Efficacy of Aqueous Extract of Saffron (*Crocus sativus* L.) in Modulating Radiation-Induced Brain and Eye Retina Damage in Rats

Abd El-Azime A. Sh., Sherif N.H. and Eltahawy N. A.

Radiation Biology Department, Drug radiation research Department, National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority(AEA), Cairo, Egypt.

ABSTRACT

**Background:** Saffron (*Crocus sativus* L.) is a plant of the iris family (Iridaceae). Its stigma contains crocin, anthocyanin, carotene and lycopene which are known to have pharmacological effects on various illnesses. The aim of present study was to investigate the role of aqueous extract of saffron on the radiation-induced changes in rat (eye retina, brain) tissues and blood. **Material & methods:** Saffron was supplemented orally, via gavages to rats at dose of 100 mg/kg body wt/day for 2 weeks pre-exposure to 6.5 Gy (one shot dose) of whole body gamma-irradiation. Animals were sacrificed at the 1st day post radiation exposure. **Results:** whole body gamma irradiation of rats induce oxidative stress in eye retina and brain tissues and blood identified by significant elevation in the level of malondialdehyde (MDA), advanced oxidation protein products (AOPP) and protein carbonyl (PC) contents associated with significant decreases of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (Gpx) activities, and glutathione (GSH) content. Oxidative stress was concomitant with a significant decrease in brain dopamine (DA), norepinephrine (NE) and epinephrine (EPI) contents. Supplemented with Saffron extract pre- irradiation have significantly reduced the severity of radiation-induced oxidative stress and catecholamines alteration in the organs under investigations. **Conclusion:** Saffron exerts its modulating effect in the organs under investigations due to the presence of associated bioactive compounds with antioxidant properties. **Keywords:** Saffron, eye retina, brain, gamma rays, blood, rats, Lipid peroxidation, Oxidative stress

Introduction

Exposure of animals to ionizing radiation as a one shot dose causes a series of physiological changes known as acute radiation syndrome, which is dependent mainly on the exposure dose and may lead to death. One of the basic mechanisms of radiation damage is the production of free radicals, leading to the formation of peroxides and oxidative reactive species that damage protein, lipids and nucleic acids. Free radicals are also known to cause a pronounced decrease in antioxidant capacity and an excessive increase in oxidative stress. Radiation damage in biological systems is largely caused by the overproduction of reactive oxygen species (ROS), including superoxide anion (O$_2^-$), hydroxyl radical (‘OH), and hydrogen peroxide (H$_2$O$_2$), that overwhelm the levels of antioxidants, resulting in oxidative stress. One of the most important consequences of oxidative stress is lipid peroxidation. Damaged effects of ionizing radiation, free radicals or oxidative stress on the molecules and cells of Retina and brain are approved.

Effects of ROS are usually repaired before mutation by two important pathways, one of them, when the body's natural defense system and antioxidants consumed in the diet. The role of alternative medicine is a phyotherapy in various diseases. Phyotherapy is broadly defined as the use of natural therapeutic agents derived from plants or crude herbal drugs.

*Crocus sativus* L. commonly known as Saffron is a stemless herb of the Iridaceae family. Its pharmacologically active and important constituents are safranal, crocin, crocrocin and crocetin. Saffron has been constantly used in traditional medicine, as therapy for several conditions such as insomnia, depression, bronchospasm, cardiovascular diseases, gastrointestinal disorders, menstrual pain, menopausal problems, as analgesic, and even against cancer. Some of the medicinal-biologic properties of Saffron or its components are attributed to its antioxidant
features, which have been highlighted in several studies (7,8).

Saffron is a monoterpenic aldehyde which is the major constituent of the essential oil of Saffron and is responsible for the Saffron odor and aroma (5,9). Saffron and its constituents are widely evaluated for their pharmacological activities such as, antidepressant (10).

