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Evaluation of antidiabetic properties of *Momordica charantia* in streptozotocin induced diabetic rats using metabolomics approach

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Abstract

Momordica charantia, also known as bitter melon or 'peria katak' in Malaysia, is a member of the family Cucurbitaceae. Bitter melon is an excellent source of vitamins and minerals that made it extensively nutritious. Moreover, the seed, fruit and leave of the plant contain bioactive compounds with a wide range of biological activities that have been used in traditional medicines in the treatment of several diseases, including inflammation, infections, obesity and diabetes. The aim of this study was to evaluate changes in urinary metabolite profile of the normal, streptozotocin-induced type 1 diabetes and *M. charantia* treated diabetic rats using proton nuclear magnetic resonance (^{1}H-NMR) -based metabolomics profiling. Study had been carried out by inducing diabetes in the rats through injection of streptozotocin, which exhibited type 1 diabetes. *M. charantia* extract (100 and 200 mg/kg body weight) was administrated to the streptozotocin-induced diabetic rats for one week. Blood glucose level after administration was measured to examine hypoglycemic effect of the extract. The results obtained indicated that *M. charantia* was effective in lowering blood glucose level of the diabetic rats. The loading plot of Partial Least Square (PLS) component 1 showed that diabetic rats had increased levels of lactate and glucose in urine whereas normal and the extract treated diabetic rats had higher levels of succinate, creatine, creatinine, urea and phenylacetylglycine in urine. While the loading plot of PLS component 2 showed a higher levels of succinate, citrate, creatine, creatinine, sugars, and hippurate in urine of normal rat compared to the extract treated diabetic rat. Administration of M. charantia extract was found to be able to regulate the altered metabolic processes. Thus, it could be potentially used to treat the diabetic patients.

Introduction

Prevalence of diabetes mellitus is increasing at an alarming rate globally. Recent estimates indicates 171 million people in the world with diabetes in the year of 2000 and this is projected to increase to 366 million by the year of 2030 (Wild *et al*., 2004) The health consequences of diabetes become increasingly overwhelming the health care systems due to the severity of the long term complications of diabetes (Noor *et al*., 2008). Diabetes mellitus is a metabolic disorder that is characterized by hyperglycemia resulting from inadequate insulin secretion (type 1 diabetes mellitus) or insulin insensitivity (type 2 diabetes mellitus) (Bastaki *et al*., 2005).

Several synthetic drugs such as sulfonylureas and biguanides are available as an oral hypoglycemic agent to lower blood glucose level in diabetics. However, their administration might give side effect to the patients (Noor *et al*.,2008). Therefore, the finding of new prevention strategies and treatments for diabetes is urgent. Nowadays, many researches are carried out to explore plant natural products that contain certain phytochemicals with anti-diabetic potential as the alternative therapy (Noor *et al*., 2008). One of such herbal plants is Momordica charantia.

Momordica charantia, also known as bitter melon, is a member of the cucumber family, Cucurbitaceae. It is grown in the tropical and subtropical regions of the world. All parts of the plant, including the fruit, taste bitter. Fruits and seeds of bitter melon possess medicinal properties such as anti-HIV, anti-ulcer, anti-inflammatory, anti-leukemic, anti-microbial, anti-tumor and anti-diabetic activity. It can be used as alternative therapy for lowering blood glucose levels in diabetic patients (Taylor *et al*., 2002).

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This is attributed to the complex action of multiple compounds in bitter melon extract (Basch *et al*., 2003). The bioactive compounds responsible for its hypoglycemic effect are a mixture of steroidal saponins known as charantins, insulin-like peptides and alkaloids (Raman *et al.*, 1996).

Metabolomics is the comprehensive measurement of all endogenous metabolites in a biological fluid (Lindon *et al.*, 2007). Through this approach, changes in metabolites profile of the biofluids can be identified. Therefore, metabolomics have been applied to diagnose diabetic patients and to identify the potential biomarkers (Van Doorn *et al*., 2007). The same approach can be used to study the medicinal properties of herbs as well. Thus, the underlying mechanisms of alteration in metabolic pathway associated with mechanism of medicinal herbs to treat a disease can be identified (Zhang *et al*., 2012).

