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Medicinal effects of Saffron on obese animals by reducing Castelli's risk index to protect from cardio-pathology and psychological distress

Saara Ahmad[1*], Hamna Rafiq[1], Asra Khan[1], Prashant Tikmani[1], Saima Khaliq[2], Saad Bilal Ahmed[3] and Muhammad Misbah ur Rehman[4]

[1]Department of Biological and Biomedical Sciences, the Aga Khan University, Karachi, Pakistan.
[2]Department of Biochemistry, Federal Urdu University of Science, Arts and Technology, Karachi, Pakistan.
[3]Department of Geriatrics, Saint John of God Health Care, Australia.
[4]Department of Surgery, Hussain Lakhani Hospital, Karachi, Pakistan.

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A high-fat diet is a major factor to the global obesity and diabetes epidemic. The mechanism underlying chronic fat-rich food exposure may raise cholesterol and inflammatory mediator levels. One of the key pathological hallmarks of cardiovascular disorders is the development of inflammatory markers (CVDs). The pathological action may also inhibit the insulin secretion, which triggers diabetes-associated psychological distress. Saffron is used for the beneficial effects on different diseases. Therefore, the presented study was conducted to assess the therapeutic profile of saffron on cardio-pathology and neuropsychology in rats, subjected on high-fat diet. The study used Sprague-dawley divided in normal diet (healthy control), high-fat diet treated control animals (HFDCs) and high-fat diet + Saffron (10 mg/kg). After two weeks of treatment, Castelli's risk index, glucose, and insulin levels were analysed, followed by cervical dislocation. Saffron supplements resulted in a lower Castelli's risk index and an increase in insulin level with normoglycemic effects, implying cardioprotective and antidiabetic benefits.

Key words: Cardiopathology, Castelli's risk index, depression, cholesterol, insulin, saffron.

INTRODUCTION

Cardiovascular diseases (CVDs) and depression are the leading causes of mortality and morbidity worldwide. It is known to evolve from multiple factors such as dyslipidemia, inflammation and endothelial dysfunction, the hallmark of CVD. Depression entails a cluster of transient emotions including distressed feeling and flat mood that may become severe enough to expose clinical symptoms (Mäkinen et al., 2014). It has been reported in many clinical trials and aetiological studies that depression is more frequently seen in Patients with CVD.

*Corresponding author. E-mail: saara_ahmad@hotmail.com, saara.muddasir@aku.edu. Tel: +92-21-36642030, +92-334-3448258.

The emerging trend of fat rich diets nowadays has escalated the occurrence of CVD. Fat rich diets contain trans fatty acids that alter lipid regulation crucial to maintain homeostasis of physiological systems. Augmented concentration of *trans*-fats in high fatty diets (HFD) may lead to interruption of normal mechanism and initiates risks of chronic diseases due to dyslipidemia and generation of inflammatory cytokines (Mayberry et al., 2013). Dyslipidemia is mainly characterized as the elevated levels of total cholesterol, low density lipoprotein cholesterol (LDL-C), triglycerides and low levels of high-density lipoprotein cholesterol (HDL-C). Furthermore, the HFD are also responsible for induction of stress induced activation of hypothalamic-pituitary-adrenal axis (HPA axis) which stimulate the production of glucocorticoids and long term exposure of HPA axis may contribute to vicious cycle of increased inflammatory mediators with augmented risk of depression and severity in CVDs (Liu et al., 2013).

The Castelli risk index-I (CRI-I) has a significant diagnostic value comparable to total cholesterol assessment. It reflects production of coronary plaques and has a good correlation with insulin resistance (IR). IR is characterized as the impaired activation of insulin in liver muscles and adipocytes and appeared as metabolic disturbances (Vargas et al., 2014). A fat rich diet may elevate the level of lipolysis a key contributor of IR in adipocytes. As a result, free fatty acid turnover is enhanced, resulting in raised circulating levels, which trigger the pathogenic processes of CVDs and depression (Li et al., 2015).

