International Journal of Phytomedicine 6 (2014) 448-454
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Evaluation of hypoglycemic properties and fertility effect of *Piper sarmantosum* Roxb. aqueous leaf extract in streptozotocin induced diabetic mice

Ampa Luangpirom[1*], Watchara Kourchampa[2], Pichet Somsapt[3]

***Corresponding author:**

Ampa Luangpirom

[1]Applied Taxonomic Research Center, Faculty of Science, Khon Kaen University, Khon Kaen, 40002, Thailand
[2]Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, 40002. Thailand
[3]Department of Radiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, 40002, Thailand.

Abstract

Male mice of ICR strain (6-7 week old, 30-40 g) were used for evaluation of the hypoglycemic and fertility effect of *Piper sarmentosum* Roxb. leaf extract. Diabetes was induced by intraperitoneal (i.p) injection of streptozotocin (STZ) (6.0 mg/100gBW) and glibenclamide (reference drug) 1 mg/ 100gBW and Piper sarmentosum Roxb. leaf extract (PS) at 60 and 100 mg/100gBW were orally administered (per os) for 21 days. The hypoglycemic activity of PS was 73.04 and 120.96% of the glibenclamide. Meanwhile, the blood insulin level also significantly increased by 8.25 and 50.53 % of the diabetic control. The concordant results showed that diabetic pancreatic islets were impaired and improved after extract treatment. However, the fertility status test showed that the seminal quality and blood testosterone of diabetic mice decreased significantly ($P< 0.05$) as compared to the normal mice and significantly increased when compared to the diabetic control after long-term treatment. In conclusion, Piper sarmentosum Roxb. aqueous leaf extract at doses of 60 and 100 mg/100 gBW revealed hyperglycemic properties in diabetic mice. They also improved both pancreatic islet function and fertility status of diabetic mice after 21 days of extract treatment.

Keywords: hyperglycemic properties, fertility effect, insulin, *Piper sarmentosum* Roxb

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder, inducing a state of chronic hyperglycemia and glucose tolerance impairment. It results from a defect in insulin secretion, insulin function or both [1]. Furthermore, chronic hyperglycemia causes oxidative stress [2, 3] and consequent pathogenesis in many organs such as vascular and neural dysfunction, opthalamopathies [4] and fertility problems [5]. Thus, diabetic patients need to control their blood glucose level and to improve blood glucose tolerance by using synthetic drugs such as glibenclamide, which enhances insulin secretion by the pancreatic islets [6]. Nowadays, the use of medicinal plants has gained more interest for anti-diabetes and other ailment therapies. *Piper sarmentosum* Roxb. is one of these, and is a vegetable belonging to the family Piperaceae. Its leaves contain many flavonoids such as myricetin, quercetin, apigenin and naringenin In particular, naringenin is a powerful antioxidant showing 75.7 % of superoxide scavenging activity [7] and decrease in oxidative stress [8]. In addition, it also inhibits alpha-glucosidase activity in the intestine, delaying the absorption of carbohydrate in type 2 diabetic rats and dampening postprandial blood glucose levels [9]. However, *P. sarmentosum* Roxb. aqueous leaf extract 12.5 g/kgBW increases superoxide dismutase (SOD), an antioxidant enzyme in rats after oral administration for 28 days. and insulin (5 IU/kgBW) administration also decreases oxidative stress in diabetic mice [10]. Interestingly, fertility problems also concurrently occur in diabetic patients [11] and in streptozytocin (STZ) induced diabetic rats [5]. Therefore, the lowering of hyperglycemia should reduce oxidative stress and consequently improve fertility in diabetic animals. The aims of this study were to evaluate the hypoglycemic activity and fertility improvement potential of *P. sarmentosum* Roxb. aqueous leaf extract in streptozotocin induced diabetic mice.