As diabetes has become a serious global health problem that getting more concern from nation worldwide, studies should be carried out to deliver the problem. Since there are lots of natural plant products that exhibit anti-diabetic potential, research should focus on herbal remedy as alternative therapy for diabetes treatment to reduce dependence on the synthetic drugs. One of such plants is *M. charantia.* However, the results of the previous research on *M. charantia* in the treatment of diabetes were still based on glucose and insulin analysis . More research needs to be done on a holistic analysis of metabolites in biofluids. The objective of this study was to evaluate changes in urinary metabolite profile of the normal, STZ-induced diabetes and *M. charantia* extract treated diabetic rats using 1H-NMR-based metabolomics profiling.

Materials and Method

Chemicals

Ethanol (Merck, Darmstadt, Germany), streptozotocin (Merck, Darmstadt, Germany), carboxymethylcellulose (Sigma-Aldrich, St. Louis, US), sodium azide (NaN_3) (Merck, Darmstadt, Germany), imidazole (Merck, Darmstadt, Germany), deuterated oxide (D_2O) (Sigma-Aldrich, St. Louis, US), Trimethylsilanepropionic acid sodium salt (TSP) (Merck, Darmstadt, Germany) and NaOD (Merck, Darmstadt, Germany) were used in this study.

Plant preparation

Fresh green unripe whole fruits of *M. charantia* harvested in May 2011 were collected from a farm at Teluk Intan, Malaysia. Identification of species was done by Herbarium Institut of Biosains and the voucher number is SK/ 1931/11.Cleaning of bitter melon with water was carried out before removal of seeds and cutting into small pieces. Then the sample was ground cryogenically in liquid nitrogen. The ground sample was lyophilized using a freeze dryer (Freezone 6, Labconco, USA) and stored at -80ºC until extraction.

Plant extraction

Extraction was carried out by referring to procedure of Virdi *et al.* (2003) with modification. The ground sample of *M. charantia* fruit was soaked in the ratio of 3:4 (w/v) in 80% ethanol (v/v) at room temperature for 24 h. The extract was then filtered and the residue re-extracted with 80% ethanol. The extraction process was repeated for three times. After filtration, the combined filtrates were evaporated to remove the solvent with a rotary evaporator (Buchi Rotavor R-200, Flawil, Switzerland) at 40ºC. The extract was freeze-dried and then preserved at -80ºC for later usage.

Preparation of animals

Animal preparation was carried out according to the method of Jalil *et al.* (2009). Twelve male Sprague-Dawley rats, each weighing 100-250 g, were used. A study approval was obtained from Animal Care and Use Committee (ACUC) of the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (ACUC No: UPM/FPSK/PADS/ BRUUH/00407). The rats were placed in plastic cages with stainless steel cover. They were put under acclimatization conditions for a week at room temperature (26-28 ºC) under a 12 h light/12 h dark cycle. During the acclimatization period, all the rats were subjected to normal rat chow (Gold Coin, Selangor, Malaysia) and distilled water ad libitum. Following acclimatization, body weight of the rats was measured and the rats were divided randomly into four (n=3): group1(normal diet without STZ injection), group 2 (diabetic control with STZ injection), group 3 (100 mg/kg BW treated with M. charantia with STZ injection), group 4 (200mg/kg BW treated with M.charantia with STZ injection).

Rat induction of diabetes

Following Amin *et al*. (2004), STZ was prepared in 0.05 M citrate buffer, pH 4.5. Rats were injected intravenously with STZ (25 mg/kg body weight) after acclimatization to induce diabetes. Blood was collected from the tail vein 3 days after STZ injection and its glucose level was determined with a glucometer (Roche, Mannheim, Germany) to ensure

the development of diabetes. The rats were considered diabetic if their blood glucose concentration was more than 13.9 mmol/L at that time (Thirumalai *et al*., 2011).

