

Utilization of Foods Rich in Vitamin D as Nutritional Supplements in Pasta Products and the Biological Impact on Obese Rats

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ABSTRACT

Due to the widespread prevalence of obesity on a global scale, it has emerged as a significant public health concern. The number of individuals attempting to lose weight has decreased over the last 10 years. The initial step in tackling this issue involves the adoption of a healthy lifestyle. This research endeavor is designed to explore the influence of specific dietary components on the biochemical characteristics of obese rats. In this study, 36 Wister strain rats were subjected to different dietary conditions, with half of them being fed a high-fat diet while residing in well-lit environments, and the other half in dark conditions. Before sacrifice, the rats underwent a 12-hour fast, followed by the analysis of their blood and serum to assess various biochemical parameters. This study's findings imply that enriching the diet with hulled sunflower seed powder and wheat germ, along with vitamin D supplementation, led to increased vitamin D levels in the rats. Furthermore, the analysis revealed rats' lipid profiles improved. Moreover, the rats exposed to sunlight exhibited enhanced levels of vitamin D and improved hematological parameters when compared to those housed in dark conditions. Additionally, the sensory assessment of the food products containing sunflower seeds and wheat germ showed that the panelists were satisfied with the product quality. In summary, the study's results suggest that incorporating hulled sunflower seeds powder and wheat germ into the diet may contribute to the reduction and management of obesity.

Keywords: Vitamin D; Hulled sunflower seeds; Wheat germ; Biological impacts; Food products; Sensory evaluation.

INTRODUCTION

The surge in popularity of natural, micronutrient-rich food items can be attributed to their positive impact on various health conditions (Hruby and Hu, 2015). Obesity has reached epidemic proportions in developed nations, with approximately 30 kg/m² of individuals falling into the obese category (Flegal *et al.*, 2004). Numerous decades of epidemiological research have revealed that the fat distribution in the body has a direct influence on illness risk, irrespective of body fat percentage and overall body weight (Piché *et al.*, 2018). The prevalence of obesity has developed as a significant

general health crisis, affecting individuals of all ages globally (Haththotuwa *et al.*, 2020). In Egypt, Over the last few years, obesity has considerably increased. In the country, approximately 35% of adults are now classified as obese, a significant rise from the 17% reported in 2014 (Koszowska *et al.*, 2014). In last thirty years, there has been a global rise in number of adults classified as overweight or obese (GBD 2015 Obesity Collaborators, 2017). This health condition is well-documented for elevating the risk of chronic non-communicable diseases, including cancer and diabetes (Al-Hadlaq *et al.*, 2022). To enhance their health, individuals must maintain a well-balanced diet. As indicated in a study by Huskisson *et al.* (2007), the sufficient consumption of foods rich in micronutrients is also a vital component of this overall health strategy. Enabling the provision of sufficient vitamin D through fortified food items can play a significant role in mitigating obesity and enhancing the well-being of individuals grappling with this worldwide health concern (Haddad *et al.*, 2016 and Shalaby & Elhassaneen, 2021). Vitamin D, which is fat-soluble, is spontaneously synthesized by the body when exposed to ultraviolet light (Aguilar-Shea, 2021). Its primary function includes supporting bone health by facilitating calcium absorption (Cashman, 2007). Additionally, vitamin D acts as a regulator of gene expression and may have relevance in various other facets of health, as discussed by Cashman and Kiely (2018). Foods rich in vitamin D include items like salmon, herring, trout, egg yolks, liver, milk, and plant oils. There is ergosterol significant amounts in the plant oils utilized in the human diet. Among the different plant oils, wheat germ and sunflower seeds have the highest amounts of vitamin D precursors (Baur *et al.*, 2016). Wheat germ powder and oil are good sources of important fatty acids, proteins, and minerals; moreover, vitamins "A, D, E, and B." It is also a natural antioxidant. which inhibits inflammation, reduce oxidative stress, improve lipid metabolism, and lower blood sugar and cholesterol levels (Nagib, 2018). Furthermore, vitamin D is added to various fortified products such as whole grains, nuts, dairy items, and polyunsaturated vegetable oils (Spiro and Buttriss,

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2014). The U.S. Department of Health and Human Services has established daily recommended allowances, with a specific focus on individuals aged 50 and above (Foote *et al.*, 2000). For those under 50, the daily requirement is 5 micrograms, while for those over 70, it is recommended to intake 15 micrograms daily (Nowson *et al.*, 2012). A study conducted by Holick (2011) underscored the significance of maintaining adequate vitamin D levels, as insufficient vitamin D status can lead to the development of conditions like rickets in both children and adults. Research has indicated that inadequate vitamin D levels can potentially play a role in the development of various chronic conditions, including obesity and being overweight (Vranić *et al.*, 2019). In a study conducted by Safari *et al.* (2017), it was found that an excess of fat tissue in the body could lead to reduced levels of vitamin D in individuals. While the impacts of vitamin D on the body are well-documented, there remains a gap in our understanding regarding vitamin D's exact effects consumption on obesity and overweight (Migliaccio *et al.*, 2019). Consequently, that study was conducted to assess foods rich in vitamin D as nutritional supplements in pasta products and its biological impact on obese rats.

MATERIALS AND METHODS

Materials:

The dried samples used in this study comprised a variety of food items, notably wheat germ and sunflower seeds, which were sourced from a market in Alexandria, Egypt. These samples were subsequently processed by dehulling, drying, grinding, and packaging into sealed packets. The chemicals and solvents utilized in the research were sourced from Sigma-Aldrich and Merck and were of purified and analytical grade. Additionally, commercial kits supplied by Biosystems S.A., Diamond, and Randox were used for various aspects of the analysis.

