IMPACT OF AQUEOUS EXTRACT OF NEEM LEAVES IN LOWERING BLOOD GLUCOSE AND LIPID PROFILE IN STZ INDUCED DIABETES MELLITUS MICE

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ABSTRACT

This study was aimed to investigate the effect of aqueous neem (Azadirachta indica) leaves extract in reducing the sugar level of diabetic patients in male mice. The study included four groups and 15 mice for each group. The first group represented the control group. The mice in the remaining three groups were injected with streptozotocin (STZ) at dose of 50 mg/kg body weight. The second group was considered as the induced control group for diabetic patients for comparison, while the third group of diabetic patients received neem leaves extract 250 mg/kg of body weight, and the fourth group of diabetic patients was given metformin hydrochloride 250 mg/kg of body weight. Measured Changes in blood glucose, blood triglycerides (TG), total cholesterol (TC), HDL, LDL cholesterol, VLDL cholesterol levels, and body weight. The results showed there was a significant decrease in blood glucose levels in diabetic groups and a decrease in cholesterol and triglycerides in groups treated with neem leaves extract compared with metformin hydrochloride. Thus, it can be concluded high blood sugar can be alleviated by treatment with an aqueous extract of *Azadirachta indica* leaves.

Keywords: hypoglycemia, *Azadirachta indica*, cholesterol, body weight.

مجلة العلوم الزراعية العراقية -2022 :53(5):977-984 علي

تاثيرالمستخلص المائي لاوراق النيم في خفض السكر و الدهون في الفئران المصابة بداء السكري المستحث بوساطة ال STZ

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مدرس

قسم التقنيات الاحيائية/كلية العلوم /جامعة بغداد

المستخلص

تهدف الد ارسة الى تقييم تأثيرمستخلص أوراق النيم (*Azadirachta indica*) المائي في خفض مستوى السكر لمرضى السكري في ذكور الفئران، اشتملت الدراسة على أربع مجاميع وبواقع عشرة فئران لكل مجموعة, مثلت المجموعة الأولى بمجموعة السيطرة ، وحقنت الفئران في المجاميع الثلاث المتبقية بمادة الستربتوسوزين بجرعة 50ملغم/كغم من وزن الجسم لحث مرض السكري لديها ، واعتبرت المجموعة الثانية هي مجموعة السيطرة المستحثة لمرضى السكري للمقارنة بينما تلقت المجموعة الثالثة من مرضى السكري مستخلص اوراق النيم 250 ملغم / كغم من وزن الجسم ، والمجموعة الرابعة من مرضى السكري أعطيت ميتفورمين هيدروكلوريد 250 ملغم / كغم من وزن الجسم. تم قياس التغيرات في مستوى الجلوكوز في الدم ، والدهون الثلاثية في الدم (TG) ، والكوليسترول الكلي (TC) ، والكوليسترول الجيد ، والكوليسترول الضار ، ومستويات الكوليسترول VLDL ، ووزن الجسم. بينت النتائج أن هناك انخفاظاً كبيرا في نسب الكلكوز بالدم في المجاميع المصابة بالسكري وانخفاضا في نسبة الكوليسترول والدهون الثلاثية في المجاميع المعاملة بمستخلص اوراق النيم مقارنة مع ميتفورمين هيدروكلوريد .وبهذا نستنتج أن ارتفاع السكر في الدم يمكن تخفيفه عن طريق العلاج بمستخلص مائي من أوراق *Azadirachta indic*

الكلمات المفتاحية: نقص سكر الدم، *Azadirachta indica*، وزن الجسم ، كوليسترول.

