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Research Article

Evaluation of Medicinal Effects of *GynuraProcumbens* Leave Extracts On Oxidative, Glycemic, Lipidomics, and Enzymatic Profiles in Alloxan-Induced Diabetic Mice

M. Ziaul Amin[1]*, Mitu Afrin[1], Md. Ashaduzzaman Nur[1], Md. Mashiar Rahman[1], M. Jashim Uddin[2]

[1]Department of Genetic Engineering and Biotechnology, Jashore University of Science and Technology, Jashore-7408, Bangladesh; [2]Department of Pharmacy, Jashore University of Science and Technology, Jashore-7408, Bangladesh

ABSTRACT

To evaluate the medicinal impact of G. *procumbens*, this study designed to explore the anti-oxidant, anti-diabetic, anti-lipidemic, and enzyme activities of G. *procumbens* leaves extract.

Alloxan induced diabetic mice used for anti-diabetic activity. Blood was collected and analyzed glucose level, lipid profile, hepatic performance, renal activity, and oxidative stress emblem with different hours (1-5 hours) and days (7th -28th days) interval using standards protocol. Bioactive compounds like alkaloid, tannin, phenol, flavonoid, and terpenoid were present in the extracts. Anti-oxidant activities were evaluated at concentration of 15, 20, 25, 30, 35, 40 and 45 µg/mL but aqueous extracts showed very significant free radical scavenging activity at concentration 40 and 45 µg/mL, (**p>0.001). Both the aqueous and ethanolic extracts (GPLAE-200 and GPLEE-200) exhibited a remarkable lowering of blood glucose level, compared to the control mice. Anti-diabetic results reveal that GPLEE-200 could be applicable for acute but GPLAE-200 for chronic treatment for diabetes mellitus. Aqueous extracts of G. *procumbens* have significant effects on cholesterol, triglycerides (TG), HDL and LDL level but GPLEE-200 have prominent effects on lowering LDL level. GPLAE-200 exhibit significant lowering effects of SGPT, SGOT, ALP (Alkaline phosphatase) and creatinine level. whereas; GPLEE-200 has prominent lowering effects on SGOT and creatininelevel. This study may suggest that treatment of the extracts was dose-dependent, and aqueous extract of G. *procumbens* could be a promising resource for better treatment of diabetes.

Keywords: Phytochemicals; Aqueous extracts; Scavenging; Triglycerides; Cholesterol; Alkaline phosphatase

Abbreviations: GPLAE-200: Gynuraprocumbens leave aqueous extract 200mg/dl; GPLEE-200: Gynuraprocumbens leave ethnolic extract 200mg/dl; GPLAE-100: Gynuraprocumbens leave aqueous extract 100mg/dl; GPLEE-100: Gynuraprocumbens leave ethnolic extract 100mg/dl; DMSO: Dimethylsulfoxide; DPPH : 2, 2-diphenyl-1-picryl-hydrazyl; NCM: Normal control mice; ADM: Alloxan-induced diabetes mice; M-15: 15 mg/kg b.w of Metformin treated mice; G-15: 15 mg/kg b.w of Glibenclamide treated mice; TG: Triglyceride; HDL: High density lipoprotein; LDL: Low density lipoprotein; SGPT: Serum glutamate pyruvate transaminase; SGOT: Serum glutamta oxalate transaminase; ALP: Alkaline phosphatase.

INTRODUCTION

The chronic elevation of blood glucose levels is the characteristic feature of hyperglycemia called diabetes mellitus. Within 20 years, the incidence of diabetes alleged to rise doubled, whereby 439 million people would be diabetic patients, which is 7.7% of the global mass of people by the year 2030 [1]. Therefore, it could be a global challenge to protect a large volume of the world population in third world countries like Bangladesh from diabetes. Thus, the treatment of diabetes should need special attention. Many strategies have developed for the therapies of diabetes in the past decades to lower the blood glucose level. To date, oral anti-diabetic drugs: α-glucosidase inhibitors, sulfonylureas, biguanides, dipeptidyl peptidase-4 inhibitors, meglitinides, and thiazolidinediones have utilized for the therapies of diabetes mellitus. Each oral administration of anti-diabetic drug acts on a diverse process of action, including delaying intestinal digestion and absorption of carbohydrate, stimulation of insulin secretion, increasing

***Corresponding to**: M. Ziaul Amin, Department of Genetic Engineering and Biotechnology, Jashore University of Science and Technology, Jashore-7408, Bangladesh, Tel: +8801726034288; Fax : +88-0421-6119; E-mail:ziaulamin@just. edu.bd

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peripheral insulin sensitivity, reduction of hepatic gluconeogenesis, and increasing endogenous levels of hormones, respectively [2]. Unfortunately, these drugs produce some severe adverse effects like gaining weight, the deficit of vitamin B12, hypoglycemia, edema, lactic acidosis, digestive problems, pancreatitis, and bone fractures [3,4]. Thus, the treatment of diabetes needs novel strategies and drugs that effectively cope with diabetes without any undesirable effects. The herbal remedies originated from plant products that grow tremendous attention as alternative therapies that have used anticipated periods for the therapeutic of diabetes [5]. Approximately 90% of the rural peoples of the third-world country depend mainly on ethnomedicine for curing diseases and primary health care. The usage of this plant by different ethnic people proved this plant as the disease curative and preventive plant. The ethnomedicinal use of the leaves of this plant is well known for cancer, hypertension, hyper-lipedema, and diabetes [6].

