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Roselle *(Hibiscus sabdariffa)* calyce ethanolic extract decreases insulin resistance in high carbohydrate diet fed cats

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Abstract

The objective of this study was to determine whether roselle calyce extract could modulate insulin resistance in high carbohydrate diet fed cats. Fifteen non-obese, mixed-breed, adult clinically healthy cats were used in this study. Following consumption of a commercially available control diet for 2 weeks, venous blood was collected to determine the baseline of blood glucose. Then the cats were randomly separated into 5 groups of 3 cats each. Group 1 received the control diet throughout the experiment. Groups 2-5 received high carbohydrate diets (HC) for 2 weeks to induce insulin resistance. After that, group 2 continued to receive a HC diet for further 5 weeks while groups 3 and 4 received a HC diet with roselle extract at doses of 0.1 or 1g/kg BW/day and group 5 received acarbose 25 mg/cat orally once daily. An intravenous glucose tolerance test (IVGTT) was performed after the 4th week of treatments in all groups and fasting blood were collected to measure insulin, glucose, triglycerides, cholesterol, and BUN. The fasting glucose and insulin values were used to calculate Homeostasis model assessment for insulin resistance (HOMA-IR). Then in the 5th week, blood samples were collected over 24 hours at 0 min, 30 min, 1, 2, 3, 4, 6, 8, 11, 14, 18 and 24 hr for monitoring of 24 hours glucose. The IVGTT showed the area under the plasma glucose time curve (AUC) of HC diet control group was significantly higher than that of cats receiving normal control diet ($p < 0.05$). Interestingly, the AUC glucose levels of HC diet group treated with roselle extract at both doses (180.6 ± 25.8 and 178.5 ± 10.9 min.mg/dL) were significantly lower than that of HC control group (270.1 ± 19.1 min.mg/dL). For 24 hours glucose, the AUC glucose of HC group was significantly ($p < 0.05$) higher than that of normal control diet group, however, treatment with both doses of roselle extract did not affect 24 hours AUC glucose levels of HC fed cats. In addition, the HC diet control group had a significantly higher HOMA-IR score as compared to the normal control diet group. This high HOMA-IR returned to normal level in the group that received roselle extract. In conclusion, the results indicate that roselle extract can reduce blood glucose by reducing insulin resistance in HC fed cats. Roselle calyce extract might therefore be used as alternative medicine in type 2- diabetic cats.

Keywords: Roselle; Triglyceride; Cholesterol; Blood glucose; Insulin resistance

1. Introduction

Diabetes is the most frequently diagnosed endocrinopathies in dogs and cats. A retrospective study of feline diabetes in 1990 found 1 in 400 cases [1] and a recent study in 2007 found a higher prevalence of feline diabetes in approximately 1 in 200 cases [2]. The increased incidence is found in both species due to the increased risk factors of, for example, obesity and physical inactivity [3]. The majority of diabetes in cats is type 2 (80-95%) called non-insulin dependent or adult onset diabetes which involves insulin resistance or a decreased response to insulin by target tissues and a decrease in insulin production by the beta cells of the pancreas [4, 5].

In human diabetics, diagnosis is based on fasting blood glucose concentration values of ≥ 7 mmol/L, however, in cats it is usually diagnosed when clinical signs such as polyuria, polydipsia and weight loss with polyphagia appear concomitantly with blood glucose level of approximately ≥14-16 mmol/L (250-290 mg/dL) which exceeds

the renal threshold [6, 7]. The blood glucose levels of pre-diabetic cats or humans are above the normal but lower than that considered diabetic during fasting or the glucose tolerance test. The main goal in management of pre-diabetes and diabetes is to normalize postprandial hyperglycemia rather than reducing the fasting blood glucose level. The recommended management of pre-diabetes or a persistent >6.5 mmol/L (117 mg/dL) level in cats is with a low carbohydrate diet, weight loss, and potential insulin sensitizers [7]. Current principal treatment for diabetic cats, however, comprises of insulin therapy and dietary management. Chronic or long-term hyperglycemia can cause damage to organs including eyes, kidneys, blood vessels and nerves [8].

