ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation of the *In Vivo* Hypoglycemic Effect of Neem (*Azadirachta Indica* A. Juss) Fruit Aqueous Extract in Normoglycemic Rabbits

A Vasudeva Rao*, V Ragha Sudha Madhuri and Y Rajendra Prasad

Pharmaceutical Chemistry Division, A U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530003, Andhra Pradesh, India.

ABSTRACT

Diabetes mellitus (DM), both insulin-dependent DM (IDDM) and non-insulin dependent DM (NIDDM) is a common and serious metabolic disorder throughout the world. Traditional plant treatments have been used throughout the world for the therapy of diabetes mellitus. Among many medications and other alternative medicines, several herbs have been known to cure and control diabetes; additionally they have no side effects. The aim of the present study was to evaluate the hypoglycemic activity of Fruit Aqueous Extract (FAE) of *Azadirachta indica* in rabbits. The effect of oral administration of FAE of *Azadirachta indica* on blood glucose levels was studied. The FAE of *Azadirachta indica* (500 mg/kg, oral) reduced the normal blood glucose levels in normoglycemic rabbits.
Keywords: *Azadirachta indica*, hypoglycemic activity.

Corresponding author
Email: vasudevaraoavupati@gmail.com

ISSN: 0975-8585

INTRODUCTION

Diabetes mellitus is a common and very prevalent disease affecting the citizens of both developed and developing countries. It is estimated that 25% of the world population is affected by this disease. Diabetes mellitus is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin [1]. Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. The herbal drugs with antidiabetic activity are yet to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicine [2]. The plants provide a potential source of hypoglycemic drugs because many plants and plant derived compounds have been used in the treatment of diabetes. Many Indian plants have been investigated for their beneficial use in different types of diabetes and reports occur in numerous scientific journals. Ayurveda and other traditional medicinal system for the treatment of diabetes describe a number of plants used as herbal drugs. Hence, they play an important role as alternative medicine due to less side effects and low cost. The active principles present in medicinal plants have been reported to possess pancreatic beta cells re-generating, insulin releasing and fighting the problem of insulin resistance [3]. Hyperglycemia is involved in the etiology of development of diabetic complications. Hypoglycemic herbs increase insulin secretion, enhance glucose uptake by adipose or muscle tissues and inhibit glucose absorption from intestine and glucose production from liver [4]. Insulin and oral hypoglycemic agents like sulfonylureas and biguanides are still the major players in the management but there is quest for the development of more effective anti-diabetic agents.

Neem (*Azadirachta indica* A. Juss) is perhaps the most useful traditional medicinal plant in India. Each part of the neem tree has some medicinal property and is thus commercially exploitable. Apart from the chemistry of the neem compounds, considerable progress has been achieved regarding the biological activity and medicinal applications of neem.

LITERATURE REVIEW

Hypoglycemic activity: Aqueous extract of neem leaves significantly decreases blood sugar level and prevents adrenaline as well as glucose-induced hyperglycaemia [5]. The aqueous leaf extract when orally fed, also produces hypoglycemia in normal rats and decreased blood glucose levels in experimentally-induced diabetes in rats [6]. Aqueous leaf extract also reduces hyperglycaemia in streptozotocin diabetes and the effect is possibly due to presence of a flavonoid, quercetin [7]. A significant hypoglycaemic effect was also observed by feeding neem oil to fasting rabbits. Recently, hypoglycaemic effect was observed with leaf extract and seed oil, in normal as well as alloxan-induced diabetic rabbits [8]. The possible mechanisms underlying the hypoglycaemic activity of the aqueous leaf extract have also been discussed [9].

ISSN: 0975-8585

MATERIALS AND METHODS

Collection of plant material

The fruits of neem (**Figure 1**) were collected from the Andhra University (Department of Botany) and identified them with the help of a Botanist, Department of Botany, Andhra University, Visakhapatnam.

Figure 1: Neem fruits

Preparation of Fruit Aqueous Extract (FAE) of *Azadirachta indica*

The collected plant fruits was sun dried, pulverized by a mechanical grinder, sieved through 40 mesh. About 200g of powdered material was extracted with water using soxhlet apparatus. The extraction was carried out until the extractive becomes colorless. The extract is then concentrated by distillation process and dried under reduced pressure. The semisolid mass thus obtained is used for screening hypoglycemic activity in normoglycemic rabbits.

