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Hypoglycemic Effects of Tea Extracts and Sterols from *Momordica charantia*

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Abstract

Mice administered with different doses of infusion obtained from commercial tea preparations of *Momordica charantia* fruit and leaf displayed variable hypoglycemic activity. *M. charantia* fruit tea extract at 100 mg/kg BW had demonstrated significant (*P=0.001*) blood glucose lowering during 0.5h to 1.0h observation period, while *M. charantia* leaf tea extract was most effective at 100 mg/kg BW with sustained blood glucose reduction pattern starting at 1.5h to 2.5h. The dichloromethane extract of the freeze-dried fruit of *M. charantia* afforded a mixture of clerosterol (1a) and 5α-stigmasta-7-en-3β-ol (1b) in a 2:1 ratio by silica gel chromatography. A single intraperitonial injection of 10 mg/kg BW of a mixture of 1a and 1b in normoglycemic mice significantly ($P<0.05$) reduced the blood glucose level with distinct percent blood glucose reduction from 1.5h to 2.0h compared with the negative control.

Keywords: *Momordica charantia*, Cucurbitaceae, hypoglycemic, clerosterol, 5α-stigmasta-7-en-3β-ol,

1. Introduction

Momordica charantia, commonly known as bitter melon is used by diabetics to lower their blood glucose levels. In the Philippines, commercial tea preparations of *M. charantia* fruit and leaf are marketed as antidiabetes. A number of studies on the hypoglycemic properties of *M. charantia* fruit have been conducted. Alcoholic extracts of the whole fruit of *M. charantia* administered to albino rats at doses of 25 mg, 50 mg and 75 mg in different durations decreased the blood sugar level of alloxan-induced diabetic mice [1].

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The mechanism of action of *M. charantia* fruit extract in alloxan-diabetic rats suggests that it enhances insulin secretion by the islets of Langerhans, induces glycogenesis in liver tissue, enhances peripheral glucose utilization and increases serum protein levels [2].

The aqueous extract from *M. charantia* fruit lowered the blood glucose of KK-Ay mice with type 2 diabetes five weeks after oral administration [3].

The aqueous extract powder of fresh unripe fruits of *M. charantia* at 20 mg/kg body weight reduced fasting blood glucose of diabetic rats [4].

M. charantia fruit juice reduced the STZ-induced hyperglycemia in mice, the STZ-induced apoptosis in RIN cells, and the STZ-induced lipid peroxidation in pancreas of mice, RIN cells and islets [5].

The alcohol extract of *M. charantia* fruit lowered the blood glucose level in the normoglycemic and streptozotocin-induced diabetic rats which was attributed to increased glucose utilization in the liver rather than an insulin secretion effect [6].

The aqueous extract of *M. charantia* leaves significantly reduced blood glucose in diabetic rats[7].

A previous study reported that the hypoglycemic compounds of *M. charantia* are a mixture of charantin, insulin-like peptides and alkaloids which are concentrated in fruits [8].

The fruit showed higher hypoglycemic activity[9].

A semipurified peptide from *M. charantia* produced a significant hypoglycemic effect in diabetic mice [10].

A study reported two types of hypoglycemic substances in *M. charantia* with time dependent effects, one with fast hypoglycemic activity of around 1h found in the aqueous extract and another with a slow hypoglycemic activity [11].

Another study reported the isolation of clerosterol from *M. charantia* which is of relevance to our present report [12].

A recent study reported that charantin, which is a mixture of clerosteryl glucoside and sitosteryl glucoside is responsible for the blood sugar lowering capacity of the fruit and seeds of bitter melon [13].