The main goal in treatment of diabetes mellitus is to prevent complications and reduce post-meal plasma glucose by using insulin, low carbohydrate diets and therapeutic agents such as the alpha-glucosidase inhibitors like acarbose to decrease glycemic load [9-11]. Acarbose is a complex oligosaccharide of microbial origin that competitively inhibits alpha-glucosidase and alpha-amylase, enzymes which are involved in carbohydrate digestion in the brush border of the small intestinal mucosa [12]. Diabetic remission in cats can be achieved if good management is practiced after early diagnosis, which will improve the quality of the cat's life and reduces the owners' time and financial commitments.

There are many plant species that have been recommended for treating diabetes. Only some of them such as *Momordica charantia* L., *Enicostemma littorale* Blume [13], *Gymnema inoforum*, *Stevia rebaudiana*, and *Tinospora crispa* have proven hypoglycemic activity [14-16] that can reduce plasma glucose. *Hibiscus sabdariffa* Linn (roselle), in Thailand called "Krachiap Daeng" belongs to the family Malvaceae. Roselle is an annual or woody-based subshrub, growing 2-2.5 meter tall. The leaves are deep three to five-lobed, 8-15 cm long, arranged alternatively on the stems. The flowers are 8-10 cm in diameter, white to pale yellow with a dark red spot at the base of each petal, and have a stout fleshy calyx at the base, 1-2 cm wide, enlarging to 3-3.5 cm, fleshy and bright red as the fruit matures. It takes approximately six months to mature. The important parts of roselle which are used as food and medicine are the calyces. The dried calyces contain flavonoid compounds (crysanthemin, delphinidin-3-O-sambubioside, myricetin, hibicitrin, gossypitrin), phenylpropanoid compounds (orthocoumaric acid, paracoumaric acid, ferulic acid), and anthocyanin.

H. sabdariffa Linn. calyces are used to make beverages and also used by Thai traditional physicians as diuretic, stomachic, aphrodisiac, antiseptic and antihypertensive agents [17]. It also has been reported as antidyslipidemic [17], antiatherosclerotic [18], and antihypertensive [18, 19]. The study by Wisetmuen et al., [19] showed that 1g/kg of ethanolic roselle extract is antihyperglycemic in streptozotozin-induced type 1 diabetic rats. Further study by Pannangpetch *et al*, [20] found that roselle extract enhances glucose uptake into adipocytes to help lowering blood glucose in the type 2 diabetic rats fed with high fructose and fat diets. Furthermore, they also showed that roselle water extract significantly decreases fasting blood glucose by 25-30% and also improves oral glucose tolerance test (OGTT) in high fructose and high fat diets induced type 2 diabetic rats. In addition, roselle extract (0.1-1 g/kg) significantly decreases the elevated insulin concentration. The HOMA-IR scores of roselle extract treated rats are also significantly decreased which indicates that roselle extract could reduce insulin resistance in high fructose and fat diet induced type 2 diabetic rats. Since, roselle extract has never been studied in cats even though cat has more similar characteristics of type 2 diabetes to humans than the rat. Animal models of type 2 diabetes have provided valuable information of the disease in humans. The feline model of type 2 diabetes provides more complementary information than the usually used rodent models as the disease occurs continuously and cats also share the same environment with humans. The feline model of type 2 diabetes is characterized by impaired beta cell function, insulin resistance [21], and islet amyloid deposition [22, 23].

The objectives of this study were firstly to determine the effect of roselle extract whether it has efficiency in lowering blood glucose and insulin in induced hyperglycaemic cats. Secondly, determine the effect of roselle extract for prevention of complications such as lipid profiles: triglycerides, cholesterol, and renal function: blood urea nitrogen (BUN) and creatinine among animals fed with a commercially available feline high carbohydrate (HC) diet with and without roselle extract treatment.

2. Materials and methods

2.1 Plant materials

H. sabdariffa (HS) was provided by Assoc. Prof. Dr. Arunporn Itharat, Thummasart University. *H. sabdariffa* extract (HS) was prepared in tablet of 250 mg.

2.2 Experimental animals

Fifteen non-obese, mixed-breed, adult, and clinically healthy cats (5 males, 10 females) were used in this study. Body condition ranged from 4-6 on a nine-point system [24]. All procedures complied with the standards for the care and use of experiment animals and approved by the Animal Ethics Committee of Khon Kaen University, Khon Kaen, Thailand, approval number AEKKU 69/2555. They were kept at the Department of Pharmacology

and Toxicology Laboratory, Faculty of Veterinary Medicine, Khon Kaen University. All cats were re-homed at the conclusion of the study.

