human or animal cholesterolemia. Diets low in saturated fatty acids and high in monounsaturated fatty acids effectively control blood lipid levels (Gorinstein *et al.* 2003; Ajayi *et al.* 2008). Diets high in plant foods such as fruits and vegetables are associated with a lower occurrence of coronary heart disease; legumes contain low levels of total oil and saturated fatty acids, as well as has high content of unsaturated fatty acid. Therefore, an increase in intake of plant foods such as legumes can be beneficial to human health (Ryan *et al.* 2007; Mishra and Parthan 2011). This study was designed to evaluate the toxicity, if any, of *Monodora myristica* seed oil on a short-term basis using animal experiment.

II. Materials And Methods

Sample preparation

M. myristica seeds were purchased from Ojo market in Ibadan, Oyo State, Nigeria. The seeds were winnowed, cleaned of all foreign particles and air-dried for some days. The seeds were then manually crushed

and the oil extracted with *n*-hexane in a Soxhlet extractor continuously for 8 h. The solvent was removed completely and the oil obtained was maintained in a properly labeled glass container for further study.

Fatty acid

The methyl esters of the crude oils were prepared in University of Tuebigen, Germany following the method of Lutz *et al*., (1998) and Ajayi *et al.* (2002; 2004). 5 ml of CH_3OH and 1 ml of CH_2Cl_2 were added to 0.10 g of each oil sample. Ice was used to cool the mixture and then 0.6 ml of CH_2COCl was added. 1 ml of the solution was withdrawn into a hydrolysis tube and heated for 1 h at 110 ^oC. The solution obtained was again cooled with ice and discharged into a separating funnel containing 10 ml of 100 % NaCl solution. The extraction of the organics in the solution was carried out thrice with 4 ml of hexane; a rotatory evaporator was used to reduce the volume to 0.5 ml after which it was eluted on a silica gel column successively with 5 ml hexane and 4 ml CH_2Cl_2. A separation was made of the CH_2Cl_2 fraction on a DB5 30 m x 0.25 mm capillary installed on a GC Chrompack 900l (model; Chrompack 9001; city: Middelburg; country: Netherlands) equipped with computer software and mosaic integration. The programming of the temperature was 35 ^oC for 3 min after which it was increased at 20 ^oC/min up to 230 ^oC for 5 mins. The internal standard was heptadecanoic acid. The detector used was flame ionization detector.

Animal Experiment

Ten albino rats (aged 4 weeks, weighing between 40 g and 70 g) were obtained from the Central Animal House, University of Ibadan, Nigeria. The animals were divided into three groups of 5 rats per group. They were given feed and water *ad libitum* for an experimental period of 8 weeks. The experiment rats were fed with a commercial rat diet (Ladokun Feeds Limited, Ibadan, Nigeria) mixed with 5 % of *M. myristica* oil (MMO) and groundnut oil (GSO) period of 8 weeks following the method of Khan *et al.* (1986) and Ajayi *et al.* (2008); the control rats were fed with normal rat feed (NRF) only. The physical appearances of the rats were monitored while the body weight of each rat was recorded weekly (without fasting) for the period of the experiment. Animals were sacrificed after a 14-16 h overnight fast on the last day of the experiment.

Haematological examination

For haematological analysis, 3 ml of blood were collected by cardiac puncture into heparinized vials and stored at 10 ^oC for analysis the same day. The packed cell volume (PCV), haemaglobin (Hb) concentration, red blood cell (RBC) and white blood cell (WBC) counts were determined using standard techniques as described by Dacie and Lewis (1991). The differential WBC counts mean corpuscular volume (MCV) and mean corpuscular haemaglobin concentration (MCHC) were calculated (Jain 1986; Ajayi *et al.* 2007, 2008). Microhaemocrit capillary tubes were filled to two-thirds mark with well mixed venous blood. One end was sealed with plasticine. The sealed tubes were placed in microhaematocrit centrifuge and the safety cover securely screwed on. The sealed capillary tubes were centrifuged for 5 mins at 10,000 revolutions per minutes. The volume of the red blood cell was read on the micro-haemocrit reader.