Materials and Methods

Experimental animals

Adult male mice (ICR strain, 8 week old, 35-40 g) were obtained from the National Laboratory Animal Center, Nakornprathom province, Thailand. They were housed in an animal room under 25±2 °C with dark:light cycle=12:12 h. Standard pellet food (Chareanpogapan Ltd.) and water ad libitum were freely available. The experiments were conducted after approval by the Institutional Animal Ethics Committee, Khon Kaen University, Thailand (Reference No.0514.1.12.2/77)

Plant extraction

P. sarmentosam Roxb. was collected from a cultivated plot, Sila district, Khon Kaen Province, northeast Thailand and identified by a taxonomist. Their leaves (2,833 g) were cleaned, boiled with distilled water (1:1, w/v) at 70 °C overnight (as in the method of Peungvicha et al., 1998 [12]) filtered with cotton mesh and then evaporated in a hot air oven at 45 °C until dry. The dried extract weighed 115.43 g (=8.89 % w:w) and was dissolved with distilled

water at concentrations of 120 and 200 mg/ml for oral administration at 0.5 ml/100 gBW of animal by feeding needle.

Blood glucose determination

After fasting for 16 hours with water free access, fasting blood glucose was determined by glucometer (ONE TOUCH®, Select Simple™ Life, Science, Inc. 2010. Johnson and Johnson Company, Bangkok, Thailand) via blood from the tail artery.

Chemicals

Streptozotocin (Sigma, St. Louis, MO, USA) for diabetic induction. Glibenclamide (Pharma Supply Ltd., Thailand) as reference drug. Testosterone radioimmunoassay kit (The DSL-400 ACTIVE® Testosterone Coated –Tube RIA Kits, Diagnostic system Laboratories, Inc., USA), assay sensitivity =0.14 ng/ml for blood testosterone determination. Insulin radioimmunoassay kit (Roche Ltd, USA), assay sensitivity = 5.5 µIU/L for blood insulin determination.

Experimental design

Forty-four male mice had diabetes induction by a single intraperitoneal (i.p) injection of streptozotocin (6 mg/100 gBW). Diabetes was confirmed by fasting blood glucose (FBG) determination on the third day after induction [13]. The blood glucose values were 140-200 mg/dl, which is considered as mild diabetes [14]. On the first day of the experiment, six normal mice and forty-four diabetic mice had fasting blood glucose determined. Six normal mice served as a reference group and the diabetic mice were randomly divided into 4 groups of six animals each as below:

Group I : Normal mice as reference group received 0.5 ml of distilled water/100 gBW

Group II : Diabetic mice as negative control received 0.5 ml of distilled water/100 gBW

Group III: Diabetic mice as positive control received glibenclamide 1mg/100 gBW

Group IV: Diabetic mice as treated group received *P. sarmentosum* extract (PS) 60 mg /100 gBW

Group V : Diabetic mice as treated group received *P. sarmentosum extract* (PS)100 mg /100 gBW

After 21 days of oral administration, all groups had fasting blood glucose level measured via tail artery for hypoglycemic activity evaluation. Then, blood was collected by cardiac puncture under ether anesthesia and centrifuged at 1,700 rpm for 5 minutes at room temperature. Plasma samples were kept for insulin and testosterone assay. Then, semen was collected for seminal analysis. Pancreases were dissected and slides prepared by paraffin method for histological studies.

Seminal analysis

At the end of the experiment, epididymis and vas deferens of all groups were excised, torn with a dissecting needle in 2 ml of 0.9 % of NaCl and incubated at 35 ^{o}C for sperm quality evaluation. Sperm concentration was determined in terms of total sperm count per individual, viable sperms, progressive moving sperms and abnormal morphology sperms and expressed as percentage of incidence [15].

Histological studies of pancreatic islet

Pancreases of all groups were sampled, dissected and immediately fixed in Bouin's solution for 48 hours, then processed by the paraffin method, sectioned at 5 micron thickness and stained with hematoxylin and eosin (H&E). The pancreatic slides were observed under light microscope with x40 objective and pancreatic islet sizes were measured by micrometer.

Statistical analysis

All results were expressed as mean±standard deviation ($\bar{X}$±SD). Data were analysed by one-way analysis of variance (ANOVA) and the different results among groups were compared by Duncan's multiple range test. Values of P <0.05 were considered as statistically significant [16].

