Treculia africana seed oil-compounded feed in male rats: nutritonal and toxicological evaluation.

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Abstract: *Treculia africana* seed oil was evaluated for physicochemical properties. Saponification, iodine, peroxide and acid values obtained were (212.9±4.50 mgKOH/100g; 27.5±2.32 $mgI_2/100g$; 1.75±0.07 mEq/kg oil and 8.41±1.25 mgKOH/100g) respectively. Varying percentages (3, 5 and 10) of seed oil-based feed was formulated and the effect on male rats fed for eight weeks was investigated. Effect of seed oil feed on growth, on brain and liver tissue antioxidant status, lipid peroxidation, artherogenic index and markers of tissue toxicity and tissue histological changes were examined. For body weight, test animals fed with with 10 % TA had the least percentage increase of 32 %. *Treculia africana* seed oil-based diet decreased serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities compared to control but increased serum triglyceride, total cholesterol, low density and high density lipoprotein cholesterol but decreased the artherogenic index significantly compared to control. The MDA was not statistical significant ($p<0.05$) in the groups fed with TA compared with control and activities of superoxide dismutase (SOD) in TA and VO were comparable.
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1. Introduction

The common sources of edible oils are becoming scarce and there is therefore the need for alternatives and the vast species of trees in both the humid and semi-arid tropics are good sources of oils and fats for consumption (Odoemelam 2005). The major sources of edible oils world-wide are sunflower, olive, walnut, corn while native to Nigeria are oil palm (*Eleasis guineensis*) and ground nut (*Archis hypogoea*) and the oils which are more expensive are believed to be healthier and most often contain unsaturated fatty acids. Polyunsaturated fatty acids in the diet are regarded as healthy lipids and are nutritionally essential as they lower plasma cholesterol level by redistribution between plasma and tissue (Hu *et al.,* 2001). Atherosclerosis is the root cause behind coronary artery disease, cerebral vascular disease and peripheral vascular disease (Malika *et al*., 2007) and increase in blood cholesterol levels increases the risk of coronary heart disease (CHD) and atherosclerosis (Malika *et al*., 2007). Abnormal deposition of cholesterol in the tissues is associated with several conditions including arthrosclerosis, hypertension and diabetes mellitus (Ross, 1993). Serum cholesterol level is strongly governed by dietary intakes, especially of saturated fats (Romero-Coral *et al*., 2006). Also, genetic factors are involved in the regulation of triglyceride and cholesterol levels in the plasma. Elevated serum cholesterol may induce fatty liver hepatic steatosis or hypertrophy of the liver, altered lipid profile, atherosclerosis and ischemic heart disease.

Treculia africana Decne is known commonly as African bread fruit. It is eaten traditionally either roasted as snack with coconut and palm kernel seed or eaten as porridge with a long cooking time. Previously this delicacy was not so expensive but increased awareness of its nutritional potentials has increased the economic value tremendously in Eastern Nigeria. Although this fruit grows throughout the Tropics, it is relatively unknown in South West Nigeria. *Treculia africana* leaves, twigs and stem bark have been used for dental disorders (Metuno *et al*., 2008) while the root bark has shown antidiabetic activity in alloxan-induced rats (Oyelola *et al*., 2007). *Treculia africana* has been shown to be a rich source of plant protein higher than those from animal and marine fishes could provide energy and seed oil content 18.54 % (Ajayi, 2008). Various studies have been done on either reducing the cooking time, improving the protein digestibility and alternate methods of preparation of the seed flower (Giami *et al*., 2001; Badifu *et al*., 2001; Giami *et al*., 2000).

As part of the search for alternate source of edible oil and possible oil which could modulate serum lipid levels, we evaluated the impact of varying percentages of *Treculia africana* seed oil compounded feed with commercially available Grand vegetable oil in male rats and evaluated the effect on brain and liver tissue antioxidant status, lipid peroxidation, artherogenic index and markers of tissue toxicity.

