

ORIGINAL CONTRIBUTION

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Senna alata leaf extract restores insulin sensitivity in high-fat diet-induced obese mice

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Abstract

Background: *Senna alata* (*S. alata*) has numerous pharmacological activities including anti-lipogenic effect in high-fat diet (HFD)-induced obese mice. The present study investigated the effect of *Senna alata* (*S. alata*) leaf extracts on the regulation of abnormal glucose metabolism in HFD-induced obese mice.

Methods: Male ICR mice were induced to become obese by being fed a HFD (45 kcal% lard fat) for 12 weeks. During the last 6 weeks of diet feeding, the obese mice were treated with the water extract of *S. alata* leaf at 250 and 500 mg/kg/day. After 6 weeks of treatment, blood was collected for measuring biochemical parameters. The liver, epididymal fat and skeletal muscle tissues were excised and kept for determining histology and western blot analysis.

Results: Treatment with *S. alata* (250 and 500 mg/kg) significantly reduced hyperglycemia, hyperinsulinemia, and hyperleptinemia. The glucose intolerance was improved by *S. alata*. The elevated monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor- α (TNF- α) levels in obese mice were reduced in *S. alata* treatment. The level of serum adiponectin was increased in obese mice treated with *S. alata* (250 and 500 mg/kg). The epididymal fat weight was reduced in *S. alata* treatment. The enlarged adipocyte size was smaller in obese mice treated with *S. alata*. In comparison with the obese control mice, the mice treated with *S. alata* showed a significant reduction of liver glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) proteins. Moreover, *S. alata* up-regulated the liver and muscle adenosine monophosphate-activated protein kinase phosphorylation (pAMPK) and muscle glucose transporter 4 (GLUT4).

Conclusions: The results indicate that the restoration of impaired glucose metabolism of *S. alata* may be associated with reduced hepatic gluconeogenesis and increased glucose uptake via AMPK activation.

Keywords: *Senna alata*, Obesity, Insulin resistance

Abbreviations: AMPK, Adenosine monophosphate-activated protein kinase; G6Pase, Glucose-6-phosphatase; GLUT4, Glucose transporter 4; HFD, High-fat diet; LFD, Low-fat diet; PEPCK, Phosphoenolpyruvate carboxykinase

Background

Obesity is a condition that is strongly associated with insulin resistance and diabetes. There are several underlying mechanisms which explain the association. For example, in studies using obesity-related insulin resistance models, the up-regulation of hepatic

phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) enzymes was found to induce an increase in glucose production [1, 2]. Furthermore, impairment of glucose transporter 4 (GLUT4) expression and GLUT4 translocation and/or insulin signaling may affect insulin-stimulated glucose uptake, also resulting in insulin resistance and type 2 diabetes mellitus (T2DM) [3]. Increased GLUT4 expression or translocation to the plasma membrane can be regulated by activation of adenosine monophosphate-activated protein kinase (AMPK) via insulin-independent

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mechanism [4]. In liver, AMPK controls glucose homeostasis mainly through the inhibition of gluconeogenic gene expression and hepatic glucose production [5]. AMPK α 2, a major subunit of AMPK, has been found to play an important role in glucose homeostasis [6]. In mice fed with high-fat diet (HFD), absence of AMPK α 2 in skeletal muscle subunit led to a development of glucose intolerance and insulin resistance [6]. AMPK is thus considered a therapeutic target in the prevention and treatment of T2DM [7–9]. Activation of AMPK in the liver and muscle is expected to elicit beneficial metabolic effects with the potential to improve the defects associated with T2DM and the metabolic syndrome [8].