Results

Hypoglycemic properties of *P. sarmentosum* Roxb. leaf extract in diabetic mice are presented in Table 1 and Figures 1 and 2. The *P. sarmentosum* Roxb. leaf extract (PS) at doses of 60, 100 mg and glibenclamide at a dose of 1 mg/100 gBW were orally administered to diabetic mice for 21 days and fasting blood glucose (FBG) was determined at day 22. The FBGs at day 1 of normal mice were 94.50±1.94 mg/dl and of diabetic mice were 194.84±7.70 – 195.57±7.34 mg/dl (Figure 1). The group receiving glibenclamide and the group receiving PS 100 mg/100 gBW showed a significant decrease in FBG of day 22 when compared with FBG of day 1. The diabetic control group revealed a significant increase and normal mice served as the reference group presenting a non-significant increase in FBG of day 22 as compared to FBG of day 1. These results reveal that the hypoglycemic potentials of PS 60 and 100 mg/100 gBW and gliblenclamide 1 mg/100 gBW were 65.11, 47.56 and 120.96 mg/dl respectively when compared with the diabetic control group and the hypoglycemic potentials of PS 60 and 100 mg/100 gBW were 73.04 and 120.96 % of the group treated with glibenclamide. Interestingly, the concordant result of significant increase in insulin level of the group that received glibenclamide (17.24±2.90 IU/L) and the group that received PS 100 mg/100 gWB (21.16±2.53 IU/L) were found when compared with diabetic control group (14.19±2.95 IU/L) (Table 1). Histological studies of pancreatic islet showed their improvement by increase in size and decrease in number of dead cells (Figure 2).

The fertility effect of *P. sarmentosum* Roxb. leaf extract in diabetic mice is shown in Table 2 and Figure 3. The relation of fertility impairment and hyperglycemia were found in the diabetic control group. They decreased in blood testosterone and sperm quality

(Table 1 and Figure 3). The diabetic control group significantly decreased their sperm concentration (Figure 3A), number of motile sperms (Figure 3B) and viable sperms (Figure 3C), while the number of abnormal sperms (Figure 3D) were significantly increased. However, an improvement in fertility index was found in diabetic groups treated with glibenclamide and *P. sarmentosum* Roxb. leaf extract (PS) with a significant increase in blood testosterone and sperm quality. The sperm concentration, percentage of viable sperms, motile sperms and abnormal sperms of the diabetic control were 82.74±2.08 x10^6 per individual, 70.84±1.54, 26.66±6.22 and 55.82±2.36 %, respectively, The corresponding values in the diabetic group treated with glibenclamide were 89.60±3.90 x10^6 per individual, 74.19±4.34, 39.13±6.78 and 44.38±2.20 %, respectively The corresponding values in the diabetic group treated with PS 60 mg/100 gBW were 135.54±4.15 x10^6 per individual, 83.59±2.59, 45.61±10.73 and 46.49±3.60 %, respectively. The corresponding values in the diabetic group treated with PS100 mg/100 gBW were 144.56±6.56 x10^6 per individual, 87.76±1.99, 48.44±5.42 and 42.44±0.60 %, respectively. Meanwhile, the sperm concentration, percentage of viable sperms, motile sperms and abnormal sperms of normal mice serving as a reference group were 97.30±3.05 x10^6 per individual, 84.63±1.72, 53.61±8.01 and 33.95±2.55 %, respectively.

Table 1 Hypoglycemic activity of *P. sarmentosum* leaf extract (PS) in diabetic mice after oral administration for 21 days

Treatmment(/100 gBW.)N=6	Changed blood glucose level (%)[A]	Efficiency of hypoglycemic activity [B] (%)	Insulin level (IU/L)	Testosterone level (ng/ml)
Normal: distilled water 0.5 ml	+ 17.97		20.05±4.09^{ab}	
Diabetes: distilled water 0.5 ml	+ 46.01		14.19±2.95^{c}	
Diabetes: glibenclamide 1 mg	- 19.1	65.11 (100)	17.24±2.90^{bc}	5.46±1.18^{ab}
Diabetes: PS 60 mg	- 1.55	47.56 (73.04)	15.36±2.86^{c}	3.53±1.07^{bc}
Diabetes: PS 100 mg	- 32.75	78.76 (120.96)	21.36±2.53^{a}	5.25±0.09^{ab}

N= number of experimental animals
A= % blood glucose level on day 22 - % blood glucose level on day 1 + means increase and - means decrease.
B = change of blood glucose level of diabetic control group - change of blood glucose level of diabetic group treated with glibenclamide or the extract.
same letter in column means non-significant difference (P>0.05).different letter in column means significant difference (P<0.05).