2. Materials and Methods

Treculia africana was harvested from Botanical Garden in University of Ibadan, Ibadan, identified by Mr D Esimekhuai at the Herbarium of the Department of Botany, University of Ibadan, Ibadan Nigeria and voucher specimens were deposited at the Departmental Herbarium. The fresh fruits were room dried in Nutritional Biochemistry Laboratory. Dried seeds were ground to coarse texture using the dry cup of domestic Kenwood blender and subjected to Soxhlet extraction using *n*Hexane for 6 hours to obtain the seed oil. The impure oil was purified by adding 0.73 % NaCl solution to the oil in a separating funnel, the lower aqueous layer was removed and the organic phase was further purified thrice using chloroform, methanol, 0.58 % sodium chloride solution (5:48:47 v/v) to obtain the pure oil. *Treculia africana* seed oil was evaluated for both physical characteristics and physicochemical parameters to determine its quality with those of conventional edible oils. Physical examination included colour, state of the oil at room temperature, smell, density, smoke point as well as flash point, while the physicochemical properties such as acid, peroxide, saponification and iodine values were determined as described by AOAC 1990 and results obtained are shown on Table 1.

Soybean oil marked as Grand vegetable oil, a product of Grand Cereals and Oil mills Limited, Bukuru, Jos, Nigeria. Yellow maize (*Zea mays*) grains and soy beans used in feed formulation were purchased from Bodija Market, Ibadan, Nigeria. Vitamin mix used in feed formulation was from BASF Aktiengesellschaft, Ludwigshafen, Germany. The composition of test feed per kg diet is as shown on Table 2. Briefly, Corn starch was carbohydrate source, it was cleaned, sundried and milled. Soybean was first dehusked before milling and it was used as protein source. Components of the mineral mix was from Sigma-Aldrich Co. Ltd., Poole Dorset, UK The different components of the diet were thoroughly mixed, made into pellets for easy handling by animals and was thoroughly oven dried to prevent mould growth was stored in air tight bags at 4 ^{O}C to prevent microbial contamination and auto-oxidation of the oil (Oladiji *et al*., 2010).

Feed composition using commercial vegetable oil and *Treculia africana* seed oil

composition	Test feed %	3 % TA	5 % TA	10 % TA
Soybean	27	27	27	27
Corn starch	59	59	59	59
Vitamin mix	4	4	4	4
Seed oil	nil	3	5	1
Vegetable oil	10	7	5	Nil

Twenty eight male albino rats (Wistar strain) weighing between 100 g and 120 g were obtained from the animal house in the Department of Physiology University of Ibadan. The rats were transferred and allowed to acclimatized for two weeks, been maintained on the standard rat chow (Ladokun Feed, Ibadan) with water *ad libitum* in the Biochemistry Department Animal house under normal room temperature before the commencement of the experiment.

Twenty eight rats weighing between 110 -125 g were distributed randomly into four different groups of seven animals each. The control group received compounded diet prepared with commercially available Grand soybean oil purchased from Bodija market, Ibadan. All animals had 10 % oil in their different formulated feeds except that the proportion of the seed oils and Grand vegetable oil varied as shown on Table 2 and animals were distributed into groups 1-4. Group1: A compounded diet with vegetable oil (10 % VO); Group2: A compounded diet with 10 % of *Treculia africana* seed oil (10 % TA); Group 3: A compounded diet with 5 % of *Treculia africana* seed oil (5 % TA) and Group 4: A compounded diet with 3 % of *Treculia africana* seed oil (3 % TA). Animals were fed seed oil compounded feed for eight weeks.