Methods:

1. Samples preparation:

Fresh samples (wheat germ and hulled sunflower seeds) pieces that were free of blemishes or evident flaws were cleaned and sliced, then dried in an electric oven at 40°C for 12 hours until entirely dry. Following drying, materials were pulverized in a Grinder (Moulinex LM2428EG - 400 Watt, Germany) and stored individually in Polyethylene bags in the refrigerator until use (Da Silva *et al.*, 2009).

2. Feeding experiments:

In this experiment, a total of thirty-six male albino rats of the Westar strain, weighing between 100 and 120 grams, were sourced from the Institute of Graduate Studies and Research at Alexandria University. These

Rats were kept in wire cages in standard laboratory settings, maintaining a room temperature of 25°C, and ensuring normal health conditions were upheld. During an adaptation period of two weeks, the rats were provided with a basal diet and had ad libitum access to food and water, with daily checks according to the American Institute of Nutrition (AIN) (1980). The protocol conforms to the guidelines of the National Institutes of Health (NIH).

To induce obesity, the rats were subsequently fed a high-fat diet, which was prepared based on the specifications outlined by Ezzat *et al.* (2020). It consisted of the basal diet supplemented with 20% animal fat (1% corn oil and 19% tallow). The basal diet, following the method described by Kim *et al.* (2008), included 20% protein (casein), 10% sucrose, 5% corn oil, 0.25% choline chloride, 1% vitamin mixture, 3.5% salt mixture, 5% cellulose, the remaining portion consisted of corn starch to complete 100%.

The rat's serum was analyzed at the beginning of the experiment to confirm the occurrence of vitamin D deficiency. The experimental groups were structured into six groups, each comprising six animals. The first group functioned as the controlling group in the sunlight, exposed to sunlight. The second group received an oral mixture consisting of 70% wheat germ and 30% sunflower seed powder while being exposed to sunlight according to the content of vitamin D in wheat germ and sunflower seeds each rat fed 3.5 gm of the mixture which equals the daily need of vitamin D for rats (23 IU). The third group received vitamin D supplementation orally giving 2-3 drops of vitamin D supplementation daily (Vidrop). The other three groups were the same but in the dark conditions. Weekly measurements of body weight and feed consumption were taken. The feed ratio of efficiency (FER) of the various diets was measured as body weight increase (g)/feed intake (g). After 45 days of receiving the extract, the rats fasted overnight before being ether-anesthetized, slaughtered, and blood samples obtained. Centrifugation at 3000 rpm (800 xg) for 20 minutes yielded serum samples, which were kept at -20 °C until analysis'. The liver- kidney- brain- lung- heart- testicles and spleen were promptly removed, weighed, and rinsed with 'cooled saline solution'; relative organ weights were computed based on the animal weight at the moment of slaughter and preserved at -70 °C for future research.

3. Determination of vitamin D:

A solution of 1 mg ml⁻¹ Vit D3 was prepared by dissolving 1 mg of lyophilized Vit D3 standard in 1 ml of methanol. Subsequently, solutions of various concentrations (0.5–5 ng ml⁻¹) were prepared from this stock solution for generating calibration plots. To

equilibrate the column, the mobile phase was filtered through a 0.45 μm membrane filter and pumped through the column at a flow rate of 0.4 ml min^{-1} . The baseline was continuously monitored throughout this process. Detection was performed at λ_{max} 265 nm. The prepared dilutions were injected sequentially, and the peak area for each dilution was calculated and plotted against concentration (Kumar *et al.*, 2015).

4. Determination of hematological parameters:

Hematological analysis was conducted, encompassing parameters such as red blood cells (RBC) by Turkson and Ganyo (2015), white blood cells (WBC) determined by the method of Jopling *et al.* (2009), hemoglobin level (HGB) determined according to Shawky (2015). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and packed cell volume (PCV) were determined as described by Turkson and Ganyo (2015). These analyses were performed following established and standardized methods in the field.

5. Biochemical assays:

Serum samples were employed for the assessment of various biochemical parameters, including lipid profile components such as triglycerides (TG) following the method indicated by Fossati and Prencipe (1982), total cholesterol (TC) was calculated using the method outlined by Allain *et al.* (1974), and high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels were calculated using the methodology outlined by Lopes-Virella *et al.* (1977). A spectrophotometer (Evolution 300 uv-vis) set at a wavelength of 500 nm was employed for these measurements. Additionally, Glucose concentrations were determined utilizing the method of Lott and Turner (1975). Other parameters such as urea, uric acid, creatinine, and bilirubin levels were assessed by following the procedures described by Wuepper *et al.* (2003). Albumin levels were measured following the method outlined by Bergmeyer and Bernt

(1974). Furthermore, the activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed based on the method established by Williamson (1972). Serum alkaline phosphatase (ALP) was assessed following the protocol detailed by Lavie *et al.* (2018). These methods were utilized to obtain comprehensive biochemical data from the serum samples.

6. Technology Application:

6.1. Pasta preparation:

The pasta production was carried out using a traditional approach with some ingredient modifications as shown in Table (1). The procedure unfolded as follows: A mixture of wheat flour, salt, eggs, and water was employed. Initially, the wheat flour, wheat germ and hulled sunflower seeds powders and salt were sifted and combined thoroughly. A well was then created in the center of the flour mixture, into which the eggs were added. This mixture was kneaded, and warm water was gradually incorporated to achieve a uniform consistency. The dough was shaped into a ball and placed on a floured surface. It was rolled out and left to rest for approximately 10 minutes. Afterward, the dough was cut into strips, sprinkled with a bit of flour, and allowed to dry for roughly 2 hours. Finally, the pasta was boiled in salted water for around 10 minutes (Coskun, 2013).

