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Molecular docking and antidiabetic activity of ethanol leaves extract of *Spinacia oleracea*

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Abstract
The leaves of *Spinacia oleracea* L., a significant and common leafy vegetable belonging to the family Amaranthaceae. This plant is also called Spinach. It contains a lot of fibre, which get slow digest. Hence, spinach does not immediately result in blood sugar increases, actuality the present soluble fibre lowers blood glucose levels and manages diabetes. The aim and object of the study is to find out the binding property of the present constituents in the plant by molecular docking and screen the anti diabetic activity of the ethanol leaves extract of the Spinacia *oleracea* L.

Keywords: *Spinacia oleracea*, Antioxidant, flavonoids, diabetic, extract

Introduction
Spinacia oleracea includes 2,400 species and 160 genera of flowering plants. Spinach is native to central and southwestern Asia.
Spinach is very nutritious and consists of vitamins A, B. C, E and vitamin K, also reported minerals (Iron, Calcium, magnesium and manganese), folic acid and protein. Spinach has a high source of zea xanthin and carotenoids that can flush out the free radicals from our body.
Spinach is commonly used for cancer, antioxidant, reduces blood sugar, weight loss, hypertension, inflammation and nutritive. The focus of the present study is to determine the role of dietary supplements for diabetics. The fiber contributes to a decrease in glycemic index, which can aid in hunger control and have a significant impact on reducing blood glucose levels. The selected plant *Spinacia oleracea* possesses rich dietary fiber and flavonoids since planned to screen the Molecular docking and anti-diabetic activity.

Materials and Methods
Molecular Docking
PPAR receptor gamma also known as the glitazone reverse insulin resistance receptor or NR1C3 is a type II nuclear receptor functioning as a transcription factor that in humans is encoded by the gene is the main target of thiazolidinediones used in diabetes mellitus characterized by insulin resistance. Thiazolidinediones, acting via PPARγ, influence free fatty acid flux and thus reduce insulin resistance and blood glucose levels. PPARγ agonists are therefore used to treat type2 diabetes. In the current study, the ability of the phytoconstituents present in *Spinacia oleracea* to bind with PPAR gamma was predicted using molecular docking studies.
Auto Dock Vina v.1.2.0 was used to predict the binding affinity, binding pose and interactions of the Phytoconstituents present in *Spinaciaoleracea* with 1 FM 6.

Ligand Preparation
The 2D Structures of the designed ligands were constructed and downloaded from Pub Chem database. The 2D structures were converted to the 3D structure using open Babel. The complete set of ligands was organized in a single SDF file using Open Babel (v2.3.0).

Anti-Diabetic Screening
Evaluation of anti-hyperglycemic activity of ethanol leaves extract of *Spinacia oleraceae* on Nicotinamide and Streptozotocin induced Type 2 diabetic rats

Experimental Design
Animals Used: Adult male albino Wistar rats (6 weeks), weighing 150 to 200 g were used for the present anti-diabetic study. The animals were housed in clean polypropylene cages and maintained in a well-ventilated temperature controlled animal house with a constant 12 h light/dark schedule.

The animals were fed with standard rat pellete diet and clean drinking water was made available *ad libitum*.

Reagents

Streptozotocin (500 mg, S-0130, Sigma-Aldrich), Nicotinamide (100g, N-3376, Sigma-Aldrich), Sodium Citrate (Mw: 294.10), Citric acid (Mw: 210.10), Sucrose 10%, Distillate water, Sodium chloride (NaCl 0.9%).

Induction of Diabetes mellitus

The animals were divided into five groups of six animals each. The animals were kept overnight fasting and checked the initial fasting blood glucose from tip of rat tail vein. Sterptozotocin was dissolved in citrate buffer (pH4.5) and Nicotinamide was dissolved in normal saline. Non-insulin dependent diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection of 60 mg/kg Streptozotocin, 15 min after the IP administration of 120 mg/kg of nicotinamide were administrated. Hyperglycemia was confirmed by the elevated levels of blood glucose were determined at 72 h. The animals with blood glucose concentration more than 250 mg/dl were used for the present study. The vehicle (saline), standard (3 plant extract), sample Spiach ethanolic extracts were administered the respective group animals for 28 days. Throughout the study period glibenclamide, extracts were freshly dispersed in normal saline and distilled water before to the administration. The fasting animal body weight, blood glucose level was estimated on 0, 7th, 14th and 21st day from tip of rat tail vein.

