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Effect of shallot (*Allium ascalonicum* L.) bulb juice on hypoglycemia and sperm quality in streptozotocin induced diabetic mice

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Abstract. The present study was carried out to determine the effect of red onion (*Allium ascalonicum*) bulb juice on hypoglycemic activity and its attenuating effect on the testicular damage caused by diabetes. Male mice were induced diabetes by a single intraperitoneal injection with stretozotocin (6 mg/100 gBW). A dose of glibenclamide (1 mg/100 gBW) and two doses of onion bulb juice (0.5 and 1.0 g/100 gBW) were orally administered for 14 days. After treatment, the decrease of blood glucose level of both treated groups were 43.45 and 59.18% of the value in glibenclamide treated group and consequently attenuated the impaired testicular function including the increase of gonadal index and sperm quality in streptozotocin induced diabetic mice.
Key Words: *Allium ascalonicum* L., hypoglycemic activity, streptozotocin, testicular function.

Introduction. Diabetes mellitus (DM) is a carbohydrate metabolic disorder, resulting form defect in insulin secretion, insulin function or both. Diabetic patients always revealed a state of chronic hyperglycemia and glucose tolerance impairment (Tiwari & Roa 2002). Oxidative stress also increased in diabetic patients and in stretozotocin-induced diabetic rats (Bayness 1991; Azuma et al 2007). This occurrence caused various pathologies including ophthalmopathy, neuropathy, nephropathy and cardio-vasculopathy (Atkinson & Maclaren 1994). Furthermore, there is a strong association between male infertility and diabetes (Agbaje et al 2007; Bener et al 2009). In recent years, food plants contained high flavonoids are attracted for human health. Dietary flavonoids were reported theirs potent antioxidant properties by decreasing the risk of disease caused by oxidants such as cadmium induced testicular oxidative damage in rats (Ola-Mudathir et al 2008), paracetamol induced seminal quality impairment in mice (Luangpirom et al 2012), cardiovascular disorder in diabetic patients (Banerjie & Mualik 2002) and the loss of bone density in menopausal women (Matheson et al 2009). Furthermore, some flavonoids had antiglycemic effect in diabetic animals (Dias et al 2005; El-Demerdash et al 2005).

Red onion or shallot (*Allium ascalonicum*) belongs to family of Alliaceae, is commonly used in many Asian dishes. Its medicinal properties were well known as common cold treatment. Since, it is rich of flavonoids including quercetin, a potent antioxidant (Griffiths et al 2002). This compound was reported to protect against oxidative stress induced by spontaneous hypertension (Duarte et al 2001) and also able to prevent nitric oxide increase in streptozotocin (STZ) treated rats (Coskum et al 2005), and to increased blood testosterone and sperm quality in diabetic rats (Khaki et al 2009). Therefore, the purpose of this study was to assess the hypoglycemic properties of *A. ascalonicum* bulb juice in STZ induced diabetic mice after 14 days of oral administration and associated the results to the testicular impairment caused by diabetes.

Material and Method. Adult male mice (ICR strain, 8-week age, 35-40 g) were obtained from National Laboratory Animal Center, Nakornprathom province, Thailand. They were housed in a room with 12 hours of light:dark cycle and 25 ± 1°C. Standard pellet food (Chareanpogapan Ltd, Bangkok, Thailand) and water *ad libitum* were freely available. The experiment were performed after an approval by the Institutional Animal Ethics Committee, Khon Kaen University, Thailand (Reference No. 0514.1.12.2/28).

Fresh aged bulbs of *A. ascalonocum* were obtained from local market in Khon Kaen province, Thailand. They were cleaned, weighed and extracted with juice extractor, then diluted with distilled water at dose of 1.0 and 2.0 g/ml for oral administration (0.5 ml/100 gBW).

Male mice were induced diabetes by a single intraperitoneal injection (i.p.) with STZ at dose of 6 mg/100 gBW. Diabetes was determined on the third days after induction and fasting blood glucose level which were greater than 125-200 mg% were considered as mildly diabetes (Latha & Pari 2004). These diabetic mice were used for this study.

