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Saffron Extract and Crocin Reduced Biomarkers Associated with Obesity in Rats Fed a High-Fat Diet

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ABSTRACT

Introduction: This study aimed to investigate the effect of saffron extract and crocin on blood biomarkers associated with obesity using the rat model. **Methods:** Obesity was induced by feeding a high-fat diet to 42 male Sprague-Dawley rats for 12 weeks, after which they were equally distributed into seven groups. Three groups served as controls namely, normal diet (ND), high-fat diet (HFD), and high-fat diet plus orlistat (HFD + ORL), while the remaining four treatment groups consisted of HFD added low or high dose (40 and 80 mg/kg/day) of either saffron extract or crocin in the food. At the end of 8 weeks, blood samples were collected by cardiac puncture for biochemical analysis. **Results:** Obese rats treated with a high dose of saffron extract and crocin showed significantly lower plasma glucose levels (5.26 and 5.67 mmol/L respectively) than the HFD rats (6.92 mmol/L). Saffron extract and crocin at a high dose showed significantly lower levels of plasma insulin (3.97 and 3.88 ng/mL respctively) compared to HFD control (5.41 ng/mL). Adiponectin levels significantly increased in obese rats fed saffron extract and crocin at high doses (7.44 and 7.92 µg/mL respectively) compared to HFD control (5.34 µg/mL). Ghrelin level significantly increased from 419.10 to 284.10 pg/mL,while leptin level significantly decreased from 8.08 to 5.68 ng/mL for the high dose crocin groups compared to HFD control. No significant differences in plasma serotonin levels were found among the groups. **Conclusion:** Saffron extract and crocin show potential in reducing blood biomarkers associated with obesity as well as anti-inflammatory and regulatory potential of adipocytokines in an animal model.

Key words: Crocin, high-fat diet, obesity, orlistat, saffron

INTRODUCTION

Obesity is currently the most widespread metabolic disease in the world. It is a medical disorder in which excess body fat is stored in a range that may induce an untoward impact on health, leading to reduced life expectancy or increased health complications. Obesity is known to be linked with increased risk of cardiovas-

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cular disease, dyslipidaemia, impaired glucose tolerance, insulinaemia, type 2 diabetes, hypertension, polycystic ovary disorder, nonalcoholic fatty liver disease and certain types of cancer (World Health Organisation, 2000).

Obesity is associated with changes in an array of blood biochemical parameters that contribute to the pathogenesis of obesity with the greater part being overproduced in the course of this syndrome (Katsareli & Dedoussis, 2014). Obesity has been linked with a parallel rise in glucose metabolism. Impaired fasting glucose level has been connected with insulin resistance, dyslipidaemia, hypertension and abdominal obesity (Grundy, 1999; Rasouli & Kern, 2008). The link between obesity and insulin resistance is likely a causal relationship. Meanwhile, human and animal studies show that body weight loss or gain corresponds closely with rising or diminishing insulin sensitivity respectively (Abdalla, 2010; Xu *et al*., 2003, Ye, 2013). As obesity is considered a chronic inflammatory condition, a gathering of inflammatory biomarkers and endocrine function of the adipocytes comes about chronically dysregulated (Menzo, 2012; Roos, Quax & Jukema, 2012). Pro-inflammatory cytokines, for example, TNF-α and leptin, are upregulated in obesity (Ouchi *et al*., 2011). However, other anti-inflammatory mediators, for example, adiponectin is commonly downregulated (Juge-Aubry, Henrichot & Meier, 2005). Additionally, it is demonstrated that obesity is associated with the reduced level of gastric hormone, ghrelin, which gives a peripheral signal to the brain that induces adiposity in animals (Muccioli *et al*., 2002). Obesity is furthermore connected with prolonged lipid peroxidation and diminished antioxidants (Khan, Naz & Yasmeen, 2006).

Endogenous antioxidant enzymes, such as superoxide dismutase and catalase secure the body against reactive oxygen free radicals by scavenging spare superoxide. The level of antioxidant enzymes in individuals with obesity is lower compared to healthy people (Furukawa *et al*., 2004). A further biochemical compound that is associated with obesity is serotonin, a neurotransmitter that plays a critical role in the state of mind regulation and mental performance (Buhot, Martin & Segu, 2000). Optimal level of serotonin guarantee healthy gut-brain communication and hunger control through the delivery of particular satiety messages from the digestive tract to the brain (Ashor & Al-Gareeb, 2012). Since obesity increases peripheral serotonin (de Matos Feijó, Bertoluci & Reis, 2011), the suppression of serotonin signaling or its formation in fat tissue might be an effective approach for obesity treatment and its co-morbidity.

