ORIGINAL ARTICLE

Comparison of Hypoglycemic Effects of Azadirachta indica Seeds and Leaves on Alloxan Induced Diabetes in Male Albino Rats

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ABSTRACT

Background: Azadirachta indica (neem) is a commonly found medicinal plant in Pakistan. Its leaves and seeds are traditionally used for management of various diseases. The current study was planned to see the impact of Azadirachta indica leaves and seeds extract on plasma glucose.
Aim: To find out and compare effects of Azadirachta indica leaves and seeds ethanolic extracts on level of glucose in blood of albino rats, in which diabetes mellitus has been induced by alloxan.
Methods: It was a randomized control trial study carried out on 120 male albino rats. Alloxan in monohydrate form was introduced intraperitoneally in rats to induce diabetes mellitus (DM). Division of rats was done at random in four groups of 30 each. Group A was designated as control and was given normal saline by oral route. Rats belonging to Group B were designated as diabetic control. Group C was given Azadirachta indica leaves extract orally (500 mg / kg body weight); whereas Group D was given extract of Azadirachta indica seeds orally (500 mg / kg body weight) as a single dose daily for 28 days. On 29th day, 4-5 ml intracardiac blood sample was taken from each rat. Biochemical parameter, plasma glucose was evaluated.
Results: The ethanolic extract of Azadirachta indica leaves and seeds given to diabetic rats produced highly significant ($p<0.001$) decrease in serum glucose.
Conclusion: Ethanolic extracts of Azadirachta indica seeds and leaves possess potent hypoglycemic effects.
Key words: Azadirachta indica, hypoglycemic effect.

INTRODUCTION

According to American Diabetes Association (ADA), Diabetes Mellitus is the frequent most occurring metabolic syndrome in which due to insulin deficiency, there is hyperglycemia and deficient glucose utilization by the cells[1]. Factors contributing towards rise in its incidence are thought to be due to simultaneous rise in population growth, aging, urbanization, obesity, unhealthy diet and sedentary life style.[2] Diabetes can lead to a wide range of complications that involves primarily arteries and capillaries. Type 2 diabetics are particularly at increased risk of morbidity and mortality due to their long term complications[3]. Diabetes mellitus results in various complications which include macrovascular complications leading to cerebrovascular disease, peripheral vascular disease, coronary heart disease; microvascular complications causing diabetic neuropathy, diabetic retinopathy and diabetic nephropathy; and others such as gastroparesis, diarrhea, sexual dysfunction, dermatologic diseases, infections, cataract, glaucoma and periodontal disease[4].

Medicines derived from plants are used quite commonly for the treatment and prevention of many diseases. Traditional plants treatment has been used worldwide for the therapy of DM. The effects of these medicines are helpful to slow the process leading to complications of DM. Some herbs furnish abundant resource of antioxidants to delay or prevent various diseases[5]. These plants may increase secretion of insulin, thereby increasing uptake of glucose by the fat cells and skeletal muscle cells. They may also decrease absorption of glucose from the gastrointestinal mucosa and gluconeogenesis by the liver[6]. One of the traditional plants practiced in the management of DM and other diseases is neem (Azadirachta indica). Azadirachta indica has gained global attraction due to its tremendous properties of cure for different diseases[7]. The world pharmacists are now recommending the use of less noxious herbs for the production of modern medicines. It has been noticed that every portion of Azadirachta indica plant has traditionally been used as medicines for many centuries in various parts of the world. Pharmacological and biological properties of Azadirachta indica plant known are; antioxidant, antidiabetic, antiplasmodial, antitrypanosomal, antibacterial, anticancer, immunomodulating, fungicidal, larvicidal, antiviral, immunocontraceptive, insecticidal, anthelminthic, antiulcer, anti-inflammatory, antifertility, nematicidal, spermicidal, and insect repellant effects[8,9].

The objectives of the present research were to find out and compare the effects of Azadirachta indica seeds and leaves ethanolic extracts, on blood glucose levels in albino diabetic rats.

