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Exploration of the GLUT-4 Modulation Potential of *Gynura procumbens* as a Mechanism Behind its Antidiabetic Activity

Juhaer Anjum[1+], Maniza Muni[1+], Nusrat Jahan Shawon[2], Fahima Aktar[1], Md. Mehadi Hasan[3], Mohammad Sofi[3], Md. Rafat Tahsin[4], Md Reaz Uddin[5], Abu Asad Chowdhury[1], Jakir Ahmed Chowdhury[5], Shaila Kabir[1], Md. Al Amin Sikder[1] and Md. Shah Amran[1]*

[1]Molecular Pharmacology and Herbal Drug Research Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh.
[2]Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh.
[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh.
[4]Department of Pharmaceutical Sciences, North South University, Dhaka 1229, Bangladesh.
[5]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh.
*Corresponding Author: Md. Shah Amran; E-mail: amranms@du.ac.bd
+The two authors contributed equally to this paper.

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The search for antidiabetic drugs from natural sources has been carried out since diabetes has become a prevalent chronic disease, whose conventional therapy causes several complications in long-term use. *Gynura procumbens* (GP), a well-known herb for its various activities, has shown promising results against diabetes in several studies. To find whether the mechanism of activity behind this antidiabetic effect is underpinned by the upregulation of a particular glucose transporter (GLUT-4), this experiment was conducted. The GLUT-4 expression levels in Alloxan-induced diabetic rats were compared with untreated healthy rats and rats treated with different doses of *G. procumbens* extract using enzyme-linked immunosorbent assay (ELISA) specified for rat GLUT-4. All four test groups including the metformin control group showed elevated expression of GLUT-4 in the liver tissue compared to the diabetic control group ($p < 0.05$, one-way ANOVA). The serum-glucose modulatory antidiabetic activity of ethanolic extract of *G. procumbens* was therefore mediated though the upregulation of GLUT-4 expression, and consequent increased uptake of glucose.

Keywords: Antidiabetic, blood glucose management, GLUT-4, *Gynura procumbens*, herbal medicine.

Type 2 diabetes mellitus, one of the most common non-communicable chronic diseases, has become a glaring concern for healthcare practitioners worldwide, especially due to the digitalization and a general shift towards a sedentary, less labour-intensive lifestyle[1]. In fact, it is often afflicted with other chronic diseases, resulting in co-morbid conditions and increasing the overall impact on health. According to a report by the American Diabetes Association, in patients with diabetes mellitus, hypertension is not only the greatest cause of morbidity and mortality,

but it also exerts the largest toll financially[2]. Diabetes-associated dyslipidemia only adds to the risk and furthers the possibility of cardiovascular complications[3]. Moreover, these conditions require lifelong pharmacological interventions, which can result in hepatorenal complications associated with chronic pharmacotherapy. Therefore, concurrent administration of herbal medicinal supplements that allow a less strenuous pharmacologic regimen or provide hepatoprotective effects can be therapeutically relevant.

Gynura procumbens is a plant with tremendous potential in the field of herbal medication development. The ethnomedical uses of the plant are versatile, and many of these claims have been ascertained scientifically[4]. Previously, we and other groups had reported this plant to possess significant anti-diabetic, anti-hypertensive, anti-hyperlipidemic, hepatoprotective, anti-inflammatory, antioxidant, and anticancer activities both *in vitro* and *in vivo* in preclinical trials[5-8]. In the alloxan-induced diabetic murine model, the ethanolic extract of the plant not only exerted a significant antihyperglycemic effect but also reversed several alloxan-mediated adverse biochemical changes[5]. In the streptozotocin-induced diabetic murine model, the ethanolic plant extract was observed to exert hypoglycemic activity and inactivate Glycogen synthase kinase-3 beta (GSK3â) in hepatic tissue[9]. The association between the expression of GLUT-4 and the amount of insulin utilized by the cells has been established long ago[10]. While exploring the mechanism of action behind the antidiabetic activity of *G. procumbens*, the chance of its ability to increase GLUT-4 expression can be hypothesized. In this communication, we aim to report the results of the animal experiment where the level of GLUT-4 expression among diabetes-induced rats treated with different doses of *G. procumbens* extract have been compared with healthy control, disease control, and metformin-controlled rats using enzyme-linked immunosorbent assay (ELISA) technique.