The animals were fasted at least 16 hours, and determined fasting blood glucose from tail artery by glucometer (ONE TOUCH® Horizon™, LifeScan, Inc. 2006, Johnson and Johnson Company, Bangkok, Thailand).

The experiments were performed on 5 groups of 6 animals each. Group I and II serves as normal mice control and diabetic mice control, were orally fed with distilled water 0.5 ml/100 gBW, group III received glibenclamide (reference drug) at dose of 1.0 mg/100 gBW and groups IV and V were given with 0.5 and 1.0 gram of *A. ascalonocum* L. juice, respectively.

All groups were recorded blood glucose level before and after 14 day treatment and the changes of blood glucose were evaluated. Hypoglycemic efficiency of *A. ascalonocum* bulb juice was assessed and compared the results with group treated with glibenclamide (reference drug) (modified method of Saenbuaphan 2005).

At the end of 14 day treatment, body weight and testicular weight of all groups were recorded. Blood were collected by cardiac puncture under anaesthetic condition with ether. Plasma was obtained after blood centrifugation at 3,000 rpm for 10 minutes at room temperature for testosterone determination by radioimmunoassay (The DSL-400 ACTIVE® Testosterone coated-Tube RIA kits, Diagnostic Laboratories, Inc.). After blood sampling, epididymis and vas deferens of all groups were excised and tore with syringe needle (No. 25) in 2 ml of 0.9 % NaCl and incubated at 37°C for sperm quality analysis. Sperm concentration and viable sperms were evaluated by modified method of Yokoi et al (2003), motile sperms were detected by the method of Sonmez et al (2005) and abnormal sperms were investigated by the method of Atessahim et al (2006).

All results were expressed as mean and standard deviation ($\overline{X} \pm SD$). Data was analysed by one-way ANOVA and Duncan's multiple range test for comparison the difference among groups. Values of $P<0.05$ were considered significance (Zar 1999).

Results and Discussion. The effect of oral administration of red onion bulb juice on blood glucose level in diabetic mice is presented in table 1. The normal group control and diabetic group control revealed the non-significant change of blood glucose level on day 15 when compared to the value on day 1 ($P>0.05$). While, group treated with glibenclamide (1 mg/100 gBW) and both groups treated with onion juice (0.5 and 1.0 g/100 gBW) significantly reduced their blood glucose level by 26.18, 8.37 and 13.30%, respectively. The results of glucose tolerance of glibenclamide treated groups and onion juice treated groups were 31.55, 13.17 and 18.67%, respectively, when compared to the diabetic control. The hypoglycemic efficiency of onion juice at doses of 0.5 and 1.0 g/100 gBW were 43.45 and 59.18% glibenclamide treated group.

Table 1

The hypoglycemic properties of *Allium ascalonicum* bulb juice in diabetic mice

Treated groups (g/100 gBW) N=6	*FBG ($\bar{X}$ ± SD, mg%) (% of blood glucose)*		*Change of glucose[A] (%)*	*Glocose tolerance[B] (hypoglycemic efficiency)[C]*
	Day 1	*Day 15*		
Normal : 0	74.50 ± 6.50 (100)	75.67 ± 7.42^{NS} (101.57)	+1.57	-
DM : 0	128.60 ± 22.75 (100)	135.50 ± 18.74^{NS} (105.37)	+5.31	-
DM : Glibenclamide 0.001	176.33 ± 16.85 (100)	130.17 ± 11.50* (73.82)	-26.18	31.55 (100)
DM : Onion juice 0.5	135.33 ± 32.60 (100)	124.00 ± 8.56^{NS} (91.63)	-8.37	13.71 (43.45)
DM : Onion juice 1.0	146.67 ± 12.48 (100)	127.17 ± 16.46^{NS} (86.71)	-13.30	18.67 (59.18)

N – number of experimental animals, FBG – fasting blood glucose, DM – diabetes mellitus, A – % of blood glucose on day 15 minus % of blood glucose on day 1, B - % of blood glucose in diabetic control minus % of blood glucose in diabetic treated group, C – glucose tolerance of onion juice treated group / glucose tolerance of glibenclamide treated group x 100, * - significant difference ($P<0.05$); NS – non-significant difference ($P>0.05$).