Saffron extract contains several different polyphenol-phytochemicals including hydrolysable tannins, condensed tannins, flavonoids, anthocyanins and ellagic acid with several biological activities (11, 12). Also, its stigma aqueous extract was showed to have inhibitory activities against acute and chronic inflammation in animal models (5). The phytochemistry and pharmacological actions of saffron components suggest a wide range of clinical applications for the treatment and prevention of cancer, as well as other diseases where chronic inflammation is believed to play an essential etiologic role (13).

The neuroprotective potential of the ancient spice Saffron was explored (14, 15). Saffron may protect photoreceptors from retinal stress, maintaining both morphology and function and probably acting as a regulator of programmed cell death, in addition to its antioxidant and anti-inflammatory properties (15). In a randomized, double blind, placebo-controlled study showed that three months of dietary Saffron supplementation significantly improved the focal estimated retinal flicker sensitivity in early patients (16, 17). In the present study, the role of Saffron against gamma irradiation-induced brain and eye retina damage has been studied.

Material and Methods

Animals (male Wistar albino rats) 10 ± 2 weeks old; 140 ± 20 g were purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt) were used. The animals were maintained under standard conditions of light, ventilation, temperature, and humidity and allowed to free access to standard pellet diet and tap water. All animal procedures were carried out in accordance with the Ethics Committee of the National Research Centre.

Radiation Facility

Animals were irradiated by using a Canadian Gamma Cell-40 (137Cs), manufactured by Atomic Energy of Canada Ltd., located at the National Center for Radiation Research and Technology (NCRRT), Nasr city, Cairo, Egypt. Animal whole bodies were exposed to 6.5 Gy applied in a single shot dose at a dose rate of 0.48Gy/min, was calibrated in Dosimetry department in (NCRRT).

Preparation of aqueous Saffron extract

Saffron (Crocus sativus L.) stigmas were collected from Ghaen (Khorasan Province, northeast of Iran). Saffron was soaked in 100 ml distilled water. After 2 hours, it was homogenized in the same distilled water, stirred for 1 hour and filtered. The residue was re-extracted with fresh distilled water. This aqueous extract was lyophilized and stored at (4°C until further use) (18). Animals received orally via gavages 100 mg/Kg body wt/day (19).

Experimental animals

Animals were randomly divided into 4 groups each of 10 rats as follows: Control Group: Normal healthy rats receiving distilled water via gavages for 2 weeks. Saffron extract Group: Rats daily received Saffron extract (100 ml/ kg body weight) for 2 weeks via gavages. Irradiated Group: Rats exposed to whole body gamma rays at one a single shot dose 6.5 Gy. Saffron extract +Irradiated Group: Rats received Saffron extract (100 ml/ kg body weight/day) daily for 2 weeks before exposure to whole body gamma rays and exposed to one single shot dose 6.5 Gy.

Preparation of Samples and biochemical analysis:

Animals were sacrificed at the 1st day post-irradiation. The whole brain and eye retina tissues were excised rapidly washed in ice cold saline and the cerebral hemispheres were separated out and wiped dry with a filter paper and weighed for the biochemical analysis. Blood was collected by heart puncture and centrifuged at 4,000 g for 15 min to separate serum for biochemical analysis. The cerebral hemispheres and eye retina were weighed, and 10% (w/v) tissue homogenates were prepared in 0.1 M phosphate buffer (pH 7.4). The
homogenates were centrifuged at 10,000 g for 15 min and aliquots of supernatants were separated and used for the different biochemical analysis. Superoxide dismutase activities (SOD), glutathione content (GSH) and glutathione peroxidase (GPx) were estimated according to Minami and Yoshikawa (20), Beutler et al (21) and Lawrence and Burk (22) respectively. Catalase (CAT) activity was assayed by the method of Sinha (23). Lipid peroxidation was assayed by the measurement of MDA content according to Yoshioka et al. (24). Advanced oxidation protein products (AOPP) level was determined according to Witko–Sarsat et al. (25). PC was determined as described by Reznick and Packer (26).

