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Effect of Safflower Flower (Carthamus tinctorius L) Extract on Serum Superoxide Dismutase Activity in Rats

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Original Article

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Abstract:

Background and aims: In recent years, due to side effects of chemical drugs, some people hsve turned to traditional medicine. Safflower is a medicinal plant that is used as a relatively cheap dye for making sweets and cooking in addition to its therapeutic effects. Superoxide dismutase (SOD) is one of the important enzymatic antioxidants that plays a role in fighting reactive oxygen species .In this study, the effects of safflower flower on serum activity of SOD in male wistar rats were studied to determine the adverse and beneficial effects of the plant on this important enzymatic antioxidant.

Methods: Sixty rats were divided into four groups consisting of three experimental groups and one controle group. The extract of *safflower* at concentrations of 100, 200 and 300 mg/kg body weight was subcutaneously injected to rats of experimental groups 1, 2 and 3, respectively, for two periods of 14 and 28 days, and then blood samples were collected from their hearts and serum SOD level was measured spectrophotometrically.

Results: SOD level increased after adminstration of safflower extract at low and medium doses in the short term and at hight doses in the long term.

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Conclusion: The safflower extract has beneficial effects on SOD, with no adverse effects on this enzyme at different doses.

Keywords: *Safflower flower extract; Superoxid dismutase; Rat.*

INTRODUCTION

As the economic development has substantially contributed to the widespread use of chemical substances for manufacturing food products, the incidence of diseases resulting from use of these chemicals is inevitable. Saffron is the most expensive spice in the world and may be used as a natural food dye in restaurants instead of artificial dyes, while its consumption in most foods for the long term may have adverse effects on human health.

The oxidative damage caused by free radicals is naturally neutralized by the antioxidant defense system. Antioxidants protect the body against reactive oxygen species (ROS) through direct modification or through inhibiting the activity of oxidizing enzymes (1). This system consists of a series of enzymatic and non-enzymatic components (2,3,4). Superoxide dismutase (SOD) acts in the frontline of defence against ROS and oxidative stress in cells (5,6) Changes in SOD encoding gene transcript level and SOD activity (7) are regarded as indicators of the level of the produced ROS and oxidative stress (8). Food antioxidants produce a substantial protective effect against these diseases. An essential mechanism for the protection of host cells against excess levels of free radicles is the enzymatic antioxidant defense system, of which SOD is a key enzyme (9). For example, Sarija Bajaja and Afreen Khan (2012) reported that antioxidants serve as essential tools for the investigation of oxidant stress-related diabetic pathologies and despite the obvious potential merit of a replacement therapy, the safety and efficacy of antioxidant supplementation remain to be established (10). The changes in plasma antioxidants and malondialdehyde can be due to cellular damage caused by the activities of free radical molecules.

Free radicals are chemically active molecules, mostly ROS (superoxide and hydrogen peroxide), and are normally produced during biological processes in various organisms (11, 12). The unstable compounds of free radicals induced by infections can react with cellular lipids, proteins, nucleic acids, and carbohydrates, among which lipids are the most sensitive (13). It has been established that an excessive amount of free radicles, particularly hydrogen peroxide, in cells induces elevation of

antioxidant enzymes activity to neutralize ROS, and prevents internal cellular damage. Conversely, decreased severity of oxidative stress (and thus the free radical level) may result in the reduction of antioxidant enzymes activity (11).

Safflower flower is used to colour foods instead of saffron, and its oil is used as a low-risk fat. This plant with the scientific name of *Tinctorious Carthamus* is native to the Far East and is currently being cultivated in many countries. The plant at an age of 1-2 years has a height of 30 to 60 cm and serrated leaves that end to the delicate and sharp spines. This plant has yellow-reddish flowers. The active ingredient of its flower is kartamyn (kartamyk acid). There are six species of this plant in Iran, which have many health benefits including wound healing, anti-inflammatory, cough soothing, antibacterial, platelet aggregation reducing, hypocholesterolemic, laxative and expectorant; and its oil contains large amounts of unsaturated fatty acids (n- polyunsaturated) and approximately 75% linoleic acid, 13% oleic acid, 0.6% palmitic acid, and 3% stearic acid. The species also have a direct small chain of fatty acid that is *γ-tocopherol*. Safflower oil is a rich source of linoleic acid. In the seeds of this plant, glycosaminoglycans exist as trachelosides (14, 15).

