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Subchronic toxicity and behavioural effects of Glycine max (L.) oil emulsion in male rats

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Abstract

The oil of Glycine max commonly known as soybean oil has over the decades grown popularity for its low cholesterol hence its use within the household and commercially for food production has grossly increased. This study was aimed to determine the effects of long term consumption of soybean oil toxicologically and behaviourally.

Male albino rats were administered with the vehicle, 5 and 10% oil emulsion for 30 days orally. The rats were subjected to behavioural tests such as novelty-induced behaviour (NIB), learning and memory tests and food intake measurement weekly. At the end of 30 days, rats were anesthetized and carefully dissected and blood sample was taken and analyzed haematogically and biochemically. The liver sample was also taken for biochemical analysis. Histopathological examinations were carried out on the brain, spleen, liver, kidney, lungs and testis samples.

The results showed that oral administration of oil caused an increase in food intake, significant effect on NIB but had no effect on learning and memory. There was a significant ($p<0.05$) reduction in the level of both haemoglobin and PCV in the grouped administered with high dose. Biochemical analysis revealed a significant ($p<0.05$) increase in triglyceride, ALT, AST levels, with no effect on cholesterol. Histopathological analysis revealed no significant effects on the essential organs tested when compared with the vehicle treated rats.

This study conclusively showed that soybean oil has central excitatory effects and there is need for caution when used for a long period since it has significant effects on biochemical parameters.

Keywords: Glycine max, soyabean oil, locomotion, rearing, toxicity, rats.

Introduction

Glycine max (L.) Merr., Leguminosae (Fabaceae) is a legume, native to China, that has become a major source of vegetable protein and oil for human and animal consumption and for industrial usage. The valued portion of the plant is the seed, which contains about 40% protein and 21% oil. So important is the soyabean to the Chinese that it is considered one of the five sacred grains ("Wu Ku") along with rice, wheat, barley and millet. The soya bean is also called soy bean, soja, soi, soy pea and Manchurian bean. Synonyms of soyabean include; Soja japonica Savi in Pisa Nuov., Glycine hispida (Moench) Maxim., Soja max (L.) Piper Glycine gracilis Skvorc., Landw.

Glycine max is cultivated and grown traditionally in East, South-East and South Asia. Nowadays, it is also grown in many countries of the sub-tropical to warm-temperate zones in most continents (North and South America, Asia, South-East Europe, Africa-mainly in the East). The composition of soyabean: Protein (40%); oil (20%); Cellulose and Hemicellulose (17%); Sugars (7%); Crude fiber (5%) and ash (dry weight basis) (6%) [7].

The old Chinese herbals suggest that the soyabean was a specific remedy for the proper functioning of the bowels, heart, kidney, liver, and stomach. Commercial grades of natural lecithin, often derived from soybean, are reported to contain a potent vasodepressor. Medicinally, lecithin is indicated as a lipotropic agent. Soybean is listed as a major starting material for stigmasterol, once known as an antistiffness factor. Sitosterol, also a soy byproduct, has been used to replace diosgenin in some antihypertensive drugs [5].

Soyabean is a source of complete protein. Soy products are also a good source of iron and contain vitamins B_1 and B_2 and an essential oil-linoleic acid, one of the Omega-3 fatty acids. Soyabeans also contains isoflavones which have been suggested to help prevent cancers and heart diseases. Because soyabean is inexpensive and nutrition-packed, it is used to produce a wide variety of products including kecap, miso, natto, okara, tamari, tempeh, tofu and yuba which are mostly eaten in South Asia. Others include meat analogs, soybean oil, soy cheese, soy flour, soy ice cream, soy margarine, soy mayonnaise, soymilk, soy nuts, soy sauce, soy sour cream, soy yogurt, chocolates, biscuits etc.

Previous reports showed that adding soy protein to the diet can moderately decrease blood levels of total cholesterol and low-density lipoprotein ("bad" cholesterol) while high-density lipoprotein ("good" cholesterol) does not seem to be significantly altered. Some scientists have proposed that specific components of soybean, such as the isoflavones, genistein and daidzein, may be responsible for the cholesterol-lowering properties of soy. This may reduce the incidence of cardiovascular diseases e.g. myocardial infarction. Omega-3 fatty acids, such as linolenic acid, are special fat components that benefit many body functions e.g., they inhibit blood clotting. Soybean oil is one of the only common vegetable oils that contain a significant amount of omega-3s. Soyabean oil is the fixed oil derived from the seeds of Glycine max (L.) Merr. Leguminosae after it has been refined, deodorized and clarified by filtration at about 0^0C [8]. Soyabean oil is the dominant vegetable oil used domestically in edible oil products. Almost all margarines and shortenings contain soyabean oil. It is also found in mayonnaise, salad creams, frozen foods, chocolates, biscuits, etc. The reported chemical constituents of soybean oil are as follows: polyunsaturated fatty acids which include 44-62% linoleic acid, 19-30% oleic acid, and 4-11% linolenic acid. 7-14% palmitic acid and 1.4-5.5% stearic acid constitutes the saturated fatty acid content of soybean oil [7]. However, other studies showed that there are some problems with soyabean oil. Nunes et al. [14] found that wistar rats treated with soyabean oil presented insulin resistance and defective islet insulin secretion when compared with untreated wistar rats. Ima-Nirwana et al. [10] found that repeated heating of soyoil destroyed the tocopherols causing raised serum interleukin 6 and osteocalcin levels leading to increased bone resorption and osteoporosis in the long term. Rueda-Clausen et al. [16] reported that soybean, olive and palm oil oils, fresh and deep fried produced an increase in triglyceride plasma levels in healthy subjects. A study carried out by Lin et al. [12] among Taiwan women showed that there is a possible relationship between the high levels of ethylene oxide emitted during frying of food using soyabean oil and the high incidence of lung cancer in Taiwanese women engaged in traditional cooking. In a previous research, it was observed that soyabean oil emulsion decreased lymphocyte proliferation and provoked neutrophil and lymphocyte apoptosis and necrosis, therefore enhanced the susceptibility of

the patients to infections [3]. Zhao et al. [18] found out that a soy diet (20% soybean protein and 5% soybean oil) significantly exacerbates the clinical course of systemic lupus erythematosus (autoimmune disease) in patients. Therefore, the aims and objectives of this study are to investigate the central nervous system effects, haematological, biochemical and histopathological potentials toxicity of sub-chronic oral administration of soyabean oil emulsion in rats.

