

Full Length Research Paper

Evaluation of antioxidant and hypolipidaemic effects of fermented *Parkia biglobosa* (Jacq) seeds in tyloxapol-induced hyperlipidaemic rats

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Globally, fermented foods form an intricate part of the staple diet of people. This study investigated the hypolipidaemic potential of fermented seeds of *Parkia biglobosa* (African locust bean/*iru*), a popular condiment by supplementing (20% w/w) in animal feed. Animals (n=5) in six treatment groups received; standard rat diet (control); *iru*-supplemented feed; standard rat feed with tyloxapol administered at the end of the experimental period (tyloxapol control); *iru* supplemented feed and triton at the end of the experimental period; standard feed with administration of fluvastatin sodium (40 and 80 mg/kg body weight) accordingly. Hyperlipidaemia was induced and ascertained by single intraperitoneal injection of 250 mg/kg triton WR 1339 (tyloxapol) constituted in normal saline. It was administered after six weeks experimental period to respective groups. The results revealed that addition of the fermented condiment into animals' feed mitigated increased lipid levels [total cholesterol (TC) and Low-density lipoprotein-cholesterol (LDL-C); triglyceride (TG)] triggered by injection of tyloxapol. On the other hand, *iru* caused a significant decrease in plasma and liver total cholesterol (TC), triglyceride (TG), LDL-C ($p < 0.05$) and increased high-density lipoprotein-cholesterol (HDL)-C levels ($p < 0.05$). The condiment showed a competitive hypotriglyceridaemic and greater hypocholesterolemic activity in the plasma when compared with fluvastatin at both concentrations. The condiment showed reasonable activities for the entire *in vitro* antioxidant assays done. Histopathologic examination revealed its hepatoprotective capability. Regular consumption of this condiment may represent a good dietary alternative for control of hyperlipidaemia and associated conditions.

Key words: *Parkia biglobosa*, hyperlipidaemia, total cholesterol, high density lipoproteins-cholesterol, low density lipoproteins-cholesterol, triglyceride, fluvastatin, tyloxapol.

INTRODUCTION

Fermentation is described as the process of anaerobic or partial anaerobic oxidation of carbohydrate material, during which process; enzymes elaborated by micro-organisms break down carbohydrates or carbohy-drate-

like materials (Odufa, 1985). The deliberate fermentation of foods by man predates written history and is possibly the oldest method of preserving perishable foods. Evidence suggests that fermented foods were

consumed as far back as 7,000 years ago in Babylon (Battcock and Aza-Ali, 1998).

Fermented foods are generally produced using plant or animal ingredients in combination with fungi or bacteria which are either sourced from the environment, or carefully kept in cultures maintained by humans. Fermented foods are noted for enhancement of diet through development of flavour, aroma and texture in food substrates. They also preserve foods and increase their shelf-life through production of various chemical substances such as lactic acid, alcohol, acetic acid and alkaline fermentation. Fermented foods enhance food quality with protein, essential amino acids, essential fatty acids and vitamins, improving digestibility and nutrient availability. Very often, detoxification of anti-nutrients in food occurs through food fermentation processes, and there can also be a decrease in cooking time and fuel requirement. Some inedible seeds and fruits are made edible through fermentation processes (Evans et al., 2013).

Globally, fermented foods whether from plant or animal origin form an intricate part of the staple diet of people and the raw ingredients are indigenous in that particular place. In Africa, the art of fermentation is widespread including the processing of fruits and other carbohydrate sources to yield alcoholic and non alcoholic beverages (Adewusi et al., 1991, 1992), they therefore play a very important role in the socioeconomics of developing countries as Evans et al. (2013) reported.

Fermented African locust beans (Dadawa or *Iru*) are made by a natural un-inoculated solid - substrate fermentation of the boiled and dehulled cotyledon of the seeds of *Parkia biglobosa* (African locust-bean). They are one of the most important food condiments in Nigeria and many countries of West and Central Africa (Evans et al., 2013). Dadawa or *Iru* are used in much the same way as bouillon cubes are used in the Western world as nutritious flavouring additives. They are rich in fat (39 to 40%) and protein (31 to 40%) and contributes significantly to the energy intake, protein and vitamins, especially riboflavin, in many countries of West and Central Africa (Achi, 2005; Daramola et al., 2009). The major fermenting organisms are the *Bacillus* and *Staphylococcus* species (Omafuvbe et al., 2004; Achi, 2005). Fermented seeds of *P. biglobosa* are used in all parts of Nigeria and, indeed, the west coast of Africa to season traditional soups (Ajaiyeoba, 2000; Agunu et al., 2005).

