

Ethanollic Extract of Azadirachta Indica Seeds and Leaves and its impact on Lipid Profile in Diabetes Mellitus

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Abstract

Objective: To evaluate and compare the outcomes of Azadirachta indica on serum lipids in alloxan induced diabetic albino rats.

Method: Randomized controlled trial (RCT) carried out in Physiology Department of Services Institute of Medical Sciences, Lahore from November 2018 to April 2019. 120 male albino rats were randomly and uniformly divided into four groups (n=30). The control group is designated as G1 and kept on normal saline only. The Diabetic control (G2) and the other two experimental groups (G3 & G4) were administered with alloxan monohydrate intraperitoneal injections (120mg/kg) to induce diabetes mellitus. While G1 and G2 were used as the normal control and the diabetic control groups, only G3 and G4 were treated with Neem leaves (500 mg/kg body weight once a day) and Neem seeds (500 mg/kg body weight once a day) respectively for 28 days. Subsequently, blood samples (4-5ml intracardiac) were collected from each group member on the 29th day to evaluate the biochemical parameters of serum lipids.

Results: The ethanol-based extracts of Neem leaves and seeds lead to highly significant (p<0.001) reduction in lipid profile of G3 and G4.

Conclusion: Azadirachta indica leaves and seeds can significantly contribute in lowering serum lipid parameters.

Keywords: azadirachta indica, lipid lowering effect.

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Introduction

The prevalence of diabetes mellitus is predicted to increase globally from an estimated 382 million in 2013 to 592 million by 2035.¹ Type 2 diabetes has already attained epidemic level, while the incidence of type 1 diabetes also increases. It initially emerges as a group of disorders with defective or deficient insulin secretory process culminating in glucose underutilization, hence, leading to hyperglycemia.² Patients with

diabetes may suffer with wide range of microvascular complications such as stroke, ischemic heart disease, diabetic retinopathy and nephropathy.³ Others complications include periodontitis, neural disorders, gastroenteritis, delayed gastric emptying, renal disorders, dermatological manifestation, erectile dysfunction, diabetic retinopathy and diabetic macular edema.⁴ Medicinal herbs have played a significant role in treating and preventing a variety of diseases worldwide for centuries. Herbal remedies are tried globally to treat diabetes mellitus proactively or to delay any further complications. They are considered a significant source of antioxidants which help in preventing or delaying many diseases and their adverse outcomes.⁵ Such remedies not only seem to increase the insulin secretion and cellular glucose uptake but also decrease the intestinal glucose absorption and hepatic glucose production.⁶ One of the conventional herb used to treat diabetes mellitus is Azadirachta indica commonly known as

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neem. It's found in abundance in Asian subcontinent.²⁰ It has gained worldwide attraction in recent years, owing to its wide range of medicinal properties⁷. Many pharmacological and biological effects are attributed to various parts and extracts of this plant, including anti-diabetic, anti-inflammatory, antioxidant, antiplasmodial, antitrypanosomal, anticancerous, antimicrobial, spermicidal, antihelminthic, antifertility, immunomodulating, nematocidal, immunocontraceptive, insecticidal, and insect repellent effects.^{8,9} The present study is the experimental type and aims to add value to future research in elaborating the protective properties of ethanolic extracts of neem leaves and seeds specifically on serum lipid profile. Although many researches have been carried out that uses neem as their subject of interest to study blood pressure, obesity, and hyperlipidemia but our study is unique in that it uses ethanolic extracts of both seeds and leaves on diabetic grounds to assess the fluctuation in lipid profile.

Materials and Methods

Adult and healthy male albino rats (One hundred and twenty) were housed in groups of 30 per cage for minimally one-week prior the commencement of experiment. Dwelling environment was kept at $26\pm 2^{\circ}\text{C}$ with 12-hour light/dark cycle¹⁰ The rats were categorized in four groups (each group containing 30 rats). Group 1: Normal control provided with normal saline orally. Group 2: Diabetic control was given normal diet. Group 3 (Experimental 1): got treatment with extract of *Azadirachta indica* leaves orally (500 mg/kg) daily for 28 days. Group 4 (Experimental 2): got treatment with extract of *Azadirachta indica* seeds orally (500 mg/kg) daily for 28 days. Alloxan monohydrate achieve its diabetogenic results by specifically destroying the pancreatic beta cells, but other endocrine cells and exocrine parenchymal cells were unaffected. The cytotoxic agent exerts its diabetogenic effects by reactive oxygen species which promptly destroys beta cells¹¹. To induce diabetes, a single dose of alloxan monohydrate was given to overnight fasting rats of diabetic control and experimental groups before commencement of experiment.¹¹ At this dose (120 mg/kg), there is incomplete destruction of pancreatic beta cells which results in type 2 diabetes mellitus (NIDDM).¹² As Alloxan can lead to fatal hypoglycemia because of tremendous release of pancreatic insulin, rats were infused with 15-20 ml of 20% glucose solution intra peritoneally after 6 hours. To prevent hypoglycemia for next 24 hours the rats were kept on 5% glucose solution bottles.¹³ Blood glucose was eva-

