Effects of *Gynura procumbens* Extract and Glibenclamide on Sperm Quality and Specific Activity of Testicular Lactate Dehydrogenase in Streptozotocin-induced Diabetic Rats

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Abstract

Diabetes mellitus is believed to bring negative effects on the male reproductive system through an increase in oxidative stress. *Gynura procumbens,* a local herb with anti-hyperglycemic and anti-diabetic properties, has been traditionally used to treat the disease. The present study aimed to assess the anti-diabetic activity of aqueous *G. procumbens* and the effect of treatment on the reproductive system in streptozotocin-induced male diabetic rats. Glibenclamide, an established anti-diabetic drug, was used as a positive control in the study. Diabetic rats (n=5) force-fed with *G. procumben*s aqueous extract with 100 mg/kg dosage (n=5) showed increased sperm count and motility by 25.12% and 23.97±1.09% respectively while sperm mortality decreased by 38.43% as compared to the controls. Testicular LDH specific activities in *G. procumbens* aqueous extract treated rats were increased by 72.53%. On the other hand, diabetic rats force-fed with glibenclamide with 5 mg/kg dosage (n=5) showed decreased sperm count and slightly increased sperm motility by 4.65% and 19.94±1.26%, respectively, while sperm mortality increased significantly ($p<0.05$) by 38.43% as compared to the controls. Testicular LDH specific activities in glibenclamide treated rats were increased by 26.58%.

Keywords: *Gynura procumbens*, sperm quality, testicular lactate dehydrogenase, streptozotocin, diabetes mellitus

Introduction

Diabetes mellitus (DM) is a metabolic disorder that can be characterized by hyperglycemia due to abnormal insulin production and secretion, resistance towards insulin or both. About 90% of male diabetic patients experienced sexual dysfunction, impotence and infertility caused by testicular failure associated with prolonged hyperglycemia [1].

Experimental models using streptozotocin (STZ)-induced rats supplied the evidence that supported the association between erectile dysfunction and DM in addition to better understand sexual dysfunction in male diabetic patients [2]. Growing evidence indicates that oxidative stress is increased in diabetes, due to the overproduction of reactive oxygen species (ROS), and decreased efficiency of antioxidant defences [1]. Numerous experimental evidences have also emphasized a potential relationship between oxidative damage in testis or sperm and testicular dysfunction leading to male infertility [3].

Hyperglycemia leads to the increased production of reactive oxygen species via at least four different routes: increased glycolysis; intercellular activation of the sorbitol pathway; auto-oxidation of glucose and non-enzymatic protein glycation [4], which in turn, causes an increase in oxidative stress to the body. The lipids in sperm are the main substrates for peroxidation and researches have shown that excess amounts of ROS and free radicals have adverse effects on sperm quality and function. This condition will ultimately cause infertility in men where it is characterized by low sperm count and motility and also high percentage of sperm mortality. Mammalian sperm cells consist of a specific lipid composition, which is high in polyunsaturated fatty acids, plasmalogens and sphingomyelins. The lipids in sperm are the main substrates for peroxidation [5] and researches have showed that excess amounts of ROS and free radicals have adverse effects on sperm motility. Furthermore, oxidative damage to lipids and DNA of sperm is associated with declining motility and diminished fertility of human sperm [6].

Testicular lactate dehydrogenase (LDH) catalyzes the reversible conversion of lactate to pyruvate in the cytosol of Sertoli cells. The resulting pyruvate can then enter the tricarboxylic acid or TCA cycle as one of the intermediates meanwhile the reduced NADH formed during this reaction will be oxidized in the mitochondria through oxidative phosphorylation where molecules of ATP are generated [7]. ATPs will serve as the energy supply for the processes in the germ cells such as spermatogenesis and sperm movement. In Sertoli cells, glucose is metabolised via cytosolic glycolysis to lactate, which is then used primarily by the germ cells as a substrate for ATP production in mitochondrial oxidative phosphorylation [8]. Testicular

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LDH plays an important role in the process of spermatogenesis and has been shown to be vital for sperm survival and motility [3]. This enzyme is found in germ cells and is present in cells of the mouse gametogenic line from pachytene primary spermatocytes to sperm. Therefore, reduced activity of testicular LDH caused by oxidative stress can consequently alter the quality and function of sperms and contribute to the decline in the fertility of diabetic male patients.

