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Antidiabetic effect of *Gynura procumbens* leaves extracts involve modulation of hepatic carbohydrate metabolism in streptozotocin-induced diabetic rats

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Gynura procumbens is an annual evergreen shrub found in Southeast Asia, especially Malaysia, Thailand and Indonesia, which is of considerable medicinal value. The present study aims to investigate the antidiabetic properties of *G. procumbens* leaves aqueous and ethanolic extracts and its possible underlying antihyperglycemic mechanisms of action involving liver carbohydrate metabolism in streptozotocin-induced diabetic rats. Experimental diabetes was induced via single intravenous injection of streptozotocin (55 mg/kg of body weight). *G. procumbens* leaves aqueous and ethanolic extracts were administered orally at 3 different doses (50, 100 and 150 mg/kg of body weight) for 42 consecutive days. Administration of *G. procumbens* leaves aqueous and ethanolic extracts significantly ($P<0.05$) reduced fasting blood glucose and HbA1c levels of diabetic rats after 42 days of treatment, in which ethanolic extract showed better improvement than aqueous extract in a dose-independent manner. Treatment of *G. procumbens* extracts also protected the diabetic rats from further body and liver weight loss, and showed slight increment on liver glycogen content in *G. procumbens* ethanolic extract-treated diabetic rats as well as significant ($P<0.05$) improvement on liver hexokinase, phosphofructokinase and fructose-1,6-bisphosphatase specific activities. However, administration of both extracts did not produce significant changes on plasma insulin concentration of diabetic rats when compared with diabetic controls. In conclusion, *G. procumbens* exerts its antidiabetic effect by promoting glucose metabolism via the glycolytic pathway and inhibiting hepatic endogenous glucose production via the gluconeogenic pathway.

Key words: Diabetes mellitus, *Gynura procumbens*, glucose profile, carbohydrate metabolism, streptozotocin.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease recognized by chronic elevation of blood glucose level (hyperglycemia) and it is often accompanied by symptoms of polyuria, polydipsia, polyphagia and weight loss. These abnormalities are due to deficiency of β-cells of the endocrine pancreas causing insulin deficiency and/or from subsensitivity to insulin in peripheral cells (Rajasekaran et al., 2005), primarily leading to changes in carbohydrate metabolism and secondarily of lipids and proteins (Al-Attar and Zari, 2010). It had been reported that the incidence of diabetes mellitus had increased over the years and there are 285 million people with diabetes mellitus in year 2010, which is about 6.4% of the world population. The prevalence of diabetes mellitus is predicted to increase by two-fold within 20 years, whereby total number of diabetic patients would reach 439 million people, that is 7.7% of world population by year 2030 (Shaw et al., 2010). Chronic hyperglycemia in diabetes mellitus is associated with various complications such as microangiopathy, retinopathy, nephropathy and neuropathy, all of which had contributed to the increase

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in mortality rate around the world (Altan, 2003; The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003).

Many diabetic treatments have been developed in the past decades with the same underlying goal to maintain adequate blood glucose concentration. To date, there are six classes of oral antidiabetic agents which have been used in the treatment of diabetes mellitus, such as sulfonylureas and meglitinides, biguanides, thiazolidinediones, α-glucosidase inhibitors and dipeptidyl peptidase-4 inhibitors. Each of these drug classes works on different mechanism of action, including stimulation of insulin secretion, reduction of hepatic gluconeogenesis, increasing peripheral insulin sensitivity, delaying intestinal digestion and absorption of carbohydrate, and increasing endogenous levels of incretin hormones that act in the presence of glucose to stimulate insulin secretion, respectively (Donner, 2006; Jain and Saraf, 2010; Klonoff, 2010). Unfortunately, these agents could produce various side effects, such as hypoglycemia, weight gain, lactic acidosis, vitamin B12 deficiency, edema, bone fractures, gastrointestinal problems and pancreatitis (Chiasson et al., 2002; Kar and Holt, 2008; Kirpichnikov et al., 2002; Klonoff, 2010; Loke et al., 2009; Nesto et al., 2003; Wulffele et al., 2003).

Gynura procumbens (Lour.) Merr (family Compositae), also known locally as "Sambung Nyawa", is found easily in Southeast Asia, especially Malaysia, Thailand and Indonesia. Traditionally, *G. procumbens* leaves have been known to possess high medicinal values and was used to treat illnesses such as eruptive fevers, rash, kidney diseases, migraines, constipation, hypertension, cancer and diabetes mellitus (Perry, 1980). Recently, pharmacological studies have indicated that *G. procumbens* has anti-inflammatory (Iskander et al., 2002), anti-herpes simplex virus (Jiratchariyakul et al., 2000; Nawawi et al., 1999), anti-hypertensive (Hoe et al., 2007; Kim et al., 2006), anti-hyperlipidemic (Zhang and Tan, 2000), anti-sterility (Halimah et al., 2008; Pusparanee et al., 2008), and anti-oxidative capabilities (Akowuah et al., 2009; Puangpronpitag et al., 2010). Studies have also shown that n-butanol fraction of *G. procumbens* leaves methanolic extract possessed blood glucose lowering activity and the possible active chemical constituents from this plant had been isolated and identified to be flavonoids, saponins, tannins and terpenoids (Akowuah et al., 2001, 2002). Although it is known that *G. procumbens* contains anti-diabetic properties, however little information on the mechanism of action involved in the antidiabetic activity of *G. procumbens* is available. Moreover, most of the antidiabetic studies on *G. procumbens* were conducted on methanolic and aqueous extracts, but little is known on the antidiabetic effect of ethanolic extract of *G. procumbens* leaves. Therefore, the aim of the present study is to evaluate the antihyperglycemic properties of aqueous and ethanolic extracts of *G. procumbens* leaves *in vivo* and to investigate its possible underlying antidiabetic mechanism involving hepatic carbohydrate metabolism.

MATERIALS AND METHODS

Plant materials

Leaves of *Gynura procumbens* (Lour.) Merr. (Compositae) were collected and identified from the Green House Facility of Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia.

Preparation of *Gynura procumbens* extracts

The leaves of *G. procumbens* were extracted in two different methods to produce crude aqueous and ethanolic extracts. Aqueous extraction was performed according to the method of Peungvicha et al. (1998). Fresh leaves of *G. procumbens* were washed, weighed and dried in oven (Sheldon Manufacturing, Inc., USA) at 45°C for 3 days. Upon drying, dried leaves were blended into powder form, then 20 g of dried leaves powder was mixed with 400 ml of distilled water (ratio of 1:20) and heated in water bath (50°C) for 3 h with stirring of the extract at every 20 min interval. The extract was then cooled and centrifuged at 3000 rpm for 10 min (Kubota Corp., Japan). Supernatant obtained was separated and the pellet was subjected to another round of centrifugation at 3000 rpm for 10 min. Both of the supernatants were combined and freeze-dried (LabConco Corporation, USA) to yield brown powder of *G. procumbens* crude aqueous extract.

Ethanolic extract of *G. procumbens* leaves was prepared according to the method of Zhang and Tan (2000). Fresh leaves of *G. procumbens* (1 kg) were washed, blended and mixed with 95% ethanol (1.5 L) for 7 days at room temperature. The extract was then filtered and centrifuged at 3000 rpm for 20 min. Supernatant obtained was concentrated using rotary evaporator at 40°C (BUCHI Rotavapor R-200, BUCHI Labortechnik AG, Switzerland) and freeze-dried (LabConco Corporation, USA) to yield yellowish dark green powder of *G. procumbens* crude ethanolic extract. The extracts were stored at -20°C until needed in the experiment.

Experimental animals

Male *Sprague Dawley* rats aged 6 weeks (weighed 200 to 280 g) were obtained from the Animal House Facility, Faculty of Science and Technology, Universiti Kebangsaan Malaysia. The animals were acclimatized to laboratory conditions for 14 days prior to the experiments. Five rats were housed per polycarbonate cage and maintained on a standard laboratory rat chow diet (Barastoc, Australia) with water *ad-libitum*. The rats were maintained at ambient room temperature (25 ± 2°C) under a light/dark cycle of 12 h. The experiment was approved by Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC Approval Number: FST/SBB/2010/HALIMAH/24-AUGUST/322-SEPTEMBER-2010-NOVEMBER-2011). All procedures in this study were performed according to the guidelines stated by UKMAEC.

Induction of diabetes

Diabetes was induced via intravenous injection of streptozotocin

(Calbiochem EMD Biosciences, Inc., USA) (55 mg/kg body weight freshly dissolved in cold sodium citrate buffer 0.1 M, pH 4.5 and kept in ice prior to use) to overnight fasted rats (16 h). Diabetes was identified by polyuria, polydipsia and high fasting blood glucose level 7 days after injection of streptozotocin. Rats with fasting blood glucose level exceeding 13 mmol/l were considered to be diabetic and were used in the experiment (Ravi et al., 2004).

