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Research Article

SCREENING OF ANTI OBESITYACTIVITY OF *ACTNIDIA DELECIOSA* FRUITS

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ABSTRACT

The present work was aimed at the evaluation of antiobesity activity of fresh fruits of *Actnidia deleciosa*. The fresh fruits were fed to the experimental animals using appropriate animal models viz., acute models of food intake and chronic models of food intake and body weight for evaluating the anti-obesity activity.[1] The tests have shown to be exhibit significant anti-obesity activity.

Keywords: *Actnidia deleciosa* and Antiobesity activity.

INTRODUCTION

Obesity is an abnormal accumulation of body fat, usually 20% or more an individual's ideal body weight. Obesity is associated with increased risk of illness, disability and death. More recent guidelines for obesity use a measurement called BMI(body mass index) which is the individual's weight multiplied by 703 and then divided by twice the height in inches.BMI of 25.9-29 is considered overweight; BMI over 30 is considered obese. Excessive weight can result in many serious, potentially life-threatening health problems, including hypertension, Type II diabetes mellitus (non-insulin dependent diabetes), increased risk for coronary disease, increased unexplained heart attack, hyperlipidemia, infertility, and a higher prevalence of colon, prostate, endometrial and possibly breast cancer.[2, 3]

Actinidia deliciosa is a plant, belonging to the family Actinidiaceae is a true perennial with oval, ovoid or oblong fruits up to 2-2.5 inches long with russet brown skin covered short brown hairs. It has soft, small and dark purple or nearly black colored seeds.

It is indigenous to the mountainous regions of southwestern and central china. It is mainly cultivated in Central Europe (New Zealand Chile, Turkey, Portugal, Italy, Greece, France and Japan), United States and China.[4]

The genus Actinidia (Actinidiaceae) are widely used in Chinese folk medicines to treat such diseases as hepatitis, edema, rheumatoid arthritis, gastric cancer and breast cancer etc.[5] Twelve compounds have been isolated from the root of A. deliciosa, and identified as (1) β-sitosterol (2) n-stearic acid (3) isoscopoletin (4) 2, 2-dimethyl-6-chromancarboxylic acid (5) fraxetin (6) aesculetin (7) umbelliferone (8) vanillic acid, (9) protocatechuic acid (10) vanillic acid 4-O-β-D-glucopyranoside (11) 5, 7-ihydroxychromone, and (12) tachioside [6,7,8,9] The review of the scientific literature did not reveal any information on the anti-obesity properties of this plant fruits. In this work, an attempt was made to assess the efficacy of this plant fruits by the High Fat Diet-Induced Obesity methods in experimental animals.

MATERIALS AND METHODS

Collection of Plant Material

The fruits of *Actinidia deliciosa* were collected from the local market of Karimnagar city, authenticated by a taxonomist. A voucher specimen is preserved in the laboratory herbarium. The collected plant material was thoroughly checked and freed from foreign matter.

Preparation of the extract

Fresh fruits of *Actinidia deliciosa.*

Animals
The male Wistar rats (150-200g) were procured from animal house facility of Prathima Institute of Medical Sciences, Karimnagar, Telangana and then housed in standard polypropylene cages and maintained under controlled room temperature (22 ± 2°C) and humidity (55 ± 5%) with 12 h light and 12 h dark cycle. After 1 week of acclimatization with free access to rodent chow diet and water, animals were used in the study. The animals were treated strictly according to the CPCSEA guidelines and the study was conducted after obtaining permission from Institutional Animal Ethics Committee (IAEC) for the period of 12 weeks[10].

Drugs and Chemicals
Orlistat was obtained from Biocon Ltd, Bangalore, India; all other reagents used in this study were of analytical grade.

Acute toxicity and gross behavioral study
The rats were divided into groups (n=6) and were orally fed with increasing doses (50, 150, 300 and 600mg/kg body weight) of fruits. After administration of the extract, the animals were observed during first 2h for their gross behavioral changes and once in 30 min for next 4h and then once in 24h for next 72h to find out percentage mortality. Acute toxicity of all the doses was determined by LD50values.