Introduction of foreign high technology products especially processed ones because of globalization and liberalization of the economy is radically changing the Nigerian food culture into a mixed grill of both foreign and local dishes (Ojo, 1991). Achi (1992), reported an

ambivalent attitude in terms of consumers' tastes and preferences for fermented foods in Nigeria. Arogba et al. (1995), reported that the traditional condiments in Nigeria have not attained commercial status, yet anecdotal evidences abound in areas where these indigenous condiments are heavily consumed that there are low incidences of dyslipidaemias. Antihypertensive (Assane et al., 1993; Ajaiyeoba, 2000), analgesic, anti-inflammatory (kouadia et al., 2000), antidiabetic, antidiarrhoeal (Agunu et al., 2005; Odetola et al., 2006) activities of different extracts from *P. biglobosa* seeds and trees have been reported. Ognatan et al. (2011), reported a low incidence of hypertension cases in a community where this condiment is heavily consumed in Togo, within the African continent.

The present study was therefore designed to rationally investigate the hypolipidaemic effects of regular intake of this condiment in tyloxapol-induced hyperlipidaemia in rats. The effects were compared with that from fluvastatin, a commonly prescribed antilipidaemic drug. This is with a view to establishing scientifically, the benefits (if any) of its intake as a means of controlling hyperlipidaemia and related conditions given the importance and widespread popularity of this indigenous condiment. Figure 1 shows clumps of *iru* seeds.

MATERIALS AND METHODS

Chemicals

Triton WR 1339 (tyloxapol) was obtained from Sigma Chemical Company, St. Louis Missouri, USA. Diagnostic kits for cholesterol, triglycerides and high-density lipoprotein (HDL) precipitants were purchased from Randox Laboratories (Antrim, United Kingdom). All other reagents were of analytical grade and of purest quality.

Fermented condiment

Traditional method of production of locust bean condiment (*iru*) as reported by Omafuvbe et al. (2004) was adopted.

Preparation of feed

Feeds supplemented with *iru* was prepared by thoroughly mixing weighed condiment and standard rodent feed in a ratio 1:4 respectively to give a twenty percent (20% w/w) *iru*-supplemented feed. The feed was pelletized by adding 4-5 drops of water, and passed through a roller to cake; and thereafter placed in the oven set at about 40°C for drying and caking.

Experimental animals

Thirty albino rats (both sexes), weighing between 75 and 110 g, were used. After 2 weeks acclimatization, rats were kept in

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Figure 1. Clumps of *iru* seeds.

standard animal cages in an animal house maintained at 26-29°C with a 12 h light–dark cycle. Animals were fed on standard laboratory chow purchased from Bendel Feeds and Flour Mills limited, Benin Road, Ewu, (Edo State, Nigeria). The feed contained 14.5% crude protein, 4.8% crude fat, 7.2% crude fiber, 8.0% crude ash, 0.6% phosphate, 0.3% available phosphorus, 0.6% lysine, 0.3% methionine, 0.5% methionine + Cystine, 8,000 I.U vitamin A, 2,400 I.U vitamin D₃, 15.0 mg vitamin E, 4.0 mg vitamin B₂, 50.0 mg Vitamin C, 30.0 mg Manganese, 30.0 mg Zinc, 0.2% sodium. Rats had access to feed and water *ad libitum*. Animals were distributed randomly into six different groups with five animals in each group. The experimental/treatment period was six weeks and animals were weighed daily.

Group A served as the control group and received standard feed (no treatment). Group B received *iru* supplemented feed; C was given standard feed with tyloxapol administered at the end of the experimental period (tyloxapol control), D received *iru* supplemented feed with tyloxapol administered; Groups E and F received standard feed with tyloxapol administered and fluvastatin sodium (40 and 80 mg/kg body weight respectively).

Administration of tyloxapol

At the end of the six weeks experimental period, animals were deprived of food for 24-h, after which hyperlipidaemia was induced by a single intraperitoneal injection of 250 mg/kg body weight of rats (5) to groups C, D, E and F.

Administration of fluvastatin sodium

Two doses of the drug, that is, 40 mg and 80mg/kg body weights of rats were prepared and administered 12 h after injection of tyloxapol to two different groups (E and F) orally with an intubator. The caplet was dissolved in distilled water; the needed concentrations were prepared based on weights of the rats.

Sample collection

Blood samples were withdrawn from 48-h fasted rats by cardiac puncture using needles and syringes into heparinised bottles.