MATERIALS AND METHODS

Drugs, Chemicals, and Instruments

Ethanol for extraction, chloroform for euthanasia and alloxan for diabetes induction were purchased from Sigma Aldrich (Germany). Metformin (Standard antidiabetic drug) as control, syringe, gloves, lancets and strips were purchased from nearby pharmacy of Dhaka Medical College. Rat GLUT-4 ELISA kit for 96 well plate was purchased from MyBioSource (Cat. No.: MBS765109).

Plant Collection and Extract Preparation

G. procumbens leaves were gathered from the medicinal plant garden of the University of Dhaka, Bangladesh. Then, taxonomic identification and authentication were completed. The National Herbarium of Bangladesh maintained plant specimens in accordance with their regulations. The herbarium administrators assigned accession number 47380, dated 11-2-2019, for future reference. After a 7-10 day period of shade drying, the leaves were coarsely crushed. Powdered leaves soaked in ethanol (70%) were forcefully agitated for 96 hours. Following the extraction's soaking period, the filtered liquid was collected. The extracted solution was then concentrated using a rotary evaporator. The dried extract was then meticulously gathered and diluted with water. Tween 20 was used to solubilize the extract in water. The concentration of the extract was determined and then stored at -20°C.

Experimental Animal Handling

Adult and healthy Albino rats of either sex weighing between 100 and 200 g, were collected from the Jahangirnagar University in Savar, Dhaka, Bangladesh. They were kept with sufficient facilities at the Institute of Nutrition and Food Science, University of Dhaka, Bangladesh. These rats were kept at a steady temperature of 25 °C in a 12-hour cycle of light and dark. A standard pellet diet and sufficient pure water were provided regularly. They were left there to become accustomed to the new environment before the investigation began. Rats were used in all of the experiments, and they were all conducted in accordance with the rules established by the Institutional Animal Ethics Committee (IAEC). The Swiss Academy of Medical Sciences (SAMS) and the Swiss Academy of Sciences (SCNAT) guidelines were followed in the care and handling of the animals.

Experimental Guidelines

The 2013 Declaration of Helsinki's ethical guidelines were followed in the execution

of all tests. The "3R" principles, a cornerstone of Swiss and international guidelines governing the exploitation of animals for experimental purposes, were strictly maintained during the whole length of this study.

Sample Size Estimation for the Animal Model

The sample size was kept at six rats per group as it is sufficient to obtain statistical significance according to previous literature.

Dose Selection

At a dose of 250 mg/kg, the plant extract (*G. procumbens*) started to exert its pharmacological effect, according to a previous study, indicating that its MEC (minimum effective concentration) value was higher than 250 mg/kg. A steady increase in this effect was observed as the dosage was increased. Eventually, there was no discernible change in the pharmacological effects when the dosage was increased from 750 mg/kg to 1000 mg/kg[5]. The three doses were therefore chosen accordingly. The standard dose of Metformin which is 500 mg/kg was chosen based on a previous literature review.

Experimental Design

Rats were separated into six groups, each consisting of six rats, based on their body weight. The oral glucose tolerance test (OGTT) and the enzyme-linked immunosorbent assay were used to measure the peak plasma glucose concentration (Table 1).

Alloxan administration and treatment

To induce diabetes in the rat model alloxan was applied at 150 mg/kg dose. The desired amount was calculated for each rat and was measured carefully. Immediately it was dissolved in normal saline and then administered into the rat by intraperitoneal route.

Following the administration of alloxan, blood glucose levels were measured to check for hyperglycaemia. The majority of the rats treated with alloxan had blood glucose levels ranging from 7 to 11 mmol/L on average after 3–4 days, which was a clear sign of hyperglycaemia or diabetes. Then, for a total of 28 days, oral treatment was initiated with Metformin and three doses of *G. procumbens* extract administered by oral gavage.

Biological Sample Collection

Blood samples were taken by puncturing the tip of rats' tails to evaluate blood glucose levels. After 4 weeks of treatment, each rat was euthanized using chloroform, and immediately after, the abdomen was dissected and the liver was collected in a glass vial. The samples were flash frozen using dry ice and then stored at a -20° C freezer for future use.