Experimental design

A total of 81 rats (36 normal and 45 diabetic rats) were used. The normal and diabetic rats were divided randomly into five groups containing 4 rats per normal group (n = 4) and 5 rats per diabetic group (n = 5). Group 1 consisted of control animals treated with vehicle (distilled water, 1 ml/rat); Group 2 animals were treated with 50, 100 or 150 mg/kg body weight (b.w.) of *G. procumbens* leaves aqueous extract; Group 3 animals were treated with 50, 100 or 150 mg/kg b.w. of *G. procumbens* leaves ethanolic extract; Group 4 animals were treated with 5 mg/kg b.w. of glibenclamide; and Group 5 animals were treated with 500 mg/kg b.w. of metformin. Both glibenclamide- and metformin-treated groups served as positive controls in this experiment. *Gynura procumbens* extracts, glibenclamide and metformin were suspended in distilled water and administered daily (1 ml/rat) via oral gavage for 42 consecutive days to the experimental animals. Fasting blood glucose level and body weight were measured prior to (day 0) and after treatment (day 43). At the end of 42 days treatment (day 43), rats were fasted overnight (16 h) and sacrificed via diethyl ether inhalation to obtain aortal blood and liver samples for analysis. Aortal blood samples were used to measure hemoglobin concentration and then processed immediately to obtain plasma for determination of plasma insulin concentration and packed erythrocyte for HbA1c assay. Liver organs were frozen in liquid nitrogen and stored at -20°C. These tissues were used for the assays of glycogen content, hexokinase, phosphofructokinase and fructose-1,6-bisphosphatase activities.

Determination of fasting blood glucose level

Blood was obtained from tail vein of rats fasted overnight (16 h) and fasting blood glucose concentration was measured on day 0 (before the start of treatment) and day 43 (end of treatment) using AccuChek® Performa glucometer (Roche Diagnostics GmbH, Germany).

Preparation and determination of plasma insulin concentration

Aortal blood samples (~1 ml) were collected into Vacuette EDTA tubes and centrifuged at 3000 rpm for 10 min at 4°C. The plasma obtained was separated into polypropylene microtubes and stored at -20°C until analysis. Insulin concentration in the plasma samples were assayed by enzyme-linked immunosorbent assay (ELISA) using the Ultra Sensitive Rat Insulin ELISA kit purchased from Crystal Chem, Inc. USA (Webster et al., 1990).

Determination of HbA1c and hemoglobin concentrations

Packed erythrocytes obtained from the preparation of aortal blood samples (~1 ml) collected into Vacuette EDTA tubes were used for determination of HbA1c concentration performed according to the method of Sudhakar Nayak and Pattabiraman (1981). The packed erythrocytes were washed with 2 ml 0.9% NaCl, through repeated

centrifugation (3000 rpm, 10 min, 4°C) and washing for four times. Then, 2 ml of distilled water and 0.5 ml of carbon tetrachloride (CCl$_4$) were added to the packed erythrocytes and incubated overnight at 4°C. The mixture was centrifuged at 3000 rpm for 15 min at 4°C to obtain hemolysate. Aliquot of hemolysate (0.2 ml) was made up to 2 ml with distilled water, followed by the addition of 4 ml of 1 M oxalic acid in 2 N HCl. The mixture was heated in boiling water bath (100°C) for 4 h and cooled before the addition of 2 ml 40% TCA. After adding 40% TCA, the hydrolysate was centrifuged at 3000 rpm for 10 min. Aliquot of hydrolysate (1 ml) was added with 0.05 ml 80% phenol, followed by 3 ml 95% H$_2$SO$_4$ and incubated at room temperature for 30 min before the measurement of absorbance. Absorbance was read at 480 nm wavelength using spectrophotometer (Hitachi U-1800 Spectrophotometer, Japan). Hemoglobin concentration in the blood samples were measured using Reflotron® Hemoglobin Strips (Roche Diagnostics GmbH, Mannheim, Germany). Glucose (200 µg/ml) was used as standard and HbA1c level was expressed as milligram of glucose per gram of hemoglobin.

Determination of liver glycogen content

Glycogen content was measured in the rat liver samples according to the method of Carroll et al. (1956). The frozen liver (50 mg) was hydrolysed with 2 ml 30% KOH for 15 min in boiling water bath (100°C). Liver hydrolysate was cooled and added with 2.4 ml 95% ethanol. The mixture was incubated overnight at 4°C and then centrifuged at 3000 rpm for 15 min. Supernatant was discarded and the tubes were allowed to drain in inverted position for 10 min to obtain glycogen pellet. Then, the glycogen pellet was dissolved in 1 ml of distilled water via vigorous shaking, followed by the addition of 5 ml of anthrone reagent (0.05% anthrone, 1% thiourea, 72% (v/v) H$_2$SO$_4$) with the tubes placed in cold water to prevent overheating. After cooling, the reaction mixture was heated in boiling water bath (100°C) for 15 min, and then cooled under running tap water. Absorbance was then read at 620 nm wavelength using spectrophotometer (Hitachi U-1800 Spectrophotometer, Japan). Glucose (0.25 mg) was used as standard and glycogen content was expressed as milligram of glycogen per gram of wet liver tissue.

Hexokinase activity assay

Activity of liver hexokinase was assayed according to the method of Salas et al. (1963). The frozen liver was minced and homogenized with 2 volumes of ice-cold 0.1 M Tris-HCl, pH 7.4. Homogenates were immediately centrifuged at 20,000 × g for 30 min at 4°C (Eppendorf Centrifuge 5810R, Germany). The supernatant was then used for the measurement of hexokinase activity by the coupled enzyme assay procedure in a reaction mixture containing, in total volume of 2 ml, 50 mM Tris (pH 7.4), 0.2 IU glucose-6-phosphate dehydrogenase, 0.25 mM β-NADP, 5 mM ATP, 5 mM MgCl$_2$, 5 mM β-mercaptoethanol, 0.5 mM glucose and 25 µl of fresh homogenate. Control assay without ATP and glucose by replacing with distilled water was carried out in parallel. Reaction mixture was incubated at room temperature (21 to 25°C) for 5 min prior to assay. Reaction was started with the addition of liver homogenate into each assay and absorbance was read at 340 nm wavelength at every 1 min interval for 10 min using spectrophotometer (Hitachi U-1800 Spectrophotometer, Japan). Hexokinase activity was calculated by subtracting the activity with the activity of control. Activity was expressed in micromoles of glucose phosphorylated per minute per ml enzyme at room temperature (21 to 25°C).

Phosphofructokinase activity assay

Phosphofructokinase activity in liver was assayed according to the method of Sakakibara and Uyeda (1983). The frozen liver was minced and homogenized with 3 volumes of ice-cold 0.1 M Tris-HCl (pH 7.4). Homogenates were immediately subjected to centrifugation at high speed (20,000 × g) for 20 min at 4°C (Eppendorf Centrifuge 5810R, Germany). Supernatant obtained was used for the measurement of phosphofructokinase activity by the coupled enzyme assay procedure. Reaction mixture was prepared, in total volume of 1 ml, containing 50 mM Tris-HCl (pH 8.0), 1 mM fructose-6-phosphate, 1 mM ATP, 2 mM $MgCl_2$, 0.16 mM NADH, 2.5 mM dithiothreitol (DTT), 1 mM EDTA, 5 mM $(NH_4)_2SO_4$, 0.4 IU aldolase, 2.4 IU triosephosphate isomerase, 0.4 IU α-glycerophosphate dehydrogenase and 25 µl of fresh liver homogenate. Control assay was carried out in parallel and contained all the chemicals, except fructose-6-phosphate and ATP which was replaced by distilled water. Reaction mixture was incubated for 5 min at room temperature (21 to 25°C) prior to assay. Reaction was started with the addition of liver homogenate into each assay and absorbance was read at 340 nm wavelength at every 1 min interval for 10 min using spectrophotometer (Hitachi U-1800 Spectrophotometer, Japan). Phosphofructokinase activity was calculated by subtracting the activity with the activity of control. Activity was expressed in micromoles of fructose-1,6-bisphosphate produced per minute per ml enzyme at room temperature (21 to 25°C).

Fructose-1,6-bisphosphatase activity assay

The method of Riou et al. (1977) was adopted for the measurement of fructose-1,6-bisphosphatase activity in the liver samples. The frozen liver was minced and homogenized with 4 volumes of ice-cold 0.1 M Tris-HCl (pH 7.4). Homogenates were immediately centrifuged at 20,000 × g for 30 min at 4°C using Eppendorf Centrifuge 5810R (Germany). Supernatant obtained was subjected to coupled enzyme assay for the measurement of fructose-1,6-bisphosphatase activity. Reaction mixture was prepared, in total volume of 1 ml, containing 100 mM Tris-HCl (pH 7.5), 2 mM $MgCl_2$, 2 mM $(NH_4)_2SO_4$, 2.5 mM β-mercaptoethanol, 0.05 mM EDTA, 0.2 mM β-NADP, 3 IU glucose-6-phosphate dehydrogenase, 3 IU phosphoglucose isomerase, 70 µM fructose-1,6-bisphosphate and 10 µl of fresh liver homogenate. Control assay containing all the chemicals, except fructose-1,6-bisphosphate was carried out in parallel. Reaction mixture was incubated for 5 min at 30°C prior to assay. Reaction was started with the addition of liver homogenate and fructose-1,6-bisphosphate into each assay. Absorbance was read at 340 nm wavelength at every 1 min interval for 10 min using spectrophotometer (Hitachi U-1800 Spectrophotometer, Japan). Fructose-1,6-bisphosphatase activity was calculated by subtracting the activity with that of control. Activity was expressed in micromoles of fructose-1,6-bisphosphate hydrolyzed per minute per ml enzyme at 30°C.

Protein determination

Protein content of liver homogenate samples were determined according to the method of Bradford (1976) using bovine serum albumin as standard.