Crocin is a herbal carotenoid that constitutes the main component of the *Crocus sativus* flower known as saffron. It is a diester made from the disaccharide gentiobiose and the dicarboxylic acid crocetin. Crocin or saffron extract has been shown to possess several health properties (Christodoulou *et al*., 2015) such as antioxidant (Chen *et al*., 2008), hypolipidaemic (Sheng *et al*., 2006), hypotensive (Imenshahidi, Hosseinzadeh & Javadpour, 2010), hypoglycaemic (Mohajeri, Mousavi & Doustar, 2009) and satiety enhancer effects (Gout, Bourges & Paineau-Dubreuil, 2010). The rationale behind the potential role of crocin and saffron extract in the prevention of obesity is mostly related to its high antioxidant properties (Mashmoul *et al*., 2013). In the present study, we aimed to determine the effect of crocin and saffron extract on obesity-associated biochemical testing, including the plasma level of glucose, insulin, ghrelin, serotonin, leptin, adiponectin, TNF-α and catalase compared with orlistat in obese rats subjected to a high-fat diet.

METHODS

Materials

Saffron used in this study was of Iranian origin (Mashhad, Iran). Crocin powder

was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Orlistat (Xenical) was purchased from a local pharmacy. Rat insulin EIA kit (SPI-BIO, French), ghrelin ELISA (Abnova, Taiwan), serotonin ELISA kit (Abnova, Taiwan), leptin EIA kit (SPI-BIO, French), adiponectin (Abnova, Taiwan), TNF-α ELISA kit (Abnova, Taiwan) and catalase assay kit (Cayman Chemical, USA) were all supplied by i-DNA Biotechnology Sdn. Bhd. (Malaysia). Moreover, blood glucose was analysed by the hexokinase enzymatic reference method using the COBAS Integra® 800 automated analyser (Rocha Diagnostic, Basel Switzerland).

Preparation, extraction and quantification of saffron extract

Preparation and extraction of crude ethanolic extract of saffron were done according to our previously published method (Mashmoul *et al*., 2013). The presence of crocins including alpha-crocin, crocin 2, crocin 3, crocin 4, crocin 5 and crocin 6 was detected at 440 nm and safranal determined at 308 nm in the extract. The saffron extract used in this study contained 29 g/100 g DW (dry weight) of total crocins and 1.9 g/100 g DW of safranal (Mashmoul *et al.,* 2014). It is estimated that high dose (80 mg/kg body weight, BW) and low dose (40 mg/kg BW) of saffron extract treatment groups received daily 23.2 and 11.6 mg/kg BW of crocin respectively for the high and low doses.

Experimental diet

High-fat diet consisted of 560 kcal/100 g with 30% carbohydrate, 20% protein and 40% fat. Meanwhile, the normal rat diet had 385 kcal/100 g with 65% carbohydrate, 20% protein and 5% fat. Formulations of normal and high-fat diets are tabulated in Table 1. Beef tallow was used as the main source of fat in the high-fat diet and was given to the rats for 12 weeks to induce obesity. The normal rat diet referred to the normal control diet while high-fat diet and orlistat taken by rats served as negative and positive control, respectively. Crocin, as the bioactive compound and saffron extract were added to the high-fat diet and were fed to animals. Saffron extract and crocin were given in two different doses of 40 and 80 mg/kg BW.

