

Antioxidant Effect of Aqueous Extract of (*Crocus Sativus L.*) on Rats Exposed to Oxidative stress

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Abstract: The aqueous extraction of Saffron *Crocus sativus* were studied against H₂O₂ induced oxidative stress in the serum of albino rats for 15 day. Twenty male rats with age (3-4) months and weight (200-300) g were divided into 4 groups (1) is control group received drinking tap water and ideal diet, group (2) received 1% H₂O₂ drinking tap water group (3) received 1% H₂O₂ and i.p dose of 60 mg/kg from B.W Saffron extract once daily group (4) received 60mg/kg from B.W Saffron extract H₂O₂ treated group should elevated malondialdehyde (MDA) and reduction in glutathione (GSH) level and super oxidizedismutase (SOD) level compared with control group in P<0.05. Treatment with saffron extract should decreasing in the (MDA) level and increasing in the (GSH) and (SOD) levels, the result of this study provided the protective effects of Saffron extract by increasing antioxidant defense and suppression free radicals production in serum.

INTRODUCTION

Antioxidant compounds in food play an important role as health protecting factor, Scientific evidence suggests that antioxidant reduce the risk for chronic disease including cancer and heart disease primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables plant sourced food antioxidants like vitamin C and E, carotenes, phenolic acids (1,2). Free radical scavengers in the body include super oxide dismutase (SOD) catalase, glutathione peroxidase and malondialdehyde. Many of approaches used in free radicals studies provide in aggregate assessment of oxidative stress (3,4). (GSH) is a tripeptide consisting of glutamate cysteine and glycine which is present in most mammalian tissues and concentration of 0.1-10mM. In contrast to tissue glutathione is present in plasma at much lower concentration. (GSH) roles inducing its functions as an important physiological antioxidant which protects cell from oxidative damage by its ability to donate hydrogen atom from thiol groups of its cysteine residue to most carbon (5).

Crocus Sativus L. commonly known as saffron is a plant cultivated in various parts of the world such as Iran, China, Spain, Italy and Greece, chemical analysis of its stigmas has shown the presence of water soluble carotenoids, small amounts aldehyde and its glucosids (safranal and picrocrocin) and flavonoids. The pistils of *Crocus sativus L.* are used traditional medicine as an antispasmodic, eupeptic, nerve sedative and crude extract have been demonstrated to prevent tumor formation, atherosclerosis or hepatic damage (6,7). The aim of this study was to further investigate the role of saffron in some antioxidant enzymes activity.

MATERIALS AND METHODS

Animals:

Adult male Wistar rats weighting 200-300 gm., age of 3-4 months were used through the study. All of them were kept in the same room under a constant temperature (22 °C ± 2) with food pellets and water available ad libitum. The animals were divided into four groups, each of one contained "5" rats. Group (1) was control in which water enough, group (2) was exposure to oxidative stress H₂O₂ in 1% concentration, group (3) was given aqueous saffron extract (60mg /kg) BW. through i.p. injection with H₂O₂ 1%, group (4) treated with saffron (60mg/kg) for 5 days only.

Preparation of plant extract :

The dried petals of saffron were obtained from local markets, about 50 g of stigmas were ground to powder and dried at room temperature, dried stigmas were decocted in water for 30min. then after the extract was filtered and concentrated using rotary evaporation apparatus and the residual extract were dissolved in normal saline whenever used in experiment (8).

Sample Preparation for (MDA), (GSH) and (SOD) measurement.

Blood sample were collected after 2weeks by Jugular vein then centrifuged for 15 minutes with 3000 rpm to separate plasma and kept frozen at (-20) C° until assay. Malondialdehyde (MDA) levels of the plasma were measured according to this procedure: 0.5ml plasma was shaking with 2.5ml of 20%trichloroacetic acid (TCA) in a10ml centrifuge tube. 1ml of 0.6% 2-thiobarbituric acid (TBA) was added to the mixture shaken and wormed for 30 m in aboiling water bath followed by rapid cooling. Then it was shaken with 4ml of n-bytyle-alcohol layer in a separation tube and MAD content in the plasma was determined from the absorbance at 535 nm by Spectrophotometer against butanal (9).

While (GSH) level was measured by alternative method y (10). This method depend on Ellman's solution content the 5,5-dithiobis 2-nitrobenzoic acid (DTMB) detector. The reduction is thus measured by the decrease in absorbance at 412 nm thus providing a spectrophotometric which is directly proportional to GR activity in the sample. The activity of (SOD) levels in blood serum was determined using photochemical method described by (11) this methods depends on an indirect approach to determine the SOD activity through the change in formazene absorbance formed from the reduction of O₂ (which is produced by radiating the sample of serum with light). fornitrobluetetrazolum (NBT) decreased difference in formazene absorbance means increased SOD activity.

