ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2015; 1(13): 518-522
www.allresearchjournal.com
Received: 14-10-2015
Accepted: 17-11-2015

Rubab Tarannum Islam
Master of Pharmacy, Department of Pharmacy University of Science and Technology Chittagong (USTC), Chittagong- 4202, Bangladesh

Dr. Ahmed Tanjimul Islam
Medical officer, Department of Medicine Chittagong Medical College and Hospital, Chittagong- 4203, Bangladesh

Dr. Kishor Mazumder
Associate Professor and Chairman, Department of Pharmacy, University of Science and Technology Chittagong (USTC) Chittagong- 4202, Bangladesh

Correspondence
Rubab Tarannum Islam
Master of Pharmacy, Department of Pharmacy University of Science and Technology Chittagong (USTC), Chittagong- 4202, Bangladesh

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

Rubab Tarannum Islam, Dr. Ahmed Tanjimul Islam, Dr. Kishor Mazumder

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.

Extraction procedure of plant material

Dried powder of seed (300 gm) was weighed & taken in a 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 1: Phytochemical test of H. annuus seeds

Phytochemicals	Test	Inference
Alkaloids	Wagner's test	+
Carbohydrates	Fehling's test	+
Flavonoids	Alcoholic test	+
Tannins	Ferric chloride test	-
Glycosides	Keller-Killiani test	-
Saponins	Foam test	+
Steroids	Libermann-Burchard test	+
Triterpenoids	Libermann-Burchard test	+
Gum	Molisch's test	-
'+' = Present; '-'= Absent.		

Drugs and chemicals

The following drugs were used: Atorvastatin (10 mg/kg), biochemical kits for total cholesterol, triglycerides, HDL cholesterol (Transania 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). Cafeteria diet consists of 3 diets, which includes

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 42 day 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]

Body mass index (BMI) = Body weight in gm/ (Height in cm)^2

Lee index of obesity (LIO) = Body weight in gm (1/3)/Nasoanal length in cm.

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,

LDL-c = total cholesterol– very low density lipoprotein (VLDL-c – HDL-c)

VLDL-c estimated by-- VLDL-c = Triglyceride/5

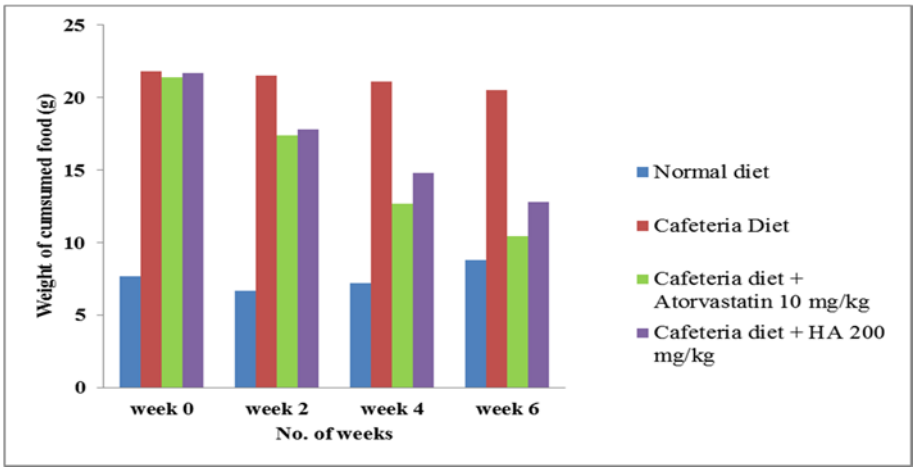
Statistical analysis

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 week 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)



All values are expressed as a mean ± SEM, n= 4.

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)

Table2: Effect of *Helianthus annuus* on locomotor behavior in normal and experimental group of mice.

Groups	Rearing	Grooming	Ambulation
Normal diet	5.9 ± 0.25	16.2 ± 0.93	149.7 ± 10.5
Cafeteria diet	3.6 ± 0.14	8.7 ± 0.56	62.8 ± 3.8
Cafeteria diet + Atorvastatin	2.7 ± 0.22	12.3 ± 0.92	137.8 ± 10.3
Cafeteria diet + HA 200mg/kg	2.61 ± 0.20	11.7 ± 0.83	125.7 ± 9.3

All values are expressed as a mean ± SEM, n= 4.

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)

Table 3: Effect of *H. annuus* on body weight of mice in the cafeteria diet-induced obesity model.

