Exploiting anti-obesity mechanism of *Clerodendrum phlomidis* against two different models of rodents

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Roots of *Clerodendrum phlomides* are used by the local people of Dibrugarh district of Assam state India as a dietary supplement for treating weight issues and are also mentioned in the traditional system of Indian medicine as a remedy for obesity. We examined the anti-obesity effect of *Clerodendrum phlomides* (family Verbenaceae) L. roots against cafeteria diet (CD) and progesterone-induced obesity. In CD-induced model obesity was induced by feeding CD for 48-days and increase in body weight and fat storage was suppressed co-administration with methanolic extract of *Clerodendrum phlomides* (MECP) at 400 mg/kg. Blood analysis showed that the levels of triglyceride and cholesterol were significantly lowered by MECP administration and there is subsequent rise in HDL-cholesterol level. From this experiment, we demonstrated that MECP is effective in ameliorating the CD-induced hyperglycemia, hyperinsulimenia, dyslepidemia, increase in wet weight of white adipose tissue, and hypertrophy of fat cells. In drug induced obesity model hyperphagia was induced by progesterone (10 mg/kg s.c.) for 28 days and was suppressed by co-administration with MECP in dose dependent manner. It is tempting to speculate that these protective effects shown by *Clerodendrum phlomides* is by multiple mechanisms. MECP contains β-sitosterol in the abundant quantity because of the structural similarity it do the physical competition with natural sterols while absorption of food stuffs from GIT and moreover the crude saponin and flavonoid has been reported for it’s the appetit suppressant property and hence reduces hyperphagia produced by progesterone. This is the first report demonstrating that *Clerodendrum phlomides* is effective in ameliorating insulin resistance and visceral obesity induced by CD and Progesterone.

Key words: Adipocyte, cafeteria diet, *clerodendrum phlomides*, hyperphagia, hypertrophy, progesterone

INTRODUCTION

Chronic obesity is a problem of epidemic proportions, and is rapidly increasing in prevalence in both the West and the Asia-Pacific region.[1-4] Obesity is often associated with type 2 diabetes mellitus, hypertension, dyslipidemia, and other cardiovascular disorders in the disease cluster termed the “metabolic syndrome.” In white populations, overweight and obesity are conventionally defined as a body mass index (BMI) ≥25 kg/m² and >30 kg/m², respectively.[1] Obesity is likely multifactorial in origin, with genetic, environmental, and physiologic factors contributing to various degrees in different individuals. Many attempts have been made to correct the metabolic disparity of the obesity condition, producing a number of reagents including Sibutramine (appetite suppressor), Orlistat (gastrointestinal lipid uptake inhibitor), and Fibrates (PPARα agonists).[5,6] However, administration of these drugs is known to often cause undesirable side effects such as dry mouth, anorexia, constipation, insomnia, dizziness, and nausea.[7] Therefore, there has been a high demand for therapeutically potent, and yet safe, anti-obesity reagents.

A large study of literature indicates that substantial progress has been made concerning our knowledge of bioactive components in plant foods and their links to obesity. For the present research protocol, we have chosen roots of *Clerodendrum phlomides* Linn to evaluate its anti-obesity activity and related complications like T2DM etc. As per the literature, flavonoids, sitosterols, tannins and saponines show promising effects to tackle obesity by various mechanisms, the selected plant have shown the presence of triterpenoids, flavonoids, and saponins etc. in their extract.[8] Moreover, traditional Indian medicine system also claims for its anti-obesity activity with this

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back ground we have selected these plants for its screening its anti-obesity. It has reported that the roots of plants are used for inflammation, swelling of the body, a chronic enlargement of the spleen or any glandular enlargement in the abdomen and various urinary disorders. The plant also used as bitter tonic, antidote, analgesic, anti-asthmatic for inflammatory disease, and in rheumatism. The root barks are used in cough, asthma, cold, oedema, and nervous disorders. Whole plant have reported for its anti-diabetic activity.

The neuroactive steroid, progesterone, is a female reproductive hormone. Its level increases during the later phase of the menstrual cycle and controls the secretory phase of the endometrium. Substantial evidence links progesterone excess in pathophysiology of eating and affective disorders. Some reports suggest the use of progesterone containing preparations as contraceptive or for the hormone replacement therapy to cause sufficient weight gain by causing hyperphagia and increased fat deposition in the body. Reports also suggest that progesterone can produce these effects by inducing myriad of neurotransmitter changes of which alterations of serotonin level can have important pathophysiological implications.

Cafeteria diets (CD) animal models of obesity have been reported to bear close resemblance to human obesity. It is well known that high fat intake and sedentary lifestyle, white collar jobs, lack of exercise etc. causes fat acclamation and increase in body weight. Cafeteria diet is the combination of different composition like supermarket highly palatable food. Cafeteria diets have been previously reported to increase energy intake and cause obesity in humans as well as animals.

With these settings we have selected progesterone and CD to induce obesity to anti-obesity activity of C. phlomidis in female mice and Wistar rats, respectively.

MATERIALS AND METHODS

Plant Authentication
Plant samples of the C. phlomidis (L) were collected in July 2007 from Amargardh-bichari Rajkot, India and verified by Prof. (Dr.) H.B. Singh, Head, Raw Materials Herbarium and Museum, NISCAIR, New Delhi, India. Duplicate herbariums were also retained in the Department of Pharmacognosy of Shree H. N. Shukla Institute of Pharmaceutical Education and research, Rajkot, for the future reference, the voucher no. of specimen is HNSIPER/herb/04.

