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ANTIHYPERGLYCAEMIC, ANTIOXIDANT POTENTIAL OF THE AQUEOUS EXTRACT OF LEAF OF *INDIGOFERA SUFFRUCTICOSA* MILL

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ABSTRACT

This desertion was designed based on the traditional claim to emphasize the antihyperglycaemic, antioxidant potential of the aqueous extract of leaf of *Indigofera suffructicosa* Mill. Preliminary phytochemical analysis of the AEIS showed that the plant has a rich possession of phytochemicals like alkaloids, reducing sugars, tannins and phenols. Terpenoids, gums and mucilage were absent in the extracts. Acute oral toxicity studies reveal that AEIS did not produce any mortality or signs of toxicity at the dose of 2000 mg/kg b.w.p.o, in experimental rats. Treatment of AEIS shown moderate hypoglycaemic effect on normal animals and significant improvement in glucose tolerance on glucose fed hyperglycaemic rats. No significant reduction of blood glucose level was observed o the acute treatment of AEIS in STZ induced diabetic rats. In the sub acute study a steady decrease in blood glucose level was observed on AEIS treatment in STZ induced diabetic rats. The treatment of AEIS showed marked increase in body weight, decrease in glycosylated haemoglobin, marked increase in protein; HDL cholesterol levels in serum of STZ induced diabetic animals. At the same time significant decrease in total cholesterol, LDL-cholesterol, VLDL-cholesterol, triglycerides and creatinine levels was observed in serum of diabetic animals. The antioxidant study revealed that the hepatic antioxidant enzyme levels (SOD, CAT and GSH-Px) are significantly decreased in STZ induced diabetic animals with high degree of lipid peroxidation. The enzyme levels increased significantly on treatment with AEIS

Keywords: STZ induced diabetic animals, antihyperglycaemic, antioxidant potential

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INTRODUCTION

In drug discovery and development, medicinal herbs have consistently been considering the leading source of pharmaceuticals employed in the treatment of various human diseases due to their high chemical diversity and broad biological functionality. There is a worldwide 'green revolution' and is reflected in the belief that herbal remedies are safe and less damaging to the human body than synthetic drugs. Hence there is an increasing interest in herbal remedies. According to WHO herbal medicines are defined as finished, labeled medicinal products that contain active ingredients, aerial or underground part of plants or other plant material or their combinations. The annual

herbal sales have skyrocketed and the global traditional market is growing at a rate of 7-15% annually. In the present scenario, herbal drugs are claimed for almost every disorder ranging from diabetes to rejuvenators. In recent years, there has been renewed interest in plant medicine for the treatment against different diseases as herbal drugs are out of toxic effect reported from research work conducted on experimental model animal. The use of medicinal plants for the treatment of diabetes mellitus dates back from the Ebers papyrus of about 1550 B.C. Ethno botanical knowledge played a particular important role in historical diabetes therapies, with over 1200 species of medicinal plants recognized throughout the world for their ability to treat diabetic indications and received scientific scrutiny, for which World Health Organization (WHO) has also recommended attention. The medicinal plants might provide a useful source of new oral hypoglycaemic compounds for development of pharmaceutical entities or as a dietary adjunct to existing therapies (1-4). Diabetes mellitus (DM) is the most common endocrine disorder. It affects more than 100 million person's worldwide and its incidence is increasing steadily with changes in life styles. It is not a single disease entity, but rather a group of metabolic disorders sharing the common underlying feature of hyperglycaemia. Hyperglycaemia result from an absolute deficiency of insulin caused by pancreatic β-cell destruction or by a combination of peripheral resistance to insulin action and an inadequate secretary response by the pancreatic β-cells. Diabetes mellitus causes disturbances in carbohydrate, protein and lipid metabolism damaging multi organ systems, especially the kidneys, eyes, nerves, and blood vessels which lead to complications such as diabetic nephropathy, retinopathy, neuropathy and microangiopathy. It has been postulated that the etiology of the complication of diabetes involves oxidative stress perhaps as a result of hyperglycemia. Approximately 50% of diabetic cases can be adequately controlled by diet alone, 20-30% will need an oral anti diabetic medication and 20-30% will require insulin. Insulin has proved to be effective to some extent in increasing the life expectancy of diabetic patients, but not a permanent solution since there are many draw backs of this therapy. Also the therapy with oral hypoglycemic agents is not satisfactory. Thus, the search for a new therapeutical agent devoid of adverse effects originating from plants used in traditional medicines would be of interest. Recent years have witnessed a renewed interest in plants as pharmaceuticals because they synthesize a variety of secondary metabolites with antioxidant potential which can play a major role in protection against molecular damage induced by reactive oxygen species (ROS). Several anti-inflammatory, digestive, antinecrotic, antidiabetic, neuroprotective and hepatoprotective drugs have recently been shown to have antioxidant or radical scavenging mechanism as part of their activity. Keeping this in view, one such plant *Indigofera suffructicosa* Mill which possesses good antioxidant property was chosen for study and evaluated for its antidiabetic and antioxidant potential (5-8).