2.3 Diet and drugs

There were two types of diet used in this experiment. Firstly, the control diet which was a healthy adult commercial cat's food (Purina One, Nestle Purina PetCare, Rhodes, NSW, Australia), with moderate carbohydrate and protein content, providing 32%, 34%, and 34% metabolize energy (ME) from carbohydrate, protein and fat, respectively, [25]. Secondly, a high carbohydrate diet (HC) which had a carbohydrate of 51% ME. Acarbose (Glucobay® Bayer Thai, Bangkok) is a hypoglycemic drug in the group of alpha glucosidase inhibitors.

2.4 Experiment design

Following consumption of a controlled diet for 2 weeks, venous bloods of all cats were collected to determine baseline blood glucose. Then cats were randomly separated into 5 groups of 3 cats each. Group 1 received the commercially available control diet throughout the experiment. Groups 2-5 received HC to induce hyperglycaemia throughout the experiment. After 2 weeks of HC consumption, group 3 and 4 rats were treated with roselle extract at dosages of 0.1 or 1 g/kg/day, and group 5 cats were treated with acarbose (alpha glucosidase inhibitor) at 25 mg/cat PO once daily for the next 5 weeks.

2.4.1 Intravenous glucose tolerance test (IVGTT)

IVGTT was performed at the end of the 4th week in all groups. Blood samples were collected before (0 min) and at 5, 10, 15, 30, 45, 60, 90, 120 and 180 min after 1 g/kg body weight glucose administration. Fasting blood glucose (FBG) levels were collected (at time 0) and used to measure blood glucose, insulin, triglycerides, cholesterol and BUN.

2.4.2 Blood samples collected over 24 hours

Twenty four hours blood samples were collected from cephalic veins (0.5-1 mL) at -5 and 30 min, and 1, 2, 3, 4, 6, 8, 11, 14, 18 and 24 hr on the 5th week.

2.5 Sample handling and analysis

Blood samples for glucose analysis were placed into sodium fluoride tubes, kept on ice for 8 min, and then centrifuged at 1500 g for 10 min, and plasma aliquots were stored at -70^{o}C in micro-vials until analysis. Plasma glucose concentrations were measured by the Khon Kaen Veterinary Hospital laboratory (Khon Kaen, Thailand) on a chemistry analyzer (Roche Integra 800 chemistry analyzer; Roche Diagnostics, Indianapolis, IN, USA). The glucose and insulin values were used to calculate HOMA-IR according to the formula below.

$$HOMA - I = \frac{Glucose\ (mg/dL)\ x\ Insulin\ (mU/L)}{405}$$

Values of blood glucose from IVGTT and 24 hr test were used to calculate AUC for comparison among groups using the trapezoidal method [26] then dividing by 24.

2.6 Statistical analyses

All values were presented as mean ± S.E.M. Blood glucose and serum insulin levels were compared between cats received control diet and HC by unpaired *t*-test. The effects of HS extract on blood glucose, insulin, triglycerides, cholesterol, BUN were evaluated using analysis of variance (ANOVA). P values < 0.05 were considered statistical significance. Statistical analyses were performed using SPSS® (StataCorp; College Station, TX, USA). Baseline concentrations were the concentration that measure at 0 minute before feeding.

3. Results

3.1 IVGTT

IVGTT showed the AUC glucose levels of cats that received HC-diet (270.1 ± 19.1 min.mg/dL) were significantly higher than those of cats receiving normal control diets group (172.0 ± 21.0 min.mg/dL) ($p < 0.05$),

(**Figure 1**). This validates that blood glucose levels in those cats that receiving HC-diet were higher than those receiving the normal control diet in the experiment. Similarly, the AUC of glucose levels of HC cats treated with both doses (0.1 and 1 g/kg BW/day) of roselle extract (180.6 ± 25.8 and 178.5 ± 10.9 min.mg/dL) were significantly lower than those of the HC group (270.1±19.1 min.mg/dL).

