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Effects of *Gynura procumbens* leaf-based meal on glucose level, lipid profile and mineral content of alloxan-induced diabetic mice

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Abstract

Gynura procumbens leaves are used as an anti-diabetic medicinal plant in Southeast Asia. A 28-day feeding trial was conducted to evaluate the effects of *Gynura procumbens* (GP) leaf meal on growth performance, glucose level, lipid profile and mineral content in alloxan-induced diabetic mice. A total of thirty-six mice at 14 days old were assigned to four treatment groups: NC (Normal Control), T_0 = Control (Basal diet), T_1 = (Basal diet + 0.5% Dry leaf on DM basis), T_2 = (Basal diet + 1.0% Dry leaf on DM basis) having three replications consisting of nine mice in each in a completely randomized design. The results showed that glucose level was significantly ($P<0.01$) reduced during the last 3 weeks compared to control of alloxan-induced mice. Three weeks of dietary supplementation of GP leaf at 0.5% and 1.0% levels significantly ($P<0.05$) increased body weight during the second and the third week whereas decreased, feed intake and water intake of alloxan-induced diabetic mice. A significant reduction of cholesterol, triglycerides (TG), low density lipoproteins (LDL) while increased high density lipoproteins (HDL) of induced mice compared to control ($P<0.001$). There were no significant variations in blood phosphorus, Ca and total protein. Moreover, in the proximate CHO, crude protein, crude fat increased while ash, moisture, crude fibre decreased in treatment groups compared to the basal diet. Overall, GP leaf showed trustworthy results on anti-diabetic effects included lipid profile.

1. Introduction

Diabetes is a lifelong metabolic disease and one of the non-communicable diseases (NCDs) that make the greatest contribution to morbidity and mortality worldwide is characterized by means of improved glucose levels in the blood due to insulin action (American Diabetes Association, 2004). Insulin, which is synthesized through β-cells of the pancreas, helps in keeping glucose concentration normal in the blood (Ahmad, 2014). Hence, insulin resistance leads to a flip pathway of glucose metabolism in the body causing diabetes mellitus (Kahn *et al*., 2014). Environmental, genetic factors, way of life changes and excessive fat, and weight loss program consumption (Ozougwu *et al*., 2013) are also accountable for diabetes mellitus. According to WHO 2016, about 422 million people had diabetes globally, where 70% of people live in low-middle income countries and Bangladesh is one of them. In Bangladesh, type 2 diabetes is assorted from 4.5% to 35% and increases healthcare use, and expenditure and imposes a big monetary burden on the healthcare system. Bangladesh used to be ranked as the 8th best possible diabetic populous (13.7 million) country in the time length of 2010-2011. About 129,000 deaths have been attributed to diabetes in Bangladesh in 2015, as pronounced with the aid of the main lookup company International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) (Mohiuddin, 2019). In the modern day, many anti-diabetic drugs have been produced by way of chemical/biochemical agents, but cures with anti-diabetic tablets are costly. Hence, lookup focuses on medicinal plant research for the treatment of diabetes.

Gynura procumbens (Lour.) Merr (family

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Compositae) (Figure 1), also regionally known as "SambungNyawa" is viewed to be beneficial for hypertension, anti-inflammation, anti-herpes simplex virus, prevention of rheumatism, and therapy of eruptive fevers, kidney troubles, colon cancer, haemorrhoids and diabetes (Perry and Metzger, 1980). It was found that 25% ethanolic extract of GP gave the best result in lowering blood glucose levels. It was also determined that the GP extract stimulates the metformin activity (Algariri *et al*., 2013) by elevating glucose consumption in the muscle cells (Zurina *et al*., 2010). Based on these findings, the objectives of this study were to observe the effects of GP leaf meal on glucose levels, growth performance and lipid profile in diabetic-induced mice. The research was also performed for taking the leaf as the easiest way to control blood sugar and lipid profile.

Figure 1. *Gynura procumbens*.