Preparing tissue samples for ELISA

The frozen liver samples were thawed to room temperature and then 120 ± 30 mg of liver tissue was taken into a 2 mm Eppendorf tube after measurement and 300 µL of RIPA buffer was added into the tube. Each of the tissue samples was homogenized using an ultrasonic homogenizer for 1 minute and then centrifuged at 4°C, for 10 minutes at 10000 rpm. The supernatant was taken into another Eppendorf tube and stored at -20°C for further use.

Running ELISA and Collecting absorbance data

96 well-plate Rat GLUT-4 ELISA kit purchased from MyBioSource was used for performing the assay. A manual with detailed

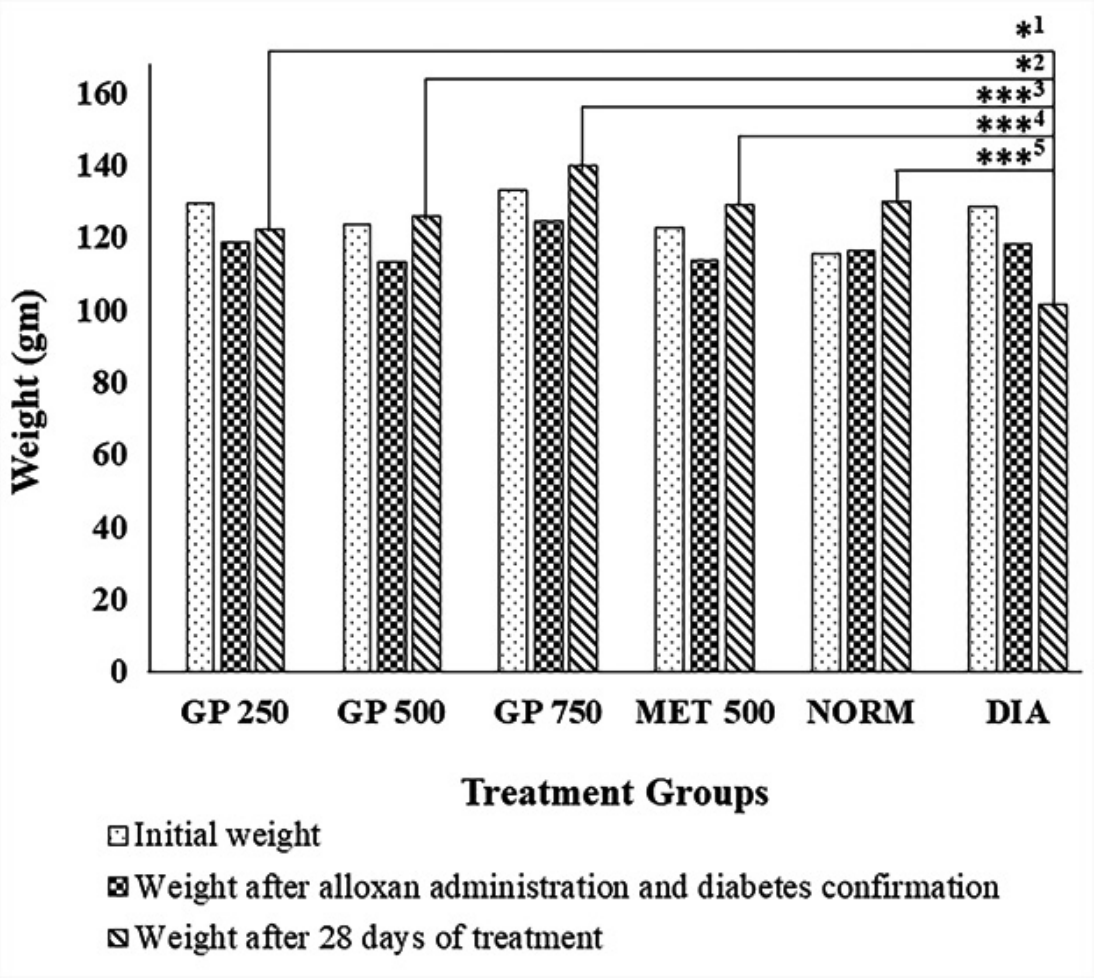
Table 1. Group distribution of rats for the experiment