6.2. Sensory evaluation:

The evaluation of the prepared products; including appearance, color, taste, odor, texture and overall acceptance was conducted with the participation of 20 of well-trained panelists from the Department of Food Science and Technology, Agriculture Faculty, and the Faculty of Specific Education Department of Home Economics at Alexandria University. This assessment was carried out following the guidelines established by Watts *et al.* (1989) and Ranganna (2000).

Table 1. Formula of preparation pasta

% of Fortification	Control	10	20	30	40
Ingredients (g)					
Wheat flour	300	270	240	210	180
Egg	70	63	56	49	42
Salt	3	2.7	2.4	2.1	1.8
Water (%)	35 %	35 %	35 %	35 %	35 %
Wheat germ	–	7	14	21	28
Sunflower powder	–	3	6	9	12

7. Statistical analysis:

This study aimed to examine the collected data utilizing IBM's SPSS 20.0 software program. The data were summarized using standard deviation and mean standard error. Subsequently (Kirkatrick and Feeney, 2013).

RESULTS AND DISCUSSIONS

1. Effect of vitamin D supplementation and a mixture of wheat germ and sunflower seeds powder "on feed intake, body weight gain (BWG) and feed efficiency ratio (FER) of rats":

The data presented in Table (2) indicate that the consumption of vitamin D, wheat germ, and sunflower seeds powder can contribute to a reduction in body weight gain and an improvement in feed intake and efficiency ratios among obese rats. The results showed an insignificant decrease in feed intake for the rats in group B₁ (fed with 70 % wheat germ and 30% sunflower seed; in sun light) and group C₁ (fed with vitamin D supplementation; in sun light) compared with group control A (daily intake, 30.1 g/day). Also, there was insignificant decrease in feed intake for the rats in group B₂ (fed with 70 % wheat germ and 30% sunflower seed; in the dark) and group C₂ (fed with vitamin D supplementation; in the dark) compared with group control D (daily intake, 31.8 g/day). Furthermore, the results showed insignificantly decrease in feed intake for both the sunlight-exposed and dark groups when compared to the controls groups (A and D). Additionally, the study demonstrated that the body weight gain of the control groups (A and D) was higher than that of the supplemented groups (B₁, C₁, B₂ and C₂). It's evident that the rats fed with a diet consisting of

70% wheat germ and 30% sunflower seeds powder (group B₁) exhibited a insignificant decrease in body weight gain (BWG g/day) in the sunlight compared to the control group (A). Conversely, the lowest BWG was observed in the group B₂ which fed in the dark. The mean values for these two groups were 8.01 and 7.97 g/day, respectively. Among the obese supplemented rat groups, the results showed that the highest feed efficiency ratio (FER) was recorded for the vitamin D-supplemented group in the sunlight (group C₁), while the lowest FER was recorded for the group with 70% wheat germ and 30% sunflower seeds powder in the dark (group B₂), and this difference was not statistically significant. The mean values for these groups were 0.31 and 0.25, respectively.

2. Effect of vitamin D supplementation and a mixture of wheat germ and sunflower seeds powder on relative organ weights and body weight of obese rats:

Table (3) shows the changes in body weight and the relative organ weights of obese rats (such as liver, kidney, spleen, pancreas, lungs, and brain) after they were fed with vitamin D-rich foods. Group B₁ (fed with 70 % wheat germ and 30% sunflower seed) rats that were exposed to sunlight had a significantly higher liver weight than the other groups (control A, C₁, control D, B₂, C₂). The mean values of the liver weight and the weight of the rats that were fed on 70 % wheat germ and 30% sunflower seed were 8.56 and 272.8 g, respectively. On other hand, the groups 'which were fed on vitamin D supplements (C₁ and C₂)' had an insignificantly lower liver weight as compared with control A and control D, respectively.

Table 2. Effect of vitamin D supplementation and a mixture of wheat germ and sunflower seeds powder on feed intake, body weight gain (BWG) and feed efficiency ratio (FER) of rats

Parameter	Feed intake (g/ day /rat)	BWG (g/7day)	FER
Experimental groups			
Control (A)	30.1 ^a ± 2.48	76.1 ^a ± 27.02	0.36 ^a
B ₁	29.5 ^a ± 2.42	56.1 ^a ± 10.7	0.27 ^a
C ₁	28.8 ^a ± 2.63	61.9 ^a ± 17.3	0.31 ^a
Control (D)	31.8 ^a ± 1.72	74.8 ^a ± 9.92	0.34 ^a
B ₂	31.0 ^a ± 2.52	55.8 ^a ± 21.3	0.25 ^a
C ₂	30.5 ^a ± 2.42	56.2 ^a ± 11.03	0.26 ^a

Control A: control group (in sunlight), B₁: Fed with 70% wheat germ and sunflower seeds powder 30% (in sunlight), C₁: Vitamin D supplementation (in sunlight), Control D: control group (in the dark), B₂: Fed with 70% wheat germ and sunflower seeds powder 30% (in the dark), C₂: Vitamin D supplementation (in the dark) Values followed by the same letter in the same column are not significantly different (P≤0.001).

