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Effect of Daily Intake of a Saffron Infusion on Blood Cholesterol Levels

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Abstract: Saffron (*Crocus sativus* L.), as well as having notable culinary value, has been traditionally used as a medicinal plant due to its bioactivity. Furthermore, its antioxidant properties have been evaluated, and it may have cardiovascular benefits. Multiple sclerosis (MS) is a chronic disease of the central nervous system that causes a diminution in the daily physical exercise of patients. This sedentary lifestyle may contribute to an increased incidence of dyslipidaemia in this population, and could explain the increased cardiovascular morbidity. The aim of this study was to assess the effect of the intake of a saffron infusion over 96 days on the lipid profile of MS patients. Considering the extraction kinetics of bioactive metabolites, the best way to prepare the infusion was by using ground saffron (PDO "Azafrán de La Mancha"), passed through a 0.5 mm sieve and infused with water at the boiling point (95–100 °C). A total of 35 MS patients took a midafternoon infusion for 96 days; 16 of them completed the treatment. A significant decrease in total blood cholesterol levels from 199.5 to 179.5 mg/dL was observed. Additionally, a significant diminution of triglyceride levels, from 124.0 to 101.0 mg/dL, was observed. In conclusion, the intake of an infusion of 50 mg of "Azafrán de La Mancha" for 96 days had a lipid-lowering effect in patients with MS.

Keywords: saffron; dyslipidaemia; multiple sclerosis; crocetin esters; safranal

1. Introduction

Multiple sclerosis (MS) is a chronic autoimmune central nervous system (CNS) disease mediated by the activation of T lymphocytes through different antigens of the oligodendrocyte–myelin complex. This activation induces a cascade of inflammation that damages myelin, slowing or blocking neurological transmission, with devastating effects on motor, sensory and cognitive functions. MS is the most frequent chronic neurological disease in young adults [1]; approximately 90% of cases occur in individuals aged 15–55 years, and it is more common in women. It causes a decrease in daily physical activity that leads to a sedentary lifestyle, which may contribute to an increase in the incidence of dyslipidaemia and the risk of cardiovascular morbidity in this population.

Cholesterol homeostasis is important for the formation and maintenance of myelin and neural membranes. Some authors have suggested a relationship between dyslipidaemia and vascular

comorbidity in these patients [2,3]. These hypotheses also suggest that the proinflammatory and thrombogenic state associated with dyslipidaemia may contribute to the progression of MS [4], hence, a therapeutic proposal to decrease the immune response in MS is the use of statins—drugs that inhibit hepatic cholesterol synthesis [2,5]. The World Health Organization (WHO) considers obesity, especially abdominal obesity, and physical inactivity to be cardiovascular risk factors. The goal set within the "Global Plan of Action for the Prevention and Control of Non-Communicable Diseases 2013–2020" is to decrease levels of total cholesterol (TC) below 190 mg/dL (50 mmol/L), LDL-cholesterol (LDL-C) below 115 mg/dL (3 mmol/L), body mass index (BMI) below 25 kg/m^2, and waist/hip ratio below 0.8 in women and 0.9 in men.

Saffron (*Crocus sativus* L.) is one of the most prized spices in the world due to its coloring, and savoring, and flavoring properties. Alongside its food value, it is traditionally used as a medicinal plant because of its bioactive properties, without undesirable secondary effects [6]. In the last decade, numerous scientific studies highlighted the effect of the biomedical and pharmacological properties of saffron and its metabolites on the gastrointestinal, cardiovascular, endocrine, genital, immune, and nervous systems [7–16]. Although the mechanism of action has not been thoroughly studied, it is known that the bioactivity of saffron is based on the antioxidant character of its carotenoids, which reduces oxidative stress. The main components of saffron, which are glucoside esters of crocetin (crocins), picrocrocin, and safranal, contribute to reducing the incidence of atherosclerosis [17]. Crocin, the most common carotenoid of saffron and the substance responsible for the color of the spice [18], reduces oxidative stress [19,20] and the levels of TC, triglycerides (TG), and plasma LDL-C [21]. Preclinical studies indicated that crocins and saffron extracts may reduce the levels of TC and TG, and improve the atherogenic index (LDL-C/HDL-C) [22]. Several animal studies showed the effect of extracts of saffron, crocetin, and crocins on the cardiovascular system. Hänsel (1977) [23] exposed the effects of saffron and its metabolites on lipid assimilation in vitro and in vivo, suggesting an improvement in the lipid profile and avoidance of atherosclerosis [22]. However, there is not enough evidence to attribute this hypolipidemic effect to a particular saffron metabolite; previous publications have assigned this effect to crocetin, crocetin esters, safranal, and their synergistic actions.

