

Assessment of Glycaemic Effect of *Benincasa hispida* Aqueous Extract in Streptozotocin Diabetic Rats

Fatariah Zakaria^a, Wan Rosli Wan Ishak^{a*}, Ng Sze Han^a, Mohd Shazwan Zain^a,
and Wan Amir Nizam Wan Ahmad^b

^aNutrition Programme, School of Health Science, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan

^bBiomedicine Programme, School of Health Science, Universiti Sains Malaysia,
16150 Kubang Kerian, Kelantan

*Corresponding author: wrosli@usm.my

Abstract : *Benincasa hispida*, Bh (winter melon) is a good source of natural sugars, amino acids, organic acids, mineral elements and vitamins. This study was designed to explore the glycaemic effect of Bh aqueous extract in diabetic rats. Male Sprague-Dawley rats (250-350g) were induced to become diabetic with streptozotocin (STZ, 45mg/kg intraperitoneal). The Bh aqueous extracts of 100, 250 and 500 mg/kg and metformin were administered through oral gavage to determine their effects on fasting blood glucose (FBG) and oral glucose tolerance test. The dose of 250 mg/kg was found to be the most effective dose and it decreased blood glucose level (BGL) by 11.8% in normal healthy rats after 6H of administration. The same dose also caused a marked reduction in BGL of 33.1% in normal rats, 38.7% in severe diabetic rats and 64.7% in mildly diabetic rats in OGTT after 3H of oral administration of the extract. The changes of BGL were not significant compared to normal rats. It shows that the extract is diabetic-friendly since the blood glucose levels were stable throughout the study period. These trends of BGL reduction show that Bh aqueous extract is diabetic friendly.

Keywords: Diabetes mellitus; glucose tolerance test; hypoglycaemic; rats; *Benincasa hispida*.

Introduction

The occurrence and prevalence of diabetes is increasing especially in developing and newly industrialized countries. It continues to increase due to population growth, aging, urbanization and lifestyle changes which lead to lack of physical activity and weight gain (Wild *et al.*, 2004). The disease is predicted to rise from 171 million in 2000 to 366 million in 2030 (Amos *et al.*, 1997). Chandra *et al.* (2004) defined diabetes mellitus as an endocrine disorder that is characterized by hyperglycaemia. Diabetes occurs when the homeostasis of carbohydrate and lipid metabolism is improperly controlled by insulin. This primarily results in raised fasting and post prandial blood glucose levels. If this imbalanced homeostasis does not return to normal and persists for a long period, it leads to hyperglycaemia that in due course turns into a disease called diabetes mellitus (Haslett *et al.* 1999).

Benincasa hispida (Bh) or winter melon is one of the famous fruits in Asian countries that is recognized for its nutritional and medicinal properties because it provides a good source of natural sugars, amino acids, organic acids, mineral elements and vitamins (Zaini *et al.*, 2010). This fruit has also been ascribed to possess numerous medicinal properties such as anti-diarrheal, anti-obesity, anti-ulcer, and antioxidant diuretic. A study by Fatariah *et al.* (2014) showed that aqueous extract of Bh contains gallic acid, a bioactive compound. Gallic acid (3,4,5-trihydroxybenzoic acid) is a type of phenolic acid and has received much attention because of its effective free radical scavenging action and antioxidant properties. Previous studies reported that oral administration of gallic acid to diabetic rats for 21 days significantly decreased the levels of blood glucose and increased the levels of plasma insulin, compared to diabetic control rats (Punithavathi *et al.*, 2011). Thus, the present study was designed to explore the glycaemic effect of Bh aqueous extract in diabetic rats.

Materials and Methods

Preparation of plant extracts

The fresh fruit, *Benincasa hispida* (Bh) which is the same kind as the one used by Zaini *et al.* (2010) was purchased at the local market in Kota Bharu, the capital of Kelantan, a state

located in Malaysia. The fruit was cleaned to remove any foreign substances on the surface of the fruit. The cleansed fruit was then manually peeled to separate its seeds, inner pulp and pulp. The pulp of Bh was homogenized with a food processor and mixed with distilled water in 1:1 ratio in a beaker. The mixture was heated with low heat ($60 \pm 2^{\circ}\text{C}$) using a hot plate for 30 minutes and the mixture was then cooled at room temperature. The mixture was then filtered with muslin cloth and centrifuged (2790 g, 10 minutes) before it was freeze-dried for further analyses.

