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Antihyperglycemic, Hypolipidemic and Antioxidant Enzymes Effect of *Strobilanthes crispus* Juice in Normal and Streptozotocin-Induced Diabetic Male and Female Rats

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Abstract: The aim of the present study was to investigate the effect of *Strobilanthes crispus* juice on glucose, lipid profile, glutathione peroxidase and superoxide dismutase in normal and streptozotocin-induced diabetic male and female albino Sprague-Dawley rats. This study was conducted on normal and streptozotocin-induced diabetic male and female Sprague-Dawley rats fed with basal diet and *S. crispus* juice with different doses 1.0, 1.5 and 2.0 mL kg^{-1} b.wt. for 30 days. The results showed that significant ($p<0.05$) decrease in serum glucose levels in male and female diabetic and normal rats with treated *S. crispus* juice (1.0, 1.5 and 2.0 mL kg^{-1} b.wt.). Cholesterol and triglyceride level significantly ($p<0.05$) decreased in diabetic rats treated with 1.0, 1.5 and 2.0 mL kg^{-1} b.wt. of *S. crispus* juice. Cholesterol, triglyceride and LDL-cholesterol level showed reduction in treated male and female normal rats. HDL-cholesterol showed the increasing but not significant ($p<0.05$) difference in treated diabetic and normal male and female rats. Glutathione peroxidase and superoxide dismutase activities significantly ($p<0.05$) increased in treated diabetic and normal male and female rats. In conclusion, *S. crispus* juice possesses antihyperglycemic, hypolipidemic and antioxidant effect in streptozotocin-induced diabetic rats. Thus, *S. crispus* juice could be the alternative treatment for lowering glucose, cholesterol and triglyceride for diabetic patients in the future.

Key words: *Strobilanthes crispus* juice, glucose, lipid profile, antioxidant

INTRODUCTION

Diabetes mellitus is a major and growing health problem in most countries. It causes considerable amount of disability, premature mortality, lost of productivity as well as increased demands on health care facilities. It is the fourth or fifth leading cause of death in most developed countries and there is substantial evidence that it is epidemic in many developing and newly industrialized nations such as Malaysia (Shafie *et al*., 2004). The number of people with diabetes is rapidly increasing. WHO estimated 150 million people worldwide with diabetes in 2002 and projected rise to 300 million by 2025 (Jin, 2004). Statistics records from the Ministry of Health, Malaysia shows that the number of admissions to Government hospitals in Peninsular Malaysia for diabetes mellitus had increased from 19,629 cases in 1991 to 30,661 cases in 2001. Mortality due to diabetes has also increased from 254 deaths in 1991 to 380 deaths in 2001 which is an increase of 50% (Shafie *et al*., 2004).

In modern medicine, no satisfactory effective therapy is still available to cure the diabetes mellitus. Though insulin therapy is also used for the management of diabetes mellitus, there are several drawbacks like insulin resistance (Piedrola *et al*., 2001), anorexia nervosa, brain atrophy and fatty liver (Yaryura-Tobias *et al*., 2001) after chronic treatment. The drugs currently available for type 2 diabetes have a number of limitations, such as significant side effects and high rates of secondary failure. As the knowledge of the heterogeneity of this disorder increases, there is a need to look for more effective agents with fewer side effects (Rang and Dale, 1991). This has led to the search for alternative therapies that may have a similar degree of efficacy without the troublesome side effects associated with conventional drug treatment.

For centuries, herbs have been used for the treatment of many ailments including chronic diseases like diabetes mellitus. One of the herbs that has great potential is *Strobilanthes crispus* or pecah beling. *Strobilanthes*

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crispus (Acanthaceae) plant is native to countries from Madagascar to Indonesia (Sunarto, 1977). It is commonly known as daun pecah beling in Jakarta or enyoh kilo, kecibeling, or kejibeling in Java (Sunarto, 1977) as well as pecah kaca or jin batu in Malaysia. Traditionally, *S. crispus* leaves were boiled with water and has been used as antidiabetic, diuretic, antilyti and laxative (Sunarto, 1977). The study showed that the leaves of *S. crispus* possessed anti-AIDS, anti-leukemia (Kusumoto *et al*., 1992), high antioxidant activity (Ismail *et al*., 2000; Abu Bakar *et al*., 2004; Asmah *et al*., 2006a), anticarcinogenic (Suherman *et al*., 2004; Fauziah *et al*., 2005; Asmah *et al*., 2006b; Fadzelly *et al*., 2006a) and antidiabetic (Fadzelly *et al*., 2006b).

This plant has many cystoliths of calcium carbonate and an infusion is mildly alkaline (Perry and Metzger, 1980). A study showed that the dried leaves of *S. crispus* contained high amount of minerals including potassium (51%), calcium (24%), sodium (24%), iron (1%) and phosphorus (1%). These leaves also contained high content of water soluble vitamins (C, B1, B2) and it also contains catechins, alkaloids, caffeine and tannins. Catechins of *S. crispus* leaves showed highest antioxidant activity compared to vitamin E (Ismail *et al*., 2000). Study of *S. crispus* tea showed that it contained considerably high amount of mineral content, phenolic content and displayed high antioxidant activity especially unfermented tea from old or matured leaves (Abu Bakar *et al*., 2004). Recent study showed that *S. crispus* juice heated at 60°C for 30 min had highest total phenolic content and antioxidant activities. Therefore, the aim of this study is to investigate the antihyperglycemic, hypolipidemic and antioxidant effect of *S. crispus* juice in normal and streptozotocin-induced diabetic male and female albino Sprague-Dawley rats.

