Antiobesity Effect of Polyherbal Formulations in Cafeteria and Atherogenic Diet Induced Obesity in Rats

*Jain Vikas Kumar, Badjatya Vishal, Nema Rajesh Kumar*

Rishiraj College of Pharmacy, Revati, Sanwer Road, Indore

ABSTRACT
A weight loss supplement containing *Cissus quadrangularis* and other ingredients including, *Glycine angustifolia, Avena sativa* and *Spinacia oleracea* was evaluated in a 6-week trial in female Wistar rats fed on cafeteria and atherogenic diets. Polyherbal Formulation (PHF) was prepared and evaluated for physicochemical parameters. Female Wistar rats were fed cafeteria diet (highly palatable, energy rich animal diet that includes a variety of human snack foods) and atherogenic diet for 6-week. Polyherbal formulation was administered in a dose of 400 mg/kg, p.o., once daily to the drug treatment groups. The effect of Polyherbal formulation was recorded on the parameters like body weight, food and water intake, behavioral activity and various biochemical parameters like serum glucose, total cholesterol and triglyceride levels. Significant reduction in body weight, behavioral activity and serum glucose levels after treatment was observed with Polyherbal formulation in cafeteria diet and atherogenic diet fed rats. Treatment with Polyherbal formulation also significantly decreased total cholesterol and triglyceride in rats fed with atherogenic diet. Polyherbal formulation had no adverse effect on behavioral parameter. The Polyherbal formulation helped reduce body weight by approx 20 - 25 % in animal fed on cafeteria and atherogenic diets.

Keywords: Obesity, Cafeteria diet, Atherogenic diet, Diet induced obesity, Polyherbal formulation (PHF), antiobesity effect

INTRODUCTION
Obesity is a serious health problem. Among the multiple factors contributing to its etiology, the sedentary life styles, white collar jobs, lack of exercise, psychological factors, and the consumption of energy rich diets are the major ones. The incidence and prevalence of obesity are rising both in developed and developing countries. Obesity is excessive accumulation of fat in the body associated with numerous complications such as cardiovascular disease, insulin resistance; type 2 diabetic mellitus, cancer and osteoarthritis. Due to obscure etiology, the pharmacological treatment of obesity has been a particularly challenging task. Further, the cause of concern is the non-availability of drugs for its treatment and the short-term efficacy and limiting side effects of the available drugs.

A Polyherbal formulation containing aqueous extracts of *Cissus quadrangularis, Glycine angustifolia, Avena sativa* and *Spinacia oleracea* was prepared and evaluated. The ingredients in the formulation have been reported to possess thermogenic, hypcholesterolemic, body weight lowering, antidiabetic and digestive stimulant properties. Thus, the present study was carried out with an aim to investigate the antiobesity activity of Polyherbal formulation in diet induced obesity in rats.

MATERIALS AND METHODS
Animals: Thirty Female Wistar rats (80 to 120 g) bred at Animal House, Rishiraj College of Pharmacy, Indore were used in this study. They were housed five per cage under standard laboratory conditions at a room temperature at ± 2°C with 12h light/dark cycle. The animals were acclimatized to the environment for a week prior to experimentation with free access to water and pellet diet for rats (week 0). The study was conducted in accordance with the Indian national science academy guidelines for care and use of animals in scientific research. All the experiments were conducted between 0900 and 1700 h. Procurements of Drugs: The crude drugs were purchased from the local crude drug market, Rajwada Indore and their identity was confirmed by correlating their morphological characteristics with those given in literature. The ingredients were identified and confirmed with the in house authentic specimens of the Matria Medica.

Preparation of Polyherbal formulation: The ingredients (*Cissus quadrangularis, Glycine angustifolia, Avena sativa* and *Spinacia oleracea*) were individually dried in shade, powdered and then mixed in mentioned proportion with help of suspending agent. The finished formulation was a fine white color suspension.

Acute toxicity studies: Healthy Female Wistar rats (180-250 gm), starved overnight were divided into five groups (n=5) and were orally fed with the Polyherbal formulation in increasing dose levels of 100, 200, 400, 600 and 800 mg/kg body weight (all four herbs in equal proportional). The rats were observed continuously for 2 h for behavioral, neurological and autonomic profiles and after 24 and 72 for any lethality.