Materials and Methods

Reagents

Absolute ethanol (BDH), Formal saline solution 10%, Anaesthetic ether (Pharmadrug), Sodium hydroxide pellets (BDH), 0.25 M sucrose, normal saline and lactate dehydrogenase, cholesterol triglyceride, aspartate transaminase (AST), alanine transaminase (ALT), assay kits by RANDOX® (RANDOX laboratories Ltd., Autrium UK) and reagents for protein assay.

Animals

Seventeen albino rats weighing between 101.5 ± 6.2 g were procured from the Animal House of National Agency for Food and Drug Administration and Control (NAFDAC), Yaba, Lagos, Nigeria. They were transferred to the animal house of the Department of Pharmacology, Faculty of Pharmacy, OAU, Ile-Ife and allowed to acclimatize to the new environment for a week. All the animals were kept under standardized environmental conditions and had free access to food and water ad libitum. All behavioral tests were carried out between 9:00 and 16:00 h. All experiments were carried out in accordance with NIH guideline for the care and use of laboratory animals.

Preparation and administration of soyabean oil

The emulsifiers, Span 80 and Tween 80 at a total blend of 1%^{w}/v were used for soyabean oil emulsion. The 50 ml emulsion was prepared using the bottle method. The required amount of span 80 was dissolved in the soyabean oil making up the oil phase. Also the required amount of Tween 80 was dissolved in distilled water making up the aqueous phase. The oil phase was added in portions to the aqueous phase in a 200 ml screw cap bottle and shaken vigorously after each addition. The emulsion formed was then passed three times through a laboratory hand homogenizer. The 5% and 10%^{v}/v soyabean oil are equivalent to 250 and 500 mg/kg dose level respectively.

Animals were divided into three groups (Vehicle, 250 and 500 mg/kg) and were administered soyabean oil or vehicle for 30 days. The behavioural study was carried out at the end of each week. The drug was administered as a 5% soy oil emulsion (250 mg/kg), low dose and 10% soy oil emulsion (500 mg/kg), high dose. Each animal received the drug via the oral route with the aid of a stainless steel ball-tipped gavage needle attached to a 1 ml syringe. The control animals received a placebo containing only 1% Tween solution 80 at 5 µl/kg. The first day of administration was considered Day 1 of the study. The animals were administered drugs or vehicle at 8:00 h every day for 30 days. After administration, the animals were observed immediately for any signs of toxicity and an hour later behaviourally. Animals were monitored during the study for mortality, clinical signs and body weight. At the end of every week behavioural (Novelty-induced behaviour test and spatial working memory test (Y-maze model)] studies were carried out in rats. At the end of the 30 days, the animals were anesthetized and carefully dissected. The blood used in this study was obtained using cardiac puncture technique. The blood was transferred into heparinized bottles, gently rolled to enable the blood mix thoroughly with heparin to prevent coagulation. Biochemical analysis was carried out on the blood alongside with samples of the liver obtained from each animal. Samples of the brain, spleen, liver, kidney, lung and testis were also obtained and preserved in 10% neutral buffered formalin for histopathological examination.

Behavioural Studies

Novelty-induced behaviour (Open field test)

The structure used consisted of a square shaped area composed of a white painted box (60 x 60 x 64 cm) with a sheet of glass making up the front panel. The floor was divided by permanent red markers into nine squares of (20 x 20 cm). An hour after administration of the test sample, the rats were introduced into the box and total locomotion (number of floor units entered; crossed with all the paws), the frequency of grooming (the number of body cleaning with paws, picking of the body and pubis with mouth and face washing actions) and the frequency of rearing (number of times the animal stood on its hind limbs or with the forearm against the wall of the observation box or in free air. These behavioural states: locomotion, grooming and rearing were observed and scored weekly for 10 min. Before each session, the rats were allowed to acclimatize to the testing environment (a quiet and well ventilated room) for 30 min before the actual test began. Before introducing each animal, the wooden box was cleaned with 5% ethanol to eliminate possible bias due to the odour left by the previous animal.

Learning and memory test (Y-MAZE)

Spontaneous alternation performance was assessed using a wooden Y-maze composed of 3 equally spaced arms (50 x 10 x 40 cm). The total height of the apparatus was 90 cm from the ground. This test was done to assess memory using spontaneous alternation performance and locomotor activity. One hour after the administration of vehicle (control) and the test samples (250 and 500 mg/kg soyabean oil), each rat was placed in one of the arm compartments and was allowed to move freely for 6 min. An arm entry is defined as the body of a rat except for its tail completely entering into an arm compartment. The sequence of arm entries is manually recorded. An alternation is defined as an entry into all three arms on consecutive choices. For instance, each alternation is scored following the sequence of arm entries (each arm is labelled A, B, or C): ACBCABCACABCA. In this example, the rat entered 14 arms, eight of which are alternations. The number of maximum spontaneous alternations is then the total number of arms entered minus 2, and the percent alternation is calculated as (actual alternations / maximum alternations) x 100. The apparatus is cleaned with 5% ethyl alcohol and allowed to dry between sessions.

Toxicology Studies

Blood and organs collection

After 30 days, the animals were each anaesthetized in the anaesthetizing chamber already saturated with anaesthetizing ether. When the animals were confirmed completely anaesthetized, they were carefully dissected. Blood was obtained using cardiac puncture technique, transferred into pre-labelled heparinised sample bottles, and gently rolled to allow the blood to mix thoroughly with the anticoagulant. Liver sample was obtained from each rat and transferred into another sample bottle that was kept in a freezer until needed for biochemical assay. The brain, kidney, lung, liver, spleen and testis samples were also removed and placed into sample bottles containing 10% formal saline solution. All the samples collected were accurately placed in the pre-labelled sample bottles designated for the individual animal.

Haematological analysis

Packed cell volume determination

With the aid of capillary tubes, blood samples of each animal were collected separately from their respective heparinised bottles. The tubes were allowed to fill via capillary action. The tubes were then placed in the micro haematocrit centrifuge and spinned for 15 minutes after which the volume of the cellular components were read using the micro haematocrit reader calibrated in the % of total height of the blood in the tube.