MATERIALS AND METHODS

Collection and authentication of plant material

The leaf of *Indigofera suffructicosa* Mill was purchased from abhirami botanicals, tuticorin, Tamilnadu.

Experimental animals

Inbred adult wistar albino rats (150-280 g) of either sex were selected and the animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. Standard pellet fed and tap water was provided *ad libitum* through out experimentation period. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. Fasting refers to that the animals were deprived of food for 16 hours but were allowed to free access for water.

Preparation of plant extracts

The leaves were chopped to small pieces and dried in shade. The dried leaves were powdered and a weighed quantity of the powder (890 g) was passed through sieve number 20 and subjected to hot solvent extraction in a soxhlet apparatus using water, at a temperature range of 60-70˚c. Before and after every extraction the marc was completely dried and weighed. The extract was concentrated to dryness at 40˚c under reduced pressure in a rotary vacuum evaporator. The aqueous extract yielded brown semi-solid residues, weighing 7.0g (7.0%) and the extract was preserved in a refrigerator till further usage.

Toxicological evaluation

Acute oral toxicity study (9)

The procedure was followed by using OECD guide lines (Organization of Economic Cooperation and Development) 423(Acute Toxic Class Method).The acute toxic class method is a step wise procedure with 3male animals per step. Depending up on the mortality and/or moribund status of animals, on the average 2-3 steps may be necessary to allow judgment on the acute toxicity of the test substance.This procedure results in the use of minimal number of animals while allowing for acceptable data based scientific conclusion. The method uses defined doses (5, 50, 300,2000mg/kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemicals which cause acute toxicity.

Experimental procedure

Wistar rats of male sex weighing 120-180g were used for the study. The starting dose level of AEIS was 2000mg/kg body weight p.o. As most of crude extracts possess LD_{50} value more than 2000mg/kg p.o. Dose volume was administered 0.5ml/100mg body weight to over night fasted rats with 0.5%w/v SCMC. Food was with held for further 3-4 hours after administration of AEIS and observed for signs of toxicity. Body weight of rats before and after termination were noted and any changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous system and somatomotor activity and behavior pattern were observed, and also signs of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity also noted.