EVALUATION OF ACTIVITY
Animal models used in the discovery of treatment of obesity
The regulation of the body weight is mainly dependent on two factors i.e., food intake and energy expenditure. The interaction between food intake and energy expenditure determines weight gain and weight loss. An increase in the daily intake of energy accompanied by a sedentary life style leads to the development of obesity. This relationship between food intake, energy expenditure and body weight leads to the different mechanism by which a drug may cause weight loss and is also of relevance in the selection and development of appropriate animal models for evaluating the anti-obesity potential. The various animal models are:

High Fat Diet-Induced Obesity

Experimental Design
In this study, a total of 30 rats were used and divided into five groups of 06 rats each

Group I
Normal Control rats were maintained on standard chow diet and water ad libitum for twelve weeks. No treatment was given to these rats.

Group II
High Fat Diet Control rats were maintained on high fat diet for twelve weeks to induce obesity.

Group III
Orlistat (Standard) (30 mg/kg/day p.o., 6 weeks) was administered to rats along with high fat diet at the end of sixth week and continued up to the end of the twelve weeks.

Group IV-V
Fresh fruits of *Actinidia deliciosa* (200 & 400 mg/kg/day p.o., 6 weeks) was administered to rats along with high fat diet at the end of sixth week and continued up to the end of the twelve weeks.
All the doses were administered orally once a day. Food intake was measured daily for the period of 12 weeks at the same time on per cage basis and the average food consumed were calculated. At the end of the experimental period (on 85thday), the animals were anesthetized with Diethyl ether, following overnight fasting. Blood was drawn by retro-orbital method into a tube and the serum was obtained by centrifugation. After collection of blood, rats were sacrificed; Retroperitoneal (RET), epididymal (EPI), mesenteric (MES) adipose tissue and liver were excised immediately, rinsed with phosphate buffer saline and weighed. The serum, liver and adipose tissue samples were stored at –70°C until analysis.

Morphological Parameters to measure Obesity
The body weights were determined once a week. Body mass index (BMI), Waist-Hip Ratio WHR, Adiposity index, Obesity index was calculated from formula:

BMI = body weight (g)/ length^2 (cm^2)[11]

Waist-hip ratio[12]

Adiposity index = (sum of the weights of perirenal white adipose tissue (WAT), retroperitoneal WAT, and epididymal WAT divided by body weight ×100)[12]

Obesity index = (body weight of rat/nasoanal length (mm) × 104)[12]

Sample Collection

At the end of the experimental period, all rats were sacrificed and blood samples were collected. Sera were separated and stored in aliquots at -20°C till used for estimation of lipid profile including; total cholesterol, triglycerides, LDL-cholesterol, and HDL-cholesterol by enzymatic colorimetric methods using commercial kits. Then the abdomen were opened, liver and adipose tissues (Retroperitoneal, epididymal and mesenteric) were removed, washed three times in ice cold saline and blotted individually on ash-free filter paper, used for preparation of tissue homogenates for estimation of tissue Malondialdehyde (MDA), superoxide dismutase(SOD), reduced glutathione (GSH) levels and for histological sections.

Biochemical Estimation

Estimation of Total Cholesterol, High Density Lipoprotein Cholesterol and Triglycerides

Total serum cholesterol was estimated by using Cholesterol Quantitation kit and triglycerides level Triglyceride Quantification kit (Sigma Aldrich Chemicals Pvt. Ltd.).

Estimation of Serum Glucose

Total serum glucose was estimated by glucose-peroxidase method.

Methods for Assessment of Oxidative Stress

Estimation of Malondialdehyde (MDA)

This method based on the formation of MDA as an end product of lipid per oxidation which reacts with thiobarbituric acid producing thiobarbituric acid reactive substance (TBARS), a pink chromogen, which can be measured spectrophotometrically at 532 nm and MDA standard was used to construct a standard curve against which readings of the samples were plotted.[13]

Estimation of Superoxide Dismutase (SOD)

The SOD activity was spectrophotometrically measured using a modified version of the method developed by Marklund and Marklund.[14] Briefly, SOD activity was detected based of its ability to inhibit superoxide-mediated reduction. One unit of SOD activity was defined as the amount of enzyme that inhibited the oxidation of pyrogallol by 50% and was expressed as unit/g Hb and that from the tissue as unit/mg protein.

Estimation of Reduced Glutathione (GSH)

The method is based on the reduction of 5, 5 dithiobis (2-nitrobenzoic acid) (DTNB) with reduced glutathione (GSH) to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 405 nm by using a commercial kit was used (Glutathione assay kit, Sigma Aldrich Chemicals Pvt. Ltd.).[15]

Histopathological Analysis

For histological examination adipose tissue was collected and fixed in 10% neutral buffered formalin, embedded in paraffin. Standard sections of 5 mm thickness were cut, which were then stained with haematoxylin and eosin, and examined by light microscopy.

