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Evaluation of Hypoglycemic Activity of Neem (Azadirachta Indica) In Albino Rats

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Abstract: *Diabetes mellitus is a complex metabolic disorder resulting from either insulin deficiency or insulin dysfunction. Based on the recent advances and involvement of oxidative stress in complicating diabetes mellitus, efforts are on to find suitable antidiabetic and antioxidant therapy. Medicinal plants are being looked upon once again for the treatment of diabetes. Neem, Azadirachta Indica is a widely grown plant in the Indian subcontinent. It has been found to have various medicinal properties, so this study was carried out.*
Rats were used as animal models to study the antidiabetic effects of neem. Diabetes was induced in rats by alloxan monohydrate. The assessment was done by fasting blood glucose levels and oral glucose tolerance test.
The results of the study indicate that neem oil has got the potential to reduce blood glucose levels within a short period of time and also it has potential to improve the glucose tolerance after a treatment period of 4 weeks. Azadirachta Indica may have beneficial effects in diabetes mellitus and holds the scope of new generation of antidiabetic drug.

Key words: *Diabetes Mellitus, Neem, Azadirachta Indica, Alloxan, Hypoglycemic, Antidiabetic.*

I. Introduction

Diabetes mellitus is a complex metabolic disorder resulting from either insulin insufficiency or insulin deficiency dysfunction. Type 1 diabetes (insulin dependent)is caused due to insulin insufficiency because of lack of functional beta cells ,patients suffering from this are therefore totally dependent on exogenous source of insulin while patients suffering from type ii diabetes(insulin independent)are unable to respond to insulin and can be treated with dietary changes ,exercise and medication. Type II diabetes is more common form of diabetes constituting 90% of the diabetic population.[1]

The global prevalence of diabetes is estimate to increase from 4% in 1995 to 5.4 % by the year 2025.WHO has predicted that the major burden will occur in developing countries .studies conducted in India in the last decade have highlighted that not only is the prevalence of diabetes is high but also it is increasing rapidly in the urban population .[2]it is estimated that there are approximately 33 million adults with diabetes in India .this number is likely to increase to 57.2 million by the year 2025.[3]drugs used routinely to treat diabetes are sulfonylurea ,biguanides,meglitinides,thiazolidinediones ,alpha glucosidase inhibitors ,insulin etc.[4]

Based on the recent advances and involvement of oxidative stress in complicating diabetes mellitus, efforts are on to find suitable antidiabetic and anti oxidant therapy.[5] The WHO expert committee on diabetes has recommended that traditional herbal medicines be further investigated.[6,7] There are many herbal remedies suggested for diabetes and its complications. Medicinal; plants form the main ingredients of the formulation. However insufficient research has been done to access the purported benefits of neem. Neem (Azadirachta Indica) also known as nim, nimtree and Indian lilac is a tree in the mahogany family meliaceae. The products made from neem tree have been used in India for over two millennia for their medicinal properties. Neem products are believed by ayurvedic practitioners to have antihelminthic, anti fungal, anti diabetic, antibacterial, antiviral, contraceptive and sedative properties.[8,9]

Neem oil is also used for healthy hair, to improve liver function, detoxify the blood and balance blood sugar levels. Hydroalchoholic extract of this plant showed antihyperglycemic activity in streptozocin treated rats and this effect is because of increase in glucose uptake and glycogen deposition in isolated rat hemidiaphrgm. Hence the present study was planned to see the anti diabetic activity of neem in albino rats

II. Materials And Methods:

Materials

Chemicals: Alloxan monohydrate, Glibenclamide hydrochloride [(Tab.Daonil, 5mg (Aventis Pharma Limited)], sodium chloride, Neem oil (Asian herbex limited).

Animals: Albino rats

Equipments: Mouth gag, polythene feeding tube, tuberculin syringe and insulin syringe (each 1ml), glucometer (Roche diagnostics).

Animals: Animals used are albino rats, of wistar strain, weighing between 250-300grams of either sex. The animals were fed with standard laboratory food and water.

Equipments :

Mouth gag: To facilitate the introduction of feeding tube in to the stomach of the rat and administer the drugs.