We investigated the effects of diabetes on sperm quality and testicular LDH enzyme in diabetic rats and compared the effects of *Gynura procumbens* aqueous leave extract with glibenclamide.

Materials and Methods

Gynura procumbens

The leaves of *G. procumbens* were collected from the Plant House, Faculty of Science and Technology, Universiti Kebangsaan Malaysia.

Animals

Male Sprague dawley rats, aged two months were obtained from Animal House, Faculty Science and Technology, Universiti Kebangsaan Malaysia. Animals were maintained on standard laboratory rat chow with water *ad libitum.* In this study, 40 rats were divided into two groups, normal (20) and diabetic (20) rats.

Induction of diabetes

Diabetes was induced after rats were fasted overnight by a single intravenous injection of STZ, dissolved in sodium citrate buffer pH 4.5. Fasting blood glucose levels of the rats were determined using Glucometer Accucheck Active® Roche Diagnostic (Canada) on the seventh day after the induction. Rats were considered diabetic if the fasting blood glucose levels exceeded 8 mmol/L. Treatments of *G. procumbens* extract (100 mg/kg) and glibenclamide (5 mg/kg) were administered daily for 14 consecutive days via force-feed method.

Extraction of Gynura procumbens leaves

G. procumbens leaves extraction was done by the method of Peungvicha [9]. Briefly, leaves were dried in oven at 45°C for two days. Dried samples weighing 100 g were mixed with one litre of distilled water and heated in water bath for two hours until the sample volume was reduced to 100 ml and then filtered. After filtration, the filtrate was centrifuged at 10 000 g for ten minutes. The supernatant was collected and freeze-dried, yielding a light green powder. The extract was suspended in distilled water before administration.

Sperm sample preparation

After animal sacrifice by chloroform, testes and epididymis were removed to obtain the sperm sample [10] and were put into 15 ml prewarmed rat sperm isolation medium known as Biggers, Whitten and Whittingham (B.W.W) medium [11]. Sperm sample was then incubated at 37°C with 5% CO_2 for 30 minutes. The sperm sample was then assessed based on three parameters; sperm count, motility and mortality.

LDH Assay

LDH activity was measured spectrophotometrically according to a previously described procedure by Racher [12] following the rate of conversion of NADH to NAD^+. Testes obtained after sacrifice were washed with PBS and homogenized in 0.03 mM PBS (pH 7.4) in the ratio of 1:2. Homogenate was then centrifuged at 10 000 g for 30 minutes using Sorvall® RC-58 Refrigerated Superspeed Centrifuge. Enzymatic assay was then performed on the supernatant. LDH activity was calculated by monitoring the absorbance change/min of NADH at 340 nm, using a molar extinction coefficient of 0.63 l $nmol^{-1}$ mm^{-1} for NADH, and expressed as nmol NADH/min/mg protein while the protein content was determined by the Biuret assay.

Results

Fasting blood glucose levels

Administration of *G. procumbens* extract and glibenclamide to diabetic rats significantly decreased the blood glucose levels by 56.5% and 30.12%, respectively, when compared to the negative control rats (Figure 1). On the other hand, both treatments did not have any significant effect on the blood glucose levels of normal rats.

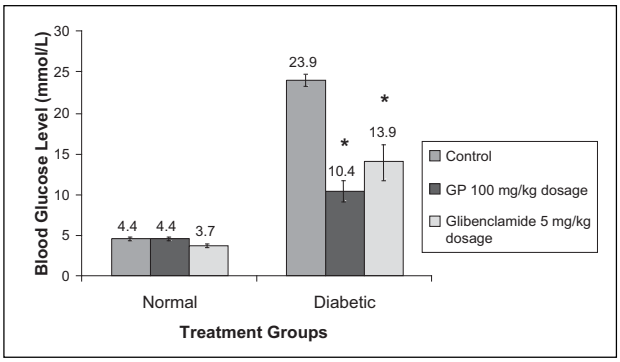

Figure 1: Effects of *Gynura procumbens* (100 mg/kg) and glibenclamide (5 mg/kg) supplementation on fasting blood glucose levels in normal and diabetic rats. (* shows significant difference p<0.05)

Sperm quality assessment

Figure 2 shows the effects of *G. procumbens* aqueous extract and glibenclamide on sperm count. The sperm counts of normal rats were not significantly affected by both the treatments (Figure 2). After treatment with

