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ARTICLE

Effects of ethanolic extract of *Azadirachta indica* leaves on lipid peroxidation and serum lipids of diabetic Wistar rats

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ABSTRACT The effects of ethanolic extract of *Azadirachta indica* leaves on lipid peroxidation and serum lipids of alloxan-induced diabetic rats were examined to assess the role of the extract in the management diabetic complications. Serum Malondialdehyde (MDA), total cholesterol, triglyceride and blood glucose were measured in diabetic and normal rats with or without 400 mg/kg leaf extract of *A. indica.* The result showed that blood glucose, total cholesterol (TC), triglyceride and MDA concentrations in serum of untreated diabetic rats were significantly ($P<0.05$) higher than that of normal control and diabetic rats treated with the extracts. The extract caused a 55.8%, 27.1%, 16.8% and 64.7% reduction in blood glucose, TC, TG and MDA respectively when compared to untreated diabetic rats. The extract also demonstrated growth promoting effect on normal and diabetic rats. The anti-lipid peroxidative, antihyperglycaemic and serum lipid modulating activities justify the possible use of extract of *A. indica* leaves in the management of diabetic complications. **Acta Biol Szeged 51(1):17-20 (2007)**

KEY WORDS

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Lipid peroxidation, a known mechanism of cellular injury, is a chain reaction initiated by oxidant radicals. It is an index of oxidative stress in cells and tissues. The free radical oxidation of lipid molecules proceeds from the fatty acylmethylene group adjacent to a double bond (Frankel 1980) to form lipid hydroperoxides which are capable of further reactions to yield various classes of peroxides. Lipid peroxides are unstable and decompose to form series of complex products including reactive carbonyl compounds. Polyunsaturated fatty acids on oxidation decompose to malondialdehyde (MDA) and 4-hydroxyalkenals (HAE). The levels of these products in serum or tissues have been widely accepted as a measure of lipid peroxidation.

Biological membranes which contain phospholipids rich in polyunsaturated fatty acids are continuously being oxidized by superoxides, hydroperoxyl and peroxyl radicals emanating from exposures to radiations and oxidative reactions involving endogenous and exogenous compounds in the body (Droge 2002). There are, however, some defense systems to prevent injury caused by these free radicals (Hensley et al. 2000). These systems include antioxidant vitamins, reduced glutathione, transport and storage proteins for binding divalent metal irons, and antioxidant enzymes. Oxidative stress which is associated with cellular injury in many pathological conditions occurs when there is an imbalance between free-radical generating and radical scavenging systems (Hensley et al. 2000).

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The complications of diabetes mellitus are morphologic consequences of many metabolic pathways which may be associated with increased free radicals and serum lipid production (Lee and Chung 1999; Sheetz and King 2002; Goldberg 2001; Smaoui et al 2004). Intracellular advanced glycated end products (AGE) may stimulate the generation of increased level of reactive oxygen species (ROS) and the activation of NF-kB following binding to specific receptors (Kahler et al. 1993; Stitt et al. 2002). Glucose can be converted to Sorbitol by the enzyme, aldose reductase and eventually to fructose. In this process, intracellular NADPH is used as cofactor and this reduces the availability of NADPH required for the regeneration of reduced glutathione (GSH), a major antioxidant molecule in cells and tissues (Lee and Chung 1999). Sustained hyperglycemia as in diabetes mellitus and progressive depletion of intracellular NADPH by aldose reductase may lead to a compromise of GSH regeneration and thus increase cellular susceptibility to oxidative stress. Protein glycosylation in cells also affect antioxidant reactions catalysed by ROS scavenging enzymes in diabetes mellitus (Stitt et al. 2002).

Accelerated atherosclerosis among diabetics is a major pathologic cause of microvascular and macrovascular complications resulting in increased risk of myocardial infarction, stroke and lower extremity gangrene. Experimental and clinical evidences suggest that these complications are promoted by dyslipidemia (Goldberg 2001). Defects in insulin action have been shown to be responsible for the changes in plasma lipoprotein fractions in patients with diabetes mellitus (Haff-

ner et al. 2000).The oxidized form of low density lipoprotein (LDL)-cholesterol is of prime importance in the formation of foam cells in atherosclerotic plaque (Jilial and Devaraj 1996). Chemical changes of LDL- cholesterol by free radicals generated in macrophages or endothelial cells in the arterial wall yields oxidize LDL thereby implicating oxidative stress in the pathogenesis of atherosclerosis (Reardon and Getz 2001). Antioxidant treatment has however been shown to protect against the development of atherosclerosis in hypercholesterolemic experimental animals (Reardon and Getz 2001) and diabetic models (Jilial et al. 1990; Jilial and Grundy 1992).

