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Anti-Hyperglycemic Evaluation of Extracts of *Spinacia oleracea* Linn. and *Acacia nilotica* Linn. in Alloxan induced Diabetic Rats

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Abstract

Aim of the study- The study was designed to investigate the Anti-hyperglycemic activity of extracts of *Spinacia oleracea* Linn. and *Acacia nilotica* Linn. in Alloxan induced diabetic Rats. **Material and Methods**- The leaves of *Spinacia oleracea* Linn. were extracted using petroleum ether, chloroform, ethyl acetate and methanol and pods of *Acacia nilotica* Linn. were extracted using petroleum ether, chloroform, ethyl acetate and ethanol, all the extracts were screened for presence of various phytoconstituents. Diabetes was induced by single dose of intraperitoneal injection of Alloxan monohydrate 120 mg/kg. The test extract were given from day 0 to 21 and on day 0,7,14 and 21 blood glucose and body weight was checked. **Results**- on day 21 standard drug Glimepiride treated rats showed highly significant $p<0.001$ while *Spinacia oleracea* leaves extracts and *Acacia nilotica* pods extracts showed significant reduction in blood glucose level from day 7 onwards over diabetic control. **Conclusion**- the antihyperglycemic activity of the extracts may be through its insulinogenic effects as it may have the ability to enhance the activity of insulin within the body.

Keywords
Alloxan, *Spinacia oleracea* leaves and *Acacia nilotica* pods extracts, Diabetic rats.

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INTRODUCTION

Diabetes mellitus also known as simply diabetes is a group of metabolic diseases in which high blood sugar levels over a prolonged period can be seen. This high blood sugar produces the symptoms of frequent urination, increased thirst, and increased hunger. Serious long-term complications include heart disease, stroke, kidney failure, foot ulcers and damage to the eyes.

Several pathogenic processes are involved in the development of diabetes; these ranges from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. Deficient action of insulin on target tissues and hyperglycemia are the basis of the abnormalities in carbohydrate, fat, and protein metabolism, causing diabetes characteristic clinical features,

micro and macro vascular complications and increased risk of cardiovascular disease.

Diabetes is due to either the pancreas not producing enough insulin, or the cells of the body not responding properly to the insulin produced. It is of two types Type one DM results from the body's failure to produce enough insulin and another is Type two DM which begins with insulin resistance, a condition in which cells fail to respond to insulin properly.

As Diabetes is a disorder in which blood sugar (glucose) levels are abnormally high because the body does not produce enough insulin to meet its needs, it damages the nerves and causes problems with sensation also damages blood vessels and increases the risk of heart attack, stroke, and kidney failure. Physician diagnoses diabetes by measuring blood sugar levels. People with diabetes need to follow a diet that is low in carbohydrates and fat, exercise, and regularity in taking drugs to lower blood sugar levels.

In 2010 there were 285 million people worldwide with diabetes, with considerable disparity between populations and regions. Population growth, ageing of populations and urbanization with associated lifestyle change is likely to lead to a 54% increase in worldwide numbers with diabetes by 2030.

Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease. In 2000, India (31.7 million) topped the world with the highest number of people with diabetes mellitus followed by China (20.8 million) with the United States (17.7 million) in second and third place respectively.

Type 1 Diabetes may cause due to the immune destruction of the beta cells of the pancreas, antibodies to islet cells and insulin are present at diagnosis, insulin secretion gradually diminishes, genetic predisposition, environmental triggers (infection or other stress).

Type 2 Diabetes may cause by insulin resistance in the liver and skeletal muscle, increased glucose production in the liver, over production of free fatty acids by fat cells and relative insulin deficiency, insulin secretion decreases with gradual beta cell failure, reductions in blood glucose levels often can be achieved with changes in food intake and physical activity patterns, other contributing factors as obesity, age, lack of physical activity or exercise, genetic predisposition, racial or ethnic background, conditions associated with insulin resistance like polycystic ovary syndrome.

