ORIGINAL ARTICLE

Anti-Obesity Effect of Methanolic Extracts of Local *Punica granatum* in High-Fat Diet-Induced Obese Rats

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ABSTRACT

Introduction: Obesity has been linked to coronary artery disease, type II diabetes, metabolic syndrome, stroke, and cancer. Pomegranate (Punica granatum; PG) has been used extensively in folk medicine for several therapeutic purposes. The goal of this study is to investigate the anti-obesity effect of PG peel and pulp methanolic extracts in high-fat diet (HFD)-induced Sprague-Dawley rats. **Methods:** The animals were separated into 7 groups namely Normal control group (normal diet); HFD-induced only as negative control group, and positive control group (HFD-induced + orlistat); treatment group included HFD-induced + peel 125 mg/kg, HFD-induced +peel 250 mg/kg, HFD-induced + pulp 125 mg/kg and HFD-induced + pulp 250 mg/kg. **Results:** It was observed that methanolic extract of peel and pulp PG 250 mg/kg showed low increment of body weight with a reduction in weight of liver, visceral fat, and subcutaneous fat. Compared to the negative control group, total cholesterol, triglyceride, and low-density lipoprotein levels were shown to be lowered for PG peel and pulp groups also showed higher values in high-density lipoprotein. **Conclusion:** PG reduced obesity-related markers in blood, liver, and adipose tissue and inhibited obesity caused by a high-fat diet probably because of its antioxidant properties.
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Keywords: Anti-obesity, *Punica granatum*, Lipid profile, Glucose level

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INTRODUCTION

Obesity is one of the 21st century's most serious public health issues. In several nations, the frequency has tripled, and the number of people inflicted is increasing at an alarming rate. Excess weight raises a person's chance of getting a variety of noncommunicable illnesses, such as heart disease, cancer, and diabetes (1). One of the factors that can lead to obesity is the oxidative stress in the body due to an imbalance in reactive oxygen species as tissue oxidises free radicals. Due to lower levels of antioxidant enzymes, such as catalase, glutathione peroxidase, and glutathione reductase, there is a reduction in antioxidant capacity in obese people (2). Since it is a major health problem, prevention and treatment of obesity are important and should be emphasised to reduce obesity and overweight prevalence (3).

A gastric and pancreatic lipase inhibitor, orlistat, through reducing dietary fat absorption by approximately 30%, has been used for about 10 years (4). However, it is less effective as its use is often associated with side effects on the gastrointestinal, cardiovascular, and central nervous system such as elevated blood pressure, dry mouth, headache, insomnia and constipation. Due to the various side effects of existing drugs, there is a need for new natural products in treating obesity to produce better results in terms of weight loss (5).

Pomegranate (*Punica granatum* (PG)) is a fruit that has been cultivated since ancient Egypt and in early

Greece and Italy. Affectionately known as the "jewel of winter", pomegranate belongs to the Punicaceae family (6). Human beings have cultivated pomegranate for its medicinal and nutritional properties for over 4000 years. It has a significant importance in the ancient cultures of Mediterranean countries (7).

Traditional medicines have attracted many researchers with the aim of treating obesity due to the failure of many conventional medicines. Different parts of herbal plants have been reported to have anti-obesity effects due to the content of minerals or chemicals from plant extracts. Reduced lipid absorption and calorie intake, increased energy expenditure, decreased adipocyte proliferation and lipogenesis, and enhanced lipolysis are all anti-obesity actions associated with herbal plants (8). The focus of this research is to observe how PG peel and pulp extracts influenced on food intake, weight gain, organ weight, lipid profile (triglyceride, total cholesterol, high-density lipoprotein and low-density lipoprotein), adipose tissue weight, and blood glucose levels in animal model.

MATERIALS AND METHODS

Sources and Preparation of Peel and Pulp Extracts of PG

Local PG was collected from Nilai, Negeri Sembilan. Authentication was carried out by a plant taxonomist from Institute of Bioscience, UPM. The voucher (SK 2396/14) has been deposited in the herbarium for future reference. 24 local PG fruits, weighing between 200 - 300 g, were separated into peel and pulp in different containers. PG was peeled manually and immersed liquid nitrogen before being grounded to fine powder. 1000 g of each peel and pulp were extracted with 1000 ml of methanol at the ratio of 1:1. The mixture was placed in an incubator shaker with continuous stirring and swirling for 1 hour at 30°C. The mixture was filtered using cotton wool and was concentrated in a rotary evaporator for 6 hours. Finally, the pure methanol extracts were freeze-dried for three days (9) and kept at -20 °C until further use.

