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An Assessment of Anti-diabetic Effect of *Gymnema sylvestre* in Alloxan-induced Rat Model

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Since the inception of human civilization, mankind has used herbal medicine for the purpose of healing. This study aimed to examine the antidiabetic efficacy and lipid profile of Gymnema sylvestre. We assessed the antidiabetic activity using the alloxan-induced diabetic technique. Regarding its antidiabetic effect, only the dosage of 900 mg/kg demonstrated statistically significant results ($p<0.05$). Regarding total cholesterol, High density Lipoprotein (HDL) and Low density Lipoprotein (LDL), the HDL level exhibited statistically significant findings in groups 3, 5, and 6. For triglyceride, SGOT (Serum glutamic oxaloacetic transaminase) and SGPT (Serum glutamate

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pyruvate transaminase), groups 4, 5, and 6 at doses of 300, 600, and 900 mg/kg, respectively, exhibited statistically significant results ($p<0.05$). The kidney function test revealed statistically significant outcomes ($p<0.05$) in groups 4, 5, and 6, which were given doses of 300, 600, and 900 mg/kg, respectively. The results suggest that *Gymnema sylvestre* has promise in developing standardized phytomedicine for treating individuals with diabetes, cardiovascular disease, liver disease, and renal sickness.

Keywords: Gymnema sylvestre; HDL; LDL; herbal medicine; triglyceride.

1. INTRODUCTION

Diabetes is a medical condition characterized by elevated levels of blood glucose, also known as blood sugar. In this scenario, the body fails to produce sufficient insulin or adequately respond to insulin. Diabetes mellitus is a metabolic disorder that leads to consistently high blood sugar levels due to disruptions in the way the body processes carbohydrates, lipids, and proteins. Insulin production, insulin action, or both can cause this [1]. Insulin resistance and pancreatic beta cell failure cause type 2 diabetes [2]. In 2017, the global prevalence of type 2 diabetes was significant. Diabetes ranks as the tenth leading cause of death, resulting in more than one million fatalities each year. The global prevalence of diabetes mellitus is on the rise, with the highest rate of increase observed in Western Europe and other developed nations [3]. Several oral medications are used in the treatment of diabetes, including sulphonylureas, meglitinides, biguanides, thiazolidinedione, alpha-glucosidase, acarbose, and others [4]. Nevertheless, it is important to note that these medications can have significant side effects, such as hypoglycemia, heart failure, gastrointestinal issues, fractures, and liver damage [5]. Aside from the serious and potentially life-threatening side effects, the exorbitant cost of these synthetic medications can pose a significant barrier for patients to successfully complete their entire treatment regimen. The utilisation of plants for medicinal purposes dates back to the earliest days of human civilization. Various forms of evidence, such as documents, historical structures, and the use of original plant remedies, provide ample proof of humanity's enduring quest for medicinal substances found in nature [6]. Medicinaal plant scientists suggest that certain chemical compounds found in plants may possess therapeutic properties. Scientists continuously seek plant-based herbal medications as alternatives to treat various ailments. The medicinal plants possess a diverse array of chemical constituents, including phenols, alkaloids, terpenoids, saponins, glycosides, tannins, flavonoids, resins, polysaccharides, plant lipids, essential oils, and more. These constituents contribute to the plants' pharmacological and therapeutic effects [7]. The concentration of the plant's chemical components, whether increasing or decreasing, can potentially lead to the desired therapeutic effect. Plant genetic manipulation achieves this. Using reverse genetics, it is possible to enhance the production of secondary metabolites like alkaloids [8].

