Original paper

Reduction of Lipid Profile and Adipocyte Size in Rats Fed on High-fat Diet Using Camel Milk and Whey Protein Mixture

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The aim of this study was to examine the effect of oral administration of Camel milk (CM) and whey protein (WP) (1.5 % and 3.0 % in water) - either alone or in a mixture - on obesity parameters and lipid profile in rats fed on a high-fat diet. The results revealed that feeding rats on CM or WP significantly reduced body weight gain, adipocyte size and the weight of perirenal adipose tissue, especially when CM was mixed with 1.5 % WP. Feeding rats on CM, WP or a mixture of CM and WP led to a significant reduction ($p < 0.05$) in the levels of LDL and total cholesterol and an improvement ($p < 0.05$) in the fasting glucose levels. In conclusion, consumption of CM, WP or a mixture of CM and WP reduced the number of large adipocytes and increased the number of small adipocytes, resulting in reduction of the body weight of the rats.

Keywords: camel milk, whey protein, high-fat diet, obesity, adipocyte size.

Introduction

Obesity is a condition in which the excess energy intake is stored in the form of fat in adipose tissues in the human body. To store fat, adipose tissue increases either in cell size or in cell number. In the past, the adipose tissue was considered as storage tissue only; however, it was recently discovered that adipose tissue secretes several products (e.g., leptin, adiponectin, resistin, etc.) that may negatively affect other organs like the heart, pancreas and liver (Haslam and James 2005). Nowadays, obesity is considered as a medical health problem. The risk of becoming overweight or obese is affected by different factors, including genetics, inactive lifestyle, consumption of high-fat and high-carbohydrates diets, certain medications, social and economic issues and lack of sleep (Haslam and James 2005). Generally, overweight and obesity are linked to health problems such as cardiovascular disease, hypertension, diabetes and certain cancers, (e.g., colon, rectum and esophageal cancer) (NIH 1998).

Camel milk (CM) is a common and popular food in Saudi

Arabia, with an annual production of 89 000 tons (FAO 2013). It is an important nutritional source that contains bioactive components which may prevent various diseases and promote health (Abrhaley and Leta 2018). Since ancient times, the medicinal properties of CM have been observed due to its higher hepatoprotective effect, insulin-like peptides, antibacterial and antiviral efficacy and anti-cancer, hypoallergenic, antioxidant, antihypertensive and anti-diabetic features (Maqsood *et al.*, 2019; Abrhaley and Leta, 2018). In an earlier report, Govindasamy *et al.* (2014) found that administration of CM may have therapeutic potential for obese diabetic patients. The study observed that consumption of CM by diabetic rats resulted in reduction of total cholesterol, triglycerides (TG), free fatty acids, and phospholipid levels in plasma and tissues, accompanied by a significant increase in plasma high-density lipoprotein (HDL)-cholesterol. Similarly, a significant reduction in the body weight of diabetic rats fed on CM was also reported (Khan *et al.*, 2013).

Whey protein (WP) is another food component that is

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considered to have functional properties. It exhibits several beneficial properties such as antioxidant (Marshall, 2004), antihypertensive, anticarcinogenic, hypolipidemic, antiviral and antibacterial characteristics and chelating agents (Marshall, 2004; Hoffman and Falvo, 2004; Saleh *et al.*, 2007). Numerous reports have pointed to the effect of WP on food intake due to a mechanism that might be related to an increase in plasma gastrointestinal hormones and metabolites (Chungchunlam *et al.*, 2014).

Therefore, the objective of the present work was to examine the effect of CM, WP and a mixture of CM and WP on some obesity parameters in rats fed on a high-fat diet.

Materials and Methods

Instantized bovine WP concentrates (76.0 % WP, 6.0 % fat, and 7.0 % carbohydrate) were purchased commercially (Davisco, Eden Prairie, MN 55344, USA). The CM was obtained from the Agriculture Research and Experimental Station at Qassim University. The approximate composition of fresh CM (3.71 ± 1.1 % fat, 2.97 ± 0.6 % protein, 4.81 ± 0.22 % lactose) was determined using a LactoStare milk analyzer (Funke-Gerber Labortechnik GmbH, Berlin, Germany). After heat treatment, the milk was stored at -20°C until used for the entire duration of the experiment. The components of the normal diet were locally purchased. All chemicals used in the study were of analytical grade.