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INTRODUCTION

There are two types of diabetes mellitus (DM): when the pancreas does not generate enough insulin or the body cannot use the insulin it does release. There are several symptoms of diabetes, including hyperglycemia, dehydration, frequent urination and weight gain or decrease. Type 1 insulin-dependent DM, type 2 (non-insulin-dependent DM), and gestational DM are the most common forms of diabetes. Type 2 diabetes is the most common and is caused by insulin resistance. (4) Cardiovascular disease, high blood pressure, and neuropathy are just a few of the diabetes consequences that might contribute to an increase in the overall risk of death for diabetic individuals. (3) Amputation of the legs, renal failure, nerve damage, and loss of vision are some of the other problems that might occur as a result of the disease. (2) Uncontrolled DM can lead to high levels of TC, LDL –C, and high levels of VLDL-C, TG, and low levels of HDL-C. As a result of insulin insufficiency, significant changes in lipid metabolism and lipoproteins are seen. Hypoglycemic medications are available to treat DM in a variety of forms. But their hazardous side effects and perhaps diminished responsiveness after extended usage are worrisome, it's still a struggle to manage DM to prevent these issues. (1) Drugs used to treat DM are expensive, and their side effects have made it difficult to treat and maintain diabetes in modern times. Also, none of the antidiabetic drugs could give long-range glycemic control. Nowadays, there is a lot of concern in alternative medicine that will have few or no side complications and at the same time will be affordable for everyone .Although the mechanism of action of these herbal medicines has yet to be determined scientifically, poor nations utilize them as an alternative remedy. (24) *Azadirachta. indica* is a popular name for " *A indica "*, a plant frequently used in African classical medicine to treat DM. It's a medicinal herb that originated in India, but is now produced in virtually every country in the globe, including the United States. There are several uses for this plant. Each of the alkaloids in the plant contributes to the overall therapeutic qualities of the plant. These include azadirachtin, nimbin and salannin. De Jussieu described the neem tree as A. indica in 1830. (31) as well as a taxonomic position is as follows:

Class:Magnoliophyta
Order: *Rutales*
Suborder: *Rutinae*
Family: *Meliaceae*
Genus: *Azadirachta*
Species: *indica*

These plants exhibit antibacterial and wound-healing properties in different sections of the plant and activity that treats skin diseases (8). There are many studies on the biological activities and pharmacological effects of A. indica based on contemporary scientific investigations. This study was conducted to investigate the comparative effect of hypoglycemia, dyslipidemia, body weight, and histological changes of the pancreas between aqueous extract of neem leaf and metformin in mice with STZ-induced diabetes.

MATERIALS AND METHODS

Collection and identification of plant

The leaves of *A. indica* were collected from Al-Nahrain University, Jadiriyah, Baghdad. The sample was identified and classified by the University of Baghdad grassland, college of Science, Department of Biology.

Preparation of extract fresh neem leaves were washed and air-dried at room temperature, crushed, 100 gm of neem was weighed and blended with 500 ml distilled water at Ratio (5:1) v / w and preserved in a hot air oven through 24 hours, the extract was purified through no.1 filter paper (whitman International Ltd., Kent, UK) using a Buchner funnel, the filter was concentrated with a rotary evaporator beneath reduced pressure. *A.indica* leaves aqueous extract was created by freezing the concentration and then drying it to produce the dried powder (11).

Chemicals

Streptozotocin was obtained from Sigma Aldrich (Germany), metformin hydrochloride was gained from drug stores, Metformin hydrochloride an oral anti-diabetic pill, was crushed, diluted in distilled water, and the resulting concentration was 500 mg/10ml.

Experimental animals

A total of 60 male mice were used in this study were albino at the age of (8-10) weeks and average weight (21±6 g), the mice were

housed in polypropylene cages under controlled conditions at temperature (25-28) C° with a 12/12 hr light/ dark cycle. Mice were acclimatized condition for 14 days before the commencement of the experiment.

Experimental design

Sixty albino mice were randomly divided into four groups 15 mice in each one, Diabetes was induced in 45 mice for B, C, D groups by STZ (50 mg/kg) diluted in 5 ml citrate buffer having pH 4.5 which was injected intraperitoneally (IP) to mice and preserved fasting condition for 18 hours to stimulate diabetic situation. (17)

Group A: Control group without STZ administration as normal control has administered 0.1 ml D.W daily for 40 days.

Group B: Diabetic control

Group C: Diabetic group received neem extract orally 250 mg/kg (bwt) daily for 40 days. The dose selected was less than (LD_{50}) of aqueous extracts of *A. indica* leaves. (37)

Group D: Diabetic group received metformin hydrochloride orally 250 mg/kg (bwt) daily for 40 days. the dose selected was less than (LD_{50}) of aqueous extracts of *A. indica* leaves. (25).

Determination of blood glucose

By Accu CHEK(R) Active blood glucose system (strip method): A drop of blood was collected from the tail vein. At the same time the Accu CHEK(R) Active monitor was started with a single soft press. After the monitor showed the code number the strip was inserted into the monitor. A drop of the blood was poured on the test zone of the strip. The values were expressed in m mol/L.