Statistical analysis

Results were expressed as mean ± standard error of mean (S.E.M.). Statistical analysis was performed by paired T test using Minitab version 15. Differences with $P<0.05$ were considered to be statistically significant.

RESULTS

Effect of *G. procumbens* extracts on body and liver weight of normal and diabetic rats

Table 1 summarizes the changes in body and liver weight of normal and diabetic rats after 42 days of *G. procumbens* leaves extracts administration. Daily oral administration of *G. procumbens* ethanolic extract at 50, 100 and 150 mg/kg b.w., as well as glibenclamide (5 mg/kg b.w.) and metformin (500 mg/kg b.w.) produced slight increment in body weight of normal rats, but did not show much difference on liver weight of normal rats when compared with normal control. Administration of *G. procumbens* aqueous extract at 50 and 100 mg/kg b.w. did not affect body and liver weights of normal rats, however normal rats given 150 mg/kg b.w. of *G. procumbens* aqueous extract showed significantly ($P<0.05$) lower body and liver weight as compared to normal control. As for the diabetic rats, body weights of *G. procumbens* aqueous and ethanolic extract-treated groups (50, 100 and 150 mg/kg b.w.), as well as glibenclamide- (5 mg/kg b.w.) and metformin- (500 mg/kg b.w.) treated groups were not significantly different from the diabetic control. However, the extract-treated groups, as well as glibenclamide and metformin-treated groups have shown decrease in body weight loss after 42 days of treatment when compared with the weights of the corresponding groups before the start of treatment. On the other hand, no significant changes were observed on the liver weight of all treated diabetic rats.

Effect of *G. procumbens* extracts on fasting blood glucose concentration of normal and diabetic rats

Changes in fasting blood glucose concentration after administration of *G. procumbens* leaves extracts are summarized in Table 2. Daily oral administration of *G. procumbens* leaves aqueous and ethanolic extracts at 3 different doses (50, 100 or 150 mg/kg b.w.) to normal rats did not cause any changes in fasting blood glucose at the end of study. As for the positive controls, administration of glibenclamide (5 mg/kg b.w.) had significantly ($P<0.05$) reduced fasting blood glucose concentration of normal rats. Administration of metformin (500 mg/kg b.w.), on the other hand, did not show any changes in fasting blood glucose of normal rats. In diabetic rats, oral administration of *G. procumbens* extracts for 42 days resulted in significant ($P<0.05$) reduction of fasting blood glucose. Diabetic rats treated with ethanolic extract of

Table 1. Effect of *Gynura procumbens* extracts on body and liver weights of normal and diabetic rats.

Test models	Groups	Dose (mg/kg)	Body weight (g)		Liver weight (g)
			Day 0 (Before treatment)	Day 43 (End of treatment)	Day 43 (End of treatment)
Normal	Control	-	231.76 ± 5.3	346.83 ± 17.4	12.88 ± 0.79
	Glibenclamide	5	313.66 ± 14.2	399.66 ± 10.8	14.43 ± 0.61
	Metformin	500	284.81 ± 11.8	372.80 ± 12.1	13.57 ± 0.41
	Aqueous extract	50	207.06 ± 7.5	339.84 ± 7.7	11.57 ± 0.22
	Aqueous extract	100	278.61 ± 7.9	365.16 ± 14.6	13.85 ± 0.64
	Aqueous extract	150	222.70 ± 5.6	308.98 ± 9.3*	9.87 ± 0.72*
	Ethanolic extract	50	253.18 ± 2.8	389.96 ± 10.4	12.52 ± 0.63
	Ethanolic extract	100	279.29 ± 14.2	405.17 ± 17.3	14.17 ± 1.23
	Ethanolic extract	150	275.92 ± 7.7	377.27 ± 14.7	11.93 ± 0.91
Diabetic	Control	-	265.40 ± 13.7	197.84 ± 10.6 (-25.5)	9.55 ± 0.56
	Glibenclamide	5	214.79 ± 8.5	218.55 ± 19.7 (1.8)	10.50 ± 0.73
	Metformin	500	246.55 ± 16.7	229.23 ± 24.4 (-7.0)	11.31 ± 0.71
	Aqueous extract	50	208.64 ± 18.6	219.33 ± 14.5 (5.0)	9.60 ± 0.59
	Aqueous extract	100	226.18 ± 27.2	208.85 ± 20.0 (-7.7)	11.20 ± 1.11
	Aqueous extract	150	229.63 ± 15.8	198.14 ± 11.8 (-13.7)	10.13 ± 0.57
	Ethanolic extract	50	242.97 ± 15.6	210.87 ± 20.1 (-13.2)	9.12 ± 0.62
	Ethanolic extract	100	220.67 ± 24.4	193.23 ± 18.8 (-12.4)	9.77 ± 1.35
	Ethanolic extract	150	227.04 ± 14.7	187.51 ± 18.3 (-17.4)	8.68 ± 0.45

Values are given as mean ± S.E.M. for five rats in each diabetic group (n=5) and four rats in each normal group (n=4). Treated groups were compared with controls at corresponding time-interval. Values in brackets show percentage change at the end of treatment (Day 43) as compared to before treatment (Day 0) for each corresponding group. Values are statistically significant at *$P<0.05$.

G. procumbens leaves at 50, 100 and 150 mg/kg b.w. showed 49.0, 56.8 and 55.7% reduction in fasting blood glucose, respectively. While oral administration of *G. procumbens* aqueous extract at 50, 100 and 150 mg/kg b.w. caused 24.6, 19.5 and 17.4% reduction in fasting blood glucose of diabetic rats, respectively. Administration of metformin (500 mg/kg b.w.) had also reduced ($P<0.05$) fasting blood glucose of diabetic rats by 41.4%, but no significant changes on fasting blood glucose were seen after the treatment of glibenclamide (5 mg/kg b.w.).

Effect of *G. procumbens* extracts on HbA1c level of normal and diabetic rats

Figure 1 shows the changes in HbA1c level of normal and diabetic rats after administration of *G. procumbens* leaves extracts for 42 days. Administration of *G. procumbens* aqueous or ethanolic extract at 50, 100 or 150 mg/kg b.w. did not show significant changes on HbA1c level of normal rats. The administration of metformin (500 mg/kg b.w.) also did not produce significant changes on HbA1c level of normal rats.

However, glibenclamide-treated normal rats (5 mg/kg b.w.) showed significant ($P<0.05$) reduction in HbA1c level of normal rats when compared with normal control (Figure 1A). Meanwhile, oral administration of *G. procumbens* aqueous and ethanolic extracts for 42 days resulted in significant ($P<0.05$) reduction on HbA1c levels of diabetic rats. *G. procumbens* aqueous extract-treated diabetic rats with doses of 50, 100 and 150 mg/kg b.w. showed 8.7, 12.8 and 14.7% reduction in HbA1c levels, respectively when compared to diabetic control. While administration of *G. procumbens* ethanolic extract at 50, 100 and 150 mg/kg b.w. resulted in 12.3, 13.7 and 15.7% reduction in HbA1c levels of diabetic rats, respectively. As for the positive controls, glibenclamide (5 mg/kg b.w.) and metformin (500 mg/kg b.w.) treated diabetic rats also showed significant ($P<0.05$) 15.4 and 23.9% reduction in HbA1c level of diabetic rats, respectively as compared to diabetic control (Figure 1B).

Effect of *G. procumbens* extracts on plasma insulin concentration of normal and diabetic rats

Table 3 summarizes the result on plasma insulin

Table 2. Effect of *Gynura procumbens* extracts on fasting blood glucose of normal and diabetic rats.

Test models	Groups	Dose (mg/kg)	Fasting blood glucose (mmol/l)	
			Day 0 (Before treatment)	Day 43 (End of treatment)
Normal	Control	-	4.75 ± 0.20	4.53 ± 0.05
	Glibenclamide	5	4.18 ± 0.33	3.93 ± 0.17*
	Metformin	500	4.35 ± 0.13	4.48 ± 0.10
	Aqueous extract	50	4.30 ± 0.25	4.23 ± 0.13
	Aqueous extract	100	4.28 ± 0.34	4.08 ± 0.19
	Aqueous extract	150	4.13 ± 0.13	4.50 ± 0.24
	Ethanolic extract	50	4.55 ± 0.13	4.55 ± 0.24
	Ethanolic extract	100	4.50 ± 0.26	4.45 ± 0.25
	Ethanolic extract	150	4.15 ± 0.06	4.35 ± 0.19
Diabetic	Control	-	18.76 ± 1.13	21.02 ± 2.00
	Glibenclamide	5	22.24 ± 0.60	18.68 ± 2.08
	Metformin	500	18.42 ± 0.70	12.38 ± 2.75 (41.4)*
	Aqueous extract	50	16.36 ± 1.14	15.84 ± 0.70 (24.6)*
	Aqueous extract	100	18.26 ± 2.00	16.92 ± 1.98 (19.5)*
	Aqueous extract	150	18.16 ± 1.59	17.36 ± 1.02 (17.4)
	Ethanolic extract	50	19.06 ± 0.88	10.72 ± 1.46 (49.0)*
	Ethanolic extract	100	18.74 ± 0.75	9.08 ± 1.95 (56.8)*
	Ethanolic extract	150	17.88 ± 0.51	9.32 ± 1.15 (55.7)**

Values are given as mean ± S.E.M. for five rats in each diabetic group (n=5) and four rats in each normal group (n=4). Treated groups were compared with controls at corresponding time-interval and values in brackets show percentage change as compared to control. Values are statistically significant at *P<0.05 and **P<0.01.

concentration of normal and diabetic rats after 42 days treatment with *G. procumbens* leaves extracts. There were no significant changes observed on plasma insulin concentration of all treated normal rats as compared to normal control. Oral administration of *G. procumbens* aqueous and ethanolic extracts at 50, 100 and 150 mg/kg b.w., as well as metformin (500 mg/kg b.w.) also did not increase plasma insulin concentration of diabetic rats as compared to diabetic control. However, administration of glibenclamide (5 mg/kg b.w.) caused significant (P<0.05) increase on plasma insulin concentration of diabetic rats.