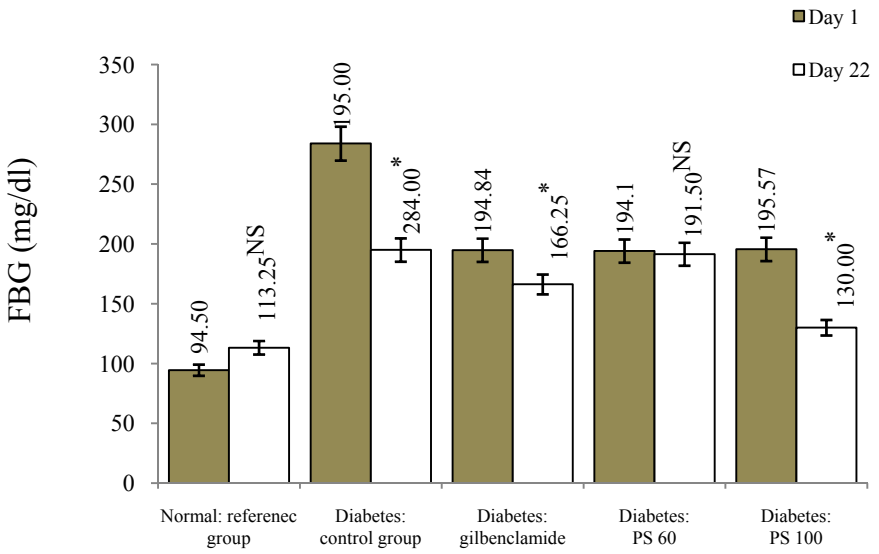

Figure 1 Fasting blood glucose level (FBG) on day 1 and day 22 of group treated with glibenclamide 1 mg, groups treated with *P. sarmentosum* extract 60 mg (PS 60) and 100 mg (PS 100)/100gBW
* = blood glucose level on day 22 differs significantly from day 1 (P<0.05) NS = blood glucose level on day 22 differs non-significantly from day 1 (P>0.05)