luated after 72 hours to confirm hyperglycemia.¹⁴ Rats with hyperglycemia (>200 mg/dL) were considered diabetic and incorporated in experiment.¹⁵ Then diabetic rats of group 3 and 4 were treated with leaves and seeds (ethanolic extract) of *Azadirachta indica* for 28 days.¹⁶ On 29th day, intracardiac blood sample (4-5ml) was obtained to evaluate the effects of plant extract on lipid profile.

Freshly matured leaves and seeds (5kg each) of *Azadirachta indica* were fetched locally from Lahore. Botanical identification of the leaves and seeds was completed in the Botany Department, Punjab University. An 80% ethanol extract of the air-dried and coarsely ground *Azadirachta indica* leaves and seeds was obtained via standardized Soxhlet extractor in Applied Chemistry Research Centre, PCSIR Labs, Lahore. The extract thus acquired, was subjected to filtration and ethanol (solvent) evaporation in a rotary evaporator in a vacuum. A blackish-brown concentrate, obtained post-evaporation, was then preserved at 4°C . Preceding to every dose, the crude extract was liquefied in sterilized distilled water and diluted to the required concentration.¹⁷

Initial blood sample was drawn aseptically from tail vein 72-hours after alloxan injection to confirm hyperglycemia. Sampling was repeated on the 29th day of the experiment after ensuring the animals were fasting overnight. Each rat was anesthetized using ether before drawing 5-milliliter blood from their tail vein. Four ml of each sample was allowed to coagulate at room temperature in the test tube for 30 minutes followed by centrifugation at 5000 rpm for 20 minutes. Post-centrifugation, the serum was collected and preserved in labeled tubes. It was kept at -20°C to be test cholesterol, triglycerides, HDL later on.¹⁸ PASW (formerly SPSS) was used to conduct data analysis. ANOVA test was carried out for descriptive analysis to find the arithmetic mean \pm SD values of obtained data. Post hoc Tukey's HSD test (multiple comparisons) was applied to find any significant value (p-value less than 0.05) among the four groups existed. The values were appraised highly significant when the p-value was less than 0.001.

Results

In this randomized controlled trail, the effects of *Azadirachta indica* (neem) leaves and seeds on the serum lipid profile of a total of 120 male diabetic albino rats were evaluated. The serum cholesterol, triglyceride, LDL, and VLDL in diabetic control group was found to be highly significantly ($p=0.000$) greater than in the control group (**Table-1**). Similarly, serum HDL level was

also significantly higher in normal controls than in the dia-betic controls (**Table-1**). After administering neem leaves and seeds extract, the mean difference showed a highly significant ($p=0.000$) drop in cholesterol, triglyceride, LDL, and VLDL levels (Table 2 and 3); and highly sig-nificant ($p=0.000$) rise in serum HDL level in treated group compared to the untreated diabetic control group (**Table-2**). The experimental group treated with neem leaves extract had highly significantly lower ($p=0.000$) serum triglyceride and VLDL levels than in the experi-mental group treated with neem seeds. However, the decline serum cholesterol and LDL levels in experi-mental group (G3) treated with neem leaves versus the experimental group treated with seeds (G4) did not show such significance. Similarly, Difference of increased serum HDL between the two groups was non-significant (**Table-2**). Figure 1 shows mean lipid values in normal control, diabetic, and treatment groups.

Table 1: Comparison of Serum Lipid Profile in Normal Control and Diabetic Control Groups Values are given as Mean \pm SD

Lipids	Normal control	Diabetic control	Mean difference	p-value
Cholesterol (mg/dL)	92.30 \pm 28.60	227.77 \pm 15.76	135.47	0.000*
Triglyceride (mg/dL)	83.60 \pm 7.39	216.20 \pm 10.20	132.60	0.000*
HDL(mg/dL)	25.83 \pm 1.91	19.47 \pm 2.29	6.37	0.000*
LDL(mg/dL)	49.75 \pm 29.04	165.06 \pm 15.85	115.31	0.000*
VLDL(mg/dL)	16.72 \pm 1.48	43.24 \pm 2.04	26.52	0.000*

