

# Evaluation of antiobesity action of herbal candy prepared from extracts of fruits of *Fortunella japonicum*

Rajni Deshpande<sup>1\*</sup>, Narendra Pratap Singh Sengar<sup>1</sup>

<sup>1</sup>Faculty of Pharmaceutical Science, Sanjeev Agrawal Global Education University, Bhopal, Madhya Pradesh, India

\*Corresponding Author

Rajni Deshpande

Faculty of Pharmaceutical Science, Sanjeev Agrawal Global Education University, Bhopal, Madhya Pradesh, India

diplomaprincipal1976@gmail.com

## Abstract

Obesity is a global health challenge associated with increased morbidity and mortality. The present study evaluates the anti-obesity potential of herbal candy formulated from ethanolic extracts of *Fortunella japonicum* fruits, which are rich in flavonoids and phenolic compounds. The candy was prepared using sucrose, gelatin, and extract, and assessed for organoleptic properties, pH, ash value, and stability. Anti-obesity activity was studied in female Swiss albino mice fed a high-fat diet (HFD). Treatment groups included various solvent extracts, herbal candy at two doses (25 mg/kg and 50 mg/kg), and orlistat as a standard. Results demonstrated that the ethanolic extract and herbal candy significantly reduced food intake compared to the HFD group, with the 50 mg/kg candy showing hypophagic effects comparable to orlistat. These findings suggest that *Fortunella japonicum* extract-based candy may serve as a novel, palatable, and effective functional food for obesity management.

## Keywords

Obesity, herbal candy, *Fortunella japonicum*, high fat diet, phenolics

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## Introduction

Obesity is a term applied to excess body weight with an abnormally high proportion of body fat.<sup>1</sup> According to the World Health Organization (WHO) reports, about 2.8 million deaths occurring throughout the world due to complications arising from obesity and overweight.<sup>2</sup> Globally 650 million adult population, 42 million children under the age of 5 years are overweight or obese; interestingly the prevalence of obesity is higher in women (about 297 million) when compared with men (205 million).<sup>3</sup>

*Fortunella japonica*, a member of the Rutaceae family, is abundant in limonene,  $\beta$ -sitosterol, umbelliferone, epigenin, and other compounds.<sup>4</sup> *Fortunella japonica* has recently been studied for a number of pharmacological properties, including as antibacterial, anti-inflammatory, antioxidant, and hypotensive properties<sup>5-8</sup>. In our previous study, we reported the antioxidant impact of the different solvent extracts of *Fortunella japonica* fruits since the plant extract included flavonoids and phenolics.<sup>9</sup>

## Material and Methods

### Preparation of herbal candy

Soft herbal candy was prepared using ethanol extract rich in phenolic content and possibly containing linalool, limonene and  $\alpha$ -pinene.

### Formula

- Extract (ethanolic) – 0.6g
- Sucrose – 4.0g
- Gelatin – 0.5g
- Water – 6.0 mL

Sugar was added to a beaker and dissolved in 2 mL of water. The solution was then heated to prepare a syrup, which was stirred continuously while the extract was incorporated to ensure even dispersion. Separately, a gelatin solution was prepared in 4 mL of water and subsequently added to the syrup solution under constant stirring. The mixture was stirred for 5 minutes to achieve proper mixing, after which it was poured into candy molds and allowed to set and cool, resulting in the desired candy.<sup>10</sup>

### Evaluation of candy<sup>11</sup>

**Sensory evaluation:** The candy were examined in terms of the different organoleptic characteristics i.e., color, appearance, taste, texture, flavour, mouth feel and overall acceptability.

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**pH measurement:** The acidity or alkalinity of a candy was indicated by using lab pH meter, a scale from 1 to 14. 1%w/v solution of candy was prepared by dissolving 1 gm candy in 100ml distilled water and its pH was recorded.

**Ash value:** Weigh accurately about 3gm of the powdered drug in silica crucible. Place the powdered drug in Muffle furnace until the sample is turned into ash. and allow it to cool. Weigh the ash and calculate the % of the total ash in contrast to the air dried sample.

**Shelf-life study:** Shelf-life study was started from the 2nd day of making the product. Candy was stored under the refrigerated condition for 4 weeks in its packaging materials. The product was observed at frequent intervals for any change in appropriate color, odour, texture, taste and moisture.

### Evaluation of anti-obesity potential

#### Animals Used

Female Swiss albino mice weighing 20–25 g, were housed six per cage with free access to food and water at laboratory conditions. The animals were used following at least a 2-day period of adaptation to the laboratory conditions. The experiments were carried out between 10.00 and 17.00 h at room temperature ( $22 \pm 2^\circ\text{C}$ ). All the experimental protocols have been approved by the Institutional Animals Ethics Committee and conducted according to the Indian National Science Academy Guidelines on the Use and Care of Experimental Animals.

