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Phytochemical and Anti-Diabetic Activity of Indigofera Species

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ABSTRACT

Over the past 30 years, the status of diabetes has changed from being measured as a kind disorder of the old to one of the main causes of morbidity and mortality disturbing the childhood and middle aged people. It is essential to note that the increase in prevalence is seen in all six populated continents of the globe. Diabetes is deadly disease in both developed and developing countries. *Indigofera* is a varied genus that has shown unique characteristics making it an interesting candidate as a potential perennial crop. Specifically, there is diverse variation among species with a number of unique characteristics. Entire plants of *I. astragalina* were collected from Tiruchengode, Tamilnadu. The plant was authenticated by Dr. G.V.S. Murthy, Joint Director, Botanical Survey of India, Coimbatore, Tamilnadu, India. The extraction yield of the extracts from plant species is vastly depends on the solvent polarity, which find out both qualitatively and quantitatively the extracted compounds. Ethanol and water are the commonly used solvent for the extraction because of their low toxicity and high extraction yield with the advantage of modulating the polarity of the solvent by using mixtures at different ratios (Jackson et al, 1996). Ethanol soluble fractions were analyzed by TLC. These fractions constituted of mainly nonvolatile mixtures of compounds. The visualizations were aided by either observing the TLC under an UV lamp or by exposing the developed TLC plates to iodine vapor. The plant extracts at both the dose level of 200 and 400 mg/kg registered 79.87 to 85.83 mg/dl of fasting blood glucose level at the end of 10h of the study, while the standard drug, glibenclamide showed 71.63 mg/dl at the same time, with a low degree of significance while compared with the solvent treated group.

Keywords: Diabetes Mellitus, *I. astragalina,* glibenclamide, alloxanisation.

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INTRODUCTION

Diabetes Mellitus Scenario in World

The occurrence of diabetes is hastily rising all over the world at a frightening rate (Huizinga and Rothman, 2006). Over the past 30 years, the status of diabetes has changed from being measured as a kind disorder of the old to one of the main causes of morbidity and mortality disturbing the childhood and middle aged people. It is essential to note that the increase in prevalence is seen in all six populated continents of the globe. Diabetes is deadly disease in both developed and developing countries. In 2000, there were a probable 175 million people with diabetes universal and by 2030, the projected estimate of diabetes is 354 million (Wild et al., 2004). The worldwide increase in the popularity of diabetes is owed to population growth, aging, urbanization and an augment of obesity and physical inactivity. The prime determinants of the epidemic are the rapid epidemiological transition associated with alteration in dietetic patterns and reduced physical activity. Unlike in the West, wherever older populations are mainly affected, the burden of diabetes in Asian countries is excessively high in young to middle-aged adults (Chan et al, 2009; Ramachandran et al, 2010). Healthcare expenditures on diabetes are expected to account for 11.6% of the total healthcare expenditure in the world in 2010. Expected global healthcare expenditures to treat and avert diabetes and its complications are expected to total as a minimum 376 billion U.S. Dollars (USD) in 2010. By 2030, this number is expected to go above some USD490 billion (IDF Diabetes Atlas, 2009)

Medication or Chemical-Induced Diabetes

Drug-induced diabetes occurs due to a variety of drugs and mechanisms (Comi, 2004). An underlying and often unsuspected abnormality in carbohydrate metabolism in the patient or a family history of diabetes greatly increases the risk for developing drug induced diabetes. A lot of drugs can damage insulin secretion. These drugs can not cause diabetes by themselves, except they may impulsive diabetes in individuals with insulin resistance.

Indigofera astragalina

Indigofera is a varied genus that has shown unique characteristics making it an interesting candidate as a potential perennial crop. Specifically, there is diverse variation among species with a number of unique characteristics. Some examples of this diversity include differences in pericarp thickness, fruit type, and flowering morphology. The unique characteristics it has displayed include potential for mixed smallholder systems with at least one other species and a resilience that allows for constant nitrogen update despite varying conditions.

Fig-1: Plant of *Indigogera astragalina*

MATERIAL AND METHODS:

Plant Selection: Drug discovery from medicinal plants has evolved to include numerous fields of inquiry and various methods of analysis. The process typically begins with a botanist, ethno botanist, ethnopharmacologist, or plant ecologist who identifies the plant of interest. Collection may involve species with known biological activity for which active compound(s) have not been isolated (e.g., traditionally used herbal remedies) or may involve taxon collected randomly for a large screening program. On the basis of intensive literature survey; *I. astragalina* was selected for present study.

