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ORIGINAL CONTRIBUTION

Effect of Dietary Substitution of Groundnut Oil on Blood Glucose, Lipid Profile, and Redox Status in Streptozotocin-diabetic Rats

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The effect of groundnut oil on blood glucose, lipid profile, lipid peroxidation, and antioxidant status in streptozotocin-diabetic rats was investigated and compared with diabetic and drug-treated rats. Diabetes was induced in adult female Wistar rats by intraperitoneal administration of streptozotocin (40 mg/kg b-wt). Normal and diabetic rats were fed an oil-free diet containing 2 percent oil supplemented with groundnut oil (6g per 94g diet), to give 8 percent oil content, for 42 days. Diabetic rats had elevated levels of glucose (322.61 ± 9.49), glycosylated hemoglobin (HbA_{1c})†, vitamin E, thiobarbituric acid reactive substances (TBARS), and lipid hydroperoxides (HP) and decreased levels of hemoglobin (Hb), vitamin C and reduced glutathione (GSH). An increase in the activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase and a decrease in hexokinase activity also were observed in the liver and kidney. When diabetic rats were fed groundnut oil, a significant reduction in glucose (244.04 ± 11.66), HbA_{1c}, TBARS, HP levels, glucose-6-phosphatase, and fructose-1,6-bisphosphatase activities and an elevation in Hb, vitamin E, GSH levels, and hexokinase activity were observed. Diabetic rats had elevated total cholesterol (TC), VLDL-cholesterol, LDL-cholesterol, and triglycerides (TG) and decreased HDL-cholesterol. Diabetic rats fed groundnut oil showed a small but significant reduction in TC, VLDL-C, LDL-C, and TG and an elevation in HDL-C. Groundnut oil consumption slightly but significantly decreases the blood glucose, HbA_{1c}, lipid peroxidation, and lipid profile and increases antioxidant levels in diabetic rats.

Currently, there are 150 million diabetics worldwide, and this number is likely to increase to 300 million or more by the year 2025 due to increases in sedentary lifestyles, consumption of energy-rich diets and obesity [1,2]. While the management of diabetes mellitus includes diet, exercise, oral hypoglycemic agents, and insulin, these do not effectively prevent the complications of diabetes mellitus [3]. In modern medicine, there is still no satisfactory effective therapy available to cure diabetes [4]. Therefore, it has become necessary to search for an economically and therapeutically effective treatment, especially for usage in developing and under-developed

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†Abbreviations: ANOVA, analysis of variance; DMRT, Duncan's Multiple Range Test; LDL, low-density lipoprotein; GSH, glutathione; Hb, hemoglobin; HbA_{1c}, glycosylated hemoglobin; HP, hydroperoxides; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; STZ, streptozotocin; TBARS, thiobarbituric acid reactive substances; TC, total cholesterol; V-LDL, very-low-density lipoprotein.

countries. Many indigenous medicinal plants have been found to be useful to successfully manage diabetes [5].

Groundnut oil is widely used in south India for cooking. Groundnut oil contains 46 and 32 percent of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), respectively [6]. Rasmussen et al. noted a reduction in peak plasma glucose concentration with the consumption of a monounsaturated fatty acid-rich diet [7]. Reports are available on the effect of groundnut oil on normal and high-fat diets [8] and myocardial infarction [9]. No investigation has been carried out to study the effect of groundnut oil on glycemic control in diabetic rats. Since groundnut oil contains 46 percent of MUFA, we were interested in exploring the role of groundnut oil in diabetes.

In diabetes, hyperglycemia results in the generation of free radicals [10] due to autoxidation of glucose [11] and glycosylation of proteins [12]. Free radicals react with lipids and cause peroxidative changes that result in enhanced lipid peroxidation [13]. The level of lipid peroxidation in cells is controlled by various cellular defense mechanisms consisting of enzymatic and nonenzymatic scavenging systems [14]. The efficiency of the antioxidant defense mechanism is altered in diabetes [15]. Hence, in this study, we investigate the effect of groundnut oil on blood glucose, carbohydrate metabolic enzymes, lipid profile, lipid peroxidation, and nonenzymatic antioxidants in normal and STZ-diabetic rats.