Statistical Analysis

Data are expressed as the mean ± standard error (SE). The biochemical data for random glucose, lipid profile and fat pad weights were statistically analyzed using one-way analysis of variance (ANOVA) and feed intake, body weight, BMI, and Obesity index at different time points were statistically analyzed using repeated measure two way ANOVA followed by Bonferroni multiple comparison test $p < 0.05$ was set to be statistically significant.

RESULTS

Morphological Parameters

Effect of Orlistat and *Actinidia deliciosa* fruits on Body Weight, Body Mass Index (BMI), Waist-Hip Ratio and Feed Intake of Rats

Obesity was induced in normal rats by feeding a high-fat diet for 12 weeks. The mean body weights of the five experimental groups were similar at the start of the experiment. A significant increase in body weight, body mass index (BMI) and waist hip ratio along with decrease in feed intake was observed in rats of HFD control group after 12 weeks, as compared to normal control group. On the other hand, treatment with standard drug Orlistat (30mg/kg, p.o.) once daily for six weeks, significantly ($p<0.05$) decreased the body weight, BMI, waist hip ratio and feed intake as compared to HFD control group. Whereas, once daily treatment for six weeks with *Actinidia deliciosa* fruits (50, 150, 300 & 600 mg/kg; p.o.), resulted in significant attenuation of body weight, BMI, waist hip ratio and feed intake as compared to HFD control group.

Effect of Orlistat and *Actinidia deliciosa* fruits on Fat Pad Weights, Total Fat, and Obesity Index and Adiposity Index of rats

The fat pad weights (Epididymal, Mesenteric, Retroperitoneal and Total fat) significantly increased in HFD control rats, as compared to those of normal, control rats. The once daily

oral treatment of animals with standard drug (Orlistat), *Actinidia deliciosa* fruits (50, 150, 300 & 600 mg/kg, p.o.), for six weeks significantly ($p<0.01$) attenuated the fat pad weights, total fat, obesity index and adiposity index as compared to HFD control group (Table2-3).

Biochemical Parameters

Effect of Orlistat and *Actinidia deliciosa* fruits treatment on High Fat Diet Induced Changes in Lipid Profile of Rats

The evaluation of serum lipid profile of experimental animals was carried out for all groups. There was statistically significant ($p<0.01$) increase in total cholesterol (TC), triglycerides (TG) along with decreased high density lipoprotein (HDL) in HFD control group, as compared to normal control group. The once daily oral administration of Orlistat for six weeks along with HFD significantly decreased the levels of TC and TG with increase in HDL as compared to HFD control group. Also, the once daily treatment with *Actinidia deliciosa* fruits **(5**0, 150, 300 & 600 mg/kg, p.o.), for six weeks significantly attenuated the levels of TC and TG with increase in HDL as compared to HFD control group and comparable to standard drug (Orlistat) treatment

Effect of Orlistat and *Actinidia deliciosa* fruits on High Fat Diet Induced Changes in Blood Glucose Level of Rats

Random blood glucose levels were measured at the end of study. Feeding with high fat diet for 12 weeks significantly increased the blood glucose level in HFD control group as compared to normal control group. Further, the once daily per oral treatment with Orlistat (standard drug) 30 mg/kg for six weeks significantly decreased blood glucose level as compared to HFD control group. Also, the treatment of animals with *Actinidia deliciosa* fruits (50, 150, 300 & 600 mg/kg, p.o.), for six weeks show significant ($p<0.01$) difference in blood glucose level, as compared to HFD control rats.

Oxidative Stress Assessment

Effect of Orlistat, *Actinidia deliciosa* fruits on HFD Induced changes in MDA, SOD & GSH level of rats

A significant ($p<0.05$) decrease in reduced glutathione (GSH), superoxide dismutase (SOD) and along with increased malondialdehyde (MDA) in HFD control group, as compared to normal control group ($p<0.005$).The once daily oral administration of Orlistat for six weeks along with HFD significantly increased the levels of GSH and SOD with decrease in MDA when compared to HFD control group. Also, the once daily treatment with *Actinidia deliciosa* fruits (50, 150, 300 & 600 mg/kg, p.o.), for six weeks significantly attenuated the levels of GSH and SOD with decrease in MDA ($p<0.005$) as compared to HFD control group and comparable to standard drug (Orlistat) treatment.