Haemoglobin determination

This was done using the Sahli method. The dilution tube was filled with freshly prepared 0.1N hydrochloric acid to the mark. 10.20 mm^3 of the heparinised blood was sucked into the pipette

and released into the acid in the dilution tube. A deep brown solution was formed. Distilled water was added drop-wisely with stirring in between each drop. The colour of the solution in the tube was then compared with that in a standard contained in a sealed tube. The volume (A) of the solution in the sample tube was taken when the colour intensity was a bit darker than that of the standard. Distilled water was then added until the colour of the fluid was slightly lighter than that in the standard solution. The volume (B) of the solution at this point was taken. The average of A and B was taken and designated Y. The amount of haemoglobin was calculated as shown:

$$\text{Amount of haemoglobin} = \frac{X \times Y}{100} \text{ g/dl}$$

where X is 100% haemoglobin

Biochemical Analysis

Before commencement of biochemical analysis, the plasma was separated from the whole blood and the liver homogenized. The preparation of samples for analysis was as follows:

Collection of plasma

The heparinised blood samples were centrifuged at 3000 G for 10 minutes. The supernatant (containing the plasma) was aspirated using a 1000 µl micropippete and transferred into a labelled bottle with a well fitted stopper and kept in the freezer until needed.

Preparation of the liver homogenate

A liver sample was taken out of the freezer, blotted out of any blood in it and weighed using the electronic balance. It was homogenized using an electronic homogenizer (Stir-R) at 1600 rpm. A 10% homogenate was prepared in 0.25 M sucrose. The homogenates were kept in the freezer until needed. The samples (plasma and liver homogenates) were used for biochemical assay for the determination of aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH) using standard methods and kits prepared by RANDOX® (RANDOX laboratories Ltd., Autrium UK) and protein concentration was estimated using Lowry's method [13]. Cholesterol and triglyceride levels were also determined in plasma using the standard methods and kits prepared by RANDOX®. The amount of glutathione (GSH) in the liver homogenate was determined in accordance with the modified Ellman method [6].

Histopathological studies

Tissue biopsies for the brain, kidney, liver, lung and testis of each animal were processed with automated tissue processor (Shandon Citadel 2000). Sections were cut at 4 microns with the rotary microtome and stained with haematoxylin and eosin. Additional thin sections of the kidney cut at 3 microns were stained with periodic acid (Schiff). Histological sections were examined using Leica light microscope.

Statistical analysis

Mean and standard deviations were calculated for all quantitative data. The treated and control groups were compared using analysis of variance (ANOVA) and post hoc tests (Dun) were carried out to determine the source of significant main effect or interaction. Results are expressed as mean ± SEM, $p<0.05$ is taken as accepted level of significant difference.

Results

Behavioural test

In the first week, there was a dose-dependent increase in locomotor activity that was significant [$f(2,16) = 13.01$; $p = 0.0001$] at high dose 10%^{V}/v when compared with the control and the low dose treated rats. Similar trend was also observed during the second week with a significant [$f(2,16) = 6.38$; $P = 0.011$] increase at the high dose when compared with the control. However, by the end of the third and fourth weeks, there was an insignificant increase in locomotor activity (Fig.1). For the rearing activity, a level of significant increase was noted in the treatment

groups as compared with the control on week 1 [f (2,16) = 9.23; p = 0.003] while a non-significant increase was observed during the remaining three weeks time interval (Fig. 2). There was no significant difference observed in grooming activity (Fig. 3). In the spontaneous alternation performance test using Y-maze, a significant [f (2,15) = 8.02; p = 0.005] increase in locomotion was also observed in the treatment groups when compared with vehicle-treated group (control) (Fig.4). However, there was no significant effect on working memory due to soyabean oil oral administration (Fig. 5).

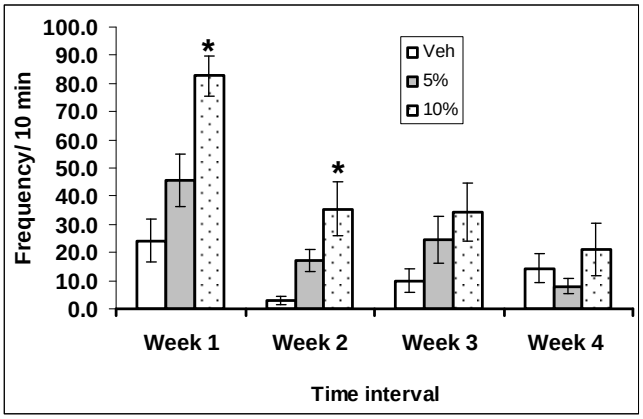

Fig.1: The effects of oral administration of soyabean oil emulsion (5 and 10%^{V}/v) on locomotor activity in male rats. Values are presented as mean ± S.E.M (n=5-6); *p<0.05 compared to the untreated (control) group.

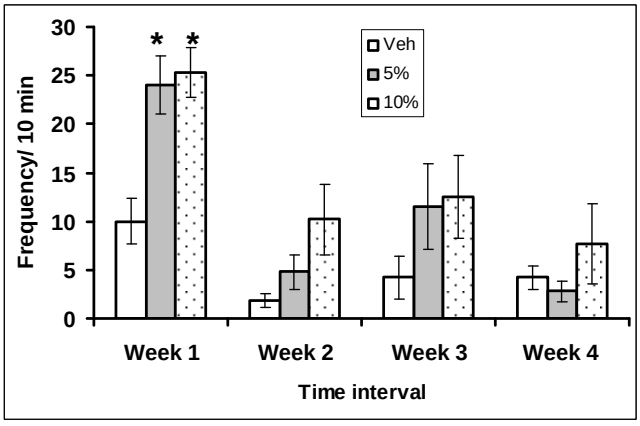

Fig. 2: The effects of oral administration of soyabean oil emulsions (5 and 10%^{V}/v) on rearing activity in male rats. Values are presented as mean ± S.E.M (n=5-6); *p<0.05 compared to the untreated (control) group.