MATERIALS AND METHODS

Oil and treatment diet

Standard pellet diet and oil-free diet were purchased from Pranav Agro Industries Ltd., Pune, India. Groundnut oil (SVS groundnut oil, trade name) was purchased from the local market. The oil was stored in a jar at 4 °C. The standard pellet diet contained 8 percent oil. Since the oil-free diet contained 2 percent oil, 94 g of this diet was mixed with 6 g of groundnut oil to make an 8 percent oil content.

Chemicals

Streptozotocin was procured from Sigma-Aldrich, St. Louis, United States, and glibenclamide from Hoechts, Frankfurt, Germany. All other chemicals used were of analytical grade and obtained from E. Merck, Darmstadt, Germany, and Hi-media, India.

Experimental animals

Adult albino female Wistar rats with body weight of 180 to 200 g bred in Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University, were used in this study. The feed and water were provided ad libitum to the animals.

Experimental induction of diabetes

The animals were made diabetic with an intraperitoneal injection of STZ at a dose of 40 mg/kg b-wt dissolved in citrate buffer (0.1 M, pH 4.5). STZ-injected animals exhibited massive glycosuria and hyperglycemia within a few days. Diabetes was confirmed in the overnight-fasted rats by measuring blood glucose concentration 96 hr after injection with STZ. The rats with blood glucose above 240 mg/dl were considered to be diabetic and used for the experiment.

Experimental design

The animals were randomly divided into five groups of six animals each.

Group I: Normal

Group II: Normal + groundnut oil diet

Group III: Diabetic control

Group IV: Diabetic + groundnut oil diet

Group V: Diabetic + glibenclamide (600 µg/kg b-wt)

After 42 days of treatment, the 12 hr-fasted animals were sacrificed by cervical decapitation between 8 a.m. and 9 a.m. Blood was collected in tubes containing a mixture of potassium oxalate and sodium

Table 1. Effect of groundnut oil in diet on blood glucose and body weight in normal and diabetic animals.

Group	Body weight (g)		Blood glucose (mg/dL)	
	Before treatment (0 day)	After treatment (42 days)	Before treatment (0 day)	After treatment (42 days)
Normal	185.16 ± 3.31	208.66 ± 4.22^{a}	80.95 ± 5.82	88.09 ± 4.32^{b}
Normal + groundnut oil diet	176.50 ± 2.25	198.16 ± 3.18^{a,b}	75.54 ± 4.23	72.61 ± 3.68^{a}
Diabetic control	178.50 ± 3.01	128.16 ± 3.12^{c}	251.78 ± 9.78	322.61 ± 9.49^{e}
Diabetic + groundnut oil diet	173.50 ± 3.14	159.66 ± 3.55^{b}	259.51 ± 11.44	244.04 ± 11.66^{d}
Diabetic + glibenclamide (600 µg/kg b-wt)	181.50 ± 4.37	201.50 ± 5.39^{a,b}	258.33 ± 7.37	122.11 ± 3.61^{c}

Values are means ± SD for six rats in each group. Values sharing a common superscript in a column are not significant with each other ($p < 0.05$, Duncan's Multiple Range Test [DMRT]).

fluoride (1:3) for the estimation of blood glucose and in tubes with ethylenediaminetetra acetic acid (EDTA) for the estimation of HP, TBARS, GSH, vitamins C and E, and lipid profile. Tissues such as liver and kidney were collected and stored at 4 °C for the estimations of enzymes.

Biochemical estimations

Blood glucose was estimated by the method of Sasaki et al. [16]. Hb and HbA_{1c} were estimated by the methods of Drabkin and Austin [17] and Sudhakar and Pattabiraman [18], respectively. The activities of glucokinase, glucose-6-phosphatase and fructose-1, 6-bisphosphatase were assayed by the methods of Brandstrup et al. [19], Koide and Oda [20], and Gancedo and Gancedo [21], respectively. Plasma vitamin C, vitamin E, GSH, TBARS, and HP were estimated by the methods of Roe and Kuether [22], Baker et al. [23], Ellman [24], Nichans and Samuelson [25] and Jiang et al. [26], respectively. TC [27], HDL-C [28] and TG [29] were measured. LDL-C was calculated by Friedwald's formula [30].