Animal study design

All research protocols were approved by the Institutional Animal Care and Use Committee, Universiti Putra Malaysia with the approval number UPM/FPSK/PADS/BR-UUH/00473. Eight-week old male Sprague-Dawley rats (n = 42) weighing 200-250 g, were acclimatised for two

Table 1. Formulations of normal and high-fat diets

Ingredient	*Normal diet (g/kg diet)*	*High-fat diet (g/kg diet)*
Corn starch	650	150
Casein	200	200
Beef tallow	0	400
Corn oil	50	0
Sucrose	0	150
Cellulose	50	50
Mineral mix	35	35
Vitamin mix	10	10
DL-methionine	3	3
Choline bitarate	2	2

weeks at room temperature (22 ± 2°C) with the usual light-dark cycle. Following acclimatisation, all rats were randomly allocated into seven groups (n = 6 per group). The rats were given free access to food and water. After 12 weeks of obesity induction, obese rats received saffron extract and crocin treatment following treatment phase for 8 weeks. High-fat diet was continuously given to the rats to induce obesity during the treatment period of crocin and saffron extract at a low dosage (40 mg/kg) (HFD+L-CRO, HFD+L-SAF) and a high dosage (80 mg/kg) (HFD+H-CRO, HFD+H-SAF), via mixing with the diet. Normal control, negative control and positive control groups received normal diet (ND), high-fat diet (HFD) and orlistat (HFD+ORL) (20 mg/kg) respectively. The schematic diagram of this study is shown in Figure 1.

Body weight and food intake

Body weight was monitored weekly and food intake was recorded daily until the end of the experiment. Weighing time (8:00 am to 9:00 am) was fixed for all experimental days.

Blood sampling and biochemical test

Blood samples were taken from the overnight unfed rats into EDTA tubes via cardiac puncture. The tubes were centrifuged at 3000 rpm at 4°C for 10 min and plasma obtained was stored at −80°C until biochemical analysis. In this study, blood glucose was analysed by the hexokinase enzymatic reference method using the COBAS Integra® 800 automated analyser (Rocha Diagnostic, Basel Switzerland). Rat insulin EIA kit, ghrelin ELISA kit, serotonin ELISA kit, leptin EIA kit, adiponectin ELISA kit, TNF-α ELISA kit and catalase assay kit

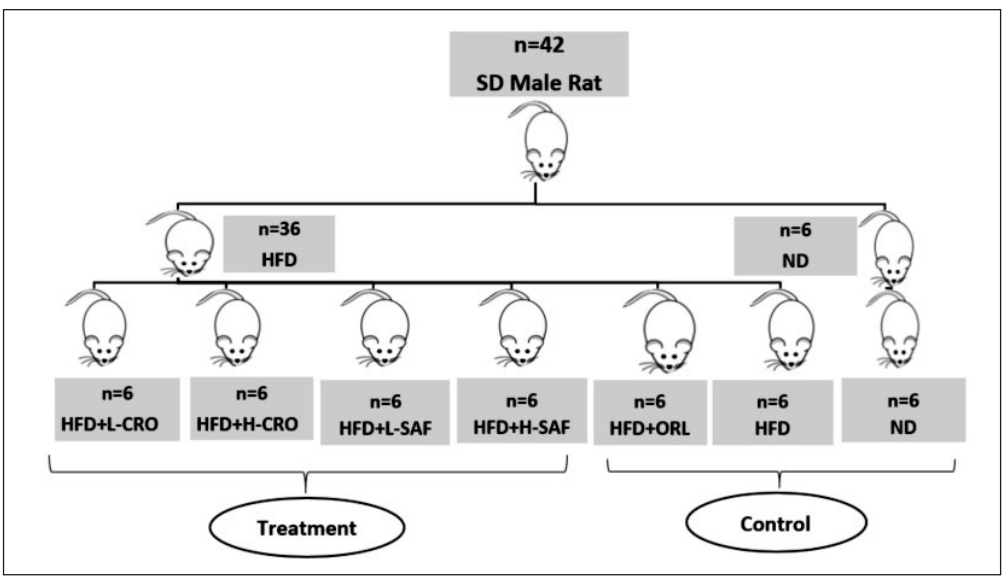

Figure 1. Animal study design. Sprague-Dawley(SD), normal diet (ND), high-fat diet (HFD), high-fat diet + crocin 40 mg/kg (HFD+L-CRO), high-fat diet + crocin 80 mg/kg (HFD+H-CRO), high-fat diet + saffron extract 40 mg/kg (HFD+ L-SAF), high-fat diet + saffron extract 80 mg/kg (HFD+H-SAF), high-fat diet + orlistat 20 mg/kg (HFD+ORL)

were used according to manufacturer's instructions.

Statistical analysis

Data were expressed as mean ± SEM (standard wrror of the mean). Data were analysed using one-way ANOVA by SPSS for Windows version 21. Duncan's multiple range test was used to test whether there were significant differences between the experimental groups. A significant difference was considered at $p<0.05$.