MATERIALS AND METHODS

This research was performed on 120 healthy and adult albino male rats which were collected from the National Institute of Health (NIH), Islamabad. These rats were placed in four cages. In each cage, 30 rats were housed for a period of one week before the start of experimentation. The conditions were maintained at temperature 26±2^{0}C and 12 hour dark/light cycle[10].
Grouping: The rats were split at random into 4 groups of 30 each,

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Group A (Normal control): received normal diet
Group B (Diabetic control): was made diabetic (Type-2 DM model) by injecting alloxan 120 milligram/kilogram body weight intraperitoneally and provided with normal diet.
Group C (Experimental 1) was given *Azadirachta indica* leaves ethanolic extract, 500 milligram/kilogram of body weight orally once a day for 28 days.
Group D (Experimental 2) was given *Azadirachta indica* seeds ethanolic extract, 500 milligram/kilogram of body weight orally once a day for 28 days.
Induction of experimental diabetes by alloxan monohydrayte: A single dose of alloxan in monohydrate form (120 milligram / kilogram) was administered to overnight fasting rats of group B, C, D to induce diabetes before start of experimentation.[11] With this dose of alloxan, all the beta cells of pancreas were not destroyed, so type 2 diabetes mellitus (NIDDM) was produced.[12] As Alloxan can produce lethal hypoglycemia as a consequence of excessive release of insulin from pancreas, rats were given 15-20 milliliters of 20% glucose intraperitoneally after six hours. For the upcoming 24 hours the rats were given five percent glucose in water to avoid hypoglycemia.[13] Hyperglycemia was confirmed by elevated blood glucose determined after 72 hours.[14] Normal fasting level of glucose in blood of rats ranges from 80-110 milligram /deciliter. Rats with greater than 200 milligram /deciliter of glucose in their blood were designated diabetic for our research purpose.[15] Management of these rats suffering from DM and belonging to group C and D was started with ethanolic extract leaves and seeds respectively of azadirachta indica on daily basis at a single dose of 500 milligram / kilogram body weight per day by a smooth tipped needle inserted into the posterior part of pharynx for 28 days[16]. On 29th day of the experiment, 4-5 ml of intracardiac blood sample was collected for plasma glucose levels by glucose oxidase method to see the in-vivo effect of the plant extract.

Data were analyzed using PASW18 (formerly SPSS). Descriptive analysis was done by using one way analysis of variant (ANOVA) test to determine arithmetic mean ± SD values of the obtained data. One way ANOVA and after that post hoc Tukey's HSD test (multiple comparisons) was used to assess the statistically significant values in all the designated groups. Pearson's correlation, $p < 0.05$ was taken as significant, whereas $p < 0.001$ was taken as highly significant.

RESULTS

This randomized controlled study was conducted on 120 male albino rats. The effects of ethanolic extracts of *Azadirachta Indica* leaves and seeds on serum glucose was evaluated and compared in diabetic male albino rats.

As shown in Table 1, Serum glucose levels in diabetic control rats were significantly highly (p=0.000) raised than the levels in normal control rats. Table 2 represents the mean difference and p-value of levels of glucose in diabetic control group and group managed by neem leaves and it reveals highly significant (p=0.000) reduction in blood glucose levels in the diabetic rats managed by neem leaves extract in comparison with the diabetic control group. Similarly the group managed by neem seeds extract revealed highly significant (p=0.000) reduction of glucose levels in comparison with the diabetic control group (table 3). However the difference in blood glucose levels between the diabetic groups managed by neem leaves and neem seeds respectively was not significant as evident from table 4. All the above results are collectively represented in table 5 and fig 1.

Table 1: Glucose levels in sera of normal control and diabetic control groups. Mean ± SD is given. Figure in parenthesis indicate number of cases is each group

Parameter	Normal control (30)	Diabetic control (30)	Mean difference	p-value
Glucose	106.47±17.74	225.23±32.16	-118.7*	.000

*p < 0.001 highly significant

Table 2. Serum glucose level in diabetic control group and group treated with neem leaves. Mean ± SD is given. Figure in parenthesis indicate number of cases is each group.

Parameter	Diabetic control (n=30)	Neem Leaves (n=30)	Mean difference	p-value
Glucose	225.23±32.16	110.20±11.84	115.03333*	.000

*p <0.001 highly significant

Table 3: Serum glucose level in diabetic control group and group treated with neem seeds. Mean ± SD is given. Figure in parenthesis indicate number of cases is each group

Parameter	Diabetic control (n=30)	Neem Seeds(n=30)	Mean difference	p-value
Glucose	225.23±32.16	118.70±14.38	106.53333*	.000

*p <0.001 highly significant

Table 4: Serum glucose level in diabetic groups treated with neem leaves and neem seeds. Mean ± SD is given. Figure in parenthesis indicate number of cases is each group

Parameter	Neem Leaves (n=30)	Neem Seeds(n=30)	Mean difference	p-value
Glucose	110.20±11.84	118.70±14.38	-8.50000	.383

