Effects of *Tiliacora triandra* Leaf Water Extract in High-Fat Diet Fed Mice

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Background: *Tiliacora triandra (T. triandra) leaf is widely used as an ingredient in Thai cuisine, but the activity of T. triandra leaf water extract (TTW) in the regulation of metabolic syndrome is still little known.*
Objective: *To examine the effects of TTW in high-fat diet (HFD)-induced obese mice.*
Material and Method: *Male ICR mice were induced to be obese by HFD feeding (45 kcal % lard fat) for 12 weeks. During the last 6 weeks of diet feeding, the obese mice were treated with TTW at 250 and 500 mg/kg/day. The biochemical parameters and histology analysis were measured at the end of treatment period.*
Results: *After 6 weeks of TTW treatment, the hyperglycemia, hyperinsulinemia hyperleptinemia and hyperlipidemia were significantly decreased. Hepatic lipid accumulation and adipocyte hypertrophy were also reduced. Serum adiponectin was increased in TTW-treated obese mice. TTW treatment could reduce the malondialdehyde in serum and liver tissue. Furthermore, the elevated serum inflammatory cytokines, tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein-1 were reduced (MCP-1) by TTW.*
Conclusion: *These results suggest that T. triandra leaf is a beneficial plant in alleviating hyperglycemia, hyperlipidemia, oxidative stress, and inflammation in the obese condition induced by HFD.*

Keywords: *Tiliacora triandra, Obesity, Insulin resistance, Hyperlipidemia, Oxidative stress, Inflammation*

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Obesity is related to the development of insulin resistance and type 2 diabetes mellitus (T2DM). Obesity-related insulin resistance is the cause of the development of fatty liver. The fatty liver is associated with high-fat diet (HFD) consumption, with common occurrence of the elevation of triglyceride in plasma and liver[1,2].

An elevation of oxidative stress and inflammation in obesity can be found in persons who receive the diet containing high fat and carbohydrate[3]. Malondialdehyde (MDA), an end product of lipid peroxidation, is a significant marker of the increases in hyperglycemia-related with hyperlipidemia[4]. There has been report that the obesity-associated insulin resistance is caused by stimulating inflammatory cytokines, tumor necrosis factor-α (TNF-α)[5]. Furthermore, the oxidative stress is a result of the alteration of cytokines including decreased expression of adiponectin, and elevated expressions of plasminogen activator inhibitor-1 (PAI-1), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1)[6].

Tiliacora triandra (*T. triandra*) is a plant that is used as an ingredient in Thai cuisines. The administration of *T. triandra* has neuroprotective and cognitive enhancing effects in ethanol dependent rats via suppressing the oxidative stress and acetylcholinesterase activity in hippocampus[7]. A single oral administration of water extract of *T. triandra* leaf at 5,000 mg/kg possesses no toxicity effect in rats[8]. Moreover, the subchronic toxicity test in the rats continuously received the extracts at doses of 300, 600, and 1,200 mg/kg for 90 days, did not show any illnesses[8]. However, the pharmacological activity of *T. triandra* leaf in the regulation of metabolic syndrome has not been reported yet. Therefore, this study was designed to investigate the role of *T. triandra* leaf extract on the hyperglycemic, hyperlipidemic, oxidative stress, and inflammatory conditions in HFD-induced obese mice.

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Material and Method

Extraction of T. triandra

The leaves of *T. triandra* were collected from Buriram, Thailand, between July and September 2014. A voucher specimen (SKP 114 20 20 01) was presented by the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. The dried leaves were extracted three times with water (1: 10, w/v) at 100°C for 30 min. This extract was concentrated and subsequently freeze-dried. The yield obtained was 9.82% of the starting dry weight of the leaves.

Animals, diet, and experimental design

This study was approved by the Animal Ethics Committee of Thammasat University, Pathumthani, Thailand (Rec. No. AE 003/2015). Male ICR mice weighing 20 to 25 g were obtained from the National Laboratory Animal Center of Mahidol University (Nakhon Pathom, Thailand). The animals were allowed to acclimatize in standard conditions with free access to water and normal diet for a week. After 1 week acclimation, the mice were fed with 10% low-fat diet (10% lard fat LFD with total energy of 3.85 kcal/g diets from Research Diets, NJ, USA) or 45% high-fat diet (45% lard fat HFD with total energy of 4.73 kcal/g from Research Diets, NJ, USA) for 12 weeks. After 6 weeks of feeding, the mice were randomly divided into 4 groups of 8 mice each: group I normal control mice were fed with LFD throughout the experiment, group II obese control mice were fed with HFD throughout the experiment, group III and IV obese mice were treated with *T. triandra* water extract (TTW 250 and 500 mg/kg, respectively) for 6 weeks. The control groups were treated with distilled water. The TTW were dissolved in distilled water. The body weight and food intake of the animals were measured every week.