Statistics

Results were expressed as means ± SD, for six rats in each group. Data were analyzed using one-way analysis of variance (ANOVA), and group means were compared with Duncan's Multiple Range Test (DMRT) [31] using SPSS-10.

RESULTS

Table 1 shows the effect of groundnut oil on body weight and blood glucose in normal and STZ-diabetic rats. A significant weight loss was observed in the diabetic control group. The weight loss was minimal in the oil-treated group, but significant improvement of weight was observed in the group treated with standard drugs. A drastic increase in blood glucose level was found in the diabetic control group. A small but significant reduction in blood glucose level was found in the diabetic animals fed groundnut oil. The reduction was highly significant in the drug-treated group.

Table 2 illustrates the effect of substitution of oil on Hb and HbA_{1c} levels in normal and STZ-diabetic rats. Hb level decreased significantly while an increase in HbA_{1c} was observed in diabetic rats when compared with normal rats. Significant improvement in Hb levels and a decrease in HbA_{1c} were observed in diabetic animals treated with groundnut oil and glibenclamide, though it

Table 2. Effect of groundnut oil in diet on Hb and HbA_{1c} in normal and diabetic animals.

Group	Hb (g/dL)	HbA_{1c} (mg/g of Hb)
Normal	10.92 ± 0.37^a	0.39 ± 0.01^b
Normal + groundnut oil diet	10.85 ± 0.49^a	0.34 ± 0.02^a
Diabetic control	6.12 ± 0.15^d	1.10 ± 0.03^e
Diabetic + groundnut oil diet	6.79 ± 0.49^c	0.99 ± 0.02^d
Diabetic + glibenclamide (600 µg/kg b-wt)	9.73 ± 0.49^b	0.49 ± 0.03^c

Values are means ± SD for six rats in each group. Values sharing a common superscript in a column are not significant with each other ($p < 0.05$, Duncan's Multiple Range Test [DMRT]).

was more prominent in the drug-treated group.

Table 3 shows the effect of groundnut oil on carbohydrate metabolic enzymes in tissues of normal and STZ-diabetic rats. The hexokinase activity decreased, whereas the activities of gluconeogenic enzymes such as glucose-6-phosphatase and fructose-1, 6-bisphosphatase increased in the liver and kidney of diabetic rats when compared with normal rats. A significant increase of hexokinase and reduction in glucose-6-phosphatase and fructose-1, 6-bisphosphatase activities were observed in the groups treated with groundnut oil and glibenclamide when compared with diabetic controls.

Table 4 illustrates the effect of groundnut oil on TBARS and HP in the plasma of normal and STZ-diabetic rats. A significant increase in TBARS and HP was observed in diabetic rats when compared with normal rats. TBARS and HP decreased significantly in the plasma of diabetic rats fed with

Table 3. Effect of groundnut oil in diet on the activities of carbohydrate metabolic enzymes in liver and kidney of normal and diabetic animals.

	Glucokinase (Hexokinase D) (Unit*/h/ mg protein	Glucose-6-phosphatase (Unit**/min/ mg/protein)		Fructose-1,6-phosphatase (Unit***/h/ mg/protein)	
Group	Liver	Liver	Kidney	Liver	Kidney
Normal	0.260 ± 0.023^a	0.165 ± 0.011^a	0.187 ± 0.036^a	0.412 ± 0.027^a	0.814 ± 0.063^a
Normal + groundnut oil diet	0.270 ± 0.012^a	0.156 ± 0.022^a	0.176 ± 0.024^a	0.395 ± 0.028^a	0.757 ± 0.061^a
Diabetic control	0.079 ± 0.009^d	0.457 ± 0.014^d	0.272 ± 0.016^d	0.714 ± 0.029^d	1.169 ± 0.042^c
Diabetic + groundnut oil diet	0.116 ± 0.009^c	0.397 ± 0.014^c	0.236 ± 0.016^c	0.656 ± 0.037^c	1.016 ± 0.151^b
Diabetic + glibenclamide (600 µg/kg b-wt)	0.216 ± 0.014^b	0.244 ± 0.014^b	0.203 ± 0.018^b	0.481 ± 0.035^b	0.802 ± 0.037^a