Feeding tube: The polythene infant feeding tube was used for oral administration of the drugs.

Tuberculin syringe: Used for injecting alloxan in to peritoneal cavity and for administering the drugs at proper dosage.

Insulin syringe: For drawing blood from tail vein of rats.

Glucometer: The glucometer used in this study is Accu-Chek sensor from Roche Diagnostics Corporation.

Methodology:The method employed in this study to induce diabetes was chemical method using alloxan monohydrate, gives intraperitoneally. Blood glucose estimations were made by using glucometer.Albino rats of wistar strain with body weight of 250-300grams were used for the study.[10]

Inclusion criteria: Body weight 250-300grams

Exclusion criteria: Rat weighting more than 300 grams and less than 250 grams. Pregnant female rats and those which have delivered once.

Fasting blood glucose readings were recorded in all rats after an overnight fasting. Blood samples were obtained from rat tail vein, after applying xylene to make veins prominent. Blood glucose was estimated by using glucometer. After 28 days of treatment oral glucose tolerance test was performed in each group.

Procedure of oral glucose tolerance test in rats: At the end of experimental period, OGTT was performed after overnight fast in order to assess the effects of A.indica on alloxan induced diabetes. On the day of the OGTT, animal were given an oral dose of glucose (3g/kg body weight) after collecting sample blood for fasting blood glucose estimation. Blood samples were collected at 45min intervals for 3 readings, with the first sampling commencing after 45 min of glucose load. Oral glucose was given 30 min after administration of the drug to facilitate absorption to take place[11].

Induction of diabetes:Alloxan monohydrate was used to induce diabetes mellitus. After an overnight fasting, the rats were injected freshly prepared 2% solution of alloxan monohydrate in 0.9% sodium chloride solution (normal saline). The dose injected was 150mg/kg body weight[12,16].

Following injection, animals were observed for 24-48 hours for evidence of any allergic reaction, behavioral changes, convulsions and hypoglycemic symptoms. No untoward reaction was observed in any animal. Fasting blood glucose was estimated at around 9.30am daily until stable hyperglycemia with FBS of more than 200mg/dl were selected for the study.

They were divided in to four groups as follows: Non –diabetic control group, Diabetic control, Standard control and Test group

Non diabetic control: This group consisted of non-diabetic rats, used as controls for the study. This group received 0.5ml of normal saline daily for 30 days. FBS was recorded on the day 1,3,7,14,21 and 28. After 30 days of treatment oral glucose tolerance test was performed.

Diabetic control: The rats in this group were rendered diabetic by injection of 2% solution of alloxan monohydrate, intraperitoneally in a dose of 150 mg/kg body weight. This group also received 0.5ml of normal saline daily for 30 days. FBS was recorded on day 1,3,7,14,21 and 28. After 30 days of treatment oral glucose tolerance test was performed.

Standard control: The rats in the group were rendered diabetic by injection of 2% solution of alloxan monohydrate, intraperitoneally in a dose of 150 mg/kg body weight. This group also received glibenclamide in a dose of 0.5mg/kg body weight suspended in normal saline, orally, daily for 30 days. FBS was recorded on the day 1,3,7,14,21 and 28. After 30 days of treatment oral glucose tolerance test was performed. Additionally on day 1, blood glucose was estimated at every half hour interval from the time of administration of the drug, for 3 hours to known the peak time of action.

Test group: The rats in this group were rendered diabetic by injection of 2% solution of alloxan monohydrate, intraperitoneally in a dose of 150 mg/kg body weight. This group also received oily extract of Azadirachta Indica linn in a dose of 500mg/kg body weight orally, daily for 30 days. The dose was determined by pilot study wherein a gradually increasing dose from 200mg to 1000mg of the extract was given to diabetic rats and the effect was observed. FBS was recorded on the day 1,3,7,14,21 and 28. After 30 days of treatment oral glucose tolerance test was performed. In this group also, on day 1blood glucose was estimated at every half hour interval from the time of administration of the drug, for 3hours to know the peak time of action.