Table 1: Grouping of animals for STZ and NIC induced diabetic model

Group	Sample	Group specification
GPI	Normal	Only Food
GPII	Negative Control	Only STZ + NIC
GPIII	Positive Control	STZ & NIC+ Standard 500 mg/kg (p.o)
GPIV	Sample	STZ & NIC + Sample 500 mg/kg (p.o)

Dose and Route

STZ (65 mg/kg) Nicotinamide120 mg/kg (i.p) used for induction of diabetes.

Standard: Mixture of ethanol extract of three plants which already reported antidiabetic activity were used as standard. The equal quantity of mixture of *Syzygium cumini*, *Gymnema sylvestre*, *Trigonella foenum* at the dose of 500/kg body weight were given by oral.

Sample: Ethanol leaves extract of *Spinacia oleracea* at dose of 500 mg/kg body weight (oral)

Estimation of blood glucose

Blood sample were collected from tip of rat tail vein and Glucose levels were estimated using a glucose oxidase-peroxidase reactive strips and glucometer Accu-chek, Roche Diagnostic USA.

Evaluation of anti-diabetic activity

Pharmacological Screening

Anti-diabetic activity of ethanol leaves extract of *Spinach oleracea* was screened against Streptozotocin and Nicotinamide (STZ+ NIC) induced diabetic on wistar rats model, the study was carried out for 21 days.

Results and Discussion

Molecular docking

The target PPAR gamma (1FM6) was docked with the major active phyto constituents present in *spinach oleracea.* The grid box (X: 18.015; Y:-19.519; Z: 10.42) was generated around the activesite of the protein. The docking pattern of each phytoconstituent was predicted using Auto dockvina software. Among the different phytoconstituents of *spinach oleracea* the present Astragaline, Hyperoside and Patuletin (-7.5 kcal/mol) were exhibited equal binding affinity and Neochlorogenic acid (-6.9) compared to the standard Rosuvastatin. As concern of the binding affinity screened the anti-diabetic activity.

Table 2: Body weight of the animals

Group	Control	Only STZ&NTC	STZ+STD 3 Plant extract	STZ+ Sample
Initial Body Weight	221±45.23	228.5±50.1	207±46.33	191.7±39.01
Final Body Weight	242±48.4	160.3±34.96	226±49.36	202.3±40.97

Values are expressed as the mean± S.D; Statistical significance (p) calculated by oneway ANOVA followed by dunnett's $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ calculated by comparing treated group with Control group

Table 3: Shows Blood Glucose Level

Group	Control	Only STZ & NIC	STZ + STD	STZ + Sample
0day	73±15.210	68.33±15.79	42.5±8.728	61±12.86
3rdday	74±15.348	323.3±82.73	316.7±83.69*	325±74.64**
7thday	70±14.118	328.3±75.12	265±56.61**	253.3±54.87**
14thday	65±12.432	313.3±70.6	208.3±42.62**	180±36.61***
21stday	73±13.071	276.7±60.59	136.7±30.07^{ns}	101.7±21.51***

Values are expressed as the mean ±S. D; Statistical significance (p) calculated by oneway ANOVA followed by dunnett's $^{***}p<0.001$, $^{**}p<0.01$,$^{*}p<0.05$ calculated by comparing treated group with control.