MATERIALS AND METHODS

Plant material and preparation of *S. crispus* juice: The leaves of *S. crispus* were grown and collected from the herbal garden of Faculty of Medicine and Health Science, Universiti Putra Malaysia, Serdang, Selangor. The plant was identified by taxonomist of Department of Botany, Faculty of Science and Technology, Universiti Kebangsaan Malaysia. The voucher number of *S. crispus* was AZ-6803. Malaysian Agricultural Research and Development Institute, Serdang, Selangor prepared the *S. crispus* juice. Concentration of juice was 4% of *S. crispus* leaf.

Experimental rats and study design: Ninety Sprague-Dawley strain male (45) and female (45) albino white rats, weighing 150 to 200 g were used in this study. The rats were supplied by Institute of Medical Research (IMR), Kuala Lumpur, Malaysia. They were housed in standard cages at an ambient temperature of 25±2°C with 12 h-light/12 h-dark cycle and placed in Animal House at Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. They were fed with commercial rat chow (Barastoc, Australia) and tap water *ad libitum*. Rats were acclimatized to the laboratory conditions for 1 week on average before any experimental work was undertaken. The experiment was designed and conducted according to ethical norms approved by Animal Care and Use Committee (ACUC), Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor.

Study design and dosage: The rats were divided into 9 groups with 10 rats (5 male and 5 female) in each group and the groups were as follows (40 normal and 50 streptozotocin-induced diabetic):

- Diabetic untreated rats (diabetic control)
- Diabetic rats treated with glibenclamide (10 mg kg^{-1} b.wt.)
- Diabetic rats treated with *S. crispus* juice (dose: 1.0 mL kg^{-1} b.wt.)
- Diabetic rats treated with *S. crispus* juice (dose: 1.5 mL kg^{-1} b.wt.)
- Diabetic rats treated with *S. crispus* juice (dose: 2.0 mL kg^{-1} b.wt.)
- Normal untreated rats (normal control)
- Normal rats treated with *S. crispus* juice (dose:1.0 mL kg^{-1} b.wt.)
- Normal rats treated with *S. crispus* juice (dose: 1.5 mL kg^{-1} b.wt.)
- Normal rats treated with *S. crispus* juice (dose: 2.0 mL kg^{-1} b.wt.)

Rat were fed with *S. crispus* juice by oral feeding daily for 30 days.

Induction of experimental diabetes: Streptozotocin (Sigma, USA; 55 mg kg^{-1} b.wt.) was freshly dissolved in 0.1 mol L^{-1} cold sodium citrate buffer, pH 4.5 and injected intraperitonealy to 50 rats. Rats were fasted for 16 h prior to induction of diabetes. Third day after streptozotocin administration, the fasting blood glucose concentration was determined using Accu-chek Go (Roche Diagnostic). Animals having glucose level >11 mmol L^{-1} were selected in this study for hyperglycaemic or diabetic model.

Blood preparation: On day 0 (baseline), 15 and 30 days of treatment, the rats were anesthetized with diethyl ether following a 12 h fast. Blood was drawn by cardiac

puncture into plain serum tube. The blood was then centrifuged at 3000 rpm for 10 min using refrigerated centrifuge at 4°C. The serum was used for determination of glucose, total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol.

Determination of glucose and lipid profile: Determination of serum glucose and lipid profile (total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol) were done enzymatically using automated clinical chemistry analyzer (Roche Diagnostic - Hitachi 902, USA).

Determination of glutathione peroxidase enzyme: Diluted 0.05 mL heparinized whole blood with 2 mL diluting agent. Mixed well and run the samples using Selectra E Chemical Analyser. This method was based on that of Paglia and Valentine (1967). Glutathione peroxidase (GPX) catalysis the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of Glutathione Reductase (GR) and NADPH the oxidised glutathione (GSSG) was immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm was measured.

Determination of superoxide dismutase enzyme: Centrifuged 0.5 mL of whole blood for 10 min at 3000 rpm and then aspirated off the plasma. Then washed the erythrocyte four times with 3 mL 0.9% NaCl solution and centrifuged for 10 min at 3000 rpm after each wash. The washed centrifuged erythrocytes was then made up to 2.0 mL with cold redistilled water, mixed and left to stand at +4°C for 15 min. Diluted 125.0 μL of erythrocytes with 3 mL diluent. Samples were run using Selectra E Chemical Analyser.

Statistical analysis: All data were presented as Mean±Standard Error of Mean (SEM) using SPSS version 13.0. The data for various biochemical parameters were analyzed using ANOVA and the means were compared by Duncan's multiple range test. Values were considered statistically significant when $p<0.05$.