Experimental animals

Male albino Sprague–Dawley rats of body weight 250-350 g were obtained from the Animal Research and Service Centre (ARASC), USM Kubang Kerian, Kelantan. Animals were kept in ARASC at an ambient temperature of $24\text{--}30^{\circ}\text{C}$ with a 12 H dark and light cycle respectively. Animals were fed pellet diet and water *ad libitum*. Approval for this study was obtained from the Animal Care and Ethics Committee, Universiti Sains Malaysia.

Experimental design

Initial screening of Bh extract for evaluating hypoglycaemic effects was done in normal healthy rats and diabetic rats with a range of different doses of 100, 250 and 500 mg/kg given orally by gavage. The study was conducted on fasting blood glucose (FBG) and oral glucose tolerance test (OGTT). BGL was estimated by using Accucheck Performa for regular check-up.

Induction of diabetes in rats

Freshly prepared streptozotocin (45 mg/kg bw) in 0.1 M citrate buffer (pH 4.5) was injected intraperitoneally to a group of overnight-fasted rats. Diabetes was confirmed after 3 days if $\text{FBG} \geq 6.6 \text{ mmol/l}$. Rats were divided according to their FBG levels into two groups: mildly diabetic rats (FBG: 6.6–13.8 mmol/l) and severely diabetic (FBG > 13.8 mmol/l) (Kesari *et al.*, 2006).

Assessment of hypoglycaemic activity in normal healthy rats

Twenty normal rats were fasted overnight and divided into five equal groups. The control group (group A) was given vehicle (distilled water) only while groups B, C and D received Bh aqueous extract orally at doses 100, 250 and 500 mg/kg, respectively. Group E was given reference drug, Metformin at 175 mg/kg. BGL was estimated before and after 2, 4 and 6H of extract administration.

Assessment of hypoglycaemic activity by OGTT in normal healthy rats

Five groups of four rats each were treated with the same treatment as mentioned above. FBG was checked initially and after 90 min of treatment. Time of 90 min was denoted as 0H. Rats were then administered orally with 2 g/kg of glucose and the BGL was tested up to 3H at regular interval of 1H each as glucose tolerance test.

Assessment of hypoglycaemic activity by OGTT in severely diabetic rats

Twenty overnight-fasted severely diabetic rats were divided into five groups of four. Group A which served as a negative control received vehicle (distilled water) only, whereas group B, C and D were treated with different doses of Bh extract at 100, 250 and 500 mg/kg, respectively. For positive control (group E), a dose of 175 mg/kg drug Metformin was administered orally which is the same as OGTT test for normal healthy group as mentioned above. The rats of all groups were given glucose (2 g/kg) after 90 min of the extract and drug administration. BGL was tested at 1, 2 and 3 H after glucose loading.

Assessment of effective dose on blood glucose level of mild diabetic rats

From the above mentioned test, 250 mg/kg as effective dose of Bh extract was selected for treatment of mildly diabetic rats model. Group A was served as the control which received vehicle (distilled water) only, whereas group B was treated with effective dose of Bh extract and group C was given 175 mg/kg of Metformin. All of the rats were given glucose (2 g/kg)

after 90 min of treatment which was considered as 0H and BGL was tested at 1, 2 and 3H after glucose administration.

Lethal Dosage (LD₅₀)

A group of six animals, three females and three males (180-220 g), was administered orally with a single dose of 10 times of effective dose of Bh aqueous extract while another group of rats was given a single dose of 15 times of effective dose of Bh aqueous extract. The rats were observed for gross behaviour and toxic effects at 2, 6, 12 and 24H. Food consumption, faeces and urine were also examined in the short interval of time.

Statistical analysis

All data were statistically evaluated using GraphPad Prism (Version 6.03) software using two-way repeated measured analysis of variance. All tests were two-tailed and significant value was set at $p \leq 0.05$.