Senna alata (*S. alata*) is a tropical plant found in many countries, including Thailand. It has numerous pharmacological activities such as antitumor [10], anthelmintic [11], anti-bacterial [12], anti-oxidant and anti-inflammatory [13], and anti-diabetic activities [14]. A recent study found that *S. alata* (250 and 500 mg/kg) improved abnormal lipid metabolism by suppressing hepatic lipogenic genes, sterol regulatory element binding protein 1c (SREBP1c), fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) [15]. However, the effect of *S. alata* in regulating glucose metabolism in obesity-related insulin resistance is little known. The present study investigated the effect of *S. alata* on impaired glucose metabolism using HFD-induced obese mice and measuring the parameters associated with hepatic glucose production, AMPK phosphorylation, and GLUT4 translocation.

Methods

Chemicals and reagents

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). The low-fat diet (LFD) and high-fat diet (HFD) were purchased from Research Diets (New Brunswick, NJ, USA). Anti-G6Pase, anti-GLUT4, and anti- β actin were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Anti-phospho-AMPK α Thr172 (pAMPK), anti-AMPK α , and polyvinylidene difluoride (PVDF) membranes were purchased from EMD Millipore (Billerica, MA, USA).

Plant preparation and extraction

The *S. alata* leaves were collected between July and September 2013 in Buriram, Thailand. A voucher specimen (SKP 034 19 01 01) was given by the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. The dried leaves were extracted three times with water at 100 °C for 30 min. The extract was filtered and lyophilized. The yield of dried extract was 15.58 % of the starting dry weight of the leaves. Distilled water was used for dissolving *S. alata* extracts prior to feeding to mice.

Animals, diets, and experimental design

The animal experimental protocol was reviewed by the Animal Ethics Committee of Thammasat University, Pathum Thani, Thailand (Rec. No. AE 009/2014). ICR male mice weighing 20–25 g (National Laboratory Animal Center, Mahidol University, Nakhon Pathom, Thailand) were housed under standard conditions with free access to water and fed with LFD for a week (3.85 kcal/g total energy, 10 % lard fat, 20 % protein, and 70 % carbohydrate). Then they were fed with LFD (normal control mice) or HFD (obese mice) containing 4.73 kcal/g total energy, 45 % lard fat, 20 % protein, and 35 % carbohydrate for 12 weeks. During the last 6 weeks period of diet, normal control mice (NC, $n = 8$) were daily given distilled water (2 mL/kg) via oral gavage, and 24 obese mice, divided into three groups ($n = 8$ per group) were orally administered with 2 mL/kg of distilled water (obese control mice: OB), or 250 mg/kg, or 500 mg/kg of *S. alata* extracts. At the 5th week of treatment period, all mice were subjected to given oral glucose tolerance test as detailed below. After the 6-weeks treatment period, all mice were sacrificed using isoflurane anesthesia. Whole blood samples were collected by cardiac puncture, and blood glucose level was measured. The serum samples were further analyzed for insulin, leptin, adiponectin, monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor- α (TNF- α) levels. Liver, epididymal fat, and skeletal muscle tissues were taken for histology and western blot analysis.

Oral glucose tolerance test (OGTT)

An OGTT was performed on the mice after 6 h of fasting. Mice were orally loaded with 50 % glucose (2.0 g/kg). Tail vein blood samples were collected before, and after glucose loading at 20, 60 and 120 min to determine glucose concentrations using an Accu-Check glucometer (Roche Diagnostics, Mannheim, Germany). Area under the curve (AUC) for glucose over time was calculated using a trapezoidal method.

Serum insulin, leptin, adiponectin, and MCP-1 levels

Serum insulin, leptin, and adiponectin concentrations were measured using ELISA kit assay (EMD Millipore, Billerica, MA, USA). Serum MCP-1 was measured using ELISA kit assay (Thermo Scientific, Rockford, IL, USA). The index of the homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as an indicator of insulin sensitivity according to the following formula: $[\text{insulin } (\mu\text{IU}) \times \text{glucose (mM)}] / 22.5$ [16].

