ANTIOBESITY EFFECT OF ETHANOLIC EXTRACT OF MUNTINGIA CALABURA LEAVES ON HIGH FAT DIET-INDUCED OBESITY IN RATS

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ABSTRACT

Obesity is a major health concern both in developed and developing countries. The goal of the present investigation was to determine whether the ethanolic extract of Muntingia calabura leaves affects body weight in an obese animal model induced by a high-fat diet. A treatment group of rats was given an ethanolic extract of Muntingia calabura leaves (250 mg/kg and 500 mg/kg) orally for 49 days to test its antiobesity potential in comparison to normal, obese control, and standard groups. Various parameters were used to assess the effect (body weight, lipid profile, and liver function tests). Our findings suggest that both doses of Muntingia calabura leaves extract exhibited a significant antiobesity activity by reducing physical and biochemical parameters with increased High density lipoproteins levels and it could be used as an antiobesity agent in the future.

KEYWORDS: High-fat diet, Muntingia calabura, Obesity, Lipid profile, Liver function test.

INTRODUCTION

Obesity is described as an abnormal or excessive fat build-up induced by an energy intake and expenditure imbalance. It is anticipated to be the world's greatest cause of mortality and morbidity in the twenty-first century. An abnormal build-up of body fat caused by excessive growth and expansion of adipose tissue as a result of an energy intake/expenditure imbalance is medically defined. There are 400 million people in the globe who are obese, with a BMI of 30 or more. A person's BMI is calculated by dividing their weight in kilograms (kg) by their height in meters squared (m²). A BMI of less than 18.5 is considered underweight, 18.5-24.9 is considered normal weight, 25.0-29.9 is considered overweight,
30.0-34.9 is considered class I obesity, 35.0-39.9 is considered class II obesity, and over 40.0 is considered class III obesity.\[^{[4]}\]

Obesity is a chronic disease with a variety of causes, including genetics, environment, metabolism, lifestyle, and behavioural variables. Obesity is combated in part by lifestyle factors such as proper nutrition, regular physical activity, and dietary alterations. Obesity has been connected to a number of diseases. Type 2 diabetes (TIIDM), hypertension, dyslipidaemia, ischemic heart disease, stroke, obstructive sleep apnea, asthma, non-alcoholic steatohepatitis, gastroesophageal reflux disease, degenerative joint disease of the back, hips, knees, and feet, infertility and polycystic ovary syndrome, various malignancies, and depression are just a few of the conditions. Obese people are more likely to get cancers of the colon, breast, prostate, gall bladder, ovary, and uterus.\[^{[5,6]}\]

As a result, obesity prevention is crucial. Obesity can be controlled or managed through a variety of methods, including diet, exercise, and medication. On the other hand, anti-obesity drugs such as Orlistat and Sibutramine have been related to constipation, mouth drying, hypertension, headache, cardiac arrest, and insomnia.\[^{[7]}\] The majority of the world's population in poor countries still rely herbal medicines to meet their health needs.\[^{[8]}\]

*Muntingia calabura* is a popular tropical and edible fruit belonging to the Muntingiaceae family. It is a fast-growing tree with slender proportions that grows to a height of 25-40 feet in 6-8 years with spreading, nearly horizontal branches. The leaves are evergreen and range in length from 5 to 12.5 cm, with 2 to 3 leaf axils and a width of 1.25 to 2 cm. Despite the fact that the bushes give fruit all year, the best flowering and fruiting seasons are from April to July.\[^{[9]}\] Flowers have been used for antibacterial, antispasmodic, antidyspeptic, tranquilizer, diaphoretic, tonic, and headache treatment for centuries.\[^{[10]}\] Roots are commonly used as an emmenagogue and abortifacient. Headaches, colds, and stomach ulcers can all be relieved with the leaves.\[^{[11]}\]

*Muntingia calabura* leaves contain alkaloids, flavonoids, anthraquinones, triterpenoids, tannins, steroids, tannins, and saponins. According to a literature review, the leaves of *Muntingia calabura* have been claimed to have anti-inflammatory, anti-oxidant, antipyretic, anti-microbial, and cardioprotective properties.\[^{[12]}\]
More study is needed in the future to find novel medication therapies that can be utilized to lower obesity prevalence. The purpose of this study was to look into the active components and antiobesity efficacy of an ethanolic extract of *Muntingia calabura* leaves in rats that had been given a high-fat diet.