Values are means ± SD for six rats in each group. Values sharing a common superscript in a column are not significant with each other ($p < 0.05$, Duncan's Multiple Range Test [DMRT]). * µmoles of glucose phosphorylated; ** µmoles of inorganic phosphorous liberated; *** µmoles of inorganic phosphorous liberated.

Table 4. Effect of groundnut oil in diet on TBARS and HP in plasma of normal and diabetic animals.

Group	TBARS (mmol/dl)	HP (x 10^{-5} mmol/dl)
Normal	0.175 ± 0.038[a]	9.81 ± 0.98[b]
Normal + groundnut oil diet	0.162 ± 0.030[a]	8.32 ± 0.91[a]
Diabetic control	0.350 ± 0.038[c]	24.15 ± 1.07[e]
Diabetic + groundnut oil diet	0.262 ± 0.041[b]	22.48 ± 0.74[d]
Diabetic + glibenclamide (600 µg/kg b-wt)	0.187 ± 0.041[a]	11.06 ± 0.59[c]

Values are means ± SD for six rats in each group. Values sharing a common superscript in a column are not significant with each other ($p < 0.05$, Duncan's Multiple Range Test [DMRT]).

groundnut oil and treated with glibenclamide when compared with diabetic control rats. The decrease in the levels of TBARS and HP was more remarkable in glibenclamide-treated rats.

Table 5 shows the effect of groundnut oil on antioxidants in the plasma of normal and STZ-diabetic rats. A significant reduction of plasma vitamin C and GSH and increase of vitamin E were observed in diabetic rats when compared with control rats. Significant increase in vitamin E and GSH were found in diabetic rats fed with groundnut oil when compared with diabetic rats. The levels of vitamin C and GSH significantly increased while vitamin E decreased in glibenclamide-treated rats when compared with diabetic controls. An increase in vitamin E also was observed in normal rats fed with oil when compared with the control group.

Table 6 shows the effect of groundnut oil in the diet on plasma TC, VLDL-C, LDL-C, HDL-C, TG, and TC/HDL-C ratio in STZ-diabetic rats. In our study, diabetic rats had elevated levels of TC, VLDL-C, LDL-C, and TG and decreased levels of HDL-C when compared with normal rats. Diabetic rats fed with groundnut oil showed a small but significant reduction in the levels of TC, VLDL-C, LDL-C .and TG and elevation in HDL-C level when compared with diabetic controls, but diabetic animals treated with glibenclamide showed better improvement.

DISCUSSION

STZ is a commonly employed compound for the induction of diabetes mellitus in experimental rats [32]. It causes DNA strand breaks in pancreatic islets, stimulates

Table 5. Effect of groundnut oil in diet on vitamin C, vitamin E, and GSH in plasma of normal and diabetic animals.

Group	Vitamin C (mg/dl)	Vitamin E (mg/dl)	GSH (mg/dl)
Normal	1.83 ± 0.08[a]	6.32 ± 0.24[a]	31.47 ± 2.00[b]
Normal + groundnut oil diet	1.36 ± 0.08[b]	7.42 ± 0.24[b]	34.26 ± 2.21[a]
Diabetic control	0.93 ± 0.08[c]	14.66 ± 0.22[d]	18.62 ± 2.37[d]
Diabetic + groundnut oil diet	0.66 ± 0.08[d]	18.92 ± 0.27[e]	23.83 ± 1.99[c]
Diabetic + glibenclamide (600 µg/kg b-wt)	1.40 ± 0.08[b]	9.57 ± 0.27[c]	28.81 ± 3.10[b]

Values are means ± SD for six rats in each group. Values sharing a common superscript in a column are not significant with each other ($p < 0.05$, Duncan's Multiple Range Test [DMRT]).