Materials
Remi research centrifuge (R-24), Soxhlet extractor, OLYMPUS iNEA 5X, 10X/0.2; India, and 100X/1.25 oil India, HPTLC (CAMAG, Switzerland), Shimadzu UV-visible Spectrophotometer (UV1800), micr tone, Separate Open field model was fabricated based on earlier standard literature for rats and mice, Stat Fax autoanalyser (2000), Afco set digital balance (ER-180A), 250 μ pore nylon mesh, micro-pipette etc.

Chemicals
Sibutramine was gifted by the Ranbaxy Laboratory Ltd, Devas, MP (India), progesterone injection purchased from local market, Bovine Serum Albumin, Collagenase Type-1, Methylene blue, Oil red O, Trypsin, biochemical kits of Span diagnostics. All the chemicals used in the study were of analytical grade.

Methods
Preparation of extract
The dried roots of C. phlomidis were collected and powdered by using the pulverizer. The powdered drug was then passed through sieves # 40 and used for extraction process. Successive solvent extraction was carried out; a dried material is extracted with different solvents, starting from solvent of low polarity first with ethanol (95%) and then methanol (90%). After extraction by one solvent, material is removed from thimble, dried and recharged, extracted with solvent of successively high polarity. Successive solvent extraction was done by using methanol (Kokate, 1994).

The extract was filtered and concentrated by using rotary flash vacuum evaporator (ROTEVA, EQUITRON, Mumbai, India). The extract was dried in vacuum drier and stored below 10°C. The results of extractive values are presented in Table 1.

Animals
Forty-five (forty five) female Swiss albino mice and 5 months-old 45 (Forty five) Wistar female rats weighing around 80 to 120 g were uses for progesterone and CD-induced obesity respectively. All the animals were bred at central animal house Shree H. N. Shukla Institute of Pharmaceutical Education and Research. Rajkot- Gujarat and used in this experiment. Animal grouping and treatment schedule mentioned in Table 2a and b. Animals were housed in a standard controlled animal care facility in cages (5 animals/cage) and maintained in a temperature-controlled room (22–25°C, 45% humidity) on a 12:12-h dark–light cycle. The animals were maintained under standard nutritional and environmental conditions throughout the experiment. All the experiments were carried out between 9:00–16:00 hours.

Table 1: Extractive values

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Extractive values</th>
</tr>
</thead>
<tbody>
<tr>
<td>MECP</td>
<td>2.4±0.11</td>
</tr>
<tr>
<td>AECP</td>
<td>3.39±0.17</td>
</tr>
</tbody>
</table>

MECP – Methanolic extract of C. phlomidis; AECP – Alcoholic extract of C. phlomidis
at ambient temperature. Nations CPCSEA guidelines were strictly followed and all the studies were approved by the Institutional Animal Ethical Committee (IAEC), (Ref: IAEC/HNSIPER/RJK/05/2009) Shree H. N. Shukla Institute of Pharmaceutical Education and Research, Rajkot-Gujarat.

**Phytochemical investigation**

Phytochemical investigation was carried out as per the method prescribed by Kokate (1994).\[^{19}\] Results of phytochemical investigations are given in Table 3.

**Estimation of total flavonoid content**

Flavonoid concentration was determined as per Chang,\[^{20}\] et al. Known volume of methanolic extract was diluted with 80% aqueous ethanol (0.9 ml). Aliquot of 0.5 ml was added to test tube containing 0.1 ml of 10% aluminum nitrate, 0.1 ml 1 M aqueous potassium acetate and 4.3 ml of 80% ethanol. After 40 min at room temperature the absorbance was determined at 415 nm with UV spectrophotometer. Total flavonoid content was calculated according to a standard curve established with Quercetin. Results are displayed in Table 4.

**Estimation of total saponin glycosides**

Total saponin content of both the extract was carried out by method prescribed by Rajpal, 2002.\[^{21}\] Total saponin content was found to be 0.121% w/w and 0.108 %w/w for methanolic and ethanolic respectively. Results are displayed in Table 4.

**Thin layer chromatography study of MECP**

The stationary phase and mobile phase set for β-sitosterol as per the Harborne, J.B.\[^{22}\] Thin layer chromatography (TLC) for β-sitosterol - Plate dimension : 5 × 15 cm.

Stationary phase : Silica gel G for TLC.

Sample preparation : 5 mg methanolic extract was dissolved in 5 ml of acetone

Mobile phase : Various solvent systems have been tried for optimization of better resolution mainly using chloroform and ethyl acetate as the methanol extracts contain sterols, which are non-polar to semi polar in nature

Visualisation : Spraying by 20% Sulphuric acid in methanol.

Treatment after spraying : Heated in oven at 105°C for 2-5 min.

TLC profile of methanolic extract of *C. phlomidis* (MECP) for β-sitosterol is given in Table 5.

**Toxicity Study**

**Procedure**

Acute toxicity studies were performed according to OECD-423 guidelines category IV substance (acute toxic class method). Albino mice (n=3) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 hrs with free access to water only. The plant extracts of *C. phlomidis* were administered orally with an initial dose of 1000 mg/kg body weight. The mortality was observed for three days. If mortality was observed in 2/3 or 3/3 of animals, then the dose administered was considered as a toxic dose. However, if the mortality

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### Table 2a: Animal grouping and diet

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Vehicle + normal standard diet</td>
</tr>
<tr>
<td>02</td>
<td>Vehicle + CD</td>
</tr>
<tr>
<td>03</td>
<td>AECP (100 mg/kg) + CD</td>
</tr>
<tr>
<td>04</td>
<td>AECP (200 mg/kg) + CD</td>
</tr>
<tr>
<td>05</td>
<td>AECP (400 mg/kg) + CD</td>
</tr>
<tr>
<td>06</td>
<td>MECP (100 mg/kg) + CD</td>
</tr>
<tr>
<td>07</td>
<td>MECP (200 mg/kg) + CD</td>
</tr>
<tr>
<td>08</td>
<td>MECP (400 mg/kg) + CD</td>
</tr>
<tr>
<td>09</td>
<td>Sibutramine 10 mg/kg + CD</td>
</tr>
</tbody>
</table>

