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ScienceDirect

Food Chemistry 106 (2008) 458–465

Food Chemistry

www.elsevier.com/locate/foodchem

Short-term toxicological evaluation of *Terminalia catappa*, *Pentaclethra macrophylla* and *Calophyllum inophyllum* seed oils in rats

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Received 4 September 2006; received in revised form 17 February 2007; accepted 29 May 2007

Abstract

The purpose of this study was to evaluate the toxicological effects of feeding the oils of *Calophyllum inophyllum*, *Pentaclethra macrophylla* and *Terminalia catappa* to rats. The effects on physical appearance, feed intake, weight gain, plasma and tissue cholesterol and triacyglycerol levels in rats with 5% of the oils in normal rat feed were determined. Weekly monitoring of the rats showed good physical appearance and steady weight gain, with no mortality recorded for the period of the study. Haematological analysis of the rats indicated that they were not anaemic. Histopathotogical examination of the sections of the heart, liver, kidney and spleen revealed moderate (*T. catappa* oil) to severe fatty change and necrosis in the liver. Glomerulonephrotic changes in the kidneys of rats fed with *T. catappa* oil were moderate, while it was severe in the group fed with *P. macrophylla* oil. Severe myocardiac necrosis as well as atherosclerotic clefts in vasa vasori was observed in the vasa vasori of the hearts of rats fed with *P. macrophylla* oil. This change was moderate in the heart of rats fed with *C. inophyllum*, while no such observation was made in the group fed with *T. catappa* oil. There was a significant difference in the plasma cholesterol levels of the rats fed with *C. inophyllum* and *T. catappa* oils when compared with the control rats, while those fed with *P. macrophylla* oil had no significant difference. The oil of *T. catappa* appears more suitable for consumption than the oils from *C. inophyllum* and *P. macrophylla*. Fatty acid analysis of the oils showed that they have high amounts of unsaturated fatty acids with linoleic and oleic acids as the major ones.
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Keywords: Toxicological effect; *Terminalia catappa*; *Calophyllum inophyllum*; *Pentaclethra macrophylla*

1. Introduction

It is now known that diet plays an important role in preventing certain diseases. The relationship between dietary fat and certain chronic illnesses including cardiovascular, neoplastic and endocrine diseases is also recognized (Walker and Ball, 1993). Consumption of saturated dietary fats has been associated with increased plasma cholesterol concentration, potentially increasing the risk of cardiovascular diseases (Npales et al., 1996). Monounsaturated fatty acids (MUFAS) such as oleic acid are known to reduce blood cholesterol levels in non-hypertriglyceridemic individuals. Because of the importance placed on dietary MUFA, it has been recommended that MUFA intake be as high as half of the total recommended dietary intake of calories from fat as a means of reducing the risk of cardiovascular disease (Nee and Foglia, 2000). It is also considered that essential fatty acids (linoleic, linolenic and arachidonic acids) play a natural preventive role in cardiovascular diseases and in the alleviation of some other health problems basically because they promote the reduction of both total and LDL cholesterol (De Hoya and Mata, 1989; Grande, 1988). The most common and useful of these is linoleic acid, and if consumed could prevent coronary heart disease (CHD).

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doi:10.1016/j.foodchem.2007.05.093

Calophyllum inophyllum Linn, a Guttiferae, is a beautiful ornamental tree which occurs mainly in the tropics. The leaves are reported to contain tannin while the embryo which becomes large on maturity contains oil and resin. The oil is one of the oldest known to be used for illumination in India and across the Pacific (Burkill, 1994). The seed contains 49.15 ± 1.20% oil. *Pentaclethra macrophylla* Benth, known in English as oil bean tree, is of the family Mimosaceae. The bark is strongly astringent, contains tannin and a decoction of it is an effective lotion for washing sores and psorias. It has been used as a laxative, for dysentery and as an anti-helmintic. The bark also has analgesic properties. Sap from the inner-bark is used as a sedative for paroxysmal coughing and asthma and to quell fits of insanity (Sandberg & Cronlund, 1982).