Observation for side effects: Throughout the experiment, the animals were keenly observed for occurrence of any visible side effects like tremors, loss of body weight, behavioral changes, convulsions, sedation etc. For testing the acute toxicity of the test compound, albino rats of either sex weighing 250-300 grams were selected. They were grouped in to 5 groups of 6 each and test drug was administered as follows[13],

Group no.	Dosage (in mg/kg body weight, orally)
10	
30	
100	
300	
1000	

Dosage could not be increased beyond 1000mg/kg due to paucity of test compound. The rats were observed for 6-8 hours for any signs of toxicity like variations in motor activity, behavior changes, tremors, convulsions, sedation, lacrimation etc. after 24 hours of drug administration, the rats were sacrificed and viscera like stomach, liver, intestine etc were inspected under magnifying glass for any visible ulcerations and hemorrhages.

III. Results

During the whole study process, the animals were observed for any visible side effects. None of them showed any signs of side effects. Acute toxicity testing showed that the test compound was safe up to the dose of 1000mg/kg body weight in rats. None of the rats showed any signs of toxicity at this dosage and the viscera appeared normal for naked eye examination using magnifying glasses.

Statistical analysis: Mean, standard deviation was calculated for each group. One way ANOVA was used for multiple group comparisons followed by studentized range test for statistical significance between groups. P values less than 0.05 were considered to be significant.

Table 1 : Mean(+, - SD) Values of Blood Glucose levels in different group of animals

Groups	Day 1	Day 3	Day 7	Day 14	Day 21	Day 28
1. NDC	49.7+ 5.9	50.3 + 6.5	48.8 + 5.5	49.8 + 4.5	47.8 + 6.0	46.7 + 3.7
2. DC	233.7 + 12.5	327.3 + 11.9	242.0+ 11+6	246.2 + 10.4	249.0 + 11.9	250.8 + 7.4
3. SC	2158 + 9.4	237.0 + 7.1	225.7 + 6.4	176.7 + 16.9	129.5 + 7.9	83.7 + 7.6
4. TG	233.6 + 11.8	117.7 + 57.3	101.8 + 45.8	95.5 + 38.5	121.3 + 30.7	91.0 + 24.8

NDC ---Non- Diabetic control group
DC --- Diabetic Control Group
SC --- Standard Control Group (Received Glibenclamide 0.5 mg/kg body weight
TG--- Test Group (received Neem oil extract 500 mg/kg body weight

Table 2: Comparison of Percentage of reduction in Blood glucose level between standard and test groups

Groups	Day 1	Day 3	Day 7	Day 14	Day 21	Day 28
Standard Control	22.1%	14.4%	18.5%	36.3%	53.2%	69.9%
Test Group	20.7%	60.1%	65.5%	67.6%	58.8%	69.2%

Table 2a: Overall Percentage reduction of blood glucose level in standard and test group

Group	Mean (%) reduction
Standard	35.70%
Test Group	65.90%

Table 3: Statistical Analysis showing comparison of blood glucose level between different groups on day 1

Groups	BGL (mean +/- SD)	Groups Compared	Mean Difference	P- value
Control	49.7 +/- 5.9	1-2	184.0	<0.01
		1-3	166.1	<0.01
Diabetic Control	233.7 +/- 12.5	1-4	183.9	<0.01
		2-3	17.9	<0.05
Standared Contro	215.8 +/- 9.4	2-4	0.1	N.S
		3-4	17.8	<0.01
Test	233.6 +/- 11.8			

F- value *	F= 457.6	P < 0.01 = highly Significant

Studentized Range Test: Least Statistical Difference (LSD) = 16.6, P <0.05 = 21.0, P <0.01

Table 4: Statistical analysis showing comparison of blood glucose levels between different groups on day 3

Groups	BGL (mean +/- SD)	Groups Compared	Mean Difference	P- value
Control	50.3 +/- 6.5	1-2	187.0	<0.01
		1-3	186.7	<0.01
Diabetic Control	237.3 +/- 11.9	1-4	67.4	<0.01
		2-3	0.3	N.S
Standard Control	237.0 +/- 7.1	2-4	119.6	<0.01
		3-4	119.6	<0.01
Test	117.7 +/- 57.3			
F- value *	F= 58.4,	P < 0.01 = highly Significant		