CD – Cafeteria diet; MECP – Methanolic extract of *C. phlomidis*; AECP – Alcoholic extract of *C. phlomidis*

### Table 2b: Treatment schedule

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Vehicle + Normal standard diet</td>
</tr>
<tr>
<td>02</td>
<td>Vehicle + Prog.</td>
</tr>
<tr>
<td>03</td>
<td>AECP (100 mg/kg) + Prog.</td>
</tr>
<tr>
<td>04</td>
<td>AECP (200 mg/kg) + Prog.</td>
</tr>
<tr>
<td>05</td>
<td>AECP (400 mg/kg) + Prog.</td>
</tr>
<tr>
<td>06</td>
<td>MECP (100 mg/kg) + Prog.</td>
</tr>
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<td>MECP (200 mg/kg) + Prog.</td>
</tr>
<tr>
<td>08</td>
<td>MECP (400 mg/kg) + Prog.</td>
</tr>
<tr>
<td>09</td>
<td>Sibutramine 10 mg/kg + Prog.</td>
</tr>
</tbody>
</table>

Number of groups with their treatment schedule is presented; MECP – Methanolic extract of *C. phlomidis*; AECP – Alcoholic extract of *C. phlomidis*; Prog – Progesterone

### Table 3: Phytochemical investigation

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>MECP</th>
<th>AECP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Anthocynidine</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

+indicates presence of constituents; –indicates absence of constituents; MECP – Methanolic extract of *C. phlomidis*; AECP – Alcoholic extract of *C. phlomidis*

### Table 4: Total flavonoids and saponin content

<table>
<thead>
<tr>
<th>Secondary metabolites</th>
<th>MECP %w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total flavonoid content</td>
<td>0.388</td>
</tr>
<tr>
<td>Total saponin content</td>
<td>0.121</td>
</tr>
</tbody>
</table>

MECP – Methanolic extract of *C. phlomidis*
was observed only one mouse out of three animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher dose.[23] Results are presented in results section.

Induction of experimental obesity by using cafeteria diet
The cafeteria diet consisted of 3 diets (condensed milk, 48 g + bread, 48 g), (chocolate, 18 g + biscuits, 36 g + dried coconut, 36 g), (cheese, 48 g + boiled potatoes, 60 g). The three diets were presented to group of 6 rats on day 1, 2 and 3 respectively and then repeated in same succession till 48 days. The diet was provided in addition to normal pellet chow. The test drugs were administered half hour earlier to cafeteria diet presentation. The control animals received only the vehicle in the same volume.[24]

Induction of experimental obesity by using progesterone
Progesterone vial contents were dissolved in arachis oil and dose of 10 mg/kg was administered subcutaneously in the dorsal neck region to mice for 28 days, control group received the vehicle. All drugs were given at a dose of 0.4 ml/100 gm body weight. The test drugs were injected 30 minutes before to progesterone administration.

Test drug preparation
MECP, Methanolic extract of C. phlomidis (AECP), and standard sibutramine are soluble in water so distilled water was used as media to dissolve. For progesterone arachis oil was used as a vehicle and diluent for appropriate doses. All the drug concentration were prepared freshly just before administration. All the test drugs including standard were given by oral gavages by p.o. route.

Evaluation
Assessment of food consumption behavior in mice
Food consumption behavior was studied only in mice model. The food intake studies were carried out on days 1, 7, 14, 21 and 28. The mice were deprived of food 1 h prior to experimentation and the test feed for the feeding experiments was standard mice chow modified for palatability by adding 10% of sucrose. On experimental days, 30 min after last drug administration, 10 g of sweetened chow was presented to groups of mice in glass Petri dishes and food intake was recorded at 0.5, 1 and 2 h time intervals. Nearest to 0.1 g with correction for spillage and the amount of food consumed/20 g body weight was calculated.[25]

Body weight
The body weight of rats and mice (g) were recorded for every week for 48 days and 28 days, respectively. Weighing was done in each group just before dosing by using precision balance of 10 mg sensitivity.

Exploratory behaviour
In rat it was recorded on day 48 using open field behaviour test apparatus and 30 min after test drug administration to treatment groups. The apparatus consists of floor of white tiles dividing field into 25 squares (15×15 cm) and wall with a height of 25 cm and in mice it was recorded on day 28 using open field behaviour test apparatus and 30 min after test drug administration to treatment groups. Fabricated chess board (black and white squares) was used for mice consists of 64 squares (5×5 cm) with wall of 15 cm. Open field test was performed by placing the rat/mouse in the center and recording the ambulatory activity (squares crossed by horizontal movement), the frequency of rearing (standing up vertically) and grooming (face washing and repetitive licks directed to body). The rat/mouse was placed in the center of the field and observed for 5 min. Animals were kept under laboratory condition 1 h prior to test. Between the trials the field was cleaned with wet sponge and tissue papers. Behavior was recorded by a handy cam (Sony) for noting exact reading and storage of data.

Body temperature
The body temperature of rats and mice was recorded on 49th and 29th day, respectively by using rectal telethermometer, before and after drug administration at 1 and 2 hrs. After measuring the body weight, each animal was placed in specially designed restricter to measure rectal temperature. A Yellow Spring Instrument telethermometer with a series 500 probe was used. The probe was lubricated using petroleum jelly prior to use and was inserted between 2.0 to 2.5 and 1.0 and 1.3 cm in rats and mice, respectively, into the rectum and held in position for 10 seconds before temperature was determined. Measurements were made once for each animal and were conducted during 2-hour period 4 hours before light offset.