Many studies reported that crocetin increases the diffusion of oxygen, and thus oxygenation in various tissues. By increasing the oxygen in the blood plasma, crocetin compensates for the reduction of oxygenation caused by cholesterol and reduces cholesterol levels [24]. Zheng et al. (2005) [25] proved in animals that a group fed with a high-fat diet and crocetin showed a reduction in their TC levels compared to a control group, in addition to reducing their atheroma plaques, foam cells, and atherosclerosis. Another study conducted in rats with induced atherosclerosis showed that crocetin inhibited atherosclerotic plaque and decreased levels of TC, TG, and LDL-C [19]. Sheng et al. (2006)[26] proved, in hyperlipidaemic rats, a significant reduction in TC, TG, and LDL-C levels compared to a control group, with an increase of faecal excretion of fats and cholesterol, without influencing the elimination of bile acids. The proposed mechanism of action is the competitive inhibition of pancreatic lipase by the crocins, decreasing the absorption of fats and cholesterol. Moreover, Asdaq and Inamdar (2010) [22] evaluated the hypolipidemic potential of saffron extracts and crocins in hyperlipidaemic rats. Both groups had reduced weights and TC and TG levels compared to the control group, but the group given saffron extracts showed greater reductions than the group supplied with only crocins. The hypothesis they suggest is a synergistic effect of the bioactive components of saffron in the prevention of cardiovascular risk.

In vivo studies in type 2 diabetic rats showed that saffron extracts are associated with a significant decrease in oxidative stress and glucose, TC, TG, and LDL-C levels, and an increase in HDL-C [27]. The safranal component also reduced TC and TG in mice and rats in studies intended to examine the toxicity of the compound [28]. Saffron also showed a hepatoprotective effect against aluminum chloride in mice, minimizing its toxicity and improving the lipid profile [29]. The lipid-lowering effect of saffron and/or its metabolites is extensively demonstrated in animal studies, but unfortunately human trials are scarce.

The aim of this quasi-experimental study was to assess the effect of the intake of an infusion of saffron on the lipid profile of MS patients. Different variables were evaluated before and after the experimental intervention, with each subject acting as his own control.

2. Materials and Methods

2.1. Saffron Samples and Reagents

In all phases of the experiment, the saffron used was of the last harvest belonging to Protected Designation of Origin (PDO) ''Azafrán de La Mancha." It was provided by the company Agrícola Técnica de Manipulación y Comercialización, S.L., Minaya (Albacete, Spain). All reagents and solvents were of analytical grade. Standards: safranal with a purity of ≥88% was obtained from Sigma-Aldrich (Madrid, Spain), and crocetin esters, trans-crocetin di(β-D-gentiobiosyl) ester (trans-4-GG), and *trans*-crocetin (β-Dglucosyl)-(β-D-gentiobiosyl) ester (*trans*-3-Gg), with a purity of ≥99%, were supplied from Phytolab GmbH and Co. KG (Vestenbergsgreuth, Bavaria, Germany). Solvents: acetonitrile was obtained from Panreac (Barcelona, Spain), while the water that was used was purified through a Milli-Q system (Millipore, Bedford, MA).

2.2. Preparation of the Saffron and Infusions

A bibliographical review was carried out to determine the amount of saffron that would be incorporated into the infusion. This search was performed in PubMed, Scopus, and SciFinder databases, the descriptors were "clinical trials and saffron," "dose saffron," and "toxicity saffron." The clinical trial databases Embase, Clinicaltrials, and Opengregi were also consulted.

Three particle sizes of saffron were considered: filaments, crushed, and ground. The filaments sample consisted of the whole dehydrated stigmas of *Crocus sativus* L., as marketed by the PDO ''Azafrán de La Mancha." The crushed sample was the result of passing the saffron through a crusher (domestic Moka SS), producing a particle size that could not pass through a 0.5 mm sieve. The ground sample resulted from grinding the stigmas with a grinder (Moligrano Becken) until all the product passed through a 0.5 mm sieve.

To determine the infusion time, 50 mg of saffron (crushed and ground filaments) was added to 200 mL of drinking water at 95–100 °C, similar to the conditions the patients used when preparing infusions themselves. A 3 mL aliquot of each infusion was taken at 1, 2, 3, 5, 7, 10, 13, and 15 min, and the solutions were collected without any solid parts. The solutions were filtered through a filter made of hydrophilic polytetrafluoroethylene (PTFE) with a pore size of 0.45 μm (Millipore, Bedford, MA), and were monitored by scanning from 200 to 700 nm using a PerkinElmer Lambda 25 spectrophotometer (Norwalk, CT) with UV WinLab 2.85.04 software (PerkinElmer). A part of each solution was transferred into a vial for HPLC−DAD analysis according to García-Rodriguez et al. (2014) [30]. Determination of the minimum extraction time was carried out in triplicate.