Studentized Range Test : LSD = 48.0 at P<0.05, 60.8 at P<0.01 One way ANOVA

Table 5: Statistical analysis showing comparison of blood glucose levels between different groups on day 7

Groups	BGL (mean +/- SD)	Groups Compared	Mean Difference	P- value
Control	48.8 +/- 5.5	1-2	193.2	<0.01
		1-3	176.9	<0.01
Diabetic Control	242.0 +/- 11.6	1-4	53.9	<0.01
Standared Control	225.7 +/- 6.4	2-3	16.3	N.S
Test	101.8 +/- 45.8	2-4	140.2	<0.01
		3-4	123.9	<0.01
F- value *	F= 92.4,	P < 0.01 = highly Significant		

Studentized Range Test: Least Statistical Difference (LSD) = 38.8, P <0.05= 49.2, P <0.01One way ANOVA

Table 6: Statistical analysis showing comparison of blood glucose levels between different groups on day 14

Groups	BGL (mean +/- SD)	Groups Compared	Mean Difference	P- value
Control	46.7 +/- 3.7	1-2	187.0	<0.01
		1-3	186.7	<0.01
Diabetic Contrlo	250.8 +/- 7.4	1-4	67.4	<0.01
		2-3	0.3	N.S
Standared Control	83.7 +/- 7.6	2-4	119.6	<0.01
		3-4	119.6	<0.01
Test	91.0 +/- 24.8			
F- value *	F= 265.9,	P < 0.01 = highly Significant		

Studentized Range Test : LSD = 22.0 at P<0.05, 27.9 at P<0.01 One way ANOVA

Table 7: Statistical analysis showing comparison of blood glucose levels between different groups on day 21

Groups	BGL (mean +/- SD)	Groups Compared	Mean Difference	P-value
Control	47.8 +/- 6.0	1-2	201.2	<0.01
Diabetic Control	249.0 +/- 11.9	1-3	81.7	<0.01
Standared Control	129.7 +/- 7.9	1-4	73.5	<0.01
Test	121.3 +/- 30.7	2-3	119.5	<0.01
		2-4	127.7	<0.01
		3-4	8.2	N.S
F- value *	F= 140.9,	P < 0.01 = highly Significant		

Studentized Range Test: Least Statistical Difference (LSD) = 27.8, P <0.05 = 35.2, P <0.01

Table 8: Statistical analysis showing comparison of blood glucose levels between different groups on day 28

Groups	BGL (mean +/- SD)	Groups Compared	Mean Difference	P- value
Control	49.8 +/- 4.5	1-2	196.4	<0.01
Diabetic Contrlo	246.2 +/- 10.4	1-3	126.9	<0.01
Standared Control	176.7 +/- 16.9	1-4	45.7	<0.01
Test	95.5 +/- 38.5	2-3	69.5	<0.01
		2-4	150.7	<0.01
		3-4	81.2	<0.01
F- value *	F= 95.7,	P < 0.01 = highly Significant		

Sudentized Range Test : LSD = 35.2 at P<0.05, 44.6 at P<0.01 One way ANOVA

Table 9: The effect of Azadirachta Indica and glibenclamide on blood glucose levels on day 1 for the initial 3 hours

Group	0 hr	½ hr	1 hr	1 ½ hr	2 hr	2 ½ hr	3 hr
Std. Control	277.0	312.3	379.5	296.7	252.2	279.2	313.7
Test Group	294.7	292.7	290.7	331.7	372.0	322.2	2879.0

Table 10: The effect of azadirachta indica and glibenclamide on oral glucose tolerance test in different study groups