Biochemical Parameters
Preparation of serum
Twenty-four hours (49th day of study for rats; 29 day of study for mice) after the last administration of test drug animals were anaesthetized under light ether anesthesia and blood for serum preparation was collected by retro orbital puncture, using 10 µl × 20 mm (L) × 0.8 mm (2R)

Table 5: Description of thin layer chromatography pattern for methanolic extract of C. phlomidis

<table>
<thead>
<tr>
<th>Color of spot (after spray)</th>
<th>MECP</th>
<th>Std.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow</td>
<td>0.34</td>
<td>–</td>
</tr>
<tr>
<td>Brown</td>
<td>0.48</td>
<td>–</td>
</tr>
<tr>
<td>Pink</td>
<td>0.59</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Best resolution and separation was observed with chloroform and ethyl acetate (8:0.6). The TLC profile has revealed the presence of seven spots after spraying with 20% methanolic sulphuric acid. MECP – Methanolic extract of C. phlomidis
glass capillary into sterile EDTA-coated tube (3 mg/ml) for the estimation. Blood was kept in wet ice for 30 min, centrifuged for 5 min at 4000 rpm at 4°C (REMIMAK, India) and plasma was aspirated out for the analysis of lipid profile and glucose. The serum was stored in the refrigerator for the analysis of biochemical parameters. All analyses on serum were completed within 24 h of sample collection. Serum samples were analyzed for glucose, triglyceride and total cholesterol, using biochemical kits of Span diagnostics Glucose: GOD/POD method;[26] Cholesterol: one step method of Wybenga and Pilleggi;[27] Triglycerides: GPO-PAP, end point method[28] and HDL-C by CHOD-PAP method.[27] Results are given in Table 6.

**Effect on white adipose tissue**

The rats and mice were euthanised by ether over dose on day 50 and 29, respectively. Then white adipose tissue (WAT) (periovarian, perirenal, mesenteric fat and Omental fat pad) were isolated. After weighing of fat content it was preserved for the further study. Adiposity index, a quantitative measure of total fat mass was calculated by using the previously determined equation derived by[29] Adiposity Index (%) = [Σ (fat pad)/Body weight] × 100

**Isolation of fat pads and experimental procedure**

Four regions of adipose tissue were carefully dissected:

1. The periovary fat, ovaries were taken out by gentle squeezing from the peripheral fat and then by horizontal cut from all sides, fat was isolated; care has been taken that too much traction was avoided on ovaries and fat.
2. The retroperitoneal, by first separating the perirenal fat and then dissecting the retroperitoneal pad in toto.
3. The mesenteric, all fat found along the mesentery starting at the lesser curvature of the stomach and ending at the sigmoid colon was considered mesenteric fat; obtained by cutting the intestine below the duodenal-jejunum junction and stripping the fat by gently pulling the intestinal loops apart.
4. The Omental, by separating its large fold which hangs down from the stomach and extends from the stomach to the posterior abdominal without producing any harm to the peripheral portion of the tissue.

**Table 6: Effect of AECP and MECP on progesterone modulated serum biochemical parameters in female mice**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glucose (mg/dl)</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Vegetable oil)</td>
<td>128.67±14.01</td>
<td>100.17±16.75</td>
<td>79.905±7.29</td>
<td>27.06±5.055</td>
<td>57.29±16.61</td>
<td>15.81±1.457</td>
<td>137.23±6.51</td>
<td>68.88±6.99</td>
</tr>
<tr>
<td>Progesterone</td>
<td>168.33±10.19</td>
<td>133.17±11.37</td>
<td>147.15±13.23</td>
<td>19.28±0.713</td>
<td>84.45±13.01</td>
<td>29.43±2.646</td>
<td>153.68±11.77</td>
<td>97.25±9.31</td>
</tr>
<tr>
<td>AECP (100 mg/kg) + Pro.</td>
<td>165±10.88</td>
<td>135.42±11.39</td>
<td>126.73±4.81</td>
<td>19.75±0.889</td>
<td>90.31±10.52</td>
<td>25.34±0.962</td>
<td>148.47±8.03</td>
<td>108.23±2.21</td>
</tr>
<tr>
<td>AECP (200 mg/kg) + Pro.</td>
<td>155±16.96</td>
<td>123.12±11.60</td>
<td>127.93±8.30</td>
<td>20.12±0.989</td>
<td>77.406±25.815</td>
<td>25.586±1.659</td>
<td>134.33±16.27</td>
<td>101.97±5.04</td>
</tr>
<tr>
<td>AECP (400 mg/kg) + Pro.</td>
<td>152.8±11.52</td>
<td>124.85±13.6</td>
<td>130.4±8.03</td>
<td>20.21±0.856</td>
<td>78.543±12.84</td>
<td>26.093±1.606</td>
<td>143±14.64</td>
<td>100.9±6.37</td>
</tr>
<tr>
<td>MEC (100 mg/kg) + Pro.</td>
<td>159.6±16.24</td>
<td>156.23±10.9</td>
<td>150.45±16.84</td>
<td>19.59±1.013</td>
<td>106.55±9.224</td>
<td>30.09±3.368</td>
<td>175.11±9.17</td>
<td>142.67±24.82</td>
</tr>
<tr>
<td>MEC (200 mg/kg) + Pro.</td>
<td>137.35±9.63</td>
<td>147.18±14.65</td>
<td>113.43±9.28</td>
<td>21.31±1.024</td>
<td>103.18±14.63</td>
<td>22.68±1.856</td>
<td>144.2±14.04</td>
<td>141.58±14.54</td>
</tr>
<tr>
<td>MEC (400 mg/kg) + Sibutramine</td>
<td>118.3±5.69</td>
<td>126.01±7.67</td>
<td>94.6±6.11</td>
<td>26.89±1.045</td>
<td>80.165±8.00</td>
<td>18.96±1.22</td>
<td>109.12±21.06</td>
<td>107.03±18.35</td>
</tr>
<tr>
<td>10 mg/kg + Pro.</td>
<td>135.33±6.63</td>
<td>54.4±4.75</td>
<td>57.37±5.95</td>
<td>30.97±0.841</td>
<td>30.97±0.841</td>
<td>11.47±1.18</td>
<td>114.7±12.16</td>
<td>80.97±7.56</td>
</tr>
</tbody>
</table>

