Biochemical studies of *Piper betle* L leaf extract on obese treated animal using 1H-NMR-based metabolomic approach of blood serum samples

Zuleen Delina Fasya Abdul Ghani a,b,⁎, Juani Mazmin Husin b, Ahmad Hazri Ab Rashid b, Khozirah Shaari c, Zamri Chika a

a Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur
b Industrial Biotechnology Research Centre, Sirim Berhad, 40700 Shah Alam, Malaysia
c Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Malaysia

A R T I C L E   I N F O

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A B S T R A C T

*Piper betle* L. (PB) belongs to the Piperaceae family. The presence of a fairly large quantity of diastase in the betel leaf is deemed to play an important role in starch digestion and calls for the study of weight loss activities and metabolite profile from PB leaf extracts using metabolomics approach to be performed. PB dried leaves were extracted with 70% ethanol and the extracts were subjected to five groups of rats fed with high fat (HF) and standard diet (SD). They were then fed with the extracts in two doses and compared with a negative control group given water only according to the study protocol. The body weights and food intakes were monitored every week. At the end of the study, blood serum of the experimental animal was analysed to determine the biochemical and metabolite changes. PB treated group demonstrated inhibition of body weight gain without showing an effect on the food intake. In serum bioassay, the PB treated group (HF/PB (100 mg/kg and 500 mg/kg) showed an increase in glucose and cholesterol levels compared to the Standard Diet (SD/WTR) group, a decrease in LDL level and increase in HDL level when compared with High Fat Diet (HF/WTR) group. For metabolite analysis, two separation models were made to determine the metabolite changes via group activities. The best separation of PCA serum in Model 1 and 2 was achieved in principle component 1 and principle component 2. SUS-Plot model showed that HF group was characterized by high-level of glucose, glycine and alanine. Increase in the β-hydroxybutyrate level similar with SD group animals was evident in the HF/PB(500 mg/kg) group. This finding suggested that the administration of 500 mg/kg PB extracts leads to increase in oxidation process in the body thus maintaining the body weight and without giving an effect on the appetite even though HF was continuously consumed by the animals until the end of the studies and also a reduction in food intake, thus maintaining their body weight although they were continuously consumed HF.

1. Introduction

Obesity is a chronic, stigmatized and costly disease that is rarely curable and is increasing in prevalence throughout most of the world (Bray and Tartaglia, 2000). Obesity is a condition in which an abnormally large amount of fat is stored in the adipose tissue, resulting in an increase in body weight. It is one of the major public health problems in developed countries. It is the result of an energy imbalance caused by an increased ratio of caloric intake to energy expenditure. It gives an important impact on lifestyle-related diseases such as coronary heart disease, glucose intolerance, diabetes, and elevated blood pressure (Hu et al., 2008). It has been suggested that dietary fat promotes body fat storage more effectively than dietary carbohydrate. Consistent with these suggestions, high fat diets can increase body weight and adiposity in human and animals. This inhibition of digestion and absorption of dietary fat is a key to treating obesity (Han et al., 2002).

Metabolomics, which is the study of metabolite profiles in a biological system under a given set of conditions, has become an approach to understanding the basic principles of relating chemical patterns in biology as well as biological systems (Nicholson and Lindon, 2008). With the capability of simultaneous analysis of hundreds and thousands of variables, metabolomics meets the requirements for the evaluation of multi-component herbal medicines in vivo, and therefore, bridges the gap between herbal medicine and molecular pharmacology (Wang et al., 2005). Obesity is a disorder of the whole body and obviously involves metabolic changes, but the actual alterations in metabolism during obesity and any dysfunction associated with

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obesity at the level of individual organs or cellular organelles are not yet clearly understood (Kussmann et al., 2006). As metabolomics can readily detect subtle changes in the metabolic network, it is uniquely poised to increase our understanding of obesity and obesity-related disease.

