

# Effect of Afghan chehelghoza (*Pinus gerardiana* L.) on Food Consumption, Body, and Brain Weight of Male Rats

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## ABSTRACT

**Aim:** To investigate the effect of Afghan chehelghoza (*Pinus gerardiana* L.) on food consumption, body, and brain weight of male rats.

**Methods:** This study was conducted in 2019. Rats were randomly divided into five groups: Control group, 2%, 6%, 12%, and 25% chehelghoza-treated groups, which consumed chehelghoza in their diet for 28 days. During this period, the daily food consumption was monitored and the body weight of each rat was assessed once a week. After treatment, their brain weight was also measured.

**Results:** This study showed that the food consumption and body weight decreased in all chehelghoza-treated groups, especially in 2% and 6% groups during weeks 1 to 4 of the treatment period ( $P < 0.0001$ ) and at the end of weeks 3 and 4 in the 12% and 25% groups ( $P < 0.01$ ). However, an increase in the brain weight was only recorded in the 12% and 25% chehelghoza-treated group when compared with the control group ( $P < 0.05$ ).

**Conclusion:** Chehelghoza pine nut consumption in high doses was effective on brain weight and in low doses was effective on body weight and food consumption parameters. Thus, consumption of chehelghoza may be considered as an effective dietary ingredient to prevent obesity with a beneficial effect on the brain.

## KEYWORDS:

Afghanistan; body weight; brain weight; food consumption; *Pinus gerardiana*

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## INTRODUCTION

Obesity, a complex disorder, is related to several factors and is a major threat to people's health.<sup>[1,2]</sup> It results in multiple adverse effects on health, including exertion of excess stress on the heart, alternation in pulmonary function, increased load on weight-bearing joints, immune impairment, and alternation in functions of the endocrine system, and so on.<sup>[3]</sup> Overnutrition and obesity are spreading

at an alarming rate in the last three to four decades in many parts of the world.<sup>[1]</sup> Studies have shown that the leading cause of obesity is behavioral and dietary factors, and role of genetic factors is minimal. Nutritionists often highlight the important preventive role of many essential nutrients for age-related weight gain.<sup>[4]</sup> Prevention of dietary fat is the main focus of dietary guidelines to overcome or inhibit obesity. The amount of saturated fatty acids intake needs to be limited because

of their adipogenic effect, whereas increased unsaturated fatty acids intake hinders obesity.<sup>[5,6]</sup> Nuts are the reservoir of unsaturated fatty acids and many other biologically active compounds.<sup>[7]</sup> For a long time, people assumed that high fat-containing nuts are unhealthy and are the culprits that aid in weight gain and obesity.<sup>[8,9]</sup> But many pieces of evidence have shown that nuts improves appetite and also prevent body weight gain.<sup>[10-12]</sup>

Chehelghoza is one of the edible pine nuts from the Pinaceae family<sup>[13]</sup> and is widely found in India, Pakistan, Tibet, and Afghanistan<sup>[13-16]</sup> Eastern and South-eastern forests of Afghanistan are rich sources of Chehelghoza trees.<sup>[17,18]</sup>

The Chehelghoza nut is a good source of energy (628kcal)<sup>[16]</sup> that contains 49.9% unsaturated fatty acids,<sup>[13]</sup> proteins,<sup>[15]</sup> minerals, vitamins, antioxidants,<sup>[16]</sup> and phytosterols.<sup>[19]</sup> It is shown that linoleic acid (51.3%), oleic acid (39.7), palmitic acid (7.2%), arachidic acid (2.1%), linolenic acid (1.5%), and stearic acid (0.3%) are found in chehelghoza oil.<sup>[13,14]</sup> In addition, chehelghoza is considered a good reservoir of plant protein.<sup>[15]</sup>

Despite the valuable constituents and biological activities of chehelghoza and the considerable effect of nuts and diet on body weight, to date no study has checked the effect of chehelghoza on food consumption and body weight. Therefore, this study evaluates its effect on food consumption, body, and brain weight of rats.

## MATERIALS AND METHODS

### ANIMALS

Thirty adult Sprague Dawley male rats (with bodyweight of 180–220g) were randomly selected from Khatam Al-nabieen university research and technology center (KNURTC) for this study. The rats were housed in plexy glass cages, with a 12 hour light/dark cycle in a room with stable temperature ( $23 \pm 2^\circ\text{C}$ ). Animals had access to food and water ad libitum. The study was approved by the Ethic research board of Khatam Al-Nabieen University and was conducted following the ethical guidelines set by the eighth edition of National Institute of Health (NIH) guide for the care and use of laboratory animals. During the housing and treatment period, the rats were carefully handled to minimize unwanted stress.