Oxidation is playing a vital role in living organisms. It has noted that many diseases, namely diabetes, coronary heart diseases, Alzheimer's disease, cancer, and aging caused by the uncontrolled formation of oxygen free radicals and impairments of the antioxidant activity [7]. Since antioxidants reduced oxidative damage, antioxidants are deemed as promising protective agents in the human body. It is well recognized that the most commonly found active constituents include terpenes, coumarins, lignans, flavonoid, tannins, stibens, and curcuminoids have antioxidant activity [8]. The leaves of G. *procumbens* contain several secondary metabolites like phenolic compounds (flavonoids and tannins), saponins, and terpenoids, which have antioxidant activity. The health benefit effects of G. *procumbens* leaves have already been proved due contain these secondary metabolites [9]. The secondary metabolites that are present in the G. *procumbens* leaves are the vital factors for antioxidant activity. Thus, there is a rising concern in the materials exposing antioxidant effects that are accessible to human and animal organisms as a food ingredient or in particular pharmaceutics.

It has been reported that G. *procumbens* can drop the cholesterol level, reduce hypertension, be utilized as a medicinal drug for kidney failure, throat infection, and dysentery [10]. The liver enzymes such as aminotransferase, alanine aminotransferase, and alkaline phosphatase increased upon diabetes [11]. Aminotransferase, alanine aminotransferase, and alkaline phosphatase are also elevated in the streptozotocin-induced diabetic rats [12]. In severe diabetes, the lipid profile, creatinine level, and the activities of aminotransferase, alanine aminotransferase, and alkaline phosphatase increased as marked by many researchers. Therefore, the present study focused on the effects of the various extracts of G. *procumbens* leaves on lipid profiles, creatinine, and some enzyme activity in alloxan-induced diabetic mice. Although the disease protecting capability of the leaves of this plant well recognized, what has not yet been done to the assessment of the dose-dependent biological potency of different extracts of the leaves of G. *procumbens* on the animal model. Therefore, evaluation of the medicinal effects of the various extracts of G. *procumbens* leaves on oxidative, glycemic, lipidomics, and enzymatic profiles in alloxan-induced diabetic mice have captured our interest.

MATERIALS AND METHODS

Drugs and chemicals

Glibenclamide and DMSO (Dimethylsulfoxide) procured from Merck, Germany. DPPH (2, 2-diphenyl-1-picryl-hydrazyl), ascorbic acid, and anhydrous sodium sulfate were from Sigma Aldrich, Germany. Ethanol, methanol, n-hexane, ether, sodium chloride, and Alloxan and metformin obtained from Merck, India.

Sample collection and processing

The fresh leaves of *Gynuraprocumbens* were harvested from the Daratana nursery in Jashore, Bangladesh, and identified by Botany Department, Rajshahi University, Rajshahi-6205, Bangladesh. The leaves were cleaned, weighed, and air drying at RT (room temperature) until drying. Upon drying, dried leaves were powdered, dried at 55°C and stored at 4°C to use in this study.

Gynuraprocumbens leaves extracts preparation

Aqueous extraction of G. *procumbens* leaves powder conducted by the procedure depicted by Peungvicha et al. 1998[13]. Briefly, 50 g of powder was mixed with 400 ml of distilled water (1:10) and warmed up in the water bath at 50°C for three hours with shaking of the extract at every 20 min interval. Ethanol and n-hexane extractions were undertaken following the procedure rendered by Kabir et al. 2019 with slight alternation [14]. Shortly, 100 g of leaves powder was stirring with 500 mL ethanol (90%) and 500 mL of n-hexane separately. The extraction was performed for seven days in a shaking incubator at 200 rpm and 37°C. All the extraction mixtures were centrifuged at 8000 rpm for 15 min. The resultant supernatants filtered through Whatman no.1 filter paper. The filtrates from ethanol and n-hexane extractions were intensified through a rotary vacuum evaporator (Stuart, UK) at 40°C. The filtrate from aqueous, ethanol and n-hexane extractions cooled at -20°C for 48h and freeze-dried (Lab Conco Corporation, USA) to obtain powdery material of solvent extractions. The powders were preserved at -20°C to use in this study.

Phytochemical screening

The attitude of various phytochemical materials in the different solvent extracts of leaves of G. *procumbens* screened by the standard conventional method [15].

In vitro antioxidant activity

The antioxidant activity assessed through DPPH (2, 2-diphenyl-1-picryl-hydrazyl) free radical chelating action partially modification was mentioned by Sakat et al. 2010 [16]. The color change of DPPH into yellow reduced form (DPPH/H+) was noticed concurrently. The ascorbic acid and the extract dissolved in methanol for preparing test sample solutions. 500 µL of each sample solution mixed to 9.5 mL of newly made DPPH solution at the concentration of 50 µg/mL prepared in absolute methanol. Ascorbic acid is used as a positive control, and DPPH is used as a negative control. The constituents of each solution were mixed adequately and kept in the dark at RT for 10 min. The optical density is estimated at 517 nm in a UV-spectrophotometer (V-1100, Mapada Instruments Co. Ltd., Shanghai). All tests have more than three readings. The free radical chelating capability of the extract determined as a percent of DPPH free radical chelated based on the following formula.

Free radical scavenging activity (%) = [(Ac–As)/Ac] x 100.

Where AC is the absorbance of the control, and AS is the absorbance of the test sample.