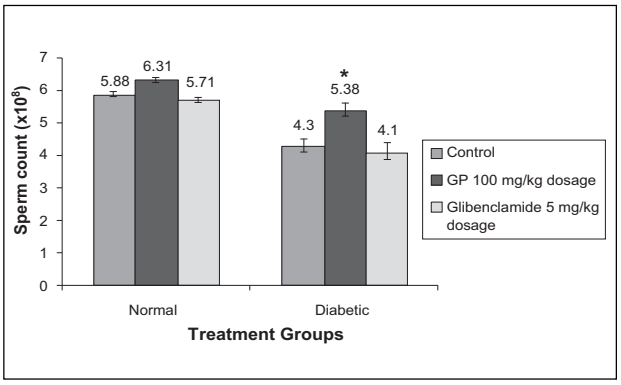

Figure 2: Effects of *Gynura procumbens* (100 mg/kg) and glibenclamide (5 mg/kg) supplementation on sperm counts in normal and diabetic rats. (* shows significant difference p<0.05)

G. procumbens aqueous extract, the sperm count of diabetic rats showed significant (p<0.05) increase by 20%. On the other hand, the sperm count of glibenclamide-fed rats did not show any large deviation from the control diabetic rats.

Table 1 displays the percentage of sperm motility based on motility grades for normal and diabetic rats. There are four sperm motility grades, where grade a (>25 μm/s) depicts the most progressive sperm movement, grade b (5-24 μm/s) and c (<5 μm/s) represent the category of intermediate movement of sperms meanwhile immotile or non-moving sperms are placed in grade d (0 μm/s). In reference to Table 1, normal rats treated with *G. procumbens* aqueous extract and glibenclamide did not show any significant changes in the percentage of sperm motility for both grades. Administration of *G. procumbens* has succeeded in increasing the percentage of sperm in grade a by more than triple fold in diabetic rats while the glibenclamide treatment resulted in a slight increment in the category.

Table 1: Effects of *Gynura procumbens* (100 mg/kg) and glibenclamide (5 mg/kg) supplementation on sperm motility of normal and diabetic rats. (* shows significant difference p<0.05)

	Percentage of Sperm Motility based on Motility Grades (%)			
Treatment Group (n=5)	**a**	**b**	**c**	**d**
NORMAL				
Control	25.93	27.50	23.14	23.43
Gynura procumbens 100 mg/kg	31.57	22.75	14.28	23.57
Glibenclamide 5 mg/kg	30.62	24.68	20.69	24.01
DIABETIC				
Control	6.96	20.29	23.03	49.72
Gynura procumbens 100 mg/kg	23.97 *	20.65	17.88	37.50
Glibenclamide 5 mg/kg	19.94	22.51	9.57	47.98

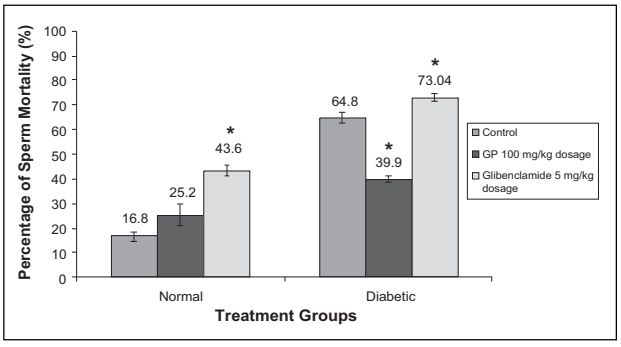

Figure 3: Effects of *Gynura procumbens* (100 mg/kg) and glibenclamide (5 mg/kg) supplementation on percentage of sperm mortality in normal and diabetic rats. (* shows significant difference p<0.05)

Administration of *G. procumbens* aqueous extract has no significant effect on the sperm mortality of normal rats but it has significantly (p<0.05) reduced the percentage of sperm mortality in diabetic rats by 38% (Figure 3). In contrast, the sperm mortality in both normal and diabetic rats treated with glibenclamide showed significant (p<0.05) increase by 61% and 11%, respectively.

Specific activity of LDH

After administration of *G. procumbens* aqueous extract (100 mg/kg), specific activities of testicular LDH in both normal and diabetic rats increased significantly (p<0.05) by 25% and 42%, respectively, as shown in Figure 4. Meanwhile, treatment with glibenclamide did not show any distinct change in the LDH activity in both normal and diabetic rats.

