

## A STUDY OF HYPOGLYCAEMIC EFFECTS OF *AZADIRACHTA INDICA* (NEEM) IN NORMAL AND ALLOXAN DIABETIC RABBITS

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**Abstract :** Hypoglycaemic effect was observed with *Azadirachta indica* when given as a leaf extract and seed oil, in normal as well as diabetic rabbits. The effect, however, was more pronounced in diabetic animals in which administration for 4 weeks after alloxan induced diabetes, significantly reduced blood glucose levels. Hypoglycaemic effect was comparable to that of glibenclamide. Pretreatment with *A. indica* leaf extract or seed oil administration, started 2 weeks prior to alloxan, partially prevented the rise in blood glucose levels as compared to control diabetic animals. The data suggests that *A. indica* could be of benefit in diabetes mellitus in controlling the blood sugar or may also be helpful in preventing or delaying the onset of the disease.

**Key words :** *Azadirachta indica*  
alloxan diabetes

hypoglycaemic effect  
blood glucose

### INTRODUCTION

In recent years, herbs are being effectively tried in a variety of pathophysiological states. *Azadirachta indica* (Neem) is one of them. It is a large evergreen tree which freely grows all over India. It has been used in traditional medicine for its several medicinal properties. Isolated studies have reported anti-inflammatory, immunostimulant and hypoglycaemic effects of *A. indica* leaf extract (1-3). Seed oil has been shown to have antifertility, hypoglycaemic and antibacterial properties (4-6).

*A. indica* leaf extract has been reported to lower blood glucose in normal rats by Sen et al (3). However, they have reported a marginal increase in blood glucose levels in restraint stress group of rats. Murty et al (7) have shown a decrease in blood glucose when leaf extract was given intravenously in a dose of 0.15 ml/kg in dogs. On the other hand, Pillai and Santhakumari (5) have shown that decoction of tender leaves failed to produce any significant effect on blood glucose levels in fasting rabbits at the three dose levels used i.e. 1, 5, 10 ml/kg (1 g leaves/ml decoction), while *A. indica* seed oil showed

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significant reduction in blood glucose at a lower dose 2.5 ml/kg but percentage reduction in blood glucose was less with a higher dose.

The aim of the present study was to investigate the effects of *A. indica* given in two forms i.e. leaf extract and seed oil on blood glucose in normal and alloxan diabetic rabbits given before as well as after establishment of diabetes to evaluate its role as a therapeutic agent and to see its influence, if any, on prevention of the disease.

## METHODS

### A. *indica* leaf extract

**Method of preparation :** One kg of freshly collected, shade dried, powdered leaves of *A. indica* were ground in 4 litres of distilled water and allowed to soak overnight. The suspension was centrifuged at 5000 rpm for 20 min and filtered through a Whatman No. 1 filter paper. The supernatant fluid was allowed to evaporate in sterile, glass petri dishes under tubelight. When completely dry, the extract was collected by scraping and stored. Stock solution of aqueous extract was prepared by dissolving 500 mg of the extract in 5 ml of distilled water (8).

**A. *indica* seed oil:** *A. indica* seed oil (Unjha Ayurvedic Pharmacy, Gujrat) was procured from the market from an Ayurvedic Drug's Stockist and oil of the same Company was used throughout the study period to avoid any variation.

**Dosage :** *A. indica* leaf extract was given in a dose of 500 mg/kg and seed oil 5 ml/kg, by

an intragastric tube (dosages were selected from the literature and after doing the pilot study).

**Animal experiments:** Albino rabbits of both sexes, weighing 1.5-2.0 kg were used. Animals were allowed standard diet and tap water *ad libitum*. The animals were divided into groups A and B. Group A consisted of 14 normal rabbits. They were further divided into 2 subgroups of 7 each ( $A_1$ ,  $A_2$ ). Subgroup  $A_1$  and  $A_2$  were given *A. indica* leaf extract and seed oil respectively, daily for 4 weeks.