Chemicals and Reagents

Methanol, liquid nitrogen, 0.1% of Tween 20, 1% of dimethylsulphoxide (DMSO) and 0.1N sodium hydroxide were used in this study. Methanol and liquid nitrogen were used for extraction purposes whereas 0.1% of Tween 20 and 1% of DMSO were used as a vehicle to dilute Orlistat (ChemoLab, USA). All chemicals were purchased from Sigma Aldrich, USA. Normal rat chow pellet, egg yolk, full cream milk powder (Fernleaf, Fonterra), corn oil (Mazola), monosodium glutamate (Aji-No-Moto) and sugar were used for the induction of obesity.

Animal

35 adult male Sprague Dawley rats (150 - 200g) were kept in the animal laboratory of University of Cyberjaya. The rats were acclimatised at room temperature to the animal room conditions for one week and were maintained on standard pellet and water ad libitum. All studies were conducted in accordance with the Animal Care Committee, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang (UPM/FPSK/PADS/BR-UUH/00254).

Anti-obesity Study

All rats were divided into 7 groups (n=5) (Table I). 6 groups were given a high-fat diet (HFD) and the normal control group was given normal rat chow pellet and water ad libitum for 35 days. PG peel and pulp extracts were given at 2 different doses each: 125mg/kg and 250 mg/kg. PG peel and pulp extracts were dissolved in 5ml/kg normal saline. PG and orlistat 10mg/kg treatment were given once daily to the rats via oral gavage for 28 days from day 8 to day 35. Peel and pulps extracts, orlistat and vehicle were administered at a fixed time (10.00 am - 11.00 am) of the day to avoid circadian variation, if any. After 35 days, all rats were sacrificed.

Table I: Depiction of each group and its respective treatment

Group	Treatment
NC	Normal pellet and water *ad libitum*
NeC	High-fat diet (HFD) + normal saline (5ml/kg)
PC	HFD + Orlistat (10mg/kg)
HFD + peel 125	HFD + peel (125mg/kg)
HFD + peel 250	HFD + peel (250mg/kg)
HFD + pulp 125	HFD + pulp (125mg/kg)
HFD + pulp 250	HFD+ pulp (250mg/kg)

Obesity Induction in Rat

Induction of obesity was achieved by feeding a high-fat diet (HFD) preparation. A normal rat chow pellet was purchased from Seri Kembangan, Malaysia. HFD was prepared following the previous method (9) with modification by mixing a mixture of 50% of normal rat chow pellet, 15% egg yolk, 15% full cream milk powder, 10% corn oil, 5% monosodium glutamate and 5% sugar. All the ingredients were carefully combined before being baked at 60 °C in the oven. The pellet was then cut into smaller pieces and each rat was provided with a 25 g pellet daily. The remaining food was then weighed on the following day to measure the amount consumed. The body weight of all were measured weekly throughout the experiment.

Blood and Organ Collection

All rats were sacrificed using a mixture of ketamine and xylazine followed by cervical dislocation. The organs were recovered after the blood was taken by heart puncture. Blood samples were kept at 4°C overnight and were centrifuged (5000 rpm; 10 min) for serum collection. Total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein

(HDL), and glucose levels were determined in the serum at UPM's Hematology Laboratory. The liver and adipose tissues which were visceral fats (retroperitoneal and epididymal) and subcutaneous fat were collected and weighed (10).

Statistical Analysis

Statistical Package for Social Science (SPSS) version 26 was used to analyse the data. All results were reported as mean ± standard error of the mean (SEM). For statistical significance, the results were analysed using One-Way ANOVA and a post-hoc Tukey test. The level of significance was set at 0.05 or less for all probability values.

RESULTS

Effects of PG on Food Intake and Body Weight

Table II indicates variations in food consumption and body weight. When compared to the NC group, there is no significant difference in food consumption with the NeC group. Food consumption is considerably lower ($p<0.05$) in the HFD + Pulp 250 group compared to the NeC group, followed by HFD + Peel 250 and the lowest for PC group. Meanwhile, HFD + Pulp 125 and HFD + Peel 125 groups show no significant difference in food intake when compared to NeC group. For bodyweight, the highest increment was NeC group (78.35%; $p < 0.05$), followed by PC group (52.40%), HFD + Peel 125 (46.15%; $p < 0.05$), HFD + Peel 250 (42.72%), HFD + Pulp 125 (40.80%) and HFD + Pulp 250 (35.44%), consecutively. NC group shows the lowest percentage of increment in body weight at 27.06% ($p < 0.05$).

Effects of PG on Liver and Adipose Tissue Weight

Weight of Liver

Figure 1 shows the mean liver weight for all rats. When compared to the NC group, liver weight of the NeC group was considerably higher ($p<0.05$). When compared to the NeC group, the HFD + Pulp 250 group has considerably reduced ($p<0.05$) liver weight, followed by HFD + Peel 250, HFD + Pulp 125, PC and HFD + Peel 125 groups, consecutively.

