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Raspberry Ketone versus Calorie Restriction on Modulating Metabolic Disorders in Obese Rats

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Abstract

Obesity management regimens suffer from discomfort, undesirable side effects and/or unsuccessful outcomes. This study compared normal diet (ND) regimen with raspberry ketone (RK) supplementation and calorie restriction (CR). 40-Male Wistar rats received high fat diet (HFD) for 16 weeks. Obese control group was maintained on HFD for another 6 weeks (n=10). Other obese groups (n=10 each) received ND, CR diet or ND with RK oral supplement (44mg/kg) for 6 weeks. HFD increased body and visceral white adipose tissue (WAT) weights, serum glucose, total cholesterol, TG, LDL-C, inflammatory markers (MCP-1, IL-6 and TNF- α) in WAT, while decreased serum HDL-C and WAT adiponectin. Like CR diet, ND with RK supplementation restored the changes attained by HFD.

Conclusion: RK supplement with ND regimen effectively attenuated obesity-related changes. Such approach could be an alternative to CR in the management of obesity, thereby overcome the side effects of applying CR for long time.

Keywords: Raspberry ketone; White adipose tissue; High fat diet; Adiponectin

Abbreviations

WAT: White Adipose Tissue; RK: Raspberry Ketone; g: Gram; HFD: High Fat Diet; BW: Body Weight; N: Normal Control Group; O: Obese Control Group; ON: Obese Rats Submitted to Normal Diet; OCR: Obese Rats Kept on Calorie Restriction; ONRK: Obese Rats Fed Normal Diet and Treated with RK; MDA: Malondialdehyde; TAC: Total Antioxidant Capacity; MCP-1: Monocyte Chemoattractant Protein-1; IL-6: Interleukin-6; TNF- α : Tumor Necrosis Factor Alpha; TC: Total Cholesterol; TAG: Triacylglycerol; HDL-C: High-Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; ELISA: Enzyme Linked Immuno Sorbent Assay.

Introduction

Obesity is a multi-factorial disorder that is no longer considered to be a cosmetic problem, but a more serious one, complicated with many co-morbidities that affect all body systems [1]. Worldwide, obesity has nearly tripled since 1975 [2] with current estimates of overweight and obesity affecting 2.1 billion people [3]. Overweight and obesity are the world's fifth leading cause of death, responsible for about 3.4 million deaths per year [4]. Moreover, these figures are still predicted to be increased continuously [5].

Excess energy consumption over the body needs leads to unhealthy expansion of adipose tissue. The latter is achieved through enlargement of existing adipocytes (hypertrophy), the predominant mechanism in adults, or by increasing the number of adipocytes (hyperplasia) [1,6]. The pathological white adipose tissue (WAT) expansion is associated with many deleterious complications, including inflammation, insulin resistance, atherosclerosis, fibrosis, hypoxia, dysregulated adipokine secretion, hypertension, other heart diseases and cancer [7,8]. Obesity is therefore a highly complicated disorder with a challenging management. It requires continuous efforts and a long-term management, including dietary adjustment, physical activity and may require pharmacological agent or even surgery; bariatric surgery [9].

Diet adjustment and physical activity are effective ways and are the first advice for management of obesity, but are difficult to be followed by most obese subjects and weight regain often occurs [10]. Bariatric surgery, although useful, is costing and cannot be applied for all patients. Moreover, it has many distressing surgical complications and weight regain also may occur [7,9]. Pharmacological

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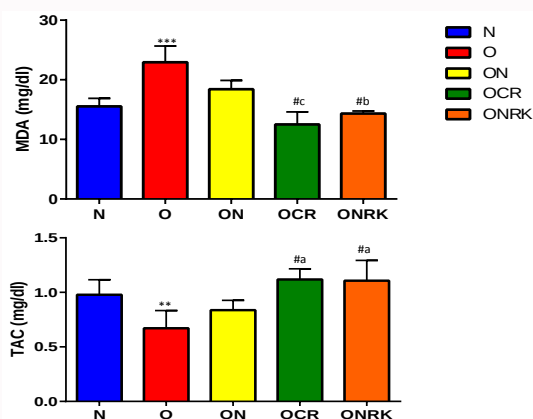


Figure 1: Oxidative stress [MDA and TAC] in serum of obese rats. Results were expressed as mean $\pm$ SD; n=6. **p< 0.01 and *** p< 0.001 compared to normal (N) group, #p< 0.001 compared to obese (O) group, #p< 0.05, #b p< 0.01 and #c p< 0.001 compared to ON group.