Collection and identification:

Entire plants of *I. astragalina* were collected from Tiruchengode, Tamilnadu. The plant was authenticated by Dr. G.V.S. Murthy, Joint Director, Botanical Survey of India, Coimbatore, Tamilnadu, India. A voucher specimen is preserved in our laboratory for future reference (Voucher No.: PCIAS 007).

Preparation of plant material:

The fruits of plant were shade dried, reduced to coarse powder with the help of grinder and stored in airtight container till further use.

Extraction and fractionation:

The extraction yield of the extracts from plant species is vastly depends on the solvent polarity, which find out both qualitatively and quantitatively the extracted compounds. Ethanol and water are the commonly used solvent for the extraction because of their low toxicity and high extraction yield with the advantage of modulating the polarity of the solvent by using mixtures at different ratios (Jackson et al, 1996). The plant materials (1 kg) were initially defatted with petroleum ether and then extracted with alcohol and water using a Soxhlet apparatus. The yield of the plant extracts ethanol (95%) and aqueous measured about 20 g each after evaporating the solvent using water bath. The standard extracts obtained from *Indigofera astragalin* were then stored in a refrigerator at 4°C for further use for phytochemical investigation and pharmacological screening (Akueshi et al, 2002).

Preparation of plates:

Slurry of silica gel G and distilled water was prepared in a pestle with continuous triturating with mortar. The slurry was spread evenly on clean grease free glass plates. The plates were dried in air and thereafter heated in oven at 110 ^{0}C for about 30 minutes to activate them[23].

Preparation of samples*:*

Approximately 10 mg of material was dissolved in respective solvents and was used for spotting on TLC plates.

Application of samples on TLC plates:

Samples were applied on the TLC plates with the help of a capillary tube at a distance of about 0.5 cm from the developing solution. The solvent from the plate was removed by air-drying and position of the spot was marked.

Saturation of TLC chamber:

The inner wall of the chamber was lined with filter paper on three sides, the solvent system was poured up to a height of about 1 cm from the base, grease was applied on the rim of the chamber and it was covered with a glass plate. The chamber was allowed to stand for about 30 minutes and by that time the filter paper inside the chamber was completely drenched by the solvent system, making the chamber completely and evenly saturated with solvent system.

Development of TLC plates: The plates were placed vertically into a solvent vapor saturated TLC chamber and allowed to develop till the mobile phase had moved about 80% from the spotting line; the plate was removed from the developing chamber and dried.

Detection of TLC plates: The eluted spots, representing various fractions/compounds, were visualized by different detection methods.

i. The plate were exposed to iodine vapor and observed.

The TLC profile was examined to determine variation in band size and color intensity between the Ethanolic extract and its two fraction .The observation indicate that variations are present amongst Ethanolic extract, Ethanolic fraction, Hexane fraction and chloroform fraction. The TLC study was done by using different solvent system for each fraction separately and the compounds were detected by sun light, in iodine vapours, vanillin sulfuric acid reagent and long UV.

Optimization of solvent system:

Ethanol soluble fractions were analyzed by TLC. These fractions constituted of mainly non volatile mixtures of compounds. The visualizations were aided by either observing the TLC under an UV lamp or by exposing the developed TLC plates to iodine vapor. The TLC was repeatedly improved by changing the solvent systems until a system that gave the best separation was obtained.

Column Chromatography (CC):[52]

Column chromatography is an example for liquid-solid chromatography. It operates on the principle that different substances will "adsorb" or adhere onto the surface of fine particles of a solid adsorbent (e.g., alumina or silica gel). Intermolecular forces, which vary in strength according to their type, cause organic molecules to bind to the stationary phase. The stronger the intermolecular force, the stronger the binding to the stationary phase, the longer the compound takes to elute from the column. Stationary phase, the longer the compound takes to elute from the column.

Antidiabetic of *I. Astragalina:*

Animals:

Healthy adult Male albino Wistar rats, weighing 150–200 g and Swiss albino mice, weighing 20–25 g were used for the Screening methods.

Investigational model for induction of diabetes:

Diabetes was induced by intra-peritoneal injection of Alloxan monohydrate (150 mg/kg b.w.) dissolved in the in normal saline (Viana et al, 2004). Blood was withdrawn (0.1 ml) from the tip of the tail of each rat under mild ether anaesthesia. The blood glucose level was checked before alloxanisation and after alloxanisation regularly in 24h intervals. Animals were considered diabetic when the blood glucose level was raised beyond 200 mg/100 ml of blood. This condition was observed at the end of 72 h after alloxanisation.