**Effect of *G. procumbens* extracts on liver glycogen content of normal and diabetic rats**

According to the results obtained (Figure 2A), oral administration of *G. procumbens* aqueous extract for 42 days on normal rats did not produce significant changes in liver glycogen content although 50 and 150 mg/kg b.w. *G. procumbens* aqueous extract-treated groups showed lower liver glycogen content when compared with normal control. *G. procumbens* ethanolic extract-treated normal rats at doses of 50 and 150 mg/kg b.w. also showed lower glycogen content as compared to normal control

and the glycogen content reduction in 50 mg/kg b.w. ethanolic extract-treated normal rats was found to be significant (P<0.05). However, the glycogen content of 100 mg/kg b.w. *G. procumbens* ethanolic extract-treated normal rats did not differ much as compared to normal control. Glibenclamide- and metformin-treated normal rats also did not show significant difference in liver glycogen content when compared with normal control. As for the diabetic rats (Figure 2B), the increment on liver glycogen content was seen highest (68.3% increment) in 100 mg/kg b.w. *G. procumbens* ethanolic extract-treated diabetic rats, followed by 150 mg/kg b.w. ethanolic extract-treated diabetic rats (39.2% increment) as compared to diabetic control. Oral administration of 100 mg/kg b.w. *G. procumbens* aqueous extract also produced 34.5% increment in liver glycogen content of diabetic rats. Glibenclamide (5 mg/kg b.w.) administered to diabetic rats resulted in 53.1% increment on liver glycogen content, but not in metformin- (500 mg/kg b.w.) administered diabetic rats.

Although there were increments on liver glycogen content observed in the *G. procumbens* aqueous and ethanolic extract-treated diabetic rats, as well as glibenclamide-treated diabetic rats, however the increments observed were not significant as compared
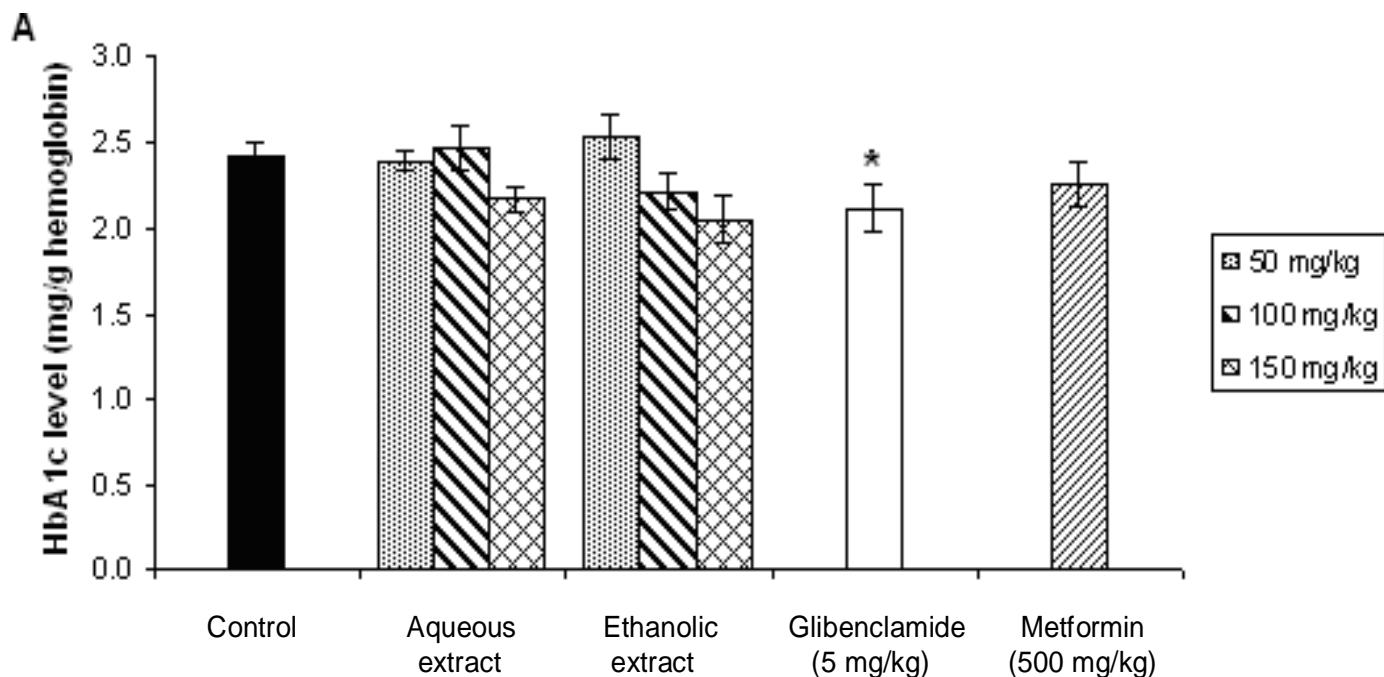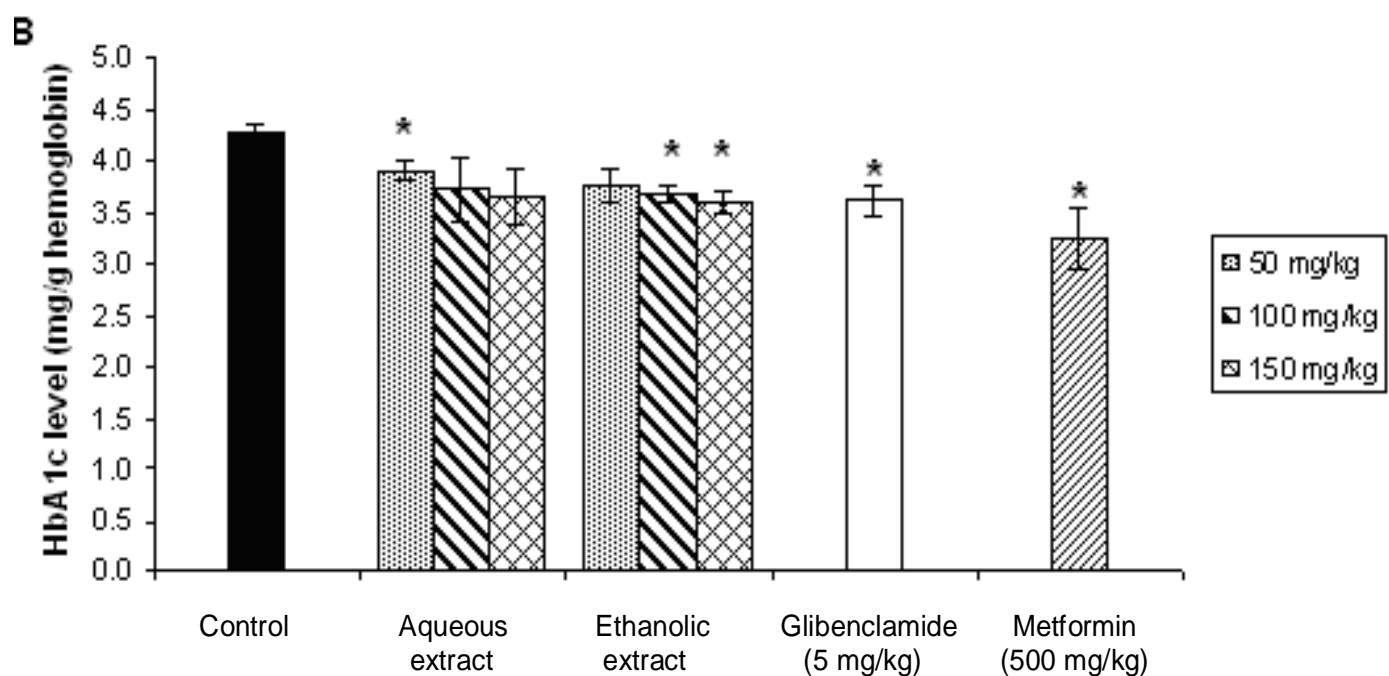
Figure 1. Effect of *Gynura procumbens* aqueous and ethanolic extracts on HbA1c level of normal (A) and diabetic rats (B). Results are expressed as mean ± S.E.M. with five rats in each diabetic group (n=5) and four rats in each normal group (n=4). Treated groups were compared with controls at corresponding time-interval. Values are statistically significant at *P<0.05.

Table 3. Effect of *Gynura procumbens* extracts on plasma insulin concentration of normal and diabetic rats.