Induction of diabetes mellitus in experimental animals (10-12)

Adult inbred wistar albino rats (32 numbers) of either sex were over night fasted and received a freshly prepared solution of streptozotocin (STZ), [Sigma Chemical Co, St Louis, MO, USA], (45 mg/kg) in 0.1 M sodium citrate buffer, PH 4.5, injected intraperitonially in a volume of 1 ml/kg .After injection the animals had free access to food and water and were given 5% glucose in their drinking water for the first 24 hours to counter any initial hypoglycemia. Normal rats (6 numbers) received 1ml citrate buffer as vehicle. The development of diabetes was confirmed after 48 hours of the streptozotocin injection. The animals with fasting blood glucose level more than 200 mg/dl were selected for the experimentation. Out of 32 animals subjected for diabetes induction, 6 animals died before grouping and two animals were omitted from the study, because of sub diabetic condition (118mg/dl) and (122mg/dl). Of the remaining 24 animals,4 groups of 6 animals were formed and used for the experimentation. In the present study, glibenclamide (0.4 mg/kg body weight) was used as the standard drug.

Determination of the blood glucose levels

Blood was collected from tip of the tail vein and fasting blood glucose level was measured using single touch glucometer (Ascensia ENTRUST, Bayer) based on glucose oxidase method.

Estimation of antioxidant enzyme levels

Preparation of liver homogenate (13)

Liver was removed, homogenized with buffer contaning (0.25 M sucrose and 0.1 M Tris-Hcl buffer, pH 7.4) to prepare 10% homogenate by using in Teflon pestle and glass homogenizer and centrifuged at 600 x g for 10 min. To obtain post mitochondrial supernatant (PMT), this was used to analyse the protein concentration by biuret's method. The post mitochondrial supernatant was again centrifuged at 8000 x g for 15 min. This supernatant was used to analyze the antioxidant enzyme level in the liver.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of aqueous leaf extract of *Indigofera suffructicosa* Mill.

Aqueous extract showed the presence of various phytochemical constituents like alkaloids, flavanoids, steroids, proteins, carbohydrates and indigo. However the extract showed negative results for anthocyanins, saponins, gums and mucilage.

Acute oral toxicity study

The acute oral toxicity study was done according to OECD 423 guide lines (Acute toxicity class method). A single administration of a starting dose of 2000 mg/kg bw/p.o, of was administered to 3 male rats and observed for 14

days. There was no considerable change in body weight before and after treatment of the experiment and no signs of toxicity were observed (Table-1).

Table-1 Acute oral toxicity studies (OECD 423 guideline)

S. No.	Treatment group	Dose	Weight of animal in gms		Signs of toxicity	Onset of toxicity	Reversible or irreversible	Duration
			Before test	After test				
1.	AEIS	2g/kg	160	170	No signs of toxicity	Nil	Nil	14 days
2.	AEIS	2g/kg	164	175	No signs of toxicity	Nil	Nil	14 days
3.	AEIS	2g/kg	165	180	No signs of toxicity	Nil	Nil	14 days

Effect of AEIS on blood glucose levels in normoglycaemic and glucose induced hyperglycaemic rats [NG-OGTT]

The AEIS at a dose level 100mg/kg b.w/p.o did not exhibit significant hypoglycemic effect in fasted normal rats after 30 minutes of administration and a high dose of 200mg/kg b.w/p.o reduced blood glucose in normal rats significantly after 60 min of drug administration ($p<0.01$). In the same group of rats which are loaded with glucose (2gm/kg b.w/p.o) after 60 min of drug administration a low dose of 100mg/kg bw reduced blood glucose level with less significance ($p<0.05$) but a high dose of 200mg/kg/b.w reduced blood glucose significantly ($p<0.01$) .The standard drug glibenclamide (0.4 mg/kg b.w/p.o) treatment showed significant reduction in blood glucose levels in both normal and glucose induced hyperglycaemic rats ($p<0.01$). Results are shown in Figure-1.

