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The Impact of Pomegranate Peel-fortified Cupcakes on Weight Loss

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ABSTRACT

Pomegranate peel is considered as an antioxidant and a nutritive by-product. It is easily available after the production of pomegranate juice. The purposes of the current research were to use the powder of pomegranate peel (PPP) as an antioxidant and a fibre-rich source to make a nutritious cupcake based on 5%, 10%, 15% and 20% PPP and investigate its effect on the weight loss of rats fed by a high-fat diet (HFD). In total, 42 female albino adult rats were included and divided into 7 groups (each group contained 6 rats). A basal diet was fed to Group 1 (G1) (negative control), a HFD was given to (G2), a HFD+ control cupcake was given to (G3), (G4) received a HFD + 5% PPP-fortified cupcake, (G5) was fed a HFD + 10% PPP-fortified cupcake, (G6) was fed a HFD + 15% PPP-fortified cupcake and (G7) was fed a HFD + 20% PPP-fortified cupcake. Food consumption, food efficiency, and body weight gain (g), the serum lipid profile and liver function were monitored. The results revealed a significant decline ($p>0.05$) in the weight gain of rats in G7 followed by G6, G5 and G4 and compared to G2 and G3. VLDL-C, LDL-C, total cholesterol, triglycerides, and liver enzymes (AST, ALT) were decreased by increasing the amount of PPP in the cupcake; whereas, HDL-C was increased significantly. In conclusion, pomegranate peel powder-fortified cupcakes can help with weight loss and avoid the risk of obesity, which could be incorporated into a range of foods.

Key words: *Obesity, Pomegranate Peel Powder, Rats, Cupcake, Lipid Profile, Liver Function.*

INTRODUCTION

Obesity and overweight are the most common and pressing health concerns facing today's society [1]. A recent study on the topic reveals that more than a billion adults are overweight, with approximately 300 million of them being reported as obese, which indicates that more people are at risk of falling victim to health issues caused by this condition [2]. According to a WHO report, higher levels of obesity among adults and children have been observed in nine Middle Eastern countries, including Saudi Arabia and Egypt [3]. Globally, it is estimated that approximately 3.4 million people die annually from obesity or instances of being overweight [1]. Weight loss in people reported to be obese (despite their age) can decrease medical complications that are associated with obesity and at the same time increase their physical functioning and thus improve their quality of life [4].

Diet intervention is considered the first step in weight loss as well as weight maintenance to reduce the prevalence of overweight and obesity [5]. It is reported that approximately 50% of the total weight of fruits corresponds to the entire peel, which have different nutritional values since they contain bioactive compounds [6]. These compounds include proanthocyanidin, phenols, ellagitannins, and flavonoids, which fight oxidation when compared to the extracts of pulp [6]. Food processing by-products may cause essential sanitary problems, but recently, more studies have been conducted to assist in the conversion of this refuse into products that can be valuable [7].

Pomegranate peel is one of the by-products that has attracted the attention of researchers due to its ability to heal wounds [7] and participate in immunomodulatory activities [8] and ultimately due to its antioxidative capacities

[9] and anti-atherosclerotic ability [9]. Although several scientific studies have demonstrated pomegranate medicinal effects and the biological activities of the edible part of the pomegranate, very few data exist regarding the bioactivity of pomegranate peel [10-13].

Pomegranate peel is considered a food processing refuse that can be converted into products that can be valuable, such as bakery products. They are considered as one of the most acceptable and viable carriers of nutritious supplements [14]. Baked products essentially have high dietary fibre, which is vital in the total consumption of food due to its digestion benefits [15]. Cakes are the most consumed owing to the fact that they are used to mark certain milestones in life such as birthday celebrations and other festivals that run throughout the year [16, 17]. The consumption rate has increased by approximately 1.5% annually [18], which requires enhancing the cupcake with beneficial ingredients such as fibre due to the health benefits.

Therefore, the purpose of the current study was to use pomegranate peel as a great source of antioxidant compounds and dietary fibre in cupcakes and to make a nutritious cupcake with different amounts of pomegranate peel powder and investigate the impact of cupcakes substituted with PPP on weight loss and the lipids profile of hypercholesteremic rats.

MATERIALS AND METHODS

Pomegranate Peel Powder (PPP) Preparation

The pomegranates used in the current research were purchased from Egypt. The ripened pomegranates were washed under running water, peeled, and then manually separated before being divided into small pieces for drying under the shade in the open air. The dried peels were then processed into powder using a 40 mesh size with a hammer before they were further evaluated. The resulting product was kept under refrigeration at -20 ℃ until ready for use [19].