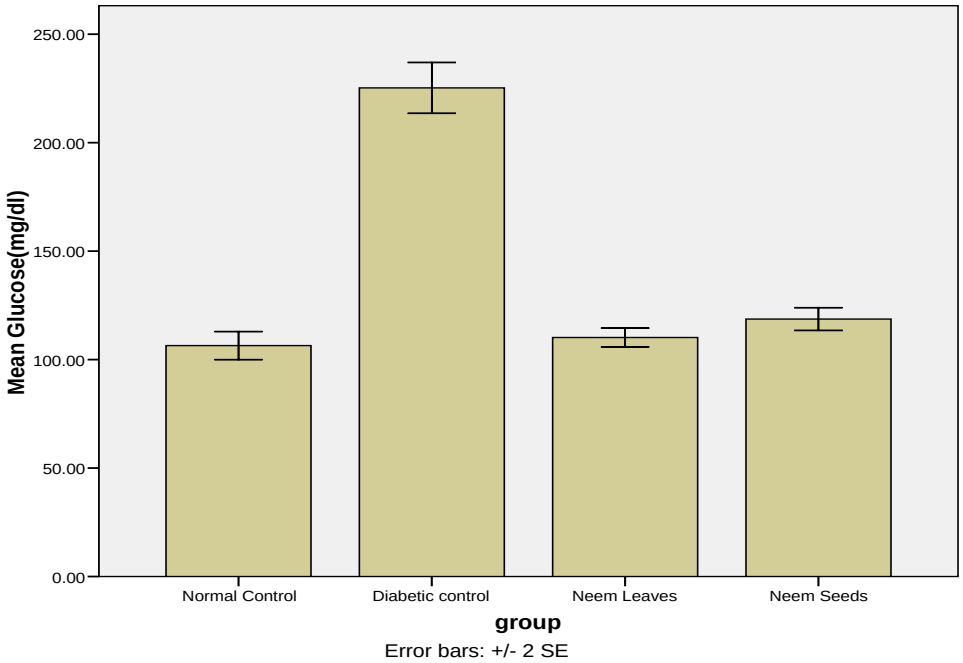
p >0.05 non-significant

Table 5: Comparison of serum glucose level in control and experimental group. Mean ± SD is given. Figure in parenthesis indicate number of cases is each group

Parameter	Normal control (n=30)	Diabetic control(n=30)	Neem leaves(n=30)	Neem seeds(n=30)
Serum glucose (mg/dl)	106.47±17.74	225.23±32.16*	110.20±11.84$^{\#}$	118.70±14.38$^{\#\$}$

p <0.05 significant in comparison of normal control group with diabetic control*, Neem leaves$^{\#}$ and neem seeds$^{\$}$ groups p >0.05 insignificant between neem leaves$^{\#}$ and neem seeds groups$^{\#}$

Fig. 1: Mean ± SEM serum glucose of the four groups (Error bars show SEM values)



DISCUSSION

The present study has been undertaken with the aim to evaluate and compare hypoglycemic effectiveness of neem leaves and seeds ethanolic extracts in rats, in which DM has been induced by alloxan. These rats showed highly significant increase of glucose in blood in comparison with the normal control. Treatment with ethanolic extracts of leaves and seeds produced highly significant reduction of elevated glucose in blood in rats suffering from DM. These results are comparable with those of Dholi et al, who also used ethanolic extract of neem leaves (dose 100 milligram / kilogram, and 250 milligram / kilogram) for single dose study and multiple dose study for fifteen days in rats with DM induced by alloxan; both having common outcome of lowering of glucose in blood.[17] Patel et al also evaluated these effects by using polyherbal formulation Dihar containing neem leaves and different parts of enicostemma littorale, syzygium cumini, tinospora cordifolia, embelica officinalis, momordica charantia, gymnema sylvestre, curcuma longa; and proved that its administration (100 milligram / kilogram) for six weeks to rats with DM, produced significant decrease in blood glucose, urea, lipids, and creatinine levels.[18] Waheed et al investigated the effects of aqueous, powdered, and alcoholic extract of seeds of neem, administered for fourteen days at low (0.5 grams tds) and high (2grams tds) doses in type-2 diabetic patients, and concluded that Azadirachta indica seeds could be added in high doses with oral hypoglycemic medicines to normalize the serum glucose levels in diabetic patients.[19] In short, our research results support the use of Azadirachta indica leaves and seeds in traditional medicine for the management of DM.

CONCLUSION

The present study concludes;

1. Ethanol extracts of neem leaves and seeds possess hypoglycemic lowering effects.
2. On comparison, neem leaves and seeds extracts show non-significant difference in hypoglycemic effects.

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