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Effect of Saffron aqua Extract on Angiogenesis in Chick Chorioalantoic Membrane

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Abstract

***Background*:** Studies confirmed anticancer properties of saffron extract. Angiogenesis, formation of new blood vessels which is necessary in many physiological stages and pathological events such as tumor growth. So it would be an effective strategy to inhibit angiogenesis to treat many cancers and metastasis. In this experimental study, effects of saffron on angiogenesis in chick chorioalantoic membrane (CAM) were investigated.

***Materials and Methods*:** Fifity ross fertilized eggs divided in 5 groups, including: control, sham exposed, experimental group 1, 2 and 3. In second day of incubation window was opened on eggs. In day 8 gelatin sponges contain gelatin and albumin was put on chorioalantoic membrane and was soaked with Saffron aqua extract in concentration 100, 400 and 800 µg/ml. In 12th day all cases were photographed by photo stereomicroscope. Numbers and lengths of vessels around the sponges were measured by Image J software. Data were analyzed with SPSS-16 in significant level $p<0.05$.

***Results*:** According to data analysis, changes had no correlation on the average length of blood vessels in the first experimental group (41.5±5.5 mm), compared with the control group, (44.5±2.4 mm). While in the second and third experimental group (40.2±2.1 mm) and (38.4±3.8 mm) these changes were significant ($p=0.001$). On the other hand, the average number of blood vessels in the first experimental group (22.07±5.2) in compare with the control group (27.46±4.4) shows a significant reduction ($p=0.02$), this decline between the second (18.80±4.4) and third (15.87±3.8) experimental groups was significant at the level of $p=0.001$.

***Conclusion*:** Saffron extract has a dose dependent inhibitory effect on angiogenesis in chick chorioalantoic membrane.

Introduction

Angiogenesis means creation of new capillaries from existing vessels, firstly used by Hertig in 1935. In 1971, Judah Folkman published a paper that discusses his new theory about angiogenesis. He reported that "tumors never grow beyond a certain size unless their vessels increase." He stated that "by inhibition the angiogenesis, tumors remain small in size and will not be harmful" [1]. Angiogenesis is important in physiological processes such as wound healing, growth and development of organs and also menstrual cycles may play a role in various pathological conditions including tumor growth and metastasis, rheumatoid arthritis [2].

Cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives each year and an extremely promising strategy for cancer prevention today is chemoprevention with natural products. Some plants, vegetables, herbs and spices used in folk and traditional medicine have been accepted currently as one of the main sources of cancer chemopreventive drug discovery and have the ability to inhibit cancer and considered as one of the main resources of cancer drug inhibitors. Saffron is the dried stigma of the plant *Crocus sativius L.* used both as a spice in food industry and as a drug in traditional medicine which shows variety of antioxidant and anti cancer properties. The active metabolites are: safranal, crocin, and dimethyl crocetin, crocetin and anthocyanin, carotene and lycopene, and pharmacologic effects of these compounds for the treatment are eligible [3].

Recently it has been reported that saffron extract has anticancer properties and was effective on different types of leukemia, osteosarcoma, fibrosarcoma, and carcinoma [4]. With regard to the fact that anti-angiogenesis effect of saffron extract has not been studied so far, in this study consider this matter.

Materials and Methods

This experimental research had been done in animal developmental biology laboratory of Mashhad Azad University in 2012 and for this research study of chick chorioalantoic membrane (CAM) is used as an appropriate model of study angiogenesis in vivo [5, 6].

Ross fertilized eggs was purchased from Toos company, then 50 of those eggs randomly divided in 5 groups, including group of control group (which were stored in the normal conditions), sham exposed (treated with vehicle PBS), experimental group 1, 2 and 3 (treated with concentrations of 100, 400 and 800 micrograms per milliliter aqua saffron extract), fertilized eggs were incubated at 38ºC and 55-65% humidity with automatic rotation in incubation system. In day 2 of incubation a

window was opened on eggs in sterile condition, which prepared by laminar hood (Telstar, Spain), part of its shell remove and small window opened which covered by sterile paraffin and lamellas (Iran Fara).

Then the eggs are transferred to an incubator and rotated manually twice a day for normal development of embryos. In day 8 of incubation in sterile condition a gelatin sponge contain gelatin in normal salin and albumin with 200 µl of penicillin streptomycin which is fresh, cut into 4×4 mm and put on chorioalantoic membrane and In samples treated with aqueous extract of saffron, the song was soaked by 10 µl of Saffron aqua extract then its place covered again and return to incubator.

In 12th day of incubation, all cases were photographed by research photo stereomicroscope (Ziess, Germany) and take appropriate photos. Variables include the number and length of blood vessels that for all samples was measured around gelatin sponge. As chorioalantoic membrane is an anatomic disk like with 400 µm diameter, so all blood vessels around gelatin sponge are countable. Quantitative data were obtained using SPSS-16 with significance level of $p<0.05$ and the *t*-test and ANOVA analysis and post hoc Tukey test was performed in cases of need.