Blood samples were collected initially (control) and after 5 hours, 1, 2 and 4 weeks of drug administration. Group B consisted of 42 diabetic rabbits. They were divided into six subgroups of 7 each ( $B_1$ - $B_6$ ). These were made diabetic by injecting alloxan as single dose of 140 mg/kg body weight, intravenously (9). Rabbits having blood glucose levels >200 mg/100 ml after 3 days of alloxan administration were included in the study.

Subgroup  $B_1$  - Served as control.

Subgroup  $B_2$  - Was given *A. indica* extract daily for 2 weeks before and 4 weeks after alloxan administration.

Subgroup  $B_3$  - Was given seed oil daily for 2 weeks before and 4 weeks after alloxan administration.

Subgroup  $B_4$  - In diabetic rabbits, *A. indica* leaf extract was given daily for 4 weeks.

Subgroup  $B_5$  - In diabetic rabbits, seed oil was given daily for 4 weeks.

Subgroup B<sub>6</sub>— In diabetic rabbits, glibenclamide (40 µg/kg) was given daily, orally, for 4 weeks as standard drug for comparison.

Blood samples were collected for estimation of blood glucose initially, after 3 days of alloxan administration and then after 1, 2 and 4 weeks.

**Collection of blood sample:** Blood samples were collected from the marginal ear vein of rabbits, in a test tube containing heparin 20 U/ml and sodium fluoride 2 mg/ml.

**Blood sugar estimation:** Plasma was separated by centrifuging the tubes at 3000 rpm. Plasma glucose was estimated by glucose oxidase/peroxidase method as described by Trinder (10).

**Statistical analysis:** Results are expressed as mean ± SE and Student's 't' test was used to check their significance. A value of P<0.05 was taken as significant.

## RESULTS

*A. indica* leaf extract produced a significant decrease in blood glucose levels

in normal rabbits. The reduction observed was gradual and the maximum fall was observed at 4 weeks i.e. at the end of study period. Similar effects were observed with *A. indica* seed oil (Table I).

Leaf extract was, however, slightly more than seed oil. The percentage fall in blood glucose was 29% and 34% after 2 and 4 weeks of *A. indica* leaf extract administration whereas it was 25% and 32% after seed oil.

In diabetic rabbits, where the leaf extract was given 2 weeks prior to alloxan administration (B<sub>2</sub>), it prevented the rise in blood glucose levels since the rise in levels was less as compared to control diabetic rabbits (B<sub>1</sub>). Similarly, seed oil pretreatment (B<sub>3</sub>) prevented the rise in blood glucose levels (Table II, Fig. 1).

In another series of experiments in diabetic rabbits (B<sub>4</sub> and B<sub>5</sub>) where the drug administration was started after 3 days of alloxan administration, a significant fall in the blood glucose levels was observed with *A. indica* leaf extract and seed oil. The percentage fall observed was 47% with leaf extract and 44% with seed oil. Effects were comparable to that of glibenclamide given in Group B<sub>6</sub> (Table II, Fig. 2).

TABLE I: Effect of *A. indica* leaf extract (500 mg/kg, po) and seed oil (5 ml/kg, po) on blood glucose levels in normal rabbits.

Groups	Drugs	Blood glucose (mg/100 ml)				
		Control	After 5 hours	1 week	2 weeks	4 weeks
A <sub>1</sub>	LE for 4 weeks	88.57±0.94	72.28±0.96*	67.28±1.55*	62.82±1.52*	57.85±1.20*
A <sub>2</sub>	SO for 4 weeks	91.57±0.69	75.42±0.67*	72.72±1.33*	68.00±1.27*	63.00±0.96*

Values are mean ± SE; n=7;  
\*P<0.001 when compared with control;  
LE-leaf extract, SO-seed oil.

TABLE II: Effect of *A. indica* leaf extract (500 mg/kg, po) and seed oil (5 ml/kg, po) on blood glucose levels in alloxan diabetic rabbits.