Gymnema Sylvestre R. Br. is an herb that is highly regarded in the academic community. It belongs to the esteemed family Asclepiadaceae and has a wide distribution across various countries, including India, Malaysia, Sri Lanka, Australia, Indonesia, Japan, Vietnam, tropical Africa, and the southwestern region of the People’s Republic of China. The plant is known by various names in different languages, such as Periploca of the Woods in English, Gurmar in Hindi, Meshashringi and Madhunashini in Sanskrit, Kavali and Kalikardori in Marathi, Dhuleti and Mardashingi in Gujrathi, Adigam and Cherukurinja in Tamil, Podapatri in Telgu, and Sannagerasehambu in Kannada [9–13]. Susruta mentions *G. sylvestre* as an effective remedy for madhumeha (glycosuria) and other urinary disorders. The extract of *G. sylvestre* is known for its various medicinal properties, including its bitter taste and ability to reduce inflammation, relieve pain, aid digestion, support liver health, induce vomiting, increase urine production, promote thermogenesis, stimulate the stomach, act as an anthelmintic, promote bowel movements, strengthen the heart, facilitate expectoration, reduce fever, and tonify the uterus. The plant has been found to have medicinal properties and can be used to treat various conditions such as jaundice, constipation, cardiopathy, asthma, bronchitis, amenorrhoea, conjunctivitis, renal and vesical calculi, dyspepsia, leucoderma, and Parkinsonism [14]. The composition of this substance includes various compounds such as

anthraquinones, tannins, phenolics, carbohydrates, flavonoid, alkaloid, saponin, resins, albumin, chlorophyll, carbohydrates, tartaric acid, formic acid, butyric acid, anthraquinone derivatives, inositole alkaloids, and organic acids [15,16]. The compound exhibits a wide range of activities, including anti-arthritic, antimicrobial, anti-inflammatory, anticancer, immunostimulatory, hepatoprotective, wound healing, antidiabetic, and antioxidant properties [17,18]. These plants contain phenolic compounds that exhibit antidiabetic properties by inhibiting the enzymes alpha-amylase and alpha-glucosidase [19]. It is believed that the total phenolic content (TPC) in these plants may play a role in their potential antidiabetic activity [20].

The present study aimed to investigate the potential anti-diabetic effects and lipid profile of an ethanolic extract from *Gymnema sylvestre.*

2. MATERIALS AND METHODS

2.1 Drugs, Chemicals, and Instruments

We obtained the ethanol and alloxan from Sigma Aldrich in Germany. Healthcare Pharmaceutical Limited provided us with a complimentary sample of metformin, a commonly used medication for diabetes. The blood serum analysis kits for various biomarkers were acquired from Plasmatic Laboratory Products Ltd. in the United Kingdom. Alere Inc. glucometer is used in this study. We acquired it from Shahbag in Dhaka, Bangladesh. We assessed the biochemical parameters using the Humalyzer 3000, a semiautomated clinical chemistry analyzer.

2.2 Plant Collection and Extract Preparation

Three distinct regions in Bangladesh were used to gather *Gymnema sylvestre* plants: North Bengal, a hill-track area, and a low-land area. Following that, the subsequent step involved authentication and taxonomic identification. Bangladesh's National Herbarium kept the plant specimen in compliance with the relevant regulations. The leaves were dried in a shaded area for seven to ten days, then finely grind them. The powdered leaves were vigorously stirred for 96 hours while being soaked in a solution of 70% ethanol. After the soaking process, the extract was filtered, and the resulting liquid was collected. We concentrated it using a rotating evaporator. We collected and stored the dried extract stored in the refrigerator for future use.

2.3 Experimental Animal Handling

50 male Wistar rats weighing 125-200 grams were obtained from the Pharmacy Department of Jahangirnagar University in Dhaka, Bangladesh. The rats were maintained in a controlled environment at the Institute of Nutrition and Food Science, University of Dhaka, with a 12-hour dark/light cycle and a constant temperature of 25°C. We regularly provided the subjects with a standard pellet meal and clean water. The rats were housed in the facility to acclimatise prior to the commencement of the study. The rat trials adhered to the guidelines set forth by the Institutional Animal Ethics Committee (IEAC). The ethical approval was taken from the Dhaka University, Department of Zoology with the issue no 147/pharm.science.ewu. The researchers cared for and managed the animals in accordance with the guidelines set by the Swiss Academy of Medical Sciences (SAMS) and the Swiss Academy of Sciences (SCNAT).