Statistical analysis

The results were presented as mean ± SD. All data were analyzed using one way analysis of variance (ANOVA). Duncan’s multiple range test was used to determine significant differences between means. The statistical analysis systems (SAS) package was used for statistical analysis. Differences between means were considered significant at P ≤ 0.05.

RESULTS

In the present study, rats treated with Saffron (100 ml/kg body weight/day) during a period of 2 weeks, showed insignificant changes (P>0.05) in the oxidant/antioxidant status of serum, cerebral hemispheres and eye retina values, insignificant changes in catecholamines (dopamine, epinephrine and norepinephrine) were recorded compared to control group, the level of MDA, AOPP and PC showed insignificant changes (P>0.05), compared to levels of control group (Tables 1-7). Radiation exposure of male albino rats provoked oxidative stress demonstrated by significant increase (P≤0.05) of MDA, AOPP and PC levels (Tables 1-3) associated with significant decreases (P≤0.05) of SOD, CAT and GPx activities and GSH content (Tables 4-6). Catecholamine contents (dopamine, epinephrine and norepinephrine) showed a significant decrease compared to their respective values of the control group on the 1st day post-irradiation (Table 7). The supplementation of Saffron extract has improved the oxidant/antioxidant status of cerebral hemisphere, eye retina, serum and catecholamines content in the cerebral hemispheres.

Table (1): Effect of Saffron extract on MDA (nmol/g fresh tissue), AOPP (µmol/g fresh tissue) and PC (nmol/g fresh tissue) levels in brain cerebral hemispheres in different animal groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA</th>
<th>AOPP</th>
<th>PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>56.92±12.06 a</td>
<td>0.91±0.07 a</td>
<td>75.24±13.72 a</td>
</tr>
<tr>
<td>Saffron</td>
<td>57.45±12.05 a</td>
<td>0.93±0.08 a</td>
<td>76.35±13.80 a</td>
</tr>
<tr>
<td>Irradiated (6.5Gy)</td>
<td>109.45±13.54 b</td>
<td>1.81±0.09 b</td>
<td>98.3±14.63 b</td>
</tr>
<tr>
<td>Irradiated + Saffron</td>
<td>68.16±12.78 c</td>
<td>1.42±0.08 c</td>
<td>83.5±12.91 c</td>
</tr>
</tbody>
</table>

Values are expressed as means of 10 records ± standard deviation (SD). Means with different superscripts are significantly different at the 0.05 level.

Table (2): Effect of Saffron extract on MDA (nmol/g fresh tissue), AOPP(µmol/g fresh tissue) and PC nmol/g levels in eye retina in different animal groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA</th>
<th>AOPP</th>
<th>PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>47.91±10.76 a</td>
<td>0.71±0.06 a</td>
<td>71.21±10.72 a</td>
</tr>
<tr>
<td>Saffron</td>
<td>46.84±10.55 a</td>
<td>0.65±3.05 a</td>
<td>69.85±10.90 a</td>
</tr>
<tr>
<td>Irradiated (6.5Gy)</td>
<td>98.54±12.04 b</td>
<td>1.67±0.07 b</td>
<td>88.32±13.63 b</td>
</tr>
<tr>
<td>Irradiated + Saffron</td>
<td>56.56±11.08 c</td>
<td>1.30±0.06 c</td>
<td>75.53±12.41 c</td>
</tr>
</tbody>
</table>

Legends as in table 1
Table (3): Effect of Saffron extract on MDA (nmol/ml), AOPP (mg/dl) and PC (nmol/ml) levels in serum in different animal groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA</th>
<th>AOPP</th>
<th>PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.39±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.85±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.59±11.72&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Saffron</td>
<td>0.38±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.86±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.12±10.80&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Irradiated (6.5Gy)</td>
<td>0.19±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.93±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.3±12.63&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Irradiated + Saffron</td>
<td>0.28±0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.51±0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>71.5±12.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Legends as in table 1

