

Palm oil and ground nut oil supplementation effects on blood glucose and antioxidant status in alloxan-induced diabetic rats

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Abstract: This study investigated the effects of two common cooking oils (palm oil, PO) and (groundnut oil, GO) supplementation on the antioxidant status and diabetic indices in Alloxan (100mg/kg) induced diabetic Wistar rats. A total of forty-eight Wistar rats of both sexes were used for this study. They were divided into four groups of 12 animals each as: control, diabetic non-supplemented, diabetic supplemented with PO (200mg/kg/day) and diabetic supplemented with GO (200mg/kg/day) rats. Blood glucose, plasma vitamin E, SOD, Total Protein and Albumin levels were measured using standard laboratory procedures. After three weeks of supplementation there was a significant ($p < 0.05$) reduction in blood glucose of supplemented groups compared with the diabetic non-supplemented group. Plasma Vitamins C and E, SOD, and Albumin levels were significantly ($p < 0.05$) increased in the supplemented groups when compared with the diabetic non-supplemented group. However, the plasma levels of these parameters were found to be significantly ($p < 0.05$) higher in the GO supplemented rats compared with the PO supplemented group. The plasma vitamin C levels in the diabetic groups were lower than in other groups while increased levels in the plasma total protein were not significant. There was no significant difference in the measured parameters in reference to the gender of the animals. It was concluded from this study that GO exhibited superior antioxidant activities and that the supplementation of red palm oil and ground nut oil as a source of antioxidant was beneficial in diabetic state as it reduced blood glucose and enhance antioxidant status.

Keywords: Palm oil, groundnut oil, antioxidant, diabetes, supplementation.

INTRODUCTION

Oxidative stress and damage to tissues are common end points of chronic diseases, such as atherosclerosis, diabetes and rheumatoid arthritis (Ceriello, 2000; Soumya, 2011). Oxidative stress is currently suggested as mechanism underlying diabetes and diabetic complications (Baynes, *et al.*, 1999; Yang, *et al.*, 2011). Elevated glucose level is known to promote oxidative stress due to increase or overproduction of mitochondrial reactive oxygen species (ROS), non-enzymatic glycation of proteins and glucose auto-oxidation (Wolf *et al.*, 1991; Fiorentino *et al.*, 2013). The increase in the level of ROS in diabetes could be due to their increased production and/or decreased destruction by non-enzymatic and enzymatic antioxidants. Reduced glutathione content (Yoshida *et al.*, 1995; Matough *et al.*, 2012), superoxide dismutase (SOD), erythrocyte catalase, α -lipoic acid, as well as vitamin E (Yue *et al.*, 1989) have been reported in diabetic patients. The level of these antioxidants critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of complications in diabetes (Baynes, 1991, Matough *et al.*,

2012). Under normal circumstances, free radicals are rapidly eliminated in the body by these antioxidants defence systems, however when these aforementioned antioxidant defence fails, oxidative stress ensued which can lead to tissue damage. It is therefore expedient to identify exogenous antioxidants that may be important in preventing the activation of oxidative stress.

Vegetable oils and fats are principally used for human consumption but are also used in animal feed, for medicinal purposes, and for certain technical applications. They are rich source of energy; they contain fatty acids, antioxidants, antifoaming and anti-surfactant. Vegetable oils play important functional and sensory roles in food products and they act as carriers of fat-soluble vitamins (A, D, E and K) (Fasina *et al* 2006; Driss *et al.*, 2009). Various studies have shown that vegetable oils affect lipid peroxidation and antioxidant parameters, and lead to favourable changes in the plasma lipid status (Scaccini *et al.*, 1992; Visioli *et al.*, 1995). Lipids are protected against oxidation by addition of antioxidants that remove the free radicals and reactive oxygen species (Driss *et al.*, 2009). Antioxidants found in vegetable oils include: phosphatides (Ismail *et al.*, 1993; Rubalya and Neelamegan, 2012), carotenes (Lichenstein *et al.*, 1994),

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tocopherols (Orthofer, 1996), tocotrienols (Peuchant *et al.*, 2004) and ascorbic acid (Driss *et al.*, 2009).

