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Full Profiling of the Orange By-Products and Their Potential Effects with Chromium on Obesity Complications in Rats

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Abstract

Obesity is characterized by an excess of body fat and is considered a therapeutic condition that heightens the danger of various syndromes and health complications. Globally, approximately 19.5% of adults are classified as obese, making it a significant health issue. This work aims to investigate the chemical component, bioactive ingredients, antioxidant properties, and effects on obesity-related issues in rats using orange by-products—specifically, peel and pulp—in conjunction with chromium. Orange pulp is richer in carbohydrates, essential minerals (such as zinc, magnesium, and iron), vitamins (including E, B1, and B6), and bioactive constituents compared to orange peel. Conversely, orange peel contains higher levels of total protein, dietary fiber, minerals (such as sodium, potassium, calcium, and phosphorus), vitamins (A and C), and total polyphenols, flavonoids, and carotenoids. Compared to the normal control group, rats on a diet intended to induce obesity (DIO) exhibited significant increases in body weight (416.76%), blood leptin levels (142.84%), glucose (95.17%), and markers of hyperlipidemia (cholesterol at 219.77%, triglycerides at 131.22%, and low-density lipoprotein cholesterol at 341.59%). The antihyperlipidemic and antidiabetic properties of orange peel and pulp contribute to the reduction of serum total cholesterol, triglycerides, and low-density lipoprotein cholesterol, while simultaneously increasing high-density lipoprotein cholesterol levels. The combination of these by-products with chromium showed significant synergistic effects, partially reversing negative health changes and reducing atherogenesis risk. These findings support the utilization of by-products from the food industry, with or without chromium, for early intervention and management of obesity. Furthermore, the study recommends the integration of orange by-product powders and/or extracts into daily diets, beverages, dietary supplements, and pharmaceutical products.

Keywords: Orange peel & pulp; Composition; Serum lipid profile; Atherogenic index; Leptin; Insulin.

1. Introduction

A metabolic disease known as obesity is brought on by an excess of calories consumed and a lack of physical exercise [1]. Obesity is associated with several chronic circumstances, containing hypertension, dyslipidemia, and coronary heart ailments [2,3]. Body mass index (BMI), which is expressed as the weight in Kgs divided by the square of the height in meters (kg/m2), is a standard tool for determining the prevalence of bulky and obesity. Corpulence is described as a BMI of over 25 kg/m2, and obesity as a BMI of over 30 kg/m2. These indicators serve as common reference points for evaluation; however, illness risks in all groups might gradually rise with decreasing BMI levels [4,5]. Over half of adults in developing countries are overweight, and 19.5% of adult populations are obese, according to the Organization for Economic Co-operation and Development member countries (OECD) [6]. Additionally, according to the World Health Organization (WHO), there are more than a billion overweight individuals worldwide [7]. At least 115 M of them are from developing nations, and there are at least 300 M of them who are clinically obese. The obesity problem has also increased, according to statistics data, from 12 : 20% for men and 16 : 25% for women [8]. According to several studies, obesity affects around 15-20% of middle-aged people in Europe [9], while it affects up to 40% of people in the USA as 3,00,000 untimely fatalities each year [10]. Patients who are obese have a higher risk of morbidity and death than those who have an appropriate body weight [11]. Significant improvements in a variety of co-morbid illnesses are linked to even moderate weight loss in the scale of 5–10% of the starting body weight [12].

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Accordingly, seeking effective methods to prevent and/or treat obesity and its associated metabolic diseases is highly demanded. Meanwhile, many pharmaceuticals have been used in this direction, but unfortunately, lots of them were not effective, alongside their harmful side effects and excessive cost. Therefore, valorizing the natural sources to be used as a safe alternative to those substances was recently spotlighted. Amongst, the plant parts, especially, food processing by-products are the most commonly and most important sources to be used and most [13,14,15].

Estimates indicate that industrialized agriculture in the Arab world generates 18.14 million tons of garbage yearly, with by-products from vegetables and fruit production around 6.14%. Processed vegetables and fruits generate substantial byproduct materials, such as seeds, peels, and pulp. Agricultural by-products are well acknowledged for their high dietary fiber content, with some additionally including substantial quantities of coloring agents, antioxidants, or other compounds that confer health benefits [16]. Citrus fruits, particularly oranges, are a substantial source of food by-products.