Experimental design

Diets The composition of the control basal diet (20 % protein, 63.3 % carbohydrates, 7 % fat, 5 % fiber, 3.5 % mineral mix, 1.25 % vitamin mix) was based on AIN-93 guidelines (Reeves *et al.*, 1993). The high-fat diet (HFD) contained 24 % total fat by weight to represent 45 % energy from fat. This HFD (20 % protein, 46.25 % carbohydrates, 24 % fat, 5 % fiber, 3.5 % mineral mix, 1.25 % vitamin mix) was prepared according to the standard formula of Research Diets Inc. (Hyeran, 2017).

Animals and Ethics Seventy six-week-old male Wistar rats weighing about 120 ± 10 g were purchased from the Animal house, College of pharmacy, King Saud University, Saudi Arabia. The animals were housed in clean regular plastic cages and allowed to acclimatize to the laboratory environment for one week (12-h light:12-h dark cycle, relative humidity of 40–45 %, room temperature of $23 \pm 2^{\circ}\text{C}$). The experiment was carried out in accordance with the Qassim University College of Agriculture and Veterinary Medicine guidelines for animal experimentations. The experimental protocol was approved by the Ethical Committee at Qassim University.

Experimental groups Rats were randomly distributed into seven groups ($n = 10$ per group) as follows:

NC-Group: normal control group (rats fed on normal diet).

PC-Group: positive control group (rats fed on HFD).

CM-group: Rats fed on HFD + oral administration of CM.

WP-1 Group: Rats fed on HFD + oral administration of 1.5 % WP concentrate solution.

WP-2 Group: Rats fed on HFD + oral administration of 3.0 % WP concentrate solution.

CMWP-1 group: Rats fed on HFD + oral administration of a mixture of CM containing 1.5 % WP concentrate.

CMWP-2 group: Rats fed on HFD + oral administration of a mixture of CM containing 3.0 % WP concentrate.

Each animal in all treatment groups received CM, WP or a mixture of CM and WP (1 mL /100 g of body weight per day) through oral gavage for six successive weeks. The calculation was based on a consumption of 275 mL/d for a 70 kg human as reported by Rouanet (2010). The amounts of WP (1.5 and 3.0 %) were chosen after consulting several previous animal and clinical studies (Sousa *et al.*, 2012).

The body weight of the rats was measured at the beginning of the experiment and at seven-day intervals. At the end of the experiment, the rats were killed by decapitation after an overnight fast, and blood samples were collected in plain tubes and centrifuged at 3 000 rpm to harvest the serum, which was stored at -20°C for biochemical analysis. A perirenal fat pad (the fat pad extending from a vertex in the inguinal region up the midline and across at the lower pole of the kidney as well as that around the kidney) was dissected and weighed by using a lab scale.

Growth parameters and blood biochemical analysis Food consumption and conversion ratio for each animal and its weight were recorded for six weeks from the start of experiment. Initial and final body weights were recorded and body weight gain was calculated. Organ weights (liver and perirenal adipose tissue) were recorded.

Glucose, albumin, triglycerides, total cholesterol and HDL-cholesterol were measured via commercial kits (HUMAN Inc., Germany).

The low-density lipoprotein (LDL)-cholesterol was calculated using the Friedewald formula (Friedewald *et al.*, 1972):

$$\text{LDL-cholesterol} = \text{Total cholesterol} - (\text{HDL-cholesterol}) - (\text{Triglycerides}/5)$$

Adipocyte cell size Adipocyte cell size in the perirenal adipose tissue was measured as described previously (Hamad *et al.*, 2009). Briefly, the adipose tissue was rinsed with saline solution and fixed in a 10 % neutral formalin-buffered solution, and then embedded in paraffin, cut into 10-mm sections and stained with hematoxylin. Cell size was measured using the National Institutes of Health (Bethesda, MD, USA) image J software (100 cells per rat).