Group number	Group status	Treatment specimen	Dose of the treatment specimen	Group abbreviation
1	*G. procumbens*	*G. procumbens* ethanolic extract	250 mg/kg	GP 250
2	*G. procumbens*	*G. procumbens* ethanolic extract	500 mg.kg	GP 500
3	*G. procumbens*	*G. procumbens* ethanolic extract	750 mg/kg	GP 750
4	Metformin control	Metformin	500 mg/kg	MET 500
5	Normal Control	Physiological saline	10 mL/kg	NORM
6	Diabetic Control	Physiological saline	10 mL/kg	DIA

directions was supplied with the kit which was followed to the point for conducting the experiment. To determine the dilution factor for samples, a trial batch was run using different concentrations of the standard kit against 2-200 times diluted homogenized tissue samples. It was shown that the 2X dilution gave the absorbance value within the accepted range of the test kit. So, the final assay was performed by using 2X dilution of the samples. ELISA plate reader was used to measure the absorbance value.

Data processing and statistical analysis

The blood glucose measurement data obtained from the oral glucose tolerance test (OGTT) were analyzed by independent sample t-test using SPSS software. The absorbance data obtained from the ELISA plate reader were adjusted for the dilution factor and weight of the tissue sample taken. Then amount of GLUT-4 per unit of tissue was determined using the standard curve. Finally, these data were analyzed by one-way ANOVA test to interpret intergroup



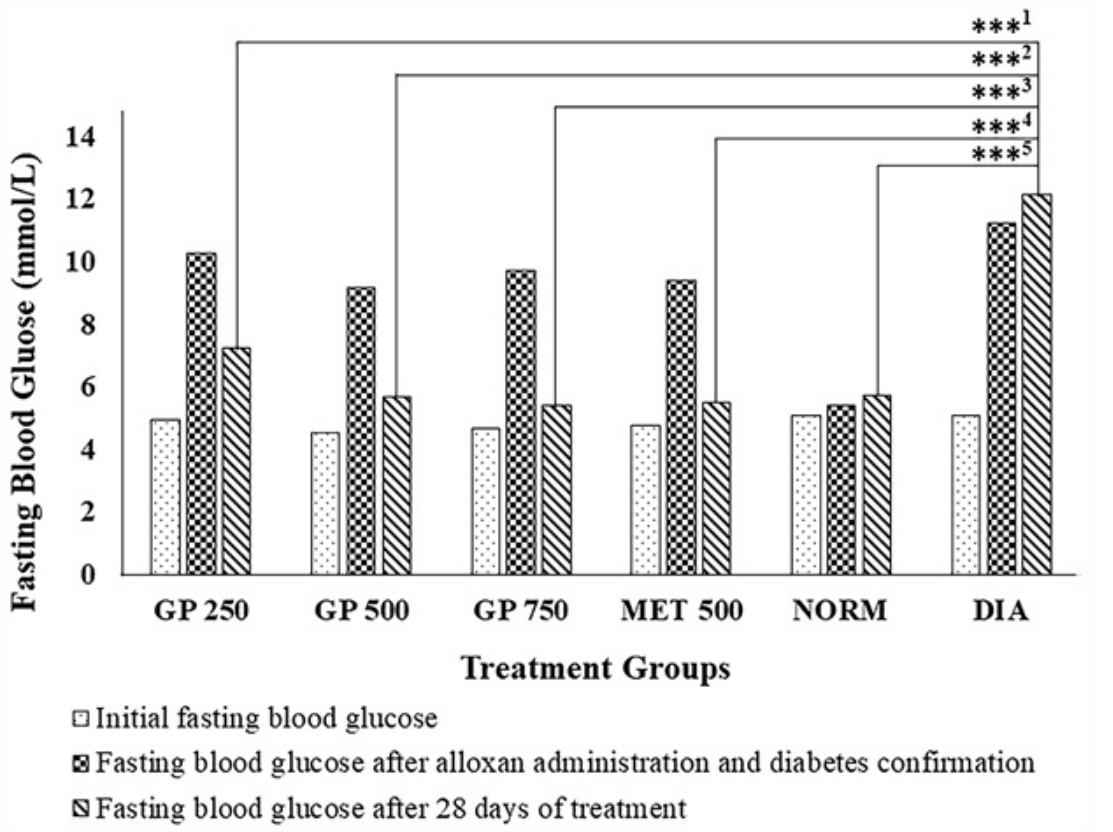
Graph 1. Weight of experimental animals over the course of the experiment. GP 250= Diabetic rats treated with 250 mg/kg *G. procumbens* extract, GP 500= Diabetic rats treated with 500 mg/kg *G. procumbens* extract, GP 750= Diabetic rats treated with 750mg/kg *G. procumbens* extract, MET 500= Rats treated with 500 mg/kg Metformin, NORM= Normal rats treated with physiological saline, DIA= Diabetic rats treated with physiological saline. Independent variables T-test was used to assess the statistical significance of the differences between the groups, as the data was considered suitable for this test. *Indicates $p<0.05$, **indicates $p<0.01$ and ***indicates $p<0.005$ level of statistical significance. The P values for the statistical tests are: $*^{1}= 0.016$, $*^{2}= 0.020$, $***^{3}= 0.000638$, $***^{4}= 0.002005$, $***^{5}= 0.000777$. Each bar shows the average weight of six rats in the group.

