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TOXICOLOGICAL STUDIES OF THE LAGENARIA SICERARIA SEED OIL

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ABSTRACT. The toxicological effect of feeding rats with 5% L. siceraria seed oil for the period of eight weeks was studied. Biochemical and haematological parameters examined in the animal shows normal values except for some enzymes activities and blood count which were below the normal range for rats. The oil appeared to have some hypocholesterol activity suggesting it to be heart friendly. Histological features of heart and kidney are normal except for nonspecific mild infiltration and necrosis. The oil may therefore be employed as supplement to conventional edible ones.

Keywords: oil, seed, lagenaria siceraria, toxicological effect, rats

INTRODUCTION

The current scarcity of conventional edible oils coupled with increase in demand for both oil and oil meal has necessitates the need to investigate alternative oil sources (Ajayi et al., 2007). Inview of these, several plants/seeds have been harness for their oil potentials in order to augment the conventional oil-seeds (Gandhi et al., 1995; Epler et al., 2010). These include rice brand (Rukmini, 1988), cotton seed (Mann, 2010), ratanyout (Gandhi et al., 1995), thunba, grape, niger, tobacco and onion (Eromosele and Eromosele 2002).

Toxicological studies carried out on some unconventional oils shows that the oils have some health effects. Rukmini (1990) observed sterility in male albino rats fed with 10% Muhua oil (Manorama, et al., 1993). Rukmini (1988) reported rice brand oil to have hypercholesterol effect on both experimental animals and human being. Phorbol ester was identified in ratanyout oil, which is responsible for its toxic and irritating effects (Gandhi et al., 1995). In China in the 1950s, it was found that crude cottonseed oil was associated with male infertility. This was attributed to the presence of "gossypol" which when administered to humans, reduces sperm count to less than 1million sperm per ml of seminal fluid (Mann, 2010). In view of these and other reasons (Radu et al., 2011), unconventional oils should be subjected to toxicity studies for it to qualify as nutritionally safe for consumption. Thus, this research aimed at investigating the toxic effect of consuming L. siceraria oil using albino rats as experimental animals.

MATERIALS AND METHODS

Sample Collection

L. siceraria fruits were collected from a farm land in Zauma village, Bukkuyum Local Government, Zamfara State, Nigeria. Seeds were removed from the spongy pulp of the fruit, dried and dehulled manually. The oil content of the seed was extracted using the traditional method as described by Hassan and Sani (2008).

Animals' Diets and Feeding

Fifteen male albino rats (aged 6 weeks) were obtained from the Faculty of Pharmaceutical Science, Ahmadu Bello University, Zaria, and allowed to acclimatize for a week. The rats were randomly divided into three groups (five rats per group) and kept in a wire cage for 8 weeks. Group A was fed with commercial rat feed (Bendel mash) mixed with 5% groundnut oil; group B 5% *L. siceraria* oil, and group C commercial rat feed only. Group A and C were taken as controls. The weakly increase in the animals' body weight was recorded. Animals were sacrificed after 14hrs fasting (Ajayi *et al*., 2007).

The animals were anaesthetized in a container saturated with chloroform vapour and then slaughtered. Blood sample used for heamatological assay was collected in a labelled EDTA bottles while that for biochemical assay was placed in labelled bottle without the anticoagulant. The kidney and liver of the animals were fixed in 4% formalin-saline for histological examinations (Umar, 2010).

Biochemical Assay

The blood samples were centrifuged at 2000rpm for 10min and sera were decanted into clean $5cm^3$ –sample bottles and kept at -20^0C until assay. The sera were used for the determination of urea (Burtis and Ashwood, 2001), creatinine (Henry, 1974), total protein (Cheesbrough, 1991), albumin (Doumas *et al*.,1971),total and direct bilirubin (Randox, 1997), aspartate aminotransferase (AST) activity, alkaline phosphatase (ALP) activity and alanine aminotransferase (ALT) actitivity (Reitman and Frankel,1957), triglyceride, sodium and potassium content (Cheesbrough,1991). Haematological parameters such as pack cell volume, red blood cell, haemoglobin, mean cell concentration, white blood cell,

lymphocytes, netrophils and monocytes were analysed using coulter counter.

