

ORIGINAL ARTICLE

CLINICAL INVESTIGATION OF HYPOGLYCEMIC EFFECT OF SEEDS OF *AZADIRACHTA-INDICA* IN TYPE-2 (NIDDM) DIABETES MELLITUS

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ABSTRACT

The present study was designed to investigate clinically the hypoglycemic effect of seeds of *Azadirachta indica* in Type-2 diabetes mellitus. After assaying fasting plasma and urinary glucose, 10 patients of type-2 diabetes mellitus with no previous medication, 10 patients of type-2 diabetes mellitus taking oral hypoglycemic agents with history of inadequate control and six control subjects were given low (0.5 g tid) and high (2 g tid) doses of powdered part, aqueous extract and alcoholic extract of *Azadirachta indica* for 14 days. On 15th day blood and urine samples for glucose were taken. Based on results obtained it was found that *Azadirachta indica* has significant hypoglycemic activity in high dose and can be successfully combined with oral hypoglycemic agents in type-2 diabetic patients whose diabetes is not controlled by these agents.

Keywords: Type-2 diabetes mellitus, *Azadirachta indica*, hypoglycemic agents, hypoglycemic activity.

INTRODUCTION

This tree, in Sanskrit, Nimba and Arishta, is a native of India, and is cultivated in all parts of the Subcontinent on account of its medicinal properties. The leaves, bark and other products of Neem have been articles of the Indian materia medica since antiquity and are mentioned in the Ayurveda of Sushruta. Chakradatta recommends a poultice of the leaves mixed with sesamum seeds for unhealthy ulcerations. The fruit is described as purgative, emollient, and anthelmintic. Oil of the seeds is applied to suppurating scrofulous glands, given in leprosy, rheumatism, and a variety of diseases. A twig with leaves used like a hand fan is supposed to bring about cure of syphilis. According to Al-Biruni and Razi, the substitute for *Azadirachta* leaves are hemp leaves. It is just possible that the herb may be a hair growth promoter, as it is so reputed. This useful tree naturally attracted the attention of the Arabs upon arrival in the Subcontinent and the tree was named as *Azad-daracht* Hindi from its resemblance to *Melia azedarach* (or the Persian lilac-Bakayan). Neem as isolated tree is cultivated in grave yards to keep the atmosphere clean. Its leaves have been used to keep the insects away and its oil is considered as one of the best natural phago-deterrents especially for crop insects. It is also considered as good antifertility agent in application and world-wide natural insecticide or at least insect-repellant. *Melia azadirachta* Linn has been taken as synonym for *Azadirachta indica* (L) A. Juss. in Flora of West Pakistan (1972). The various parts of the plant used medicinally are fruit, seed, seed oil, leaves, bark, fruit and sap (Ruskin 1993; Said *et al.*, 1996). In the present

investigation, the effect of powdered, aqueous and alcoholic extracts of seeds of *Azadirachta indica* were investigated clinically in type-2 diabetic patients.

MATERIALS AND METHODS

The present study was carried out in the laboratories of Hamdard Institute of Pharmaceutical Sciences, Islamabad and Army Medical College, Rawalpindi. The following criteria was used to include or exclude the patients in the study.

Inclusion criteria

1. Type 2 diabetic patients with fasting plasma glucose level equal to or greater than 140 mg/dl of blood (WHO study group on diabetes mellitus, 1985, Geneva technical report series 727) (WHO, 1985) with out any detectable/visible complications.
2. Type 2 diabetic patients taking oral hypoglycaemic agents with history of inadequate control of blood glucose with these agents.
3. Normal healthy subjects with no family history of diabetes mellitus.
4. The patients and control subjects were of either sex (male or female) between the ages of 35-60 years.

Exclusion criteria

1. Pregnant or nursing patients.
2. Smokers
3. Patients with GIT, hepatic, cardiovascular, renal or endocrine disorder (other than diabetes mellitus) which

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can interfere with the absorption, metabolism and excretion of the study plant.

4. Patients with any complication of diabetes mellitus.
5. Patients suffering from type 1 (IDDM) diabetes mellitus.

Subjects

The selected subjects (patients and controls) were medically examined and given code numbers and were asked to present themselves on a specified date for sample collection. They were requested to come with fasting (no food before 12 hours) and to void their morning urine and to drink 250ml glass of water before coming for testing (Bahajiri *et al.*, 2000). Patients already taking oral hypoglycaemic agents were requested to take their usual medicine and food after sampling.