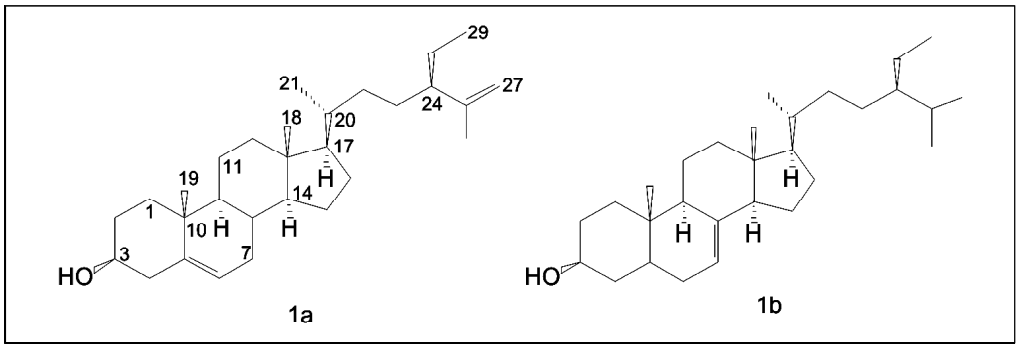
Bioassay guided fractionation of the methanol extract of *M.charantia* led to the isolation of cucurbitane triterpenoids which showed hypoglycaemic effects in the diabetes-induced male ddY mice strain at 400 mg/kg [14].

A review on the anti-diabetic and hypoglycaemic effects of *Momordica charantia* has been provided [15].

We report here the hypoglycemic effects of fruit and leaf tea extracts of *M. charantia*. Many polar substances have already been reported from *M. charantia.* This study was conducted to isolate relatively non-polar compounds which may have hypoglycemic potential. The dichloromethane extract of the freeze-dried fruits of *M. charantia* afforded a mixture of clerosterol (1a) and 5α-stigmasta-7-en-3β-ol (1b) whose structures were elucidated by extensive 1D and 2D NMR spectroscopy. The hypoglycemic potential of a mixture of 1a and 1b was evaluated by a intraperitonial injection of 10 mg/kg BW in normoglycemic mice. To the best of our knowledge this is the first report on the isolation of 5α-stigmasta-7-en-3β-ol from *M. charantia* and the hypoglycemic potential of clerosterol and 5α-stigmasta-7-en-3β-ol.

2. Materials and Methods

2.1 General Experimental Procedures



NMR spectra were recorded on a VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^{1}H-NMR and 150 MHz for ^{13}C-NMR spectra. Column chromatography was performed with silica gel 60 (70-230 mesh); TLC was performed with plastic backed plates coated with silica gel F_{254}; plates were visualized by spraying with vanillin sulfuric acid and warming.

2.2 Sample collection

The fruit was collected from Tanauan, Batangas, Philippines in February 2009. It was authenticated at the Institute of Biology, University of the Philippines, Diliman, Quezon City, Philippines and a voucher specimen (#14603) is deposited at the Institute of Biology, University of the Philippines - Diliman. The commercial tea preparations of *M. charantia* fruit and leaf were bought from local supermarkets in Metro Manila.

2.3. Isolation

The freeze-dried fruits of *M. charantia* (1 kg) were soaked in CH_2Cl_2 for three days, then filtered. The filtrate was allowed to concentrate under vacuum to afford a crude extract (17.5 g), which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment as eluents. The 10% to 40% acetone in CH_2Cl_2 fractions were combined and rechromatographed (5x) eluting with increasing proportions of EtOAc (5% to 10%) in light petroleum, to afford a mixture of 1a and 1b (12 mg).

Clerosterol (1a): ^{1}H-NMR ($CDCl_3$) δ: 1.05, 1.80 (H_2-1), 1.50, 1.80 (H_2-2), 3.50 (H-3), 2.20, 2.30 (H_2-4), 5.35 (dd, 2.0, 4.5) (H-6), 0.94, 1.25 (H_2-7), 1.98 (H-8), 1.80 (H-9), 1.00, 1.47 (H_2-11), 1.14, 1.90 (H_2-12), 0.90 (H-14), 1.00, 1.54 (H_2-15), 1.25, 1.80 (H_2-16), 1.30 (H-17), 0.65 (s, H_3-18), 0.98 (s, H_3-19), 1.35 (H-20), 0.88 (d, 6.0, H_3-21), 0.94, 1.25 (H_2-22), 1.20, 1.30 (H_2-23), 1.82 (H-24), 1.55 (br s, H_3-26), 4.62 (d,1.5), 4.70 (d, 1.5, H_2-27), 1.35 (H_2-28), 0.78 (t, 6.0, H_3-29); ^{13}C-NMR ($CDCl_3$) δ: 37.1 (C-1), 31.6 (C-2), 71.8 (C-3), 42.5 (C-4), 140.7 (C-5), 121.7 (C-6), 33.7 (C-7), 31.9 (C-8), 50.1 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (C-12), 42.3 (C-13), 56.7 (C-14), 24.3 (C-15), 28.2 (C-16), 56.0 (C-17), 11.8 (C-18), 19.8 (C-19), 35.6 (C-20), 18.6 (C-21), 33.7 (C-22), 29.4 (C-23), 49.6 (C-24), 147.6 (C-25), 17.8 (C-26), 111.4 (C-27), 26.5 (C-28), 12.1 (C-29).