Animal models have provided a fundamental contribution to the investigation of the onset and progression of complex multifunctional disease such as obesity (Speakman et al., 2008). The advantage of animal models is that they allow strict control of factors such as diet, environmental conditions or genetic background. Thus, animal models have been widely used in metabolomics for exploring the metabolic changes and potential biochemical mechanism of obesity development.

Piper betle L. the name of a species in the genus Piper (family Piperaceae). The betel plant is an evergreen and perennial creeper, with glossy heart-shaped leaves and white catkin. The betel plant originated from South East Asia. Betel leaf has a strong pungent aromatic flavor and is widely used as masticators (Dasgupta and De, 2004). The presence of a fairly large quantity of diastase in the betel leaf is likely to play an important part in starch digestion. P. betle leaf consists of juice which cures pharyngitis, abdominal pain and abdominal distension. It also possesses an anti-oxidant action (Saravanan et al., 2002) anti-hypercholesterol (Venkadeswaran et al., 2014), and give a reduction of body weight after 2–3 month of consumable (Chiang et al., 2004; Sengupta et al., 2012). The effect is due to the presence of phenols particularly hydroxylchavicol (4-allyl pyrocatechole). Hydroxichavicol also founds to inhibit activity against pancreatic lipase which is effective for a prevention of obesity ( Kato et al., 2013). The leaf produces an aromatic volatile oil containing a phenol called chavicol which has powerful antiseptic properties (Tewari SN, 1991). The essential oil present gives rise to a sensation of warmth and well-being in the mouth and stomach. It is also known to produce a primary stimulation of the central nervous system.

In this study, the significant metabolites in the blood serum were identified and metabolic changes were plotted in the animal groups fed with P. betle extracts and compared to obese and lean groups which can then be used to predict the usability of the extract for obesity treatment.

2. Material and methods

2.1. Chemicals

Ethanol was purchased from ChemPur (Karlsruhe, Germany). Normal rat chow was purchased from Gold Coin, Kuala Lumpur, Malaysia. The following chemicals were used for the NMR analysis: Imidazole (Merk, Darmstadt, Germany), Deuterated oxide (D2O) (Sigma Aldrich, St. Louis, US), Trimethylsilanepropionic acid sodium salt (TSP) (Merk, Darmstadt, Germany), KH2PO4 (Merk, Darmstadt, Germany) and NaOD (Merk, Darmstadt, Germany).

2.2. Preparations of Piper betle leaves extract

Leaves of Piper betle L. were collected from Rembau region, Negeri Sembilan, Malaysia. The samples were authenticated for their correct botanical identity by The Forest Research Institute of Malaysia as Sample No: PID 481113-25. About 5 kg of the dried leaves were ground to powder. Each of 500 g of the leaves powder were placed in a cellulose thimble and refluxed for 8 cycle in a soxhlet extractor with 500 mL of 70% (v/v) ethanol. The final extract was then evaporated using rotary evaporator and lyophilized to remove the remaining water (yield 14.3%, w/w, dry weight basis). The extraction was repeated three times and the extracts were stored at −80 °C prior to analysis.

2.3. Animal study

Twenty eight Sprague-Dawley rats (weight range of 100–150 g) were purchased from Sapphire Enterprise Sdn Bhd. All experimental procedures were approved by the Institutional Animal Care and Use Committee University of Malaya (UM-ACUC) with Ethic Reference No: FAR/31/01/2013/ZDFAG (R). During the acclimatization period of one week (room temperature, 12/12 h light/dark cycle), the rats were given normal rat chow (Gold Coin, Malaysia) (7% fat, 69% carbohydrate and 24% protein from total energy in kcal) and distilled water ad libitum. After acclimatization period, the rats were randomly divided into 4 groups of seven rats (n=7) per group labelled as control.
group (Standard Diet (SD/WTR), High Fat Diet (HF/WTR) and *P. betle* treated Group (Low Dose (HF/PB (100 mg/kg), High Dose (HF/PB (500 mg/kg). The dosage have been given below the toxicity level (< 2000 mg/kg) referring to in-vivo Up and Down Toxicity testing. The experimental design is described below (Fig. 1):

The obese animal model condition was developed according to Abbe Maleyki et al. (Jalil et al., 2008) with a slightly modification. The high-fat diet (HF) was formulated with 57% fat from ghee (milk fat and corn oil, 34% carbohydrates and 9% protein).