### EXPERIMENTAL GROUPS

The study was conducted in 2019. We randomly divided the rats into five groups (n=6): Group 1, non-treated rats; Group 2–5, rats received chehelghoza (2, 6, 12 and 25%) in the standard rodent diet for 28 days.

### CHEHELGHOZA SEEDS PREPARATION

Raw seeds of chehelghoza were purchased from a local market of Ali-sheng, Laghman Province of Afghanistan. The nuts were carefully screened, dried, packed in the 250g capacity aluminum laminate pouch, and stored at ambient temperature ( $25^\circ\text{C}$ ) for further use with one of the suitable methods for

drying and packing of nuts, as discussed previously.<sup>[20,21]</sup> Later, these piled nuts were manually grounded and combined with the standard rodent diet.

### FOOD CONSUMPTION AND BODY WEIGHT MONITORING

The daily food intake of rats was monitored during the study, and their body weight was assessed once a week.

### BRAIN EXTRACTION AND WEIGHING

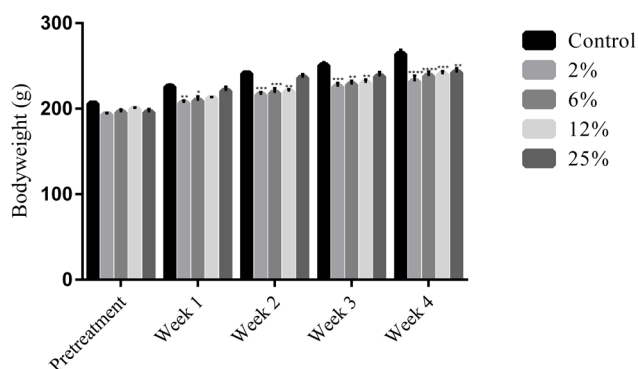
For measurement of wet brain weight, the rats were decapitated. Their brains were extracted, cleaned in a petri dish filled with ice and were weighed immediately after cerebellum removal.<sup>[22,23]</sup>

### STATISTICAL ANALYSIS

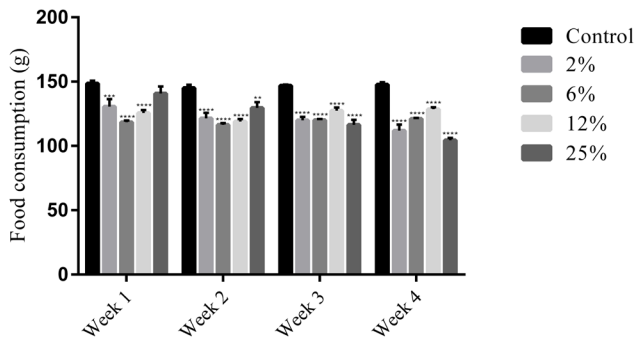
The statistical analysis was performed using the Graph pad prism software, version 6.07. Data of food consumption and body weight were analyzed by the two-way analysis of variance (ANOVA) test, and a one-way ANOVA test was used to analyze the data of brain weight ( $P < 0.05$ ).

## RESULTS

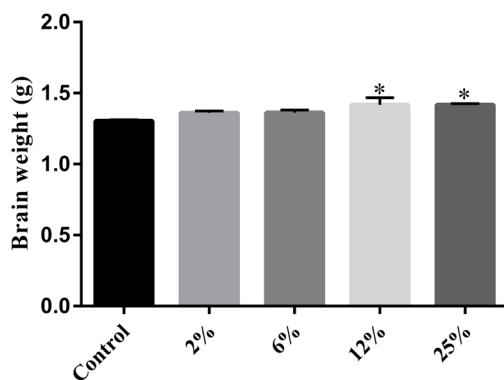
The data of body weight, food consumption, and brain weight of chehelghoza-treated groups were analyzed in comparison with the control group. At the end of week 1, the bodyweight of 2% and 6% groups were considerably reduced versus the control group. A significant reduction was also detected in the bodyweight of 2%, 6%, and 12% groups at the end of weeks 2 and 3. At the end of week 4, the bodyweight of each 2%, 6%, 12%, and 25% groups was decreased in comparison with the control group (see Figure 1). The food consumption significantly reduced in 2%, 6%, and 12% groups at the end of week 1; and all chehelghoza-treated groups at the end of weeks 2 to 4 (see Figure 2). Besides, as shown in figure 3, the increase in brain weight was significant in each 12% and 25% groups, in comparison with the control group ( $P < 0.05$ ).



**Figure 1** Effect of dietary chehelghoza (2%, 6%, 12%, and 25%) on body weight. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; and \*\*\*\* $P < 0.0001$  in comparison with control group. Data are shown as Mean  $\pm$  SEM.