Statistical analysis Results were presented as means $\pm$ SD and the data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons by using SPSS ver. 22.0 statistical package software. Differences were

Table 1. Effect of whey protein and camel milk either alone or in a mixture on some growth parameters in rats fed high-fat diet

Treatment	Total Food Intake	IBW(g)	FBW (g)	BWG (g)	%BWG
NC	1125 ± 33 ^a	187 ± 15 ^a	260 ± 8 ^b	73 ± 8 ^b	40 ± 7 ^{ab}
PC	1072 ± 12 ^a	180 ± 14 ^a	307 ± 10 ^a	127 ± 4 ^a	72 ± 8 ^a
CM	977 ± 13 ^a	187 ± 15 ^a	265 ± 10 ^b	77 ± 4 ^{ab}	42 ± 5 ^{ab}
WP-1	976 ± 45 ^a	187 ± 14 ^a	238 ± 12 ^{cb}	51 ± 16 ^b	29 ± 11 ^b
WP-2	994 ± 75 ^a	188 ± 13 ^a	244 ± 7 ^{cb}	57 ± 14 ^b	31 ± 9 ^b
CMWP-1	979 ± 53 ^a	179 ± 8 ^a	223 ± 8 ^c	44 ± 15 ^b	25 ± 10 ^b
CMWP-2	1064 ± 9 ^a	187 ± 13 ^a	249 ± 7 ^{cb}	63 ± 7 ^b	34 ± 6 ^{ab}

Data are mean ± SD (n=10 per group).

NC = normal control, PC = positive control, CM = camel milk, WP-1 = whey protein 1.5 %, WP-2 = WP 3.0 %, CMWP-1 = camel milk + WP 1.5 %, CMWP-2 = camel milk + WP 3.0 %.

IBW = Initial body weight, FBW = Final body weight, BWG = Body weight gain, %BWG = [(FBW-IBW)/IBW × 100].

Means in the same column with different letters differ significantly at $p < 0.05$.

Table 2. Effect of whey protein and camel milk either alone or in a mixture on serum lipids of rats fed high-fat diet.

Treatment	TG (mg/dL)	TC (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
NC	54 ± 13 ^c	78 ± 4 ^b	31 ± 1 ^b	36 ± 3 ^a
PC	128 ± 10 ^a	121 ± 4 ^a	70 ± 4 ^a	27 ± 2 ^a
CM	85 ± 18 ^{abc}	79 ± 6 ^b	32 ± 6 ^b	30 ± 2 ^a
WP-1	106 ± 10 ^{abc}	90 ± 14 ^b	42 ± 12 ^{ab}	26 ± 5 ^a
WP-2	111 ± 5 ^{ab}	75 ± 3 ^b	25 ± 4 ^b	28 ± 2 ^a
CMWP-1	90 ± 13 ^{abc}	77 ± 2 ^b	31 ± 3 ^b	28 ± 1 ^a
CMWP-2	71 ± 6 ^{bc}	82 ± 3 ^b	35 ± 4 ^b	33 ± 1 ^a

Data are mean ± SD (n=10 per group).

NC = normal control, PC = positive control, CM = camel milk, WP-1 = WP 1.5 %, WP-2 = WP 3.0 %, CMWP-1 = camel milk + WP 1.5 %, CMWP-2 = camel milk + WP 3.0 %.

TG: triglycerides, TC: total cholesterol, LDL: low-density lipoprotein, HDL: high-density lipoprotein.

Means in the same column with different letters differ significantly at $p < 0.05$.

considered to be statistically significant at $p < 0.05$.

Results

Growth parameters of rats The effect of WP, CM and a mixture of CM and WP on some growth parameters in rats fed on HFD are presented in Table 1. As shown, all groups had similar body weight and similar food intake at the beginning of the experiment ($p > 0.05$). The final body weight (FBW) of the CM, WP-1, WP-2, and CMWP-2 groups was similar to that of the normal control diet group. The lowest FBW was recorded for the CMWP-1 group (27 % less than that of the PC, $p < 0.05$). Moreover, the lowering effect of the CM treatment on the FBW of the rats was less than that of the WP and CMWP treatments, but still significant ($p < 0.05$).