2. Materials and methods

2.1 Study area and climate condition

The study was carried out at Chattogram Veterinary and Animal Sciences University, Khulshi, Chattogram, Bangladesh. The experimental shed was used for animal trials including analysis under the Department of Animal Science and Nutrition and different analyses was carried out in the Department of Physiology, Biochemistry and Pharmacology. The standard research protocol was used to carry out from March 2019 to July 2019. The weather of the Chattogram was not too warm or too cold (30°C to 40°C). The humid summer season started in March and at the end of the experiment, the climate was rainy. The animal trial had to conduct avoiding excessive climate conditions.

2.2 Preparation of leaf meal

Fresh, mature healthy GP leaves were collected and sorted to reject unwanted and insect-affected leaves. Then they were washed and left at room temperature for 2-3 days. The washed leaves were dried in a cabinet drier at 60°C for three days. The dried leaves had been grounded in a clean blender and formed coarse powder. After that, the course leaves powder was sieved using a sieve of 80 µm mesh size to acquire quality leaves powder and kept at 25°C in an airtight container.

2.3 Layout of the experiment

A whole of thirty-six (divided into 4 groups with 9 animals each) (Table 1) female healthy laboratory Swiss albino mice weighing between 23-27 g was bought from the animal residents of the Department of Pharmacy, Jahangir Nagar University, Bangladesh. The study had the approval of the CVASU Institutional Animal Ethical Committee and research had been carried out with scrupulous recommendations for the care of laboratory animals. For the experiment, thirty-six mice were randomly distributed in a completely randomized design with the following treatments: NC as Normal Control, T_0 as Diabetic control, T_1 (basal diet with 0.5% dry leaf supplement), T_2 (basal diet with 1.0% dry leaf supplement). All dietary treatments had been given orally for three weeks.

2.4 Management and feeding

2.4.1 Preparation of the shed

Extra care was given to the experimental animal. It was cleaned, washed and then disinfected by spraying phenyl solution. The shed was dried out and then fumigated using formalin and potassium permanganate and sealed for 24 hrs. Lime was spread around the shed to maintain strict biosecurity of the shed. Feeders and drinkers were cleaned using water, and detergent followed by 0.3% potassium permanganate solution.

Table 1. Layout of the experiment.

Dietary treatment group	Replication	No. of mice per replication	No. of mice per treatment
NC (Normal Control)	R1	3	9
	R2	3	
	R3	3	
T_0 = Diabetic Control (Basal diet)	R1	3	9
	R2	3	
	R3	3	
T_1 = Basal diet + 0.5% dry leaf powder	R1	3	9
	R2	3	
	R3	3	
T_2 = Basal diet + 1.0% dry leaf powder	R1	3	9
	R2	3	
	R3	3	

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Personal hygiene was maintained properly by using a face mask, hand gloves, footwear and clothing. Strictly maintained everything to avoid contamination.

2.4.2 Housing

The mice house was well-ventilated, wire-floored, closed cages. Room humidity was 30-70% and the temperature was 18-26°C. Each cage was solid-bottom caging with wood-shaving bedding. Each cage was provided with a drinker to ensure *ad-libitum*. Standard laboratory conditions with 12 hrs light: 12 hrs dark cycle were maintained.

2.4.3 Feeding and watering

The mice were supplied with self-made feed which is defined as a basal diet. The supplements were mixed uniformly with the feed before feeding to the mice. In Control, only a basal diet was offered. In treatment groups, 0.5% dry leaf supplement (T_1), 1.0% dry leaf supplement (T_2) dry matter basis were mixed with a basal diet. Feed and water were supplied ad-libitum to all groups of mice at two different times in a day (10.00 am and 6.00 pm) throughout the experimental period. Cucumber slice was added to fill up their water balance during the dry season. Mice were acclimatized for 7 days before the commencement of the study. For the first week, the basal diet was offered to all. Fresh drinking water was supplied to the mice at free choice. The body weights and fasting blood glucose levels of the mice were recorded every week during the experiment period. Feed and water consumption were also recorded during the study period. The basal diet is presented in Table 2.