Dose selection: The Polyherbal formulation of the four herbs (viz. *Cissus quadrangularis, Glycine angustifolia, Avena sativa* and *Spinacia oleracea*) was prepared...
accor

1. **Table: 1 Effect of Poly-herbal formulation (Dose 400 mg/kg/day, orally, after 6 weeks) on body weight**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameter</th>
<th>Change in body weight (%)</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control Diet</td>
<td></td>
<td>8.2±2.1</td>
<td>8.7±2.2</td>
<td>9.1±3.1</td>
<td>12.3±3.8</td>
<td>15.2±4.1</td>
<td>20.8±3.2</td>
</tr>
<tr>
<td>2</td>
<td>Cafeteria Diet</td>
<td></td>
<td>9.1±2.2</td>
<td>11.7±2.6</td>
<td>15.0±2.9*</td>
<td>21.2±3.2*</td>
<td>29.8±3.8*</td>
<td>38.1±3.4*</td>
</tr>
<tr>
<td>3</td>
<td>Cafeteria Diet + Poly herbal formulation</td>
<td></td>
<td>8.7±1.8</td>
<td>10.2±2.4</td>
<td>14.4±3.2</td>
<td>18.2±3.4</td>
<td>25.2±3.8</td>
<td>28.4±4.1</td>
</tr>
<tr>
<td>4</td>
<td>Atherogenic Diet</td>
<td></td>
<td>9.8±2.2</td>
<td>12.9±2.6</td>
<td>17.4±2.8*</td>
<td>24.8±3.2*</td>
<td>31.2±4.1 *</td>
<td>44.4±4.3*</td>
</tr>
<tr>
<td>5</td>
<td>Atherogenic Diet + Poly herbal formulation</td>
<td></td>
<td>8.6±2.3</td>
<td>11.1±2.4</td>
<td>16.4±1.8</td>
<td>22.2±2.1</td>
<td>26.6±2.2</td>
<td>32.2±3.2*</td>
</tr>
</tbody>
</table>

*Values are mean ± SD of 5 animals each P<0.05, * as compared to group I (Control diet), ** as compared to group IV (Atherogenic diet)*

2. **Table: 2 Effect of Poly-herbal formulation on food intake (Dose 400 mg/kg/day, orally, after 6 weeks treatment)**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameter</th>
<th>Food intake (g)/100 g body weight</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control Diet</td>
<td>11.4±1.4</td>
<td>12.8±1.6</td>
<td>13.6±2.2</td>
<td>14.8±1.2</td>
<td>15.6±1.4</td>
<td>16.6±2.4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Cafeteria Diet</td>
<td>11.6±1.2</td>
<td>13.2±1.2</td>
<td>14.8±1.6</td>
<td>15.8±1.1</td>
<td>16.8±2.1</td>
<td>18.6±2.2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Cafeteria Diet + Poly herbal formulation</td>
<td>11.4±1.6</td>
<td>13.2±1.4</td>
<td>14.6±1.8</td>
<td>15.0±1.6</td>
<td>15.2±1.4</td>
<td>15.0±2.1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Atherogenic Diet</td>
<td>12.2±1.0</td>
<td>14.8±1.2</td>
<td>16.2±1.4</td>
<td>17.2±1.5</td>
<td>18.2±2.1</td>
<td>19.2±1.2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Atherogenic Diet + Poly herbal formulation</td>
<td>10.8±1.2</td>
<td>13.4±1.4</td>
<td>14.9±2.1</td>
<td>16.2±2.4</td>
<td>16.4±1.7</td>
<td>16.0±1.2</td>
<td></td>
</tr>
</tbody>
</table>

*Values are mean ± SD of 5 animals each*

3. **Table: 3 Effect of Poly-herbal formulation on water intake (Dose 400 mg/kg/day, orally, after 6 weeks treatment)**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameter</th>
<th>Water intake (g)/100 g body weight</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control Diet</td>
<td>10.2±1.4</td>
<td>12.2±1.0</td>
<td>14.1±1.3</td>
<td>13.2±1.4</td>
<td>15.2±1.5</td>
<td>16.8±1.7</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Cafeteria Diet</td>
<td>11.2±1.5</td>
<td>12.4±1.2</td>
<td>14.2±1.2</td>
<td>15.2±1.3</td>
<td>16.1±1.5</td>
<td>17.0±1.6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Cafeteria Diet + Poly herbal formulation</td>
<td>11.4±1.4</td>
<td>12.2±1.2</td>
<td>14.8±1.4</td>
<td>15.1±1.5</td>
<td>15.4±1.2</td>
<td>14.8±1.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Atherogenic Diet</td>
<td>10.8±1.1</td>
<td>12.4±1.2</td>
<td>15.3±1.3</td>
<td>16.8±1.5</td>
<td>17.2±1.2</td>
<td>17.0±1.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Atherogenic Diet + Poly herbal formulation</td>
<td>10.4±1.0</td>
<td>11.8±1.1</td>
<td>12.4±1.2</td>
<td>14.8±1.4</td>
<td>15.2±1.3</td>
<td>15.0±1.2</td>
<td></td>
</tr>
</tbody>
</table>