Administration of Momordica charantia *extract*

M.charantia extract was suspended in 0.03% (w/v) of carboxymethylcellulose (CMC) and administered daily by gastric intubation using a force-feeding needle for the duration of one week (Jalil *et al*., 2009). Rats in other groups received 0.03% (w/v) CMC throughout the experiment (Jalil *et al.*, 2009). After 1 week administration of *M. charantia* extract or CMC to the respective groups, blood glucose level was determined at 0, 2, 4 and 6 h (Yuan *et al*., 2008).

Collection of urine sample

Urine samples before and after treatment were collected in the tubes containing 0.1% sodium azide (as anti-microbial) by placing the rats in metabolic cages overnight. The collected urine samples were then stored frozen at -80°C until analysis (Zhoa *et al*., 2011).

Preparation of urine samples and acquisition of 1H-NMR spectra

Thawed urine sample was centrifuged at 5000 rpm for 10 minutes. 0.01 M phosphate buffer (KH_2PO_4) was made in D2O (pH 7.4) containing 0.1% TSP and 0.1% of imidazole. The pH of the D2O was adjusted to 7.4 using a 1 M NaOD solution. Then 400 µl of the centrifuged urine sample was mixed with 200 µl of 0.01 M phosphate buffer (pH 7.4) into 1.5 ml of eppendorf tube and sonicated for 5 minutes (room temperature) to remove air bubbles. The urine solution was transferred into standard 5 mm NMR tube (Beckonert *et al*., 2007). The NMR tube containing the urine sample was then inserted into a Varian INOVA 500 spectrometer (Illinois, USA). 1H-NMR spectra was recorded at 26°C and 500 MHz with the following parameters: pulse width (PW) 21.0 µs (90°) and relaxation delay (RD) 2.0 s. Deuterium oxide was used as the internal lock. Each 1H-NMR spectrum consisted of 64 scans requiring 3 min and 26 s acquisition time. A pre-saturation sequence was used to suppress the residual water signal with low power selective irradiation.

Data processing and statistical analysis

The resulting NMR spectra were manually phased and baseline-corrected and calibrated to TSP (internal standard) at 0.0 ppm. A spectral region from 0.50-10.00 ppm was bucketed into 0.04-ppm-wide using Chenomx NMR software (Chenomx NMR Suite 5.1 Professional, Edmonton, Canada). Water (4.68–5.00 ppm) and imidazole (7.23-7.40, 8.43-8.46 ppm) signals were excluded from the data. The spectra data were then converted to Microsoft Excel format and imported into SIMCA-P software (version 12.0, Umetrics, Umea, Sweden) for multivariate data analysis. The bucketed data were perpetrated using MSC Filter and Pareto scaled. The data were statistically analyzed using partial least squares discriminant analysis (PLS-DA). The PLS-DA model was validated by permutation testing with describing R2 and Q2 values. Samples located out of Hotelling's eclipses in the PLS-DA score plots were considered as outlier and excluded for further analysis.

Results of blood glucose level measured by glucometer were expressed as mean±standard deviation of 3 rats in each group. Statistical evaluation was done using one-way ANOVA, followed by Tukey's test using Minitab (Minitab 16). Differences of p-value less than 0.05 were considered statistically significant.

Table 1. Blood glucose level of rats at 3 days after STZ injection and one week after administration of *M. charantia* extract

