**Smilax chinensis** Linn. (*Liliaceae*) root attenuates insulin resistance and ameliorate obesity in high diet induced obese rat

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**Abstract**

High fat diet a prominent reason for obesity leads to overweight and insulin resistance. The objective of the present study was to evaluate antiobesity of *Smilax chinensis* through attenuating insulin resistance inn high fat diet induced obese rat. High fat diet induced obese rats were treated with *S. chinensis* methanol extract (SCME) for the 8 weeks at two different doses 100 and 200 mg /kg orally. Administration of SCME significantly decreased body weight, food intake and fat depot. Furthermore SCME reversed lipid metabolism to near normal and significantly decreased glucose, plasma insulin and leptin levels and decreased insulin resistance. Histological analysis of adipose tissue decreased the size of adipose tissue further proves the anti-obesity property of SCME.

**1. Introduction**

Obesity is characterized by excessive fat accumulation and a resultant imbalance in energy intake and expenditure. Excessive consumption of high fat diet (HFD) rich western food is one of the major factors contributing to obesity (Jequier 2002). Mounting data suggest that obesity is central dogma for altered lipid and protein metabolism results in various lives threatening disease (Cheetham et al., 2004; You et al., 2014). Several strategies have been implicated to combat and prevent obesity which includes medicinal plant source aiming to minimize the side effect. Natural products have aroused considerable interest in recent years as vital therapeutic agents to combat obesity. Several phytocompounds exhibits antiobesity property via different metabolism leads to shrinks adipocyte progress (Braithwaite et al., 2013).

*Smilax chinensis* Linn. (*Liliaceae*) is widely distributed in tropical America, Mexico, and South America. It was introduced into India in rain forests of South India, subsequently in Annanagar (w), Chennai, Mysore, Kerala, and Tamilnadu (Anonymous 1976). The extract prepared from root of *Smilax chinensis* L. has been used as analgesic, and to treat various diseases including anti-inflammation. Furthermore the roots of *Smilax chinensis* are used traditionally in the treatment of pyrexia and its antipyretic activity were found experimentally (Jana et al., 2009). Recently, some authors reported the hypoglycemic property of *S. chinensis* (Raju et al., 2012, Bhati et al., 2011). However, no scientific available on antiobesity property of *S. chinensis* root. HFD obese model is renowned model shows similar character of obesity related problems (Mooradian et al., 2000; Brown et al., 2002). Hence, the objective of the study was to ameliorate obesity through attenuation of insulin resistance using *S. chinensis* methanol extract (SCME) in HFD obese rats.

**2. Materials and methods**

2.1. Plant material

Roots of *S. chinensis* were acquired from the herbal market in Chennai. Dr. S. Somusundaram, National Institute of Siddha, Chennai, authenticated the root part. The voucher specimen was deposited in the herbarium at Entomology research Institute, Loyola College, Chennai.
2.2. Preparation of plant samples and extraction
The plant materials were washed thoroughly with water to remove the soil particles, air dried and powdered. One kilogram of plant material was extracted sequentially with methanol solvent (3 L for three times). The filtrates were concentrated under reduced pressure at 40°C and the extracts were stored in the refrigerator.

2.3. Animals and diet-induced obesity
Male Sprague Dawley (SD) rats (3 weeks of age) were obtained from Koatech, South Korea. The animals were housed in polypropylene cages, maintained at a constant temperature (22 ± 2°C) with rat chow and water. After 1 week of adaptation, the rats were divided into two groups, one fed with normal chow diet (Purina Co. Korea) and the other with a 60% high fat diet (HFD) (Research Diets, Inc., USA) for 12 weeks in order to induce obesity. All the experiments were carried out according to ethical procedures of Chonbuk National University (CBU-2014-002-1).

2.4. Experimental design
After 12 weeks, the rats were divided into four groups of six rats in each group according to the following design. Group I: normal control received normal rat chow
Group II: HF control
Group III: HFD + S.china 100 mg/kg
Group IV: HFD + S.china 200 mg/kg.
The extract was dissolved in water and administered orally once a day for the period of 8 weeks. A day before animal decapitation oral glucose tolerance and insulin tolerance test was carried out. At the end of 8th week the rats were sacrificed, the rats were kept fasted overnight; and then anesthetized with 400 mg/kg chloral hydrate. Initial and final body weight and food intake was calculated. Blood was collected; adipose tissue was collected and

2.7. Estimation of fasting plasma glucose and plasma insulin levels
Fasting blood glucose was estimated by the method of Trender, 1969. Serum insulin was determined by ELISA method. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as fasting glucose (mg/dL) x fasting insulin (µU/mL/243). Serum leptin was measured by immuno assay using a commercially available ELISA assay kit from BioVendor (Modrice, Czech Republic).

