ANTI-DIABETIC ACTIVITY OF NEEM OIL - AN INVITRO STUDY

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ABSTRACT:

AIM:

To evaluate the anti-diabetic activity of neem oil.

OBJECTIVE:

The research was undertaken to evaluate the anti-diabetic activities of neem oil.

BACKGROUND:

Neem leaf extracts and leaves are used as an active ingredient as an effective cure for diabetes. Neem extract shows beneficial effects of maintaining blood glucose levels in glucose fed hyperglycemic and diabetic patients and it also protects metabolic aberrations. Neem has a vital role in various problems associated with human health. The chemical constituents present in the neem plant makes it a doctor tree due to its wide scope in the biological activities associated with it and has become a global context today. Neem has been extensively used in Ayurveda, unani and homeopathic medicine. It has become a centre of attraction of modern medicine.

REASON:

This research was done to establish anti-diabetic activities of neem oil which can be effectively used in drug preparations for the the diabetic patients.

RESULT:

Anti-diabetic activity of neem oil was evaluated.

KEYWORDS: Azardiarchta indica, anti-diabetic activity, phytochemical analysis, yeast cells.

INTRODUCTION:

Type 2 diabetes mellitus is one of the most common chronic diseases in most countries. The prevalence of the disease is estimated to double by 2030 with 69% increase in developing countries and 20% increase among adults in developed countries (15). Conventional treatments for the management of diabetes mellitus include: enhancement of the action of insulin at the target tissues, with the use of sensitizers (biguanides, thiozolidinediones); stimulation of endogenous insulin secretion, with the use of sulfurnylureas (glibenclamide, glimipiride), and reduction of the demand for insulin using specific enzyme inhibitors (Acarbose, meglitol) [16].However, there is a burden of unwanted side- effects that may among others, include; hypoglycaemia, diarrhoea, nausea, dyspepsia, myocardial infarction, peripheral oedema and dizziness, with the use of these drugs. Also, the incalculable costs as well as unavailability of these drugs are also deterrent factors to drug adherence. These challenges call for concern and therefore underscores the need for appropriate and effective therapies in the management of the disease and its complications. Different medicinal plants from all over the world have been explored for their anti-diabetic properties, and several scientific research has collaborated these claims [17].

6

Neem (Azardiarchta indica)is a medicinal plant.Neem is one of the very few trees known in the Indian subcontinent. Neem belongs to Meliceae family, and grows rapidly in the tropic and semi-tropic climate. It is also observed that this tree could survive in very dry and arid conditions. Neem tree is an evergreen tree, but it may become leafless for a short period in certain conditions [1]. All parts of Neem plant such as leaves, bark, flower, fruit, seed and root have advantages in medical treatment and industrial products. Its leaves can be used as drug for diabetes, eczema and fever. Neem roots has an ability to heal diseases and fight against insects [1, 2]

Plants are natural reservoir of bioactive compounds that may be source of lead compounds with α -glucosidase inhibitory potentials. Some of these compounds have been shown to inhibit α -glucosidase activity [18]. Herbal extracts used in the study area has been reported for their anti-diabetic activities [17]. The hypoglycaemic, toxicity and hypolipidemic effect of the aqueous leaf extract of A. chevalieri has been reported extensively by Saidu et al[17]. Bilbis et al[19]also reported the hypoglycaemic and hyperlipidaemia effect of A. hypogea seed in alloxan induced diabetic rats. V. amygdalina, C. procera, and M. indica aqueous extracts were also reported to possess significant hypoglycaemic effects [17].

Aqueous extract of neem leaves significantly decreases blood sugar level and prevents adrenaline as well as glucose-induced hyperglycaemia [20]. Aqueous leaf extract also reduces hyperglycaemia in streptozotocin induced diabetes and the effect is possibly due to presence of a flavonoid or quercetin [21] A significant hypoglycaemic effect was also observed by feeding neem oil to fasting rabbits [22].

The main objective of this research was to evaluate the anti-diabetic activities of neem oil and extracts.

MATERIALS AND METHODS:

Neem oil used in this study was purchased from cyprus enterprises arumbakka,, Chennai,India. The reagents required for the procedure was procured from Himedia

IN VITRO ANTI-DIABETIC ACTIVITY:

Essential oil samples of Neem leaves were assessed for in vitro anti-diabetic activity by the α -amylase, glucose uptake by yeast cells and α -glucosidase inhibition.