Weight of Visceral Fats

Figure 2 shows the weight of visceral fats for all groups. When comparing the NeC and HFD + Peel 125 groups to the NC group, the weight of visceral fats was considerably higher ($p<0.05$). For meanwhile, HFD + Pulp 250 and HFD + Peel 250 groups show significantly lower ($p<0.05$) visceral fats weight compared to the NeC group and HFD + Peel 125 group. PC and HFD + Pulp 125 groups show considerably lower ($p<0.05$) visceral fats weight compared to NeC group. Meanwhile, HFD + Peel 125 mg/kg group show no substantial difference ($p>0.05$) in visceral fats weight when compared to the NeC group.

Table II: Mean ± SEM values of food intake (g/rat/day) from week 1 to week 5 for all the groups of rats and body weight changes of all rats

Group	Food intake (g/rat/day)	Body weight		
		Initial body weight (g)	Final body weight (g)	Percentage (%) of weight gain
NC	22.93±0.49	190.48 ± 3.67	242.38 ± 11.08 [b]	27.06
NeC	24.22±0.20	182.06 ± 10.04	319.52 ± 10.47 [a]	78.35
PC	19.24±1.06 [b]	165.67 ± 7.29	251.05 ± 6.85 [b]	52.40
HFD + Peel 125	20.72±0.99	187.23 ± 5.92	275.58 ± 6.93 [b]	46.15
HFD + Peel 250	19.20±1.60 [b]	179.46 ± 4.28	254.99 ± 10.3 [b]	42.72
HFD + Pulp 125	19.86±1.08	190.75 ± 4.02	270.62 ± 13.41 [b]	40.80
HFD + Pulp 250	17.67±1.00 [a b]	181.13 ± 5.20	245.02 ± 6.01 [b]	35.44

[a] significant when compared to the NC group ($p<0.05$). [b] significant when compared to the NeC group ($p<0.05$).

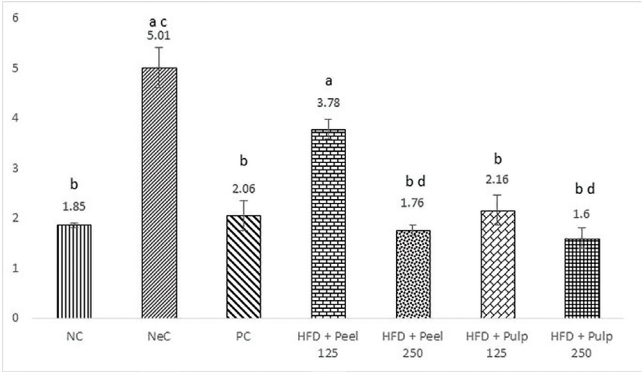

Figure 1: Mean ± SEM values of liver weight (g) of all rats. [a] significant when compared to the NC group ($p<0.05$). [b] significant when compared to the NeC group ($p<0.05$). [c] significant when compared to the PC group ($p<0.05$). [d] significant when compared to the HFD + peel 125 group ($p<0.05$).

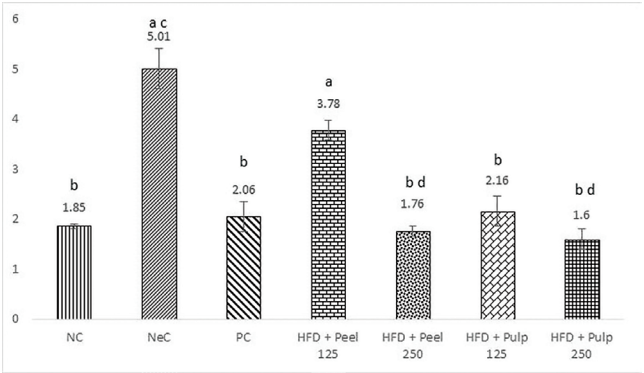

Figure 2: Mean ± SEM values of visceral fats (g) of all rats. [a] significant when compared to the NC group ($p<0.05$). [b] significant when compared to the NeC group ($p<0.05$). [c] significant when compared to the PC group ($p<0.05$). [d] significant when compared to the HFD + peel 125 group ($p<0.05$).

Weight of Subcutaneous Fat

Figure 3 shows the weight of subcutaneous fat for all group. The weight of subcutaneous fat was significantly higher ($p<0.05$) in NeC group compared to NC group. Meanwhile, HFD + Pulp 250 mg/kg group shows significantly lower ($p<0.05$) subcutaneous fat weight compared to NeC group and followed by HFD + Peel 250 mg/kg, HFD + Pulp 125mg/kg, PC, and HFD + Peel 125 mg/kg groups consecutively.