Table 4: Effect of Saffron extract on SOD (U/mg protein), CAT(U/mg protein) and GPx (U/g) activities and GSH (U/g) content in brain cerebral hemispheres in different animal groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD</th>
<th>CAT</th>
<th>GSH</th>
<th>Gpx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.38±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.09±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.24±9.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.5±1.98&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Saffron</td>
<td>0.37±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.02±10.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.3±1.74&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Irradiated (6.5Gy)</td>
<td>0.19±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.05±0.006&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.35±8.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.59±1.34&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Irradiated + Saffron</td>
<td>0.32±0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.075±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>33.01±7.41&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.1±1.34&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Legends as in table 1

Table 5: Effect of Saffron extract on SOD (U/mg protein), CAT(U/mg protein), and GPx (U/g) activities and GSH (U/g) content in eye retina in different animal groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD</th>
<th>CAT</th>
<th>GSH</th>
<th>Gpx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.92±2.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.59±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.54±2.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.54±1.72&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Saffron</td>
<td>7.41±2.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.60±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.54±2.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.60±1.92&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Irradiated (6.5Gy)</td>
<td>3.45±1.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.34±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.36±1.93&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.54±1.52&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Irradiated + Saffron</td>
<td>5.01±1.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.49±0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>39.52±1.41&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.76±1.72&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Legends as in table 1

Table 6: Effect of Saffron extract on SOD (U/ml), CAT(U/ml), GPx (U/ml) activities and GSH (mg/dl) content in blood in different animal groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD</th>
<th>CAT</th>
<th>GSH</th>
<th>Gpx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45.91±10.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.78±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.02±3.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.54±2.72&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Saffron</td>
<td>47.45±10.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.77±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.62±3.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.54±2.72&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Irradiated (6.5Gy)</td>
<td>23.95±8.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.40±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.35±1.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.54±2.72&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Irradiated + Saffron</td>
<td>40.64±7.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.62±0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>52.59±2.71&lt;sup&gt;c&lt;/sup&gt;</td>
<td>38.54±2.72&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Legends as in table 1
**Discussion**

Radiation has been proposed as environmental factors that may affect several enzymes and other biomolecules related to neurotoxicity and Alzheimer's disease. Radiations are commonly used in a number of medical and industrial situations; however, their prooxidative effects limit their applications (1,3).

Oxidative stress with the subsequent production of ROS was postulated as one of the mechanisms of radiation toxicity (28). Experimental evidences have considered the brain a radiosensitive organ because of its high O$_2$ utilization rate, its high content of polyunsaturated fatty acids, which are prone to lipid peroxidation, its high content of iron, which through the Fenton reactions increase the formation of free radicals (29). In addition, relative to other organs brain tissues are poor in antioxidants (30).

In the present study, whole body exposure of male albino rats to gamma radiation (6.5 Gy) provoked an imbalance between oxidant and antioxidant species in the cerebral hemispheres of rats. Significant increase in the level of MDA, AOPP and PC accompanied by significant decreases of total SOD, CAT and GPx activities and GSH content were recorded the 1st day post-irradiation. Oxidative stress was concomitant with significant decreases in the level of catecholamine (dopamine, epinephrine and norepinephrine).

The elevation of brain lipid peroxidation in this study, which was evidenced by the increased production of MDA, suggests the participation of free-radical-induced oxidative cell injury in mediating the toxicity of IR(31). The increase of TBARS level is probably due to the interaction of ('OH ) resulting as a by-product of water radiolysis, upon exposure of rats to ionizing radiation, with the polyunsaturated fatty acids present in the phospholipids portion of cellular membranes initiating the lipid peroxidation chain reaction(2). In the same way, the increase of PC and AOPP levels might be attributed to the interaction of proteins with ROS(32). The decrease of antioxidants might result from their increased utilization to neutralize the excess of free radicals generated in the body after exposure to ionizing radiations, where SOD catalyzes the reduction of O$_2^-$ to H$_2$O$_2$. The majority of which is broken down to oxygen and water by CAT. In addition to CAT, GSH-Px effectively removes H$_2$O$_2$ in presence of adequate amount of GSH (33). In addition, radiation-induced cell membrane damage causing their release to the blood stream (34) and protein oxidation might contribute to the partial inactivation of enzymes (35).