These were centrifuged with a bench centrifuge at 3000 revolutions per minute for 15 min to separate the plasma from the blood cells. Plasma samples were used for the assays. Animals were sacrificed by cervical dislocation and their livers were removed.

Preparation of liver homogenate

Liver samples were washed with normal saline and blotted with soft tissue paper. 1 g each was homogenized in 10 ml of 0.25 M sucrose solution to give 10% (w/v) homogenates.

Lipid profile estimation

Plasma triglyceride and cholesterol levels were assayed using commercial kits (Randox Laboratories). The HDL was measured using the enzymatic colorimetric method. After centrifugation at 3000 g for 10 min at 25°C, the clear supernatant contained the HDL fraction, which was assayed for cholesterol using the kit. Low-density lipoprotein-cholesterol (LDL-C) was calculated according to the formula of McNamara et al. (1990).

In vitro antioxidant assays

The antioxidant activity of the condiment was determined by different *in vitro* methods such as, the DPPH free radical scavenging assay (Blois, 1958; Brace, 2001), nitric oxide radical inhibition activity (Marcocci et al., 1994), lipid peroxidation assay (Nabasree and Bratati, 2002), ascorbic acid equivalent (Benzie and Strain 1999) methods. All the assays were carried out in triplicates and average values were considered.

Histopathological investigation

Histopathological investigation was performed by fixing the representative liver tissues from each group in 10% formal saline by total immersion for 48 h after which they were processed via paraffin wax embedding method of Drury and Wallington (1980). The staining procedure of H and E as described by Drury and Wallington (1980) was also adopted. This was done at the Department of Anatomy and Cell Biology of the Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

Statistical analysis

Statistical evaluation of the data was done with Graph Pad Prism version 4.0 for Windows, Graph Pad Software, San Diego California USA (Ansarullah et al., 2009). The difference between groups was analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's Multiple Comparison Test with 5% level of significance ($P < 0.05$) considered significant (Gaudiot et al., 2000). Values are expressed as mean \pm SEM (Standard Error of Mean).

RESULTS

Effect of condiment supplementation on animal weekly weight changes

Average weights of rats' increased with the progression in experimental week based on the diet received.

Table 1 Effect of *iru* supplementation on animal weekly weight changes.

Time (weeks)	Control	<i>Iru</i> -supplemented feed	Tyl. control	<i>Iru</i> -supplemented feed +Tyl.	<i>Iru</i> -supplemented feed + Tyl. + FLU (40 mg)	<i>Iru</i> -supplemented feed+Tyl.+FLU (80mg)
1	93.25 ± 5.51	85.73 ± 3.39	117.90 ± 1.99	77.14 ± 3.02	110.80 ± 5.09	115.20 ± 3.31
2	104.40 ± 5.30	98.73 ± 2.33	125.90 ± 2.09	89.27 ± 3.81	110.90 ± 3.78	120.40 ± 4.09
3	108.20 ± 3.42	107.40 ± 2.91	127.70 ± 3.44	100.80 ± 2.06	111.30 ± 4.47	120.50 ± 4.81
4	110.20 ± 4.01	105.70 ± 5.01	129.50 ± 2.41	111.90 ± 2.27	111.00 ± 2.48	122.00 ± 3.41
5	105.30 ± 3.30	103.90 ± 3.64	123.30 ± 3.51	114.80 ± 5.41	110.90 ± 5.13	117.50 ± 5.50
6	107.20 ± 5.19	110.10 ± 2.11	124.00 ± 3.98	128.90 ± 3.99	111.10 ± 2.39	121.20 ± 2.05

Table 2. Antioxidant assay result of *iru*.

DPPH		Nitric oxide		Lipid peroxidation		Ascorbic acid equivalent	
Concentration (mg/ml)	Inhibition (%)	Concentration (mg/ml)	Inhibition (%)	Concentration (mg/ml)	Inhibition (%)	Concentration (mg/ml)	(µgVitCequiv/g)
25.00	64.65 ± 4.27	100.00	46.73 ± 2.67	100.00	24.93 ± 2.06	100.00	63.86
12.50	41.92 ± 1.11	50.00	40.43 ± 3.12	50.00	18.79 ± 3.79	50.00	62.84
6.25	25.15 ± 0.12	25.00	36.41 ± 0.10	25.00	10.10 ± 2.04	25.00	60.03
IC ₅₀	17.41		128.03		229.96	Mean ± SEM	62.24 ± 1.15

Values are Mean±SEM of triplicate tests.

Supplementation of rats feed with *iru* resulted in improved growth better than the control group in this study (Table 1). While weight change declined from the 4th week and gradually normalizes in control rats, sharp weight gain was seen in *iru*-fed rats. The increase became evident in test rats from the 3rd week.