The management of CVDs involves therapeutics known as beta blockers, calcium channel blocker and anticoagulants; however, for depression antidepressant like selective serotonin reuptake inhibitors (SSRIs) are the most often. It is also important to mention here that some of the CVDs medications are reported to cause depressive symptoms as a side effect and may exaggerate the clinical symptoms of depression. Likewise, there are many controversial studies providing evidence of compromised cardiac health by the use of antidepressants (De Souza et al., 2015). As a result, the use of natural herbs as medicine, which has been practiced for millennia, has recently advanced in current CVD and depression treatments. Researchers are interested in studying the possible impacts of numerous natural substances and their bioactive compounds, which operate on several targets to alleviate chronic illnesses and their comorbidities.

Crocus sativus L. is a useful spicy herb for the treatment of various ailments. Many clinical studies demonstrated its efficacy against depression and found it quite comparable with SSRI (Fluoxetine) as the administration of saffron increases the level of monoamine neurotransmitters in the synapse (Faridi et al., 2019). Emerging research also indicates that saffron has a significant potential to prevent CVDs by inhibiting platelet aggregation and lowering LDL-C, since herbs with medicinal characteristics are now widely regarded as an important treatment option with fewer side effects. The main chemical constituents of saffron, causing modulation of the monoamine levels in brain and reduction of LDL-c in the body, are crocetin, crocin, saffranal, carotenoids, lycopene, anthocyanins and saponins (Lopresti and Drummond, 2014).

Likewise, saffron, is known for its natural antioxidant properties. The existence of anthocyanins has gotten a lot of attention in this respect, and they, together with other ingredients, allow for a reduction in prostaglandin production by inhibiting the cyclooxygenase 1 and 2 enzymes. Anthocyanins have also been shown to inhibit the endoplasmic reticulum's ability to create inflammatory cytokines (Gwarzo et al., 2014). Therefore, the present study intended to evaluate the therapeutic effects of saffron in rats fed with high fat diet in related with neuropsychology and cardio-protection.

METHODOLOGY

Collection of plant

Dried stamens of saffron were bought from local market of Karachi. Specimen was preserved in herbarium with voucher number CS-ST-08-18-05 in Natural Products Research Division, Department of Biological and Biomedical Sciences, The Aga Khan University, Karachi.

Methanolic extract preparation

After cleaned of adulterants, weighed approximately 40 g saffron were soaked in 2 L of 70% methanol for 3 days with intermittent shaking followed by filtration with muslin cloth and then “whatman qualitative grade-1 filter paper.” All filtrate were then combined and subjected to rotary evaporator in order to concentrate the filtrate with maintained temperature 35-40°C and the subnormal pressure. The ultimate product was crude (Wt.Cr) with 13% yield (wt/wt) was obtained which was soluble in saline as well as in distilled water. Fresh dilutions were prepared every time prior the administration.

Experimental animals

The experiment was carried out using eighty 200 g Sprague-dawley rats from the Aga Khan University's animal house in Karachi, according to a protocol authorized by the Ethical Committee of Animal Care and Use under number 68 ECACU-BBS-17. The guidelines made by National Institute of Health (NIH) Care and Use of laboratory animals were also strictly followed (Publication No. 85-23, revised 1985. All animals were kept in clean plastic cages in a strictly regulated environment with a 12 h light/dark cycle and a temperature of 22°C, with free access to a standard rodent feed and clean tap water. Animals were individually treated respective to their group name as Normal diet (Healthy control), High fat diet treated control animals (HFDC) and High fat diet+ Saffron (Saff). Saffron were given to animals individually at dose 10 mg/kg (orally).

Composition of normal and high fat diet

Normal diet (ND) was developed at the animal house of Aga Khan

University. ND consists of (gram of dietary constituents per kilogram body weight of rats): fiber 380, flour 380, molasses 12, powdered milk 150, sodium chloride 5.8, vegetable oil 38, potassium metabisulphate 1.2, nutrivet-L 2.5, and fish meal 170.

In addition to typical diet components, a high fat diet (HFD) were constructed using a combination of cholesterol, cholic acid, and butter fat (2, 0.5, and 5% w/w, respectively).

Experimental protocol

Animals were randomly divided into 3 group (n=10) as healthy control (HC), HFDC and High fat diet test group of methanolic extract of saffron (Saff). All animals treated according to their groups for two weeks. At the end of experiment blood and brain samples of animals were collected through cervical dislocation. Blood samples were collected in heparin-containing test tubes and stored at room temperature for 30 min before being centrifuged at 15,000 g for 10 min. Brain samples were promptly washed with saline and kept at -20°C until analysis.