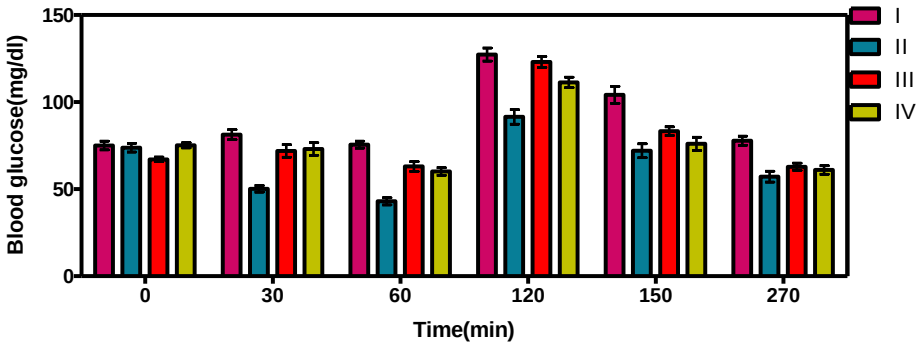

Fig-1 Effect of AEIS on blood glucose in STZ induced diabetic rats [NG-OGTT]

Effect of single dose treatment of AEIS on blood glucose level in STZ induced diabetic rats

The effect of AEIS was evaluated at a single dose administration of 100mg/kg and 200mg/kg orally at the 2nd, 4th and 6th hour. Two concentrations of the extract (100mg and 200mg/kg b.w/p.o) did not produce significant reduction in the blood glucose levels in STZ induced diabetic rats. Only at the 6th hour of administration dose level of 200mg/kg and of standard (glibenclamde 0.4mg/kg orally) shows slight significant in blood glucose levels of STZ induced diabetic rats.($p<0.01$) .Results are shown in Figure-2.

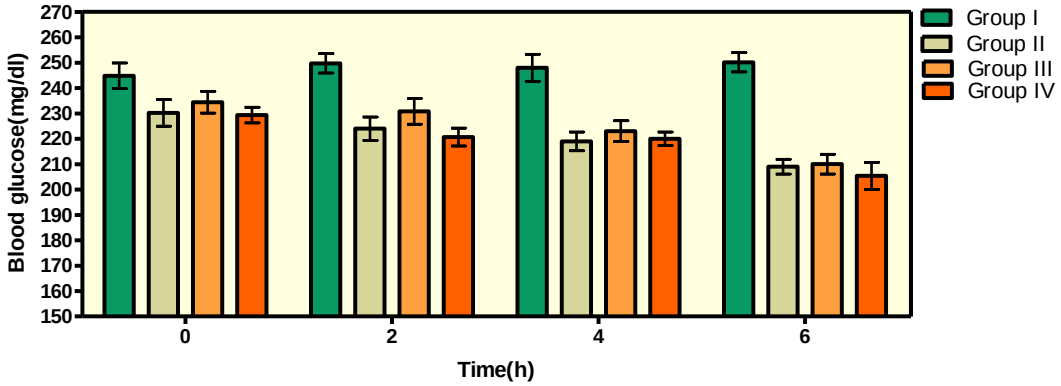

Fig-2 Effect of single dose treatment of AEIS on blood glucose in STZ induced diabetic rats

Superoxide dismutase (SOD)

A significant ($p<0.01$) decrease in the liver SOD was observed in STZ induced diabetic animals when compared to control animals. The liver SOD levels of diabetic animals treated with AEIS (100mg and 200mg/kg b.wt/p.o) and glibenclamide (0.4 mg/kg b.w/p.o) showed significant ($p<0.01$) increase when compared to STZ induced diabetic animals. Results are shown in Figure-3.

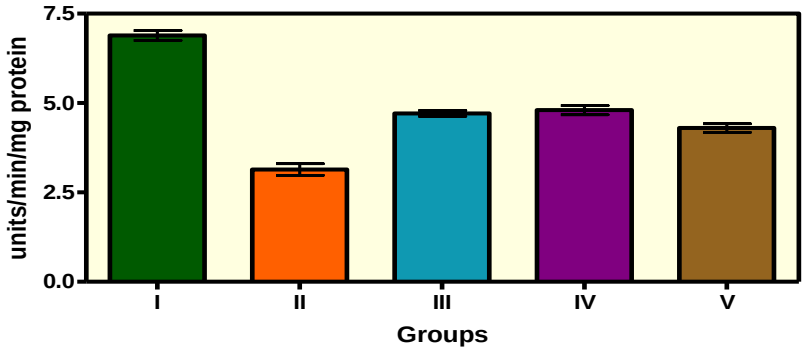

Fig-3 Effect of AEIS on SOD in STZ induced diabetic rats

Catalase(CAT)