Preparation of Interventions

The measured quantity of extracts and fractions of *Indigofera astragalin* and the standard drug glibenclamide (5 mg/kg) was suspended in 25% Tween-20 in distilled water. The solvent, test samples and standard drugs were administered by oral route based on dose and corresponding weight of the animals. For oral administration of test, standard as well as Solvent Feeding needle no 21 was used.

Maintenance of animals and Exposure Conditions

Earlier to the experiments, the selected animals were housed in acrylic cages in standard environmental conditions (conditions (temp: 20–25 0C; relative humidity: 45-55 % under 12 h light/dark cycle), fed with standard rat feed for 1 week in order to adapt to the laboratory conditions and water *ad libitum.* They were fasted overnight (12 h) before experiments, but were allowed free access to water. Six animals were used for each group of study. All the experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use and as per the experimental protocols duly approved by the Institutional Ethical Committee.

Blood glucose level determination

Fasting blood glucose concentration was determined using a Glucometer (Optium), based on the glucose oxidase method. Blood samples were collected from the tip of tail at the defined time patterns (Aslan et al, 2007a, Aslan et al, 2007b)

Antihyperglycemic activity of extracts in glucose-loaded animals (oral glucose tolerance test)

The oral glucose tolerance test (OGTT) measures the body's ability to use main source of energy i.e. glucose. OGTT is to simplify and facilitate the diagnosis of diabetes (Luzi, 1998). This method is frequently referred to as physiological induction of diabetes mellitus because the blood glucose level of the animal is fleetingly increased with no damage to the pancreas. An oral glucose tolerance test (OGTT) was performed on diabetic rats by feeding glucose (5 g/kg) per os. Animals were deprived of food 18 h before and during the experiment, but were allowed free access to water. They were divided into 7 groups of 6 rats each. Group I served as normal control, Group II served as solvent control and received only vehicle (Tween + water - 2 ml/kg b.w.) through the oral route. Group III received glibenclamide (5 mg/kg b.w.). Groups IV to VII received the alcohol and aqueous extracts of *Indigofera astragalin* at a dose of 200 and 400 mg/kg b.w., respectively, through oral route. The blood glucose level was determined before drug and glucose administration (−1 and 0 h, respectively) and subsequently at 0.5, 1, 2 and 3h after.

RESULTS AND DISCUSSION:

Preliminary phytochemical study of the *I. astragalin* extracts/fractions

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. The preliminary phytochemical screening was carried out to assess the qualitative chemical composition of crude extracts and fractions from *I. astragalin* by using precipitation and coloration reaction to identify the major natural chemical groups (Harborne, 1998; Kokate et al., 2003). General reactions in this analysis revealed the presence or absence of these compounds in the crude extracts and fractions tested. Summary of preliminary phytochemical screening of different extracts and fractions is depicted in Table-1.

Table-1: Phytochemical screening of extracts and fractions of *I. astragalin*

Chemical constituents	Chemical Test	Extracts/Fractions			
		Ethanol extract	Aqueous extracts	Chloroform fraction	Aqueous fraction
Alkaloids	Mayer's	+	+	+	+
	Dragendorff's	+	+	+	+
	Wagner's	-	-	-	-
	Hager's	+	+	+	+
saponin	Foam	-	+	-	-
	Haemolytic	-	-	-	-
Phenolic compounds and Tannins	Ferric Chloride	+	+	+	+
	Gelatin	-	-	-	
	Lead acetate test	+	+	+	+
Proteins	Million's	+	-	+	-
	Biuret	+	+	+	-
	Xanthoprotein	-	-	-	-
Flavonoids	Ferric Chloride	+	+	-	+
	Shinoda	-	-	-	-
	Lead Acetate	+	+	+	+
Glycoside	Baljet's	-	-	-	-
	Legal's	-	-	-	-
	Borntrager's	-	-	-	-
	Killer killani	-	-	-	-
Fixed oil	Spot	-	-	-	-
Carbohydrate	Molisch's	-	-	-	-
	Fehling's	+	+	+	+
	Benedict's	-	-	-	-
	Barfoed's	+	+	+	+
	Cobalt-chloride	-	-	-	
Gums and mucilage	Swelling Index	-	-	-	-
Amino Acids	Ninhydrin	-	-	-	-
	Tyrosin	-	-	-	-
	Tryptophan	-	-	-	-
Sterols and triterpenes	Liebermann-Burchard's	+	+	+	-
	Salkowski's	+	-	+	-

