African Journal of Biotechnology Vol. 10(41), pp. 8093-8100, 3 August, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.406
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Determination of antioxidant activity of saffron taken from the flower of *Crocus sativus* grown in Lebanon

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Accepted 19 May, 2011

Since oxidative stress has been implicated in most common cause of death, especially in case of cancer and cardiovascular disease, natural substances and spices that show antioxidant effects merit a closer examination. Saffron is the yellow natural spice derived from the flower of *Crocus sativus* and used as a coloring agent in many foods worldwide. In this study, we determined the total polyphenols content in the Lebanese saffron and the antioxidant effects of different extracts from this saffron *in vitro* using electrolysis of physiological solution for generation of free radicals (FR) in the presence of colorimetric indicator N,N-di-ethyl-P-phenylenedialanine; the absorbance was measured spectrophotometrically at 515 nm. Histophathological studies allowed us to observe the damages caused by FR in the isolated organs of hamsters (kidney, liver, lungs, and heart) and on the other hand the protection that saffron provided to these vital organs. By using assay kits, we evaluated the levels of lipid peroxidation and superoxide dismutase activity, the important free radical scavenging enzyme. The results showed that both boiled and soaked saffron at 0.45 mg/ml are highly effective against FR generated by electrolysis and against the damages caused to the organs tested as observed by light microscopy. Moreover, saffron significantly ($p < 0.05$) decreased lipid peroxidation and increased superoxide dismutase activity in all tissues used as compared to control. We concluded that Lebanese saffron strongly protects vital organs against oxidative stress.

Key words: *Crocus sativus*, oxidative stress, free radicals, Lebanese saffron, antioxidant activity, free radicals scavengers.

INTRODUCTION

Modern lifestyle habits cause many people to develop abnormally high levels of oxidative stress. Although, most of us are not in a continuous state of oxidative stress, we generally experience this unhealthy condition on a regular basis as it can be precipitated by a wide range of factors. These include psychological stress, alcohol, medication, trauma, a cold environment, toxins, automobiles and industrial fumes, asbestos, bacterial, fungal or viral infections, drugs, smoking, pollution, obesity and mainly the exposure to ionizing radiation and free radicals (FR) (Roberts et al., 2010). For protection against FR, organisms are endowed with endogenous (catalase, superoxide dismutase, glutathione peroxidase/reductase) and exogenous (vitamins C and E, carotene, uric acid) defense systems. However, these systems are not sufficient in situations (oxidative stress, contamination, UV-exposure etc) where the production of FR significantly increases (Mondon et al., 1999).

In the last decades, much attention has been focused on naturally derived substances and spices that provide antioxidant effects. Concerning saffron, this is an important spice that is derived from the flower *Crocus sativus* which is a plant from the Iridaceae family. It possesses red-orange tripartite stigmas. This triploid

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Abbreviations: FR, Free radicals; **LP,** lipid preoxidation; **Els,** electrolysis; **ROS,** reactive oxygen species; **SOD,** superoxide dismutase.

sterile monocot species is not known to grow in the wild, but has been cultivated for its stigma for a long time. Saffron is a perennial crop well adapted to arid and semi-arid lands which produce stigmas annually. It is also adaptable to temperate and sub-tropical climates, and can be grown on soils varying from sandy to well-drained clay loams. It blooms in autumn and spends a long period of dormancy (aestivation) in the summer. It is said to be native to the Mediterranean environment which is characterized by cool to cold winters, with autumn–winter-spring rainfall, and warm dry summers with very little rainfall (Negbi, 1999).

Commercial saffron comprises the dried red stigma with a small portion of the yellowish style attached. Compounds considered pharmacologically active and important are volatile agents (example safranal), bitter principles (example picrocrocin) and dye materials (example crocetin and its glycoside, crocin) (Rios et al., 1996). It is highly valued as a culinary spice for its flavoring and coloring properties, and is the subject of ongoing scientific research for its potential medicinal properties. Interest in the impact of saffron carotenoids on human health is growing due to its high antioxidant capacity (Abdullaev, 2002; Nair et al., 1995; Verma and Bordia, 1998; Bathaie and Mousavi, 2010).

In modern pharmacological studies, saffron, or its active constituents, has demonstrated anticonvulsant (Hosseinzadeh and Khosravan, 2002), antidepressant (Hosseinzadeh et al., 2004), anti-inflammatory and antitumor agents. Learning, memory improving properties and promotion of the diffusivity of oxygen in different tissues have also been reported (Hosseinzadeh et al., 2009).