MATERIAL AND METHODS

Collection, Authentication of the plant material

Leaves of *Spinacia oleracea* and pods of *Acacia nilotica* had been selected for present study, both the crude drugs were collected from local region of Nanded District, Maharashtra, India, Identified and authenticated by senior botanist, Department of Botany, Yeshwant Mahavidyalaya, Nanded.

Successive solvent extraction method

Extraction of *Spinacia oleracea* leaves and *Acacia nilotica* pods was carried out through successive solvent extraction using Soxhlet apparatus. For *Spinacia oleracea* leaves solvents used were petroleum ether (SOL-PE) chloroform (SOL-CH), Ethyl Acetate (SOL-EA) and methanol (SOL-ME). For *Acacia nilotica* p*ods* petroleum ether (ANP-PE), chloroform (ANP-CH), Ethyl Acetate (ANP-EA) and ethanol (ANP-ET). All the extracts were dried and percent yield was calculated.

Phytochemical screening of extracts

All the extracts were screened for presence of phytoconstituents *viz.* alkaloids, flavonoids, tannins, steroids, saponins, triterpenoids, fixed oil and sugars as per standard procedure as given under (Trease & Evans, 1983). **Evaluation of Anti-hyperglycemic activity**

Acute toxicity determination

Acute oral toxicity study (LD_{50} determination and Safe Dose Calculation) and Sub-acute toxicity study in rats (Repeated Dose 28-Day Oral Toxicity Study) for the extracts SOL-EA, SOL-ME, ANP-EA, and ANP-ET was carried out to find out safe experimental dose of extracts and observed for toxicity according to OECD Guideline No: 423 (Acute Oral Toxicity- Class Method) in which Wistar rats were used, received a single dose of 2000 mg/kg of different extracts observed over various parameters like behavioural change, hypersensitivity, mortality, etc. Further repeated Dose Oral Toxicity Study as per OECD Guideline No: 407 was carried out.

Preparation of doses

Dose equivalent to 200 mg/kg and 100 mg/kg of crude drug body weight were calculated and suspended in DMSO.

Alloxan induced diabetes in rats

Diabetes was induced by single i.p. injection of 120 mg/kg body weight of alloxan monohydrate in sterile physiological saline (Mohesun 2014), diabetic state was confirmed 48 hours after alloxan injection. Rats with blood glucose level higher than 200 mg/dl were selected for the study. The alloxan induced rats were allowed to drink 5% glucose solution to overcome drug induced hypoglycemia, evaluation was carried out over blood glucose using GOD-POD kit (glucose

oxidase-peroxidase method) procured from Ambika Diagnostics, Parbhani, Maharashtra, over semi-auto analyzer and morphological parameter viz., body weight of animal at 7th, 14th and 21st day of treatment.

Treatment groups and schedule

All the animals were divided in eleven groups as group A for normal or negative control, group B as diabetic or positive control, group C as diabetic standard group, group D as SOL-EA extract, dose 100 mg/kg, group E as SOL-EA extract, dose 200 mg/kg, group F as SOL-ME extract, dose 100 mg/kg, group G as SOL-ME extract, dose 200 mg/kg, group L as ANP-EA extract, dose 100 mg/kg, group M as ANP-EA extract, dose 200 mg/kg, group N Test group as ANP-ET extract, dose 100 mg/kg, group O as ANP-ET extract, dose 200 mg/kg. Treatment was given to animals for 21 days.

Blood glucose using GOD-POD kit and change in body weight in rats

In present study initially SOL-EA, SOL-ME, ANP-EA and ANP-ET was studied for its antidiabetic activity where diabetes was induced by single i.p. injection of 120 mg/kg body weight of alloxan monohydrate in sterile physiological saline and treatment continued for 21 days in which blood glucose and change in body weight was observed. Blood glucose was measured using glucose oxidase peroxidase method on day 0, 7, 14 and 21 and over the same duration change in body weight was observed and recorded.

Statistical analysis

Values were expressed as Mean ± SEM (n=6), ANOVA followed by Tukey test. *p<0.05 significant difference, **p<0.00 highly significant difference when compared with Diabetic-control. #p>0.05 non-significant difference when compared with standard.