The citrus fruit known as orange (Citrus sinensis L.) is a member of the Rutaceae family and an evergreen declining tree. In tropical and subtropical climes, orange trees are frequently grown for their fruit, which is skinned or chopped (to prevent the bitter rind) and consumed entire, or treated to obtain orange juice, as well as their aromatic peels. Around the world, 68.5 M tons of oranges were cultivated in 2008, mostly in Brazil and the US state of Florida. Oranges were grown in China by 2500 BC and came from Southeast Asia [17]. Orange fruits are entirely peeled and utilized for food, orange juice production, or fragrance production. It includes a variety of vital vitamins, minerals, and nutrients that support healthy growth and development. Peels, seeds, and pulp are the waste products of the citrus processing business. Orange peels comprise around 50% of the weight of the fresh fruit and are high in pectin, cellulose, and hemicellulose but low in protein (5.8%) [18]. 50–70% of the fresh weight of the initial fruit is invented of the pulp. The peel (60-65%), interior tissues (30-35%), and seeds (0.5-10%) are all parts of it. The essential oils from the peel are abundant in bioactive substances such coumarins, flavonoids, carotenes, terpenes, and linalool [19]. Natural phenolic chemicals, especially flavanone glycosides, principally naringin, hesperidine, narirutin, and neohesperidine, are abundant in it, making it stand out as a particularly rich source [20]. Numerous biological processes, including antidiabetic, antibacterial, anticancer, and antioxidant properties, have been associated with citrus flavonoids [21]. Additionally, Lv et al. [22] looked at how orange-peel essential oil affected acute otitis media rats' oxidative stress. The blood levels of malondialdehyde (MDA), immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) could all be decreased, and antioxidant enzyme activity could be increased by using orange peel essential oil. This research was conducted to investigate the potential effects of chromium intake in conjunction with orange byproducts (peels and pulps) on obesity-related problems in rats. The scope of this study will also include the chemical composition, minerals, vitamins, and bioactive substances of such plant sections.

2. Materials and Methods

2.1. Materials

2.1.1. Orange fruits

During the 2020 harvesting season, orange (Citrus sinensis L.) fruits were purchased from Saudi Arabian regional marketplaces. The gathered samples were taken to the lab and utilized right away to prepare the peels and pulps.

2.1.2. Chemicals

The caseinate was collected from obtained from Sigma (St. Louis, USA). The chromium picolinate (CHROMIUM ® capsules) were purchased from EPACO Company. The typical bioactive chemicals (gallic acid (GA), Butylated hydroxytoluene (BHT), $CusO_4$, β-carotene, and α-tocopherol) and vitamins (B_1, B_2, B_6, C, and E) were purchased from Sigma Chemical Co., St. Louis, MO. All other chemicals and solvents were obtained from analytical or HPLC-rating suppliers (Fisher, UK). The mobile parts, reagent solutions, and standards were all made with dH_2O (Milli-Q 18.2 M).

2.1.3. Machines

Throughout this investigation, the UV-160A from Shimadzu Corporation in Kyoto, Japan, was used to detect absorbance for several assays. A Perkin-Elmer Model 2380 atomic absorption spectrophotometer from Waltham, Massachusetts, USA, was used to analyze the minerals. Furthermore, a micro diaphragm pump, Pneumatic pressure and vacuum, Flow Rate 0.4 L/min, a Spectra System UV 1000 UV/Visible and PC 1000 system software were employed in conjunction with liquid chromatography (Thermo Separation Products, San Jose, CA, USA) to separation, with columns used were Adsorbosil C18 (5 µM, 100 mm × 4.6 mm I.d.) for the analysis of hydrophilic vitamins and ordinary Ultrasphere Si (5 µM, 250 mm × 4.6 mm I.d.) for the analysis of vitamins soluble in fat (Alltech, Deerfield, IL, USA).

2.2. Methods

2.2.1. Preparation orange peels and pulps

Orange fruits were manually cleaned, peeled, and pressed to get pulp from the orange flesh. In two phases, at 50 ºC for 4 h and then 40 ºC for 6 h, the collected peels and pulps were dehydrated in a

horizontal hot-air oven (Air-Drier, Proctor and Schwartz Inc., Philadelphia). The dehydrated peels and pulps were processed at a high blender rapidity to create a fine powder. The items kept for usage must pass through an 80-mesh filter.

2.2.2. Preparation of orange peel & pulp extracts

For the extraction of orange peel and pulp powder, 20 g of dried orange peels or pulps were combined with others with of 80% ethanol to complete 200 mL(v/v) in a beaker. The mixture was homogenized and shaken at 200 round /min for 1 60 min at 25 ºC using a Unimax 1010 shaker (Heidolph Instruments GmbH & Co. KG, Germany). Then filtered by Whatman No. 1 filter paper to separate the extract from the remaining waste. The residual substance was extracted twice more, and the resultant extracts were amalgamated. The solvent was then removed using a rotary evaporator (Laborata 4000; Heidolph GmbH & Co., Germany) at 45 °C under reduced pressure.

