

Antiobesity and Lipid Lowering Activity of *Vigna unguiculata* (L) Walp. Seed in High Fat Diet Induced Obese Mice

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Abstract: Seed of *Vigna unguiculata* is commonly used as culinary in Bangladesh and many parts of the world. Besides this, seed of *V. unguiculata* possesses some medicinal values like being an antioxidant etc. In this study, seeds of *V. unguiculata* were used to determine the link between its total phenolic content and lipid lowering activity. Phytochemicals presents in the seeds were evaluated. Total phenolic content (TPC) was determined by using folin-ciocalteu method. High fat diet induced obese mice were used for anti-obesity and antihyperlipidemic test. Body weight gain, total cholesterol and triglyceride levels were analyzed. Preliminary phytochemical screening showed that amino acid, phenol, alkaloid and terpenes were very strongly present in cowpea seed, while carbohydrate, cardiac glycoside, was moderately present. The *V. unguiculata* seeds showed significant total phenolic content with a value of 119.716mg of GAE/gm equivalent. Cowpea seeds, with a dose of 400mg/kg body-weight, showed significant reduction in body weight gain when treated with fat diet compared with normal diet. Cowpea seeds are also capable of lowering the serum cholesterol ($p < 0.01$) and serum triglyceride level ($p < 0.01$) more significantly in high fat diet induced obesity. *V. unguiculata* seeds have a significantly high total phenolic content while the other phytochemicals are moderately present in them. It has anti-obesity activity that can interfere with serum cholesterol and triglyceride metabolism. So, it seems like a healthy diet for the management of obesity and other heart related disease.

Keywords: Antihyperlipidemia, *Vigna unguiculata*(Cowpea), Total phenolic content, Cholesterol, Triglyceride.

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1. INTRODUCTION

Obesity and overweight are a major concern in this current world. Attenuated energy expenditure triggers the accumulation of abnormal or excess fat in the body [2]. In addition to excess energy intake, over activity of pancreatic lipase enzyme plays a vital role in the pathogenesis of obesity [3]. Furthermore, obesity has been proved to be linked with different life style diseases like atherosclerosis, [4] diabetes, [5] and hypertension [6] etc. Right now, orlistat, a pancreatic lipase inhibitor [7] and Sibutramine, an appetite suppressant [8] are available in the market for management of obesity. Because of having different side effects [9] of these two drugs including increased blood pressure, dry mouth, constipation and insomnia, the scientists are trying to develop innovative nutritional agents for the management of obesity and overweight.

Human beings have started to use the plants successfully to treat the illnesses from the ancient period of life and the discovery of new molecules from plant sources has renewed the popularity of herbal medicine. *Vigna unguiculata* L. Walp, commonly known

as cowpea, is a legume which is widely cultivated all over the world and their seeds are used as human food as a cheap source of high-quality proteins along with different types of phytonutrients like vitamin, minerals and insoluble dietary fibres. Beside this, *V. unguiculata* has been studied for different medical benefits. Cowpea seeds are considered as nematocidal and antifungal [10]. Powder of seeds when mixed with oil is used in treating stubborn boils [11]. Several studies have demonstrated that the compound having antioxidant activity is considered to be a good candidate for the management of obesity [12]. Cowpeas have been documented because of their antioxidant activities [13] but nothing is known for its effect on obesity. Thus, after taking all these things in consideration, we aimed to study the anti-obese potentiality of cowpea seeds.

2. METHODS

2.1. Collection of Plant

For this present investigation *V. unguiculata* Linn (seeds) were collected from Noakhali, Bangladesh on April, 2015. After collection, the seeds were thoroughly washed with water and then dried under sunlight. The plant was identified by an expert of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. (Accession number: 37752).

