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The Anti-Diabetic Effect of Neem Leaves (Azadirachta indica,) in Alloxan-Induced Diabetic Rats

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Abstract

The traditional medicinal herb neem (Azadirachta indica) may contribute intriguing bioactive compounds to the present diet in many countries. Herbal substances are being used as medicinal agents. This study evaluated the effects of different concentrations of neem leaves powder 2.5 and 5% and ethanolic extract 250 and 500 mg/kg body weight on diabetic rats. Thirty-six white male albino rats weighing 140±10g were divided into six groups of six rats each. Diabetic rats were infected with alloxan (150 mg/kg body weight). The following tests were performed: glucose, serum liver functions (ALT, AST, and ALP), T.G., T.C., LDL-c, HDL-c, VIDL-c, and kidney functions (urea, uric acid, and creatinine). HPLC was also used to determine the phenolic components in neem leaves. The findings showed that rats given neem leaves powder or extract improved their serum glucose levels, liver functions, kidney functions, and lipid profile. The best results were obtained with 500 mg/kg neem leaf extract. As a result of the findings, it is suggested that neem leaves contain numerous phytochemicals and can be used as an antioxidant to lower glucose levels in diabetic rats, apart from the fact that it offers multiple health advantages.

Key words: Neem plant, Biochemical analysis, Rats, Hyperglycemic.

Introduction

Diabetes mellitus (DM) is a class of metabolic illnesses marked by a persistent hyperglycemic state caused by insulin production, insulin action, or both. Glucokinase deficiency causes permanent neonatal diabetes, which is an inborn defect in the glucose insulin signaling system [(1)]. The prevalence of diabetes is increasing rapidly worldwide and, according to the World Health Organization [(2)], the number of adults with diabetes would have more than doubled by 2030, from 177 million in 2000 to 370 million.

Diabetes is expected to increase by 64 percent by 2025, according to experts. Insulin dependent diabetes mellitus is caused by a shortage of insulin secretion by the pancreatic beta cells in all kinds of diabetes mellitus [(3)]. Regardless of the fact that large randomized trials show that tight blood glucose control reduces microvascular and macrovascular problems, many diabetics do not or do so ineffectively [(4)]. Diabetes mellitus is a disease in which the pancreas does not produce enough insulin or the body's insulin is not utilized properly. As a result, the glucose level in the blood increases (hyperglycemia) [(5)].

In many countries, medicinal plants have been the primary source of primary health care. Traditional medicines are still used by almost 80% of the world's population. Due to a lack of or limited access to modern health care in underdeveloped nations, folk medicines have become increasingly important. Plants have been a rich source of efficient and safe remedies since ancient times. Indigenous treatments are popular among people in both urban and rural areas in China and India because they are safe, effective, and economical[(6)]. Neem (*Azadirachta indica*, A. Juss) is a tropical and subtropical evergreen woody plant belonging to the Meliaceae family that is native to the Indo-Pak subcontinent. Limonoids (e.g., azadirachtin, salanin), flavonoids, essential oil, and other secondary metabolites are found in neem plants. Almost every part of the neem tree, including the seeds, blossoms, leaves, bark, trunk, and branches, is utilized for shade, construction material, medicine, and pesticides [(7)]. In India, neem is possibly the most beneficial traditional medicinal herb. Each portion of the neem tree has medical properties and can thus be utilized commercially. Apart from the chemistry of neem compounds, significant progress has been made in the biological activities and therapeutic applications of neem over the previous five decades. Neem is widely utilized in Ayurveda, Unani, and Homoeopathic treatment, and has become a modern medical cynosure. Neem produces a wide range of physiologically active chemicals that are both chemically and structurally varied. It is today regarded as a valuable source of unique natural components for the creation of medications and commercial products for a variety of ailments [(8)].