**Figure 2** Effect of dietary chehelghoza (2%, 6%, 12%, and 25%) on food consumption. \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; and \*\*\*\* $P < 0.0001$  in comparison with control group. Data are shown as Mean $\pm$ SEM.



**Figure 3** Effect of dietary chehelghoza (2%, 6%, 12%, and 25%) on brain weight. \* $P < 0.05$  in comparison with control group. Data are shown as Mean $\pm$ SEM.

## DISCUSSION

In the present study, the effect of chehelghoza in combination with food in different proportion was evaluated on food consumption, body, and brain weight of rats. Results indicate that dietary chehelghoza influences food consumption and body weight in a dose- and time-dependent manner. As the effect of chehelghoza on food consumption and body weight was greater in the lower (2% and 6%) over higher concentrations (12% and 25%). Besides, the scant amounts of chehelghoza combined with food decrease the food consumption and body weight of rats, even at the end of week 1 of the treatment period. However, the effect of a high concentration (25%) of dietary chehelghoza on food consumption was initiated at the end of week 2, much lesser than the effect of its meager amounts. The high amount (25%) of chehelghoza combined with food also decreased the body weight of rats, but its effect was initiated at the end of week 4. Therefore, the lower amounts of chehelghoza were effective in short- and a long-term period of consumption also, whereas the higher amounts of chehelghoza had a greater effect in the long- than a short-term period of consumption.

Likewise, many previous studies have shown that nuts reduce hunger, and appetite, causes early satiety and body weight loss. Because of the presence of polyunsaturated fatty acids

(PUFAs), monounsaturated fatty acid (MUFAs), high protein, and dense energy.<sup>[10,11]</sup> Chehelghoza like other nuts mostly contains linoleic acid (a PUFA) and oleic acid (a MUFA),<sup>[13,14]</sup> which may lead to a decrease in food consumption and body weight. Multiple mechanisms explain these effects. Consumption of nuts as energy-dense nutrients can increase the daily energy intake and suppresses hunger. In other words, it increases energy availability and effect on energy metabolism.<sup>[3,24]</sup> Unsaturated fatty acids have a great effect on appetite, hunger, and body weight and aids in the secretion of satiety hormones, including cholecystokinin and glucagon-like peptide-1. It is known that high fat of nuts increases the release of these hormones, which causes early satiety, gastric emptying delay, and reduction in body weight.<sup>[4,10]</sup> Another mechanism involved in weight gain prevention by nuts may be the thermogenic response of the body to fatty foods.<sup>[10,12]</sup> Animal and human studies showed that consumption of unsaturated fatty acids causes an increase in diet-induced thermogenesis, fat oxidation, and a decrease in body fat accumulation, as compared with the saturated fat intake.<sup>[24]</sup> Besides, multiple studies also have shown that PUFA enriched diet changes the metabolism of adipose tissue and thus favor the deposition of fat. For example, a diet containing n-6 PUFA can reduce the mRNA of fatty acid synthase in adipose tissue and liver in rats. It could lead to a reduction in lipogenesis capacity.<sup>[25]</sup> Also, high protein and unsaturated fatty acids in nuts increase the resting energy expenditure. This hinders the fat deposition in the body.<sup>[11,26]</sup>

Therefore, one can say that consumption of chehelghoza may lead to a decrease in body weight and food consumption through a reduction in appetite and early satiety mechanisms. However, its preventing effect on body weight gain is minimal in high amounts. The possible explanation for this finding may be the fact that chehelghoza also contains some saturated fatty acids including palmitic acid (7.2%).<sup>[13,14]</sup> The adipogenic effects of saturated fatty acids on one's body are well known.<sup>[5]</sup> So, it is postulated that the high ratio of saturated fatty acid in the higher concentrations of chehelghoza consumption may reduce the anti-obesity potential.

Besides, this study results also indicated that the brain weight of rats was increased by consumption of high amounts of chehelghoza (12% and 25%). However, low quantities of the nut did not have a considerable effect on brain weight. Chehelghoza pine nuts constituents also explain this effect that goes with previous researchers that support the role of PUFAs in the growth and development of the brain.<sup>[27,18]</sup> Oleic acid is also effective on brain growth. It acts as a neurotrophic factor in nerve cells and causes neuronal migration. This may lead to an increase in gene expression of pre- and postsynaptic proteins and dendrite and axon growth, and aids in the formation of a synapse.<sup>[29,30]</sup>

## CONCLUSION

All in all, consumption of chehelghoza pine nuts in high amounts can increase brain weight. Besides, dietary chehelghoza is

effective on body weight and food consumption, especially in the lowest concentrations. Thus, consumption of chehelghoza can be considered as an effective diet ingredient for the prevention of obesity, with a beneficial effect on the brain.

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## CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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