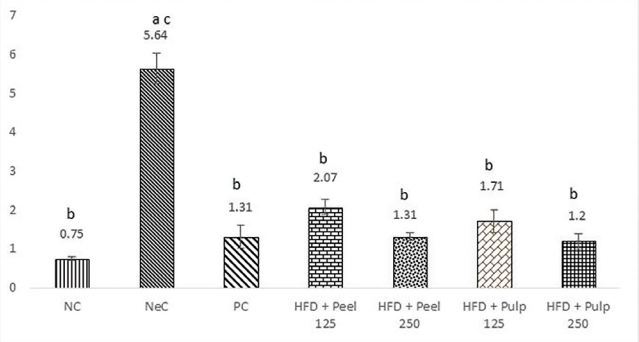

Figure 3: Mean ± SEM values of subcutaneous fats (g) of all rats. [a]significant when compared to the NC group ($p<0.05$). [b]significant when compared to the NeC group ($p<0.05$). [c] significant when compared to the PC group ($p<0.05$).

Effects of PG on Lipid Profile

Total cholesterol

Table III shows total cholesterol value for all groups. Total cholesterol level was significantly higher ($p<0.05$) in NeC group compared to NC group. HFD + Pulp 250 mg/kg group show significantly lower ($p<0.05$) level of total cholesterol compared to NeC group followed by HFD + Peel 250 mg/kg, HFD + Pulp 125mg/kg, PC, and HFD + Peel 125 mg/kg groups consecutively.

Triglyceride Level

Triglyceride level was significantly higher ($p<0.05$) in NeC and HFD + Peel 125 mg/kg groups compared to NC group. HFD + Pulp 250 mg/kg group showed a significantly lower ($p<0.05$) level of triglyceride level compared to NeC group followed by HFD + Peel 250 mg/kg, HFD + Pulp 125mg/kg groups consecutively. Meanwhile, HFD + Peel 125 mg/kg and PC groups show no significant difference ($p>0.05$) in triglyceride level when compared to NeC group.

Low-Density Lipoprotein Level (LDL)

Low-density lipoprotein level was significantly higher ($p<0.05$) in NeC group compared to NC group (Table III) . HFD + Pulp 250 mg/kg group shows significantly lower ($p<0.05$) level of low-density lipoprotein compared to NeC group and followed by HFD + Peel 250 mg/kg, PC, HFD + Pulp 125mg/kg, and HFD + Peel 125 mg/kg groups, consecutively.

High-Density Lipoprotein Level (HDL)

Table III shows high-density lipoprotein value for all the groups of rats. The amount of high-density lipoprotein was substantially higher ($p<0.05$) in the HFD + Peel 125 mg/kg, HFD + Peel 250 mg/kg, HFD + Pulp 125 mg/kg and HFD + Pulp 250 mg/kg groups when compared to NC group and PC groups. HFD + Pulp 250 mg/kg, HFD + Peel 250 mg/kg, HFD + Pulp 125 mg/kg and HFD + Peel 125 mg/kg groups show significant difference ($p>0.05$) in high density lipoprotein level when compared to NeC group.

Table III: Total cholesterol level (mmol/L), triglyceride level (mmol/L), low density lipoprotein level (mmol/L) and high density lipoprotein level (mmol/L) of all the groups of rats at the end of day-35 (n = 5)

Group	Total cholesterol	Triglyceride level	Low Density Lipoprotein	High Density Lipoprotein
NC	1.59 ± 0.05 [b]	0.47 ± 0.01 [b]	0.32 ± 0.01 [b]	1.14 ± 0.02
NeC	2.86 ± 0.25 [a]	1.53 ± 0.05 [a]	0.58 ± 0.05 [a]	1.01 ± 0.01
PC	1.53 ± 0.07 [b]	1.07 ± 0.07	0.30 ± 0.03 [b]	1.17 ± 0.02
HFD + Peel 125	1.91 ± 0.10 [b]	1.25 ± 0.06 [a]	0.31 ± 0.02 [b]	2.02 ± 0.03 [a,b]
HFD + Peel 250	1.39 ± 0.07 [b]	0.82 ± 0.01 [b]	0.26 ± 0.01 [b]	2.06 ± 0.01 [a,b]
HFD + Pulp 125	1.45 ± 0.85 [b]	0.86 ± 0.02 [b]	0.31 ± 0.02 [b]	2.04 ± 0.03 [a,b]
HFD + Pulp 250	1.24 ± 0.10 [b]	0.83 ± 0.03 [b]	0.23 ± 0.03 [b]	2.08 ± 0.04 [a,b]

Each bar represents the mean ± SEM of total cholesterol. [a] significant when compared to the NC group ($p<0.05$). [b] significant when compared to the NeC group ($p<0.05$).

