In vivo anti-obesity activity of methanolic extract of Helianthus annuus seeds

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Abstract
The aim of the present study was to investigate the constituents and anti-obesity activity of the methanolic extract of Helianthus annuus seeds in mice model. Parameters such as food consumption, locomotor activity, body weight, body mass index (BMI), lee index of obesity (LIO), total cholesterol, triglyceride, LDL, HDL and glucose were studied in swiss albino mice. The mice received cafeteria diet, atorvastatin (10 mg/kg) and Helianthus annuus 200 mg/kg daily for 6 weeks. Phytochemical analysis revealed the presence of carbohydrate, alkaloid, terpenoids, saponin and flavonoid. Significant increase in locomotor activity (rearing, grooming, ambulation) with HDL and significant decrease in food consumption, body weight, BMI, LIO, total cholesterol, triglyceride, LDL and glucose was seen with Helianthus annuus which is opposite to the result of cafeteria diet. Present findings suggest that the methanolic extract of Helianthus annuus have significant anti-obesity activity by maintaining the normal levels of physical and biochemical parameters.

Keywords: Helianthus annuus, Asteraceae, Cafeteria diet, Obesity.

1. Introduction
In the developed world obesity is the most common nutritional disorder and is a risk factor for the genesis or development of various diseases including hyperlipidemia, type 2 diabetes mellitus, hypertension, stroke, cardiovascular disease and osteoarthritis (Yun JW, 2010) [1]. The metabolic disparity of obesity can be minimized by the application of inhibitors of appetite, gastrointestinal lipid uptake and peroxisome proliferator activated receptor. However, these drugs can produce adverse effects. Therapeutically potent and safe anti-obesity reagents such as botanical drugs are required. The sunflower seed is the fruit of the sunflower (Helianthus annuus, family: Asteraceae). The term “sunflower seed” is actually a misnomer when applied to the seed in its pericarp (hull). These seeds are usually pressed to extract their oil. It is a potential protein supplement for human diet.

In the literature survey, it was found that flavonoids, sterols, tannins, and alkaloids have shown promising effects to tackle obesity by various mechanisms (Rohit Gundamaraju et al., 2012) [2]. In the present study we have done the phytochemical analysis of Helianthus annuus seeds for the presence of sterols, flavonoids and alkaloids in the extracts and also investigated for antiobesity activities in mice. Furthermore, the activities of this extract on motor coordination were investigated by using mice model.

2. Materials and methods
Animal
Swiss albino mice (22-26 g) obtained from Bangladesh Council of Scientific and Industrial Research (BCSIR) Chittagong, were used in this experiment. The mice were kept at constant temperature of 22±2 °C and 12-h light/12-h dark. Mice were fed Hind Lever diet pellets (standard laboratory food) and water was given ad libitum. In the behavior tests each animal was used once. The Institutional Ethical Committee of University of Science and Technology Chittagong (USTC) (USTC/2015/1823/07) following the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) approved the experimental protocols for this study.
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**Extraction procedure of plant material**
Dried powder of seed (300 gm) was weighed & taken in an aspirator (2.5 liter). The jar was washed properly with acetone before placing powders into the aspirator and then dried. 800 ml of methanol (solvent) was added gradually. The container with its content was sealed & kept for 20 days with occasional shaking & stirring. The major portion of the extractable compounds of the plant materials were dissolved in the solvent. Then whole mixture was filtered through cotton wool and the filtrate was concentrated by evaporation in dry & clean air. And it was kept for 15 days to get the final extract of the seed.

**Phytochemical test**
Preliminary Phytochemical screening of the powdered seed was performed for the presence of alkaloids, carbohydrates, flavonoids, steroid and triterpenoids.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Test</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Wagner’s test</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Fehling’s test</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Alcoholic test</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric chloride test</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam test</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>Libermann-Burchard test</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>Libermann-Burchard test</td>
<td>+</td>
</tr>
<tr>
<td>Gum</td>
<td>Molisch’s test</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1: Phytochemical test of H. annuus seeds

*+*= Present; *-*= Absent.

**Drugs and chemicals**
The following drugs were used: Atorvastatin (10 mg/kg), biochemical kits for total cholesterol, triglycerides, HDL cholesterol (Transsania bio-medicals ltd, solam, HP-ERBA diagnostics) methanolic extract of *Helianthus annuus* seed.

**Anti-obesity activity protocol for experiment**
22-26 gm Swiss Albino mice were selected randomly and divided into four groups of four mice in each and treated (orally) are as follows:

- **Group A**: Normal diet.
- **Group B**: Cafeteria diet in pellets forms.
- **Group C**: Cafeteria diet + Atorvastatin (10 mg/kg, orally) daily.
- **Group D**: Cafeteria diet + HA (200 mg/kg, orally) daily.

**Cafeteria diet for the experiment**
According to the method of Harris and Kulkarni there are few modifications in the cafeteria diet (human snack foods containing highly energy rich, palatable diet) (Kaur G, Kulkarni SK, 2000; Harris RB, 1993) [3, 4]. Cafeteria diet was given to the 3 groups of 4 mice in each for 6 weeks (42 day).

- **I. Bread 48 g + Condensed milk 48 g**
- **II. Dried coconut 36 g + Chocolate 18 g + Biscuits 36 g**
- **III. Boiled potatoes 60 g + Cheese 48 g**

3 groups of 4 mice in each for 42 days were given cafeteria diet in the form of pellets.