Blood sample

Blood samples (3-5 ml) were drawn from each patient and control subject by vene-puncture through plastic disposable syringes. The blood samples were collected in clean oven dried glass bottles which were previously rinsed with 1% sodium fluoride, 3% potassium oxalate solution to prevent coagulation and glycolysis. The plasma was separated after centrifugation. Any sample showing haemolysis was discarded. After separation of plasma, it was transferred to clean, previously acid rinsed, washed and oven dried glass bottles with plastic caps. The plasma glucose estimation was done immediately on the same day by kit method (Trinder, 1969).

Plant

Azadirachta indica seeds were purchased from the local market. Dr. Mir Ajab Khan department of biological sciences Quaid-i-Azam University Islamabad identified the selected plant. The seeds were shade dried, pulverised by a mechanical grinder and passed through 40-mesh sieve. Filtrate from powdered samples of plants soaked overnight in 95% ethanol and distilled water, were used as alcoholic and aqueous extract respectively for studied plant.

Aqueous extract

After grinding in an electric grinder, the powder was soaked in equal amount of water and stirred intermittently and then left overnight. The macerated pulp was then filtered through a coarse sieve and the filtrate was dried at reduced temperature. This dry mass served as aqueous extract of respective plant for experimentation (Vats *et al.*, 2002).

Alcoholic extract

Alcoholic extract was prepared by powdering 1 kg plant material in an electric grinder. The powder was then mixed with 500 ml of alcohol and kept at room temperature for 36 hours. The slurry was stirred intermittently for 2 hours and left overnight. The mixture was then filtered and the filtrate was freed from solvent under partial vacuum (71 mmHg) at

35-45°C to yield pulp. A few drops of silicon emulsion were added near the end of distillation to avoid frothing. The final residue collected was a thick paste. This was dried at reduced temperature. This dried mass served as alcoholic extract for experimentation (Vats *et al.*, 2002).

General Plan of Study

The fasting blood and urine samples from patients and control subjects were assayed for respective glucose levels. 10 patients of type-2 diabetes mellitus with no previous medication and 10 type-2 diabetic patients taking oral hypoglycaemic agents (with history of inadequate control) were given dry powdered seeds of *Azadirachta indica* for fourteen days. On 15th day blood and urine samples of glucose were taken.

After an interval of one-week fasting blood and urine samples were again taken from these patients. These patients were given aqueous extract of *Azadirachta indica* seeds for 14 days. On 15th day blood and urinary samples of glucose were taken. After an interval of one-week fasting blood and urine samples for the monitoring of glucose level were again taken from these patients. These patients were given alcoholic extract of the *Azadirachta indica* seeds for 14 days. On 15th day blood and urinary samples of glucose were taken.

Out of the ten patients five received low dose 0.5 g tid and five received high dose 2 g tid of the plant. Six healthy subjects were kept as control. Three subjects received low dose and three subjects received high dose of powdered, aqueous and alcoholic extracts of *Azadirachta indica* seeds as described above. All the patients and control subjects were monitored for any adverse reaction of the plant. Plasma assay of glucose was done by kit method and urinary glucose was estimated by strip method (Burtis *et al.*, 1998). The data was statistically evaluated (Glazer, 1995).

RESULTS

The results are summarized in the table and graph.

Untoward effects

Mild headache initially was complained by some patients belonging to all the groups. The basis of headache was psychological.

DISCUSSION

Diabetes mellitus has been defined as a syndrome of abnormal carbohydrate metabolism, resulting in hyperglycaemia with acute metabolic complications and chronic vascular, neurogenic and orthopedic complications affecting many organs of the body (Jamil *et al.*, 2001). A significant decrease was noted in mean concentration of plasma glucose two weeks after administration of high (2 g t.i.d)

Table showing effects of dry powdered part, aqueous extract and alcoholic extract of seeds of *Azadirachta indica* on blood and urinary glucose in type-2 diabetic patients with no previous medication, taking oral hypoglycemic agents with history of inadequate control and control subjects before and two weeks after administration. The values are given as Mean $\pm$ SD (n=10+10+6)