2.3. Saffron Dispensing to Volunteers

The ground saffron was packed in single-dose envelopes of 50 mg weight to protect it from light and moisture, ensuring safety, efficacy of administration, and conservation. Infusions were prepared by pouring the contents of one sachet into one cup with 200 mL of water at 95–100 ºC. A control was performed at 0, 45, and 96 days to determine their commercial category according to ISO 3632 [31], and the concentration of the bioactive metabolites according to García Rodríguez et al. (2014) [30].

2.4. Subjects

Thirty-five Caucasian patients with the diagnosis of MS based on the McDonald criteria were included. We included a unique group of volunteers without comparison with placebo or control groups because saffron, in our environment, is a common nutritional compound whose flavor, odor, and color are unique and characteristic. It cannot be masked with another type of infusion. It is not administered in tablets or encapsulated because in an infusion, the extraction minimizes the

destructive effect of heat on the active substances, and its chemical structure is not altered. This was designed as a quasi-experimental study without random assignment (a before–after study), where each individual was his own control.

Saffron intake was the controlled intervention. Volunteers were told how to prepare the infusion and when they should ingest it: in the middle of the afternoon with an empty stomach, to increase absorption and to avoid interactions. The diet of each participating subject was controlled and measured by surveys at the beginning and end of the study period.

The saffron infusion was administered daily to patients for 93 days (3 months); 16 of them completed the study (6 males and 10 females, on average 49.8 ± 12.1 years). The study obtained ethics approval from the Research Ethics Committee of the University of Murcia (Spain). At the beginning and after the intervention, blood levels of TC, LDL-C, HDL-C, and TG were determined. Due to the type of pathology, the sample was small, which made it difficult obtain a working group and a control group, and it was decided not to deprive all participants of the possible benefits. Cholesterol levels in patients may vary over time, and this could have occurred in both those who took the saffron and those who did not. Parallel to the biochemical study, the patient's diet was followed in our study. A placebo was not used because tablets or capsules were not used, and an infusion's characteristics of color, smell, and taste are difficult to mask. Most studies in this area have used saffron packed into capsules, but our study aimed to demonstrate the effects of saffron as a nutritional intervention, in an infusion.

2.5. Statistical Analysis

The data variables are presented as the mean (standard deviation). Differences in blood levels of TC, LDL-C, HDL-C, and TG before and at the end of the follow-up period were tested with a Student's t-test for paired data and Wilcoxon-test for a nonparametric data distribution. A two-sided $p < 0.05$ was considered significant.

All the statistical analyses were performed using the Statistical Package for Social Sciences V.22.0 statistical software (SPSS, Chicago, Illinois, USA).

3. Results and Discussion

3.1. Preparation of the Saffron and Infusions

Saffron has been safely used in food for centuries [32]. Several studies have been carried out to evaluate its toxicity in experimental animals and in vitro [33], proving its safety [34]. Table 1 shows some of the findings obtained from the data we compiled.

Table 1. Compilation of saffron studies and pathologies, 2009–2014. Dose in mg of saffron per day, period of administration, and references.

Route of Administration	n [1]	Pathology	Dose	Days	Reference
Capsules	46	Alzheimer	30	112	[7]
Saffron	35	Macular degeneration	20	84	[8]
Tablets	45	Immunological disease	100	21	[9]
Saffron	260	Infertility	60	182	[10]
Saffron	36	Sexual dysfunction	30	28	[11]
Tablets	60	Macular degeneration	43	7	[12]
Saffron	34	Sexual dysfunction in women	30	42	[13]
Oral supplements	33	Macular degeneration	20	84	[14]
Aqueous extracts	17	Primary open angle glaucoma	30	28	[15]
Capsules	40	Depression	30	42	[16]

[1] n = number of patients.

Table 1 shows the route of administration, number of patients, pathology, dosage, period of administration, and reference for each trial. Since effectiveness is achieved with 30–50 mg per day

[32], it was decided that the patients in this study would take an infusion of 50 mg saffron in a single dose for 93 days.

Sheng et al. (2006) [26] proposed that crocins are involved in the lipid-lowering effect of saffron. The maximum absorbance of these metabolites in aqueous solutions is at 440 nm [18]. For this reason, the best way to determine the crocins from the saffron in the infusion was with absorbance at 440 nm. This quick measurement shows how the crocins are extracted with the infusion time. Figure 1 shows the evolution of this average value of absorbance at 440 nm for each of the saffron types used to prepare the infusion: whole filaments, crushed, or ground. The results show that the highest absorbance values are for the infusion prepared with ground saffron. Therefore, this was the chosen presentation for our research. It was also observed (Figure 1) that after 7 min of infusion, the absorbance value (0.671) was constant as the infusion time increased. There were no significant differences in absorbance values at infusion times of 7, 10, 13, and 15 min, but there were significant differences between absorbances at 1, 2, 3, 5, and 7 min of infusion. For this reason, a minimum infusion time of 7 min was proposed. However, after 7 min of infusion, the temperature of the drink was still too hot, which could cause slight burns to the most sensitive volunteers. Therefore, it was decided to recommend an infusion time of 10 min, by which time the temperature would be lower and there were no significant differences with respect to the absorbance at 440 nm.