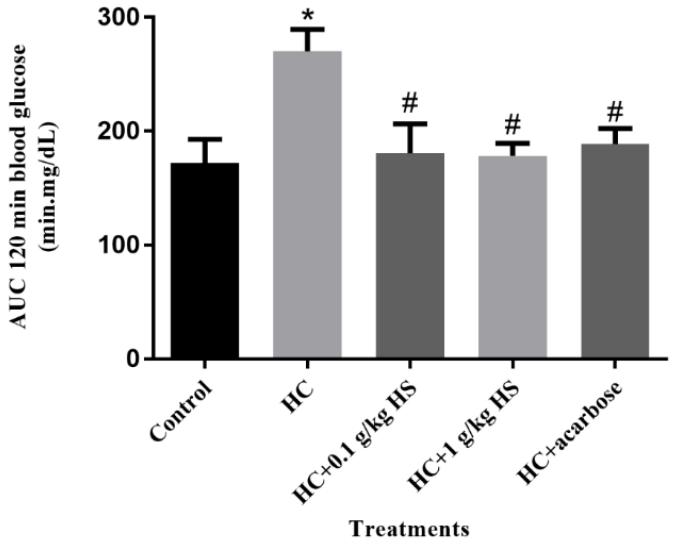

Figure 1. Effects of roselle extract on AUC glucose in IVGTT at 120 mins after injection of 1 g/kg of glucose in HC treated cats.
Control = normal diet group; HC= high carbohydrate group; HS = roselle extract
*p<0.05: as compared to control diet group.
#p<0.05: as compared to HC group

3.2 Twenty four hours plasma glucose test

AUC of 24 hours glucose of control diet group (85.7 ± 4.8 mg.hr/dL) was significantly lower than HC group (98.2 ± 2.9 mg.hr/dL) ($p < 0.05$). Although treatment with both roselle extract could reduce the 24 hours AUC glucose but did not reach significant levels. For acarbose treated group, the AUC (78.5 ± 11.4 mg.hr/dL) was significantly lower than that of HC diet group ($p < 0.05$).

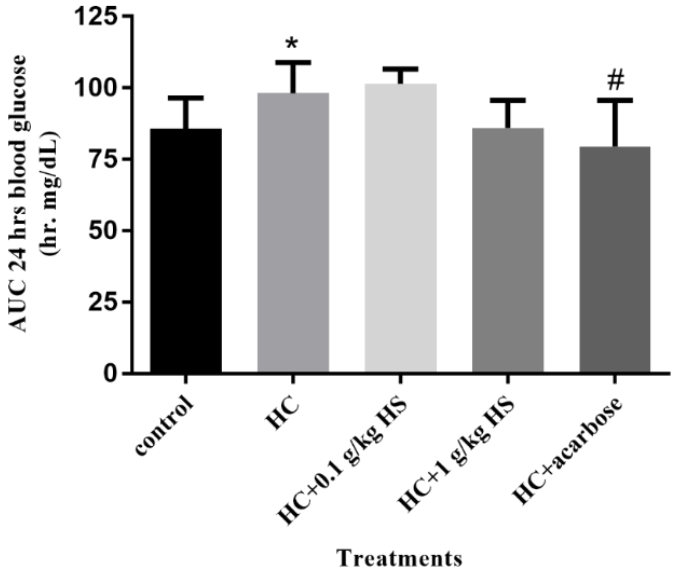

Figure 2. Effects of roselle extract on AUC 24 hours blood glucose in HC treated cats.
Control = normal diet group; HC= high carbohydrate group; HS = roselle extract
*p<0.05: as compared to control diet group.
#p<0.05: as compared to HC group

3.3 HOMA-IR

Treatment with HC markedly and significantly increased HOMA-IR (0.44 ± 0.08) compared with control diet treated group (0.1 ± 0.0) ($p < 0.05$). Treatment with roselle extract (1 g/kg) reduced HOMA-IR (0.14 ± 0.03) to near control diet group (**Figure 3**).