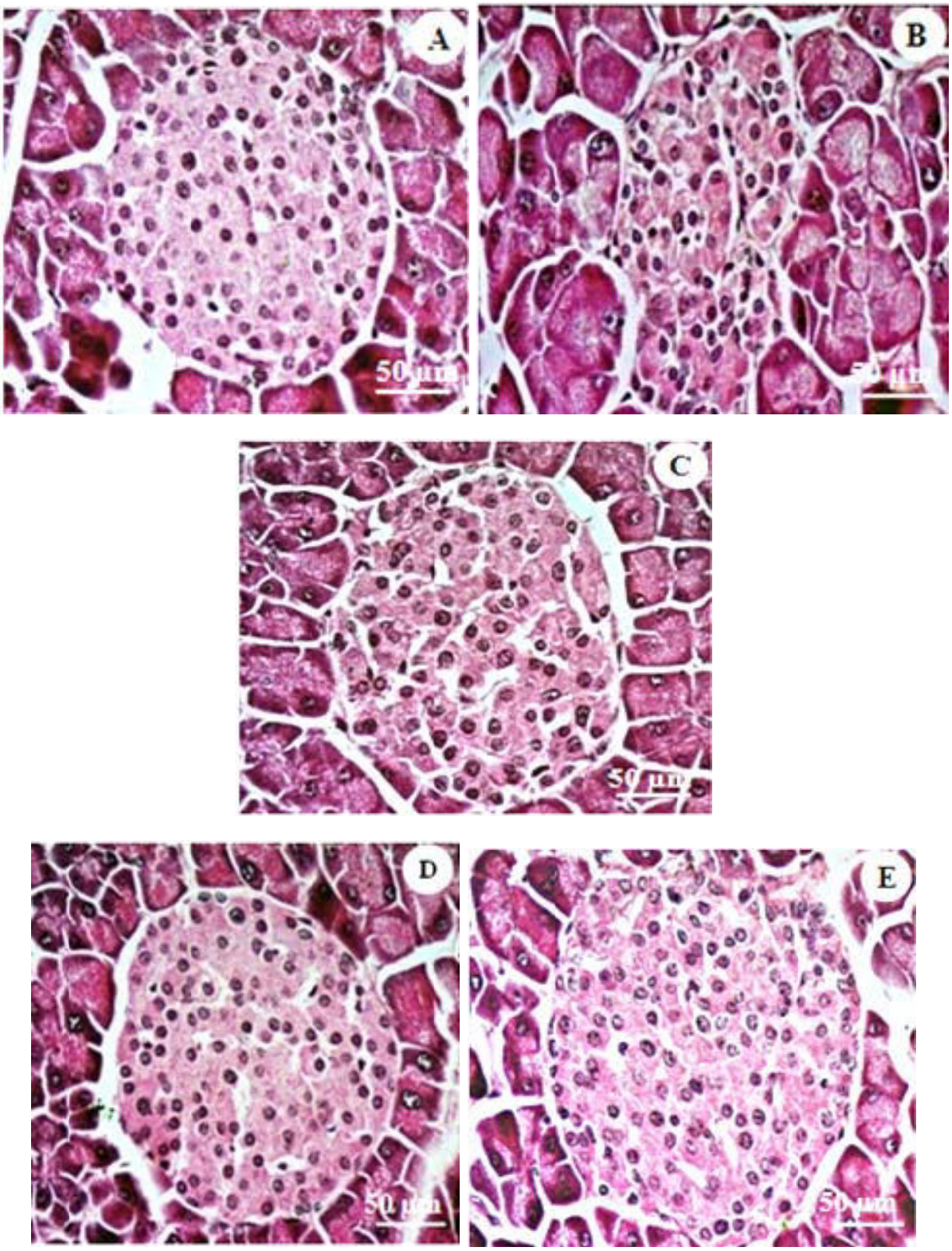

Figure 2 Cross-section of pancreas showing islets of Langerhann, H&E, bar = 50 µM; A, normal control group; B, diabetic control group; C, diabetic group treated with glibenclamide and D & E, diabetic groups treated with *P. sarmentosum* extract 60 mg and 100 mg /100gBW