Determination of anti-diabetic efficacy

Experimental animals and ethical approval

Sixty-four Swiss albino mice (25-30g) aged six weeks acquired from the Animal Center of Jahangirnagar University, Savar, Dhaka, Bangladesh. The mice were retained under a healthy environment with a maximum of 6 mice in each polypropylene cage and housed under controlled environmental conditions of 12 hs light and dark at 23 ± 2°C with open admittance to standard feed and water. The care, handling, maintenance of mice, and experimental procedures were validated by the Institutional Ethical Committee of Faculty of Biological Science of Jashore University of Science and Technology, Jashore-7408, Bangladesh.

Experimental design

The mice were distributed randomly into eight groups, each with eight mice as follows: group-1 normal control (NCM), group-2 alloxan-induced diabetes mice (ADM), group-3 ADM dealt with aqueous extract (100 mg/kg b.w) of *Gynuraprocumbens* leaves (GPLAE-100), group-4 ADM treated with aqueous extract (200 mg/kg b.w) of *Gynuraprocumbens* leaves (GPLAE-200), group-5 ADM treated with 100 mg/kg b.w of ethanol extract of *Gynuraprocumbens* leaves (GPLEE-100), Group-6 ADM treated with 200 mg/kg b.w of *Gynuraprocumbens* leaves (GPLEE-200), group-7 ADM treated with 15 mg/kg b.w of metformin (M-15), and group-8 ADM treated with 15 mg/kg b.w of glibenclamide (G-15).

GPLAE and GPLEE reconstituted in distilled water and fed orally every day at 9.00 a.m at the doses of 100, and 200 mg/kg b.w through the stomach tube continued for 28 days. Mice in group-1 (NCM) and group-2 (ADM) received the vehicle of the extract. Group-7 and group-8 received metformin and glibenclamide for 28 days. Mice weighed in every week. The glucose level in the fasting blood of mice and body weight were assessed before (at day 0) and after 28 days of treatment. After 28 days of treatment, mice were withheld for food but not water for 16 hs and killed via chloroform to collect cardiac blood and liver sample for further investigation.

Induction of diabetes in mice

Mice in group-2 to 8 fasted for 16 hs and freshly prepared Alloxan Hydrate in 0.9% saline solution at a single dose of 55 mg/kg b.w injected i.p to persuade diabetes mellitus. The glucose level in the blood taken from the tail vein measured on days 7, 14, 21, and 28 by a glucometer (On-call EZII, ACON, USA) to demonstrate confirmed diabetes mellitus. The mice showed a glucose level 14 mmol/L or higher deemed as diabetic mice employed them for evaluating the anti-diabetic activities of the extract [17].

Plasma glucose level measurement

The blood of aged-match mice was collected from the lateral tail vein, and the glucose level was measured by a glucometer (On-call EZII, ACON, USA).

Determination of Biochemical parameters and enzyme activity

The mice were anesthetized by diethyl ether after 28 days of treatment, and blood was picked up from the thoracic artery of the mice. Then blood was left for 10 minutes at room temperature for clot formation, and serum was obtained by centrifugation at 3000 rpm for 10 minutes at 4°C and kept at -20°C before required for enzymatic analysis. The Erba Chem 5v3 Clinical Chemistry Analyzer (Mannheim, Germany) was employed for the determination of serum indexes by commercial kits following the manufacture's protocol. The level of Cholesterol, HDL, LDL, triglyceride, creatinine, ALT, AST, and alkaline phosphatase assessed, and serum samples analyzed triplicates for determining mean values.

Experimental analysis of the data

The data was analyzed by Origin Lab Version 7. The values are expressed as mean ± STDEV. The significance of among different parameters are evaluated by ANOVA after Tukey'sposthoc test. $P<0.05$ (*), and $p<0.01$ (**) denoted as the levels of significance.

RESULTS

The phytochemical investigation unveiled the existence of flavonoids, alkaloids, steroids, and saponins in the aqueous, ethanol, and n-hexane extracts of *Gynuraprocumbens* leave. The distinct solvent extracts of *Gynuraprocumbens* leaves contain various phytochemicals showed in Table 1. From table 1, it exhibited that aqueous extract comprises carbohydrates, fat and fixed oil, alkaloid, tannin, and phenol and flavonoid. The ethanol extract contains carbohydrates, fat, and fixed oil, alkaloid, flavonoid, and glycosides. The hexane extract retains carbohydrates, fat and fixed oil, alkaloid, and flavonoid.

To evaluated the free radical chelating capacity of the different extracts of *Gynuraprocumbens* leaves, DPPH assays were carried out. The concentration of 15, 20, 25, 30, 35, 40, and 45 µg/mL were used to carry out the DPPH assay, all of the experimental extracts (aqueous, ethanolic, and n-hexane) showed antioxidant activity by chelating DPPH free radicals (Table 2). The aqueous extracts (GPLAE) exhibited antioxidant activity ranged between 78.32±0.06% to 94.49±0.06%. In contrast, ethanolic (GPLEE) and n-hexane (GPLHE) extracts showed antioxidant activity from 76.68±0.12% to 89.08±0.06% and 77.21±0.06% to 92.23±0.12% respectively. Ascorbic acid is a positive control, presented antioxidant activity with the range from 78.26±0.13% to 96.12±0.12%.

From Table 3, GPLAE-100 and GPLAE-200 showed anti-diabetic activity within 1-5 hs of treatment by lowering blood glucose levels. The diabetic mice treated with GPLAE-200 showed the highest

Table 1: Preliminary phytochemical screening of the different extracts of *Gynuraprocumbens* leaves.

