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Antiobesity effect of Phytolacca berry in rats

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Abstract

Extract of berries from *Phytolacca americana* L. plant (Phytolacca berry) is widely used in alternative systems of medicine i.e. in homeopathy for the treatment of obesity. A study was made to evaluate its anti-obesity effect in the rat model. The experiement was conducted in rats using the diet-induced obesity model in which cafeteria and atherogenic diets were used. The activity was tested by measuring body weight, glucose, total cholesterol and triglyceride levels in comparison with a control group. The results revealed that the Phytolacca berry extract had significant anti-obesity activity by reducing excess body weight, and cholesterol and triglyceride concentrations. This activity may be due to its appetite suppressant activity and also by increasing the metabolic rate. Further studies are needed to be conducted to prove its utility in humans.

Key words: atherosclerosis, homoeopathy, obesity, *Phytolacca americana*, Phytolacca berry, rat model.
Abbreviations: AD, atherogenic diet; CD, cafeteria diet.

Introduction

Maintaining energy homeostasis is fundamental for survival. However, obesity is due to over-nutrition and an increasing worldwide public health problem (Friedman 2000; Wilborn et al. 2005). The World Health Organization recognized the obesity epidemic as one of the top 10 global health problems. In developed countries it is estimated that 5% of total health costs are related to obesity (Kopelman 2000; Odgen 2002) and is often considered a problem of the belly rather than of the brain. Epidemiological studies from India suggest a rise in morbid obesity, close to 5% (Misra 2001). Obesity is the excess accumulation of fat in the body and an imbalance in energy intake and energy expenditure, which is the most common nutritional disorder in the developed world and is considered to be a risk factor associated with the development of the major human diseases. Various factors may lead to obesity, such as sedentary life style, increased intake of high calorie (energy and fat) food, genetic determinants and psychologic and behavioural determinants (Wilborn et al. 2005).

Phytolacca americana L. (Phytolacca berry) is a common perennial native plant found in Northern and Central North America. It is widely used to treat obesity due to its appetite suppressant activity, and hypocholesterolemic and excess body weight reducing properties (Haider et al. 2006). Phytochemical investigations of the plant revealed the presence of triterpene saponins, triterpene alcohols, betacyanins and lignanes etc. (Wang 2008). No extensive pharmacological studies have been conducted on Phytolacca berry. Therefore, the aim the present study was to conduct an evaluation of the antiobesity activity of Phytolacca berry extract on diet-induced obese rats.

Materials and methods

Forty two female wistar rats (180 to 220 g) were obtained from the animal house facility of St. Peters Institute of Pharmaceutical Sciences, Warangal, India. They were divided into seven groups and housed in polypropylene cages under standard laboratory conditions at a room temperature 23 ± 2 °C with 12/12 h light/dark cycle. The animals were provided with pellet chow and water ad libitum. The study protocol was approved by Institutional Animal Ethical Committee. Standard kits used for the estimation of total cholesterol, triglyceride, glucose and total protein were purchased from Crest Biosystems, Goa, India. Cholesterol and cholic acid were from SD Fine Chemicals (Mumbai, India), and Lard oil from Sigma Chem. Co. (USA). The entire experimental work was conducted in the department of pharmacology, St. Peters Institute of Pharmaceutical Sciences, Warangal, Andhra Pradesh, India.

Phytolacca berry tablets were obtained from Dr. Willmar Schwabe India Pvt. Ltd. (Noida, India). Each tablet contained 20% of the Phytolacca berry extract.

Obesity was induced by using two types of diets: cafeteria diet (CD; Harris 1993) and atherogenic diet (AD; Jiao et al. 1991). Cafeteria diet was a high carbohydrate/sugar diet and consisted of condensed milk (40 g), bread (40 g), chocolate (15 g) biscuits (30 g), dried coconut (30 g), cheese (40 g) and boiled potatoes (50 g). The atherogenic diet consisted of 1% cholesterol, 0.5% cholic acid, and 5%

lard oil. These high calorie diets were provided to each rat through oral ingestion along with a normal pellet chow.

The animals were divided in seven groups containing six animals each. Group I served as control and received only normal pellet diet; Group II received AD; Group III received AD and Phytolacca Berry extract high dose (2.850 mg kg^{-1}); Group IV received AD and Phytolaccca Berry low dose (0.285 mg kg^{-1}); Group V received CD; Group VI received CD and Phytolacca Berry high dose; and Group VII received CD and Phytolacca berry low dose for 40 days. The normal pellet diet was provided to all groups of rats ad libitum. Cafeteria and atherogenic diets were orally fed to rats twice a day in the quantity of 10 mL kg^{-1}.

Evaluation of physiological parameters were performed according to Kaur and Kulkarni (2000).