Vitamin E compounds; tocopherols and tocotrienols are important minor constituents of most vegetable oils, they are well recognized for their effective inhibition of lipid oxidation in food and biological systems (Kang *et al.*, 2001; Egbal *et al.*, 2011). They serve as antioxidants to retard rancidity and are sources of the essential nutrient vitamin E. Tocotrienols are mainly present in palm oil, rice bran oil but can also be found in mustard and sesame oil (Orthofer, 1996), while tocopherols (α , β , γ , δ etc. tocopherol) are the most important antioxidants present high concentration in sunflower oil, soya bean oil, groundnut oil, rice bran oil (Rubalya and Neelamegam, 2012). The purpose of this study was to comparatively assess the effect of groundnut oil (GO) and palm oil (PO) supplementation on some antioxidant parameters in Alloxan-induced diabetic rats.

MATERIALS AND METHODS

Experimental Animals

Forty-eight Wistar rats of both sexes, weighing 100-180g obtained from animal house of Achievers University, Owo, Nigeria were used for this study. The animals received a chow pellet diet and water *ad libitum*. The study was approved by Achievers University Animal Research Ethical Committee constituted for the purpose of ethics in research (Ref. AUO/IREC/VOL 1/001).

Drugs and chemicals

Alloxan was obtained from Sigma (St, Louis, MO) while albumin, total protein kits were obtained from Randox laboratory limited United Kingdom. All other chemicals used were of analytical grade and obtained from either Sigma–Aldrich or Merck.

Animal care

The rats were housed individually in stainless steel wired-bottom cages fitted with polypropylene houses in an experimental animal holding facility maintained at a temperature of between 21-24°C, with a 12h light dark cycle. The rats were fed standard rat pellet *ad libitum* and had free access to tap water. After acclimatization in the experimental animal holding facility for one week, the rats were randomized into four groups. Animals used in the study received humane care in accordance with the Principle of Laboratory Animal Care of the National Medical Research and the Guide for the Care and Use of Laboratory Animals of the National Academy of Sciences (National Institute of Health Publication no. 80-23, revised 1978). The general conditions of the rats were monitored daily throughout the study and body weights recorded weekly and just before sacrifice. Fluid intake was monitored at intervals of 2 days for the duration of the study period.

Induction of diabetes

Diabetes was induced by a single intraperitoneal injection of 100mg/kg of Alloxan monohydrate. Diabetes was confirmed by glucose oxidase method using One touch glucometer (LifeScan, Inc. Milipitas, CA 95035, USA) after 72 hours of Alloxan injection. Rats with plasma glucose level ≥ 12 mmol/L were separated and used as diabetic in this study. Control rats received distilled water and standard rat pellet. At the end of the experimental and supplementation period and following 16 hours fasting, animals in all the groups were sacrificed and specific parameters determined.

Supplementation

Palm Oil (PO) and Groundnut Oil (GO) were bought from the Oba's Market in Owo, Ondo state, Nigeria. Red palm oil and groundnut oil were administered orally at doses of 200 mg/kg/day by gavage for three weeks.

Experimental procedure

The forty-eight rats (12 control rats, 12 diabetic rats without supplementation, and 24 supplemented diabetic rats) used in this experiment, were divided into four groups of twelve rats each as follows:

Group 1: Control group.

Group 2: Diabetic rats without PO or GO supplementation

Group 3: Diabetic rats received supplemented palm oil PO (200mg/kg/day) in a volume of 0.23ml/kg orally by gavage.

Group 4: Diabetic rats received Groundnut oil GO (200mg/kg/day) in a volume of 0.23ml/kg orally by gavage.

After three weeks of supplementation, the rats were fasted overnight and sacrificed under deep anaesthesia with diethyl ether and blood was drawn by cardiac puncture and collected into Lithium Heparin bottles for total protein, albumin, SOD, vitamin E and vitamin C. The blood samples were centrifuged; the supernatant were kept frozen for analysis.

Biochemical analysis

Blood glucose was measured using ONETOUCH Basic Blood Glucometer (Life Scan, inc. Milipitas, CA 95035 USA). The levels of plasma albumin, uric acid and total protein were determined using Randox reagent kit.

Determination of Vitamin E

Plasma vitamin E level was determined by the method of (Baker and Frank, 1968). 1.5ml of plasma and standard was treated with 1.5ml of ethanol and water respectively. Then 1.5ml of xylene was added to the mixture, mixed vigorously and centrifuged. The supernatants were transferred to a new set of test tubes and 1ml α, α' -dipyridyl was added to each tube and mixed. The absorbance of 1.5 ml of the mixture was measured against reagent blank at 460nm. Then in turn beginning with the blank, 0.33ml ferric chloride was added to all test tubes and the absorbance was read against blank after 10min at 520nm.