#### Drugs Used and Grouping of Animals

The extracts were suspended in distilled water and administered at a dose of 50mg/kg; the candy was dissolved in distilled water and administered at dose of 25mg/kg and 50g/kg p.o. Orlistat was dissolved in distilled water and administered orally at constant volume of 10 mg/kg body weight.

**Table 6.2 Experimental Design for anti-obesity study**

| Group | No. of animal | Treatment                           |
|-------|---------------|-------------------------------------|
| I     | 6             | Control (Normal diet)               |
| II    | 6             | Obese (High Fat Diet (HFD))         |
| III   | 6             | Orlistat (10 mg/kg, per oral) + HFD |
| IV    | 6             | Extract 1 +HFD                      |
| V     | 6             | Extract 2 +HFD                      |
| VI    | 6             | Extract 3 +HFD                      |
| VII   | 6             | Extract 4 +HFD                      |

|      |   |                        |
|------|---|------------------------|
| VIII | 6 | Candy (25 mg/kg + HFD) |
| IX   | 6 | Candy (50 mg/kg + HFD) |

### Study of effect of extract on food intake<sup>12</sup>

Mice were kept in groups of six in test cages and included a vehicle-treated control group and the various drug treatment groups. The food and water were withheld 1 h before the experimentation. Extract was administered orally and after 15 min preweighed sweetened chow (5 g) was presented to the different groups of mice in petri dishes in the test cages. The amount remaining was weighed after 0.5, 1, 2, 3 and 4 h intervals and the cumulated food intake/mouse (g/20 g body weight) was calculated.

### Results and Discussion

The candy was prepared using the ethanolic extract (highest antioxidant action) and the organoleptic parameters, ash value, pH and stability were evaluated (Table 1).

**Table 1. Properties of herbal candy**

| Parameter       | Observation         |
|-----------------|---------------------|
| Color           | Dark Brown          |
| Odor            | Characteristic      |
| Taste           | Sour                |
| Shape           | Uniform oval shaped |
| Texture         | Jelly like          |
| pH              | 2.8                 |
| Total Ash value | 39.33%              |

The candies were uniform in shape and size as they were prepared in molds (Figure 7.23).

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**Figure 1.** Candies prepared from the ethanolic extract

The candies were stored at 25°C and exhibited no change in pH, taste, odor and color. No microbial growth occurred over the candies during the storage duration. The texture of the candy was slightly hardened over a period of 3 months of storage.

### Anti-obesity action

The ability of rodent to consume sweetened chow or high fat diet is a highly effective model for study of anti-obesity action of drugs. A standard chow diet provides 12.98% fat energy, 58.62% carbohydrates and 28.40% proteins, contributing to total of 1243.6 kcal/100g. On the contrary, a high fat diet (sweetened chow) usually contains 50.52% fat, 31.50% carbohydrates, and 17.98% proteins, contributing to 1765.8 kcal/100g.

The anti-obesity effect of the extract was assessed by observing the effect of the extract on the food intake habit of experimental mice. The amount of food remained unconsumed post 4 hours of treatment in each group was weighed and the food intake in grams was calculated per 20 g body weight (Table 2 & 3).

**Table 2.** Weight of food unconsumed by mice

| Treatment Group | Weight of HFD remaining (g) |    |    |    |    |
|-----------------|-----------------------------|----|----|----|----|
|                 | 0.5 h                       | 1h | 2h | 3h | 4h |

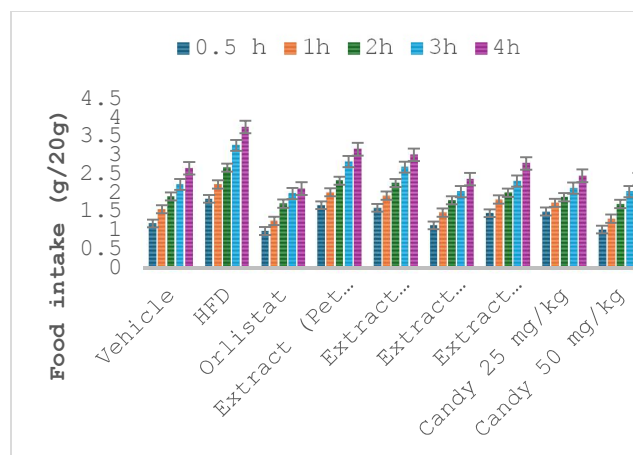
|                      |      |      |      |      |      |
|----------------------|------|------|------|------|------|
| Vehicle              | 3.81 | 3.43 | 3.09 | 2.77 | 2.34 |
| HFD                  | 3.15 | 2.77 | 2.32 | 1.73 | 1.24 |
| Orlistat             | 4.01 | 3.74 | 3.28 | 3.01 | 2.88 |
| Extract (Pet ether)  | 3.32 | 2.98 | 2.67 | 2.16 | 1.83 |
| Extract (chloroform) | 3.39 | 3.07 | 2.73 | 2.31 | 1.98 |
| Extract (ethanol)    | 3.86 | 3.52 | 3.19 | 2.96 | 2.63 |
| Extract (water)      | 3.54 | 3.17 | 2.99 | 2.68 | 2.21 |
| Candy 25 mg/kg       | 3.49 | 3.26 | 3.11 | 2.87 | 2.54 |
| Candy 50 mg/kg       | 3.97 | 3.68 | 3.29 | 2.96 | 2.63 |