A significant ($p<0.01$) decrease in the liver CAT was observed in STZ induced diabetic animals when compared to control animals. The liver CAT levels of diabetic animals treated with AEIS (100mg and 200mg/kg b.wt/p.o) and glibenclamide (0.4 mg/kg b.w/p.o) showed significant ($p<0.01$) increase when compared to STZ induced diabetic animals. Results are shown in Table-2.

Table-2 Effect of AEIS on catalase in STZ induced diabetic rats

Group	Treatment	Dose (Kg^{-1} Body Weight)	CAT Liver tissue
I	Control (0.5% SCMC)	5 ml	3.656± 0.247
II	Disease control (STZ)	45mg	$2.352 \pm 0.046^{a**}$
III	Standard (Glibenclamide+STZ)	0.4mg	$3.653 \pm 0.132^{b**}$
IV	Test I (AEIS+STZ)	100mg	$2.475 \pm 0.162^{b*}$
V	Test II (AEIS+STZ)	200mg	$3.612 \pm 0.176^{b**}$

The values are expressed as mean ± SEM. Statistical significance test was done by ANOVA followed by Dunnet's test. a-Group II is compared with Group I ; b-Group III, IV and V are compared with Group II . ** -p<0.01;*-p<0.05.

Glutathione peroxidase(GSH-Px)

A significant (p<0.01) decrease in the liver GSH-Px level was observed in STZ induced diabetic animals when compared to control animals. The liver GSH-Px levels of diabetic animals treated with AEIS (100mg and 200mg/kg b.wt/p.o) and glibenclamide (0.4 mg/kg b.w/p.o) showed significant (p<0.01) increase when compared to STZ induced diabetic animals. Results are shown in **Table-3**.

Table –3 Effect of AEIS on GP_X in STZ induced diabetic rats

Group	Treatment	Dose (Kg^{-1} Body Weight)	GSH-P_X Liver tissue
I	Control (0.5% SCMC)	5 ml	17.14 ± 0.04
II	Disease control (STZ)	45mg	8.54 ± 0.02^{a}**
III	Standard (Glibenclamide+STZ)	0.4mg	15.16 ± 0.14^{b}**
IV	Test I (AEIS+STZ)	100mg	13.56 ± 0.12^{b}**
V	Test II (AEIS+STZ)	200mg	17.15 ± 0.13^{b}**

The values are expressed as mean ± SEM. Statistical significance test was done by ANOVA followed by Dunnet's test. a-Group II is compared with Group I ; b-Group III, IV and V are compared with Group II. ** -p<0.01.

Lipid peroxidation (LPO)

A significant (p<0.01) increase in the liver LPO was observed in STZ induced diabetic animals when compared to control animals. LPO level of diabetic animals treated with AEIS (100mg and 200mg/kg b.wt/p.o) and glibenclamide (0.4 mg/kg b.w/p.o) showed significant (p<0.01) decrease when compared to STZ induced diabetic animals. Results are shown in **Figure-4.**