**Parameters for experiment**

**Food consumption study**
On week 0 (day 1), week 2 (day 14), week 4 (day 28) and week 6 (day 42) study of food consumption was done and it was recorded at 1 h, 2 h and 3 h of time intervals. By subtracting the amount of food left on the grid from initial food weight (gram) the measurement food consumption was done. (Gallou-Kabani C et al., 2007) [5]

**Locomotor activity study**
On day 42 after 30 min of drug administration open field behavior test was done for locomotor activity study. Open field test was done by placing albino mice in the center of apparatus and frequency of rearing, grooming and ambulation for a 5 min test period was estimated. In this test ambulation was recorded by counting the number of horizontal and vertical compartments traversed by animal.

**Body weight of mice**
On week 0 (day 1), week 2 (day 14), week 4 (day 28) and week 6 (day 42) body weights of albino mice (g) were recorded in each group.

**Body mass index (BMI) and lee index of obesity (LIO) study**
On day 1 and day 42 of study, BMI and LIO of mice were recorded. For this experiment first and last body weight and body height was taken. Following formulas were used for this purpose. (Kanarek RB, Orthen-Gambill N, 1982) [6]

\[
\text{BMI} = \frac{\text{Body weight in gm}}{\text{Height in cm}^2}
\]

\[
\text{LIO} = \frac{\text{Body weight in gm}}{(\text{Nasoanal length in cm})^2}
\]

**Biochemical parameter study**
The blood samples were taken on day 42 by penetrating the retro-orbital plexus with a fine glass capillary. The blood samples were centrifuged (2500 rpm for 15 min) to separate the serum and preserved (−20 °C) for estimation of glucose by Trinder's Method, total cholesterol by Modified Roeschau's Method, triglycerides (Glycerol phosphate oxidase (GPO) by Trinder Method, End Point), high density lipoprotein-c (HDL-c) by Phosphotungstic Acid Method, Low density lipoprotein-c (LDL-c) by Calculated using Friedewald's equation,

\[
\text{LDL-c} = \text{Total cholesterol} - \text{VLDL-c} = \frac{\text{Triglyceride}}{5} - \text{HDL-c}
\]

**Statistical analysis**
The results were expressed as mean ± S.E.M. Statistical analysis of difference between groups was evaluated by ANOVA test (SPSS software; version 16). The p value less than 0.01 and 0.05 were considered high significant and moderate significant.

3. Results

**Food consumption with H. annuus**
On day 0 (day 1), week 2 (day 14), week 4 (day 28) and week 6 (day 42) food consumption was increased significantly \(P < 0.001\) comparison to normal diet group. On day 28 and 42 when atorvastatin 10 mg/kg (Group C) added there was significant \(P < 0.001\) decrease in food consumption in comparison to cafeteria diet group. On day 28 and 42 when *H. annuus* (200 mg/kg) (group D) was added, it showed significant \(P < 0.05, P < 0.01\) decrease in food consumption in comparison to cafeteria diet group. (Figure 1)
Fig 1: Effect of *Helianthus annuus* on food consumption in normal and experimental group of mice.

**Locomotor activity with *H. annuus***

In comparison to normal diet group (Group A), cafeteria diet group (group B) showed significant ($P < 0.001$) reduction in rearing, grooming and ambulation. In comparison to cafeteria diet group (group B), atorvastatin 10 mg/kg orally treated group (group C) and *H. annuus* (200 mg/kg) groups (Group D) showed a significant increase in rearing ($P < 0.05$) and grooming ($P < 0.001$) and ambulation ($P < 0.001$). (Table 2)

**Body weight changes with *H. annuus***

In comparison to normal diet group (group A), cafeteria diet group (group B) showed significant ($P < 0.001$) body weight increase on week 0 (day 1), week 2 (day 14), week 4 (day 28) and week 6 (day 42). When atorvastatin 10 mg/kg orally added with cafeteria diet to mice (group C), it showed significant ($P < 0.001$) body weight reduction on week 2, 4 and 6 in comparison to cafeteria diet group. Oral administration of *H. annuus* group (200 mg/kg) (group D) showed significant ($P < 0.05$) decrease in body weight at week 1, 2, 3, 4, 5 and 6 in comparison to cafeteria diet group. (Table 3)

**Body mass index (BMI) and Lee index of obesity (LIO) with *H. annuus***

In comparison to normal diet, cafeteria diet significantly ($p < 0.001$) increased the last body mass index and lee index of obesity. When atorvastatin 10 mg/kg orally added with cafeteria diet (group C) it showed a significant ($P < 0.001$) decrease in the last BMI and LIO. Group D also showed significant ($p <0.05$) decrease in BMI and LIO in comparison to cafeteria diet (group B) (Table 4)

**Biochemical parameter with *H. annuus***

In comparison to normal diet group (group A), cafeteria diet group (group B) showed significant ($P < 0.001$) increase in the total cholesterol, triglycerides, LDL, serum glucose and significant ($P < 0.001$) reduction in the level of HDL. In comparison to cafeteria diet group (group B), atorvastatin 10 mg/kg treated group (group C) and *H. annuus* (200 mg/kg) groups (Group D) showed a significant reduction ($P < 0.05$) in the levels of total cholesterol, triglycerides, LDL and serum glucose with significant increase in the levels of HDL. (Table 5)
significant increase in HDL. This suggests cardioprotective and antiatherogenic activity of H. annuus. Further validation is needed for anti-obesity potential to ascertain the exact molecular mechanism involved and to find out the particular components responsible for these activities of H. annuus.

5. Conclusion
As obesity is becoming a serious health issue and due to side effects of currently available anti-obesity drugs, there is a need for further development of drugs by identifying potential phytochemicals from the traditional medicinal plants. It can be suggested that active Phytochemical component of H. annuus would give a positive lead in the successful management of obesity. Further studies are needed to prove the clinical safety of H. annuus.

6. References
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