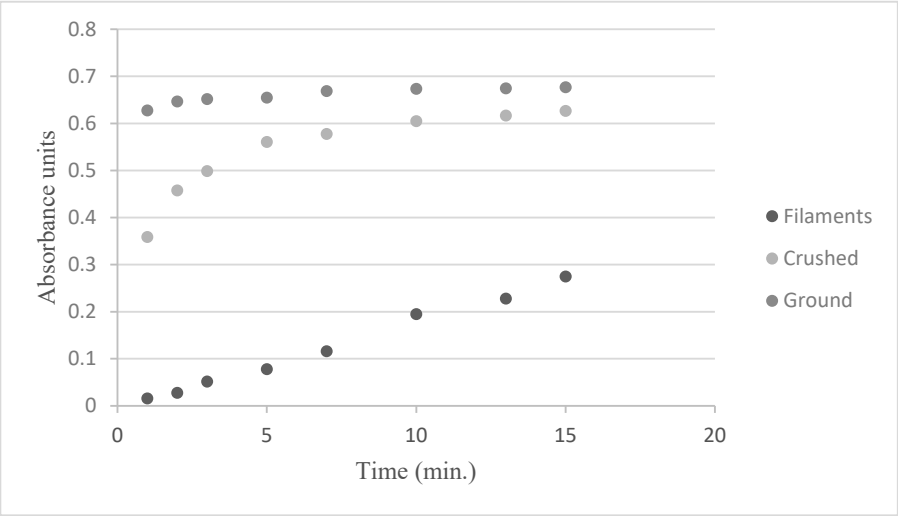

Figure 1. Absorbance values at 440 nm versus infusion time of saffron presented as whole filaments, crushed, and ground (standard deviations were less than ± 0.02).

3.2. Saffron Dispensing to Patients

The saffron administered to patients was of the highest quality according to ISO 3632-1 [30] (Category I), and did not vary during the 93 days (Table 2). The spectrophotometric parameters that determine the quality of saffron are A_{257}, A_{330}, and A_{440}, which are defined according to this standard as the strength of flavor, aroma, and coloring, respectively. A_{257} is not related to the concentration of picrocrocin, the main metabolite responsible for the bitter taste of saffron, and A_{330} is not related to the concentration of safranal, the main metabolite responsible for the aroma of saffron [30]. A_{440} is related to the crocin concentration. Table 2 shows the results of the quality parameters based on ISO 3632, and the percentage composition of the main metabolites of saffron administered to patients.

Table 2. Controls of the samples administrated to the patients and spectrophotometric parameters according to ISO 3632 (A_{257}, A_{330} and A_{440}) and percentage (*w*/*w*) of bioactive compounds by HPLC: picrocrocin (Picr), safranal (Saf), sum crocins (ΣCrc), and sum of *trans*-crocins (Σ*t*-Crc).

Control	Days	A_{257}	A_{330}	A_{440}	Picr	Saf	ΣCrc	Σ*t*-Crc
1	0	84.4 ± 1.2 ^{b}	31.6 ± 0.2 ^{b}	229 ± 6	12.3 ± 0.5	0.11 ± 0.00 ^{c}	21.2 ± 0.8 ^{a}	18.9 ± 0.5 ^{a}
2	45	87.1 ± 1.1 ^{a}	33.6 ± 0.3 ^{a}	226 ± 5	12.9 ± 0.6	0.28 ± 0.01 ^{b}	20.8 ± 0.7 ^{a}	17.8 ± 0.4 ^{b}
3	96	66.2 ± 0.9 ^{c}	20.2 ± 0.1 ^{c}	219 ± 4	13.0 ± 0.6	0.30 ± 0.01 ^{a}	17.9 ± 0.9 ^{b}	17.0 ± 0.6 ^{b}

One-way analysis of variance (ANOVA). Different letters (a, b, c) within each column represent statistically significant differences ($p < 0.05$).