2.8. Estimation of lipid profile
Serum triglycerides (T.G), total cholesterol (T.C) and free fatty acids (FFAs) were determined using a commercially available kit (Asan Pharmaceutical Co, Seoul, Korea).

2.9. Histopathological analysis
For histopathological studies, the adipose fat tissues were collected from the adipose tissue and analyses were done by following the method of Kang et al. (2011).

2.9. Statistical analyses
Results are presented as mean ± S.D and comparison between groups was performed by one-way ANOVA. P<0.05 was considered significant.

3. Result
3.1. Effect of SCME on oral glucose tolerance test
Fig 1 (A) Oral administration of SCME 100 and 200 mg/kg with glucose gradually increased blood glucose levels in all the groups at 30 min and it remained unchanged over the period of 120 min in HFD control. Whereas SCME 100 and 200 mg/kg treated rats significantly (p≤0.05) suppressed the blood glucose levels (at 60 and 120 min) after glucose administration.

3.2. Effect of SCME on oral insulin tolerance test
In insulin tolerance test, oral administration of SCME 100 and 200 mg/kg significantly reduced the plasma glucose levels. After 60 min of insulin treatment the HF control rats exhibit only slight decrease in plasma glucose levels, whereas obese rats treated with SCME 100 and 200 mg/kg reduced plasma glucose level significantly Fig 1 (B).

3.3. Effect of SCME on serum glucose, plasma insulin, leptin
HFD fed rats exhibited significant increase in serum glucose, insulin, and leptin compared with normal control. In contrast, treatment with SCME 100 and 200 decreased serum glucose, insulin and leptin compared with HFD control rats.

3.4. Effect of SCME on body weight and food intake
The changes in body weight and food intake of differ-
ent groups of animal are summarized in (Table 1). HFD control groups exhibits enhanced body weight and food intake was observed in HFD control group compared to normal control. Furthermore obese rats treated with SCME 100 and 200mg/kg for eight weeks significantly decreased body weight and food intake in dose dependent manner compared to HFD control.

**3.5. Effect of SCME on plasma lipid profile**

Table 3 summarizes the TC, T.G, FFA of normal, HFD and SCME treated groups. Significant increase in lipid profile was absorbed in HF control compared with normal control rats.

**Table 1. Effect of SCME 100 and 200 mg/kg on body weight of obese rat.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Body weight (g)</th>
<th>Food Intake (g/day)</th>
<th>Fat Pad (% bw)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
</tr>
<tr>
<td>Normal control</td>
<td>330.10±20.32</td>
<td>417.20±23.30</td>
<td>57.56 ± 9.25</td>
</tr>
<tr>
<td>HF – control</td>
<td>512.30±40.30</td>
<td>569.40±33.30</td>
<td>11.10±0.28</td>
</tr>
<tr>
<td>HF+PLE 200mg/kg</td>
<td>504.20±41.40</td>
<td>537.30±39.60</td>
<td>8.14±0.10</td>
</tr>
<tr>
<td>HF+PLE 400mg/kg</td>
<td>516.40±49.30</td>
<td>521.30±35.30</td>
<td>6.4±0.15</td>
</tr>
</tbody>
</table>

*p < 0.05 vs HFD rats; **p < 0.01 vs HFD rats; # p < 0.05 vs Normal control rats; ## p < 0.01 vs Normal control rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal control</th>
<th>HFD - control</th>
<th>HFD- SCME100 mg/kg</th>
<th>HFD- SCME 200 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol(mg/dL)</td>
<td>63.04± 4.59</td>
<td>110.10± 3.25##</td>
<td>81.50± 7.18*</td>
<td>59.01±3.54*</td>
</tr>
<tr>
<td>Free fatty acid(mg/dL)</td>
<td>0.85 ± 0.10</td>
<td>2.07± 0.31#</td>
<td>1.31± 0.41*</td>
<td>1.07± 0.25*</td>
</tr>
<tr>
<td>Triglycerides(mg/dL)</td>
<td>67.45±3.54</td>
<td>114.10±7.52#</td>
<td>115.20±7.24*</td>
<td>87.52±5.24*</td>
</tr>
<tr>
<td>HDL-C(mg/dl)</td>
<td>42.84±4.59</td>
<td>30.34±6.14 b</td>
<td>33.50±3.98 c</td>
<td>37.81±4.93 d</td>
</tr>
</tbody>
</table>