Results

Effects on fasting blood glucose level of normal healthy rats

Table 1 shows the hypoglycaemic effect of different doses of *Benincasa hispida* aqueous extract on FBG of normal rats. Rats treated with 500 mg/kg of Bh aqueous extract showed a maximum fall of 13.5% in FBG after 6H of oral administration, whereas the falls of 9.1% and 11.8% were observed with the doses of 100 mg/kg and 250 mg/kg, respectively.

Effect on OGTT of normal healthy rats

Figure 1 displays the hypoglycaemic effect of a single oral administration with different doses of 100, 250 and 500 mg/kg on OGTT of normal rats. The dose of 500 mg/kg depicted a maximum fall of 39.2% at 3H after glucose administration, whereas falls of 33.4% and 33.1% were observed with the dose of 100 and 250 mg/kg, respectively.

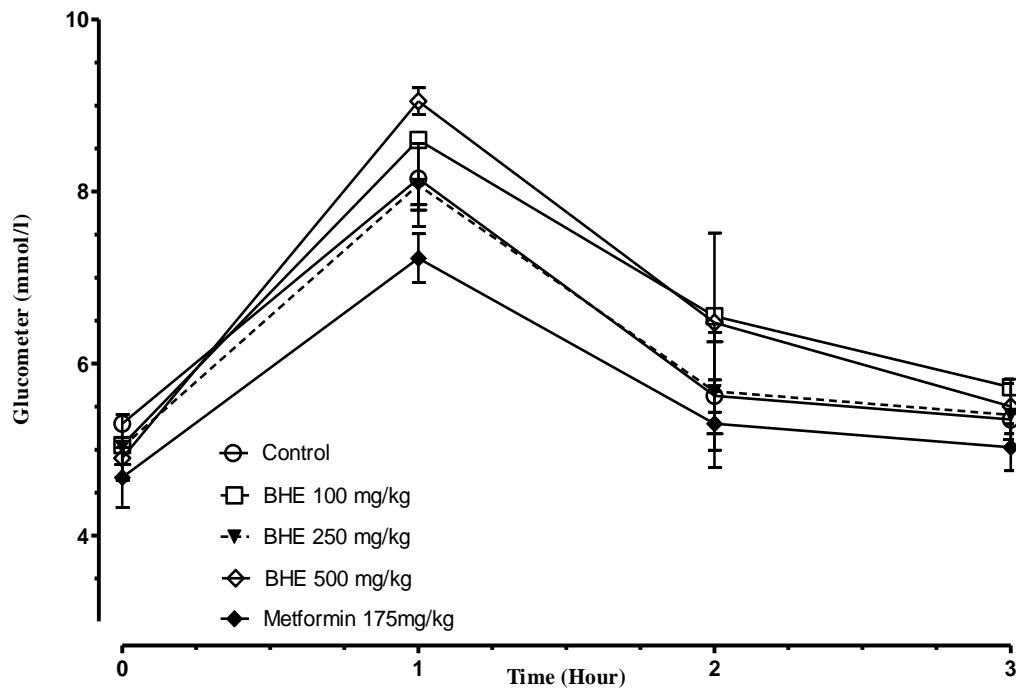


Figure 1: Effect of different doses of aqueous *Benincasa hispida* extract on glucose tolerance in normal rats

In this present study, the treatment of Bh extract on normal rats did not significantly reduce or increase the glucose level (**Table 1**). There was no great drop in BGL up to 500 mg/kg Bh extract. Even after 6H, the BGLs of the normal rats were stable compared to the control group which was given distilled water. In comparison to the control group, BGL level of Metformin group (175mg/kg)dropped after 2H and significantly reduced the blood sugar at 4H ($P<0.05$) and 6H ($P<0.01$).

Table 1: Effect of different doses of aqueous *Benincasa hispida* (Bh) extract on blood glucose level of normal rats (mean ± S.D)

Group	Doses	Blood glucose levels (mmol/l)			
		FBG	2H	4H	6H
A (control)	DW	4.6 ± 0.4	4.4 ± 0.6	4.6 ± 0.1	4.5 ± 0.4
B (extract)	100 mg/kg	5.5 ± 0.3	4.6 ± 0.4	4.7 ± 0.4	5.0 ± 0.3
C (extract)	250 mg/kg	5.1 ± 0.5	4.6 ± 0.4	4.6 ± 0.3	4.5 ± 0.3
D (extract)	500 mg/kg	5.2 ± 0.1	4.9 ± 0.2	4.9 ± 0.2	4.5 ± 0.1
E (metformin)	175 mg/kg	5.5 ± 0.4	3.9 ± 0.3	3.9 ± 0.5*	3.6 ± 0.5**

* $P < 0.05$ as compared with control.