Groups of rats	Blood glucose level (mmol/L)				
	3 days after STZ injection	* One week after administration			
		0 h	2 h	4 h	6 h
Normal control (0.03% w/v CMC) [x]	$4.13^{b}A \pm 0.12$	$3.73^{b}A \pm 0.38$	$4.03^{a}A \pm 0.61$	$3.53^{b}A \pm 0.40$	$3.37^{b}A \pm 0.31$
Diabetic control (0.03% w/v CMC)	$26.20^{a}A \pm 1.71$	$27.07^{a}A \pm 6.21$	$16.20^{a}A \pm 4.74$	$19.93^{a}A \pm 7.86$	$27.47^{a}A \pm 2.59$
Diabetic (MC 100mg/kg bw)	$29.27^{a}A \pm 0.38$	$19.30^{a}AB \pm 5.37$	$15.10^{a}AB \pm 11.65$	$11.10^{ab}AB \pm 9.21$	$7.93^{b}B \pm 6.50$
Diabetic (MC 200mg/kg bw)	$25.00^{a}A \pm 4.62$	$17.03^{a}B \pm 0.95$	$11.27^{a}BC \pm 1.00$	$8.13^{ab}CD \pm 1.86$	$4.40^{b}D \pm 0.76$

Values are expressed as mean±standard deviation for 3 rats in each group.

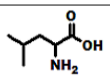
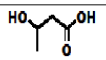
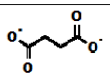
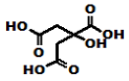
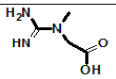
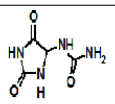
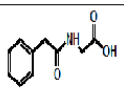
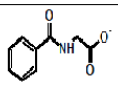
Means that do not share the same letter are significantly different ($p<0.05$).

Upper case after mean values represents a statistical comparison through rows while lower case after mean values in superscript represents a statistical comparison through columns.

Results

As shown in Table 1, there was a significant difference in the mean blood glucose level between STZ-induced diabetic groups and normal control group ($p < 0.05$). The normal control group showed normal blood glucose level at 4.13±0.12 mmol/L,

Table 2. 1H-NMR chemical shifts of metabolites in urine from normal, diabetic control and MC extract treated diabetic rats (in ascending order of the signals in spectra)

Compound	Structure	^{1}H-NMR chemical shift (ppm) and splitting pattern
1: 2-hydroxybutyrate		0.86(t)
2: leucine		0.93(d)
3: 3-hydroxybutyrate		1.19(d)
4: lactate		1.32(d)
5: alanine		1.47(d)
6: acetate		1.91(s)
7: succinate		2.40(s)
8: citrate		2.53(d), 2.66(d)
9: dimethylamine		2.71(s)
10: creatine		3.01(s), 3.92(s)
11: creatinine		3.02(s), 4.05(s)
12: glucose		3.20-3.90(m), 4.64(d), 5.24(d)
13: mixture of sugars		3.20-3.90(m)
14: galactose		4.64(d)
15: allantoin		5.38(s), 6.00(s)
16: urea		5.78(s)
17: phenylacetylglycine		7.38(m)
18: hippurate		7.54(t), 7.62(t). 7.82(d)

* s = singlet, d = doublet, t = triplet, m = multiplet

whereas the blood glucose level of the diabetic groups was ranged from 25.00±4.62 mmol/L to 29.27±0.38 mmol/L. Thus, it was evident that injection of STZ intravenously at a dosage of 25 mg/kg body weight caused a significant diabetogenic response in the