Test models	Groups	Dose (mg/kg)	Plasma insulin (ng/ml) Day 43 (End of treatment)
Normal	Control	-	1.476 ± 0.144
	Glibenclamide	5	1.841 ± 0.102
	Metformin	500	1.475 ± 0.217
	Aqueous extract	50	1.304 ± 0.199
	Aqueous extract	100	1.516 ± 0.522
	Aqueous extract	150	0.864 ± 0.129
	Ethanolic extract	50	1.338 ± 0.191
	Ethanolic extract	100	1.394 ± 0.354
	Ethanolic extract	150	1.587 ± 0.239
Diabetic	Control	-	0.145 ± 0.016
	Glibenclamide	5	0.189 ± 0.008*
	Metformin	500	0.148 ± 0.022
	Aqueous extract	50	0.152 ± 0.012
	Aqueous extract	100	0.149 ± 0.015
	Aqueous extract	150	0.158 ± 0.020
	Ethanolic extract	50	0.144 ± 0.025
	Ethanolic extract	100	0.159 ± 0.017
	Ethanolic extract	150	0.156 ± 0.016

Values are given as mean ± S.E.M. for five rats in each diabetic group (n=5) and four rats in each normal group (n=4). Treated groups were compared with controls at corresponding time-interval. Values are statistically significant at *$P<0.05$.

to diabetic control.

Effect of *G. procumbens* extracts on liver hexokinase activity of normal and diabetic rats

According to the results obtained (Figure 3A), oral administration of *G. procumbens* ethanolic extract at 50, 100 and 150 mg/kg b.w. and glibenclamide (5 mg/kg b.w.) to normal rats did not produce changes on liver hexokinase specific activity. However, normal rats treated with 50, 100 and 150 mg/kg b.w. of aqueous extract showed lower liver hexokinase specific activity as compared to normal control, in which the reduction on liver hexokinase specific activity of 100 and 150 mg/kg b.w. aqueous extract-treated rats was significant ($P<0.05$). Similar reduction ($P<0.05$) was observed with the administration of metformin (500 mg/kg b.w.) on normal rats. On the contrary, oral administration of 100 mg/kg b.w. of aqueous extract had shown 17.8% increase ($P<0.05$) in liver hexokinase specific activity of diabetic rats, but aqueous extract at doses of 50 and 150 mg/kg b.w. did not show significant changes. Meanwhile, oral administration of 100 and 150 mg/kg b.w. of ethanolic extract resulted in more pronounced significant ($P<0.05$) increase on liver hexokinase specific activity of

diabetic rats by 36.6 and 34.9%, respectively as compared to diabetic control. However, oral administration of ethanolic extract at 50 mg/kg b.w. did not show any significant changes on liver hexokinase specific activity of diabetic rats. The positive controls, glibenclamide (5 mg/kg b.w.) and metformin (500 mg/kg b.w.) also did not produce significant improvement on liver hexokinase specific activity of diabetic rats (Figure 3B).

Effect of *G. procumbens* extracts on liver phosphofructokinase activity of normal and diabetic rats

Figure 4A shows significant ($P<0.05$) reduction on liver phosphofructokinase specific activity of normal rats administered with 50, 100 and 150 mg/kg b.w. of aqueous extract of *G. procumbens* leaves. In contrast, oral administration of *G. procumbens* ethanolic extract, glibenclamide (5 mg/kg b.w.) and metformin (500 mg/kg b.w.) did not produce significant changes on liver phosphofructokinase specific activity of normal rats as compared to normal control (Figure 4A). Meanwhile, oral administration of *Gynura procumbens* to diabetic rats resulted in improvement on liver phosphofructokinase
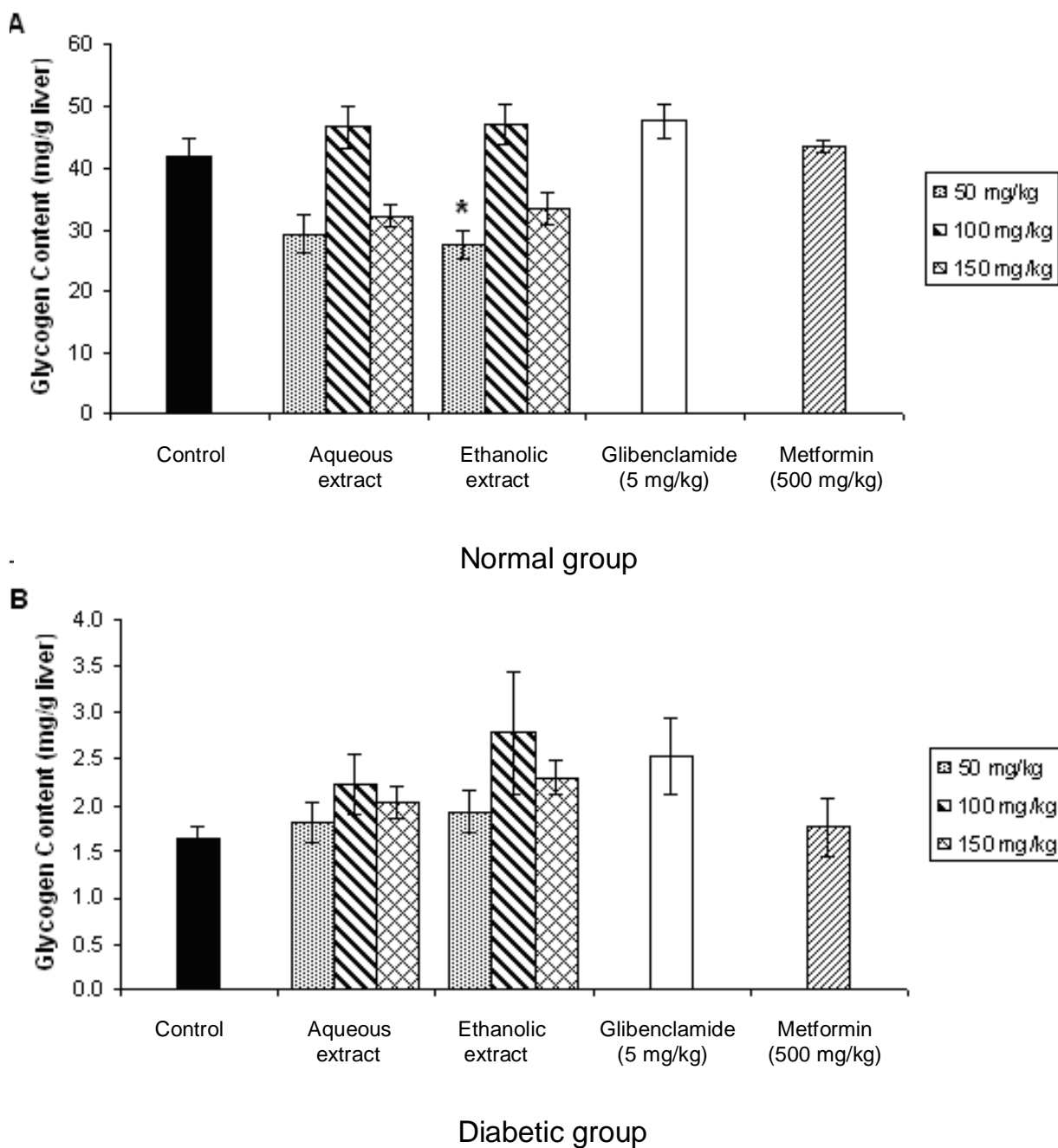
Figure 2. Effect of *Gynura procumbens* aqueous and ethanolic extracts on liver glycogen content of normal (A) and diabetic rats (B). Results are expressed as mean ± S.E.M. with five rats in each diabetic group (n=5) and four rats in each normal group (n=4). Treated groups were compared with controls at corresponding time-interval. Values are statistically significant at *P<0.05.

specific activity. Ethanolic extract of *G. procumbens* at doses of 100 and 150 mg/kg b.w. resulted in significant (P<0.05) increase on liver phosphofructokinase specific activity of diabetic rats by 90.2 and 77.7%, respectively. Administration of 100 mg/kg b.w. of *G. procumbens* aqueous extract had also significantly (P<0.05) increased

