Original Research Article

An Assessment of Anti-Diabetic Potential of Ethanolic Extract of *Gymnema lactiferum* in Alloxan Induced Rat Model.

Abstract

Endocrinological disease Diabetes mellitus has become a wide spread disease nowadays. To ameliorate diabetes many synthetic drugs are available but those drugs possess numerous adverse effects. Medicinal plants are a rich source of medications. Plant extracts, like those from *Gymnema lactiferum*, can be utilized as an alternate means of treating hyperglycemia. This study investigated the effects of *Gymnema lactiferum* extract on alloxan induced diabetic rats.. In comparison to the group that did not receive *Gymnema lactiferum* extract treatment, rat in the *Gymnema lactiferum* extract administration groups demonstrated a significant ($p<0.05$) reduction in body weight and diabetes levels after 60 days intragastric *Gymnema lactiferum* extract gavage. The hyperglycemia rat's blood sugar levels were significantly ($p<0.05$) reduced by the *Gymnema lactiferum* extract therapy. In this study with *Gymnema lactiferum* extract therapy, medium and higher doses of *Gymnema lactiferum* extract showed significant decrease in blood sugar level proving the plant having efficient anti-diabetic activity. Moreover, the lipid test resulted in a significant decrease in the total cholesterol level including significant reduction in urea and creatinine level in the diabetic rats by different dosages of Gymnema lactiferum in a dose-dependent manner.

keywords: medicinal plant, hyperglycemia, *Gymnema lactiferum*, ameliorate, alloxan

Introduction

Diabetes mellitus (DM) is a growing health problem brought on by an inability to produce enough insulin or by an intolerance to insulin. Serious damage to the heart, blood vessels, eyes, kidneys, and nerves can result from diabetes overtime. Approximately 422 million individuals globally suffer from diabetes, with the majority residing in low- and middle-income nations. The disease is directly responsible for 1.5 million fatalities annually (Gamage Piyumi Wasana et al., 2021). Over the past few decades, there has been a steady

rise in both the number of cases and the incidence of diabetes. Based on data from the International Diabetes Federation (IDF), 463 million persons aged 20 to 79 had diabetes in 2019, and by 2045, it is expected that this figure would have increased to 700 million (Dahlén et al., 2022).

Diabetes is being treated aggressively by reducing circulating blood glucose and preventing postprandial hyperglycemic rises, in addition to greater medical vigilance. The current approaches to treating diabetes include the use of Metformin, under the drug class Biguanides that causes inhibition of hepatic glucose production and promotion of skeletal muscle glucose reuptake; Sulfonylureas and meglitinides (Glipizide, Glyburide, Glimepiride, Nateglinide) depolarizes the beta cell membrane to increase insulin secretion; Activation of nuclear receptor peroxisome proliferator activated receptor gamma (PPAR) to increase adiponectin and improve insulin resistance through Thiazolidinediones (Pioglitazone, Rosiglitazone); Alpha glucosidase inhibitors (Acarbose, Miglitol, Voglibose) inhibits hydrolysis of starches and carbohydrates in the gut to decrease absorption (Samson, S. L., & Garber, A. J.,2018).

However, the anti-diabetic medications are also associated with several side effects. These include Metformin causing Gastrointestinal upset (diarrhea), Lactic acidosis, B12 or folate deficiency; Hypoglycemia, weight gain, acceleration of loss of beta cell function during treatment with Sulfonylureas and meglitinides; Fluid retention, chronic heart failure exacerbation, osteoporosis, bladder cancer, suppressed hematopoiesis with Thiazolidinediones etc (Samson, S. L., & Garber, A. J., 2018).

Consequently, there is a need for alternative medicine and novel strategies to address this health issue. One such strategy is the use of medicinal plants with an antihyperglycemic effect, which is attributed to their capacity to either stimulate insulin secretion or inhibit the absorption of glucose, or to facilitate metabolites in insulin-dependent processes with fewer adverse effects (Karimi, A., Majlesi, M., & Rafieian-Kopaei, M., 2015). In addition, a single medicinal plant can be associated with several healing potential like antidiabetic, analgesic, antilipidemic etc. Moreover, genetical modifications can be performed on medicinal plants which alter the concentration of the undesired and desired compound according to the requirement of the treatment (SAITO, K., 1994).

A few medicinal plants that have been shown to have anti-diabetic properties and their mechanisms of action have been explained. These include Morus alba L., Trigonella foenum graecum L., Cinnamomum zeylanicum J.Presl, Zingiber officinale Rosc., Phaseolus vulgaris L., Panax ginseng C.A.Meyer and others (Przeor, M., 2022).