Behavioral assessment

Forced swim test (FST)

Forced swim test is the assessment of animal depressive behavior. The apparatus for this test was consist of a container with 56 cm height and 30 cm width. The container was filled with water at the height of 22 cm and temperature of 25°C. The depth of water was adjusted to avoid the contact of tail to the bottom as well as to escape from the apparatus. Animals were subjected to swim and struggling time (Latency to immobilize) recorded for six minutes.

Biochemical estimations

Estimation of blood TG/LDL/HDL-cholesterol

The determinants of lipid content of TG/LDL/HDL-cholesterol were done by using Roche kits Cobas c 111 (Roche, Pakistan).

Determination of Castelli's risk index

The indices were calculated using the following formulae:

Castelli's risk index-I (CRI-I) = TC/HDL-c
Castelli's Risk Index (CRI-II) = LDL-c/HDL-c.

Estimation of insulin

Insulin level (U/l) determination was done by Ultra-Sensitive Rat Insulin ELISA Kit (Crsytal Chem, USA).

Estimation of fasting blood glucose levels

The animals were fasted overnight, and blood was collected from their tails to calculate fasting blood glucose levels (mmol/lit) using an Accu-Check Performa glucometer.

Estimation of brain derived neurotropic factor (BDNF) level

Serum BDNF was quantified using an ELISA method (Cloud Clone Corp. BDNF Sandwich ELISA kit, SEA011Mi, USA) following the instructions of the manufacturer.

Statistical analysis

Results are represented as mean ± SD. Data of behavioral and biochemical analysis evaluated by one-way ANOVA of SPSS version 20. Tukey's test was used for post hoc analysis. Non-significant was defined as p >0.05.

RESULTS

Effects on Castelli's risk index

Figure 1 presents the effects of saffron for two weeks on CRI-I in high fat diet treated rats. Statistical analysis by one-way ANOVA revealed a significant effect of saffron ($F_{2.27}$ = 33.5, p<0.05). The *post hoc* Tukey's test revealed that the ratio of TC/HDL-cholesterol was significantly higher in high fat diet treated rats (HFDC) than normal diet treated rats (HC) (p<0.05). However, the saffron group showed significantly (p<0.05) lower CRI-I than HFDC group.

Figure 2 presents the effects of saffron for two weeks on CRI-II in high fat diet treated rats. Statistical analysis by one-way ANOVA revealed the significant effect of saffron on CRI-II ($F_{2.27}$ = 59.795; p<0.05). The Tukey's test showed that the ratio of LDL/HDL-cholesterol was significantly higher in high fat diet treated rats (HFDC) than normal diet treated rats (HC) (p<0.05). The effects of saffron reduced the CRI-II in Saff group, the effects were significant in HFDC (p<0.05) and non-significant relation was found between HC and Saff group.

Effects on insulin level

Figure 3 presents the effects of saffron for two weeks on insulin in high fat diet treated rats. The effect of therapy on insulin ($F_{2.27}$ = 41.99; p0.05) was significant, according to statistical analysis using ANOVA (one-way). The level of insulin in HFDC was substantially greater than in HC (p<0.05) after post hoc analysis using Tukey's test. However, saffron was significantly (p<0.05) reduced the insulin level than HFDC rats.

Effects on glucose level

Figure 4 presents the effects of saffron for two weeks on glucose in high fat diet treated rats. Statistical analysis by ANOVA (one-way) revealed that the effect of treatment on the glucose ($F_{2.27}$ = 10.740; p<0.05) was significant. Results of *post hoc* analysis by Tukey's test revealed that long term consumption of high fat diet in HFDC group increased the level of glucose (p<0.05) than HC group. However, saffron reduced the glucose level than HFDC rats.

