RESEARCH ARTICLE

Exploring Antidiabetic Potential of *Gymnema sylvestre*

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Abstract

Context: *Gymnema sylvestre* (GS) is a plant species that is mostly found in the tropical regions ofAsia, Africa and Australia; it is widespread in India and Sri Lanka. According to various scientificstudies, GS contains a chemical constituent that is known to suppress the taste for sugar; this kindled our interest to study the various extracts of GS for their antidiabetic potential.

Aim: The current study emphasizes screening the leaves extracts of *Gymnema sylvestre* using chloroform, ethyl acetate, and water for their phytoconstituents and evaluating their Antidiabetic property. All the individual concentrates wereexposed to subjective qualitative examinations for the distinguishing proof of the phytoconstituents, which was followed by evaluating the effect of different extracts of GS on bloodglucose level against Streptozotocin induced Diabetic rats as GS had been reported for its antidiabetic activity.

Material and Methods: The plant material *Gymnema sylvestre* 1.5 kg was macerated with 3.75 liter of chloroform, ethyl acetate and Wwater for comparative antidiabetic activity. A total of 36 male wistar rats were utilized to induce diabetes by means of intraperitoneal injection with 60 mg/kg STZ. The acute toxicity study adhered to the guidelines set by the Organization for Economic Co-operation and Development (OECD 423, 2001). Throughout a period of 21 days, the animals received various treatments. Glucose levels were measured on day 1, which marked the initiation of any treatment, as well as on the 7th, 14th, and 21st days, utilizing a one-touch glucometer. Hematoxylin and eosin staining were employed for histopathological examination of the pancreas. Statistical analysis was conducted using SPSS software, employing one-way ANOVA followed by Dunnet's multiple comparisons.

Results: Blood glucose levels in streptozotocin induced diabetic rats fed with different GS extractsdecreased to normal levels. When the reduced percentage of blood glucose levels of the various extracts was compared with that of glibenclamide (62%), aqueous extract showed maximal (59%) decline, thus confirming the potentiality of the GS as the most significant antidiabetic agent.

Keywords: *Gymnema sylvestre*, Anti-diabetic, Streptozotocin, Phytochemical

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Introduction

Gymnema sylvestre is considered a therapeutic plant and belongs to the family Asclepiadaceae, and is local to the tropical woodlands of south and local India. It has been utilized as a naturopathic treatment for Diabetes for almost two centuries and it is spread over most parts of India and Africa. It is very much considered in conventional drug as a solution for Diabetes, stomachic and anti-diarrheal agent. The plant is prevalently known as 'Gurmar' for its particular property of incidentally annihilating the flavor of sweetness. A magical constituent namely, gymnemic acids were acquired from Gymnema leaves.[1] The herb has been found to assist in frequent urination and diminish hyperglycemia in both animals and human investigations. The primary bioactive constituents of *G. sylvestre* are triterpenoid glycosides called gymnemic acids, which have been accounted for as antidiabetic and anti-cholesterol agents in the *in-vitro*-studies.[2]

Diabetes mellitus (DM) is a chronic metabolic disorder, resulting in insulin deficiency- characterized by hyperglycemia. The metabolic abnormalities lead to symptoms of Polyurea (frequent urination), Polydypsis (excessive thrust), polyphagia (excessive hunger), Long term untreated DM might lead to gangrene, retinopathy, myocardial infarction & sometimes increase blood pressure.[3]

DM is of two types Diabetes Mellitus Type-1 and Diabetes Mellitus Type-2. Oral Hypoglycemic agents are used in the treatment of patient who has type-2 Diabetes & cannot be managed by diet alone.[4] Patient who has developed Diabetes after the age of 40 & have Diabetes less than 5 years respond well to the oral hypoglycemic agent, while those with long standing disease may require a combination of oral hypoglycemic agent with or without insulin to control their hyperglycemia. Oral hypoglycemic agent should not be given to patients with Type1 Diabetes. As GS contains an array of chemical constituents that can be used as potential antidiabetic agents, proper study and investigation of all its extracts and phyto constituent is essential.[5]

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Materials And Methods

Materials

Glibenclamide, chloroform, ethyl acetate, distilled water, hexane, methanol, silica gel g, silica gel mesh 60-120, Sodium hydroxide pellets, citric acid, sodium citrate, acetone, ethanol, formaldehyde. All the chemicals obtained were of analytical grade.