Collection of blood and tissues

At the end of experiment, all mice were fasted overnight and then sacrificed by isoflurane anesthesia. The blood samples were collected by cardiac puncture. The whole blood was used for measuring the blood glucose level. Then, the serum samples were collected by centrifugation and kept for biochemical analysis. The epididymal fat and liver samples were removed for biochemical and histology analysis.

Serum insulin, leptin, adiponectin, TNF-α, and MCP-1 determination

The concentrations of serum insulin, leptin, adiponectin, and TNF-α were measured using ELISA kit assay (EMD Millipore, MA, USA). The serum MCP-1 was measured using ELISA kit assay (Thermo Scientific, IL, USA).

Serum lipid profile and liver triglyceride determination

The concentrations of serum total cholesterol (TC), triglyceride (TG), and non-esterified fatty acid (NEFA) were measured using the commercial kits (Wako, Osaka, Japan).

The hepatic TG was extracted with isopropanol (1: 20, w/v), centrifuged at 8,000 rpm for 15 min. The supernatant was collected for TG measurement using the colorimetric kit (Wako, Osaka, Japan).

Serum and hepatic MDA determination

The serum and hepatic MDA were measured using thiobarbituric acid reactive substances assay kit (Cayman Chemical, MI, USA). For the hepatic MDA concentration, the liver was homogenized in 1.15% cold potassium chloride. The supernatant was collected for MDA assay, after 10 min of centrifugation (10,000 rpm, 4°C). The concentration of hepatic MDA was normalized against the protein concentration. The protein concentration was measured using the Bradford's method[9].

Epididymal fat and liver histology

The fat and liver tissues were fixed in 10% neutral buffered formalin solution and embedded in paraffin. The sections of about 3 μm thick were stained with hematoxylin and eosin stain (H&E). The histological changes were explored using a light microscope (Olympus, Tokyo, Japan).

Statistical analysis

All results were expressed as mean ± standard error of the mean (SEM) for each group. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to analyze the statistical significance between the groups. The differences were considered significant at $p<0.05$. The statistical analyses were performed using computer-based software SigmaStat (Systat Software, CA, USA).

Results

Body weight, organ weight, food intake, and biochemical parameters

After the first 6 weeks of diets feeding, the body weight of all obese groups was significantly increased as compared with the normal control group

(Table 1). However, at the end of treatment period, no significant difference was observed in the body weight among the obese groups. The body weight of the obese mice treated with TTW (250 and 500 mg/kg) was slightly decreased as compared to the obese control group (Table 1). No significant difference in food intake was observed among the groups (Table 1). However, the energy intake and liver weight in the obese control group was significantly increased as compared to the normal control group (Table 1). In comparison with the obese control mice, the treatment with TTW leaf extract significantly decreased the liver weight (Table 1). The epididymal fat weight of obese control mice was significantly increased as compared to normal control mice, but the fat weight of TTW groups of was not decreased as compared to obese control mice (Table 1).

The fasting blood glucose (FBG) level of the obese control group was found to be significantly higher than the normal control group (Table 2). However, the treatment with TTW (250 and 500 mg/kg) significantly reduced the FBG as compared to the obese control group. The obese control group had a high serum insulin level as compared to the normal control group (Table 2) whereas a significant reduction in insulin level was observed in the case of obese mice treated with TTW (250 and 500 mg/kg). The serum leptin level of obese control group was significantly higher than that of the normal control group (Table 2), but the obese mice treated with TTW (250 and 500 mg/kg) showed a significant reduction in the leptin level when compared to the obese control group. More importantly, the level of serum adiponectin was significantly increased in the obese mice treated with 250 and 500 mg/kg TTW

Table 1. Effect of *T. triandra* leaf water extract (TTW) on body weight, organs weight, and food intake in HFD-induced obese mice

Groups	NC	OB	OB + TTW (mg/kg)	
			250	500
Body weight at 6th week (g)	37.1±0.72	42.1±1.15#	43.9±0.73#	42.0±1.45#
Body weight at 12th week (g)	44.0±0.56	49.9±1.70#	50.1±1.35#	48.8±1.31#
Liver weight (mg/g BW)	36.5±0.94	43.3±1.78#	37.5±0.77*	34.0±0.43*
Fat weight (mg/g BW)	20.5±2.13	45.2±1.50#	41.4±1.42#	40.5±2.10#
Food intake (g/day)	4.0±0.34	3.5±0.05	3.6±0.05	3.7±0.03