Studies have shown that consumption of safflower and soy reduces lipid and liver cholesterol and enhances neutral steroids. Previous studies have shown that safflower oil is effective on the levels of platelet and *thromboxane B2* (15). There are reliable evidence on safflower therapeutic effects, but the effects of different dosese of this plant on SOD activity is unknown.Therefore, the aim of this study was to investigate the adverse and beneficial effects of safflower on serum SOD activity.

MATERIALS AND METHODS

Study design:

In this study, 60 Wistar adult male rats weighing 180±20 g were procured from the Opioid Breeding Laboratory Animals Department of Islamic Azad University, Kazerun Branch. All the procedures were approved by the Institutional Animal Care and Use Committee of the University (approval code: IR.IAU.KAU.REC.1398.162).

The animals were transported to the laboratory one week before the test to acculturate to the laboratory conditions. During the study, the rats were kept under identical conditions (22-24 °C and 12:12 light-dark cycle) and had ad *libitum* access to food and water.

The rats were divided into four groups, groups 1, 2 and 3 received, respectively, 100, 200, and 300 mg /kg of the extract subcutaneously once a day (16,17). Group 4 was considered as controls that received normal saline alone. The total period of the intervention was 28 days and after 12 hours from the last injection, the blood samples were collected from the hearts at completion of 14th and 28th days of intervention. Serum was isolated by centrifugation at 2500 rpm for 15 minutes at 4 °C and transported to the laboratory on ice.

SOD activity was assayed using one diagnostic ZellBio kit (ZellBio, Germany) according to Arthur and Boyne protocol (1985) and expressed in U of SOD /ml of serum (18).

Extraction:

Extraction was performed in the Nutrition Laboratory of Shiraz University of Medical Sciences.To this end, 500 g of the prepared powder in 50% of ethanol was put under pressure for 72 hours. Afterwards, the tap of the extracting machine was opened to collect the extract drop by drop at the bottom of the machine.

The solvent (alcohol) was constantly added by pipette to the top of the container to prevent the liquid level loweing and the plant powder drying. It should be noted that while the extract was collected by the separating funnel of the percutaneous device, the alcoholic solvent was added drop by drop from the top of the device until the extractsbecame colorless, indicating that the extract was fully collected. Next, the prepared liquid containing ethanol in percutor, its solvent removed by rotary device to be fully concentrated. Then, the brown extract was collected by means of a desiccating and vacuum pump and weighted according to the doses in required quantities and was solved in normal saline. Then, the resulting extract was administered according to the body weights of the rats.

Data analysis

Data analysis was done by SPSS 10 using ANOVA, Tukey's test, and *t*-test.

RESULTS

According to the results of this study (Tables 1 and 2), there was a statistically significant difference in the mean activity of SOD between experimental groups 1 and 2 and control group on the 14th day (P<0.05).

Table 1: Mean±standard deviation of superoxide dismutase (U/ml) in the different groups on 14th and 28th days of intervention

Groups	Day 14	Day 28
Control	92.19±2.08	92.19±2.08
Experimental 1	45.94±3.0	35.13±2.04
Experimental 2	45.12±3.9	30.74±0.53
Experimental 3	40.20±4.12	48.97±2.38

Table 2: The presence or absence of significant diffrence in superoxide dismutase between experimental groups and control group on 4th and 28th days of intervention

Groups	Day 14	Day 28	Day 14 compared to day 28
Experimental 1	0.016	0.262	0.021
Experimental 2	0.024	0.989	0.015
Experimental 3	0.187	0.0005	0.095

Moreover, there was a significant difference in the mean activity of the enzyme between experimental group 3 and control group on the 28th day (P<0.05). There was also a significant difference in the mean activity of SOD between the 14th and 28th days in experimental groups 1 and 2 (P<0.05).