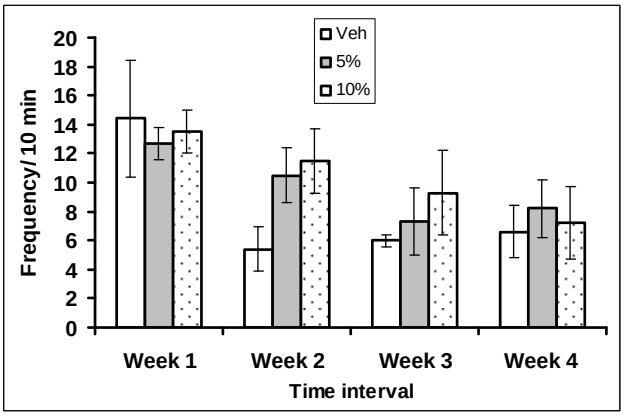

Fig. 3: The effects of oral administration of soyabean oil emulsion (5 and 10%^{V}/v) on grooming activity in male rats. Values are presented as mean ± S.E.M (n=5-6).

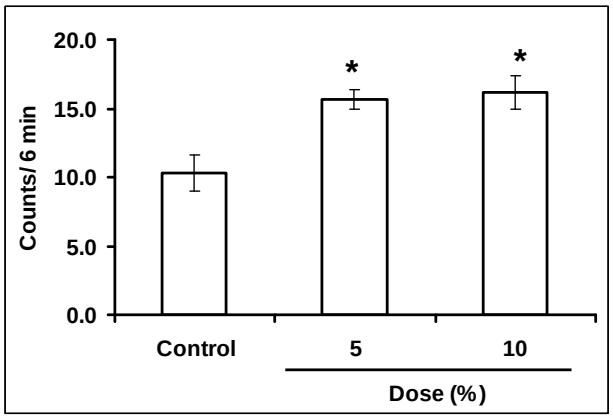

Fig. 4: The effects of oral administration of soyabean oil emulsion (5 and 10%^{V}/v) on locomotor activity in male rats (n=5-6 per group) (Y-Maze model). Each bar represents mean ± SEM.*p<0.05 compared to vehicle-treated rats.

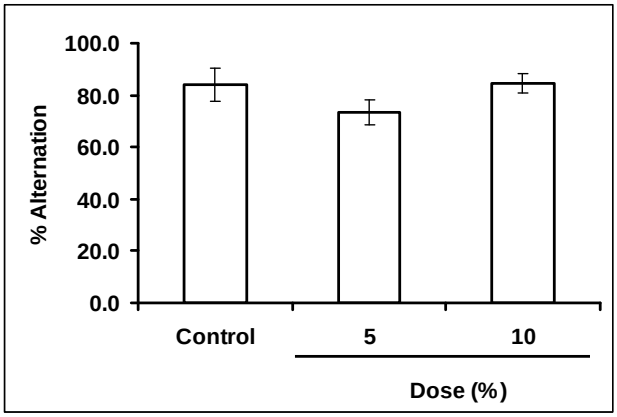

Fig. 5: The effects of oral administration of soyabean oil emulsion (5 and 10%^{V}/v) on memory using spontaneous alternation performance in male rats. Values are presented as mean ± S.E.M (n=5-6).

Haematological analysis

After 30 days of treatment, there was a significant [f (2,16) = 3.861; p<0.05] decrease in the level of haemoglobin at the high dose (Fig. 6), however, there was a non-significant [f (2,16) = 2.793; p=0.095] decrease on packed cell volume level when compared with control (Fig. 7).

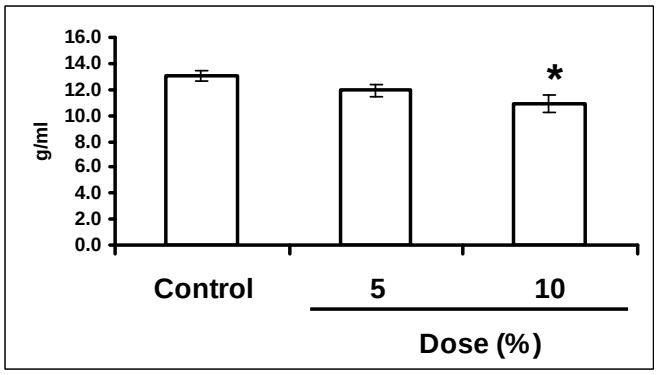

Fig. 6: The effects of oral administration of soyabean oil emulsion (5 and 10%^{V}/v) on Haemoglobin level. Values are presented as mean ± S.E.M (n=5-6). *p<0.05 compared to vehicle-treated rats.

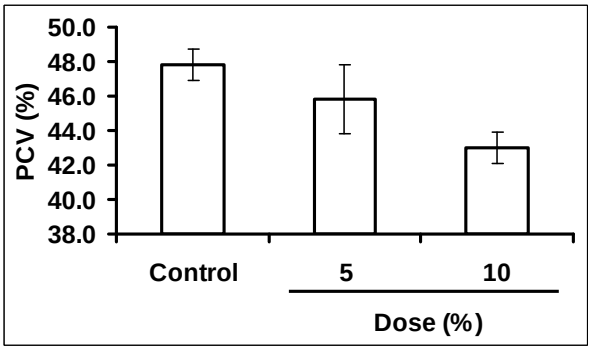

Fig. 7: The effects of oral administration of soyabean oil emulsion (5 and 10%^{V}/v) on PCV level. Values are presented as mean ± S.E.M (n=5-6).

Biochemical analysis

No significant change was observed in the cholesterol assay (Fig. 8). However, there was a significant [f (2,16) = 15.05; p = 0.0001] increase in triglyceride level in the high dose group when compared with the low dose and control groups (Fig. 9). The level of LDH in the plasma was not significantly affected (Fig.10a), however, in the liver, the level of LDH in the low dose group was significantly [f (2,16) = 20.55; p = 0.0001] higher than those of the high dose and the control groups (Fig.10b). In the alanine transaminase (ALT) analysis, the high dose treatment group showed a significant [f (2,16) = 19.86; P = 0.0001] increase when compared with the control and the low dose treatment groups in the plasma (Fig. 11a) and showed no significant effect in the liver (Fig. 11b). Although no significant difference was observed in the liver AST (Fig. 12b), however, in the plasma there was a significant [f(2,16) = 15.07; p = 0.000] increase in the level of AST with the high dose administration when compared with the control and the low dose treatment groups (Fig. 12a). For the plasma protein, an insignificant increase was observed between the control and treated groups (Fig. 13a). In the analysis of liver protein, there was a significant [f (2,16) = 10.63; p = 0.002] decrease in the level of protein in the low dose group (Fig. 13b). In the gluthathione analysis, a significant [f (2,16) = 8.23; P = 0.004] decrease was observed in the high treatment group when compared to the low dose and control treatment groups (Fig. 14).