Blood samples: After 40 days, animals were sacrificed by cervical dislocation. Then open the inverted T-shaped cavity and draw blood directly from the heart by stabbing the heart to get as much blood as possible. Blood was collected for glucose estimation just before drug administration on the 1st day and after drug administration on days 10, 20, 30, and 40. For estimating serum lipids profile, serum was isolated from the blood collected on 40 th day of all groups.

Determination of body weight

The bodyweight of the mice was determined using a compact electronic scale.

Statistical analysis: The Statistical Analysis System (28) was used to affect different factors in study parameters. A low significant difference (LSD) test at ($P<0.01$) was used to significant contrast between means in this study.

RESULTS AND DISCUSSION

Effect of STZ on blood glucose, lipids profile and bodyweight: Table (1) shows the effect of STZ which caused a significant increase ($p<0.001$) in blood glucose level in groups B, C, D compared with group A. In the present study, STZ was used in a dose of 50 mg/kg bwt. in mice to induce hyperglycemia. Toxic effects on islet beta cells make STZ a popular way to generate insulin-dependent DM in animals (18). Because of permanent damage to the pancreatic beta cells, STZ causes degranulation and insulin secretion impairments (40). Multiple sub-diabetogenic dosages of STZ administered intravenously or intraperitoneally to laboratory mice results in pancreatic insulitis and eventual death of insulin-secreting beta cells (33). DM is associated with hyperlipidemia, which occurs in conjunction with hyperglycemia. Table (2) shows serum TG, TC, LDL –C, and VLDL-C were significantly elevated ($P>0.01$), HDL cholesterol was non significantly decreased ($P>0.01$) in diabetic mice compared with a control group. This could be due to insulin insufficiency, which alters the liver apolipoprotein synthesis in diabetic mice. Moreover, LpL and cholesterol ester transport protein are two enzymes that insulin can control. So, insulin shortage decreases hepatic lipase activity and many stages in the synthesis of biologically active LpL (13). Furthermore, lipase was responsible for converting triglycerides into free fatty acids and glycerol, and insulin shortage prevented this. The acetyl CoA is produced when free fatty acids are broken down in the liver, and the elevated scale of acetyl CoA leads to triglyceride, cholesterol, and ketone bodies as a result of ketosis (5) in the liver, an increase in the flow of free fatty acids promotes the production of VLDL particles, which in turn are transformed to LDL. In type 1 diabetics, higher levels of VLDL were found as a result of reduced clearance and over-production. An increase in VLDL-C and triglycerides in the bloodstream as a result of impaired clearance (30) increased triacylglycerol production is likely the result of

altered adipose tissue lipolysis or intrahepatic processes involving additional changes to fractional esterification of fatty acids or changes to the assembly or secretion of VLDL. Therefore, lipid profile is altered, as well as plasma levels of low density lipoprotein (LDL), very low density lipoprotein (VLDL), and chylomicron (22). As a result of the increased amount of reactive oxygen radicals, specifically polyunsaturated fatty acids in DM, there was an aberrant lipid metabolism as well as lipid peroxidation (36). The abnormally high lipid level ameliorates antioxidant capability and increases oxidative stress levels, resulting in different organ damage (12). As the smallest lipoprotein, HDL cholesterol contains roughly 20% cholesterol ester and relatively little triglyceride, Diabetics have been demonstrated to have a lower HDL turnover rate. HDL catabolism can be accelerated in Guinea pigs by non-enzymatic glycosylation of HDL; this process may also be responsible for decreased HDL levels in diabetic mice (14). There is evidence from several research that glycated HDL clearance accelerates from the circulation, in contrast to glycated LDL catabolism, which is slowed down. Because of this, it has been hypothesized that low levels of HDL in diabetic patients are due to a faster HDL clearance, which is another factor contributing to the increased risk of atherosclerotic disease in diabetics (23). The animals in group B that were treated with STZ seemed sickly with significantly (P>0.01) loss of their body weights compared with group A as shown in table (3) as a result of STZ's harmful consequences, which included alkylation of DNA, hyperglycaemia, and necrotic lesions, as well as a possible connection to structural protein aggregation and muscle loss (15).