2.2.3. Chemical examination of powdered orange peel and pulp

Employing the methodologies delineated in the AOAC, samples of orange peel and pulp were analyzed for their chemical characteristics, which included protein (total nitrogen 6.25, determined via micro-Kjeldahl assay using an autoloading device from Velp, Italy), moisture, fiber, fat, ash, and dietary fiber content (assessed using a Soxhlet semiautomatic system from Velp, Italy), [23]. The estimations of carbs based on differences were as follows:

Carbohydrates (%) = 100 - (%moisture + %protein + %fat + %Ash + % fiber)

2.2.4. Determination of minerals content

Using the method explained by Singh *et al.* [24] the minerals content of orange peel and pulp powder samples was tested as follows: The Kjeldahl digestion unit received 0.5 g of the defatted material to be processed, and each tube received 6 mL of the tri-acid combination (HNO_3, $HClO_4$, and H_2SO_4 acid in the ratios of 20: 4: 1 v/v, orderly). The tubes' innards were slowly absorbed as observed: for 30 min / 70 °C, for 30 min / 180 °C, then for 30 min / 220 °C. The combination was digested, chilled, and then dispersed in DH_2O. The quantity was then raised to 50 mL in a volumetric beaker. A selection of minerals was examined in aliquots following filtering *via* ashless filter paper with the aid of measuring device for atomic absorption.

2.2.5. Vitamins determination

Water soluble vitamins (B and C) were extracted from orange peel and pulp powders in accordance with Moeslinger *et al.* and Epler *et al.* procedures [25,26], while fat soluble vitamin (E) was done so in accordance with Epler *et al.* [26] and Hung *et al.* methods [26,27]. Vitamins C, B_1, B_2, B_6, and E recoveries were 89.98±3.76, 85.67±2.98, 88.65±2.09, 84.99±1.83, and 90.03±1.21%, separately under the chromatographic conditions utilized in those procedures.

2.2.6. Bioactive compounds determination

Singleton, Rossiand and Wolfe *et al.* used the Folin-Ciocalteu reagent to evaluate the total phenolics in orange peel and pulp powder samples [28,29]. Results are presented as equivalents and gallic acid (GAE). Utilizing the assay described by Lichtenthaler [30], the sum carotenoids in the 80% acetone extract were calculated and represented as g of dry extract/g of carotenoid. Utilizing the colorimetric technique established by Zhishen *et al.* [31], the value of total flavonoids was calculated and represented as Milligrams of catechin equivalent (CAE) per gram of dried extract as follows:

$Y = (0.0003x - 0.0117, r^2 = 0.9827)$, mg of CA/g of dehydrated extract

2.2.7. Antioxidant activity (AA)

A modified version of the process Marco outlined was used to assess the AA of orange peel and pulp extracts and standards (α-tocopherol and BHT) [32]. Using the Al-Saikhan *et al.* equation [33], antioxidant activity (AA) was all measured as a percentage inhibition compared to control as follows:

$$AA = (R_{control} - R_{sample}) / R_{control} \times 100$$

$R_{control}$ and R_{sample} were the levels of β-carotene bleaching in the reactant blend in the absence of antioxidants and with byproducts' extract, orderly.

2.3. Biological experiments

2.3.2. Animals

Animals utilized in this study, adult male albino rats (100±10 g per each) were collected from Animal Lab, King Saud University, Riyadh, Saudi Arabia.

2.3.3. Basal Diet

As per AIN guidelines, the basal diet should include the following components: 1% vitamin mixture, 10% protein, 4% mineral blend, 10% corn oil, 0.2% choline chloride, 5% cellulose, 0.3% methionine, with the remaining 69.5% being cornstarch. The mineral mixtures and vitamin used were formulated according to AIN specifications [34].

2.3.4. Experimental design

The National Research Council, Commission on Life Sciences, and Institute of Laboratory Animal Resources regulations were followed in all biological research [35]. Rats (n=48 rats) were kept in separate rope cages in a room that was kept at a constant temperature of 25±2 ºC and at a damp level of 50–60%. Prior to the trial, all rats were given a baseline

diet for one week to help them become used to it. After one week, the rats were divided into two main groups: Group 1 (6 rats) maintained a baseline diet, while Group 2 (42 rats) adhered to a diet-induced obesity (DIO) protocol (Product No. D1245, Research Diets, Inc., NJ) for a duration of one month. The DIO diet comprised 24 g protein/100 g (20% kcal), 41 g carbohydrates/100 g (35% kcal), and 24 g fat/100 g (45% kcal). Group 2 was further divided into seven sub-groups: Group 2 served as a positive control receiving DIO; Group 3 received DIO plus Chromium Picolinate at 80 g/kg/d via stomach tube; Group 4 received DIO with 15% orange peel powder (OPP); Group 5 received DIO with 15% orange pulp powder (OPuP); Group 6 received DIO containing a mixture of OPP and chromium; Group 7 and Group 8 received variations of the DIO mixture. Rats were weighed before the trial, weekly during the study, and at the end of the experiment.