In this study, we are interested in searching both the antioxidant and free radical scavenging properties of the Lebanese saffron taken from *C. sativus* as well as analyzing the other effects that it may have on isolated organs of hamsters by histopathological and biochemical methods. In addition, we aimed to determine the total dosage of polyphenols in saffron extracts.

MATERIALS AND METHODS

Electrolysis

The physiological solution is first prepared and it is constituted of: NaCl (137 Mm), KCl (2.7 mM), $MgCl_26H_2O$ (1 mM), $CaCl_22H_2O$ (1.5 mM), $NaH_2PO_42H_2O$ (0.4 mM), $NaHCO_3$ (12 mM). The apparatus of electrolysis is made up of a stimulator adjusted at 10 mA by a sensitive multimeter, wires that connect it with 2 platinum electrodes which are in turn introduced into 20 ml of the prepared physiological solution, and a magnetic stirrer for rapid mixing and homogenizing the medium (Lecours et al., 1998). During electrolysis, each minute, 1 ml is taken from the physiological solution and added to 2 ml of N,N-diethyl-P-phenylendiamine (DPD) (25 mg/ml) in a specific tube, vortexed and measured using spectrophotometer at 515 nm (control). Similarly, the electrolysis of the physiological solution is performed in the presence of saffron. The DPD solution gives a pink color proportional to the FR generated. Experiments were repeated at different doses (200, 400 and 600 µl) and for different forms of extracts from the stigmata.

Saffron extractions

The following extracts were prepared:

(1) Boiled water + saffron: 30 ml of distilled water was boiled and then 0.5 g of saffron was added to the boiled water for 5 min.
(2) Boiled saffron: 0.5 g of saffron was added to 30 ml of distilled water and boiled for 5 min.
(3) Soaked saffron: 0.5 g of saffron was introduced in 30 ml of distilled water and kept for 12 h.
(4) Boiled and soaked saffron: 0.5 g of saffron was put in 30 ml of distilled water and boiled for 5 min. The mixture was kept at 18°C for 12 h.
(5) Water extraction: 3 g of saffron was put in 300 ml distilled water and kept for 24 h at 4°C then lyophilized. The remaining mass was 1.41 g. A stock solution containing 0.235 g of this mass was dissolved in 20 ml of distilled water.
(6) Methanol extraction: 1 g of saffron was put in 50 ml of methanol and 50 ml distilled water and kept for 24 h at 4°C then lyophilized. The remaining mass was 0.36 g. A stock solution containing 0.235 g of this mass was dissolved in 20 ml of distilled water. The solutions were then used for experimental protocols *in vitro*.

Isolated organs

Male golden hamsters (150 to 160 g) were used for this study. The animals purchased from a local breeder were maintained for one week on 12 h- light-dark cycles at room temperature, and fed on a standard laboratory pellet diet with water supplied *ad libitum*. These animals were sacrificed by cervical dislocation: their kidneys, livers, lungs, and hearts were rapidly extracted, rinsed with a physiological solution and maintained separately in a bath containing oxygenated physiological solution at 37°C. After 5 min of electrolysis, the organs were removed from the electrolyzed physiological solution and put either in 20 ml of formaldehyde (10%) or congealed at -80°C for biochemical assay. Similarly, the same experimental protocol was repeated in the presence of boiled and soaked saffron extract. A control group was also used without any intervention.

Histopathological studies

For these studies, we used the organs that are preserved in formaldehyde. They were fixed on paraffin and counterstained with hematoxylin-eosin, then cut into slices of 5 µm with microtome so that they can be observed using light microscope.

Biochemical assays

Lipid hydroperoxide assay

Lipid peroxidation (LP) levels were determined using a lipid hydroperoxide assay kit which measures the redox reactions with ferrous ions from Cayman's Chemical Co USA. Briefly, hydroperoxides are highly unstable and reacted with ferrous ion to produce ferric ions. The resulting ions are detected using thiocyanate ion as the chromogen. To avoid over-estimation of lipid