DISCUSSION

To obtain bioactive compounds from the plant, there are several steps including grinding, milling, homogenization, and extraction. Among these steps, extraction is the important step to recover and isolate bioactive compounds from the materials. Solvent is recognized as one of the most important parameters. The present study used methanol to extract bioactive compounds from PG as previous study by Mahesar et al (5) showed methanol showed best extraction yield compared to other solvent. Other studies (8) and (25) also reported methanolic extraction possess best antioxidant and other pharmacological effect.

Obesity is defined by an increase in adipose tissue mass because of the increase in adipocyte number and size. It is a metabolic disorder that develops from genetic and environmental factors. Even though genetic is the major leading cause of obesity, many researchers suggest that the common environmental factors may be exacerbating the problem (11). The greatest element that contributes

to the development of obesity is a high-fat diet and the long-term intake of HFD causes a significant increase in body weight. HFD induction is an established method for animal model obesity which is associated with the imbalance between energy intake and expenditure. In this study, rats fed with HFD had significantly increased body weight, liver weight, adipose tissue, glucose level and lipid profile after 35 days of induction. The HFD food intake is related to body weight gain, according to the findings.

In the study, the mean body weights of normal control, negative control and other treated groups were similar at the start of the experiment. Bodyweight of negative control group was considerably greater than the normal control group after 35 days on HFD. It was observed that the normal pellet diet, used as a low-fat control diet that contained plant-derived ingredients, did not cause a significant increase in body weight while HFD, which contained unsaturated fat from 10% corn oil, has been associated with the significant increase in body weight. This finding is in line with previous study (12) that showed a significant increment of body weight after HFD. Moreover, body weight of the groups administered with PG peel and pulp extracts had also shown increment but were significantly lower in comparison to the negative control group. When compared to the negative control group, pulp 250mg/kg and positive control groups had significantly reduced body weight. This is followed by groups of peel 250 mg/kg, pulp 125 mg/kg and peel 125 mg/kg. The preparation of HFD also contained 5% monosodium glutamate, a flavour-enhancing food additive. The oral ingestion of monosodium glutamate increases body weight and significantly increases serum glucose and lipid profile level, in agreement with a previous study (13). Based on previous literature, it is expected that PG with peel extract of 250 mg/kg would be the most effective in terms of anti-obesity effect since it has a higher antioxidant capacity compared to pulp extract in scavenging free radicals. Furthermore, peel extract contains higher polyphenol contents compared to pulp extract. A large amount of phenolic content in peel extract could be the contributor to its strong antioxidant ability (14).

Adipose tissue is an organ that function to balance energy intake and expenditure as well as changes in mass based on human metabolic requirements (15). The development of obesity is due to excess energy intake and a decrease in energy expenditure resulting in abnormal excessive growth of white adipose tissue (16). After 28 days of oral administration of PG peel and pulp extracts, lower weights of epididymal and retroperitoneal tissue (visceral fats) in addition to subcutaneous adipose tissue (subcutaneous fats) were observed in HFD-induced rats. The weight of visceral fats and subcutaneous fat in pulp 250 mg/kg, peel 250 mg/kg, Orlistat group, pulp 125 mg/kg and peel 125 mg/kg groups were significantly lower compared to the negative control group. Meanwhile, the weight of visceral and subcutaneous fats in negative control group was slightly higher than in the normal control group. Lower weight of adipose tissue of the HFD-induced rats treated with PG extracts might be due to the anti-obesity effects of the PG extracts. Currently, there are no other similar studies done on the anti-obesity effect of local PG of peel and pulp extracts, but previous research (17) had shown that oral administration of PG leaf extracts containing active compounds significantly reduce abdominal adipose tissue weight. The excess of visceral fats in the abdominal cavity will lead to cardiovascular diseases, insulin resistance and other metabolic syndromes (18). Thus, one previous study (20) suggested that oleanolic acid isolated from flower extracts of PG contributes to the significant reduction of visceral abdominal fat via inhibition of pancreatic lipase activity and appetite suppression.

Obesity is linked to the development of fatty liver disease. In this study, the weight of the liver in the negative control group was considerably greater than that of the normal control group after 35 days of HFD. This significant difference is suggested to be associated with hepatic steatosis, indicating accumulation of adipocytes in the liver. Accumulation of fatty droplets is a sign of fatty liver in HFD-induced obese subjects (21). In comparison to the normal control group, liver weight of negative control group was considerably higher. Meanwhile, the liver weight was significantly lower in pulp 250 mg/kg, peel 250 mg/kg, pulp 125 mg/kg, Orlistat 10 mg/kg and peel 125 mg/kg treated groups compared to negative control group. This result suggests that the administration of PG extracts can suppress the development of the HFD-induced fatty liver.