Plasma and tissue cholesterol and tissue triacyglycerol determination

The method of Searcy and Berquist was followed in estimating the plasma concentration of total cholesterol while the cholesterol in the heart homogenate was measured using the method outlined by Gottfried (1973). In determining total cholesterol concentration, 0.2 ml of the plasma sample was placed into a centrifuge tube and 5 ml of chloroform:methanol mixture was added to it. The tube was vortex to allow proper mixing of the content and left to stand for 30 mins after which the content was spun at 2,000 rpm for 3 mins. The control and pooled sera were treated similarly. I ml of the supernatant was introduced into a test tube. To this were added 4 ml of acetic acid and ferrous sulphate reagent and 2 ml of conc. H_2SO_4. The resulting solution was mixed using vortex mixer and the solution was allowed to cool. I ml of high and low working standard cholesterol and 1 ml of chloroform methanol mixture for blank were treated similarly. The absorbance of the cooled test was read at 490 nm in a 60-200 Unicam spectrophotometer.

2.6 Statistical analysis

Results are expressed as the means of five separate contents except for the total cholesterol and triglyceride where only three determinations were made. The data were statistically analyzed by SAS (1987) 2-way analysis of variance (ANOVA). Means were compared by Duncan's (1955) at 5 % level of significance ($P \leq 0.05$).

III. Results And Discussion

Fatty acids

Reports from clinical studies tend to suggest that plasma cholesterol levels are significantly elevated by high levels of saturated fat in the diet; (Grundy and Ahrens 1970). Fatty acid analysis of the oils showed that they have high amounts of unsaturated fatty acids with linoleic (35.52 %) and oleic acids (33.15 %) as the major ones; both accounts for 68.67 % (Table 1). It has been reported by many authors El-Adawy and Taha, 2001; Ramadan *et al.* 2006 and Melgarejo (1994) that oils containing unsaturated fatty acids especially linoleic and oleic acids can be used to lower plasma cholesterol. Oleic acid is very important in nervous cell construction; it has fundamental role in cardiovascular diseases prevention (Nasri *et al*. 2005). The high percentage of oleic acid in the oil makes it desirable in terms of nutrition and high stability cooking and frying oil (Anwar *et al.* 2006). A higher intake of oleic acid is associated with decreased risk of coronary heart disease caused by high cholesterol level in blood (Corbett 2003). Linoleic acid helps to relieve flaky or rough skin and maintain smooth moist skin (Ariffin *et al*. 2009). The consumption of diets containing high levels of polyunsaturated fatty acids has been reported to be immensely correlated to mortality from certain systemic disease. The evidence that the intake of saturated fatty acids and cholesterol are causally related to atherosclerotic cardiovascular disease is convincing. Thus the oil of *M. myristica*, because of its high level of unsaturated fatty acid is likely to reduce coronary heart disease if consumed (Hansen *et al.* 1992; Nielsen *et al.* 1992; Thompson *et al*. 1993). *M. myristica* seed oil was characterized by a polyunsaturated/saturated (P/S) ratio of 2.18. A high ratio of P/S is regarded favourable for the reduction of serum cholesterol and atherosclerosis and prevention of heart diseases (Oomah *et al.* 2002 and Nehdi 2011).

Physical appearance of test and control rats

The result of the examination of the physical appearance of the rats in NRF, MSO and GSO groups is presented on **Table 2**. All the rats were healthy throughout the period of study. Their body did not smell oil and they all showed normal hair structure and sheen. No mortality was recorded in any of the groups. Ajayi *et al.* (2007) gave similar report for *Garcinia mangostana* seed oil. However, the nails of the rats in NRF group showed some abnormality while those of the control did not show any; the eyes and skin of the NRF rats were normal while those of the MSO rats were not.

Body weights

All the rats had steady weight gain during the period of study (**Fig. 1**). The differences in the weight gain of the rats from the different groups are significant. This is in close similarity to the report given by Oliveira *et al.* (2000). The rats fed with GSO showed a higher body weight change than the ones in the NRF and MSO groups. Longvah *et al.* (2000) reports that there was no difference in the body weight gain of animals fed with groundnut oil and Perilla seed oil.