RESULTS

Figure1 shows the weekly increase in weight of experimental animals used for the toxicological studies. Generally, there was increase in the body weight of the experimental animals in the first two weeks; the rate was higher in the animals fed with 5% *L. siceraria* oil, followed by 5% Ground nut oil, and commercial rat feed. During the period of third to fourth week, there was a general decline in the growth rate in all the groups, but the effect was more pronounced in those fed with oil. The body weight kept increasing in fifth to last week of the experiment.

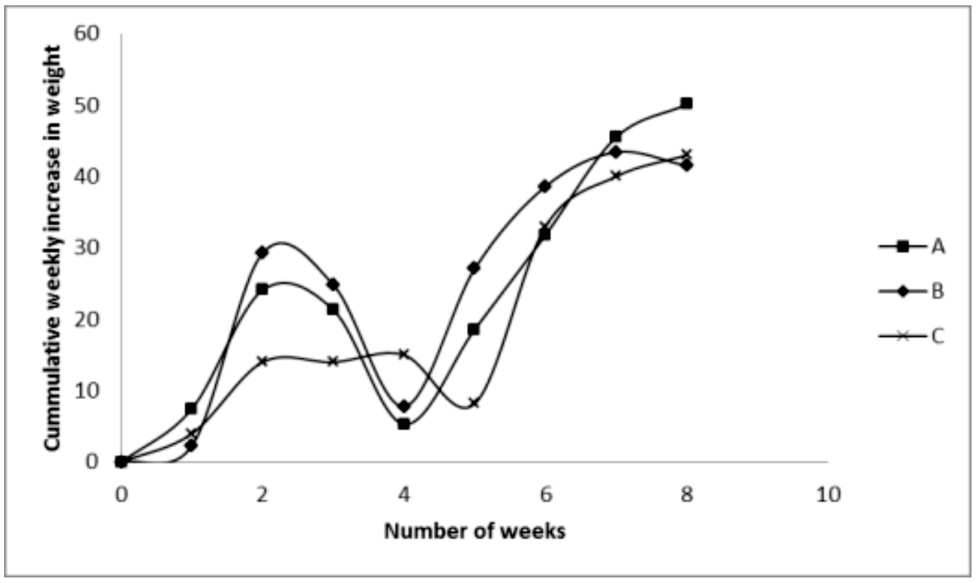

Fig. 1 Graph showing the weekly increase in weight of the experimental animals.
A: Groundnut oil, B: *L. siceraria* oil and non-oil treated group.

Table 1 shows the biochemical parameters of the animals fed with the L. siceraria oils, groundnut oil and commercial rat feed. The parameters analysed varied significantly ($p<0.05$) between the groups except those of sodium, potassium, bilirubin and albumin contents of the serum which shows no significant difference ($p<0.05$).

Table 1

Biochemical parameters of the rats fed with *L. siceraria*, Groudnut oils and rat feed

Parameters	Groundnut$^{\neq}$	*L. siceraria*	Control$^{\neq}$
Total cholesterol (mg/dl)	170.67 ± 2.52^{b}	146.0±3.61^{c}	200.0±1.00^{a}
Total protein (mgL^{-1})	5.50±0.50^{c}	6.50±0.78^{b}	7.70±0.4^{a}
Albumin (mgL^{-1})	3.33±0.15	3.47±0.57	3.60±0.70
Total Bilirubin (mgdL^{-1})	0.33±0.15	0.63±0.15	0.43±0.15
Direct Bilirubin (mgdL^{-1})	0.12±0.02	0.14±0.06	0.20±0.10
Urea (mmolL^{-1})	7.07±0.21^{c}	9.53±0.76^{b}	12.37±1.39^{a}
Creatinine (mgdL^{-1})	1.27±0.25^{c}	1.90±0.27^{b}	2.30±0.20^{a}
AST(IUL^{-1})	27.03±0.15^{c}	33.67±2.08^{b}	62.43±3.29^{a}
ALT (IUL^{-1})	34.07±1.00^{b}	20.07±0.25^{c}	39.53±141^{a}
ALP (IUL^{-1})	117.67±5.13^{b}	58.20±1.61^{a}	119.27±3.61^{b}
Sodium (mmolL^{-1})	139.67±1.53	139.67±1.53	140.0±9.84
Potassium (mmolL^{-1})	4.50±0.46	5.03±0.21	3.93±0.12
Chloride (mmolL^{-1})	91.87±1.85^{a}	86.73±1.55^{b}	79.80±2.30^{c}

Values with different superscripts on the same raw are significantly different at $p<0.05$. $^{\neq}$= control groups
ALT = Alanine aminotransferare, AST = Aspartate aminotransferase, ALP = Alkalinephosphatase, IUL^{-1} = International unit.