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2.2. Preparation of Aqueous Suspension of *V. unguiculata* Seeds for Anti-Obese Activity

The dried seeds (500 gm) were powdered and the required amount of powder, at the dose of 400 mg/kg body weight, was calculated and weighed accurately. Afterwards, it was suspended in the water, containing few drops of Tween 80, to make a suspension of 10mg/ml. One (1) ml of this suspension was administered to each mouse, where applicable.

2.3. Preparation of Methanolic Extract of *V. unguiculata* Seeds

Adequate amount of powdered seeds was soaked in methanol with occasional shaking for 15 days. Solution was then filtered using a filter cloth and the Whatman® filter paper No. 1. The resulting filtrates were then evaporated in a rotary evaporator below 40°C to dryness and thus a concentrated semisolid mass of the extract was obtained.

2.4. Experimental Animals

Swiss albino mice, used for this study were collected from Jahangir Nagar University, Savar, Dhaka, Bangladesh and were kept in polypropylene cages where they were exposed to alternate cycles of 12hr dark and 12hr light at a temperature of $25 \pm 2^{\circ}\text{C}$ and relative humidity of $55 \pm 10\%$. Mice were fed with standard laboratory pellet diet and water *ad libitum*, and were allowed to acclimatize for 7 days to the laboratory conditions before the experiment. Mice were given adequate human care throughout the experimental period.

2.5. Study Design

Twenty experimental healthy mice were randomly selected and divided into four groups with five mice in each group.

Group-1: Control group, where all of the mice were fed with normal food.

Group-2: Mice fed with normal diet along with aqueous extract at 400mg/kg body weight.

Group-3: Obese group, mice fed with high fat diet; 20% (w/w) cow fat along with normal diet (HFD group).

Group-4: Mice fed with high fat diet with aqueous extract at 400mg/kg body weight.

All groups of mice were treated daily with their respective food and water for a period of 11 days and

each mouse was weighed on alternate days. At the end of the experimental study, they were anaesthetized before being killed by decapitation and their blood was collected for further analysis.

2.6. Determination of Total Phenolic Content

The total phenolic content of the *V. unguiculata* seeds extract was determined by using Folin-Ciocalteu reagent following a slightly modified method of Ainsworth [14]. Gallic acid was used as a reference standard for plotting the calibration curve. A volume of 0.5 mL of the plant extract (100 µg/mL) was mixed with 2 mL of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and were neutralized with 4 mL of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for colour development. The absorbance of the resulting blue colour was measured at 765 nm using double beam UV-VIS spectrophotometer (UV Analyst-CT 8200). The total phenolic contents were determined from the linear equation of a standard curve prepared with Gallic acid. The content of total phenolic compounds was expressed as mg/g Gallic Acid Equivalent (GAE) of the dry extract.

2.7. Determination of Serum Cholesterol

Serum cholesterol level were calculated by enzymatic end point (CHOD-PAP) method [15] by using a double beam spectrophotometer (Shimadzu, Japan). In detail, the cholesterol concentration was determined through oxidation of enzymatic hydrolysis. Due to the chemical changes occurring in the presence of phenol and peroxidase, hydrogen peroxide is produced which then reacts with 4-aminophenazine and produces colorquinoneimine as an indicator. During this study, three washed test tube were taken at first and labelled as 'Blank', 'Standard' and 'Serum sample' respectively. Then, 10µl of serum sample was taken to a test tube labelled 'sample serum' with the help of micropipette. After that 10 ml of standard Cholesterol sample was taken into the 'standard' labelled test tube. After that 1 ml of enzyme reagent was taken into each test tube respectively and kept for incubation at 37°C for 20 minutes, in dark place. The absorption of the solution was measured at 510nm against blank. Then absorbance value was put in the cholesterol measuring equation against standard sample and then the cholesterol level was determined. Cholesterol concentration = {OD of sample /OD of standard} × 200(mg/dl) = {OD of sample/OD of standard} × 5.17 (mmol/l).