STATISTICAL ANALYSIS

Statistical analysis was performed using one way analysis of variances (ANOVA)P-values of 0.05 or less were considered significant, statistical analysis was performed using Duncan multiple test. (12)

RESULT AND DISCUSSION

The MDA level increased significantly following H₂O₂ treatment (P≤ 0.05) compared with the control group (fig 1) then group 3 Saffron &H₂O₂ increased significantly with control group 4 Saffron alone MDA level decreased compared with control group. GSH&SOD concentration fig 2&3 showed a significant decreased in GSH&SOD levels in H₂O₂ treated group (p≤ 0.05) compared with control group Then Saffron &H₂O₂ and Saffron group alone GSH&SOD levels increased significantly with control group. This results was explain the important role of H₂O₂ asapotent oxidizing agent which cause the oxidative stress by increasing the reactive oxygen species (ROS) or decreasing the oxidative defense (13,5) The evidence of involvement of free radicals and reactive oxygen species(ROS) in the pathogenesis of number of disease and toxicity . They are involved in the biological damage induced by number of Therapeutic molecules ,Poisonous chemicals and toxins (14). Saffron treated groups reversed the increasing of MDA level to consider able extent(fig. 1) Saffron a scavenging activities by having chemical components present in stigma these components include carbohydrates, minerals, vitamins such as riboflavin and thiamin , color pigments such as crocin, anthocyanin ,carotene, lycopene, Zeaxanthin and aromatic terpenic essence called (Saffranal) (15).

GSH levels fig(2) and SOD levels fig(3) showed a significant decrease with H₂O₂ treated group (P≤0.05) compared with the control group this result agreement with that obtained by (16) that used omega 3 against acrylamid toxicity and (17) that used protective effect of aqueous extract and crocin its active constituent on renal ischemia –reperfusion - induced oxidative damage in rats. Saffron and its characteristic component possess anticarcinogenic and antitumor activities in vivo and in vitro it was shown that Saffron extract and its purified characteristic compounds crocin, safranin, different types of tumor cell growth it was reported that anovalgluco conjugate isolated from corms and callus of Saffron possessed cytotoxic activity against different tumor cells.(18)Saffron have defense effect against oxygen toxicity in this investigation used method were compared for the measurement of Super oxid dismutase (SOD) activity in crocus sativus effect(19). The methods based on a competition between the enzyme itself and another superoxide scavenger involved respectively cytochrome C reduction, nitro blue tetrazolium reduction and pyroyallol oxidation (20).The treatment of mice with aq. extract of saffron can significantly inhibit genotoxicity produce by cisplatin, cyclophosphamide , mitomycin these genotoxins alone can inhibit glutathione S- transferase (GST) activity , it was also observes that inhibitory effects Of genotoxins on (GST) activity (21).

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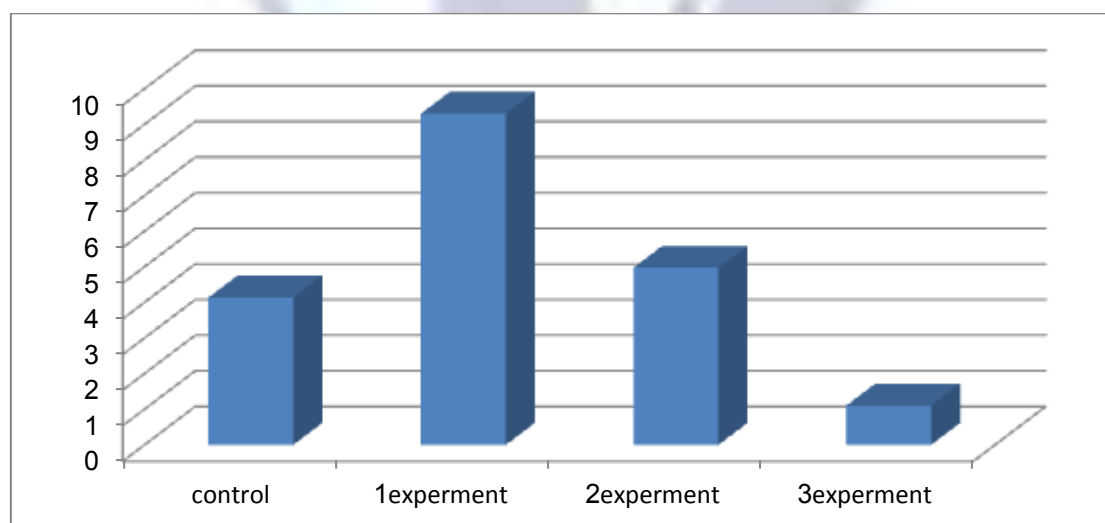


Fig. (1): Effect Saffron extract on MDA levels (micromole/L)