Terminalia catappa, a Combretaceae is a tree that in Nigeria, is grown commonly as a shade tree in towns, villages and cities. In fact, there is hardly any residential or public quarter where the tree is not found in southern Nigeria. The plant is widely distributed throughout the tropics in Asia and South America (Hutchinson & Dalziel, 1972). The outer flesh, which contains 95.9 ± 5.8 mg ascorbic acid/100 g fruit (Keshinro, 1985), is usually consumed by children in the tropics, but where the tree has been introduced as an ornamental and shade tree, as in Southern Florida, and there is little familiarity with the fruit, vast quantities lie on the ground and the flesh is consumed by land crabs, scavenger beetles, birds or other animals. Eventually the fruits are raked up and discarded and this constitutes a serious waste of nutritious food. Several species of the genus *Terminalia* have long been used in the traditional medicine in both East and West African countries to treat infectious diseases (Fabry et al., 1996). The oil yield of the seeds of *C. inophyllum*, *P. macrophylla* and *T. catappa*, has been reported in literature to be 49.12 g/100 g, 40.29 g/100 g and 55.05 g/100 g, respectively (Ajayi et al., 2002; Oderinde and Ajayi, 1998, 2000). In view of the properties and uses of these seeds and oils and the proximate, physiochemical and metal content of the seeds that compare favourably with conventional edible oils, this study was designed to examine the fatty acid composition of the seed oils and evaluate the toxicity, if any, in a short-term animal experiment in order to determine the suitability of the oils as edible oils.

2. Materials and Methods

2.1. Plant materials and sample preparation

C. inophylum seeds were collected from the Botanical Garden, University of Ibadan. *P. macrophylla* seeds were purchased from Ojo market in Ibadan, while *T. catappa* seeds were picked from trees grown within the premises of the University of Ibadan, Nigeria. The seeds were shelled by cracking to remove the kernels inside. The kernels were ground to powder in a Hammer mill and stored in airtight sample bottle in a refrigerator (4 °C) until needed for analysis. Seed oils were extracted using the continuous Soxhlet extraction technique with petroleum ether (40–60 °C) for 8 h. The solvent was removed completely and the oils obtained were used for this study.

2.2. Fatty acid analysis

The determination of the fatty acids content of the oils was carried out following the method of Ajayi, Adebowale, Dawodu, and Oderinde (2004a). To 0.10 g of the oil was added 5 ml of CH_3OH and 1 ml of CH_2Cl_2. The mixture was cooled in ice and 0.6 ml of CH_3COCl was added. One milliliter of the solution was withdrawn into the hydrolysis tube and heated for 1 h at 110 °C. The solution was cooled and discharged into 10 ml of 100% NaCl solution in a separating funnel. The organics were extracted with 3 × 4 ml of hexane and the volume was reduced to 0.5 ml using a rotatory evaporator. This was eluted on silica gel column successively with 5 ml hexane and 4 ml CH_2Cl_2. The CH_2Cl_2 fraction was separated on a DB5 30 m × 0.25 mm capillary installed on a GC Chrompack 9001 equipped with computer software and Mosaic Integration. A flame ionization detector was used. The temperature was programmed as follows: 35 °C for 3 min; temperature was then increased at 20 °C/min up to 230 °C for 5 min. Heptadecanoic acid was used as an internal standard.

2.3. Animals and diets

Twenty weanling 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 four groups of 5 rats/group and were given feed and water *ad libitum* for an experimental period of 8 weeks. The experimental rats were fed with a commercial rat diet (Ladokun Feeds Limited, Ibadan, Nigeria) mixed with 5% of *C. inophyllum* oil (CIO), *P. macrophylla* oil (PMO) or *T. catappa* oil (TCO) following the method of Khan et al. (1986), while the control rats were fed with commercial feed alone. The physical appearance of the experimental rats was monitored while the body weight of each rat was recorded weekly (without fasting) for the period of the experiment. Animals were sacrificed under mild anesthetics with chloroform after 14–16 h overnight fast.