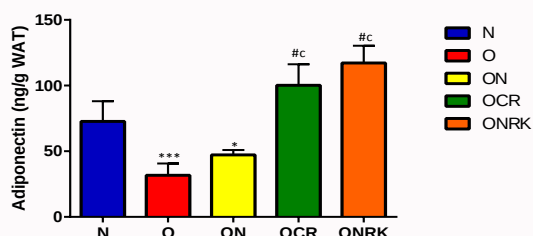


Figure 2: Adiponectin in visceral WAT of obese rats. Results were expressed as mean $\pm$ SD; n=6. *p< 0.05 and *** p< 0.001 compared to normal (N) group, #p< 0.001 compared to obese (O) group and #p< 0.001 compared to ON group.

agents are another option for the management of obesity. However, most of these agents are no longer used due to their deleterious side effects, including hypertension, constipation, headache, and insomnia [11,12]. Hence, it is urgently required to find a safe and effective agent for the management of obesity. For this reason, a large number of natural products have been tested for their potential benefits in obesity management [11,12].

Raspberries (red raspberry, *Rubus idaeus*, family Rosaceae) exert antioxidants, along with anti-inflammatory, antimicrobial, and anticancer activities due to their high content of phenolic compounds [13]. Among these compounds is raspberry ketone (RK) [4-(4-hydroxyphenyl) butan-2-one] which is an aromatic compound ($C_{10}H_{12}O_2$ and a molecular weight of 164.2g/mol), responsible for the aroma of raspberries [14]. It has long been used as a flavoring agent in food and beverages, but is currently tested for potential health benefits [15]. The RK was first reported to be protective and a useful treatment for nonalcoholic steatohepatitis *in vivo* and *in vitro* [16]. Previous studies suggested that RK may prevent the elevation in the body weight as well as the WAT weight in mice and rats fed high fat diet (HFD) [17,18]. Moreover, the only randomized, placebo-controlled, double-blind human eight-week trial added RK to five other ingredients known to stimulate weight loss showed that supplementation with the RK combination encouraged body weight loss compared to control obese group. However, it was unknown how much of this effect was attributable to the RK alone [19]. The current study was undertaken to evaluate to what extent the influence of RK in the management of obesity is effective in comparison with calorie

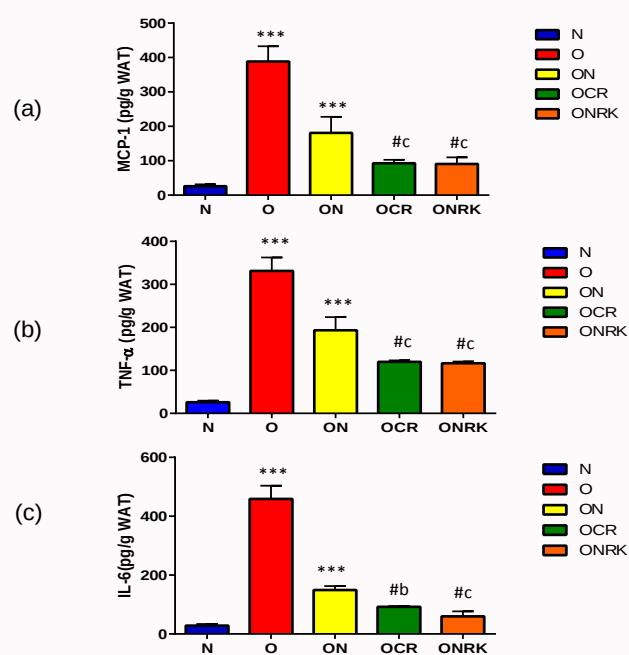


Figure 3: Inflammatory markers in visceral WAT of obese rats. Level of (a) MCP-1, (b) TNF-α and (c) IL-6. Results were expressed as mean $\pm$ SD; n=6. ***p< 0.001 compared to normal (N) group, #p< 0.001 compared to obese (O) group, #p< 0.01 and #c p< 0.001 compared to ON group.

restriction regimen.

Materials and Methods

Experimental animals

Fifty adult male Wistar albino rats (140–160 g), obtained from the faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt, were used in the current study. Rats were acclimatized for one week prior to experiments in polypropylene cages with stainless steel wire-mesh tops (five rats/cage) in the animal unit facility of the Faculty of Pharmacy, Zagazig University, Zagazig, Egypt. They were kept at controlled temperature ($23 \pm 2^\circ\text{C}$), light/dark (12/12 h) cycle and humidity ($60 \pm 10\%$) and supplied with normal chow diet and water *ad libitum*. Experimental design and animal handling procedures were approved by the Ethical Committee for Animal Handling at Zagazig University, Zagazig, Egypt (approval number P2-6-2016).

Drugs and diets

Raspberry Ketone (Raspberry Ketones; RK) capsules were purchased from Holland and Barrett, UK.