The effect of red onion bulb juice on testicular function in diabetic mice is shown in table 2 and 3. The gonadal index of diabetic control group was significantly decreased as compared to the value in normal control group ($P>0.05$). After treatment the diabetic groups with glibenclamide and onion bulb juice, the value of gonadal index significantly increased ($P<0.05$). While, the blood testosterone of all treated groups were non-significantly increased (Table 2).

Table 2

Effect of *Allium ascalonium* juice on gonadal index and blood testosterone level in diabetic mice

Treated groups (g/100 gBW) N=6	*Gonadal index ($\bar{X}$ ± SD, x10^{-2})*	*Testosterone (X ± SD, ng/ml)*
Normal : 0	0.04±0.0004^{a}	0.20±0.07^{a}
DM : 0	0.33±0.0003^{b}	0.18±0.10^{a}
DM : Glibenclamide 0.001	0.37±0.0003^{ab}	0.25±0.05^{a}
DM : Onion juice 0.5	0.37±0.0005^{ab}	0.23±0.09^{a}
DM : Onion juice 1.0	0.37±0.0006^{ab}	0.20±0.17^{a}

N – number of experimental animals, DM – diabetes mellitus, same alphabet within column means non significant difference ($P>0.05$), different alphabet within column means significant difference ($P<0.05$).

Table 3

Effect of *Allium ascalonium* bulb juice on sperm quality in diabetic mice

Treated groups (g/100 gBW) N=6	*Sperm count ($\bar{X}$ ± SD,x10^{6} cells/individual)*	*$\bar{X}$ ± SD (%)*		
		Viable sperms	*Motile sperms*	*Abnormal sperms*
Normal : 0	97.47±2.87^{ab}	73.10±7.15^{ab}	68.12±5.95^{a}	12.33±1.12^{a}
DM : 0	63.08±2.82^{a}	69.98±5.84^{a}	57.59±5.16^{b}	35.28±4.73^{bc}
DM : Glibenclamide 0.001	93.22±1.38^{ab}	79.06±3.33^{bc}	59.02±4.37^{b}	30.83±5.87^{b}
DM : Onion juice 0.5	100.62±2.34^{b}	78.98±2.34^{bc}	57.77±9.16^{b}	28.89±2.51^{b}
DM : Onion juice 1.0	113.47±4.03^{b}	81.07±3.81^{c}	59.89±6.89^{ab}	26.77±3.48^{c}

N – number of experimental animals, DM – diabetes mellitus, same alphabet within column means non significant difference ($P>0.05$), different alphabet within column means significant difference ($P<0.05$).

However, the sperm quality of diabetic control were significantly decreased such as number of sperms count, number of motile sperms and number of viable sperms, while the number of abnormal sperms were increased when compared to the normal control group. After treatment diabetic mice with glibenclamide and onion juice, their sperm quality significantly increased as compared to the diabetic control (Table 3).