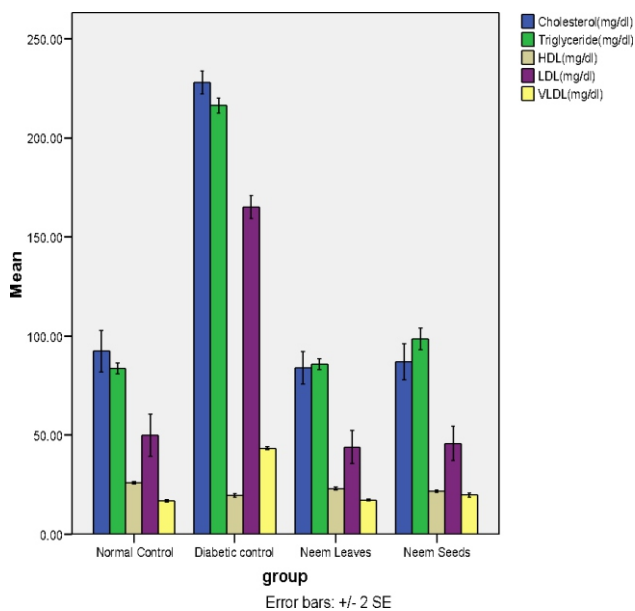


Figure1: Mean \pm SEM lipid profile of the four groups

Table 2 : Comparison of serum lipid profile in diabetic control group and group treated with neem leaves. Values are given as Mean \pm SD

Lipids	Diabetic control	Neem leaves	Mean difference	p-value
Cholesterol (mg/dL)	227.77 \pm 15.76	83.90 \pm 22.68	143.86	0.000*
Triglyceride (mg/dL)	216.20 \pm 10.20	85.70 \pm 7.32	130.50	0.000*
HDL(mg/dL)	19.47 \pm 2.29	22.90 \pm 1.81	3.43	0.000*
LDL(mg/dL)	165.06 \pm 15.85	43.86 \pm 23.00	121.20	0.000*
VLDL(mg/dL)	43.24 \pm 2.04	17.14 \pm 1.46	26.10	0.000*

*p <0.001 highly significant

Discussion

Recently, more research is being focused on unveiling the anti-diabetic properties of neem in search of some harmless herbal alternative to allopathic medicines. For this purpose, meta studies are being carried out to assess the effects of various aqueous, ethanolic, methanolic, and petroleum extracts from all parts of the plant on blood pressure, hyperlipidemia and obesity.²⁰ The current study is focused to evaluate and compare the lipid lowering outcomes of ethanolic neem leaves and seeds extracts in alloxan induced diabetic rats.

When induced with Alloxan, diabetic rats showed a rise in serum triglyceride, total cholesterol, VLDL- cholesterol, LDL- cholesterol levels ($p=0.001$) compared to normal controls. Also, serum HDL levels was higher in normal controls ($p=0.000$) than in the diabetic controls. The serum triglyceride, total cholesterol, & LVLDL-cholesterol reduced; and HDL-cholesterol increased in the experimental groups treated with the ethanolic extracts of neem leaves & seeds versus the untreated diabetic controls. Furthermore, the leaves extract was shown to have more potent triglyceride and VLDL lowering effect than the seed extracts ($p=0.000$). Similar results were obtained by Dholi et al¹⁷, when alloxan induced diabetic rats were administered ethanolic extract of neem leaves for single dose therapy and multiple dose therapy for two weeks both leading to decline of cholesterol and triglyceride levels. Patel et al¹⁸, administered neem extracts among few other herbal extracts for 42 days to diabetic rats that resulted in notable decline in the serum lipids levels. Ashafa et al¹⁹, evaluated effects of ethanolic extract of stem bark extract of Azadirachta indica that seemed to significantly decrease the serum triglyceride and HDL-cholesterol but increased the serum concentration of cholesterol, LDL- cholesterol at all tested doses. Hence, the results of our study indicate the potential lipid lowering benefits of using the

Azadirachta indica in herbal medicine and warrants further research and human trials.

Conclusion

The current research decides; Ethanol based extracts of neem seeds and leaves are potent lipid lowering agents. Leaves of neem may have more potent effects in lowering the serum triglycerides and VLDL than the neem seeds.

Conflict of Interest *None*

Source of Funding *None*

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Authors Contribution

TS: Conceptualization of Project
TS, AS, NK: Data Collection
TS, NZ, AZ: Literature Search
AS: Statistical Analysis
TS, NS: Drafting, Revision
TS, NZ: Writing of Manuscript