RESULTS

Phytochemical screening

Among the extracts *Spinacia oleracea* leaves Ethyl Acetate (SOL-EA) and methanol (SOL-ME) and *Acacia nilotica* p*ods* Ethyl Acetate (ANP-EA) and ethanol (ANP-ET) shown better presence of presence of flavonoids, saponins and tannins compared to other extracts hence for further pharmacological screening these extracts were used.

Toxicity study of plant extracts

No significant toxic effect was observed in the case of body weight, food and water consumption, in blood analysis of haemoglobin, red blood cells, and white blood cells,and extracts did not show any toxic symptoms, changes in behaviour or mortality in animals even at the maximum dose 2 g/kg p.o. This suggests that the plant extracts are safe even at the highest tolerable doses suggesting a LD_{50} above 2.0 g/kg by p.o. route. The substances that present LD_{50} higher than 2.0 g/kg by oral route can be considered practically non-toxic. No toxicity signs or deaths were recorded during the 28 days. No sign of toxicity was observed in case of haematological parameter. Hence 1/10th dose is considered as therapeutic dose or effective dose.

Alloxan induced anti-hyperglycemic activity in rats

Effect on blood glucose level and change in body weight

The induction of diabetes using Alloxan 120 mg/kg significantly increases blood glucose level in rats compared to normal control group rats. On 21st day glimepiride treated group showed highly significant (p<0.001) reduction in blood glucose level compared to diabetic control group while the extract treated groups shown significant reduction in blood glucose from day 7 onwards and in case of body weight all the standard and extract treated groups shown significant recovery in body weight compared to diabetic control group. The results for change in body weight in Table No.1 and for blood glucose are shown in Table No.2

Table 1.: Effect of SOL-EA, SOL-ME, ANP-EA and ANP-ET on change in body weight in alloxan-induced diabetic rats (120mg/kg)

Groups	Change in Body Weight (gm)		
	Day 7	Day 14	Day 21
Control	02.37±0.47	03.83±0.32	04.01±0.13
Diabetic Control	-11.31±1.09	-14.44±0.04	-16.32±0.32
D + Glimepiride	-3.61±0.03**	1.17±0.07**	2.03±0.13**
D + SOL-EA 100	-11.03±0.05	-9.11±0.02**	-6.72±0.31**
D + SOL-EA 200	-8.61±0.04**	-7.35±0.04**	-5.31±0.47**
D + SOL-ME 100	-8.77±0.08**	-7.68±0.08**	-3.41±0.43**
D + SOL-ME 200	-6.03±0.02**	-2.03±0.03**	-0.21±0.31**
D + ANP-EA 100	-14.55±0.08	-11.43±0.07*	-9.41±0.45**
D + ANP-EA 200	-11.33±0.06	-9.33±0.04**	-7.23±0.57**
D + ANP-ET 100	-11.49±0.07	-9.28±0.04**	-6.17±0.32**
D + ANP-ET 200	-9.77±0.03*	-7.72±0.02**	-5.24±0.34**

Values are expressed as Mean±SEM. (n=6), ANOVA followed by Tukey test. *p<0.05 significant difference, **p<0.00 highly significant difference when compared with Diabetic-control. #p>0.05 non-significant difference when compared with standard; **D**- Diabetic**, SOL-** ***Spinacia oleracea leaves*** extract**, ANP-** ***Acacia nilotica pods*** extract, **EA-** ethyl acetate, **ET**- ethanol, **ME-** Methanol.

Change in body weight was measured on day 7, 14 and 21. Normal control shown body weight change 4.01 gm on day 21 while Diabetic control shown body weight change -16.32 gm, Glimepiride (2.03), SOL-ME 200 (-0.21), shown significant (p<0.001) change in body weight on day 21. Significant change in body weight is seen in SOL extract treated groups from day 7 onwards while ANP extracts treated groups shown significant change from day 14 onwards.

