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Full Length Research Article

Effect of *Azadirachta indica* leaf extract on serum lipid profile changes in normal and streptozotocin induced diabetic rats

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Abstract

Effects of *Azadirachta indica* leaf extract on serum lipid profile changes in normal and streptozotocin - induced diabetic rats have been studied with a view to elucidate its possible effect on cardiovascular disease induced by hyperglycemia. It was observed that *A. indica* leaf extract significantly reduced the total cholesterol, LDL- and VLDL-cholesterol, triglycerides and total lipids of serum in streptozotocin-induced diabetic rats but HDL-cholesterol levels remained unchanged when compared with streptozotocin- induced diabetic control animals.

Key words:
Azadirachta indica; Streptozotocin induced diabetes; Serum lipid profiles; cardiovascular disease.

INTRODUCTION

Diabetes is one of the major degenerative disease in the world today. It is a major risk factor for the development of cardiovascular disease. About 70-80% of deaths in diabetic patients are due to vascular disease. In particular, hyperglycemia, the primary clinical manifestation of diabetes, is thought to contribute to diabetic complications by altering vascular cellular metabolism, vascular matrix molecules and circulating lipoproteins . For instance hyperglycemia increases diacylglycerol levels and activates protein kinase C activity in the aorta of streptozotocin (stz.) induced diabetic rats (Inoguchi *et al*, 1994)) and dogs (Xia P *et al*, 1994) . Thickening of the basement membranes in renal glomeruli and peripheral capillaries has been observed in stz. induced diabetic rats (Olgemoller *et al*, 1993) and hyperlipidemia is a feature of drug induced diabetes in rats (Still *et al*, 1964) and rabbits (Nordestgaard *et al*, 1988; Miller and Wilson, 1984).

Many minor components of foods, such as secondary plant metabolites, have been shown to alter biological processes which may reduce the risk of chronic diseases in humans. *Azadirachta indica* (Meliaceae) popularly known as neem is an indigenous plant widely available in India and Burma. Different parts of this plant have been reported to have antiseptic, wound healing and skin disease curing activity (Kirtikar and Basu, 1975; Biswas *et al*, 2002) . Studies conducted in our laboratory reveals that water soluble portion of alcoholic extract of leaves of *Azadirachta indica* possesses significant antiinflammatory, antiserotonin, antifertility and hepatoprotective activity (Chattopadhyay R.R.*et al*, 1987a, 1986, 1993, 1992). Significant hypolipidemic activity in rats fed on atherogenic diet and antihyperglycemic as well as hypotensive activity have also been repoted by us (Chattopadhyay R.R., 1995, 1997 ; Chattopadhyay R.R. *et al*, 1987b). Significant blood sugar lowering effect of *A. indica* in alloxan and streptozotocin induced diabetic rats have also been reported by several workers (Dixit *et al*, 1978; Murty *et al*, 1978; Pillai *et al*, 1984; Sukla *et al*, 1973). It is well documented that cardiovascular disease induced by hyperglycemia is associated with alterations in serum lipid profiles (Laakso M, 1996; Steiner G, 1999; Massing *et al*, 2001). In this study, we investigated the effects of *A. indica* leaf extract on serum lipid profile changes in normal and stz induced diabetic rats with a view to

finding out its possible effect on cardiovascular disease induced by hyperglycemia.

MATERIALS AND METHODS

Collection of plant material: Fresh Matured leaves of *A. indica* were collected from our Institute's (Indian Statistical Institute, Kolkata, India) garden and were identified by a pharmacognosy expert. At the time of collection standard herbariunm record sheets were completed with the name of the collector, collection number, date, locality and local name.

Extraction of plant material:
Air-dried powder (1 kg) of fresh matured *A. indica* leaves were extracted by percolation at room temperature with 70% EtOH. Leaf extract of *A. indica* was concentrated under reduced pressure (bath temp. 50^{o} C) and finally dried in a vacuum desiccator. The residue was dissolved in distilled water and filtered. The filtrate was evaporated to dryness. The dried mass (yield = 50.2 g) was suitably diluted with normal saline and used in experiments.

Animals: Male albino rats of Wistar strain (100-150g; 4-6 weeks old) were maintained under controlled conditions of light (12h/24h) and teperature (23 $\pm$ 1^{o}C). Food pellets (Hindustan Lever Ltd., India) and tap water were provided *ad libitum* .

