Nephroprotective Effects of Ethanolic Extracts of Azadirachta Indica Seeds and Leaves in Diabetic Rats

Tahira Saleem, Abida Saleem, Nusrat Jabeen, Naila Saleem, Naima Khalid, Omaila Ikram

Abstract

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

Method: The study was a randomized controlled trail carried out in Physiology department of services institute of medi-cal sciences, Lahore from November 2018 to April 2019. 120 male albino rats were randomly and uniformly divided in four groups (n=30). Diabetic control and experimental groups were administered with alloxan monohydrate intraperitoneal injection of (120mg/kg) to induce diabetes mellitus. G1 (control) received normal saline orally; G2 was (diabetic control), group 3 received Neem leaves extract (500 mg/kg body weight); and group 4 received Neem seeds extract (500 mg/kg body weight) as a single dose for four weeks. Subsequently blood samples (4-5ml intracardiac) were collected from each group member on 29th day to evaluate the biochemical parameters of serum urea and creatinine.

Results: The ethanol based extracts of Neem seeds and leaves lead to highly significant ($p<0.001$) reduction in urea and creatinine levels of G3 and G4.

Conclusion: Azadirachta indica leaves and seeds can significantly contribute in lowering serum urea and creatinine

Key words: Azaditachta Indica, urea and creatinine lowering Effect.

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Introduction

The prevalence of diabetes mellitus is predicted to rise globally from an estimated 382 millions in 2013 to 592 million by 2035.[1] Type 2 diabetes has already attained epidemic level, while incidence of type1 diabetes is also increasing. It initially emerges as group of disorders with a defective or deficient insulin secretory process, glucose underutilization leading to hyperglycemia.[2] Patients with diabetes may suffer with wide range of complications that involves microvasculature related 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 throughout the world. Conventional herbal therapy is tried globally to treat diabetes mellitus to delay the onset of diabetic complications and it also supply a great source of antioxidants which is helpful in preventing or delaying different diseases and their outcomes.[5] Their mechanism of action is based upon increasing insulin secretion, enhancing glucose uptake by adipose and skeletal muscle tissue, inhibiting intestinal glucose absorption and inhibiting hepatic glucose production.[6] One of the conventional herb used to treat diabetes mellitus is neem (Azadirachta indica). Azadirachta indica commonly

1. *Department of Physiology, King Edward Medical University, Lahore.*
2. *Department of Dental Materials, Shifa College of Dentistry, Islamabad.*
3. *Department of Oral Biology, Shifa College of Dentistry, Islamabad.*
4. *University of North Dakota, USA.*
5. *Al Aleem Medical Colleg, Lahore.*
6. *Department of Anaesthesia, Mayo Hospital Lahore.*

Correspondence:
Dr Tahira Saleem, *Associate Professor, Department of Physiology, King Edward Medical University, Lahore. Pakistan.*
E mail: drtahirasaleem1@gmail.com

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known as neem has gained worldwide attraction in recent years, owing to its wide range of medicinal properties.[7] The world prefers the development of such drugs which have minimum toxic potential. [Azadirachta indica A. Juss] reportedly has various medicinal properties and has been in use in many continents for centuries. Pharmacological and biological effects are attributed to all parts and extracts of this plant, including antidiabetic, anti-inflammatory, antioxidant, antiplasmodial, antitrypanosomal, anticancerous, antimicrobial, spermicidal, anthelminthic, antifertility, immunomodulating, nematicidal, immunocontraceptive, insecticidal, and insect repellant effects.[8,9] Diabetic nephropathy is a very common complication of diabetes mellitus which is associated with high mortality and morbidity.[10] This study was aimed to determine and compare the nephroprotective effect of ethanolic extracts of leaves and seeds of azadirachta indica on serum urea and creatinine in diabetic albino rats. The assessment of the effects of this potential herbal medicine will allow clinicians to redesign preventive and therapeutic regime of a fairly common health disorder.

Material and Methods

The study was a randomized controlled trail carried out in Physiology department of services institute of medical sciences, Lahore from November 2018 to April 2019.

Adult and healthy male albino rats (One hundred and twenty) were housed in four groups of 30 per cage for minimally seven days prior the commencement of experiment. Dwelling environment was kept at 26±2 0C with 12-hour light/dark cycle.[11] 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.

Results

In this randomized controlled trial, the effects of neem leave and seeds on the serum urea and creatinine profile of a total of 120 male diabetic albino rats were evaluated.

The serum urea and creatinine in diabetic control group was found to be highly significantly (p=0.000) greater than in the control group (Table 1). After administering neem leaves and seeds extract, the mean difference showed a highly significant (p=0.000) drop in urea and creatinine quantity in treated group compared to the untreated diabetic control group. (Table 2, 3).

The experimental group treated with neem leaves extract had non-significantly lower (p=0.000) serum urea levels than in the experimental group treated with neem seeds. However, difference of decreased serum creatinine between the two groups was non-significant (Table 4). Figure 1 shows mean urea and creatinine values in normal control, diabetic, and treatment groups.