The animals were fasted for 24 h after the last compounded meal and were sacrificed by cervical dislocation. Blood was obtained using 2 ml syringe by cardiac puncture into clean bottles without anticoagulant and were left to stand for 1 h for complete coagulation. The clotted samples were spun at 3000 rpm for 10 minutes, the supernatant serum was removed and it was stored at 4 ^{0}C. The visceral organs (liver and brain) were quickly removed, washed with 1.15 % KCl, homogenized in 56 mM Tris-HCl buffer (pH 7.4) containing 1.15 % potassium chloride and the homogenate was centrifuged at 10,000 rpm for 15 minutes at 4 ^{0}C. Supernatant was stored until needed. Small pieces of liver and brain sections were fixed in 10 % formal saline and sent to Veterinary Anatomy Department, University of Ibadan, Ibadan for histopathological examination.

Organ protein concentration was done using the Biuret method (Gornal *et al.,* 1949) with bovine serum albumin (BSA) as standard. Lipid peroxidation was assayed by measuring thiobarbituric acid reactive substances (TBARS), by colorimetric reaction of the lipid peroxidation product malondialdehyde (MDA) with thiobarbituric acid (TBA) to form a pink precipitate, which was read at 532 nm by spectrophotometry. Catalase (CAT) activity was done by measuring the rate of decomposition of hydrogen peroxide at 570 nm as described by Sinha, (1971). Reduced glutathione (GSH) level was determined by measuring the rate of formation of chromphoric product in a reaction between DTNB (5,5´-dithiobis-(2-nitrbenzoic acid) and free sulphydryl groups at 412 nm (Beutler et al., 1963). Superoxide dismutase (SOD) activity was assayed using the method of Misra and Fridovich 1972, Cholesterol was determined using Randox kit. The lipoproteins were assayed using enzymatic colorimetric method for very low density lipoprotein (VLDL) and low density lipoprotein (LDL) by precipitation using phosphotungistic acid and magnesium chloride. After centrifugation at 3000 g for 10 min at 25 ^{0}C, the clear supernatant contained high density lipoprotein (HDL) fraction using HDL-cholesterol precipitant kit. The LDL-cholesterol (LDL-c) was calculated using the formulae of Friedwald *et al*., (1972). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed using Randox kits.

At the time of sacrifice, the liver tissues were removed and fixed in 10 % formaldehyde solution and sent to Veterinary Anatomy Department, University of Ibadan, for histopathology examinations. Briefly, the tissues were washed by dehydration in increasing gradient of ethanol and finally cleared in toluene. The tissues were then embedded in molten paraffin wax. Sections were cut at 5 µm thickness and stained with hematoxylin and eosin. The slides were photographed with an Olympus UTU1X-2 camera connected to an Olympus CX41 microscope (Tokyo, Japan).

Data was expressed as mean ± S.D of seven determinations, except for the proximate analysis which was done in triplicate. All data was analyzed using one way analysis of variance (ANOVA) and complimented with student t-test. Values for $p < 0.05$ were considered to be statistically significant.

3. Results

Table 1: Physiochemical properties of *Treculia africana* oil

Parameter	TA oil
Saponification value (mg KOH/100g)	212.90±4.40
Iodine value (mg I_2/100g)	27.50±2.32
Peroxide value (m Eq/kg oil)	1.75±0.07
Acid value (mg KOH/g oil	8.41±1.25
Specific gravity (g/ml)	0.89±0.03
Solidification point (^{0}C)	20-24
Smoke point (^{0}C)	201
Flash point (^{0}C)	232

Table 2: Effect of *Treculia africana* seed oil based formulated feed on body weight and organ weight of rats for eight weeks

Treatment Group	Initial weight(g)	Final weight (g)	% Weight gain	Liver (g)	Brain (g)
Group 1 (10% VO)	123.33±1.66	193.33±6.66	36.20	5.693±0.046	2.15±0.712
Group 2 (10% TA)	123.33±1.66	181.66±1.66^a	32.1	5.72±0.070^a	1.98±0.556^a
Group 3 (5% TA)	125.0±0.00	200.0±5.77^b	37.5	6.75±0.093^b	2.116±0.338^b
Group 4 (3% TA)	119.0±2.08	190.0±10.00	38.9	5.53±0.208^b	2.05±0.305^b