Table 3. Effect of vitamin D supplementation and a mixture of wheat germ and sunflower seeds powder on relative organ weights and body weight of obese rats

Treatments groups	Organs weight (g)	Body weight	Liver	Kidney	Heart	Brian	Lungs	Spleen	Pancreas	Testicular
Control (A)		337.0 ^a ±10.6	5.99 ^{cd} ± 0.22	1.55 ^a ±0.98	0.77 ^a ± 0.39	1.52 ^a ± 0.03	1.22 ^b ± 0.07	0.58 ^b ± 0.05	0.43 ^b ± 0.05	2.31 ^a ± 0.23
B ₁		272.8 ^c ±9.38	8.56 ^a ±0.51	1.78 ^a ±0.69	0.86 ^a ±0.61	1.59 ^a ± 0.09	1.51 ^a ± 0.04	0.82 ^a ± 0.05	0.44 ^b ± 0.04	2.80 ^a ± 0.20
C ₁ :		276.8 ^c ±8.79	5.90 ^d ± 0.10	1.77 ^a ±0.53	0.86 ^a ± 0.022	1.65 ^a ± 0.05	1.42 ^{ab} ± 0.03	0.63 ^b ± 0.04	0.53 ^{ab} ± 0.07	2.14 ^a ± 0.23
Control (D)		331.5 ^a ±6.34	6.90 ^{bc} ± 0.26	1.58 ^a ±0.13	0.90 ^a ±0.084	1.60 ^a ± 0.06	1.11 ^{ab} ± 0.14	0.77 ^{ab} ± 0.08	0.30 ^a ± 0.19	2.39 ^a ± 0.10
B ₂ :		290.6 ^b ±16.6	7.02 ^b ± 0.26	1.69 ^a ±0.96	0.85±0.048	1.56 ^a ± 0.07	1.35 ^{ab} ± 0.05	0.70 ^{ab} ± 0.04	0.51 ^{ab} ± 0.04	2.71 ^a ± 0.16
C ₂ :		293.3 ^b ±13.8	6.56 ^{bcd} ± 0.29	1.59 ^a ±0.88	0.75 ^a ±0.026	1.51 ^a ± 0.08	1.40 ^{ab} ± 0.07	0.61 ^b ± 0.07	0.51 ^{ab} ± 0.06	2.19 ^a ± 0.24

Control A: control group (in sunlight), B₁: Fed with 70% wheat germ and sunflower seeds powder 30% (in sunlight), C₁: Vitamin D supplementation (in sunlight), Control D: control group (in the dark), B₂: Fed with 70% wheat germ and sunflower seeds powder 30% (in the dark), C₂: Vitamin D supplementation (in the dark). Values followed by the same letter in the same column are not significantly different (P≤0.001).

The rats that were exposed to sunlight (B_1 and C_1) had a insignificant increase in the kidney, heart, brain and pancreas weights compared with the control group (A). On the other hand, the rat groups which were exposed to dark (B_2 and C_2) had an insignificant decrease in the heart, brain and spleen weight and an insignificant increase in the kidney, lungs and pancreas weight compared with control groups (D). It is clear that the higher lungs, spleen and testicular weights were to the group (B_1) which were exposed to sunlight and fed wheat germ and sunflower seeds powder and this is due to their vitamin D contents. The highest pancreas weight was recorded in the rats that were given vitamin D supplements in sunlight (C_1).

The mean values of the data revealed that the total body weight of the rats that were exposed to sunlight (B_1 and C_1) was significantly lower than those that were exposed to dark (B_2 and C_2). The rats that were given wheat germ and sunflower seeds powder in the sunlight (B_1) had the lowest body weight, on the other hand, those which were given vitamin D supplements in the dark (C_2) had the highest levels. The results of this study revealed that the rats that were exposed to dark had obesity, which is characterized by increased body weight and some organ weights. Also, the effect of sunlight on the decrement of rat body weights was significant.

This findings is agreement with results of Bozic *et al.* (2016), who noted that the effects of vitamin D on obesity were not very accurate. Its experiment on animal models indicated that a vitamin D receptor (VDR) might be involved in the development of obesity. The addition of wheat germ was associated with a decrease in lean mass and an increase in fat mass. On the other hand, HFS was not able to affect the liver weight. In conclusion, the effects of sunlight on these organs are not related to liver weight.

Andruszkow *et al.* (2013) noted that the rats which were exposed to dark had developed obesity, which is characterized by increased body weight, fat mass, and some organ weights, and the effect of sunlight on the decrement of rat body weights was significant.

3. Effect of vitamin D supplementation and a mixture of wheat germ and sunflower seeds powder on glucose level of obese rats:

The data presented in Table (4) showed that taking vitamin D supplements and adding sunflower seeds and wheat germ powder can help decrease the glucose levels of obese rats. The results indicated that the control group had a higher glucose level 'in the dark compared to the control group in sunlight'. The difference between the groups was not significant. The mean values were 117.0 and 112.8 mg/dl, in order. The rats that were fed

with vitamin D supplements in the dark had higher glucose levels than those that were given wheat germ and sunflower seeds powder in sunlight. On the other hand, the rats that were given sunflower seeds powder and wheat germ in sunlight had lower glucose level than those that were given wheat germ and sunflower seeds powder in the dark. Both of these can help maintain a high level of energy even when the calories are reduced. Wheat germ, which is a byproduct of wheat milling, is regarded as a high-protein and essential fatty acid source that can be utilized as a dietary supplement. Because of its palatability and nutritional value, it is an ideal source of human requirements. According to a study conducted by Vangoitsenhoven *et al.* (2016), vitamin D has been known to have detrimental effects on the endocrine pancreas. In the absence of a vitamin D receptor (VDR), the secretion of insulin by pancreatic cells in rats is impaired. In addition, the reduction in the secretion of glucose-induced insulin in the mouse islets was caused by VDR defects. Compared to the use of chemical-based medicines, food treatment can result in fewer side effects. This is because of how the extract and micro- and macronutrients of sunflower seeds contain compounds that can regulate insulin metabolism and glucose levels.