This present results showed that the hypoglycemic properties of onion bulb juice at doses of 0.5 and 1.0 g/100 gBW were 43.45 and 59.18% of gliblenclamide (1 mg/100 gBW) after oral administration for 14 days in STZ induced diabetic mice. Glibenclamide is a synthetic anti-diabetic drug working by stimulating insulin secretion *in vivo* and *in vitro* (Serrano-Martin et al 2006; Youl et al 2010). Our results were consistent with the study of El-Demerdash et al (2005) on the hypoglycemic effect of onion at dose of 0.4 g/100 gBW after oral administration for 28 days in alloxan diabetic rats. Red onion is popular folk remedy and benefit for human health. It is rich of flavonoids, especially quercetin approximate 1,960 mg/kg (Smith et al 2003). Quercetin, a high potent antioxidant was capable to increase glucose tolerance in STZ induced diabetic rats and increased their hepatic glucokinase activity as an insulin like effect (Vessal et al 2003), protected in diabetes by increasing antioxidative enzyme activities (Coskum et al 2005), potentiated in insulin secretion in insulin secreting cell line and protected pancreatic beta cell function against oxidative stress induced by hydrogenperoxide (H_2O_2) *in vitro* (Youl et al 2010). These results were suggested that the hypoglycemic properties of red onion (*A. ascalonicum*) may be due to the quercetin present, which involved by stimulating hepatic glucokinase activity and potentiated in insulin secretion. Our studies also assessed the toxicity of STZ on testicular function in diabetic animals. Streptozotocin is a potent alkylating agent damaging DNA of pancreatic beta cells and induced oxidative stress by generating reactive oxygen species (ROS). Furthermore, ROS caused testicular dysfunction leading to infertility (Bolzan & Bianchi 2002; Agarwal & Said 2005). Our studies also found the sperm quality impairment in STZ induced diabetic mice including decrease of gonadal index, sperm concentration, number of viable sperms and number of motility sperms. After 14 days of oral administration of onion bulb juice, the gonadal index and sperm quality of both treated groups were significantly restored. These results were consistent with the study of Gholamhosin et al (2009) on recovery of sperm quality and reduced ROS level in testis tissue of STZ induced diabetic rats after 4 week treatment with onion 1 ml/kgBW. Previous studies revealed that STZ induced oxidative damage in testis leading to alter steroidogenesis and impaired spermatogenesis (Muralidhara 2007), Benitez & Perez Diaz (1985) reported that STZ decreased plasma LH and testosterone level, as well as these levels completely restored after insulin treatment. Furthermore, quercetin, a major component of red onion exhibited protective effect on testicular damage induced by oxidative stress in STZ induced diabetic rats after quercetin (15 mg/kgBW) intraperitoneal injection for 28 days (Khaki et al 2009).

Conclusions. Long term oral administration of red onion (*A. ascalonicum*) bulb juice is able to decrease blood glucose level in STZ induced diabetic mice. In addition, it also attenuated the testicular dysfunction caused by diabetes.

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References

Agarwal A., Said T. M., 2005 Oxidative stress, DNA damage and apoptosis in male infertility: a clinical approach. Br J Urol 95:505-507.

Agbaje I. M., Rogers D. A., McClure N., Atkinson A. B., Mallidis C., Lewis S. E. M., 2007 Insulin dependent diabetes mellitus: implications for male reproductive function. Hum Reprod 22(7):1871-1877.