Key (+) = Presence, (-) = Absent

Effect of various extracts of *Indigofera astragalin* on Blood Glucose Level of normoglycaemic rats (hypoglycemic activity)

The effects of ethanol and aqueous extracts of aerial parts of *I. astragalin* on fasting blood glucose levels of normal rats are presented in table 2. The plant extracts at both the dose level of 200 and 400 mg/kg registered 79.87 to 85.83 mg/dl of fasting blood glucose level at the end of 10h of the study, while the standard drug, glibenclamide showed 71.63 mg/dl at the same time, with a low degree of significance while compared with the solvent treated group. The percentage change of blood sugar of test extracts treated groups at the end of 10 h showed 3 to 12% fall when compared with initial BGL in a dose dependent manner. The potency order of the test extracts towards the falling of BGL is an aqueous extract followed by ethanolic extract.

Effect of various extracts of *Indigofera astragalin* on BGL of glucose loaded hyperglycemic rats (oral glucose tolerance test, OGTT)

The effect of the test extracts on blood glucose level (BGL) in OGTT is depicted in Table 3. The ethanol and aqueous extracts at 200 mg/kg dose level registered 92.13, 95.50 mg/dl at the end of 3 h of the study, while it was 91.50, 96.53 mg/dl with dose level of 400 mg/kg. However, at the same time the standard drug glibenclamide at 5mg/kg showed 62.51 mg/dl of BGL. However the calculated percentage fall of BGL demonstrated 6.22, 18.26 and 14.73, 24.79% with respect to 200 and 400 mg/kg dose levels when measured at the end of the 3 h of the study, while at the same time glibenclamide showed a 30.10% fall of BGL. The progressive fall of BGL of the test extracts, in the different test hour showed a statistical significant of $p<0.05$ to $p<0.01$, while analyzed by using ANOVA followed by Dunnett's t-test. The aqueous extract possesses more BG lowering potency than that of the ethanol extract in a dose dependent manner. The test extracts at tested dose levels also showed a significant fall of BGL while compared with solvent control group during the study period of 30, 60 and 120 min.

Table-2: Effect of ethanolic and aqueous extracts of *I. astragalin* on blood glucose level in normoglycemic rats.

Groups	Treatment and dose	Blood Glucose Levels(mg/dl)							% decrease at 10 hrs
		0 hr	1 hr	2 hr	4 hr	6 hr	8 hr	10 hr	
I.	Solvent Control (Tween + Water)	94.6 ± 1.1	87.2 ± 4.62	91.43 ± 1.86	89.56 ± 0.81	91.58 ± 2.23	89.66 ± 0.46	92.67 ± 3.22	--
II.	Glibenclamide (5mg/kg)	91.43 ± 1.31	81.22 ± 2.63	67.53 ± 2.34*	58.12 ± 2.61**	54.72 ± 2.44**	73.83 ± 1.42**	71.63 ± 2.81**	21.65
III	Eth. Ext. (200mg/kg)	89.13 ± 1.2	87.8 ± 1.1	86.93 ± 2.65	86.73 ± 1.46	86.57 ± 1.43	86.29 ± 0.89*	85.83 ± 1.51	3.70
IV	Eth. Ext. (400mg/kg)	88.4 ± 2.43	87.31 ± 2.16	86.13 ± 1.87	85.78 ± 1.67	84.97 ± 2.69	84.11 ± 1.43*	82.21 ± 2.49*	7.0
V	Aq. Ext. (200mg/kg)	92.53 ± 1.27	91.46 ± 1.68	89.88 ± 1.09	87.19 ± 0.91	86.07 ± 2.13	85.78 ± 1.18*	83.66 ± 1.89*	9.58
VI.	Aq. Ext. (400mg/kg)	91.18 ± 0.93	87.19 ± 0.78	85.71 ± 2.61	85.23 ± 1.37	84.83 ± 2.38*	83.11 ± 1.21**	79.87 ± 2.73**	12.40

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test (t-value denotes statistical significance at * p <0.05, and ** p <0.01 respectively, in comparison to group-I).