RESULTS

Effects on body weight and food intake

Body weight and food intake at the initial and end of the 8-week treatment of rats are shown in Table 2.

Effect on plasma blood glucose level

Obese rats exhibited a significantly higher ($p<0.01$) hyperglycaemia (6.92 ± 0.61 mmol/L) than normal control rats (5.42 ± 0.67 mmol/L) (Figure 2a). After the 8-week treatment, saffron extract dose-dependently decreased blood glucose level in the obese rats compared to the negative control rats (Figure 2). Both saffron extract ($p<0.001$) and crocin ($p<0.05$) at the high dose (80 mg/kg/day) significantly decreased glucose level compared to untreated obese rats (Figure 2a).

Effect on plasma insulin level

As shown in Figure 2b, there was a significant increase ($p<0.05$) in insulin level between the normal and HFD control

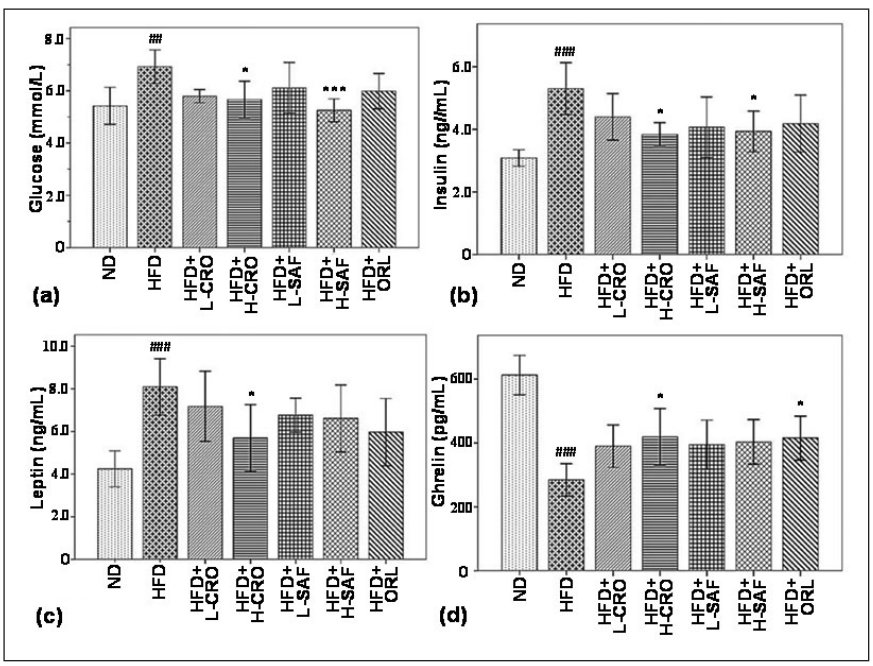

Figure 2. Mean concentrations of (a) glucose, (b) insulin, (c) leptin and (d) ghrelin, in normal diet (ND), high-fat diet (HFD), high-fat diet + crocin 40 mg/kg (HFD+L-CRO), high-fat diet + crocin 80 mg/kg (HFD+H-CRO), high-fat diet + saffron extract 40 mg/kg (HFD+ L-SAF), high-fat diet + saffron extract 80 mg/kg (HFD+H-SAF) and high-fat diet + orlistat 20 mg/kg (HFD+ORL) after the period of treatment (8 weeks).

Each value is the mean ± SEM. Values of the high-fat diet group were significantly different compared to normal control: ###$p<0.001$, ##$p<0.01$ and #$p<0.05$, and values of treatment group were significantly differed from high-fat diet group (negative control): ***$p<0.001$, **$p<0.01$ and *$p<0.05$.