Development of induced diabetes and assessment of changes in serum lipid profile: Following four groups of rats, six rats in each group were taken.
Group I : Normal Control
Group II: Stz. induced diabetic control
Group III: Stz. induced diabetic Control + *A. indica*
Group IV: Normal rats treated with *A. indica* leaf extract

Diabetes was induced in Grp II and Grp. III animals by intraperitoneal injection of streptozotocin (50 mg/kg body weight) dissolved in 0.01M citrate buffer (pH 4.5). 72h after streptozotocin injection diabetes was confirmed in rats showing blood sugar level 262 $\pm$ 17 mg/dl. *A.indica* leaf extract at a fixed dose (500 mg/kg, po), which was found to be the working dose from our previous experiments was fed daily to stable hyperglycemic (Gr. III) rats (72h after stz. injection) for 7 days consecutively. The normal control (Gr. I) and stz. induced diabetic control (Gr. II) animals received normal saline in place of leaf extract. Gr.IV animals were treated with *A. indica* leaf extract (500 mg/kg,po) alone for consequitive 7 days. Animals from each group were deprived of food overnight but with free access of water before taking the samples of blood. Blood samples were collected at the end of 7 days of *A. indica* treatment from orbital plexus by pricking a needle. Serum was separated quickly for estimation of serum lipid profiles. Total cholesterol, total lipids , and triglycerides of serum were estimated using standard methods (Zlatkis *et al*, 19533; Folch *et al*, 1957; Varley, 1988). HDL-cholesterol was determined by phosphotungstate/magnesium method (Burstein *et al*, 1970). VLDL-cholesterol was calculated as triglycerides/5 and LDL-cholesterol was calculated by the equation :

LDL-cholesterol= Total serum cholesterol – (HDL + VLDL).

Statistical analysis: Results were statistically analysed by analysis of variance (F-test) and multiple comparison procedure (Woolson, 1987). Significance between the groups were estimated using Student's t-test.

RESULTS

Table 1 shows the results of total cholesterol, total lipids, triglycerides and fractions of cholesterol of serum of Gr. I, II, III and IV animals. It was observed that the levels of total cholesterol, total lipids , triglycerides and fractions of cholesterol except HDL-cholesterol were significantly higher in case of stz induced hyperglycemic animals (Gr. II) when compared with normal control (Gr. I) animals. On the other hand values of above mentioned serum lipid parameters were near to normal in case of animals receiving both *A. indica* leaf extract and streptozotocin (Gr. III Vs. Gr. II). But HDL-cholesterol levels remained unchanged. It was also observed that *A. indica* leaf extract failed to alter the levels of serum lipid profiles significantly of normal rats (Gr. IV Vs. Gr. I).

DISCUSSION

Premature and extensive atherosclerosis involving renal, peripheral and cardiovascular sites remain major complications of diabetes mellitus. In addition to hyperglycemia, systemic or local elevations in insulin may contribute to aberrant lipid metabolism and vascular wall function (Lyons, 1992).

Since alteration in serum lipid profiles are known in diabetics, which are likely to increase the risk of coronary heart disease (Laakso, 1996; Steiner, 1999; Massing *et al*, 2001) , a reduction in serum lipids, particularly LDL and VLDL fractions and triglycerides levels should be considered as beneficial in the long-term prognosis of these patients.

Table 1.
Effect of *A. indica* leaf extract on serum lipids in normal and induced diabetic rats

Group	Serum lipid profiles (mg/dl)					
	Total **Cholesterol**	Total **lipids**	Tri-**glycerides**	HDL **cholesterol**	LDL **cholesterol**	VLDL **cholesterol**
I	90.00 ± 5.17	148 ±5.74	112.00 ±6.42	24.00 ±2.92	44.00 ± 4.12	22.00 ±1.84
II	128.00 ± 6.82[b]	187.00 ±6.88[b]	141.00±7.34[a]	21.00 ±2.38	77.00 ± 5.76[b]	29.00 ±2.14[a]
III	94.00 ±3.24[d]	157.00 ±5.47[d]	109.00 ±6.37[d]	24.00 ±3.17	47.00 ±3.75[c]	21.00 ±2.06[c]
IV	92.00 ± 3.74	146.00 ±6.14	110.00 ±5.61	23.00 ±2.47	46.00 ±3.64	22.00 ±1.92

Values are mean of six observations ± S.E.M.
[a]P < 0.05 when compared with normal control animals (Gr.II Vs. Gr. I)
[b]P < 0.01 when compared with normal control animals (Gr. II Vs.Gr. I)
[c]P < 0.05 when compared with stz. induced diabetic animals (Gr. III Vs.Gr. II)
[d]P < 0.01 when compared with stz. induced diabetic animals (Gr.III Vs.Gr. II)
Gr. IV Vs. Gr. I : Not Significant.

Chemical analysis revealed that A. *indica* leaf extract contains following six compounds: Quercetin-3-O-B-D-glucoside, Myricetin-3-O-rutinoside, Quercetin-3-O-rutinoside, Kaempferol-3-O-rutinoside, Kaempferol-3-O-B-D-glucoside, Quercetin-3-O- L-rhamnoside (Chattopadhyay, 1999).

It is presumed that these compounds either wholly or partly may be responsible for antihyperlipidemic activity. Thus, it can be concluded from our findings that the levels of total serum cholesterol, triglycerides, total lipids, VLDL and LDL-cholesterol which are actually raised in diabetes can be lowered with *A. indica* leaf extract. Moreover, its antihyperlipidemic effect could represent a protective mechanism against the development of atherosclerosis.

Thus, *A. indica* leaf extract may be helpful in controlling the development of hyperlipidemia as well as atherosclerosis in diabetic subjects in view of its antihyperlipidemic activity. Further studies both on the extract and/or its chemical constituents are needed to pinpoint the findings. This report may serve as a footstep on this aspect.

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