To study the effect of water extract of Solanum torvum fruit in high-fat fed male rats

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1. Introduction

Obesity is defined as a medical condition where excess body fat has accumulated over time and is associated with high levels of cholesterol and triglycerides (hyperlipidemia). Hyperlipidemia has been classified as one of the greatest risk factors contributing to the incidence and severity of coronary heart disease [1]. Moreover, obesity is also a risk factor associated with metabolic syndrome and reproductive dysfunction, where reproductive function is reduced in obese people. It has been reported that obese males have decreased testosterone and gonadotropin levels and increased circulating estrogen levels [2,3].

Hyperlipidemia can be decreased by lifestyle change, medication, or a combination of both. In recent times, products from medicinal plants have become more widely used for the prevention and treatment of illness. Several medicinal plants have an antihyperlipidemic effect such as Hibiscus sabdariffa [4] and Ammi majus seeds [5].

Solanum torvum (S. torvum), is commonly known as Turkey berry (or “makhuaphuang” in Thai). This plant is found in tropical Africa, Asia and South America. In several countries, S. torvum is widely used as a vegetable and food ingredient. S. torvum has a
wide spectrum of pharmacological activities such as antimicrobial and antifungal [6], anti-inflammatory [7], antioxidant [8], antidiabetic [9,10], antihelminthic [11] and nephroprotective activities [12]. However, the effect of *S. torvum* fruit extract on the sex hormones of obese subjects is still little known. Thus, this study aimed to evaluate its effects on the regulation of lipid profiles and sex hormones in high-fat fed male rats.

2. Materials and methods

2.1. Animal and experimental design

Male Wistar rats (6 weeks old; 200–250 g; the National Laboratory Animal Center, Nakorn Pathom, Thailand) were kept in an animal room where the temperature was maintained at (25 ± 1) °C and 12 h light–dark cycle. They were fed a standard diet (SD) and water ad libitum for a week to be acclimatized before starting the experiment. All experimental procedures involving animals were conducted in accordance to the Association for Assessment and Accreditation of Laboratory Animal Care (Frederick, MD, USA) and approved by the Animal Ethics Committee, Faculty of Medicine, Thammasat University, Thailand (AE011/2552). Animals were randomly separated into six groups (six rats per group). Group 1 was control rats fed with SD. Group 2 was control rats fed SD and treated with *S. torvum* at 400 mg/kg. Group 3 was obese control rats fed with HFD. Group 4–6 were obese rats fed HFD and treated with *S. torvum* at 100, 200 and 400 mg/kg, respectively. Control groups (groups 1 and 3) were treated with distilled water which was used as vehicle for all test substances. *S. torvum* and distilled water were orally administered in an equivalent volume of 2 mL/kg body weight of the rats during the last 4 weeks (7th–10th week). The SD contained 70 kcal% carbohydrate, 20 kcal% protein, and 10 kcal% lard fat (D12450B, Research Diets, New Brunswick, NJ, USA). The HFD had 20 kcal% carbohydrate, 20 kcal% protein, and 60 kcal% lard fat (D12492, Research Diets, New Brunswick, NJ, USA). Body weight was recorded weekly for 10 weeks.

2.2. Plant material and extract

Fresh unripe fruit of *S. torvum* was collected from Nakorn Sawan, Thailand. The identity of the medicinal plant was verified by a taxonomist at the Faculty of Pharmacy, Chiang Mai University, and the voucher specimen (No. 023203) was deposited in the Herbarium of Faculty of Pharmacy, Chiang Mai University. The unripe fruits of *S. torvum* (1000 kg) were boiled in water for 6 h and filtered. The filtrate was concentrated by spray drying to obtain crude water extract which yielded 13 kg powder. The percentage yield was 1.3% of the starting weight of the fruits. The quality control of crude water extract was evaluated in the areas of physical properties, chemical identification, water content, total ash, acid-insoluble ash, extractive value heavy metal and microbial contamination following Thai herbal pharmacopoeia.