DISCUSSION

In the present study, anti-obesity effect of *Actinidia deliciosa* fruits investigated using a HFD-induced obese rat model. In epidemiological studies, BMI is widely used as a measure of fatness, because it is highly correlated with body fat and is nearly independent of height. Reduction in body weight gain of HFD-fed rats was accompanied by a utilization of body fat stores, since treatment with *Actinidia deliciosa* fruits significantly reduced the weight of adipose tissues (Epididymal, retroperitoneal and mesenteric fat) as compared with that of HFD-fed rats. These data confirmed that *Actinidia deliciosa* fruits are rich in weight loss friendly nutrients, such as dietary fibre, water-soluble antioxidants and vitamins. Also calcium may help maintain the weight, Folic acid and Vitamin C promote fat burning, aids protein digestion[16]. They are also low in calories, thus this investigation claim that the fruits are particularly helpful in weight loss efforts. Research shows that dietary fibre may lower your food intake (e.g. it makes you eat less by promoting the feeling of satiety) [17]. When you eat less, you also consume fewer calories, which results in weight loss[18]. They lowers blood triglycerides, decreases insulin resistance, show low glycemic index[19, 20].

CONCLUSION

In this study, it was investigated that fresh fruits of *Actinidia deliciosa* have been significantly reduced the body weight, thus proved to possess antiobesity activity. Further studies are desirable to isolate the active constituents responsible for this activity.

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I express my gratitude to my husband in making me succeed through all my work.

Table 1: Effect of various doses of *Actinidia deliciosa* fruits aqueous and ethanol extracts on HFD-induced changes on BMI, feed intake in kilocalories (Kcal) and in gram, WH Ratio, obesity index, adiposity index (%) on Day 84

Parameter	Normal chow diet control	High fat diet control	Orlistat (30mg/kg)	*Actinidia deliciosa* fresh fruits			
				50 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
BMI	1.09 ± 0.094	1.74 ± 0.11a	0.99 ± 0.09b	1.2 ± 0.11b	1.4 ± 0.04b	1.52 ± 0.12b	1.65 ± 0.10b
Feed intake (gm)	24.66 ± 5.21	15.18 ± 1.78a	4.52 ± 1.45b	10.30 ± 1.20b	12.86 ± 1.42b	13.98 ± 0.61b	14. 11± 1.22b
Feed intake (Kcal)	68 ± 32.74	51.65 ± 3.513a	17.0 ± 4.20b	32.85 ± 3.50b	30.92 ± 2.36b	31.14 ± 3.82b	25.06 ± 5.42b
WH Ratio	0.75 ± 0.021	1.17 ± 0.018a	0.72 ± 0.21b	0.58 ± 0.17b	0.52 ± 0.43b	0.50 ± 0.11b	0.47 ± 0.02b
Obesity Index	295.8 ± 7.09	289.4 ± 5.07a	284.5 ± 2.25b	286.2 ± 6.67b	283.2 ± 5.14b	281.3 ± 3.37b	277.7 ± 4.22b
Adiposity Index (%)	2.438 ± 0.0	4.00 ± 0.22a	1.96 ± 0.24 b	4.20± 0.11b	3.34 ± 0.12b	3.22 ± 0.20b	2.80 ± 0.15b

All values are represented as mean ± S.E; a = p< 0.05 vs. Normal Chow Diet control, b = p< 0.05 vs. HFD control

Table 2: Effect of various doses of *Actinidia deliciosa* fruits aqueous and ethanol extracts on HFD-induced changes on various fat pads on Day 84

Parameter	Normal chow diet control	High fat diet control	Orlistat (30mg/kg)	*Actinidia deliciosa* fresh fruits			
				50 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
Epididymal Fat (gm)	2.35 ± 0.49	1.45 ± 0.11a	1.01 ± 0.08b	1.4 ± 0.11b	1.13 ± 0.05b	1.40 ± 0.12b	1.1 ± 0.15b
Retroperitonial Fat (gm)	1.30 ± 0.44	4.26 ± 0.65a	1.50 ± 1.50b	3.30 ± 1.20b	1.26 ± 1.41b	3.98 ± 0.81b	2.52 ± 1.52b
Mesenteric Fat (gm)	2.75 ± 0.25	6.65 ± 3.51a	2.57 ± 0.52b	6.48 ± 0.90b	2.91 ± 0.78b	5.75 ± 0.84b	2.99 ± 0.60b
Total Fat (gm)	5.75 ± 0.49	15.75 ± 1.24a	5.52 ± 0.40b	15.68 ± 1.02b	14.53 ± 0.84b	13.71 ± 0.90b	13.30 ± 0.52b