1.α-amylase inhibition activity:

The α -amylase inhibition assay was performed using the 3, 5-dinitrosalicylic acid (DNSA) method (Miller, 1959)[3]. A starch solution (0.25% w/v) was prepared by stirring 0.125 g of tapioca powder in 50 mL of 20mM sodium phosphate buffer containing 6.7mM sodium chloride at pH 6.9. One unit of α -amylase enzyme solution was prepared by mixing 0.0253 g of α -amylase in 100 mL of cold distillation water. Neem oil was dissolved in DMSO to give concentrations (50, 100, 150µg/ml). The colour reagent was prepared by mixing sodium potassium tartrate solution (12 g of sodium potassium tartrate tetra hydrate in 8.0 mL of 2 M NaOH) and 96mM of 3,5-dinitrosalicylic acid solution (0.4381 g of 3,5-dinitrosalicylic acid in 20 mL of deionized water). One unit of α -amylase solution and Neem oil were mixed thoroughly in a tube and incubated for 15 min. Then 500µL of the starch solution was added into each tube and incubated for 15 min. The reaction was terminated by addition of 500µL DNSA reagent, placed in boiling water bath for 5 min. The mixture was cooled to ambient temperature, diluted with 5 mL distilled water, and the absorbance was measured at 540 nm using a visible spectrophotometer. The blank control of reaction showing 100% enzyme activity was conducted by replacing the essential oil with DMSO (1.0 mL). To eliminate the absorbance effect of essential oil, a blank solution was also used and the reaction was terminated by DNSA before adding the starch solution. Acarbose solution (diluted in DMSO to $80 - 400 \ \mu L/mL$) was used as a positive control. The production of maltose will decrease with α -amylase inhibitory activity which will result in reduced absorbance intensity. The α -amylase inhibitory activity was expressed as percent inhibition and was calculated using the following equation[3].

% Relative enzyme activity = (Enzyme (Maltose) activity of test/enzyme activity of control)*100 % of α -amylase inhibition activity = 100 - % Relative enzyme activity

$2.\alpha$ -glucosidase inhibition activity:

Alpha-glucosidase inhibitory activity of essential oil was carried out according to method of Bachhawat et al [4]with slight modification. In a 96-well plate, reaction mixture containing 50µl phosphate buffer (50mM, pH= 6.8), 10µl alpha-glucosidase (1U/ml) and 20µl of varying concentrations of oils was pre-incubated at 37°C for 15 min. Then 20µl p-nitrophenyl- α -DGlucopyranoside (PNPG) (1mM) was added as a substrate and incubated further at 37°C for 30 min. The reaction was stopped by adding 50µl sodium carbonate (0.1M). The yellow colour produced was read at 405nm using visible spectrophotometer. Acarbose at various concentrations (50-150 µg/ml) was included as a standard. The control samples were prepared without any essential oil. The result is expressed as percentage inhibition, which was calculated as,

% Inhibition= ((Absorbance of Control-Absorbance of test sample) /Absorbance of control) X100

Acarbose (Sigma, U.S.A) a well-known α -glucosidase and α -amylase inhibitor was used as reference drug for the inhibitory activity.

3.Glucose uptake by Yeast cells:

Yeast cells were prepared according to the method of Cirillo, 1962[5].Briefly, commercial baker's yeast was washed by repeated centrifugation $(3,000 \times g; 5 \text{ min})$ in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of oil (50, 100, 150µg/ml) were added to 1 mL of glucose solution (5, 10 and 25mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 µl of yeast suspension, vortex and further incubated at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant by DNSA method. Metronidazole was taken as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula,

Inhibition % = Abs sample – Abs control /Abs sample × 100

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbance of the test sample. The amount of glucose lingering in the medium after a specific time serves as a marker of the glucose uptake by the yeast cells(5).

RESULTS

1.α-amylase inhibition activity:

O				% Relative	% Relative	% of a-amylase inhibition activity			
(µg)	Control	Need oil	Acarbose	Neem oil	Acarbose	Need oil	Acarbose		
50	0.871	0.579	0.528	66.47531573	60.61997704	33.52468427	39.38002296		
100	0.871	0.462	0.408	53.04247991	46.84270953	46.95752009	53.15729047		
150	0.871	0.388	0.305	44.54649828	35.01722158	55.45350172	64.98277842		

Table 1: % of a-amylase inhibition activity

Both neem oil and glucose uptake showed α -amylase inhibition activity (table1) for a concentration of 50 µg neem oil showed 33% α -amylase inhibition activity as compared to 39% incase of the standard drug acarbose. As the concentration of neem oil increased , there was a concomitant increase in the α -amylase inhibition activity. Neem oil showed a significant α -amylase inhibition activity as compared with standard drug acarbose.

2.α-glucosidase inhibition activity:

Table 2: a-glucosidase inhibition activity

Concentration (µg)	Control	Need oil	Acarbose	% inhibition activity neem oil	% inhibition activity acarbose	
50	0.796	0.484	0.508	39.1959799	36.18090452	
100	0.796	0.396	0.388	50.25125628	51.25628141	
150	0.796	0.294	0.251	63.06532663	68.46733668	

 α -glucosidase inhibition activity of neem oil was estimated and compared (table 2) with the standard drug acarbose. The . α -glucosidase inhibition activity of neem oil was almost equal to the α -glucosidase inhibition activity exhibited by the acarbose. The result was significant as the neem oil showed 39% inhibitory activity with 50 µg concentration as compared to 36% inhibitory activity exhibited by acarbose for the same concentration.