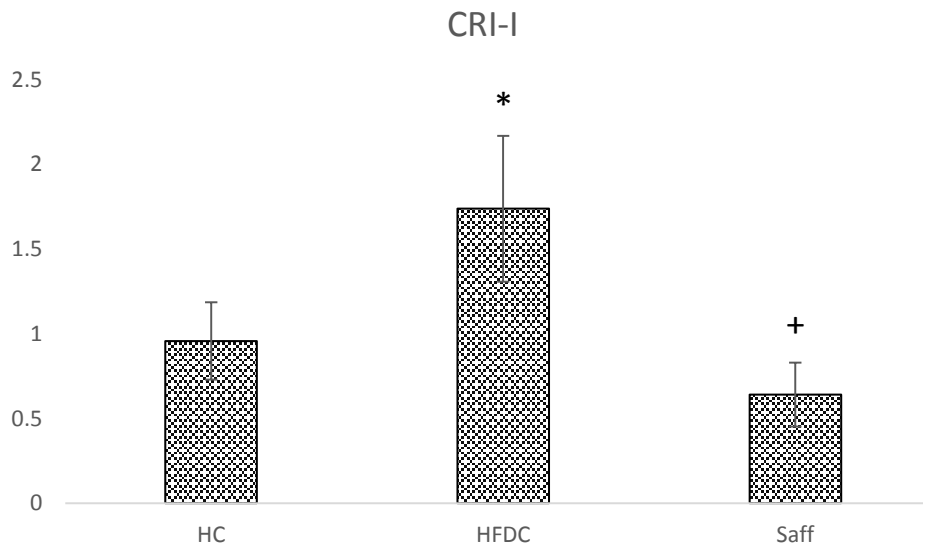

Figure 1. Effects of saffron on CRI-I in High fat diet treated rats. Values are presented as means ± SD (n=10). Tukey's test Significant values:* p<0.05 from control HC group, + p<0.05 from HFDC group

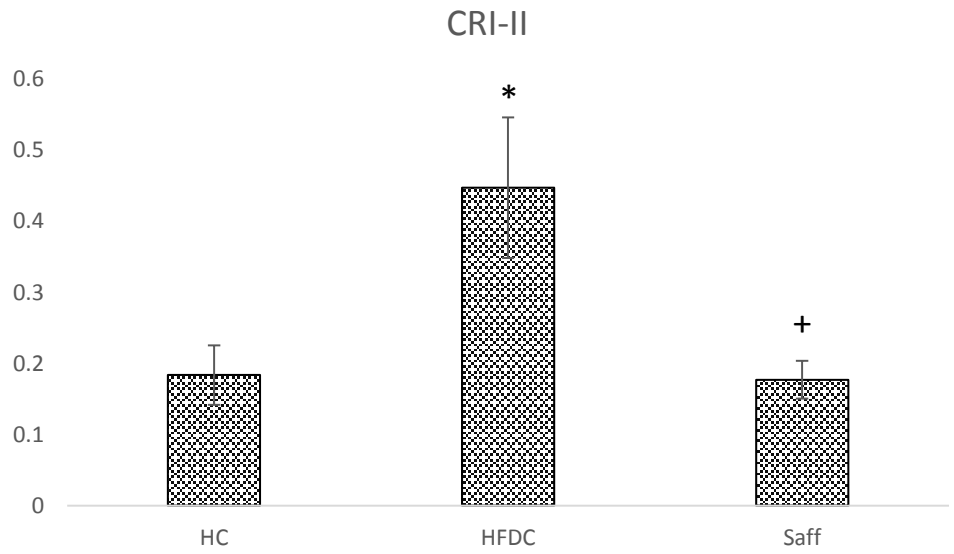

Figure 2. Effects of saffron on CRI-II in High fat diet treated rats. Values are presented as means ± SD (n=10). Tukey's test Significant values:* p<0.05 from control HC group, + p<0.05 from HFDC group.

Effects on FST

Figure 5 presents the effects of saffron for two weeks on FST in high fat diet treated rats. Statistical analysis by one-way ANOVA revealed the effect of saffron ($F_{2.27}$ = 40.9, p<0.05) was significant. The *post hoc* Tukey's test revealed that the latency time to immobilize was significantly reduced after the consumption of high fat diet in HFDC group as compared to normal diet treated rats (HC) (p<0.05). However, the saffron group showed significantly (p<0.05) increased latency time to immobilization time in HFDC group.