**Isolation and Sizing of Fat Cells**

**Adipocyte isolation**

Periovarian adipocytes were isolated by using trypsin and centrifuged as pre the previous method described by Honnor et al. with slight modifications. The samples were provided with (5% CO₂ and 95% O₂), capped and incubated at 37°C with shaking until digestion was complete (30–40 min).

**Sizing of fat cells**

Add 0.2 to 0.4 ml aliquots of the stirred suspension of stained cells were placed on a siliconized glass slide and examined with a Zeiss microscope equipped with a Polaroid camera attachment. The insertion of a micrometer disc in a focusing eyepiece placed in the phototube of the camera attachment produced a projected caliper scale. At a magnification of ×200, the caliper scale was calibrated so that the unit marks had a constant interval of 7 μ. The free fat cells, floating on...
the surface of the medium, were recognized by the spherical shape, the stained nucleus with one or two nucleoli, and the stained cytoplasm; the latter features readily distinguished the fat cells from occasional droplets of floating lipid. One hundred cells one by one from the same population were brought in the caliper field with systematic motion of the stage control knobs. The cells were aligned on the caliper scale, the equatorial plane of the cell was brought into focus and the fat cell diameter was determined with accuracy. The sizing and grouping of 100 fat cells was performed by one observer in approximately 15–20 min. From this, the mean diameter and the standard deviation about the mean could be rapidly calculated by the usual formulas.

**Histology of fat pad**
The periovarian fat was selected for histological study. The periovarian fat of each group including rat and mice were excised and rinsed in 0.9% saline blotted dry of saline and excess blood. They were fixed in 12% formalin for 24 hr. The tissues, after fixation, were washed in water to remove excess fixative. Washed tissues were then dehydrated through a graded series of ethyl alcohol, cleared with xylene and embedded in paraffin wax. Sections were cut at 3 µm with microtone blade and mounted on clean glass slide. The sections were routinely stained with haemotoxyllin and eosin. The stained slides were observed (×200) in research microscope and photographed.

**Statistical Analysis**
The results are expressed as mean±SEM. Comparisons between the treatment groups and positive control; positive control and control were performed by one way analysis of variance (ANOVA) followed by Dunnett’s t-test. In all tests the criterion for statistical significance was \( P<0.05 \) (95% level). The analysis was performed by using GraphPad Prism 4.

\( P \) value <0.05 is considered as significant \(*P<0.05, **P<0.01\).

**OBSERVATIONS AND RESULTS**

**Toxicity Study**
The plant extracts of *C. phlomidis* did not show any mortality and toxicity even at highest dose of 2000 mg/kg body weight employed so \( LD_{50} \) value is expected to exceed 2000 mg/kg body weight.

**DISCUSSION**

Obesity is a chronic metabolic disorder that results from the imbalance between energy intake and energy expenditure characterized by enlarged fat mass and elevated lipid concentration in blood. Many attempts have been made to correct the metabolic disparity of the obesity condition, producing a number of reagents including fibrates, sibutramine (an anorectic or appetite suppressant) and Orlistat but no drug is free from severe side effects.\[^{31-33}\]

At present, because of dissatisfaction with high costs and potentially hazardous side effects, the potential of natural products for treating obesity is under exploration and this may be an excellent alternative strategy for developing future effective, safe anti-obesity drugs. A variety of natural products including crude extracts and isolated compounds from plants, can induce body weight reduction and prevent diet-induced obesity. Therefore, they have been widely used in treating obesity.\[^{34,35}\]

Several infusions or decoctions of plants used in traditional medicine to reduce obesity could be utilized to delete the clinical side effects of the current chemically formulated anti-obesity agents; examples include *Camellia sinensis* (L.) Kuntze (Theaceae), *Chlorella pyrenoidosa* Chick. (Oocystaceae), *Citrus aurantium* L. (Rutaceae), *Garcinia cambogia* L. (Clusiaceae), *Lagerstroemia speciosa* (L.) Pers. (Lythraceae), *Panax ginseng* C.A. Meyer (Araliaceae), *Salix matsudana* Koidzumi (Salicaceae), *Nelumbo nucifera* Gaerthn. (Nymphaeaceae), and *Hibiscus sabdariffa* L. (Malvaceae).\[^{36-38}\]

*Clerodendrum phlomidis* Linn. (Family: Verbenaceae) is a large bush or small tree, reported to possess potent spasmyotic and anti-diabhoerial effect. The plant is commonly used as an anti-fungal and anti-pyretic agent.\[^{39}\] The juice of leaves is used as an alternative and bitter tonic. The decoction of root is slightly aromatic and astrigent is used as a demulcent in gonorrhea.\[^{40}\] The plant has been found to possess hypoglycemic activity.\[^{41}\] Rural people of Tamil Nadu use fresh juice of the leaves of the plant to treat mental tension and mental disturbance. Methanolic extract of leaves of *Clerodendrum phlomidis* was found to have an effect on alteration in general behavioral profiles, including alertness, awareness, spontaneous activity, touch, pain and sound responses in mice and showed its characteristics as minor tranquilizer.