Normal chow diet: 5% total fat (including 2% unsaturated fat), 62% carbohydrate, 20% protein and 13% fiber, ash and other ingredients like vitamins and minerals.

High fat diet (HFD): 25% total fat (including 11% unsaturated fat), 44% carbohydrate, 18% protein, and 13% fiber, ash and other ingredients like vitamins and minerals [20].

Calorie restricted diet: 25% food restriction (commercial chow) [21].

Induction of obesity

One week after acclimatization, rats were fed on HFD for 16 weeks to induce obesity. Animals which achieved $\geq 30\%$ increase in their body weight (BW) were considered obese and were selected for

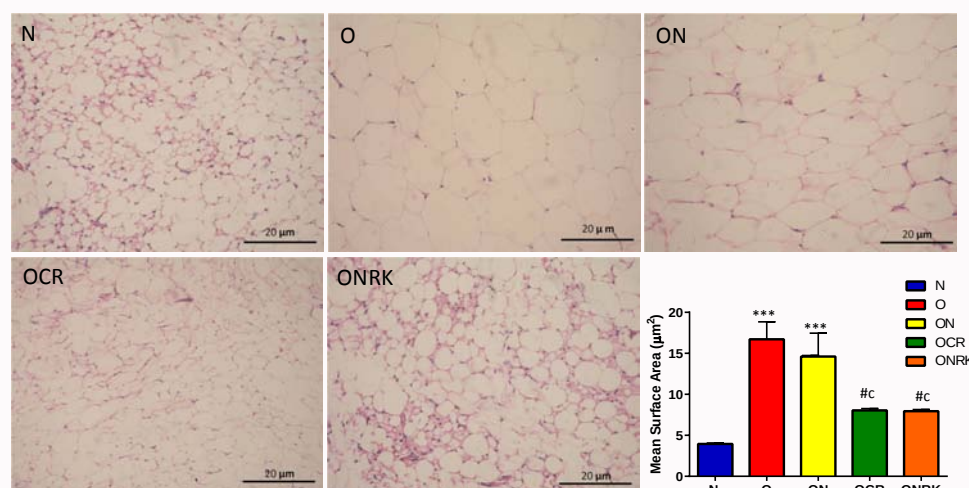


Figure 4: Representative H & E stained sections of visceral WAT. Visceral WAT sections from normal rats (N), obese fed HFD for 16 weeks (O), obese rats shifted to normal diet for 6 weeks (ON), obese rats shifted to calorie restricted diet for 6 weeks (OCR), and obese rats shifted to normal diet and received RK for 6 weeks (ONRK). The surface area of adipocytes was measured using image J software. Results were expressed as mean $\pm$ SD. * $p < 0.05$ and *** $p < 0.001$ compared to normal (N) group, # $p < 0.001$ compared to obese (O) group and # $p < 0.001$ compared to ON group.

the current study.

Experimental design

Normal and obese rats were assigned into the following experimental groups $n=10$ rats each).

- Normal control (N) group: normal rats kept on normal chow diet throughout the experimental period.
- Obese control (O) group: obese rats kept on HFD for 6 weeks.
- ON group: obese rats submitted to normal diet for 6 weeks.
- OCR group: obese rats kept on calorie restricted diet for 6 weeks.
- ONRK group: obese rats fed normal diet and treated with RK (44mg/kg body weight, daily; orally) for 6 weeks.

Sampling

At the end of the experiment, overnight fasted rats were weighed and blood was collected and centrifuged at 3000 rpm for 15 minutes for serum separation. Fresh serum samples were processed immediately for determination of glucose level. The remainder serum samples were stored as aliquots at -20°C for determination of lipid profile and oxidative stress markers (malondialdehyde; MDA and total antioxidant capacity; TAC).

Following blood collection, rats were killed by decapitation. Visceral WAT were removed instantly, rinsed with cold normal saline, weighed and divided into two parts. One of them was quickly frozen in liquid nitrogen (-170°C) for 5 minutes then stored at -20°C for further determination of adiponectin, monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α). The other part was kept in 10% formalin for at least 1 week and processed for histological study.

Biochemical analysis

Serum glucose and lipid profile: Serum glucose level, total cholesterol (TC), Triacylglycerol (TAG), and High-density lipoprotein cholesterol (HDL-C) were determined colorimetrically

by spinreact kit (Spinreact, Girona, Spain). Serum low-density lipoprotein cholesterol (LDL-C) level was calculated according to Friedewald formula [22] where $\text{LDL-C (mg/dl)} = \text{TC} - [\text{HDL-C} + \text{TAG}/5]$.