Groups	Drugs	Blood glucose (mg/100 ml)				
		Initial	After 3 days of alloxan	After treatment		
				1 week	2 weeks	4 weeks
B <sub>1</sub>	Control	88.43±1.14	281.71±6.31	307.14±5.51	246.42±4.26	172.85±1.50
B <sub>2</sub>	LE, 2 weeks prior & 4 weeks after ALLO	90.14±0.79	190.00±3.15*	198.00±3.42*	170.00±1.40*	119.42±2.22*
B <sub>3</sub>	SO, 2 weeks prior & 4 weeks after ALLO	88.57±0.64	195.50±3.42*	202.00±1.34*	165.00±3.30*	112.42±3.32*
B <sub>4</sub>	LE for 4 weeks after ALLO	90.71±0.62	285.00±2.63	265.57±3.77*	188.00±3.15*	149.14±3.13*
B <sub>5</sub>	SO for 4 weeks after ALLO	91.28±1.17	275.50±0.52	255.00±3.03*	191.14±3.42*	152.57±2.43*
B <sub>6</sub>	Glibenclamide for 4 weeks after ALLO	89.57±1.20	270.40±1.12	251.85±4.01*	188.85±0.71*	145.42±4.05*

Values are mean ± SE; n=7;

\*P<0.001 when compared with control;  
LE-leaf extract, SO-seed oil, ALLO-alloxan.

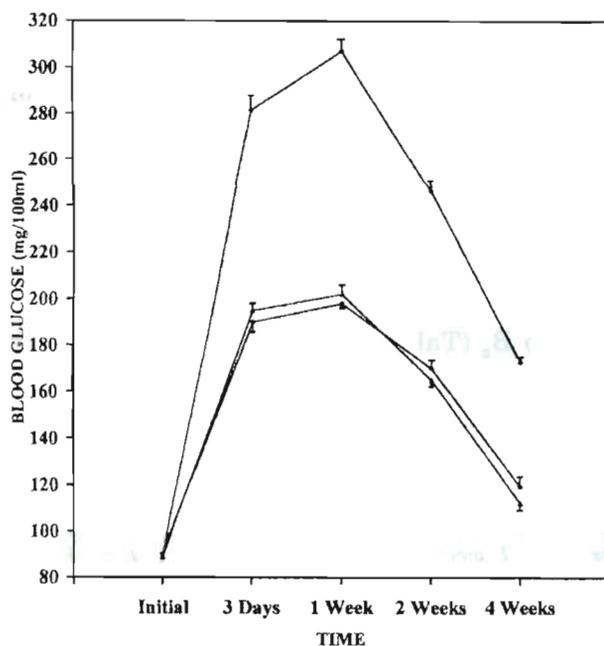


Fig. 1: Effect of *A. indica* leaf extract (●) and seed oil (▲) given 2 weeks before and 4 weeks after alloxan on blood glucose levels [Control diabetic (◆)].

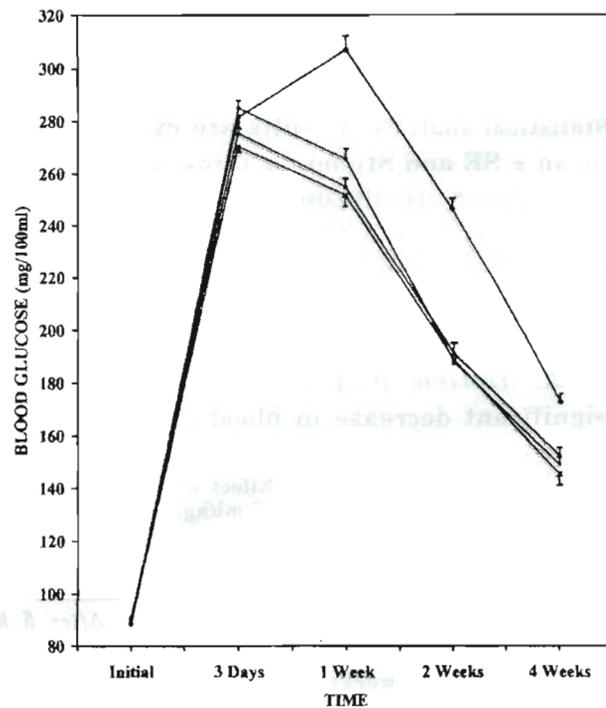


Fig. 2: Effect of *A. indica* leaf extract (■), seed oil (▲) and glibenclamide (×) on blood glucose levels in alloxan induced diabetic rabbits [Control diabetic (◆)].