Table 2. Ingredients used in basal diet.

Composition of diet	Quantity (%)
Wheat flour	26.30
Wheat bran	19.00
Fish meal	10.00
Mustard oil cake	4.00
Maize powder	22.00
Rice police	3.00
Milk powder	13.06
Full fat soy	12.00
Soybean oil	6.00
Salt	0.25
Choline chloride	0.10
Enzyme	0.01
Vitamin mineral premix	0.25
DCP	1.00
Soybean meal	12.00

2.5 Induction of diabetes

Selected mice (twenty-seven mice) were kept overnight and fasted for making diabetic were intraperitoneally administered alloxan monohydrate (150 mg/kg body weight) dissolved in ice-cold saline (0.9% NaCl). To prevent hypoglycemia, the animals were given 5% glucose solution for the next 24 hrs. After 4 days of observation blood glucose level was figured out by using Glucometer. Mice having more than the 3-4-fold increase in their blood glucose level were considered diabetic (Tao Bu *et al*., 2012).

2.6 Feed intake

Feed intake is determined by subtracting the refusal feed collected every morning before supplying feed from the weighed feed provided to the birds for *ad-libitum* feeding. The average daily feed intake was calculated using the formula:

Average daily feed intake = (Weight of supplied feed- Weight of refused feed) / Number of mice.

2.7 Collection of blood samples

At the end of the experiment, blood samples were collected by cardiac puncture from overnight fasted anaesthetized (by diethyl ether) animals. Serum was separated from blood after 40 to 60 mins by centrifugation at 3500 rpm for 10 mins. Obtained serum samples were stored at -20°C until analysis.

2.8 Biochemical tests

The blood of three mice from each replication was collected and serum was separated from the blood. The separated serums were stored in a freezer at –20°C and from these different biochemical tests such as total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) were determined using a biochemical analyzer (Semi Automatic Erba Chem 5×, India) in the Post Graduate laboratory of Department of Animal Science and Nutrition by following the directions supplied with the kits (Randox® Laboratories Limited, UK). The low-density lipoprotein (LDL) levels were calculated according to the formula (Friedewald *et al*., 1972):

LDL = Total Cholesterol − (HDL+ Triglyceride/5)

2.9 Proximate analysis of basal diet

From the stored samples, chemical analysis was carried out in according to AOAC (2006). The analysis of proximate components was carried out in Animal Nutrition Laboratory under the Department of Animal Science and Nutrition, CVASU, to determine the Dry Matter (DM), crude protein (CP), crude fibre (CF) and ash. Total carbohydrate contents were determined by subtracting method:

% Carbohydrate = 100 – %(Protein + Moisture + Fiber +

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Fat + Ash)

2.9.1 Moisture content

The per cent of moisture was calculated by the following formula:

%Moisture = (W1-W2) × 100/ wt. of sample.

Where W1 = Initial weight of crucible + Sample and W2 = Final weight of crucible + Sample. For the determination of ash, a clean empty crucible was placed in a muffle furnace at 600°C for an hr, cooled in a desiccator and then the weight of the empty crucible was noted (W1). Each sample (1 g) was taken in a crucible (W2). The sample was ignited on a fibertherm heater until it is charred. Then the crucible was placed in a muffle furnace at 550°C for 2-4 hrs. The appearances of grey-white ash indicate the complete oxidation of all organic matter in the sample. After the ashing furnace was switched off. The crucible was cooled and weighed (W3). Per cent ash was calculated by the following formula:

% Ash = Difference wt. of ash ×10/wt. of sample

Difference in wt. of Ash = W3-W1

2.9.2 Crude protein content

Crude protein in the sample was determined by the Kjeldahl method. The samples were digested by heating with concentrated sulphuric acid (H_2SO_4) in the presence of a digestion mixture and made alkaline. Ammonium sulphate thus formed, released ammonia which was collected in a 2% boric acid solution and titrated against standard HCl. Crude protein was calculated by multiplying the amount of nitrogen with the appropriate factor (6.25) and the amount of protein was calculated. 0.5-1.0 g of dried samples were taken in a digestion flask. Add 10-15ml of concentrated H_2SO_4 and 8 g of digestion mixture i.e. K_2SO_4: $CuSO_4$ (8: 1). The flask was swirled in order to mix the contents thoroughly then placed on a heater to start digestion till the mixture become clear (blue-green in colour). It needs 2 hrs to complete. The digest was cooled and transferred to a 100 mL volumetric flask and the volume was made up to mark by the addition of distilled water. Distillation of the digest was performed in Markam Still Distillation Apparatus. Ten millilitres of digest were introduced in the distillation tube then 10 mL of 0.5 N NaOH was gradually added in the same way. Distillation was continued for at least 10 mins and NH_3 produced was collected as NH_4OH in a conical flask containing 20 mL of 4% boric acid solution with a few drops of modified methyl red indicator. During distillation yellowish colour appears due to NH_2OH. The distillate was then titrated against standard 0.1 N HCl solution till the appearance of pink colour. A blank was also run through all steps as above. Per cent crude protein content of the sample was calculated by using the following formula:

% Crude Protein = 6.25* × %N (*Correction factor)
% Protein = (S-B)×N×0.014×D×100 / wt. of the sample
Where S = Sample titration reading, B = Blank titration reading, N = Normality of HCl, D = Dilution of sample after digestion, V = Volume taken for distillation, 0.014 = Milli equivalent weight of Nitrogen.

2.9.3 Ether extract

A dry extraction method for fat determination was implied. It consisted of extracting dry samples with some organic solvent since all the fat materials e.g. fats, phospholipids, sterols, fatty acids, carotenoids, pigments, chlorophyll and more are extracted together therefore, the results are frequently referred to as crude fat. Fats were determined by intermittent soxhlet extraction apparatus. Crude fat was determined by ether extract method using the Soxhlet apparatus. Approximately 1 g of moisture-free sample was wrapped in filter paper, placed in a fat-free thimble and then introduced in the extraction tube. Weighed, cleaned and dried the receiving beaker was filled with petroleum ether and fitted into the apparatus. Turned on the water and heater to start extraction. After 4-6 siphoning the ether was allowed to evaporate and was disconnected from the beaker before the last siphoning. The extract was transferred into a clean glass dish with ether washing and evaporated in a water bath. Then the dish was placed in an oven at 105°C for 2 hrs and cooled in a desiccator. The per cent crude fat was determined by using the following formula:

%Crude Fat = wt. of ether extract × 100 / Wt. of sample

2.9.4 Crude fibre content

Weighed 0.153 g sample (W0) transferred it to the porous crucible and placed the crucible into the fibre unit and kept the valve in the “OFF” position. After 150 mL of preheated H_2SO_4 solution was added and a few drops of foam-suppresser to each column. Then the cooling circuit was opened and the heating elements were turned on (power at 90%). When it started boiling power was reduced to 30% and left for 30 mins. Valves were opened for drainage of acid and rinsed with distilled water thrice to completely ensure the removal of acid from the sample. The same procedure was used for alkali digestion by using KOH instead of H_2SO_4. The sample was dried in an oven at 150°C for 1 hr. Then allowed the sample to cool in a desiccator and weighed (W1). Kept the sample crucibles in a muffle furnace at 55°C for 3-4 hrs. Cooled the samples in a desiccator and weighed again (W2).

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% Crude Fiber = (W1-W2)×100 / W0

2.10 Statistical analysis

All statistical analysis was completed by the use of a statistical package for social sciences (SPSS) model 16. A one-way analysis of variance was used to evaluate the data. Data are presented as the mean ± SD (Standard Deviation). Differences in mean have been compared using the Tukey test. P-values≤0.05 had been considered significant.