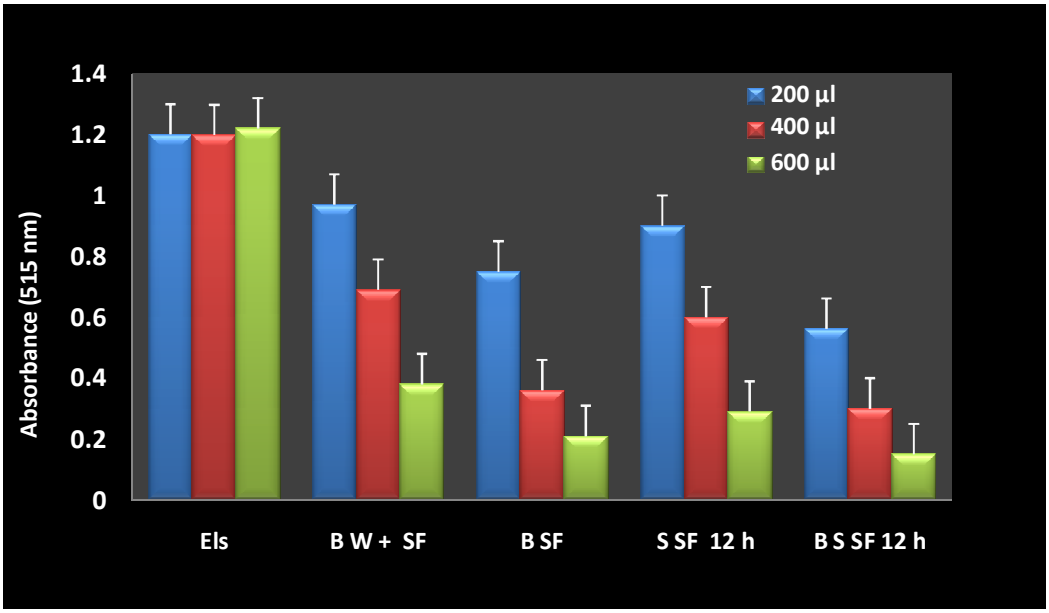

Figure 1. Levels of free radicals generated after 5 min electrolysis (Els) of physiological solution and represented in term of absorbance. Effects of different forms of saffron at 200, 400 and 600 µl corresponding respectively to 0.15, 0.30 and 0.45 mg / ml saffron added to the physiological solution during electrolysis: Saffron added during 5 min to Boiled water (BW + SF), Boiled saffron with water for 5 min (B SF), Soaked saffron for 12 h (S SF 12 h), Boiled saffron with water for 5 min then soaked for 12 h (B S SF 12 h).

hydroperoxides, they must be extracted from samples into chloroform; then the absorbance of each tube is measured at 500 nm.

Superoxide dismutase assay

Superoxide dismutase (SOD) levels were determined using an SOD assay kit that utilizes a tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine from Cayman's Chemical Co USA. Briefly, one unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. This assay measures all the three types of SOD (Cu / Zn, Mn and FeSOD). Tissue sample from each organ was homogenized in 5 to 10 ml of cold 20 mM HEPES buffer of pH 7.2, containing 1 mM EGTA, 210 mM mannitol and 70 mM sucrose per gram tissue. A plate was incubated on the shaker for 20 min at room temperature and the absorbance measured at 440 to 460 nm using a plate reader.

Determination of total polyphenols

500 mg of saffron were dissolved in 500 ml of distilled water and agitated for 24 h at 4°C in the dark. 2 ml from this solution were then put in a 100 ml volumetric flask with 2.5 ml folin-Ciocalteu's phenol reagent and 5 ml sodium bicarbonate 4.25% completed with distilled water until 100 ml; and was then left in the dark for 1 h. For the standard, 2.5, 25, 50, 75 and 100 mg of galic acid were put in 5 different volumetric flasks respectively with distilled water until 100 ml volume for each. 0.5 ml of each prepared galic acid were taken and introduced in another 5 different volumetric flasks with 2.5 ml folin-Ciocalteu's phenol reagent and 5 ml sodium bicarbonate and distilled water till 100 ml. Samples from the prepared flasks were put in specific tubes to measure the absorbance of equivalent polyphenols at 725 nm.

Statistical analysis

Data was expressed as ± SEM. Statistical analysis was performed using one-way ANOVA and all values were analyzed by t-test. The p-values less than 0.05 were considered statistically significant.

RESULTS

As shown in Figure 1, the electrolysis of the physiological solution (Els) generates a rapid increase of the amount of FR after 5 min (control) represented in term of absorbance (1.2 ± 0.1). When different forms of saffron extracts were added, the absorbance decreased, indicating fewer amounts of FR generated. The effect of each extract is dose-dependent and the most powerful extract is the soaked and boiled saffron; it is more efficient at 600 µl (corresponding to 0.45 mg/ml saffron). At lower doses, the antioxidant effects of saffron are not significant after 5 min from starting electrolysis as compared with the higher dose. Thus, the antioxidant effects of saffron are dose-dependent. Concerning water and methanol extractions, results are not represented; their ability to scavenge FR was less than the