Methods

Collection and Authentication of Plant Material

Gymnema sylvestre plant was collected in August 2018 from M/S Global Herbs, New Delhi. The formal authentication was done by Dr. Sunita Garg, the Emeritus Scientist, CSIR-NISCAIR, Delhi and voucher specimen number was NISCAIR/RHMD/Consult/2018/3302-03. The leaves were allowed to air dry. The dried material was coarsely powdered and stored in brown bottles.

Animals

Male wistar albino rats (150-200 gm; 8-11 weeks old) were obtained from AIIMS New Delhi (India). The animals were housed in the Animal house of I.T.S College of Pharmacy, Ghaziabad, India in polycarbonate cages, in a room maintained under controlled room temperature 22 ± 2°C, relative humidity 60-70% and given food and water ad libitum. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee (Register Number: 1044/PO/Re/S/07/CPCSEA) and the care of laboratory animals was taken as per the guidance of CPCSEA, Ministry of Forests and Environment, Government of India. Before the experimentation, the animals were deprived of food for 24 hours but were allowed free access to water throughout. The studies were carried out by using five animals in one group for Antidiabetic activity.

Extraction Procedure

GS leaves were dried in shade and powdered. Cold maceration technique was employed for extraction. In cold maceration powdered plant drug was kept in contact with the solvent in a closed container for 72 hr. It was agitated frequently to dissolve the soluble matter. Powdered GS leaves were extracted with three solvents of increasing polarity in order of chloroform (Extract-1), Ethyl acetate (Extract-2) and Water (Extract-3), respectively at room temperature twice.[6]

The extracts thus obtained were concentrated by distilling off the solvent under reduced pressure by using Rota vacuum Evaporator (Buchi, Germany).[7] The marc thus obtained was air-dried and then the percentage yield of all the three extracts of Chloroform, Ethyl acetate and Aqueous of *Gymnema sylvestre* was calculated.

All the extracts were subjected to Phytochemical screening[8] and Antidiabetic evaluation. Thin Layer Chromatography carried out the separation and identification of the components present in various extracts of GS. Solvent systems for the extracts were:

Extract-1: Hexane: Ethyl acetate (1:15)
Extract-2: Hexane: Ethyl acetate (1:10)
Extract-3: Chloroform: Methanol (6:5)

Acute Toxicity study

The acute toxicity was done following OECD guidelines (OECD 423, 2001). Extracts were administered orally to rats at the graded doses of 1000, 2000 & 4000 mg/kg body weight. Then the animals were monitored continuously for the first four hours to observe any behavioral changes and mortality and then daily up to 14 days. The mortality rate was zero even after 14 days. This indicated that the extracts were safe up to a single 4000 mg/kg body weight dose. Hence the selected doses for administration in experimental animals were considered 1/10th of maximum safe dose.

Antidiabetic activity (STZ induced Diabetes model)
Induction of Diabetes in animals

Streptozotocin (STZ) was freshly prepared using 10 mM/ citrate buffer, pH 4.5. The male Wistar rats fed a standard diet weighing about 150–220 gm were induced with diabetes by injecting with 60 mg/kg STZ intraperitoneally. In the initial three hours the blood glucose level was found to be increased, reaching up to 150-220 mg, after 6-8 hours of STZ injection the serum insulin values were increased up to 4 times, resulting in a hypoglycemic phase which was followed by persistent hyperglycemia. After 72 hours of STZ administration, the blood glucose levels were measured and the rats showing blood glucose level > 250mg/dl were considered to be diabetics and were used in the study. The animal groups and their dosage schedule are mentioned in Table 1.

Animals were treated for 21 days. Glucose levels were measured on day 1 i.e., just initiation of any treatment on day 7th, 14th and 21st using one touch glucometer.

Histopathological Examination

Under Anesthesia pancreas was carefully removed from rats and immediately placed in formalin solution. According to Lillie's method, tissues were prepared histologically using hematoxylin and eosin recoloring.

Statistical Analysis

The statistical analysis of all the results was carried out using one-way ANOVA followed by Dunnet's multiple comparisons using SPSS software (version 20). The significance level was determined in comparison with the control group.

Table 1: Animal groups and their Dosage schedule

S. No.	*Groups*	*Treatments*	*Dosage schedule*
1	I	Normal saline	0.5 ml/kg
2	II	Normal saline + STZ	0.5 ml/kg + 60 mg/kg
3	III	Extract-1 + STZ	400 mg/kg
4	IV	Extract-2 + STZ	400 mg/kg
5	V	Extract-3 + STZ	400 mg/kg
6	VI	Glibenclamide + STZ	5 mg/kg

RESULTS

Extraction

Gymnema sylvestre was serially extracted with Chloroform, Ethyl acetate and Water solvents. The percentage yield of all the extracts is given in Table 2.