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The results of heamatological analysis conducted on the blood samples obtained from the experimental animals are shown in Table 2. With the exception of monocytes and MCHC, all the parameters analysed varied significantly between the control groups and *L. siceraria* oil.

Table 2

Heamatological parameters of the rats fed with *L. siceraria* seed, Groudnut oil and rat feed

Parameters	Groundnut[≠]	*L. siceraria*	Control[≠]
Hb(g/L)	9.27±0.64[a]	7.93±0.25[ab]	7.23±0.68[ab]
WBC(10^9dL)	0.90±0.10[a]	1.30±0.05[b]	1.10±0.20[ab]
RBC(10^{12}dL)	6.01±1.23[a]	4.18±0.64[b]	4.50±1.05[b]
PCV (%)	27.33±2.52[ac]	23.66±1.53[ba]	20.33±1.53[b]
MCV(fL)	50.90±0.26[a]	57.47±0.47[c]	53.33±2.32[b]
MCHC(g/dL)	36.13±0.81	35.67±0.31	35.53±1.0
LYMPOCYTES (%)	78.67±1.53[a]	82.33±0.57[b]	81.67±1.52[c]
NEUTROPHILS (%)	11.00±1.73[a]	7.33±1.53[b]	7.33±0.58[c]
MONOCYTES (%)	11.00±1.00	11.00±2.0	12.00±1.00

Values with different superscripts on the same raw are significantly different at $p<0.05$. [≠]= control groups
PCV = Packed Cell Volume, RBC = Red Blood Cell, Hb = Haemoglobin, WBC = White Blood Cell, MCV = Mean Cell Volume, MCHC = Mean Cell Haemoglobin Concentration, fL = femtoliters (10^{-15} liter).

Plate 1-6 shows the histological appearance on both the liver cells (hepatcytes) and kidney cells.

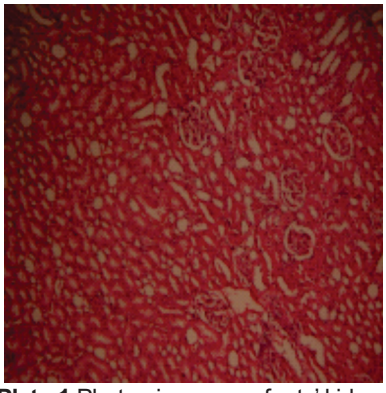

Plate 1 Photomicrogram of rats' kidney fed with groundnut oil showing normal tubules and glomerelus.

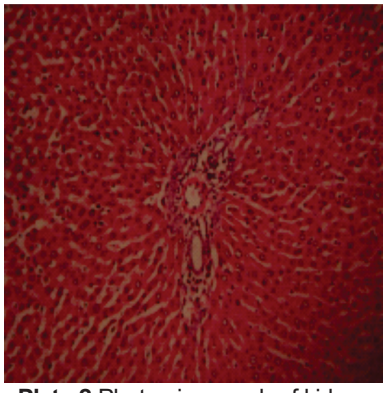

Plate 2 Photomicrograph of kidney cells of the rat fed with *l.siceraria* oil showing normal tubules and glomerelus.