Groups	Week 0	Week 2	Week 4	Week 6
Normal diet	4.3±0.04	5.7±0.04	7.3±0.05	9.8±0.04
Cafeteria diet	4.7±0.05	6.4±0.12	10.7±0.05	16.7±0.04
Cafeteria diet + Atorvastatin	4.4 ± 0.04	6.2 ± 0.15	10.1 ± 0.08	11.3 ±0.08
Cafeteria diet+ HA 200 mg/kg	4.5±0.04	5.3±0.05	8.4±0.04	13.8±0.06

All values are expressed as a mean ± SEM, n= 4.

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)

Table 4: Effect of *Helianthus annuus* on BMI and LIO in normal and experimental group of mice.

Groups	First BMI	Last BMI	First LIO	Last LIO
Normal diet	0.36± 0.03	0.40 ±0.04	57.4± 0.03	62.7± 0.05
Cafeteria diet	0.39± 0.04	0.52±0.04	60.9± 0.06	81.6±0.07
Cafeteria diet + Atorvastatin	0.38± 0.04	0.31±0.03	60.3±0.06	48.3±0.08
Cafeteria diet + HA 200 mg/kg	0.39± 0.04	0.34±0.03	61.6±0.02	53.6± 0.03

All values are expressed as a mean ± SEM, n= 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)

Table 5: Effect of *Helianthus annuus* on biochemical parameter in normal and experimental group of mice.

Groups	Total cholesterol (mg/dl)	TG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	S. Glucose (mg/dl)
Normal diet	169.6 ± 1.13	81.7 ± 1.05	118.3±1.08	38.7±1.08	85.3±1.02
Cafeteria diet	201.7 ±1.18	106.3 ±1.03	154.3±1.02	29.3±1.41	101.2±1.06
Cafeteria diet+ Atorvastatin	179.1 ± 3.15	77.8±2.03	130.6±1.02	34.1±1.49	87.1±1.07
Cafeteria diet + HA 200mg/kg	184.7±3.02	81.5±2.01	135.7±1.05	35.2±2.14	91.8±1.07
TG = Triglyceride, LDL= Low Density Lipoprotein, HDL= High Density Lipoprotein					

All values are expressed as a mean ± SEM, n= 4.

4. Discussion

According to World Health Organization (WHO) overweight and obesity are defined as excessive or abnormal accumulation of fat that presents a risk to health in human being. Body Mass Index (BMI) of 30 and above is defined as obesity by the National Institutes of Health (NIH) (Swinburn BA *et al.*, 2004) [7]. The BMI, a key index for relating body weight to height, is a person's weight in kilograms (kg) divided by their height in meters (m) squared. Since the body mass index describes the body weight relative to height, it correlates with the total body fat content. Some very muscular people may have a high body mass index without undue health risks.

Obesity is the risk factor for developing hyperlipidemia, diabetes, stroke, cardiovascular diseases which causes the increase in overall mortality. The cafeteria diet has been reported to increase body weight and organ weight. Obesity model with cafeteria diet is the simplest obesity induction model which closely resembles the reality of obesity in human being (Sclafani A, Springer D, 1976) [8]. In our study we found a significant increase in food consumption, body weight, BMI and LIO in cafeteria diet induced mice. We also investigated for locomotor activity and found significant reduction in rearing, grooming and ambulation with cafeteria diet induced mice. With our plant extract of *H. annuus* we found a significant increase in locomotor activity with significant reduction in food consumption, body weight, BMI and LIO. So it suggests a clear beneficial role in maintaining a healthy physical and locomotor behavior.

Insulin resistance can occur with high fat diet induced obesity. There have been reports suggesting that obesity can lead to imbalance in insulin and glucose homeostasis. (Patel MJ, Patel JK, 2011) [9]. our study showed increase in serum blood glucose level with cafeteria diet. Treatment with *H. annuus* (200 mg/kg) significantly decreased the blood glucose level which suggests its antidiabetic or hypoglycemic effect. Abnormal lipid profile is also associated with obesity. It causes increase in total cholesterol, triglyceride, LDL and decrease In HDL. Cafeteria diet increases both cholesterol and triglyceride levels in blood by inhibiting its uptake and clearance with preventing the catabolizing enzymes like lipoprotein lipase and lecithin cholesterol acetyl transferase. Fatty diet also increases LDL that results in atherosclerotic plaque formation in the vessels. HDL which is anti atherogenic, decreases with cafeteria diet (Byers SO *et al.*, 1963) [10]. These findings are in supportive with our study results. With cafeteria diet we found a significant increase in total cholesterol, triglyceride, LDL and significant decrease in HDL. With *H. annuus* (200mg/kg) there was significant decrease in total cholesterol, triglyceride and LDL and significant increase in HDL. This suggests cardioprotective and anti atherogenic 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