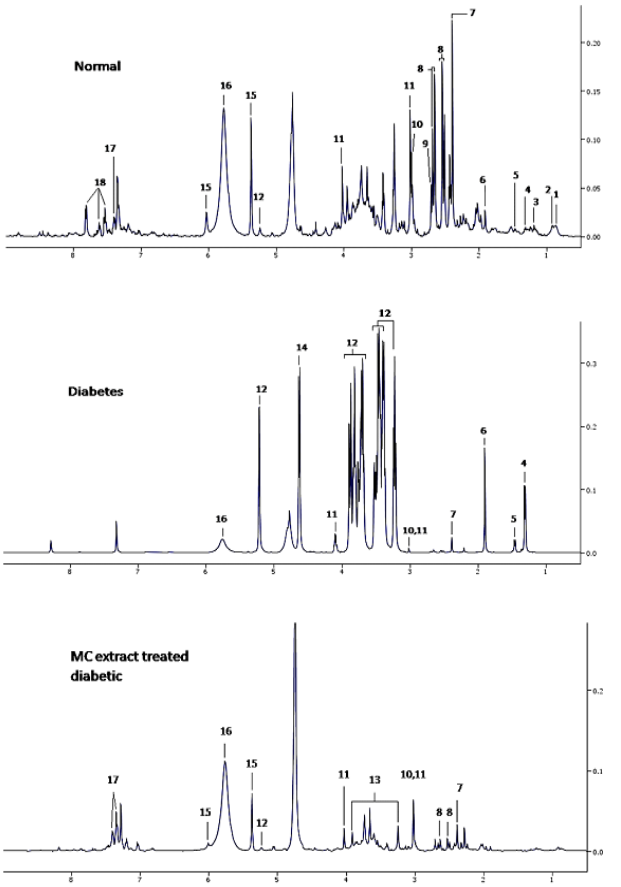


Figure 1. ^{1}H-NMR spectra of urine from a normal, diabetic control and MC extract treated diabetic rat. Identified metabolites: 1: 2-hydroxybutyrate; 2: leucine; 3: 3-hydroxybutyrate; 4: lactate; 5: alanine; 6: acetate; 7: succinate; 8: citrate; 9: dimethylamine; 10: creatine; 11: creatinine; 12: glucose; 13: mixture of sugars; 14: galactose+glucose; 15: allantoin; 16: urea; 17: phenylacetylglycine; 18: hippurate

rats. Administration of *M. charantia* extract resulted in a statistically significant reduction in mean blood glucose level in the diabetic rats ($p < 0.05$). The untreated diabetic rat had significantly higher blood glucose level than that of treated diabetic rats ($p<0.05$). Administration of *M. charantia* extract 100 and 200 mg/kg body weight lowered blood glucose level of the diabetics to 7.93±6.50 mmol/L and 4.40±0.76 mmol/L, respectively, with no significant difference between both dosage groups and normal control group at 6 h after second administration of *M. charantia*.

Urine samples collected from the normal, STZ-induced type 1 diabetic and *M. charantia* treated diabetic rats were subjected to 1H-NMR. The bucketed data of all 1H-NMR spectra were used in conjunction with multivariate data analysis to examine metabolic changes in rat's urines associated with diabetes and *M. charantia* treatment. The assignments of spectra

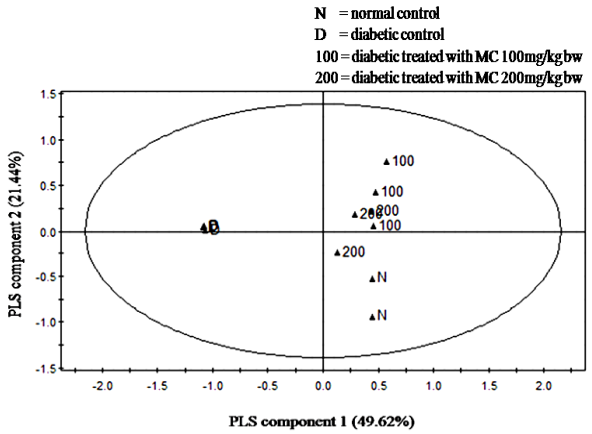


Figure 2. PLS-DA score plot obtained using 1H-NMR data for the normal, diabetic control and MC treated diabetic groups. Outliers located out of Hotelling's eclipse were excluded

were made based on the database of Chenomx NMR software (Chenomx NMR Suite 5.1 Professional, Edmonton, Canada) and comparison to previous reports on the NMR spectra of urine metabolites (Zhao *et al*., 2010, Zhao *et al*., 2011; Liu *et al*., 2012).

1H-NMR spectra of urine from a normal, diabetic control and *M. charantia* extract treated diabetic rat are shown in Figure 1along with the metabolites assignment. The spectra contained a number of signals such as 2-hydroxybutyrate, leucine, 3-hydroxybutyrate, lactate, alanine, acetate, succinate, citrate, dimethylamine, creatine, creatinine, glucose, sugars, galactose, allantoin, urea, phenylacetylglycine and hippurate. From the spectra, it is obvious that the urine of diabetic rat contained higher amount of glucose compared to those of normal and *M. charantia* treated diabetic rats. Higher levels of lactate and acetate were also observed in urine of diabetic rat. However, levels of citrate, succinate, urea and hippurate were lower in urine of diabetic rat compared to those of normal and *M. charantia* treated diabetic rats.