2.4. Administration of *P. betle* extracts to experimental animals

*P. betle* leaf extracts were suspended in 0.01% (w/v) and 0.05% (w/v) of distilled water and administered daily (100 mg/kg and 500 mg/kg) to the experimental animals by gastric intubation using a force-feeding needle. The treatment was continued over a period of 4 weeks. To monitor the development of obesity, basal food consumption together with body weight gain were collected weekly (0, 1st, 2nd, 3rd and 4th week). Food intake was measured and corrected for spillage by weighing the jars containing food (to the nearest 0.1 g).

2.5. Preparation of serum samples and acquisition of $^1$H NMR spectra

Blood withdrawal of the rat was performed by cardiac puncture under general anesthesia procedure. The blood sample was collected in a vacutainer tube for serum collection. After collection, the whole blood was allowed to clot by leaving it undisturbed at room temperature for 15–30 min. The clotted blood was then centrifuged at 1000 g for 10 min, after which the resulting supernatant (serum) was immediately transferred into a clean polypropylene tube using a sterile pipette. The serum samples were stored at −80 °C prior to analysis.

200 µl of thawed serum sample was mixed with 400 µl of 0.01 M phosphate buffer (pH 7.4) containing 0.2% trimethylsilyl propionate (TSP). Water signals and broad protein resonance were suppressed by a combination of PRESAT and the Carr-purcell-Meiboom-Gill (CGMP) pulse sequence. $^1$H NMR spectra were measured at frequency 499.91 Hz with 128 scan to obtained 8012.576 data points over the set spectral width. Total acquisition time was 480 s

2.6. Biochemical analysis

Biochemical analysis of serum samples was determined spectrophotometrically using a Chemistry Analyzer (Hitachi 902 Automatic Analyzer, Hitachi, Tokyo, Japan) using appropriate kits for measurement of the following serum parameters: glucose, cholesterol, LDL, LDH and HDL.

2.7. Data processing and statistical analysis

The resulting NMR spectra were manually phased, baseline-corrected and calibrated to TSP as internal standard at 0.0 ppm. The spectral region from 0.50 to 10.00 ppm were binned (0.04 ppm) using Chenomx NMR software (Chenomx NMR Suite 5.1 Professional, Edmonton, Canada). Each spectrum was integrated after excluding water (4.68–5.00 ppm) regions.

Fig. 2 shows the overlayed binned $^1$H NMR spectra for serum sample of SD/WTR and HF/WTR rats. Based on chemical shift data of standard compounds available in the Chenomxlibrary, the identified metabolites in both animal group rats were identified visually by inspecting each of the spectra.

Multivariate data analyses, namely principle component analysis (PCA) and orthogonal projection to latent structure analysis-discriminant analysis (OPLS-DA) performed using SIMCA-P software (version 13.0, Umetrics, Umea, Sweden). OPLS-DA was used to discriminate and identify the metabolites contributing to the changes occurring in the serum samples. Three models were developed; for SD/WTR versus HF/WTR (Model 1), SD/WTR versus HF/PB(100 mg/kg) (Model 2) and SD/WTR versus HF/PB(500 mg/kg) (Model 3). PCA was used to evaluated the effect of *P. betle* treatment on HF rats. OPLS-DA with Pareto scaling were performed using SIMCA-P+13. 0 software (Umetrics, Umea, Sweden).