Western blot analysis

Liver and skeletal muscle tissues were homogenized and extracted with TPER[®] mixed with Halt[®] protease inhibitor (Thermo Scientific, Rockford, IL, USA). Protein samples (40 μg) were subjected to 12 % SDS-polyacrylamide

gel electrophoresis and transferred to PVDF membranes, which were then probed with anti-PEPCK, anti-G6Pase, anti-pAMPK, anti-AMPK, anti-GLUT4, and anti-actin primary antibodies (diluted 1:500) overnight at 4 °C. Following the incubation with horseradish peroxidase-conjugated secondary antibody (diluted 1:5000) for 2 h at room temperature, and the blots were developed in Clarity™ Western ECL substrate (Bio-rad, Brea, CA, USA). Images were obtained with an Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE, USA), and band intensities were quantified by densitometry using a Gel-Pro™ Analyzer version 3.1 software (MediaCybernetics, Rockville, MD, USA). All relative target proteins were normalized to actin.

Epididymal fat histology

The epididymal fat tissues were fixed with 10 % formalin, embedded in paraffin, and sectioned and stained with hematoxylin and eosin (H&E). The histological changes were explored using a light microscope (Olympus, Tokyo, Japan). The adipocyte size was determined using an ImageJ software program (National Institute of Health, Bethesda, MD, USA).

Statistical analysis

All values were expressed as mean ± standard error of the mean (SEM). Following the performance by one-way analysis of variance (ANOVA) followed by a Tukey's post-hoc test for multiple comparisons using SigmaStat (Systat Software, San Jose, CA, USA). $P < 0.05$ was considered to be significant.

Results

Effect of *S. alata* extracts on metabolic parameters

As shown in Table 1, after 12 weeks of HFD feeding, the body weight of obese control mice was significantly increased as compared to normal control mice. There was a slight decrease in body weight between the obese control

group and *S. alata*-treated groups, but this data is not statistically significant. Obese mice treated with *S. alata* (250 and 500 mg/kg) significantly decreased FBG and serum insulin, with improved HOMA-IR. *S. alata* (250 and 500 mg/kg) significantly reduced the serum leptin whereas the serum adiponectin was significantly increased compared with obese control mice (Table 1). Moreover, the elevated serum MCP-1 and TNF- α were significantly reduced by *S. alata* treatment (Table 1). In OGTT, obese control mice had high blood glucose level at 20, 60, and 120 min after glucose loading compared with normal control mice (Fig. 1a), but obese-treated *S. alata* groups (250 and 500 mg/kg) significantly suppressed the elevated blood glucose at 120 min. The average AUC of OGTT of *S. alata* groups was less than that of obese control mice (Fig. 1b), suggesting that *S. alata* improved glucose tolerance ability. However, these parameters were not significantly different between two *S. alata* doses.

Effect of *S. alata* extracts on hepatic PEPCK, G6Pase and pAMPK protein expressions

Hepatic gluconeogenic enzymes, PEPCK and G6Pase were elevated in OB mice relative to NC mice (Fig. 2a and b). *S. alata* (250 and 500 mg/kg) significantly reduced PEPCK and G6Pase protein expression compared to the OB mice. Furthermore, the AMPK phosphorylation was increased in obese mice treated with *S. alata* compared with obese control mice (Fig. 2c). While *S. alata* at both doses had a significant effect on these parameters, *S. alata* at 500 mg/kg especially reduced gluconeogenic enzymes and increased AMPK phosphorylation protein expression to a level near the control.

Effect of *S. alata* extracts on muscle pAMPK and plasma membrane GLUT4 protein expressions