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 °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 °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. A sample of the heart weighing about 0.2 g was cut into pieces and homogenized (Shandon Equipment, UK) in 5 ml of phosphate-buffered saline (PBS). The homogenate was centrifuged at 1200 rpm for 10 min at 37 °C and the supernatant was then used for the determination of tissue cholesterol level. A specimen of the heart was then stored at 20 °C for the determination of total cholesterol and triglyceride levels.

2.5. Haematology, plasma and tissue cholesterol determination

The microhaematocrit method was used for the analysis of packed cell volume and the white blood counts as described by Dacie and Lewis (1991). The plasma concentrations of total cholesterol were estimated according to the method of Searcy and Berquist (1960), while the cholesterol level in the heart homogenates was measured according to Gottfried (1973).

2.6. Histopathology

A sample of the heart, liver, kidney and spleen for each animal in the various groups was fixed in 10% phosphate-buffered formalin, embedded in paraffin wax and sectioned at 6 μ following the methods of Longvah, D'eosthale, and Uday Kumar (2000) and Raghumuraulu, Nair, and Sundaram (1983). The stained (Hematoxylin and Eosin, HE) sections were examined under a light microscope for any changes in the tissues due to the consumption of *C. inophyllum*, *P. macrophylla* and *T. catappa* seed oils.

2.7. Statistical analysis

Results are expressed as the means of five separate determinations, except for the fatty acid contents, total cholesterol and triglyceride. The data were analyzed using the 2-way analysis of variance (ANOVA) according to SAS (1987). Significant differences between mean values of the parameters studied were determined at the 5% level (Duncan, 1959).

3. Results and discussion

3.1. Fatty acids

The consumption of diets containing high levels of polyunsaturated fatty acids has been reported to be immensely correlated to mortality from certain systematic diseases (Hansen et al., 1992; Thompson et al., 1993). The fatty acid composition of the seed oils examined in this study (Table 1) reveals that oleic and linoleic acids are the predominant unsaturated fatty acids. The $C_{18:1}$ and $C_{18:2}$ fatty acids together account for 67.42% *C. inophyllum* oil; 63.93% *P. macrophylla* and 53.47% *T. catappa* seed oils. This particular finding is encouraging because it is a desirable feature in human food (Vijayakumari, Siddhuraju, & Janardhanan, 1997). Recent studies have demonstrated that MUFAS (monounsaturated fatty acids) are better contributors to plasma cholesterol lowering effects than SFAS (saturated fatty acids). The high linoleic acid content of the seed oils, 27.60% *C. inophyllum*, 35.76% *P. macrophylla* and 23.44% *T. catappa* is significant since linoleic acid is undoubtedly one of the most important polyunsaturated fatty acids in human food due to its prevention of distinct cardiovascular disease. According to Vles and Gottenbos (1989) and Dagne and Johnson (1997), cardiovascular disorders such as coronary heart diseases, atherosclerosis and high blood pressure are prevented by dietary fats rich in linoleic acid. All the seed oils used in this study contain higher percentages of unsaturated fatty acid than saturated ones.

Table 1
Fatty acid composition (%) of *C. inophyllum*, *P. macrophylla* and *T. catappa* seed oils

Fatty acid	*C. inophyllum*	*P. macrophylla*[a]	*T. catappa*
C_{16}:0	14.62	3.83	34.82
C_{18}:2	27.60	35.76	23.44
C_{18}:1	39.82	28.17	30.13
C_{18}:0	16.48	0.67	6.79
C_{20}:3	–	4.34	1.04
C_{20}:2	–	0.88	1.00
C_{20}:1	0.86	0.74	0.49
C_{20}:0	0.38	1.47	2.31
C_{22}:2		3.71	–
C_{22}:1	0.24	1.60	–
C_{22}:0	–	3.94	–
C_{24}:0	–	12.52	–
C_{26}:0	–	2.37	–
Saturated	31.72	24.80	43.92
Unsaturated	68.28	75.20	56.08

[a] Ajayi et al. (2002).