Many findings have reported that a high-fat diet will induce hypercholesterolemia in animal subjects (22). Moreover, excessive dietary intake of fats causes serum cholesterol levels to be elevated by downregulating LDL receptor synthesis which reduces the uptake of LDL in the bloodstream via LDL receptor, hence resulting in an increase of blood cholesterol level. The consequence of diabetes might also result in elevated blood lipid levels. A substantial rise in total cholesterol, triglycerides, and LDL blood levels were seen in HFD-induced rats, resulting in a shift in HDL blood level. The results from the present study showed that rats fed with a HFD for 35 days have significantly high total cholesterol, triglycerides and LDL levels with significantly low HDL level when compared to normal control group. The efficacy of local PG hypolipidemic activity is generally evaluated based on the decrease of total cholesterol, triglycerides and LDL levels with the increase of HDL level. A decrease in blood cholesterol and triglycerides have been associated with the flavonoid content of PG extracts (23). The protective effect of flavonoids has also been reported to be of benefit against the incidence of cardiovascular event due to their free radical scavenging property.

In comparison to normal control and other treated groups, negative control group had a high level of blood cholesterol. This is due to the direct effect of HFD containing 10% of corn oil. The high cholesterol absorbed was transported to the liver via chylomicron remnants. An increase in blood cholesterol level suppresses the synthesis of LDL receptors, hence, raising the LDL concentration by decreasing the uptake of VLDL remnants. The conversion of VLDL to LDL causes a delay in the clearance of circulating LDL. Meanwhile, total cholesterol level was significantly lower in pulp 250 mg/kg, peel 250 mg/kg, pulp 125 mg/kg, positive control and peel 125 mg/kg treated groups than the negative control group. This is due to the presence of flavonoid contents in the PG extracts (24). The results obtained indicate that PG acts as a hypolipidemic agent.

When compared to the normal control and other treatment groups, LDL level in the negative control group was shown to be considerably higher. Moreover, all treated groups administered with PG of peel and pulp extracts showed a significantly low level of serum LDL when compared with negative control group. When comparing the groups treated with pulp 250 mg/kg to negative control group, the group treated with pulp 250 mg/kg showed the greatest reduction in LDL levels. The low LDL level might be due to the suppression of LDL receptor activity leading to inhibition of LDL oxidation and reduction of oxidative stress by PG constituents. PG has been reported to contain numerous antioxidants in their peel, pulp and juice fractions (25) and is known to be a source of polyphenols and other antioxidants (26). The contents of total phenolics, proanthocyanidins and flavonoids are higher in peel extracts compared to pulp extracts and phenolics content in peel extracts has been reported to have strong antioxidant ability. Both PG pulp and peel contain many different kinds of antioxidants, possibly some that are not so well-characterized. It has been reported (23) that pulp extract of PG contain some chemical constituents which are not found in peel extracts. Polyphenols and flavonoids have been shown to reduce LDL oxidation by shielding LDL from cell-mediated oxidation (26) in a prior investigation. Thus, the risk of atherosclerosis can also be reduced by lowering the level of LDL.

HDL is the main cholesterol carrier from body cells to the liver and arterial walls. HDL particles pick up and process excess cell cholesterol before delivering it to the liver for metabolism (27). Blood HDL level is dependent on dietary fat and cholesterol. In this study, the level of HDL is increased by about 50% in the groups treated with PG of peel and pulp extracts (28). The group treated with pulp extract at 250 mg/kg showed the highest increment in HDL level of about 50.9% compared to the negative control group followed by group treated with peel extract at 250 mg/kg (50.5%), pulp extract at 125 mg/kg (50.2%) and peel extract at 125 mg/kg (50.1%). Therefore, another beneficial effect of PG is lowering the risk of atherosclerosis which is also one of the complications of obesity (29).

CONCLUSION

PG is one of the functional foods that is beneficial to the human body. It has been safely consumed for centuries without any adverse effects. Several studies have demonstrated its potential effects. The present study shows the beneficial health effect of pulp and peel of local PG as an anti-obesity agent as compared to the conventional drug, orlistat. This may be due to the presence of abundant antioxidants such as phenolics and flavonoids. Rats supplemented with a HFD were successfully induced with obesity causing increase in body weight, visceral and subcutaneous adipose tissues, TC, TG, LDL, glucose level and a decrease in HDL level. As compared to peel extracts, the PG pulp extracts showed better results in blood lipid profile values. PG pulp extracts of 250 mg/kg demonstrated significant effect in obese rats when compared to Orlistat, the positive control group. Furthermore, the extract reduces body weight, visceral and subcutaneous adipose tissues, and improves lipid profile. Pulp and peel extracts of PG do reduce appetite in the treated groups. Thus, this concludes that local PG possess anti-obesity properties. However, further studies are recommended to identify the exact mechanism of the pulp and peel extracts in reducing obesity as well as identifying their active constituents. A profiling of metabolites using HPLC or NMR is also needed to identify the metabolites of interest that gives anti-obesity effects.

