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Anti-Hyperglycemic And Antidyslipidemic Potential Of *Azadirachta indica* Leaf Extract In STZ- Induced Diabetes Mellitus

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Abstract:

Azadirachta indica , an Indian medicinal plant, has been studied for its role in diabetes and its effect on lipid profile. This study was conducted to elucidate whether treatment of *Azadirachta indica* leaf extract after streptozotocin (STZ) - induced diabetes has anti-hyperglycemic and anti-dyslipidaemic action or not. The experiment involved four groups of rat; one group was control group, second diabetic control, third diabetic group received alcoholic extract of *Azadirachta indica* and fourth diabetic group received Glibenclamide as a reference standard. Oral glucose tolerance test was performed before induction of diabetes. Blood was collected by retro-orbital puncture for glucose estimation, and to evaluate serum triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol levels. Blood Glucose level as well as serum lipid profile parameters such as total-cholesterol, triglyceride, low-density lipoprotein and very low-density lipoprotein cholesterol were also elevated, whereas, the level of high-density lipoprotein-cholesterol was reduced significantly ($P<0.05$) in diabetic rats . Ethanolic extract of *A.indica* after induction of diabetes, normalized glucose level and lipid profile. It can be concluded that STZ-induced hyperglycaemia can be ameliorated by treatment with ethanolic extract of A indica. A.indica ethanolic leaves extract after diabetic induction, reverses dyslipidaemia.

Key words: *Diabetes mellitus, serum lipid, Streptozotocin, Azadirachta indica.*

Introduction:

India has one of the oldest, richest and diverse cultural traditions associated with the use of the plants and herbs for human, liverstock and plant health. Many of the ingredients of Indian cooking which have been handed down from ages contain medicinal properties. A vast ethnobotanical knowledge exists in India from ancient times. However, very few plants used by locals for medicines are subjected to scientific investigation. The need for conservation of medicinal plants and traditional knowledge, particularly in developing countries like India, taking into account the socio cultural and economic conditions is urgent.[1]

Diabetes mellitus (DM) is a serious metabolic disease which has several complications including diabetic nephropathy, diabetic neuropathy, coronary heart disease and hypertension.[2] It has been estimated that by the year 2010, the prevalence of DM worldwide will reach approximately 240 million.[3] Patients with DM are more likely to develop and die from microvascular and macrovascular complications than the nondiabetic population.[4] There is usually an association between coronary heart disease or atherosclerosis and dyslipidaemia.[5, 6] Dyslipidaemia is a frequent complication of DM and is characterized by low levels of HDL-cholesterol and high levels of LDL-cholesterol and triglyceride. Several groups of hypoglycaemic drugs are currently available to treat DM. However, their toxic side effects and sometimes diminution in response after prolonged use are problematic. Management of DM to avoid these problems is still a major challenge. In the indigenous Indian system of medicine, good number of plants was mentioned for the cure of diabetes and some of them have been experimentally evaluated and the active principles isolated.[7] However search for new antidiabetic drugs were continues.

Neem (*Azadirachta indica* A. Juss) is perhaps the most useful traditional medicinal plant in India. Each part of the neem tree has some medicinal property and is thus commercially exploitable. During the last five decades, apart from the chemistry of the neem compounds, considerable progress has been achieved regarding the biological activity and medicinal applications of neem. It is now considered as

a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products.[8]

Every part of the tree has been used as traditional medicine for household remedy against various human ailments, from antiquity[9-14]. Neem has been extensively used in ayurveda, unani and homoeopathic medicine and has become a cynosure of modern medicine. The importance of the neem tree has been recognized by the US National Academy of Sciences, which published a report in 1992 entitled 'Neem – a tree for solving global problems'. The advancement of neem research has earlier been documented [15, 16]. The neem tree has been described as *A. indica* as early as 1830 by De Jussieu [17] and its taxonomic position is as follows:

Order	Rutales
Suborder	Rutinae
Family	Meliaceae (mahogany family)
Subfamily	Melioideae
Tribe	Melieae
Genus	*Azadirachta*
Species	*indica*

Neem oil, bark and leaf extracts have been therapeutically used as folk medicine to control diseases like leprosy, intestinal helminthiasis, respiratory disorders, constipation, and skin infections.[18] However, apart from these uses, there are several reports on the biological activities and pharmacological actions based on modern scientific investigations, such as antiviral [19], antibacterial [20], antifungal [21], anti-inflammatory and antipyretic [22], antiseptic, antiparalitic [23], antioxidant [24, 25]etc.

In this study, we investigated the effects of *A. indica* leaf extract on blood glucose level, serum lipid profile changes in normal and stz induced diabetic rats with a view to finding out its possible effect on cardiovascular disease induced by hyperglycemia.