Partial least square discriminant analysis (PLS-DA) was selected among multivariate data analysis techniques in order to discriminate urine sample from different rat groups. The rat groups were treated as y variables in this regression technique. PLS-DA score plots (Figure 2) showed clustering of the rat groups in the first and second PLS components, with separation between the diabetic group of normal and *M. charantia* treated diabetic groups along the PLS component 1 that accounts for 49.62% of the variation. On the other hand, normal group separated from *M. charantia* treated diabetic groups along PLS component 2 that accounts for 21.44% of the variation. These two PLS component account for 71.06% of the total variation.

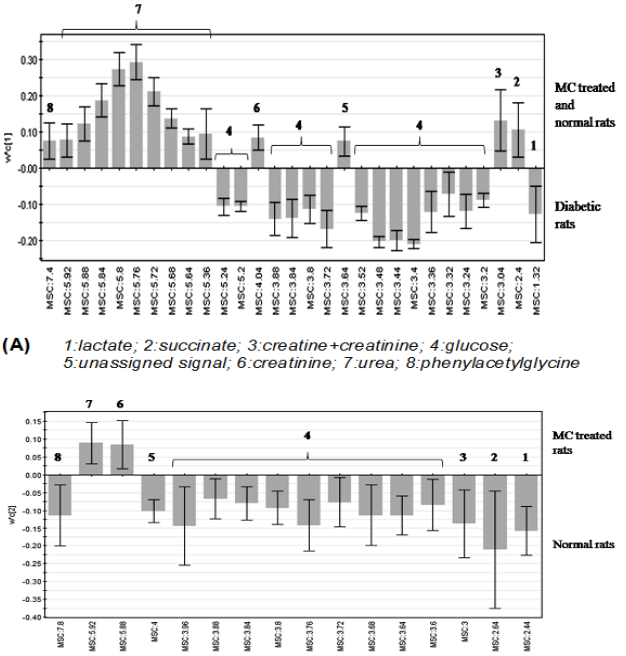


Figure 3. The loading column plot for (A) PLS component 1 and (B) PLS component 2 with jack-knifed confidence intervals. 'MSC' represents Multiplicative Signal Correction defined as normalization of each observation (spectra) by regressing it against the average spectrum and the value in x-axis represents the bucketed ^{1}H-NMR chemical shift (ppm).

A value of R^2Y shows the goodness of fit for the components, while Q^2 indicated the predictive ability (Barker *et al*., 2003). R^2Y is the percent of variation of the response explained by the model; Q^2 is the percent of the variation of the response predicted by the model according to cross validation. This PLS-DA model had R^2Y value of 0.58 and Q^2 of 0.41. The model was considered good since the difference between R^2Y and Q^2 was less than 0.3 (Eriksson *et al.*, 2006). Permutation test was performed to validate the PLS-DA model. R^2 intercept less than 0.3-0.4 and Q^2 intercept less than 0.05 indicated the models are valid (Mazzara *et al*., 2011). The PLS-DA model had R^2 intercept value of 0.161 and Q^2 intercept value of -0.396. Thus, this PLS-DA model was considered valid.

The loading column plot for PLS component 1 is shown in Figure 3(A). The buckets in Figure 3(A) correspond to the 1H-NMR chemical shift binned in every 0.04 ppm interval, which leads to separation of the diabetic group from normal and *M. charantia* treated diabetic groups along PLS component 1 of the model. These buckets are assigned with metabolites based on the chemical shifts in Table 2.