2.3. Phytochemical screening by thin layer chromatography (TLC)

Thin layer chromatography was performed using 5 × 10 cm TLC plate. The process was carried out with solvent system of ethyl acetate:methanol:water (50:13.5:10) as previously described with modifications [13]. Dried plate was sprayed with vanillin-sulphuric acid spraying reagent. Spot detection was carried out under UV light at 254 and 366 nm.

2.4. Evaluation of plasma biochemical parameters

The animals were sacrificed after 4 weeks of treatment. Blood was collected from the cardiac puncture and centrifuged for collecting plasma. The serum total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), blood urea nitrogen (BUN) and creatinine were measured using enzymatic colorimetric assay (Wako, Osaka, Japan). Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using Max Discovery™ assay kit (Bioo Scientific, Austin, TX, USA). Serum estradiol and testosterone were measured using ELISA kit (Abcam, Cambridge, MA, USA).

2.5. Liver histology

After blood collection, the liver was excised, weighed, and fixed in 10% neutral buffered formaldehyde solution for pathological examination.

2.6. Statistical analysis

Results were expressed as mean ± standard error of mean (SEM). Statistical significance was determined by one-way analysis of variance (ANOVA), Dunnett test and student’s *t*-test. *P* values less than 0.05 were considered significant.

3. Results

Quality of *S. torvum* was evaluated following Thai herbal pharmacopoeia. The results showed that the appearance of the *S. torvum* was brownish powder, water content with the value of (8.68 ± 0.07)% w/w, total ash with the value of (16.00 ± 0.06)% w/w, acid-insoluble ash with the value of (20 ± 0.02) w/w and water extractive value (88.43 ± 0.93)% w/w. The chemical identification of *S. torvum* was done using phytochemical screening and TLC technique. The phytochemical screening revealed that phenolic compounds and steroidal glycosides found in *S. torvum*. TLC chromatogram revealed steroidal glycoside in the *S. torvum* (Figure 1). The chemical extraction and TLC technique. The phytochemical screening revealed that phenolic compounds and steroidal glycosides found in *S. torvum*. TLC chromatogram revealed steroidal glycoside in the *S. torvum* (Figure 1). Heavy metal and microbial contamination were found to be below the quality control specifications.

![Figure 1. TLC chromatogram of *S. torvum* extract.](image-url)
Condition: Stationary phase: Silica Gel GF254, Mobile phase: EtOAc:MeOH:H2O = 50: 13.5:10; Detection: (A) UV 254 nm (B) UV 365 nm (C) Vanillin sulphuric acid spraying reagent.)
The effect of HFD and the *S. torvum* extract on body weight is shown in Table 1. At weeks 6th and 10th, all groups which received the HFD had a significant increase in body weight when compared with the SD control group. At week 10th (after receiving the *S. torvum* extract for 4 weeks), rats in the HFD-treated *S. torvum* groups had slightly reduced body weight compared to the HFD control group, but not in a statistically significant way.

At week 10th, the serum LDL, TC and TG concentrations of HFD control group were higher than that of the SD control group (Figure 2A–C, respectively). *S. torvum* extract at 100 mg/kg significantly reduced serum TC compared with HFD control group. The HFD group treated with *S. torvum* extracts showed a significant increase in liver weight. In liver histopathological change, lipid accumulation was found in the HFD control group as compared with the SD control group (Figure 4), but HFD treated with *S. torvum* showed lesser lipid accumulation.