liver phosphofructokinase specific activity of diabetic rats, although to a lesser extent as compared to ethanolic extract at corresponding dose (Figure 4B). No significant changes were observed on liver phosphofructokinase specific activity of glibenclamide- and metformin-treated diabetic rats.
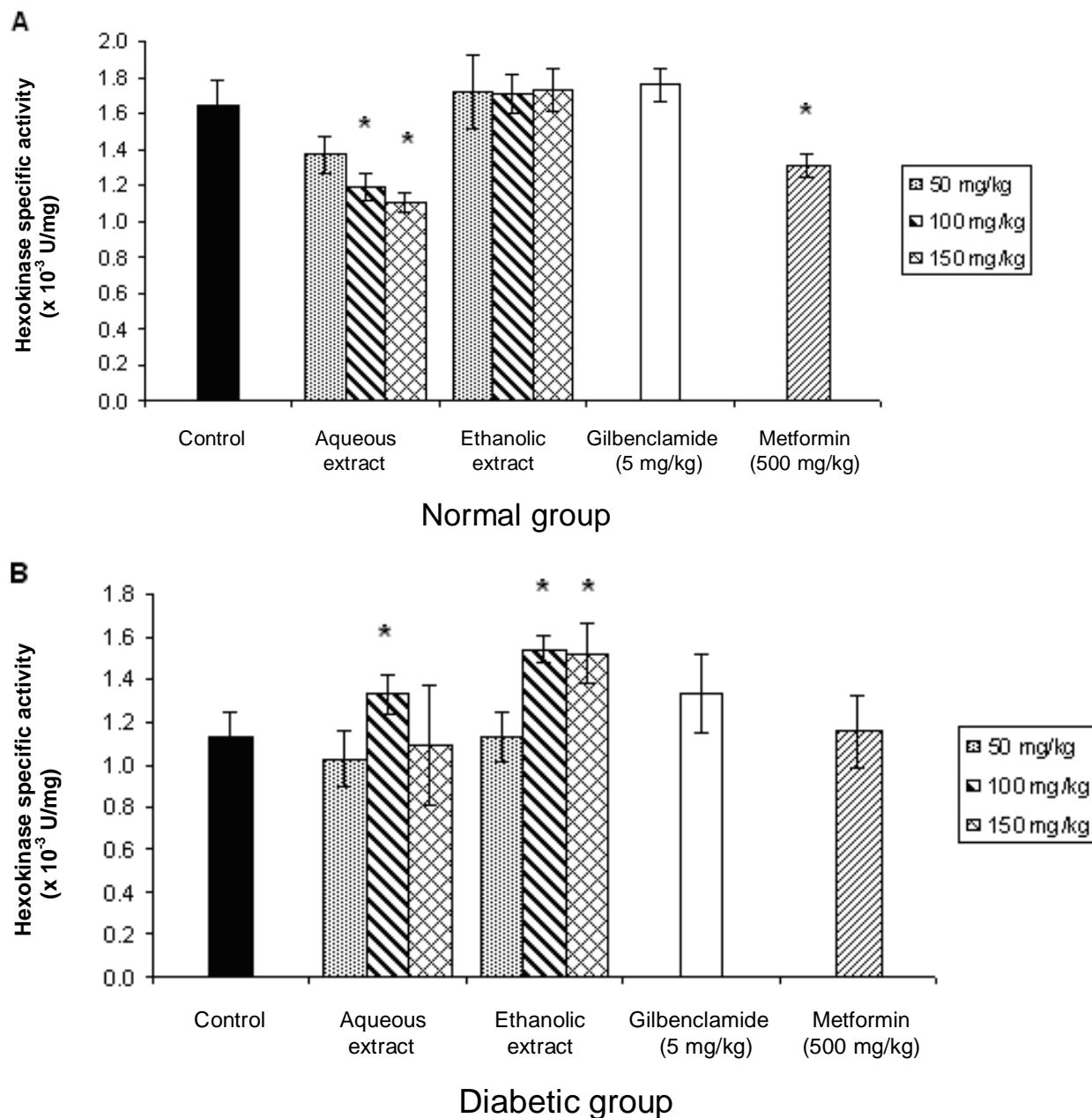
Figure 3. Effect of *Gynura procumbens* aqueous and ethanolic extracts on liver hexokinase specific activity of normal (A) and diabetic rats (B). Results are expressed as mean ± S.E.M. with five rats in each diabetic group (n=5) and four rats in each normal group (n=4). Treated groups were compared with controls at corresponding time-interval. Values are statistically significant at *P<0.05.

Effect of *G. procumbens* extracts on liver fructose-1,6-bisphosphatase activity of normal and diabetic rats

As seen in Figure 5A, there were no significant changes on liver fructose-1,6-bisphosphatase specific activity in all of the treated normal rats as compared to normal control. However, oral administration of *G. procumbens* extracts

produced significant (*P<0.05*) improvement on liver fructose-1,6-bisphosphatase specific activity of diabetic rats. Oral administration of 50, 100 and 150 mg/kg b.w. of aqueous extract reduced (*P<0.05*) liver fructose-1,6-bisphosphatase specific activity of diabetic rats by 13.9, 19.5 and 16.6%, respectively as compared to diabetic control. Diabetic rats administered with 100 and 150 mg/kg b.w. of ethanolic extract resulted in significant
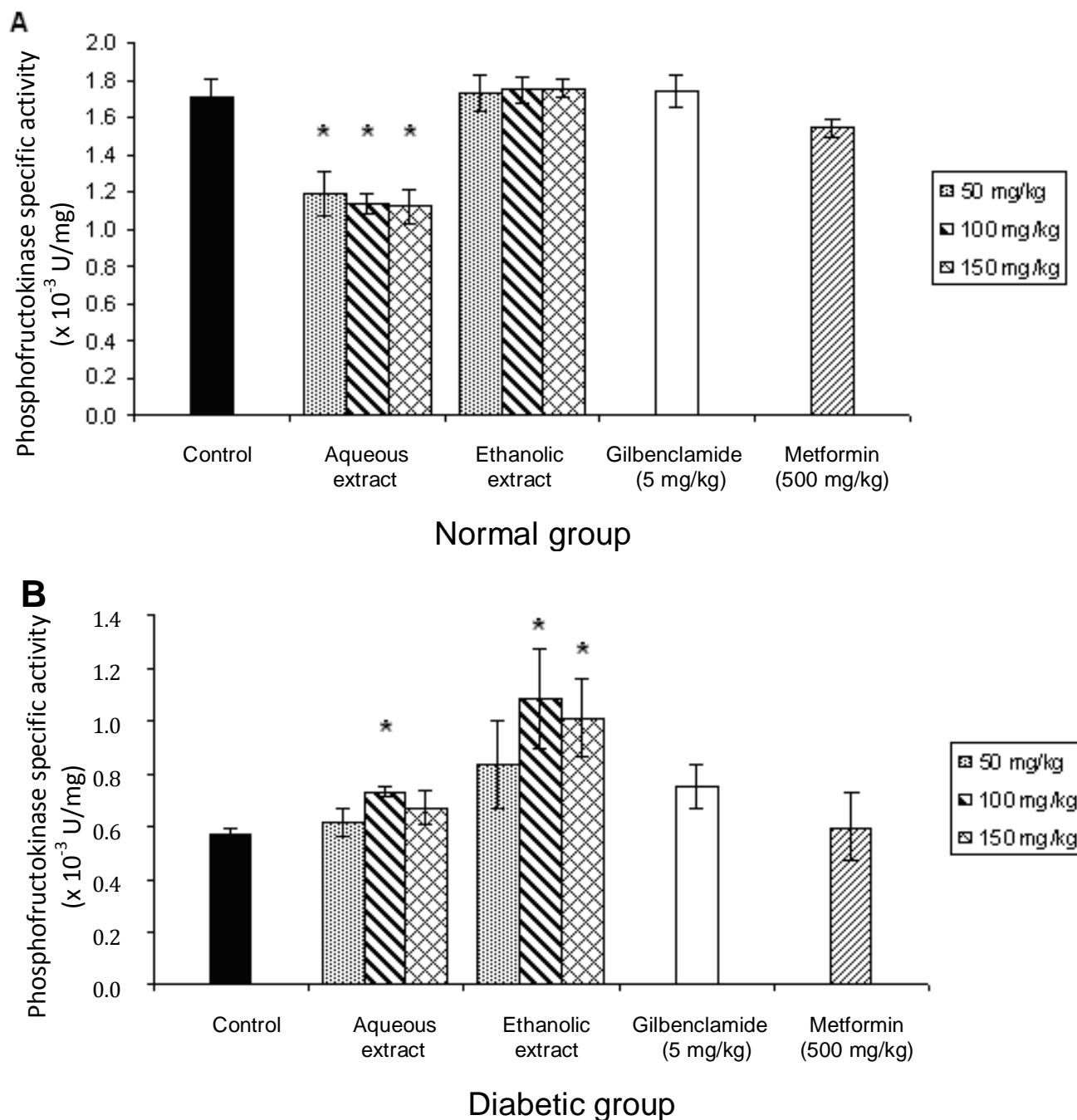
A

Normal group

B

Diabetic group

Figure 4. Effect of *Gynura procumbens* aqueous and ethanolic extracts on liver phosphofructokinase specific activity of normal (A) and diabetic rats (B). Results are expressed as mean ± S.E.M. with five rats in each diabetic group (n=5) and four rats in each normal group (n=4). Treated groups were compared with controls at corresponding time-interval. Values are statistically significant at *P<0.05.