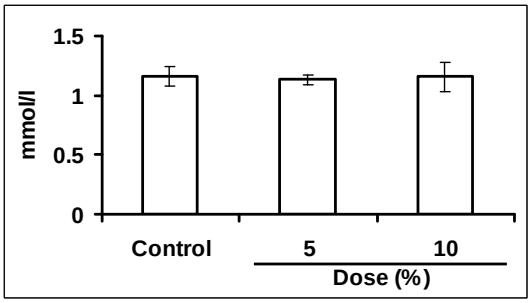

Fig. 8. The effects of oral administration of soyabean oil emulsion (5 and 10%^{V}/v) on cholesterol level. Values are presented as mean ± S.E.M (n=5-6).

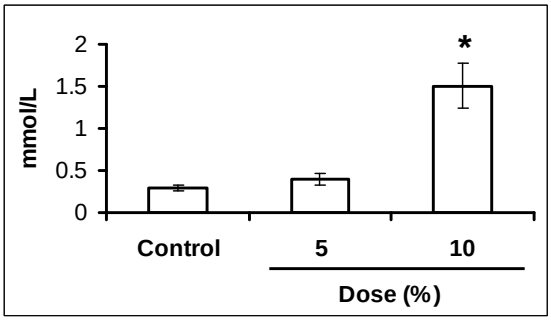

Fig. 9: The effects of oral administration of soyabean oil emulsion (5 and 10%^{V}/v) on triglyceride level. Values are presented as mean ± S.E.M (n=5-6); *p<0.05 compared to the untreated (control) group.

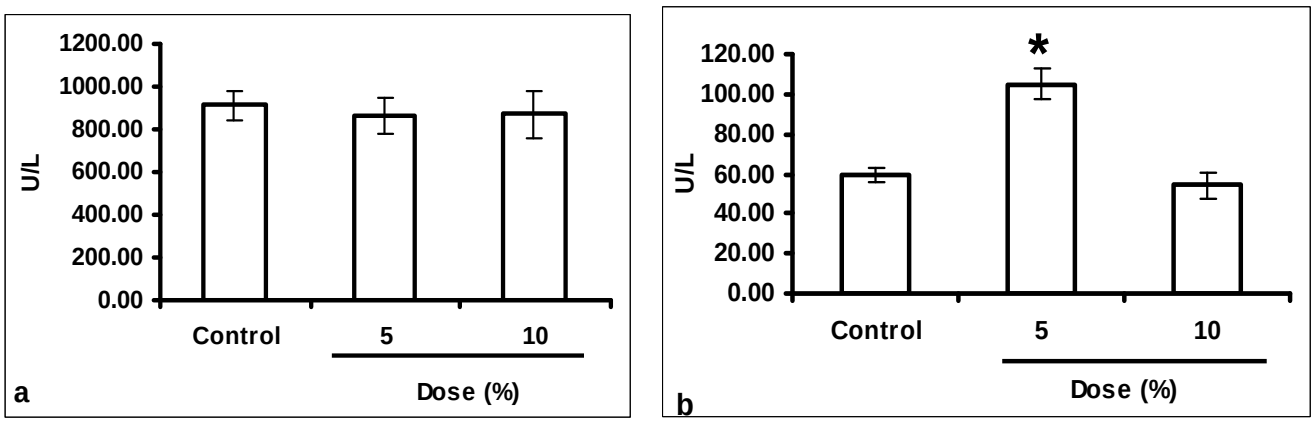

Fig.10 Bar chart showing the amount of Lactate dehydrogenase (LDH) in the plasma and liver of both soyabean oil emulsion (5 and 10%^{V}/v) orally administered rats and untreated rats (control). Values are presented as mean ± S.E.M (n=5-6); *p<0.05 compared to the control group.

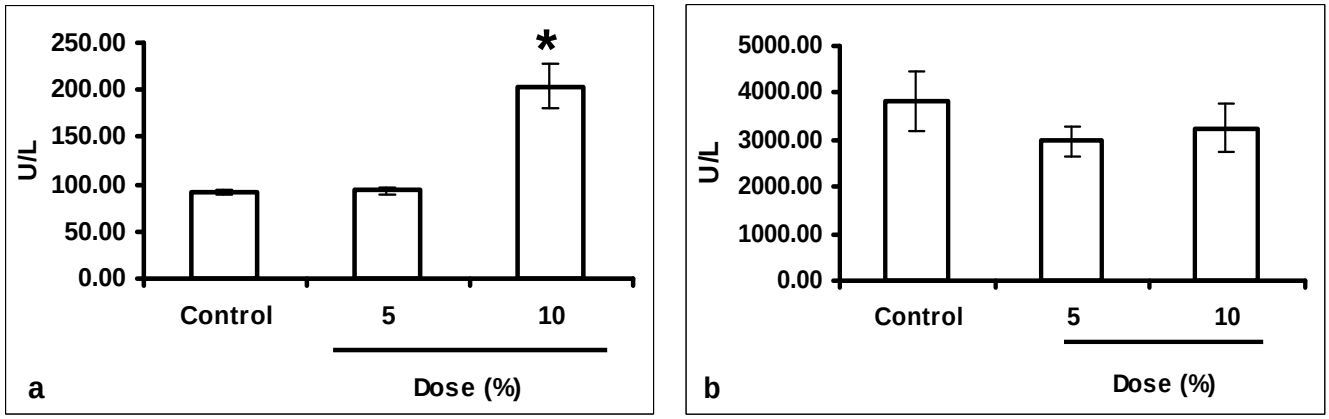

Fig. 11: The amount of alanine transaminase (ALT) in the plasma and liver of both soyabean oil emulsion (5 and 10%^{V}/v) orally administered rats and untreated rats (control). Values are presented as mean ± S.E.M (n=5-6); *p<0.05 compared to the control group.