In addition, *S. torvum* extract had an effect on testosterone and estradiol levels. The SD group which received the *S. torvum* extract at the dose of 400 mg/kg showed a slight increase whereas the HFD control group showed a decrease of testosterone and estradiol levels compared with the SD control group (Figure 3A and B). *S. torvum* extract at a dose of 100 mg/kg significantly increased levels of both sex hormones as compared to the HFD control group. In contrast, *S. torvum* extract at a dose of 200 and 400 mg/kg slightly decreased these sex hormones.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Weight gain in 4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Week 6</td>
</tr>
<tr>
<td>SD + DW</td>
<td>306.67 ± 5.58</td>
<td>420.00 ± 9.57</td>
</tr>
<tr>
<td>SD + ST 400</td>
<td>287.50 ± 2.81</td>
<td>424.17 ± 10.12</td>
</tr>
<tr>
<td>HFD + DW</td>
<td>294.17 ± 6.76</td>
<td>517.50 ± 8.54*</td>
</tr>
<tr>
<td>HFD + ST 200</td>
<td>288.33 ± 5.11</td>
<td>518.33 ± 15.42*</td>
</tr>
<tr>
<td>HFD + ST 400</td>
<td>294.17 ± 6.76</td>
<td>518.33 ± 15.42*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n = 6).*P < 0.05 when compared with SD group. SD: standard diet group; HFD: high-fat diet group; DW: distilled water.

### Table 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>Organ weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>SD + DW</td>
<td>11.80 ± 0.41</td>
</tr>
<tr>
<td>SD + ST 400</td>
<td>11.42 ± 0.66</td>
</tr>
<tr>
<td>HFD + DW</td>
<td>17.55 ± 0.63³</td>
</tr>
<tr>
<td>HFD + ST 100</td>
<td>16.41 ± 1.32³</td>
</tr>
<tr>
<td>HFD + ST 200</td>
<td>17.52 ± 1.15²</td>
</tr>
<tr>
<td>HFD + ST 400</td>
<td>16.53 ± 0.88⁴</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n = 6). *P < 0.05 when compared with SD group. SD: standard diet group; HFD: high-fat diet group; DW: distilled water.

**Figure 2.** Effect of *S. torvum* (ST) extract on serum LDL (A), total cholesterol (B) and triglyceride (C) in HFD fed male rats. Values are expressed as mean ± SEM (n = 6); ^*^ P < 0.05 when compared with SD control group; ^*^ P < 0.05 when compared with HFD control group; SD: Standard diet group; HFD: High-fat diet group; DW: Distilled water.

**Figure 3.** Effect of *S. torvum* (ST) extract on serum testosterone (A) and estradiol (B) in HFD fed male rats. Values are expressed as mean ± SEM (n = 6); ^*^ P < 0.05 when compared with SD group; ^*^ P < 0.05 when compared with HFD group; SD: Standard diet group; HFD: High-fat diet group; DW: Distilled water.
4. Discussion

Values are expressed as mean ± SEM (n = 6). SD: Standard diet group; HFD: High-fat diet group; DW: Distilled water.

Table 3

<table>
<thead>
<tr>
<th>Groups</th>
<th>BUN (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD + DW</td>
<td>19.98 ± 1.45</td>
<td>0.56 ± 0.01</td>
<td>108.00 ± 5.84</td>
<td>33.83 ± 3.02</td>
</tr>
<tr>
<td>SD + ST 400</td>
<td>18.17 ± 0.84</td>
<td>0.58 ± 0.03</td>
<td>120.67 ± 8.15</td>
<td>34.00 ± 2.34</td>
</tr>
<tr>
<td>HFD + DW</td>
<td>22.73 ± 1.25</td>
<td>0.57 ± 0.03</td>
<td>113.50 ± 12.19</td>
<td>46.17 ± 4.50</td>
</tr>
<tr>
<td>HFD + ST 100</td>
<td>18.78 ± 2.11</td>
<td>0.51 ± 0.03</td>
<td>112.50 ± 9.40</td>
<td>41.17 ± 6.34</td>
</tr>
<tr>
<td>HFD + ST 200</td>
<td>21.20 ± 0.90</td>
<td>0.51 ± 0.03</td>
<td>114.00 ± 6.52</td>
<td>39.50 ± 1.65</td>
</tr>
<tr>
<td>HFD + ST 400</td>
<td>17.72 ± 1.57</td>
<td>0.51 ± 0.03</td>
<td>108.33 ± 2.01</td>
<td>37.33 ± 2.23</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n = 6). SD: Standard diet group; HFD: High-fat diet group; DW: Distilled water.