($P<0.01$) 28.6 and 25.3% reduction of liver fructose-1,6-bisphosphatase specific activity, respectively. These effects in the ethanolic extract-treated diabetic rats were comparable to metformin-treated rats (500 mg/kg b.w.), which showed 25.2% reduction ($P<0.05$) on liver fructose-1,6-bisphosphatase specific activity. Glibenclamide (5 mg/kg b.w.), on the other hand, did not cause significant reduction on liver fructose-1,6-bisphosphatase specific activity of diabetic rats (Figure 5B).
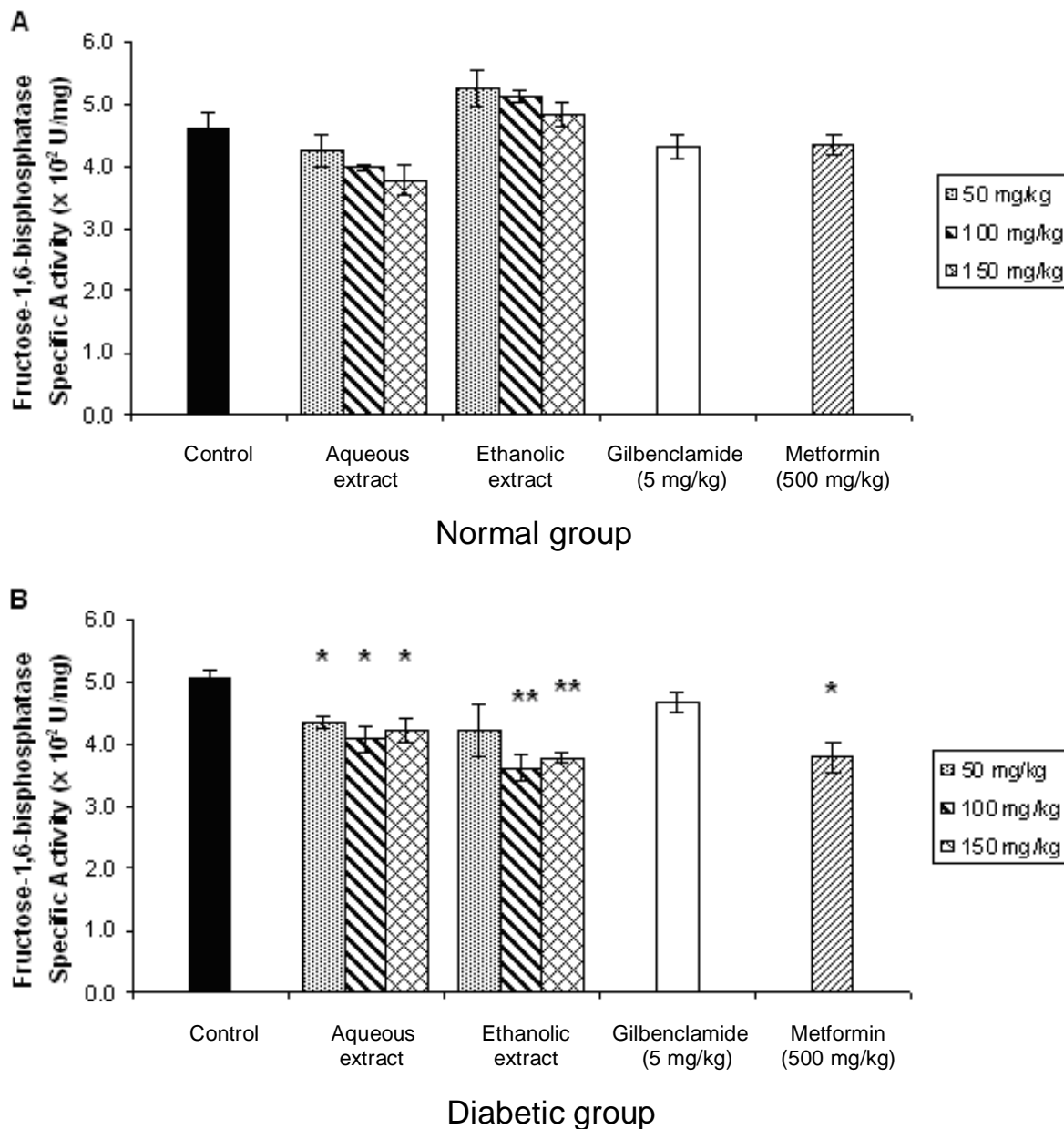
Figure 5. Effect of *Gynura procumbens* aqueous and ethanolic extracts on liver fructose-1,6-bisphosphatase specific activity of normal (A) and diabetic rats (B). Results are expressed as mean ± S.E.M. with five rats in each diabetic group (n=5) and four rats in each normal group (n=4). Treated groups were compared with controls at corresponding time-interval. Values are statistically significant at *$P<0.05$ and **$P<0.01$.

DISCUSSION

In the present study, streptozotocin (STZ) has been used as diabetogenic agent in the experimental animals. Streptozotocin, 2-deoxy-2-(*N*-methyl-*N*-nitrosourea)-1-D-glucopyranose, is a potent alkylating agent that enters the pancreatic β cells via glucose transporter-GLUT2, induces methylation of DNA and damages DNA, which activates poly(ADP-ribose) polymerase, leading to NAD$^+$

depletion in pancreatic β cells (Murata et al., 1999). STZ also acts as nitric oxide donor in pancreatic islets, enhances O_2^- radical generation by xanthine oxidase system of pancreatic β cells and stimulates H_2O_2 generation, causing DNA fragmentation in islet cells (Bolzán and Bianchi, 2002; Spinas, 1999). As a result of STZ action, pancreatic β cell death by apoptosis and necrosis occurs, which in turn decreases proinsulin synthesis (Fröde and Medeiros, 2008). Therefore,

selective destructive action of STZ on pancreatic β cells renders it a good diabetogenic agent and STZ-induced hyperglycemia in rodents is considered to be a good preliminary screening model for the study of antidiabetic agents (Singh et al., 2001).

The limitations of currently available oral pharmacological agents for control of blood glucose have stimulated research on novel antidiabetic agents with different mechanism of action. Plant-derived antidiabetic agents are gaining popularity around the world for its effective antihyperglycemic activity and minimal side effects, such as resveratrol found in grapes (Palsamy and Subramanian, 2008), catechin from *Cassia fistula* (Daisy et al., 2010), myricetin from *Abelmoschus moschatus* (Liu et al., 2006) and berberine from *Cortidis rhizoma* (Chen et al., 2010). There are a few scientific reports relating on the antidiabetic potential of various extracts from *G. procumbens* demonstrated that *G. procumbens* extracts could normalize blood glucose level in diabetic animals (Akowuah et al., 2001, 2002; Zurina et al., 2008, 2010). This study was conducted to evaluate the antihyperglycemic effect of aqueous and ethanolic extracts of *G. procumbens* leaves in normal and STZ-induced diabetic rats by investigating the effect of these extracts on glucose profile, activity of hepatic enzymes such as hexokinase, phosphofructokinase and fructose-1,6-bisphosphatase, and liver glycogen content, as well as the ability of these extracts to stimulate insulin secretion.

One of the parameters to consider the amelioration of diabetic state is to ascertain the effect of treatment on the body weight (Al-Attar and Zari, 2010). In diabetes mellitus, deranged glucagon-mediated regulation of cyclic 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 carbohydrate for energy metabolism in diabetes mellitus results in decreased body weight (Gireesh et al., 2009; Palsamy and Subramanian, 2008; Zurina et al., 2010). Normalization of carbohydrate, protein and fat metabolism would alleviate the diabetic symptom of body weight loss; therefore body weight holds one of the key in evaluating the effectiveness of an antidiabetic treatment (Al-Attar and Zari, 2010). In the present study, treatment on diabetic rats with *G. procumbens* extracts showed decrease in body weight loss, which indicates the prevention of muscle tissue damage and protein wasting that is due to hyperglycemic condition, suggesting the potential of *G. procumbens* extracts in ameliorating diabetic state in STZ-induced diabetic rats. In addition, administration of *G. procumbens* extracts to normal rats did not produce significant changes in the body weight of normal rats, except 150 mg/kg b.w. *G. procumbens* aqueous extract-treated normal rats which showed significant reduction in

body weight gain. Body weight is a sensitive indicator that reflects the state of health of experimental animals and decrease in body weight correlates with defects in body metabolism that is due to toxicity (Heywood, 1983). The reduced body weight gain which was only seen in 150 mg/kg b.w. aqueous extract-treated normal rats might be due to the presence of antagonistic substances at higher doses of the aqueous extract.

Present study also demonstrated that *G. procumbens* extracts lowered fasting blood glucose in diabetic rats, in which the ethanolic extract of *G. procumbens* leaves displayed higher extent of diabetic state amelioration than aqueous extract. The data presented in Table 2 displayed that fasting blood glucose was significantly lower for 100 mg/kg b.w. *G. procumbens* ethanolic extract-treated diabetic rats than 50 and 150 mg/kg when compared with diabetic control. This suggested that the antihyperglycemic activity of *G. procumbens* occurs in a dose-independent manner. The decrease in antihyperglycemic activity at higher doses could be due to reduced or no effect of the components present in the extracts at higher doses and/or the presence of other antagonistic components in the extract. Since the extracts did not produce any hypoglycemic effect in normal rats, therefore the extracts may be considered to have good antihyperglycemic active principles without causing any hypoglycemic effect (Fatima et al., 2010). Furthermore, the blood glucose lowering effect of *G. procumbens* leaves ethanolic extract is comparable to that of metformin, showing the potential of *G. procumbens* ethanolic extract as plant-derived antidiabetic agent.

Due to the diabetogenic nature of STZ, STZ-induced diabetic rats have diminished insulin secretion (Sefi et al., 2011). As seen in this study, oral administration of *G. procumbens* extracts did not stimulate insulin secretion as demonstrated by the low levels of plasma insulin concentrations in the diabetic rats after 42 days of treatment. There were also no changes in the plasma insulin concentration of normal rats administered with *G. procumbens* extracts. The results obtained in this study is consistent with those reported by Zurina et al. (2008, 2010), whereby oral administration of *G. procumbens* water extract did not improve the viability of pancreatic β cell and did not induce regeneration of the remaining partially destroyed pancreatic β cell of diabetic rats. According to Zurina et al. (2010), immunochemical staining for insulin of the pancreas of *G. procumbens* water extract-treated rats demonstrated minimal immunochemical staining for insulin, showing no activation of the β cells of pancreas. These findings together with the results obtained in our current study suggested that *Gynura procumbens* extracts do not possess insulinotropic activity.