However, the decrease of monoamines level may be due to alterations in the dopaminergic system (36), or might be attributed to decreased synthesis resulting from radiation-induced damage to the ileal mucosa and reduction in net ilea absorption, where a decrease in the absorption of tryptophan would reduce the synthesis of serotonin, while a decrease in the absorption of L-tyrosine may diminish the production of DA, NE and EPI (37).

It is well documented that antioxidants play an important role in mitigating the damaging effects of oxidative stress. In the present study, Saffron (100 mg/kg body weight/day) administered to rats via gavages for 2 weeks pre-irradiation, significantly attenuated radiation-induced oxidative stress in the cerebral hemispheres serum and eye retina. The decrease of MDA, PC and AOPPs, suggests its free radical scavenging activities. Furthermore, the significant amelioration of SOD, CAT and GPx activities and GSH content suggest its potential effect in enhancing antioxidant defense. The results corroborate the findings which stated that Saffron...
possesses antioxidant activity and radical scavenging properties \((38, 39)\). SOD and CAT make up a primary line of defense against superoxide anion \((O_2^-)\) and hydrogen peroxide \((H_2O_2)\), respectively. More precisely, SOD is a specific scavenger of superoxide anion. It converts harmful superoxide radicals to \(H_2O_2\), which is detoxified by CAT to harmless by products. GPx, which belongs to the family of selenoproteins, is present in relatively large amounts in the epithelium of the lens \((40)\).

The decrease of catecholamine levels observed in the cerebral hemispheres of irradiated rats could be attributed to radiation-induced oxidative stress causing their oxidation and increased catabolism \((41)\). In addition to decreased synthesis resulting from radiation-induced damage to the ileal mucosa and reduction in net ilea absorption \((42)\), where a decrease in the absorption of L-tyrosine may diminish the production of catecholamine.

According to the results obtained in the current study, administration of Saffron \((100 \text{ mg/kg body weight/day})\) via gavages 2 weeks pre-irradiation induced a significant increase of catecholamine levels, compared their respective levels in the irradiated group. The results support the role of Saffron in scavenging free radicals \((19)\), Hosseinzadeh and Younesi \((43)\) stated that improvement in macular function is an effect of integrated activities of Saffron’s chemical compounds, mainly of crocin, and crocetin, antioxidant derivatives of carotenoids, which may act through a protective mechanism similar to that seen with carotenoid supplementation \((6, 44)\). Our results demonstrate that Saffron possesses antioxidant activity and radical scavenging properties in the homogenate of Retina tissues, where the efficacies of Saffron supplementation was already demonstrated in a previous blind, randomized, placebo-controlled study \((16)\). Saffron may protect photoreceptors against retinal stress, maintaining both morphology and function and probably acting as a regulator of programmed cell death. To identify the genes and noncoding RNAs (ncRNAs) involved in the neuroprotective actions of Saffron, Natoli et al. \((15)\) used continuous bright light as a standardized assay of photoreceptor damage in albino Sprague Dawley rats. RNA from the eye of exposed and unexposed bright light animals was hybridized to Affymetrix rat genome ST arrays.

Protective effect of Saffron and its main constituent crocin and safranal have been shown in different in vivo and in vitro models \((45, 46)\). It was shown that Saffron and its constituents could decrease lipid peroxidation in renal \((47)\), hippocampal \((46)\) and skeletal muscle \((48, 49, 18)\).

**Conclusion**

Based on the results obtained in the current study, it could be concluded that Saffron would attenuate the severity of biochemical disorders, mainly attributed to its free radical scavenging and antioxidant properties especially in Brain and Retina.

**References:**


8. Asdaq SM and Inamdar MN(2010): Potential of Crocus sativus (saffron) and its Constituent, Crocin, as...
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