Group	0 min	45 min	90 min	135 min
Non Diabetic control	46.7 +/- 3.7	80.0 +/- 3.6	84.8 +/- 2.7	72.8 +/- 5.2
Diabetic Control	250.8 +/- 7.4	442.7 +/- 9.9	579.8 +/- 18.9	387.3 +/- 12.8
Standard Control	83.7 +/- 7.6	420.2 +/- 10.7	463.3 +/- 5.2	312.0 +/- 6.5
Test Group	91.0 +/- 24.8	240.8 +/- 25.4	220.7 +/- 28.4	167.8 +/- 48.9
One way ANOVA 'F' value	265.8 P<0.01	795.7 P<0.01	1014.6 P<0.01	182.7 P<0.01

Analysis of Results:

The results have been statistically analyzed for significance by using one way analysis of variance (ANOVA) for multiple group comparisionsflowed by stidentized range test. 'F' value was calculating usingformula.

$$F = \frac{\text{Between group Variance}}{\text{Within group Variance}}$$

Table 1 shows the variation in blood glucose levels on day 1,3,7,14,21, and 28 in each group. Mean blood glucose level in non- diabetic control group varied from 46.7 to 50.3 mg/dL, with not much of variation during the study period.In diabetic control group, there was a gradual increase from mean value of 233.7 mg/dL on day 1 to 250.8 mg/dL on day 28.

In standard control group, there was a fall in blood glucose level on day 1 followed by a rise on day 3 which was later followed by a steady decline through the study period to reach its minimum value in the study on day 28 (83.7 mg/dL).Test group behavior was some what different, with a reduction on day 1, it was followed by a steep fall on day 3 that was maintained up to day 14. This was followed by

On day 14, the difference between the groups is significant. On day 21 and 28 the difference is maintained in all the groups except for the comparison between the standard control and test group, which is not significant. On day 21, the test drug is showing a better glycemic control then the standard drug though it is statistically insignificant. On day 28 the trend is reversed with a better glycemia in standard group than in test group but is statistically insignificant.

Table 9 gives an account of the peak time of action after single close of the drug in standard and test groups. Standard control shows the peak action at 2 hours after drug administration and test group has its peak action at 3 hours after drug administration. Table 10 shows the effect of drugs on oral glucose tolerance test (OGTT) after completion of treatment. The test group shows better glucose tolerance than the standard group.

All the above results have been represented in bar and line diagrams. A rise in blood glucose level on day 21 followed by fall on day 28 to reach its minimum value during the study (91.0 mg/dL).

Table 2 shows the percentage of reduction in blood glucose levels of standard and test groups. In standard group, there was 22.1 % reduction in blood glucose on day 1 and it changed to 14.4 % on day 3. Then there was a steady rise in percentage of reduction from day 7 to day 28. Maximum percentage of reduction was observed on day 28 (69.8%) In test group, the reduction on day 1 was 20.7 % which was slightly less than that of control group. Then there was a steep rise in percentage of reduction of blood glucose to 60.1 % on day 3

IV. Discussion:

The current study was performed to evaluate the hypoglycemic effects of Azadirachta Indica in alloxan induced diabetic albino rats. This study shows that oily extract of Azadirachta Indica produces a marked decrease in blood glucose levels in alloxan diabetic rats.

Azadirachta Indica has been shown to possess hypoglycemic activity in some previous studies both in normal and experimental animals.[14,15] These studies were conducted using aqueous extract of neem leaf and in some the neem oil has been used.

Present study goes in accordance with the previous studies of bopanna et al and khosla et al with respect to outcome on day 28.it shows that Azadirachta Indica has hypoglycemic activity, with its peak effect at the end of 4 weeks. But in the intervening period ,particularly on day 3,7 and 14 ,neem has shown better hypoglycemic control than the standard drug glibenclamide, which is statistically very significant(<0.01).this is not so in khosla et.al,where the decline in blood glucose is steady and follows a uniform trend throughout the study period. This is probably due to varied procedures of extraction and parts used .in the study by khosla et.al.[10]The oil extracted was from the seeds whereas in our study the extract was derived only from kernel of the seeds. The procedure followed was cold press technique which may cause retention of active ingredients of neem. Similar is the case with the study by Sharma et al., where the neem oil was obtained from a local stockiest and procedure of extraction was not described. One more reason for variation in the results may be due to the use of different species of animals. In our study we used albino rats where as in the study by khosla et al the animals used were rabbits.