Antioxidant assay results

Antioxidant assay results are presented in Table 2. The condiment showed nitric oxide and DPPH radical scavenging activities. It also inhibited lipid peroxidation *in vitro*. For each of the assays, the results were concentration dependent, in other words, the higher the concentration, the more the radical scavenging potential. Ascorbic acid equivalent test also showed that antioxidant activity from 1 µg ascorbic acid (standard antioxidant) is equivalent to that from 62.24±1.15 g of *iru*.

Effects of condiments supplementation on plasma and liver lipids of rats

Plasma and liver lipid profile

Total cholesterol estimation in plasma (Figure 2), shows that triton induced a statistically significant increase ($P < 0.05$) in the plasma total cholesterol, causing a 23.86% increase. Fluvastatin administered at 40 and 80 mg/kg

body weight induced reductions of 2.92 and 6.88%, respectively. In group D animals that were fed the *iru*-supplemented diet, the percentage reduction in total cholesterol was found to be 32.29. This implies that pretreatment of the rats with *iru* resulted in reduction of total cholesterol that was much better than what was obtained with Fluvastatin.

Triton again induced an increase of 88.19% in liver total cholesterol concentration (Figure 3) which was again ameliorated in the group pre-treated with the condiment. Fluvastatin at both concentrations of 40 and 80 mg/kg body weight caused significant reductions of 4.37 and 34.04% respectively. There was no significant difference ($P > 0.05$) between groups D and E (3.8 and 4.37% decrease, respectively), this suggests the percentage reduction elicited by 40 mg/kg body weight, fluvastatin can be likened to that of the condiment supplemented group in the liver.

In the case of plasma triglycerides (Figure 4), injection of triton elicited a percentage increase of 38.96% in triglyceride level (group C). This increase was mitigated in group D pre-treated with condiment supplemented feed (5.46% decrease). This implies pre-treatment of rats with condiment-containing diet prevents the rise in plasma triglycerides level elicited by administration of triton. Interestingly, in the group fed the condiment alone without the induction of hyperlipidaemia, there was a 23.77% reduction in triglycerides concentration. A similar result was obtained with liver triglycerides concentrations (Figure 5). Triton induced a 38.39% increase in liver

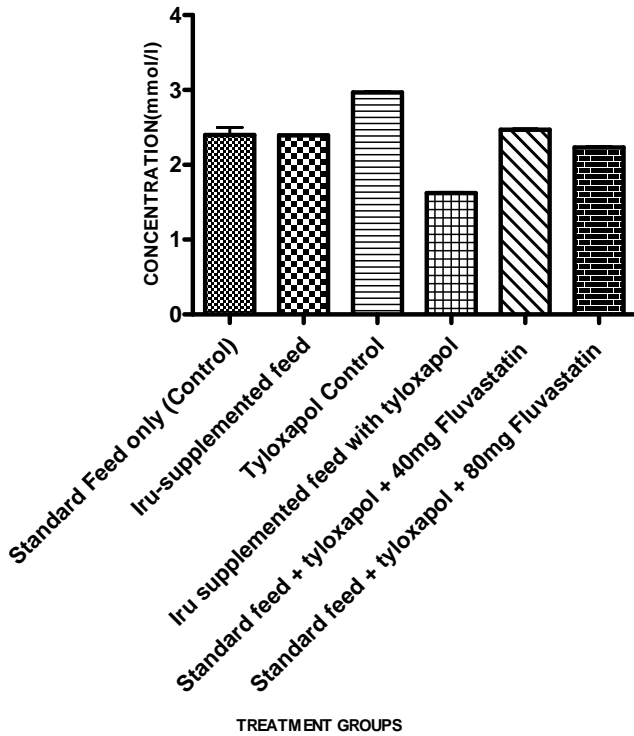


Figure 2. Effect of *iru* supplementation on plasma total cholesterol levels following triton-induced hyperlipidaemia. All values are expressed as mean \pm SEM. n=5.