Material and Methods:

Animal preparation:

stz- induced model :

Male Wistar albino rats weighing between 180-220g were used in the study with the approval of the animal ethical committee of Bhopal Nobels College of Pharmacy, Udaipur, Rajasthan. Rats were housed in a 12-hr light-dark cycle at 25 ± 2 °C. The animals were provided standard rat pellet feed and tap water *ad libitum*. All animals were cared for in accordance with the principles and guidelines of the Institutional Animal Ethics Committee of B.N.College of pharmacy, Udaipur. Diabetes was induced in rats by tail vein injection of streptozotocin (50 mg/kg, *i.v.*) (Sigma chemicals) dissolved in normal saline. (One group of identical rats was kept without streptozotocin administration as normal control, group I). Forty eight hours after streptozotocin administration blood samples were drawn by retroorbital puncture and glucose levels determined to confirm diabetes. The diabetic rats exhibiting blood glucose levels in the range of 275 to 300 mg/100 ml were selected for the studies. Glibenclamide (500 µg/kg) was used as reference standard. The dose of Glibenclamide was selected based on previous reports.[26]

Following four groups of rats, were taken.

Group I : normal control (NC)

Group II: diabetic control (DC) -given (untreated rats) 0.5 ml of 5% Tween 80.

Group III: diabetic rats given (200 mg/kg) ethanolic extract of *A.Indica* (ET) in 0.5 ml 5% Tween 80

Group IV: diabetic rats treated with glibenclamide (500 µg/kg) (GT) in 0.5ml 5% tween 80 (GT)

Preparation of Azadirrachta indica leaf ethanol extract:

Fresh leaves of A.indica obtained from the local market of Jaipur, were washed in tap

water and then left to dry at room temperature for 2-3 days. The dried leaves were then ground to fine powder in a mixer. The dried leaf powder was then extracted with 95% ethanol using a soxhlet apparatus for 15 hr. after filtration through cotton wool; the filtrate was concentrated at 65^0C by a rotavapor. The concentrate was then freeze dried to yield dried powder and were designated as *A.indica* leaf ethanol extract. [27]

Experimental design:

oral glucose tolerance test [28]:

The oral glucose tolerance test was performed in overnight fasted (18-h) normal animals. Rats divided into three groups were administered 2% gum acacia solution, ethanolic extract of A.*Indica* (200 mg/kg), and Glibenclamide (0.25 mg/kg), respectively. Glucose (2 g/kg) was fed 30 min after the administration of samples. Blood was withdrawn from the retro-orbital sinus at 0, 30, 60, 90 and 120 min of samples administration.[29] Fasting blood glucose levels were estimated by glucose oxidase-peroxidase reactive strips (Accu-check, Roche Diagnostics, USA).

biochemical estimation in stz- induced model:

The diabetic rats exhibiting blood glucose levels in the range of 275 to 300 mg/100 ml were selected for the studies. The treatments were continued daily for 40 days. Blood was collected by retro-orbital puncture for glucose estimation just before drug administration on the 1st day and 1 h after drug administration on days 1, 10, 20, 30, 40**.** Blood glucose (FBG) concentration of all the four experimental groups was determined by glucometer during different phases of the experiment by withdrawing blood from the retero orbital vein. For estimating serum lipid profile, serum was isolated from the blood collected on 40^{th} day of *A. indica* leaf ethanol extract treatment and serum total cholesterol (TC), triglyceride (TG) and HDL-cholesterol were estimated by using diagnostic kits (Erba Mannheim Cholesterol kit, Transasia Bio-Medicals Ltd., Daman). VLDL and LDL cholesterol were calculated as per Friedevald's equation [30]:

VLDL-cholesterol = Serum triglyceride-Cholestrol

LDL-cholesterol = Serum total-cholesterol – VLDL-cholesterol – HDL-cholesterol.

Results were expressed in mg/dl

Data and statistical analysis:

Results are expressed as mean ±Standard Error of Mean (SEM). Statistical analysis was performed using one-way Analysis of Variance (ANOVA) using SPSS (version 10.0) and student's 't'-test using Sigma Plot (version 8.0). The values of $P<0.05$ were considered as statistically significant.

Results:

effects of a.indica leaf ethanol extract on blood glucose level in ogtt:

Table 1 shows the changes in fasting blood sugar level during oral glucose tolerance test. Fasting Blood sugar level was determined by collecting the blood from retro orbital sinus in 0, 30, 60, 90, 120 minute. There was significant increase in blood sugar level in animal of control group having 2% gum acacia solution from 82.33±2.58 to 90±.1.78mg/dl. Group receiving Glibenclamide (0.25 mg/kg) showed a significant ($P<0.05$) decrease in blood glucose level in every 30 minutes interval from 84.66±2.16 to 78.33±1.36mg/dl, group receiving ethanolic extract of *A.indica* leaves showed a significant and continuous decrease from 81.5±2.73 to 768.33±1.21 in blood sugar level till the 90 min. When it was observed after 120 min it was reached near about normal level i.e 81.33±2.25 mg/dl.

effect of a.indica leaf ethanol extract on blood glucose level in diabetic rats:

As shown in Table 2, the extract (group III) produced gradual and moderate

Table 1: Blood glucose concentration (mg/dl) in OGTT

Group	Treatment	Initial	30 min	60 min	90 min	120 min
I	Control	82.33±2.58	85.83±2.48	89.5±1.87	91.83±1.47	90±1.78
II	Extract treated	81.5±2.73	79.5±2.34	77.5±2.42	78.33±1.21	81.33±2.25
III	Glibenclamide treated	84.66±2.16[a]	76.83±1.47[a]	71.66±1.63[a]	68.5±1.64[a]	78.33±1.36[a]

Student's 't'-test is significant at $P<0.05$. a-significant ($P<0.05$) difference compared to C; b-significant ($P<0.05$) difference compared to GT.