The percentage of picrocrocin did not vary significantly throughout the study. The level was high compared to that found by Lage and Cantrel [35], and in a similar range to the level reported by del Campo et al. [36]. The percentage of safranal in the study (0.11, 0.28, and 0.30%) showed a slight but significant increase over time. The values were similar to those obtained by García-Rodríguez et al. [37] for Spanish saffron. The percentage of the sum of crocins slightly decreased (21.2, 20.8, and 17.9%) during the period of administration, showing significant differences between the first and the third control. As expected, the sum of the percentage of *trans*-crocins (18.9, 17.8, and 17.0%) showed a similar behavior. The concentration of *trans* esters, higher than 83% of the total of crocins, contributes to a greater bioactive effect [36]. The percentage ratio of *trans*/*cis* crocins was variable but remained below 12.5.

3.3. Effect on the Lipid Profile

Each patient's nutritional status was monitored by anthropometry at the beginning of the study and at the end. Two anthropometric measurements were carried out. The patients responded to dietary questionnaires on their food consumption frequency (CFC), which directly estimated the dietary intake, allowing us to establish an individual diet profile and the caloric and nutritional contribution; we used a 24 h reminder to assess the consumption of food and nutrients and the Nutritional Screening Initiative check list (NSI) to detect nutritional status and assess nutritional risk. Only one of the patients was on the lipid-lowering drug simvastatin at the start of the study, and they continued this treatment for 96 days of the study. All patients were receiving MS treatment at the start of the study, and continued without variation for the duration of the study.

Of the 35 MS patients included, 16 completed the study. The reason for the high dropout rate was that many of the volunteers did not like the taste of the infusion. One subject was eliminated because he made a change to his diet, and therefore the effect we observed could not be confirmed to be due to the intake of the infusion. The mean age of these patients was 49.8 ± 12.1 years, and 62% were women.

Table 3 shows the results of TC, TG, LDL-C, and HDL-C. There was a decrease in TC levels in 13 of the 16 patients (87.5%), and TG level decreased in 11 of the 16 patients (69.0%). The TC level at the start of the study was 199.5 ± 27.6 mg/dL, and it was 179.2 ± 19.8 mg/dL after administration of the saffron infusion for 93 days. This decrease of 20.3 ± 22.0 was statistically significant ($p = 0.002$). This means that 44% of the sample had a TC level above 200.0 mg/dL at the beginning of the study, which decreased by 19% after the saffron administration. In women, the mean TC at baseline was 199.0 ± 24.6 mg/dL, and in men it was 201.0 ± 34.4 mg/dL. After saffron intake, it was 178.1 ± 16.7 mg/dL in women and 180.9 ± 25.7 mg/dL in men.

Table 3. Lipid profile before and after use of a saffron infusion.

	Pre Treatment	Post Treatment	*p*-Value
TC level	199.5 ± 27.6 [a]	179.5 ± 19.8 [b]	0.002
TG level	124.0 ± 80.0 [a]	101.0 ± 51.6 [b]	0.002
LDL-C level	124.86 ± 35.76	115.86 ± 27.04	-
HDL-C level	56.57 ± 12.42	60.14 ± 16.34	-

TC: total cholesterol (mg/dL); TG: triglyceride (mg/dL), LDL-C: low density lipoprotein cholesterol (mg/dL), HDL: high density lipoprotein cholesterol (mg/dL). A one-way analysis of variance (ANOVA) was undertaken. Different letters (a, b) within each row represent statistically significant differences ($p < 0.002$).

Regarding the level of TG in the blood, the mean before the study was 124.0 (80.0) mg/dL, and after treatment with saffron it was 101.0 ± 51.6 mg/dL. The mean decrease was 23.0 ± 42.1 mg/dL. Thirty-one percent of the patients had a TG level greater than 150 mg/mL at baseline, and after ingestion this decreased by 19%. The mean TG in women at baseline was 127.4 ± 79.0 mg/dL and in men was 118.8 ± 89.1 mg/dL. In the end, it was 97.9 ± 47.9 mg/dL and 107.0 ± 61.6 mg/dL; the mean decreases in TG were 29.5 ± 36.2 mg/dL and 11.7 ± 52.2 mg/dL in women and men, respectively.

Although no significant differences were found in LDL-C and HDL-C after ingestion of the saffron infusion, the results point to a trend of improvement in these values. Thus, a slight decrease in the level of LDL-C was observed, from 124.86 ± 35.76 mg/dL to 115.86 ± 27.04 mg/dL, while HDL-C increased from 56.57 ± 12.42 mg/dL to 60.14 ± 16.34 mg/dL.