The neem phytochemical nimbidin (200 mg/kg) significantly delayed the rise in blood glucose after oral glucose administration. It reveals the antihyperglycemic benefits of neem [(9)]. The use of neem lowered blood glucose levels considerably. The mechanism behind the extract's anti-diabetic benefits is unknown. Jelodar *et al.,* [(10)] hypothesized that the extract's anti-diabetic qualities stemmed from its ability to induce sufficient insulin secretion by the pancreas, which aided in the peripheral consumption of glucose in the cells, or from the extract's ability to renew cells to carry out its activities. 70% ethanolic alcoholic of neem root extract (NRE) showed anti-diabetic activity, due to the glibenclamide reduced blood sugar levels significantly in the glucose tolerance test. Only an 800 mg/kg dosage of NRE produced statistically meaningful results [(11)]. The upregulated blood glucose, total plasma cholesterol such as LDL-c, and triglyceride levels

in the three treatment groups of mice were considerably (P≤0.001) reduced (TG). As a result, researchers suggested that neem leaves and/or spirulina could be an effective diabetic alternative therapy [(12)].

The purpose of this study was to see how different concentrations of neem leaves powder (2.5 and 5%) and its solvent extract (250 and 500 mg/kg bw.) affected alloxan-induced diabetic rats.

Materials And Methods

Materials:

Fresh neem leaves (*Azadirachta indica*) were obtained from the Agriculture Research Center in Giza, Egypt, and transported to the laboratory, where they were kept at 5 ^{0}C until testing.

The chemicals and kits

El-Gomhoria Company for Trading Chemical, Drugs and Medical Instruments, Cairo, Egypt, provided casein, cellulose, choline chloride powder, and DL methionine powder. In Menoufia, Egypt, oil and corn starch were purchased from a local market. Bio Diagnostics Company in Cairo, Egypt, provided the kits.

Experimental animals

The Vaccine and Immunity Organization, Ministry of Health, Helwan Farm, Cairo, Egypt, provided a total of 36 adult normal male albino rats of the Sprague Dawley strain weighing 140±10g.

Methods

Preparation of neem leaves extract

The leaves were rinsed under running water before being dried in a 45ºC oven for 24 hours. Dried leaves that have been ground into flour with a blender and then sifted. The leaves were extracted with 70% ethanol in brown bottles for 5 days in a dark atmosphere at room temperature (25-30ºC), shaking gently every day. The mixture was filtered using suction pumping and bokhnnar cone throw filter paper. Heating the ethanolic extract under vacuum at 62°C to concentrated it **[(13)]**.

Experimental design

In the experiment, 36 male albino rats weighing 140±10g were tested. Rats were housed in separate stales steel cages in the animal home under regulated environmental conditions and fed a basal diet (casein diet) for 7 days before starting the experimental diet for adaptation, according to [(14)]. The rats are divided into six groups, each of which contains six rats. Group 1 (-ve): fed on basal diet only, as negative control. Group 2 (+ve): fed on basal diet and injected by a single dose of freshly prepared solution of alloxan (150mg/kg) and was used as a positive control group according to the procedure described by Desai and Bhide, [(15)]. Group 3: Diabetic rats fed on basal diet and neem powder by

2.5% of the weight of the diet. Group 4: Diabetic rats fed on basal diet and neem powder by 5%of the weight of the diet. Group 5: Diabetic rats fed on basal diet and treated with oral administration of neem extract by 250 ml/kg. Group 6: Diabetic rats fed on basal diet and treated with oral administration of neem extract by 500 ml/kg. The experiment lasted 28 days, and at the end of that time, each rat was weighed independently, and the rats were slaughtered and blood samples were taken. To separate blood serum, blood samples were centrifuged at 4000 rpm for 10 minutes and then maintained in a deep freezer until needed.

Biochemical analysis

Lipids profile

Serum total cholesterol was determined according to the colorimetric method described by **(16)**. Serum triglyceride was determined by enzymatic method using kits according to the **(17and 18)**. HDL-c was determined according to the method described by **(19 and20)**. VLDL-c was calculated in mg/dl according to **(21)** was using the following formula:

VLDL-c (mg/dl) = Triglycerides / 5. LDL-c was calculated in mg/dl according to **(21)** as follows:

LDL-c (mg/dl) = Total cholesterol – HDL-c – VLDL-c

Liver functions

Determination of serum alanine amino transferase (ALT), serum asparatate amino transferase (AST), serum alkaline phosphatase (ALP) was carried out according to the method of **(22,23and 24)**, respectively.