*Values are mean ± SD of 5 animals each*

4. **Table: 4 Effect of Polyherbal formulation open field behavior of rats fed on cafeteria diet and atherogenic diet. (Dose 400 mg/kg/day, orally, after 6 weeks treatment)**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment</th>
<th>Frequency of open field behavior++</th>
<th>Ambulation</th>
<th>Rearing</th>
<th>Grooming</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control Diet</td>
<td>65.4 ± 6.43</td>
<td>14.4 ± 3.75</td>
<td>6.6 ± 1.17</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Cafeteria Diet</td>
<td>81.3 ± 7.65</td>
<td>33.0 ± 5.76</td>
<td>6.0 ± 0.91</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Cafeteria Diet + Poly herbal formulation</td>
<td>119.0 ± 8.88</td>
<td>32.0 ± 1.73</td>
<td>10.8 ± 1.32</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Atherogenic Diet</td>
<td>55.8 ± 6.18</td>
<td>18.0 ± 2.74</td>
<td>6.0 ± 1.05</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Atherogenic Diet + Poly herbal formulation</td>
<td>97.0 ± 6.65</td>
<td>23.5 ± 3.71</td>
<td>12.3 ± 2.95</td>
<td></td>
</tr>
</tbody>
</table>

*Values are mean ± SD of 5 animals each, ++Tested for 5 minutes duration*

according to their effective doses. They were well mixed equal proportional in a mortar and pestle till the stable and homogeneous suspension formed and then administered orally in a dose of 400 mg/kg, per oral per day for 6 weeks. This dose was selected on the basis of our preliminary studies. The control animals received only the vehicle in the same volume and through the same route. Polyherbal formulation was quantitatively evaluated for any incompatibility by visible observation of precipitation and separation.

Experimental induction of obesity in rats: Cafeteria and atherogenic diets

The cafeteria diet consisted of 3 diets:
Table 5: Effect of Polyherbal formulation on biochemical parameters in rats fed on cafeteria diet and atherogenic diet. (Dose 400 mg/kg/day, orally, after 6 weeks treatment)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment</th>
<th>Biochemical Parameters (mg/dl)</th>
<th>Week 0</th>
<th>Week 6</th>
<th>Total Cholesterol</th>
<th>Week 0</th>
<th>Week 6</th>
<th>Triglyceride</th>
<th>Week 0</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control Diet</td>
<td>Glucose</td>
<td>51.1±2.1</td>
<td>52.1±3.1</td>
<td>84.7±1.3</td>
<td>85.7±2.4</td>
<td>83.7±2.1</td>
<td>84.9±3.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Cafeteria Diet</td>
<td></td>
<td>52.3±3.2</td>
<td>62.3±6.7*</td>
<td>82.4±4.5</td>
<td>83.4±2.1</td>
<td>84.8±1.9</td>
<td>85.8±2.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Diet + Poly herbal formulation</td>
<td>Atherogenic Diet</td>
<td>51.8±1.8</td>
<td>64.8±3.8</td>
<td>82.7±4.1</td>
<td>84.7±3.2</td>
<td>83.5±5.2</td>
<td>84.5±4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Diet + Poly herbal formulation</td>
<td>Atherogenic Diet</td>
<td>50.7±3.1</td>
<td>58.7±4.1*</td>
<td>84.4±6.1</td>
<td>128.4±8.1*</td>
<td>86.7±4.3</td>
<td>87.9±6.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td>52.7±2.8</td>
<td>61.9±3.8</td>
<td>85.7±3.3</td>
<td>105.7±6.4**</td>
<td>84.5±5.1</td>
<td>69.5±7.8**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD of 5 animals each P<0.05, * as compared to group I (Control diet), ** as compared to group IV (Atherogenic diet)

Effect of Polyherbal formulation on biochemical parameters in rats fed on cafeteria diet and atherogenic diet. (Dose 400 mg/kg/day, orally, after 6 weeks treatment)

1. Condensed milk 40g & Bread40g
2. Chocolate 15g, Biscuits 30g & Dried coconut 30g
3. Cheese 40g & Boiled potatoes 50g

The three diets were presented to group of 5 rats on day 1, 2 and 3 respectively and then repeated in same succession. The atherogenic diet consisted of cholesterol 1%, cholic acid 0.5% and lard oil 5%. These diets were provided in addition to normal pellet chow.