Serum oxidative stress markers: Serum MDA and TAC were determined by commercially available colorimetric methods using kits supplied by Biodiagnostics (Dokki, Giza, Egypt) following the manufacturer's instructions.

Adiponectin in visceral WAT: Adiponectin content of visceral WAT was determined by solid phase Enzyme Linked Immuno Sorbent Assay (ELISA) using rat adiponectin kit (RayBio® Inc., Norcross, Georgia, USA) and a microtiter plate reader capable of reading at 450nm. The instructions of the manufacturers were followed.

MCP-1, IL-6 and TNF- α in visceral WAT: The levels of inflammatory markers in WAT were measured by ELISA kits; MCP-1 (Abcam Trading (Shanghai) Company Ltd., Shanghai, China.), IL-6 (R&D Systems, Inc., Minneapolis, MN, USA) and TNF- α (Sigma Aldrich Co. LLC, USA) according to the manufacturers' instructions.

Histological study

Formalin-fixed visceral adipose tissue specimens were dehydrated with a series of ascending grade ethanol from 75 to 100%. Tissues were placed thereafter in xylol and embedded in paraffin. Cross sections of about 4µm thickness were processed on slides and stained with Hematoxylin and eosin (H&E) stain as a routine method for studying the histological structure of fat cells [23]. Sections were captured using (Leica Qwin 500, England) image analyzer [24]. The surface area of adipocytes were measured from sections using image J software.

Statistical analysis

Data were expressed as mean $\pm$ standard deviation (SD). They were statistically analyzed using one-way analysis of variance (ANOVA) test followed by Tukey's post hoc test using Graphpad prism software v.7 (GraphPad Software Inc., La Jolla, CA, USA) for comparison between the studied groups. For correlation study, Pearson's correlation analysis was performed. For all analysis, $p < 0.05$ was considered a significant difference.

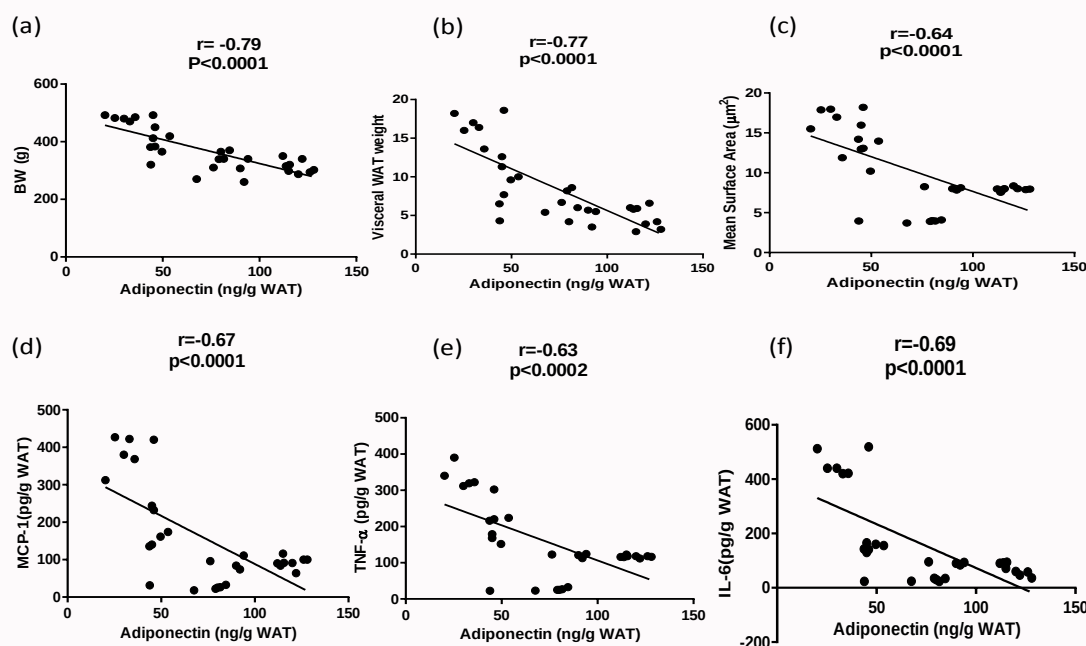


Figure 5: Correlation between the level of adiponectin in visceral WAT and (a) body weight; (b) WAT weight; (c) adipocyte surface area; visceral WAT content of; (d) MCP-1, (e) IL-6, (f) TNF- α (n=30, all $p < 0.001$).

Results

Body and visceral WAT weights

Table 1 shows that rats in obese group had significant increase in body and visceral WAT weights compared to normal control group. Obese rats shifted to normal diet for 6 weeks also had their BW and visceral WAT weights similar to that of obese group maintained on HFD for these 6 weeks. On the other hand, BW and WAT weight for rats in OCR and ONRK groups were significantly lower than those from (O) and (ON) groups ($p < 0.001$).