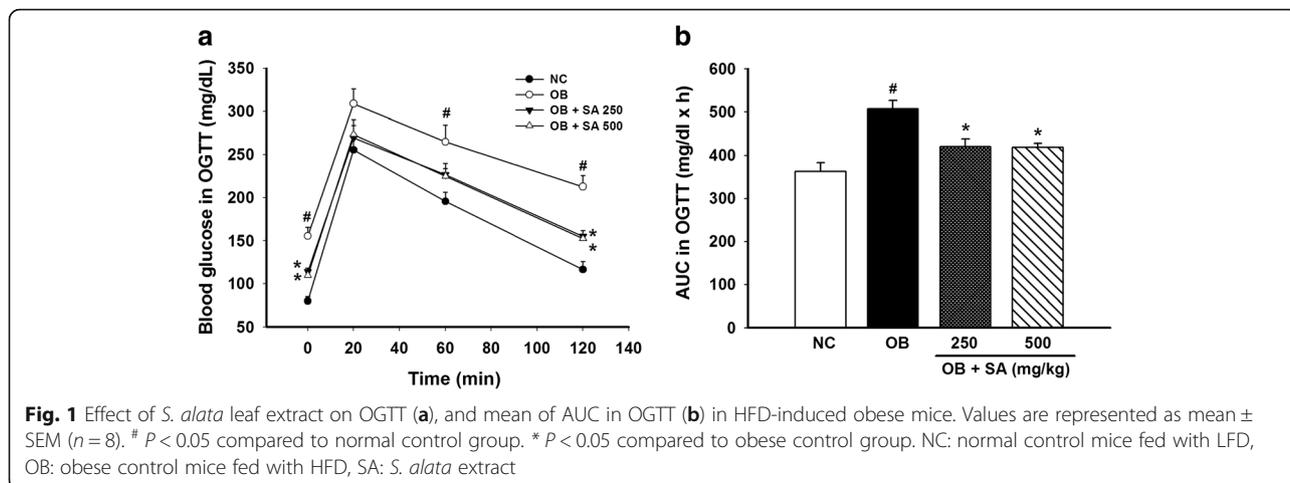
Expression of pAMPK and GLUT4 proteins were significantly decreased in the OB group compared with NC group (Fig. 3a and b). The expressions of these two

Table 1 Effect of *S. alata* leaf extract on metabolic parameters in HFD-induced obese mice

Groups	NC	OB	OB + <i>S. alata</i> (mg/kg)	
			250	500
Body weight (g)	44.0 ± 0.70	50.0 ± 1.68 *	48.6 ± 1.20 *	48.7 ± 1.13 *
FBG (mM)	5.5 ± 0.14	9.9 ± 0.61*	7.5 ± 0.28 * **	7.4 ± 0.16 * **
Insulin (ng/mL)	0.6 ± 0.06	3.6 ± 0.23 *	2.4 ± 0.26 * **	2.3 ± 0.25 * **
HOMA-IR	3.8 ± 0.41	37.7 ± 3.08 *	19.2 ± 1.84 * **	18.7 ± 2.96 * **
Leptin (ng/mL)	1.3 ± 0.28	15.0 ± 1.15 *	10.3 ± 0.72 * **	10.0 ± 0.72 * **
Adiponectin (pg/mL)	7.6 ± 0.25	5.9 ± 0.44 *	9.1 ± 0.31 * **	9.6 ± 0.26 * **
MCP-1 (pg/mL)	3.9 ± 0.46	16.2 ± 1.10 *	5.0 ± 0.67 *	3.9 ± 0.50 *
TNF- α (pg/mL)	5.1 ± 0.70	18.5 ± 1.52 *	10.6 ± 0.72 * **	8.7 ± 0.61 * **

Values are represented as mean ± SEM (n = 8). * $P < 0.05$ compared to normal control group

** $P < 0.05$ compared to obese control group. NC normal control mice fed with LFD, OB obese mice fed with HFD, FBG fasting blood glucose, MCP-1 monocyte chemoattractant protein-1, TNF- α tumor necrosis factor- α



proteins were up-regulated in the OB mice treated with *S. alata* (250 and 500 mg/kg) compared to the OB control group.

Effect of *S. alata* extracts on epididymal fat histology

The epididymal fat weight of OB group was significantly increased compared with NC group (Fig. 4a). However, treatment of *S. alata* (250 and 500 mg/kg) significantly decreased the fat weight as compared with OB group. The average size of fat cells of OB group was also larger than NC group (Fig. 4b–f), but treatment with *S. alata* significantly decreased the enlarged size of adipocytes compared with the OB group.