2.3.5. Biological assessment

Throughout the trial time, daily dietary records and weekly body weight records were kept (8 weeks). According to Chapman *et al.* [36], food intake (FI), the body weight gain (BWG, %), and food efficacy ratio (FER) were calculated by the subsequent equations:

BWG (%) = (Ending weight – Preliminary weight) / Preliminary weight ×100

FER = g that increase in body weight (g/28 d)/ g that feed consumption (g/28 d)

2.3.6. Blood testing

The abdominal aorta was used to collect blood samples after 12 h of fasting at the conclusion of the 28-d trial, and rats were sacrificed while being sedated with ether. Blood samples were put in clean, dry separator tubes and allowed to clot at RT before being spun for 10 min at 3000 rpm to split up the serum, using the Drury and Wallington [37]. The serum was meticulously extracted, moved to sterile, clean tubes, and then placed at -20 ºC until analysis.

2.3.7. Hematological analysis

Hematological analysis involving serum total cholesterol (CHO), triglycerides (TGs), high-density lipoprotein cholesterol (HDL-C) and total lipids (T. lipids); blood leptin, insulin, and glucose; and liver cholesterol, TGs, T. lipids and glycogen using the methods of Everds and, Ramaiah [38]. According to Lee and Nieman [39], the following formulas were utilized to multiply very-low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-c), and phospholipids as follow: VLDL-C = TG / 5, LDL-C = CHO - HDL-C - VLDL-C, and phospholipids = T. lipids - (TGs – CHO). Also, the atherogenic index was determined using the formula provided by Castelli and Levitar [40]: Atherogenic index = (CHO / HDL-C and LDL-c / HDL-C

2.4. Statistical analysis

Using the Student *t*-test and the statistical software MINITAB 12, all data were statistically evaluated (Minitab Inc., State College, PA). The data was shown as means with a standard deviation (SD). $P \leq 0.05$ was utilized to verify if differences between treatments were significant [41].

3. Results and Discussion

3.1. Proximate chemical composition of orange by-products

The approximate composition of orange by-products was tabulated in **Table 1**. It could be noted that the peel contained higher values of total protein and fiber, while the pulp had the higher values of carbohydrates and moisture. Both contained similar amounts of ash and fat. These data are partially matched with that reported by Abd-Elwahed [16]. Related results have been reported in different studies, while many factors affect the contents of orange's peel and pulp like climate, citrus variety, agricultural, and analytical methods [42]. Owing to their extra nutritional value, rich resources of protein, fibre, and ash, and data from this study and others, these plant components might be employed effectiveness in food technology and/or nutrition purposes. In terms of nutrition and cardiovascular disorders, it is also a lower-fat calorie meal. The high fiber content of orange by-products is another noticeable feature. Despite being indigestible, fibers serve a crucial nutritional function by contributing bulk to stool and facilitating passage through the digestive system.

Table 1. The approximate composition of orange by-products (on dry weight, DW).

Parameters (g/100g)	OPP	OPuP
Ash	5.18 ± 0.35	5.05 ± 0.51
Total fats	2.28 ± 0.21	2.18 ± 0.32
Total proteins	11.58 ± 1.56	7.63 ± 0.77
Total carbohydrates	57.73 ± 3.25	59. 17 ± 2.21
Crude fiber	12.66 ± 0.65	9.29 ± 0.74
Total moisture	10.57 ± 1.32	16. 68 ± 1.67

Each value signifies the Mean of four replicates ± stander division SD. Means under the same row with different superscript letters are significantly different at $p \leq 0.05$. OPP, orange peel powder; OPuP, orange pulp powder.

3.2. Minerals' concentration of orange by-products

Data of mineral contents of orange by-products has been illustrated in **Table 2**. Data indicated that the peel contained higher values of Na, Ca, and P, while the pulp had higher values of K, Mg, Fe, and Zn. Due to their large quantities of orange by-products, minerals, including K, Mg, P, and Ca, are suggested to be the main elements. Nevertheless, orange by-products include lower sodium levels; hence, they may be suggested as a beneficial source for those with hypertension. Trace metals are present in significant quantities in the inorganic form within orange by-products. All trace elements are physiologically essential to the human body to prevent and treat

several disorders, including anemia, immunodeficiency, cancer, and atherosclerosis [43]. Zn's role in cellular growth, division, maturation, membrane integrity, and the synthesis of DNA and RNA underscores its importance in the immune system [44, 45]. Hemoglobin in erythrocytes contains iron, which is responsible for carrying oxygen from the lungs to all of the body's organs' tissues. Furthermore, it is necessary for the human immune system to function and for DNA creation [45].

Table 2. Minerals' concentration of orange by-products (on dry weight, DW).