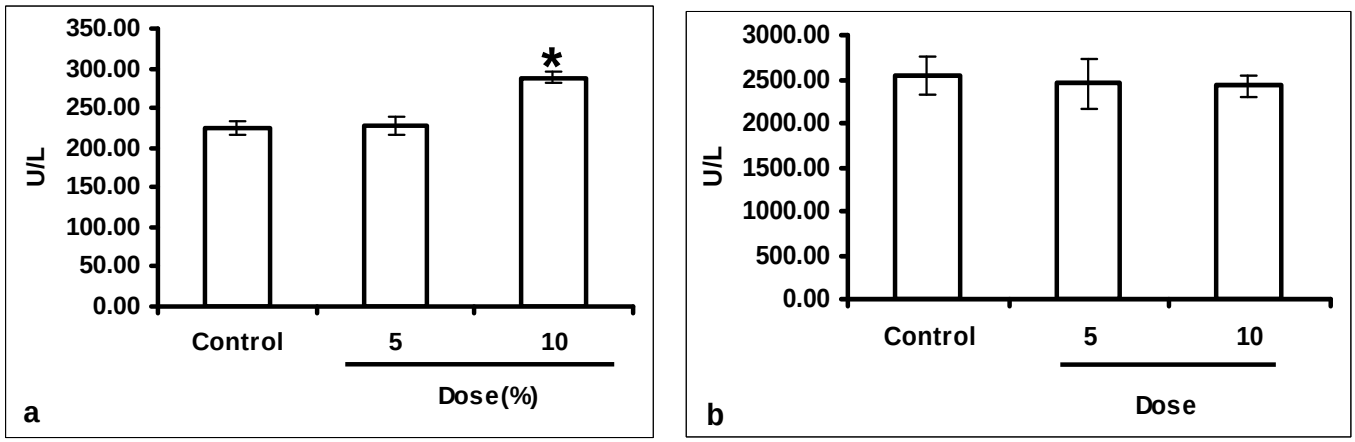

Fig. 12: The amount of aspartate transaminase (AST) in the plasma and liver of both soyabean oil emulsion (5 and 10%^{V}/v) orally administered rats and untreated rats (control). Values are presented as mean ± S.E.M (n=5-6); *p<0.05 compared to the control group.

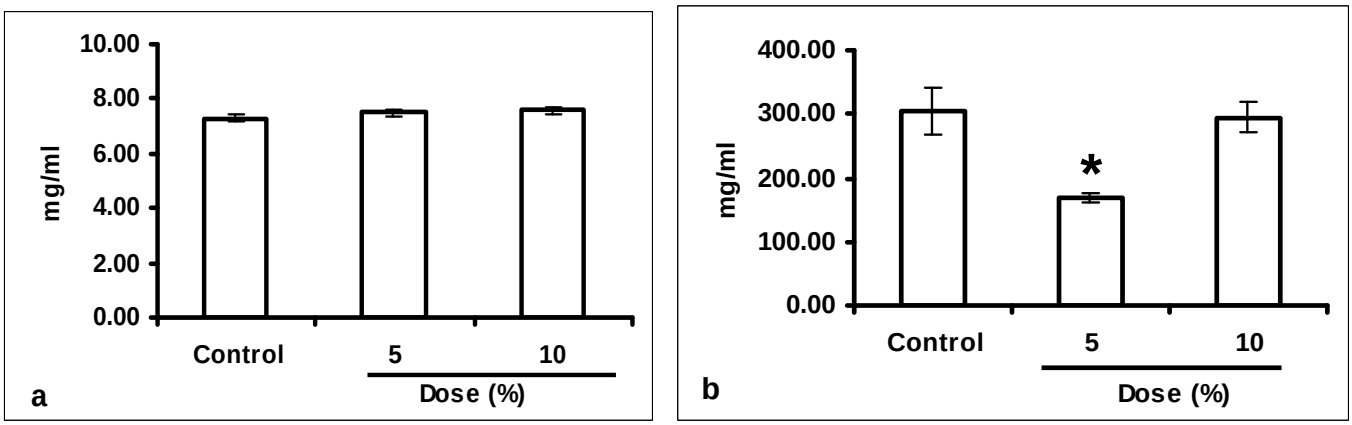

Fig. 13: The concentration of protein in the plasma and liver of both soyabean oil emulsion (5 and 10%^{V}/v) orally administered rats and untreated rats. Values are presented as mean ± S.E.M (n=5-6); *p<0.05 compared to the untreated (control) group.

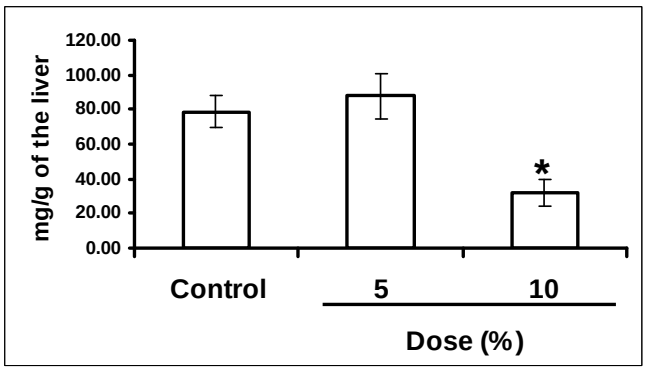

Fig. 14: Glutathione level in the liver of soyabean oil emulsion (5 and 10%^{V}/v) orally administered rats and untreated (control) rats. Values are presented as mean ± S.E.M (n=5-6); *p<0.05 compared to the untreated (control) group.

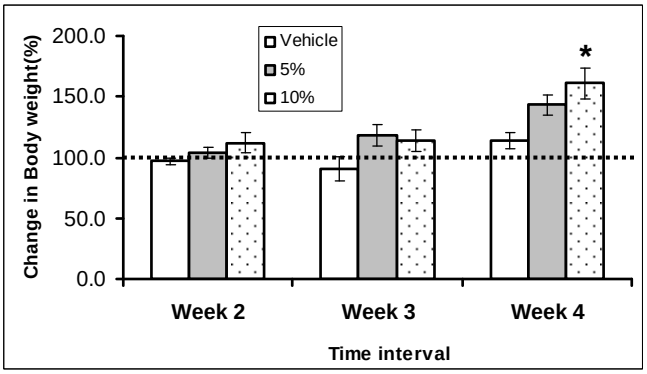

Fig. 15: Effects of soyabean oil emulsion (5 and 10%^{V}/v) on body weight in male albino rats. Values are presented as mean ± S.E.M (n=5-6); *p<0.05 compared to the untreated (control) group.