Table 2: Blood glucose level (mg/dl) in STZ – induced diabetic rats

Group	Treatment	1 Day	10 Day	20 Day	30 Day	40 day
I	Control	84.83± 1.47	85.33± 1.21	84.5± 1.04	84.33± 1.21	84.33± 0.51
II	Diabetic control	283.16± 3.31[a]	370.16± 3.12[a]	404.5± 1.87[a]	373.83± 2.87[a]	372.16± 3.31[a]
III	Extract Treated	293.5± 1.87[a]	263.5± 3.27[a bc]	230.83± 3.06[abc]	214.83± 3.6[abc]	198.83± 3.97[abc]
IV	Glibenclemide (500 µg/kg)	286.6± 3.01[a]	213.83± 2.31[a b]	176.83± 3.06[a]	176.83± 3.06[a b]	125.16± 2.63[a b]

Student's 't'-test is significant at $P<0.05$. a significant ($P<0.05$) difference compared toC; b significant ($P<0.05$) difference compared to DC; c significant ($P<0.05$) difference compared to GT.

Table 3: Effect of treatment of A.indica leaf extract on serum lipid profile (mg/dl) in streptozotocin -induced diabetic rats:

Parameters	Normal Control	Diabetic Control	Extract treated	Glibenclamide Treated
TC	87.7 ± 2.7	217.5±3.5[a]	89.6±3.7[b]	78.1±3.7[b]
TG	75.6± 2.9	161.1±4.2[a]	73.7±5.6[b]	61±5 [b]
HDL	29.7±3	18.7±0.7[a]	32±2.47[b]	49.2±3.6[b]
LDL	46.3±3.4	170±3.1[a]	48±3.7[bc]	19.7±1.7[b]
VLDL	17.4±0.3	34.5±0.7[a]	18.01±2[b]	14.4±1[b]

Student's 't'-test is significant at $P<0.05$. a significant ($P<0.05$) difference compared to NC; b significant ($P<0.05$) difference compared to DC; c significant ($P<0.05$) difference compared to GT.

antihyperglycemic effect on day 10, 20, 30 and 40. The plasma glucose level on these days was significantly less than the pretreatment level. Further the antihyperglycemic effect produced by the extract was significant ($p<0.05$) as compared to what observed in group I and II (control and diabetic control) on different days. However, the antihyperglycemic effect produced by Glibenclamide (group IV) was more pronounced than *A.Indica* extract.

effects of a. indica leaf extract on lipid profile in diabetic rats:

A highly significant increase in total cholesterol level was recorded in normal control and diabetic control group (untreated animals). However, the cholesterol level of extract treated animals were significantly less than the animals of group I and II indicating antihypercholesterolemic effect of the extract.

The plasma triglyceride levels of group III were significantly less than the contol and diabetic control group. Intergroup comparison revealed that the Glibenclamide treated and extract treated exerted antihypertriglyceridemic effect, as the plasma triglyceride levels were significantly less than that in the untreated control and diabetic group. *A.Indica* leaf extract and Glibenclamide reduced the level of serum TC, TG, LDL and VLDL-cholesterol reduced significantly ($P<0.05$) whereas, the level of serum HDL-cholesterol was significantly increased. *A.indica* treated group were showed the increased level of HDL as compare to Glibenclamide treated group after the 40 days.

Discussion and Conclusion:

Evaluation of the ethanolic extract of *A.Indica* leaves in normoglycemic and STZ –hyperglycemic rats indicated that the extract possesses hypoglycemic and antihyperglycemic activities.

Hyperlipidemia has been reported to accompany hyperglycemic states[15] and the most common lipid abnormality observed was hypertriglyceridemia. A significant increase in the total cholesterol, TG, LDL and VLDL levels were in accordance to earlier studies.[16, 17] Repeated administration of ethanolic extract of *A. indica* prevented the elevation of TC,TG, LDL and VLDL-cholesterol level in diabetic rats indicating the *A. indica* had a beneficial effect on the hyperlipidemia induced by STZ.

Our finding indicates that the ethanolic extract of A. indica leaves may be useful for treatment of diabetes associated with hyperlipidemia. Further chemical and pharmacological investigations are required to elucidate the exact mechanism of action of this extract and to isolate the active principles responsible for such effects.

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