heterogeneity using Prism software. The "p" value margin was set to less than 0.05 ($p<0.05$) for statistical significance.

RESULTS AND DISCUSSION

G. procumbens extract restores the weight of diabetic animals

In the current experiment, it was observed that the weight of the experimental animals took a downward turn in all experimental groups after the administration of alloxan and consequent induction of diabetes. This is to be expected, as not only is alloxan a toxic substance that stresses out the physiological system in the course of diabetes induction, but also diabetes itself is a condition that has been reported to cause muscular damage[11,12]. The latter statement is also reflected heavily in the case of the diabetic control rats, where body weights took a further drop after 28 days of observation. The diabetes-mediated reduction in body weight was prevented in all three test groups, i.e., GP 250, GP 500, and GP 750, as well as the metformin control group. In all four groups, the reduction in body weight due to alloxan administration was recovered, and body weights continued to rise gradually under treatment. In a previously reported study, the aqueous extract of

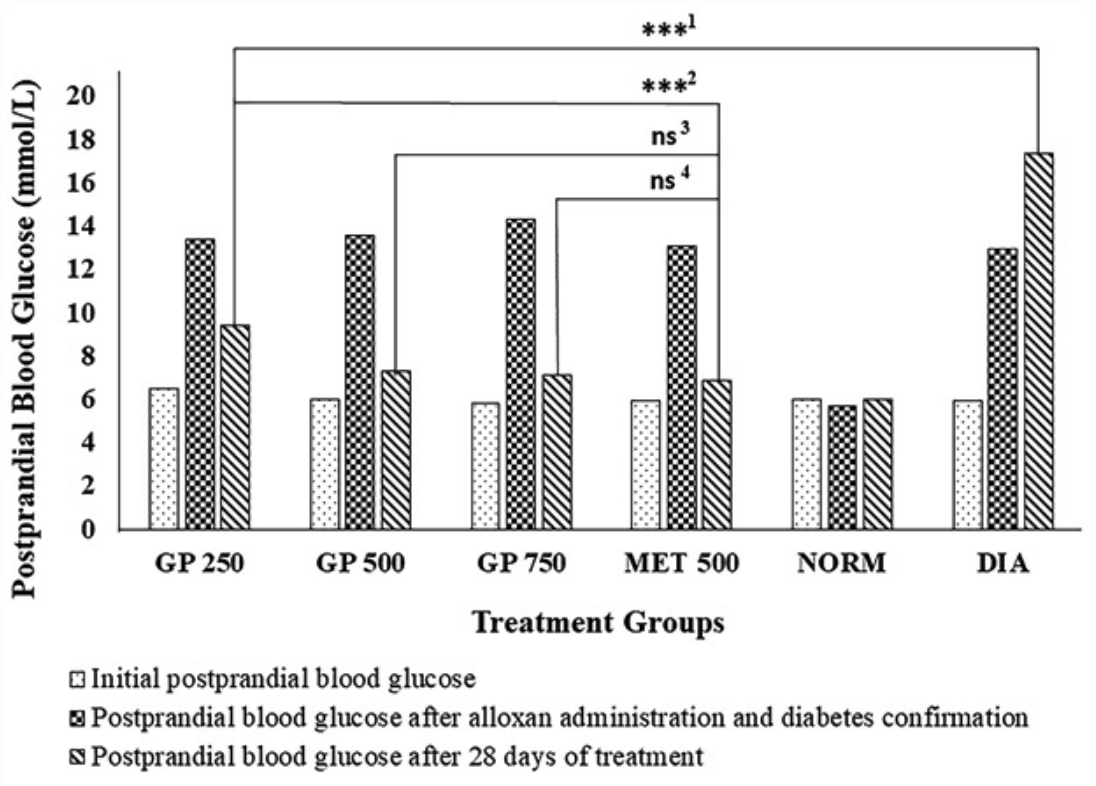


Graph 2. Fasting blood glucose of experimental animals over the course of the experiment. GP 250= Diabetic rats treated with 250 mg/kg *G. procumbens* extract, GP 500= Diabetic rats treated with 500 mg/kg *G. procumbens* extract, GP 750= Diabetic rats treated with 750mg/kg *G. procumbens* extract, MET 500= Rats treated with 500 mg/kg Metformin, NORM= Normal rats treated with physiological saline, DIA= Diabetic rats treated with physiological saline. Independent variables T-test was used to assess the statistical significance of the differences between the groups, as the data was considered suitable for this