Essential fatty acids are useful in alleviating fatty acid syndrome and in preventing coronary heart disease (Gurr, 1985). *C. inophyllum* and *T. catappa* oils contain palmitic and stearic acids as the main saturated fatty acids; *P. macrophylla* oil has long chain saturated fatty acids, the most prevalent one being lignoceric acid, 12.52%. Seed oils of *C. inophyllum* and *P. macrophylla* contain erucic acid, 0.24% and 1.60%, respectively and these values are within the allowable limits for edible oils (Rossel & Pritchard, 1991). Data on the fatty acid composition of the seed oil suggest (even though not conclusive) that the oils might help in the prevention of cardiovascular disease if included in the diet.

3.2. Physical appearance of test and control rats

Weekly inspection of the physical appearance of the rats revealed that the rats were healthy. Throughout the period of study, the hair of the rats did not fall out neither did any of them show any skin defects. Their body did not smell of oil and they all showed normal hair structure and sheen. No mortality was recorded in any of the experimental groups. The result of the body smell is in contrast to the report by Khan et al. (1986).

3.3. Body weights

Table 2 shows the body weight changes of rats in both the test and control groups. All the test rats steadily gained weight during the period of study (except for *P. macrophylla* group that stopped gaining weight after five weeks). This observation indicates a possible negative effect of *P. macrophylla* oil on the rats. However, rats fed with *C. inophyllum* seed oil showed lower weight gain potentials than those fed the seed oils from *P. macrophylla* and *T. catappa* and those of the rats on normal diet ($p < 0.05$). The average weight of the rats fed with *T. catappa* was significantly higher ($p < 0.05$) than the rats fed with a normal diet. This is similar to the data reported by Oliveira et al. (2000).

3.4. Organ weights

The organs whose weights were taken are heart, liver, kidney and spleen (Table 3). At sacrifice, similar organs of the test and control rats have comparable weights showing that consumption of the seed oils have no significant effect ($p > 0.05$) on these weights of similar organs of the test and the control groups. However there was a significant difference between different organs of both test and control rats.

Table 2
Body weight changes (g) of test and control rats*

Week	*C. inophyllum* oil	*P. macrophylla* oil	*T. catappa* oil	Normal diet
1	53.5 ± 26.5^{a}	$62.5. \pm 22.5^{a}$	50.0 ± 10.0^{a}	47.5 ± 7.5^{a}
2	97.5 ± 32.5^{b}	84.6 ± 24.0^{b}	134.0 ± 46.0^{b}	55.0 ± 15.0^{b}
3	97.5 ± 27.5^{c}	85.0 ± 35.0^{c}	117.5 ± 2.5^{c}	77.5 ± 22.5^{c}
4	117.5 ± 17.5^{d}	100.0 ± 20.0^{d}	137.5 ± 22.5^{d}	115.0 ± 10.0^{d}
5	145.0 ± 25.0^{e}	150.0 ± 30.0^{e}	157.5 ± 22.5^{e}	147.5 ± 12.5^{e}
6	140.0 ± 30.0^{f}	160.0 ± 40.0^{f}	170.0 ± 20.0^{f}	155.0 ± 12.5^{f}
7	135.0 ± 25.0^{g}	165.0 ± 40.0^{g}	180.0 ± 25.0^{g}	180.0 ± 20.0^{g}
8	147.5 ± 27.5^{h}	165.0 ± 35.0^{h}	187.5 ± 22.5^{h}	175.0 ± 10.0^{h}

Data presented as mean ± standard deviation ($n = 5$).
* Values with different superscripted letters are significantly different ($p < 0.05$).

Table 3
Organ weights (g) of test and control rats*

Organ	*C. inophyllum* oil	*P. macrophylla* oil	*T. catappa* oil	Normal feed
Heart	1.15 ± 0.35^{a}	0.95 ± 0.35^{a}	1.50 ± 0.55^{a}	1.20 ± 0.20^{a}
Liver	6.95 ± 0.95^{a}	6.55 ± 0.45^{a}	7.90 ± 0.60^{a}	7.85 ± 0.45^{a}
Kidney	1.20 ± 0.20^{a}	0.75 ± 0.25^{b}	1.05 ± 0.50^{ab}	1.00 ± 0.20^{a}
Spleen	1.15 ± 0.45^{a}	0.80 ± 0.20^{b}	1.30 ± 0.40^{a}	1.00 ± 0.20^{ab}

Data presented as mean ± standard deviation ($n = 5$).
* Values on the same row with different superscripted letters are significantly different ($p < 0.05$).