OPLS-DA is a supervised classification method which finds correlated (predictive) and uncorrelated (orthogonal) variations between X and Y components (Wiklund, 2008). It improves the separation of class discriminant through the score and loading plots, and interpretation of variables responsible for class discrimination through SUS-plot. In the current analysis, a binary vector with the value 0 was designated for SD/WTR group and 1 for HF/WTR or HF/PB (100 mg/kg) or HF/PB (500 mg/kg) groups. The score plot displays the predicted variation, $t$ which represents the variation between class, and the orthogonal variation, $to$, which constitutes the variation within the class. In the loading plot, the potential biomarkers are shown by the most positive and negative loadings. The column loading plot provides the jack-knifing confidence interval where high and low confidence intervals show uncertain and significant variable, respectively.

The SUS-plot compares biomarkers from two models, where the correlation from the predictive component, ($p$corr) of Model 1 was plotted against that of Model 2 or Model 3. The shared structure shows biomarkers which vary in the same or opposite direction for both

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**Fig. 2.** Binned 1H NMR spectra overlay (ppm) of Standard Diet and High Fat Diet rats. Selected biomarker (ID:ppm): α-hydroxybutyrate (1:0.90), β-hydroxybutyrate (2:1.18), Acetoacetate (3:2.26), Acetone (4:2.22), Alanine (5:1.46), Arginine (6:1.90), Choline (7:3.10), Citrate (8:2.54/2.66), Creatine (9:3.02), Creatinine (10:4.06), Glucose (11:4.62/5.22), Glutamate (12:2.34), Glutamine (13:2.42), Glycine (14:3.54), Isoleucine (15:0.98), Lactate (16:1.34), Leucine (17:0.94), Methionine (18:2.14), Pyruvate (19:2.38), Serine (20:3.94), Succinate (21:2.38) and Valine (22:1.02).
Fig. 3. Changes of body weight in the control group (HF/WTR, SD/WTR) and treated group HF/PB (100 mg/kg) and HF/PB (500 mg/kg) by weeks. * value of $p < 0.05$ compared to the SD/WTR group. ** value of $p < 0.05$ and *** value of $p < 0.005$ compared to the HF/WTR group.

Fig. 4. Changes of food intake of control group (HF/WTR, SD/WTR) and treated group HF/PB (100 mg/kg) and HF/PB (500 mg/kg)) by weeks. * value of $p < 0.05$ compared to the SD/WTR group. ** value of $p < 0.05$ compared to the HF/WTR group.

Table 1
Biochemical analyses of control group (HF/WTR, SD/WTR) and treated group HF/PB (100 mg/kg) and HF/PB (500 mg/kg)).

<table>
<thead>
<tr>
<th>Biological parameter</th>
<th>SD/WTR $^{1}$</th>
<th>HF/WTR $^{1}$</th>
<th>HF/PB (100 mg/kg) $^{2}$</th>
<th>HF/PB (500 mg/kg) $^{2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>3.400 ± 0.118</td>
<td>9.180 ± 0.408$^{aaa}$</td>
<td>8.140 ± 0.280$^{aaa,bb}$</td>
<td>7.740 ± 0.297$^{aaa,bb}$</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>0.810 ± 0.080</td>
<td>1.372 ± 0.102$^{aaa}$</td>
<td>1.304 ± 0.111$^{aaa}$</td>
<td>1.340 ± 0.087$^{aaa}$</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>0.114 ± 0.007</td>
<td>0.684 ± 0.153$^{aaa}$</td>
<td>0.404 ± 0.035$^{aa,bb}$</td>
<td>0.468 ± 0.049$^{aa,bb}$</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.344 ± 0.578</td>
<td>0.770 ± 0.373$^{aa}$</td>
<td>0.998 ± 0.708</td>
<td>1.058 ± 0.845</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>1317.8 ± 126.56</td>
<td>1379.8 ± 262.67</td>
<td>1362.4 ± 256.63</td>
<td>1144.4 ± 226.60</td>
</tr>
</tbody>
</table>