Kidney functions

Determination of serum urea and creatinine

Serum urea, uric acid and creatinine were determined by enzymatic method according to **(25, 26&27)**.

Determination of glucose level

Serum glucose was measured using the modified kinetic method according to **(28)** by using kit supplied by spin react. Spain.

Statistical analysis

The data were analyzed using a completely randomized factorial design **(36)** when a significant main effect was detected; the means were separated with the Student-Newman-Keuls Test. Differences between treatments of ($P \leq 0.05$) were considered significant using Costat Program. Biological results were analyzed by One Way ANOVA**(29)**.

Results And Discussion

The impact of neem leaves powder and its extract on glucose levels of diabetic rats' is shown in table (1). The obtained results revealed that the positive control group had the

higher glucose level, while the negative control group had the lower level, with significant differences (P≤0.05). The mean blood sugar levels were 210.00 mg/dl and 95.60 mg/dl, respectively.

The greatest glucose level was obtained in rats fed 2.5 percent neem leaves powder, while the lowest glucose level was recorded in rats fed 500 mg/kg neem leaves extract, with mean values of 121.0 and 103.0 mg/dl, respectively. It was discovered that increasing the amount of neem leaves resulted in the greatest decrease in glucose levels. These findings are consistent with those of Khosla *et al.,* [30], who found that giving rabbits an aqueous extract of neem leaf (500 mg/kg orally) for 4 weeks after alloxan induced diabetes lowered blood glucose levels significantly (P≤0.001). Kar *et al.,* [31] reported that in alloxan-induced diabetes in rats, 95 percent alcoholic extract of neem leaf in the dose of 250 mg/kg twice daily orally for one-week lowered blood sugar levels by 55 percent and urine sugar by 100 percent (p≤0.05).

The usage of neem in controlling blood glucose levels has long been in practice in many countries. Neem leaf extract dilate the blood vessels in diabetic patients and the neem leaves and seed is found to reduce the amount of insulin required to be administered to a diabetic patient. These actions are supposed to be exhibited due to cumulative effect of glycosides, terpenoids and flavonoids present in the leaf and seed extracts [32].

Table (1) Effect of neem leaves and its extract on glucose levels of diabetic rats

Parameters / **Groups**	**Glucose (mg/dl) Mean±SD**
G1 C (-)	95.60 ± 0.40e
G2 C (+)	210.00± 1.10a
G3 2.5% neem leaves powder	121.00± 0.10b
G4 5% neem leaves powder	118.30± 0.30b
G5 (250 mg/kg neem extract)	114.50± 0.30c
G6(500 mg/kg neem extract)	103.00± 0.20d
LSD (P ≤ 0.05)	4.101

Each value is represented as mean ± standard deviation (n = 3). Mean under the same column superscript with different letters are different significantly (P≤0.05).

The effect of neem leaves and their extract on ALT, AST, and ALT in diabetic rats is shown in table (2). The results showed that the ALT liver enzyme in the positive control group was greater than in the negative control group, with a significant difference at (P≤0.05), which were 199.0 and 97.0 U/L, respectively. While the highest ALT liver enzyme of the treated group was found in those who were administrated 250 mg/kg neem leaves extract, the lowest value was found in those who were fed 5 percent neem leaves powder, with a significant difference at (P≤0.05). The mean values were127.0 and 115.0 U/L, respectively.

When compared to the negative control group, the AST liver enzyme of positive control rats had a higher value, with a significant difference at (P≤0.05). The averages were 57.40 and 10.80 U/L, respectively. While the highest AST liver enzyme of the treated group was found in the group given 2.5 percent neem leaves powder, the lowest value was found in the group fed 500 mg/kg neem leaves extract, with a significant difference of (P≤0.05). The mean values were 40.98 and 16.79 U/L, respectively.