In our study initially pharmacognostic and phytochemical analysis was carried out; data not shown here. In the phytochemical investigation it was clear that *C. phlomidis* contains triterpenoids, glycoside, carbohydrates, proteins, flavonoids, anthocynidine and saponins as shown in Table 1. Some of the chemical constituents like saponines, flavonoids and some triterpenoids have reported for its anti-obesity effect in various plants.\[^{38}\] Based on this phytochemical study and ethnobotanical claims this plant was selected to carry out this study.

In the present study we have studied the effects of methanolic and alcoholic extracts of *C. phlomidis* against progesterone and CD induced obesity for 28 and 48 consecutive days.
The neuroactive steroid progesterone is a female reproductive hormone. Its level increases during the second part of the menstrual cycle and control the secretory phase of endometrium. As the name suggests, (Pro = for, gest = gestation), the higher endogenous levels of progesterone and its metabolites in pregnant women are reported to enhance food ingestion throughout pregnancy and conserve energy for the growing fetus. Progesterone also exerts anti-esterogenic effects, which also been shown to increase in food intake. Further, some reports suggest that use of progesterone containing preparation as contraceptive or for hormonal replacement therapy to cause significant weight gain by increasing fat deposition. Furthermore, progesterone has been reported as the most fattening of steroids hormone that promotes synthesis and storage of fats. Therefore, progesterone-induced hyperphagia causes weight gain and fat deposition is useful as animal model of drug-induced obesity. Our results demonstrated that progesterone (10 mg/kg) induced hyperphagia the results are consistent with the reported dose dependent increase in food intake with progesterone and maximum effect at 10 mg/kg dose.[42]

Rats that are fed cafeteria diet (CD) are a widely used model of obesity. The so called ‘cafeteria diet’ involves feeding experimental animals a mixture of palatable commercially available supermarket foods to stimulate energy intake.[14] Characteristic for such diets is the combination of the high fat content with high carbohydrate content. Further, the components of the cafeteria diet are a variety of foods high in fat and sugar but usually low in protein, vitamins and minerals. Such diets have pronounced implications in the development of obesity, leading to significant body weight gain, fat deposition and also insulin resistance resembling to human beings.[43] It has been suggested that rats become more obese with cafeteria diets than with pure high fat diets and normal chow diet, indicating a greater hyperphagia arising from the food variety, texture and palatability.[44] Because of the above mentioned facts the ‘cafeteria diet’ model is considered for the study.

In the study conducted by Kaur G and Kulkarni SK, 2000,[25] have also reported the subchronic treatment with progesterone for 4 weeks to significantly increase food intake and body weight in female mice. The progesterone induced group has showed significant increase in food consumption as compared to the normal control group animals at 30 min, 1 hr and 2 hr, which was significantly decreased by the co-administration of MECP 100, 200 and 400 mg/kg compared to plane progesterone treated group, standard sibutramine was most significant compared to plane progesterone treated and control group [Figure 1].

It is believed that progesterone producing hyperphagia via progestin receptors, which have been reported to be expressed on the serotoninergic neurons[44] and sibutramine suppresses the progesterone-induced hyperphagia by inhibiting reuptake of 5-HT (serotonin) at the hypothalamic site which regulate the food intake, which suggests the possible interaction exists between the neurosteroid and serotonin receptor system in regulating food intake and body weight. Further, these data implicate that disturbances in the ovarian hormone levels may predispose females to eating disorders by causing alterations in the serotonin level or serotoninergic receptor function.[25] The reduction in the food intake by the administration of MECP at medium and high dose is may be due to its saponin and flavonoid content; these phytoconstituents are present in abundant quantity which is confirmed by total saponin and total flavonoid contents of the extract as given in Table 4. Crude saponin and flavonoid has been reported for its the appetite suppressant property.[9] From this study we are predicting that saponin and flavonoids after absorption from GIT it cross the blood brain barrier (BBB) and enter in the brain and amplify signaling in the basal hypothamus energy sensing function, which is the master regulator of food intake and energy expenditure or it may also possible that saponin inhibits the re-uptake of 5-HT in the hypothalamus. Some flavonoids also cause to activate β-adrenergic receptors which are involved in the burning of fats.[10]
Plane progesterone induced group have shown significant increase in body weight compared with control group but co-administration with medium and high dose of MECP have shown significant reduction in the body weight compared with plane progesterone induced group as given in Figure 2. The reduction in the body weight by MECP may be because of its anorexic property as explained above.

In CD-induced obesity model there is significant weight gain compared with normal chow diet feed rats, which is significantly decrease by co-administration with MECP in a dose dependent manner. This increase in weight gain in CD-treated group is may be because of its more palatability. Standard sibutramine have shown most significant results in treated group is may be because of its more palatability. This increase in weight gain in CD-induced obesity model there is significant weight gain as shown in both the models, i.e., progesterone and CD-induced obesity, as shown in Figures 3 and 4.

The thermogenic effect of natural progesterone is well known and progesterone treatment has also been reported to increase the body temperature, so measurement of rectal temperature recording was considered one of the parameter in this study. Drugs which demonstrating such changes indicating the thermogenic property of the drugs, but in our study we have not observed any significant changes in body temperature even in the standard sibutramine as well as in plane progesterone induced group [Figure 5].