RESULTS AND DISCUSSION

Serum glucose level: Table 1 shows serum glucose level in male and female diabetic rats. The result showed that significant decrease of serum glucose levels in male and female diabetic rats with treated glibenclamide and all groups treated with 1.0, 1.5 and 2.0 mL kg^{-1} b.wt. of *S. crispus* juice at day 15 and 30 when compared to control group and baseline data (zero day). *Strobilanthes crispus* juice at dose 2.0 mL kg^{-1} b.wt. showed the highest reduction of serum glucose in male (5.16 mmol L^{-1}) and female diabetic rats (6.98 mmol L^{-1}) after 30 days treatment (Table 1). Serum glucose level at day 30 of treatment with glibenclamide, 1.0 and 1.5 mL kg^{-1} b.wt. of *S. crispus* juice in male diabetic rats were 9.43, 9.89 and 7.48 mmol L^{-1} and female diabetic rats were 8.28, 8.65 and 7.75 mmol L^{-1}. The *S. crispus* juice at doses 1.5 and 2.0 mL kg^{-1} b.wt. showed better reduced glucose levels compared with the hypoglycemic drug (glibenclamide).

The highest reduction of glucose level was 80.9% at day 30 in male diabetic group treated of 2.0 mL kg^{-1} b.wt. *S. crispus* juice followed by 78.9% reduction in group treated of 1.5 mL kg^{-1} b.wt. and 67.4% reduction in group treated with 1.0 mL kg^{-1} b.wt. of *S. crispus* juice. In female diabetic groups, reduction of glucose level were 78.2, 68.9 and 68.6% in groups treated 2.0, 1.5 and 1.0 mL kg^{-1} b.wt. of *S. crispus* juice, respectively (Table 2).

Table 1: Serum glucose level (mmol L^{-1}) in male and female STZ-induced diabetic rats

	Day		
Groups	0	15	30
Male diabetic control	19.33±2.84^{a,1}	35.92±1.7^{b,2}	42.65±0.34^{c,3}
Male diabetic + glibenclamide	34.09±4.78^{a,3}	17.23±4.37^{a,2}	9.43±1.11^{a,1}
Male diabetic + SCJ 1.0 mL kg^{-1}	30.32± 5.47^{a,2}	17.16±4.5^{a,1,2}	9.89±1.9^{b,1}
Male diabetic + SCJ 1.5 mL kg^{-1}	35.53±6.76^{a,2}	15.10±2.45^{a,1}	7.48±0.48^{a,1}
Male diabetic + SCJ 2.0 mL kg^{-1}	27.04±6.06^{a,2}	12.81±2.22^{a,1}	5.16±0.83^{a,1}
Female diabetic control	20.76±4.12^{a,1}	33.11±4.91^{b,1,2}	41.37±4.01^{b,2}
Female diabetic + glibenclamide	20.16±2.85^{a,2}	13.90±3.91^{a,1,2}	8.28±2.29^{a,1}
Female diabetic + SCJ 1.0 mL kg^{-1}	27.63±3.83^{a,2}	12.91± 3.01^{a,1}	8.65±1.15^{a,1}
Female diabetic + SCJ 1.5 mL kg^{-1}	24.99±4.55^{a,2}	15.71±2.7^{a,2}	7.75±2.77^{a,1}
Female diabetic + SCJ 2.0 mL kg^{-1}	32.06±4.75^{a,2}	14.50±3.25^{a,1}	6.98±0.58^{a,1}

Data are expressed as Mean±SEM, n = 5-6. Values within a group followed by the different letter(s) are significantly different at $p<0.05$. Values within a day followed by the different number are significantly different at $p<0.05$. SCJ: *S. crispus* Juice

Table 2: Percentage of glucose reduction in treated male and female STZ-induced diabetic rats at day 15 and day 30

	Day	
Groups	15	30
Male diabetic + glibenclamide	49.45	72.33
Male diabetic + SCJ 1.0 mL kg^{-1}	43.73	67.38
Male diabetic + SCJ 1.5 mL kg^{-1}	57.50	78.94
Male diabetic + SCJ 2.0 mL kg^{-1}	52.62	80.91
Female diabetic + glibenclamide	31.05	58.92
Female diabetic + SCJ 1.0 mL kg^{-1}	53.27	68.69
Female diabetic + SCJ 1.5 mL kg^{-1}	37.13	68.98
Female diabetic + SCJ 2.0 mL kg^{-1}	54.77	78.22

SCJ: *S. crispus* Juice

Table 3: Serum glucose level (mmol L^{-1}) in male and female normal rats

Groups	Day 0	15	30
Male normal control	7.60±0.17^{b,1}	7.52±0.07^{c,1}	7.29±0.07^{c,1}
Male normal + SCJ 1.0 mL kg^{-1}	7.10±0.03^{a,b,1}	6.84±0.22^{b,c},	16.07±0.62^{b,1}
Male normal + SCJ 1.5 mL kg^{-1}	7.65±0.12^{b,3}	6.35±0.36^{a,b,2}	5.20±0.25^{a,b,1}
Male normal + SCJ 2.0 mL kg^{-1}	6.72±0.30^{a,3}	5.77±0.20^{a,2}	4.31±0.01^{a,1}
Female normal control	7.27±0.21^{a,1}	7.56±0.25^{b,1}	7.60±0.14^{c,1}
Female normal SCJ 1.0 mL kg^{-1}	7.02± 0.26^{a,2}	6.82±0.28^{a,b,2}	5.67±0.21^{b,1}
Female normal + SCJ 1.5 mL kg^{-1}	7.58± 0.17^{a,2}	6.82±0.33^{a,b,2}	5.50±0.16^{a,b,1}
Female normal+ SCJ 2.0 mL kg^{-1}	7.19±0.32^{a,2}	5.83±0.30^{a,1}	4.85±0.27^{a,1}