5α-stigmasta-7-en-3β-ol (1b): ^{1}H-NMR ($CDCl_3$) δ: 1.05, 1.80 (H_2-1), 1.45, 1.80 (H_2-2), 3.59 (H-3), 1.25, 1.70 (H_2-4), 1.38 (H-5), 1.76 (H-6), 5.16 (br s, H-7), 1.62 (H-9), 1.40, 1.50 (H_2-11), 1.18, 2.00 (H_2-12), 1.78 (H-14), 1.48 (H-15), 1.22, 1.80 (H_2-16), 1.25 (H-17), 0.78 (s, H_3-18), 0.51 (s, H_3-19), 1.35 (H-20), 0.90 (d, 6.0, H_3-21), 0.95, 1.26 (H_2-22), 1.30 (H-23),

0.92 (H-24), 1.75 (H-25), 0.82 (d, 6.0, H_3-26), 0.83 (d, 6.0, H_3-27), 1.48 (H-28), 0.80 (t, 6.0, H_3-29); ^{13}C-NMR ($CDCl_3$) δ: 37.1 (C-1), 31.5 (C-2), 71.1 (C-3), 38.0 (C-4), 40.2 (C-5), 29.7 (C-6), 117.4 (C-7), 139.6(C-8), 49.5 (C-9), 34.2 (C-10), 21.5 (C-11), 39.5 (C-12), 43.4 (C-13), 55.0 (C-14), 23.0 (C-15), 27.9 (C-16), 56.1 (C-17), 13.0 (C-18), 12.0 (C-19), 36.5 (C-20), 19.1 (C-21), 33.9 (C-22), 26.5 (C-23), 45.8 (C-24), 29.1 (C-25), 19.0 (C-26), 19.4 (C-27), 23.0 (C-28), 12.1 (C-29).

2.4 Experimental animals

A total of 126 male albino mice (*Mus musculus* L.) of an inbred ICR strain (8 weeks old) weighing 23.0 ± 2.0 g were acclimatized for 7 days prior to conducting the bioassay. The animals were procured from the Bureau of Food and Drugs, Muntinlupa City, Philippines, and housed at the animal containment unit of DLSU-Manila with 12h daylight and 12h darkness with free access to food pellets and water. A 16h fasting period was conducted prior to each treatment procedure. Cervical dislocation was performed at the end of the animal treatment procedure. All procedures involving animal handling were in accordance with the Philippine Association of Laboratory Animal Science (PALAS) code of practice for care and use of laboratory animals and with administrative order 40 of the Bureau of Animal Industry relative to Republic Act No. 8485.

2.5 M. charantia *Tea Aqueous Extract Preparations*

M. charantia fruit tea weighing 1.75 g was steeped in 175 mL hot water for 10 minutes and vigorously stirred. The doses administered to mice were based on the weight of tea/kg BW of mice as follows: 200 mg/kg BW (F200H), 100 mg/kg BW (F100H) and 50 mg/kg BW (F50H). The 100 mg/kg BW (F100L) dose obtained from steeping was prepared by soaking the tea bag in hot water for 10 minutes with occasional swirling.

M. charantia leaf tea weighing 2.00 g was steeped in 200 mL hot water for 10 minutes and vigorously stirred. The doses administered to mice were based on the weight of tea/kg BW of mice as follows: 200 mg/kg BW (L200H), 100 mg/kg BW (L100H) and 50 mg/kg BW (L50H). The 100 mg/kg BW (L100L) dose obtained from steeping was prepared by soaking the tea bag in hot water for 10 minutes with occasional swirling.