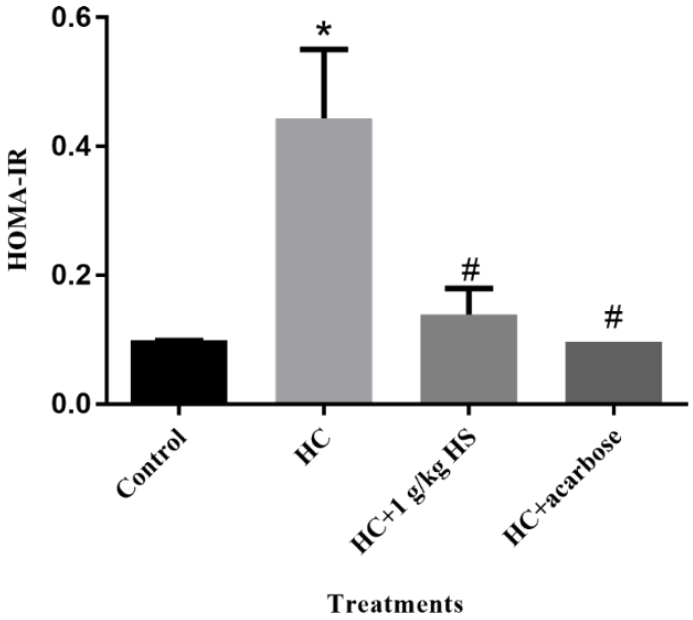

Figure 3. Effects of roselle extract on HOMA-IR in HC treated cats. or insulin resistance, comparing between groups. HOMA-IR of HC group: 0.44 ± 0.08; HOMA-IR of control diet group: 0.1 ± 0.0; HOMA-IR of HC + 1g/kg of HS group: 0.14 ± 0.03; HOMA-IR of HC + acarbose group: 0.1 ± 0.0.
Control = normal diet group; HC= high carbohydrate group; HS = roselle extract
*p<0.05: as compared to control diet group.
#p<0.05: as compared to HC group

3.4 Triglycerides and cholesterol

HC did not increase triglyceride and cholesterol levels compared with control diet groups. Although 0.1 and 1 g/kg doses of roselle extract could reduce triglyceride (24.0 ± 6.0 and 27.0 ± 3.0 mg/dL) compared with HC treated only group (36.0 ± 1.7 mg/dL) and cholesterol levels (59.0 ± 4.0 and 55.5 ± 4.5 mg/dL) compared with HC treated only group (73.0 ± 4.0 mg/dL) but these lowering effect did not reach significant levels **(Figures 4A and 4B)**. Acarbose, however, could significantly reduce triglyceride level to 25 ± 2.65 mg/dL ($p < 0.05$) but not cholesterol levels (68.0±4.4 mg/dL) **(Figures 4A and 4B)**.

3.5 BUN and creatinine

There were no significant differences in BUN and creatinine levels among control diet, HC, and roselle extract and acarbose plus HC treated groups (**Figures 5A and 5B**).

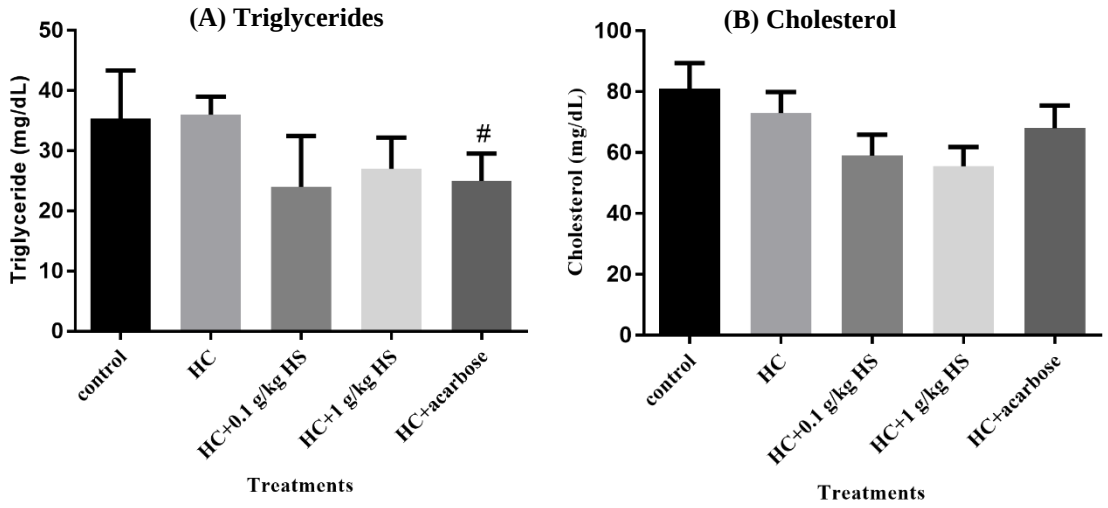

Figure 4. Effects of roselle extract on triglycerides (A) and cholesterol (B) in HC treated cats.
Control = normal diet group; HC= high carbohydrate group; HS = roselle extract
#p<0.05: as compared to HC group