Phytochemical compounds	Extracts		
	Aqueous extract	Ethanol extract	n-hexane extract
Carbohydrates	+	+	+
Proteins	+	+	+
Alkaloids	+	+	+
Phenols	+	_	_
Tanins	+	_	_
Saponins	+	+	+
Steroids	+	+	+
Flavonoids	+	+	+
Terpenoids	_	+	_
Glycosides	_	+	_
Caffeine	_	_	_

Table 2: DPPH free radical scavenging activity of the different extracts of *Gynuraprocumbens* leaves.

Concentration (µg/mL)	Percent (%) of DPPH inhibition			
	L-Ascorbic acid (M ± SE)	n- hexane extract (GPLHE)	Ethanol extract (GPLEE)	Aqueous extract (GPLAE)
15	78.26± 0.13	77.21 ± 0.06**	76.68 ± 0.12	78.32 ± 0.06
20	84.37 ± 0.20	85.37 ± 0.12	82.04 ± 0.46*	85.17 ± 0.12
25	86.64 ± 0.17	86.06 ± 0.06*	83.61 ± 0.17**	86.85 ± 0.06
30	88.46 ± 0.12	87.75 ± 0.06*	85.86 ± 0.12**	88.74 ± 0.23
35	92.32 ± 0.06	89.32 ± 0.17	87.56 ± 0.12**	90.60 ± 0.06*
40	94.20 ± 0.06	91.18 ± 0.12	88.24 ± 0.12	92.47 ± 0.12**
45	96.12 ± 0.12	92.23 ± 0.12	89.08 ± 0.06	94.49 ± 0.06**

Each value represents the mean ± STDEV (n = 3). * and ** indicates a significant and highly significant difference between the scavenging activity of n-hexane extract and ethanol of same the same concentration with aqueous extract at p<0.05 and <0.01.

Table 3: Anti-diabetic activity of different extracts (aqueous and ethanol) with different doses of G.*procumbens* before and after treatment 21.1 ± 0.17.

Treatment groups	Blood sugar level (mmol/L)			
	Before treatment	After treatment		
	Fasting	1 hour	3 hours	5 hours
Normal control mice (NCM)	6.7± 0.12*	6.7± 0.12	6.8 ± 0.12	6.6 ± 0.12
Alloxan-induced diabetic mice (ADM)	20.5 ± 0.17	16.1 ± 0.12	16.1 ± 0.6	16.0 ± 0.06
ADM+ GPLAE-100	20.1 ± 0.06	14.0 ± 0.12*	8.7 ± 0.12*	7.5 ± 0.12*
ADM + GPLAE-200	21.6 ± 0.12	13.4 ± 0.12*	7.3 ± 0.12**	6.9 ± 0.12** (68%)
ADM + GPLEE-100	20.3 ± 0.17	12.1 ± 0.06*	9.1 ± 0.06*	7.9 ± 0.06*
ADM + GPLEE-200	20.6 ± 0.17	11.5 ± 0.15*	8.5 ± 0.23*	7.6 ± 0 .12* (63%)
ADM+ M-15	21.7 ± 0.06	10.2 ± 0.12**	7.7 ± 0.12**	6.8 ± 0.06** (69%)
ADM+ G-15	21.1 ± 0.17	9.1 ± 0.12**	7.5 ± 0.12**	6.9 ± 0.17** (67%)

Each value represents the mean ± STDEV (n = 3); * and ** indicates a significant and highly significant difference between hour 0 and hour 5 of the same treatment group with UDMC at p< 0.05 and <0.01. GPLAE-100 and GPLAE-200 indicate aqueous extract of *Gynuraprocumbens* leaves at the dose of 100 and 200 mg/kg body weight, respectively. GPLEE-100 and GPLEE-200 denote ethanol extract of *Gynuraprocumbens* leave at the doses of 100 and 200 mg/kg body weight, respectively.M-15, and G-15 indicates metformin and glibenclamide at the dose of 15 mg/kg b.w.

Table 4: Body weight and anti-diabetic activities measurement after (7th, 14th, 21th, 28th days) treatment with different doses of GPLAE and GPLEE.

Treatment groups	Body weight (g)		Blood sugar level (mmol/L)			
	1st day	28th days	7th days	14th days	21th days	28th days
Normal control mice (NCM)	26.8 ± 0.23	23.0 ± 0.17 (12.5%)	7.0 ± 0.12	6.0 ± 0.17	5.5 ± 0.06	5.1 ± 0.06
Alloxan-induced diabetic mice (ADM)	27.2 ± 0.12	21.2 ± 0.12 (22%)	16.0 ± 0.12	15.0 ± 0.17	14.5 ± 0.17	14.0 ± 0.12
ADM+ GPLAE-100	25.6 ± 0.12*	23.0 ± 0.17 (10%)	6.27 ± 0.02*	4.6 ± 0.12	4.2 ± 0.12	4.1 ± 0.06**
ADM + GPAE-200	25.1 ± 0.12*	22.5 ± 0.06 (10.3%)	4.6 ± 0.17**	3.8 ± 0.12	3.62 ± 0.01	3.36 ± 0.01**
ADM + GPEE-100	26.8 ± 0.06	23.4 ± 0.23(12.6%)	6.3 ± 0.17*	5.63 ± 0.02	5.2 ± 0.12	4.7 ± 0.12**
ADM + GPEE-200	26.2 ± 0.12*	24.1 ± 0.17 (8%)	5.7 ± 0.17*	4.92 ± 0.06	4.5 ± 0.17	4.0 ± 0.12**
ADM+ M-15	27.3 ± 0.06	23.7 ± 0.12 (13.2%)	6.8 ± 0.06*	4.6 ± 0.12	4.3 ± 0.09	3.9 ± 0.06**
ADM+ G-15	26.2 ± 0.12*	24.2 ± 0.12 (7%)	5.1 ± 0.17**	4.68 ± 0.2	4.4 ± 0.12	4.1 ± 0.06**