Consumption of CD results in overweight animals but also increased heat production through diet-induced thermogenesis, so measurement of rectal temperature recording was considered one of the parameter in this study. It is well known that an increase in food ingestion results in the activation of both heat production and deposition of reserves mainly fat. The drugs which demonstrating such changes indicating the thermogenic property of the drugs. In CD model as presented in

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**Figure 2:** Progesterone induced showed significant % increase in the body weight as compared to normal control animals which was significantly decreased in MECP 100, 200 and 400 mg/kg in dose dependent manner and most significantly by Sibutramine.

**Figure 3:** Animals fed with CD showed significant increase in the body weight as compared to normal control animals which was significantly decreased in MECP 100, 200 and 400 mg/kg in dose dependent manner and most significantly by Sibutramine compared to normal control and disease control animals.

**Figure 4:** Animals fed with CD showed significant increase in the body weight as compared to normal control animals which was significantly decreased in MECP 100, 200 and 400 mg/kg in dose dependent manner and most significantly by Sibutramine compared to normal control and disease control animals.

**Figure 5:** There wasn’t any significant change found in the body temperature in any of the group of animals.
Figure 6, administration of high dose of MECP have shown significant rise in body temperature after 1 hr and 2 hr of administration then normal control and plane CD feed rats. Medium dose of MECP have shown rise in body temperature after 1 hr and 2 hr of administration compared to normal control rats. Treatment with standard sibutramine has shown most significant result in rise in body temp. compared with normal control and plane CD-treated group.

Exploratory behavior was performed on 28 th and 48 th day of the study in drug and food induced obesity, respectively. No significant changes have been observed in the exploratory behavior in progesterone induced obesity as seen in Figure 7. In CD-induced obesity administration high dose of MECP and standard sibutramine have shown significant increase in ambulation and grooming compared to plane CD feed and normal control rats. Administration of low and medium dose of MECP have shown significant rise in rearing and grooming compared with normal control rats. The increases in rectal temperature and ambulatory activity by MECP may be attributed to the overall stimulant and thermogenic property of phyto-constituents of the extracts [Figure 8].

Progesterone is also reported to exert various metabolic effects such as rising basal insulin levels, stimulating lipoprotein lipase activity and enhancing fat storage in the body. In this study progesterone modulated various biochemical parameters in female mice. It caused significant increase in the serum glucose, Triglycerides (TG) and very low density lipoprotein cholesterol (VLDL-C) levels and decrease in HDL-C levels as compared to the normal control animals which were significantly reversed by co-administration of MECP 100, 200 and 400 mg/kg as well as standard sibutramine as given in Table 6.

Feeding with CD caused significant increase in the serum glucose, insulin, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), TG, total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and fall in HDL-C levels as compared to normal control animals which was significantly decreased by the administration MECP 200 and 400 mg/kg. Sibutramine was most significant in this case. Increase in the glucose as well as serum insulin level indicates obesity induced insulin resistance DM (T2DM) Table 7.

Several studies show that an increase in HDL cholesterol is associated with a decrease in cardiovascular risk which is a major complication of obesity associated dyslipidemia and most of the drugs that decrease total cholesterol also decrease HDL cholesterol.[48,49] But in the present study the extract decreased the total cholesterol and LDL cholesterol
and enhanced the HDL cholesterol significantly. This is an important advantage in treatment of hypercholesterolemia especially among Indians where low HDL cholesterol is the prevalent lipoprotein abnormality.\cite{50,51}

The decrease of serum TG level is an important finding of this experiment. Recent studies shown that triglycerides are independently related to obesity induced cardiovascular complications\cite{52,53} and most of the anti-hypercholesterolemic drugs (can also be used to correct obesity associated dyslipidemia) do not decrease triglycerides levels but MECP 400 mg/kg has lowered it significantly by 21.83% compared to CD group.

There was significant increase in the organ/body weight ratio of liver, left kidney and right kidney in progesterone treated animals which were significantly decreased by the co-administration of MECP 200 and 400 mg/kg compared to plane CD feed rats. Sibutramine was the most significant in this regard. \*Comparison of test and disease control with normal control

From the histological study it was clear that adipocyte diameter is clearly reflected high in plane progesterone and plane CD feed rats compared to normal control groups. Sibutramine was the most significant in this regard [Figures 11 and 12].

In both the models correction in lipid profile, decrease in adipocyte diameter and adiposity index by MECP is may be because of \(\beta\)-sitosterol content which is confirmed by TLC.