Table-3: Effect of ethanolic and aqueous extracts of *I. astragalin* on oral glucose tolerance in normal rats

Gr.	Treatment and dose	Blood glucose concentration (mg/dl)					% decrease at end of 3hr
		0 min	30 min	60 min	120 min	180 min	
I	Normal Control	83.75 ± 0.47	86.50 ± 0.84	88.50 ± 1.56	83.50 ± 0.98	86.50 ± 1.47	--
II	Solvent control	90.50 ± 0.64	135.52 ± 0.64**	118.83 ± 0.85**	98.50 ± 0.61**	91.50 ± 0.24**	--
III	Glibenclamide (5mg/kg)	89.43 ± 0.40	95.50 ± 1.04**	81.53 ± 0.91**	72.50 ± 0.64**	62.51 ± 0.66**	30.10
IV	Eth. Ext. (200mg/kg)	83.62 ± 1.78	98.25 ± 0.85**	97.61 ± 1.91**	93.50 ± 1.63**	92.13 ± 0.95	6.22
V	Eth. Ext. (400mg/kg)	87.50 ± 0.89	107.31 ± 1.37**	102.32 ± 1.10**	94.50 ± 0.54*	91.50 ± 0.54	14.73
VI	Aq. Ext. (200mg/kg)	91.50 ± 0.64	116.84 ± 1.10**	109.83 ± 0.85**	102.65 ± 1.91*	95.50 ± 0.64*	18.26
VII	Aq. Ext. (400mg/kg)	84.87 ± 0.91	128.36 ± 0.85**	117.36 ± 1.70	103.51 ± 0.77**	96.53 ± 1.27**	24.79

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test (t-value denotes statistical significance at * p <0.05, ** p <0.01 respectively, in comparison to group-II)

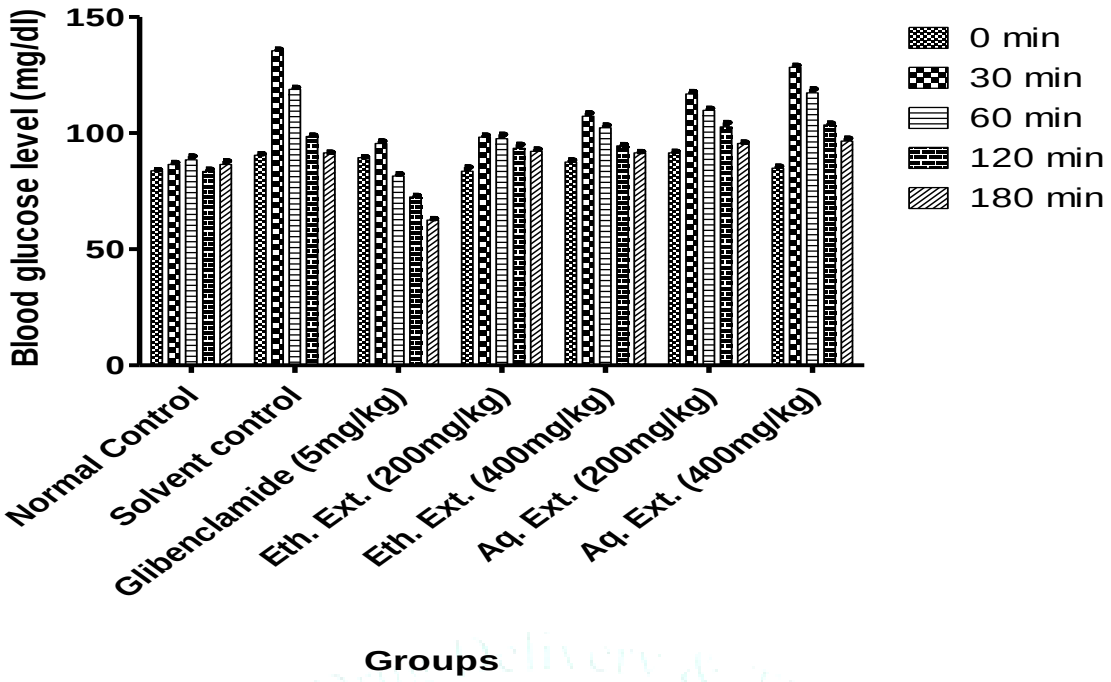

Fig.2: Effect of ethanolic and aqueous extracts of *I. astragalin* on oral glucose tolerance in normal rats

CONCLUSION

The present investigations concluded that the ethanolic and aqueous extracts of aerial parts of I. astragalin endowed with potential antidiabetic activity which could be attributed by their possible multiple effects on both pancreatic and extra-pancreatic site by influencing either the metabolism and/or absorption of glucose, which in turn also influence the lipid metabolism.

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