The results are shown as means ± SE for each group of seven rats per group.

a= $p < 0.05$ when compared with the control; V.O= Vegetable oil

b= $p < 0.05$ when compared with 10% TA; T.A= *Treculia africana*

On Tables 1 are data obtained for the physiochemical properties of TA seed oil. Physicochemical properties examined were saponification, iodine, peroxide and acid values and (212.9±4.50 mgKOH/100g; 27.5±2.32 mgI_2/100g; 1.75±0.07 mEq/kg oil and 8.41±1.25 mgKOH/100g) were obtained for each of the parameters. In all treatment groups, for the control rats on 10 % VO and those on varying proportions of TA oils, we observed increase in body weight of rats. Changes in body weight and visceral organs of rats on VO and TA diets are shown on Table 2. *Treculia africana* seed oil compounded feed (10 % TA oil) elicited the least increment in percentage body weight increase of 32 %, compared to both control (10 % VO) and other test groups. Similar trend was seen in brain tissue while the liver was slightly enlarged. Liver tissue in group 3 rats was significantly increased compared to control and only in group 4 animals was there a slight decrease. There was significant ($p<0.05$) increase in the body weight of animals fed with 3 % and 5 % of or *Treculia africana* oil compared to 10 % *Treculia africana*. 10 % *Treculia africana* had the least increase in body weight and was significant compared to 10 % VO. There was no significant ($P<0.05$) difference in the weight of liver of rats fed on *Treculia africana* when compared with the control. However, in the brain, there was significant ($P<0.05$) decrease in the brain weight of the animals fed with *Treculia africana* seed oil compared to control.

Figure 1 has data for results obtained for serum lipids in animals fed varying percentages of *Treculia africana* seed oil based diet for eight weeks. The result showed that rats fed with seed oil based formulated feed had their total cholesterol and triglyceride increased significantly (p<0.05) when compared with the control and animals fed with 3 % *Treculia africana* were significantly (p<0.05) increased when compared with those fed with 10 % *Treculia africana* respectively. Serum HDL-c level was higher in animals fed with seed oil diet compared to 10 % vegetable oil. The level of LDL-c in the rats fed with control diet (10 % vegetable oil) and 10 % *Treculia africana* group were similar. Moreover, when compared with 10 % *Treculia africana* based formulated diet, the groups of animals fed *Treculia africana* with varying composition of *Treculia africana*, their LDL levels increased significantly (p<0.05).

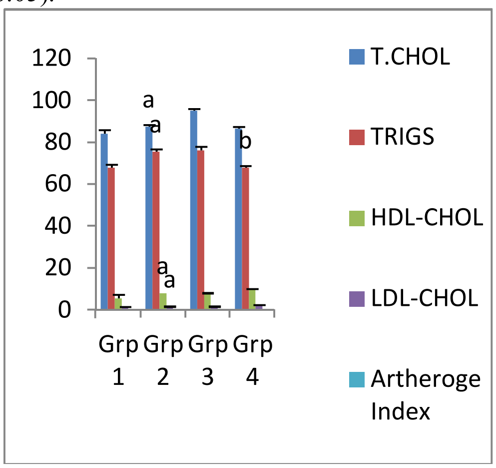


Fig 1: Serum lipid levels of rats on *Treculia africana* seed oil diet for eight weeks

Data obtained for antioxidants levels in rats fed *T. africana* seed oil formulated diet for eight weeks are shown on Figs 2a and 2b respectively for liver and brain tissues respectively. *T. africana* seed oil feed caused a significant increase in the activity of SOD in the brain of rats on 10 % TA fed group compared to control, 3 and 5 % TA oil supplemented groups significantly lowered SOD activity compared to control. GSH did not increase significantly in the test groups (2, 3 and 4) compared to control in both liver and brain tissue homogenate. CAT in both liver and brain in rats fed 5 % and 3 % *Treculia africana* were incidentally significantly (p<0.05) reduced when compared to 10 % TA treated rats in group 2. MDA was significantly increased in the groups fed with seed oil diet compared to control, 5 % *Treculia africana* elicited greater increase in MDA levels.