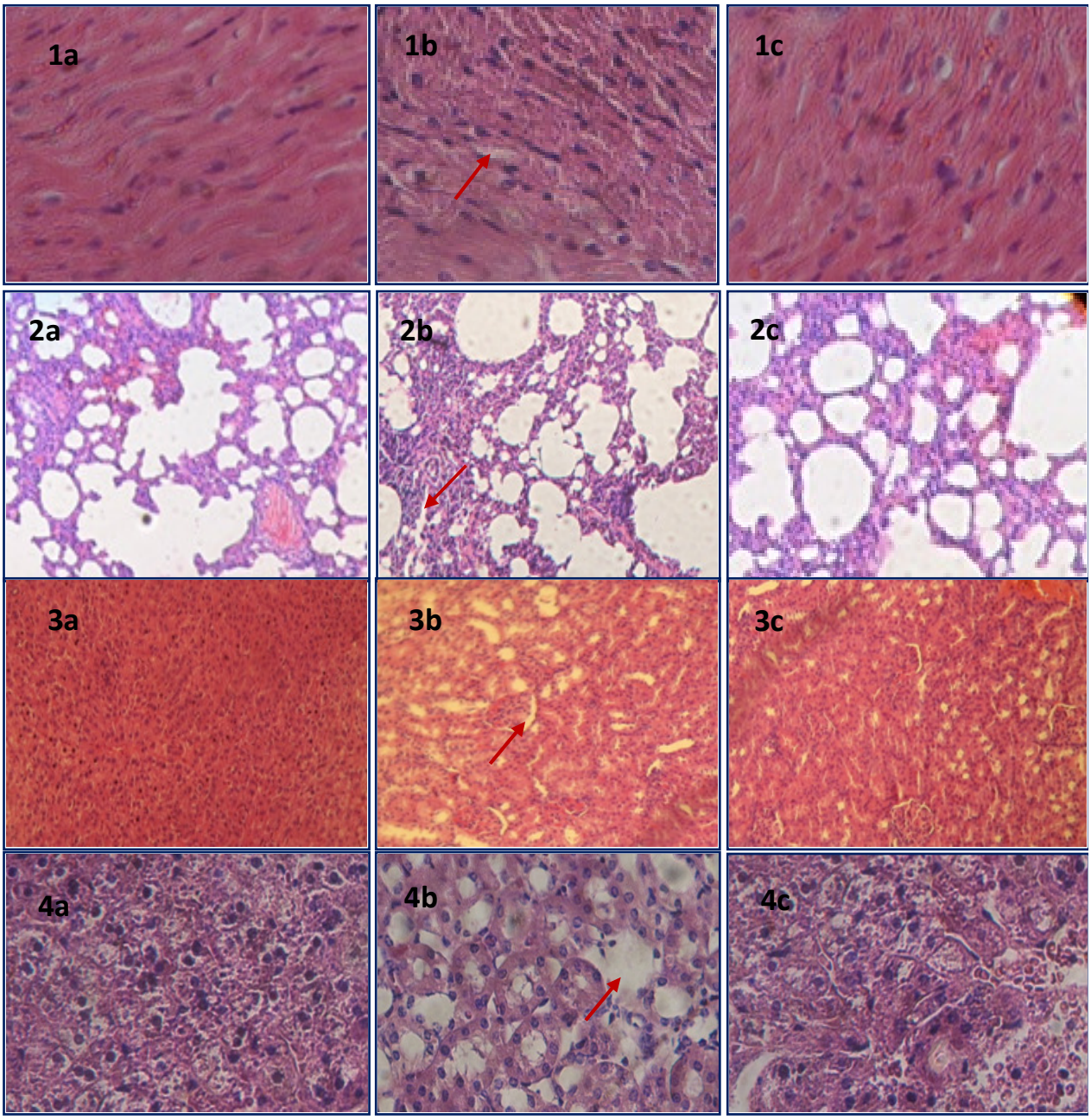

Figure 2. Micrographs of the studied organs (original magnification ×200): 1-heart: 1a-Control heart, 1b-Electrolyzed heart, 1c-Electrolyzed heart + Saffron; 2-lung: 2a-Control lung, 2b-Electrolyzed lung, 2c-Electrolyzed lung + Saffron; 3-liver: 3a-Control liver, 3b-Electrolyzed liver, 3c-Electrolyzed liver + Saffron; 4-kidney: 4a-Control kidney, 4b-Electrolyzed kidney, 4c-Electrolyzed kidney + Saffron. Red arrow: extracellular space (Boiled and soaked saffron concentration: 0.45 mg/ml).

represented solutions. Histopathological studies on the isolated organs (heart, lung, liver and kidney) submitted to 5 min of electrolysis resulted in structural damages caused by FR as compared to control. With the heart and the liver, there were losses of striations, hyalinization, and extracellular spaces became larger (Figures 2: 1b, 3b). Also, in the lungs and kidneys extracellular spaces became larger (Figures 2: 2b, 4b); whereas in the presence of saffron, boiled and soaked at 600 µl (0.45 mg), a protection is provided to these organs (Figure 2c).