* Values are mean ± SE. Notes: LDL: low-density lipoprotein, HDL: high-density lipoprotein, LDH: lactate dehydrogenase.
** Value of $p < 0.005$.
*** Value of $p < 0.001$ compared to the SD/WTR group.
$^{a}$ Value of $p < 0.05$.
$^{ab}$ Value of $p < 0.005$ compared to the HF/WTR group.
The changes in body weight of control group (HF/WTR, SD/WTR) and treated group HF/PB (100 mg/kg) and HF/PB (500 mg/kg)) from the 13th week to the 16th week is shown in Fig. 3. Results showed that the animals treated with HF/PB (100 mg/kg) showed a high concentration of lactate, while the lower cluster contained high levels of serine. The HF treated group by weeks 3.1. The changes in body weight of control group and PB treated group by weeks

The changes in body weight of control group (HF/WTR, SD/WTR) and treated group HF/PB (100 mg/kg) and HF/PB (500 mg/kg)) from the 13th week to the 16th week is shown in Fig. 3. Results showed that the animals treated with HF/WTR showed a steady increased in body weight till the end of the study. However, the group of rats which received P. betle extract (HF/PB (100 mg/kg) and (HF/PB (500 mg/kg)) was found to be clustered together with HF animals (HF/WTR) on the upper side of the score plot. Interestingly our P. betle treated group (HF/PB (100 mg/kg) and (HF/PB (500 mg/kg)) showed a decreasing level of glucose, cholesterol, LDL and DHLD and increase in HDL content compared to HF/WTR even though they consumed the HF continuously until the end of the studies (Table 1).

3.2. Changes of food intake of control group and PB treated group by weeks

The changes in food intake of control group (HF/WTR, SD/WTR) and treated group HF/PB (100 mg/kg) and HF/PB (500 mg/kg)) is shown in Fig. 4. After 12 weeks of HF, the food intake of the animal was measured continuously until 16th week. The result showed that food intake was maintained for the rats treated with HF/PB (100 mg/kg) from 13th week to 16th week when compared to the 12th week and showed no significant difference with the control group (HF/WTR and SD/WTR). However, the group of rats treated with HF/PB (500 mg/kg) showed a reduction of food intake from the 14th week until the end of the studies, with the p < 0.05 compared to HF/WTR group.

3.3. Biochemical analyses of control group and PB treated groups

Table 1 shows the results of biochemical analysis for glucose, cholesterol, LDL, and HDL levels in all the studied rats. The rats in HF/WTR group demonstrated higher content of glucose, cholesterol, and LDL but lower content of HDL. However the rats treated with SD/WTR showed lower content glucose, cholesterol, LDL and higher HDL content. High level of LDL in blood serum will lead to a high lipid profile, an indicator of developing heart disease (Zhang et al., 2013). While increased HDL will lead to removal of excess cholesterol from tissues and carries it to the liver for disposal (Weissglas-Volkov and Pajukanta, 2010). Interestingly our P. betle treated group (HF/PB (100 mg/kg) and (HF/PB (500 mg/kg)) showed a decreasing level of glucose, cholesterol, LDL and DHLD and increase in HDL content compared to HF/WTR even though they consumed the HF continuously until the end of the studies (Table 1).

3.4. PCA - fitted to the binned NMR spectra of serum from the SD/WTR, HF/WTR, HF/PB (100 mg/kg) and HF/PB (500 mg/kg)

Fig. 5 shows the PCA - fitted to the binned NMR spectra of serum from the SD/WTR, HF/WTR, HF/PB (100 mg/kg) and HF/PB (500 mg/kg). PCA was used to evaluate the effect of P. betle treatment against obese and lean animals. The score and loading plots for Principal component shown in Fig. 5. Principle component 1 and 2 of Fig. 5, respectively described 27% and 18.2% of total variation of the data. The data set of HF animal treated with PB (HF/PB(100 mg/kg) and HF/PB(500 mg/kg)) was found to be clustered together with HF animals (HF/WTR) on the upper side of the score plot. However, lean animal (SD/WTR) was clustered on the lower side of the score plot. Interestingly, HF/PB(100 mg/kg) was clustered on the right side of score plot while HF/PB(500 mg/kg) was on the left side of the score plot. A scrutiny of the loading plot showed that the upper cluster was discriminated from the higher cluster by high glucose and glycine; while the lower cluster contained high levels of serine. The HF treated with PB(100 mg/kg) showed a high concentration of lactate, while PB(500 mg/kg) a high concentration of β-hydroxybutyrate.

