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Effect of Saffron Extract on Proteins Biochemical Parameter of Serum

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Due to widespread use of saffron as food colourant and flavour and its reputation in folk medicine as a drug, this study was designed to assess the efficacy of saffron on serum proteins fractions in the male mice. Five group including eight adult male mice were used in this study. Normal saline administered as placebo to control group and saffron extract in doses of 25, 50, 100 and 200 mg/Kg/48 h were injected intraperitoneally for 20 days to the experimental groups. Albumin, α-1, α-2, β- and γ-globulins were separated electrophoretically and albumin/globulin ratio was calculated from the electrophoretogram. The result indicated that the levels of albumin increased significantly in two experimental groups that had received 50 and 100 mg/Kg/48 h of saffron extract as compared to that of control group. The levels of α-1 didn't have any remarkable changes in any group. The injection of saffron extract decreased ($p < 0.05$) the α-2 level in plasma as compared to the control group and levels of β-globulins increased significantly in these three groups. The levels of γ-globulins increased significantly in 100 and 200 mg/kg-treated groups as compared to the control group. Albumin/globulin ratio were significantly ($p < 0.05$) lower than control group in any groups that received saffron extract in a dose-dependent manner. Albumin were significantly increased in two groups and albumin/globulins ratio were decrease in any groups this can be interpreted that in the absence of antigen stimulation, serum globulins increased markedly by saffron. The study shows that since albumin synthesis occurs in the liver cells, thus administration of saffron may improve the status of liver function significantly.

Key Words: Albumin, Globulin, Serum, Mice, Saffron.

INTRODUCTION

The dried stigmata of *Crocus sativus* L (saffron) are commercially available in superstores. It is popular because of its delicate aroma and attractive colour. Saffron also is used in traditional medicine for various purposes as aphrodisiac[1-3], anti spasmodic[4] and hypolipidemic effects as well as radical scavenging properties[4,5], anti

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depressant[6] and antiinflammatory[7]. Saffron also reported to be useful in treating various human disorders such as heart and blood disorders[8] and neuronal injury[9]. Recently pharmacological studies have been revealed that the extract of saffron has antitumor and antimutagenic activities and inhibits nucleic acid synthesis in human malignant cells[10,11]. It is also reported that saffron petals are rich in polyphenolic flavonoids that exhibit antioxidant properties and prevent DNA damage[2,12] and inhibits genotoxicity in mice[13,14].

Saffron extract consists of many compounds such as α-crocetin, carotenoid, crocins (crocin, di-crocin and tri-crocin), picrocrocin and safranal[15]. It has been demonstrated that antioxidant flavonoids bind to human serum albumin and interaction of human serum albumin with flavonoids causes protein unfolding at high pigment concentrations[12]. Because serum albumins are the major carrier of flavonoid pigments in blood, the present study was undertaken to investigate the effect of various concentration of saffron extract on the electrophoretc pattern of serum proteins in male mice.

EXPERIMENTAL

This study was carried out on adult male Balb/C mice (30 ± 5 g) from Pasteur Institutes, Tehran, Iran. Animals were randomly divided into five experimental groups (8 mice per group), the control and four experimental groups were housed in plexiglas cages (40 cm long × 20 cm high × 30 cm wide) four per cage, in a regulated environment (25 ± 1 ºC; 50-55 % relative humidity; 12 h light/dark cycle), with free access to food plets (Pasteur Institutes) and water.

Extraction of saffron: Air-dried and powdered stigmata of saffron (100 mg) was macerated with 5 mL of normal saline and incubated for 2 h at room temperature. The mixture was then, centrifuged at 5000 rpm for 5 min. The upper solution carefully aspirated in a test tube. The dried sediments were weighed and the amount of extracted saffron was calculated and adjusted to 10 mg/mL.

Administration: Saffron extract was injected intraperitoneally in doses of 25, 50, 100 and 200 mg/kg every 48 h for a period of 20 days. The day after last injection the animals were mildly anesthetized and blood collection was performed directly from heart.

Serum protein electrophoresis: The serum samples were applied on cellulose acetate paper strips and the distribution of serum proteins obtained on electrophoresis at pH 8.6 for a period of 25 min with 160 mA. The proteins were then stained and the intensity of the separated protein bands on the cellulose acetate strip was obtained graphically. The relative amounts of the various proteins were calculated on the basis of the total protein of each sample

Statistical analysis: Probability test, as well as one way analysis of variance, followed by Duncan test, was used for statistical evaluation. The P-values less than 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Serum protein electrophoresis in mice normally yields six bands (Fig. 1). The first band is pre albumin and most intense band is albumin followed by four globulins (α-1, α-2, β and a broad γ band), each of which can contain a number of different proteins (Fig. 1).

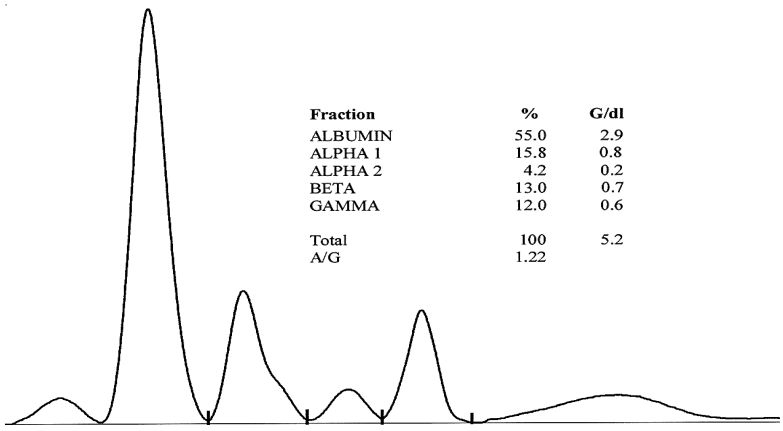

Fig. 1. Electerophoretic patterns of serum proteins in mice (control group). The first band is pre albumin that is not seen in human samples

The effect of saffron extract on serum proteins in control and experimental groups are summarized in Table-1. Analysis of post-hoc comparisons ($p < 0.05$) showed that the levels of pre albumin and albumin increased significantly in two experimental groups that received 50 and 100 mg/Kg/48 h of saffron extract.