Data are expressed as Mean±SEM, n = 5-6. Values within a group followed by the different letter(s) are significantly different at p<0.05. Values within a day followed by the different number are significantly different at p<0.05. SCJ: *S. crispus* Juice

Table 4: Total cholesterol level (mmol L^{-1}) in male and female STZ-induced diabetic rats

Groups	Day 0	15	30
Male diabetic control	1.56±0.31^{a,1}	1.80±0.28^{a,1}	2.02±0.01^{c,1}
Male diabetic + glibenclamide	2.06±0.16^{a,2}	1.79±0.15^{a,2}	1.46±0.17^{b,1}
Male diabetic + SCJ 1.0 mL kg^{-1}	1.95±0.26^{a,1}	1.52±0.27^{a,1}	1.34±0.28^{b,1}
Male diabetic + SCJ 1.5 mL kg^{-1}	1.78±0.25^{a,1}	1.52±0.24^{a,1}	1.14±0.18^{a,b,1}
Male diabetic + SCJ 2.0 mL kg^{-1}	1.55±0.19^{a,2}	1.11±0.19^{a,1,2}	0.67±0.11^{a,1}
Female diabetic control	1.84±0.06^{a,1}	1.91±0.49^{a,1}	2.22±0.4^{b,1}
Female diabetic + glibenclamide	1.21±0.11^{a,1}	2.14±0.37^{a,1}	1.42±0.25^{a,b,1}
Female diabetic + SCJ 1.0 mL kg^{-1}	1.78±0.22^{a,2}	1.04±0.22^{a,1,2}	0.77±0.31^{a,1}
Female diabetic + SCJ 1.5 mL kg^{-1}	2.01±0.53^{a,1}	1.79±0.48^{a,1}	1.11±0.41^{a,1}
Female diabetic + SCJ 2.0 mL kg^{-1}	1.96±0.25^{a,2}	1.68±0.20^{a,2}	0.99±0.10^{a,1}

Data are expressed as Mean±SEM, n = 5-6. Values within a group followed by the different letter(s) are significantly different at p<0.05. Values within a day followed by the different number are significantly different at p<0.05

Table 5: Total cholesterol level (mmol L^{-1}) in male and female normal rats

Groups	Day 0	15	30
Male normal control	1.6450±0.24^{a,1}	1.4600±0.37^{a,1}	1.4967±0.24^{a,1}
Male normal + SCJ 1.0 mL kg^{-1}	2.5800±0.04^{b,2}	1.9850±0.16^{a,1}	1.5250±0.04^{a,1}
Male normal + SCJ 1.5 mL kg^{-1}	2.2450±0.13^{b,2}	2.0975±0.13^{a,2}	1.4350±0.12^{a,1}
Male normal + SCJ 2.0 mL kg^{-1}	2.0867±0.08^{a,b,1}	1.8933±0.07^{a,1}	1.5867±0.13^{a,1}
Female normal control	1.5200±0.12^{a,1}	1.8500±0.12^{a,1}	2.3800±0.23^{b,1}
Female normal + SCJ 1.0 mL kg^{-1}	2.8000±0.14^{c,2}	2.1600±0.14^{a,1}	2.0800±0.02^{a,b,1}
Female normal + SCJ 1.5 mL kg^{-1}	2.1233±0.04^{b,1,2}	2.2533±0.13^{a,2}	1.6900±0.21^{a,1}
Female normal+ SCJ 2.0 mL kg^{-1}	2.1767±0.09^{a,b,1}	1.9167±0.1^{a,1}	1.5100±0.09^{a,1}

Data are expressed as Mean±SEM, n = 5-6. Values within a group followed by the different letter(s) are significantly different at p<0.05. Values within a day followed by the different number are significantly different at p<0.05. SCJ: *S. crispus* Juice

Table 6: Triglyceride level (mmol L^{-1}) in male and female STZ-induced diabetic rats