3.5. Haematological parameters

The results of the packed cell volume (PCV) and total white blood cell (WBC) counts of the control and test rats are shown on Table 4. The results indicate that the rats were not anaemic, as their PCV values are similar to those reported for healthy rats and related murine species (Oyewale, Olayemi, & Oke, 1988; Ogunsanmi, Ozegbe, Ogunjobi, Taiwo, & Adu, 2002). The similarity of WBC counts in all the groups (both the test and control) suggests that the rats had no infection. This is similar to previous report on *T. occidentalis* by Ajayi, Oderinde, Taiwo, and Agbedana (2004b).

3.6. Cardiac and plasma lipids

The result of the total organ cholesterol and total triacyglycerol of the hearts of the rats from both the test and control groups are presented in Figs. 1a and 1b. There are significant differences ($p < 0.05$) in the cholesterol levels in the hearts of the test groups and the control groups and even from one test group to another. Previous reports by Agbedana et al. (1993) revealed abnormal lipid deposition in the heart tissue of rats with puromycin amino nucleoside induced nephrosis. There are no previous reports suggesting specific excessive cardiac lipid deposition in rats consuming *C. inophyllum*, *P. macrophylla* and *T. catappa* seed oils, but excessive cardiac lipid deposition was reported in rats fed on certain athregenic diets (Hung, Umemuna, Yamashiro, & Slinger, 1976). The triglyceride levels of the organs of the rats in the test groups were not significantly different from those of the control. This report is similar to that of Dong, Barban, Gazzaz, Venaventura, and Holcomb (1990) who reported that the triglyceride concentration in the heart of the rats was essentially the same for all dietary groups. The result of the plasma total cholesterol (Fig. 1b) showed that the cholesterol levels in the plasma of the rats in both the test and control groups are below the recommended limit. The plasma cholesterol of rats from test groups is significantly lower ($p < 0.05$) than those from the control groups. These results are comparable to that of Kritchevsky (1995) 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

Table 4
Packed cell volume (PCV) and total white blood cell (WBC) counts of test and control rats

Parameters	*C. inophyllum* oil	*P. macrophylla* oil	*T. catappa* oil	Normal feed
PCV (%)	42.0 ± 1.0^{a}	39.0 ± 4.0^{a}	40.5 ± 2.5^{a}	39.5 ± 4.5^{a}
WBC ($\times 10^3$ μ/l blood)	7.25 ± 2.75^{a}	3.85 ± 1.85^{a}	5.35 ± 1.95^{a}	5.50 ± 2.50^{a}

Data presented as mean ± standard deviation ($n = 5$). Values on the same row with different superscripted letters are significantly different ($p < 0.05$).