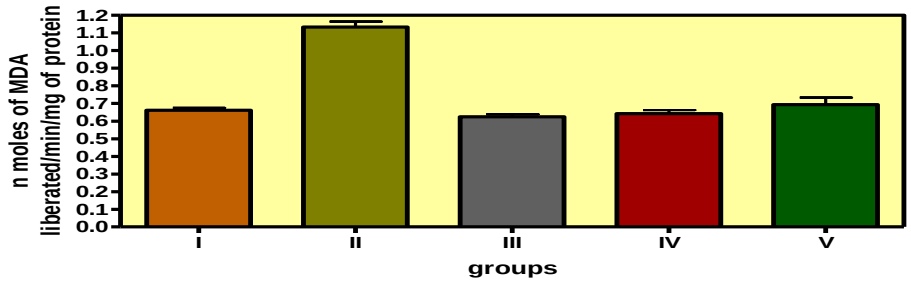

Fig-4 Effect of AEIS on LPO in STZ induced diabetic rats

Plants have been used as source of drugs for the treatment of diabetes mellitus in developing countries where the cost of conventional medicines represents a burden to the population. Many species have been reported to present antidiabetic activity.[50] Working on the same line, we have undertaken a study on *Indigofera suffructicosa* Mill *for* its antidiabetic property along with its anti oxidant potential. Preliminary phytochemical analysis of the AEIS showed that the plant has a rich possession of phytochemicals like alkaloids, reducing sugars, tannins and phenols. Terpenoids, steroids, gums and mucilage were absent in the extracts. Acute oral toxicity studies reveal that AEIS did not produce any mortality or signs of toxicity at the dose of 2000 mg/kg b.w. p.o, in experimental rats. The AEIS at doses 100 and 200 mg/kg bw.po.did not significantly suppress blood glucose levels in overnight fasted normoglycaemic animals but showed significant improvement in glucose tolerance in glucose fed hyperglycaemic normal rats. Such an effect may be accounted for, in part, by a decrease in rate of intestinal glucose absorption, achieved by an extra pancreatic action including stimulation of peripheral glucose utilization or enhancing glycolytic and glycogenic process. Streptozotocin is the most commonly employed agent for the induction of experimental diabetic animal models of human insulin –dependent diabetes mellitus. There is an increasing evidence that streptozotocin causes diabetes by rapid depletion of β-cells, by DNA alkylation and accumulation of cytotoxic free

radicals that is suggested to result from initial islet inflammation, followed by infiltration of activated macrophages and lymphocyte in the inflammatory focus (14, 15). A single dose of two concentrations of aqueous extract did not bring about significant hypoglycaemic action. In the sub-acute study, glebenclamide treatment brought down the sugar levels from the first day of the treatment. AEIS 100 mg and 200mg treatment produces significant reduction in blood glucose levels from 10^{th} hr of treatment and a steady decrease was observed thereafter. Another possibility for the activity may be due to presence of phytochemicals like flavanoids, phenolics and alkaloids etc.[53] Histopathological studies that showed prominent islets cell hyperplasia and regeneration of islet cell show a proof for the possible antidiabetic property of the of *Indigofera suffructicosa* Mill. Increased non-enzymatic and autooxidative glycosylation is one of the possible mechanisms linking hyperglycaemia and vascular complications of diabetes.In the present study diabetic rats had shown higher levels of HbA_1 compared to those in normal rats indicating their poor glycaemic control. Treatment with AEIS, showed a significant decrease in HbA_1 levels in diabetic rats. This property provides a practical and objective means of assessing average blood glucose levels over a time frame of about 2 months and has proven to be a very useful adjunct to SMBG. The enzymatic antioxidant defense mechanism contains various forms of superoxide dismutases, catalase, and Glutathione peroxidases. A marked decrease in all these was observed in STZ diabetic rats. Glibenclamide, AEIS 100mg and AEIS 200mg showed a marked increase of these enzymes.

CONCLUSION

Based on the obtained results and observations, we can infer that the leaf of the plant *Indigofera suffructicosa* Mill under study could be used for the supportive treatment of diabetes mellitus, as the plant also offers effective protection against free radicals that form the basis for the development of diabetic complications. Further studies are required to establish the anti hyperglycaemic activity of *Indigofera suffructicosa* Mill in terms of molecular mechanism(s) involved in the activity.

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