Results

Comparing mean of number (27.46±4.4) and length of blood branches (44.5±2.4 mm) in the control samples with the number (27.37±4.5) and the length (43.8±2.5 mm) in sham-exposed samples did not show any significant difference, so all experimental samples were compared with control. The mean number of branches and length in samples which treated with 100, 400 and 800 mg/ml saffron aqueous extract to the number and length of branches in the control samples showed a dose dependent significant decrease ($p<0.05$) (Fig. 2, 3 & Table 1).

According to data analysis, changes had no correlation on the average length of blood vessels in the first experimental group (41.5±5.5 mm), compared with the control group, (44.5±2.4 mm). While in the second and third experimental group (40.2±2.1 mm) and (38.4±3.8 mm) these changes were significant ($p=0.001$). On the other hand, the average number of blood vessels in the first experimental group (22.07±5.2) in compare with the control group (27.46±4.4) shows a significant reduction ($p=0.02$), whereas this decline between the second (18.80±4.4) and third (15.87±3.8) experimental groups was significant at the level of $p=0.001$.

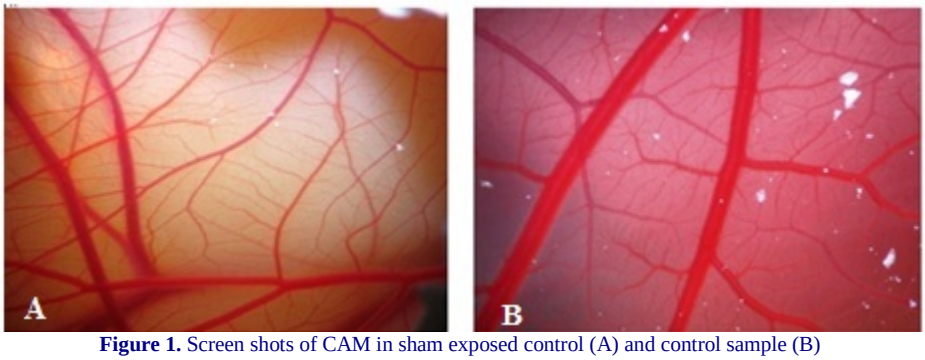

Figure 1. Screen shots of CAM in sham exposed control (A) and control sample (B)

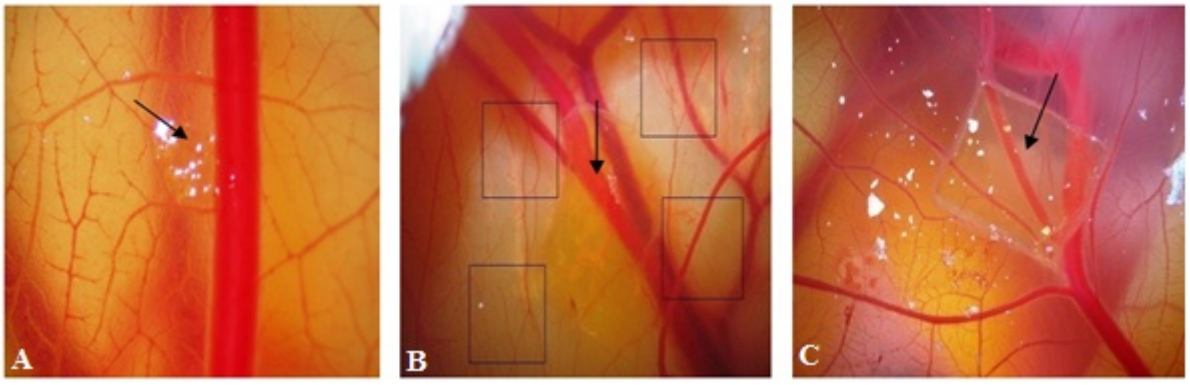

Figure 2. Reduction of angiogenesis in the samples treated with different concentrations, respectively, from A to C, 800, 400 and 100 mg/ ml saffron, squares shows the place of counting around the gelatin sponge and flash shows song site

Table 1. Mean of variables include the number and length of blood vessels, standard deviation and *p*-Values in different groups

Groups	Mean of length of blood vessels (mm)±SD	*p*-Value	Mean of number of blood vessel±SD	*p*-Value
Control	44.5±2.4	-	27.4±4.4	-
Sham-exposed	42.8±2.6	>0.05	26.6±3.7	p>0.05
First group	41.5±5.5	0.132	22.0±5.2	0.02
Second group	40.2±2.1	0.001	18.8±4.4	0.001
Third group	38.4±3.8	0.001	15.8±3.8	0.001