Haematology parameters

There was no significant difference ($P < 0.05$) in the result of the haematological analysis of the NRF, MSO and GSO rats (**Table 3**). The rats were not anaemic; their PCV values are similar to those reported for healthy murine species (Oyewale *et al.* 1998; Ogunsami *et al.* 2002). The WBC counts of the rats from the three groups, which are similar, indicate that the rats had no infection. There is a similar report in literature for *T. occidentalis* by Ajayi *et al.* (2004).

Cardiac and plasma lipids

The result of the total cholesterol and total triacyglycerol of the hearts of rats from NRF, MSO and GSO are presented in **Fig. 2**. There were differences in the cholesterol levels in the hearts of the rats from these groups. Kaplan and Pesce (1989) reported that diets high in plant foods such as fruits and vegetables are associated with a lower occurrence of coronary heart disease. The oil from *M. myristica* seeds, being of vegetable origin, might thus likely lower the occurrence of coronary heart diseases if consumed.

IV. Conclusion

Monodora myristica seed oil appeared not to have any toxic effect on the albino rats hence might be suitable as edible oil. However the oil will require refining so as to improve on the colour and further work needs to be carried out so as to establish whether the oil is actually nutritionally suitable or not.

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Table 1 Fatty acid composition of *Monodora myristica* seed oil (% of dry matter)

Fatty acid composition[a]	**MSO**
$C_{16:0}$	5.96
$C_{18:0}$	4.44
$C_{18:1}$	33.15
$C_{18:2}$	35.52
$C_{20:0}$	9.52
$C_{20:1}$	2.96
$C_{20:2}$	5.43
$C_{20:3}$	2.87
$C_{24:0}$	0.15
Saturated	20.07
Unsaturated	79.93
P/S[b]	2.18

[a]Ajayi *et al*., 2004
[b]Polyunsaturated /unsaturated fatty acid

Table 2 Physical appearance of NRF, MSO and GSO albino rats

Parameter	**NRF**	**MSO**	**GSO**
Nails	-	+	++
Eyeballs	+	-	++
Skin	+	-	++
Whiskers	+	+	+
Smell	+	+	++

+ = normal
++ = very normal
- = abnormal

Table 3 Result of Haematological analysis of NRF, MSO and GSO albino rats

Paramaters[a]	**NRF**	**MSO**	**GSO**
[b]PCV	40.00 ± 3.21^a	41.67 ± 1.67^a	39.00 ± 4.62^a
[c]RBC	6.82 ± 0.39^a	7.15 ± 0.27^a	6.72 ± 0.49^a
[d]Hb	12.97 ± 0.96^a	13.67 ± 0.57^a	12.87 ± 1.62^a
[e]WBC	1.08 ± 0.14^a	0.74 ± 0.14^a	0.75 ± 0.23^a
[f]MCV	58.45 ± 1.74^a	58.27 ± 0.69^a	57.66 ± 2.81^a
[g]MCHC	32.47 ± 0.67^a	32.79 ± 0.15^a	32.93 ± 0.39^a
Platelets	2.21 ± 0.18^a	1.85 ± 0.24^a	1.98 ± 0.27^a

[a]Data on the row with same superscripts are not significantly different at P<0.05.
[b]PCV-Packed Cell Volume [c]RBC-Red Blood Cell [d]HB-haemoglobin [e]WBC – White Blood Cell.
[f]MCV – Mean Corpuscular Volume [g]MCHC