Increased non-enzymatic glycosylation is one of the possible mechanism linking hyperglycemia and vascular complications of diabetes. Prolonged hyperglycemia

promotes non-enzymatic glycosylation of enzymes and proteins, forming advanced glycation end-products (AGEs). One of the AGEs most commonly produced upon chronic hyperglycemia is glycosylated hemoglobin (HbA1c), which resulted from the reaction between excess glucose in the blood with hemoglobin (Al-Yassin and Ibrahim, 1981). Therefore, the concentration of glucose in the blood shows positive correlation with the level of HbA1c. Insulin exhibits anabolic effect on protein metabolism in which it stimulates protein synthesis and retards protein degradation (Devlin, 2006). However, diminished insulin response in diabetes mellitus retards protein synthesis and stimulates protein degradation, and thus decreasing the synthesis of hemoglobin. In the present study, STZ induction caused an increment in the level of HbA1c in the diabetic rats which is consistent with other studies (Kesari et al., 2007; Rajasekaran et al., 2005). Oral administration of *G. procumbens* aqueous and ethanolic extracts have shown significant reduction on the HbA1c level of the STZ-diabetic rats which was due to improved glucose metabolism as well as increased hemoglobin synthesis (Rajasekaran et al., 2005). These observations suggested the ability of *G. procumbens* extract in improving glycemic index of diabetic rats and the reduction of HbA1c level by *Gynura procumbens* extract further confirmed its ability in prevention of oxidative damage resulted from protein glycosylation reactions during diabetic condition, and thus reducing the risk of diabetic complication pathogenesis (Palsamy and Subramanian, 2008).

The liver, which accounts for approximately 80% of endogenous glucose production, is an important organ that plays a pivotal role in the homeostasis of blood glucose and is primarily responsible for increased fasting blood glucose in diabetes mellitus (Xing et al., 2009). Glycogen is the primary intracellular storable form of glucose and its levels in various tissues directly reflect insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Since STZ selectively destroys β-cells of pancreas, markedly decreasing insulin biosynthesis and its concentration in the circulation, it is rational that glycogen levels in tissues especially liver and skeletal muscle decrease as they depend on insulin for glucose influx (Kalaiarasi and Pugalendi, 2009). Our findings that STZ-induced diabetic rats showed diminished glycogen storage in the liver are consistent with those of others (Adisakwattana et al., 2005; Bavarva and Narasimhacharya, 2008; Singh and Kakkar, 2009).

Results of the present study demonstrated slight increment of liver glycogen content in STZ-diabetic rats after *G. procumbens* ethanolic extract administration for 42 days, showing minimal effect of *G. procumbens* on liver glycogen of diabetic rats. In addition, our results also revealed that the antihyperglycemic effect of *G. procumbens* do not involve stimulation of insulin secretion by the pancreatic β-cells. This implies that the antihyperglycemic activity of *G. procumbens* does not mainly occur via improvement in glycogen metabolism, which is consistent with the results obtained on liver weight of diabetic rats as glycogen deposition in liver accounts up to 60% of liver weight in diabetic animals (Anderson, 1974). However, it is suggested that the slight improvement in liver glycogen storage observed plays a minor role in the improvement of blood glucose by *G. procumbens*. According to Daisy et al. (2010), catechin isolated from methanolic extract of *C. fistula* does not possess insulinotropic activity, but this flavan compound exerts antihyperglycemic effect by promoting glycogen storage in liver and muscle tissues of STZ-induced diabetic rats after 45 days of administration.

The authors suggested that catechin possess insulinomimetic effect by acting as a molecule which mimics insulin in stimulating insulin-dependent processes, such as activation of glycogen synthase, and thus promoting glycogen synthesis in liver and muscle of diabetic rats. The lower glycogen content observed in the liver of *G. procumbens* aqueous and ethanolic extract-treated normal rats at doses of 50 and 150 mg/kg b.w. might be due to the presence of different types of active principles with a diverse range of biochemical activities which suppressed glycogen synthesis in the liver of normal rats. However, the suppression of glycogen storage in these normal rats did not significantly affect the fasting blood glucose and HbA1c levels of these normal rats, suggesting that the changes observed on glycogen storage in normal rats do not pose negative impact and does not affect blood glucose homeostasis in these rats. Besides glycogen metabolism, the liver is also an essential organ in glycolysis and gluconeogenesis for the regulation of blood glucose homeostasis. A partial or total deficiency of insulin causes derangement in carbohydrate metabolism that decreases the activity and expression of a few carbohydrate metabolic key enzymes such as hexokinase and phosphofructokinase, resulting in impaired peripheral glucose utilization and augmented hepatic glucose production contributing to hyperglycemia (Kalaiarasi and Pugalendi, 2009). In the present study, the decrease in activities of hepatic hexokinase and phosphofructokinase in STZ-induced diabetic rats which might be due to insulin deficiency is consistent with other studies on hexokinase (Kondeti et al., 2010; Rajasekaran et al. 2005) and phosphofructokinase (Adisakwattana et al., 2005). Chronic administration of *G. procumbens* extract to the diabetic rats markedly raised the activity of these two glycolytic enzymes, suggesting that antidiabetic action of *G. procumbens* is the result of increased glucose utilization by the liver. The lower hepatic glycolytic enzymes activity observed in *G. procumbens* aqueous extract-treated normal rats but not in ethanolic extract-treated normal rats might be due to the presence of antagonistic substances present in the

aqueous extract which suppressed the activity of glycolytic enzymes in the liver of normal rats. However, there were no significant changes observed on the fasting blood glucose of these normal rats, suggesting that these changes in activity of hepatic glycolytic enzymes in normal rats do not pose negative effect on the rats and does not affect blood glucose homeostasis in these rats.

Insulin integrates hepatic carbohydrate metabolism by increasing the biosynthesis of enzymes of glycolysis and by inhibiting gluconeogenesis. Therefore, insulin deficiency that occurs in diabetes mellitus causes activation and expression of gluconeogenic enzymes (Kalaiarasi and Pugalendi, 2009). Activity of gluconeogenic enzyme, fructose-1,6-bisphosphatase in diabetic rats was significantly reduced by the administration of *G. procumbens* extract when compared with diabetic control. The reduction by *G. procumbens* ethanolic extract, especially 100 and 150 mg/kg b.w., was also comparable to that of metformin. The antidiabetic mechanism of metformin has been known to act mainly by inhibiting hepatic gluconeogenesis, resulting in decreased endogenous glucose production, and also by promoting peripheral glucose uptake via glucose transporters (Kim et al., 2008; Kirpichnikov et al., 2002). Thus, it is suggested that the antidiabetic effect of *G. procumbens* involves inhibition of hepatic gluconeogenesis.

G. procumbens extracts did not show insulinotropic activity in this study, therefore the amelioration of diabetic state observed which occurs via stimulation of hepatic glucose utilization and inhibition of gluconeogenesis could be due to the presence of active principles in the extracts that possess insulinomimetic activity at the cellular level. Preliminary phytochemical analysis on *G. procumbens* methanolic extract conducted by Akowuah et al. (2001, 2002) led to the isolation of flavonol and flavonol glycosides, including rutin, quercetin, kaempferol, as well as their glycosides quercetin-3-O-rhamnosyl(1-6)glucoside, quercetin-3-O-rhamnosyl(1-6)galactoside, kaempferol-3-O-glucoside and kaempferol-3-O-rhamnosyl(1-6)glucoside. High performance thin layer chromatography (HPTLC) analysis performed by Rosidah et al. (2009) and Zurina et al. (2008, 2010) revealed that methanolic and water extract of *G. procumbens* leaves contains 0.76 and 2.65% of kaempferol-3-O-rutinoside and astragalin, respectively. These flavonoids and their glycosides have been found to be responsible for blood glucose lowering activity in experimental animals (Chattopadhay, 1999; Zhang et al., 2010) and might be responsible for promoting glucose uptake by muscle tissues in streptozotocin-induced diabetic rats (Zurina et al., 2010). Treatment of diabetic rats with ethanolic extract of *G. procumbens* seemed to exert better antihyperglycemic activity than aqueous extract as seen in improvement on glucose and glycogen metabolism in the diabetic rats. Ethanolic extract of *G. procumbens* had been previously reported to contain higher amount of polyphenols compared to aqueous and methanolic extracts (Puangpronpitag et al., 2010). Therefore, it is suggested that the antihyperglycemic activity in the ethanolic extract of *G. procumbens* could possibly due to the presence of higher amount of flavonoids and their glycosides which possess insulinomimetic activity. The antihyperglycemic effect of the *G. procumbens* ethanolic extract is comparable to that of metformin, suggesting its potential as an antidiabetic agent.

Conclusion

In conclusion, our findings demonstrated that the antidiabetic mechanism of *G. procumbens* extract is extra-pancreatic, which involves stimulation of glycolysis and inhibition of gluconeogenesis in the liver, leading to increased hepatic glucose utilization and decreased hepatic endogenous glucose production. The antidiabetic effect of *G. procumbens* extract may be due to the insulinomimetic ability of flavonoids present in the extract. Ethanolic extract of *G. procumbens* leaves showed promising antidiabetic effect and is comparable to metformin, suggesting its high potential to be developed as a plant-derived antidiabetic agent.

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