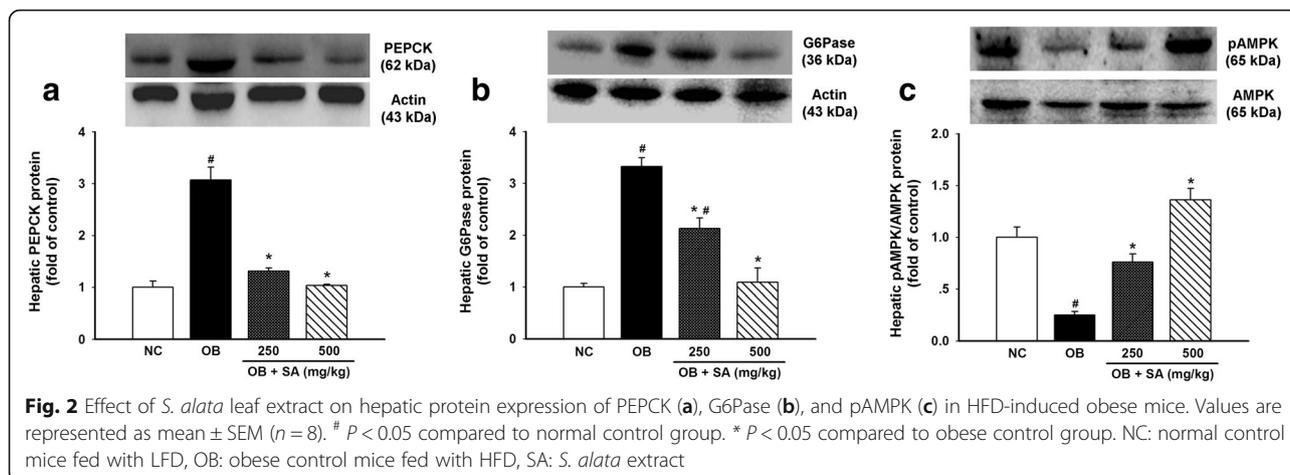
Discussion

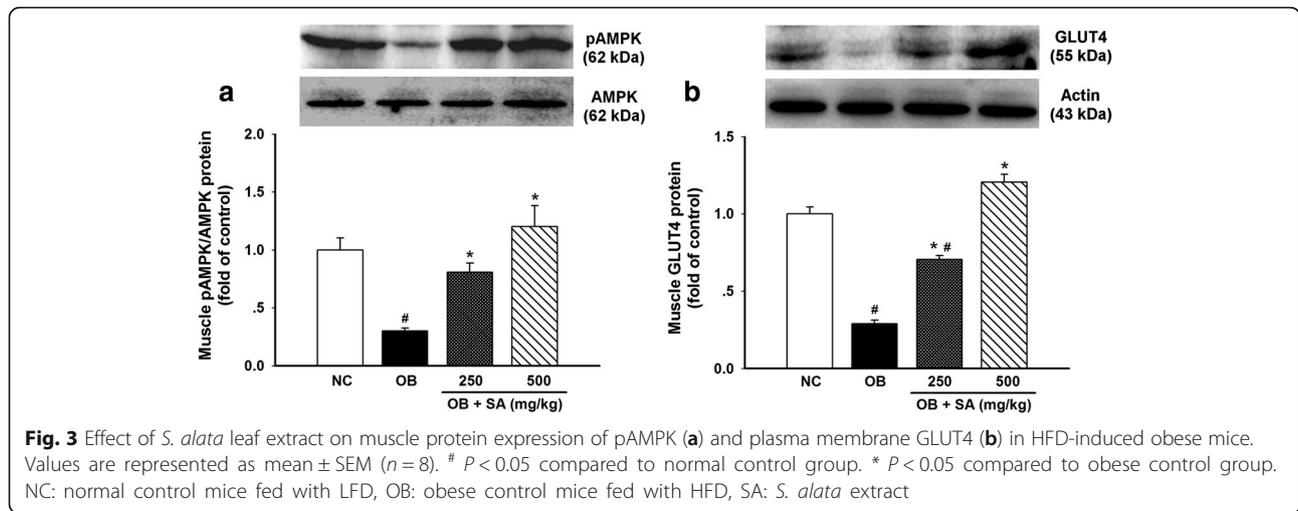
A previous study showed that treatment with *S. alata* leaf extract has potent anti-lipogenic activity in mice fed with HFD [15]. However, its activity in improving impaired glucose metabolism in obesity-related insulin resistance is unclear. In the present study, we demonstrated that treatment with *S. alata* leaf extract also improved glucose