1. Yun JW. Possible anti-obesity therapeutics from nature–A review. Phytochemistry. 2010; 71:1625-1641.
2. Rohit Gundamaraju, Sartaj Banu Mulaplli, Dr. Ramesh C. Evaluation of Anti-Obesity Activity of Lantana camara Var Linn. by Progesterone Induced Obesity on Albino Mice. International Journal of Pharmacognosy and Phytochemical Research. 2012-13; 4(4):213-218.
3. Kaur G, Kulkarni SK. Anti-obesity effect of a polyherbal formulation, OB200G in female rats fed on cafeteria and atherogenic diets. Indian J Pharmacol. 2000; 32:294-9.
4. Harris RB. The impact of high-or low-fat cafeteria foods on nutrient intake and growth of rats consuming a diet containing 30% energy as fat. Int J Obes Relat Metab Disord. 1993; 17:307-15.
5. Gallou-Kabani C, Vigé A, Gross MS, Boileau C, Rabes JP, Fruchart-Najib J *et al.* Resistance to high-fat diet in the female progeny of obese mice fed a control diet during the periconceptual, gestation, and lactation periods. Am J Physiol Endocrinol Metab. 2007; 292:E1095-100.
6. Kanarek RB, Orthen-Gambill N. Differential effects of sucrose, fructose and glucose on carbohydrate-induced obesity in rats. J Nutr. 1982; 112:1546-54.
7. Swinburn BA, Caterson I, Seidell JC, James WP. Diet, nutrition and the prevention of excess weight gain and obesity. Public Health Nutr. 2004; 7:123-46.
8. Sclafani A, Springer D. Dietary obesity in adult rats: Similarities to hypothalamic and human obesity syndromes. Physiol Behav. 1976; 17:461-71.
9. Patel MJ, Patel JK. Evaluation of the anti-hyperlipidemic activity of Premna integrifolia using experimental animal model. Int J Res Phytochem Pharmacol. 2011; 1:146-9.
10. Byers SO, Fiedman M, Sugiyama T. Triton hypercholesteremia: Cause or consequence of augmented cholesterol synthesis. Am J Physiol. 1963; 204:1100-2.
11. Augusti KT, Mattew BC. Bio chemical effects of garlic protein and garlic oil on glycosaminoglycan metabolism

in cholesterol fed rats. Indian journal of Experimental biology. 1996; 34:346-350.
12. Bagchi C, Tripathi SK, Hazra A, Bhattacharya D. Evaluation of hypolipidemic activity of Premna integrifolia Linn. Bark in rabbit model. Pharmbit. 2008; 18:149-53.
13. Kaur G, Kulkarni SK. Anti-obesity effect of a polyherbal formulation, OB200G in female rats fed on cafeteria and atherogenic diets. Indian J Pharmacol. 2000; 32:294-9.
14. Basarkar PW, Nath N. Hypocholesterolemic and hypolipidemic activity of quercetin: A vitamin P-like compound in rats. Indian J Med Res. 1983; 77:122-6.
15. Erhardt E, Zibetti LC, Godinho JM, Bacchieri B, Barros HM. Behavioral changes induced by cocaine in mice are modified by a hyperlipidic diet or recombinant leptin. Braz J Med Biol Res. 2006; 39:1625-35.
16. Bjursell M, Gerdin AK, Lelliott CJ, Egecioglu E, Elmgren A, Törnell J *et al.* Acutely reduced locomotor activity is a major contributor to Western diet-induced obesity in mice. Am J Physiol Endocrinol Metab. 2008; 294:E251-60.
17. Lemhadri A, Hajji L, Michel JB, Eddouks M. Cholesterol and triglycerides lowering activities of caraway fruits in normal and streptozotocin diabetic rats. J Ethnopharmacol. 2006; 106:321-6.
18. Ahmed R, Ifzal SM, Saifuddin A, Nazeer M. Studies on *Punicagranatum* L.-I Isolation and Identification of Some Constituents From the Seeds of Punicagranatum. Pakist. J of Phar Sc.1995; 8(1):69-71.
19. Lei F, Zhang XN, Wang W, Xing DM, Xie WD, Su H *et al.* Evidence of anti-obesity effects of the pomegranate leaf extract in high-fat diet induced obese mice. Int. J of Obesity. 2007; 31:1023-1029.