In contrast, Sahraian et al. (2016) [38] found no significant differences in the lipid profile when saffron was given in a double-blind placebo-controlled clinical trial. This difference in the effectiveness of saffron ingestion may be due to dose and presentation. While in our study 50 mg/day was supplied, in their study a smaller quantity was given of 30 mg/day. The presentation used to administer the saffron was also different; Sahraian et al. (2016) [38] supplied it in capsules, and in our study it was administered as an infusion, in which the metabolites were already extracted from the saffron before ingestion. On the other hand, Rahmani et al. (2019) [39], in a meta-analysis on the effect of saffron intake on the lipid profile, found significant differences in TC, TG, and LDL-C, but found no significant differences in HDL-C. The effect of saffron intake on the lipid profile could represent a new nutritional tool to battle cardiovascular disease. Cardiovascular disease remains a first order issue for national health systems all over the world. Approximately 9% of health expenditure in Europe goes to cardiovascular disease [40], and it is estimated that the United States will triple this in the next three decades [41]. It is estimated that by optimizing risk factors, including cholesterol, 80% of cardiovascular disease could be prevented [42]. Diet and exercise play an important role in dyslipidaemia control, but saffron has no specific role in these recommendations. The effect reported in this study, that saffron may impact the cholesterol and triglyceride level, could add another tool to the fight against atherosclerosis, although it needs to be confirmed in larger clinical trials.

4. Conclusions

In conclusion, daily intake of 50 mg ground saffron of the PDO "Saffron La Mancha" type, with a content of at least 12.3% picrocrocin, 0.11% safranal, and 21.2% total crocins, infused in 200 mL of drinking water and taken for 93 days, has a lowering-cholesterol effect in multiple sclerosis patients. This effect and its clinical impact must be confirmed in larger studies.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

BMI	Body Mass Index
CNS	Central Nervous System
HDL-C	High Density Lipoproteins Cholesterol
LDL-C	Low Density Lipoprotein Cholesterol
MS	Multiple Sclerosis
PDO	Protected Designation of Origin
PTFE	Polytetrafluoroethylene
TC	Total Cholesterol
TG	Triglycerides
WHO	World Health Organization

References

1. Fernández, O.; Fernández, V.E.; Guerrero, M. *Esclerosis Múltiple*; McGraw-Hill/Interamericana: Madrid, Spain, 2011.
2. Tettey, P.; Simpson, S., Jr.; Taylor, B.; Blizzard, L.; Ponsonby, A.L.; Dwyer, T.; Kostner, K.; Van Der Mei, I. Adverse lipid profile is not associated with relapse risk in MS: Results from an observational cohort study. *J. Neurol. Sci.* **2014**, *340*, 230–232.
3. Zhornitsky, S.; McKay, K.A.; Metz, L.M.; Teunissen, C.E.; Rangachari, M. Cholesterol and markers of cholesterol turnover in multiple sclerosis: Relationship with disease outcomes. *Mult. Scler. Relat. Disord*. **2016**, *5*, 53–65.
4. Mandoj, C.; Renna, R.; Plantone, D.; Sperduti, I.; Cigliana, G.; Conti, L.; Koudriavtseva, T. Anti-annexin antibodies, cholesterol levels and disability in multiple sclerosis. *Neurosci. Lett.* **2015**, *606*, 156–160.
5. Ulivieri, C.; Baldari, C.T. Statins: From cholesterol-lowering drugs to novel immunomodulators for the treatment of Th17-mediated autoimmune diseases. *Pharmacol. Res.*, **2014**, *88*, 41–52.
6. Ulbricht, C.; Conquer, J.; Costa, D.; Hollands, W.; Iannuzzi, C.; Isaac, R.; Jordan, J.K.; Ledesma, N.; Ostroff, C.; Serrano, J.M.G.; et al. An evidence-based systematic review of saffron (*Crocus sativus*) by the Natural Standard Research Collaboration. *J. Diet. Suppl.* **2011**, *8*, 58–114.
7. Akhondzadeh, S.; Shafiee-Sabet, M.; Harirchian, M.H. A 22-week, multicenter, randomized, double-blind controlled trial of *Crocus sativus* in the treatment of mild-to-moderate Alzheimer's disease. *Psychopharmacology* **2010**, *207*, 637–643.
8. Falsini, B.; Piccardi, M.; Minnella, A.; Savastano, C.; Capoluongo, E.; Fadda, A.; Balestrazzi, E.; Maccarone, R.; Bisti, S. Influence of saffron supplementation on retinal flicker sensitivity in early age-related macular degeneration. *Invest. Ophthalmol. Vis. Sci.* **2010**, *51*, 6118–6124.
9. Kianbakht, S.; Ghazavi, A. Immunomodulatory effects of saffron: A randomized double-blind placebo-controlled clinical trial. *Phytother. Res.* **2011**, *25*, 1801–1805.
10. Safarinejad, M.R.; Shafiei, N.; Safarinejad, S. A prospective double-blind randomized placebo-controlled study of the effect of saffron (*Crocus sativus* Linn) on semen parameters and seminal plasma antioxidant capacity in infertile men with idiopathic oligoasthenoteratozoospermia. *Phytother. Res.* **2011**, *25*, 8–516.
11. Modabbernia, A.; Sohrabi, H.; Nasehi, A.A.; Raisi, F.; Saroukhani, S.; Jamshidi, A.; Tabrizi, M.; Ashrafi, M.; Akhondzadeh, S. Effect of saffron on fluoxetine-induced sexual impairment in men: Randomized double-blind placebo-controlled trial. *Psychopharmacology*, **2012**, *223*, 381–388.
12. Ayatollahi, H.; Javan, A.O.; Khajedaluee, M.; Shahroodian, M.; Hosseinzadeh, H. Effect of *Crocus sativus* L. (saffron) on coagulation and anticoagulation systems in healthy volunteers. *Phytother. Res.* **2014**, *28*, 539–543.