2.8. Determination of Serum Triglyceride

Serum triglyceride was estimated by enzymatic colorimetric GPO-PAP method [16] by using a double beam spectrophotometer (Shimadzu, Japan). In detail, three washed test tube were taken at first and labelled as 'Blank', 'Standard' and 'Serum sample' respectively. 10 ml of serum sample was taken to a test tube labelled 'sample serum' with the help of micropipette. Then 10 μ l of standard triglyceride sample was taken into the 'standard' labelled test tube. After that 1 ml of enzyme reagent were taken into each test tube respectively and kept for incubation at 37 $^{\circ}$ C for 20 minutes in dark place. The absorption of the solution was measured at 530nm against blank. Then the absorbance value was put in the triglyceride measuring equation against standard sample and the cholesterol level was determined.

Triglyceride concentration = {OD of sample /OD of standard} \times 200(mg/dl) = {OD of sample/OD of standard} \times 5.17 (mmol/l)

2.9. Statistical Analysis

All the results were expressed as mean \pm SEM. P-value and were calculated by one-way ANOVA using SPSS software, version 22.0 (IBM Corporation, New York, NY, U.S.A.). Where $P \leq 0.05$ was considered as statistically significant.

3. RESULTS

3.1. Phytochemical Analysis of Methanolic Extract of *V. unguiculata* Seed

Results of phytochemical screening presented in Table 1 revealed that the seeds of *V. unguiculata* possessed high amount of phenol, terpenes and protein followed by alkaloids and carbohydrates (Table 1). Seeds extract were subjected for the determination of total phenolic content and the results was obtained as 119.716 \pm 5.40mg of GAE (Gallic acid equivalent)/gm dry extract.

3.2. Effect of *V. unguiculata* Seed on Body Weight

Animals were grouped randomly and with their average body weight being without considerable differences among the groups at the beginning of the study. Mice fed on normal diet maintained their body weights without fluctuation. Weight of mice of group-3, fed on high fat, started to increase considerably after day 5 and continued till the end of the experiment. Whereas, when the plant's aqueous extracts were

Table 1: Phytochemical Groups Present in *V. unguiculata* Seed

Phytochemicals	Test	Observation
Alkaloid	Hager's test	++
	Meyars test	++
Phenol	Ferric chloride test	+++
Carbohydrate	Molisch's Test	+
	Benedict's Test	+
Saponines	Froth test	-
	Foam test	-
Tannin	Gelatin test	+
Flavonoid	Alkaline reagent test	+
Protein	Xanthoproteic Test	+++
	Ninhydrin Test	+++
Terpenes	Copper acetate test	+++

Here, +++ very strongly present, ++ strongly present, + weakly present and - absent.

administered, regardless of normal diet or high fat, they caused a significant reduction of body weight. The reduction was more prominent in group-4, where the mice were fed with high fat diet with aqueous extract. Mice in group-4, showed a continuous reduction in body weight after the day 6 (Figure 1). There were no remarkable differences in food intake behaviour among the groups.