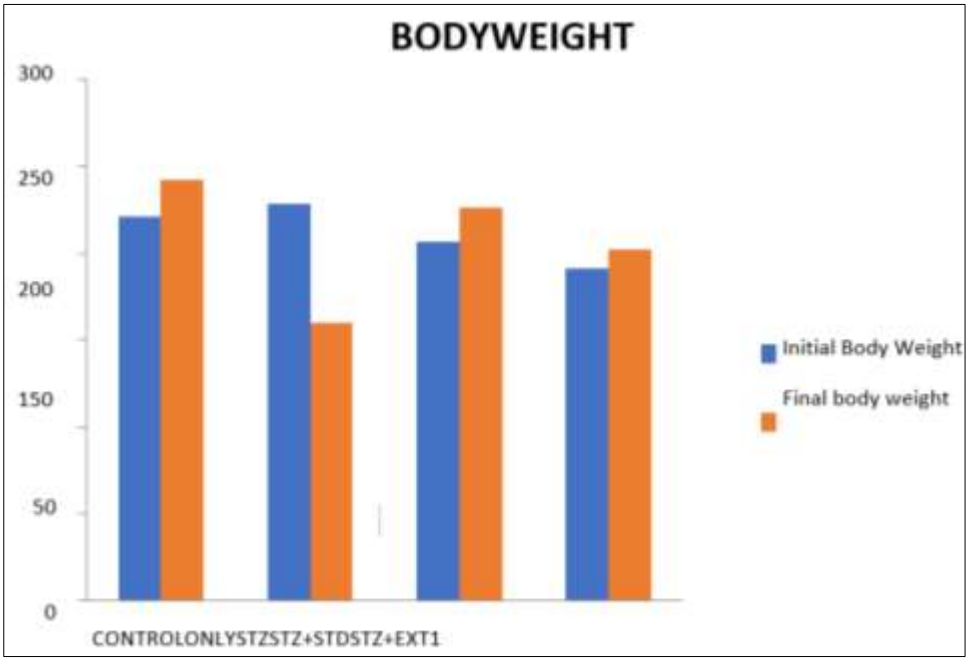

Fig 1: Bodyweight of the used animals

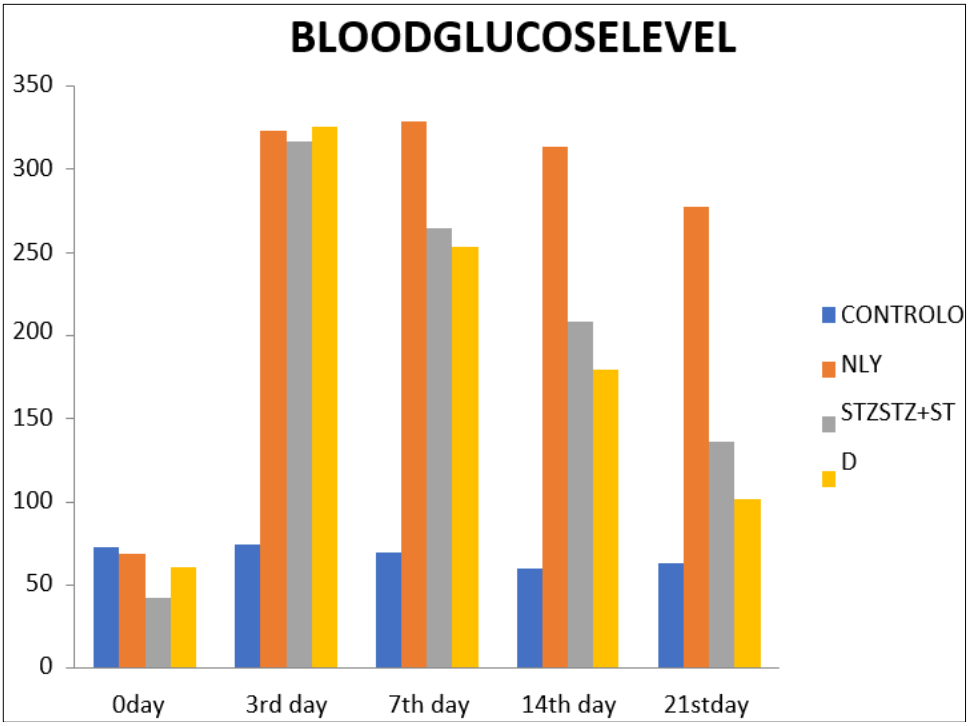

Fig 2: Blood glucose level

The ethanolic leaves extract of *Spinach oleracea* was screened against streptozotocin & nicotinamide (STZ + NIC) induced diabetic model for 21 days. The ethanolic extract of the sample when compared with standard plant extract (*Gymnema sylvestre*, *Fenugreek*, *Syzygium cumini*) and controlled group at a dose level of 500 mg/kg body weight.

The blood glucose was significantly higher in negative, standard and sample treated group's on 3rd and 7th day. The increased blood glucose was significantly declined from 14th day after treatment and found that significant hypoglycemic activity for the ethanol leaves extract of Spinach than that of standard extract on 21st day.

Based on its fiber content, nutritional value and molecular docking the plant *Spinach oleracea* selected and screened hypoglycemic activity. We concluded that the nutritionally dense herb *Spinach oleracea* posses significant hypoglycemic activity based on its fibers, carotenoids and flavonoid content. To confirm the physiological relevance of our *in-vitro* data, these encouraging results call for in-*vivo* research and potentially clinical trials needed.

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