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Original article

Crocus sativus L. (saffron) extract reduces the extent of oxidative stress and proinflammatory state in aged rat kidney

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Summary. *Aim:* This study evaluated whether saffron hydro-ethanolic extract, has a protective effect on kidney through reducing the oxidative stress and inflammatory response in aged rats. *Methods:* In this study the changes in activities of antioxidant enzymes, lipid peroxidation, glutathione (GSH) levels and the expression of pro-inflammatory cytokines in the serum and renal tissues were evaluated by ELISA and RT-PCR, respectively. The middle and aged rats were given intraperitoneal injections of the saffron hydro-ethanolic extract for 4-week. After 4-week, animals were anesthetized with diethyl ether. The kidney samples were taken for biochemical analysis. *Results:* The results revealed the aging was associated with a significant decrease in the activities of antioxidant enzymes, and GSH content accompanied with increase in lipid peroxidation level in kidney of the aged rats. The increased levels of serum renal functional parameter, oxidative parameters and also pro-inflammatory cytokine levels were significantly reduced by the saffron hydro-ethanolic extract. The aged rats exhibited a dysregulation of the oxidative stress, and inflammation in the kidneys, but the saffron hydro-ethanolic extract treatment significantly reduced the expression of the inflammatory genes. *Conclusions:* These results provide a pivotal documentation that the saffron hydro-ethanolic extract has a renoprotective effects against the development of oxidative stress and inflammation in the kidney of old rats.

Key words: saffron extract, antioxidant, inflammation, kidney, aged rat

Introduction

Aging causes deleterious modifications at genetic, cellular, tissue, and system levels in all organisms (1). The molecular mechanisms underlying this age-associated loss of functional cells are poorly understood, but a growing body of documents support the idea that oxidative stress and inflammatory pathways are the important contributing factors to deterioration of organs and cell function that are associated with aging (2). The age-associated loss of renal mass and its physiological function are unavoidable part of aging. The kidneys show greater age-associated deterioration than most organs, even in the absence of cardiovascular disease (3). Several ideas have been implicated to show the aging process containing the free radical thesis (4), oxidative stress theory of aging (5), the mitochondrial hypothesis (6), and the molecular inflammatory pathways (7). These are all distinct to a specific reason of the physiological modifies appearing with aging, however, the exact mechanisms remain unclear. Aging is associated with an imbalance between production of reactive oxygen species (ROS) and its elimination by the antioxidant defense system in the body. Increased oxidative damage to cellular macromolecules in the kidney has been observed in aging (8). Free radicals may adversely affect cell survival because of membrane damage through the oxidative damage of lipid, protein and irreversible DNA modification (9).

Oxidative stress is postulated to be one of the most important mechanisms behind the age related depletion in enzymatic and non-enzymatic antioxidant content (10). To protect cells against oxidative damage by oxidants, produced during the oxygen metabolism, an antioxidant system has presumably involved in aerobic organisms. Major antioxidants like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione (GSH) are important for cellular protection due to their ability to detoxify free radicals, such as reactive oxygen species (ROS) (11). New normal aging study has emerged on oxidatively-induced, chronic inflammatory processes. Evidence have recently focused that at the molecular level, the maximum chronic disorders, such as cancer, are induced by an impaired inflammatory pathways and age-related disease (12, 13).