Table 1. Effects of STZ, Neem Leaves Aqueous Extract and Metformin Hydrochloride on Glucose Level of Mice (Means±SE)

Group	Mean ± SE (mg/dl)				
	Day 1	Day 10	Day 20	Day 30	Day 40
Control (A)	89.20 ±4.04 a	90.20 ±1.24 b	89.40 ±2.76 b	90.20 ±3.51 b	89.80 ±4.21 b
STZ (B)	111.00 ±6.37 a	162.80 ±12.70 a	285.80 ±28.42 a	427.20 ±22.74 a	467.40 ±35.93 a
Neem (C)	112.00 ±7.39 a	105.00 ±6.74 b	100.00 ±3.70 b	96.80 ±2.78 b	75.20 ±2.24 b
Metf (D)	104.80 ±7.47 ab	96.20 ±5.12 b	89.20 ±3.77 b	82.00 ±2.02 b	90.80 ±1.93 b
LSD value	19.026 *	22.97 **	43.53 **	34.87 **	54.424 **
P-value	0.0844	0.0001	0.0001	0.0001	0.0001

Means having with the different letters in same column differed significantly.
* (P≤0.05), ** (P≤0.01).

Table 2. Effects of STZ, Neem Leaves Aqueous Extract and Metformin Hydrochloride on Lipid Profile of Mice (Means±SE)

Group	Mean ± SE(mg/dl)				
	Cholesterol	LDL	VLDL	Triglyceride	HDL
Control (A)	76.00 ±3.61 b	49.98 ±0.75 b	10.64 ±0.57 d	53.40 ±2.06 b	22.62 ±1.56 a
STZ (B)	96.71 ±2.14 a	68.60 ±1.70 a	16.34 ±0.35 a	77.28 ±2.01 a	19.00 ±0.83 a
Neem (C)	79.40 ±2.76 b	49.37 ±1.09 b	12.24 ±0.30 c	57.03 ±2.75 b	22.61 ±1.56 a
Metf (D)	91.82 ±1.90 a	68.47 ±1.44 a	13.84 ±0.25 b	83.13 ±2.92 a	19.32 ±0.71 a
LSD value	8.068 **	3.897 **	1.172 **	7.412 **	3.703 NS
P-value	0.0001	0.0001	0.0001	0.0001	0.114

Means having with the different letters in same column differed significantly.
** (P≤0.01).
NS:non-significant

Effect of *A.Indica* leaves extract on blood glucose, lipids profile and body weight in diabetic mice:

Table (1) shows an antihyperglycemic impact was seen in group C on days 10, 20, 30, and 40. The plasma glucose level on these days was significantly less (P>0.01) than the pre-treatment mice in group B, and non-significantly increase compared with group D. Bioactive in neem leaves, meliacinolin inhibits A-glucosidase and beta-amylase, this one of the Mechanisms of antihyperglycemic activity. (38) A possible explanation for neem leaf extract's blood sugar lowering action is that inhibits the glucose-induced inhibitory impact of serotonin on insulin release (26). An additional benefit of consuming *A. indica* in diabetic mice improves the insulin signaling molecules and increases the expression of glucose transporter 4 (GLUT4) in muscle tissue (22) .Anti-dyslipidemic effect of aqueous *A. indica* leaves extract in STZ-induced diabetic mice was clear significantly decrease (P>0.01) TC, LDL-C, VLDL-C and TG levels in group C compared with groups B and D, and had non-significant increase in HDL level compared with groups B and D As shows in Table (2). Cholesterol esterase deficiency in the pancreas causes the accumulation of triglycerides and cholesterol esters in cells and tissues, where cholesterol esters are broken down into non-esterified cholesterol and free fatty acids. (29) Pancreatic cholesterol esterase activity was inhibited by the use of the neem extract. (27) This plant's leaf includes alkaloids and glycosides, as well as flavonoids and polyphenols. As well as these compounds, the aqueous neem extract also indicated the presence of saponin and steroids. (35) Quercetin-3-O-B-D-glucoside, Myricetin-3-Orutinoside, and other beneficial chemicals found in *A. indica* leaf extract showed antihyperlipidemic activity (10). Inhibition of pancreatic cholesterol esterase activity was achieved by neem extract polyphenols, which resulted in reduced lipid digestion and intestinal cholesterol absorption (9). Because of the β-sitosterol in neem extract, cholesterol absorption might be reduced by disrupting micelle formation (19).

Bodyweight was improved with the use of a neem extract, table (3) shows non-significant increase (P>0.01) in body weights of diabetic mice in group C compared with group B and significant increase compared with group D. It is possible that the mice's increased feed consumption has boosted their perception of taste and hunger after they have consumed it. This is in line with the findings of the study in the reference (34).