2.6 Anti-diabetes assay

The OGTT (5 g/kg BW) was performed on normoglycemic mice, followed by measurement of blood glucose level (mg/dL) using OneTouch Horizon Glucometer (Lifescan, Johnson & Johnson, USA). The blood sample was obtained by clipping and bleeding the tail vein of the mice. Distilled water as the negative control and Glimepiride Solosa (16.7 μg/kg BW, Aventis, Italy) dissolved in distilled water as the positive control. An increasing dose of 50, 100, and 200 mg/kg BW extracts obtained from vigorous stirring were administered to the test animals 30 minutes after administration of 5 g/kg BW glucose. The median dose obtained from light stirring was also administered to glucose fed mice.

A single intraperitonial injection of a mixture of 1a and 1b was performed on normoglycemic mice, followed by measurement of blood glucose level (mg/dL) using OneTouch Horizon. 10% DMSO in 2% Polysorbate 80 (Tween-80, AJAX, Finechem Pty. Ltd., Australia) in 88% distilled H_2O (25 mL/kg BW) was administered as the negative control and vehicle. The mixture of 1a and 1b (10 mg/kg BW) dissolved in the vehicle (25 mL/kg BW) was administered intraperitonially (IP) as the test drug.

Blood glucose was measured within a 3h period at 30 minutes intervals. Blood glucose reduction was computed and was used in the statistical analysis.

2.7 Statistical analysis

The results were analyzed using SPSS ver. 10.5 for Windows. One way analysis of variance was performed to determine significant effects of the tea extracts on blood glucose reduction. Post hoc analysis was also performed at 0.05 Tukey's test to determine differences between group variables. One sample T test was performed to compare the means of the test compound and control while paired sample t test was used to determine that the significant reduction in each blood glucose measurement period was not due to chance and that the significant changes were due to the effects of the test substances (mixture of 1a and 1b) at 95% confidence interval. The data were presented as Mean ± SD.

3.Results and Discussion

3.1 Anti-diabetes assay of tea extracts.

Mice administered with different doses of infusion obtained from commercial tea preparations from fruit and leaves displayed variable hypoglycemic activity. *M. charantia* fruit tea had demonstrated significant (P=0.001) blood glucose lowering during 0.5h to 1.0h observation period. Blood glucose lowering in OGTT challenged mice was highest in those mice administered with the 100 mg/kg BW of fruit tea extract produced under light stirring (F100L) during 0.5h observation period (Table 1). The observed reduction persisted until 1.0h and started to diminish in the succeeding observation periods. The results obtained for F100L was quite comparable with the percent blood glucose reduction obtained in the positive control (Glimepiride Solosa) during 0.5-1.0h observation period.

Linear regression analysis of the percent blood glucose reduction in F100H (R^2=0.0117) treated mice however, indicates that blood glucose lowering was sustained until 2.5h observation period (Figure 1). *M. charantia* F100H had minimal but constant blood glucose reduction, though such was considered insignificant in the statistical analysis. On the other hand, F100L had immediate potency, but relatively short duration of hypoglycemic activity in the animals tested.

Mice orally administered with *M. charantia* leaf tea extracts had significantly reduced the blood glucose level of OGTT challenged mice across a 3h observation period. Blood glucose reduction patterns in the experimental groups demonstrated irregular signs of reduction which is indicative of poor hypoglycemic activity. *M. charantia* leaf tea extract produced under light stirring (L100L) on the other hand was the most effective and had sustained blood glucose reduction pattern starting at 1.5h to 2.5h (Table 2). Concomitant observation is evident in the linear regression analysis of the reduction pattern of L100L (R2=0.00003) (Figure 2).

*3.2 Identification of **1a** and **1b** from M. charantia fruit*

The dichloromethane extract of the freeze-dried fruit of *M. charantia* afforded a mixture of clerosterol (1a) and 5α-stigmasta-7-en-3β-ol (1b) in a 2:1 ratio by silica gel chromatography. The structures of these compounds were elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by comparison of the ^{13}C NMR data of 1a and 1b with clerosterol [16] and 5α-stigmasta-7-en-3β-ol, respectively [17]. The spectra matched in all essential respects.