## DISCUSSION

The present study was conducted to study the hypoglycaemic effects of *A. indica* in normal as well as alloxan diabetic rabbits when given in the form of leaf extract and seed oil.

This study shows that *A. indica* leaf extract produces a marked decrease in blood glucose in normal as well as alloxan diabetic rabbits. Our findings are in agreement with those reported by Murty et al in dogs (7). The hypoglycaemic effect of *A. indica* leaf extract increased gradually and was observed to be maximum at the end of the study period i.e. 4 weeks. The effect was observed both in normal and the hyperglycaemic rabbits (Table I, II; Fig. 1, 2). However, the percentage fall in blood glucose levels in normal rabbits was 34% while in hyperglycaemic rabbits, it was 47% after 4 weeks of extract administration.

Similar results were observed with *A. indica* seed oil administration. Our results are supported by some earlier reports which show the hypoglycaemic effect of *A. indica* seed oil in both the normal and hyperglycaemic animals (11-14). The percentage fall in blood glucose levels in normal rabbits was 32% while in hyperglycaemic rabbits, it was 44% after 4 weeks of seed oil administration in our study.

It is evident from this study that both *A. indica* leaf extract and seed oil have a stronger hypoglycaemic effect in hyperglycaemic as compared to normal rabbits. The data shows that both leaf extract and seed oil could have therapeutic usefulness in disease states where blood glucose levels are high like diabetes mellitus.

However, *A. indica* oil was found to have slightly less hypoglycaemic effect as compared to the extract. Moreover, the oil is difficult to take orally because it is unpalatable and it has a disagreeable odour. For this reason, the oil probably is not a suitable preparation for clinical use. It seems that the recommended formulation for use should be *A. indica* leaf extract.

In order to see any preventive effect of *A. indica* on the rise of blood glucose the leaf extract and the oil were given before producing experimental diabetes. It was interesting to note a marked attenuating effect, since a much less significant rise in blood glucose was observed as compared to the control diabetic animals. Our observations indicate a preventive role in the rabbits pretreated with *A. indica* leaf extract and seed oil before the induction of diabetes where they have shown reduction in the severity of onset of experimental diabetes and improvement in the diabetic status when followed by leaf extract and seed oil administration. This type of effect could be of use in the prevention of diabetic states since the interventions made at the earliest sign of abnormal glucose metabolism may prevent or delay the onset of the disease.

The hypoglycaemic effect of *A. indica* may be due to increased release of insulin from beta cells of pancreas similar to that observed after sulphonylureas administration. Sulphonylureas, however, do not decrease blood glucose in alloxan diabetic animals (15). In contrast, parenteral administration of insulin is well known to produce hypoglycaemia in normal as well as alloxan diabetic rabbits (16). In our study with *A. indica*, the fall in blood glucose was

observed in normal as well as alloxan diabetic rabbits. This would indicate that mechanism of hypoglycaemia produced by *A. indica* may be similar to that of insulin. As suggested by Sharma et al (12), the hypoglycaemic action of *A. indica* may partly be due to extrapancreatic sites of action i.e. by increased peripheral glucose utilization or by direct metabolic effect on tissues particularly on liver.

In conclusion, the data in our study suggests that *A. indica* may have beneficial effects in established diabetes mellitus, and it may also delay or prevent the onset of the disease. *A. indica* thus holds the hope of a new generation of drugs. However, there is need for further studies on experimental animals and human beings with the various active principles to establish its usefulness and exact mode of action.

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