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<th>HFD- SCME 200 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum glucose (mg/dL)</td>
<td>100.60±8.65</td>
<td>165.46±7.70#</td>
<td>145±8.15*</td>
<td>121±6.53*</td>
</tr>
<tr>
<td>Plasma Insulin (µU/mL)</td>
<td>41±2.54</td>
<td>81.28±6.52#</td>
<td>63.42±5.21*</td>
<td>47.65±5.25*</td>
</tr>
<tr>
<td>HOMO IR</td>
<td>1.69±0.14</td>
<td>5.52±0.45#</td>
<td>3.78±0.25*</td>
<td>2.37±0.21*</td>
</tr>
<tr>
<td>leptin (pg/mL)</td>
<td>312.5±112</td>
<td>450±98.20</td>
<td>401±8.40</td>
<td>350±61.70</td>
</tr>
</tbody>
</table>

*p < 0.05 vs HFD rats; **p < 0.01 vs HFD rats; # p< 0.05 vs Normal control rats; ## p< 0.01 vs Normal control rats
3.6. Histopathological analysis
Intake of high fat diet for 8 weeks resulted in a significant increase in the size of adipocytes and mesenteric and retroperitoneal fat deposits when compared to rats fed normal diet, which showed normal adipocyte architecture (Fig 1A, B). Treatment with vasicine acetate 20 and 50 mg/kg significantly decreased size of adipocytes compared to HF control (Fig 1, C, D).

4. Discussion
Several synthetic antiobesity drugs works with various metabolic pathways often coupled with adverse side effect (Lee et al., 2015). Presently, there has been growing interest in herbal products for treating diseases which shows promising potential in healing the disease enhance the functional food market globally (Braithwaite et al., 2013; Jung et al., 2014).

In the present study, anti-obesity effect of SCME was explored using a HFD-induced obese rat model. We observed drastic increase in body weight, food intake and fat deposit which is often considered as character of obesity. Administration of SCME decreased body weight gain decreased food intake and fat deposit associated with decreased fat cell size which clearly evidenced by histology of adipose tissue. Thus a decrease in body weight might be leads to reduction in fat mass and size of adipose tissue (Joo et al., 2010). Moreover, administration of SCME decreased leptin level plays vital role in regulating appetite control (Kang et al., 2011). Reports states that serum leptin concentration is accompanied with adipocyte and body fat content (Staiger and Haring 2005).

In addition to its weight reducing effect, SCME was also able to modulate some lipid metabolites including total cholesterol, triglycerides, and high density lipoprotein-cholesterol concentration-cholesterol and free fatty acid levels in HFD control rats. Obesity is often related with altered lipid profile, characterized by elevated triglycerides and High density lipoprotein-cholesterol concentration. Triglycerides are tangled in the accruing of lipid stores in the liver coupled with a metabolic syndrome (Bhandari et al., 2013). High TC and lower HDL-C level are risk factors for coronary heart disease (Chang et al., 2011). Thus, this result suggested that SCME would be obilging in the prevention of obesity complications via improving hyper lipidaemia. Further, SCME treatment showed improvements in the histological features of adipose tissue by reducing its size induced by HFD in rats. These results seemed to correspond to the white adipose tissue weights and serum lipid profiles. Obesity is associated with an impaired ability of tissue to respond to insulin and effectively store glucose. Further, in response to insulin resistance production is increased, leading to hyper-insulinemia, hyperglycemia and ultimately type 2 diabetes (Knight and Imig 2007). In our study, decrease in the serum glucose and insulin levels after the treatment with SCME in HFD-fed rats was observed. These results shows that SRME exert modulate insulin resistance leads to attenuate increased blood glucose and insulin in obese subject.

5. Conclusion
Conclusively, observed reduction in body weight gain, fat depots, serum lipids, glucose, insulin, leptin levels, and improved insulin resistance associated with decreased adipose tissue size which suggests that SCME possesses significant anti-obesity potential.

Conflict of interest statement
We declare that we have no conflict of interest.

Acknowledgment
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