3. Results

The yield from the GP leaves was 100 g/kg. The chemical composition of GP leaves is represented in Figure 2.

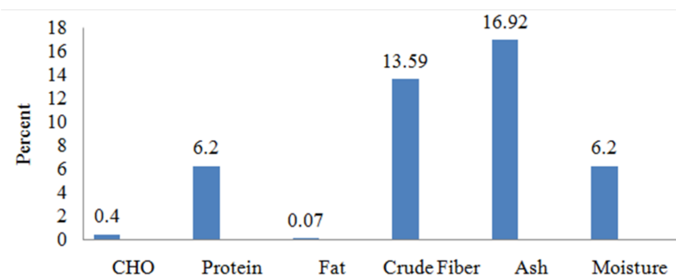

Figure 2. The graph shows that the chemical composition of GP leaves.

3.1 Effects of Gynura procumbens leaves on blood glucose levels in mice

Fasting blood glucose was measured in mice every week (Table 3). A distinct boost in blood glucose level was observed in all samples induced by alloxan-monohydrate. The fasting blood glucose level was around 4 mmol/L in all groups at the initial stage. The glucose concentration in blood, however, decreased from 7.6±0.25 to 5.13±1.05 mmol/L in different groups after alloxan induction. Moreover, the hypoglycemic effect in the diabetic control group changed significantly (P<0.001) every week compared with the normal control. T_0 which was the diabetic control group demonstrated a higher blood glucose level for using only basal diet. But T_1 and T_2 were the treatment group where dry leaf powder was used to see the effectiveness of diabetes.

3.2 Effects on body weight of mice

The average body weight of all mice in various groups was about 25g at the beginning of the experiment. The weight of normal control mice continued to increase evenly and the diabetic control group lost weight consistently to the end of the experiment as shown in (Table 4). In the first week, no significant variation was noted between the NC and other treatment groups. However, at the end of the experiment, all mice under treatment exhibited a significant (P<0.001) increase in body weight in contrast to diabetic control.

3.3 Food and water consumption of alloxan-induced diabetic mice

Food intake per day among different groups showed considerable variation (Figure 3). The NC mice consumed around 4.6 g/day of food whereas the consumption rate in T_0 was statistically significantly higher (P<0.001) at 11.1 g/day. However, the food intake was significantly lower (P<0.001) and at T_1 and T_2 at 7.8 g/day and 8.06 g/day food respectively in contrast to the

Table 3. Effects of GP leaf on blood glucose level.

Parameters	Blood glucose (mmol/L)				P-value
	NC	T_0	T_1	T_2	
Initial	4.06±0.47	6.1±0.55	5.13±1.05	5.4±1.05	0.083
Week 0	4.10±0.50^{b}	6.0±0.75^{a}	6.23±0.55^{a}	5.5±0.83^{a}	0.006
Week 1	4.06±0.25^{c}	7.4±0.26^{a}	5.3±0.50^{b}	5.9±0.51^{b}	<0.001
Week 2	4.4±0.15^{c}	7.6±0.25 ^{a}	5.5±0.58^{b}	5.9±0.60^{b}	<0.001
Week 3	4.3± 0.45^{c}	7.2±0.20^{a}	6.06±0.51^{b}	6.5±0.28^{ab}	<0.001

Values are presented as mean±SD. Values with different superscripts within the same row are significantly different. NC: Normal control, T_0: Diabetic Control without treatment, T_1: 0.5% GP leaf (Basal diet + 0.5% GP leaf on DM basis), T_2: 1.0%GP leaf (Basal diet + 1.0 % GP leaf on DM basis).

Table 4. Effects of GP dry leaves on body weight of mice.