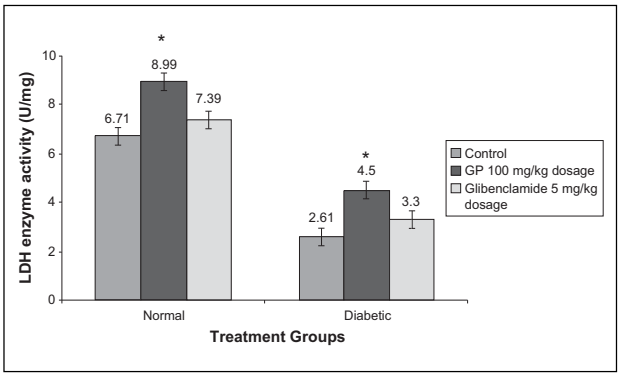

Figure 4: Effects of *Gynura procumbens* (100 mg/kg) and glibenclamide (5 mg/kg) supplementation on specific activities of testicular LDH in normal and diabetic rats. (* shows significant difference $p<0.05$)

Discussion

The significant decrease in fasting blood glucose level of diabetic rats treated with *G. procumbens* aqueous extract serves as evidence of the anti-diabetic and anti-hyperglycemic activity of this plant [13]. The same result shown in the rats treated with glibenclamide confirms the role played by this drug in the control of hyperglycemia in diabetes. *G. procumbens* aqueous extract treatment managed to restore the sperm quality that has been affected by diabetes. Even though the mechanism of action of *G. procumbens* extract is still unknown, it can be hypothesized that its mode of action can be likened to that of other anti-diabetic plants such as *Ginkgo biloba* [14], where the flavonoid components of the herb act to neutralize the increased activity of reactive oxygen species and also to inhibit the lipid peroxidation by blocking the peroxyl radical activity [15].

On the other hand, the results shown by treatment with glibenclamide can be explained by the relations between the mode of diabetes induction by streptozotocin (STZ) and mechanism of glibenclamide action. STZ is selectively toxic to the pancreatic β cells and act to destroy these cells so that insulin secretion in rats is impaired, giving rise to the diabetic state [16]. Even with the treatment of glibenclamide, the remaining functional pancreatic β cells may not be able produce and secrete enough insulin to regulate the blood glucose level. Thus, the effects of glibenclamide on sperm quality could be attributed to the state of prolonged diabetes or the adverse effects of the drug itself.

Increasing evidence suggests that diabetes has an adverse effect on male reproduction function and that oxidative stress may be involved [17]. According to Mahboob et al. [18], antioxidant enzyme-dependent defences may play an important role by scavenging free radicals produced under oxidative stress. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. Antioxidant may also play important roles in health care, such as by acting as cancer chemopreventive and anti-inflammatory agents and by reducing the risk of diabetes [19].

Sperm are particularly susceptible to oxidative stress-induced damage because their plasma membranes contain large quantities of polyunsaturated fatty acids (PUFA) [20] and their cytoplasm contains low concentrations of scavenging enzymes [21]. Oxidative stress-mediated damage to the sperm plasma membrane may account for defective sperm function observed in a high proportion of infertility patients [22], where it attacks not only the fluidity of the sperm plasma membrane but also the integrity of DNA in the sperm nucleus. Oxidative stress-induced DNA damage may accelerate the process of germ cell apoptosis leading to the decline in sperm counts associated with male infertility and the apparent deterioration of semen quality.

The increase in the activity of LDH after treatment with G. procumbens aqueous extract can be used as a correlation to the increase of sperm quality of the diabetic rats. Due to the important role played by the enzyme in spermatogenesis and complete development of sperm, the increased activity of this enzyme in the testis is able to recover the sperm function and quality and subsequently the fertility of male diabetic rats.

The study concerns the effects of *G. procumbens* and glibenclamide on sperm quality and specific activity of testicular LDH in streptozotocin-induced diabetic rats. Taken together these results lead us to conclude that diabetes mellitus and hyperglycemia have adverse effects on sperm count, motility and mortality as well as testicular LDH specific activity in model animals for type II diabetes. *G. procumbens* aqueous extract supplementation at the dosage of 100 mg/kg is beneficial in decreasing blood glucose level, restoring the fertility of diabetic rats by increasing sperm count and motility, decreasing sperm mortality as well as increasing the specific activity of testicular LDH. Meanwhile, glibenclamide supplementation at the dosage of 5 mg/kg did not manage to demonstrate similar effects in diabetic rats.

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