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REFERENCES

1. Camacho S, Michlig S, de Senarclens-Benzencon C, Meylan J, Meystre J, Pezzoli M, Markram H, le Coutre J.. Anti-obesity and anti-hyperglycemic effects of Cinnamaldehyde via altered Ghrelin secretion & functional impact of food intake & gastric emptying. Scientific Reports. 2015;5:7919. doi: 10.1038/srep07919.
2. Rusdiana Rusdiana, Siti Syarifah, Yunita Sari Pane, Sry Suryani Widjaja, Dwi Rita Anggraini, The Effects of High Fat Diet on the Liver of the White Rat Model Obesity. Scientific Foundation SPIROSKI, Macedonian Journal of Medical Sciences. 2022; 10(A):709-714. doi:10.3889/oamjms.2022.9383
3. Yusra Azhar, Ashish Parmar, Colette N. Miller, Janaiya S. Samuel and Srujana Rayalam: Phytochemicals as novel agents for the induction of browning in white adipose tissue. Nutrition and Metabolism. 2016; 19: 717-726. doi: 10.1186/

s12986-016-0150-6
4. Taltia A, Roy A. Orlistat - an anti-obesity drug - An overview. J Adv Pharm Edu Res 2017;7(3):190-193.
5. Mahesar, S.A., Kori, A.H., Sherazi, S.T.H., Kandhro, A.A., Laghari, Z.H. (2019). Pomegranate (*Punica granatum*) Seed Oil. Fruit Oils: Chemistry and Functionality. Springer, Cham. doi: 10.1007/978-3-030-12473-1_37
6. Industrial Pomegranate Wastes and their Functional Benefits in Novel Food Formulations. In: Ramadan, M.F., Farag, M.A. (eds) Mediterranean Fruits Bio-wastes. Springer, Cham. doi:10.1007/978-3-030-84436-3_31
7. Müller, T.D., Blüher, M., Tschup, M.H. et al. Anti-obesity drug discovery: advances and challenges. Nat Rev Drug Discov 21, 201–223 (2022). doi:10.1038/s41573-021-00337-8
8. Rahman SA, Suhaimi SM, Hakeem WA, Adam Z. Antioxidant, antidiabetic, and antiglycation properties of methanolic extracts of local and imported *Punica granatum*. Journal Natural Product and Biomedical Research. 2015;1(1).
9. Azman KF, Amom Z, Azlan A, Esa NM, Ali RM, Shah ZM, Kadir KKA.. Antiobesity effect of Tamarindus indica L. pulp aqueous extract in high-fat diet-induced obese rats. Journal of Natural Medicine. 2011;66(2):333-42. doi: 10.1007/s11418-011-0597-8
10. Guilherme Fleury Fina Speretta,I Marisa Cristina Rosante,I Fernanda Oliveira Duarte,II Richard Diego Leite,IIIAnderson Diogo de Souza Lino,I Rafael Arquias Andre,I Joa˜ o Guilherme de Oliveira Silvestre,I HeloisaSobreiro Selistre de Araujo,II Ana Claudia Garcia de Oliveira DuarteI The effects of exercise modalities on adiposity in obese rats. The effects of exercise modalities on adiposity in obese rats. CLINICS2012;67(12):1469-1447 doi:10.6061/clinics/2012(12)19
11. Xinghua L and Hong L. Obesity: Epidemiology, Pathophysiology, and Therapeutics. Frontiers in Endocrinology. 2021; 12:706978 doi:10.3389/fendo.2021.706978
12. Supriya K, Kotagiri S, Vrushabendra Swamy BM, Archana Swamy P, Vishwanath KM. Anti-obesity activity of Shorea robusta G. leaves extract on monosodium glutamate induced obesity in albino rats. Res J Pharm Biol Chem Sci. 2012;3(3):555-565.
13. Chi, Q., Wang, G., Sheng, Y., Xu, W., Shi, P., Zhao, C., & Huang, K. (2017). Ethanolic extract of the golden oyster mushroom, Pleurotus citrinopileatus (Agaricomycetes), alleviates metabolic syndrome in diet-induced obese mice. International Journal of Medicinal Mushrooms, 19(11), 1001–1008. doi:10.1615/IntJMedMushrooms.20.
14. Wang LY, Chen C. Energy metabolism homeostasis in cardiovascular diseases. J Geriatr Cardiol. 2021 Dec 28;18(12):1044-1057. doi: 10.11909/j.issn.1671-5411.2021.12.006.