Food intake

Figure 16 shows the effect of change in body weight in the animals every week. In the second and third weeks, there was increase in the body weight of the low dose (5% soybean oil) and high dose (10% soybean oil) treatment groups however this was not statistically significant. In the fourth week there was a significant [f(2,16) = 5.28; P = 0.020] increase in the high dose treatment group when compared with the control. The low dose group however showed an insignificant increase in body weight.

Histopathological analysis

In the vehicle treated group, the spleen, liver, kidney, brain and testes were essentially normal while there was a moderate expansion of the interstitium with moderate infiltration by lymphocytes in the lungs. In the low dose group, the spleen, liver, kidney, brain and testes were essentially normal, however, a moderate expansion of the interstitium with moderate infiltration by lymphocytes was observed in the lungs. In the high dose treatment group, a mild expansion of the interstitium with moderate infiltration by lymphocytes was observed in the lungs, whereas, the spleen, liver, kidney, brain and testes were essentially normal (Fig. 16).

Discussion

Soyabean oil is used generally by the general populace and therefore decided to carry out the general central nervous system effect and potential toxicity studies acutely and sub-chronically. There was a significant increase in locomotor activity with both doses of soyabean oil administered in the first week and this effect was more pronounced in the high dose treatment group. This trend was the same throughout the four weeks period of the experiment, however the weekly analysis showed that there was a decrease in locomotion from the second to the fourth week, which may have resulted from habituation. The spontaneous motor activity of rodents exposed to a new environment is characterized by an initial period of hyperactivity i.e. the exploratory period, followed by hypoactivity i.e the habituation period [2]. Increase in novelty-induced rearing is considered a central excitatory vertical locomotor behaviour [1,2]. In the first week, there was a significant increase in rearing in both treatment groups that decreased drastically from the second to fourth week. However, the same trend of increase was observed across the groups i.e. the control and treatment groups. This decrease in rearing pattern across the time intervals may be as a result of habituation as earlier stated. Both locomotion and rearing behaviour is known to be mediated via D_2 receptor. Grooming has been primarily regarded as a behaviour involved in the care of the body surface of rodents. Grooming in rodents is associated with the stimulation of the dopamine receptors D_1 and D_5 in the brain [4]. In the 30-day experiment, there was no significant variation in the grooming pattern of the treated animals even though it was non-significantly increase. The increase in locomotor and rearing activities of the rats suggests that soybean oil is excitatory in nature.

Memory is a highly complex process that involves several brain structures as well as the rate of several neurotransmitters and neuropeptides [17]. In the Y-maze test, there was no significant effect on spontaneous alternation in both treatment groups when compared with control. This indicates that soyoil has no effect on memory. There was a significant increase in the Y-maze locomotion pattern. This correlates with the excitatory effect observed in the novelty induced behaviour further emphasizing that soyabean oil is excitatory in nature.

There was a decrease in the haematocrit level of the blood of the rats. The results showed that haemoglobin level was significantly decreased at the high dose administered. However, there was no significant difference on PCV level between the test groups and the control. Reimold [15] found that the haemoglobin and haematocrit levels decreased in soyabean oil treated beagles. A reduction in the haematocrit and haemoglobin levels indicates anaemia [11]. Therefore high levels of soyabean oil in the blood may aggravate anaemia or cause anaemia in individuals who may be prone to it.

Biochemical Analysis

The results obtained for cholesterol levels indicate no significant difference in the cholesterol level in both treatment groups. This confirms the claim that soyabean oil does not increase the level of cholesterol in the blood. A significant increase was observed for the triglyceride levels of the high dose group. Triglycerides belong to the class of the very low density lipoproteins. High level of triglycerides in the body is known as hypertriglyceridaemia. Hypertriglyceridaemia tends to occur in association with a reduced high density lipoprotein concentration [11]. There is a relatively weak independent link between raised concentration of very low density lipoprotein particles and risk of cardiovascular disease, however, hypertriglyceridaemia can cause a greatly increased risk of acute pancreatic and retinal vein thrombosis [11]. There was an insignificant variation in the level of plasma protein in contrast to the liver protein in which the low dose produced a significant decrease. The liver is the principal site of synthesis of all circulating proteins in the plasma apart from

gamma globulins which are produced in the reticuloendothelial system [11]. Reduced level of protein in the plasma can be associated with hepatotoxicity. Reduction in liver protein may be associated with the suppression in the synthesis of protein. However, this cannot be justified since the high dose group showed no significant decrease.

Alanine transaminase (ALT) and aspartane transaminase (AST) are enzymes present in hepatocytes and they leak into the blood with liver cell damage. AST is primarily a mitochondrial enzyme in the liver. It is also present in the heart muscles, kidney and brain hence high serum concentration may not be specifically indicative of liver damage. On the other end, ALT is a cytosol enzyme and it is principally found in the liver hence, it is more indicative of damage to hepatocytes. Kumar and Clark [11] had reported that liver disease can lead to an increase in ALT levels. From the results, there was a significant increase in the level of ALT and AST in the plasma of animals treated with high dose level of soyabean oil indicating possible damage to the hepatocytes and to an extent some other tissues. No significant variance was observed in ALT and AST of the liver. High concentration of soybean oil may, therefore, be indicated or may cause hepatic damage.

There was an insignificant variance in the level of plasma LDH in both low and high dose groups. However, in the liver, the low dose group showed a significant increase in the LDH value which was not reflected in the high dose treated group. This may indicate damage to hepatocytes in the low dose group but, it cannot be conclusively stated that the oil resulted in hepatic damage. There was a significant decrease in the glutathione level in the high dose treatment group. Glutathione (GSH) is the general term for glutathione sulphydrl. It is a peptide that occurs naturally within the body especially in the liver. It is the organ's most abundant antioxidant enzyme. It functions as a substrate for key detoxification process in the liver in phase II reactions. GSH has been shown to play a crucial role in cardiovascular diseases such as stroke, artherosclerosis and reperfusion injury. Elevated GSH levels help to combat the infirmities of ageing such as Alzheimer's disease, cancers of ageing (e.g. prostate cancer). The functions of glutathione can be summarised as an antioxidant, a detoxifier and an immune system enhancer in the body. GSH deficiency accompanies liver damage. Also, impaired GSH effectiveness increases the risk of developing multifactorial illnesses and other infections [9]. From the results, high concentrations of soy oil may deplete the body of its glutathione stores which can imply liver damage. Also depletion of the stores can invariably makes the body prone to infections and diseases.