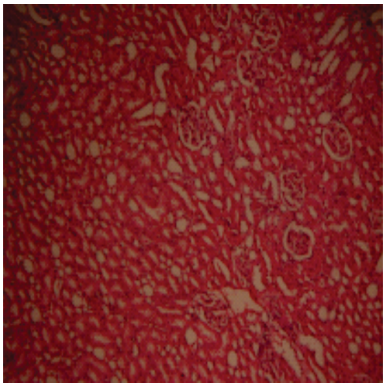

Plate 3 Photomicrogram of rats' kidney fed with normal rat feed showing normal tubules and glomerelus

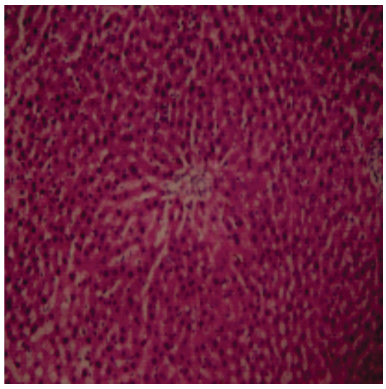

Plate 4 Photomicrograph of liver cells of the rat fed with groundnut oil showing normal hepatocytes

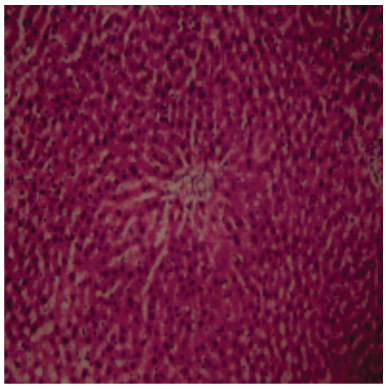

Plate 5 Photomicrograph of liver cell of the rats fed with *l.siceraria* oil showing moderate infiltration on the hepatocytes.

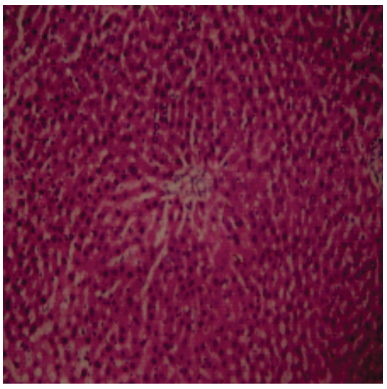

Plate 6 Photomicrograph of liver cells of the rat fed with rat feed only showing normal hepatocytes.

DISCUSSIONS

The weekly change in weight is shown in Figure 1. The observed decline in growth could be attributed to the harsh weather condition (45^0C) experienced during the period of the experiment (Feb-Apr). It may also be due to some disease conditions such as yellow fat disease which has been reported to occur naturally in wild life and domestic species as well as in toxicological studies of rats, rabbits, mink and pigs administered with diets rich in polyunsaturated fatty acids containing oils (Kroes *et al*., 2003), considering the fact that, *L. siceraria* oil contain 69.4% polyunsaturated fatty acid (Hassan *et al*., 2011). This decrease in weight was observed in both the treatment and control groups, indicating that it may not be treatment related. The variation in the weight could be due to increase in adipose tissues in the animals which consequently melt under high temperature resulting in rapid loss of weight in the animals. The growth rate also witnesses an increase in the subsequent week and continued declining in the last three weeks.

Cholesterol level of the groups fed with *L. siceraria* oils and groundnut oil were lower than that of group fed without any oil. However, the serum cholesterol of rats fed with 5% *groundnut,* and *L. siceraria* oils are within the range of (198-34.4 mg/dl) reported by Rukmini (1988) on rats fed with similar quantity of rice brand oil and groundnut oil. Similarly, Kroes *et al*., (2003) observed a decrease in serum concentrations of total cholesterol, phospholipid and free fatty acid levels on animals fed with oily feed compared with non oil-treated control group. This indicates that, the oil under investigation has some hypocholesterol activity which may be responsible for decrease in serum cholesterol and therefore not a potential source of heart diseases.

Serum total protein of the rats fed with *L. siceraria* oil (6.5±0.78 mg/L) is within the permissible limit of 5.6-7.6g/dl. The two control groups: rats fed with normal rat feed (5.50±0.50 mg/l) and those fed with groundnut oil (7.70±0.4 mg/L) were lower and higher than the acceptable limit respectively. However, albumin contents of the three groups are all below (3.8-4.8 mg/l) which is the accepted albumin limit for rats (The rat fan club, 2010). The main functions of albumin beside being a source of amino acids, are to maintain the water balance in serum and plasma and to transport and store a wide variety of ligands e.g. fatty acids, calcium, bilirubin and hormones such as thyroxine (Doumas *et al*., 1987). Hypoalbuminaemia is associated with impaired albumin synthesis in the liver; liver disease; malnutrition or malabsorption; kidney disease and intestinal malfunctioning (Doumas *et al*., 1987). Considering the result obtained, the oil extracted from the three varieties may be a potential source of one of these disease conditions.

