Effect of Safflower Flower Extract (*Carthamus tinctorius L*) on Glutathion Peroxidase and Catalase Activity on Serum in Rats

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Abstract

Due to side effects of chemical drugs, some people again turned to traditional medicine in recent years. One of medicinal plants is *Carthamous tinctorius* L (safflower) which is used as a relatively cheap dye for making sweets and cooking in addition to its therapeutic effects. Antioxidants are enzymes from body defense systems that play an important role against free radicals. In this study, the effects of safflower flower on serum activity of three important antioxidant enzymes(catalasand glutathion peroxidas) in sixty male wistar rats were studied. These rats randomly were divided into four groups including experimental 1, 2, and 3and also the control group. They were kept in standard cages with equal parts of water, light, temperature and food. The extract of safflower in concentrations of 100, 200 and 300 mg /kg BW respectively were subcutaneously injected to rats of experimental groups 1, 2 and 3 in two periods of 14 and 28 days and blood samples were taken from their hearts and the catalase, and glutathione peroxidase activity were carried out on their serum with spectrophotometry method by Zell Bayou kits. The data was analized using t-test and ANOVA, Tukey's and T tests. No significant difference wes observed among activity of catalase and glutathione peroxidase in controle group in comparision to experimental groups at 14^{th} and 28^{th} of study ($p>0.05$).

Keywords:*Safflower,flower extract, Catalase,Glutathione peroxidase, Rat*

Introduction

Since the economic development of different communities maximizes the widespread use of chemical substances to manufacture food products for people, the incidence of diseases resulting from use of these chemicals is inevitable. Saffron is the most expensive spice in the world. It is used as the color of the food in different restaurants instead of artificial colors. In spite of all its benefits, continuous use of Safron as a food supplement in long term may have adverse effects on human health.

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Followed by lipid peroxidation and a series of external factors such as stress, free radicals are always produced in the body which must be neutralized as quickly as possible, otherwise they destroy the body cells. Antioxidants are the most important protective system in body against free radicals that reduce oxidative stress. They are involved in prevention and treatment of many diseases, including: cancer, heart disease, cardiovascular and inflammatory diseases. Food antioxidants have a large protective effect against these diseases. One of the important antioxidants is glutathione peroxidase and catalase. Free oxygen radicals are known as one of the most important causes of cancer.

Safflower flower is used to color food instead of saffron, and its oil is used as a low-risk oil. This plant with the scientific name of "*Carthamus tinctorious*" is a native plant from the orient which is now planted in many countries. A one or two year plant has a height of 30 to 60 cm and serrated leaves that end to the delicate and sharp spines. The plant has yellow-red or reddish flowers. The active material in this flower is Kartamyn (Kartamyk acid). There are six species of this plant in Iran, which have many health benefits including wound healing, anti-inflammatory, soothing cough, and anti-bacterial, reducing platelet aggregation, lowering serum cholesterol, laxative and expectorant. Its oil contains large amounts of unsaturated fatty acids (n-polyunsurated) and approximately 75% linoleic acid, 13% of oleic acid, 0.6% of palmitic acid, and 3% of stearic acid. They also have direct chain fatty acids which is small and Gamma-tocopherol. The oil of safflower is a rich source of Linoleic acid. In the seeds of this plant glycosidoglycan is as Tracheloside (Mozaffarian,1996; Duke,2002).

Therefore we decided to analyze the effect of safflower flowers on the activity of glutathione peroxidase and catalase in blood of rats which are taking these plants in the current study. Moreover, the study indirectly analyzes the effect of the plant on reducing the incidence of cancer .

Materials and Methods

For the purpose of this study 60 wistar adult male rats with an average weight of 180±20 were obtained from the opioid animal breeding laboratory department in Islamic Azad University of Kazeroun. The Animals were brought to the laboratory a week before the test for compatibility with the environment. During the study, rats were

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kept under identical conditions with temperature of 22-24 ^{0}C and 12 hours of darkness and 12 hours of light. The food and water storage was sufficiently provided for them. The Rats were divided into four groups, Group 1 to 3 intraperitoneally received 100, 200 and 300 mg /kg of the extract, respectively. The fourth group was control group that received no extract. The period of the research was 28 days and after 12 hours from the last meal, the Blood samples were taken from the hearts of animals. The sera were separated with 2,500 rpm for 15 minutes and transferred to the laboratory on ice.

Then, using laboratory automation methods, the activity of, catalase and glutathione Peroxidase were measured by spectrophotometry method and by Zell Bayou kit from Zell Bayou Company in Germany, and the results of tests were analized using ANOVA, Tukey and t- tests.

Extraction

A certain amount of prepared powder in 50% of solvent ethanol was put under pressure for three days or 72 hours. After three days the tap of the extract machine opened to collect the extract drop by drop at the bottom of the machine.

Continuously, the solvent (alcohol) was added by pipette to the top of the container in order not to let the low liquid level dry plant powder. It should be noted that while the extract was collected by the separating funnel of percutaneous device, the alcoholic solvent was added drop by drop from the top of the device until the extracts were colorless, meaning that the extract has been taken entirely. Next, the prepared liquid containing ethanol inpercutor (its solvent) was removed by rotary device to be fully concentrated. Then with the help of desiccating and vacuum pump, the brown extract was obtained and weighted according to the doses in required quantities, and was solved in normal saline solution. The administration was based on the body weight of the rats in the next stage.

Results

The results of this study are tabulated in tables 1 to 3, it was demonstrated that there were no significant differences between the mean activity of glutathione peroxidase and catalase activity in control group in comparision to experimental groups ($P>0.05$). There was a significant differences beween the mean concentration of

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glutathione peroxidase in experimental group 2 and Catalase in experimental Group 1 at 14th in comparision to 28th day of study (P <0.05).