test. *Indicates $p<0.05$, **indicates $p<0.01$ and ***indicates $p<0.005$ level of statistical significance. The P values for the statistical tests are: ***^{1}= 5.5811E-8, ***^{2}= 7.7727E-9, ***^{3}= 3.3996E-9, ***^{4}= 3.3577E-9, ***^{5}= 1.048E-8. Each bar shows the average fasting blood glucose level of six rats in the group.

the plant was reported to retard diabetes-induced body weight loss[13]. The change in body weights is depicted in Graph 1.

***G. procumbens* extract reduces fasting blood glucose of diabetic animals**

Fasting blood glucose (FBG) and postprandial blood glucose (PBG; blood glucose 2 hours after eating) are two important measures for monitoring diabetic status. In the current study, we observed that the initial fasting blood glucose of all experimental animals had a mean 4.84 ± 0.37 mmol/L, which has been reported to be within the normal range for rats[14]. After the administration of alloxan, the mean fasting blood glucose value increased to 9.18 ± 1.99 mmol/L, indicating the attainment of diabetic status of the experimental animals. After the 28-day treatment period, reductions in blood glucose were recorded in all three GP treatment groups along with the metformin control group. The differences in data between the diabetic control group and the other groups; including the GP test groups, the metformin control group, and normal control group; were found to be statistically significant using the independent variables T-test. No statistical significance was obtained between the metformin control group, the normal control group, and the GP 500 and GP 750 groups, although GP 250 was deemed to have a statistically significant difference. Algariri and others have also communicated the fasting blood glucose-lowering activity of the ethanolic extract of the plant leaf, although a higher dose of the extract