Table 2. Changes in body weight and food intake at initial and at the end of 8-week treatment

Group[a]	*Food intake (g/kg BW)*				*Body weight (g)*			
	Week-0	*Week-8*	*Change*	*p value*	*Week-0*	*Week-8*	*Change*	*p value*
ND	380.1±9.24	420.1±10.1	40	0.02	132.4±1.2	132.3±3.7	-0.1	0.85
HFD	505.1±17.5	575.8±23.1	70.7	0.02	115.6±3.8	120.0±1.6	4.4	0.85
HFD+L-CRO	506.8±24.3	563.8±31.5	57	0.25	106.8±3.2	104.7±0.2	-2.1	0.23
HFD+H-CRO	505.1±24.2	524.0±16.6	18.9	0.06	114.2±2.3	103.6±1.5	-10.6	0.01**
HFD+L-SAF	504.3±28.7	556.1±35.9	51.8	0.09	111.3±6.6	103.5±2.4	-7.8	0.12
HFD+H-SAF	504.6±22.5	555.5±22.6	50.9	0.10	114.5±5.6	100.5±0.5	-14	0.01**
HFD+ORL	504.6±18.1	501.8±21.6	-2.8	0.001***	102.0±4.7	124.3±3.3	22.3	0.001***

Significance level: ***p<0.001, **p<0.01

normal diet (ND), high-fat diet (HFD), high-fat diet + crocin 40 mg/kg (HFD+L-CRO), high-fat diet + crocin80 mg/kg (HFD+H-CRO), high-fat diet + saffron extract 40 mg/kg (HFD+L-SAF), high-fat diet + saffron extract 80 mg/kg (HFD+H-SAF), high-fat diet + orlistat 20 mg/kg (HFD+ORL).

groups (3.08 ± 0.25 ng/mL and 5.3 ± 0.79 ng/mL respectively). The 80 mg/kg doses of saffron extract and crocin significantly (p<0.05) decreased insulin levels of the rats to 3.93 ± 0.62 ng/mL and 3.84 ± 0.29 ng/mL respectively compared to the negative control (Figure 2b).

Effect on plasma leptin level

Plasma leptin level of the HFD group (8.08 ± 1.25 ng/L) was elevated significantly compared with the normal control ND rats (4.23 ± 0.8 ng/mL). As shown in Figure 2c, crocin treatment significantly (p<0.05) decreased leptin level up to 5.68 ± 1.4 ng/mL at the dose of 80 mg/kg compared to untreated groups.

Effect on plasma ghrelin level

After obesity induced by the HFD, plasma ghrelin level was lower in the HFD (284.10 ± 48.19 pg/mL) compared to normal control (612.81 ± 58.62 pg/mL). More importantly, the ghrelin level increased significantly (p<0.05) to 419.10 ± 83.95 pg/mL after the 8-week administration of 80 mg/kg of crocin (Figure 2d).

Effect on plasma adiponectin level

Untreated obese rats showed significant decreases in the plasma adiponectin level (5.34 ± 0.64 µg/mL) compared to the normal control (10.16 ± 1.6 µg/mL) (Figure 3a). High dose (80 mg/kg/day) of crocin (7.92 ± 0.85 µg/mL, p<0.01) and saffron extract (7.44 ± 0.76 µg/mL, p<0.05) significantly increased the plasma levels of adiponectin. However, orlistat showed a more significant (p<0.001) difference in plasma adiponectin level in positive control rats (Figure 3a).