Each value represents the mean ± STDEV (n=3); * and ** indicates a significant and highly significant difference between day 0 and day 28 of the same treatment group at p<0.05 and <0.01.

anti-diabetic activity (p<0.01) by lowering 68% blood glucose whereas GPLEE-200 fall blood glucose level (p<0.05) by 63% in the ADM. The metformin (M-15) and glibenclamide (G-15) treated mice at a dose of 15 mg/kg b.w significantly (p<0.01) reduce blood glucose were 69% and 67%, respectively.

The effect of different dosages of different extracts of G. *procumbens leaves* on body weight and fasting blood glucose (FBG) levels of ADM and NCM showed in Table 4. It was observed that the bodyweight of all groups of mice was reduced 7-13% after 28 days of the treatment except untreated ADM, lost weight 22%. It was also observed that ADM treated with GPLAE-200 and GPLEE-200 showed gradually decreasing the blood glucose level after 7th. 14th, 21th and 28th days of the treatment and the values were ranging from 4.6 ± 0.17 to 3.36 ± 0.01 mmol/L, and 5.7 ± 0.17 to 4.0 ± 0.12 mmol/L, respectively, and these results were significant (p<0.05 and p<0.01). Whereas at the same duration, the blood glucose level of ADM was reduced by metformin and glibenclamide at the dose of 15 mg/kg b.w, and the values were

Table 5: Determination of lipid profile after 28thdays treatment with GPLAE and GPLEE.

Treatment groups	Cholesterol (mmol/L) (M ± SE)	Tryglycerides (mmol/L) (M ± SE)	HDL (mmol/L) (M ± SE)	LDL(mmol/L) (M ± SE)
NCM	5.16 ± 0.02	2.19 ± 0.02	2.89 ± 0.02	2.52 ± 0.02
Alloxan-induced diabetic mice (ADM)	5.96 ± 0.02**	3.13 ± 0.01**	1.92 ± 0.01**	3.81 ± 0.01
ADM+ GPLAE-100	5.29 ± 0.02**	2.16 ± 0.01**	2.72 ± 0.01**	3.01 ± 0.09
ADM+ GPLAE-200	5.22 ± 0.02**	2.13 ± 0.01**	2.78 ± 0.02**	2.90 ± 0.01*
ADM + GPLEE-100	5.52 ± 0.01*	2.36 ± 0.01*	2.68 ± 0.02**	3.12 ± 0.06
ADM+ GPLEE-200	5.46 ± 0.02*	2.32 ± 0.01*	2.72 ± 0.01*	2.51 ± 0.02**
ADM+ M-15	5.64 ± 0.01*	2.94 ± 0.01	2.76 ± 0.01**	2.92 ± 0.01*
ADM + G-15	5.68 ± 0.01	2.95 ± 0.01	1.84 ± 0.01	3.79 ± 0.02

Each value represents the mean ± STDEV (n = 3); * and ** indicates a significant and highly significant difference between day 0 and day 28 of the same treatment group at p< 0.05 and <0.01.

Table 6: Determination of Enzyme activity and serum creatine level after 28th days of treatment with GPLAE and GPLEE.

Treatment groups	SGPT(ALT) U/L	SGOT(AST) U/L	ALP U/L	CRATE mg/dL
NCM	7.3 ± 0.29	32.4 ± 0.23	13.5 ± 0.23	0.4 ± 0.06
ADM	17.6 ± 0.12	116.4 ± 1.28	20.3 ± 0.29	1.5 ± 0.24
ADM+ GPLAE-100	10.6 ± 0.32*	72.6 ± 0.34*	15.36 ± 0.14	0.86 ± 0.02
ADM+ GPLAE-200	6.4 ± 0.29**	40.4 ± 0.23**	12 ± 0.23**	0.4 ± 0.06**
ADM+ GPLEE-100	14.4 ± 0.22	64.8 ± 0.26*	16.46 ± 0.14	0.74 ± 0.02
ADM+ GPLEE-200	13.6 ± 0.12	36.12 ± 0.20**	14.6 ± 0.46*	0.3 ± 0.06**
ADM+ M-15	13.8 ± 0.16	82.4 ± 0.44	16.6 ± 0.18	0.98 ± 0.04
ADM + G-15	14.2 ± 0.08	84.6 ± 0.36	17.2 ± 0.06	0.84 ± 0.02

Each value represents the mean ± STDEV (n = 3); * and ** indicates a significant and highly significant difference between day 0 and day 28 of the same treatment group at p< 0.05 and <0.01.

ranging from 6.8 ± 0.06 to 3.9 ± 0.06 mmol/L and 5.1 ± 0.17 to 4.1 ± 0.06 mmol/L, respectively. The normal control mice (NCM) and untreated ADM blood glucose level were ranging from 7.0 ±0.12 to 5.1 ± 0.06 mmol/L and 16.0 ± 0.12 to 14.0 ± 0.12 mmol/L, respectively. The initial values of the FBG level of untreated ADM were ranging from 20.05 ± 0.17 to 21.7 ± 0.06 mmol/L, and NCM were 6.7 ± 0.12 mmol/L (Table 3).