Effects on BDNF level

Figure 6 presents the effects of saffron for two weeks on BDNF in high fat diet treated rats. Statistical analysis by

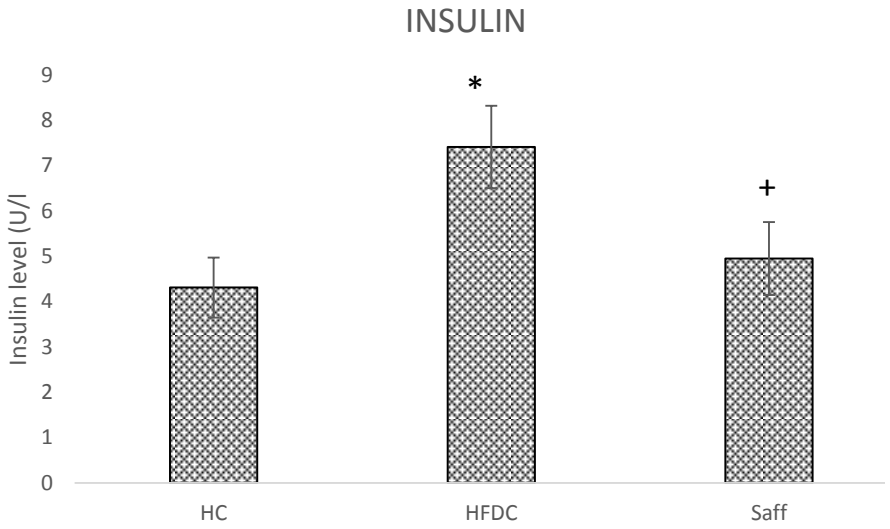

Figure 3. Effects of saffron on insulin in High fat diet treated rats. Values are presented as means ± SD (n=10). Tukey's test Significant values:* p<0.05 from control HC group, + p<0.05 from HFDC group.

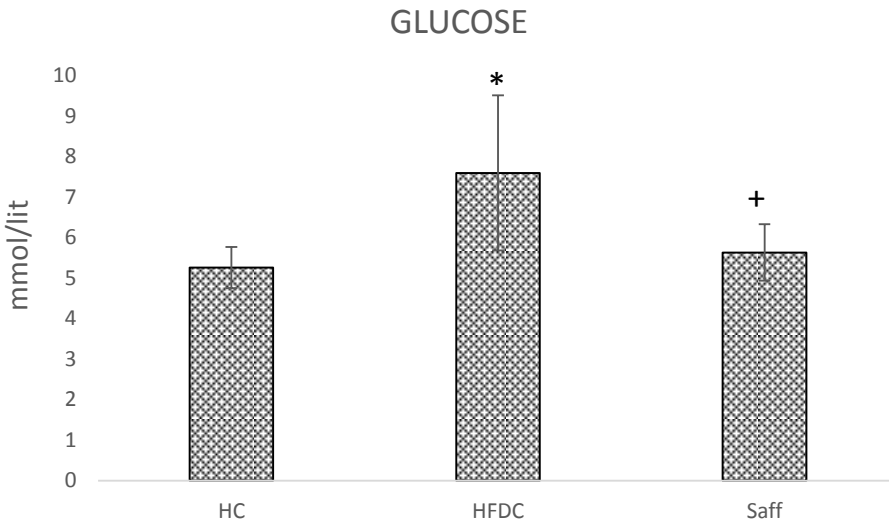

Figure 4. Effects of saffron on glucose in High fat diet treated rats. Values are presented as means ± SD (n=10). Tukey's test Significant values:* p<0.05 from control HC group.

one-way ANOVA revealed the effect of saffron ($F_{2.27}$ = 67.3, p<0.05) was significant. The *post hoc* Tukey's test revealed that the consumption of high fat diet reduced the BDNF level in HFDC group as compared to normal diet treated rats (HC) (p<0.05). The long term administration of saffron showed significantly (p<0.05) increased BDNF level in HFDC group and provided the antidepressant effects.

DISCUSSION

Insulin resistance, diabetes, obesity, and CVDs are all linked to a high-fat diet, particularly trans-fats. CVDs are becoming a serous life threatening cause of modern ages and they offer a challenging task for clinical managements. Beside the chronic metabolic diseases the behavioral deficits may also appear which mainly include depressive symptoms. The aim of the presented study was to be evaluated the effects of methanolic extract of saffron to treat high fat diet induced metabolic and neuropsychological deficits.