3.5. OPLS-DA of control group and PB treated groups

Fig. 6 shows the OPLS-DA of model 1, 2 and 3, which involves HF/WTR vs SD/WTR, HF/PB(100 mg/kg) and HF/PB(500 mg/kg). The total variation in Y explained by Model 1 are R2(Y(cum)) is 0.999 and Q2(cum) is 0.882 and the predictive variation, t[1], corresponded to 25% of all variation in the data and uncorrelated or orthogonal variation, to[1] corresponded to 14.2%. The total variation in X explained by Model 1 is 39.2%. For model 2, R2(Y(cum)) is 1 and Q2(cum) is 0.811. The predictive variation, t[1], corresponded to 28.4% of all variation in the data and uncorrelated or orthogonal variation, to[1] corresponded to 15.1%. The total variation in X explained by model 2 is 43.5%. Lastly, for model 3, the R2(Y(cum)) is 0.816 and Q2(cum) is 0.447 and the predictive variation, t[1], corresponded to 22.9% of all variation in the data and uncorrelated or orthogonal variation, to[1] corresponded to 29.7%. The total variation in X explained by model 3 is 52.6%. Furthermore, based on the misclassification table of OPLS-DA, all models were able to classify
A. Model 1: OPLS-DA of SD/WTR vs. HF/WTR

B. Model 2: OPLS-DA of SD/WTR vs. HF/PB(100mg/kg)

C. Model 3: OPLS-DA of SD/WTR vs. HF/PB(500mg/kg)

Fig. 6. OPLS-DA of serum metabolite. Metabolite ID: α-hydroxybutyrate (1), β-hydroxybutyrate (2), Acetoacetate (3), Acetone (4), Alanine (5), Arginine (6), Choline (7), Citrate (8), Creatine (9), Creatinine (10), Glucose (11), Glutamate (12), Glutamine (13), Isoleucine (15), Lactate (16), Leucine (17), Methionine (18), Pyruvate (19), Serine (20), Succinate (21) and Valine (22). A. Model 1: OPLS-DA of SD/WTR vs. HF/WTR. B. Model 2: OPLS-DA of SD/WTR vs. HF/PB(100 mg/kg). C. Model 3: OPLS-DA of SD/WTR vs. HF/PB(500 mg/kg).

3.6. SUS-Plot of the correlation from the predictive component p(corr) of control group and PB treated groups

The SUS-Plot in Fig. 7 (Model 1) shows the correlation from the predictive component p(corr) of SD/WTR vs. HF/WTR (Model 1 in Fig. 6) on the x-axis and SD/WTR vs. HF/PB(100 mg/kg) (Model 2 in Fig. 6) on the y-axis. As seen in Model 1, changes in the “unique metabolites” such as increase of lactate and citrate was clearly observed for HF treated PB(100 mg/kg) rats. “shared metabolite” in same
Ketone body production by the liver is dependent on FFA concentration. Cholesterol, fatty acid and complex lipids especially during starvation are synthesized from acetyl-CoA in the liver for the synthesis of the ketone bodies (acetoacetate, hydroxybutyrate, and acetone), which fatty acid derivatives and cholesterol in blood have been used as an important source and store of energy for metabolism. Blood lipids, derived from food intake or adipose tissue and liver are mainly fatty acid, glucose, TCA cycle, amino acid and creatine metabolism. Lipids such as citrate, creatine and glycine can be generated from serine, an amino acid derived from pyruvate. Both glutamine and glycine are related to glucose metabolism. Our finding demonstrated increased of isoleucine, leucine and valine levels in blood in lean animal (SD/WTR) and depletion in obese rats (HF/WTR). Depletion of serum level of BCAAs was reported in HF-fed obese animals in other metabolomics studies (Shearer et al., 2008). An HF with lower protein content may decrease supply to BCAAs. Obesity may play a role in the regulation of BCAs catabolism.