**MATERIALS AND METHODS**

**Plant material**
The leaves of *Muntingia calabura* were collected from the Medicinal garden, SJM College of Pharmacy, Chitradurga. The plant material was identified and authenticated by Dr. Lingannaiah, Professor, Head of the department, Department of Botany, Govt. Science College, Chitradurga, Karnataka.

**Preparation of plant extract**
The plant leaves were cleaned and shade dried at room temperature and pulverized. The powder obtained was then subjected to Maceration with 95% ethanol at a ratio of 1:10 (w/v) for 72 hrs. The mixtures were decanted and filtered. The filtrate will be concentrated under reduced pressure using a rotary flash evaporator with the temperature set at 40°C.[13] The yield was about 10%w/w and stored at 4°C in the refrigerator. The stock solutions of ethanolic extracts were prepared using 0.5% Sodium Carboxy Methyl Cellulose (CMC) and used for oral administration to animals.

**Experimental animals**
Healthy adult Wistar rats of either sex weighing about 150 to 180g and Female albino mice weighing 20 -25g were procured from Biogen Laboratory animal facility Sy. No-162, TVS-Anekal road cross, Attibele-Hobli, Anekal-Taluk, Bangalore - 562107. The animals were kept under standard environmental conditions such as temperature (26±2°C), relative humidity (45-55%), and 12 hrs light/dark cycle was maintained. All the animals had free access to a standard pellet diet with water *ad libitum*. Animals were kept in the animal house of SJM College of Pharmacy, Chitradurga. Before the studies, the animals were housed in polypropylene cages for a 10-day acclimatization period. The experimental protocol was approved by the Intuitional Animal Ethics Committee (IAEC) and conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Ref. No. 02 SJMCP/IAEC/2020-21.
Acute toxicity study
The acute toxicity of ethanolic extract of *Muntingia calabura* leaves was determined as per the OECD guideline no. 423. It was observed that the extract was not lethal to the rats even at 2000mg/kg dose and LD$_{50}$ is 2500 mg/kg. Hence 1/10$^{th}$ (250 mg/kg) and 1/5$^{th}$ (500 mg/kg) of LD$_{50}$ cut-off values of the leaves extract were selected as screening doses for the Anti-obesity activity.

Phytochemical screening of the extract
The phytochemical screening, as well as identification of the plant extract, was done by standard chemical methods.

Pharmacological studies
High-fat diet-induced obesity in experimental rats
Preparation of diet\[14\]
A high-fat diet is a hypercaloric diet that was prepared by mixing Indian Vanaspati ghee and coconut oil in the ratio of 3:1 (v/v). The feed was prepared and administered orally (3ml/kg) everyday morning to animals with water *ad libitum*. Diet was administered and weight gain was observed in rats every day for 49 days.

Anti-obesity studies\[15\]
The obtained Wistar albino rats were randomly divided into 5 groups each group containing six animals. Group I was fed with a normal diet and the remaining groups fed with the high-fat diet for 49 days. The schedule of dose and diet administration in experimental groups was followed as:

**Group I:** Negative control rats (Normal pellet chow and water *ad libitum* for 49 days)

**Group II:** Positive control group (HFD for 49 days)

**Group III:** Standard group (HFD with Orlistat 30 mg/kg b.w. orally from 16$^{th}$ to 49$^{th}$ day)

**Group IV:** Treated group (HFD with EEMC 250 mg/kg b.w. orally from 16$^{th}$ to 49$^{th}$ day)

**Group V:** Treated group (HFD with EEMC 500 mg/kg b.w. orally from 16$^{th}$ to 49$^{th}$ day)

Evaluation parameters
**Bodyweight:** The body weight (gram) of each rat in all five groups was recorded on day one and then on alternate days for 49 days using digital weighing balance.
Biochemical estimation: At the end of the experiment, on day 50 animals blood samples were collected from overnight fasted animals under inhalation of anaesthesia by retro-orbital puncture. Plasma was separated by centrifugation at 3000 rpm for 15 mins. and was used for biochemical investigation such as Total cholesterol, Triglycerides, LDL-C, HDL-C, VLDL-C, SGOT, and SGPT.

Statistical analysis
The results are expressed as mean ± SEM. Comparisons between the treatment groups and positive control will be performed by analysis of variance (ANOVA) followed by Tukey’s multiple comparisons tests using GraphPad Prism version 9.0.1. p values <0.05 were considered as significant.