Phytochemical analysis

Preliminary phytochemical qualitative analysis of various extracts of *Gymnema sylvestre* indicated the presence of alkaloids, terpenoids, saponins, flavonoids, tannins and phenolic compounds in the extracts (Table 3).

Using Thin layer chromatographic technique (Table 4) and other spectral analytical methods, flavanol, terpenoid and triterpene, saponin were identified from the extracts of chloroform, ethyl acetate and water, respectively.

Antidiabetic Activity

The effects of different extracts of GS on blood glucose levels against STZ induced Diabetic rats as tabulated under

Table 2: Percentage yield of the extracts of *Gymnema sylvestre*

S. No.	*Gymnema sylvestre extracts*	*Amount of extract (in gms)*	*Percentage yield*
1.	Extract-1	7g	1.4%
2.	Extract-2	4g	0.8%
3.	Extract-3	12g	2.4%

Table 3: Phytochemical screening of the extracts of *Gymnema sylvestre*

S. No	*Phytoconstituents Present*	*Extract-1*	*Extract-2*	*Extract-3*
1	Glycosides	(-)	(-)	(+)
2	Flavonoids	(+)	(-)	(-)
3	Alkaloids	(-)	(-)	(-)
4	Steroids	(-)	(+)	(-)
5	Terpenoids	(-)	(+)	(-)
6	Saponins	(-)	(-)	(+)

Table 4: Rf value of extracts of *Gymnema sylvestre*

No. of Extracts	*Extracts of Gymnema sylvestre*	*Rf value*
1.	Chloroform extract	0.8
2.	Ethyl acetate extract	0.7
3.	Aqueous extract	0.66

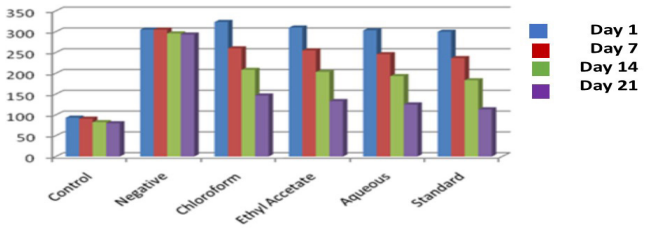

Figure 1: Histography represents the effect of different extracts of *Gymnema sylvestre* on blood glucose level against STZ induced Diabetes.

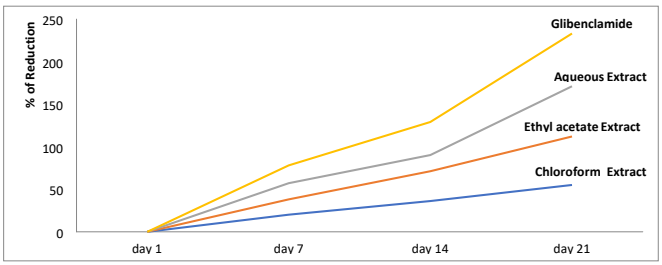

Figure 2: Percentage reduction of blood sugar in comparison with standard drug.

Table 5, shows a significant reduction of blood glucose in the treatment group when compared with the standard group. All values were expressed as mean ± SD (n=5). *p<0.001 when compared to control group, #p<0.001 when compared to standard (One way ANOVA followed by Dunnett's Test). Histogram representing the effect of different extracts of *Gymnema sylvestre* on blood glucose level against STZ induced Diabetes is presented in Figure 1. Percentage reduction of blood sugar in comparison with that of Glibenclamide (62%) is presented in Figure 2. Extract 3 showed 59% reduction,

Table 5: Effect of different extracts of *Gymnema sylvestre* on blood glucose level against STZ induced diabetic rat

Group	*Treatment (mg/kg b.w.)*	*Blood glucose (mg/dl)*			
		Day 1	*Day 7*	*Day 14*	*Day 21*
I	Normal saline (0.5ml/kg)	93.33±5.1	90.83±4.9	82.50±4.1	80.00±6.3
II	Normal saline+ STZ (0.5ml/kg + 60mg/kg)	340.0 ±15.1	341.0±16.4	341.8±12.0	341.9±12.1
III	Chloroform extract + STZ (400mg/kg)	323.3 ±17.5	260.0±8.9#	208.3±4.0#	146.6±5.1#
IV	Ethyl acetate extract + STZ (400mg/kg)	310.0 ±17.8	255.0±10.4#	203.3±8.1#	133.3±5.1#
V	Aqueous extract + STZ (400mg/kg)	303.0 ±17.5	245.8±10.2*	193.3±8.1*	125.0±5.4*
VI	Glibenclamide + STZ (0.5mg/kg)	300.0±26.0	236.6±10.3*	183.3±8.1*	113.3±8.1*