Discussion

The mean level of SOD activity increased in different experimental groups on the 14th day as compared to the control group and this increase was statistically significant in experimental groups 1 and 2, so that it caused a significant difference in SOD activity in these groups compared to that in the control group.

Despite the increase in the mean activity of SOD in the three experimental groups compared with the control group on the 28th day, this increase was statistically significant only in experimental group 3 so that a significant difference in the mean level of the enzyme was observed compared to that in the control group. It can be argued that hydroalcoholic extract of safflower at low and medium doses

significantly increases the mean level of SOD in the short term, and the body will adjust to the low and medium doses in the long term and only high doses of the extract lead to increased mean SOD level on the 28th day.

Therefore, despite the increasing effect of safflower extract on the mean level of SOD, the extract cannot be used in the long term as a stimulant for the production of SOD because of the acquired adaptation to consumption of this extract. It can be used as an additive of SOD from safflower extract in the short term.

The antioxidant effects of safflower are due to a powerful antioxidant compound of its oil named p-n control or serotonin (19). It is known that the antioxidant causes the fibroblast's progressive activity of mice and humans in the environment and prevents proinflammatory cytokine production in human monocytes. The antioxidant activity and preventing role in the production of the proinflammatory cytokine from human monocytes is another effect of this plant (20,21).

SOD is the first enzyme of the antioxidant defense system and it seems that the extract of safflower flower has the greatest effect on this antioxidant. SOD is the first natural antioxidant acting against the toxicity of free radicals. This enzyme is a powerful antioxidant that protects the body from damage caused by superoxide, which is the toxic radical generated in the mitochondria. Free radicals are highly reactive molecules that are produced by cells during normal metabolism. Free radicals can accumulate and cause damage to mitochondrial DNA, nuclei and proteins inside the cell.

One of the iron-sulfur containing hydrates in metabolic pathways is disabled by superoxide.

Regarding the antioxidant properties of medicinal plants, Meena et al. (2012) reported that the plant protects brain cells against free radicals and treats the neurological problems caused by free radicals by producing antioxidant effects (22). Arjmandi et al (2018) observed no significant differences between activities of catalase and GPX in control group in comparision to experimental groups on the 14th and 28th days of study ($p>0.05$) (23).

Khoshvaghti et al. (2013) reported that after 14 days of administering hydroalcoholic safflower flower extract, the activities of glutathione peroxidase and SOD increased in a dose-dependent situation (24).

In this regard, Khorasani et al. (2012) observed walnut's effect in increasing serum glutathione peroxidase level, and that walnut and lemon juice significantly reduced MDA level in blood plasma (25).

Regarding the medicinal effects of the plant, Asgary et al. (2012) reported that the hydroalcoholic extract of safflower flower was effective in the treatment of diabetes (26). Dehkordi et al. (2014) investigated the effect of aqueous extract of safflower on the reproductive system in female rats and observed that the extract was more effective on ovarian activity compared with wounds and had a positive impact on the fertility of female rats (27). Khoshvaghti et al. reported that the continuous short-term use of the safflower could decrease the level of thyroid hormones temporarily, while in the long term, it did not show the same effect (28).

Ellis et al. (2000) found that concomitant use of safflower oil and a high-fat diet caused insulin resistance and increased acyl coenzyme-A (29). The other advantage of safflower is lack of producing any harmful effect on blood pressure, heart rate, and contraction of the pulmonary artery (22,30). Rahimi et al (2014) reported the effect of safflower seed oil in the prevention of diabetes-related complications (31).

CONCLUSION

The results of this study showed a dramatic increase in SOD after short-term use of safflower extract at low and medium doses. Therefore, this plant can be used to boost the antioxidant immune-enzyme system, because SOD is one of the most important antioxidant enzymes in the body, and it is therefore useful to add this plant to our diet.

CONFLICT OF INTERESTS

Authors have no potential commercial or financial conflicts of interest to disclose.

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