Table 3: Effect of various doses of *Actinidia deliciosa* fruits aqueous and ethanol extracts on HFD-induced changes on antioxidant enzyme activities on Day 84

Parameter	Normal chow diet control	High fat diet control	Orlistat (30mg/kg)	*Actinidia deliciosa* fresh fruits			
				50 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
MDA (nmol/g protein)	20.22 ± 0.68	28.48 ± 2.12a	22.34 ± 0.60b	27.21 ± 0.91b	24.56 ± 1.25b	26.94 ± 1.43b	23.54 ± 1.12b
GSH (µg/mg protein)	28.74 ± 2.65	10.46 ± 1.25a	26.57 ± 1.12b	22.20 ± 1.64b	24.34 ± 1.25b	23.65 ± 1.24b	25.24 ± 1.33b
SOD (unit/mg protein)	8.44 ± 0.15	6.54 ± 0.03a	7.64 ± 0.11b	4.82 ± 0.19b	7.25 ± 0.25b	5.90 ± 0.62b	7.41 ± 0.24b

REFERENCES

1. Clapham JC and Arch JRS. Thermogenic and metabolic anti-obesity drugs. rationale and opportunities. Diabetes Obes Metab. 2007;9(3):259-75.
2. Pi-Sunyer and Xavier F. Obesity, in Cecil Textbook of Medicine, edited by Russel L. Cecil, et al. Philadelphia, PA: W.B. Saunders Company. 2000.
3. National institute of health. Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in adults- The Evidence Report. Ode Res.1998;6(2):2209-51.
4. Sotiropoulos T, Petridou MK, Petridis A, Egnatia DS, Almaliotis D,

Papadakis L, Therios I, Molassiotis A and 'Tsechelidis' Kiwifruit. The Journal of Horticultural Science. 2009;44(2): 466-468.
5. Jiangsu New Medical College. Dictionary of Chinese Herbal Medicines. Shanghai: Shanghai Science and Technology Press. 1977;2210.
6. Fu Wen-Wei, Tan Chang-Heng, Lu Lu-Lu, Meng Xiang-Xue, LuoHong-Feng and Zhu Da-Yuan. Chemical Constituents from the Root of Actinidia deliciosa, Chinese Journal of Natural Medicines. 2010;247-249.
7. Takeoka GR, Guntert M, Flath RA, Wurtz RE and Jennings W. Volatile constituents of kiwifruit (Actinidia deliciosa). J Sci Food Agric. 1986;34:576-578.
8. Bolzoni L, Careri M and Gherardi S. Study of the aroma components of kiwifruit. Ind Conserve. 1988;63(4):347-353.
9. Cossa G, Trova C and Gandolfo G. Extraction and identification of kiwifruit volatile constituents. Ind Ailment. 1988;27(261):531-534.
10. Srinivasan K, Viswanad B, Asrat L, Kaul CL and Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. Pharmacol Res. 2005;52:313-320.
11. Friedewald WT, Levy RI and Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry. 1972;18:499–502.
12. Singh R, Singh M and Krishan P. Modulation of Impact of Obesity in Pathological and Physiological Cardiac Hypertrophy by Orlistat Lat. Am J Pharm. 2010;29:754-762.
13. Ohkawa H, Ohishi N and Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95:351-364.
14. Marklund S and Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Euro J Biochem. 47(974):469-474.
15. Tietez F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal Biochem. 2011;27:502-502.
16. https://www.livestrong.com>article>43.
17. Clark MJ et al, The effect of fibre on satiety and food intake a systematic review. 2013;32(3):200-211.
18. Joanne L Slavin. Dietary fibre and body weight, The International Journal of Applied and Basic Nutritional Sciences. 2005;21(3):411-418.
19. Recico-Rodrguez JI. Effects of kiwi consumption on plasma lipids, fibrinogen and insulin resistance in the context of a normal diet. Nutrition Journal. 2015;14:97.
20. Boudewijn Klop. Dyslipidemia in obesity; Mechanisms and potential targets. 2013;5(4):1218-1240.