*3.3 Anti-diabetes assay of **1a** and **1b***

Intraperitonial injection ensures faster absorption of the compounds as compared to oral

administration. The obtained quantity of the mixture of 1a and 1b was only 12 mg, thus the intraperitonial route was chosen. Mice administered with the 1a and 1b did not exhibit behavioral signs of toxicity within a 3.5h observation period. There was a slight increment in fasted blood glucose levels observed during 1.0h measurement period. Such can be accounted to the establishment of homeostatic condition in mice related to the

Table 1: Percent glucose reduction in mice orally administered with *M. charantia* fruit tea.

Group	0.5h	1.0h	1.5h	2.0h	2.5h
Control (water)	-0.17±23.66^{c}	36.70±17.35^{a}	26.20±18.71	18.16±15.64	-0.26±12.64
Glimepiride Solosa	19.94±16.59^{ab}	41.68±14.80^{a}	28.13±9.77	6.71±21.97	0.21±12.91
50 mg/kg BW (F50H)	9.47±20.17^{bc}	41.96±15.32^{a}	20.67±46.64	4.88±12.34	-0.14±13.83
100 Light (F100L)	32.29±12.11^{a}	44.89±11.58^{a}	12.20±11.55	9.49±15.31	-3.52±10.97
100 Heavy (F100H)	0.65±15.22^{c}	27.73±16.64^{ab}	30.18±24.34	10.42±9.85	13.53±19.12
200 mg/kg BW (F200H)	13.57±7.71^{bc}	17.19±26.66^{b}	37.68±17.32	12.77±18.33	-1.10±12.73

Note: *Means followed by the same letter superscript are not significantly different at 95% DMRT (α = 0.05).

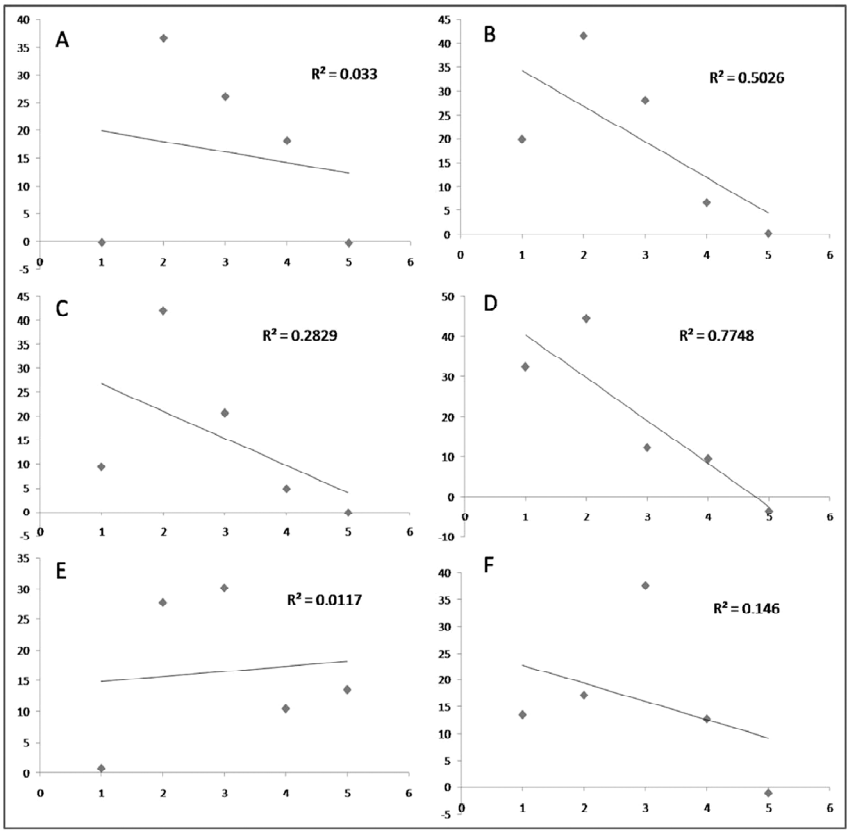


Figure 1. Percent blood glucose reduction in mice administered with control (A), Glimepiride Solosa (B) and an infusion of *M. charantia* fruit tea at 50 mg/kg BW (C), steeped 100 mg/kg BW (D), heavily stirred 100 mg/kg BW (E) and 200 mg/kg BW (F).