Serum lipids As can be seen in Table 2, there were

significant differences among all treatments in the rats' serum lipid profiles ($p < 0.05$), except for HDL-cholesterol, which was similar in all groups ($p > 0.05$). The CM, WP-1, WP-2, CMWP-1, and CMWP-2 groups exhibited significantly decreased ($p < 0.05$) total cholesterol and LDL-cholesterol levels. However, the WP-1 showed LDL-cholesterol levels similar to those of the PC group ($p > 0.05$). Only the CMWP-2 group showed a significant reduction in the rats' serum TG levels when compared with the PC group ($p < 0.05$). The other experimental groups showed an insignificant reduction in serum TG levels.

Fasting glucose and serum total proteins It can be noticed that feeding rats on HFD (PC group) resulted in an elevation in glucose levels ($p < 0.05$) and a reduction in serum total protein and albumin concentrations ($p > 0.05$) (Table 3). On the other

Table 3. Effect of whey protein and camel milk either alone or in a mixture on fasting glucose and serum proteins of rats fed high-fat diet.

Treatment	FG (mg/dL)	TP (mg/dL)	ALB (mg/dL)
NC	92 ± 1 ^b	7.06 ± 0.31 ^{ab}	3.43 ± 0.06 ^a
PC	119 ± 4 ^a	6.03 ± 0.23 ^b	2.90 ± 0.05 ^a
CM	76 ± 5 ^b	6.76 ± 0.17 ^{ab}	3.10 ± 0.10 ^a
WP-1	88 ± 8 ^b	6.53 ± 0.14 ^{ab}	2.96 ± 0.06 ^a
WP-2	87 ± 2 ^b	7.20 ± 0.30 ^a	3.23 ± 0.31 ^a
CMWP-1	80 ± 4 ^b	6.60 ± 0.17 ^{ab}	3.10 ± 0.20 ^a
CMWP-2	81 ± 2 ^b	7.28 ± 0.07 ^a	3.13 ± 0.12 ^a

Data are mean ± SD (n=10 per group).

NC = normal control, PC = positive control, CM = camel milk, WP-1 = WP 1.5 %, WP-2 = WP 3.0 %, CMWP-1 = camel milk + WP 1.5 %, CMWP-2 = camel milk + WP 3.0 %.

FG: fasting glucose, TP: total protein, ALB: albumin.

Means in the same column with different letters differ significantly at $p < 0.05$.

hand, all experimental treatments showed an improvement in the serum glucose levels ($p < 0.05$), which were similar to those in the normal control group. Although none of the experimental diets had any effect on serum albumin levels ($p > 0.05$), the CM, WP-1, and CMWP-1 groups exhibited total protein levels similar to those of the NC group ($p < 0.05$).

Liver and white adipose tissue weight and adipocyte cell size Liver and white adipose tissue (WAT) weight and adipocyte cell size are presented in Table 4 and Figure 1.

The results showed a significant difference between the NC and PC groups in the weight of WAT and adipocyte size. The liver weight was quite similar among all groups ($p < 0.05$); however, there was a slight decrease in the liver weight of the WP-2 and CMWP-1 group rats ($p > 0.05$).

There were no significant differences between the NC and the CM, WP-1, WP-2, CMWP-1 or CMWP-2 groups in either WAT or adipocyte size, even though rats in these groups were fed on HFD. Thus, all of these treatments (CM, WP-1, WP-2, CMWP-1 and CMWP-2) had a significant effect in decreasing WAT weight and adipocyte size ($p < 0.05$).