Body weight (g)	Treatments				P-value
	NC	T_0	T_1	T_2	
Week 0	24.97±1.2	25.53±1.1	25.57±0.6	25.46±1.2	0.63
Week 1	26.64±1.2^{a}	24.62±1.5^{b}	25.14±0.9^{b}	24.95±1.2^{b}	<0.001
Week 2	29.12±1.13^{a}	23.00±1.5^{c}	26.22±1.2^{b}	25.35±1.1^{b}	<0.001
Week 3	31.46±1.5^{a}	21.94±1.4^{c}	27.66±1.7^{b}	26.60±1.3^{b}	<0.001

Values are presented as mean±SD. Values with different superscripts within the same row are significantly different. NC: Normal control, T_0: Diabetic Control without treatment, T_1: 0.5% GP leaf (Basal diet + 0.5% GP leaf on DM basis), T_2: 1.0%GP leaf (Basal diet + 1.0 % GP leaf on DM basis).

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diabetic group (Figure 3).

The normal control group drank only 5.7±1.3 mL/day of water, which was statistically significant ($P<0.05$) in contrast to diabetic control groups (13.5±0.8 mL/day) (Figure 4). All other alloxan-induced groups - T_1 (8.3 mL/d) and T_2 (9.3 mL/d) consumed a considerably lower amount of water when compared to non-treated diabetic control groups.

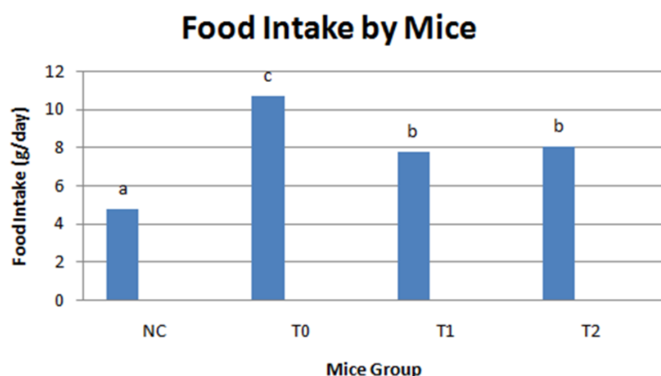

Figure 3. Food intake by mice. Values are presented as mean±SD. Bars with different superscripts are significantly different. NC: Normal control, T_0: Diabetic Control without treatment, T_1: 0.5% GP leaf (Basal diet + 0.5% GP leaf on DM basis), T_2: 1.0%GP leaf (Basal diet + 1.0 % GP leaf on DM basis).

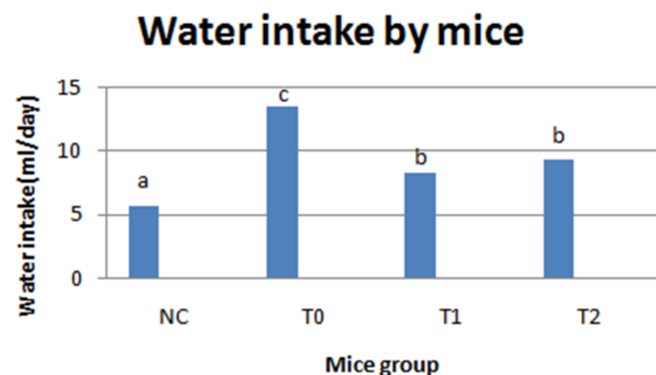

Figure 4. Water intake by mice. Values are presented as mean±SD. Bars with different superscripts are significantly different. NC: Normal control, T_0: Diabetic Control without treatment, T_1: 0.5% GP leaf (Basal diet + 0.5% GP leaf on DM basis), T_2: 1.0%GP leaf (Basal diet + 1.0 % GP leaf on DM basis).