13. Kashani, L.; Raisi, F.; Saroukhani, S.; Sohrabi, H.; Modabbernia, A.; Nasehi, A.A.; Jamshidi, A.; Ashrafi, M.; Mansouri, P.; Ghaeli, P.; et al. Saffron for treatment of fluoxetine-induced sexual dysfunction in women: Randomized double-blind placebo-controlled study. *Hum. Psychopharmacol*. **2013**, *28*, 54–60.
14. Marangoni, D.; Falsini, B.; Piccardi, M.; Ambrosio, L.; Minnella, A.M.; Savastano, M.C.; Bisti, S.; Maccarone, R.; Fadda, A.; Mello, E.; et al. Functional effect of Saffron supplementation and risk genotypes in early age-related macular degeneration: A preliminary report. *J. Transl. Med.* **2013**, *25*, 11–228.
15. Jabbarpoor Bonyadi, M.H.; Yazdani, S.; Saadat, S. The ocular hypotensive effect of saffron extract in primary open angle glaucoma: A pilot study. *BMC Complement. Altern. Med.* **2014**, *15*, 14–399.
16. Shahmansouri, N.; Farokhnia, M.; Abbasi, S.H.; Kassaian, S.E.; Tafti, A.A.N.; Gougol, A.; Yekehtaz, H.; Forghani, S.; Mahmoodian, M.; Saroukhani, S.; et al. A randomized, double-blind, clinical trial comparing the efficacy and safety of *Crocus sativus* L. with fluoxetine for improving mild to moderate depression in post percutaneous coronary intervention patients. *J. Affect. Disord.* **2014**, *155*, 216–222.
17. Kamalipour, M.; Akhondzadeh, S. Cardiovascular effects of saffron: An evidence-based review. *J. Tehran Heart Cent.* **2011**, *6*, 59–61.
18. Carmona, M.; Zalacain, A.; Sánchez, A.M.; Novella, J.L.; Alonso, G.L. Crocetin esters, picrocrocin and its related compounds present in *Crocus sativus* stigmas and *Gardenia jasminoides* fruits. Tentative identification of seven new compounds by LC-ESI-MS. *J. Agric. Food Chem.* **2006**, *54*, 973–979.
19. He, S.Y.; Qian, Z.Y.; Wen, N.; Tang, F.T.; Xu, G.L.; Zhou, C.H. Influence of Crocetin on experimental atherosclerosis in hyperlipidamic-diet quails. *Eur. J. Pharmacol.* **2007**, *554*, 191–195.
20. Yoshino, F.; Yoshida, A.; Umigai, N.; Kubo, K.; Masaichi, C.I.L. Crocetin reduces the oxidative stress induced reactive oxygen species in the stroke-prone spontaneously hypertensive rats (SHRSPs) brain. *J. Clin. Biochem. Nutr.* 2011, 49, 182–187.
21. Samarghandian, S.; Azimi-Nezhad, M.; Farkhondeh, T. Crocin attenuate Tumor Necrosis Factor-alpha (TNF-α) and interleukin-6 (IL-6) in streptozotocin-induced diabetic rat aorta. *Cytokine* **2016**, *88*, 20–28.
22. Asdaq, S.M.; Inamdar, M.N. Potential of *Crocus sativus* (saffron) and its constituent, crocin, as hypolipidemic and antioxidant in rats. *Appl. Biochem. Biotechnol.* **2010**, *162*, 358–372.
23. Hänsel, R. Medizinische Empirie und moderne Arzneimittelforschung. *Dtsch. Apoth. Zeitag* **1977**, *117*, 1672–1679.
24. Gainer, J.L. Trans-sodium crocetinate for treating hypoxia/ischemia. *Expert Opin. Investig. Drugs* **2008**, *17*, 917–924.
25. Zheng, S.; Qian, Z.; Tang, F.; Sheng, L. Suppression of vascular cell adhesion molecule-1 expression by crocetin contributes to attenuation of atherosclerosis in hypercholesterolemic rabbits. *Biochem. Pharmacol.* **2005**, *70*, 1192–1199.
26. Sheng, L.; Qian, Z.; Zheng, S.; Sun, S. Mechanism of hypolidemic effect of crocin in rats: Crocin inhibits pancreatic lipase. *Eur. J. Pharmacol.* **2006**, *543*, 116–122.
27. Shirali, S.; Zahra Bathaie, S.; Nakhjavani, M. Effect of crocin on the insulin resistance and lipid profile of streptozotocin-induced diabetic rats. *Phytother. Res.* **2013**, *27*, 1042–1047.
28. Hosseinzadeh, H.; Nassiri-Asl, M. Acute and subacute toxicity of safranal, a constituent of saffron, in mice and rats. *Phytother. Res.* **2013**, *27*, 475–483.
29. Shati, A.A.; Alamri, S.A. Role of saffron (*Crocus sativus* L.) and honey syrup on aluminum-induced hepatotoxicity. *Saudi Med. J.* **2010**, *31*, 1106–1113.
30. Valle Garcia-Rodriguez, M.; Serrano-Diaz, J.; Tarantilis, P.A.; Lopez-Corcoles, H.; Carmona, M.; Alonso, G.L. Determination of saffron quality by high-performance liquid chromatography. *J. Agric. Food Chem.* **2014**, *62*, 8068–8074.
31. ISO 3632. *Saffron* (*Crocus Sativus*, L.). *Part 1 (Specification) and Part 2 (Test Methods*); International Organization for Standardization: Geneva, Switzerland, 2011.
32. Moshiri, M.; Vahabzadeh, M.; Hosseinzadeh, H. Clinical Applications of Saffron (*Crocus sativus*) and Its Constituents: A Review. *Drug Res.* (Stuttg), **2014**, *65*, 287–295.
33. Srivastava, R.; Ahmed, H.; Dixit, R.K.; Dharamveer. *Crocus sativus* L.: A comprehensive review. *Pharmacogn. Rev.* **2010**, *4*, 200–208.
34. Modaghegh, M.H.; Shahabian, M.; Esmaeili, H.A.; Rajbai, O.; Hosseinzadeh, H. Safety evaluation of saffron (*Crocus sativus*) tablets in healthy volunteers. *Phytomedicine* **2008**, *15*, 1032–1037.
35. Lage, M.; Cantrel, C.L. Quantification of saffron (*Crocus sativus* L.) metabolites crocins, picrocrocin and safranal for quality determination of the spice grown under different environmental Moroccan conditions. *Sci. Hortic*. **2009**, *121*, 366–373.