Groups	Day 0	15	30
Male diabetic control	2.44±1.66^{a,1}	1.75±0.25^{a,1}	1.52±0.004^{c,1}
Male diabetic + glibenclamide	2.65±0.95^{a,2}	1.49± 0.44^{a,1,2}	0.58± 0.11^{b,1}
Male diabetic + SCJ 1.0 mL kg^{-1}	1.26±0.36^{a,1}	0.74± 0.13^{a,1}	0.57±0.11^{b,1}
Male diabetic + SCJ 1.5 mL kg^{-1}	1.59±0.2^{a,1}	1.45± 0.77^{a,1}	0.37±0.06^{a,b,1}
Male diabetic + SCJ 2.0 mL kg^{-1}	0.93±0.13^{a,2}	0.67±0.14^{a,1,2}	0.29±0.03^{a,1}
Female diabetic control	0.82±0.13^{a,1}	1.21±0.2^{a,1}	1.22±0.17^{b,1}
Female diabetic + glibenclamide	0.72±0.18^{a,1,2}	1.06±0.003^{a,2}	0.46±0.03^{a,1}
Female diabetic + SCJ 1.0 mL kg^{-1}	1.18±0.26^{a,b,2}	0.72±0.15^{a,1,2}	0.34±0.03^{a,1}
Female diabetic + SCJ 1.5 mL kg^{-1}	1.59±0.36^{b,2}	1.25±0.18^{a,2}	0.47±0.2^{a,1}
Female diabetic + SCJ 2.0 mL kg^{-1}	1.05±0.05^{a,b,1,2}	1.41±0.41^{a,2}	0.47±0.1^{a,1}

Data are expressed as Mean±SEM, n = 5-6. Values within a group followed by the different letter(s) are significantly different at p<0.05. Values within a day followed by the different number are significantly different at p<0.05. SCJ: *S. crispus* Juice

The results showed that significant (p<0.05) reduction of serum glucose levels in male and female normal rats after day 15 and 30 treated with 1.0, 1.5 and 2.0 mL kg^{-1} b.wt. of *S. crispus* juice as compared to control group and zero day. In male and female normal rats treated with 2.0 mL kg^{-1} b.wt. of *S. crispus* juice showed significantly (p<0.05) lower serum glucose level than *S. crispus* juice 1.0 and 1.5 mL kg^{-1} b.wt. (Table 3).

Cholesterol level: Supplementation of *S. crispus* juice significantly (p<0.05) reduced the level of total cholesterol at day 30 (Table 4). In male diabetic rats treated with 2.0 mL kg^{-1} b.wt. of *S. crispus* juice showed the lowest of total cholesterol level (0.67 mmol L^{-1}) and female diabetic rats (0.99 mmol L^{-1}).

Normal rats (both male and female) also showed decreased total cholesterol level treated with *S. crispus* juice (Table 5).

Triglyceride level: Results of triglyceride level showed that groups treated with 1.0, 1.5 and 2.0 mL kg^{-1} b.wt. of *S. crispus* juice in male and female diabetic rats showed reduction of triglyceride level. Significantly (p<0.05) highest decrease was noticed 2.0 mL kg^{-1} b.wt. *S. crispus* juice treatment at day 30 in male diabetic rats (0.29 mmol L^{-1}) (Table 6). Table 7 shows that triglyceride

Table 7: Triglyceride level (mmol L^{-1}) in male and female normal rats

	Day		
Groups	0	15	30
Male normal control	1.53±0.33^{a,2}	0.69± 0.16^{a,1}	0.47±0.06^{a,1}
Male normal +SCJ 1.0 mL kg^{-1}	1.18±0.28^{a,1}	0.81±0.08^{a,1}	0.49±0.05^{a,1}
Male normal + SCJ 1.5 mL kg^{-1}	0.91±0.15^{a,1}	0.69±0.12^{a,1}	0.55±0.12^{a,1}
Male normal + SCJ 2.0 mL kg^{-1}	0.93±0.03^{a,1}	0.82±0.12^{a,1}	0.48±0.005^{a,1}
Female normal control	0.81±0.17^{a,1}	0.72±0.13^{a,1}	0.50±0.07^{a,1}
Female normal + SCJ 1.0 mL kg^{-1}	0.80±0.04^{a,2}	0.65±0.07^{a,2}	0.41±0.01^{a,1}
Female normal + SCJ 1.5 mL kg^{-1}	0.90±0.13^{a,2}	0.66±0.10 ^{a,1,2}	0.47±0.03^{a,1}
Female normal+ SCJ 2.0 mL kg^{-1}	1.08±0.20^{a,1}	0.74±0.10^{a,1}	0.53±0.05^{a,1}

Data are expressed as Mean±SEM, n = 5-6. Values within a group followed by the different letter(s) are significantly different at $p<0.05$. Values within a day followed by the different number are significantly different at $p<0.05$. SCJ: *S. crispus* Juice

Table 8: Glutathione peroxidase activity (μ mL^{-1}) in male and female diabetic rats