metabolism in HFD-induced obese mice and led to reduction in the hypertrophic adipocyte size.

In this study, we found that administration of *S. alata* extract can decrease FBG, insulin, leptin, and MCP-1 levels, indicating that *S. alata* extract may lead to an improvement of insulin resistant condition. When further examining the effect of *S. alata* extract on the glucose tolerance test, it was found that treated obese mice relieved glucose intolerance and insulin sensitivity as shown by decreasing HOMA-IR index. These findings support the effects of *S. alata* in regulating glucose metabolism in obese-related insulin resistant mice.

To better understand the underlying mechanism by which *S. alata* mediates the beneficial effects on obese animals, the expressions of proteins involving in energy metabolism were investigated. There have been several studies indicating that insulin resistance and T2DM are associated with the increase of two key enzymes involved in the hepatic glucose production, PEPCK and G6Pase [17, 18]. In accordance with the other studies, we found the correlation of PEPCK and G6Pase expression in obese

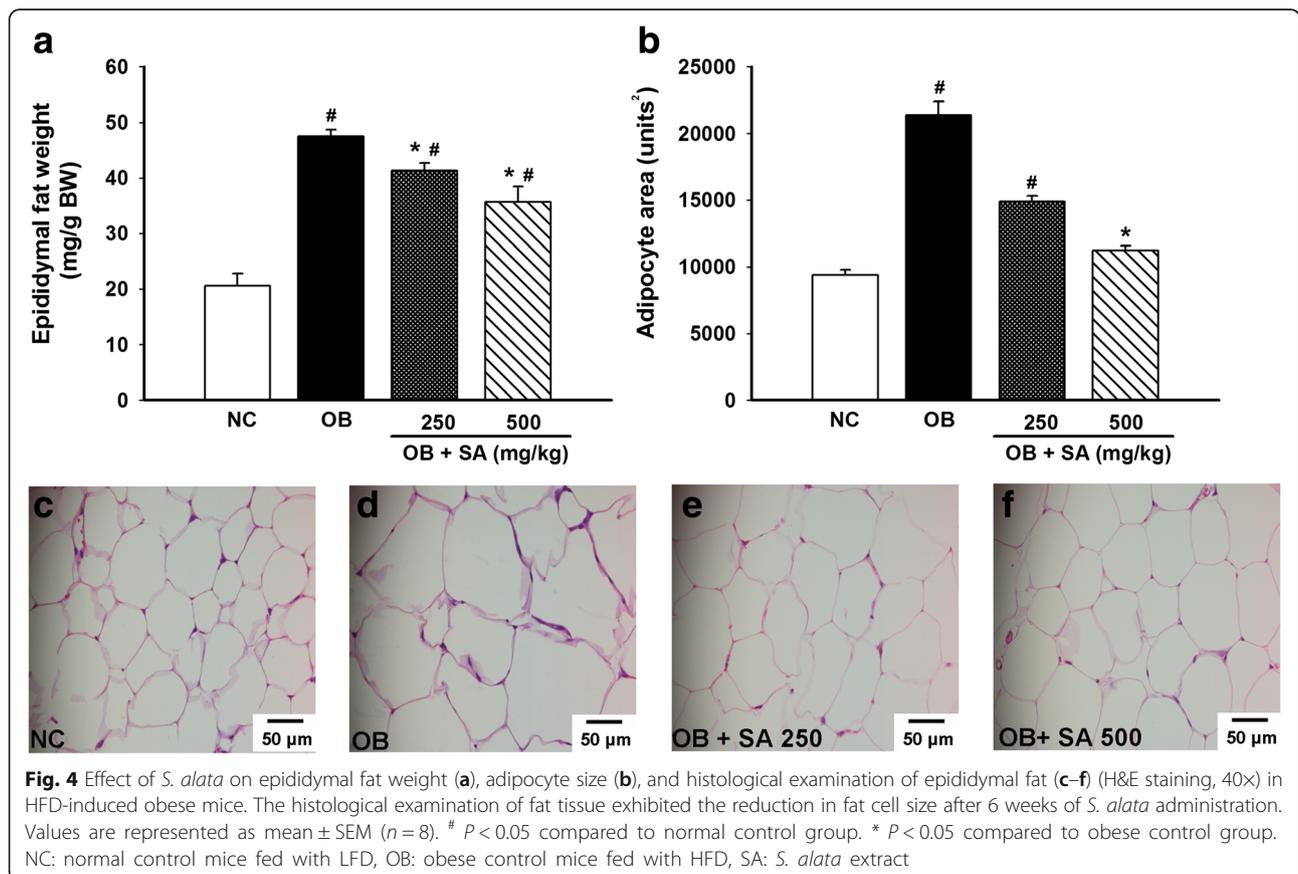




mice; however, our results showed that obese mice receiving the *S. alata* extracts had decreasing expressions of PEPCK and G6Pase in liver tissue. These results indicate the possible mechanism of *S. alata* in improving glucose metabolism via suppressing hepatic glucose production.

AMPK is a major enzyme that regulates glucose and lipid metabolism in tissues including liver, adipose, and muscle. Activation of AMPK can inhibit lipid synthesis

and improve insulin action [19, 20]. Important mechanisms of AMPK involved in the control of diabetes and obesity include prevention of (glucose or lipid) overproduction through down-regulation of SREBP1 gene, and inhibition of lipogenic and gluconeogenic enzymes as evidenced by the liver-specific AMPK α 2 knockout mice developed hyperglycemia and glucose intolerance as a result of increased hepatic glucose production [21]. A