In biochemical studies, the LP level in the isolated organs is represented as lipid hydroperoxide nmol/mg and SOD activity is represented as units/mg. Results are summarized in Tables 1 and 2 in which the levels of lipid

Table 1. Effects of saffron (0.45 mg/ml) on the level of lipid peroxidation (LP) in the organs that are subjected to free radicals (through electrolysis). The level of lipid peroxidation is equivalent to that of lipid hypdroperoxide.

LP (nmol / mg)	Control	Electrolysis	Electrolysis + saffron
kidney	241.24 ± 16.26	423.64 ± 15.38 *	276.84 ± 11.27**
Liver	122.50 ± 11.53	189.66 ± 8.76*	143.20 ± 13.63**
Lung	59.88 ± 7.60	108.64 ± 7.61*	78.38 ± 7.18**
Heart	73.00 ± 5.87	126.26 ± 10.10*	89.40 ± 9.80**

n = 6. *, p-value < 0.05 vs. Control; **, p-value < 0.05 vs. Electrolysis.

Table 2. Effects of saffron (0.45 mg/ml) on the level of superoxide dismutase (SOD) in the organs that are subjected to electrolysis.

SOD (U /100 mg)	Control	Electrolysis	Electrolysis + saffron
Kidney	206.26 ± 10.16	127.50 ± 8.31*	180.64 ± 12.97**
Liver	520.08 ± 10.72	395.22 ± 19.47*	476.52 ± 10.03**
Lung	78.84 ± 6.98	59.06 ± 7.31*	66.82 ± 6.84**
Heart	113.16 ± 10.62	88.22 ± 5.81*	97.94 ± 11.57**

n = 6. *, p-value < 0.05 vs. Control; **, p-value < 0.05 vs. Electrolysis.

hydroperoxide significantly increased after electrolysis, while SOD activity decreased. Lipid hydroperoxide level is equivalent to that of lipid peroxidation; it exits at higher levels in kidneys (241.24 nmol/mg) and livers (122.5 nmol/mg) than that of lungs and hearts and this is normal because kidneys and livers are more exposed to FR than hearts (73 nmol/mg) and lungs (59.88 nmol/mg). Follo-wing electrolysis, FR increased and SOD activity was significantly affected; as seen in the kidney it is (127.5 U/100 mg ±10.169 vs. 206.26 U/100 mg ± 8.307, $p< 0.05$), liver (395.22 U/100 mg ± 10.719 vs. 520.08 U/100 mg ±19.473 , $p< 0.05$), lungs (78.84 U/100 mg ±6.976 vs. 59.06 U/100 mg ±7.315, $p< 0.05$), and heart (88.22 U/100 mg ±10.626 vs. 113.16 U/100 mg ±5.816, $p< 0.05$).

For determination of total dosage of polyphenols, we take the galic acid as a standard. After measuring the absorbance at 725 nm and at different concentrations, we can plot the obtained values in a graph through which we can determine the amount of polyphenols (equivalent to galic acid) in our saffron (Figure 3).

DISCUSSION

In last decade, much attention was focused on the biological and medical properties of an ancient spice, saffron and its ingredients. Recent scientific findings have been encouraging, uniformly showing that saffron and its components (carotenoids) can affect carcinogenesis and have currently been studied extensively as the most promising cancer chemopreventive agents (Abdullaev, 2002). Different hypotheses for the modes of anti-carcinogenic and antitumor actions of saffron and its components have been proposed (Nair et al., 1991, 1995; Abdullaev and Frenkel, 1992; Abdullaev, 1994); one of them is the inhibitory effect on free radical chain reactions. In fact, most carotenoids are lipid-soluble and might act as membrane-associated high-efficiency free-radical scavengers, which is connected with their anti-oxidant properties (Molnar et al., 2000; Palozza and Krinski, 1992). The results obtained in this study emphasize on such properties of saffron that significantly decreased the levels of free radicals and act as an antioxidant.

There are many sources of FR; however, it is very difficult to simulate the conditions of the body *in vitro*. The electrolysis of a physiological solution is a reproducible model that supplies an important amount of FR (Lecours et al., 1998). In these adopted conditions, all saffron solutions showed a decrease in the FR but the boiled and soaked saffron had a significant decrease in the FR levels and a great antioxidant effect as compared with the other forms after 5 min of electrolysis. After analyzing the results, we observe that the antioxidant property of saffron increases and becomes more valuable as we change the applied dose.