	Day		
Groups	0	15	30
Male diabetic control	2296.10±205.53^{a,1}	2085.42±195.63^{a,1}	1963.17±218.26^{a,1}
Male diabetic + glibenclamide	2373.74±182.65^{a,1}	2599.96±324. 92^{a,1,2}	2397.30±324.62^{a,b,1}
Male diabetic + SCJ 1.0 mL kg^{-1}	2131.70±274.34^{a,1}	2128.10±349.89^{a,1}	2544.23±369.37^{a,b,1,2}
Male diabetic + SCJ 1.5 mL kg^{-1}	1773.45±171.51^{a,1}	2288.37±235.35^{a,1}	2808.62±209.24^{a,b,1,2}
Male diabetic + SCJ 2.0 mL kg^{-1}	2279.70±60.80^{a,1}	2794.80±36.50^{a,1,2}	3195.79±39.01^{b,1,2}
Female diabetic control	2462.70±326.78^{a,1,2}	2403.06±307.38^{a,b,1,2}	2298.66±250.50^{a,1,2}
Female diabetic + glibenclamide	2469.02±306.76^{a,1,2}	1734.05±288.54^{a,1}	2430.85±195.43^{a,1,2}
Female diabetic + SCJ 1.0 mL kg^{-1}	2632.73±353.71^{a,1,2}	1912.30±161.84^{a,b,1}	3024.26±495.77^{a,b,2}
Female diabetic + SCJ 1.5 mL kg^{-1}	2823.37±174.08^{a,1,2}	2634.27±351.50^{a,b,1}	3250.97±132.42^{b,2}
Female diabetic + SCJ 2.0 mL kg^{-1}	2425.17±186.30^{a,1}	2776.22±144.67^{b,1,2}	3314.12±112.21^{b,2}

Data are expressed as Mean±SEM, n = 5-6. Values within a group followed by the different letter(s) are significantly different at $p<0.05$. Values within a day followed by the different number are significantly different at $p<0.05$. SCJ: *S. crispus* Juice

Table 9: Glutathione peroxidase activity (μ mL^{-1}) in male and female normal rats

	Day		
Groups	0	15	30
Male normal control	1960.80±179.47^{a,1}	1672.57±273.12^{a,1}	1547.50±764.92^{a,1}
Male normal + SCJ 1.0 mL kg^{-1}	1971.57±126.71^{a,1}	2776.72±259.22^{b,1,2}	3245.95±320.36^{c,2}
Male normal + SCJ 1.5 mL kg^{-1}	2123.97±134.06^{a,1}	2874.85±506.06^{b,1,2}	3224.35±136.87^{c,2}
Male normal + SCJ 2.0 mL kg^{-1}	1880.22±185.21^{a,1}	2603.57±63.00^{b,1,2}	3175.30±61.97^{c,2}
Female normal control	2341.83±403.19^{a,1}	2223.73±192.53^{a,1}	2241.33±216.61^{a,1}
Female normal + SCJ 1.0 mL kg^{-1}	2423.03±249.35^{a,1}	2434.13±213.07^{a,1}	2813.55±541.85^{a,b,1,2}
Female normal + SCJ 1.5 mL kg^{-1}	2052.93±199.14^{a,1}	2950.97±180.64^{a,b,1,2}	3401.10±181.60^{b,2}
Female normal+ SCJ 2.0 mL kg^{-1}	1788.07±109.51^{a,1}	2029.57±447.30^{a,1}	3061.47±323.87^{a,b,2}

Data are expressed as Mean±SEM, n = 5-6. Values within a group followed by the different letter(s) are significantly different at $p<0.05$. Values within a day followed by the different number are significantly different at $p<0.05$. SCJ: *S. crispus* Juice

level in male and female normal rats treated with *S. crispus* juice also were decreased compared to zero day.

HDL-cholesterol level and LDL-cholesterol level: *S. crispus* juice treated groups showed not significantly different ($p<0.05$) at day 15 and 30 in male and female normal and diabetic rats.

Glutathione peroxidase activity: Table 8 shows the activity of glutathione peroxidase in blood of male and female diabetic rats. During diabetes there was a reduction in the activity of glutathione peroxidase. Administration of *S. crispus* juice and glibenclamide increased the activity of glutathione peroxidase in male and female diabetic rats. The effect of *S. crispus* was more prominent when compared with glibenclamide. Administration of 2.0 mL kg^{-1} b.wt. *S. crispus* juice showed the highest increase of glutathione peroxidase activity on day 30 in male (3195.79±39.01 μ mL^{-1}) and female (3314.12±112.21 μ mL^{-1}) diabetic rats when compared with control group.

Administration of 1.0, 1.5 and 2.0 mL kg^{-1} b.wt. *S. crispus* juice for 30 days showed significant ($p<0.05$) increase in the activity of glutathione peroxidase in male (3245.95±320.36, 3224.35±136.87 and 3175.30±61.97 μ mL^{-1}, respectively) and female normal rats (2813.55±541.85, 3401.10±181.60 and 3061.47±323.87 μ mL^{-1}, respectively) when compared to control group and zero time (Table 9).

Superoxide dismutase activity: Superoxide dismutase activity increased significantly ($p<0.05$) in treated 1.0, 1.5

Table 10: Superoxide dismutase activity (μL^{-1}) in male and female diabetic rats