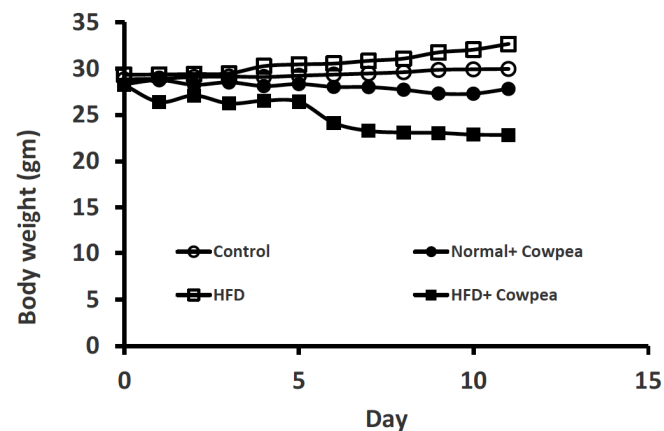
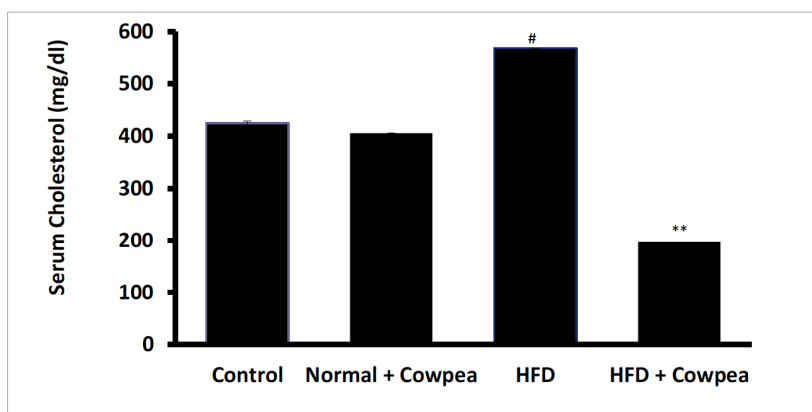


Figure 1: Weight gain by different group of swiss albino mice.

3.3. Effect of *V. unguiculata* Seed on Serum Cholesterol

As predicted, serum cholesterol level was significantly increased in the mice kept on high fat diet compared with normally fed mice. Although, aqueous suspensions were not effective in reducing the cholesterol level in normal fed mice but interestingly, they were able to reverse the effect of high fat induced



p < 0.05, versus control, ** p<0.01, versus HFD

p < 0.05, versus control, ** p<0.01, versus HFD

Figure 2: Serum cholesterol level in different groups of mice.

hypercholesterolemia significantly ($p < 0.01$) (Figure 2). Cowpea seeds reduced the cholesterol level by around 60% in HFD group.

3.4. Effect of *V. unguiculata* Seed on Serum Triglyceride

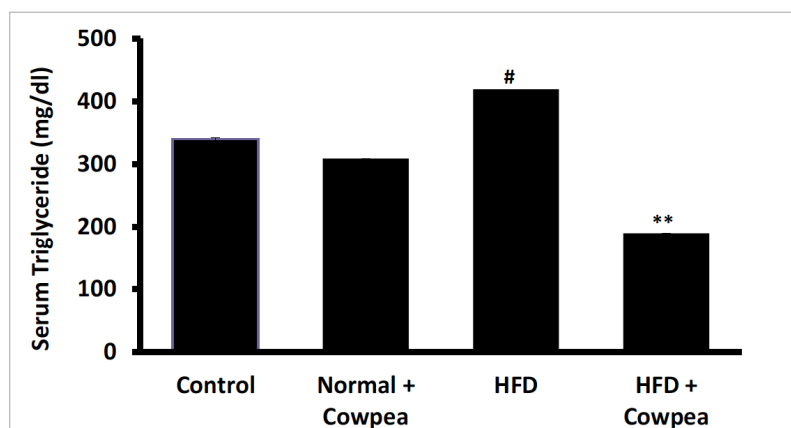
Supporting the notion of fat induced obesity, hypertriglyceridemia was observed in mice of HFD group when compared with normal fed diet. Seeds of *V. unguiculata* showed the triglyceride lowering effect in normal fed mice insignificantly but significant ($p < 0.01$) reduction was documented in HFD induced hypertriglyceridemia (Figure 3). Cowpea contributed about 50% triglyceride reduction in HFD group but in the case of mice with normal diet, it was only 10%.

4. DISCUSSION

As like other legumes, *V. unguiculata* seeds are generally used as food and culinary in Bangladesh and

many other parts of the world. Several health-related beneficial effects of this plant have been studied. Here we aimed to explore the lipid lowering activity of this legume. Dose of the extract applied in this study did not cause the death of any experimental mice.