All values were expressed as mean ± SD (n=5)
*p<0.001 when compared to control group
#p<0.001 when compared to standard (one way ANOVA followed by Dunnett's Test)

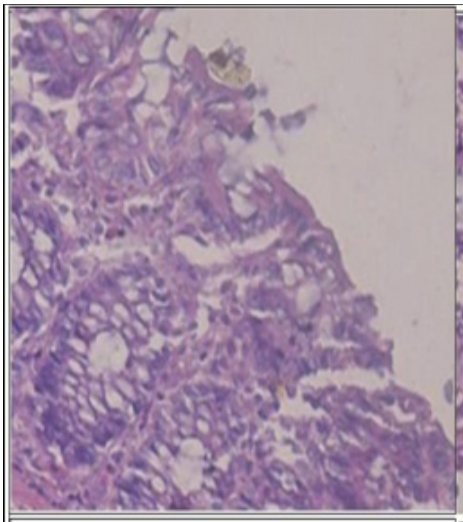

Figure 3. Histopathological examination of the rat pancreas (Control Group)

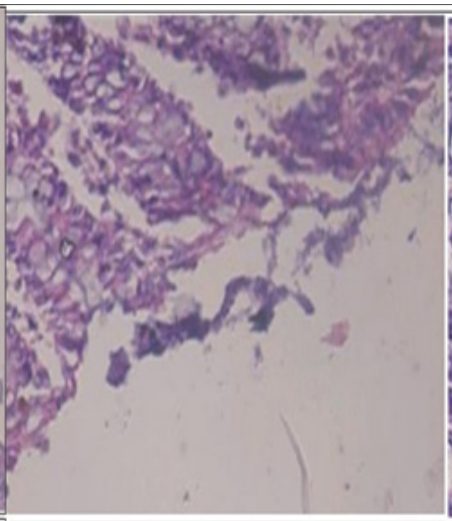

Figure 4. Histopathological examination of the rat pancreas (Negative Group)

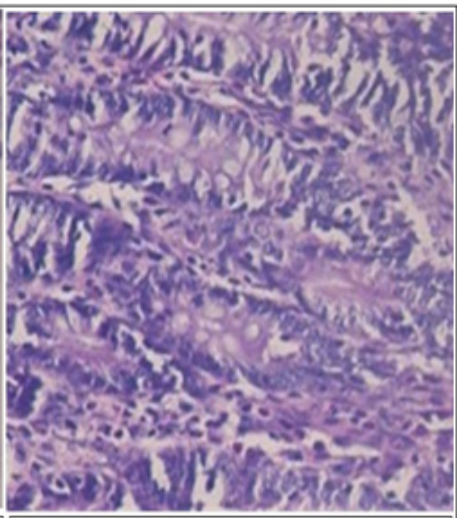

Figure 5. Histopathological examination of the rat pancreas (Extract-1) of GS Group

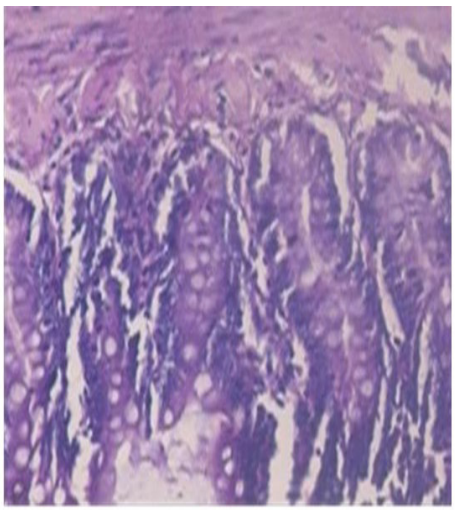

Figure 6. Histopathological examination of the rat pancreas (Extract-2) of GS Group