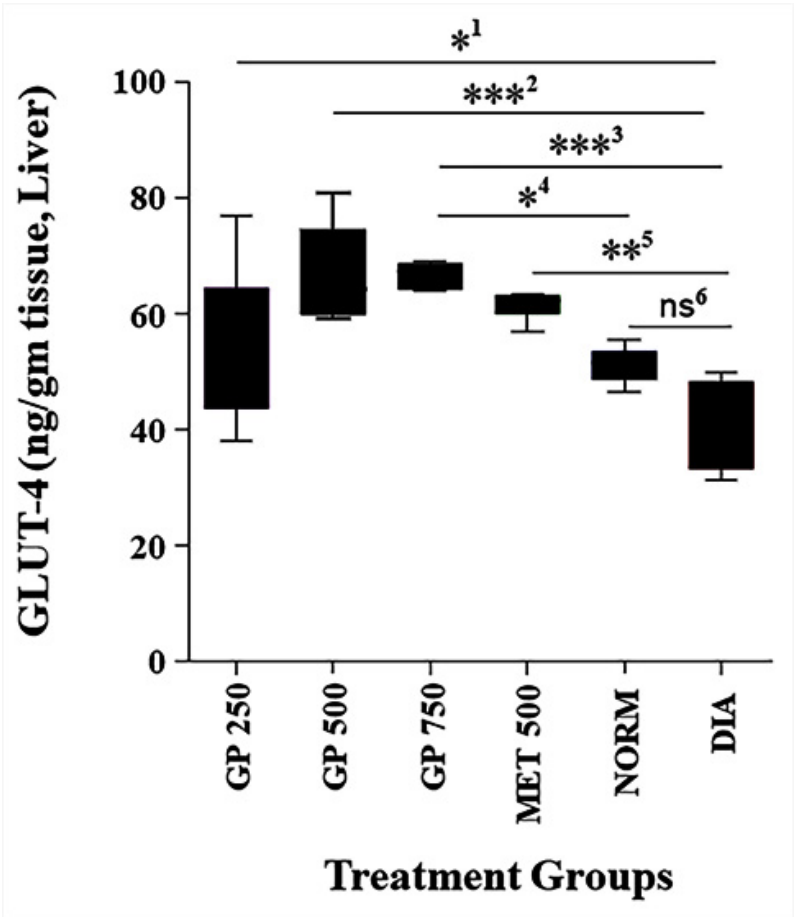
Graph 3. Postprandial blood glucose data of the experimental animals over the course of the experiment. GP 250= Diabetic rats treated with 250 mg/kg *G. procumbens* extract, GP 500= Diabetic rats treated with 500 mg/kg *G. procumbens* extract, GP 750= Diabetic rats treated with 750mg/kg *G. procumbens* extract, MET 500= Rats treated with 500 mg/kg Metformin, NORM= Normal rats treated with physiological saline, DIA= Diabetic rats treated with physiological saline. Independent variables T-test was used to assess the statistical significance of the differences between the groups, as the data was considered suitable for this test. *Indicates $p<0.05$, **indicates $p<0.01$ and ***indicates $p<0.005$ level of statistical significance, while ns indicates no significance. The P values for the statistical tests are: ***[1]= 4.9001E-8, ***[2]= 0.000047, ns[3]= 0.416, ns[4]= 0.518295. Each bar shows the average postprandial blood glucose level of six rats in the group.

(1 gm/kg) was employed in the mentioned study, and a different concentration of ethanol (50%, 25% and 0%) was utilized for the extract preparation[15]. Lee and others reported the ethanolic extract to exert a similar effect on the fasting blood glucose levels of the streptozotocin-induced diabetic murine models; where 50, 100, and 150 mg/kg doses of the ethanolic extract reached statistical significance compared to the control[16].

The fasting blood glucose data over the course of the experiment is depicted in Graph 2.

***G. procumbens* extract reduces postprandial blood glucose of diabetic animals**

The postprandial blood glucose data of the experimental animals followed a similar pattern as the fasting blood glucose data. Prior to alloxan administration, the postprandial blood glucose of all experimental animals displayed a mean value of 6.019 ± 0.66 mmol/L. Administration of alloxan resulted in this increasing to a mean value of 12.166 ± 3.19 mmol/L. GP treatment brought this data down, and statistical significance was achieved



Graph 4. GLUT-4 expression level in the liver tissue of the experimental animals. GP 250= Diabetic rats treated with 250 mg/kg *G. procumbens* extract, GP 500= Diabetic rats treated with 500 mg/kg *G. procumbens* extract, GP 750= Diabetic rats treated with 750mg/kg *G. procumbens* extract, MET 500= Rats treated with 500 mg/kg Metformin, NORM= Normal rats treated with physiological saline, DIA= Diabetic rats treated with physiological saline. The one-way ANOVA test was used to assess the statistical significance of the differences between the groups, as the data was considered suitable for this test. *Indicates $p<0.05$, **indicates $p<0.01$ and ***indicates $p<0.005$ level of statistical significance, while ns indicates no significance. The P values for the statistical tests are: *[1]= 0.0353, ***[2]< 0.0001, ***[3]< 0.0001, *[4]= 0.0126, **[5]= 0.0014, ns[6]= 0.3329. Each box and whisker plot indicates the expression level of GLUT-4 in each gram of liver tissue of six rats in the group.