Animals

Healthy Albino rabbits of either sex weighing in between to 1-1.5 kg body weight were procured from the Ghosh Enterprises, kolkata. Throughout the study protocol the animals were group-housed in polypropylene cages. Standard Laboratory Conditions with 10-15 air changes per hour, continuously monitored environment, room temperature of 24 ± 3 °C with a relative humidity of 30-70 % and 12/12 hours of day light / dark cycle were maintained.

Selected route of administration

Oral administration

Different groups of rabbits as considered as follows

Group I: Normal, receive normal fed and water, *ad libitum*
Group II: Control, receive sodium CMC, with normal fed and water, *ad libitum*
Group III: Standard, treated with Gliclazide (5mg/kg, oral suspension).
Group IV: Test, treated with the Fruit Aqueous Extract (FAE) of *Azadirachta indica* (500 mg/kg, oral suspension)

Experimental protocol (Single dose study)

The standard (**Gliclazide**) was administered with a single dose (5mg/kg, oral suspension) for 1 day and the test (Fruit Aqueous Extract (FAE) of *Azadirachta indica*) was also treated with a single dose (500 mg/kg, oral suspension). The rabbits were then subjected to blood sample collection from marginal ear vein using disposable insulin syringe for the time intervals of 0, 1, 2, 3, 4, 6, 8, 10, and 12 hrs respectively. The collected blood samples are further processed for the estimation of plasma blood glucose levels using Glucometer (ACCU CHEK ACTIVE).

Statistical analysis

The data obtained after completion of the study protocol was analyzed using statistical and graphical representations. All the data are expressed as Mean ± S.E.M shown in **Tables 1-8**.

Table 1: Blood glucose levels (mg/dL) in normoglycemic rabbit (Control).

Control	Rabbit-1 (mg/dL)	Rabbit-2 (mg/dL)	Rabbit-3 (mg/dL)	Rabbit-4 (mg/dL)	Mean ±SEM
0	115	115	115	115	115
1	115	115	115	115	115
2	115	115	115	115	115
3	114	114	114	114	114
4	113	113	113	113	113
6	111	111	111	111	111
8	109	109	109	109	109
10	109	109	109	109	109
12	106	106	106	106	106

Table 2: Percentage blood glucose reduction values in normoglycemic rabbit (Control).

Control	Rabbit-1 (%)	Rabbit-2 (%)	Rabbit-3 (%)	Rabbit-4 (%)	Mean ± SEM
1	0	0	0	0	0
2	0	0	0	0	0
3	0.86	0.86	0.86	0.86	0.86
4	1.79	1.79	1.79	1.79	1.79
6	3.47	3.47	3.47	3.47	3.47
8	5.21	5.21	5.21	5.21	5.21
10	5.21	5.21	5.21	5.21	5.21
12	7.82	7.82	7.82	7.82	7.82

ISSN: 0975-8585

Table 3: Blood glucose levels (mg/dL) with Sodium CMC (Vehicle) in normoglycemic rabbits.

Sodium CMC	Rabbit-1 (mg/dL)	Rabbit-2 (mg/dL)	Rabbit-3 (mg/dL)	Rabbit-4 (mg/dL)	Mean ±SEM
0	118	117	118	115	117±0.70
1	118	117	118	115	117±0.70
2	118	117	118	115	117±0.70
3	116	115	117	114	115.5±0.64
4	115	114	116	113	114.5±0.64
6	115	114	116	113	114.5±0.64
8	111	111	113	110	111.25±0.62
10	115	115	115	112	114.25±0.75
12	116	116	116	113	115.25±0.75

Table 4: Percentage blood glucose reduction values with Sodium CMC in normoglycemic rabbits.

Sodium CMC	Rabbit-1 (%)	Rabbit-2 (%)	Rabbit-3 (%)	Rabbit-4 (%)	Mean ± SEM
1	0	0	0	0	0
2	0	0	0	0	0
3	1.69	0	0.84	0.86	0.84±0.34
4	2.54	0.84	1.69	1.79	1.71±0.34
6	3.89	0.84	1.69	1.79	2.05±0.64
8	5.93	5.12	4.23	4.34	4.90±0.39
10	3.89	3.41	2.54	2.60	3.11±0.32
12	1.69	0.84	1.69	1.79	1.50±0.22

Table 5: Blood glucose levels (mg/dL) with Gliclazide (5mg/kg) body weight in normoglycemic rabbits.