** $P < 0.01$ as compared with control.

Effect on OGTT of severe diabetic rats

In order to choose the optimum dose for severe diabetic animals, different doses of aqueous extract at 100, 250 and 500 mg/kg were evaluated on glucose tolerance of severe diabetic rats. **Figure 2** displays the effect of variable doses of aqueous extract of Bh fruit on BGL of severely diabetic rats during OGTT analyses. After 3H of glucose administration, the BGL falls observed for the doses of 100, 250 and 500 mg/kg were 28.6, 38.7 and 21.2%, respectively. The reference drug, metformin produced the greatest BGL fall (48.5%). Thus, 250 mg/kg of Bh aqueous extract was selected as the most effective dose in lowering BGL of diabetic rats.

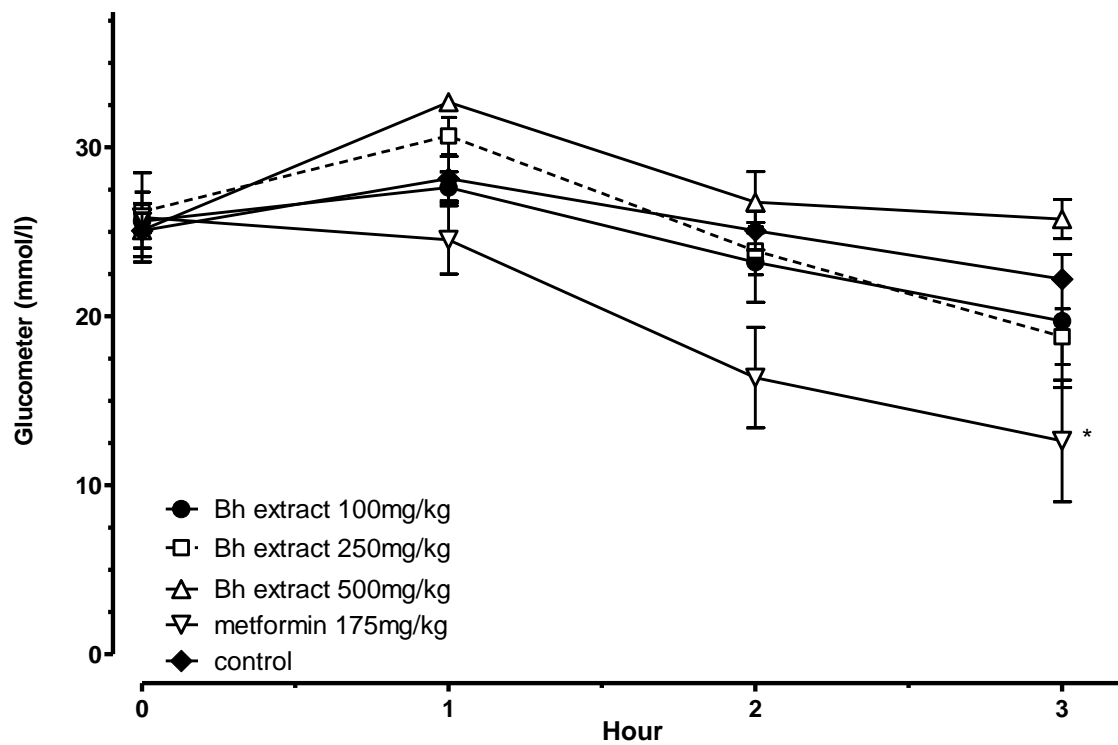


Figure 2: Effect of variable doses of *Benincasa hispida* aqueous extract on OGTT of severe diabetic rats

Effect of effective dose on blood glucose level of mild diabetic rats

From the OGTT analyses on severe diabetic rats, the nominated effective dose was 250 mg/kg (Table 2). The study was extended to observe the effect on the mild diabetic group. After 3H of glucose administration, the rats treated with 250 mg/kg of Bh aqueous extract showed a higher reduction in BGL from 19.0 (1H) to 6.7 mmol/L (3H). This reduction was equivalent to 64.7% of decrement. In contrast, the reference drug, Metformin produced a fall of 42.0% which was lesser than the fall produced by the most effective dose of the extract.