Bilirubin index of a serum is known to be associated with the breakdown product of red blood cell (Anosike *et al.,* 2008; Phillips, 2010). Abnormal level of serum bilirubin signifies improper bile excretion; therefore an obstruction may be present in the bile duct or gall bladder. It also signifies the ability of liver to actively treat its haemoglobin (Phillips, 2010). In this work, no significant difference ($p<0.05$) was observed between the control groups and one fed with *L. siceraria* oil. However, the results are within the permissible limit (0.2-0.55mg/l), hence it can be said that, the oil is not a potential causes of liver malfunctioning.

The result for the serum urea and cretinine of the experimental animals is shown in Table 1. The urea content ranges from 7.07±0.21 to 12.37±1.39 $mmolL^{-1}$ with the two control groups having the least and highest values respectively. Urea is useful in assessing kidney performance (Boonprong *et al*., 2007). In comparison with acceptable range for urea (2.50-22.5$mmolL^{-1}$) (the rat fan club, 2010), both the controls and experimental group are not having any symptoms of kidney failure, indicating the oils have no negative effect on the organ.
Unlike the urea content, creatinine content of both the control groups and those fed with *L. siceraria* oil were above the normal range for experimental rats (0.2-0.8 mg/dL)(Umar,2010). The increase in the creatinine content (1.27±0.25 – 2.3±0.20 mg/dL) may not be as a result of the oil fed to the experimental animals since the group fed with normal rat feed registered highest creatinine content (2.3±0.20 mg/dL). Increase in the creatinine content might be due interference with creatinine metabolism leading to its increased synthesis (Yakubu *et al*., 2008).

The AST activities in rats treated with *L. siceraria* (33.67±2.08 U/L) and that treated with groundnut oils (27.03±0.15 U/L) are significantly ($p>0.05$) lower than group fed with non-oil feed (62.43±3.29 U/L). AST is an enzyme responsible for transfer of amino group from an alpha-amino to alpha-oxoacid. Increase AST activities may signifies damage in the heart, liver, skeletal muscles and kidney (Zilva and Pannall, 1978).The reduction in the level of this enzyme might serve as an indication of inhibitory activity of the enzyme by the oils. Similarly, the activity in the oil-treated groups is lower than the normal range of (45.7-80.8U/L) for rats (The rat fan club, 2010).

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ALT activity in rats treated with groundnut (34.07±1.00 U/L) and that treated with *L. siceraria* oils (20.07±0.25 U/L) are significantly lower ($p>0.05$) than group fed with non-oil feed (39.53±1.41 U/L). This may serve as an indication of lower enzymes activity in the oil-fed animals. However the values obtained in this work are above the rat's normal range of (17.5-30.2U/L) except for *L. siceraria* treated group with ALT value of (20.07±0.25U/L) (The rat fan club, 2010).

ALP acts as marker used in assessing the integrity of plasma membrane (Yakubu *et al*., 2008). The ALP values obtained in this analysis varies significantly ($p<0.05$) between the groups; the groups fed with Groundnut oil and without oil are significantly higher than the group fed with *L. siceraria* oil. However, the results are within the normal range of 56.8-128 U/L (Umar, 2010). The abnormal pattern of ALP might be due individual metabolic differences (Wabiho, 2007). This is an indication that the oils may not have any damaging effect on the plasma membrane of the rat organs (Ashafa *et al*., 2009).