Minerals (mg/100 g)	OPP	OPuP
Sodium	3 .09 ± 0.12	2.66 ± 0.20
Potassium	212 .89 ± 14.10	131.66 ± 9.16
Calcium	161 .40±10.65	109.05 ± 7.57
Phosphorous	21 .94 ± 1.67	19.80 ± 2.02
Magnesium	22 .88 ± 1.03	42.99 ± 3.27
Iron	108 .65 ± 9.68	175.88 ± 14.29
Zinc	47.25 ± 3.09	90.43 ± 6.10

Each value signifies the Mean of four replicates ± stander division (SD). At $p < 0.05$, there is a significant difference between the means under the same row with different superscript letters. OPP, orange peel powder; OPuP, orange pulp powder.

3.3. Vitamins content of orange by-products

The vitamin content of orange by-products is displayed in **Table (3)**. The peels had the highest concentrations of vitamins C and β-carotene, whereas the pulp held the highest concentrations of vitamins E, B_1, and B_6. From a nutritional perspective, vitamins are necessary for life since they support development and good health. Vitamin B_1, B_2, and B_6 are all found in significant amounts in orange byproducts. The B vitamins represent a diverse group in terms of function and structure. It plays a part in the metabolism of glycine, histidine, serine, and methionine, among other amino acids. On the other hand, there are the anti-inflammatory minerals, such β-carotene (found in orange byproducts) and vitamins C, E, and Nutritional Reference Values (DRVs).

Table 3. Lists the vitamins that orange by-products contain (on dry weight, DW).

Vitamins	OPuP	OPP
Vitamin A (β-carotene, µg/100g)	292 .55 ± 17.56	180.79 ± 10.21
Vitamin C (mg/100g)	39.18 ± 3.76	23.38 ± 2.42
Vitamin E (µg/100g)	1.72 ± 0.23	5.71 ± 0.53
Vitamin B_1 (mg/100g)	0.29 ± 0.05	0. 42 ± 0.07
Vitamin B_2 (mg/100g)	0.10 ± 0.01	0.15 ± 0.04
Vitamin B_6 (mg/100g)	0.264 ± 0.031	0.360 ± 0.028

Each value signifies the Mean of four replicates ± stander division (SD). At $p < 0.05$, there is a significant difference between the means under the same row with different superscript letters. OPP, orange peel powder; OPuP, orange pulp powder.

3.4. Bioactive compounds of orange by-products

Bioactive compounds of orange by-products are illustrated in **Table (4)**. Data noticed that the total polyphenols, flavonoids, and carotenoids were higher in the pulp. Our data partially agreed with that noted by Abd-Elwahed [16]. Numerous of our earlier studies, as well as other research, have demonstrated the critical role those bioactive substances, such as the phenolics, flavonoids, and carotenoids found in orange by-products in this study, play in averting and/or treating several ailments, including diabetes, atherosclerosis, cancer, obesity, bone, and ageing [14, 46]. The miraculous antioxidant properties of these chemicals are primarily responsible for all the benefits. Due to their rich supply of both nutritional and non-nutritional substances, all these elements combined to make orange by-products a full bundle of nutritious food.

Table 4. Bioactive compounds of orange by-products (on dry weight, DW).

Parameters (mg/100g)	OPP	OPuP
Total polyphenols	0.37 ± 0.09	0.44 ± 0.11
Flavonoids	0.54 ± 0.16	0.91 ± 0.15
Carotenoids	0. 274 ± 0.03	0.389 ± 0.04

Each value signifies the Mean of three replicates ± SD. Means under the similar row with different superscript letters are significantly different at $p \leq 0.05$. OPP, orange peel powder; OPuP, orange pulp powder.

3.5. Antioxidant activity (AA) of orange by-products

Table (5) displays the antioxidant activity (AA) of orange by-products. It was evident from such data that changes in the orange by-products ranged from 73.65-80.67%, which was different ($p \leq 0.05$). Due to the abundance of phytochemicals and nutrients in all the examined sections, they all showed robust activity (phenolics, flavonoids, carotenoids, essential oils, vitamins, and other components). These outcomes are constant with those described by Abd-Elwahed [16], who discovered that various by-products of food processing, such as orange peels and others, have a strong antioxidant activity because of their high presence of phenolic constitutes. In a related study, Velioglu *et al.* revealed that there was a statistically significant association between the total phenolic content and antioxidative activity of twenty-eight plant products, including plant by-products [47]. Additionally, several investigations revealed a favorable and substantial ($p \leq 0.01$) correlation between total phenolics and AA in various plant sections

[46,48,49,50]. Antioxidants aid in defending cells against "oxidative stress," a physiological condition that may be harmful (injury to healthful cells or DNA by peculiar electrons recognized as free radicals). Chronic disorders such as cancer, heart syndrome, diabetes, rheumatoid arthritis, obesity, and age-related ailments such as neurodegenerative diseases like Parkinson's and Alzheimer's are influenced by oxidative stress [13,15,51,52,53,54]. The orange by-products were advised to be employed successfully in several medicinal applications for all of these and other reasons.

Table 5. Antioxidant activity (AA) of orange by-products and standards.