Atessahim A., Turk G., Karaham I., Yilmaz S., Ceribast A. O., Bulmus O., 2006 Lycopene prevent adriamycin-induced testicular toxicity in rats. Fertil Sterili 85(suppl 1):1216-1222.
Atkinson M. A., Maclaren N. K., 1994 The pathgenesis of insulin-dependent diabetes mellitus (review). N Engl J Med 331:1428-1436.
Azuma K., Minami Y., Ippoushi K., Terao J., 2007 Lowering effects of onion intake on oxidative stress biomarkers in streotozoticin induced-diabetic rats. J Clin Biochem Nutr 40(2):131-140.
Banerjee S. K., Maulik S. K., 2002 Effect of garlic on cardiovascular disorder, a review. Nutr J 1(4):1-14.
Bayness J. W., 1991 Role of oxidative stress in development of complications in diabetes. Diabetes 40:405-412.
Benitez A., Perez Diez J., 1985 Effect of stretozotocin diabetes and insulin treatment on regulation of Leydig cell function in the rat. Horm Metab Res 17(1):5-7.
Bener A., Al-Ansari A. A., Zirie M., Al-Hamaq A. O., 2009 Is male fertility associated with type 2 diabetes mellitus? Int Urol Nephrol 41:777-784.
Bolzan A. D., Bianchi M. S., 2002 Genotoxicity of streptozotocin. Mutat Res 7690:1-14.
Coskum O., Kanter M., Korkmaz A., Oter S., 2005 Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin induced oxidative stress and beta cell damage in rats pancreas. Pharmacol Res 51:117-123.
Dias A. S., Porawski M., Alonso M., Marroni N., Collado P. S., Gnzaley-Gallego J., 2005 Quercetin decrease oxidative stress, NF-*k*B activation and iNOS overexpression in liver of streptozotocin induced diabetic rats. J Nutr 135:2299-2304.
Duarte J., Galistco M., Ocete M. A., Perez-Vizcaino F., Zarzuelo A., Tamago J., 2001 Effect of chronic quercetin treatment on hepatic oxidative stress in spontaneously hypertensive rats. Mol Cell Biochem 221:155-160.
El-Demerdash F. M., Yousef M. I., Abou El-Naga N. I., 2005 Biochemical study on the hypoglycemic effect of onion and garlic in alloxan induced diabetic rats. Food Chem Toxicol 3:57-63.
Gholamhosini B., Khaki A., Ahmadi-Ashtiani H. R., Rezazadeh S., Rastegar H., Fathiazad F., Ghanbari M., 2009 Treatment effect of onion on spermatogenesis in stretozotocin induced diabetic rats. Journal of Medicinal Plant 8(32):153-161.
Griffiths G., Trueman L., Crowther T., Thomas B., 2002 Onions a global benefit to health. Phytother Res 17(7):603-615.
Khaki A., Nouri M., Fathaizad F., Ahmadi-Ashtiani H. R., Rastgar H., Rezazadeh Sh., 2009 Protective effect of quercitin on spermatogenesis in streptozotocin induced diabetic rat. Journal of Medicinal Plants 8(5):57-64.
Latha M., Pari L., 2004 Effect of an aqueous extract of *Scoparia duleis* on blood glucose, plasma insulin and some polyol pathway enzyme in experimental rat diabetes. Braz J Med Res 37:577-586.
Luangpirom A., Kourchampa W., Junaimuang T., 2012 Attenuating effect of *Allium ascalonicum* L. on paracetamol induced seminal quality impairment. Journal of Medicinal Plant Research 6(13):2655-2659.
Matheson E. M., Mainous A. G., Carnemolla M. A., 2009 The association between onion consumption and bone density in perimenopuasal and post menopausal non-Hispanic white women 50 years and older. Menopause 16(4):756-759.
Muralidhara B. S., 2007 Early oxidative stress in testis and epididymis sperm in streptozotocin induced diabetic mice: Its progression and genotoxic consequences. Reprod Toxicol 23(4):875-587.
Ola-Mudathir K. F., Suru S. M., Fafunso M. A., Obioha U. E., Faremi T. Y., 2008 Protective roles of onion and garlic extracts on cadmium-induced changes in sperm characteristics and testicular oxidative damage in rats. Food Chem Toxicol 46:3604-3611.
Saenbuaphan N., 2005 Hypoglycemic effect of *Cassia siames* leaf extract and effect on histopathology of pancreatic islets of diabetic rats. M SC. Thesis, Khon Kaen University, Thailand, ISBN: 974-284-733-9.

Serrano-Martin X. Payares G., Mendoza-Leon A., 2006 Glibenclamide, a blocker of K^+-ATP channels, shows antileish manial activity in experimental murine cutaneous luishmaniasis. Antimicrob Agents Chemother 50(12):4214-4216.

Smith C., Lombard K. A., Peffley E. B., Lui W., 2003 Genetic analysis of quercetin in onion (*Allium cepa* L.) "Lady Rider". The Texas Journal of Agriculture and Natural Resource 16:21-28.

Sonmez M., Turk G., Yuce A., 2005 The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats. Theriogenology 63:2063-2072.

Tiwari A. K., Roa M., 2002 Diabetes mellitus and multiple therapeutic approaches of phytochemicals pesent status and future aspects. Curr Sci 83:30-38.

Vessal M., Hemmati M., Vasei M., 2003 Antidiabetic effects of quercetin in streptozotocin-induced diabetic rats. Comp Biochem Physiol C Toxicol Pharmacol 135(3):357-364.

Yokoi K., Uthus E. O., Nielsen F. H., 2003 Nickel deficiency diminishes sperm quality and movement in rats. Biol Trace Elem Res 93:141-153.

Youl E., Baedy G., Magons R., Cros G., Sezaion F., Virsolvy A., Richard S., Quinard J. F., Gross R., Petit P., Bataille D., Oiry C., 2010 Quercetin potentiates insulin secretion and protect INS-1 pancreatic beta cell agonist oxidative damage via ERK ½ pathway. Br J Pharmacol 161:799-814.

Zar J. H., 1999 Biostatistical analysis. 4th edition, Prince Hall International, Inc. New Jersey, USA.

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