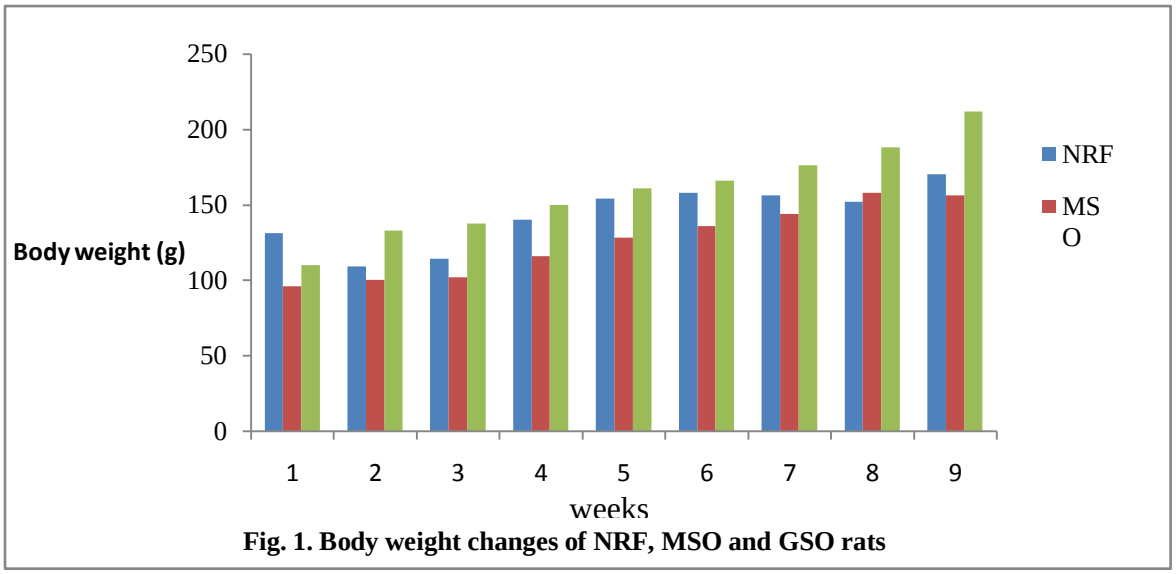

Fig. 1. Body weight changes of NRF, MSO and GSO rats

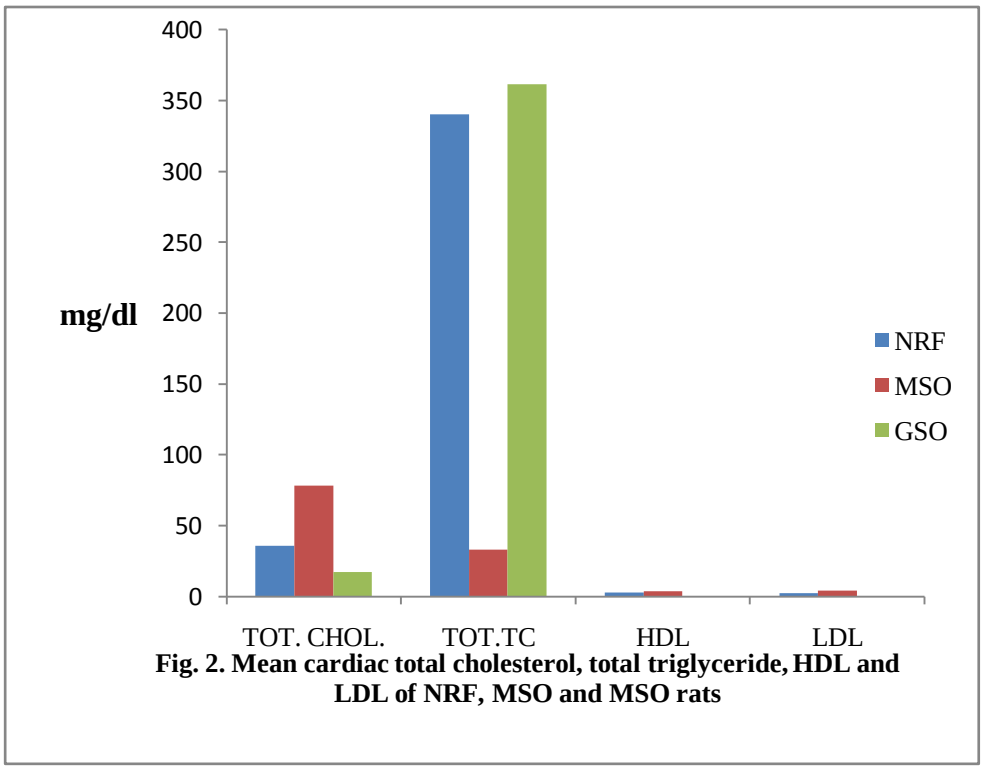

Fig. 2. Mean cardiac total cholesterol, total triglyceride, HDL and LDL of NRF, MSO and MSO rats