Parameters	OPP	OPuP
AA (%)	73.56 ± 4.52	80.67 ± 3.09
AA [% of BHT (50 mg/mL)]	83.01 ± 2.31	91.03 ± 0.99
AA [% of BHT (100 mg/mL)]	87.28 ± 1.45	84.81 ± 2.03
AA [% of α –tocopherol (50 mg/mL)]	75.05 ± 2.91	82.30 ± 1.79
BHT (50 mg/mL)	88.62 ± 0.49	88.62 ± 0.49
BHT (100 mg/mL)	95.11 ± 0.81	95.11 ± 0.81
α-tocopherol (50 mg/mL)	98.02 ± 0.57	98.02 ± 0.57

Each value signifies the mean of ten replicates ±SD. Mean values with the different superscript letters in the similar row mean significantly different at level p≤0.05. OPP, orange peel powder; OPuP, orange pulp powder; BHT, butalyted hydroxutoluene.

3.6. Biological studies

3.6.1. Effect of orange by-products on BWG, FI, and FER of obese experimental rat groups

The obesity experimental rat groups' FER, FI, and BWG were shown in Table (6). The values of these parameters had increased in the positive control obese rat group consuming the DIO, while they had significantly (p≤0.05) decreased in the chromium, orange peel, orange pulp, orange peels with chromium, orange pulps with chromium, and mixed orange peels and pulps with chromium groups. The mixed orange peel and pulp with chromium group listed as the lowest values compared to the positive control group. This study demonstrates obesity-reducing effects of the dietary parameters of chromium and both orange peels and pulps. Chromium is vital for maintaining the ordinary carbohydrate and lipid metabolism and attenuating certain DIO impacts, primarily body fat accretion. Orange by-products contain considerable amounts of bioactive compounds (phenolics, carotenoids, flavonoids, etc.) and vitamins (A, C, and E) that have strong anti-inflammatory and antioxidant features and exhibited the positive effects of such plant parts in regarding the control of the obesity [14,15,16,55,56]. Orange peel and pulp also contain high amount of fiber which positively affects the regulation of intestinal capacity, disease prevention and as a therapeutic factor in diverse diseases, especially the metabolic disorders [57,58].

Table 6. BWG, FI, and FER of obese investigational rat groups.

Parameters	Negative control	Positive control	Chromium	OPP	OPuP	OPP + chromium	OPuP + chromium	OPP+ OPuP+ chromium
BWG	34.67±1.12e	179.16±4.61a	104.55±3.33c	110.33±3.66b	113.07±3.11b	49.66±2.01d	45.44±2.11d	36.99±2.05e
FI	22.98±2.98c	39.05±3.41a	30.33±3.05b	31.01±3.10b	31.51±3.33b	24.44±3.21c	24.60±3.71c	23.59±3.61c
FER	0.024±0.003d	0.085±0.002a	0.057±0.01b	0.059±0.001b	0.060±0.003b	0.033±0.004c	0.30±0.001c	0.026±0.002cd

Mean ± SD (n = 6) is shown by each value. At p≤0.05, there is a significant difference between the means under the same row with different superscript letters. Feed intake (FI); body weight gain (BWG); feed efficacy rate (FER); orange pulp powder (OPuP); and orange peel powder (OPP).

3.6.2. Impact of orange by-products on serum lipid profile of obese investigational rat groups

Data in Table (7) demonstrated how orange byproducts affected the groups of obese experimental rats' blood lipid profiles. The positive control obese rat group that ingested the DIO exhibited high values for the blood lipid profile (TGs, CHO, VLDL-c, LDL-c, and low HDL-c, according to the current findings. Chromium, orange peels, and orange pulps treatment decreased their prominent levels and elevated HDL-c. When related to the positive control group, the administration of chromium with orange peels or pulps combination obviously had synergistic effects on lowering CHO and LDL-c levels. Citrus flavonoids may have a lipid-lowering effect as because of their antioxidant properties, suppression of the absorption of oxidized LDL by macrophages, and decreased LDL aggregation and oxidation of LDL cholesterol. When compared to a hypercholesterolemic diet, diets supplemented with 10 and 20% orange powder significantly lowered T. lipids, CHO, TGs, and LDL-c in rats [59]. Modeling derived from systematic reviews of randomized controlled trials suggests that modest and sustained weight loss (5–10 kg) in individuals who are overweight or obese correlates with increased HDL-c and decreased levels of TGs, CHO, and LDL-c [60,61,62].

Table 7. Serum lipid profile of the obese experimental rat groups.