**Table 3.** Weight of food consumed by mice

| Treatment Group      | Cumulative Food intake/mouse (g/20g body weight) |      |      |      |      |
|----------------------|--------------------------------------------------|------|------|------|------|
|                      | 0.5 h                                            | 1h   | 2h   | 3h   | 4h   |
| Vehicle              | 1.19                                             | 1.57 | 1.91 | 2.23 | 2.66 |
| HFD                  | 1.85                                             | 2.23 | 2.68 | 3.27 | 3.76 |
| Orlistat             | 0.99                                             | 1.26 | 1.72 | 1.99 | 2.12 |
| Extract (Pet ether)  | 1.68                                             | 2.02 | 2.33 | 2.84 | 3.17 |
| Extract (chloroform) | 1.61                                             | 1.93 | 2.27 | 2.69 | 3.02 |
| Extract (ethanol)    | 1.14                                             | 1.48 | 1.81 | 2.04 | 2.37 |
| Extract (water)      | 1.46                                             | 1.83 | 2.01 | 2.32 | 2.79 |
| Candy 25 mg/kg       | 1.51                                             | 1.74 | 1.89 | 2.13 | 2.46 |
| Candy 50 mg/kg       | 1.03                                             | 1.32 | 1.71 | 2.04 | 2.37 |

The graphical representation of the food consumed by various groups is presented in figures 2.

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**Figure 2. Effect of extract and orlistat on food intake in mice**

As clearly visible from the figure above, orlistat and candy are hypophagic and restrict the intake of food by the mice. The reduction in food intake was calculated with reference to the HFD only group exhibited by hyperphagic animals (Table 4).

**Table 4. Percent reduction in food intake**

| Treatment Group      | % reduction in food intake compared to HFD |             |             |             |             |
|----------------------|--------------------------------------------|-------------|-------------|-------------|-------------|
|                      | 0.5 h                                      | 1h          | 2h          | 3h          | 4h          |
| Orlistat             | 46.4<br>9**                                | 43.5<br>0** | 35.8<br>2** | 39.1<br>4** | 43.6<br>2** |
| Extract (Pet ether)  | 9.19<br>ns                                 | 9.42<br>ns  | 13.0<br>6*  | 13.1<br>5*  | 15.6<br>9*  |
| Extract (chloroform) | 12.9<br>7*                                 | 13.4<br>5*  | 15.3<br>0*  | 17.7<br>4*  | 19.6<br>8*  |
| Extract (ethanol)    | 38.3<br>8**                                | 33.6<br>3** | 32.4<br>6** | 37.6<br>1** | 36.9<br>7** |
| Extract (water)      | 21.0<br>8**                                | 17.9<br>4*  | 25.0<br>0** | 29.0<br>5** | 25.8<br>0** |
| Candy 25 mg/kg       | 18.3<br>8*                                 | 21.9<br>7** | 29.4<br>8** | 34.8<br>6** | 34.5<br>7** |
| Candy 50 mg/kg       | 44.3<br>2**                                | 40.8<br>1** | 36.1<br>9** | 37.6<br>1** | 36.9<br>7** |

\*p<0.05, \*\*p<0.01, ns-not significant

As expected, hyperphagic animals consumed the most food (HFD group). Orlistat and Candy (50 mg/kg), both showed robust hypophagic effects, nearly matching each other in efficacy. Ethanolic extract demonstrated strong appetite suppression, approaching the effect of candy and orlistat.

### Conclusion

The study demonstrated that herbal candy prepared from ethanolic extracts of *Fortunella japonicum* exhibits significant anti-obesity activity in mice fed a high-fat diet. Both the extract and candy reduced food intake, with the 50 mg/kg candy dose showing effects comparable to orlistat. The candy was stable, palatable, and retained its organoleptic properties during storage. These findings support the potential of *Fortunella japonicum*-based formulations as functional foods for obesity management. Further clinical studies are warranted to validate efficacy in humans and explore mechanisms underlying appetite suppression.

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