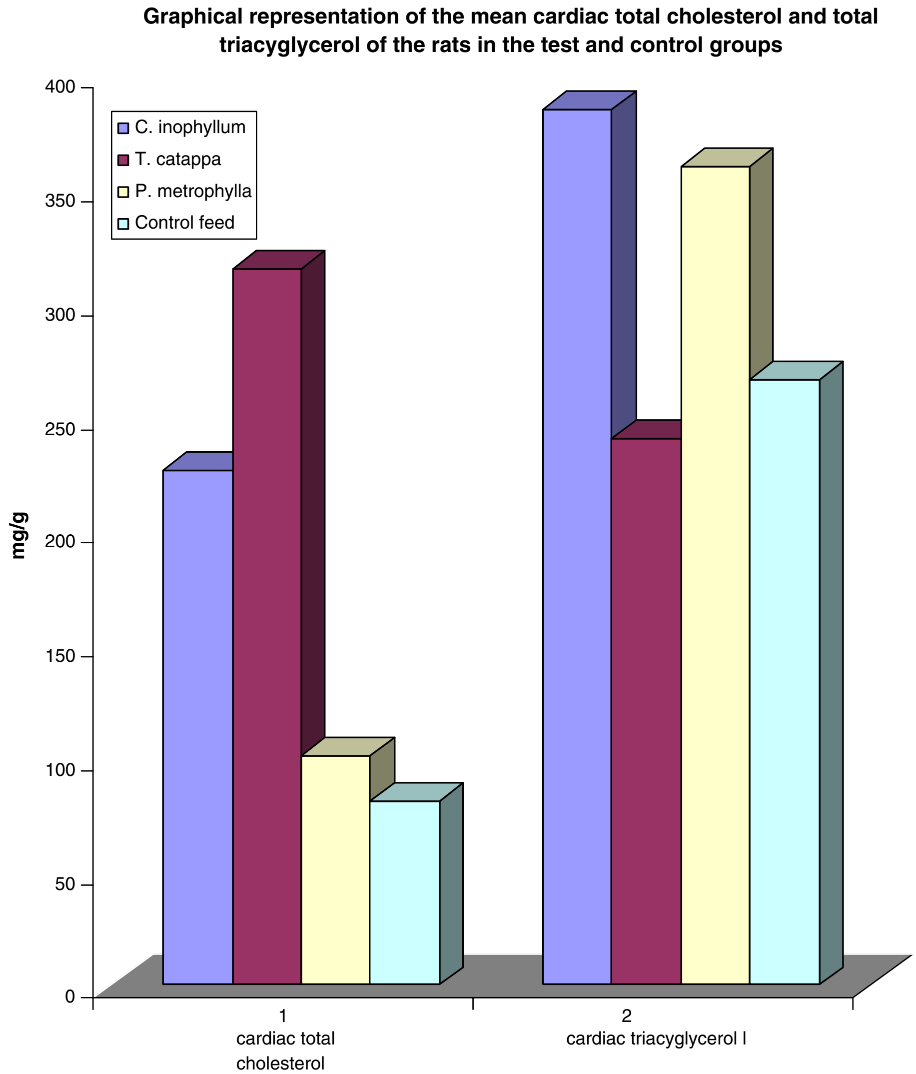

Fig. 1a. Graphical representation of the mean cardiac total cholesterol and total triacyglycerol of the rates in the test and control groups.

to show that the type of fat (saturated or unsaturated) played an important role in human or animal cholesterolemia (Kritchevsky, Davidson, Weight, Kriek, & Du Pleiss, 1982). Results of the present study show a good indication that the consumption of *C. inophyllum*, *P. macrophylla* and *T. catappa* seed oils can likely be used to lower cholesterol levels in blood. This is because of the high unsaturated fatty acid content of the test oils, since it has been reported by many authors (Ahmed & Young, 1982; Kritchevsky et al., 1982; Melgarejo, Gee, & Knight, 1994) that oils containing unsaturated fatty acids, especially linoleic and oleic acids, can be used to lower plasma cholesterol. 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 oils from *C. inophyllum*, *P. macrophylla* and *T. catappa* seeds, being of vegetable origin, are thus likely to lower the occurrence of coronary heart diseases, if consumed. The range of the plasma cholesterol: 67.70 mg/dl (*T. catappa*) to 93.00 mg/dl (control group) is within the 73.0–100.0 mg/dl reported in literature by Dong et al. (1990). According to Magne (1971), 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 dietary groups is less than 275 mg/dl, which suggests that the oils from *C. inophyllum*, *P. macrophylla* and *T. catappa*, if consumed, cannot raise the plasma cholesterol of the individual consuming the oil to above the specified limit.