The loading column plot for PLS component 2 is shown in Figure 3(B). It shows the corresponding

variables that leads to separation of *M.charantia* treated diabetic groups from normal group along PLS component 2 of the model. As shown in Figure 3(B), there were higher levels of succinate, citrate, creatine, creatinine, sugar and hippurate in urine samples of normal rat compared to *M. charantia* treated diabetic rat. However, there were two unassigned metabolites with increased level in urine of *M.charantia* treated diabetic rats. The sugar signals were difficult to be assigned since it could be a mixture of different sugars

Discussion

The significant reduction of blood glucose level showed *M. charantia* extract had blood glucose lowering effect in the diabetics. *M. charantia* extract at 200 mg/kg body weight was more effective than 100 mg/kg body weight since the former dosage reduced blood glucose level to near normal value. Thus, the *M.charantia* extract at the dosage of 200 mg/kg body weight had a higher hypoglycaemic effect in this study.

Takasu *et al.* (1991) reported that streptozotocin induces diabetes in rat by β cell destruction, through generation of free radicals, which cause alkylation of DNA and subsequently increase blood glucose level. The rats were considered diabetic if their blood glucose levels were more than 13.9 mmol/L after 3 days due to β cell destruction, through generation of free radicals which cause alkylation of DNA and subsequently increase blood glucose level (Thirumalai *et al.*, 2011). In the present study, injection of STZ induced diabetic condition since the blood glucose levels of rats were more than 13.9 mmol/L.

Previous study by Yuan *et al*. (2008) showed that a water-soluble hypoglycemic peptide in *Momordica charantia* L. Var. *abbreviata* Ser. (MCV) aqueous extract at a dose of 2 mg/kg significantly lowered blood glucose level in alloxan-induced diabetic mice. Zhao *et al.* (2005) also reported crude saponins in MCV could significantly lower the blood glucose level in alloxan-induced diabetic mice.

As shown in Figure 3(A), there were higher levels of lactate and glucose in urine samples of diabetic rat. On the other hand, normal and *M. charantia* treated diabetic rats had higher levels of succinate, creatine, creatinine, urea and phenylacetylglycine. Diabetic rat exhibited higher levels of glucose in urine due to decreased glucose metabolism and increased glucose production resulting from STZ injection and subsequently hyperglycemia . Zhang *et al.* (2008) also reported increased levels of glucose in urine of STZ-induced diabetic rat due to gluconeogenesis and glycogenolysis. Besides that, Zhang *et al*. (2008) reported a significantly or moderately increased level of urea in diabetic rats and this relationship suggests enhanced amino acids-fueled gluconeogenesis in the diabetic that lead to increased nitrogen load to the liver where urea is formed. However, the present study demonstrated contrary result to that of Zhang *et al*. (2008) in which urea level reduced in diabetic rats but support the study by Guan *et al*.(2013) where the urea level was significantly decreased after the 15th week of STZ injection. Low concentration of urea suggested a reduced turnover of protein in diabetic treated rats (He *et al.*, 2012). Thus, it indicated a reflect in glomerular filtration rate and worsened the renal function (Guan *et al*., 2013).

Enhanced lactate level in urine of diabetic indicates reduced consumption of lactate compared to its production. It was consistent with previous work by Zhang *et al.* (2008) that demonstrated severely increased lactate level in the diabetic urine. Elevated level of lactate in diabetic urine indicates an increased glycolysis. This resulted from a deficiency of muscle glucose that lead to oxidation of all the carbon from glucose through oxidative phosphorylation, higher level of lactate instead came from the glycolic production of lactate in red blood cells, which express a variant of the enzyme lactate dehydrogenase (Wiback *et al.*, 2002).

Succinate, which is an intermediate of Krebs cycle, presented in decreased level in urine samples of diabetic rat. The present result supported by previous studies by Zhao *et al*. (2010) that also reported reduction in level of succinate in urine samples of diabetic rats. Since succinate that involved in energy metabolism was perturbed resulted from hyperglycemia and hypoinsulinemia after STZ injection, energy metabolism was also altered, as well as impairment in mitochondrial function or activity due to increased blood glucose (Waters *et al*., 2005).