Table 4. Effect of vitamin D supplementation and a mixture of wheat germ and sunflower seeds powder on glucose level of obese rats

Treatments groups	Glucose mg/dl
Control (A)	112.8 ^a ± 6.40
B_1	89.3 ^d ± 3.38
C_1	105.6 ^b ± 3.77
Control (D)	117.0 ^a ± 2.60
B_2	97.6 ^c ± 4.80
C_2	107.5 ^b ± 10.16

Control A: control group (in sunlight), B_1 : Fed with 70% wheat germ and sunflower seeds powder 30% (in sunlight), C_1 : Vitamin D supplementation (in sunlight), Control D: control group (in the dark), B_2 : Fed with 70% wheat germ and sunflower seeds powder 30% (in the dark), C_2 : Vitamin D supplementation (in the dark). Values followed by the same letter in the same column are not significantly different ($P \leq 0.001$).

4. Effect of vitamin D supplementation and a mixture of wheat germ and sunflower seeds powder on the lipid profile of obese rats:

Table (5) shows oral intake of vitamin D-rich foods to male rats for 45 days showed a reduction in the cholesterol levels and (LDL-c) levels. Rats given vitamin D supplementation and a mixture of wheat germ and sunflower seeds powder showed that HDL levels rose in both conditions as compared to the control group. Mean values were 97.00 and 30.6 mg/dl, in order. Saini and Sharma (2013) reported that sunflower

ethanol extract had a lower effect on plasma total cholesterol in diabetic obese rats when compared to the control group. Results revealed that treatment with wheat germ and sunflower seeds and vitamin D supplementation caused a decrease in VLDL cholesterol as compared to the controls groups with a significant difference ($p \leq 0.001$). Hertog *et al.* (1993) reported that the drop in VLDL cholesterol levels in the treated group might be directly connected with the decline in levels of triglycerides in these groups since VLDL particles are the principal transporter of triglycerides in plasma.

5. Effects of vitamin D supplementation and a mixture of wheat germ and sunflower seed powder on liver functions:

The data in Fig. (1) showed that the supplement of vitamin D and a mixture of sunflower seeds powder and wheat germ reduced the liver functions of obese rats. The results indicated that the control group's liver enzyme in the dark had a higher value than that of the group in sunlight. The mean values were 37.33 and 35.66 U/L, in order. The highest levels of liver enzyme were observed in the group that received vitamin D supplements in the dark, while the lowest levels were observed in the group that received sunflower seeds powder and wheat germ supplements in sunlight. The control group's liver enzyme in the dark had a higher value than that of the group in sunlight.

Table 5. Effect of vitamin D supplementation and a mixture of wheat germ and sunflower seeds powder on the lipid profile of obese rats' mg/dl

Lipid profile	Cholesterol	Triglycerides	HDL-c	LDL-c	V-IDl
Control (A)	105.8 ^{cd} ± 8.44	67.0 ^a ± 5.44	42.6 ^c ± 2.16	47.9 ^c ± 7.72	15.2 ^a ± 1.08
B ₁	99.33 ^d ± 12.11	65.1 ^b ± 3.10	50.5 ^b ± 2.88	35.8 ^d ± 10.1	13.1 ^b ± 0.62
C ₁	97.00 ^d ± 8.34	58.6 ^c ± 3.17	54.6 ^a ± 2.80	30.6 ^d ± 10.8	11.7 ^c ± 0.62
Control (D)	121.6 ^a ± 4.76	77.3 ^a ± 6.08	29.8 ^e ± 2.99	76.4 ^a ± 5.98	15.4 ^a ± 1.22
B ₂	115.8 ^{ab} ± 4.26	74.3 ^a ± 1.86	32.5 ^{de} ± 3.27	68.4 ^a ± 6.00	14.8 ^a ± 0.37
C ₂	108.6 ^{bc} ± 3.01	72.5 ^a ± 8.50	35.2 ^d ± 3.65	59.0 ^b ± 4.65	14.5 ^a ± 1.70

‘TG: Triglyceride, HDL high-density Lipoprotein, LDL: Low-Density Lipoprotein and VLDL: Very Low-Density Lipoprotein. Control A: control group (in sunlight), B₁: Fed with 70% wheat germ and sunflower seeds powder 30% (in sunlight), C₁: Vitamin D supplementation (in sunlight), Control D: control group (in the dark), B₂: Fed with 70% wheat germ and sunflower seeds powder 30% (in the dark), C₂: Vitamin D supplementation (in the dark)’. Values followed by the same letter in the same column are not significantly different ($P \leq 0.001$).