Table 2: Percent blood glucose reduction in mice orally administered with *M. charantia* leaf tea.

Group	0.5h	1.0h	1.5h	2.0h	2.5h
Control (water)	-0.17±23.66^{b}	36.70±17.35^{ab}	26.20±18.71^{ab}	18.16±15.64^{a}	-0.26±12.64^{b}
Glimepiride Solosa	19.94±16.59^{a}	41.68±14.80^{a}	28.13±9.77^{ab}	6.71±21.97^{ab}	0.21±12.91^{b}
50 mg/kg BW (L50H)	20.88±19.17^{a}	23.12±15.74^{bc}	17.43±12.77^{bc}	-2.45±10.17^{b}	20.74±10.16^{a}
100 Light (L100L)	8.75±13.09^{ab}	17.31±15.34^{c}	39.12±21.07^{a}	16.86±27.85^{a}	8.75±28.32^{a}
100 Heavy (L100H)	14.43±14.51^{ab}	37.75±16.72^{ab}	2.73±13.73^{c}	8.22±19.95^{ab}	15.57±14.18^{a}
200 mg/kg BW (L200H)	9.75±18.31^{ab}	39.44±10.89^{a}	8.54±9.32^{c}	11.20±6.96^{ab}	7.19±11.49^{ab}

Note: *Means followed by the same letter superscript are not significantly different at 95% DMRT (α = 0.05).