Glutamine and glycine are non essential amino acids that can be synthesized in the body. Glutamine is the most abundant amino acid in plasma (Oberbach et al., 2011) and glutamate. Glycine can be generated from serine, an amino acid derived from pyruvate. Both glutamine and glycine are related to glucose metabolism. Our finding showed an increase of glycine in all HF treated animals. Elevated level of serine in lean animal (SD/WTR) might be due to two pathways involved in glyconeogenesis. One involves the direct formation of pyruvate from l-serine. The other pathway involves the formation of carbon dioxide in the mitochondria. The main function of TCA cycle is to metabolize acetyl-CoA (the product glycolysis) into two molecules of CO₂ (Gaster et al., 2012). In a simple definition TCA cycle refers to a series of chemical reactions used by all aerobic organisms to generate energy through the oxidation of acetate derived from carbohydrates, fats and proteins into carbon dioxide and chemical energy in the form of adenosine triphosphate (ATP). Besides its catabolic function, TCA cycle also has relationship with other metabolic pathways such as the source of precursors for anabolic processes in the synthesis of fatty acid, amino acids and gluconeogenesis. Metabolites include citrate, fumarate, succinate and oxoglutarate, which are intermediates of TCA.

In this study, succinate in the blood serum were increased in lean animals and decreased in all HF treated animals. This is consistent with the results from previous studies (Sickmann et al., 2010) which concluded that depletion of TCA intermediates in obese conditions were due to mitochondrial dysfunction.

The branch chain amino acids (BCAAs), leucine, isoleucine and valine are among the nine essential amino acids for humans. The BCAAs play important roles in protein synthesis (Holecck, 2001), improve glucose metabolism and oxidation (Dei et al., 2007) and regulate leptin secretion from fat and food intake (Lynch et al., 2006). Our finding demonstrated increased of isoleucine, leucine and valine levels in blood in lean animal (SD/WTR) and depletion in obese rats (HF/WTR). Depletion of serum level of BCAAs was reported in HF-fed obese animals in other metabolomics studies (Shearer et al., 2008). An HF with lower protein content may decrease supply to BCAAs. Obesity may play a role in the regulation of BCAAs catabolism.

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hydroxypyruvate. The relative contributions of these two gluconeogenic pathways depend upon the dietary composition of the food consumed by the animals (Koning et al., 2003).

Creatine is a degradation product of creatine, which is biosynthesized from arginine and glycine in the muscle (Wyss and Kaddurah-Daouk, 2000). Creatine production is generally proportional to body mass and is subsequently filtered from the blood to the kidney. Thus, creatine level in the blood and urine have been used as a non-invasive indicator of renal function. Thus, caution is required in interpreting changes in creatinine clearance in obesity. Our studies showed creatine level was increased in obese animals and decreased in lean animals.

5. Conclusion

Metabolite fingerprinting of obese, lean and high fat diet animals treated with PB(100 mg/kg) and PB(500 mg/kg) were compared and the biomarkers identified using SUS-Plot. Based on the results, HF/WTR group was characterized by high-level of glucose, glycine and alanine. Meanwhile, SD/WTR group was characterized with high-level of serine, isoleucine, valine and acetocacetate. Interestingly, increasing the dosage of PB to 500 mg/kg shared the same metabolite profile, especially β-hydroxybutyrate level with lean animals (SD/WTR) in SUS-plot. This finding suggest that the administration of 500 mg/kg PB extracts leads to increase in oxidation process in the body contribute to the weight loss. From the behavioural studies, the administration of 500 mg/kg PB extracts also leads to a reduction in food intake and we conclude that both factors were involved in maintaining animals body weight although they were continuously consumed HF.

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