Prevalence of obesity is becoming high in the world and considered as a major risk factor for the development of other health problems [17]. Human studies have been reported which showed that increased fat intake causes the weight gain and develops the obesity with other metabolic complications. Mimicking this episode, high fat diet induced obese animal models have been commonly used to study the obesity related changes [18]. In this current study, we also observed significant increased body weight of the mice fed with high fat as compared to those on a normal diet. The treatment with cowpea along with a normal diet or HFD, reduced the body weight. Although cowpea causes reduction in the body



p < 0.05, versus control, ** p<0.01, versus HFD

p < 0.05, versus control, ** p<0.01, versus HFD

Figure 3: Serum triglyceride level in different groups of mice.

weight regardless of diet, but cowpea is more prominent to inhibit the weight gain in HFD induced obesity. Cowpea causes about 19% of weight loss of the mice fed with HFD. During our experiment period, we did not observe any changes in the rate of food intake among the animals, thus this prevention of weight gain might be independent of inhibition of food intake by the treated mice.

Furthermore, hypertriglyceridemia and hypercholesteremia, is being linked with development of obesity and obesity related complications. In this current study, we observed considerable elevated level of triglyceride and cholesterol in the mice fed with HFD compared with normal diet group, but as predicted, treatment with cowpea extract was capable to reduce triglyceride and cholesterol level significantly. Cowpea seeds were effective in reducing cholesterol level to about 60% and triglyceride level to about 50% in HFD mice but these values were nearly insignificant in case of normal diet. This result suggests that cowpea may play a role in inhibiting the lipid absorption. This reversing effect of the cowpea confirms its ability to attenuate the obesity and lipid lowering activity. This result motivates us to use this extract for the management of obesity related cardiac complications. Because, it has already been established that drugs having the cholesterol lowering activity are frequently used in the treatment of obesity related cardiac complication [19]. Although it is ambiguous that how this extract reduces obesity and hyperlipidaemia, but some researchers studied that natural products or their derivatives like Orlistat, an anti-obese drug, act by inhibiting the pancreatic lipase to decrease the lipid absorption from gut [20]. Besides this, polyphenols present in plant is an important candidate to inhibit pancreatic lipase [21, 22]. Phytochemical screening (Table 1) of this plant shows that, among other phytochemicals, cowpea seeds possess a high content of polyphenols. The polyphenols may be accounted for the lowering of the lipids in HFD mice by inhibiting the action of pancreatic lipase. Moreover, polyphenols are accounted for the apoptosis of adipocytes [23] and the arrest of cell cycle in G1 phase to inhibit differentiation of 3T3-L1 adipocytes [24]. Along with polyphenol, alkaloid's present in the plants have also been studied for their anti-obese effect by regulating the lipid metabolism and activating AMP-activated protein kinase [25]. Thus, it could not be excluded that alkaloids present in cowpea seed, along with phenols, might interfere with the lipid metabolic pathway as a result of their anti-obese effects.

Furthermore, in the last few years, obesity is considered as an inflammatory disorder [26, 27] and oxidative stress in a key inducer for obesity mediated inflammation [28]. A diet with high antioxidant capacity has been documented for reversing the central adiposity, metabolic and oxidative stress [29, 30]. Cowpeas have been documented for antioxidant activity [13] and this antioxidant capacity might also be responsible for the management of obesity and oxidative stress in obesity.

5. CONCLUSION

Although our present study does not represent the total lipid lowering profile of *V. unguiculata*; but by considering the cholesterol, triglyceride, and body weight lowering index, our data revealed that seeds of cowpea contributed greatly to regulate the pathogenesis of obesity and lipid related metabolism pathway with a possibility of developing anti-obese and lipid lowering drug from *V. unguiculata* (seed). Further studies are warranted for finding out this bioactive metabolite.

COMPETING INTEREST

The authors declare no conflict of interest

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AVAILABILITY OF DATA AND MATERIALS

Data and materials are available in the department of pharmacy, Noakhali Science and Technology University.

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