3.4 Blood lipid profile of mice

High density lipoprotein (HDL) decreased as well as total cholesterol, Triglycerides, and Low-density lipoprotein (LDL) levels increased significantly in diabetes-induced groups compared to the normal control group. It is also apparent from the present study that mucilage as well as treatment groups significantly ($P<0.001$) decreased the HDL level and reduced the cholesterol, triglycerides, and LDL levels in alloxan-induced diabetic mice when compared with the diabetic control group (Table 5).

3.5 Mineral contents and total protein of blood

Mineral content analysis was done by blood samples of mice. Phosphorus, Ca and TP values were non-significant. In the present study, decreased total protein was observed in diabetic control mice than in normal control mice. However, Ca level was increased at T_1, phosphorus level was similar to diabetic control mice (Table 6).

3.6 Proximate analysis

The result appears that the moisture percentage differs significantly among all the dietary groups (Table 7). In Basal Diet, there was a significant value in crude protein which was observed in all dietary groups compared to others ($P<0.01$). The highest value was (20.96±1.3) at T_0. The crude fat decreased in T_1. The total ash content possessed significant change among all dietary groups.

4. Discussion

The results of this study confirm that GP leaf powder can ameliorate diabetes mellitus of alloxan-induced diabetic mice, as assessed by fasting blood glucose level, body weight, food, and water consumption. At the end week, the result of blood glucose was similar in the treatment groups and the control group showed a higher level of blood glucose because of not controlling diabetes. The efficiency of hypoglycemic activity in mice may be due to the ability of the GP powder meal to prevent free radicals which is the major cause of alloxan-

Table 5. Effects of *G. procumbens* on lipid profile of mice.

Parameters (mg/dl)	Treatments				P-value
	NC	T_0	T_1	T_2	
Cholesterol	114.07±3.09^c	169.7±3.55^a	137.07±3.09^b	132.5±2.96^b	<0.001
TG	112.4±2.08^d	175.5±3.61^a	146.4±2.90^b	135.5±4.37^c	<0.001
HDL	80.10±1.45^a	36.43±0.60^d	45.96±4.14^c	54.43±4.56^b	<0.001
LDL	11.48±1.66^d	98.14±3.83^a	61.81±2.07^b	51.02±4.62^c	<0.001

Values are presented as mean±SD. Values with different superscripts within the same row are significantly different. NC: Normal control, T_0: Diabetic Control without treatment, T_1: 0.5% GP leaf (Basal diet + 0.5% GP leaf on DM basis), T_2: 1.0%GP leaf (Basal diet + 1.0 % GP leaf on DM basis).

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Table 6. Effects of GP leaf on blood total protein and mineral content in mice.

Parameters (mg/dl)	Treatments				P-value
	NC	T_0	T_1	T_2	
Phosphorus	3.5±0.9	3.1±0.4	3.8±0.9	3.1±0.3	0.60
Calcium	8.73±0.7	9.2±1.2	10.15±0.9	9.2±0.4	0.16
Total protein	5.8±0.3	4.7±0.5	4.8±0.7	4.5±1.01	0.34

Values are presented as mean±SD. Values with different superscripts within the same row are significantly different. NC: Normal control, T_0: Diabetic Control without treatment, T_1: 0.5% GP leaf (Basal diet + 0.5% GP leaf on DM basis), T_2: 1.0%GP leaf (Basal diet + 1.0 % GP leaf on DM basis).

Table 7. Effects GP leaves on feed chemical composition.

Parameters (%)	Treatments			P-value
	T_0	T_1	T_2	
CHO	31.83±0.15^a	25.83±0.80^b	23.50±0.70^c	<0.001
Crude Protein	20.96±1.3^a	17.13±0.60^b	18.13±0.60^b	<0.001
Crude Fat	1.60±1.6^a	0.07±0.005^c	0.83±0.50^b	<0.001
Ash	10.14±0.16^c	11.20±0.17^b	12.36±0.11^a	<0.001
Moisture	18.29±0.16^a	11.16±0.15^c	12.23±0.20^b	<0.001
Crude Fibre	5.75±0.17^c	7.79±0.05^b	8.60±0.06^a	<0.001