Serum glucose and lipid profile

Obese rats showed a significant elevation in serum glucose, TC, TAG and LDL-C while HDL-C recorded a significant reduction in comparison to normal rats ($p < 0.001$). Obese rats shifted to normal diet for 6 weeks also had similar serum levels of the previous parameters as obese group maintained on HFD for 6 weeks. However, obese rats shifted to either calorie restricted diet or to normal diet with concurrent administration of RK for 6 weeks showed significant reduction in their serum glucose, TC, TAG and LDL-C levels, associated with significant elevation in HDL-C levels in comparison with (O) or (ON) groups ($p < 0.001$) as shown in Table 2.

Serum MDA and TAC

Oxidative stress (increased serum MDA and decreased TAC) was observed in obese rats, compared to normal rats ($p < 0.01$). After 6 weeks of treatment, a significant decrease in serum MDA and a significant ($p < 0.001$) increase in serum TAC were observed in (OCR) and (ONRK) groups compared to (O) and (ON) rats (Figure 1).

Adiponectin level in visceral WAT

Adiponectin levels were significantly lower in the visceral WAT of both (O) and (ON) groups than (N) group ($p < 0.001$). After 6 weeks of treatment, rats in both (OCR) and (ONRK) groups showed significant ($p < 0.001$) elevation in their adiponectin levels in

Table 1: Body and visceral WAT weights in obese rats.

Groups	Body weight (g)	WAT weight (g)
N	329.8 $\pm$ 28.53	5.890 $\pm$ 1.619
O	478.2 $\pm$ 15.81***	16.61 $\pm$ 2.659***
ON	406.9 $\pm$ 38.04***	10.43 $\pm$ 2.407***
OCR	329.4 $\pm$ 18.28 ^{ac}	5.670 $\pm$ 0.8832 ^{ac}
ONRK	312.3 $\pm$ 29.56 ^{ac}	4.280 $\pm$ 1.307 ^{ac}

Results were expressed as mean $\pm$ SD; n=10 each. N: normal control group, O: obese control group, ON: obese rats shifted from HFD to normal diet, OCR: obese rats shifted from HFD to calorie restricted diet, ONRK: obese rats shifted from HFD to normal diet supplemented with RK. Significant difference from N group at *** $p < 0.001$. Significant difference from O group at # $p < 0.001$ and Significant difference from ON group at ^a $p < 0.001$.

comparison with (O) and (ON) groups (Figure 2).

MCP-1, IL-6 and TNF- α in visceral WAT

Levels of MCP-1, IL-6 and TNF- α in visceral WAT were significantly increased in the (O) and (ON) groups compared to (N) group ($p < 0.001$). After 6 weeks of treatment, (OCR) or (ONRK) groups demonstrated a significant reduction in these inflammatory markers in comparison with O and ON groups ($p < 0.001$) (Figure 3).

Histological study

Hematoxylin and Eosin (H&E) stained sections of visceral WAT (Figure 4) showed that the WAT of the normal control group were mainly small-sized population. The consumption of a HFD markedly increased the lipid content of the adipocytes which composed mainly of largely-sized population compared to normal control group. The diet shift from HFD to normal diet showed non-significant change versus obese group. The size of the adipocytes of (ON) group still significantly higher than the normal control group and the population of this is mainly large to medium-sized ($p < 0.001$). The application of OCR did significantly decrease the adipocytes size from medium to small-sized adipocytes compared to (O) and (ON) groups ($p < 0.001$).

Table 2: Serum glucose, lipid profile in obese rats.

Groups	Glucose	TC	TAG	HDL-C	LDL-C
	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)
N	88.4 ± 6.6	78.5 ± 8.3	75.0 ± 6.4	34.9 ± 5.2	28.6 ± 8.7
O	131.7 ± 10.5***	134.1 ± 16.9***	120.3 ± 11.6***	22.4 ± 2.7***	93.1 ± 15.3***
ON	108.0 ± 2.3***	94.9 ± 5.6*	86.6 ± 6.4*	27.0 ± 6.2*	44.0 ± 3.5*
OCR	81.3 ± 4.0 ^{#c}	68.9 ± 4.7 ^{#b}	55.4 ± 4.4 ^{#c}	36.2 ± 3.7 ^{#a}	26.7 ± 4.2 ^{#a}
ONRK	85.1 ± 8.0 ^{#c}	69.0 ± 5.0 ^{#b}	52.5 ± 7.7 ^{#c}	37.2 ± 3.8 ^{#b}	25.3 ± 4.6 ^{#b}

Results were expressed as mean ± SD; n=10 each. N: normal control group, O: obese control group, ON: obese rats shifted from HFD to normal diet, OCR: obese rats shifted from HFD to calorie restricted diet, ONRK: obese rats shifted from HFD to normal diet supplemented with RK. Significant difference from N group at * $p < 0.05$ and *** $p < 0.001$. Significant difference from O group at # $p < 0.001$ and Significant difference from ON group at ^a $p < 0.05$, ^b $p < 0.01$ and ^c $p < 0.001$.