Table 5 represented the change of lipid profile by the administration of GPLAE, GPLEE, metformin, and glibenclamide in ADM. It was observed that GPLAE-100, GPLAE-200, and GPLEE-100 and GPLEE-200 showed significant (p<0.1) reduction of blood cholesterol level and this reduction level is higher than that of blood cholesterol level significantly (p<0.5) reduced by metformin and glibenclamide. Similarly, both the GPLAE and GPLEE exhibited significant(p<0.1) drop-down of the TG (triglyceride) level in ADM blood. However, metformin and glibenclamide did not show a significant decrement of the TG level in blood. The blood-HDL level was elevated significantly in all the treated diabetic mice concerning untreated ADM. Both the GPLAE and GPLEE at the concentration of 100 and 200 mg/kg b.w performed higher activity than that of metformin. In the case of the LDL level, a few different scenarios were observed where both the GPLAE and GPLEE treated mice showed a slight decrease in the LDL level, but the GPLEE-200 demonstrated significantly (p<0.05) lower the LDL level. Surprisingly, metformin-treated mice exposed significant (p<0.05) decline in the LDL level, but the glibenclamide-treated mice showed no significant changes in the LDL level.

As shown in Table 6, the level of SGPT, SGOT, and ALP were increased in untreated ADM as compared to NCM. ADM treated with GPLAE-200 showed a significant enough (p<0.01) lowering effect of SGPT, SGOT, and ALP levels. Although GPLEE-100 (p<0.05) and GPLEE-200 (<0.01) significantly decreased the level of SGOT, they did not prominently decrease the SGPT in ADM. The alkaline phosphatase (ALP) level was increased in untreated ADM (20.3 ± 0.29 U/L) in comparison to the NCM (13.5 ± 0.23 U/L). Interestingly, the ALP level was significantly decreased in the blood of the GPLAE- 200 and GPLEE-200-treated ADM in comparison to the normal control mice (NCM), and this decreasing level of ALP was highly significant (p<0.01) and significant (p<0.05) in GPLAE-200 and GPLEE-200 treated ADM.

This study, also analyzed the creatinine level of the untreated ADM and ADM treated with GPLAE-200 and GPLEE-200 (Table 6). It was observed that the creatinine level was higher in the untreated ADM (1.5 ± 0.24 mg/dl) in comparison to the normal control mice (NCM) (0.4±0.06 mg/dl). But the GPLAE-200 and GPLEE-200 treated ADM significantly (p<0.01) reduced the creatinine level, and these values were 0.4 ± 0.2 mg/dl and 0.3 ± 0.3 mg/dl, respectively. There were no significant changes in creatinine level were viewed in the GPLAE-100, and GPLEE-100 treated ADM. Interestingly, it was observed that no many differences of the SGPT, SGOT, ALP, and creatinine levels were observed in metformin (M-15) and glibenclamide (G-15) treated ADM mice.

DISCUSSION

Phytoconstituents as active components in plant extracts are amenable for the multifactorial pharmacological actions.

Plant secondary metabolites influence the therapeutic and pharmacological endeavors of meditative herbs. In this study, it was observed that all the extracts of G. *procumbens* have DPPH free radicals scavenging potentiality. The ranking of the highest antioxidant activity showed by the plant extracts at the dose of 15 to 45 µg/ml was GPLAE>GPLEE>GPLHE. Consistent with this, a comparative study of Maw et al., 2011 claimed that the ethanol extract of G. *procumbens* has the highest percentage of DPPH inhibition [18]. Similarly, Nasiruddin et al. 2020 reported that the maximum antioxidant activity has been demonstrated by 75% ethanolic extracts of G. *procumbens* leaves [19]. Besides, Riyadh et al., 2008 mentioned that G. *procumbens* appears to be a potent source of natural antioxidants due to its high phenolic content [20]. In this study, a preliminary phytochemical screening experiment, it was found that aqueous extracts of G. *procumbens* leave displayed the existence of phenolic compounds (tannins and flavonoids). But ethanol and n-hexane extract of G. *procumbens* leaves exhibited only the presence of flavonoids. It could be a reason that aqueous solvent has a strong capacity to extract phenolic compounds. Therefore, the aqueous extract showed the highest percentage of antioxidant activity. It has evident that antioxidants have a crucial role in protecting diabetes[21]. In the current study, it was also observed that G. *procumbens* leaves extract has antioxidant properties and showed significant anti-diabetic effects in alloxan-induced diabetic mice. Similarly, it has been pretended that several parts of Z. *zanthoxyloides* considerably dropped the glucose amount in the diabetic animal with the non-treated group [22], and *Heliotropiumindicum* showed significant hypoglycemic activity on STZ triggered diabetic rats [23]. The majority of medicinal plants that are used traditionally contain phytochemicals. These include phenols, flavonoids, tannins, alkaloids, glycosides, and terpenoids are commonly well known for anti-diabetic properties as well as many other therapeutic benefits [24]. This study is in good agreement with that aqueous extracts of G. *procumbens* contain all of these phytoconstituents in comparison to the other two extracts (ethanol and n-hexane), and aqueous extracts of G. *procumbens* may exert combined effects against diabetes in mice. Although G. *procumbens* leaves are customary as a primitive therapeutics for the treating of cancer, diabetes, and hypertension [6], till now, no study has addressed the dose-dependent anti-diabetic functions of the different extracts of G. *procumbens* leaves. Moreover, this work shows an anti-diabetic effect of G. *procumbens* laves *in vivo*.