Discussion

The present study examined the effect of two healthy diet ingredients (CM and WP either separately or taken together) on obesity parameters. Compared to that of other ruminants, the milk of camels is unique in its chemical composition as well as for its known health benefits (Abrehaley and Leta 2018; Maghraby *et al.*, 2005). Compared with the PC group, the CM group showed a 39 % reduction ($p > 0.05$) in the body weight gain of the rats. Similar results were reported by Korish and Arafah (2013) using Wistar rats fed on high-fat high-cholesterol diets. The ability of CM to inhibit lipase enzymes may be one possible mechanism for its effect on body weight

Table 4. Effect of whey protein and camel milk either alone or in a mixture on liver and white adipose tissue (WAT) weight and adipocyte size of rats fed on high-fat diet.

Treatment	Liver (g)	WAT (g)	Adipocyte Size ($\mu\text{m}^2 \times 10^3$)
NC	8.79 ± 0.22 ^{ab}	1.64 ± 0.22 ^b	2.99 ± 0.45 ^b
PC	9.81 ± 0.27 ^a	5.56 ± 0.82 ^a	10.64 ± 0.68 ^a
CM	7.69 ± 0.85 ^{ab}	2.51 ± 0.56 ^b	6.03 ± 1.30 ^b
WP-1	8.12 ± 0.74 ^{ab}	1.88 ± 0.61 ^b	3.35 ± 0.33 ^b
WP-2	6.85 ± 0.60 ^b	1.74 ± 0.32 ^b	4.18 ± 0.05 ^b
CMWP-1	6.87 ± 0.24 ^b	1.68 ± 0.76 ^b	2.90 ± 0.89 ^b
CMWP-2	7.22 ± 0.43 ^{ab}	2.12 ± 0.19 ^b	4.80 ± 0.33 ^b

Data are mean ± SD (n=10 per group).

NC = normal control, PC = positive control, CM = camel milk, WP-1 = WP 1.5 %, WP-2 = WP 3.0 %, CMWP-1 = camel milk + WP 1.5 %, CMWP-2 = camel milk + WP 3.0 %.

Means in the same column with different letters differ significantly at $p < 0.05$.

reduction (Mudgil *et al.*, 2018). Recently, studies have shown that CM has the ability to inhibit pancreatic lipase (Mudgil *et al.*, 2018; Jafar *et al.*, 2018). This ability could be due to the release of bioactive peptides from CM proteins showing potent lipase inhibitory activities (Mudgil *et al.*, 2018; Jafar *et al.*, 2018). In addition, it was suggested that CM might have a local effect on the stomach by increasing the gastric emptying time. Therefore, it may increase satiety and lower food intake (Korish and Arafah, 2013). Furthermore, Ziamajidi (2013) suggested another hypothesis for the lowering of body weight, which involves adjusting the ratio of the peroxisome proliferator-activated receptor (PPAR) alpha to the sterol regulatory element-binding protein 1 (SREBP-1) via CM intake. The researchers noted that this effect might lead to the increased activity of some enzymes related to fat metabolism that increase calorie expenditure and reduce storage of fat (Ziamajidi, 2013). Contrary to our results, Badkook (2013) found that CM did not affect rats' weight. These inconsistent results may have been due to the difference in the form of CM utilized. Fermented CM was used in the Badkook (Badkook 2013) study and non-fermented CM in the present study.

In addition, when rats were given 1.5 and 3.0 % WP solutions, a significant reduction in body weight gain was observed (60 and 55 % less than in the PC group, $p < 0.05$), which was similar to that of the NC group (Table 1). The lowest body weight gain was achieved by the CMWP-1 group (65 % less than the PC group, $p < 0.05$). To the best of our knowledge, the combined effects of WP and CM on obesity in a high-fat fed rat model was examined for the first time in the present study. These beneficial effects were associated with significant reduction in the body weight gain of the rats. In previous studies, when WP was added to the diet of several animal species, it led to a rapid and marked decrease in their

Fig. 1. Effect of whey protein and camel milk either alone or in a mixture on adipocyte cell. Adipocytes in paraffin sections: NC = normal control, PC = positive control, CM = camel milk, WP-1 = WP 1.5 %, WP-2 = WP 3.0 %, CMWP-1 = camel milk + WP 1.5 %, CMWP-2 = camel milk + WP 3.0 % (scale bar, 50 μ m; magnification, 40 $\times$).

body weights due to its effect on fat deposition (Pichon *et al.*, 2008; Pilvi *et al.*, 2007; Donato *et al.*, 2006). This anti-obesity effect of WP may be due to its higher levels of leucine, isoleucine and valine, which may direct the energy consumption to protein synthesis in the muscle instead of utilizing it in fat synthesis (Donato *et al.*, 2006; Zhang *et al.*, 2007).