15. Jo J, Gavrilova O, Pack S, Jou W, Mullen S, Sumner AE, Cushman, SW, Periwal V. Hypertrophy and/or hyperplasia: dynamics of adipose tissue growth. PLoS Comput. Biol. 2009;5(3): e1000324. doi: 10.1371/journal.pcbi.1000324
16. Sati SC, Sati N, Sati OP. Bioactive constituents and medicinal importance of genus Alnus. Pharmacogn Rev. 2011 Jul;5(10):174-83. doi: 10.4103/0973-7847.91115.
17. Sukandar, E.Y., Yuniarto, A., & Finna, S. Anti-obesity effect of the pomegranate leaves ethanol extract (*Punica granatum* L) in high-fat diet induced mice, Chemistry. 2014.
18. de Melo CL, Queiroz MG, Fonseca SG, Bizerra AM, Lemos TL, Melo TS, Santos FA, Rao VS.. Oleanolic acid, a natural triterpenoid improves blood glucose tolerance in normal mice and ameliorates visceral obesity in mice fed a high-fat diet: Chem. Biol. Interact. 2010;185(1):59–65. doi:10.1016/j.cbi.2010.02.028
19. Xu A,Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. The fat derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice: J. Clin. Invest. 2003;112(1):91–100. doi:10.1172%2FJCI17797
20. Cherng JY, Shih MF. Preventing dyslipidemia by Chlorella pyrenoidosa in rats and hamsters after chronic high fat diet treatment. Life Sci. 2005;76(26):3001-13. doi:10.1016/j.lfs.2004.10.055
21. Tan BK, Tan CH, Pushparaj PN. Anti-diabetic activity of the semi-purified fractions of Averrhoa bilimbi in high fat diet fed-streptozotocin-induced diabetic rats. Life Sci. 2005;76(24):2827-39. doi:10.1016/j.lfs.2004.10.051
22. Al-Muammar MN, Khan F. Obesity: The preventive role of the pomegranate (*Punica granatum*). Nutrition. 2012;28(6):595-604. doi:10.1016/j.nut.2011.11.013
23. Schummer CM, Werner U, Tennagels N, Schmol D, Haschke G, Juretschke H, Patel MS, Gerl M, Kramer W, Herling AW. Dysregulated pyruvate dehydrogenase complex in Zucker diabetic fatty rats. Am. J. Physiol. Endocrinol. Metab. 2008;294(1):88-96. doi:10.1152/ajpendo.00178.2007
24. *Punica granatum* review Linn Muhammad Fithri Nozula 1.: A phytochemical and pharmacological, Hannis Fadzilah Mohsin 1 1 , Ibtisam Abdul Wahab, Journal of Advanced Research in Materials Science 30, Issue 1. 2017 1-9.
25. P Singh Aviram M, Volkova N, Coleman R, Dreher M, Reddy MK, Ferreira D, Rosenblat M. Pomegranate phenolics from the peels, arils, and flowers are antiatherogenic: studies in vivo in atherosclerotic apolipoprotein E-deficient mice and in vitro in cultured macrophages and lipoproteins. J. Agric. Food Chem. 2008;56(3):1148–57. doi:10.1021/jf071811q

26. Michael C. Phillips, New insights into the determination of HDL structure by apolipoproteins1: Thematic Review Series: High Density Lipoprotein Structure, Function, and Metabolism, Journal of Lipid Research, Volume 54, Issue 8, 2013, Pages 2034-2048, ISSN 0022-2275, doi:10.1194/jlr.R034025.
27. M. Hajimahmoodi, G. Moghaddam, A. Ranjbar, H. Khazani, N. Sadeghi, M. Oveisi and B. Jannat, "Total Phenolic, Flavonoids, Tannin Content and Antioxidant Power of Some Iranian Pomegranate Flower Cultivars (*Punica granatum* L.)," American Journal of Plant Sciences, Vol. 4 No. 9, 2013, pp. 1815-1820. doi: 10.4236/ajps.2013.49223.
28. Jie X, Ke C, Lin Z, Zhihui F, Zhizhong D, Jianke L, and Jiankang L. The effects and mechanisms of pomegranate in the prevention and treatment of metabolic syndrome. Traditional Medicine and Modern Medicine Vol. 3, No. 4 (2020) 223–237. doi:10.1142/S2575900020300064