Table 3: Correlation between inflammatory markers (MCP-1, TNF- α and IL-6) with glucose, oxidative stress markers (MDA and TAC) and lipid profile (TAG, TC, HDL and LDL) in the tested groups.

LDL-C	HDL-C	TC	TAG	TAC	MDA	Glucose	Parameter
0.92	-0.69	0.89	0.79	-0.65	0.8	0.85	MCP-1
0.85	-0.69	0.78	0.75	-0.63	0.74	0.85	TNF- α
0.93	-0.71	0.89	0.84	-0.67	0.78	0.87	IL-6

(n=30, all $p < 0.001$).

In the (ONRK) group, there was a dramatic decrease in the size of fat cells and the population was mainly small-sized when compared to (O) and (ON) groups ($p < 0.001$). Interestingly, the fat cell size of both (OCR) and (ONRK) groups was nearly the same.

Correlations

Adiponectin content in visceral WAT was negatively correlated with body and visceral WAT weights, the surface area of adipocytes and the visceral WAT content of MCP-1, TNF- α and IL-6 ($p < 0.001$) as shown in Figure 5. Inflammatory markers (MCP-1, TNF- α and IL-6) were positively correlated with glucose, MDA, TAG, TC and LDL-C, associated with negative correlations with TAC and HDL-C ($p < 0.001$) as shown in Table 3.

Discussion

Calorie restriction regimen in the management of obesity is associated with patient deprivation and rebound weight gain rapidly occurs due to failure to follow the regimen [7]. Surgical choice has many psychological and surgical distressing complications [9]. In this study we tried to prove that, following normal diet regimen with a natural product (RK) supplementation to promote weight loss may be an alternative way comparable to that attained by calorie restriction.

Feeding rodent a HFD is a well approved model of obesity with close relation to that in human [25]. Rats maintained on HFD for 16 weeks developed obesity as indicated by significant increase in body and visceral WAT weights. Adipose tissue is considered as a highly dynamic tissue that changes its mass according to metabolic requirement [26]. It was considered as an inert organ that just stores excess energy and releases it when needed, but it is now regarded as an endocrine organ capable of synthesizing and secreting more than 600 bioactive factors termed adipokines [27]. These compounds directly affect other organ systems including the brain, liver, pancreas and skeletal muscle, controlling many physiological and pathophysiological processes as food intake, insulin sensitivity, immunity, blood pressure, inflammation, lipid metabolism and energy homeostasis [7].

In this context, our obese rats developed dyslipidemia and systemic oxidative stress due to the increased consumption of saturated fats

in HFD [28]. These effects were widely reported in previous studies [29,30]. The enlargement of WAT due to positive energy balance recruited macrophage, neutrophils, and T-cells in WAT, initiating an inflammatory response [7]. The infiltrated macrophages produce proinflammatory adipokines (TNF- α and MCP-1) in WAT. On the other hand, the level of anti-inflammatory adiponectin in WAT decreased and hence a state of chronic low grade inflammation was developed. The inflammatory state in our study showed positive correlations with glucose, oxidative stress and lipid profile. Such inflammatory state represents the “common soil” for the pathogenesis of the metabolic and cardiovascular complications of obesity. These results are in accordance with previous studies [31,32].

Obese rats maintained on calorie restricted diet for 6 weeks showed improvement in their obesity profile. Body and visceral WAT weight were reduced. Obesity-related metabolic abnormalities were also better; decreased serum glucose, TC, TAG, LDL-C, MDA and inflammatory markers MCP-1, IL-6 and TNF- α , while increased HDL-C, TAC and adiponectin levels. These results are in agreement with other reports [33,34].

In contrast, obese rats maintained on normal diet alone for 6 weeks showed slight, but not significant improvement in their obesity profile (body weight, visceral WAT weight, dyslipidaemia, systemic oxidative stress and WAT inflammatory markers). Although the saturated fats are low in this diet, the extent of calorie cut in normal diet was not enough to induce favorable effects.