In contrast to the PC diet, the CM, WP and a mixture of CM and WP significantly reduced the adipocyte size in rats (Table 4). The reduction was associated with a significant reduction in the number of large adipocytes as well as a significant increase in the number of small adipocytes (Table 5 and Fig. 1). This reduction in adipocyte size was a result of the significant reduction in the WAT mass of the experimental groups compared to the PC group (Table 4). These results are in line with other studies that have reported a reduction in adipocyte size (Pilvi *et al.*, 2008) and adipose tissue mass (Donato *et al.*, 2006; Zhang *et al.*, 2007; Gasparetto *et al.*, 2013) following administration of WP.

It is noticeable that the mixture of WP and CM resulted in a more pronounced and significant reduction in the body weight gain of the rats. Interestingly, when combined with CM,

1.5 % WP was better than 3.0 % WP at lowering body weight gain. Recently, West *et al.* (2017) reported that the ingestion of WP improved the body net protein balance. Therefore, ingestion of 3.0 % WP could result in more anabolism of protein in muscle in compensation for reduction in the body weight. However, the muscle weight was not determined in the present study. In addition, it has been suggested that the good taste of the WP itself may have encouraged the rats to eat more (non-published data). However, in the present study the total food intake among the experimental groups was found to be similar. In a previous report, a greater accumulative food intake was observed in rats fed on a diet containing WP (Pilvi *et al.*, 2007).

On the other hand, when rats fed on a higher concentration of WP, the weight of adipose tissue tended to be decreased ($p > 0.05$). This effect did not replicated when WP was mixed with CM, as the weight of adipose tissue of CMWP-2 is tended to be higher than that of CMWP-1 ($p > 0.05$). Although these differences were not significant ($p > 0.05$), but it seems that camel milk did not reduced adipose tissue weight to the same extent as WP. This effect remains unclear, but it could be due to the effect of CM on the lipogenesis in adipose tissue.

Table 5. Effect of whey protein and camel milk either alone or in a mixture on adipocyte cell size distribution profile of rats fed on high-fat diet.

Treatment	Mean adipocyte size (μm^2)	Number of cells of different sizes				
		15 μm^2	25 μm^2	30 μm^2	95 μm^2	100 μm^2
NC	29955 $\pm$ 459 ^b	10.00 $\pm$ 1.06 ^{ab}	12.67 $\pm$ 0.54 ^{ab}	12.67 $\pm$ 0.54 ^a	0.33 $\pm$ 0.20 ^b	0.33 $\pm$ 0.20 ^b
PC	106462 $\pm$ 688 ^a	0.67 $\pm$ 0.41 ^b	0.67 $\pm$ 0.20 ^c	1.67 $\pm$ 0.74 ^b	5.33 $\pm$ 0.20 ^a	3.33 $\pm$ 0.54 ^a
CM	56679 $\pm$ 1307 ^b	5.00 $\pm$ 2.47 ^{ab}	6.00 $\pm$ 0.94 ^{abc}	4.67 $\pm$ 1.08 ^{ab}	2.33 $\pm$ 0.41 ^{ab}	2.00 $\pm$ 0.61 ^b
WP-1	33522 $\pm$ 339 ^b	8.33 $\pm$ 0.20 ^{ab}	14.33 $\pm$ 2.86 ^a	9.67 $\pm$ 1.47 ^{ab}	0.00 $\pm$ 0.00 ^b	0.00 $\pm$ 0.00 ^b
WP-2	54752 $\pm$ 500 ^b	5.33 $\pm$ 1.78 ^{ab}	6.33 $\pm$ 0.41 ^{abc}	10.00 $\pm$ 2.15 ^{ab}	1.67 $\pm$ 0.74 ^{ab}	1.00 $\pm$ 0.61 ^b
CMWP-1	29063 $\pm$ 895 ^b	17.33 $\pm$ 2.16 ^a	9.33 $\pm$ 1.02 ^{abc}	8.00 $\pm$ 0.61 ^{ab}	0.67 $\pm$ 0.41 ^b	0.33 $\pm$ 0.20 ^b
CMWP-2	69403 $\pm$ 338 ^{ab}	3.00 $\pm$ 1.84 ^b	3.33 $\pm$ 0.54 ^{bc}	3.67 $\pm$ 0.89 ^{ab}	4.00 $\pm$ 0.94 ^{ab}	1.33 $\pm$ 0.54 ^{ab}