Cupcake Processing Formulas

The ingredients used in the preparation of the cupcakes are summarized in Table 1. The processing method proposed by A.A.C.C. [20] was used in the experiment and involves the following steps: the shortening was thoroughly melted, and then salt and sugar were added to the mixture and vigorously stirred. Whole eggs were mixed with vanilla and whipped for several minutes to make a cream with a smooth texture. Moreover, PPP were replaced with white flour at different levels, namely, 5.0%, 10.0%, 15.0%, and 20.0%, which were individually mixed with approximately 2.15% baking powder and skimmed milk powder at 4.30%. The resulting mixture was then gradually added to the whipped egg and gently mixed to form a homogenous dough with the help of a handheld mixer (MK-4H-W, Panasonic Co, Malaysia). The resulting homogeneous mixture was then poured into different cups of similar sizes and baked at a temperature of 180 ℃ (+/- 5) for 30 to 35 minutes. The product was cooled and then stored for use in the experiment.

Table 1: Ingredients of the cupcakes.

Ingredients	Weight (g)
Soft wheat flour (72% extraction)	255
Sugar	130
Salt	4
Skimmed milk powder	30
Shortening	60
Fresh whole egg	115
Baking powder	14
Vanilla	3

Experimental animals

This experimental study was conducted from July-August 2018. A total of 42 two-month-old white male albino Sprague Dawley rats weighing approximately 180 ± 10 grams were used in this experiment. They were obtained from Animal Health Research Institute, Egypt. Each rat was housed individually in a bottomed, stainless steel cage under standard conditions. It was well-aerated cages in an animal room and maintained in a temperature-

controlled room (23 ± 1 °C) with a 12 h light/12 h dark cycle, 55±10 % humidity. The animals were processed according to the suggested international ethical guidelines for the care of laboratory animals.

Experimental procedure

On arrival, these rats were randomly sampled into seven groups comprising 6 rats each. The rats were weighed each week before the experiment and this continued except for the first week, which included weighing every day. The basal diet proposed by the AIN-93 diet guidelines was prepared to feed these animals [21] for one week to help them adapt to the environment and induce an accelerated increase in body weight and obesity. The basal diet comprised soybean oil at 4%, casein at 14%, a salt mixture at 3.5% and cellulose at 5%. The rest of the diet was complimented with corn starch and a high-fat intake at 30 percent (28% animal fat and 7% soybean oil) [22]. After acclimation, the experimental animals were fed by different diets as presented below [23]:

G1: Negative control, fed a basal diet
G2: Positive control, fed a high-fat diet (HFD)
G3: Fed a HFD + 30% control cupcake
G4: Fed a HFD + 30% pomegranate peel powder-fortified cupcake (PPP) 5%
G5: Fed a HFD + 30% pomegranate peel powder-fortified cupcake (PPP) 10%
G6: Fed a HFD + 30% pomegranate peel powder-fortified cupcake (PPP) 15%
G7: Fed a HFD + 30% pomegranate peel powder-fortified cupcake (PPP) 20%

Sampling of Blood

Blood samples were taken from the rats three times (on day No. 1, 21 and 42) after fasting for 12 hours for all groups. At the end of the study, the weights of the rats were measured, and then the rats were scarified using a knife; the blood from the scarification was then put into dry and clean centrifuge tubes and left to clot. All the collected blood was centrifuged at 3500 rpm for 10 minutes for separation. The resulting serum was then cautiously aspirated and transferred into a clean plastic tube for biochemical analysis [24]. The serum lipid levels were then analysed to measure all the available parameters, for instance, the total cholesterol present, HDL-cholesterol, vLDL-cholesterol and triacylglycerides.

Parameter Analysis of Growth

Feed intake and body weight were assessed every two days throughout the test period (6 weeks). The calculation of the difference between the weight of the feed remaining in the feed bin (Da) and the amount placed in the bin for the feed (D) was used to determine the amount of feed intake. This is summarized by the formula below [25]:

Feed intake (g) = $\frac{D-Da}{1}$ / 1

The data collected can also be used to calculate the survival percentage and body weight gain, as shown below:

BWG (%) = [(W2 – W1) / W1] × 100.

Survival (%) = R2 / R1 ×100.

where W1 = initial weight and W2 = final weight. R1 represents the number of rats at the end of the study, while R2 is the number of rats at the beginning of the test [26].

Statistical Analysis of the Data

The SPSS (version 23.0) program was used in the present study to analyze the data. The results adapted from the collected data are presented as the mean value +/- SD. However, the statistical comparison was performed using ANOVA for the difference between groups.

RESULTS

Table 2 shows the body weight changes, amount of food intake, body weight gain and feed efficiency ratio of rats that received PPP-fortified cupcakes. The results revealed that the rats fed a HFD had a significant increase in food efficiency ratio, the amount of food intake and body weight gain compared with G1 rats (negative control) ($p<0.05$). The rats fed PPP-fortified cupcakes demonstrated a significant decline in the efficiency of food, the amount of food intake and body weight gain compared to G2 rats, which were fed a high-fat diet, and G3 rats, which were fed the control cupcake ($p<0.05$). However, G7 rats, which were fed the 20% PPP cupcake,

had significantly lower food consumption and body weight gain than rats fed the other PPP-fortified cupcakes and were followed in decreasing order by G6 then G5 and G4 ($p<0.05$).