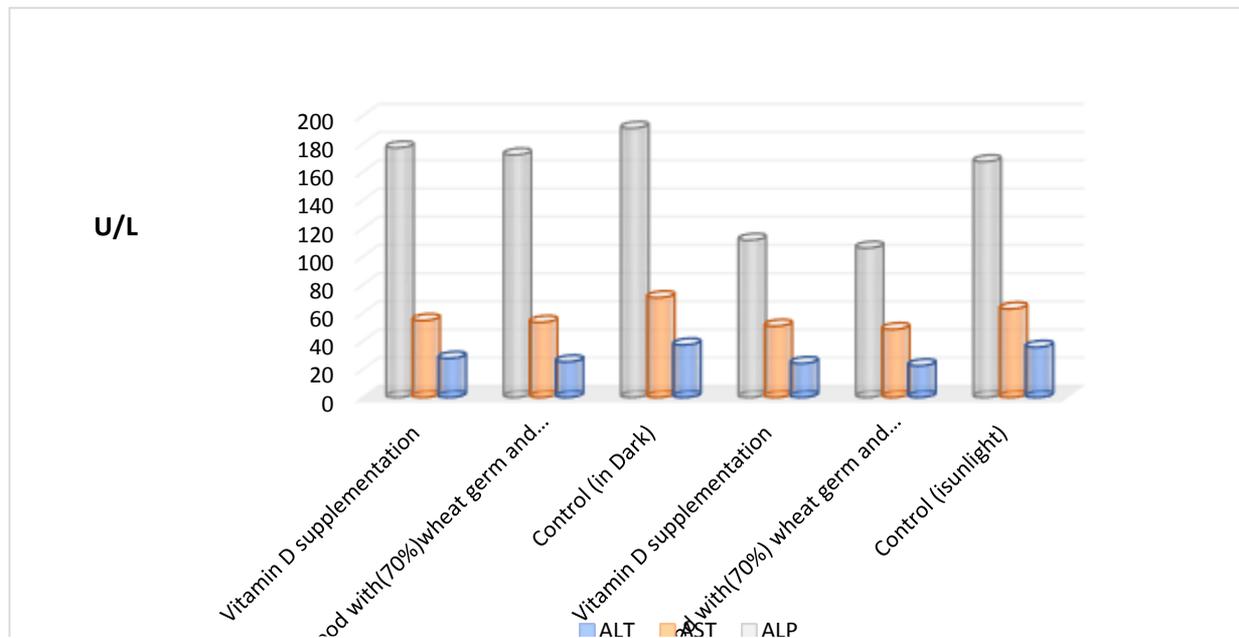


Figure 1. Effects of vitamin D supplementation and a mixture of wheat germ and sunflower seeds powder on liver functions, U/L

The mean values were 37.33 and 35.66 U/L, respectively. The difference between the groups' values was significant when it came to the ALP enzyme. The control group's liver enzyme had a higher value than that of the group in sunlight. The liver enzyme of the group that received vitamin D supplements in the dark had a higher value than that of the group that received sunflower seeds powder and wheat germ supplements in sunlight. However, the lowest value was recorded for wheat germ and sunflower seeds powder, with a non-significant difference. Most studies have examined the link between low vitamin D levels and liver dysfunction in individuals with severe liver conditions. Some studies also suggest that liver enzymes can be improved by taking vitamin D supplementation Panahi *et al.* (2017) found that sunflower seeds have effective anti-inflammatory and antioxidant properties. The results of study revealed that high-fat diets can increase the levels of liver enzymes. However, the levels of liver enzyme in the groups that took sunflower seeds were lower than those that took vitamin D supplements.

6. Effect of vitamin D supplementation and a mixture of wheat germ and sunflower seeds powder on kidney functions:

Table (6) displays the findings of the serum biochemical value analysis. The levels of uric acid, urea, and creatinine were considerably lower in all groups as compared to the control group, but there was a significant increase in the level of albumin compared to the control group. The uric acid levels for groups fed on wheat germ and sunflower seeds powder recorded a lower value when compared to groups fed on vitamin D supplementation with a significant difference ($P \leq 0.001$). The mean values were 2.22 & 2.63 and 2.54 & 2.84 mg/dl, respectively. Khalifa *et al.* (2011) reported that the addition of wheat germ to the diet caused betterment in kidney function. Results of kidney function are similar to those reported by those who determined that omega-3 fatty acids protect rats from renal failure.

7. Serum vitamin D:

The data in Fig. (2) showed the focus of serum vitamin D in the control group in sunlight reported a greater value in the dark as compared to the control group, with significant difference, ($P \leq 0.001$). The average values were (9.68) and (6.90) mg/dl, respectively. Moreover, Serum vitamin D levels for groups fed on vitamin D supplementation in sunlight recorded the highest value but the lowest fed on a mixture of wheat germ and sunflower seeds in the dark 'with a significant difference ($P \leq 0.001$). The average values were (17.5) and (8.11) mg/dl, respectively. The most efficient technique to increase vitamin D supply is by exposure to ultraviolet B (UVB) radiation in sunshine, although it is still unknown what amount of sunlight is necessary to create a particular quantity of 25(OH)D. 'Environmental and personal' variables both influence the generation of vitamin D in the skin, which is complex, making a one-size-fits-all amount of exposure for the general population impossible to propose. Our findings corroborate those of Samanek *et al.* (2006), who noted that the best estimations imply that for most people, daily casual exposure to sunshine is sufficient to generate vitamin D throughout the summer months, assuming ideal environment's conditions. Also, Rhodes *et al.* (2010) 'according to a recent research, Caucasian British subjects were given a simulated dosage of summer UV exposure while wearing in casual summer attire that exposed a third of their skin. These controlled settings (the equivalent of 13 minutes of noon summer sun exposure provided three times a week for six weeks during winter) elevated 25(OH)D levels to higher than 50 nmol/L in 90% of participants and greater than 70 nmol/L in 26% of participants'. WHO (2013), according to expert consultation, the best physiologically appropriate and effective way of acquiring vitamin D in most regions around the equator is to synthesize it endogenously from skin from 7-dehydrocholesterol present in subcutaneous fat after 30 minutes of skin exposure.