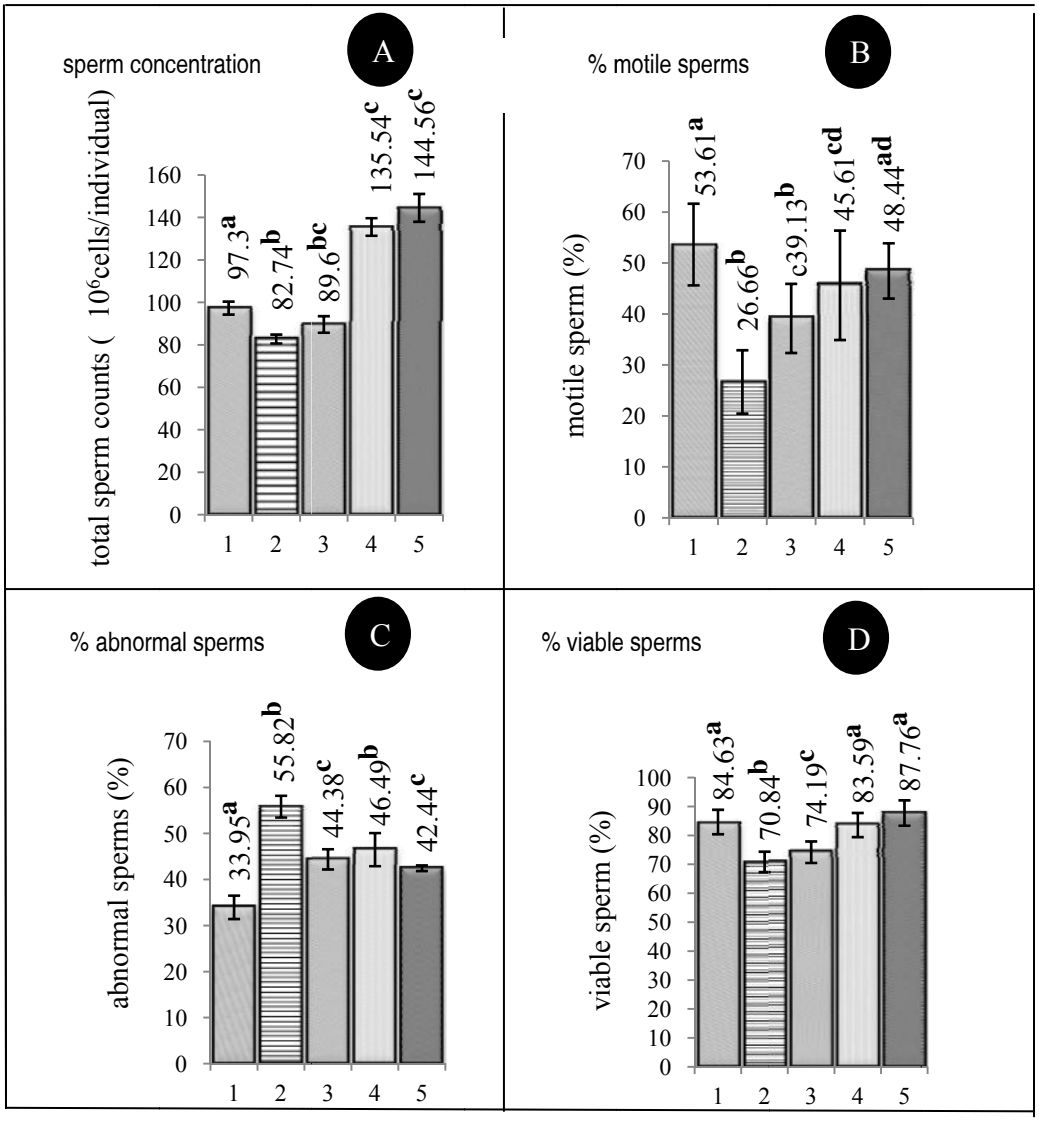

Figure 3 A, sperm concentration; B, motile sperms (%); C, abnormal sperms (%) and D, viable sperm (%) in the diabetes control group and diabetes groups that received glibenclamide or *P. sarmentosum* extract at 60 and 100 mg/ 100 gBW.
1, Normal: distilled water 0.5 ml; 2, Diabetes: distilled water 0.5 ml; 3, Diabetes:glibenclamide 1 mg; 4, Diabetes: PS 60 mg; 5, Diabetes: PS 100mg/100gBW same letter means non-significadifference (P>0.05). different letter means non-significant difference (P<0.05).

Discussion

This study used streptozotocin to induce typy II diabetes in male mice. It worked by entering into pancreatic insulin secreting beta cells via a glucose transporter, causing DNA damage and generating superoxide radicals to destroy beta cells [6]. Therefore, the hyperglycemia was concordantly incident with oxidative stress. Glibenclamide was used as reference drug. It is a commercial synthetic hypoglycemic agent and has been used as an antidiabetic drug in type II diabetic patients since 1973 [17]. This drug works by inhibiting the ATP sensitive K^+ ($K\text{-}_{ATP}$) channel in the plasma membrane of beta cells [18]. The inhibition causes cell membrane depolarization, activation of the voltage-gated Ca^+ channel, increased Ca^+ influx, a rise in cytosol Ca^+ and thereby insulin release [19]. Furthermore, this drug also behaves as an antioxidant, resulting from its amino azobicyclo-octare ring [20]. Our study found that diabetic groups that received *P. sarmentosum* leaf extract orally at 60 and 100 mg/100 gBW showed hypoglycemic potentials of 73.04 and 120.96 % of glibenclamide 1 mg/100 gBW and increased insulin levels 8.25 and 50.53 % of