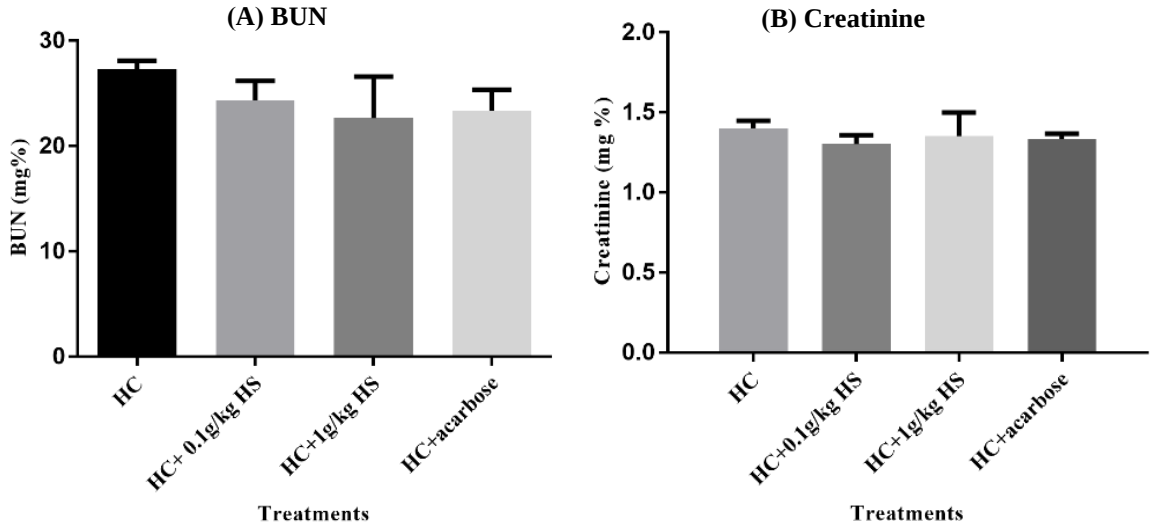

Figure 5. Effects of roselle extract on BUN (A) and Creatinine (B) in HC treated cats.

4. Discussion

Long-term HC feeding significantly caused hyperglycemia and increased HOMA-IR score and impaired IVGTT in cats that could be normalized by roselle extract but not the AUC of 24 hours glucose. Interestingly, long-term HC consumption did not increase triglycerides, cholesterol, BUN, or creatinine levels in cats. Although long-term HC diet did not increase triglycerides and cholesterol levels, roselle extract showed a trend to reduce triglycerides and cholesterol levels in HC fed cats but these seemingly beneficial effects did not reach significant levels. Roselle extract did not affect BUN and creatinine levels in HC fed cats.

Our results showed that roselle extract improved the hyperglycemia induced by HC in IVGTT probably by reducing insulin resistance as shown by the significant reduction in HOMA-IR scores. These findings are in line with previous studies that roselle extract has antihyperglycemic effect in streptozotozin-induced type 1 diabetic rats (19). Roselle extract has been found to enhance glucose uptake into adipocytes to help lowering blood glucose in the type 2 diabetic rats fed with high fructose and fat diets (20). Furthermore, they also showed that roselle water extract significantly decreases fasting blood glucose by 25-30 % and also improves oral glucose tolerance test (OGTT) in and decreases the elevated insulin concentration in type 2 diabetic rats by virtue of improving insulin resistance (20). However, roselle extract could not reduce AUC of 24 hours glucose in our study. Since in the 24 hours glucose experiment, glucose was given in divided doses spreading over 24 hours and each individual dose was therefore too small to induce a surge in blood glucose to trigger the surge of insulin release in these already acquired insulin resistance, the effect of roselle extract may not be strong enough to counteract this minimal hyperglycemia. In contrast, the IVGTT where glucose was given in bolus dose that induced sudden

surge of insulin release leading to a prominent hypoglycemic effect and in condition of improved insulin resistance induced by roselle extract.

The limitations of the present study were the small number of cats used in each treatment that power of statistical test was therefore to weak to test the significant differences among the test groups. However, this pilot study may pave the way to future study verify the beneficial antihyperglycemic and improvement of insulin resistance effects of roselle extract in humans in general and cats in particular.

5. Conclusions

In conclusion, the results indicated that roselle can reduce blood glucose in hyperglycaemic cats by reducing insulin resistance. *H. sabdariffa* extract might therefore be used as alternative medicine in type 2 diabetic cats.

6. Acknowledgements

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