Table 2: Effect of the effective dose of *Benincasa hispida* aqueous extract on OGTT of mild diabetic rats

Group	Doses	Blood glucose levels (mmol/l)			
		FBG	1H	2H	3H
Control	DW	9.9 ± 2.7	21.5 ± 5.4	17.3 ± 5.6	14.3 ± 4.6
Extract	250	13.8 ± 2.5	19.0 ± 6.3	11.6 ± 4.9	6.7 ± 2.1
Metformin	175	12.5 ± 3.8	16.3 ± 4.2	8.8 ± 4.5	9.5 ± 3.1

Lethal Dosage (LD₅₀)

No toxic effect was observed on treatment with Bh aqueous extract up to 10 and 15 times of the effective dose. The behaviours of the treated animals were observed as normal and no death occurred in any of the experimental groups.

Discussion

The purpose of this study was to evaluate the effect of Bh aqueous extract on glycaemic control of diabetic rats. The rats were initially induced to be hyperglycaemic using STZ which has been described as a suitable experimental model to analyze the activity of hypoglycaemic agents (Junod *et al.*, 1969).

Metformin is often used as a treatment for non-insulin-dependent diabetes mellitus (NIDDM) because it is an anti-hyperglycaemic agent which may reduce blood glucose level. Metformin helps in increasing glucose uptake in skeletal muscle and reducing hepatic gluconeogenesis resulting in low blood glucose level (Stumvoll *et al.*, 1995). The treatment of Bh extract on normal rats did not significantly reduce or increase the glucose level observed in the present study. This phenomenon showed that the Bh fruit, even when in high concentration will not lead to hyperglycaemia in normal group even though the fruit contains high sugar levels as there is a total of 4.0 g carbohydrate in 100 g edible portion of Bh (Zaini *et al.*, 2010). After 6H, the FBGs were shown to decrease by 38.7 and 21.2% in severely diabetic rats treated with 250 and 500 mg/kg of aqueous Bh extract, respectively. The condition of less hypoglycaemic response at higher concentration is normal in natural products and has already

been observed in *Moringa oleifera* (Jaiswal *et al.*, 2009) and *Psidium guajava* (Rai *et al.*, 2007).

The stabilisation of blood glucose level in diabetic rats in every hour may be due to the natural bioactive compound in Bh extract. Our previous study verified that the aqueous extract of Bh contains gallic acid, a typical phenolic acid found in vegetables and fruits (Fatariah *et al.*, 2014). Gallic acid is able to scavenge free radicals and inhibit lipid peroxidation resulting in decreased BGL and increased plasma insulin levels by decreasing STZ-induced oxidative stress and thus protecting the β -cells.

Considering the medicinal effect of gallic acid, more research has been focused on the compound and some properties were found such as anti-hyperglycaemic, anti-lipid peroxidation, antioxidant, insulin-secretagogue, anti-hyperlipidemic and other protective effects of gallic acid that provide a huge benefit to diabetes mellitus (Latha and Daisy, 2011; Punithavathi *et al.*, 2011). Further study on phenolic compounds in Bh extract would be beneficial in improving the health status of diabetes mellitus patients.

A high LD₅₀ of the extract observed in this study shows that Bh extract is safe and the treated rats did not show toxicity signs as the rats acted normal throughout the study period.

Conclusion

In conclusion, this study reveals that *Benincasa hispida* extract maintains blood glucose levels in normal and diabetes groups. The effective dose slightly reduces the BGL in mild and severe diabetic animals. The fruit will not cause BGL to hypoglycaemic level. Thus, the fruit can be considered as diabetes friendly and can be used in clinical study to confirm the glycaemic profile of Bh aqueous extract in diabetic patients.

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