diabetic control group. They also had an increase in blood insulin level which was extract dose dependent. *P. sarmentosum* Roxb. leaves contain many flavonoids, such as hesperitin (3´5,7-trihydroxy-4´-methoxyflavanone), quercetin (3,3´,5´,5,7-pentahydroxy flavones) and naringenin (4´,5,7 trihydroxyflavanone), which are antioxidants [7]. Naringenin especially exhibits a potent antioxidant effect with 75.7 % of superoxide scavenging activity [7]. It also shows a strong inhibition of glucose uptake by exhibiting a dose-dependent inhibition of rat intestinal -glucoxidase activity [9]. Recently, an in vitro study revealed that *P. sarmentosum* extract of 5 and 10 mg/ml could significantly lower glucose absorption ($P<0.05$) in a sodium fluoride treated group and enhanced glucose utilization of rat muscle cells by 2.0-2.5 times in comparison with the control group [21]. This implies that the hypoglycemic properties of *P. sarmentosum* leaf extract result from the inhibition of glucose absorption and increase of blood insulin. Thereby, glucose utilization of cells was increased. In addition, an increase in insulin decreased oxidative stress leading to improved pancreatic islet histology and function.

It is well-known that some diabetic males are infertile. It is induced by oxidative stress generated from chronic hyperglycemia. There is much experimental evidence emphasizing a potential relationship between oxidative stress in testicular dysfunction leading to infertility [22, 23]. Furthermore, antioxidant supplementation is able to improve sperm quality in infertile men [24]. A previous study claimed that *P. sarmentosum* aqueous leaf extract and insulin treatment decreases oxidative stress in diabetic rats [10]. In addition, insulin treatment also improves testicular steroidogenesis in streptozotocin induced diabetic rats [26]. In our study, decrease in blood testosterone and impairment of sperm quality were found in the diabetic control group in comparison to the normal control group (reference group). All parameters were improved in the diabetic group treated with glibenclamide (reference drug) and *P. sarmentosum* leaf extract of 60 and 100 mg/100 gBW. Interestingly, the increase in blood testosterone and sperm quality is related to the decrease in FBG level in a reverse manner. This result implies that *P. sarmentosum* Roxb. leaf extract exhibits hypoglycemic properties and meanwhile it also decreases the oxidative stress. The improvement in oxidative status associated with a recovery of pancreatic islet function leads to an increase in insulin concentration. Futhermore, insulin also affects testicular function via hypothalamic and/or pituitary level by increasing lutinizing hormone (LH) [26], and thereby, it stimulates Leydig cell function leading to an increase in testosterone secretion. Finally, testosterone controls spermatogenesis [27].

Conclusion

Piper sarmentosum Roxb. aqueous leaf extract at doses of 60 and 100 mg/100 gBW had hyperglycemic properties in diabetic mice. They also increased insulin secretion, improved both pancreatic islet function and fertility status in diabetic mice after 21 days of extract treatment. It may therefore be recommeneded as a medicinal plant for diabetic patients.

Acknowledgements

This work was supported by the Applied Taxonomic Research Center and Department of Biology, Faculty of Science, Khon Kaen University, Thailand.

References

[1]. Tiwari AK, Roa M. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: present status and future prospects, Curr Sci, 2002; 83: 30-38.

[2]. Baynes JW, Thorpe SR. 1999. Role of oxidative stress in diabetic complications: A new perspective on an old paradigm, Amer Diabetes Assoc, 1999; 48: 1-9.

[3]. Vijayalingam S, Parthiban A, Shanmugasundaram KR, Mohan V. Abnormal antioxidant status in impaired glucose tolerance and non-insulin-dependent diabetes mellitus, Diabetic Med, 1996; 13: 715-719.

[4]. Atkinson MA, Maclaren NK. The pathogenesis of insulin-dependent diabetes mellitus \(review), N Eng J Med, 1994; 331: 1428-1436.

[5]. Khaki A, Nouri M, Fathiazad F, Ahmadi-Ashtiani HR, Rastgar H, Rezazadeh Sh. Protective effect of quercetin on spermatogenesis in streptozotocin-induced diabetic rats, Int Med PI, 2009; 8(supplement): 57-64.

[6]. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas, Physiol Res, 2001; 50(6): 537-546.

[7]. ubramaniam V, Adenan IM, Ahmad AR, Sahdan R. Natural antioxidant *Piper* sarmentosum and Morinda ellpitc, Mal J Nutr, 2003; 9: 41-51.

[8]. Hafizah AH, Zaiton Z, Zulkhairi A, Ilham AM, Anita MMNN, Zaleha AM. *Piper sarmentosum* as an antioxidant on oxidative stress in human umbilical vein endothelial cells induced by hydrogen peroxide, J Zhejiang Uni Sci Biomed Biotech, 2010; 5: 357-365.