The long term consumption of high fat diet reduces the brown adipocytes which inhibit the synthesis of fatty acid while increasing white adipocytes which stimulates the synthesis of fatty acid and dysregulate the levels of

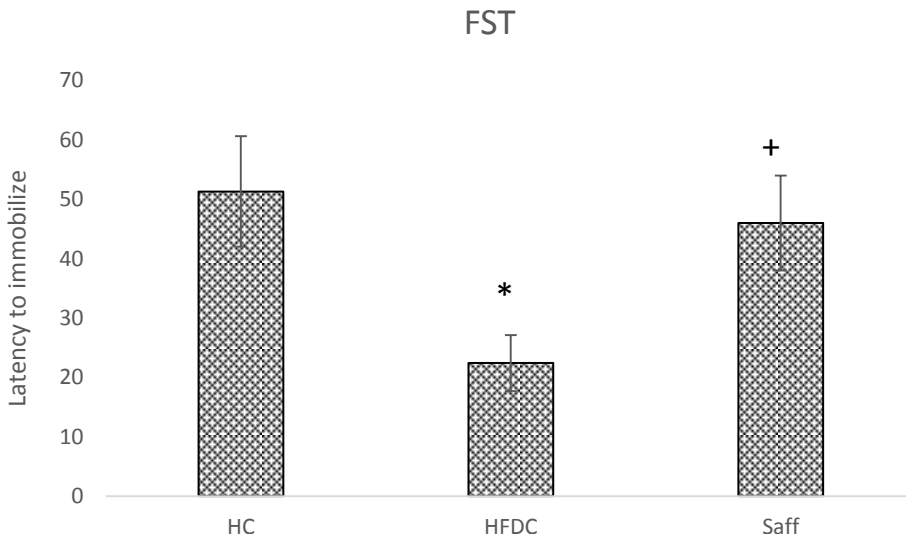

Figure 5. Effects of saffron on FST in High fat diet treated rats. Values are presented as means ± SD (n=10). Tukey's test Significant values:* p<0.05 from control HC group.

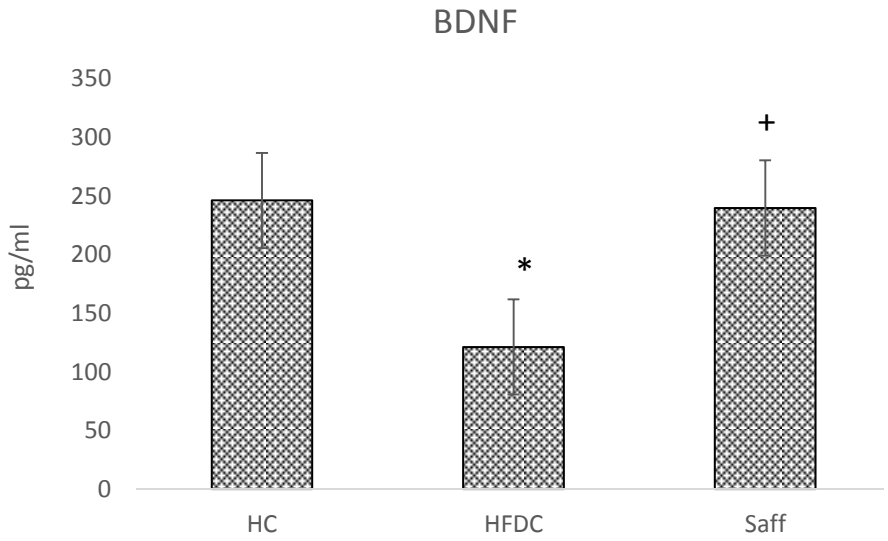

Figure 6. Effects of saffron on BDNF in High fat diet treated rats. Values are presented as means ± SD (n=10). Tukey's test Significant values:* p<0.05 from control HC group.

insulin. The pathological alterations of high fat diet in terms of hyperglycemia and hyperlipidemia reflect altered psychological behavior of animals. Variations in glycemic parameters occur when the insulin become unable to exert its affects. Excessive calories due to obesogenic diet decline the insulin synthesis and functions by the accumulation of fat in pancreatic cells. Instead of adipocytes, saturated fats accumulate in other organs, interfering with glucose metabolism. Excess fat buildup in organs causes oxidative stress by raising lipid peroxidation levels due to mitochondrial dysfunction.