On the day 1, there was no statistical significance between diabetic control and test groups which was due to higher initial mean blood glucose values in the test group (294.7) compared to diabetic control (233.7).though neem has produced fall in blood glucose on day 1 as shown in table 2 by 20.1%, it failed to produce any statistical significance compared to diabetic control.

In the present study oral glucose tolerance test was performed after completion of 268 day treatment. It shows that both glibenclamide and neem reduced the blood glucose levels and the effect of neem oil was significantly greater than that of glibenclamide. This goes in accordance with the study by Sonia Bajaj and srinivasan where they conducted the oral glucose tolerance test after 6 weeks of treatment with neem leaf

extract.[17]All the above descriptions fail to give an effective explanation to the rise in blood glucose levels on day 21.further exploration is needed in this respect..

Mechanism of action: the exact mechanism of action is still unclear. In the study by khosla et al, neem has been shown to be protective against diabetes induced by alloxan .as described earlier; alloxan produces diabetes in experimental animals by producing damage to beta cells through reactive oxygen species. It can be said that neem protects beta cells against alloxan probably through its antioxidant action.

In this study, during the oral glucose tolerance test, the animals that received neem oil have shown a low rise in blood glucose levels compared to both the diabetic control and standard control glibenclamide. This can be explained as effect of neem on glucose absorption, which is reduced in presence of neem. This mode of action still needs to be evaluated in this aspect. Azadirachta Indica may also act by increased release of insulin from beta cells of pancreas similar to sulfonylurea as suggested by khosla et al.also it may increase the uptake of glucose peripherally as suggested by Sonia Bajaj and Srinivasan[17], due to its blood glucose reducing action in type 1 diabetes model as well.Several other mechanisms have been suggested like decreased synthesis or release of glucose by liver, inhibition of proximal tubular absorption of glucose in kidney etc[18] by Sharma et al.in spite of all these explanations the exact mode of action still needs to be elicited and extensively studied in both human and animal models[15]. It is necessary to know the effect of each of the active ingredient.

Drawbacks of the study: the present study has several drawbacks .the study is very primitive in the parameters used. The study has been carried out only in one species of animals viz rats and needs to be extended to other animals as well.Only the fasting glucose and post prandial blood glucose were estimated in this study which does not give a clear picture about the effects of neem on other parameters of diabetes mellitus. No attempt was made to establish insulin and C-peptide levels due to lack of facilities which might have been useful to throw some light on the mode of action of neem. The effect of serum lipid levels, reactive oxygen species, atherogenesis, beta cell pathology etc needs to be evaluated. Owing to non availability of analytical methods in our set up, the pharmacokinetic profile has not been defined.

Acute toxicity testing has been fairly preliminary. Only oral route has been used for administration of test compounds and testing needs to be done using other routes viz intravenous, intramuscular, intraperitoneal etc as well. Chronic toxicity studies to evaluate the effect of neem on various hematological parameters, lipid profile, electrolyte profile, teratogenic and carcinogenic potential etc have to be undertaken for further evaluation.

V. Summary and conclusions:

Many workers have shown Azadirachta Indica to be effective in diabetes mellitus ,both type 1 and type 2 in various animal models .in the present study ,oily extract of neem ,obtained by cold compression technique has been used to evaluate the hypoglycemic effect in alloxan induced diabetic rats. The parameters considered in the study were fasting blood glucose post prandial blood glucose (oral glucose tolerance test).for the above purpose, the test compound was administered by oral route.

The results of the study indicate that neem oil has got potential to reduce the blood glucose levels within a short period of time and also it has potential to improve the glucose tolerance after a treatment period of 4 weeks, as suggested by oral glucose tolerance test. In conclusion our study suggests that Azadirachta Indica may have beneficial effect in diabetes mellitus and may improve glucose tolerance also. Thus it holds the scope of a new generation of antidiabetic drug. However, there is need for further studies on experimental animals and human beings, using various active principles, to establish its usefulness, exact mode of action and toxicity data.

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