[9]. Priscilla DH, Roy D, Suresh A, Kumar V,Thirumurugan K. Naringenin inhibits -glucosidase activity: A promising strategy for the regulation of postprandial hyperglycemia in high fat diet fed streptozotocin induced diabetic rats, Chemico-Biol Interact, 2014; 210: 77-85.

[10]. Rahman NA, Noor KM, Hlaing KPP, Suhaimi FH, Kutty MK, Sinor MZ. *Piper Sarmentosum* influences the oxidative stress involved in experimental diabetic rats, Internet J Herb PI Med, 2011; 1: 1-9.

[11]. Sexton WJ, Jarow JP. Effect of diabetes mellitus upon male reproductive function, Urol, 1997; 49: 508-513.

[12]. Peungvicha P, Suwan S. Hypoglycemic effect of the water extract of *Piper sarmentosum* in rats, J Ethnopharmacol, 1989; 60: 27-32.

[13]. Ali Madhi MA, Chandra A, Singh RK, Shukla S, Mishra LC, Ahmad S. 2003. Effect of herbal hypoglycemic agents on oxidative stress and antioxidant status in diabetic rats, Indian J Clin Biochem, 2003; 18(2): 8-15.

[14]. Latha M, Pari L. Effect of an aqueous extract of *Scoporia dulcis* on blood glucose, plasma insulin and some polyol pathway enzymes in experimental rat diabetes, Brazillian J Med Biol Res, 2004;37: 577-586.

[15]. Luangpirom A, Junaimuang T, Kourchampa W, Somsapt P, Sritrakool O. Protective effect of pomegranate (*Punica granatum* Linn) juice against hepatotoxicity induced by ethanol in mice, ABAH BioFlux, 2013; 8: 89-93.

[16]. Zar JH. Biostatistical analysis, International edition, 4th ed. Prentice Hall International, Inc., New Jersey, 1999, p 662.

[17]. World Health Organization. WHO list of essential medicines, 15th edition, World Health Organization, 2007, p21.

[18]. Ashcroft FM, Ashcroft SJH. The sulfonylurea receptor, Biochim Biophys Acta, 1992; 1175:45-59.

[19]. Eliasson L, Renstrom E, Ammala C, Berggran PO, Bertorello AM, Bokvist K, Chibalin A, Deeney JT, Flatt IR, Gabel J, Gromacia J, Larsson O, Linstrom P, Rhodes C, Rossman P. PKC-dependent stimulation of β-cells, Science, 1996; 271: 813-815.

[20]. Jennings PE, Belch JJF. Free radical scavenging activity of sulfonylureas: A clinical assessment of the effect of gliclazide, Metabolism, 2000; 49(2): 23-26.

[21]. Krisanapun C, Womgkrajang Y, Temsiririrkkul R, Phonrnchirasilp S, Peungvicha P. In vitro evaluation of anti-diabetic potential of *Piper sarmentosum* Roxb. extract, The FASEB Journal, 2012; 26: 686.7.

[22]. Doreswamy K, Shrilatha B, Rajeshkummar T, Muralidhara. Nickel induced oxidative stress in testis of mice, J Androl, 2004; 25:996-1003.

[23]. Shrilatha B, Muralidhara. Early oxidative stress in testis and epididymis sperm in streptozotocin-induced diabetic mice: Its progession and genotoxic consequences, Repro Toxicol, 2007; 23:578-587.

[24]. Keskes-Ammar L, Feki-Chanroun N, Rebai T, Sahnoun Z, Ghozzi H, Hammami S, Zghal K, Fki H, DAmak J, Bahloul A. Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men, Arch Androl, 2003; 49: 83-94.

[25]. Kefer JC, Agarwal A, Sabanegh F. Role of antioxidants in the treatment of male infertity, Int J Urol, 2009; 16:447-457.

[26]. Benitez A, Perez-Diaz J. Effect of steptozotocin-diabetes and insulin treatment on regulation of Leydig cell function in the rat, Horm Metab Res, 1985; 17:3-7.

[27]. Johnson HM, Everitt BJ. Essential reproduction, Blackwell Science Pty Ltd.1995, Australia.