Parameters (mg/dL)	Negative control	Positive control	Chromium	OPP	OPuP	OPP +chromium	OPuP + chromium	OPP+ OPuP + chromium
CHO	123.56±6.78e	395.11±5.77a	197.55±4.21b	191.77±3.99b	190.55±3.61b	170.81±4.11c	165.33±5.11c	155.11±5.31cd
TGs	87.12±3.02c	201.44±7.33a	115.40±6.11b	110.44±5.19b	112.20±5.31b	105.22±5.23b	103.77±5.11bc	95.16±4.01c
HDL-c	30.67±2.45a	21.11±1.77b	38.99±2.41a	38.01±2.99a	37.81±2.81a	40.31±3.01a	40.71±3.66a	34.21±3.25a
LDL-c	75.47±3.15e	333.72±7.96a	135.48±4.21b	131.67±4.77b	13.31±4.31b	109.46±5.1c	103.87±5.82c	92.87±4.71cd
VLDL-c	17.42±0.99c	40.28±2.11a	32.08±1.45b	22.9±1.66b	22.44±1.34b	21.04±1.77b	20.75±1.36bc	19.03±1.22c

Mean ± SD (n = 6) is shown by each value. At p≤0.05, there is a significant difference between the means under the same row with different superscript letters. OPP, orange peel powder; OPuP, orange pulp powder; CHO, cholesterol; TGs, triglycerides; HDL-c, high density lipoprotein cholesterol; low density lipoprotein cholesterol; LDL-c, VLDL-c, very low-density lipoprotein cholesterol.

On the other side, data in **Table (8)** illustrated that the increase of atherogenic indexes (CHO/HDL-c and LDL-c/HDL-c) of positive control obese rat group, while administration of chromium, orange peels & pulps or a combination of orange peels and/or pulps with chromium clearly showed their synergistic effects to reduce CHO and LDL-c and raised the HDL-c. Additionally, there was an increase in serum T. lipids and lower phospholipids in the positive control obese rat group, while groups administrated with chromium, orange peels, orange pulps or a combination of the two with chromium reduced T. lipid levels (**Table 9**). The chromium, orange peels and orange pulps groups showed lower values of phospholipids, while the orange pulps with chromium group and the orange peels and pulps with chromium group showed higher values of phospholipids compared to the positive control group. Several studies on diabetes illustrated that supplementation with chromium ion (III) reduced blood glucose concentrations, CHO, LDL-c, TGs, and non-esterified fatty acids levels; as well as enhanced the concentrations of HDL-c [63].

Table 8. Atherogenic parameters (CHO/HDL-c and LDL-c/HDL-c) of the obese experimental rat groups.

Parameters	Negative control	Positive control	Chromium	OPP	OPuP	OPP + chromium	OPuP + chromium	OPP+ OPuP+ chromium
CHO/HDL-c	4.03±0.32c	18.71±1.55a	5.6±0.41b	5.04±0.53b	5.03±0.46b	4.23±0.35bc	4.06±0.45bc	3.58±0.44c
LDL-c/HDL-c	2.46±0.20c	15.80±1.60a	3.47±.31b	3.46±37b	3.44±0.35b	2.71±0.22b	2.55±0.28bc	2.14±0.26c

Mean ± SD (n = 6) is shown by each value. At p≤0.05, there is a significant difference between the means under the same row with different superscript letters. OPP, orange peel powder; OPuP, orange pulp powder.

Table 9. Serum total lipids and phospholipids of the obese experimental rat groups.

Parameters (mg/dL)	Negative control	Positive control	Chromium	OPP	OPuP	OPP + chromium	OPuP + chromium	OPP+ OPuP+ chromium
T. lipids	289.67±11.39 c	703.93±33.61 a	392.11±11.77 b	387.41±13.41 b	379.75±14.55 b	380.71±19.33 b	391.79±17.7 7b	374.55±16.44 b
Phospholipids	78.99±9.42b	107.38±9.66b	79.16±7.22d	85.21±8.75c	77.01±7.42 d	104.68±10.21 b	122.69±13.6 1a	124.28±14.25 a

Mean ± SD (n = 6) is shown by each value. At p≤0.05, there is a significant difference between the means under the same row with different superscript letters. OPP, orange peel powder; OPuP, orange pulp powder; T. lipids, total lipids.

3.6.3. Effect of orange by-products on blood leptin, insulin, and glucose levels of obese investigational rat groups

According to data in **Table (10)** the positive control group of obese rats had significantly higher ($p \leq 0.05$) blood levels of leptin and glucose and lower insulin values, whereas the treatment of chromium, orange peels, and orange pulps boosted leptin and glucose levels and decreased insulin levels. Compared to the positive control group, the orange peels with chromium, orange pulps with chromium, and orange peels and pulps with chromium groups showed a drop in leptin and glucose and an increase in insulin. Research has shown that serum leptin levels are higher in obese individuals and that chromium, which enhances glucose and insulin tolerance, may decrease blood sugar and cholesterol [64].