2.4 Experimental Guidelines

The tests were conducted in adherence to the ethical principles stated in the 2013 Helsinki Declaration. The study strictly adhered to the "3R" standards, which are fundamental principles in Swiss and global legislation regarding the use of animals in research. The prefix "R" represents the concept of "replacement," encompassing both absolute replacements (such as substituting animal models with computer-generated models) and relative replacements (such as substituting live animals with cell or tissue cultures or vertebrates with invertebrates). For the purpose of conducting thorough research, an animal model was utilised. Rats were chosen as test subjects due to their distinct pancreas and beta cells, which makes them suitable for antidiabetic research. This is in contrast to invertebrates, as mammals are vertebrates. The second "R" represents "reduction," which pertains to techniques that minimise the number of animals needed to gather sufficient data for research purposes or maximise the information obtained from each animal. We selected ten rats for this study based on the sample size estimate determined by the power analysis method. We employed this approach to ensure adherence to the recommended guidelines. Refinement, the third "R," involves reducing the pain and distress

experienced by experimental animals. In order to enhance the comfort of the rats during surgery and minimise any discomfort caused by pinching, the tail tips of the rats were gently rubbed with isopropyl alcohol both before and after each blood glucose level measurement. The rats were provided with sufficient nourishment throughout the trial, and they were euthanized painlessly at the conclusion, in accordance with the 2013 amendment to the Guidelines for the Euthanasia of Animals.

2.5 Experimental Design

We divided the rats into groups based on their body weight and subsequently tested them for antihyperglycemic action (Table 1). The rodents were categorized into groups according to their body weight, with 10 rats in each group. Table 1 illustrates the alloxan control group, consisting of rats that received only alloxan therapy. N/A indicates the absence of therapeutic treatment in this group.

2.6 Biological Sample Collection

To measure blood glucose levels, we obtained blood samples by puncturing the tip of the rat's tail. By way of comparison, blood was promptly collected from the slaughtered animal following a heart puncture and transferred to amicrocentrifuge tube. Centrifuging the collected samples for 5 minutes at 5,000 rpm obtained the supernatant fluid. The fluid was subsequently transferred to another microcentrifuge tube to facilitate biochemical testing. The kidneys and liver were immediately extracted from the animal's body following sacrifice and thoroughly rinsed with an ice-cold saline solution for subsequent kidney and liver function analysis. We categorized the rats into different groups according to their body weight and then conducted tests to evaluate their antihyperglycemic action (Table 1). We grouped the rodents based on their body weight, with 10 rats in each group. The alloxan control group in Table 1 solely treated rats with alloxan. This group does not receive therapeutic treatment when N/A is indicated.

2.7 Estimation of Biochemical Parameters

By using a glucometer, the blood glucose level was ascertained. The Humaluzer 3000 was one of many tests administered, along with those for the lipid profile (HDL, LDL, Cholesterol, triglyceride|), kidneys (Urea, Creatinine), and liver (SGPT and SGOT). We also tested liver and kidney samples for gluconeogenic and glycolytic enzyme activity.

Table 1. Anti-hyperglycemic activity analysis

Group number	Group Status	Treatment specimen	Dose of treatment specimen (mg/kg)	Group Abbreviation
1	Negative Control	Physiological Saline	10 mL/kg	N
2	Alloxan control	Alloxan	150 mg/kg	A
3	Alloxan + Metformin	Alloxan + Metformin	150 mg/kg +100mg	A + M100
4	Alloxan + *Gymnema sylvestre*	Alloxan + *Gymnema sylvestre* extract low dose	150 mg/kg + 500 mg/kg	A + GS_{500}
5	Alloxan + *Gymnema sylvestre*	Alloxan + *Gymnema sylvestre* extract medium dose	150 mg/kg + 1000 mg/kg	A + GS_{1000}
6	Alloxan + *Gymnema sylvestre*	Alloxan + *Gymnema sylvestre* extract high dose	150 mg/kg +1500 mg/kg	A+ GS_{1500}
7	Metformin	Metformin	100 mg/kg	M
8	*Gymnema sylvestre*	Alloxan + *Gymnema sylvestre* extract low dose	500 mg/kg	GS_{500}
9	*Gymnema sylvestre*	Alloxan + *Gymnema sylvestre* extract medium dose	1000 mg/kg	GS_{1000}
10	*Gymnema sylvestre*	Alloxan + *Gymnema sylvestre* extract high dose	1500 mg/kg	GS_{1500}

2.8 Statistical Analysis

For each group, the mean and standard deviation (SD) of each research parameter are displayed. The "one-way ANOVA test" was used to examine the statistical significance of intergroup heterogeneity by evaluating differences across groups in terms of different biological parameters. The application "SPSS 16" was used for the analysis. The result was considered statistically significant when the "P" value was less than 0.05 ($p<0.05$) and highly significant when it was less than 0.01 ($p <0.01$).