The climbing perennial shrub Gymnema lactiferum is indigenous to Sri Lanka and India. Gymnema lactiferum is known by the colloquial term "kurighghan." It is known as ksirakakoli in Sanskrit. Gymnema lactiferum leaves are consumed in both their raw and cooked forms as a remedy for diabetes. This plant is a member of the Asclepiadaceae family, and the purpose of this study was to determine what bioactive substances this plant synthesizes. Gymnema lactiferum leaf methanolic extract contained the following chemicals: alkaloids, flavonoids, steroids, saponins, quinones, cardiac glycosides, phenol compounds, protein, and

carbohydrates (Prabodhani, W. D. M. H., 2019).

Gymnemic acid, tartaric acid, gurmarin, calcium oxalate, glucose, stigmasterol, betaine, and choline are the main chemical components of gymnema. (Kanetkar, P., Singhal, R., & Kamat, M., 2007). Gymnema lactiferum extracts and pure compounds have been shown to exhibit a variety of pharmacological properties, such as anti-inflammatory, anti-tumor, anti-inflammatory, antihyperlipidemic, immunostimulatory, and hepatoprotective properties (Tiwari, P., Mishra, B. N., & Sangwan, N. S., 2014).

In this study anti-diabetic activity of Gymnema lactiferum is going to be assessed in rat model. If this plant provides effective and potential anti-diabetic activity in this assessment, then isolation of compounds from G. lactiferum would be done for further vigorous studies to open a new remedy for herbal doorway against diabetes.

Objective

The aim of this research is to create a new, efficient treatment for hyperglycemia because the synthetic drugs that are currently on the market have a lot of side effects that could lead to further problems. Thus, the aim of this research is to minimize each of these side effects to the greatest extent feasible and develop new herbal medicines that are devoid of them. Therefore, if the plant can successfully treat hyperglycemia, it will open the door for the creation of cutting-edge, side-effect-free medications. Ultimately, this will offer a fresh perspective on anti- hyperglycemic drugs that have fewer adverse effects than those currently available.

Methods and Materials

Drugs, Chemicals, and Instruments

The purchasing of the ethanol and alloxan was from Sigma Aldrich in Germany. A free sample of metformin, a common medication for diabetes, was sent to us by Healthcare Pharmaceutical Limited. The UK-based Plasmatic Laboratory Product Ltd. provided the blood serum analysis kits for creatinine, SGOT, SGPT, HDL, LDL, triglycerides, and total cholesterol. Shahbag in Dhaka, Bangladesh provided the glucometer (Alere GI from Alere Inc., USA), and the Humalyzer 3000 was used to assess the biochemical parameters. (a clinical chemical analyzer that is semi automated).

Plant Collection and Extract Preparation

Three different regions of Bangladesh were used to gather *G. lactiferum* leaves: North Bengal, a hill track area, and a low land location. Taxonomic identification and authentication came next. The plant specimen was kept in compliance with Bangladeshi legislation at the National Herbarium. Before being finely powdered, the leaves were allowed to dry for seven to ten days in the shade. The powdered leaves steeped in 70% ethanol while being vigorously stirred every 96 hours. When the extract was done soaking, it was filtered, and the resulting liquid was collected. After being transported there, the extracted solution was concentrated using a rotating evaporator. After that, the dried extract was carefully gathered and put away

for later use.

Experimental Animal Handling

The Institute of Nutrition and Food Science at the University of Dhaka maintained adult, healthy male Wistar rats weighing between 125 and 200 grams under a 12-hour light/dark cycle at a consistent temperature of 25° C. The rats were obtained from the Pharmacy Department of Jahangirnagar University in Dhaka, Bangladesh. A standard pellet meal and fresh water were given on a regular basis. Prior to the start of the inquiry, the rats were housed there to adjust. Every rat trial adhered to the Institutional Animal Ethics Committee's (IEAC) rules. The Swiss Academy of Sciences (SCNAT) and the Swiss Academy of Medical Sciences (SAMS) both provide standards for the care and handling of animals.