RESULTS
Phytochemical test
A phytochemical test of *Muntingia calabura* was performed and it was evaluated that the ethanolic extract of the plant shows presence of following phytoconstituents.

Table 1: Preliminary phytochemical screening of leaves of *muntingia calabura* effect on body weight.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Ethanolic extract of <em>Muntingia calabura</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>+</td>
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</tbody>
</table>

(+) Present, (-) Absent

Animals fed with HFD showed significant (P<0.001) gain in body weight(g) when compared to those in the control group. Animals fed with HFD + Orlistat showed a significant decrease (P<0.0001) in weight gain. Animals fed with HFD + EEMC (250mg/kg and 500mg/kg) exhibited dose-dependent reduction in body weight gain. It is illustrated in Table. 2 and graphically represented in Fig. 1
Table 2: Effect of EEMC on HFD induced obesity.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body Weight (g)</th>
<th>Food intake (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial (0day)</td>
<td>16th day</td>
</tr>
<tr>
<td>Negative control</td>
<td>121.5 ± 3.46</td>
<td>148.2 ± 4.57</td>
</tr>
<tr>
<td>Positive control</td>
<td>121.8 ± 2.41</td>
<td>160.5 ± 2.17</td>
</tr>
<tr>
<td>Standard</td>
<td>126.2 ± 1.97</td>
<td>164.7 ± 1.30</td>
</tr>
<tr>
<td>EEMC (250 mg/kg)</td>
<td>123.5 ± 2.72</td>
<td>160.3 ± 2.78</td>
</tr>
<tr>
<td>EEMC (500 mg/kg)</td>
<td>131.8 ± 2.79</td>
<td>168.5 ± 2.56</td>
</tr>
</tbody>
</table>

All values are expressed in (mean ± SEM, n=6) *p<0.05, **p<0.01, ***p<0.001 when compared to positive control. (One way ANOVA followed by Tukey’s Kramer test)

![Fig. 1: Effect of EEMC on body weight gain in HFD rats.](image)

**Effect of EEMC extract on lipid profile**

The present study demonstrated antiobesity activity of EEMC in the Wistar albino rats and efficacy was found to be significant. The oral administration of EEMC for 33 days showed significant changes in the Biochemical parameters. Group II (positive control) animals fed with HFD exhibited a significant increase in TC, TG, LDL, and VLDL when compared to Group I (Negative control) animals. Administration of EEMC (250mg/kg and 500 mg/kg) and Orlistat (30 mg/kg) shows a significant reduction in TC, TG, LDL, and VLDL when compared with the Group II animals. Whereas decreased HDL levels observed in Group II animals were significantly increased in Group V. It is illustrated in Table. 3 and graphically represented in Fig. 2.
Table 3: Effect of EEMC on lipid profile in HFD rats.

<table>
<thead>
<tr>
<th>Lipid Profile</th>
<th>Negative control</th>
<th>Positive control</th>
<th>Standard</th>
<th>EEMC 250 mg/kg</th>
<th>EEMC 500 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>74.50 ± 3.73</td>
<td>122.5 ± 5.72</td>
<td>90.6 ± 2.88 *</td>
<td>107.5 ± 2.71 *</td>
<td>99.6 ± 1.30 *</td>
</tr>
<tr>
<td>TG’s</td>
<td>64.50 ± 3.73</td>
<td>123.0 ± 5.24</td>
<td>91.0 ± 2.74 **</td>
<td>103.2 ± 2.18 *</td>
<td>93.67 ± 2.59 *</td>
</tr>
<tr>
<td>HDL</td>
<td>39.17 ± 1.37</td>
<td>31.5 ± 1.11</td>
<td>42.67 ± 0.80 **</td>
<td>39.83 ± 0.54 **</td>
<td>33.83 ± 0.94 *</td>
</tr>
<tr>
<td>LDL</td>
<td>43.50 ± 1.89</td>
<td>74.67 ± 1.64</td>
<td>47.17 ± 1.53 ***</td>
<td>64.33 ± 1.70 *</td>
<td>60.17 ± 1.19 **</td>
</tr>
<tr>
<td>VLDL</td>
<td>12.90 ± 0.74</td>
<td>24.60 ± 1.04</td>
<td>18.20 ± 0.54 **</td>
<td>20.63 ± 0.43 *</td>
<td>18.73 ± 0.51 *</td>
</tr>
</tbody>
</table>