Level of creatine was also reduced in the urine samples of diabetic. This result was consistent with previous work by Zhao *et al.* (2011) that explained the observation might be due to factors such as creatine reabsorption, altered renal function and morphology resulted from diabetic nephropathy, which is one of the manifestations of diabetic systemic microvascular disease. The clearance of creatine and creatinine measured as an indicator of glomerular filtration rate. Decreased levels of urinary creatine and creatinine are considered as markers of renal function. STZ was reported to have a direct damaging effect on some organs, such as kidney and liver (Elsner *et al*., 2000). The present study showed that the deleterious action

of STZ on renal function might lead to alteration in urinary excretion of these metabolites in STZ-induced diabetic rats. On the other hand, normal rats had higher levels of creatine and creatinine in the urine as these metabolites passed out efficiently due to normal function of their kidneys.

Higher level of phenylacetylglycine (PAG) in urine of normal and *M. charantia* treated diabetic groups was observed in this study. According to Wikoff *et al.* (2009) production of PAG could be attributed to gut microbial activity. Moreover, liver also plays an important role in the production of PAG. PAG was formed from conjugation of their precursor acids with glycine in hepatic mitochondria. The alteration of PAG production indicated the malfunction of liver (Kasuya *et al*., 2000).

Normal rats had higher levels of citrate in urine. Citrate is excreted in urine due to its important role as an inhibitor of calcium salt crystallization. Citrate forms complex with calcium ions in urine, reducing calcium ion activity and subsequently reduces urinary supersaturation of calcium phosphate and calcium oxalate (Rodger *et al*., 2006). Low amount of citrate in urine or hypocitraturia, is a risk factor for kidney stone formation. In addition, citrate is often the most abundant organic acid in urine. Higher levels of creatine and creatinine were found in urine of normal rats. Although less creatine and creatinine were found in *M. charantia* treated rats, the amount of both metabolites was higher in *M. charantia* treated rats compared to diabetic rats as explained earlier.

According to Deguchi *et al.* (2005) hippurate is a harmful uremic toxin that accumulates during chronic renal failure. It is a glycine conjugate of benzoate and formed mainly by gut flora from aromatic amino acids. The primary pathway for its elimination from the plasma via kidney is the active tubular secretion. Functional failure of this system might lead to accumulation of hippurate in blood (Tsutsumi *et al*., 2002). Therefore, normal level of hippurate in the urine indicated healthy renal function to eliminate this uremic toxin.

In the present study, administration of *M. charantia* fruit extract to diabetic rats was able to shift the metabolites profile of diabetic rat closer to normal level as shown were clustered in the PLS-DA score plot along PLS component 1 (Figure 2).This could be attributed to the ability of *M. charantia* fruits extract in regulating of some altered metabolic pathways involved in diabetes. Although the glucose level of *M. charantia* fruits treated rats were the same as normal rats, some metabolites were not shifted to normal level. It suggested that the use of *M. charantia* fruits could not lead the urine profile back totally to normal except glucose, succinate, lactate, creatine, creatinine, urea and phenylacetylglycine level.

Conclusion

1H-NMR-based metabolomics analysis of urine samples showed some metabolism alteration involved in diabetes. Such altered metabolic pathways were glycolysis, Krebs cycle, protein and creatine metabolism. The administration of *M. charantia* fruits extract was able to partially fix the altered metabolism of diabetic rats as indicated by the shifting of some metabolites profile (glucose, succinate, lactate, creatine, creatinine, urea and phenylacetylglycine) to normal level. Hence, this extract demonstrated to be potential anti-type 1 diabetic agent, as it able to alter the main pathway leading to diabetic condition in treated rats although not all metabolites were shifted to normal level. Further studies are required to normalized those remain metabolites such as the evaluation of synergism effect of this plant with other medicinal plants on anti-diabetic activity, and enhancement of anti-diabetic compounds in *M. charantia* fruits extract by optimization of extraction, harvesting and storage conditions.

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