Reactive oxygen species (ROS) and associated FR have been implicated in the etiology of various human diseases including inflammation, metabolic disorders, cellular aging and atherosclerosis, heart disease, stroke, diabetes, cancer, malaria, rheumatoid arthritis and HIV/AIDS (Alho and Leinonen, 1999; Odukoya et al., 2005). Moreover, it causes damages to the vital organs that lead to different diseases. These damages were observed after histopathological studies on the isolated organs (Figure 2).

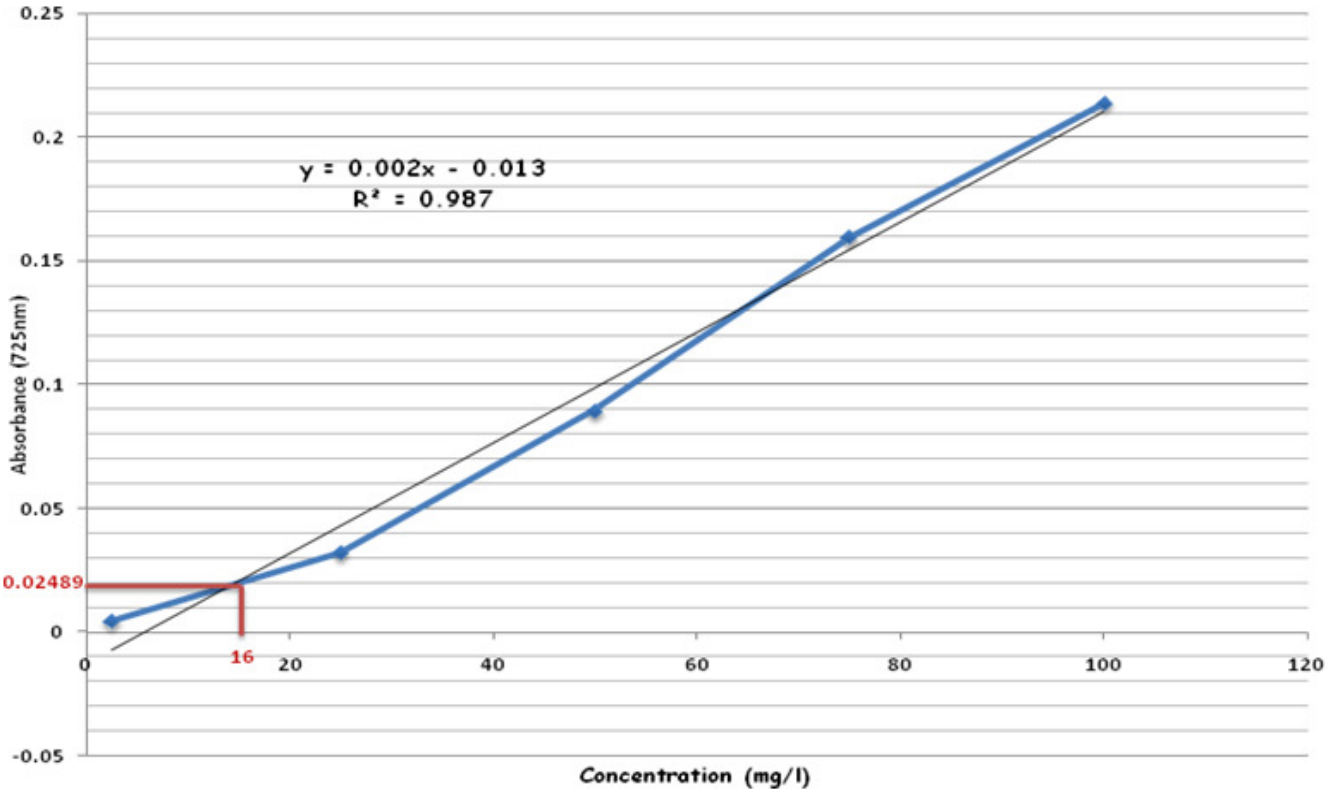

Figure 3. The standard, galic acid and the corresponding absorbance of polyphenols. 0.2489 is the absorbance of polyphenols (at 725 nm) in saffron and its concentration is 16 mg GAE/L (GAE: galic acid equivalent).

Under normal circumstances, the FR and ROS can be removed by the body's natural antioxidant defense, example glutathione peroxidase, catalase and SOD (Aruoma, 1994). However, overproduction of ROS may come from either the mitochondrial electron transport chain or excessive stimulation of NADPH or from exposure to environmental pollutants: cigarette smoke, UV-rays, radiation and toxic chemicals (Valko et al., 2006). One of the best known toxic effects of FR is the damage to cellular membranes (plasma, mitochondrial and endomembrane systems), which is initiated by a process known as lipid peroxidation in which cytochrome P450 generates extensive amounts of oxygen radicals under certain conditions. Induction with alcohol or acetone forms the cytochrome isoenzyme, which shows massive formation of ($O_2^{\cdot-}$), subsequent dismutation to H_2O_2 and the formation of other reactive species result in the oxidative breakdown of polyunsaturated fatty acids. In other words, lipid peroxidation is a general mechanism of tissue damage by free radicals and is known to be responsible for cell damage and may induce many pathological events (Kwiatkowska, 1999).