	Day		
Groups	0	15	30
Male diabetic control	2.18±0.19^{a,1}	2.28±0.21^{a,1}	1.91±0.10^{a,1}
Male diabetic + glibenclamide	2.08±0.36^{a,1}	2.79±0.54^{a,1}	2.32±0.17^{a,b,1}
Male diabetic + SCJ 1.0 mL kg^{-1}	2.91±0.09^{a,1}	2.96±0.47^{a,1}	3.36±0.59^{b,c,1,2}
Male diabetic + SCJ 1.5 mL kg^{-1}	2.46±0.51^{a,1}	2.88±0.42^{a,1}	3.68±0.60^{c,d,1,2}
Male diabetic + SCJ 2.0 mL kg^{-1}	2.37±0.29^{a,1}	3.46±0.19^{b,c,1,2}	4.77±0.10^{d,2}
Female diabetic control	2.02±1.04^{a,1}	1.35±0.79^{a,1}	1.24±0.62^{a,1}
Female diabetic + glibenclamide	2.08±0.11^{a,1}	2.03±0.41^{a,1}	2.30±0.39^{a,b,1}
Female diabetic + SCJ 1.0 mL kg^{-1}	1.03±0.23^{a,1}	2.16±0.80^{a,1}	3.40±0.64^{b,1,2}
Female diabetic + SCJ 1.5 mL kg^{-1}	2.19±0.35^{a,1}	3.20±0.24^{a,1,2}	3.67±0.31^{b,1,2}
Female diabetic + SCJ 2.0 mL kg^{-1}	1.54±0.47^{a,1}	3.03±0.55^{a,1,2}	5.39±0.37^{c,2}

Data are expressed as Mean±SEM, n = 5-6. Values within a group followed by the different letter(s) are significantly different at $p<0.05$. Values within a day followed by the different number are significantly different at $p<0.05$. SCJ: *S. crispus* Juice

Table 11: Superoxide dismutase activity (μL^{-1}) in male and female normal rats

	Day		
Groups	0	15	30
Male normal control	0.52±0.13^{a,1}	3.32±0.80^{a,2}	1.34±0.36^{a,1}
Male normal + SCJ 1.0 mL kg^{-1}	0.66±0.09^{a,1}	2.88±0.18^{a,2}	3.2 ±0.22^{b,2}
Male normal + SCJ 1.5 mL kg^{1}	0.78±0.06^{a,1}	4.96±0.77^{a,3}	3.54±0.20^{b,2,3}
Male normal + SCJ 2.0 mL kg^{-1}	0.92±0.14^{a,1}	4.78±0.97^{a,3}	3.56±0.38^{b,2,3}
Female normal control	0.89±0.17^{a,1}	2.59±0.38^{a,2}	1.46±0.05^{a,1}
Female normal + SCJ 1.0 mL kg^{-1}	0.81±0.18^{a,1}	2.59±0.78^{a,2}	1.96±0.57^{a,1,2}
Female normal + SCJ 1.5 mL kg^{-1}	1.08±0.43^{a,1}	3.45±0.74^{a,2}	3.22±0.17^{b,2}
Female normal+ SCJ 2.0 mL kg^{-1}	1.60±0.41^{a,1}	4.42±0.81^{a,2,3}	3.29±0.12^{b,2}

Data are expressed as Mean±SEM, n = 5-6. Values within a group followed by the different letter(s) are significantly different at $p<0.05$. Values within a day followed by the different number are significantly different at $p<0.05$. SCJ: *S. crispus* Juice

and 2.0 mL kg^{-1} b.wt. *S. crispus* juice at day 30 in male (3.36±0.59, 3.68±0.60 and 4.77±0.10 μL^{-1} respectively) when compared to male diabetic control (1.91±0.10 μL^{-1}) and in female diabetic rats also showed the significant ($p<0.05$) increase in treated 1.0, 1.5 and 2.0 mL kg^{-1} b.wt. *S. crispus* juice at day 30 (3.40±0.64, 3.67±0.31 and 5.39±0.37 μL^{-1} respectively) when compared to female diabetic control (1.24±0.62 μL^{-1}). In male and female treated 1.0, 1.5 and 2.0 mL kg^{-1} b.wt. *S. crispus* juice for 30 days also showed the significant ($p<0.05$) increase when compared to zero time (Table 10).

A significant increase in superoxide dismutase activity was observed in the male group treated with *S. crispus* juice, where superoxide dismutase activity in 1.0 mL kg^{-1} b.wt. at day 30 (3.24±0.22 μL^{-1}), 1.5 mL kg^{-1} b.wt. (3.54±0.20 μL^{-1}), 2.0 mL kg^{-1} b.wt. (3.56±0.38 μL^{-1}) when compared to male normal control (1.34±0.36 μL^{-1}). In female group treated 1.0 mL kg^{-1} b.wt. (1.96±0.57 μL^{-1}), 1.5 mL kg^{-1} b.wt. (3.22±0.17 μL^{-1}) and 2.0 mL kg^{-1} b.wt. of *S. crispus* juice (3.29±0.12 μL^{-1}) also showed the significant ($p<0.05$) increase when compared to female normal control (1.46±0.05 μL^{-1}). Male and female groups treated with *S. crispus* juice also showed the significant ($p<0.05$) increase when compared to zero time (Table 11).

The experimental streptozotocin-diabetes model usually involves type 1 diabetes. However, in the present study, at the dose of STZ used, the model probably involved type 2 diabetes and some functioning β cells of islets of Langerhans were present because a response to treatment was observed in group treated with glibenclamide (without insulin treatment). Streptozotocin is a valuable agent for the production of diabetes because it allows the consistent production of diabetic states with mild, moderate or severe hyperglycemia and STZ-induced diabetic rats have been widely used as a model for diabetes mellitus in experimental animal. Toxicity studies were carried out as a requirement for Ethics Committee Clearance. The animals were observed for 7 days with no evidence of mortality or abnormalities.