Determination of Vitamin C

Plasma vitamin C level was determined by the method of (Aye Kyaw, 1977). 2ml of plasma was treated with 2.0ml of phosphotungstic acid (PTA), Mixed thoroughly and allowed standing for 30 minutes at room temperature. The mixture was centrifuged at 3,000rpm for 15 minutes. The absorbance of the blue coloured supernatant was measured against a reagent blank and a standard at 700nm.

Superoxide dismutase (SOD) activity

SOD activity was determined according to the method of (Misra and Fridovich, 1989). 2.7ml of the reaction mixture contains 2.5ml of 0.1M carbonate buffer pH 10.3, 0.2ml of the suitable aliquot of enzyme extract, and then allowed standing at room temperature, and then 0.3ml of 0.3M adrenaline solution was added to each of the test solutions. Change in absorbance was recorded at 420 nm for one min at 15 sec interval. The reference (0.2ml distilled water) consisting of all the ingredients, except enzyme preparation, was run simultaneously.

STATISTICAL ANALYSIS

All results were expressed as mean \pm SD. Data were analyzed by one-way analysis of variance (ANOVA), with statistical significant level at $p < 0.05$. All analyses were performed using SPSS 17.0 package.

RESULTS

Table 1 shows that there was no significant difference ($p < 0.05$) in % change in body weight in all groups. Alloxan-induced diabetic non-supplemented group however showed a decrease in body weight but not significantly different when compared with the control and diabetic supplemented groups.

Table 2 shows the mean value of the blood glucose in all four groups (before and after 3 weeks). The baseline values of blood glucose ranged from 3.19 to 3.35mmol/L. A significant ($p < 0.05$) increase was observed in the final blood glucose of the diabetic groups (8.24mmol/L). However, rats supplemented with PO and GO showed a significant ($p < 0.05$) reduction in blood glucose level when compared with the non-supplemented diabetic rats.

Table 2, also showed the mean value of plasma albumin and total protein. Plasma albumin in GO supplemented diabetic group significantly ($p < 0.05$) increased when compared with the control and diabetic non-supplemented group. There was no significant difference in albumin between the GO and PO supplemented diabetic rats. The plasma total protein levels in the PO treated group were significantly reduced when compared with the normal control.

Table 3 represents the levels of plasma vitamin E, vitamin C and SOD of the control, diabetic non-supplemented and the supplemented groups. The plasma levels of vitamin E, vitamin C and SOD were found to be significantly ($p < 0.05$) increased in the supplemented group compared with the diabetic non-supplemented group. However GO supplemented group showed a higher increase in the plasma vitamin E, vitamin C and SOD levels than the PO supplemented group.

DISCUSSION

This study showed that palm oil and groundnut oil supplementation was able to reduce blood glucose levels in the diabetic rats, this reduction may be due to the improvement in the antioxidant activities. Luostarinen *et al.*, (1995) reported in a previous study that palm oil supplementation was able to prevent hyperglycaemic condition in fish oil intake in healthy volunteers. They also found that tocopherol supplementation could increase the production of insulin and increase insulin: glucose ratio, which could have the protective effect against β cell destruction.

In diabetic state, persistent high level of blood glucose results in increased catabolism of proteins and its decreased synthesis (Williams, 1989). In this study, the higher plasma total protein and albumin levels in GO supplemented rats when compared with the PO supplemented group, control, and diabetic groups, may be attributed to the fact that groundnuts have the highest protein concentrations (26g/99.2g nuts) than any other nuts (Sheldon, 1992).

SOD is a metalloprotein and is the first enzyme involved in the antioxidant defence by lowering the steady state of O_2 . Decreased activity of SOD leads to increased production of free radicals, e.g., superoxide anion combines with hydrogen peroxide in the presence of copper ion to form powerful hydroxyl radical (Ishikawa, 1993). This radical modifies proteins and DNA, damages cellular membranes of mitochondria, nuclear envelope and endoplasmic reticulum (Hayashi *et al.*, 2005; Kawasaki *et al.*, 2004). In this study, free radicals generated as a result of the induced hyperglycemia in the test groups caused the endogenous antioxidants to decrease in the diabetic rats as indicated in the results on Table 3. Hyperglycemia resulted in reduced SOD level, in the diabetic group but supplementation with GO and PO significantly restored the antioxidant enzyme activities.