Azadirachta indica, also called neem, belongs to the family of meliacea. It is one of the most useful medicinal plants with anti-inflammatory, antibacterial, anti-parasitic and immunomodulatory activities (Kausik and Ranajet 2002). Blood glucose lowering effect of *Azadirachta indica* seed oil and water extract have been reported in various models of diabetic animals (Dixit et al. 1986; Khosla et al. 2000; Halim 2003; Gupta et al. 204). The leaf extract of *A. indica* have been used traditionally in the control of diabetes mellitus in many countries including Nigeria, however, scientific studies on the efficacy and its mechanism of action have not been fully investigated.

This study therefore examines the effects of ethanolic extract of *A. indica* leaves on lipid peroxidation and serum lipids in diabetic rats with a view to ascertaining its involvement in the management of oxidative stress and other complications associated with diabetic mellitus.

Materials and Methods

Plant materials and extract preparation

Fresh matured leaves of *Azadirachta indica* (Neem) were harvested from Endocrine Research Farm of the Department of Biochemistry, University of Calabar, Calabar. The leaves were washed with tap water, rinsed with distilled water and sundried to remove traces of water. The leaves were ground to form paste with an electric blender (Binatone, Japan). One hundred and fifty grams of leaf paste was agitated in 500 ml of ethanol and stored overnight in a refrigerator at 4°C for complete extraction. The suspension was filtered and 50 ml aliquots of the filtrate were poured into separate beakers of known weight. The aliquots were dried at 50°C to constant weight using rotary evaporator. The dry extract was stored in a refrigerator at 4°C. Two grams of the dry extract was resuspended daily in 50 ml of distilled water for administration to the animals. 1.5 ml of the suspension, given to a 150 g rat was equivalent to 400 mg extract per kilogram body weight. The volumes of extract suspension were adjusted accordingly for various weights of the rats.

Animals

Thirty albino wistar rats, weighing 150-200 g, were obtained from the Animal House of the Department of Biochemistry, University of Calabar. The animals were kept in standards plastic cages and placed in a well ventilated room of temperature between 22°C and 27°C. The animals were acclimatized for seven days. During this time and throughout the experimental period, they were fed with commercial rat feed (Pfizer Livestock Co. Ltd, Aba, Nigeria) and tap water *ad libitum* .

Animal treatments

After acclimatization, the animals were assigned into four groups of six rats each as follows: Normal Control (NC), Diabetic Control (DC), Diabetic Treated (DT) and Normal Treated (NT) groups. The animals for DC and DT groups were made diabetic by a single intraperitoneal administration of 150 mg/kg body weight of alloxan (Sigma, St. Louis, USA) dissolved in distilled water (Battel et al. 1999). The animals were left for 7 days after which fasting glucose concentrations were determined in their tail-prick blood samples using One Touch Basic Glucometer (Life Scan, USA). Animals with consistent blood glucose greater than 200 mg/dl for 2 days were considered diabetic and randomly assigned to DC or DT group before administration of extract commenced. Groups DT and NT were treated with 400 mg/kg body weight of the extract twice daily for 7 days by oral gavage while groups DC and NC which served as controls were given distilled water orally. Body weight of the animals and their fasting blood glucose were monitored before and after extract administration.

Collection and preparation of samples

At the end of the 7-day treatment, the animals were anaesthesized under chloroform vapour and sacrificed. Blood samples were obtained by cardiac puncture and poured into plain screw-caped sample bottles and allowed to clot. Serum samples were separated from the clotted blood by centrifugation at 2000 x g for 5 min using an MSE model (England) bench centrifuge. The sera were stored at 4°C and biochemical assays conducted within 24 h.

Biochemical assays

Blood glucose was determined by pricking the tail of rats asceptically with Lancet and placing one drop of blood on a sample strip. The blood on the strip was inserted into the glucometer (Life Scan, USA) and glucose concentration was read (WHO, 1980)

Lipid peroxidation product, malondialdehyde (MDA) was measured by the method of Esterbauer et al (1991) using Bioxytech MDA reagents kit.

Total cholesterol and triglyceride concentrations were determined by enzymatic colourimetric assay using reagents kits from Dialab produktion, France. The absorbances in all analysis were read using spectrophotometer Optima SP-300.

Table 1. Mean values of body weight and blood glucose of normal and diabetic rats on or without extract of *A. indica*.

Group	Body weight(g) Before	After	% Weight gain	Blood Glucose (mg/dl) Before	After
NC	180.2± 5.15	183.5± 8.92	1.83 ± 0.31	106.6 ± 15.10	104.8 ± 18.10
DC	179.3± 6.10	181.0± 8.25	0.95 ± 0.42*	300.4 ± 10.46*	306.5 ± 12.43*
DT	177.5± 6.50	181.1± 9.50	2.03 ± 0.35	302.6 ± 16.48*	133.8 ± 13.75
NT	179.4± 4.25	185.5± 8.20	3.40 ± 0.51*	105.5 ± 18.20	104.5 ± 16.50

Mean ± SD, *Significantly different from normal control at P < 0.05.