study showed that the stimulation of AMPK in wild-type mice dramatically reduced hepatic glucose output [5]. Our study found that *S. alata* treatment reduced the protein expressions of hepatic gluconeogenic enzymes, PEPCK and G6Pase. *S. alata* treatment was also found to significantly decrease hepatic and serum lipid content via down-regulating expressions of SREBP1c and lipogenic enzyme (FAS and ACC) [15]. The present study showed that *S. alata* extract significantly up-regulated protein expression of AMPK phosphorylation in liver and skeletal muscle tissues. This finding indicates that *S. alata* has the ability to reduce hyperglycemia through AMPK activities – a similar mechanism to AMPK activators such as metformin, which decreases blood glucose level by inhibiting hepatic glucose production and stimulating glucose disposal in skeletal muscle [22].

Phosphorylation of AMPK is also a major regulator of GLUT4 translocation during exercise or in response to some anti-diabetic agents such as AICAR or metformin. It results in the stimulation of GLUT4 translocation to plasma membrane and elevation of glucose uptake in skeletal muscles [23]. In the present study, we observed that *S. alata* was able to stimulate GLUT4 translocation in muscle cells. It is possible that the enhanced GLUT4 translocation by *S. alata* extract is controlled by AMPK phosphorylation.

Secretion of adiponectin has also been reported to induce glucose uptake and GLUT4 translocation in rat skeletal muscle cells [24], and AMPK is activated by adiponectin in skeletal muscle [25]. Increased adiponectin level, which stimulates glucose uptake and fatty oxidation and inhibits the expression of gluconeogenic genes, is thus beneficial for insulin resistance [26]. In this study, it was found that serum adiponectin levels were significantly reduced in HFD-induced obese mice but were reversed significantly to the level higher than the control after the obese mice receiving *S. alata*. Thus, it is possible that *S. alata* has a beneficial effect on glucose and lipid metabolism by AMPK activation which may be mediated via increasing adiponectin secretion and/or via direct activation of AMPK.