Palm oil contains sitosterol, vitamin E, tocotrienols, which acts as a super-antioxidant and the carotenoids in red palm oil also acts as secondary antioxidants by quenching singlet oxygen (Robalya and Neelmegan, 2012). Groundnut oil contains tocopherol (α , β , γ , δ etc. tocopherol) and pantothenate (Robalya and Neelmegan,

Table 1: The mean values of body weight of control, diabetic and diabetic groups supplemented with PO and GO.

Groups	Mean baseline weight (g)	Mean final weight (g)	% change in weight
Control	127.73±28.7	157.56±25.90	19
Diabetic	135.01±37.80	158.28±38.16	14
Diabetic + PO	119.16±18.01	150.75±18.03	20
Diabetic + GO	135.08±32.15	164.26±26.39	17

Table 2: The mean blood glucose, plasma albumin and total protein of control, diabetic, diabetic groups supplemented with PO and GO.

GROUPS	Initial fasting blood glucose (mmol/L)	Final fasting blood glucose (mmol/L)	Total protein (g/L)	Albumin (g/L)
Control	3.23±0.28	5.36±0.73	99.3±2.45	37.2±1.63
Diabetic	3.25±0.28	8.24±2.77***	82.4±1.32	41.0±2.16**
Diabetic + PO	3.21±0.26	5.37±0.93	77.6±3.16*	48.0±1.43
Diabetic + GO	3.19±0.19	5.05±1.95	91.7±1.39	59.3±0.95*

***Significantly different from normal control group (p<0.05) and diabetic supplemented groups. **Significantly different from normal control and GO supplemented rats (p<0.05) *Significant different from normal control (p<0.05)

Table 3: The mean values of plasma SOD, vitamin E and vitamin C of control, diabetic and diabetic groups supplemented with PO and GO

Parameters	Control	Diabetic control	Diabetic+ PO	Diabetic +GO
SOD(U/ml)	2.01±0.13	0.17±0.05***	1.86±0.18**	1.98±0.26
VIT E(mg/L)	3.50±1.31	3.12±0.95	4.44±1.18	7.83±2.76***
VIT C(mg/L)	0.81±0.61	0.55±0.47	0.95±0.70	1.27±1.36

***Significantly different from control group and diabetic supplemented rats (p <0.05) ** significantly different from control and diabetic rats (p<0.05)

2012). The alpha (α)-tocopherol, biologically and chemically the most active form of vitamin E is the major lipid-soluble antioxidant known to break the chain of free radical mediated lipid peroxidation of PUFA (Thomas *et al.*, 1995). It functions as a potent inhibitor of lipid peroxidation in biological cells, cell membranes and plasma (Monahan *et al.*, 1993; Thomas *et al.*, 1995). This study revealed that palm oil and groundnut oil supplemented group showed increased the vitamin E levels in the diabetic rats. This may be due to the presence of unsaponifiable components like vitamin E, which may have contributed to the antioxidant defence against hyperglycemia induced oxidative stress as observed in the supplemented groups. The result revealed that a GO supplemented group has significantly higher plasma vitamin E level when compared with the PO supplemented rats. This may be due to higher content of tocopherol in GO as against tocotrienol which is the predominant form of vitamin E found in palm oil as reported by (Robalya and Neelmegan, 2012). The overall concentration of this antioxidant may not depend solely on the vitamin E composition of the oil only, but also on the content and activities of other antioxidants, such as panthothenate, polyphenols and other nutrients such as thiamine, niacin, folates, iron and magnesium which are present in groundnut oil. The higher plasma vitamin E in the GO supplemented group is in support of a previous study by (Nevada *et al.*, 2012) which reported that a higher dietary vitamin E supplementation may be more

efficient antioxidant in rats fed groundnut oil- based diets when compared with those supplemented with Palm oil and Coconut oil. However, dietary supplementation with GO and PO did not result in any significant difference in the plasma levels of vitamin C between the groups.

In conclusion, results from this study indicate that palm oil and groundnut oils may be important sources of antioxidants that can be used in the management of diabetes and stress conditions and that supplementation with Groundnut oil have higher content of biologically active components, which is superior in antioxidant property than palm oil. Further studies to elucidate possible effects of the individual components of PO and GO are suggested.

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