Measurement of change in body weight, food and water intake: Change in body weight: The body weight (g) was recorded on week 0 (before starting drug treatment) and then on alternate days for 6 weeks in each group before giving the food and water.

% change in BW = [(BW at end of ‘n’ week (g) - BW on day 1 (g))/BW on day 1] x 100

Food consumption: All animals were housed individually in cages; measured amount of food was kept in each cage daily. Next day the remaining food was weighed. For cafeteria diet, each item was provided in excess along with and excess amount of standard pellet diet. Food consumption per 100 g of body weight of animals:

Diet consumed (g) = total diet provided (g) – total diet remaining (g)

Diet consumed per 100 g BW (g) = (Diet consumed in ‘n’ week/ mean body weight in that week) x 100

Water intake: Rats were provided with measured quantity of water each morning. Left over volume was noted next morning to calculate water intake per 100 g of body weight of animals.

Water consumed (ml) = Water provided (ml) – Water remaining (ml)

Water intake per 100 g BW (g) = (Water consumed in ‘n’ week/ mean body weight in that week) x 100

Behavioral parameter: Locomotors activity: It was recorded on Week 6 using open field behavior test apparatus and 30 min after drug administration to treatment groups. The apparatus consisted of a circular wooden arena of 75 cm diameter and wall with a height of 25 cm. Open field test was performed by placing the rat in the center circle and recording the ambulatory activity, the frequency of rearing and grooming for a 5 min test period.

Biochemical parameters: Fasting blood Glucose, Total cholesterol and Triglyceride levels: On Week 6 changes in glucose, total cholesterol and triglyceride levels were measured from serum samples using the biochemical kits.

STATISTICAL ANALYSIS

The results are expressed as mean ± SD. Comparisons between the treatment groups and control cafeteria and atherogenic group were performed by Holm Sidek Method followed by multiple t-tests using Graph pad Prism 6.0 Software. In all tests the criterion for statistical significance was P < 0.05.

RESULTS

Effect of poly herbal preparation on body weight, food and water intake –There was a significance difference in percentage change in body weight among the groups. On the basis of statistical analysis, there was a significance increase in % change in body weight in group II and IV as compared to group I, three weeks onwards (Table 1). The group II (Cafeteria diet) and group IV (Atherogenic diet) significantly increase % change in body weight from 20.8 ± 3.2 in group I (Control) to 38.1 ± 3.4 in group II (Cafeteria diet) and to 44.4± 4.3 in group IV (Atherogenic diet) (P<0.05) at 6th week of study in comparison to base line. Drug (PHF) treated groups III and V with cafeteria and atherogenic diet shows % decrease in body weight as compared to group II and group IV after 6th weeks. Effect on % decrease in body weight group II (Cafeteria diet) as compared group III (cafeeteria diet with PHF) was 28.4 ± 4.1 and Effect on significantly % decrease in body weight group IV (Atherogenic diet) as compared group V (Atherogenic diet with PHF) was 32.2 ± 3.2 (P<0.05) at 6th week of study in comparison to base line (Table 1).

Food and water intake by animals was measured every day. Average food and water intake per week are given in Table 2 and 3. After statistical analysis, it was found that difference in food and water intake among the groups.
throughout the study was not significant changes (P>0.05). Poly herbal preparation also did not cause any significant change in total food and water intake in group III and V as compared to group II and IV (P<0.05).

Effect of poly herbal preparation on behavioral parameter-

There was significant increase in ambulatory and rearing activity in cafeteria diet group animals as compared to control group. Treatment with poly herbal preparation as per resulted in increase in only ambulatory activity but in cafeteria diet and atherogenic diet treated animals it enhanced all the three activities in Table 4.