Table 6. The effects of vitamin D supplementation and a mixture of wheat germ and sunflower seeds on kidney functions mg/dl

Kidney functions Treatments groups	Urea	Creatinine	Albumin	Uric acid
Control (A)	36.16 ^{ab} ± 4.53	0.68 ^a ± 0.186	2.83 ^b ± 0.23	3.13 ^b ± 0.37
B ₁	27.16 ^c ± 3.65	0.51 ^c ± 0.75	3.94 ^a ± 0.55	2.22 ^d ± 0.16
C ₁	28.66 ^c ± 6.71	0.56 ^{bc} ± 0.125	3.88 ^a ± 0.20	2.54 ^c ± 0.17
Control (D)	41.16 ^a ± 3.86	0.73 ^a ± 0.051	2.74 ^b ± 0.66	3.70 ^a ± 0.34
B ₂	36.33 ^{ab} ± 4.03	0.63 ^{ab} ± 0.116	3.60 ^a ± 0.39	2.63 ^c ± 0.14
C ₂	35.16 ^c ± 3.54	0.58 ^{bc} ± 0.039	3.57 ^a ± 0.36	2.84 ^c ± 0.17

Control A: control group (in sunlight), B₁: Fed with 70% wheat germ and sunflower seeds powder 30% (in sunlight), C₁: Vitamin D supplementation (in sunlight), Control D: control group (in the dark), B₂: Fed with 70% wheat germ and sunflower seeds powder 30% (in the dark), C₂: Vitamin D supplementation (in the dark) Values followed by the same letter in the same column are not significantly different ($P \leq 0.001$).

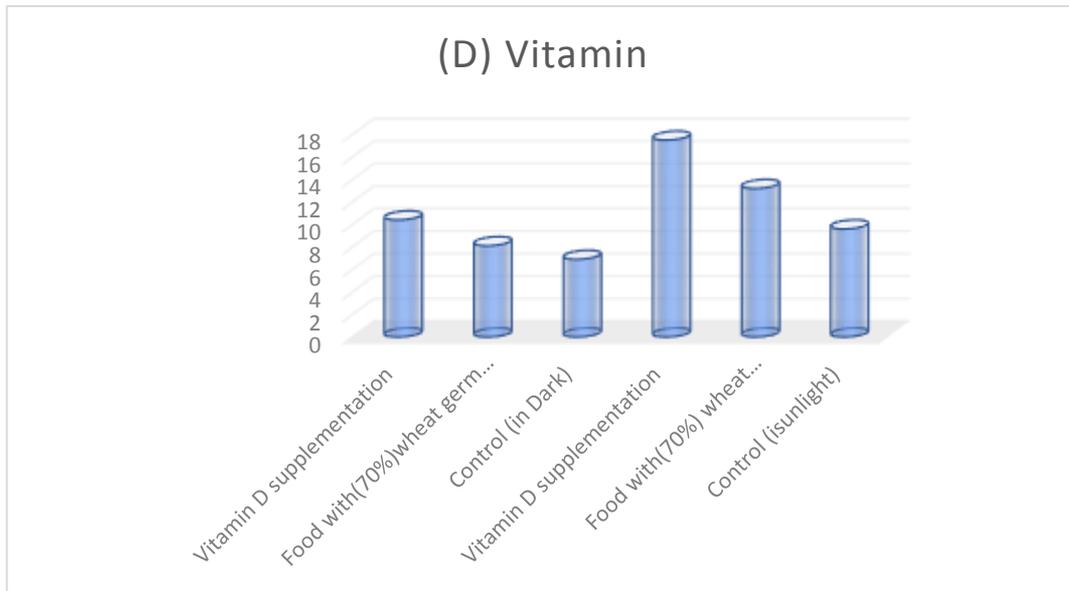


Figure 2. Effect of vitamin D supplementation and a mixture of wheat germ and sunflower seeds powder on vitamin D level, mg/dl

(Without sunscreen) of the arms and face to mid-day sun. A study conducted on Indian adults that agreed with our findings found that typical serum levels of 25(OH)D (30 ng/mL) achieved after a loading dose (250,000 – 500,000 U) could not be sustained after 1 year without additional supplementation (Goswami *et al.*, 2008).

8. Sensory evaluation of pasta with different ratios of the sensory of wheat germ and sunflower seeds mixture:

The findings provided in Table (7) and Photo (1,2,3,4,5) from the sensory assessment revealed that the pasta produced by substituting flour with a 70:30 mixture of wheat germ and sunflower seeds (T₃) resulted in the highest score in all sensory quality measures. Meanwhile, Pasta with 40% wheat germ and sunflower seeds (T₄) had lower mean scores for appearance, color, taste, odor, texture and overall

acceptance as compared to other samples. The taste of control pasta was (7.67), while the highest value recorded for pasta (30%) was (7.87) as judged by panelists. The odor of control pasta was (7.60) and the lowest value recorded for pasta 20% and 30% was (7.73). There were no statistically significant variations between control and fortified pasta. The texture of control pasta was (8.00), and the highest value recorded for pasta (10%) and (40%) was (8.07). Overall acceptance of control pasta was (8.13). The obtained data revealed no statistically significant differences between all fortified pasta and control samples in overall acceptance. Pasta is popular among customers because of its variety, simplicity of transit, handling, cooking, storage, and availability. A wide range of shapes and sizes, great digestion, strong nutritional value, and a reasonable cost.