Table 1: The Mean activity of the parameters in the different groups on 14th and 28th days

Groups	Day 14 th		Day 28 th	
	GPX	CAT	GPX	CAT
Control	44.23±0.65	12.09±0.36	44.23±0.65	12.09±0.36
Experimental 1	43.41±13.10	23.18±0.11	42.78±1.44	22.3±0.20
Experimental 2	49.19±18.4	21.88±0.38	39.31±0.16	22.53±0.34
Experimental 3	56.92±17	22.47±0/39	38.78±21.64	22.10±0.30

Table 2: The presence or absence of significant difference of each parameter between the experimental groups and the control group on 14th and 28thdays

Groups	Day 14th		Day 28th	
	GPX	CAT	GPX	CAT
Experimental 1	0.928	0.070	0.999	0.792
Experimental 2	0.431	1	0.977	0.476
Experimental 3	0.875	0.660	0.295	0.966

Table 3: presence or absence of significant difference between the Mean it of each parameter in different experimental groups on 14th day compared to 28th day

Group	GPX	CAT
Exprimental 1	0.677	0.003
Exprimental 2	0.030	0.237
Exprimental 3	0.094	0.475

Discussion

Although the Mean concentration of catalase and glutathione peroxidase is higher in experimental groups in comparison to control group numerically (table 1), there was no statistically significant difference between the Mean activity of catalase in control group and the experimental groups on 14th and 28th days (table 2). It seems that in spite of the fact that safflower contains a strong antioxidant in its oil named P-N Komtrol or serotonin, the different doses of aqueous extract from safflower had no impact on the Mean activity of catalase and glutathione peroxidase.

Free radicals are highly reactive molecules that are produced by cells during normal metabolism. Free radicals can accumulate and cause damage to mitochondrial DNA and nuclear proteins inside the cell. Glutathione Peroxidase tetramer found in most human tissues, is the most prominent form of this enzyme. Within the cell, Glutathione Peroxidase is more in cytosolic and mitochondrial and seems that it is the most important collector of peroxide hydrogen in the tissues.

The enzyme catalyzes the revived peroxide hydrogen and a number of organic hydroxides using glutathione as a regenerative. This enzyme reduces the Hydroperoxide lipids from its alcohols and changes peroxide hydrogen into water. Although the enzyme glutathione peroxidase is relatively a stable enzyme, this enzyme becomes disabling under severe oxidative stress and produces the oxidative metabolism in the body.

The plant antioxidant protects brain cells against free radicals and treats the neurological problems caused by free radicals because of its antioxidant effects (Meena *et al*., 2012). The researchers reported that a white veiled, basil, Dar peppers, nightshade and Bostan can be used as an antioxidant source in the production of medicinal compounds against free radicals(Veeru *et al*.,2009).

According to table 2 the short-term and long-term consumption of safflower extract in different doses do not have a significant impact on the Mean value of GPX activity. There seems to be no statistically significant difference between the Mean values of GPX in different experimental groups compared to the control group in the period. After 14 days of using *Carthamus tinctorios* Hydro-alcoholic extract, the activity of GPX, SOD enzymes increases in a dose-dependent situation (Khoshvaghti *et al*.,2013).

In a study about the antioxidant effect of selenium and selenium nanoparticles on testis and spermatogenesis's activity, it was indicated that the selenium nanoparticles have stronger effects on antioxidant capacity by increasing glutathione and total antioxidant capacity and an increase in glutathione reductase and lipid peroxidation reduction compared to conventional selenium (Abdollah and Hashem,2014).

About the medicine effects of the plant, has been reported that the hydro-alcoholic extract of safflower flower is effective in the treatment of diabetes(Asgary *et al*.,2012). The effect of aqueous extract of safflower on the reproductive system in female rats has

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been evaluated and stated that the extract is more effective on ovarian activity compared to wounds and has a positive impact on fertility of female rats.

The concomitant use of safflower oil with a high fat diet causes insulin resistance and increases acyl coenzyme A (Ellis *et al*.,2000). The other property of Safflower is no harmful effect on blood pressure, heart rate and contraction of pulmonary artery (Veeru *et al*,2009;Walser et al,2006).The prevention of diabetes-related complications is of the other effects of safflower seed oil (Rahimi *et al*.,2014).

The safflower extract reduces the number of follicles in the ovaries but does not affect the number and size of the corpus luteum(Dehkordi *et al*., 2014; Mozaffarian,1996).

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 surface of platelet and B_2 thromboxane(Duke,2002). There are the well-studied about antioxidant activity of the plant, but the main material of the plant which have antioxidant role are still unknown.

Moreover, the antioxidant action of the extract against hydrogen peroxide has been shown. (P-N Komtrol) Serotonin is a combination of powerful antioxidants which is in safflower oil and is known that it is effective in improving of progressive activities in rat fibroblasts and human fibroblast. The antioxidant activity and preventing role in production of the proinflammatory cytokine from human monocytes is another effect of the plant. In a research was found that consuming10 grams of safflower oil in a day has no side effects on liver enzyme and kidney parameters (such as uric acid, urea nitrogen in blood, creatinine)(Dehkordi *et al*., 2014).

Conclusions

The results of this study showed that the effect of safflower flower extract in the production of antioxidant catalase enzymes and glutathione peroxidase is not very statistically significant. But in case the objective is increasing the antioxidant defense system in certain circumstances, necessary and constantly, high doses of this extract should be used. In such a case it is advisable to use other plants with greater impacts on increasing antioxidant enzyme.

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