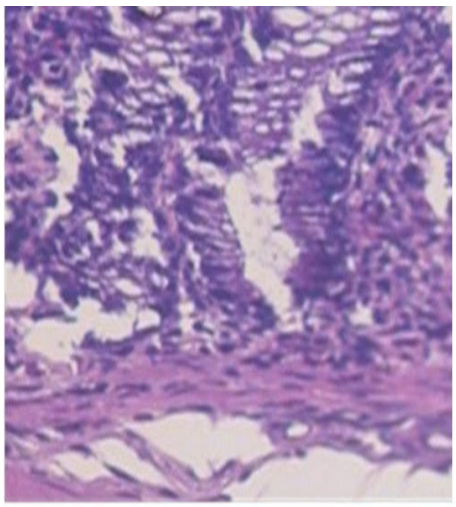

Figure 7. Histopathological examination of the rat pancreas (Extract-2) of GS Group

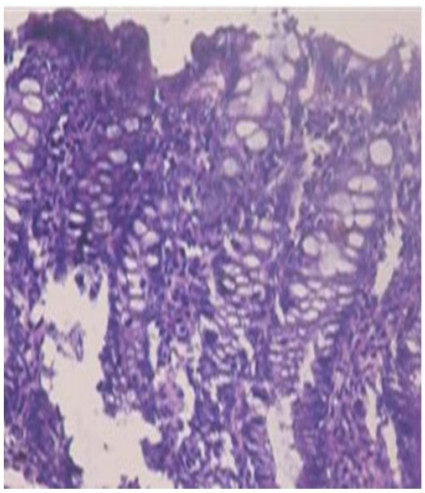

Figure 8. Histopathological examination of the rat pancreas (Standard Group)

Table 6: Percentage of reduction of blood sugar in comparison with standard drug

Group	*Day 1*	*Day 7*	*Day 14*	*Day 21*
Chloroform	0	20	36	55
Ethyl acetate	0	18	35	57
Aqueous	0	19	19	59
Glibenclamide (Standard)	0	21	39	62

Extract 2 and Extract 1 possessed 57% and 55% reduction respectively. Pancreatic histopathology of GS extracts indicated typical islet with sufficient β cell histomorphology. Relatively, in diabetic group there were atrophic and central necrotic changes seen as shown in Figures 3, 4, 5, 6, 7, and 8.

Discussion

Diabetes mellitus (DM) cause for worldwide fitness worry as the ailment is quickly advancing; likewise, the period

of beginning to more youthful age factions is alarming. The standard medication treatment has different adverse reactions and thus there is a requirement for improvement of new medications with a safer profile.[9, 10] Numerous herbal medications have been utilized since time immemorial for the treatment of DM. GS leaves contain certain phyto constituents which can annihilate the sense of taste for sugar for a brief period.[11,12] The experimentation involving rat models which are induced with diabetes by using streptozotocin are the most prevalent ones. It has been generally expressed that oxidative stress created because of free radicals[13, 14] is engaged with the advancement of diabetes by pancreatic cell annihilation.[15, 16]

Assessed the methanolic leaf extract of GS on glucose transport and insulin resistance *in vitro*. The result showed that the extract enhanced in L6 myotubes, ameliorating the insulin resistance in 3T3 murine adipocyte cell line *in-vitro*. The above and other scientific evidence thus kindled our interest in further foraying the topic further.[17, 18] All the extracts of *Gymnema sylvestre* were assessed for their Antidiabetic activity (STZ induced Diabetes) at $p<0.001$ and they inhibited the blood glucose concentration. Table 6 elaborates a comparative data elucidating the reduced percentage in the blood glucose levels of the three groups on day 1, day 14 and day 21, compared with that of the group being treated with the standard drug Glibenclamide. It was found that the aqueous extract showed maximum Antidiabetic activity with 59% on day 21 in rats followed by ethyl acetate (57%) and chloroform extracts (55%). This maximal activity could be attributed due to the presence of triterpene saponin in the aqueous extract.

Conclusions

The presence of lead triterpene saponin, Gymnemic acid, in the aqueous extract identified through phytochemical investigation. By establishing a correlation between phytoconstituent, such as Gymnemic acid, and it's antidiabetic activity in Gymnema, this study further strengthens the understanding of the mechanisms underlying the observed effects. Hence, Gymnemic acid is believed to be primarily responsible for the antidiabetic activity observed in Gymnema.

This research provides a strong foundation and serves as a stepping stone for formulating a potent antidiabetic drug that could potentially aid mankind in combating diabetes, which has become a common lifestyle disorder. However, further research and clinical trials are needed to fully explore and validate their efficacy, safety, and potential as a treatment for diabetes.

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