When it came to ALP liver enzyme, the positive control rats had a higher value than the negative control rats, with a significant difference at (P≤0.05). The average values were 21.55 and 7.35 U/L, respectively. While the greatest ALP liver enzyme of the treated group was found in those who were fed 2.5 percent neem leaves powder, the lowest value was found in those who were fed 500 mg/kg neem leaves extract, with a significant difference at (P≤0.05). The averages were16.05 and 11.80 U/L, respectively. These findings are consistent with those of Chattopadhyay and Bandyopadhyay [(33)], who observed no significant change in these enzymes in Wister rats treated with both paracetamol and neem extract, which is also consistent with published observations on neem's hepatoprotective properties.

Table (2): Effect of neem leaves and its extract on liver functions levels of diabetic rats

Parameters / Groups	ALT (U/L) Mean±SD	AST (U/L) Mean±SD	ALP (U/L) Mean±SD
G1 C (-)	97.0d ± 1.20	10.80f ± 1.10	7.35e± 0.40
G2 C (+)	199.0a ± 0.20	57.40a ± 1.35	21.55a± 0.10
G3 (2.5% neem leaves powder)	125.0b ± 0.10	40.98b ± 2.05	16.05b ± 0.20
G4 (5% neem leaves powder)	115.0c ±0.40	32.58c ± 0.60	14.65c ± 1.00
G5 (250 mg/kg neem extract)	127.0b ± 1.10	21.73d ± 1.25	14.78c± 0.10
G6(500 mg/kg neem extract)	117.0c ± 1.30	16.79e ± 0.90	11.80d ± 0.30
LSD (P ≤ 0.05)	2.30	1.49	1.10

Each value is represented as mean ± standard deviation (n = 3). Mean under the same column superscript with different letters are different significantly (P≤0.05).

Table (3) shows the effect of neem leaves powder and its extract on total cholesterol and triglycerides in diabetic rats. The obtained results revealed that the positive control group's total cholesterol was higher than the negative control groups, with a significant difference at (P≤0.05). The mean values were 135.0 and 80.0 mg/dl, respectively. While the group administrated 500mg/kg neem leaves extract had the lowest total cholesterol, the group fed 2.5 percent neem leaves powder had the highest, with a significant difference at (P≤0.05). The mean values were 113.0 and 127.0 mg/dl, respectively.

When compared to the negative control group, the triglycerides levels of the positive control group were higher, with a significant difference at (P≤0.05), which were 120.50 and 59.50 mg/dl, respectively. While the group administrated 500mg/kg neem leaves extract had the lowest triglycerides levels, the 2.5 percent neem leaves powder had the highest, with a significant difference at (P≤0.05). The average levels were 69.17 and 93.89 mg/dl, respectively. Changes in the levels of major lipids such as cholesterol and triacylglycerol could provide useful information on the predisposition of the heart of animals to atherosclerosis and coronary heart disease, according to Yakubu *et al.,* [34], who reported that changes in the levels of major lipids such as cholesterol and triacylglycerol could provide useful information on the predisposition of the heart of animals to atherosclerosis and its associated coronary heart disease. The considerable decrease in triacylglycerol may be linked to impaired lipolysis, although the decrease in HDL-C at all dosages examined may not be therapeutically helpful to the animals because the rate at which plasma cholesterol is transported to the liver will be reduced as well.

Table (3) Effect of neem leaves and its extract on serum triglycerides, and serum total cholesterol of diabetic rats

Parameters	Total cholesterol mg/dl	Triglycerides mg/dl
Groups	**Mean±SD**	**Mean±SD**
G1 C (-)	80.00f ± 0.10	59.50f ± 0.22
G2 C (+)	135.00a ± 1.30	120.50a ±1.31
G3 (2.5% neem leaves powder)	127.00b ± 0.20	93.89b ± 2.10
G4 (5% neem leaves powder)	122.00c ± 0.10	82.08c ± 1.10
G5 (250 mg/kg neem extract)	118.00d ± 0.10	79.38d± 1.25
G6(500 mg/kg neem extract)	113.00e ± 0.40	69.17e ± 0.30
LSD (P ≤ 0.05)	3.60	2.48

Each value is represented as mean ± standard deviation (n = 3). Mean under the same column superscript with different letters are different significantly (P≤0.05).