Table 2. Malondialdehyde (MDA), total cholesterol (TC) and triglyceride (TG) in serum of normal and diabetic rats on or without extract of *A. indica*.

Group	MDA (μmol/ml)	TC (mg/dl)	TG (mg/dl)
NC	2.07 ± 0.42	92.10 ± 6.17	114.20 ± 6.24
DC	11.51 ± 0.22*	132.00 ± 7.82*	145.71 ± 8.34*
DT	4.06 ± 0.43	96.24 ± 3.42	121.31 ± 7.63
NT	-	94.37 ± 3.47	110.56 ± 5.16

Mean ± SD, *significantly different from normal control at P < 0.05.

Statistical Analysis

Data are presented as mean ± SD. The differences between groups were tested using student's *t*-test. A probability of 0.05 was chosen as a level of significance.

Results and Discussion

The mean values of body weight and blood glucose of normal and diabetic rats before and after treatment with 400 mg/kg of *A. indica* leaf extract is presented in Table 1, while Table 2 shows the effects of the extract on serum malondialdehyde (MDA) and serum lipids of normal and diabetic rats. The percentage weight gain of diabetic and normal rats treated with the extract of *A. indica* leaves were respectively higher than those of diabetic and normal control rats which did not receive the extract. Serum glucose, cholesterol and triglyceride concentrations were significantly (P <0.05) higher in diabetic control rats compared to those of normal control and those receiving extract treatment.

The extract reduced serum glucose, cholesterol and triglyceride concentrations in diabetic rats to about normal levels and also appeared to promote growth of both normal and diabetic rats. The mechanism of growth promotion by the extract is not well known, but may be related to enhanced cellular anabolic processes in which carbohydrates, proteins and lipids are metabolized and stored in tissues. Insulin, a hormone with known anabolic activity, enhances peripheral uptake of glucose and triglyceride from extracellular compartment into cells (Saltiel and Kahn 2001). Thus increase in weight of animals treated with the extract may be the result of enhanced insulin action, especially since extract treatment caused the reduction of serum glucose, cholesterol and triglyceride in the diabetic rats.

The lipid peroxidation product, malondialdehyde (MDA), was significantly (P<0.05) higher in the diabetic control rats compared to normal control whereas the MDA of diabetic treated rats was not significantly different from those of normal control. This indicates that the extract of *A. indica* prevents lipid peroxidation reactions in the animals. Fresh mature leaves of *A. indica* have been shown to contain quecretin (a poly-phenolic flavonoid), nimbosterol (β-sitosterol), carotenes and ascorbic acid (Jacobson 1990). Thus, the prevention of lipid peroxidation by the leaf extract could be attributed to the antioxidant activities of the flavonoid, carotenes and ascorbic acid present in the leaves.

Alloxan used in this study for the induction of diabetes mellitus is known to mediate the destruction of β-cells by establishing redox cycles resulting in the formation of reactive free radicals which constitute the major inducer agent for the cell damage and hence diabetes in the rats (Szkuldeshi 2001). The alloxan associated radicals and those generated as a result of hyperglycemia by aldose reductase (Ceriello et al. 1992) and AGE-related (Wolff et al. 1991) activities may be responsible for the increased lipid peroxidation observed in the diabetic control rats. Earlier reports have demonstrated elevated serum lipid peroxides levels and diminished antioxidant status in diabetic subjects (Oberley 1988). It is likely that administration of the extract prevents further destruction of β-cells by terminating the progressive generation of reactive hydroperoxyl radicals. This reduction of lipid peroxidation by the extract appeared to allow for cellular repair and regeneration of β-cells which result in production of insulin as may be evident by the reduction of blood glucose, triglyceride and cholesterol in the diabetic treated rats.

Diabetes mellitus and its complications are associated with free radical mediated celluar injury (Asayama et al. 1993; Lee and Chung 1999) and impaired lipid metabolism (Goldberg 2001). This study have shown that ethanolic extract of *A. indica* leaves demonstrated anti-lipid peroxidative, anti-hyperglycemic, and anti-hypercholesterolemic activities and reduced triglyceride level in diabetic rat model, therefore justifying the possibility of using the extract in management of diabetes mellitus and its complications.

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References

Asayama K, Uchida N. Nakane T, Hayashiba H, Dobashi H, Dobashi K, Amemiya S, Kato K, Nakazawa S (1993) Antioxidants in the serum of children with insulin dependent diabetes mellitus. Free Radic Bio Med 15:597-602.