Effect on biochemical parameters: No significant difference was found in the basal levels of fasting glucose, total cholesterol and triglyceride among groups (P>0.05). Cafeteria diet (group II) had to significant increase in the fasting glucose 62.3 ± 6.7 as compared to control group I (P>0.05). Atherogenic diet (group IV) had to significant increase in the fasting glucose 58.7 ± 4.1 and total cholesterol 128.4 ± 8.1 as compared to control group I (P>0.05).

Drug (PHF) treated groups III with cafeteria diets did not shows any significant difference in basal levels of fasting glucose, total cholesterol and triglyceride (P>0.05) as compared to group II. Drug (PHF) treated groups V with Atherogenic diet did not shows any significant difference in basal levels of fasting glucose, but there was significant decline in the total cholesterol 105.7 ± 6.4 and triglyceride 69.5 ± 7.8 (P>0.05) as compared to group IV after 6th weeks treatment (Table 5).

**DISCUSSION**

Herbal medicines are being looked up for treatment of obesity due to a long standing experience with them in the traditional system of medicine and failure of many conventional medicines. There are many preclinical and clinical studies in which efficacy of herbal drugs have been reported. In the present study, evaluate the antiobesity activity of poly herbal preparation was studied using the dietary (cafeteria and atherogenic diets) animal models of obesity as they have been reported to bear close resemblance to human obesity. The results of our study showed that rats fed with a variety of highly palatable, energy rich, high carbohydrate cafeteria foods (Chocolate, coconut and butter cookies) elicited significant increase in body weights and fat pad mass. Cafeteria diets have been previously reported to increase energy intake and cause obesity in humans as well as animals. Further, the composition and variety of cafeteria foods also exert synergistic effects on the development of obesity. Increase in body weight in cafeteria diet control group started from 2nd week of the study in comparison to standard pellet diet control. The increase in body weight, lipid profile level demonstrates successfully development of obesity in the study.

High fat diet increases the expression of fatty acid catabolism related gene in small intestine which is associated with development of obesity. In this study atherogenic diet fed rats also exhibited an increased body weight along with corresponding rise in cholesterol levels. Polyherbal formulation treatment prevented the increase in body weight in both cafeteria and atherogenic diet fed groups. However the effect was significant from week 3 onwards. Lee’s index, also known as obesity index, has been shown to correlate well with % body fat especially in the diet induced obesity model. Fluctuations were observed in food and water intake over 6 week treatment period. At the end of study, the food and water intake did not significantly differ between groups suggesting the
Polyherbal formulation neither causes anorexia nor diuresis. Its anti-obesity effect might be thought to be due to improved digestion, energy metabolism or lipolysis as suggested for other antiobesity herbs.\(^{22, 21}\) High fat diets increase the expression of fatty acid catabolism related genes in the small intestine which is associated with development of obesity. Pancreatic lipase is well known for its role in fat metabolism and absorption of lipolysis products\(^{22, 23}\). Although, lipase inhibitory effect of Polyherbal formulation was not evaluated but its involvement in antiobesity effect of Polyherbal formulation cannot be ruled out. The Polyherbal formulation (\textit{Cissus quadrangularis, Glycine angustifolia, Avena sativa and Spinacia oleracea}) may offer enormous therapeutic potential for its treatment to decrease fat absorption by inhibiting digestion.

High fat diet induced hyperlipidemia, hyperinsulinemia and increased level of leptin are well reported\(^{31, 32}\). In present study also, cafeteria and atherogenic diet increased TC and TG levels. Poly herbal formulation significantly decreased the TC and TG levels as compared to control cafeteria and atherogenic diet groups. The Polyherbal formulation might be exert hypolipidemic effect and lower cholesterol levels that provide beneficial effect in diabetes and obesity.

Behavioral activity of animal were evaluated in the present study using open field behavior test. The results indicate the high fat diet does not alter behavioral activity. Poly herbal formulation also does not adversely effect on behavioral activity.

**CONCLUSION**

The weight reducing effect of the Polyherbal formulation of \textit{Cissus quadrangularis, Glycine angustifolia, Avena sativa and Spinacia oleracea} may be attributed to inhibit lipogenesis. Thus, in conclusion the study demonstrates the potential antiobesity effect of Polyherbal formulation, prevents the increase in body weight without affecting food and water intake, alter the lipid profile favorably in rats fed on cafeteria and atherogenic diets. However the study has few limitations. Further action suggest a potential antiobesity effect of Polyherbal formulation which need confirmation using lipoprotein lipase assay and other animal model like genetic model may further enhance understanding the mechanism of action the Polyherbal formulation.

**REFERENCE**

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