The rats of different groups were weighed initially on day 1 and then on alternate days for 40 days and recorded. The body temperature of the rats in different groups was recorded on day 39 using a digital telethermometer (Inco Instruments and Chemicals, Ambala, India) for a period of 3 h at 0, 30, 60, 90, 120 and 180 min using contact time of 1 min.

Locomotor activity was recorded on day 40 for all the treatment groups using the open field behavior test apparatus after 30 min of the administration of test drug. The apparatus consisted of a circular wooden area of 75 cm and a wall with a height of 25 cm. The test was performed by placing the rat in the middle of the apparatus and recorded the frequency of rearing and grooming activity and ambulatory activity for 5 min.

After exsanguination of rats by cervical dislocation, different organs (kidney, liver, heart, spleen) and fat pads (mesenteric, ovarian, perirenal and uterine) were removed and weighed.

Glucose was determined according to Trinder (1969). The blood was collected from rats immediately after exsanguinations and serum was separated using centrifugation. In all the groups on day 41 the serum glucose was estimated by using GOD/POD method with a biochemical kit manufactured by Crest Biosystems (Goa, India).

The separated serum was subjected to biochemical analysis and total cholesterol (CHOD/PAP method; Allain 1974), triglycerides (GPO/PAP method; Bucolo et al. 1973) and total protein (Biuret method; Gornall et al. 1949) were determined.

The results were expressed as means ± SE. Comparisions between treatment and control groups were performed by analysis of variance (ANOVA) followed by the Bonferroni multiple comparison test. The statistical level of significance was $P < 0.05$.

Results and discussion

Obesity results from dysregulation between energy intake and expenditure. It is believed to be associated with numerous diseases including hyperlipidemia, hypercholesterolemia and type 2 diabetes (Friedman 2000). There are many drugs currently used for the treatment of obesity, but due to the various complications which they produce, research has been carried out on identifying phytochemicals from traditional medicinal plants that can provide a safer therapy for obesity. This will open new research avenues for the future (Haider et al. 2006).

In this study, Phytolacca berry tablets were tested. They contain an extract of *Phytolacca americana* native to North America and are widely used as a homoepathic medicine to treat obesity. This study provides a suitable explanation for the anti-obesity activity of Phytolacca berry. Animal models of obesity have been reported to bear close resemblance to human obesity (Sclafani et al. 1976).

In this study obesity was induced by two types of diets: cafeteria diet (Group II), which was a high carbohydrate diet, and atherogenic diet (Group III), which was a high fat diet given along with a pellet chow.

There was a significant increase in body weight in the group of animals fed with cafeteria diet (CD) and atherogenic diet (AD) compared with the control group.

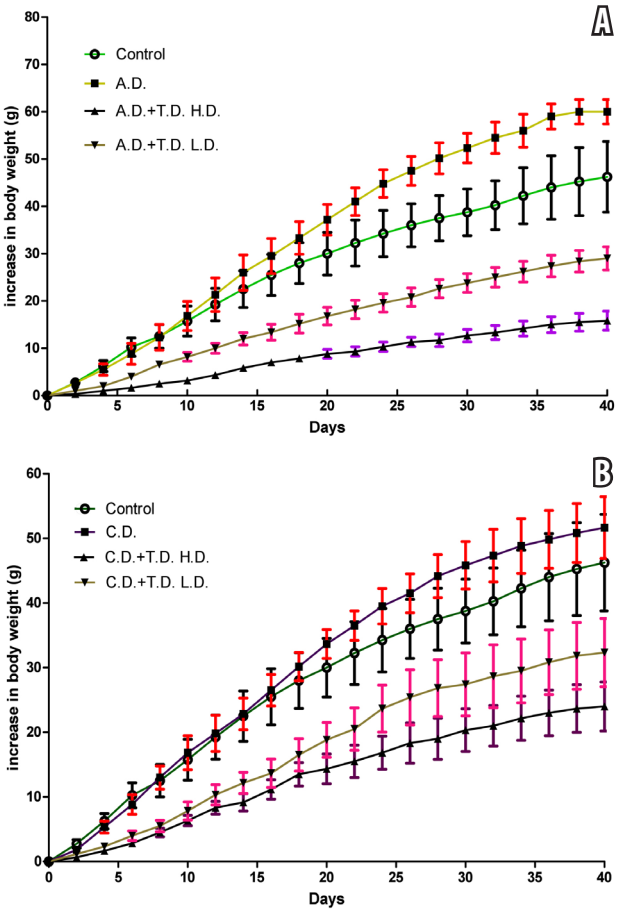

Fig. 1. Effect of Phytolacca berry tablets on body weight of rats fed with atherogenic diet (A) and cafeteria diet (B) for 40 days. A.D., atherogenic diet, C.D., cafeteria diet; T.D. H.D., test drug high dose, T.D. L.D., test drug low dose.