Medicinal plants have played a significant role in maintaining human health and improving the quality of human life for thousands a years (Shinde *et al*., 2007). *S. crispus*, a well-known herb, possesses diverse biological activities and pharmacological functions including antioxidant, antihyperglycemic, antiproliferative and antimicrobial. It has long been used traditionally to treat diabetes mellitus and related disorders. It is also commonly consumed in the form of herbal tea. Although, it has been used widely in the treatment of various ailments, scientific data on this plant is still lacking especially its biological activity (Fadzelly *et al*., 2006b).

The leaf of *S. crispus* contained high amount of minerals (potassium, calcium, sodium, iron and phosphorus); high content of water-soluble vitamins (C, B1 and B2); also contained polyphenols, catechins, caffeine, tannin, alkaloid, carbohydrate, proteins, fiber (Ismail *et al*., 2000) and bioactive compounds such as β-sitosterol and stigmasterol (Abdah *et al*., 2004).

The present study demonstrated that supplementation of *S. crispus* juice at doses 1.0, 1.5 and

2.0 mL kg^{-1} b.wt. reduced serum glucose level in STZ-induced diabetic rats. The highest reduction of glucose level was 80.9% at day 30 in male diabetic group treated with 2.0 mL kg^{-1} b.wt. *S. crispus* juice followed by 78.9% reduction in group treated with 1.5 mL kg^{-1} b.wt. and 67.4% reduction in group treated with 1.0 mL kg^{-1} b.wt. of *S. crispus* juice. In female groups, reduction of glucose level were 78.2, 68.9 and 68.6% in treated 2.0, 1.5 and 1.0 mL kg^{-1} b.wt. of *S. crispus* juice, respectively. The epicatechin properties of *S. crispus* may act as antihyperglycemic in diabetic rats. Epicatechin is a member of a group of polyphenolic compounds collectively known as catechins that are present in the tea and belong to the flavonoid family, has been reported to possess insulin-like activity (Rizvi and Zaid, 2001). Scientific studies have emphasized the antidiabetic properties of catechins in alloxan-induced diabetes. Recent study showed that *S. crispus* juice showed high total phenolic content and antioxidant activities .

Diabetes mellitus is characterized by hyperglycemia, which usually produces many complications, such as hyperlipidemia, hyperinsulinemia, hypertension, obesity, atherosclerosis and even cardiovascular disease (Defronzo *et al*., 1992; Alberti *et al*., 1997). High levels of total cholesterol and triglyceride (serum lipids) are major risk factors for atherosclerosis and coronary heart disease. An increase in HDL-cholesterol is associated with decrease in atherosclerotic and coronary risk (Chait and Brunzell, 1996). In the present study, administration of *S. crispus* juice reduced total cholesterol, triglyceride, LDL-cholesterol and increased HDL-cholesterol in STZ-induced diabetic and normal rats. The improvement of lipid profile might be contributed by plant sterols (β-sitosterol and stigmasterol) that are found in the leaves of *S. crispus* (Abdah *et al*., 2004). Recent study suggests that plant sterol esters such as sitosterol and campesterol esters or stanol (sitostanol and campestanol) esters also act as lipid-lowering agents (Law, 2000), by competing with cholesterol for incorporation into micelles, thereby decreasing the intestinal absorption of cholesterol (Law, 2000; Cater *et al*., 2005).

Diabetes has been found to be associated with indices of oxidative damage. Free radicals have been shown to cause oxidative damage to lipids (lipid peroxidation), protein and nucleic acids (Simpson *et al*., 1992). In order to combat the damaging effects by free radicals, cells have evolved a complex antioxidation system which includes both exogenous antioxidants (examples vitamin C and E) and endogenous antioxidant enzymes such as glutathione peroxidase (GPx) and superoxide dismutase (SOD) (Lee *et al*., 2005). The results of the present study demonstrated that administration of *S. crispus* juice increased the activities of GPx and SOD and may help to control free radicals, offered protection to cells against oxidative stress by scavenging free radicals generated during diabetes. The increased activities of antioxidant enzymes may act as an added compensation mechanism to maintain the cell integrity and protection against free radical damage (Murugan and Pari, 2006). The ability of *S. crispus* juice increased the activities of antioxidant enzymes in diabetic treated rats implies that *S. crispus* juice reactivates the antioxidant defense system, thereby increasing the capacity of antidiabetic activity through the enhanced scavenging of oxy radicals. Thus, findings related to *S. crispus* juice suggest that it may implicated as an antioxidant agent in addition to its antidiabetic effect.

CONCLUSION

Glucose, lipid profile, activities of glutathione peroxidase and superoxide dismutase of diabetic and normal in male and female rats were evaluated in this study. The results showed that *S. crispus* juice possesses antihyperglycemic, hypolipidemic and antioxidant effect. Thus, *S. crispus* juice could be an alternative treatment for lowering glucose, cholesterol and triglyceride for diabetic patients in the future.

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