In the case of the heart, it is normally protected by the pericardium which somehow obstructs the passage of larger amounts of free radicals. In addition to being in direct contact with airborne toxicants, the entire volume of blood in the body passes through the lung one to five times a minute, exposing the lung to toxicants and drugs within the systemic circulation; thus, the possibility of damage from both inhaled and circulating agents is enormous (Hodgson, 2004). As with the liver and kidney, the lungs possess xenobiotic metabolizing enzymes such as superoxide dismutase (78.84 U/100 mg) thus it can detoxify the exogenous chemicals. Being the largest organ in the body, the liver is often the target organ for chemically induced injuries. Most xenobiotics enter the body through the gastrointestinal tract and, after absorption, are transported by the hepatic portal vein to the liver thus it is the first organ perfused by chemicals that are absorbed in the gut (Hodgson, 2004). Obviously, one can say that it must show the highest level of lipid peroxidtion whereas the results showed that in the kidney, the LPO level is higher than that of the liver. But when we analyze the superoxide dismutase level, we found that it is maximal in the liver (520.08 U/100 mg) and higher than that of the kidney (206.26 U/100 mg).

From this data (Tables 1 and 2), we can notice the defense strategy in hamsters which is similar to that in our body; where more lipid peroxidation is expected to exist, and where SOD are more active and higher. Consequently, there was no balance between the levels of free radicals and their scavenger SOD enzyme, and

there would be a significant increase in the lipid per-oxidation level p-value < 0.05 as compared to the control (Table 2). All these conditions resulted in a weakened body defense system, hence creating the need to provide the body with a constant supply of antioxidants through dietary supplementation. Here appears the necessity for saffron as a strong exogenous antioxidant that provides protection to vital organs and acts as a scavenger for free radicals together with SOD enzyme which also becomes less affected by excess free radicals in the presence of saffron. It significantly decreases the lipid peroxidation (p-value < 0.05) and maintains an important activity of the superoxide dismutase enzyme in all the organs that are subjected to free radicals compared to the electrolyzed organs in the absence of saffron.

Plant phenolics present in fruits and vegetables have received considerable attention because of their potential antioxidant activities (Lopez-velez et al., 2003). They are the secondary metabolites of plants that are generally involved in defense against ultraviolet radiation or aggression by pathogens (Manach et al., 2004). The main groups of polyphenols are: flavonoids, phenolic acids, phenolic alcohols, stilbenes and lignans (D'Archivio et al., 2007). They are believed to prevent many degenerative diseases including cancer and atherosclerosis (Roginsky, 2003). A study done in our laboratory (not published) about the antioxidants present in traditional white grape juice is considered one of the important sources of polyphenols and a strong antioxidant. It contains 6.285 mg GAE/L of polyphenols. In this study, we found that the total dosage of polyphenols in saffron is even higher than that in white grape juice. It is 16 mg GAE/L.

Conclusion

Our study showed that Lebanese saffron taken from the flower of *C. sativus* grown in Lebanon, which contains an important amount of polyphenols, has a strong antioxidant effect against free radicals and provide a protection for the organs (liver, kidneys, lung and heart) from some damages caused by free radicals, and these effects are dose-dependent. Also, it decreases the level of lipid peroxidation that would lead to severe problems and maintain the activity of one of the important antioxidant enzymes, the superoxide dismutase.

Acknowledgments

This project was supported by a grants team from the Central Research Committee, Lebanese University.

REFERENCES

Abdullaev FI (1994). Inhibitory effect of crocetin on intracellular nucleic acid and protein synthesis in malignant cells. Toxicol. Lett., 40: 243-251.

Abdullaev FI (2002). Cancer chemopreventive and tumoricidal properties of saffron *Crocus sativus*. Exp. Biol. Med., 227: 20-25.

Abdullaev FI, Frenkel GD (1992). Effect of saffron on cell colony formation and cellular nucleic acid and protein synthesis. BioFactors, 3(3): 201-204.

Alho H, Leinonen J (1999). Total antioxidant activity measured by the chemiluminescence method. Methods Enzymol., 299: 3-15.

Aruoma OI (1994). Nutrition and Health aspect of free radical and antioxidants. Food Chem. Toxicol., 32: 671-683.