	Blood glucose (mg/dl)		Urinary Glucose	
	Before Administration	After Administration	Before Administration	After Administration
Dry Powdered Part				
No previous medication				
Low dose	209.8 $\pm$ 13.27	195.2 $\pm$ 9.14*	Glycosuria	Glycosuria
High dose	207.6 $\pm$ 16.09	124.2 $\pm$ 9.75*	Glycosuria	No glycosuria
Taking oral hypoglycemics				
Low dose	156.6 $\pm$ 12.13	148 $\pm$ 8.80*	No glycosuria	No glycosuria
High dose	153.6 $\pm$ 7.50	117.8 $\pm$ 8.75*	No glycosuria	No glycosuria
Control				
Low dose	88	81 ^{NS}	No glycosuria	No glycosuria
High dose	64	72 ^{NS}	No glycosuria	No glycosuria
Aqueous Extract				
No previous medication				
Low dose	209.4 $\pm$ 22.46	172.8 $\pm$ 4.14*	Glycosuria	No glycosuria
High dose	203.2 $\pm$ 20.14	127.4 $\pm$ 12.42*	Glycosuria	No glycosuria
Taking oral hypoglycemics				
Low dose	157.2 $\pm$ 7.25	146 $\pm$ 4.84*	No glycosuria	No glycosuria
High dose	154.6 $\pm$ 11.54	118 $\pm$ 12.82*	No glycosuria	No glycosuria
Control				
Low dose	80	84 ^{NS}	No glycosuria	No glycosuria
High dose	76	72 ^{NS}	No glycosuria	No glycosuria
Alcoholic Extract				
No previous medication				
Low dose	216 $\pm$ 22.96	196.6 $\pm$ 12.66*	Glycosuria	Glycosuria
High dose	206.2 $\pm$ 15.80	133.4 $\pm$ 3.97*	Glycosuria	No glycosuria
Taking oral hypoglycemics				
Low dose	198.8 $\pm$ 9.95	190 $\pm$ 9.13*	Glycosuria	Glycosuria
High dose	200.4 $\pm$ 14.29	128.8 $\pm$ 6.72*	Glycosuria	No glycosuria
Control				
Low dose	80	70 ^{NS}	No glycosuria	No glycosuria
High dose	72	68 ^{NS}	No glycosuria	No glycosuria

Low dose: 0.5 g tid, High dose: 2 g tid, *Significant, NS: Non significant

dose of powdered part, aqueous extract and alcoholic extract of *Azadirachta indica*. The fall in mean concentration of plasma glucose was more marked in patients taking oral hypoglycaemic agents with history of inadequate control. With addition of high dose of *Azadirachta indica* their plasma glucose fell within the normal controlled range of diabetes mellitus. Glycosuria disappeared two weeks after administration of high dose of seeds of *Azadirachta indica* while low dose did not have any effect on glycosuria. These results are related with those of Rahman & Zaman (1989) who reported the hypoglycaemic activity of leaves of *Azadirachta indica* in their review article about antidiabetic

activity of medicinal plants. In other study, the *Azadirachta indica* leaf extract was found to block significantly the inhibitory effect of serotonin on insulin secretion mediated by glucose (Chattopadhyay, 1999).

Mechanism of action

Hypoglycaemic mechanism of action of *Azadirachta indica* leaf extract is thought to be due to increase glucose mediated secretion of insulin.¹⁰ *Azadirachta indica* seeds exert only an insulin releasing effect on the beta cells of pancreas in type-2 diabetic patients (Akhtar, 1995).