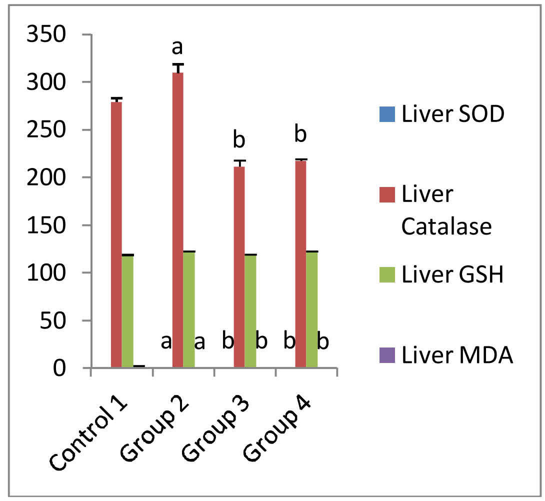


Fig 2a: Liver antioxidants and lipid peroxidation levels of rats on *Treculia africana* seed oil diet for eight weeks

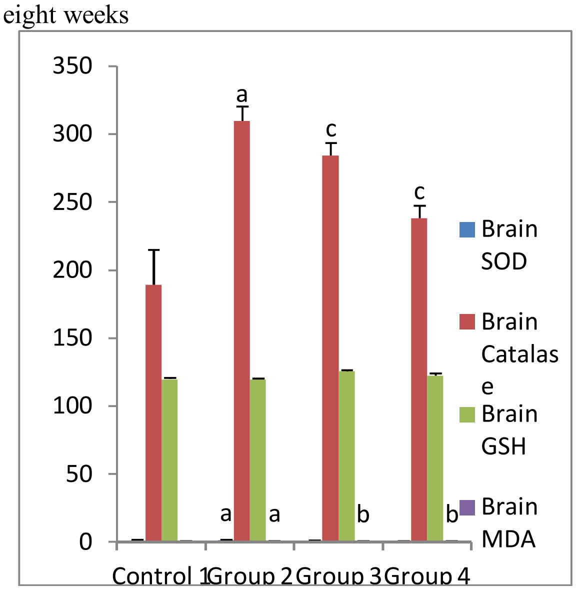


Fig 2b: Brain antioxidants and lipid peroxidation levels of rats on *Treculia africana* seed oil diet for eight weeks

Activities of aspartate amino-transferase (AST), alkaline aminotransferase (ALT) and variations in the brain and liver protein concentrations in this study are shown on Table 4. The data obtained for both parameters (AST and ALT) were significantly (p<0.05) reduced in TA when compared with control. There was no significant (p<0.05) difference when compared within groups for AST and there was significant (p<0.05) reduction when 5 % and 3 % TA (groups 3 and 4) was compared with 10 % TA (group 2) in activities of ALT. There were no significant differences in protein concentrations in liver and brain of rats in test groups compared to control group.