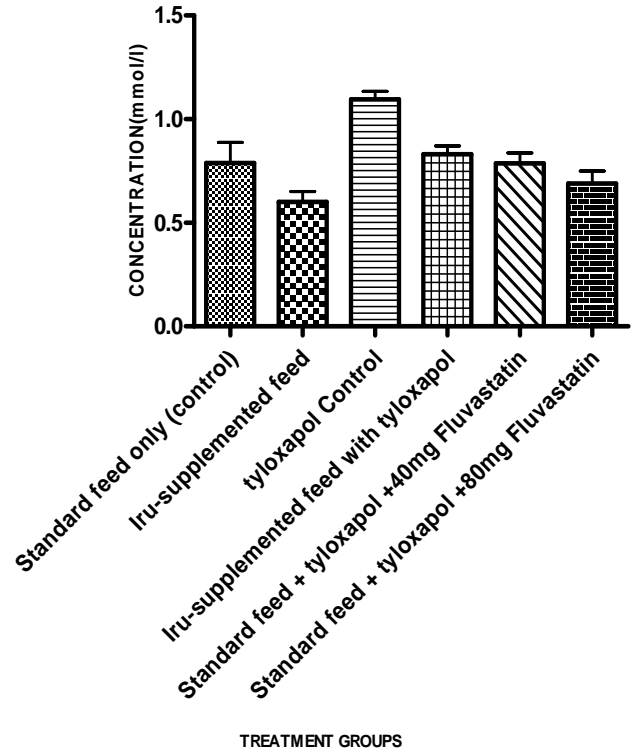


Figure 4. Effect of *iru* supplementation on plasma triglycerides (mmol) levels following triton-induced hyperlipidaemia in experimental and control rats. All values are expressed as mean \pm SEM.

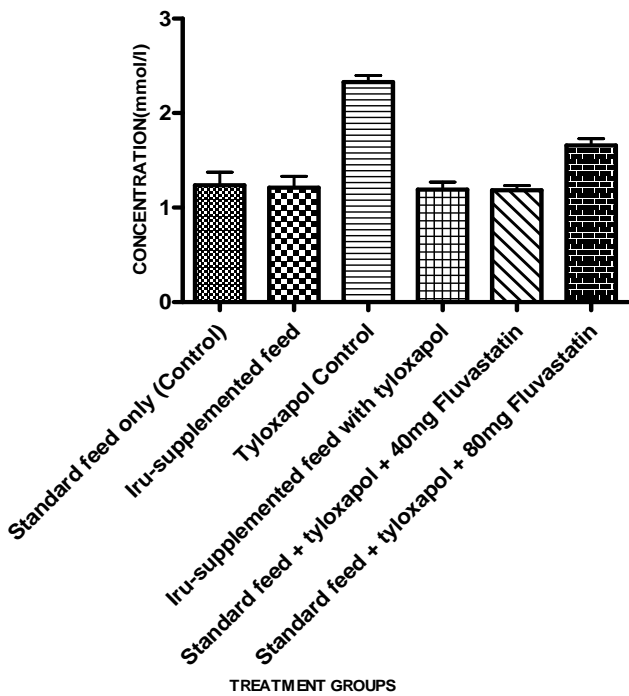


Figure 3. Effect of *iru* supplementation on liver total cholesterol concentrations in triton-induced hyperlipidaemia in experimental and control rats. All values are expressed as mean \pm SEM.

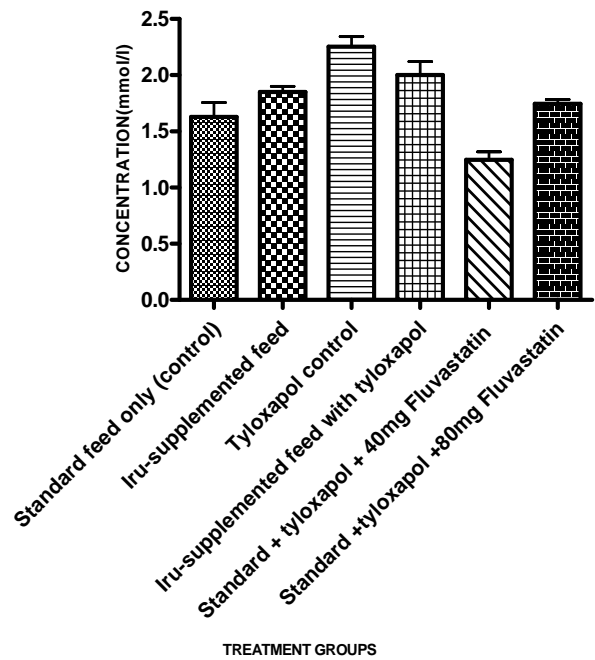


Figure 5. Effect of *iru* supplementation on liver triglyceride levels in triton-induced hyperlipidaemia in experimental and control rats. All values are expressed as mean \pm SEM. n=5.

triglycerides concentration. The condiment treated group and the fluvastatin 40 mg/kg treated groups were not significantly different from each other; there was a 22.91 and 23.40% decrease, respectively.