Data tabulated in table (4) show the effect of neem leaves and its extract on the serum lipid profiles of diabetic rats. When compared to the positive control rats, the HDL-c of the negative control rats' group had the highest value, with a significant difference at (P≤0.05). The average levels were 44.65 and 28.40 mg/dl, respectively. While the greatest HDL-c of the diabetic group was found in the group fed 500 mg/kg neem leaves extract, the lowest value was found in the 2.5 percent neem leaves powder group, with a significant difference at (P≤0.05). The average readings were 38.45 and 31.80 mg/dl.

As compared to the negative control group, the LDL-c of positive control rats had the higher value, with a significant difference at (P≤0.05). The average readings were 78.82 and 43.42 mg/dl. While the highest LDL-c of the treated group was found in the group

fed 2.5 percent neem leaves powder, the lowest value was found in the group fed 500 mg/kg neem leaves extract, with a significant difference at (P≤0.05). The average readings were 79.77 and 66.37 mg/dl.

When it came to VLDL-c, the positive control rats had a greater value than the negative control rats, with a significant difference (P≤0.05). The average levels were 27.78 and 11.93 mg/dl, respectively. While the greatest VLDL-c of the diabetic group was found in the group fed 2.5 percent neem leaves powder, the lowest value was found in the group fed 500 mg/kg neem leaves extract, with a significant difference of (P≤0.05). The mean values were 18.78 and 13.83 mg/dl, respectively. These findings support Kausik *et al.*, [35], who findings that *A. indica* leaf extract can reduce elevated levels of total serum cholesterol, triglycerides, total lipids, VLDL, and LDL-cholesterol in diabetic patients. Furthermore, its antihyperlipidemic action could be a preventive mechanism against atherosclerosis formation. As a result of its antihyperlipidemic activity, *A. indica* leaf extract may be useful in preventing the development of hyperlipidemia and atherosclerosis in diabetics.

Table (4) Effect of neem leaves and its extract on lipoprotein cholesterol of diabetic rats

Parameters / Groups	HDL-C (mg/dl) Mean±SD	LDL-c (mg/dl) Mean±SD	VLDL-c (mg/dl) Mean±SD
G1 C (-)	44.65a ± 1.10	43.42d±0.11	11.93e± 0.35
G2 C (+)	28.40d ± 1.21	78.82a± 1. 30	27.78a± 1.10
G3 (2.5% neem leaves powder)	31.80c± 0.10	69.77b ± 1.40	18.78b ± 1.32
G4 (5% neem leaves powder)	34.65c ±1.20	69.26b ± 0.40	16.42c ± 0.40
G5 (250 mg/kg neem extract)	36.32b ±1.10	67.47b± 1.20	15.88c± 1.30
G6(500 mg/kg neem extract)	38.45b±0.30	66.37c±1.30	13.83d±1.10
LSD (P ≤ 0.05)	3.00	2.60	1.65

Each value is represented as mean ± standard deviation (n = 3). Mean under the same column superscript with different letters are different significantly (P≤0.05).

The effect of neem leaves and their extract on kidney functions (urea, uric acid, and creatinine) in diabetic rats is shown in table (5). The results showed that the urea level in the positive control rats' group was greater than the negative control rats' group, with a significant difference at (P≤0.05), which were 75.80 and 44.35 mg/dl, respectively. While the greatest urea level of the treatment group was found in those fed 2.5 percent neem leaves powder, the lowest value was found in those fed 500 mg/kg neem leaves extract, with a significant difference (P≤0.05), which were 60.28 and 48.42 mg/dl, respectively.

The uric acid level of the positive control rats' group, on the other hand, was significantly greater than that of the negative control rats' group (P≤0.05). The mean values were 4.40

and 2.50 mg/dl, respectively. While the group fed 2.5 percent neem leaves powder had the highest uric acid level, the group fed 500 mg/kg neem leaves extract had the lowest, with a significant difference at (P≤0.05). The mean values were 3.71 and 2.70 mg/dl, respectively.