Treatment with the test drug caused significant decrease in body weight when compared to the control group and also significantly effective in reducing weight in the cafeteria and atherogenic diet group animals (Fig. 1).

There was a significant increase in ambulatory activity and rearing activity in the cafeteria fed group compared to control group. Treatment with the test drug resulted in promotion of ambulatory activity and rearing activity and there was an increase in ambulatory activity in AD with Phytolacca berry treatment. There was a significant increase in grooming activity in AD and CD drug treatment groups as compared to that for the control group (Table 1).

Treatment with the test drug significantly increased body temperature in both cafeteria and atherogenic diet groups (Table 2). This indicates that Phytolacca berry increased the metabolic rate in the body, which ultimately resulted in lowering of fat stores.

Administration of CD and AD increased the weight of organs and increased fat accumulation in uterine and perirenal fat pads. Treatment with the test drug in the cafeteria diet group reduced fat accumulation in uterine and perirenal fat pads (Table 3).

Treatment with the test drug showed increased glucose level, compared to that in the control group especially in CD animals im the high dose drug group, but it significantly decreased total cholesterol and triglyceride levels in CD and AD groups, which had high cholesterol and triglyceride concentration. compared to those in control group animals. Animals fed with the cafeteria diet had higher total protein content compared to that in the control, and treatment with

Table 1. Effect of Phytolacca berry on open field behavior of rats fed on different diets. Values are means ± SE. *, significant difference to control; a, significant difference to atherogenic diet group; b, significant difference to cafeteria diet group ($P < 0.05$)

No	Treatment	Frequency of open field behavior		
		Ambulation	Grooming	Rearing
1	Control	74.67 ± 3.33	8.33 ± 0.33	13.17 ± 1.42
2	Atherogenic diet	59.00 ± 0.78	5.66 ± 0.42	23.17 ± 1.99*
3	Atherogenic diet + test drug high dose	93.00 ± 2.30*	10.83 ± 0.79a	38.00 ± 2.69*
4	Atherogenic diet + test drug low dose	80.33 ± 4.86a	8.33 ± 1.08	19.5 ± 2.2
5	Cafeteria diet	84.17 ± 3.10	5.33 ± 1.25	41.17 ± 1.53*
6	Cafeteria diet + test drug high dose	96.33 ± 4.10*	12.17 ± 1.24b	27.33 ± 2.89*
7	Cafeteria diet + test drug low dose	64.5 ± 4.68b	11.83 ± 1.83b	30.67 ± 1.74*

Table 2. Effect of Phytolacca berry on body temperature (°C) of rats fed on different diets. Values are means ± SE. *, significant difference ($P < 0.05$) to control group

No	Treatment	Body temperature at time (min)					
		0	30	60	90	120	180
1	Control	38.08 ± 0.04	38.73 ± 0.17	38.67 ± 0.19	38.97 ± 0.03	38.47 ± 0.16	38.43 ± 0.13
2	AD	39.92 ± 0.08*	39.75 ± 0.17*	39.5 ± 0.18*	39.87 ± 0.12	40.0 ± 0.0*	39.75 ± 0.17*
3	AD + test drug high dose	38.08 ± 0.04	39.83 ± 0.16*	40.10 ± 0.06*	40.23 ± 0.08*	39.58 ± 0.20*	38.5 ± 0.18
4	AD+ test drug low dose	39.17 ± 0.40*	39.75 ± 0.17*	39.8 ± 0.16*	39.67 ± 0.21	39.25 ± 0.17	39.20 ± 0.38
5	CD	38.15 ± 0.20	38.27 ± 0.16	38.23 ± 0.15	38.30 ± 0.15	37.92 ± 0.20	38.17 ± 0.16
6	CD + test drug high dose	38.77 ± 0.30	38.8 ± 0.19	39.23 ± 0.40	38.83 ± 0.30	38.67 ± 0.21	38.25 ± 0.25
7	CD + test drug low dose	37.83 ± 0.41	39.0 ± 0.36	39.08 ± 0.32*	38.9 ± 0.19	38.6 ± 0.20	38.25 ± 0.30

Table 4. Effect of Phytolacca berry on various biochemical parameters in rats fed on different diets. Values are mean ± SE. *, significant difference to control; a, significant difference to AD; b, significant difference to CD ($P < 0.05$)