Plasma and liver lipoproteins

Figure 6 presents the results for the plasma LDL-Cholesterol; triton again induced a significant increase of 67.27%. Fluvastatin at 40 mg reduced plasma LDL-C by 25.28% while 80 mg/kg body weight fluvastatin reduced it by 16.43%. *Iru*-supplemented feed caused a percentage decrease of 66.32. This reduction is statistical significant ($P < 0.05$), and also higher than that from both concentrations of fluvastatin. Again in the absence of triton, the condiment elicited a reduction of 9.51%.

The plasma HDL-cholesterol results are shown in Figure 7. In the tritonised group of rats (Group C), there was a significant reduction (28.61%) in the HDL-C concentration. 40 and 80 mg/kg body weight fluvastatin induced increases of 3.75 and 8.61% respectively, while *iru* condiment supplemented feed induced an increase of 18.33%. This was much higher than the value at both concentrations of fluvastatin. Again in the absence of triton, pre-treatment with the condiment gave rise to an increase of 29.44% in HDL-cholesterol concentration. As shown in Figure 8, triton induced 20.18% decrease in liver HDL-C concentration. Fluvastatin at 40 and 80 mg/kg body weight gave rise to percentage increases of 7.45 and 75.30 respectively, while the condiment brought about an increment of 0.90%.

Histopathology results

Figure 9 shows the photomicrographs of representative liver sections from the experimental groups. The staining and magnification used is hematoxylin and eosin at 400x.

In group A rats, architecture of the liver appears normal. The hepatocytes (H) are arranged in plates around the central vein (CV) with sinusoids (Sn) in between the plates. In group B rats (treated with the condiments alone), the hepatocytes (H) are arranged in plates with poorly defined sinusoids (blue arrow) in between the plates. Architecture of the liver of group C rats (triton-treated) appears abnormal showing necrosed hepatocytes with poorly defined sinusoids in between the plates. There are also signs of fibrosis (F) and degeneration (D) in the liver section. The architecture of the liver of *iru*-fed rats appears normal. In the fluvastatin (40 mg) treated rat (group E) liver section, the hepatocytes in the periportal area of the liver section with sinusoids (Sn) in between the plates were seen, as well as the portal area (PA). The hepatocytes appear normal. In the fluvastatin (80 mg) treated rats (group F), the architecture of the liver appears normal.

DISCUSSION

High nutritive values of fermented foods have been well documented (Steinkraus, 1997; Gadaga et al., 2004).

The results of this study (Table 2) suggest that the condiment is a potential source of antioxidants of natural origin. The condiment possessed significant DPPH radical and nitric oxide scavenging potentials. This suggests that regular intake of this condiment would be of immense health benefit since oxidative processes have been proposed to have a causative and/or contributory role in an increasingly growing number of diseases, including certain types of cancers, neurodegenerative disorders, stroke, diabetes, etc.

Triton WR 1339, in agreement with literature (Otway and Robinson, 1967; Abe et al., 2007; Ngoc et al., 2008) induced significant increase in total cholesterol and triglycerides in the present study. The results of this study further showed that, the condiment caused a significant ($P < 0.05$) increase in the plasma level of HDL-C, which is usually termed the 'good cholesterol' (Agbedana, 1999). The resultant effects of increased HDL-C and decreased LDL-C (bad cholesterol), resulted in an increased HDL-C/LDL-C ratio in the test animals, this has been shown to be beneficial and is indicative of a lower risk of coronary heart diseases (Castelli, 1984).

In increasing the good cholesterol, the condiment performed much better than the standard medicine used (Figure 7). The effect on lowering plasma LDL-C was also significantly ($p < 0.05$) better than fluvastatin sodium (Figure 6), while a comparison of the hypotriglyceridemic effects of the standard drug and condiment also shows that the condiment compares favorably with the standard drug.

Hepatic examination reveals that the liver sections of *iru*-supplement fed animals were comparable with standard control rats. The effect of tyloxapol was highly pronounced on the hepatocytes of group C rats, as deadness and degeneration were observed. This is in agreement with literature (Otway and Robinson, 1967; Abe et al., 2007; Ngoc et al., 2008). This effect was however mitigated in group pretreated with the condiment and also those administered fluvastatin at both concentrations (Figure 9). This suggests that the condiment has hepatoprotective potential as well as ability to ameliorate liver cells fibrosis and necrosis due to chronic hyperlipidaemic agent in rats.