The active chemical ingredient of saffron is crocin, which contains four analogues: crocin 1, crocin 2, crocin 3 and crocin 4. Crocin is mainly known to relieve the pain and to improve the blood circulation. Crocin is bearing multiple therapeutic activities including antioxidant, cardioprotective, antidepressant and anticonvulsant effects. The extract of saffron is known to protect against the damage of lipid peroxidation, the present study provided the positive effects of saffron on CVDs by lowering the Castelli's risk index (Figures 1 and 2). The doses of saffron also found to produce antidiabetic effects by augmentation of insulin level, the mechanism behind the antidiabetic effects is the stimulation of

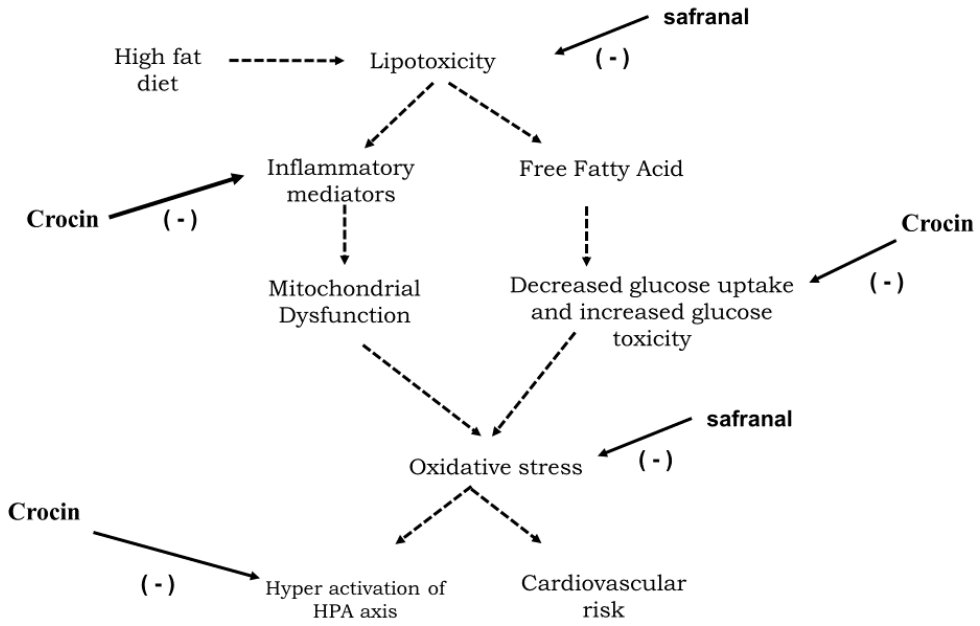

Figure 7. Summarized effects of saffron extract on high fat diet and its manifestations. (-) signs shows the inhibitory effects of saffron ingredients.

peripheral glucose uptake and liver glycogen storage. The effects of chemical constituents of saffron displayed the potential radical scavenging properties and protective effects of vital tissues as well as regenerative activity which may trigger the pancreas to produce insulin for maintaining the glucose homeostasis (Figures 3 and 4).

Long-term consumption of saturated fats disrupts the hippocampal receptor that regulates inflammatory cytokines, which are important for detecting pathogenic causes (Hanke and Kielian, 2011). Previous study has also shown that the HFD causes the creation of triglycerides, which are harmful to brain health and cause neuropsychiatric illnesses, and are detected in people with bipolar disorder (van Dooren et al., 2013). Saffron has been shown to lower oxidative stress and boost mitochondrial activity (Fathimoghadam et al., 2019). In numerous *in-vitro* and *in-vivo* investigations, prolonged doses of saffron were linked to improved mental health, demonstrating higher antidepressant and cognitive function after saffron administration in mice. Depression is also linked to an increase in insulin resistance and the Castelli's index, which is a powerful predictor of coronary artery disease (Kianbakht and Mozaffari, 2009). Depression is also linked to changes in the expression of neurotrophic factors, particularly brain derived neurotrophic factors (BDNF), which play a key role in neuronal growth and plasticity. The levels of BDNF draw broad attention in many clinical and s studies which indicate that the progression of depression is inversely related with BDNF expression and most of the antidepressant reduces the depression by increasing the levels of BDNF (Sanati et al., 2018). The measuring parameters of depression in rodents are mainly involve the time to show behavioral despair, the forced swim test (FST) is the most widely used to observe the severity of depression in rodents. The level of neurotrophic factors are indicators of healthy brain functioning however, the low levels of BDNF describe the severity of depressive symptoms. The induction of high fats in animals might interrupt the activity of HPA axis, the dysregulated HPA is often seen to increase the depressive symptoms (Figure 5), the effects of saffron in FST demonstrated the anti-depressive profile of the treatment (Dourado et al., 2020). The long term doses of saffron successfully increased the level of BDNF and ensure the antidepressant effects (Figure 6). It has been concluded from the present study that the ethanolic extract of saffron has significant pharmacological effects (Figure 7) with curative and protective potentials in CVDs and diabetes. Further experimental studies are necessary to demonstrate the molecular mechanisms.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERNCES

De Souza J, Mente A, Maroleanu A, Cozma I, Ha V, Kishibe T, Anand S. (2015). Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. British Medical Journal 351 p.