3.7. Histopathology

No lesions were observed in the heart, liver, kidney and spleen of rats from the control group fed with normal rat feed. In the rats fed with *C. inophyllum*, there was mild renal cortical congestion, severe multifocal tubular degeneration and the presence of eosinophilic proteinaceous casts in the Bowman's capsule of glomeruli. The livers of the

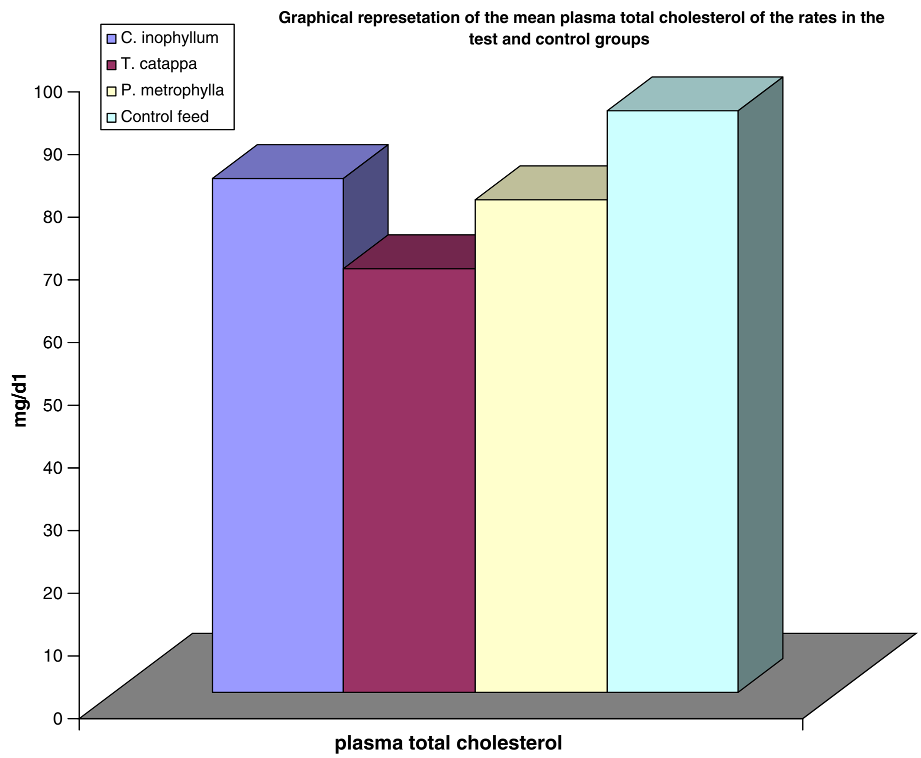

Fig. 1b. Graphical representation of the mean plasma total cholesterol of the rates in the test and control groups.

rats in this group showed severe diffuse hepatic sinusoidal congestion, widespread vacuolar degeneration of hepatocytes, periportal hepatic necrosis (Fig. 2), neutrophilic infiltration and focal areas of Kupffer cell hyperplasia. Rats given *P. macrophylla* oil had severe diffuse congestion and haemorrhage in the medulla of the kidney, with the heart showing cardiac congestion, haemorrhage, myocardiac degeneration and necrosis, intermuscular fibrosis and presence of artherosclerotic clefts within the tunica intima of vasa vasori (Fig. 3). Rats fed with *T. catappa* oil had widespread congestion of hepatic sinusoids, the kidneys had mild diffuse degeneration and presence of eosinophilic proteinaceous casts in Bowman's capsule and tubular degeneration in the cortex (Fig. 4). No lesions were observed in the heart and spleen of these rats. Enlargement of the small intestine and pancreas had been observed previously in rats fed with diets containing purified lecithins from *Canaualis brasiliensis* (Oliveira et al., 1994), *Phaseolus vulgaris* (Oliveira, Pusztai, & Grant, 1988) and *Glycine max* (Geleneser et al., 1994). Numerous factors influence the action of poisonous

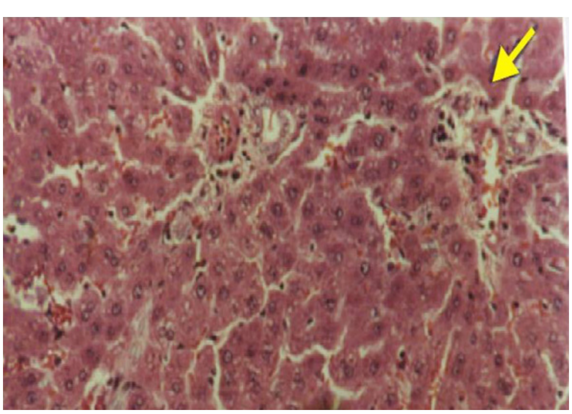

Fig. 2. Photomicrograph of the liver of rat given 5% *Calophyllum inophyllum* oil in normal rat feed showing diffuse vacuolar degeneration of hepatocytes, periportal hepatic necrosis and inflammatory cellular infiltration (arrow). H&E ×650.