Data are mean $\pm$ SD (n=10 per group; 100 cells per rat were used for adipocyte size measurement).

NC = normal control, PC = positive control, CM = camel milk, WP-1 = WP 1.5 %, WP-2 = WP 3.0 %, CMWP-1 = camel milk + WP 1.5 %, CMWP-2 = camel milk + WP 3.0 %.

Means in the same column with different letters differ significantly at $p < 0.05$

Recently, administration of CM enhanced expression of fatty acid synthase gene in adipose tissue of rats (Mansour *et al.*, 2017).

As it can be observed, feeding rats on WP resulted in a reduction in the liver weight compared to the PC. Interestingly, the liver weight of WP-2 group was lower ($p > 0.05$) than that of WP-1 group. While, the liver weight of CMWP-2 group was higher ($p > 0.05$) than that of the CMWP-1 group. Although the differences between groups are not significant ($p > 0.05$), but it seems that WP and CM have different effects on liver weight. A previous study have shown a downregulation effect of WP on some hepatic lipogenic enzymes in rats (Morifuji *et al.*, 2005). In addition, CM showed an effect of on the liver phosphorylase activity (Sadek *et al.*, 2016). However, the different effects of WP on liver weight when combined with CM could be due to the combination effect. More work is needed to unravel the underlying mechanism.

Both CM and WP lowered and LDL and TG levels significantly ($p < 0.05$) and increased the rats' serum HDL levels significantly ($p < 0.05$) (Table 2). This dyslipidemic effect of CM is in agreement with recent reports on both animals and humans (Abdalla *et al.*, 2018; Mihic *et al.*, 2016). The hypolipidemic effect of CM could have resulted from decreased cholesterol absorption due to the high L-carnitine content in CM (Karanth and Jeevaratnam 2009). Moreover, the WP found in CM showed a significant cholesteryl esterase inhibition activity when hydrolyzed by gastric and pancreatic enzymes (Jafar *et al.*, 2018).

On the other hand, the serum total cholesterol was significantly reduced by WP intake. These results are in agreement with other findings where WP reduced blood serum lipids and lipoproteins (Pal *et al.*, 2010). The underlying mechanisms are yet to be revealed, but one possibility could be through reduction of hepatic cholesterol synthesis (Baragob, 2015).

There was a significant reduction ($p < 0.05$) in the blood glucose levels as a result of feeding the rats on CM, WP or a mixture of CM and WP (Table 3). A recent study has demonstrated the ability of CM to manage postprandial glucose levels (Mudgil *et al.*, 2018). This ability could be due to the release of bioactive peptides from the CM proteins that show inhibitory activity against important carbohydrase enzymes (α -amylase and α -glucosidase). Additionally, some reports have observed an insulin-like substance in CM that supports its ability to prevent and control diabetes (Mihic *et al.*, 2016; Isa *et al.*, 2013).

Conclusion

In conclusion, WP and CM can be used as effective food supplements for preventing overweight and obesity by lowering weight gain, adipocyte size and serum levels of glucose and lipids. The CM and WP exhibited a more positive effect when administrated as a mixture than when administrated separately, especially when the combination contained 1.5 % WP.

Conflicts of Interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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