Conclusion

In conclusion, whole *iru*-supplemented feed showed hypolipidemic activity in tyloxapol-induced hyperlipidemia in rats. Regular intake of a good quantity would be useful in protecting the human body system against changes that would elicit high cholesterol, LDL-C, and triglycerides levels while significantly boosting HDL-C. Even in the absence of an hyperlipidaemic state, its consumption is

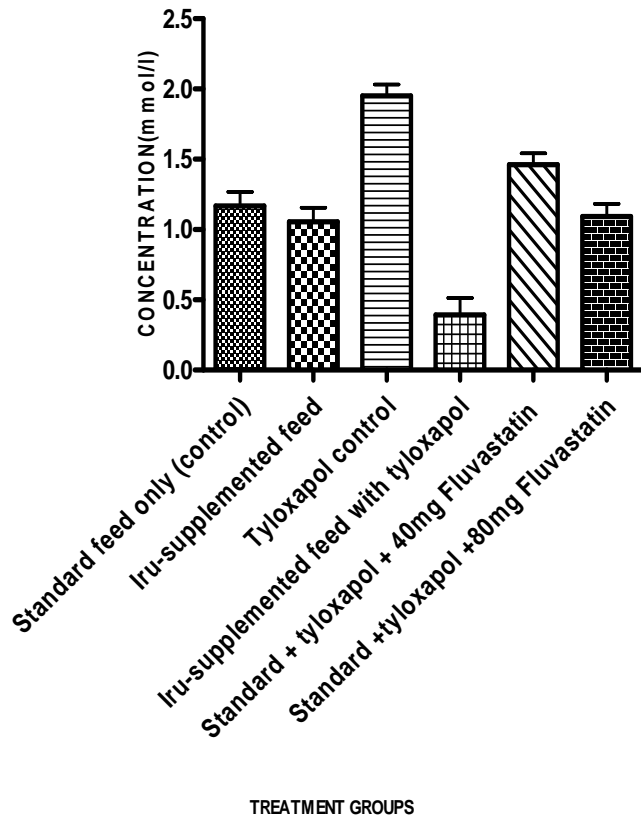


Figure 6. Effect of *iru* supplementation on plasma LDL-cholesterol concentrations in triton-induced hyperlipidaemia. All values are expressed as mean±SEM. n=5.

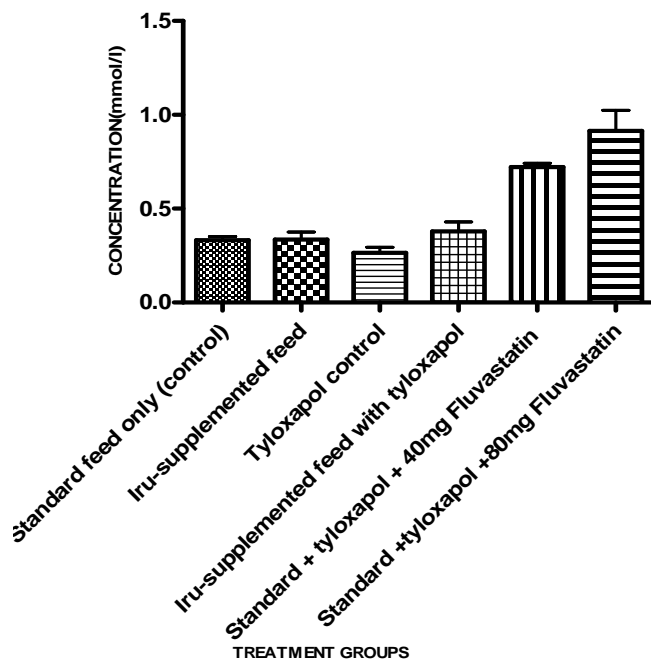


Figure 7. Effect of *iru* supplementation on plasma HDL-Cholesterol levels in triton-induced hyperlipidaemia with the various treatments administered. All values are expressed as mean±SEM. n=5.