Experimental Guidelines

Every test was conducted in compliance with the 2013 Helsinki Declaration's ethical guidelines. Throughout this investigation, the "3R" standards—a fundamental component of

Swiss and international laws governing the use of animals in research—were strictly adhered to. The prefix "R" stands for "replacement," which includes relative replacements (like switching out vertebrates for invertebrates or living animals for cell or tissue cultures) as well as absolute replacements (like replacing animal models with computer-generated models). An animal model was used to do flawless research. Due to the unique pancreas and beta cells seen in mammalian vertebrates, unlike invertebrates, rats were chosen as test subjects for ant diabetic research.

The third "R" is for "refinement," which is reducing the amount of discomfort the test animals experience by easing their suffering. Before and after each blood glucose level measurement, rats' tail tips were wiped with isopropyl alcohol to make the procedure more bearable and less painful from pinching. The rats were painlessly murdered at the end of the trial in compliance with the 2013 revision to the Guidelines for the Euthanasia of Animals, and they were fed appropriately during the experiment.

Experimental Design

Rats were weighed individually, grouped according to body weight, and tested for anti-hyperglycemic activity (Table 1). Ten rodents per group were used to split the rats according to body weight. The rats in the alloxan control group are shown in Table 1 as having only undergone alloxan therapy. N/A denotes that the rats in this group did not receive any therapeutic intervention.

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 + *G. lactiferum*	Alloxan + *G. lactiferum* leaves extract low dose	150 mg/kg + 500 mg/kg	A + GL500
5	Alloxan + *G. lactiferum*	Alloxan + *G. lactiferum* leaves extract medium dose	150 mg/kg + 1000 mg/kg	A + GL1000
6	Alloxan + *G. lactiferum*	Alloxan + *G. lactiferum* leaves extract high dose	150 mg/kg + 1500 mg/kg	A+ GL1500

7	Metformin	Metformin	100 mg/kg	M
8	*G. lactiferum*	*G. lactiferum* leaves extract low dose	500 mg/kg	GL500
9	*G. lactiferum*	*G. lactiferum* leaves extract medium dose	1000 mg/kg	GL1000
10	*G. lactiferum*	*G. lactiferum* leaves extract high dose	1500 mg/kg	GL1500

Biological Sample Collection

To evaluate blood glucose levels, the rat's tail tip was punctured to obtain blood samples. On the other hand, following a heart puncture, blood was extracted from the killed animal right away and placed in a tiny centrifuge tube. The samples that were obtained were centrifuged for five minutes at 5,000 rpm to create the supernatant fluid. After that, the fluid was moved to a different micro centrifuge tube so that it could be tested biochemically. After the animal was

sacrificed, the kidney and liver were quickly removed from the corpse and carefully cleaned in ice-cold saline in order to assess their respective functions.

Estimation of Biochemical Parameters

A glucometer was used to measure the blood glucose level. Tests for liver, kidney, and lipid profile were conducted in addition to the Humaluzer 3000. Furthermore, the activity of glycolytic and gluconeogenic enzymes in liver and kidney samples was investigated.

Statistical Analysis

For each group, the mean and standard deviation (SD) of each research parameter is 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 ($p < 0.01$).

Results and discussion:

Using rats in three different experimentally created diabetic scenarios, this study assessed the capacity of *Gymnema lactiferum*on's extract to prevent diabetes. Below displayed graph represent the blood sugar level of rat's belonged to different group throughout treatment period.