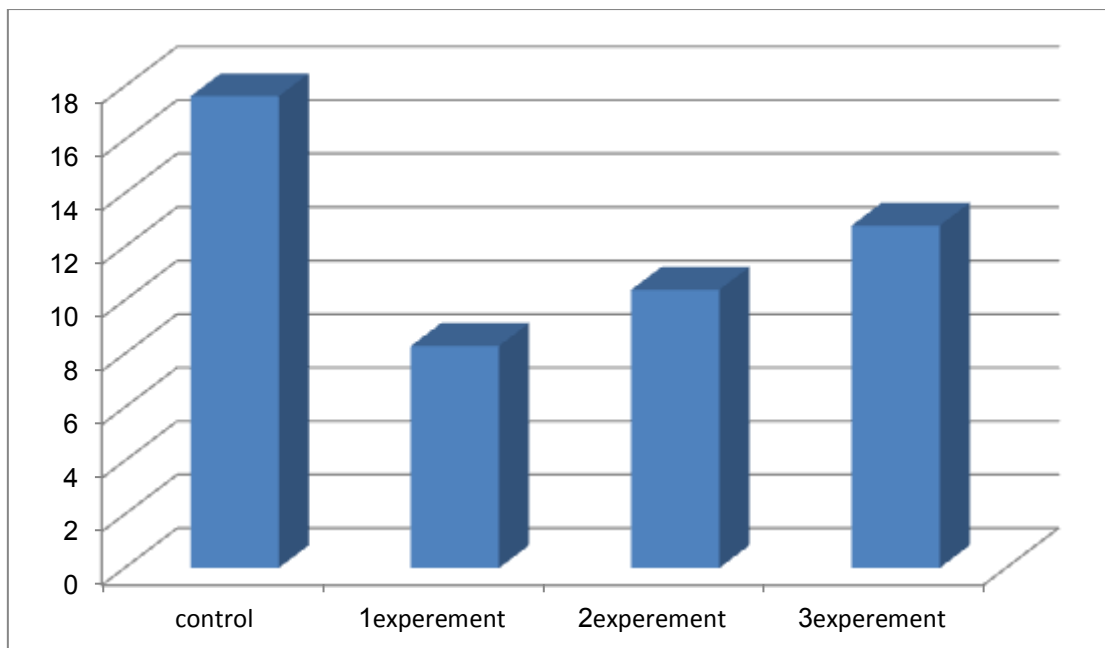


Fig. (2): Effect Saffron on GSH levels (micromole/L)

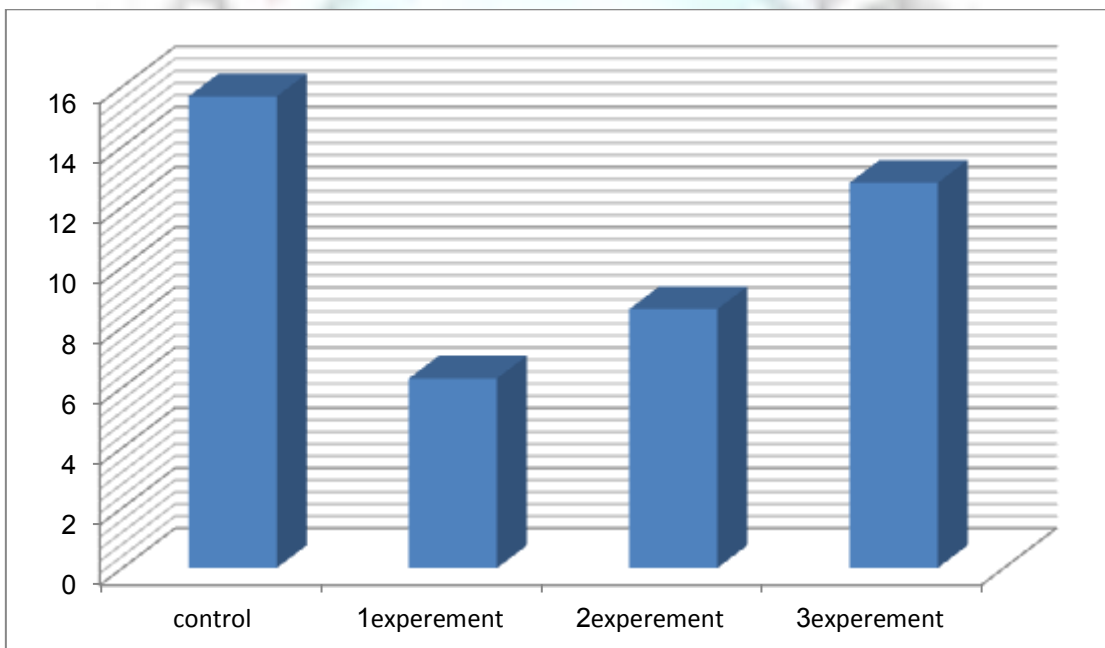


Fig. (3): Effect saffron on SOD levels (Micromole/ L)