Dourado NS, Souza CD, de Almeida MM, Bispo da Silva A, Dos Santos BL, Silva VD, De Assis AM, da Silva JS, Souza DO, Costa MD, Butt AM (2020). Neuroimmunomodulatory and neuroprotective effects of the flavonoid apigenin in in vitro models of neuroinflammation associated with Alzheimer's disease. Frontiers in Aging Neuroscience 12:119.

Faridi S, Delirezh N, Froushani SMA (2019). Beneficial effects of hydroalcoholic extract of saffron in alleviating experimental autoimmune diabetes in C57bl/6 mice. Iranian Journal of Allergy, Asthma and Immunology 18(1):38-47.

Fathimoghadam H, Farbod Y, Ghadiri A, Fatemi R (2019). Moderating effects of crocin on some stress oxidative markers in rat brain following demyelination with ethidium bromide. Heliyon 5(2):e01213.

Gwarzo MY, Ahmadu JH, Ahmad MB, Dikko AU (2014). Serum glucose and malondialdehyde levels in alloxan induced diabetic rats supplemented with methanolic extract of tacazzea apiculata. International journal of biomedical science 10(4):236.

Hanke ML, Kielian T (2011). Toll-like receptors in health and disease in the brain: mechanisms and therapeutic potential. Clinical Science 121(9):367-387.

Kianbakht S, Mozaffari K (2009). Effects of saffron and its active constituents, crocin and safranal, on prevention of indomethacin induced gastric ulcers in diabetic and nondiabetic rats. Journal of Medicinal Plants 8(29):30-38.

Li Y, Hruby A, Bernstein AM, Ley SH, Wang DD, Chiuve SE, Sampson L, Rexrode KM, Rimm EB, Willett WC, Hu FB (2015). Saturated fats compared with unsaturated fats and sources of carbohydrates in relation to risk of coronary heart disease: a prospective cohort study. Journal of the American College of Cardiology 66(14):1538-1548.

Liu CS, Carvalho AF, Mansur RB, McIntyre RS (2013). Obesity and bipolar disorder: synergistic neurotoxic effects? Advances in Therapy 30(11):987-1006.

Lopresti AL, Drummond PD (2014). Saffron (Crocus sativus) for depression: a systematic review of clinical studies and examination of underlying antidepressant mechanisms of action. Human Psychopharmacology: Clinical and Experimental 29(6):517-527.

Mäkinen VP, Civelek M, Meng Q, Zhang B, Zhu J, Levian C, Huan T, Segrè AV, Ghosh S, Vivar J, Nikpay M (2014). Integrative genomics reveals novel molecular pathways and gene networks for coronary artery disease. PLoS Genetics 10(7):e1004502.

Mayberry LS, Gonzalez JS, Wallston KA, Kripalani S, Osborn CY (2013). The ARMS-D out performs the SDSCA, but both are reliable, valid, and predict glycemic control. Diabetes Research and Clinical Practice 102(2):96-104.

Sanati S, Razavi BM, Hosseinzadeh H (2018). A review of the effects of Capsicum annuum L. and its constituent, capsaicin, in metabolic syndrome. Iranian Journal of Basic Medical Sciences 21(5):439.

van Dooren FE, Nefs G, Schram MT, Verhey FR, Denollet J, Pouwer F (2013). Depression and risk of mortality in people with diabetes mellitus: a systematic review and meta-analysis. PloS ONE 8(3):e57058.

Vargas HO, Nunes SO, Barbosa DS, Vargas MM, Cestari A, Dodd S, Venugopal K, Maes M, Berk M (2014). Castelli risk indexes 1 and 2 are higher in major depression but other characteristics of the metabolic syndrome are not specific to mood disorders. Life Sciences 102(1):65-71.