3. RESULTS AND DISCUSSION

Herbal medicine is the use of medicinal plants for illness prevention and treatment, ranging from traditional and popular treatments found in every culture to the use of standardised and tritated herbal extracts. In this research, we investigated the antidiabetic efficacy and lipid profile of the herb *Gymnemma sylvestre* in mice. Diabetes is one of the most difficult health issues of the twenty-first century. It is one of the major causes of mortality, and diabetic macro- and microvascular complications cause increasing disability and huge healthcare costs. Only the dosage of 900 mg/kg produced statistically significant ($p < 0.05$) findings in terms of antidiabetic efficacy. Several studies on *G. sylvestre* yielded similar findings [21–23].

Groups 3,5 and 6 had statistically significant outcomes for HDL levels when testing for total cholesterol, HDL, and LDL. Two other studies came to similar conclusions [24,25]. Statistically significant results ($p<0.05$) were observed for triglyceride, SGPT, and SGOT in groups 4, 5, and 6, respectively, with doses of 300, 600, and 900 mg/kg. Other research on *G. sylvestre* also came to the same conclusions [26]. Groups 4, 5, and 6 showed statistically significant results ($p<0.05$) in the kidney function test, with doses of 300, 600, and 900 mg/kg. Additional research on *G. sylvestre* yielded the same conclusions [27].