Values are expressed as mean ± SEM (n = 8). # $p<0.05$ when compared with the NC group. * $p<0.05$ when compared with the OB group.
NC = normal control mice fed with low-fat diet; OB = obese control mice fed with high-fat diet; BW = body weight

Table 2. Effect of *T. triandra* leaf water extract (TTW) on biochemical parameters in HFD-induced obese mice

Groups	NC	OB	OB + TTW (mg/kg)	
			250	500
FBG (mg/dL)	99.6±2.52	177.3±10.90#	145.6±7.20#*	144.8±4.38#*
Serum				
Insulin (ng/mL)	0.7±0.06	4.7±0.50#	2.8±0.32#*	2.7±0.33#*
Leptin (ng/mL)	1.4±0.30	14.6±1.16#	10.8±0.60#*	9.9±0.97#*
Adiponectin (pg/mL)	7.4±0.30	5.5±0.37#	10.0±0.41#*	10.1±0.32#*
MCP-1 (pg/mL)	3.6±0.37	17.0±0.98#	3.0±0.35*	2.6±0.26*
TNF-α (pg/mL)	4.9±0.64	15.6±1.55#	10.5±0.80#*	10.1±0.60#*
MDA (μM)	3.6±0.26	5.1±0.37#	3.3±0.44*	3.1±0.56*
Liver MDA (nmol/mg protein)	0.6±0.06	1.23±0.19#	0.5±0.03*	0.4±0.02*

Values are expressed as mean±SEM ($n = 8$). # $p<0.05$ when compared with the NC group, * $p<0.05$ when compared with the OB group.
NC = normal control mice fed with low-fat diet; OB = obese control mice fed with high-fat diet

(Table 2).

The serum levels of inflammatory cytokines, MCP-1 and TNF-α, were significantly increased in obese control group as compared with the normal control group (Table 2). However, TTW (250 and 500 mg/kg) significantly reduced these inflammatory markers in obese mice. Moreover, the lipid peroxidation, MDA, was also decreased in serum and liver tissue of obese mice treated with both doses TTW (Table 2).

Serum and liver lipid profiles

The serum TC showed a significant increase in obese control mice as compared to the normal control mice (Table 3). TTW (250 and 500 mg/kg) treatment showed no effect on TC level. Interestingly, the high levels of serum TG and NEFA were markedly reduced by the 250 and 500 mg/kg TTW treatment (Table 3). In addition, treatment with TTW (250 and 500 mg/kg) significantly decreased the TG storage in the liver tissue (Table 3).

Histopathological changes of epididymal fat and liver tissues

The fat cell size in obese control group showed the larger size than normal control group (Fig. 1), however treatment with TTW (250 and 500 mg/kg) could reduce the enlarged size of fat cell as compared to obese control group. In liver histological examination, the lipid accumulation in the obese control mice was observed more than that of the normal control and TTW-treated obese mice (Fig. 2), which was consistent with the results of liver TG analysis.

Discussion

In this study, the animals in all the HFD-fed groups showed significantly higher body weight than those fed with LFD. The obese mice were presented with hyperglycemia, hyperinsulinemia, and hyperleptinemia. This model also showed the condition of hyperlipidemia, and increased serum lipid peroxidation and inflammatory cytokines. These abnormal parameters are markedly observed in the obesity state. Therefore, this model is well suitable for examining the antihyperglycemic, antihyperlipidemic, antioxidant, and anti-inflammatory effects of *T. triandra* leaf extract in HFD-induced obese ICR mice.

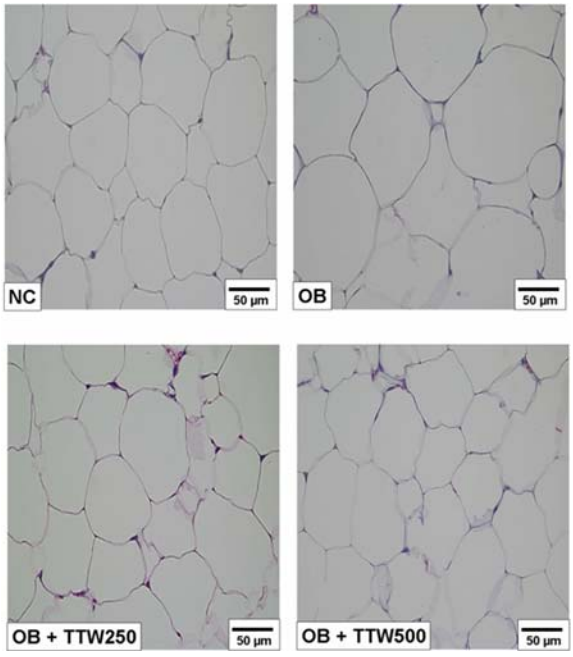
During 6 weeks of treatment with TTW, the body weight and food consumption were not a significant difference as compared with the obese control group. However, the weight of liver tissue in obese mice treated with TTW was markedly decreased as compared to the obese control mice. We further examined the lipid profiles in the serum and liver. We found that TTW effectively reduced the serum TG and NEFA as well as caused the decrease in the hepatic TG accumulation. Liver plays a major role in lipid metabolism. HFD consumption cause an increase in the liver weight due to TG accumulation[10]. This study exhibited that TTW could reduce the liver weight in obese mice. These results showed that treatment with TTW could decrease the hyperlipidemia, liver TG and fat accumulation in the liver, which is likely due to the decreasing of circulating NEFA, and inhibition of hepatic TG synthesis[11].