for each GP test group compared to the diabetic control. Only the differences between the GP 250 group and the metformin control group and the normal control group were deemed statistically significant, the other GP treatment groups displayed no significant differences with the two aforementioned groups. The ethanolic extract was previously communicated to lower postprandial blood glucose in streptozotocin-induced diabetic rat models, although the 150 mg/kg dose, not the higher 300 mg/kg dose, was considered to yield the most statistically significant effect[17]. The difference in observations in the mentioned study and the current study may be owed to the different concentrations of the solvents used for extraction (95% vs 70%), as well as other geo-ecological factors that may have contributed to changes in plant matter composition. Further analysis in this regard is warranted. The postprandial blood glucose data of the experimental animals over the course of the experiment is depicted in Graph 3.

***G. procumbens* extract stimulates GLUT-4 expression in diabetic animals.**

We observed that the concentration of the GLUT-4 protein in the liver tissue of the diabetic control group decreased to a level lower than that observed in the other groups, although no statistical significance was reported between the normal control and the diabetic control group data. All three GP test groups and the metformin control group exhibited higher GLUT-4 protein in the liver tissue compared to the diabetic control group ($p < 0.05$). Metformin has also been previously observed to upregulate GLUT-4 expression in murine metabolic disorder models[18]. Surprisingly, GP 750 group also showed expression of GLUT-4 higher than the normal control group. The GLUT-4 expression level of the experimental animals is presented in Graph 4.

Previous studies have hypothesized that *G. procumbens* exerts its antidiabetic effects by enhancing glucose uptake[13]. Sathiyaseelan and others reported that the ethyl acetate fraction of the methanolic extract enhanced glucose uptake in hepatic cell line HepG2, an incidence that can be explained by an increased expression level of the GLUT-4 protein[19]. Moreover, in murine cell line 3T3-L1, the ethanolic extract increased GLUT-4 expression significantly[20]. The serum-glucose modulatory antidiabetic activity of ethanolic extract of *G. procumbens* is therefore mediated though the upregulation of GLUT-4 expression, and consequent increased uptake of glucose.

CONCLUSION

The study demonstrated that *Gynura procumbens* leaf extract exhibits significant antidiabetic activity, with a dose-dependent effect supporting its therapeutic value. In alloxan-induced diabetic rats, *G. procumbens* effectively restored body weight and reduced fasting and postprandial blood glucose levels, comparable to metformin. The increase in GLUT-4 expression in diabetic rats, as evidenced by ELISA assay, further supports the potential mechanism of action of *G. procumbens* in diabetes management. These findings highlight *G. procumbens* as a promising candidate for antidiabetic drug development. Additional studies are necessary to isolate, characterize, and determine which secondary metabolites are accountable for the antidiabetic effects observed.

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Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability

This statement does not apply to this article

Ethics Statement

This study has been approved (Ref. no. 146/Biol. Scs.) by the Ethical Review Committee of the Faculty of Biological Sciences, University of Dhaka.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required

Clinical Trial Registration

This research does not involve any clinical trials

Author's contribution

Md. Shah Amran: Originating the concept, Supervision of the overall work; Juhaer Anjum: Literature study, Labwork, Manuscript writing; Maniza Muni: Literature study, Labwork, Manuscript writing; Nusrat Jahan Shawon: Labwork, Manuscript reviewing and editing; Md. Mehadi Hasan: Labwork, Manuscript reviewing and editing; Mohammad Sofi: Labwork, Manuscript reviewing and editing; Md. Rafat Tahsin: Critically reviewed the whole activities; Md Reaz Uddin: Critically reviewed the whole activities; Fahima Aktar: Critically reviewed the whole activities; Abu Asad Chowdhury: Critically reviewed the whole activities; Jakir Ahmed Chowdhury: Critically reviewed the whole activities; Shaila Kabir: Critically reviewed the whole activities; Md. Al Amin Sikder: Critically reviewed the whole activities.

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