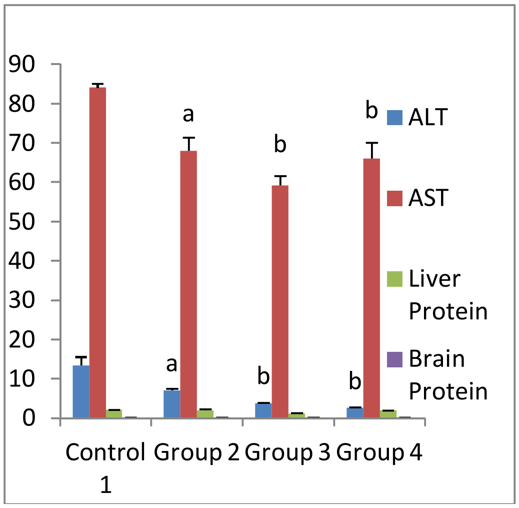
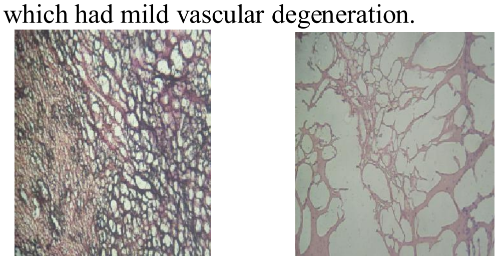
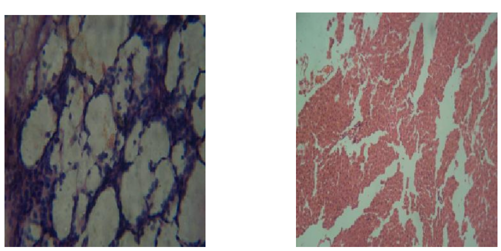


Fig 3: Liver ALT, AST, Protein and brain protein concentration levels of rats on *Treculia africana* seed oil diet for eight weeks

Photomicrographs of liver tissue in the study are shown on Fig 3. In control rats, as well as animals in groups 3 and 4, all had normal liver tissue histology with no visible lesions, however test animals on10 % TA which had mild vascular degeneration.



(A) Control rats on 10% VO: No visible lesion. (B) Rats on 10% TA: Mild diffuse vascular degeneration



(C) Rats on 5% TA: No visible lesion. (D) Rats on 3% TA: No visible lesions

4. Discussions

Fats and oils are essential nutrients in both human and animal diets because they are a concentrated source of calorie and essential fatty acids which are precursors of important hormones and prostaglandins. They are stores of fat soluble vitamins; they serve to make foods more palatable and are important in membrane fluidity and function. The knowledge of the chemical composition of fats and oils, their purification and chemical modification of oils and fats is essential for health benefits (Asif *et al.*, 2010). Some seed oil contain toxic antinutritional factors thus the need for safety evaluations, purification, and assessment on their effect on both growth and development.

Physicochemical analysis of *Treculia africana* seed oil (Table 1), parameters of thermal stability (smoke and flash points) indicate that both oils may be used at relatively very high temperatures such as in deep frying. Acid value is a measure of the age, quality and suitability for consumption and stability (Kardash and Tur'yan, 2005, Akubugwo, 2008). Low acid value of the seed oils indicates reduced susceptibility to lipolytic hydrolysis and the acid value (8.4 mg KOH/g oil) compares favourably with edible oils such as sesame, soybean, sunflower and rape seed and the value is within the allowable limits for edible oils (Eckey,1954). The low iodine values of the oil (27.20 mg I_2/100 g oil), a measure of unsaturation indicates reduced susceptibility to hydrolysis and oxidative spoilage on storage (Popoola and Yangomodou, 2006). The iodine value of the seed oil places it as non- drying and the peroxide value of (1.75 mEq/kg oil) further supports the lowered tendency to go rancid and the value is lower than those of commonly consumed oils such as palm, coconut, groundnut and even soybean oils (Oladiji *et al.*, 2009). The saponification numbers of the seed oil is low (212.70mgKOH/ 100g oil): it is not likely to be suitable for soap making.

There was increase in body weight in all tested animals on TA seed oil based feed compared to control on vegetable oil, while only in 5% *Treculia africana* fed rats was there a significant increase in body weight. The decrease in weight in 3% and 10% maybe due decreased fed intake or reduction in drinking water utilized, caused by either the reduced palatability of *Treculia africana* compounded feed, diet-induced anorexia, or systemic toxicity (Abdulazeez, *et al.,* 2008). *Treculia africana* feed rats had reduced brain weight which corresponded with the body weight but the livers were bigger except for the 3% TA group 4 rats. *Treculia africana* seed oil based diet, especially in group 2 caused decrease in both the body weight and visceral organs may probably be less palatable and acceptable to the rats unlike the vegetable oil in control group.