When it came to creatinine, the positive control rats had a higher value when compared to the negative control rats, with a significant difference at (P≤0.05). The mean values were 1.55 and 1.15 mg/dl, respectively. While the greatest creatinine level was found in the group fed 250 mg/kg neem leaves extract, the lowest value was found in the group fed 500 mg/kg neem leaves extract, with a significant difference (P≤0.05). The mean values were 1.51 and 1.44 mg/dl, respectively. These findings are consistent with those of Soliman *et al.*, [36], who found that pre, post, and co-treatments with methanolic neem (*A. indica*) leaves extract were more efficient in preventing nephrotoxicity as evidenced by improvements in histochemical parameters.

Table (5): Effect of neem leaves and its extract on kidney functions of diabetic rats

Parameters / Groups	Urea (mg/dl) Mean±SD	Uric acid (mg/dl) Mean±SD	Creatinine (mg/dl) Mean±SD
G1 C (-)	44.35f ± 2.10	2.50b± 0.20	1.15b+ 0.21
G2 C (+)	75.80a ±3.20	4.40a ± 0.90	1.55a+ 0.13
G3 (2.5% neem leaves powder)	60.28b± 1.30	3.71a ± 0.70	1.48a+ 0.01
G4 (5% neem leaves powder)	55.55c± 0.50	3.35a± 1.20	1.50a+ 0.14
G5 (250 mg/kg neem extract)	51.11d± 1.60	3.07b± 0.60	1.51a+ 0.01
G6(500 mg/kg neem extract)	48.42e ± 0.90	2.70b± 0.30	1.44a+ 0.03
LSD (P ≤ 0.05)	2.521	1.250	0.322

Each value is represented as mean ± standard deviation (n = 3). Mean under the same column superscript with different letters are different significantly (P≤0.05).

Conclusion

Overall, neem leaf powder or extract had a considerable beneficial effect on alloxan-induced diabetic rats. As a result, neem leaf powder or extract could be a therapeutic option for diabetic mullites.

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التأثير المضاد لمرض السكرلأوراق النيم في الفئران المصابة بالسكر بتأثير الألوكسان

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قسم التغذية وعلوم الأطعمة، كلية الاقتصاد المنزلي، جامعة المنوفية، شبين الكوم، مصر

الملخص العربى

يعتبر نبات النيم أكثر النباتات الطبية التقليدية فائدة ويمكن أن يوفر مركبات نشطة بيولوجيًا مثيرة للاهتمام للنظام الغذائي الحالي في العديد من البلدان. حيث يتم استخدامه فى المنتجات الطبيعية كعوامل علاجية .في هذه الدراسة تم تقييم تأثير تركيزات مختلفة لمسحوق أوراق النيم (٢,٥،٥٪) والمستخلص الإيثانولي (٢٥٠،٥٠٠ مجم / كجم من وزن الجسم) على الفئران المصابة بمرض السكر. تم استخدام ستة وثلاثين من ذكور الفئران البيضاء وزنها ١٤٠ ± ١٠ جرام وقسمت إلى ٦مجموعات، كل مجموعة (٦) فئران. تم اصابة الفئران بمرض السكر عن طريق حقنها بالالوكسان (١٥٠ مجم / كجم من وزن الجسم). تم تقديرمستوى الجلوكوز ووظائف الكبد في السيرم (ALT، AST ، ALP)وصورة دهون الدم مثلT.C T.G، LDL-c ، HDL-c ، VIDL-c ووظائف الكلى (اليوريا وحمض اليوريك والكرياتينين). أظهرت النتائج أن أوراق النيم كمسحوق أومستخلص أدى إلى تحسين مستوى الجلوكوز في الدم، وظائف الكبد ووظائف الكلى وصورة دهون الدم في الفئران. تم تسجيل أفضل النتائج خاصة لمستخلص أوراق النيم بتركيز ٥٠٠ مجم / كجم من وزن الجسم. الخلاصة, بناءً على النتائج المتحصل عليها، وجد أن أوراق النيم قد تكون مفيدة في منع أو تأخير ظهور مرض السكر.

الكلمات الدالة: نبات النيم، الفئران،التحاليل الكيمائية الحيوية، ارتفاع سكر الدم.