Battel ML, Yuen VG, Verma S, McNeil JH (1999) Other Models of type 1 diabetes. In McNeil JH (ed) Experimental models of diabetes CRC Press LHC. Florida pp. 219-226.

Ceriello A, Quatraro A, Giugliano D (1992) New Insight on non-enzymatic glycosylation may lead to therapeutic approaches for the prevention of the diabetic complications. Diabet Med 9:297-299.

Dixit VP, Sinh R, Tank R (1986) Effect of Neem seed oil on the blood glucose concentration of normal and alloxan diabetic rats. J Ethnopharmacol 17(1):95-98.

Droge W (2002) Free radicals in the physiological control of cell function. Physiol Rev 82:47-52.

Esterbauer H, Schaur RJ, Zollner H (1991) Chemistry and Biochemistry of 4- hydroxynoneral, malondialdehyde and related aldehydes. Free Radic Biol Med 11:81-87.

Frankel EN (1980) Lipid peroxidation. Prog Lipid Res 19:1-22.

Goldberg IJ (2001) Diabetic dyslipidaemia: causes and consequences. J Clin Endocrinol Metab 86(3):965-971.

Gupta S, Kataria M, Gupta PK, Murganandan S, Yashroy RC (2004) Protective role of extracts of neem seeds in diabetes caused by streptozotocin in rats. J Ethnopharmacol 90(2-3):185-9.

Haffner SM, Mykkanen L, Festa A, Burke JP, Stern MP (2000) Insulin resistant prediabetic subjects have more atherogenic risk factors than insulin-sensitive prediabetic subjects: Implication for preventing coronary heart disease during the prediabetic state. Circulation 101:975-980.

Halim EM (2003) Lowering of blood sugar by water extract of Azadirachta indica and Abroma augusta in diabetes rats. Indian J Exp Biol 41(6):636-640.

Hensley K, Robinson KA, Gabbita SP, Salsman S, Floyd RA (2000) Reactive oxygen species, cell signaling, and cell injury. Free Radic Biol Med 28:1456-1461.

Jacobson M (1990) Review of Neem research in the United States. In Proceeding of a workshop on neem potentials, pest management programme USA – ARS, Beltsville, Ad ARS 86:40-44.

Jilial I, Devaraj S (1996) The role of oxidized LDL in atherosclerosis. J Nutri 126:1053s-1057s.

Jilial I, Grundy SM (1992) Influence of antioxidant vitamins on LDL oxidation. Ann NY Acad Sci 669:237-247.

Jilial I, Lena-Veta G, Grundy SM (1990) Physiologic levels of ascorbate inhibit the oxidative modification of LDL. Antherosclerosis 82:185-191.

Kahler W, Kuklinski B, Rublman C, Plotz C (1993) Diabetes mellitus, a free radical-associated disease. Result of adjurant antioxidant supplementation 1. J Gesanite Inn Med 48(4):223-232.

Kausik BI, Ranajit KB (2002) Biological activities and medicinal properties of Neem plant. Current Science 82(11):1336-1345.

Khosla P, Bhanwra S, Singh J, Seth S, Srivastava RK (2000) A study of hypoglycaemic effects of Azadirachta indica (Neem) in normaland alloxan diabetic rabbits. Indian J Physiol Pharmacol 44(1):69-74.

Lee AY, Chung SS (1999) Contributions of Polyol Pathway to oxidative stress in diabetic cataract. FASEB J 13(1):23-30.

Oberley LW (1988) Free radicals and diabetes. Free Radic Biol Med 5: 113-124.

Reardon CA, Getz GS (2001) Mouse models of atherosclerosis. Curr Opin Lipidol 12:167-173.

Saltiel AR, Kahn CR (2001) Insulin signaling and the regulation of glucose and lipid metabolism. Nature 414(6865):799-806.

Sheetz MJ, King GL (2002) Molecular understanding of hyperglycemia's adverse effect for diabetic complications. JAMA 288(20):2579-2588.

Smaoui M, Hummani S, Chaaba R, Affia N, Handa KB, Masmoudi AS, Mahjoub S, Bousslama A, Farhat MB, Hammani M (2004) Lipid and lipoprotein (A) concentrations in Tunisian type 2 diabetic patients: Relationship to glycemic control and coronary heart disease. J Diabetes Complications 18(5):258-263.

Stitt AW, Jenkins AJ, Cooper ME (2002) Advanced glycation end products and diabetic compilations. Expert Opin Invest Drugs 11(9):1205-1223.

Szkudelski T (2001) The mechanisms of alloxan and streptozotocin action in beta-cells of the rat pancreas. Physiol Res 50:536-546.

WHO expert committee on diabetes mellitus. Tech Rep Series No. 646. World Health Organization, Geneva.