Table 3 presents the rats' lipid profiles in serum. LDL-C and VLDL-C, total cholesterol, and triglycerides were highly significant ($p<0.05$) in G2; while, a significant decrease was recorded for HDL-C in G2 compared to G1 (negative group). The levels of vLDL-c, LDL-c, total cholesterol, and triacylglycerides were significantly lower ($p<0.05$) among the rat groups fed PPP-fortified cupcakes, particularly the 20% PPP-fortified cupcake, followed by the 15% and 10% PPP-fortified cupcakes. However, the highest levels of HDL-cholesterol were recorded for G7 and G6, which were significantly higher than those of the other groups ($p<0.05$). The ratio of HDL-c to LDL-c was significantly lower ($p<0.05$) in G7 than in G3 and G2, followed by G6, G5 and G4.

Table 4 presents the estimated levels of serum AST and ALT in the rats in each group. Serum AST and ALT levels in G2 (HFD) were significantly higher than those in G1 (the control group) ($p<0.05$). AST and ALT levels were significantly lower among the rat groups fed PPP-fortified cupcakes (20%, 15%, 10%, and 5%) when compared to G2 and G1 (the positive and cupcake control groups) ($p<0.05$).

Table 2: Body weight (g), food intake (g) and food efficiency ratio body weight gain% of rats that received PPP-fortified cupcakes

No.	Rat groups	Initial weight	SD	Final weight	SD	Body weight gain	SD	Food intake (FI) g	SD	Feed efficiency ratio (FER) %	SD	Body weight gain (BWG) %	SD	Survival (%)
1	Control (-)	182.15a	1.67	243.11c	1,82	60.96f	2.50	957.71d	1.66	3.04de	1.03	33.46e	3.34	100
2	Control (+)	180.90a	1.18	297.40a	2.41	116.50a	3.04	974.62b	2.71	5.82a	1.41	0.64f	3.28	100
3	Control cupcake	182.06a	2.19	284.65a	3.76	102.59b	2.32	1027.54a	1.09	5.12ab	1.15	56.34a	2.25	100
4	Cupcake with 5% PPP	181.74a	3.95	271.19ab	3.98	89.45c	1.72	960.73c	3.07	4.47c	3.11	49.21b	1.80	100
5	Cupcake with 10% PPP	183.50a	1,84	265.03b	2.51	81.53d	2.70	948.01de	1.14	4.07cd	3.09	44.43c	2.76	100
6	Cupcake with 15% PPP	182.43a	2.10	248.12c	1,27	65.69e	1.24	940.92e	2.45	3.28d	2.76	36.00de	1.04	100
7	Cupcake with 20% PPP	180.07a	3.73	231.50d	2,09	51.43g	1.19	935.11f	2.05	2.75e	1.63	38.56d	3.65	100

Table 3: Serum lipid profile of the rat groups

No	Rat groups	Cholesterol* (mg/dl)	SD	HDL* (mg/dl)	SD	TG*	SD	VLDL** (mg/dl)	SD	LDL*** (mg/dl)	SD	LDL/HDL (mg/dl)	SD
1	**Control (-)**	103.18g	1.15	51.29c	1.86	161.17a	0.74	32.23a	1.04	19.66g	0.72	0.38e	1.56
2	**Control (+)**	135.87a	1.49	43.73d	1.50	132.24c	1.29	26.44bc	1.53	65.70b	1.15	1.50b	1.23
3	**Control cupcake**	127.43ab	0.62	30.18e	1.06	136.47bc	0.56	27.29b	0.82	89.96a	1.89	2.98a	0.40
4	**Cupcake with 5% PPP**	124.92c	1.64	42.15d	1.39	138.30b	0.42	27.66b	1.13	55.11c	1.76	1.30bc	1.85
5	**Cupcake with 10% PPP**	119.59d	1.81	45.07cd	0.74	130.25cd	1.66	26.05bc	1.90	48.47d	0.37	1.07c	1.83
6	**Cupcake with 15% PPP**	117.03e	1.09	58.13b	0.82	118.64d	0.27	23.72c	1.82	35.18e	0.80	0.60d	0.54
7	**Cupcake with 20% PPP**	108.11f	0.15	62.56a	1.93	111.59e	1.85	22.31d	0.71	23.24f	1.60	0.37e	0.21

* Each value is an average of four determinations.