Adiponectin secretion is related to improved peripheral insulin sensitivity[12]. The increased circulating adiponectin can improve insulin sensitivity in humans and rodents[13,14] and is reduced in human with obesity and T2DM[15]. Interestingly, treatment with TTW for 6 weeks could increase the level of serum adiponectin. This study supported the effect of TTW in improving insulin sensitivity and the reduction of blood glucose level in obese mice. There have been reports that the peroxisome proliferator-activated

Table 3. Effect of *T. triandra* leaf water extract (TTW) on serum and hepatic lipid profiles in HFD-induced obese mice

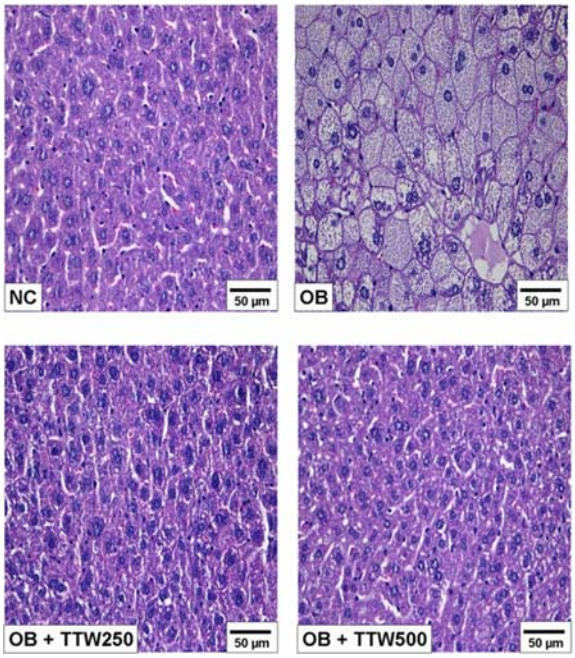
Groups	NC	OB	OB + TTW (mg/kg)	
			250	500
Serum TC (mg/dL)	121.0±10.20	225.2±5.42#	223.3±16.32#	222.6±8.36#
Serum NEFA (mEq/L)	1.5±0.09	3.0±0.11#	2.1±0.90#*	1.9±0.09#*
Serum TG (mg/dL)	61.8±8.09	152.8±6.30#	101.0±8.86#*	98.7±10.25#*
Liver TG (mg/g tissue)	10.4±1.21	42.3±4.42#	17.6±2.12#*	16.9±1.70#*

Values are expressed as mean± SEM (n=8). # p<0.05 when compared with the NC group
* p<0.05 when compared with the OB group. NC: normal control mice fed with low-fat diet, OB: obese control mice fed with high-fat diet



NC = normal control mice fed with low-fat diet, OB = obese control mice fed with high-fat diet

Fig. 1 Effect of *T. triandra* leaf water extract (TTW) on histological examination of epididymal fat tissue (H&E staining, 40x) in HFD-induced obese mice.



NC = normal control mice fed with low-fat diet, OB = obese control mice fed with high-fat diet

Fig. 2 Effect of *T. triandra* leaf water extract (TTW) on histological examination of liver tissue (H&E staining, 40x) in HFD-induced obese mice.

receptor gamma (PPARγ) synthetic agonist, may stimulate adipocyte differentiation that can increase the number of small adipocytes, thus, promoting fat cells to function as lipid storage and to secrete insulin-sensitizing adipokines, adiponectin[16,17]. Finally, the body becomes more sensitive to insulin[18]. It is interesting that TTW may have this insulin-sensitizing activity like PPARγ synthetic agonist as we found the small fat cells and increased adiponectin secretion in TTW-treated obese mice.