TABLE-1
EFFECT OF SAFFRON EXTRACT ON SERUM PROTEINS IN THE MALE MICE

Proteins (g/dl)	Saffron doses (mg/Kg/48 h)				
	Placebo controlled	25	50	100	200
Pre albumin	0.272±0.004	0.288±0.005	0.312±0.006*	0.3070±0.004*	0.282±0.009
Albumin	2.650±0.042	2.750±0.046	2.975±0.075*	3.0130±0.044*	2.800±0.088
α-1	0.750±0.018	0.825±0.025	0.738±0.032	0.7630±0.059	0.738±0.032
α-2	0.200±0.000	0.200±0.026	0.100±0.000*	0.1000±0.000*	0.113±0.012*
β	0.600±0.018	0.700±0.073	1.213±0.029*	1.2000±0.018*	1.400±0.050*
γ	0.550±0.018	0.650±0.026	0.700±0.046	1.4388±0.046*	1.275±0.016*
A/G	1.243±0.011	1.103±0.039*	1.082±0.030*	0.8610±0.030*	0.780±0.024*

Values are mean ± 1 SE; *$p < 0.05$, compared to the control.

In case of globulins, post-hoc comparisons indicated that the level of α-2 in 50, 100 and 200 mg/kg-treated mice discriminated significantly lower than control group and 25 mg/kg-treated mice. Statistical evaluation did not show any significant difference between the levels of α-1 in any experimental group.

Saffron extract administration also increased significantly the levels of β globulin in 50, 100 and 200 mg/kg and γ globulin in 100 and 200 mg/kg-treated groups.

Injection of saffron extracts caused a dose-dependent reduction in the albumin/globulin ratio as compared to the placebo controlled group.

The albumin band represents the largest protein component of the male mice serum. Although the effect of saffron on different body systems have been extensively investigated[10], few studies have examined its effect on serum proteins changes. Finding of this study is the first one to be reported in this field.

The results of this study indicated that the levels of albumin increased significantly in two experimental groups that had received 50 and 100 mg/Kg/48 h extract of saffron. Albumins synthesis occurs essentially in liver, it performs important carrier function, a physiological mechanism for the transport of many small molecules in plasma. It is very well known that diminished synthesis of albumin in liver, the plasma albumin levels fall in any liver illness[16]. Because high level of crosins flavonoid pigments in saffron may bind to albumin, the increased level of albumin in saffron treated animals is interpreted as being consistent with the improvement of liver function. This suggestion is in agreement with the previous studies that saffron and its major components such as crosins exhibit antioxidant activity and anti-free radicals' abilities[5,9,13] thus, it may stimulate albumin synthesis in liver.

Second band in electrophoretic pattern is α-1 globulin which didn't have any remarkable changes in any group. The α-1 band is made up almost entirely of α-1 antitrypsin. α-1 Antitrypsin is responsible for 90 % of serine antiprotease activity in serum. α-1 Globulins are increased in active inflammatory or neoplastic diseases[16,17]. This may suggest that saffron may not have any adverse effect leading to emphysema and cirrhosis. However, the injection of 50 mg, 100 mg and 200 mg/Kg/48 h of saffron extract significantly decreased ($p < 0.05$) the serum α-2 globulin levels. The α-2-globulin band consists of α macroglobulin and hemoglobin carrier protein, haptoglobin[18]. It is known that plasma haptogolobin concentrations fall in patients with hemolytic disorders[19], because then most of the plasma haptoglobin is converted to hemoglobin-haptogolobin complexes and their rapid clearance dose not stimulate compensatory increased haptogolobin production[18,20]. The presence of high levels of saffron may compete with the ligands of the carrier proteins to form saffron-carrier proteins complexes and may be excreted *via* renal system. However, more experiments are needed to evaluate this explanation.

The increased β-globulins levels in saffron treated animals is another finding of this study (Table-1). The β band consists mainly of transferrin, with some low-density lipoprotein (LDL). Transferrin plays an important role in the metabolism of iron, facilitating ferric iron transport from intracellular stores to the bone marrow[18,21]. Increasing β-globulins in this study revealed that saffron extract can influence iron metabolism in mice.

The levels of γ-globulins in this study increased significantly in 100 and 200 mg/kg-treated groups. The γ-globulins comprise immunoglobulins and are derived

from B cells of the immune system[17]. They may be decreased with cytotoxic or immunosuppressive drug therapy and adult common variable immunodeficiency syndrome[18]. The immunomodulatory and anti-invasive properties of proteoglycan derived from saffron corms has demonstrated. This compound promoted significant macrophage activation, by the release of nitric oxide and rapid activation of protein kinase C and specifically promoted apoptosis of macrophages[22].

In addition saffron extract decreased albumin/globulin ratio in all experimental groups in a dose-dependent manner since levels of albumin increased and also the decreased albumin/globulin ratio may be interpreted as administration of saffron extract can promote both immunoglobulins and albumin production.

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