Table 3. Effects of STZ, Neem Leaves Aqueous Extract and Metformin Hydrochloride on Body Weight of Mice (Means±SE)

	Mean ± SE (gm)				
Group	**Day 1**	**Day 10**	**Day 20**	**Day 30**	**Day 40**
Control (A)	**26.30 ±0.82**	**26.39 ±0.68**	**26.24 ±0.66 a**	**27.10 ±0.0 a**	**26.75 ±0.80 a**
STZ (B)	**27.06 ±0.54**	**25.83 ±0.41**	**24.71 ±0.41 b**	**24.17 ±0.53 b**	**21.89 ±0.63 b**
Neem (C)	**26.70 ±0.31**	**25.16 ±0.29**	**24.03 ±0.19 b**	**22.86 ±0.68 bc**	**23.71 ±0.45 c**
Metf (D)	**26.77 ±0.49**	**25.39 ±0.31**	**23.47 ±0.33 b**	**22.01 ±0.03 c**	**20.91 ±0.60 b**
LSD value	**1.724 NS**	**1.366 NS**	**1.308 ****	**1.588 ****	**1.912 ****
P-value	**0.825**	**0.237**	**0.0023**	**0.0001**	**0.0001**

Means having with the different letters in same column differed significantly.
**** (P≤0.01).**

Effect of metformin hydrochloride on blood glucose, lipids profile and body weight in diabetic mice:

In this study, the continuous treatment with Metformin hydrochloride produced a significant reduction (p<0.01) of the blood glucose level in STZ-induced diabetic mice in group D compared with group B and non-significant increase compared with group C as shown in Table (1) First-line therapy for type 2 diabetes, metformin, is the most oft0en prescribed medicine in the world (16). It reduces mitochondrial electron transfer as a result, the AMP/ATP ratio rises, resulting in the activation of AMP-activated kinase (AMPK). Improves poor glucose tolerance by

inhibiting gluconeogenesis and promoting glycolysis when AMPK is active (7). There are digestive problems, diarrhea, and vomiting reported in roughly 30% of individuals who have been taken metformin despite its well-established therapeutic advantages. This was evident in mice treated with it, Where there is a significant decrease (P>0.01) in body weight of mice in group D compared with groups B and C as shows in Table (3). Found in tissue biopsy specimens of human duodenal mucosa, a low concentration of metformin sub- threshold activating AMPK stimulates the release of 5-hydroxytryptamine (5-HT), which can explain diarrhea and vomiting via central nervous system. In addition to its ability to penetrate the blood-brain barrier and lower hunger in the hypothalamus, metformin also has other peripheral regulatory mechanisms that enhance leptin levels (6). Metformin had a non-significant effect ($p < 0.01$) in reducing TC, LDL-C and VLDL-C in group D compared to groups B and C, and significant increase ($p < 0.01$) in TG level in group D compared to group C, and non- significant increase compared with group B, and did not effect on HDL-C level compared with group B and non-significant decrease compared with group C as shows in Table (2).To do this, metformin causes an enhanced rate of fat metabolism while lowering levels of lipid production in areas such as the liver and muscle tissue. Metformin enhances AMP-activated protein kinase activity and has an important role in glucose and lipid metabolism. Also, a decrease in the intestinal absorption of bile acids caused by metformin leads to an increase in the liver's bile acid production, which requires cholesterol, as a result, the hepatocytes produce less cholesterol. Lipid uptake may be boosted by increasing the LDL-C receptor's activity, to retrieve cholesterol levels in the liver to a healthy level (20). Hereby, Metformin may reduce LDL-C and plasma total cholesterol concentrations in an indirect manner (39). Many lipogenic genes are affected by SREBP1c upregulation. . It was shown that AMPK activated the Te acetyl-CoA carboxylase ACC, which inhibits malonyl-CoA production, after metformin exposure. This resulted increased the quantity of TG (21).

CONCLUSION

The results of this study revealed that administration of *A. indica* leaves for 40 days shows an effective role in the management of DM by improving the expression of insulin signaling molecules and GLUT4 protein to enhance the oxidation in the skeletal muscle. Also, anti-dyslipidaemic action of *A. indica* leaves extract appears to involve reduce levels of TG, TC, LDL-C and VLDL-C. Consider the medicinal potential of *A. indica* leaves extract. Owing to its ability to regulate glucose and cholesterol levels, *A. indica* extract might be used as an adjunct to the drug used to lower the level of glucose in the blood and lipid levels.

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