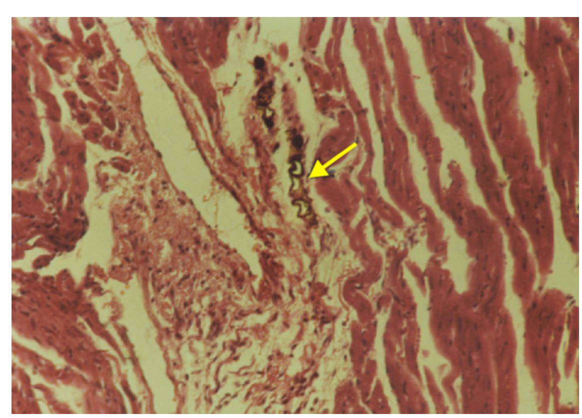

Fig. 3. Photomicrograph of the heart of rat given 5% *Pentaclethra macrophylla* oil in normal rat feed showing myocardiac degeneration, fibrosis and presence of artherosclerotic clefts in a vasa vasorum (arrow). H&E ×650.

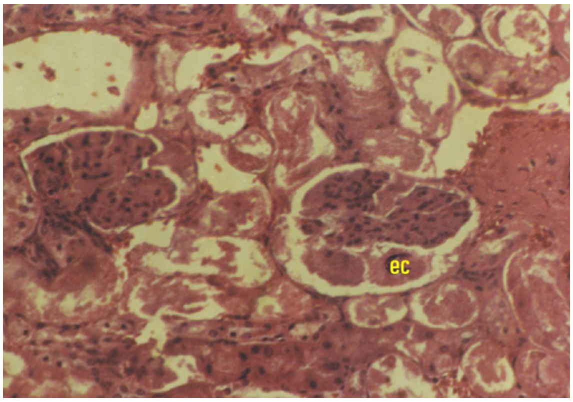

Fig. 4. Photomicrograph of the kidney of rat given 5% *Terminalia catappa* seed oil in normal rat feed showing severe glomerular and tubular degeneration and eosinophilic casts in Bowman's capsule of a glomerulus (ec) and some tubules. H&E ×650.

substances in body tissues. Such factors include the dose, the physical and chemical nature of the poison. The oils from *C. inophyllum* and *P. macrophylla* seeds probably had the effects observed on the rats because of the presence of erucic fatty acid in the oils. Although the percentage of this fatty acid in the seed oils: 0.24% and 1.60% from *C. inophyllum* and *P. macrophylla*, respectively are less than 5% allowable limit for edible oils, the rats might still find this intolerable to their system. The oil from the seeds of *T. catappa* had the least deleterious effect on the rats.

4. Conclusion

The seed oils of *C. inophyllum*, *P. macrophylla* and *T. catappa* did not have any deleterious effect on the physical appearance, body weight gain, organ weights and haemotological parameters of the test rats. However, mild, focal to severe and widespread lesions were found in the kidneys, hearts and livers of the rats fed with *C. inophyllum* and *P. macrophylla* seed oils. The oil from *T. catappa* had the least deleterious effect and seems to be the best of the three oils. If the oil from *T. catappa* is consumed, it cannot raise the plasma cholesterol of the individual consuming the oil above the specified limit; it is even likely to reduce the cholesterol level in blood and also lower the occurrence of coronary heart disease. It is suggested that the seed oils be further refined to observe whether this will improve the quality of the oils and thereby prevent the deleterious effects of the oils on some of the organs of the test rats.

Acknowledgements

The authors are grateful to the Departments of Chemistry, Veterinary Pathology and Chemical Pathology, University of Ibadan, Nigeria and also University of Tuebigen, Germany for making their facilities available for this study.

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