Potassium plays very important role, along with sodium and chloride ions, in the osmotic regulation of the body fluids and in the acid base balance in animals. Whereas sodium is the main inorganic element of extra cellular tissue fluid, potassium functions principally as the cation cell and also concerned with nerve and muscle excitability and in carbohydrate metabolism (Adeyeye *et al.,* 2005). The normal range for the animal's body electrolyte is (K = 5.4 – 7.0; Na = 143 – 156 and Cl = 100 – 110 $mmolL^{-1}$). Potassium level for all the results (3.93±0.12 - 5.03±0.21$mmolL^{-1}$) is below the acceptable limit set for rats showing the animals to be deficient in potassium. This may be an indication that, the body electrolyte regulating mechanism is affected (Yakubu *et al.*, 2008) However, sodium level (139.67±1.53 - 142.00±2.0 $mmolL^{-1}$) is within the normal range accepted for experimental rats. Similarly, the chloride content is below the accepted limit. Deficiency of these electrolytes in the body (especially sodium) leads to a lowering of osmotic pressure, which results in dehydration of the body (McDonald *et al*, 1995).

Haemoglobin estimation gives the oxygen carrying capacity of blood and helps in detecting diseases which cause deficiency or excess of haemoglobin (Ochei and Kolhatkar, 2000). The result obtained in this work (6.72±0.17- 9.33±2.51 mg/dl) is quite low in both the control groups and those fed with *L. siceraria* oil when compared with experimental animal fed with *Garcinia mangostana* seed oil (15.7 mg/dl) reported by Ajayi *et al.*, (2007).

The WBC, RBC and PCV content of the blood collected from three groups varies significantly ($p>0.05$). RBC count ranges from 3.67±0.05 x $10^{12}L^{-1}$ in group fed with *L. siceraria* oil to 6.01±1.23 x $10^{12}L^{-1}$ for control group, indicating possibility of anemia in the animals considering the normal range of 6.76-9.75 x $10^{12}L^{-1}$(Umar, 2010). The values are quite low when compared with the result obtained by Ajayi *et al*., (2007) on rats fed with *Garcinia mangostana* seed oil. Furthermore, the MCV and MCHC shows no significant difference ($p>0.05$) between the control and sampled groups except for MCV which shoes significant difference.

White Blood Cell (WBC) count gives the measure of cellular immunity against disease causing microorganisms in the body (Umar, 2010). WBC count of the groups fed with *L. siceraria* oil was higher than control groups. However, the parameters obtained in all the groups are below the normal range of 6.6-12.6 x $10^{9}L^{-1}$ for rats (Umar, 2010). Other index of cellular immunity includes the lymphocyte, monocytes and neutrophils content of the blood.

Unlike monocytes, lymphocytes and neutrophils content of the blood samples analysed varies significantly ($p<0.05$) between the samples group and controls. Their function in the blood is to protect the body against bacterial, viral and other parasitic attack. Monocytes content of all the samples are within the rat's normal range of 65-85% while that of lymphocytes is above the recommended range of 0-6% (the rat fan club 2010). However, the range of values obtained in this work for lymphocyte (78.67±1.53 – 88.00±2.08 (%)), neutrophils (4.00±1.00-11.00±1.73 (%)) and monocytes (10.67±1.53-12.00±1.00 (%)) compared favorably with that of Ajayi *et al*., (2007).

Histological appearance of kidney and liver appear normal except for mild to moderate lymphocytic infiltration around the portal area and urethra as well as within interstitum in the group fed with *L. siceraria* oil. Also observed, were congestion of central vein in the liver as well as in some interstitial blood vessels in both control and experimental group. All these findings are non-specific and may not necessarily be as a result of feeds giving to those animals. However, these may not be unlikely.

CONCLUSION

The current toxicological studies permit the following conclusion to be drawn. The animals fed with *L. siceraria* oil witnessed relatively high growth rate compared to those fed with groundnut oil and without oil. The significant reduction in serum cholesterol level of the oil-fed animals compared to control is an indication of hypocholesterol activity of the oil, hence heart friendly. The observed reduction in enzymes activity such as AST and ALP warrant further investigation. Kidney and liver function indices indicated most of the parameters to be within the acceptable range. Histological appearance of the kidney and liver in all the groups appear normal except for mild to moderate lymphocytic infiltrate around portal area of kidney and also blood congestion along

central vein of liver. These might not necessarily be due to the feed administered to the animals. Thus, the use of this oil for edible purpose in the shortage of conventional ones is recommended.

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