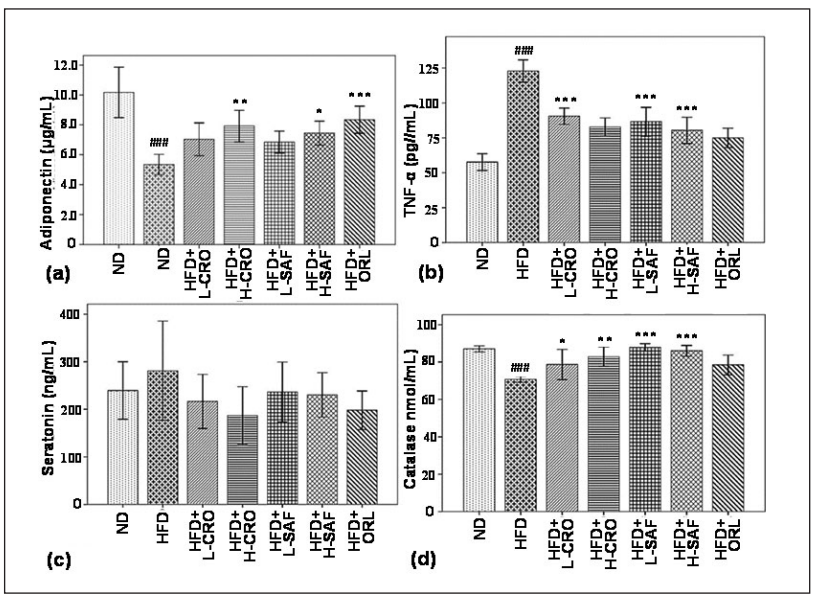

Figure 3. Mean concentrations of (a) adiponectin, (b) TNF-α, (c) serotonin and (d) catalase in normal diet (ND), high-fat diet (HFD), high-fat diet + crocin 40 mg/kg (HFD+L-CRO), high-fat diet + crocin 80 mg/kg (HFD+H-CRO), high-fat diet + saffron extract 40 mg/kg (HFD+L-SAF), high-fat diet + saffron extract 80 mg/kg (HFD+H-SAF) and high-fat diet + orlistat 20 mg/kg (HFD+ORL) after the period of treatment (8 weeks).

All values are expressed as mean ± SEM. Values of high-fat diet group were significantly different compared to normal control: ###p<0.001, ##p<0.01 and #p<0.05, and values of the treatment group significantly differed from high-fat diet group (negative control): ***p<0.001, **p<0.01 and *p<0.05.

Effect on tumour necrosis factor-alpha (TNF-α)

Plasma TNF-α level was markedly (p<0.001) increased in obese rats (122.8 ± 7.61 pg/mL) compared with normal control (57.5 ± 5.64 pg/mL). Interestingly, the plasma TNF-α level decreased significantly (p<0.001) from 86.53 ± 9.8 to 80.35 ± 8.9 pg/mL after the 8-week administration of 40 and 80 mg/kg saffron extracts. The same doses of crocin also markedly (p<0.001) decreased the plasma TNF-α level of up to 90.4 ± 5.54 and 82.8 ± 6.1 pg/mL respectively (Figure 3b).

Effect on plasma serotonin level

As shown in Figure 3c, HFD increased the serotonin level in negative control (281.1 ± 99.49 ng/mL) compared to the normal control (239.48 ± 57.5 ng/mL). However, there was no significant difference in serotonin level between the treated and control groups (p>0.05).

Effect on plasma catalase level

There was a significant decrease in catalase (CAT) level between the obese control group and normal control group (70.8 ± 1.32 and 87.1 ± 1.2 nmol/mL respectively) (p<0.001). CAT activity was significantly increased in the rats receiving 40 and 80 mg/kg of saffron (87.8 ± 1.67 and 85.9 ± 2.7 nmol/mL respectively) compared with the untreated obese group (p<0.001). Supplementation with the low and high concentrations of crocin for two months also significantly increased (p<0.05) CAT activities in the treated rats (78.6 ± 7.7 nmol/mL) compared with negative control group (82.8 ± 4.19 nmol/mL) (Figure 3d).

DISCUSSION

Our experiment showed that crocin (80 mg/kg) was able to significantly suppress the body weight gain in obese rats fed with the HFD. Saffron extract (80 mg/kg) also reduced the food intake over the 8-week treatment period. This result indicates that although the anti-obesity activity of the saffron plant contributed mostly by its bioactive compound crocin, current evidence indicates that food intake and satiety signaling are stimulated by the other bioactive compounds of saffron extract rather than crocin.

We also considered the effect of crocin and saffron extract on changes in clinical biochemical measurements that are associated with obesity and insights into pathophysiology underlying this major health issue in an animal model of HFD-induced obesity. The results obtained from this study indicated the effective plasma glucose and insulin ameliorative properties of saffron extract and crocin rather than orlistat in the obese animal model. Our results are inconsistent with previous studies demonstrating the potent hypoglycaemic effect of saffron (Mohajeri, Mousavi & Doustar; 2009; Shirali, Zahra Bathaie & Nakhjavani, 2013). The mechanism of hypoglycaemic actions of saffron through investigating its signaling pathways associated with glucose metabolism in skeletal muscle cells has been studied (Vendrell *et al.*, 2004). According to their results, the co-treatment of saffron and insulin further improved the insulin sensitivity via both insulin-independent and insulin-dependent pathways. It was also proposed that there was interference between the two signaling pathways of glucose metabolism in skeletal muscle cells.