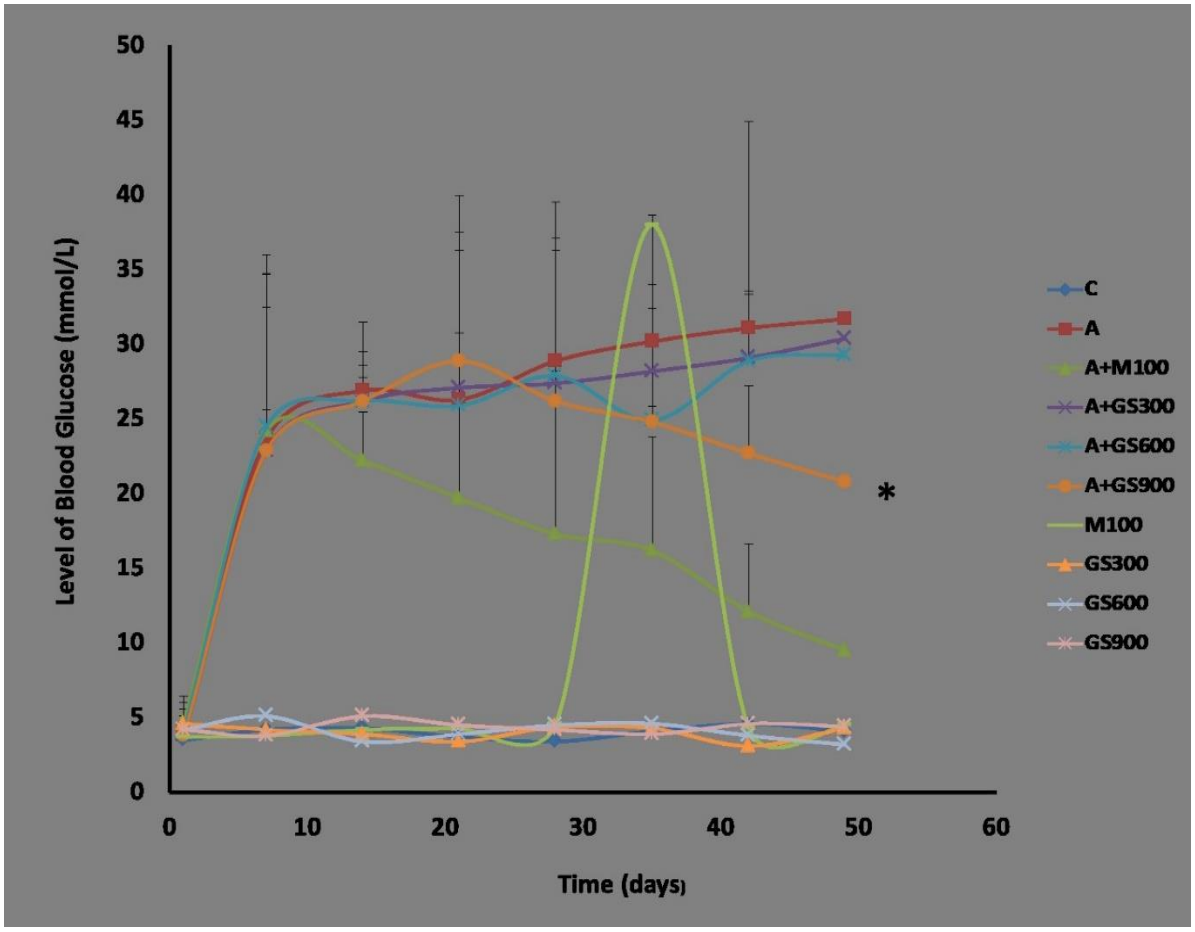

Fig. 1. Antidiabetic activity of different dose of *G. Sylvestre*

Table 2. Lipid profile after administration of different dose of *G. sylvestre*

	Total cholesterol	HDL	LDL	Triglyceride	SGPT	SGOT	Urea	Creatinine
N	95.26±4.61	72.75±4.10	36.90±2.36	56.42±2.21	39.35±2.32	4.62±4.10	20.28±1.50	0.4±0.03
A	126.5±11.32	51.33±4.18	74.37±8.29	115.19±6.14	84.41±3.90	97.33±2.90	100.46±5.43	2.6±0.09
A+M_{100}	109.7±10.40	63.56±4.73*	51.32±4.57	69.48±5.20	57.50±4.10	61.29±3.99	57.39±6.19	1.3±0.04
A+GS_{500}	124.5±9.37	53.51±6.41	73.29±4.1	112.20±6.18	82.10±3.19	91.40±4.22*	98.24±4.3	2.2±0.05*
A+ GS500	121.18±8.46	57.52±2.12*	70.19±2.50	109.41±6.22*	78.41±4.11*	85.37±3.36*	93.49±3.61*	1.19±0.08*
A+GS_{1500}	119.23±9.41	61.40±5.47*	65.26±5.30	100.20±3.25	73.60±2.40*	82.22±4.10*	85.42±4.21*	1.7±0.06*
M_{100}	94.70±5.21	75.22±4.69	39.26±4.12	60.41±3.26	38.41±3.19	42,49±3.10	26.22±2.20	0.6±0.01
GS_{500}	92.50±4.17	72.21±3.97	36.24±1.42	58.46±2.22	41.22±4.56	41.46±1.83	27.22±3.29	0.5±0.04
GS_{1000}	99.18±5.26	70.10±4.20	33.21±4.6	53.91±4.11	35.20v1.26	43.25±2.20	28.40±2.14	0.8±0.06
GS_{1500}	96.26±4.26	74.29±4.69	34.16±3.1	52.22±3.16	39.39±2.46	45.69±3.69	29.19±0.19	0.07±0.06

*Note: The results were expressed in Mean± SEM (standard mean error) *p< 0.05, **p< 0.01, and ***p< 0.001 were considered as statistically significant. The statistical analysis followed by one-way analysis of variance (Dunnett's test) compared to the control*

4. CONCLUSION

The results of this study suggest that an ethanol extract from the plant *Gymnema sylvestre* might help protect against diabetes, high cholesterol, liver damage, and poor kidney function. The plant extract did not significantly affect the target outcome, despite its anti-diabetic and anti-hyperlipidemic properties. Hence, more studies are required to identify the anti-diabetic and anti-hyperlipedemic active components in the whole extract. An extensive study may be undertaken when the active compounds have been identified.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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