Values are presented as mean±SD. Values with different superscripts within the same row are significantly different. NC: Normal control, T_0: Diabetic Control without treatment, T_1: 0.5% GP leaf (Basal diet + 0.5% GP leaf on DM basis), T_2: 1.0%GP leaf (Basal diet + 1.0 % GP leaf on DM basis).

induced hyperglycemia. This theory is also supported by the health condition of diabetic control mice. By inhibiting the glucose sensor of beta-cell referred to as glucokinase, alloxan impedes the secretion of glucose-induced insulin. Simultaneously, alloxan initiates a redox cycle to form superoxide radicals which undergo the method of dismutation to make hydrogen peroxide causing the formation of highly reactive hydroxyl radicals by Fenton reaction resulting in the death of beta cells and establishing the condition of insulin-dependent diabetes (Szkudelski, 2001). Alloxan induction in mice, within the current study, also exhibited typical visible features of diabetes mellitus including weight loss, polydipsia (excessive thirst) and polyphagia (excessive hunger). One of the parameters to consider in the amelioration of a diabetic state is to ascertain the effect of treatment on body weight (Al-Attar and Zari, 2010). In diabetes mellitus, deranged glucagon-mediated regulation of cyclic adenosine monophosphate (AMP) formation in insulin deficiency leads to accelerated proteolysis (Rajasekaran *et al*., 2005). Since structural and tissue proteins contribute to 30 to 40% of total body weight, the excessive breakdown of tissue proteins due to diminished insulin response as well as the unavailability of carbohydrates for energy metabolism in diabetes mellitus results in decreased body weight. Normalization of carbohydrate, protein and fat metabolism would alleviate the diabetic symptom of body weight loss; therefore body weight holds one of the keys to evaluating the effectiveness of an anti-diabetic treatment (Al-Attar and Zari, 2010). In the present study, treatment of diabetic mice with *G. procumbens* meal showed a decrease in body weight loss, which indicates the prevention of muscle tissue damage and protein wasting that is due to a hyperglycemic condition in ameliorating diabetic state in alloxan-induced diabetic mice. Normal mice and control groups gained weight in the last week compared to the treatment groups. It was found that there was a numerical decrease in feed intake among treatment groups compared to the control which was in agreement with Oloruntola *et al*. (2018) observed the numerical reduction in feed intake. A significant decrease in serum cholesterol, triglyceride and LDL in the treatment groups compared to the control. However, serum HDL levels increased in all treatment groups relative to the control (Karthikesan *et al*., 2010). This finding might cause the reduction in blood pressure and return to the normal range in atherosclerosis (Ruano *et al*., 2005). No significant variation was found in the blood phosphorus, calcium and total protein content of this experiment.

Proximate analysis of basal diet and GP leaf meal showed lower content of carbohydrate, crude protein, and crude fat while increased ash and crude fibre content compared to control. Previous studies (Puangpronpitag *et al.,* 2010) had a few pieces of research on the extract of *G. procumbens* and reported similar findings to this study.

5. Conclusion

In Bangladesh, diabetes is one of the most prevalent diseases which costs high and is mostly involved in medicine and hospital costs. This can be optimized by changing the lifestyle and utilization of herbal plants which will minimize the cost. *Gynura procumbens* is a

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wonderful plant with high medicinal value. The leaf meal has a good effect on the reduction of blood glucose of the alloxan-induced mice by lowering blood cholesterol, LDL and triglyceride level. It is a good indication for diabetic Mellitus patience who can control or reduce blood glucose levels by consuming this leaf meal or powder. *Gynura procumbens* is also rich in fibre. The standard value of calcium, phosphorus and total protein level found in the blood in treatment groups compared to the control group. Hence, results obtained from this study justify the traditional use of GP leaf in the treatment or management of diabetes in Bangladesh and the world as well.

Conflict of interest

The authors declare no conflict of interest.

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