Serum lipid profiles data are essential biomarkers for assessing health risk in obesity, diabetes and coronary heart diseases (CHD) (Cain, 2007). Diet induced alterations in serum lipids of animals could provide useful information on the effect of the diet on lipid metabolism as well as predisposition of the heart to atherosclerosis and other CHD (Visavadiya and Narasimacharya, 2005; Abolaji *et al*., 2007). Saturated and unsaturated fatty acids in the diet have been reported to have an influence on both serum lipid levels and lipidperoxidation (Gilani *et al*., 2002). The tested oil (*Treculia Africana* seed oil) in rats had higher total cholesterol and triglyceride values, the rats all had higher HDL-c and subsequently lower artherogenic index (Fig.1). Increase in the serum HDL-c of rats maintained on 10% T.A shows that the oil might be good for consumption. HDL-c (good cholesterol) is an essential transporter of cholesterol from cells and arteries to the liver for catabolism (Lacko *et al*., 2000). Cardiovascular disease (CVD) patients have markedly elevated levels of triglycerides and reduced HDL-c levels (Patsch, 1993). Furthermore, the equivalence in triglyceride and low density lipoprotein cholesterol levels in the same group of rats when compared with the control further suggested that the oil *Treculia africana* could offer protection against cardiovascular diseases.

The peroxide value of the oil (Table 1), showed that the oils might be susceptible to oxidative damage and might have fewer unsaturated bonds, hence we evaluated the radical generation, radical scavenging and probable radical induced damaged potential that may be attributed to *Treculia africana* seeds oil in both the brain and liver. The antioxidant system plays an effective role in protecting the various biological tissues below a critical threshold of reactive oxygen species (ROS), thus preventing organ dysfunction (Oschsendorf, 1999). The result of this study shows that *Treculia africana* seed oil based formulated feeds showed slight increase in the liver and brain compared with control (Group 1). Similarly CAT and SOD levels increased in TA treated animals compared to control, the increase was highest in 10% TA rats compared to groups 3 and 4 rats which had significantly decreased values especially in the brain. However, activities of superoxide dismutase (SOD), an enzyme that generate H_2O_2 in the brain of animals with reduced GSH were comparable with those maintained on vegetable only. 10% *Treculia africana* seed oil-based fed groups had increased SOD activities in the liver. This reflects that *Treculia africana* is not toxic to liver and brain and generation of H_20_2, in the brains through other mechanisms apart from SOD (that is, they are pro-oxidant) since catalase activities are significantly pronounced in both liver and brain of the same set of animals when compared with the control while the activities in both organs of the animals maintained on VO and *Treculia africana* are comparable with control.

Aminotransferases (ALT and AST) occupy a central position in the metabolism of amino acids as they help in retaining amino groups (to form new ones) during degradation of amino acids. They are also involved in the biochemical regulation of amino acid pool and in providing necessary intermediate to predict possible toxicity in some organs such as liver cytolysis and the heart of animals (Rahman *et al*., 2001; Shahjahan *et al*., 2004). The measurement of the activities of these enzymes is of clinical and toxicological significance. Since the enzymes will only be released into cellular flow when liver is damaged, the reduction in the levels of these enzymes in the serum of the animals maintained on formulated diets (Fig 3). Thus, the effect of the compounded feeds is insignificantly toxicologically on the liver.

Histological, the results of this study showed no lesions in both the brain and liver of all the experimental animals but one with mild vascular lesion. However, result from the brain in the T.A (10%) has mild diffuse vascular degeneration. The seed oil when fed to rats was found not to be toxic to the liver and the brain of the rats and none of the rats died throughout the period of experiment. The mild lesion might be due to other confounding factors. It could be concluded that the oil *Treculia africana* has no deleterious effects on rats but could be administered at 5% inclusion level in order to avoid possible oxidative damage to liver and brain damage.

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