Bathaie SZ, Mousavi SZ (2010) New applications and mechanisms of action of saffron and its important ingredients. Crit. Rev. Food Sci. Nutr., 50(8):761-86

D'Archivio M, Filesi C, Di Benedetto R, Gargiulo R, Giovannini C, Masella R (2007). Polyphenols, dietary sources and bioavailability. Ann. Ist Super Sanita, 43(4): 348-361.

Hodgson E (2004). A textbook of modern toxicology. Third Edition. Puplished by John Wiley and sons, Inc., Hobken, New Jersey.

Hosseinzadeh H, Karimi Gh, Niapoor M (2004). Antidepressant effects of *Crocus sativus* stigma extracts and its constituents, crocin and safranal, in mice. Acta Hortic., 650: 435-45.

Hosseinzadeh H, Khosravan V (2002) Anticonvulsant effects of aqueous and ethanolic extracts of *Crocus sativus* L. stigmas in mice. Arch. Irn. Med., 5: 44–47.

Hosseinzadeh H, Modaghegh M, Saffari Z (2009). *Crocus Sativus* L. Saffron Extract and its Active Constituents Crocin and Safranal on Ischemia-Reperfusion in Rat Skeletal Muscle. Evid. Based Complement Alternat. Med., 6(3): 343-350.

Kwiatkowska S, Piasecka G, Zieba M, Piotrowski W, Nowak D (1999). Increased serum concentrations of conjugated dienes and malondialdehyde in patients with pulmonary tuberculosis. Respir. Med., 93(4): 272-276.

Lecours S, Chahine R, Rochette L, Nadeau R (1998). Production of oxygen free radicals during electrolysis in rat heart. Free Radicals Biol. Med., 24: 573-579.

Lopez-velez M, Martinez-Martinez F, Del Valle-Ribes C (2003). The study of phenolic compounds as natural antioxidants in wine. Critical Reviews Food Sci. Nutr., 43: 233-244.

Manach C, Scalbert A, Morand C, Remesy C, Jimenez L (2004). Polyphenols: food sources and bioavailability. Am. J. Clin. Nutr., 79(5): 727-747

Molnar J, Szabo D, Pusztai R, Mucsi I, Berek L, Ocsovski I, Kawata E, Shoyama Y (2000). Membrane associated antitumor effects of crocine, ginseniside- and cannabinoid derivates. Anticancer Res., 20(2a): 861-867.

Mondon P, Leclercq L, Lintner K (1999). Evaluation of free-radical scavenger effects of *Helianthus annuus* extracts using new ex vivo stripping methods. Cosmetics, Aerosols & Toiletries in Australia, 12(4): 87-99.

Nair SC, Kurumboor SK, Hasegawa JH (1995). Saffron chemoprevention in biology and medicine: a review. Cancer Biother., 10(4): 257-264.

Nair SC, Pannikar B, Pannikar KR (1991). Antitumour activity of saffron (*Crocus sativus*). Cancer Lett., 57(2): 109-114.

Nair SC, Varghese CD, Pannikar KR, Kurumboor SK, Parathod RK (1994). Effects of saffron on vitamin A levels and its antitumor activity on the growth of solid tumors in mice. Int. J. Pharmacog., 32(2): 105-114.

Negbi M (1999). Saffron cultivation: past present and future prospects. In: Negbi M (Ed.), Saffron *Crocus sativus* L., vol. 154. Harwood Academic Publishers, Amsterdam, pp. 1-17.

Odukoya OA, Ilori OO, Sofidiya MO, Aniunon OA, Lawal BM, Tade IO (2005). Antioxidants activity of Nigerian dietary spices. Electr. J. Environ. Agric Food Chem., 4(6): 1086-1093.

Palozza P, Krinsky NI (1992). Antioxidant effects of carotenoids *in vivo* and *in vitro*: an overview. Methods Enzymol., 213: 403-420.

Rios JL, Recio MC, Giner RM, Man˜ez S (1996). An update review of saffron and its active compounds. Phytother. Res., 10: 189-193.

Roberts RA, Smith RA, Safe S, Szabo C, Tjalkens RB, Robertson FM

(2010). Toxicological and pathophysiological roles of reactive oxygen and nitrogen species. Toxicology, 276(2): 85-94.
Roginsky V (2003). Chain breaking antioxidant activity of natural polyphenols as determined during the chain oxidation of methyl linoleate in triton X-100 micelles. Arch. Biochem. Biophys., 414: 261-270.
Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J (2006). Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell. Biol., 7(1): 45-78.
Verma SK, Bordia A (1998). Antioxidant property of saffron in man. Ind. J. Med. Sci. 52: 205-207.