All values are expressed in (mean ± SEM, n=6) *p<0.05, **p<0.01, ***p<0.001 when compared to positive control. (One way ANOVA followed by Tukey’s Kramer test)

Effect of EEMC on liver function parameters

The liver marker enzyme profile SGOT and SGPT were found to be significantly increased in Group II (positive control) over Group I (negative control). Administration of EEMC (250mg/kg and 500 mg/kg) and Orlistat (30 mg/kg) shows a significant reduction in SGOT
and SGPT when compared with the Group II animals. Results are illustrated in Table 4 and graphically shown in Fig. 3.

**Table 4: Effect of EEMC on liver function test in HFD rats.**

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>Serum Glutamate Oxaloacetate Transaminase (SGOT) (IU/L)</th>
<th>Serum Glutamate Pyruvate Transaminase (SGPT) (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>131.2 ± 3.91</td>
<td>68.83 ± 2.33</td>
</tr>
<tr>
<td>Positive control</td>
<td>153.5 ± 6.70</td>
<td>77.33 ± 3.44</td>
</tr>
<tr>
<td>Standard</td>
<td>119.3 ± 2.92 *</td>
<td>43.83 ± 2.15 *</td>
</tr>
<tr>
<td>EEMC (250 mg/kg)</td>
<td>122.7 ± 4.02 *</td>
<td>55.33 ± 1.99 **</td>
</tr>
<tr>
<td>EEMC (500 mg/kg)</td>
<td>125.8 ± 5.38 *</td>
<td>51.67 ± 2.66 *</td>
</tr>
</tbody>
</table>

All values are expressed in (mean ± SEM, n=6) *p<0.05, **p<0.01, ***p<0.001 when compared to positive control. (One way ANOVA followed by Tukey’s Kramer test)

**Fig. 3: Effect of EEMC on liver function parameters.**

**DISCUSSION**

Obesity is defined as having an excess of fat in the body, which is determined by the lipid content of each fat cell and the total number of fat cells. When excess body fat is present in and around the belly, obesity dramatically raises the risk of cardiovascular disease and Type II diabetes. It should be treated as a life-threatening medical condition with a high risk of morbidity.\(^{[16]}\)

The current research examines the antiobesity activity of ethanolic extract of leaves *Muntingia calabura* was studied using a dietary animal’s model of obesity. Obesity was induced in Wistar albino rats by administrating a High-fat diet (HFD) to Group II, III, IV, and V at a dose of 3ml/kg daily bodyweight for 49 days. The present pharmacological investigation revealed that HFD elicited a significant increase in body weight, serum levels of
Total cholesterol, Triglycerides, LDL, VLDL, SGOT, and SGPT. Treatment with EEMC resulted in a reduction of body weight in HFD fed rats indicating that the extracts possess weight-reducing properties. Since obesity is associated with hyperphagia, HFD fed rats consumed more food than normal diet fed rats. EEMC effective in decreasing daily food intake in HFD fed rats, indicating that it possesses hypophagic properties.

After administration of EEMC (250 mg/kg & 500 mg/kg) showed a significant reduction in serum levels of total cholesterol, LDL cholesterol, triglycerides, VLDL cholesterol along with a significant increase in serum HDL cholesterol levels in HFD fed rats, Considering the enhancement of cardioprotective HDL cholesterol, it can be concluded that leaves of *Muntingia calabura* are a potent cardioprotective agent. The enzyme SGOT, SGPT increased in a group of animals treated with HFD, and of EEMC (250 mg/kg & 500 mg/kg) decreases the SGOT, SGPT levels were observed.

From the observations of the study performed, Because of its hypophagic, hypoglycaemic, and hypolipidemic effects in rats on a high-fat diet, it was hypothesised that an ethanolic extract of *Muntingia calabura* leaves would have significant anti-obese activity.

**CONCLUSION**

The results of the present study demonstrated that ethanolic extract of *Muntingia calabura* leaves exhibited significant antiobesity effect when compared to control. It can be concluded that active constituents such as flavonoids, alkaloids, tannins, saponins, and phenolic compounds of leaves extract are responsible for the antiobesity activity. However, further studies regarding the isolation and characterization of active constituents are needed to explore underlying mechanisms.

**ACKNOWLEDGEMENTS**

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