As shown in Table 3, no significant differences of BUN, creatinine, AST and ALT values were found, indicating normal renal and liver functions in all groups.

4. Discussion

A diet containing high levels of cholesterol leads to the development of hyperlipidemia, atherosclerosis and abnormal lipid metabolism, these being important factors in the development of male reproductive dysfunction [14]. In the present study, feeding of a diet containing high content of cholesterol to male rats could increase body weight, serum TC, TG, and LDL indicating the hyperlipidemic condition. Interestingly, *S. torvum* extract at a dose of 100 mg/kg decreased the levels of TC and extract at doses of 200 and 400 mg/kg decreased the levels of TG compared to the HFD control group. This result is consistent with studies of Mohan et al. [15] which found the effect of ethanol extract of *S. torvum* at the doses of 100 or 300 mg/kg for 6 weeks reduced blood TG and TC levels in fructose hypertensive rats. The decreases in TC and TG levels were related to the histological change of liver, the deposit of lipid displayed less in HFD-treated *S. torvum* groups. Treatment of *S. torvum* extract slightly reduced body weight in the treated groups compared to the obese control group rats. Hyperlipidemia may affect the function of the reproductive system. Testosterone, the major male sex hormone, was found to be decreased in hypercholesterolemic rodents [16-18]. According to previous studies, mice given a HFD for a long time have a decreased level of the serum testosterone hormone [18,19].

It has been reported that low serum testosterone is associated with the formation of hepatic steatosis in men [20]. Our study found that the levels of testosterone and estradiol were decreased in the control HFD group as well. Interestingly, the HFD group which received *S. torvum* extract at the dose of 100 mg/kg had decreased cholesterol levels, but the levels of sex hormones were not decreased. Instead, testosterone level was significantly increased, almost to the level of the control SD group. However, treatment of *S. torvum* at doses of 200 and 400 mg/kg tended to decrease both sex hormones. Therefore, awareness should be measured when using high doses of *S. torvum* in hypercholesterolemic condition. However, the safety of *S. torvum* extract was also demonstrated using liver and renal function tests that showed no significant difference in BUN, creatinine, AST and ALT among all groups. Moreover, the weights of liver, kidney, testis and epididymis showed no significant change in all groups.

Boiling the fruit of *S. torvum* in hot water as a drink has historically been widely used in Thai culture as a remedy against hyperlipidemia and diabetes. In this study, the water extract from fresh unripe fruit of *S. torvum* was prepared and its activity tested. Since a monograph of the unripe fruit of *S. torvum* and its extract has never been reported in any herbal pharmacopoeia, the quality of *S. torvum* was evaluated using the protocol of Thai herbal pharmacopoeia to determine chemical and physical properties and contamination of heavy metal and/or microbes. Specification of the extract reported in this study could be used as a guideline for quality control of raw materials and its extract. In the present study, chemical groups found in the *S. torvum* and its extract were identified by phytochemical screening and chromatographic fingerprint using chemical reagents as one of the detection procedures. The results of TLC and phytochemical screening suggested that *S. torvum* may contain steroidal glycosides as a major component-previously reported in another study [7]. The previous studies reported that the methanol extract of *S. torvum* contained high levels of phenolic compounds, mainly rutin (1.36% w/w), cafffeic acid (12.03% w/w), gallic acid (4.78% w/w) and catechin (0.46% w/w) [10]. There are many bioactive compounds found in *S. torvum* with a positive effect in reducing hyperlipidemia in HFD induced obese condition, such as rutin [21,22], and cafffeic acid [23-25].
Therefore, the use of *S. torvum* extract may be useful for improving hyperlipidemia in obesity.

In conclusion, *S. torvum* extract has an important effect on the regulation of hyperlipidemia and impaired sex hormone levels in obesity. Moreover, the treatment of *S. torvum* extract did not affect liver and kidney function in rat model.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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References