36. Del Campo, C.P.; Carmona, M.; Maggi, L.; Kanakis, C.D.; Anastasaki, E.G.; Tarantilis, P.A.; Polissiou, M.G.; Alonso, G.L. Effects of mild temperature conditions during dehydration procedures on saffron quality parameters. *J. Sci. Food Agric.* **2010**, *90*, 719–725.
37. García-Rodríguez, M.V.; López-Córcoles, H.; Alonso, G.L.; Pappas, C.S.; Polissiou, M.G.; Tarantilis, P.A. Comparative evaluation of an ISO 3632 method and an HPLC-DAD method for safranal quantity determination in saffron. *Food Chem*. **2017**, *221*, 838–843.
38. Sahraian, A.; Jelodar, S.; Javid, Z.; Mowla, A.; Ahmadzadeh, L. Study the effects of saffron on depression and lipid profiles: A double blind comparative study. *Asian J. Psychiatr*. **2016**, *22*, 174–176.
39. Rahmani, J.; Manzari, N.; Thompson, J.; Clak, C.C.T.; Villanueva, G.; Varkaneh, H.K.; Mirmiran, P. The effect of saffron on weight and lipid profile: A systematic review, meta-analysis, and dose–response of randomized clinical trials. *Phytother. Res.* **2019**, *33*, 2244–2255.
40. Nichols, M.; Townsend, N.; Luengo-Fernandez, R.; Leal, J.; Gray, A.; Scarborough, P.; Rayner, M. *European Cardiovascular Disease Statistics*; European Heart Network: Brussels, Belgium, 2012.
41. Heidenreich, P.A.; Trogdon, J.G.; Khavjou, O.A.; Butler, J.; Dracup, K.; Ezekowitz, M.D.; Finkelstein, E.A.; Hong, Y.; Johnston, S.C.; Khera, A.; et al. Forecasting the future of cardiovascular disease in the United States: A policy statement from the American Heart Association. Circulation. *Circulation* **2011**, *123*, 933–944.
42. Ezzati, M.; Lopez, A.D.; Rodgers, A.; Murray, C.J.L. *Comparative Quantification of Health Risks: Global and Regional Burden of Disease Attributable to Selected Major Risk Factors*; World Health Organization: Geneva, Switzerland, 2004.