Table 2.: Effect of SOL-EA, SOL-ME, ANP-EA and ANP-ET on change in blood glucose level in alloxan-induced diabetic rats (120mg/kg)

Groups	Blood Glucose (mg/ dl) at			
	Day 0	Day 7	Day 14	Day 21
Control	59.88±4.51	60.71±1.83	62.03±3.28	64.33±0.49
Diabetic Control	540.05±03.81	546.33±08.9	554.01±08.61	473.41±0.11
D + Glimepiride	557.31±8.43	375.87±13.87**	173.31±08.8**	98.31±4.8**
D + SOL-EA 100	566.23±6.71	488.71±14.61**	448.77±8.76**	344.58±0.55**
D + SOL-EA 200	493.41±7.31	449.55±10.58**	371.67±7.67**	276.51±0.43**
D + SOL-ME 100	496.43±5.11	441.51±7.31**	229.77±3.66**	199.51±0.23**
D + SOL-ME 200	491.51±4.93	323.75±2.92**	205.38±5.48**#	129.01±0.37**
D + ANP-EA 100	497.55±11.45	460.21±7.34**	395.44±7.31**	314.52±7.31**
D + ANP-EA 200	489.71±10.53	458.55±4.75**	351.55±11.32**	288.45±8.39**
D + ANP-ET 100	481.44±10.27	453.33±5.48**	328.67±6.58**	266.86±4.02**
D + ANP-ET 200	499.49±10.44	451.91±4.02**	253.78±4.74**	198.43±7.31**

Values are expressed as Mean±SEM. (n=6), ANOVA followed by Tukey test. *p<0.05 significant difference, **p<0.00 highly significant difference when compared with Diabetic-control. #p>0.05 non-significant difference when compared with standard; **D**- Diabetic**, SOL-** ***Spinacia oleracea leaves*** extract**, ANP-** ***Acacia nilotica pods*** extract, **EA-** ethyl acetate, **ET**- ethanol, **ME-** Methanol.

Blood glucose was measured using glucose oxidase peroxidase method on day 0, 7, 14 and 21. Normal control shown blood glucose 64.33 mg/dl on day 21 while Diabetic control shown blood glucose 473.41 mg/dl, Glimepiride (98.31), SOL-ME 100 (199.51), SOL-ME 200 (129.01), ANP-ET 200 (198.43) shown significant (p<0.001) reduction in blood glucose level on day 21. SOL-ME 200 and ANP-ET 200 shown significant reduction in the levels of blood glucose from day 7 onwards similar to standard.

DISCUSSION:

Consumption of fruits and vegetables is well known to have positive effects on human health and has

been correlated to a decreased risk of most of the chronic diseases such as cardiovascular disease, diabetes and diabetic complications. The present study is an attempt to establish the potential anti-hyperglycemic response of *Spinacia oleracea,* and *Acacia nilotica pods* extracts for confirming claims made in traditional system of medicine.

The preliminary phytochemical screening revealed the presence of various phytoconstituents in extracts and also earlier studies of chemical constituents and their pharmacology suggest that the plants containing flavonoids, saponins, tannins possess activity against hyperglycemia (S B Gaikwad, et al., 2014).

Acute toxicity study as per OECD guideline 423 and Sub-acute repeated dose 28-day oral toxicity study was carried out as per OECD Guideline No. 407 from which value of LD_{50} was calculated.

In the present study, the effect of extracts shown significant reduction in blood glucose which may be through its insulinogenic effects as Insulin is the main regulator of glycogenesis in muscles and liver so the extracts and fractions may have the ability to enhance the activity of insulin within the body. Also change in body weight was observed, significant improvement in the body weight of diabetic rats may be due to reduction in protein catabolism and peripheral glucose utilizing properties of the extract as decrease in body weight takes place due to deficient energy and the cellular catabolic process characterized by glycogenolysis, lipolysis and proteolysis.

CONCLUSION:

Significant glucose lowering response of extracts along with recovery in reducing body weight all this response is suggestive of anti-hyperglycemic potential. Pharmacognostic evaluations confirmed presence of various phytoconstituents which may be responsible for the anti-hyperglycemic effect. Role of extracts in hyperglycemia supports its possible claim as alternative for treatment of diabetes and may be its complications therefore *Spinacia oleracea, Acacia nilotica pods* deserves to be explored for its use in it.

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