Discussion

Alloxan monohydrate achieves 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 cells.[12] 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.[12] At this dose (120 mg/kg), there is incomplete destruction of pancreatic beta cells which results in type 2 diabetes mellitus (NIDDM).[13] 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.[14] Blood glucose was evaluated after 72 hours to confirm hyperglycemia.[15] Rats with hyperglycemia (>200 mg/dL) were considered diabetic and incorporated in experiment[16]. Then diabetic rats of group 3 and 4 were treated with leaves and seeds (ethanolic extract) of Azadirachta indica for 28 days.[17] On 29th day, intracardiac blood sample (4-5ml) was obtained to evaluate the effects of plant extract on renal 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 40^{0}C. Preceding to every dose, the crude extract was liquefying in sterilized distilled water and diluted to the required concentration.[18]

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 urea and creatinine later on.[19] PASW[18] (formerly SPSS) was used to conduct data analysis. ANOVA test was carried out for descriptive analysis to find the arithmetic mean±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.

The current study is focused to evaluate and compare the urea and creatinine 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 urea and creatinine levels (p= 0.001) compared to normal controls. The serum urea and creatinine reduced; 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 non significantly serum urea lowering effect than the seed extracts (p=0.000), but difference in decline of serum creatinine between two groups was non-significant. Similar results were obtained by Dholi et al[18], 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 urea and creatinine levels. Patel et al[19], administered neem extracts among few other herbal extracts for 42 days to diabetic rats that resulted in notable decline in the serum urea and creatinine levels. Kpela T[20], investigated the protective effects of neem on cisplatin-induced kidney damage and results showed that urea and creatinine levels were normalized by pretreatment with neem leaf extract. Hence, the results of our study indicate the potential serum urea and creatinine lowering benefits of using the Azadirachta indica in herbal medicine and warrants further research and human trials.

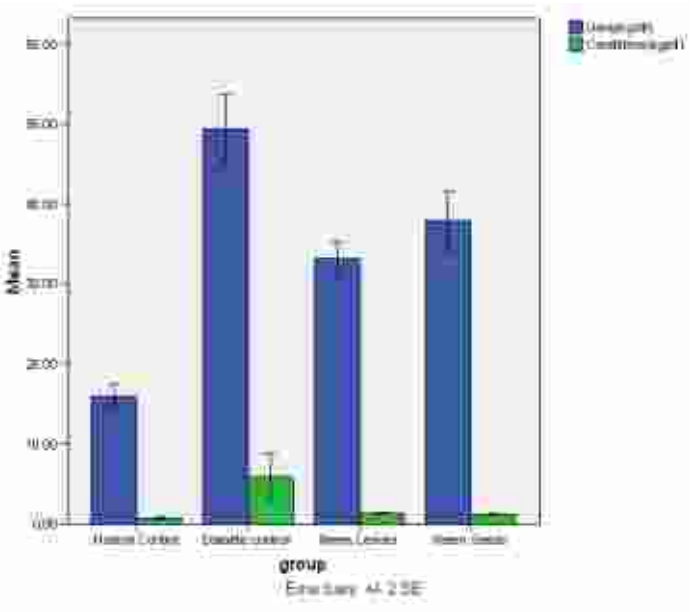

Figure 1: *Mean ± SEM serum urea and creatinine.*

Table 1: *Renal parameters in normal and diabetic control*

Renal profile	Group 1	Group 2	Mean difference	p-value
Urea(mg/dL)	16.00±4.00	49.33±11.61	33.33	0.000*
Creatinine (mg/dL)	0.71±0.28	5.95±7.86	5.24	0.000*

*Values are given as Mean ± SD: *p <0.001 highly significant*

Table 2: *Renal parameters in experimental group treated with neem leaves*

Renal profile	Group 2	Group 3	Mean difference	p-value
Urea(mg/dL)	49.33±11.61	33.20±5.62	16.13	0.000*
Creatinine (mg/dL)	5.95±7.86	1.34±0.28	4.61	0.000*

Values are given as Mean ± SD: *p <0.001 highly significant

Table 3: *Renal parameters levels in experimental group treated with neem seeds*

Renal profile	Group 2	Group 4	Mean difference	p-value
Urea(mg/dL)	49.33±11.61	37.90±10.15	11.43	0.000*
Creatinine (mg/dL)	5.95±7.86	1.21±0.30	4.74	0.000*

*Values are given as Mean ± SD: *p <0.001 highly significant*

Table 4: *Comparison of renal parameters between experimental groups (G3 and G4) treated with neem leaves seeds respectively*

Renal profile	Group 3	Group 4	Mean difference	p-value
Urea (mg/dL)	33.20 ± 5.62	37.90±10.15	4.70	0.142
Creatinine (mg/dL)	1.34 ± 0.28	1.21 ± 0.30	0.13	0.999

*Values are given as Mean ± SD: *p <0.001 highly significant*

Conclusion

The current research decides;

1. Ethanol based extracts of neem seeds and leaves are urea and creatinine lowering agents.
2. Leaves and seeds extract of Neem exhibited nephroprotective effect but do not show any significant difference in urea and creatinine lowering.

Conflict of Interest None

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Authors Contribution

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