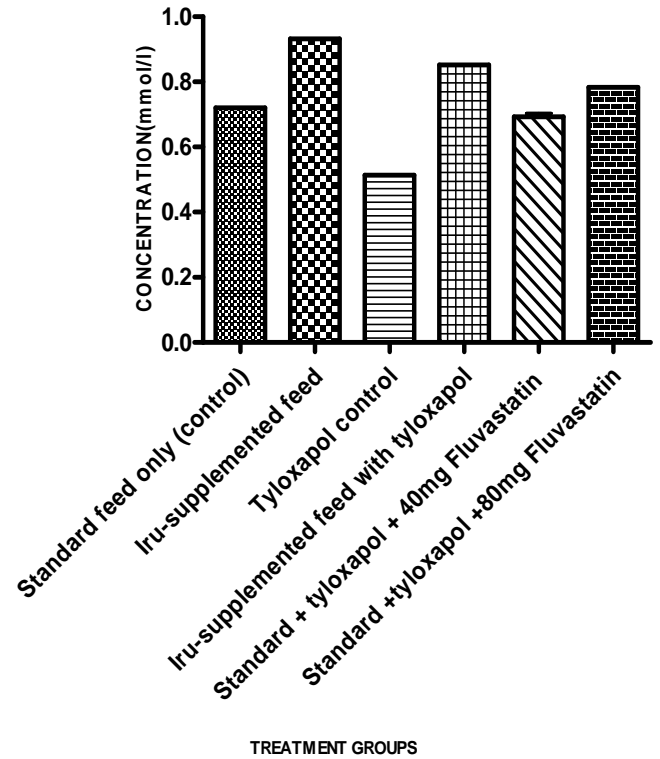


Figure 8. Effect of *iru* supplementation on liver HDL-cholesterol in triton-induced hyperlipidaemia in experimental and control rats. All values are expressed as mean±SEM. n=5.

also proven to be beneficial in the animal model used. Therefore, regular consumption of the condiment may represent a new alternative way to control or protect against common disorders of lipid metabolism which are associated with hyperlipidaemia and oxidative stress. It may also be considered a cheap and accessible source of antihyperlipidaemics. The use of the condiment could also be advocated as a food ingredient for processed and canned foods in order to further increase their acceptability, versatility and utility.

Work is ongoing in our laboratory on identification and isolation of specific antioxidant and hypolipidaemic component(s) present in the condiment as this may lead to chemical entities with huge potential for clinical use.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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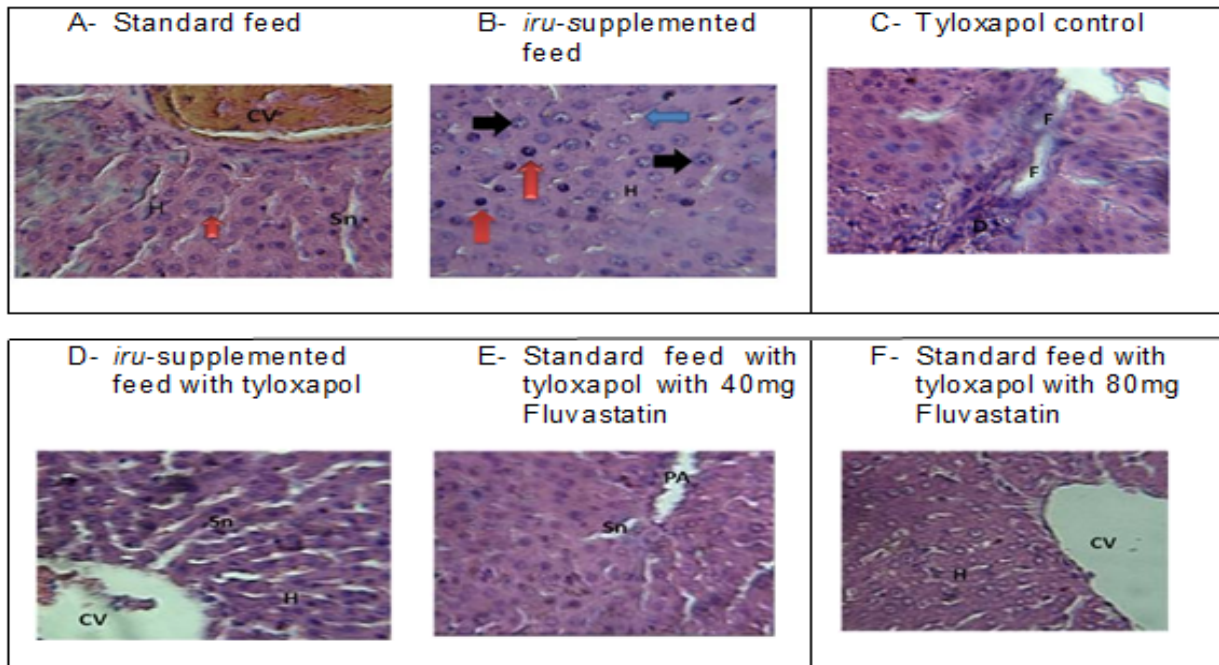


Figure 9. Changes in hepatocytes of representative rats from groups A-F in triton-induced hyperlipidaemia after treatment with condiment, triton and fluvastatin sodium. F- fibrosis, D- degeneration, Sn-sinusoids, H- Hepatocytes, CV- Central vein, PT- portal area. (hematoxylin and eosin400 x).

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