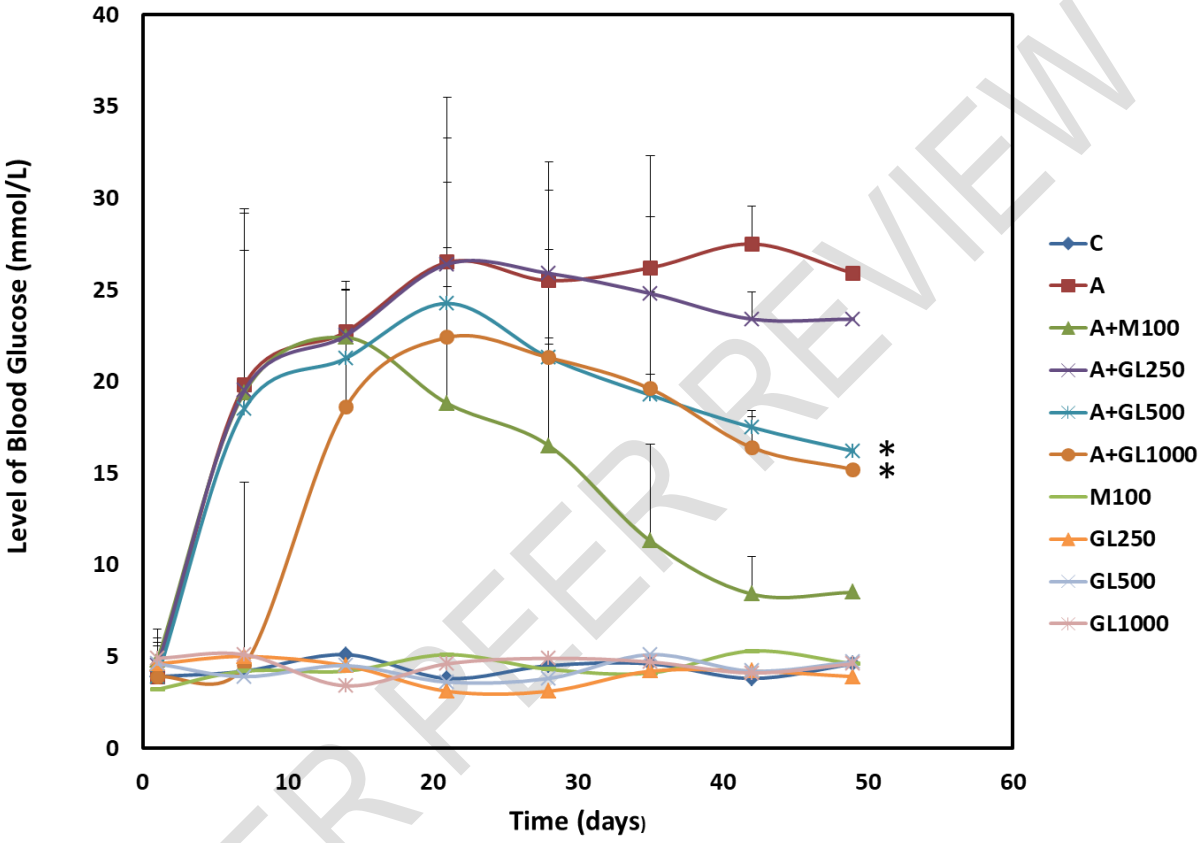

Figure 1: Blood Glucose Levels of Rats in Ten Groups over the Course of Treatment

In group 4 the plant extract was given in lower dose. As a result the blood sugar level decreased in non-significant manner comparing to group 2. On the other hand, in group 5 & 6 where plant extracts was given in medium and higher dose, the blood sugar level was decreased significantly. This indicates that our plant possesses anti-hyperglycemic activities [figure-1]

When we compared groups 8,9,10 (no disease + plant group) to group 1 (disease free rat), we couldn't find any significant differences, indicating that our plant has no side effects.

Analysis of lipid parameters in control and experimental rats following *Gymnema lactiferum* treatment indicates that *Gymnema lactiferum* may play a role in influencing lipid metabolism. A more in-depth discussion is warranted to investigate the consequences of these alterations

within the framework of cardiovascular health, disorders related to lipid metabolism, and the existing literature concerning the impact of herbal interventions on lipid profiles.

Lipid profile:

Table 2 : Lipid profile of rats belonged to different groups are displayed in

Group number	Group Status	Total Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Triglyceride (mg/dL)
1	Negative Control	**93.74 ± 4.16**	**72.43 ±2.93**	**40.22 ±2.70**	**53.24 ±3.47**
2	Alloxan control	**137.45 ± 6.13**	**49.53 ±3.74**	**79.39 ±3.46**	**115.62 ±6.89**
3	Alloxan + Metformin	**104.56 ± 5.56**	**68.79 ±4.29**	**50.51 ±2.89**	**78.89 ±5.47**
4	Alloxan + *Gymnema lactiferum*	**133.46 ± 4.89**	**55.71* ±2.50**	**77.99 ± 5.41**	**111.26 ±4.73**
5	Alloxan + *Gymnema lactiferum*	**131.22 ±7.22**	**57.56* ±2.90**	**71.21* ±44.63**	**107.46* ±4.11**
6	Alloxan + *Gymnema lactiferum*	**127.23* ± 6.29**	**62.33* ±2.63**	**65.42* ±3.26**	**101.44* ±2.37**
7	Metformin	**122.49 ± 3.46**	**70.36 ±5.23**	**31.23 ±1.79**	**49.87 ±3.41**
8	*Gymnema lactiferum*	**97.46 ± 5.57**	**70.74 ±4.45**	**38.42 ±2.28**	**55.68 ±2.42**
9	*Gymnema lactiferum*	**100.23 ± 3.21**	**74.56 ±3.19**	**40.23 ±3.45**	**54.63 ±4.50**
10	*Gymnema lactiferum*	**95.59 ± 3.45**	**69.82 ±4.21**	**37.94 ±4.50**	**51.50 ±3.49**