Gliclazide	Rabbit-1 (mg/dL)	Rabbit-2 (mg/dL)	Rabbit-3 (mg/dL)	Rabbit-4 (mg/dL)	Mean ±SEM
0	116	100	110	95	105.25±4.75
1	81	73	83	82	79.75±2.28
2	81	69	75	64	72.25±3.68
3	86	70	79	73	77±3.53
4	87	79	89	87	85.5±2.21
6	94	87	92	89	90.5±1.55
8	99	91	100	92	95.5±2.32
10	102	93	103	94	98±2.61
12	105	93	104	95	99.25±3.06

ISSN: 0975-8585

Table 6: Percentage blood glucose reduction values with Gliclazide (5mg/kg) body weight in normoglycemic rabbits.

Gliclazide	Rabbit-1 (%)	Rabbit-2 (%)	Rabbit-3 (%)	Rabbit-4 (%)	Mean ± SEM
1	30.17	27	24.5	13.68	23.83±3.57
2	30.17	31	31.8	32.63	31.4±0.52
3	25.86	30	28.1	32.6	29.14±1.43
4	25	21	19.09	8.42	18.37±3.54
6	18.96	13	16.36	6.31	13.65±2.73
8	14.6	9	10	2.10	8.92±2.58
10	12.06	7	6.36	1.05	6.61±2.25
12	9.48	7	5.45	0	5.48±2.00

Table 7: Blood glucose levels (mg/dL) with Fruit Aqueous Extract (FAE) of *Azadirachta indica* (500 mg/kg) body weight in normoglycemic rabbits (test).

AFE	Rabbit-1 (mg/dL)	Rabbit-2 (mg/dL)	Rabbit-3 (mg/dL)	Rabbit-4 (mg/dL)	Mean ±SEM
0	117	114	118	112	115.25±1.37
1	112	109	113	107	110.25±1.37
2	109	106	110	104	107.25±1.37
3	101	98	102	96	99.25±1.37
4	86	83	87	81	84.25±1.37
6	89	86	90	84	87.25±1.37
8	94	91	95	89	92.25±1.37
10	100	97	101	95	98.25±1.37
12	107	104	108	102	105.25±1.37

Table 8: Percentage blood glucose reduction values with Fruit Aqueous Extract (FAE) of *Azadirachta indica* (500 mg/kg) body weight in normoglycemic rabbits (test).

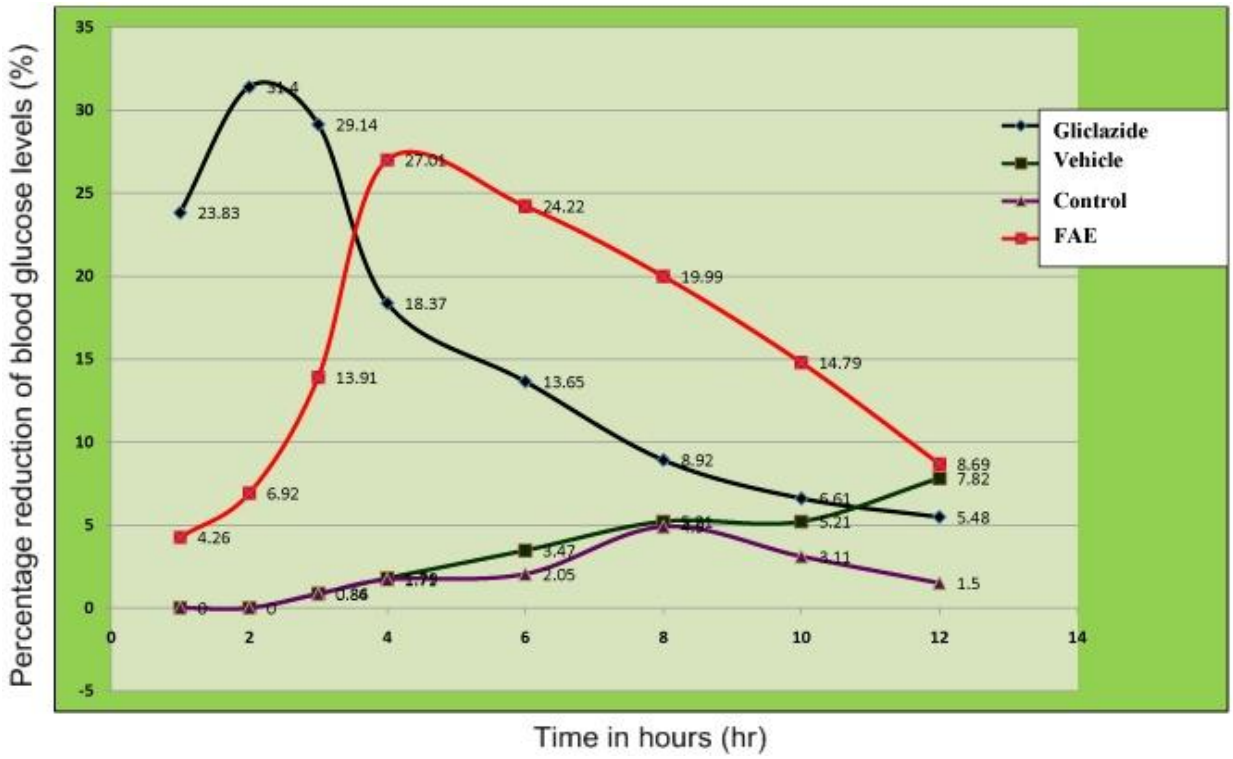
AFE	Rabbit-1 (%)	Rabbit-2 (%)	Rabbit-3 (%)	Rabbit-4 (%)	Mean ± SEM
1	4.27	4.27	4.23	4.27	4.26±0.01
2	6.83	7.07	6..87	6.87	6.92±0.06
3	13.67	14.03	14.01	13.93	13.91±0.08
4	26.49	27.19	27.39	26.99	27.01±0.19
6	23.93	24.56	23.96	24.46	24.22±0.16
8	19.65	20.17	20.17	19.97	19.99±0.12
10	14.52	14.91	14.87	14.89	14.79±0.092
12	8.54	8.77	8.69	8.76	8.69±0.053