Pro-inflammatory cytokines (such as TNF-α) are known to increase in pathological states that are associated with obesity (Menzo, 2012). TNF-α has been involved in the regulation of energy balance and is considered as the potent pro-inflammatory mediator, with explicit effects over many of the hormonal factors produced by the adipose tissue (2004). In our study, we found higher levels of TNF-α in the HFD than in normal diet groups. This increased pro-inflammatory state was markedly ameliorated after the 8-week treatment with saffron

extract and crocin. A significant inhibition of TNF-α level by saffron observed in the recent study contributed to beneficial effects in diabetic encephalopathy (Samarghandian, Azimi-Nezhad & Samini, 2014).

Adiponectin is a protein hormone that regulates some metabolic pathways, including glucose metabolism and fatty acid oxidation. It is particularly secreted from adipose tissue into the bloodstream and is extremely abundant in plasma relative to many other hormones. The levels of adiponectin are inversely correlated with body fatness in adults (Vendrell *et al*., 2004). Leptin, the "satiety hormone", is a hormone produced by adipose tissue that helps to modulate energy homoeostasis by inhibiting appetite. Leptin is diametrically opposed to the actions of the hormone ghrelin, the "hunger hormone" which is produced by ghrelinergic cells in the gastro-intestinal tract and functions as a neuropeptide in the central nervous system. Both hormones act on receptors in the hypothalamus to control appetite to get energy homoeostasis. In obesity, a declined sensitivity to leptin happens, leading to failure in detecting satiety although high energy is stored. Leptin level is closely correlated with the body fat percentage while ghrelin inversely correlated with body mass index (Vendrell *et al*., 2004). The current investigation showed that the administration of 80 mg/kg of crocin caused a significant decrease in plasma leptin while leading to a major increase in ghrelin and adiponectin levels. The findings demonstrate that crocin at 80 mg/kg can inhibit body fat gain and regulate glucose and energy homoeostasis through modulating the biochemical pathways of producing adipocytokines, leptin and adiponectin as well as ghrelin formation *in vivo.* Xi *et al. (*2007) also reported the positive impact of crocetin on adiponectin, TNF-α and leptin expression in white adipose tissue which was suggested to be involved in the improvement of insulin sensitivity observed in fructose-fed rats.

Evidently, there are two types of serotonin, the type that is known for its effect on mood and appetite only accounts for 5% of serotonin in the body while the other 95%, called peripheral serotonin, plays an important role in obesity (Crane *et al*., 2015). Apparently, too much peripheral serotonin in the blood inhibits the brown fat that burns energy and glucose to make heat which leads to obesity and the development of diabetes. In the present study, level of plasma serotonin did not differ significantly among the treated groups of crocin and saffron extract suggesting that the mechanism of anti-obesity action of saffron might not have contributed to the modifications of peripheral serotonin in the model of obese rats induced by HFD. Interestingly, a recent study by Ettehadi *et al.* (2013) concerning anti-depressant properties of saffron extract in animals found that saffron extract had no effect on brain serotonin or norepinephrine concentration. They also showed that saffron can induce production of important neurotransmitters in the brain, namely dopamine and glutamate.

Overall findings from the blood biochemical analyses in this study are in line with the anthropometrical and nutritional outcomes from our recent published work (Mashmoul *et al*., 2014) demonstrating that antioxidant-rich saffron has the potential to modulate obesity and related metabolic disorders such as hyperglycaemia, hyperlipidaemia, insulinaemia and pro-inflammatory-derived complications. It is suggested that the principal mechanisms involved are contributed by its high antioxidant and anti-inflammatory activities which can be classified into four major groups: (1) reducing energy intake via blocking dietary fat absorption by inhibiting pancreatic lipase; (2) acting as an antioxidant and regulating pro-inflammatory adipocytokines such as leptin, adiponectin and TNF-α; (3) suppressing food intake due to increasing satiety hormone or the level of neurotransmitters such as dopa-

mine; and (4) improving glucose uptake and insulin signaling.

CONCLUSION

The present study showed the pharmacological effect of saffron extract in the obese animal model. Dietary saffron and crocin have positive effects on modulating several biochemical pathways of endocrine and adipocytokine excretions which may potentially be applicable for suppression of obesity and its metabolic complications as well. Much more work is evidently needed before phytotherapy for obesity and metabolic disorders could progress to clinical trials.

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Conflict of interest

All authors declare no conflict of interest.

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