Table 10. Blood leptin, insulin, and glucose levels of the obese investigational rat groups

Parameters	Negative control	Positive control	Chromium	OPP	OPuP	OPP + chromium	OPuP + chromium	OPP+ OPuP+ chromium
Leptin (ng/l)	46.45±2.92d	112.80±6.33a	63.14±2.77b	60.79±3.41b	61.11±3.22b	55.17±2.96c	56.37±3.01c	54.11±3.19c
Insulin (mg/dL)	16.41±3.08a	10.11±1.22d	12.80±1.11c	12.59±1.61c	13.55±1.33ac	14.11±1.41b	14.03±1.92b	14.77±1.80b
Glucose (mg/dL)	110.56±4.09e	215.78±5.33a	160.71±4.55 b	168.40±5.76 b	163.33±6.01b	140.76±4.99 c	145.84±5.12c	130.77±3.98d

Mean ± SD (n = 6) is shown by each value. At p≤0.05, there is a significant difference between the means under the same row with different superscript letters. OPP, orange peel powder; OPuP, orange pulp powder.

3.6.4. Effect of orange by-products on liver cholesterol, total lipids, triglycerides (TGs), and glycogen of the obese investigational rat groups

Data presented in Table 11 indicate that changes in liver cholesterol, total cholesterol, and T. lipids were significantly different (p≤0.05) in the positive control obese rat group and significantly different (p≤0.05) in the other groups (increasing glycogen and decreasing TG). When compared to the positive-controlled group, triglycerides increased following the administration of orange peels and pulps in conjunction with chromium. Considering this, hesperidin, a flavonoid that aids in lowering cholesterol and TGs, is present in orange peels and pulps [65]. Additionally, oranges' ability to decrease cholesterol is due to the limonene component in their peels. Furthermore, polymethoxylated flavones found in citrus fruit peels can lower cholesterol more effectively and safely than certain prescription drugs. On the other hand, by lowering the levels of T. lipid, TG, and CHO, chromium supplementation has a considerable impact on the obesity index, body fat, and blood lipid concentrations. In related research, Lewicki *et al.* [66] found that adding chromium ions (III) to a diet decreased blood glucose levels, LDL-c levels, cholesterol levels, and the risk of atherosclerosis and heart attacks.

Table 11. Liver cholesterol, total lipids, triglycerides (TGs), and glycogen of the obese investigational rat groups.

Parameters (mg/g)	Negative control	Positive control	Chromium	OPP	OPuP	OPP + chromium	OPuP + chromium	OPP+ OPuP+ chromium
CHO	4.01±0.39c	8.03±1.30a	6.11±0.96b	4.95±0.33c	4.88± 0.55c	4.79±0.41c	4.99±0.53c	4.66±0.52c
T. lipids	34.77±3.14d	66.89±5.11a	55.77±5.91 b	50.11±5.31 b	46.50±4.91bc	45.61±4.77 bc	44.75±4.35bc	40.77±4.3c
TGs	4.51±0.41a	2.01±0.25c	3.19±0.37b	4.45±0.45a	4.31±0.35a	4.20±0.42a	4.01±0.47a	4.91±0.44a
Glycogen	4.02±0.42a	2.11±0.27b	3.03±0.43a	3.49±0.26a	3.71±0.39a	3.61±0.37a	3.51±0.35a	3.66±0.34a

Mean ± SD (n = 6) is shown by each value. At p≤0.05, there is a significant difference between the means under the same row with different superscript letters. OPP, orange peel powder; OPuP, orange pulp powder; CHO, cholesterol; T. lipids, total lipids; TGs, triglycerides.

Conclusion

The present research demonstrated the efficacy of certain food industry waste, namely peels, and pulps, in conjunction with chromium to address issues or anomalies in obese rats. The implications include liver function, hyperglycemia, and serum lipid profile alterations. Their substantial concentration of bioactive compounds, vitamins, and minerals contributes to significant antioxidant activity, which accounts for these advantages. These findings provide a foundation for using certain food sector by-products in the initial management of obesity. The present research recommended including orange by-product powder and/or extracts into daily meals, drinks, dietary supplements, and medicinal formulations. The study has several limitations that warrant consideration. Firstly, the research was conducted solely on rats, which may limit the applicability of the findings to human populations, as metabolic and physiological responses can differ significantly between species. Additionally, the study focused on specific orange by-products and chromium, potentially overlooking other dietary components that could influence obesity complications. The duration of the experiment was also relatively short, which may not adequately capture the long-term effects and potential side effects associated with the consumption of orange by-products and chromium supplementation. Furthermore, the sample size was limited, which could affect the statistical power and reliability of the results. For future research, it is essential to conduct studies involving diverse animal models and eventually human clinical trials to validate the findings and explore the broader implications of incorporating orange by-products into diets. Investigating the mechanisms behind the observed effects of orange peel and pulp in conjunction with chromium will also be crucial for understanding their role in obesity management. Additionally, future studies should consider a longer duration and larger sample sizes to assess long-term benefits and any adverse effects associated with these dietary interventions. Exploring combinations with other functional foods may further enhance therapeutic strategies against obesity complications.

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