In the current study, the use of RK supplement for 6 weeks along with normal diet significantly decreased the body and WAT weights compared to those of (O) and (ON) groups. Obesity-related metabolic, oxidative and inflammatory abnormalities were also corrected. All these effects were similar to those attained by calorie restriction. These findings are consistent with [17,18] who have concluded that, weight gain and the increase in WAT weight were prevented in rats and mice fed HFD supplemented with RK. This was attributed to increased norepinephrine that induces lipolysis in WAT.

A human and cell culture studies also showed that RK may promote weight loss and mitigate obesity [19]. The RK in (ONRK) group not only decreased WAT weight but also the size of adipocytes

due to the shrinkage of adipocytes of WAT to nearly normal size. This effect might indicate enhanced lipolysis in WAT. Previous *in vitro* study suggested that RK induced weight loss through its action mediated by the stimulation of the hormone adiponectin. The latter is a potent enhancer of fatty acid oxidation, glucose regulation, as well as an inhibitor of lipid accumulation. Another proposed action is thought the stimulation of naturally occurring norepinephrine-mediated lipolysis [18]. Furthermore, in cultured mammalian adipocytes, RK (10µg) induced a dramatic 300% increase in lipolysis after 24 hours and a 100% increase in adiponectin activity at 48 hours [16]. Correspondingly, obese rats treated with RK along with normal diet in the present study showed elevated adiponectin level in their visceral WAT which was negatively correlated with the weight of visceral WAT and the size of adipocytes in visceral WAT.

Adiponectin is an adipocytokine possessing potent anti-inflammatory function. It was reported to be an essential mediator in the regulation of anti-inflammatory effects through the inhibition of TNF-α and up-regulation of the anti-inflammatory cytokines [35]. The increase in its level in our (OCR) and (ONRK) groups resulted in reduction of inflammatory markers MCP-1, IL-6 and TNF-α and hence decreased inflammation. These results were further confirmed with the strong negative correlation between adiponectin and inflammatory markers of visceral WAT.

Conclusions

Collectively, the findings of this study support the recent evidence suggesting the potential of natural products RK as an effective intervention for management of obesity complications through its supplementation with normal diet. Concomitant supplementation of RK with normal diet is equivalent to calorie restriction effect in reducing body weight and obesity-related metabolic changes as oxidative stress and inflammatory markers associated with improvement in the anti-inflammatory cytokine (adiponectin).