The epididymal fat histologic results in this study also revealed that the number of large adipocytes was decreased while the number of small adipocytes was increased among mice receiving *S. alata*. The adipose tissue mass in obese mice treated with *S. alata* is lower than in obese control mice. It is of interest to note that *S. alata* efficiently reduces adipose mass and adipocyte hypertrophy in obese mice. A study showed that AMPK-knockout mice had higher body weight than wild-type mice, with a specific increase in adipose tissue mass [27]. This indicates that AMPK activation exerts an anti-lipogenic effect and AMPK might be a potential target

for the treatment of obesity. It is thus likely that the decreases in size and mass of adipose tissue of obese mice receiving *S. alata*, as found in this study, are associated with the AMPK activation. The hypertrophy of fat cell is known to be closely related to metabolic diseases [28]. Insulin resistance in adipose tissue causes the increased release of free fatty acid, which in turn, increased hepatic generation of triglyceride (TG). Moreover, the accumulation of fat and cell size in adipose tissue are mainly from the circulating TG [29]. There was a report that *S. alata* can decrease hepatic TG accumulation [15]. It is possible that decreasing mass and size of fat cells, an effect of *S. alata*, results from decreasing TG accumulation or increasing hepatic lipid metabolism.

Furthermore, AMPK activation was shown to relieve lipopolysaccharide (LPS)-induced inflammation in macrophages [30]. TNF- α is the proinflammatory cytokine that defects insulin function in the peripheral tissue and has a direct impact in obesity-related insulin resistance [31]. Our study showed that *S. alata* decreased proinflammatory cytokine, MCP-1 and TNF- α . Therefore, it appears that *S. alata*-induced AMPK might also contribute to the suppression of inflammatory responses, which would lead to improvement in metabolic disorders such as insulin resistance in obesity.

Several phytochemicals such as berberine, quercetin, and resveratrol act as AMPK activators and have effects on metabolic disorders including hyperlipidemia, insulin resistance, and obesity [32–35]. Our previous study demonstrated that *S. alata* leaf extract contained flavonoid and polyphenol [15]. Although we did not identify the specific active ingredients, we think that these contents may be associated with improvement of impaired glucose metabolism in HFD-fed mice. In addition, our previous study showed the administration of *S. alata* at 2000 mg/kg for 7 days had no toxicity in mice [15]. In the present study, mice treated with of *S. alata* did not show any side effects such as diarrhea during the 6-week study period.

Conclusions

In conclusion, this study demonstrated that *S. alata* had effects on regulation of abnormal glucose metabolism in HFD-fed mice, and such effect might be associated with increased phosphorylation of AMPK both in liver and skeletal muscle, decreased hepatic glucose production (down-regulation of PEPCK and G6Pase), and increased protein contents of skeletal muscle GLUT4. These findings indicate that *S. alata* could alleviate hyperglycemia, hyperinsulinemia, hyperleptinemia, and adiposity by activation of AMPK, which would lead to the improvement of glucose homeostasis. Therefore, *S. alata* may be beneficial for the management of obesity-related insulin resistance and T2DM. The identification of active ingredients of *S. alata* should be further studied.

Acknowledgements

The authors gratefully acknowledge the financial support provided by the Thammasat University Research Fund under the TU Research Scholar, Contract number: GEN2-36/2015. We thank Dr. Pholawat Tingpej and Mr. Sébastien Maury for proofreading and for their valuable recommendations.

Authors' contributions

JN and PP designed the experiments, collected and analyzed the data, and wrote the article. Both authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Received: 11 June 2016 Accepted: 6 September 2016

Published online: 08 September 2016

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