The loss of body weight is a common phenomenon in diabetes. In this study, it was found that alloxan-induced diabetic mice lose body weight. Indeed, loss of body weight is imputed owing to the breakdown of fat or proteins in tissues with subsequent muscle atrophy [25]. However, treatment with GPLAE and GPLEE prevented the phenomenon of body weight loss along with diabetes promisingly. Consistently, the plants that have anti-diabetic activity are reported to contribute to preventing the loss of body weight [26].

This study revealed that oral dosage of all groups of G. *procumbens* extracts from days 7 to days 28 decreased blood glucose levels remarkably in diabetic mice in comparison to the controlled diabetic mice. However, GPLAE-200 demonstrated a notable ($p<0.01$) blood glucose level lowering effect. It is noteworthy that the blood-glucose-reducing potency of GPLAE is greater than that of metformin (M) and glibenclamide (G) (Table 4). Similar results have been reported by Subhan et al. 2014 [23], where they administered methanol fraction of *Heliotropiumindicum* at a dose of 750 mg/kg b.w of diabetic mice. Thus in this study, it is found that GPLAE-200 could be a potential source for the medication of diabetes. It has also been reported in several types of research that the compounds containing steroids, alkaloids, saponin, etc. have several anti-diabetic mechanisms [27], and in this study, it was observed that aqueous extracts of G. *procumbens* contained the mentioned compounds.

After a four-week treatment, it was found that GPLAE-100, GPLAE-200, GPLEE-100, and GPLEE-200 markedly ($p<0.01$) reduced the cholesterol and triglycerides level relative to untreated diabetic mice. Although the glibenclamide (G) has high potential activity in the reduction of HDL level ($p<0.01$), there was no significant difference in triglyceride levels observed in diabetic mice be treated with metformin and glibenclamide. No significant changes in LDL level were observed by treated with different groups of G. *procumbens* extracts but, metformin (M) treatment significantly reduced LDL level ($p<0.05$), whereas glibenclamide (G) significantly increase LDL level ($p<0.05$). Thus the aqueous extracts of G. *procumbens* have a potential effect on lowering the cholesterol and triglycerides (TG) level in ADM. This finding is consistent with Murugesh et al. 2006 reported that the *HeliotropiumZeylanicum* possesses anti-hyperlipidemic activities in STZ induced rats [28].

Diabetes mellitus is associated with liver and heart disease cause a rising of SGPT, and SGOT levels are indicating heart and liver damage. It has been reported that due to the hepatotoxic effect of STZ, the level of SGOT, SGPT, and alkaline phosphatase (ALP) increase in plasma [29]. In this study, both the GPLAE and GPLEE treated ADM had significantly lowered the SGOT, SGPT, and ALP level. But the lowering level of SGPT by GPLAE is highly significant (**$p<0.01$) as compared to the GPLEE (Table 6). This reduction in SGOT and SGPT may be resulted due to the existence of phenolic compounds in GPLAE [30]. These results in suggesting improvement of the heart and hepatic function of the G. *procumbens* extracts treated diabetic mice for untreated diabetic mice. Highly significant and significant changes in alkaline phosphatase (ALP) levels were observed when ADM has treated with GPLAE-200 and GPLE-200 (**$p<0.01$;*$p<0.05$). Interestingly, the significant reduction of the ALP level in the treatment group of G. *procumbens* extracts as compared to the untreated ADM (Table 6). Therefore, the GPLAE could be an inspiring amateur for the amelioration of the heart and liver function of diabetic mice. Increased serum creatinine level is a significant marker of renal dysfunction in diabetes is also significantly corrected by GPLAE-200, and GPLEE-200 treated ADM. Thus, these results represented that the treatment of diabetic mice with GPLAE and GPLEE improved the renal function contrast to the control of diabetic mice without treating.

CONCLUSION

The results of the current investigation concluded that aqueous extracts of G. *procumbens* has potent anti-oxidant, blood glucose, cholesterol, and triglycerides lowering effect. This study also found that the aqueous extracts of G. *procumbens* have the potential to improve the heart and liver function of diabetic mice by lowering the SGPT, SGOT, and ALP levels. Finally, this study suggests that aqueous extract of G. *procumbens* is dose-dependent, and it could exploit as a potential resource in treating diabetes as well as heart and hepatic diseases.