References

- Brandao Bb, Guerra Ba, Mori Ma. Shortcuts to a functional adipose tissue: The role of small non-coding RNAs. *Redox Biol.* 2017; 12: 82-102.
- Who. Obesity and overweight (facts sheet). 2018.
- Hruby A, Hu Fb. The Epidemiology of Obesity: A Big Picture. *Pharmacoeconomics.* 2015; 33: 673-689.
- Smith Kb, Smith Ms. Obesity Statistics. *Prim Care.* 2016; 43: 121-135, ix.
- Park S. Pathways linking obesity to health-related quality of life. *Qual Life Res.* 2017; 26: 2209-2218.
- Narayanawami V, Dwoskin Lp. Obesity: Current and potential pharmacotherapeutics and targets. *Pharmacol Ther.* 2017; 170: 116-147.
- Kusminski Cm, Bickel Pe, Scherer Pe. Targeting adipose tissue in the treatment of obesity-associated diabetes. *Nat Rev Drug Discov.* 2016; 15: 639-660.
- Autieri Mv. Adipose inflammation at the heart of vascular disease. *Clin Sci (Lond).* 2016; 130: 2101-2104.
- Pucci A, Finer N. New medications for treatment of obesity: metabolic and cardiovascular effects. *Can J Cardiol.* 2015; 31: 142-152.
- Powell Ag, Apovian Cm, Aronne Lj. New drug targets for the treatment of obesity. *Clin Pharmacol Ther.* 2011; 90: 40-51.
- Lim Dw, Kim Yt, Jang Yj, Kim Ye, Han D. Anti-obesity effect of *Artemisia capillaris* extracts in high-fat diet-induced obese rats. *Molecules.* 2013; 18: 9241-9252.
- Yun Jw. Possible anti-obesity therapeutics from nature--a review. *Phytochemistry.* 2010; 71: 1625-1641.
- Burton-Freeman Bm, Sandhu Ak, Edirisinghe I. Red Raspberries and Their Bioactive Polyphenols: Cardiometabolic and Neuronal Health Links. *Adv Nutr.* 2016; 7: 44-65.
- Lee D, Lloyd Nd, Pretorius Is, Borneman Ar. Heterologous production of raspberry ketone in the wine yeast *Saccharomyces cerevisiae* via pathway engineering and synthetic enzyme fusion. *Microb Cell Fact.* 2016; 15: 49.
- Leu Sy, Chen Yc, Tsai Yc et al. Raspberry Ketone Reduced Lipid Accumulation in 3T3-L1 Cells and Ovariectomy-Induced Obesity in Wistar Rats by Regulating Autophagy Mechanisms. *J Agric Food Chem.* 2017; 65: 10907-10914.
- Wang L, Meng X, Zhang F. Raspberry ketone protects rats fed high-fat diets against nonalcoholic steatohepatitis. *J Med Food.* 2012; 15: 495-503.
- Morimoto C, Satoh Y, Hara M, Inoue S, Tsujita T, Okuda H. Anti-obese action of raspberry ketone. *Life Sci.* 2005; 77: 194-204.
- Park Ks. Raspberry ketone increases both lipolysis and fatty acid oxidation in 3T3-L1 adipocytes. *Planta Med.* 2010; 76: 1654-1658.
- Lopez Hl, Ziegenfuss Tn, Hofheins Je, et al. Eight weeks of supplementation with a multi-ingredient weight loss product enhances body composition, reduces hip and waist girth, and increases energy levels in overweight men and women. *J Int Soc Sports Nutr.* 2013; 10: 22.
- Alzoubi Kh, Abdul-Razzak Kk, Khabour Of, Al-Tuweiq Gm, Alzubi Ma, Alkadhhi Ka. Adverse effect of combination of chronic psychosocial stress and high fat diet on hippocampus-dependent memory in rats. *Behav Brain Res.* 2009; 204: 117-123.
- Luvizotto Ra, Conde Sj, Sibio Mt, et al. Administration of physiologic levels of triiodothyronine increases leptin expression in calorie-restricted obese rats, but does not influence weight loss. *Metabolism.* 2010; 59: 1-6.
- Friedewald Wt, Levy Ri, Fredrickson Ds. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 1972; 18: 499-502.
- Drury Ra, Wallington Ea. *Histological techniques* 5th ed. Oxford University press. Oxford, N.Y., Toronto. 1980; 27-29.
- Shin Ss, Jung Ys, Yoon Kh, et al. The Korean traditional medicine gyeongshingangjeehwan inhibits adipocyte hypertrophy and visceral adipose tissue accumulation by activating PPARα actions in rat white adipose tissues. *J Ethnopharmacol.* 2010; 127: 47-54.
- Hancox Rj, Milne Bj, Poulton R. Association between child and adolescent television viewing and adult health: a longitudinal birth cohort study. *Lancet.* 2004; 364: 257-262.
- Ahima Rs, Flier Js. Adipose tissue as an endocrine organ. *Trends Endocrinol Metab.* 2000; 11: 327-332.
- Lehr S, Hartwig S, Sell H. Adipokines: a treasure trove for the discovery of biomarkers for metabolic disorders. *Proteomics Clin Appl.* 2012; 6: 91-101.
- Enos Rt, Velazquez Kt, Murphy Ea. Insight into the impact of dietary saturated fat on tissue-specific cellular processes underlying obesity-related diseases. *J Nutr Biochem.* 2014; 25: 600-612.
- Yida Z, Imam Mu, Ismail M, Ismail N, Ideris A, Abdullah Ma. High fat diet-induced inflammation and oxidative stress are attenuated by N-acetylneuraminic acid in rats. *J Biomed Sci.* 2015; 22: 96.
- Charradi K, Elkahoui S, Limam F, Aouani E. High-fat diet induced an oxidative stress in white adipose tissue and disturbed plasma transition metals in rat: prevention by grape seed and skin extract. *J Physiol Sci.* 2013; 63: 445-455.
- Skurk T, Alberti-Huber C, Herder C, Hauner H. Relationship between adipocyte size and adipokine expression and secretion. *J Clin Endocrinol*

- Metab. 2007; 92: 1023-1033.
32. Weisberg Sp, Mccann D, Desai M, Rosenbaum M, Leibel RL, Ferrante Aw, Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest. 2003; 112: 1796-1808.
33. Park S, Park Ny, Valacchi G, Lim Y. Calorie restriction with a high-fat diet effectively attenuated inflammatory response and oxidative stress-related markers in obese tissues of the high diet fed rats. Mediators Inflamm 2012; 2012: 984643.
34. Ding Q, Ash C, Mracek T, Merry B, Bing C. Caloric restriction increases adiponectin expression by adipose tissue and prevents the inhibitory effect of insulin on circulating adiponectin in rats. J Nutr Biochem. 2012; 23: 867-874.
35. Tilg H, Moschen Ar. Role of adiponectin and PBEF/visfatin as regulators of inflammation: involvement in obesity-associated diseases. Clin Sci (Lond). 2008; 114: 275-288.