Table 6. Effect of groundnut oil in diet on TC, VLDL-C, LDL-C, HDL-C, and TG in normal and diabetic animals.

Group	TC (mg/dl)	VLDL-C (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	TG (mg/dl)
Normal	82.00 ± 1.74^a	16.29 ± 1.15^a	24.71 ± 2.13^a	41.10 ± 3.27^d	81.45 ± 2.00^a
Normal + groundnut oil diet	84.20 ± 2.20^a	17.91 ± 1.32^b	31.44 ± 3.14^b	35.15 ± 2.55^c	90.38 ± 2.41^b
Diabetic control	131.20 ± 1.09^d	36.09 ± 2.63^d	67.13 ± 5.24^d	27.78 ± 1.89^a	180.45 ± 4.85^d
Diabetic + groundnut oil diet	116.33 ± 1.50^c	32.00 ± 3.12^c	54.68 ± 4.33^c	30.88 ± 2.17^b	160.95 ± 3.74^c
Diabetic + glibenclamide (600 µg/kg b-wt)	91.66 ± 1.75^b	18.63 ± 1.07^b	33.59 ± 2.98^b	40.02 ± 3.95^d	93.15 ± 2.29^b

Values are means ± SD for six rats in each group. Values sharing a common superscript in a column are not significant with each other ($p < 0.05$, Duncan's Multiple Range Test [DMRT]).

nuclear poly (ADP-ribose) synthetase, and thus depletes the intracellular NAD^+ and $NADP^+$ levels, which inhibits proinsulin synthesis and induces diabetes [33]. The decrease in body weight in diabetic rats shows that the loss or degradation of structural proteins is due to diabetes, and the structural proteins are known to contribute to the body weight [34]. In diabetic rats fed with groundnut oil, the weight loss was minimized, which may be due to the reduction of blood glucose. Groundnut oil-fed diabetic rats showed a significant reduction in blood glucose level. The reduction may be due to the presence of MUFA. Rasmussen et al. have reported reduction in peak plasma glucose concentration with the consumption of a MUFA-rich diet [7]. An earlier report from our laboratory shows that dietary substitution of sesame oil showed a better reduction of blood glucose 322.61 $\pm$ 9.49 to 222.02 $\pm$ 8.27) than groundnut oil in STZ-diabetic rats [35].

Insulin generally has an anabolic effect on protein metabolism in that it stimulates protein synthesis and retards protein degradation [36]. Previous reports have shown that protein synthesis is decreased in all tissues due to decreased production of ATP and absolute or relative deficiency of insulin [37], which may be responsible for the decreased level of Hb in diabetic rats. HbA_{1c} comprises 3.4 percent to 5.8 percent of total Hb in normal human red cells, but it is increased in patients with overt diabetes mellitus [38]. It was found to increase in diabetic patients up to 16 percent [39], and the level of HbA_{1c} is monitored as a reliable index of glycemic control in diabetes [40]. Elevated levels of HbA_{1c} and reduced levels of Hb observed in our study reveal that diabetic animals had prior high blood glucose level. Groundnut oil-fed diabetic rats showed an increase in Hb level and decrease in HbA_{1c} level, which may be due to the reduction of the blood glucose level.

In experimental diabetes, enzymes of glucose metabolism are markedly altered. Persistent hyperglycemia is a major contributor to such metabolic alterations that lead to the pathogenesis of diabetic complications, especially microvascular diseases [41]. One of the key enzymes in the catabolism of glucose is glucokinase, which phosphorylates glucose to glucose-6-phosphate [42]. In our study, the glucokinase activity was decreased in the liver of diabetic rats, which may be due to the deficiency of insulin. Groundnut oil-fed diabetic rats showed an elevated activity of glucokinase, which may be associated with reduced blood glucose.