** VLDl cholesterol (mg/ld) = (Triglyceride / 5).

*** LDL cholesterol (mg/ld) = Total cholesterol – (VLDL+HDL cholesterol).

Table 4: Liver functions of the rats fed PPP-fortified cupcakes

No	Rat groups	AST (U/L)	SD	ALT (U/L)	SD
1	**Control (-)**	31.65 f	1.21	22.48 f	1.91
2	**Control (+)**	92.30 a	1.06	65.83 a	0.65
3	**Control cupcake**	81.54 b	0.75	59.07 b	0.83
4	**Cupcake with 5% PPP**	77.14 c	1.80	55.41 c	1.14
5	**Cupcake with 10% PPP**	75.09 cd	0.46	53.72 cd	1.79
6	**Cupcake with 15% PPP**	63.78 d	1.58	48.03 d	0.43
7	**Cupcake with 20% PPP**	49.32 e	1.29	33.11 e	1.15

* Each value is an average of four determinations.

** VLDL cholesterol (mg/ld) = (Triglyceride / 5).

*** LDL cholesterol (mg/ld) = Total cholesterol– (VLDL+HDL cholesterol).

DISCUSSION

Several studies are currently exploring more about pomegranate as a nutritional and medicinal product due to multiple benefits, including benefits to human health. To the authors' knowledge, the current study is a novel incorporation of pomegranate peel in cupcake ingredients and describes its role in weight loss, the lipid profile and liver enzymes of rats. The present study revealed that rats fed PPP-fortified cupcakes had significantly lower weight gain, VLDL –c, LDL-c, total cholesterol, and triacylglyceride, while HDL-cholesterol was significantly higher in rats fed PPP-fortified cupcakes.

These results are in agreement with other results, which found a significant decrease in triglycerides and total cholesterol among rats that were fed pomegranate peel extract for eight weeks [27]. Furthermore, another study revealed that the extract of pomegranate peel led to a significant decline in body weight, total cholesterol, triglycerides, and LDL-c, whereas it elevated the HDL-c levels of rats fed a high-fat diet [12]. Similar results were reported elsewhere [11].

In addition, the results of the current research demonstrated that the intake amount of PPP-fortified cupcake was significantly lower than that in other groups. Similar results were reported by Sayed, who demonstrated that the amount of food consumed by rats fed pomegranate peel was significantly lower than that of other groups [28]. This could be attributed to the higher level of fibre content in pomegranate peel [28]. Pomegranate peel can prevent fat accumulation and body weight gain by controlling hormones that are related to obesity (leptin-a diponectin pathway). The presence of tannin, especially at a high concentration, may reduce the consumption and performance of rats through their negative effect on palatability [29]. At the same time, dietary TG absorption is inhibited and thus plays an important role in regard to weight loss because a high intake of dietary TG is correlated with the development of obesity [29]. Hypercholesterolemic rats that were given various amounts of pomegranate peel powder had a significant decrease in food consumption and body weight gain ratio [30]. These results together suggest that pomegranate peel-fortified cupcakes may reduce appetite and prevent weight gain and subsequently reduce the obesity level.

In addition, the present study revealed that ALT and AST enzyme levels showed a significant decrease among pomegranate peel powder-fortified cupcake groups. These results are in agreement with previous studies that found a significant reduction in ALT and AST enzymes among rats that were fed pomegranate peel extract for eight weeks [13]. Another study demonstrated that pomegranate peel extract decreased AST and ALT levels in rats fed a high-lipid diet [12]. Cupcakes fortified with pomegranate peel powder and its compounds, such as flavonoids and phenolics, could have a positive impact, such as elevating faecal fat excretion and inhibiting lipase activity. This suggests that VLDL-c is transformed into LDL-c and the serum TG concentrations decrease due to this mechanism, which is one of multiple mechanisms [31]. Increasing the LDL-c level in plasma after HFD intake could be explained through the involvement of cholesterol ester synthetase (CES) and cholesterol ester hydrolase (CEH). These enzymes are responsible for the balancing of cholesterol levels in the blood [31]. Phenols and flavonoids are considered essential plant constituents due to their antioxidant activity [32]. Pomegranate peels contain high levels of polyphenols and demonstrate high ability in inhibiting LDL-c oxidation in vitro and in vivo as well as scavenging free radicals [33]. In the current study, pomegranate peel powder-fortified cupcakes had strong antioxidant activity (rich in total flavonoids and phenolic compounds),

and the secretion of AST and ALT was significantly lower in rats fed 20% PPP-fortified cupcakes followed by those fed 15% PPP-fortified cupcakes.

CONCLUSION

In conclusion, the consumption of pomegranate peel powder-fortified cupcakes, which are considered a high fibre source, promotes anti-oxidative activities. It is recommended for weight loss and to improve the lipids profile. Thus, PPP can be considered a protective natural agent against obesity and high lipid levels and could be incorporated into multiple foods to obtain its health benefits.

Conflict of Interest

None

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