ISSN: 0975-8585

RESULTS AND DISCUSSION

Though wide range of studies were reported on the effect of neem on diabetes in combination with other medicinal plants, so far no work has been done exclusively on the hypoglycemic activity of neem fruit aqueous extract. Present study focuses entirely on the role of neem fruit aqueous extract as a hypoglycemic agent. The actual mechanism of action that brings up on the action of hypoglycemia is not understood but it is proved to be neem fruit aqueous extract is found to have blood glucose lowering capacity at dose 500 mg/kg. Hence, with all data recorded (**Tables 1-8**) and analyzed (**Figure 2**), this study concludes that the neem fruit aqueous extract can formulate for an efficient and effective alternate complementary medicine in management of diabetes mellitus. Plots of observed percentage blood glucose reduction values vs. Time (hrs) of Fruit Aqueous Extract (FAE) of *Azadirachta indica* given in **Figure 2**.

Figure 2: Plots of observed percentage blood glucose reduction values vs. Time (hrs) of Fruit Aqueous Extract (FAE) of *Azadirachta indica*



CONCLUSION

The results obtained from the present study show that the *Azardirachta indica* fruit aqueous extract had beneficial effects on blood glucose levels in normoglycemic rabbits. It confirms to be an attractive material for further studies, leading to possible drug development for diabetes. Development of phytomedicines is relatively inexpensive and less time consuming. However, the results from this study give scientific support to the use of *Azardirachta indica*

fruit aqueous extract in folklore medicine for the treatment of diabetes, and show the potential role of hypoglycemic activity.

ACKNOWLEDGEMENTS

One of the authors, Mr. A.VASUDEVARAO is grateful and thankful to the Principal, Andhra University College of Pharmaceutical Sciences for providing the required help in carrying out the hypoglycemic activity.

REFERENCES

[1] Maiti R, Jana D, Das UK, Ghosh D. J Ethnopharmacol 2004; 92: 85-91.
[2] Wadkar KA, Magdum CS, Patil SS, Naikwade NS. Journal of Herbal Medicine and Toxicology 2008; 2: 45-50.
[3] Welihinda J, Arvidson G, Gylfe E, Hellman B, Karlsson E. Ada. Biol MetLGer 1982; 41: 1229.
[4] Hongxiang Hui, George Tang, and Vay Liang W Go. VLW. Chin Med 2009; 4: 11-14.
[5] Murty, K. S., Rao, D. N., Rao, D. K. and Murty, L. B. G. Indian J Pharmacol 1978; 10: 247–250.
[6] El-Hawary, Z. M. and Kholief, T. S. Arch Pharmacol Res 1990; 13: 108–112.
[7] Chakraborty, T., Uerotta, L. and Poddar, G. Phytother Res 1989; 3: 30–32.
[8] Khosla, P., Bhanwra, S., Singh, J., Seth, S. and Srivastava, R. K. Indian J Physiol Pharmacol 2000; 44: 69–74.
[9] Chattopadhyay, R. R. Gen Pharmacol 1996; 27: 431–434.