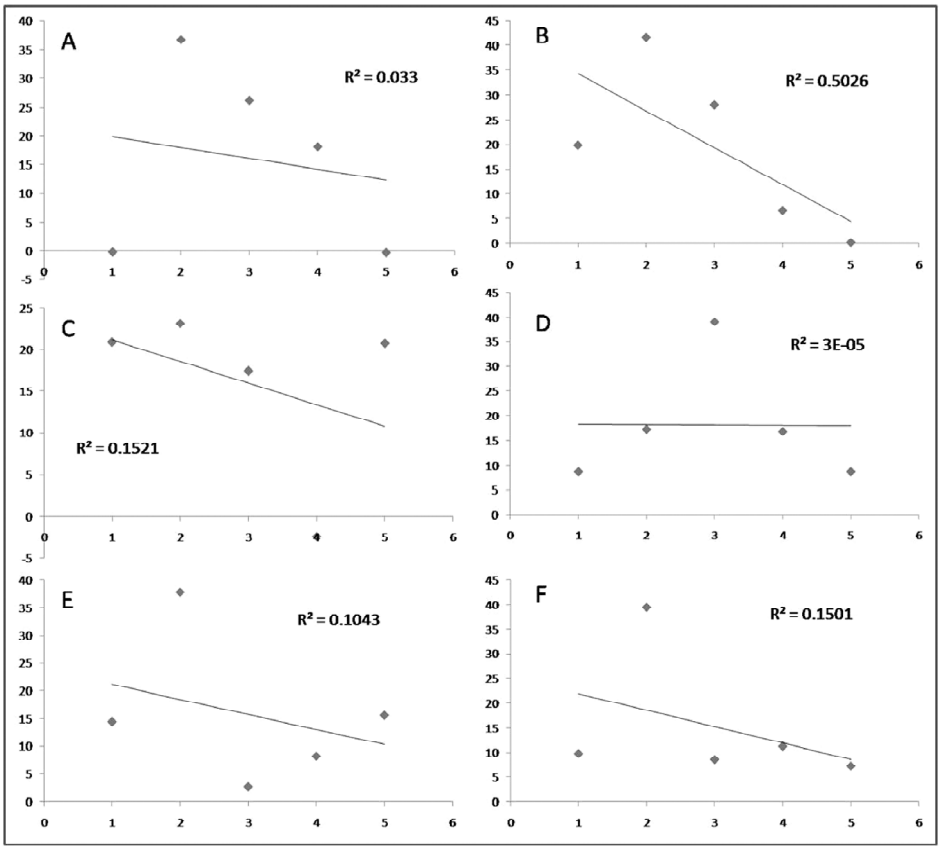


Figure 2. Percent blood glucose reduction in mice administered with control (A), Glimepiride Solosa (B) and an infusion of *M. charantia* leaf tea at 50 mg/kg BW (C), steeped 100 mg/kg BW (D), heavily stirred 100 mg/kg BW (E) and 200 mg/kg BW (F).

Table 3. Blood glucose of mice administered with a mixture of **1a** and **1b**.

	Time of blood glucose measurement (mg/dL)						
	0.5h	1.0h	1.5h	2.0h	2.5h	3.0h	3.5h
Mixture of 1a and 1b	136.11±23.29	160.78±20.52	159.78±21.29	140.22±21.06	137.44±25.38	126.11±10.95	129.89±14.95
Negative Control	106.67±11.03	122.33±14.78	107.67±10.39	116.44±16.13	117.44±13.65	110.11±15.15	95.22±8.61

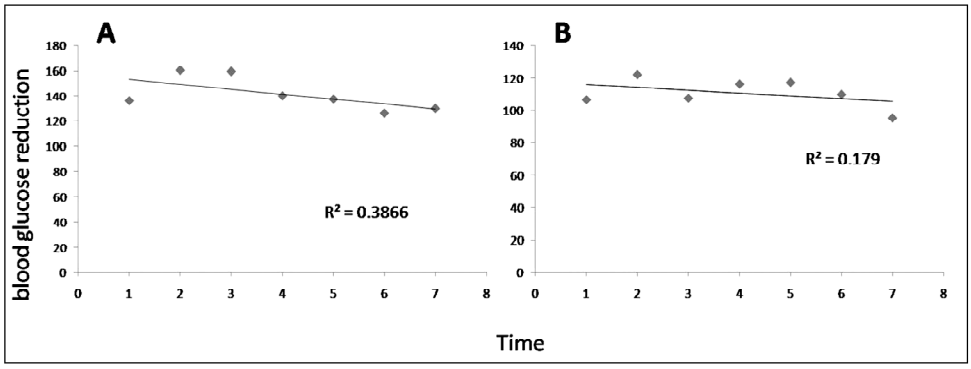


Figure 3. Blood glucose reduction of mice administered with a mixture of 1a and 1b (A) had pronounced reduction compared to control (B).

Table 4. Percent blood glucose reduction of mice administered with a mixture of **1a** and **1b**.

	Time of blood glucose measurement				
	0.5h-1.0h	1.0h-1.5h	1.5h-2.0h	2.0h-2.5h	2.5h-3.0h
Mixture of 1a and 1b	-19.72±15.62	0.51±6.21	12.13±7.00	1.80±11.50	6.13±15.35
Negative Control	-15.71±18.14	11.27±10.49	-8.09±9.52	-1.884±13.41	6.16±8.24

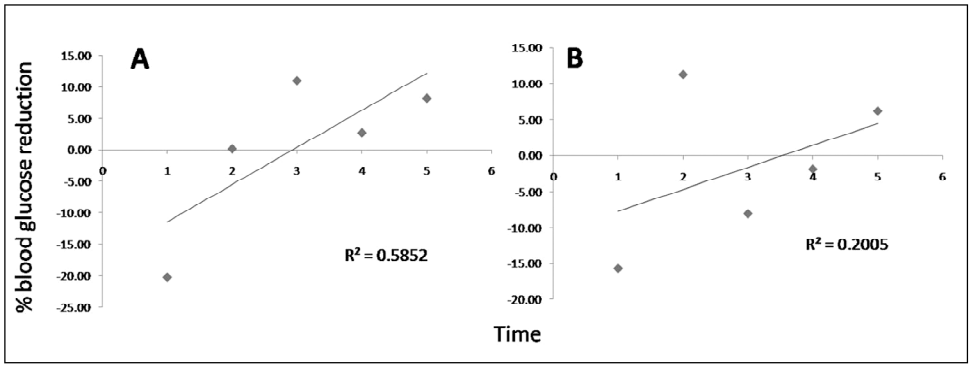


Figure 4. Percent blood glucose reduction of mice administered with a mixture of 1a and 1b (A) had increasing level of percent reduction starting from 1.0h up to 3.5h compared to control (B).