Insulin decreases gluconeogenesis by decreasing the activities of key enzymes, such as glucose-6-phosphatase, fructose-1, 6-bisphosphatase, phosphoenolpyruvate carboxykinase, and pyruvate carboxykinase [43]. Glucose-6-

phosphatase is an important enzyme in homeostasis of blood glucose as it catalyzes the terminal step both in gluconeogenesis and glycogenolysis [44]. Fructose-1, 6-bisphosphase is one of the key enzymes of gluconeogenic pathway. It is present in liver and kidney but absent from heart, muscle, and smooth muscle. In our study, the increased activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase in liver and kidney of diabetic rats may be due to insulin deficiency. In oil-fed diabetic rats, the activities of these two enzymes were significantly reduced, which is responsible for the improved glycemic control.

Diabetes mellitus has been reported to generate reactive oxygen species (ROS). ROS, such as free hydroxyl radicals ($^{\bullet}OH$) and superoxide ($O2^{\bullet-}$), can cause lipid peroxidation [45]. Membrane lipid peroxidation results in loss of PUFA, decreased membrane fluidity, and loss of enzyme and receptor activity. The products of lipid peroxidation are capable of interacting with DNA and cause oxidative damage [46]. In our study, the lipid peroxidation markers such as TBARS and HP were significantly increased in the plasma of STZ-diabetic rats as reported earlier [47]. The levels of TBARS and HP were significantly decreased in diabetic rats fed with oil, which may be associated with decreased blood glucose and the presence of vitamin E in the oil.

Oxidative stress occurs when there is an imbalance between free radical reaction and the scavenging capacity of the antioxidative defense mechanism of the organism [48]. The nonenzymatic antioxidants such as GSH, vitamin C, and vitamin E are interrelated by recycling process [49]. Glutathione is the most important non-protein compound-containing thiol group, which acts as a substrate for glutathione transferase and glutathione peroxidase involved in preventing the deleterious effect of oxygen radicals [50]. In our study, diabetic rats showed a significant decrease in the level of GSH, which may be due to increased utilization. In oil-fed diabetic rats, a significant improvement in GSH was observed. This could be due to the decreased utilization of GSH.

Vitamin C is one of the most powerful natural antioxidants [51]. It is capable of regenerating α-tocopherol from the tocopheroxyl radical that is formed upon the inhibition of lipid peroxidation by vitamin E [52]. Vitamin C has been reported to contribute up to 24 percent of the total peroxyl radical-trapping antioxidant activity (TRAP) [53]. In our study, vitamin C was decreased significantly in the plasma of diabetic rats as reported earlier [54]. Groundnut oil-fed diabetic rats did not show any variation in vitamin C levels. Among lipid soluble antioxidants, α-tocopherol plays a central role as it controls radical-induced lipoprotein lipid peroxidation [55]. In our study, vitamin E level also was elevated significantly in the plasma of diabetic rats as reported earlier [56]. The increased level of α-tocopherol could be due to the increased release from membrane damage by ROS. Groundnut oil-fed diabetic rats showed a significant elevation of vitamin E in the plasma of diabetic rats. The increased level might be due to the presence of vitamin E in the oil.

The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease [57]. Diabetic rats fed with groundnut oil showed a small but significant reduction in levels of TC, VLDL-C, LDL-C, and TG and elevation in HDL-C levels when compared with diabetic controls. This could be due to the presence of MUFA and PUFA in the oil. There have been numerous studies in humans and animals that have demonstrated that oils containing saturated fatty acids raise serum TC, TG, and, in particular, LDL-C levels, while those enriched in unsaturated fatty acids lower TC, TG, and LDL-C [58, 59]. Diets high in monounsaturated fatty acids have been found to be relatively hypocholesterolemic or hypotriacylglycerolemic, respectively [60, 61].

In conclusion, our results show that groundnut oil substitution in the diet influences blood glucose, lipid profile, lipid peroxidation, and antioxidants beneficially in STZ-diabetic rats.

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