Table 7. Sensory evaluation of pasta with different ratios of the sensory of wheat germ and sunflower seeds mixture

Pasta	appearance	Color	Taste	Odor	Texture	Overall acceptability
Control	8.07 ^a ±0.02	8.27 ^a ±0.03	7.67 ^a ±0.04.	7.60 ^a ±0.04	8.00 ^a ±0.04	8.13 ^a ±0.04
(T ₁) 10%	7.73 ^{ab} ±0.02	8.07 ^a ±0.03	7.60 ^a ±0.03	7.80 ^a ±0.03	8.07 ^a ±0.02	8.07 ^a ±0.03
(T ₂) 20%	7.87 ^{ab} ±0.03	7.87 ^a ±0.03	7.60 ^a ±0.03	7.73 ^a ±0.03	7.93 ^a ±0.02	8.07 ^a ±0.02
(T ₃) 30%	8.00 ^{ab} ±0.04	7.80 ^a ±0.04	7.87 ^a ±0.03	7.73 ^a ±0.03	8.07 ^a ±0.04	8.47 ^a ±0.02
(T ₄) 40%	7.13 ^b ±0.03	7.40 ^a ±0.03	7.40 ^a ±0.03	7.93 ^a ±0.03	8.00 ^a ±0.03	7.67 ^a ±0.03

(T₁) 10%: Pasta prepared with 10% of wheat germ and sunflower seeds powder, (T₂) 20%: Pasta prepared with 20% of wheat germ and sunflower seeds powder, (T₃) 30%: Pasta prepared with 30% of wheat germ and sunflower seeds powder, (T₄) 40%: Pasta prepared with 40% of wheat germ and sunflower seeds. 'powder Values followed by the same letter in the same column are not significantly different (P ≤ 0.05)'. 'powder



Photo (1): Control pasta



Photo (2): T₁ (10%)



Photo (3): T₂ (20%)



Photo (4): T₃ (30%)



Photo (5): T₄ (40%)

Photo 1, 2, 3, 4, 5. The pasta was prepared by replacing flour with a mixture of wheat germ and sunflower seeds powder

As a result, pasta can be utilized as a vehicle for some drugs. It is traditionally made using durum wheat semolina (Duda *et al.*, 2019). Wheat germ includes a significant amount of unsaturated fatty acids, alpha-tocopherol, calcium, potassium, zinc, arsenic, magnesium, selenium, silica, thiamine, riboflavin, lysine, and niacin which can be utilized to boost nutrient value of many food products after they've been processed. Wheat germ has been utilized for its health benefits in a variety of cereal-based products such as cake, cookies and toast bread (Majzoobi *et al.*, 2012). Sunflower seeds are a good source of unsaturated fat, vitamin D and protein, so it can be added to bakery products (Puraikalan and Sabitha, 2014).

CONCLUSION

After inducing obesity in the rats, they were divided into six groups for different dietary interventions. The first two groups received a diet consisting of wheat germ and sunflower seeds powder, while the second two groups were supplemented with vitamin D. The third two groups served as a control without any specific dietary intervention, the rats were exposed to sunlight while the other was in the dark, for 45 days before they were sacrificed biochemical analyses were conducted, focusing on the lipid profile of the rats. Additionally, the levels of vitamin D and various hematological parameters were assessed. Sensory evaluation was carried out to gauge the acceptability of food products containing sunflower seeds and wheat germ by a panel of experts or participants. The study's findings suggested that the inclusion of sunflower seeds and wheat germ products in the diet could contribute to a reduction in the weight of obese rats. This implies a potential beneficial effect of these dietary components on weight control in the setting of obesity.

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الملخص العربي

استخدام الأطعمة الغنية بفيتامين د كمكملات غذائية في منتجات المكروننة وتأثيرها البيولوجي على الفئران البدينة

نيفين الورداني، صبرى العجيزى، سلمى محمد مصطفى حسن، مينا سمير عبداللاهوت

الغذائي ببذور عباد الشمس وجنين القمح، إلى جانب مكملات فيتامين د، أدى إلى زيادة مستويات فيتامين د لدى الفئران. وعلاوة على ذلك، كشف التحليل عن تحسن في مستوى الدهون في الفئران. علاوة على ذلك، أظهرت الفئران المعرضة لأشعة الشمس مستويات معززة من فيتامين د ومؤشرات دموية محسنة بالمقارنة مع تلك الموجودة في ظروف مظلمة. بالإضافة إلى ذلك، أظهر التقييم الحسي للمنتجات الغذائية التي تحتوي على بذور عباد الشمس وجنين القمح أن أعضاء اللجنة كانوا راضين عن جودة المنتج. باختصار، تشير نتائج الدراسة إلى أن دمج بذور عباد الشمس وجنين القمح في النظام الغذائي قد يساهم في الحد من السمنة وإدارتها.

نظرًا لانتشار السمنة على نطاق عالمي، فقد برزت باعتبارها مصدر قلق كبير للصحة العامة. في السنوات العشر الماضية، كان هناك انخفاض في عدد الأفراد الذين يحاولون إنقاص الوزن. تتضمن الخطوة الأولى في معالجة هذه المشكلة اعتماد نمط حياة صحي. تم تصميم هذا المسعى البحثي لدراسة تأثير مكونات غذائية معينة على الخصائص البيوكيميائية للفئران السمنة. في هذه الدراسة، تم إخضاع ستة وثلاثون فأرًا من سلالة ويستر لظروف غذائية مختلفة، حيث تم تغذية نصفهم بنظام غذائي غني بالدهون أثناء إقامتهم في بيئات جيدة الإضاءة، والنصف الآخر في ظروف مظلمة. قبل التضحية، خضعت الفئران للصيام لمدة ١٢ ساعة، تليها تحليل الدم والمصل لتقييم مختلف المعايير البيوكيميائية. تشير نتائج هذه الدراسة إلى أن إثراء النظام