No	Treatment	Biochemical parameters (mg dL^{-1})			
		Glucose	Total cholesterol	Triglycerides	Total protein
1	Control	45.33 ± 4.716	84.67 ± 5.19	93.67 ± 3.06	7.31 ± 0.28
2	AD	65.33 ± 8.22	100.3 ± 4.36	111.7 ± 7.46	8.25 ± 0.44
3	AD + test drug high dose	63.33 ± 12.49	66.5 ± 5.84a	78.17 ± 5.91	8.73 ± 0.28
4	AD + test drug low dose	47.67 ± 6.20	80.67 ± 3.82	93.83 ± 8.35	8.08 ± 0.24
5	CD	92.67 ± 3.31*	88.33 ± 6.46	165.8 ± 4.9*	10.65 ± 0.50*
6	CD + test drug high dose	110.3 ± 21.01*	53.83 ± 4.93*ab	123.8 ± 5.43*b	9.35 ± 0.41*
7	CD + test drug low dose	97.67 ± 5.61*	62.5 ± 2.30*ab	156.5 ± 9.55*	9.45 ± 0.33*

Table 3. Effect of Phytolacca berry on organ and fat pad weights in rats fed on cafeteria and atherogenic diets. Values are means ± SE. *, significant difference to control; b, significant difference to cafeteria diet ($P < 0.05$)

No	Treatment	Organ weight (g)					Fat pad weight (g)			
		Heart	Liver	Spleen	Kidney L	Kidney R	Mesenteric	Uterine	Ovarian	Perirenal
1	Control	0.91 ± 0.09	6.63 ± 0.66	1.12 ± 0.27	0.72 ± 0.05	0.76 ± 0.02	1.23 ± 0.32	2.7 ± 0.81	1.82 ± 0.79	0.64 ± 0.20
2	AD	0.9 ± 0.07	7.59 ± 0.26	1.15 ± 0.09	0.70 ± 0.05	0.67 ± 0.11	1.69 ± 0.20	4.23 ± 0.55*	1.91 ± 0.30	0.52 ± 0.07
3	AD + test drug high dose	1 ± 0.10	6.72 ± 0.38	1.31 ± 0.16	0.69 ± 0.03	0.67 ± 0.03	1.22 ± 0.14	3.28 ± 0.34b	1.75 ± 0.28	0.45 ± 0.04
4	AD + test drug low dose	0.93 ± 0.04	7.28 ± 0.16	1.08 ± 0.10	0.72 ± 0.02	0.72 ± 0.02	2.07 ± 0.18	3.10 ± 0.51b	2.13 ± 0.23	0.52 ± 0.06
5	CD	1.14 ± 0.21	6.4 ± 0.35	1.28 ± 0.19	0.84 ± 0.04	0.76 ± 0.01	1.72 ± 0.42	5.45 ± 0.69*	1.54 ± 0.43	1.24 ± 0.21
6	CD + test drug high dose	0.93 ± 0.04	6.47 ± 0.25	1.35 ± 0.19	0.91 ± 0.27	0.84 ± 0.31	2.91 ± 0.47	5.71 ± 0.34*	0.56 ± 0.11*	1.04 ± 0.14
7	CD+ test drug low dose	1.03 ± 0.05	6.44 ± 0.34	1.23 ± 0.16	0.89 ± 0.04	1.24 ± 0.24	2.29 ± 0.05	5.77 ± 0.92*	1.71 ± 0.33	1.45 ± 0.34

the test drug lowered the total protein content in cafeteria fed animals (Table 4).

The major building block for the synthesis of triglycerides, in tissues other than adipose tissue, is glycerol. Adipocytes lack glycerol kinase, therefore, dihydroxyacetone phosphate produced during glycolysis is the precursor for triglyceride synthesis in adipose tissue. This means that adipocytes must have glucose to oxidize in order to store fatty acids in the form of triglycerides. Dihydroxyacetone phosphate can also serve as a backbone precursor for triglyceride synthesis in tissues other than adipose. Upon treatment with the test drug, triglyceride levels were significantly reduced. Total protein concentration was increased in cafeteria diet fed animals. Treatment with the test drug decreased the concentration of total protein in the cafeteria group.

The results of the study showed that rats fed with a variety of highly palatable, energy rich, high carbohydrate cafeteria foods elicited significant increase in body weight and fat pad mass. Cafeteria diet has previously been reported to increase energy intake and cause obesity in humans and animals (Rothwell et al. 1983; Bull 1988). In this study, atherogenic diet-fed rats also exhibited increased body weight along with a corresponding rise in cholesterol levels. Thus, it was found that the test drug (Phytolacca berry tablets) used with higher dose had significant effect on obesity. The weight-reducing property of this test drug may be due to its thermogenic property, by increasing metabolic activity and appetite suppressant activity, which controls feed intake.

In conclusion, alternative medicine has been proved as a good source for identification of lead compounds. In the current experimental study, antiobesity effect of phytolacca berry was conducted on rats, and the results were promising. Parameters like weight of the animal, biochemical markers and fat pad weights showed the efficacy of Phytolacca berry extract tablets against obesity in rats. Further studies need to be conducted to evaluate its efficacy in the human population.

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