### Table 7: Effect of \textit{C. phlomidis} on biochemical parameters in cafeteria diet fed Wistar rats

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Treatment</th>
<th>Glucose (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>TC (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gp I</td>
<td>Normal diet</td>
<td>42.8±2.7</td>
<td>99.2±6.3</td>
<td>91.6±4.9</td>
<td>33.8±1.06</td>
<td>39.6±4.199</td>
<td>18.1±1.27</td>
<td>65.1±1.52</td>
<td>29.4±0.719</td>
</tr>
<tr>
<td>Gp II</td>
<td>Cafeteria diet</td>
<td>76.7±3.1**</td>
<td>106±3.8</td>
<td>105.6±4.2</td>
<td>26.1±3.1**</td>
<td>58.2±2.914</td>
<td>21.2±0.76</td>
<td>101.0±0.754</td>
<td>51.4±1.239**</td>
</tr>
<tr>
<td>Gp III</td>
<td>AECP (100 mg/</td>
<td>80.8±6.3**</td>
<td>143.6±4.7</td>
<td>102.6±6.3</td>
<td>25.3±2.1**</td>
<td>48.5±6.933</td>
<td>28.7±6.94</td>
<td>98.4±3.834</td>
<td>52.3±8.954**</td>
</tr>
<tr>
<td>Gp IV</td>
<td>AECP (200 mg/</td>
<td>81.3±6.2**</td>
<td>135.9±5.9</td>
<td>110.3±5.6</td>
<td>24.1±3.18**</td>
<td>58.9±4.753</td>
<td>27.1±7.18</td>
<td>97.4±6.177</td>
<td>51.1±8.121**</td>
</tr>
<tr>
<td>Gp V</td>
<td>AECP (400 mg/</td>
<td>74.1±8.7**</td>
<td>92.6±6.2</td>
<td>106.8±6</td>
<td>26±1.32*</td>
<td>62.3±5.155</td>
<td>18.5±1.24</td>
<td>98.125±1.597</td>
<td>51.4±2.136**</td>
</tr>
<tr>
<td>Gp VI</td>
<td>MECP (100 mg/</td>
<td>89.9±9.3**</td>
<td>109.9±5.8</td>
<td>105.7±3.7</td>
<td>30.8±1.52</td>
<td>52.8±6.675</td>
<td>21.9±2.36</td>
<td>100.02±0.969</td>
<td>51.90±1.03**</td>
</tr>
<tr>
<td>Gp VII</td>
<td>MECP (200 mg/</td>
<td>73.7±6.2**</td>
<td>93.9±4.3</td>
<td>100.3±4.3</td>
<td>31.8±1.444*</td>
<td>49.6±5.365</td>
<td>18.7±0.86</td>
<td>80.26±2.863</td>
<td>48.45±1.515**</td>
</tr>
<tr>
<td>Gp VIII</td>
<td>MECP (400 mg/</td>
<td>70.6±6.5*</td>
<td>87.9±3.9</td>
<td>97.4±6.4</td>
<td>34.4±1.864</td>
<td>45.6±5.348</td>
<td>17.5±0.78</td>
<td>69.8±3.19</td>
<td>30.81±2.496**</td>
</tr>
<tr>
<td>Gp XV</td>
<td>Sibutramine (10 mg/kg)+CD</td>
<td>49.7±3.2*</td>
<td>79.5±5.5</td>
<td>73.7±4.5**</td>
<td>35.27±2.23**</td>
<td>24.6±6.012**</td>
<td>15.9±1</td>
<td>62.67±1.906**</td>
<td>28.73±0.918**</td>
</tr>
</tbody>
</table>

TG – Triglycerides; TC – Total cholesterol; HDL-C – High density lipoprotein cholesterol; LDL-C – Low density lipoprotein cholesterol; VLDL – Very low density lipoprotein cholesterol; SGOT – Serum glutamate oxaloacetate transaminase; SGPT – Serum glutamate pyruvate transaminase; GP – Group; CD – Cafeteria diet; Feeding with CD caused significant increase in the glucose, insulin, SGOT, SGPT, TG, TC and LDL-C levels in plane CD treated group animals as compared to normal control animals which was significantly decreased by the co-administration of MECP 200 and 400 mg/kg compared to disease control and normal control group. Treatment with medium and high dose of MECP has also improved the HDL-C level compared with plane CD feed rats. Sibutramine was the most significant in this regard. \*Comparison of test and disease control with normal control \#Comparison of test and disease control with normal control
Stigmasterols and β-sitosterol are the plant sterol with a structure similar to that of cholesterol. Among that β-sitosterol compound having more comparable except for the substitution of an ethyl groups at C-24 of its side chain and it is cholesterol lowering agent.

β-sitosterol reduced absorption of cholesterol by 42% in a meal containing 500 mg of cholesterol. Moreover saponin content of the extract may also responsible for its anti-obesity effect. Reports have shown that several saponins inhibits pancreatic lipase activity. Pancreatic lipase which is the most important enzyme of the human lipases for digesting fats responsible for the hydrolysis of 50–70% of total dietary fats. It is reported that dietary fat was hydrolyzed during digestion by pancreatic lipase. The two main products formed by the hydrolysis of pancreatic lipase are fatty acids and 2-monoacylglycerols. These lipolytic products are mixed with bile salts, dispersed as micelles and carried in this process.
form to the site of fat absorption. Lipid absorption takes place in the apical part of the plasma membrane of epithelial cells or enterocytes lining the gut, so inhibition of pancreatic lipase may cause decrease in fat absorption. The summary of the whole study is presented in the graphical abstract [Figure 13]. Saponins, flavonoids and β-sitosterol are the bioactive phytoconstituent in *C. phlomidis* which may responsible for its anti-obesity activity by multiple mechanisms as explained above.

CONCLUSION

Oral administration of extracts reduced the level of circulating lipids as well as the size of adipose diameter, resulting in the decrease of body weights in female albino mice and Wistar rats, which bearing close resemblance to human obesity. Extracts appear to show such activities by decreasing food consumption, modulating the lipid metabolism through the decreased absorption of dietary fats, and may be inhibition of pancreatic lipase activity. Among these two extracts MECP have shown promising results compared to AECP may be its multiple targets as mentioned above. From this we also proposed that use of MECP along the progesterone might be useful as a supplement to attenuate hyperphagic effect of progesterone. MECP can also be used as to treat obesity caused due to CD.

REFERENCES

13. Singh VP, Sharma SK and Khare VS. Medicinal plants from Ujjain district Madhya Pradesh - Part II. Indian Drugs Pharm. 1980 Ind. 5:7-12.


42. Reddy DS, Kulkarni SK. The role of GABA, and mitochondrial diazepam-binding inhibitor receptors on the effects of neurosteroids on food intake in mice. Psychopharmacology (Berl) 1998;137:391-400.


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Announcement

Android App

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