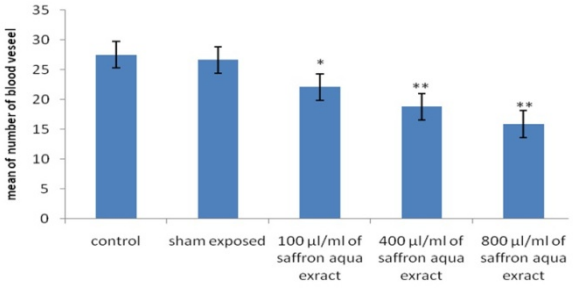

Figure 1. Average of number of vessels in the samples which treated with aqueous extract of saffron compared with the control sample and sham exposed * $p<0.05$, ** $p<0.001$

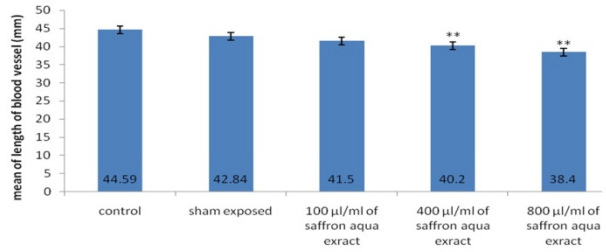

Figure 2. Average of length of blood vessels in the samples which treated with aqueous extract of saffron compared with the control sample and laboratory control * $p<0.05$, ** $p<0.001$

Discussion

According to the results of this study, the aqueous extract of saffron, reduce significantly the number and length of vessel branching in the chick chorioalantoic membrane in treated groups in compare with the control and sham exposed in a dose-dependent manner ($p<0.05$). So it seems that this plant has anti-angiogenesis properties and Saffron could be considered as a promising chemotherapeutic agent in cancer treatment in future.

Despite of many advances in the field of controlling and curing malignant diseases, including cancer, there were lots of research with the aim of understanding the mechanisms involved in their development, manufacturing, cell proliferation and angiogenesis had been done. On the other hand wide uses of medicinal plants are devoted to this research.

Various studies have shown that the important thing in cancer treatment with chemotherapy, acquired tumor resistance to these drugs and this has created major problems in cancer therapy. As mutation and genetic transitory in tumor cells are more than normal cell, they resistant to drugs so much while endothelial cells are normal with less mutations, so inhibition of angiogenesis with natural products based on these type of cells did not cause drug resistance or to provide less resistance. Inhibition of angiogenesis can also be effective not only in cancer but also in many diseases including rheumatoid arthritis, diabetic retinopathy, even in cases of obesity [7].

Apoptosis or programmed cell death is one of the anti-cancer mechanisms. Results from some studies have shown that saffron extract on hepatocyte cell line and HeLa is concentration-dependent cytotoxic effects [8]. Also, some anti-carcinogenic and anti-tumor effects of saffron extract and induction of apoptosis has revealed which shows that saffron extract concentrations, in a dose-dependent manner, induce cytotoxicity in tumor cells with an IC50 value against MCF7 and HepG2 cells in concentration 400 and 950 µg/ml respectively [9, 10]. These in vitro data coincide with our in vivo results. On the other hand, treatment of tumor cells with saffron extract shows reduction in the level of intracellular sulfidril compounds, this can also be an explanation for the cytotoxicity effect of saffron [11].

Pharmacological studies have also shown that the saffron extract perform anti-tumor effects via absorbance of free radicals from the blood which shows significant inhibitory effects of extract at higher doses [12]. Our data analysis accurately accordance this results. Yukihiro Shoyama studied anti-aging effects of crocin which is mostly through activation of superoxide dismutase [13]. Saffron extract also significantly contribute to the inhibition of human colon cancer cells and has anti-tumor activity against tumor cells in mouse skin [14].

In another study, the effects of the active ingredients of saffron, safranal, on lipid peroxidation has been considered. These experiments showed that the safranal, increased tissue oxygen and had scavenge effect on free radicals, which have been induced oxidative stress by genotoxic compounds [15]. Since one of the properties of beginning the angiogenesis in tissue is hypoxia, so at least part of the anti-angiogenesis effects of saffron can be attributed to the tissue oxygen circulation. In some articles the anti-tumor effects and biological properties of saffron like anti genotoxic, cytotoxic and anti-cancer has been mentioned [16, 17].

Study of cytotoxic effects of saffron on human liver carcinoma cell line has shown that, saffron has inhibitory effect on synthesis both RNA and DNA. Inhibitory effects caused by the interaction of free radicals and cytotoxic effects results from interaction of carotenoids and topoisomerase 2 [18]. It is also said that saffron can improve and simulate immune system [19]. Studies have shown that different plant species can be through different mechanisms may have effects on angiogenesis or anti-angiogenesis [20].

The results show that saffron aqua extract has an inhibitory effect on angiogenesis and decrease the formation of new blood vessels in chick chorioalantoic membrane dose-dependently. The results of this research could be start for another research on saffron and its active metabolites. Its inhibitory effect on cell reproduction, cytotoxicity and anti angiogenic effect of this plant, propose saffron as an appropriate with high efficiency candidate against cancers in pharmacology.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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