3.Glucose uptake by Yeast cells:

Table	3:	%	uptake	of	glucose
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Concentration (µg)	Control	At concentration of 5mM Neem extract		At concen Neem extr	tration of 10mM act	At concentration of 25mM Neem extract	
		Neem oil	% uptake of glucose 5mM Neem	Neem oil	% uptake of glucose 10mM Neem	Neem oil	% uptake of glucose 25mM Neem
50	0.458	0.626	26.8370607	0.578	20.76124567	0.509	10.01964637
100	0.458	0.784	41.58163265	0.691	33.71924747	0.596	23.15436242
150	0.458	0.915	49.94535519	0.814	43.73464373	0.704	34.94318182

The percentage of glucose uptake in yeast cells was studied with varying concentration of glucose (5mM, 10mM, 25mM) and neem oil (50 μ g , 100 μ g, 150 μ g)As the concentration of neem oil increased there was a concomitant increase in the glucose uptake . Glucose uptake was about 26.8% when 50 μ g of neem oil was added with 5mM glucose. As the glucose concentration increased , there was a gradual decrease in the glucose uptake. The rate of glucose uptake in yeast cells was linear in all the three glucose concentrations . However the highest uptake of glucose was seen in 5mM glucose concentration

DISCUSSION:

The current study was performed to evaluate the hypoglycaemic effects of Neem oil in terms of uptake of the glucose by yeast cells and the α -amylase and α -glucosidase inhibition activity. This study shows that neem oil produced a marked increase in the uptake of glucose by yeast cells and a significant amylase and glucosidase inhibitory activity.

Treatment of type II diabetes is complicated by several factors inherent to the disease, and elevated post prandial hyperglycaemia (PPHG) is one of the risk factors[21].PPHG is elevated by the action of glucosidases, a class of enzymes that helps in the breakdown of complex carbohydrates into simple sugars such as glucose. α -Glucosidase inhibitors play a major role in managing PPHG in diabetic patients by reducing starch hydrolysis which shows beneficial effects on glycemic index control in patients [20]]. The plant based α amylase inhibitors offers a prospective therapeutic approach for the management of diabetes [10]. The inhibitory activity of neem oil on α -amylase was investigated in this study and the results are shown in table 1.

Dose-dependent α -amylase and α -glucosidase inhibitory activity was also observed in the Neem oil and compared with acarbose , which is a major tool for further investigations. The inhibitory action became more significant with increasing concentration of the neem oil. There is possibility to suggest that the bioactive compounds present in the neem oil may be responsible for their α -glucosidase inhibitory activity given in table 2. However further studies would be required to isolate the bioactive compound. The rich phytochemical constituent and high α -glucosidase inhibitory activity of neem oil under study supports local claims on the efficacy of these plants and provides possible lead for isolation of active compounds.

It was studied that the glucose uptake rate increased with increasing concentration of the neem oil and decreased with increasing extracellular glucose. It is stated that transport of glucose across yeast cell membrane occurs by facilitated diffusion down the concentration gradient. Hence glucose transport occurs only if the intracellular glucose is effectively reduced [14]. The data obtained clearly suggests that the plant extract is capable of effectively enhance glucose uptake which in turn suggests that it is capable of enhancing effective glucose utilisation thereby controlling blood glucose level.

The in vitro assays of the present study concluded that neem oil possess hypoglycaemic activity and can be used in the management of diabetes. The active principles of neem oil are responsible for inhibitory action of α -amylase, α -glucosidase and increased glucose uptake in Yeast cells.

CONCLUSION:

The ethno pharmacological use of herbal medicine for the treatment of diabetes mellitus is developed potentially as a preliminary point in the development of alternative and inexpensive therapies for treating the disease. Many valuable drugs have been obtained from plants over the years.

The anti-diabetic properties of plants can be evaluated in vitro by several methods such as study of glucose uptake, effect on glycosylation of the hemoglobin and inhibition of α -glucosidase and α -amylase enzymes. The mechanism of glucose transport across the yeast cell membrane has been gaining significant importance as an in vitro screening method for evaluating the hypoglycemic effects of various medicinal plants [21]. The above conducted in vitro studies depict an appreciable increase in the glucose uptake by the yeast cells in combination with the neem oil. It was observed that the neem oil inhibited the enzyme activities and thereby helps in the inhibition of the formation of glycated end products. We can therefore conclude from this study that the presence of the phytochemicals might be the reason for these inhibitions and that the plants may essentially contain herbal bioactive compounds which require further structural elucidation and characterization methodologies to identify the bioactive constituents. Further investigations should be done for confirming the anti-diabetic activity of these oil.. The results of the study indicate that neem oil has got potential to reduce the blood glucose level. In conclusion our study suggests that neem oil may have beneficial effect in diabetes mellitus and may improve glucose tolerance also. Thus it holds the scope of a new generation of anti-diabetic drug.

ABBREVIATIONS:

- 1. DNSA 3, 5dinitrosalicylic acid (DNSA)
- 2. PPHG prandial hyperglycaemia
- 3. PNPG p-nitrophenyl-α-DGlucopyranoside

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