In this study, rats in groups 5 and 6 (diseased + plant extract treated groups) displayed significant lower ($p<0.05$) level of total cholesterol than that of group,. Additionally, LDl and triglyceride level of rats belonged to group 4, 5 and 6 also decreased significantly ($p<0.05$). Besides HDL cholesterol levels were significantly higher compared to group 2 in case of group 4, 5 and 6(disease group) ($p<0.05$).

Liver function test:

The ability of *Gymnema lactiferum* to restore the liver functions of diabetic rats was investigated by measuring the levels of two enzymes in their serum—SGPT and SGOT—which serve as the most accurate indicators of liver damage [Table 3].

Table 3 : **Result of Liver function test**

Group number	Group Status	SGPT (U/L)	SGOT (U/L)
1	Negative Control	**36.37±0.42**	**44.49±2.29**
2	Alloxan control	**73.73±5.67**	**84.56±7.41**
3	Alloxan + Metformin	**52.47±5.50**	**70.79±5.54**
4	Alloxan + *Gymnema lactiferum*	**72.78±4.26**	**82.46±5.54**
5	Alloxan + *Gymnema lactiferum*	**70.12±5.16**	**79.89*±4.53**
6	Alloxan + *Gymnema lactiferum*	**68.79*±4.22**	**75.59*±4.49**
7	Metformin	**35.59±2.01**	**40.42±3.47**
8	*Gymnema lactiferum*	**36.39±0.79**	**43.97±3.12**
9	*Gymnema lactiferum*	**33.69±2.01**	**41.79±2.26**
10	*Gymnema lactiferum*	**37.19±2.27**	**43.69±3.47**

It has been observed the plant extract are capable of reducing serum SGPT and SGOT level in different extend and dose dependent manner. SGOT levels were significantly lower

($p<0.05$).in groups 5 and 6 than in group 2. Also SGPT level was restored significantly in group 6.

Kidney Function test:

The effect of *Gymnema lactiferum* the renal functions of diabetic rats was evaluated by comparing serum creatinine and urea levels between experimental group and disease control group[Table 4].

Table 4 **: Result of Kidney Function test**

Group number	Group Status	Creatinine (mg/dL)	Urea (mg/dL)
1	Negative Control	**0.5±0.02**	**24.9±1.99**
2	Alloxan control	**2.6±0.09**	**90.57±6.79**
3	Alloxan + Metformin	**1.0±0.05**	**71.71±5.37**
4	Alloxan + *Gymnema lactiferum*	**1.8±0.09***	**89.92±6.31**
5	Alloxan + *Gymnema lactiferum*	**1.3±0.02***	**84.49*±4.82**
6	Alloxan + *Gymnema lactiferum*	**0.8±0..08***	**79.39*±6.82**
7	Metformin	**0.6±0.04**	**25.6±2.37**
8	*Gymnema lactiferum*	**0.5±0.359**	**26.20±0.86**
9	*Gymnema lactiferum*	**0.6±0.069**	**27.92±2.08**
10	*Gymnema lactiferum*	**0.6±0.04**	**27.79±2.46**

Table 4 shows that there was a significant decrease ($p<0.05$) in serum creatinine levels in rats from groups 4, 5, and 6 (disease control groups), also urea level was significantly restored in group 5 and 6 indicating that the rat's renal perfusion was compromised. However, giving these disease control groups different dosages of *Gymnema lactiferum* significantly ($P<0.05$) decreased the diabetic rat's high levels of urea and creatinine in a dose-dependent manner, indicating the plant extract's nefroprotective efficacy against biochemically generated blood sugar level changes.

Conclusion

Gymnema lactiferum contains anti-hyperglycemic chemicals, according to our findings. Furthermore, the extract restores the disturbed pathogenic state in a dose-dependent manner. The solvent used for extraction is not optimal for removing the leaf chemical with anti-hyperglycemic properties. In addition, these chemicals may not have been present in significant amounts in the plant at the time of leaf harvest. They may also exist naturally in trace amounts in the plant in the area where the plant was picked. In order to determine the type and extent of *Gymnema lactiferum*'s ability to treat anti-hyperglycemic, further research on the plant's pharmacological activity will need to be conducted.

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