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REFERENCES

1. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010and 2030. Diabetes Res Clin Pract. 2010;87:4-14.
2. Jain S, Saraf S. Type 2 diabetes mellitus-Its global prevalence and therapeutic strategies. Diabetes and Metabolic Syndrome: Clinical Research and Reviews. 2010;4:48-56.
3. Kar P, Holt RI. The effect of sulphonylureas on the microvascular and macrovascular complications of diabetes. Cardiovasc. Drugs Ther. 2008;22:207-213.
4. Wulffele MG, Kooy A, Lehert P, Bets D, Ogterop JC, Borger van der Burg B, et al. Effects of short-term treatment with metformin on serum concentrations of homocysteine, folate and vitamin B12 in type 2 diabetes mellitus: a randomized, placebo-controlled trial. J Intern Med. 2003;254:455-463.
5. Çlrak C, Radušiene J, Janulis V, Ivanauskas L, Arslan B. Chemical Constituents of Some Hypericum Species Growing in Turkey. J Plant Biol. 2007;50:632.
6. Bavarva JH, Narasimhacharya AV. Anti-hyperglycemic and hypolipidemic effects of Costusspeciosus in alloxan induced diabetic rats. Phytother Res. 2008;22:620-626.
7. Geleijnse JM, Witteman JC, Launer LJ, Lamberts SW, Pols HA. Tea and coronary heart disease: protection through estrogen-like activity? Arch Intern Med. 2000;160:3328–3329.
8. Ng TB, Liu F, Wang ZT. Antioxidant activity of natural products from plants. Life Sciences.2000;66:709-723.
9. Masoko P, Masiphephethu MV. Phytochemical investigation, antioxidant and antimycobacterial activities of Schkuhriapinnata (Lam) Thell extracts against mycobacterium smegmatis. J Evid Based Integr Med. 2019;24:2515690X19866104.
10. Burkill IH. A Dictionary of the Economic Product of Malay Peninsular. Kuala Lumpur. Ministry of Agriculture and Co-operatives, Kuala Lumpur.1966;1:11:p:2444, W.- K. Chen, Linear Networks and Systems(Book style). Belmont, CA: Wadsworth. 1993;123–135.
11. Seifter S, England S. Energy Metabolism, In: Liver, Biology and Pathology, I.M. Arias, H. Propper and D. Schachter (Eds.). Raven press, New York, USA. 1982;219-249.
12. El-Agouza, Rawy IA, Saad A, LashinA, El-Sisi. Effect of sulfur containing amino acids and insulin injection on streptozotocin diabetic rats. J Drug Res. Egypt. 2000;23:213-224.
13. Peungvicha P, Thirawarapan SS, Temsiririrkkul Watanabe RH, Prasain JK, Kodata S. Hypoglycemic effect of the water extract of Piper sarmentosum in rats. J Ethnopharmacol. 1998;60:27-32.
14. Kabir M, Noman AA, Dash BK, Hassan M, Akhter S, Rahman M. Tremaorientalis (Linn.) leaves promotes anticancer activity in Ehrlich ascites carcinoma (EAC) in Swiss albino mice. J Basic Clin Physiol Pharmacol. 2019;31.
15. Harbone JB. Phytochemical methods. A guide to modern techniques of plant analysis, Chapman and Hall. London. 1998;60.
16. Sakat SS, Juvekar AR, Gambhire MN. In vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata Linn. International J Pharma Pharmacological Sci. 2010;2:146-155.
17. Ravi K, Sivagnanam K, Subramaniam S. Anti-diabetic activity of Eugenia jambolana seed kernels on streptozotocin-induced diabetic rats. J Med Food. 2004;7:187- 191.
18. Maw SS, Mon MM, Oo ZK. Study on antioxidant and antitumor activities of some herbalextracts. World Acad.Sci., Eng. Technol.; Int. J Pharma Pharmceu Sci. 5(3) (2011) 85-91.
19. Nasiruddin M, Sinha SN. Phytochemical screening and antioxidant, antibacterial efficacy of Gynuraprocumbens (Lour.). Merr Asian J Med Biol Res. 2020;6:187-195.
20. Rosidah MF, Yam A, Sadikun A, Asmawi MZ. Antioxidant potential of Gynuraprocumbens. Pharm Biol. 2008;46:616-625.
21. Bajaj S, Khan A. Antioxidant and diabetes. Indian J Endocriol Metab. 2012;16:267- 271.
22. Aloke C, Nwachukwu N, Ugwuja EI, Idenyi JN, Nwachi EU, Obasi IO, et al. Effects of Zanthoxylumzanthoxyloides leaves on blood glucose, lipid profile and some liver enzymes in alloxan induced diabetic rats. Int J Sci Nat. 2012;3:497-501.
23. Subhan Ali M, Shaik AN, Saritha M, Krishna TT, Malaka VJK, Chippada AR. Phytochemical screening and anti-hyperglycemic activity of Heliotropiumindicum whole plant in Streptozotocin induced diabetic rats. J App Pharm Sci. 2014;4:65-71.
24. Loew D, Kaszkin M. Approaching the problem of bioequivalence of herbal medicinal products. Phytother Res. 2002;16:705-711.
25. Pupim LB, Heimburger O, Qureshi AR, Ikizler TA, Stenvinkel P. Accelerated lean body mass loss in incident chronic dialysis patients with diabetes mellitus. Kidney Int. 2005;68:2368–2374.
26. Pareek H, Sharma S, Khajja BS, Jain K, Jain GC. Evaluation of hypoglycemic andanti-hyperglycemic potential of Tridaxprocumbens(Linn.). BMC Complementary Altern Med. 2009;9:48.
27. Nazaruk J, Borzym-Kluczyk M. The role of triterpenes in the management of diabetes mellitus and its complications. Phytochem Rev. 2014;10.
28. Murugesh K, Veerendra Y, Kumar DD, Pinaki S, Chandra BM, Kumar TM. Antidiabetic, antioxidant and antihyperlipidemic status of Heliotropium Zeylanicumextract on Streptozotocin induced diabetic rats. Biol Pharm Bull. 2006;29:2202-2205.
29. El-Demerdash FM, Yousef MI, Abou El-Naga NI. Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. Food Chem. Toxicol. 2005;43:57–63.
30. Gerich JE. Physiology of glucose homeostasis. Diabetes Obes Metab. 2000;2:345-350.