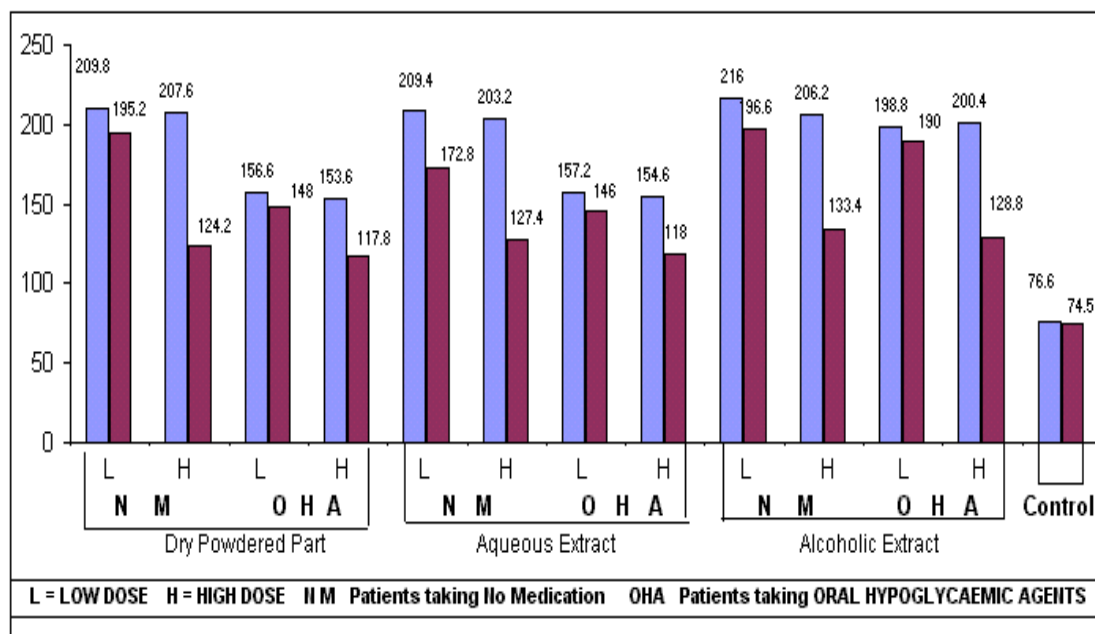


Figure: Comparison of effect on blood glucose in type-2 diabetic patients and control subjects before and two weeks after administration of dry powder part, aqueous and alcoholic extract of seeds of *Azadirachta indica*.

CONCLUSION

Azadirachta indica seeds can be combined in high dose with oral hypoglycaemic agents to bring the blood glucose to normal levels in patients whose diabetes is not controlled with these agents or in those patients in whom these drugs produce adverse effects on dose increments.

REFERENCES

- Akhtar MS (1995). Efficacy of some indigenous medicinal plants in diabetic patients. Proceedings of the 2nd Annual national symposium on health care and social development, the Aga Khan University Karachi, Pakistan, pp.232-236.
- Bahajiri SM, Mirza SA, Mufti AM and Ajabnoor MA (2000). The effects of inorganic chromium and brewer's yeast supplementation on glucose tolerance, serum lipids and drug dosage in individuals with type-2 diabetes. *Saudi Med. J.*, **21**(9): 831-837.
- Burtis CA and Ashwood ER (Editors) (1998). Tietz text book of clinical chemistry, 3rd Ed, Indira printers, New Delhi, India (W.B. Saunders Co and Harcourt Brace & Co Asia PTE LTD): 783.
- Chattopadhyay RR (1999). Possible mechanism of antihyperglycaemic effect of *Azadirachta indica* leaf extract: part v. *J. Ethno. Pharmacol.*, **67**(3): 373-6.
- Glazer AN (1995). High-Yield™ Biostatistics. Williams & Wilkins, Philadelphia, USA, 19-44.
- Jamil M, Amin Z, Chaudhry TH, Shaheen J and Ali ZR (2001). Management of diabetic foot infections. *JCPSP*, **11**(10): 6060-610.
- Rehman A and Zaman K (1989). Medicinal plants with hypoglycaemic activity. *J. Ethnopharmacol*, **26**: 1-55.
- Ruskin FR editor (1993). Neem: A tree for solving global problems. Report of an adhoc panel of the board on Science and Technology for International Development: National Research Council. National Academy Press (2nd Printing) Washington DC.
- Said M, Saeed A, D' Silva LA, Zubairi N and Bano Z (1996). Medicinal herbal. Research publication of Bait Al-Hikmah at Madinat Al-Hikmah, Karachi; **1**: 39-41.
- Trinder P (1969). Enzymatic determination of plasma glucose. *Ann. Clin. Biochem.*, **6**: 24.
- Vats V, Grover JK and Rathi SS (2002). Evaluation of anti-hyperglycemic and hypoglycemic effect of *Trigonella foenum-graecum* Linn, *Ocimum sanctum* Linn and *Pterocarpus marsupium* Linn in normal and alloxanized diabetic rats. *J. Ethno. Pharmacol*, **79**: 95-100.
- World Health Organization. (1985). Diabetes mellitus, report of study group. Geneva: WHO technical report series; 727.

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