Histopathology Analysis

In all the treated groups,the spleen, liver, kidney, brain and testes were essentially normal while there was a variation in the lungs. This indicates that no significant damage was done to these organs. The variation in the lungs can be said to be inflammation since there was expansion of the interstitium and infiltration of lymphocytes. This however, cannot be attributed to the oil since the untreated group showed a similar variation. On the contrary, the biochemical analysis indicated variation in the integrity of the liver. There was increase in the markers of tissue damage (ALT, AST and GSH), mainly in the liver which was not indicated in the histopathology examination i.e. the liver in all the treated groups were essentially normal. The duration of the experiment may not have been sufficient enough to show a significant effect on the tissue histopathologically.

There was gradual increase in the level of food intake of the rats. In the fourth week, there was a significant change in weight of animals in the high dose group when compared with the control. This suggests that increase level of soyabean oil in food may stimulate increase in food intake which leads to an increase in weight. Increase in weight was dose dependent.

Conclusion

Soyabean oil has excitatory effect centrally and its principal effect may be on the receptors that are involved in the central stimulatory effects - such as dopaminergic and cholinergic systems among others. There was a decrease in the level of haemoglobin and packed cell volume of the animals in the treated group. However, only the level of haemoglobin was significantly reduced. This indicates that prolonged use of soy oil may result in anaemia if blood producing food supplements are not taken. Long term consumption of soyabean oil did not increase the cholesterol levels which supports the claim of its beneficial effects. However, the results showed that there was hypertriglyceridaemia that can lead to detrimental effects on the body. Increased level of ALT and AST in the plasma and also decreased level of plasma protein in the treated groups are indicative of hepatocyte damage, however this was not supported by the histopathological analysis of the liver tissue. Also increased level of LDH (low dose group) in the liver is suggestive of possible liver cell damage. Depletion of GSH stores in the body can also be associated with long term consumption soy oil. No evidence of damage was seen in histopathology studies on the liver but biochemically, long term consumption of the oil may be detrimental to the liver. Long term consumption of soy oil may also lead to weight gain.

Finally, soyabean oil, while not increasing the body cholesterol levels may not be regarded as being totally devoid of toxic effects on body organs especially the liver. Its ability to reduce glutathione levels of the body may be regarded as a very high risk factor for the development of some diseases especially on prolonged consumption. Hence, a chronic study of the effects of consumption of soyabean oil should be carried out to confirm its long term effects on body organs especially the liver.

References

1. Ajayi AA and Ukponmwan OE. Evidence of angiotensin II and endogenous opioid modulation of NIR in the rat. Afr J Med Sci. 1994;23:287-90.
2. Akanmu MA, Adeosun SO and Ilesanmi OR. Neuropharmacological effects of oleamide in male and female mice. Behav Brain Res. 2007;182:88-94.
3. Cury-Boaventura MF, Gorjão R, de Lima TM, Piva TM, Peres CM, Soriano FG and Curi RToxicity of a soybean oil emulsion on human lymphocytes and neutrophils. J Parenter Enteral Nutr. 2006;30(2):115-23.
4. Drago F, Contarino A, Busal L.The expression of neuropeptide-induced excessive grooming behavior in dopamine D1 and D2 receptor-deficient mice. Eur J Pharmacol. 1999;365:125-31.
5. Duke JA. Handbook of Energy crops. Purdue University, Center for New Crops and Plant Products. Last updated 2 July 1998. URL:http://www.hort.purdue.edu/newcrop/ duke_energy/ dukeindex.html.
6. Ellman GL. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 1959;82:70-77.
7. Erickson DR, Pryde EH, Brekke OL, Mounts TL, Falb RA. Handbook of Soy oil Processing and Utilization, American Soybean Association and the American Oil Chemist's Society. St. Louis, Missouri and Chanpaign, Illinois 1980.
8. Evans WC. Pharmacognosy. 15th Edition. W.B. Saunders . 2002;pp 188:294
9. Gutman J. Glutathione (GSH) Your Body's Most Powerful Protector. 3rd edition. Kudo. CA communications. 2002;Pp 11-15:116
10. Ima-Nirwana S, Ahmad SN, Yee LJ, Loh HC, Yew SF, Norazlina M, Abdul MT., Kamsiah J. Reheating of soy oil is detrimental to bone metabolism in oestrogen deficient rats. Singapore Med J. 2007;48(3):200-6.

11. Kumar P and Clark M. Clinical Medicine. 5th edition. W.B. Saunders. 2002;pp 341:337:410, 1106.
12. Lin JS, Chuang KT, Huang MS and Wei KM. Emission of ethylene oxide during frying of foods in soybean oil**.** Food Chem Toxicol. 2007;45(4):pp. 568-74.
13. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. Protein measurement with the Folin phenol reagent. J. Biol. Chem 1951;193:265-275.
14. Nunes E, Peixoto F, Louro T, Sena CM, Santos MS, Matafome P, Moreira PI. Seiça R Soybean oil treatment impairs glucose-stimulated insulin secretion and changes fatty acid composition of normal and diabetic islets. Acta Diabetol. 2007;44(3):121-30.
15. Reimold EW. Studies of the toxicity of an intravenous fat emulsion. i. Hematologic changes and survival after administration of asoybean oil (FE-S15) in beagles. J Parenter Enteral Nutr. 1979;3(5):328-34.
16. Rueda-Clausen CF, Silva FA, Lindarte MA, Villa-Roel C, Gomez E, Gutierrez R, Cure-Cure C, López-Jaramillo P. Olive, soybean and palm oils intake have a similar acute detrimental effect over the endothelial function in healthy young subjects. Nutr Metab Cardiovasc Dis. 2007;17(1):50-7.
17. Steckler T, Drunkenburg WH, Sahgal H, Aggleton JP. Recognition memory in rats-I concepts and classification. Prog Neurobiol. 1998;54(3):289-311.
18. Zhao JH, Sun SJ, Horiguchi H, Arao Y, Kanamori N, Kikuchi A, Oguma E, Kayama F. A soy diet accelerates renal damage in autoimmune MRL/Mp-lpr/lpr mice. In Immunopharmacol 2005;5(11):1601-1610.