action of epinephrine released by the adrenal medulla to stimulate the conversion of glycogen in the muscle and liver into glucose needed by the body to perform its supposed activity. A slight blood glucose reduction in the overall trend using a time-bound hypoglycemic investigation was observed after intraperitonial injection of 1a and 1b (Figure 3). Pearson correlation between 1.0h to 1.5h (P=0.001) and 1.5 h to 2.0h indicates that there is almost perfect correlation for blood glucose reduction during such periods of blood glucose measurement (Table 3). The average loss of 1.0 ± 10.02 mg/dL during 1.0h to 2.0h was not significant (P>0.05) and cannot be attributed to the main effect of 1a and 1b. Correlation for the period 1.5h to 2.0h however with an average reduction of 19.56 ± 11.40 mg/dL is not due to chance variation and can be directly attributed to the possible hypoglycemic effect of 1a and 1b.

Determining the percent blood glucose reduction eliminates the possibility that the difference in mean was just due to the fact that some mice innately had higher blood glucose levels. Standardizing the scores revealed that mice administered with 1a and 1b was constantly increasing overtime (Figure 4). Although linear regression also shows that the % reduction observed in the negative control also had an increasing trend, the values however are very low and inconsistent which means that the normal hepato-pancreatic physiology is evident. In 1a and 1b however, consistent increase in % reduction was observed indicating an indirect but possible insulin-like activity. The percent blood glucose reduction observed from 0.5h to 1.0h and 1.0h to 1.5h indicates that mice administered with 1a and 1b had lower (P=0.463) blood glucose reduction compared to the control (Table 4). However, results obtained in 1.5h to 2.0h indicate that those mice administered with 1a and 1b had significantly (P=0.00) higher percent blood glucose reduction compared to the control, but the change obtained during 2.0h to 2.5h and 2.5h to 3.0h was not significantly different (P=0.366) with that of the percent reduction obtained in the control.

The consistent increase (R=0.59) in blood glucose reduction (Figure 4) may be a possible indication of the minimal but potential hypoglycemic activity of 1a and 1b. Positive Pearson correlation for percent blood glucose reduction at 1.0h to 1.5h measurement indicates that the average (11.61 ± 5.89%) increase in percent blood glucose was obtained and may be directly attributed to the possible hypoglycemic activity of the 1a and 1b.

4. Conclusions

This study demonstrated the hypoglycemic activity of the two types of commercial *M. charantia* tea in the Philippines. *M. charantia* fruit tea at 100 mg/kg BW had demonstrated significant (P=0.001) blood glucose lowering during 0.5h to 1.0h observation period. Meanwhile, *M. charantia* leaf tea extract was most effective at 100 mg/kg BW with sustained blood glucose reduction pattern starting at 1.5h to 2.5h. This study differentiated between two types of hypoglycemic substances in *M. charantia* with time dependent effects, the *M. charantia* fruit tea with fast hypoglycemic activity of around 1h and the *M. charantia* leaf tea with a slow hypoglycemic activity.

The current study also demonstrated minimal hypoglycemic activity in mice observed during 1.5h to 2.0h after systemic administration of the mixture of 1a and 1b. A recent study reported that charantin, which is a mixture of clerosteryl glucoside and sitosteryl glucoside is responsible for the blood sugar-lowering capacity of the fruit and seeds of bitter melon [12]. The minimal hypoglycemic activity of a

mixture of clerosterol (1a) and 5α-stigmasta-7-en-3β-ol (1b) may be attributed to the loss of the glucosides attached to C-3 of the sterols in charantin. Another difference is in 5α-stigmasta-7-en-3β-ol the olefin is at C-7, while in sitosterol the olefin is at C-5.

5. Acknowledgment

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