Low grade chronic inflammation leads to insulin resistance, increase in the production of reactive oxygen species[19] and reduction in antioxidant enzyme levels[20]. The administration of TTW could reduce the oxidative stress condition as seen by a reduction of MDA in both the serum and liver tissue. Furthermore, the treatment of TTW could suppress the elevated serum TNF-α and MCP-1 levels in the obese mice. The reductions of oxidative stress and inflammatory cytokines by TTW also play the regulatory role in HFD-induced insulin resistance in the obese mice model. There are reports showing that the TTW contained the phenolic and flavonoid compounds[7,21]. These compounds are known to have several pharmacological activities such as antihyperglycemia[22], antioxidation[21,23], anti-inflammation[24], and antihyperlipidemia activities[25]. Therefore, such effects of TTW may be associated with the compounds found in its leaf.

Conclusion

In conclusion, our data clearly showed that the treatment of *T. triandra* leaf water extract in obese mice could reduce the elevated blood glucose, serum insulin, serum leptin, serum MCP-1, and serum TNF-α levels. The high serum NEFA and TG concentration were reduced, the accumulation of TG in liver tissue was suppressed, and the level of MDA in serum and liver tissue was decreased. These findings are the first report to show a potential effect of *T. triandra* leaf water extract in the regulations of carbohydrate and lipid metabolic change in HFD-induced obesity condition.

What is already known on this topic?

Obesity-related insulin resistance is

associated with the development of type 2 diabetes mellitus.

What this study adds?

The present study suggested that *T. triandra* leaf water extract has antihyperglycemic, antihyperlipidemic, antioxidant, and anti-inflammatory effects in obese mice.

Acknowledgements

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Potential conflict of interest

None.

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ฤทธิ์ของใบย่านางที่สกัดด้วยน้ำในหนูอ้วนที่เหนี่ยวนำด้วยอาหารไขมันสูง

อุรารัตน์ แน่นหนา, จริญญาพร เนาวบุตร, ลินดา จุฬาโรจน์มนตรี

ภูมิหลัง: *ใบย่านางนิยมใช้เป็นส่วนผสมแพร่หลายในอาหารไทยแต่การออกฤทธิ์ควบคุมกลุ่มอาการทางเมตาบอลิกของใบย่านางที่สกัดด้วยน้ำยังมีข้อมูลน้อย*

วัตถุประสงค์: *เพื่อประเมินฤทธิ์ของสารสกัดใบย่านางในหนูอ้วนที่เหนี่ยวนำด้วยอาหารไขมันสูง*

วัสดุและวิธีการ: *หนูไมซ์เพศผู้ถูกเหนี่ยวนำให้อ้วนด้วยอาหารไขมันสูง (ไขมันจากน้ำมันหมูร้อยละ 45 แคลอรี่) นาน 12 สัปดาห์ ในระหว่าง 6 สัปดาห์สุดท้ายของการให้อาหารไขมันสูง หนูอ้วนจะได้รับการป้อนด้วยสารสกัดใบย่านางขนาด 250 และ 500 มก./กก./วัน ค่าชี้วัดทางชีวเคมีและการวิเคราะห์ชิ้นเนื้อจะถูกวัดเมื่อสิ้นสุดการทดลอง*

ผลการศึกษา: *หลังจากได้รับสารสกัดใบย่านางนาน 6 สัปดาห์ ระดับน้ำตาล อินซูลิน เลพทิน และไขมัน ที่สูงในเลือด ลดลงอย่างมีนัยสำคัญทางสถิติ การสะสมไขมันในตับและขนาดเซลล์ไขมันที่ใหญ่ขึ้นนั้นลดลงด้วยเช่นเดียวกัน ระดับอะดิโพเนคทินในซีรัมเพิ่มขึ้นในหนูอ้วนที่ได้รับสารสกัดใบย่านาง สารสกัดใบย่านางสามารถลดระดับมาลอนไดอัลดีไฮด์ (malondialdehyde) ในซีรัมและเนื้อเยื่อตับได้ นอกจากนี้สารสกัดใบย่านางสามารถลดการเพิ่มขึ้นของสารสื่ออักเสบ tumor necrosis factor-α (TNF-α) และ monocyte chemoattractant protein-1 (MCP-1) ได้*

สรุป: *ผลการทดลองเหล่านี้บ่งชี้ว่าใบย่านางเป็นพืชที่มีประโยชน์ในการลดระดับน้ำตาลในเลือดสูง ไขมันในเลือดสูง ภาวะเครียดออกซิเดชั่น และการอักเสบในหนูอ้วนที่เหนี่ยวนำด้วยอาหารไขมันสูงได้*