Antioxidant depletion and dysregulated inflammatory response have been identified as the important factors in the aging process occurring as a general phenomenon in tissues and blood of aging organisms concurrently with enhancements in oxidative stress related damage (10). Potential antioxidant and anti-inflammatory therapy should, therefore, include either natural antioxidant or agents, which are capable of augmenting the function of antioxidant system. Various synthetic antioxidants have been used, which restricted the use of natural antioxidants as in food, and were proven to be carcinogen (14, 15). Thus, natural antioxidants have received growing attention as potential preventive agent by scavenging ROS, detoxifying potent genotoxic oxidants in recent years and also therapeutic interventions that inhibit inflammatory pathways may be an effective strategy to attenuate the aged related-disorders (14, 15). *Crocus sativus* L. (Iridaceae), commonly known as saffron has been used in medicine as an effective for neurodegenerative disorders and related memory impairment, ischemic retinopathy and age-related macular degeneration, coronary artery disease, blood pressure abnormalities, acute and chronic inflammatory disease, mild to moderate depression, seizure, Parkinsonism (16-19). Moreover, these studies have indicated that saffron and its ingredients exert anti-mutagenic, anti-genotoxic, tumoricidal, anti-inflammatory and antioxidant activities (20-23). The main ingredients of saffron including crocin, crocetin, safranal, and picrocrocin are responsible for these pharmacological effects (16). To our knowledge, there lacks the information in literature about the capacity of saffron extract to prevent free radical formation, molecular inflammatory pathways and peroxidation in aged rat tissues. The expression of downstream inflammatory genes, such as interleukin-6 (IL-6), interleukin-1 b (IL-1b), and tumor necrosis factor-α (TNF-a) which are involved the molecular inflammatory pathways, played a pivotal role in different diseases and disorders, such as aging and survival (24, 25). Therefore, inactivating the above signaling pathway may be a pivotal target for therapeutic intervention of inflammatory response in the kidney of aged rats. However, the molecular protective pathway for the effects of the saffron hydro-ethanolic extract against the oxidative stress and inflammation involved in the aging process has not been examined. Therefore, the present study was designed to evaluate the protective effects of the saffron hydro-ethanolic extract against oxidative stress and molecular inflammatory pathways in aging.

Materials and Methods

Chemicals

All purified enzymes, coenzymes, substrates, standards, buffers, kits and other chemicals were purchased from Sigma-Aldrich Chemical (St. Louis, USA). Serum Blood Urea Nitrogen (BUN) and Creatinine diagnosis kit were purchased from Pars Azmoon Inc. (Tehran, Iran). Serum specific ELISA kit TNF-α, IL-6 and IL-1b (eBioscience, Bender MedSystems, Vienna, Austria)

Plant collection and identification

Crocus sativus was collected from previous garden (northeast Iran), and identified by botanists in the herbarium of Ferdowsi University of Mashhad (specimen number 293-0303-1).

Extraction

For preparation of hydro-ethanolic extract, three grams of chopped *Crocus sativus* stigmas were mixed

with 50 ml ethanol 70% for 72h at room temperature and the solution was separated by maceration method which was repeated for three times. The solutions were dried in room temperature and stored at -4°C away from light.

Experimental design

Male Wistar rats of different ages namely 2 (young rats), 10 and 20 (middle and aged rats, respectively) months (n = 10 for each group) were used for all the experiments. The average of life span of male Wistar rats is almost 25 months. Accordingly, the percent lifespan of 2, 10 and 20 months male Wistar rats are almost 8, 40 and 80 % respectively. The animals were kept at a constant temperature of 25˚C, humidity of 55% at 8:00–20:00 h light, and 20:00–8:00 h dark cycle. The animals were fed standard chow (Javaneh Khorasan Ltd. Iran) until treatment or time of sacrifice. After adaptation (1 week), the old rats) 10 and 20 months) were divided into two subgroups of equal number (n = 10, each) as the untreated (vehicle) and the saffron hydro-ethanolic extract -treated old rats. The saffron hydro-ethanolic extract was administered to the rats intraperitoneal (i.p.) at a doses (5, 10, 20 mg/kg body weight per day) daily for 28 consecutive days (4-week), whereas control animals received an equal volume of saline 0.9%. The body weight, food intake, and water intake were determined every day during the experimental period. After 4-week of saffron extract treatment, animals were anesthetized with diethyl ether. After anesthesia, blood was subsequently collected from the retro-orbital sinus for determination of plasma biochemical parameters. Before removing their kidneys, the right ventricle of the heart was perfused with ice-cold saline (0.9% NaCl) to remove all blood from the kidneys. The kidneys were then removed and weighed were then immediately frozen in liquid nitrogen and stored at -80˚C for biochemical analysis.

The kidney samples taken were washed in saline and kept in an ice bath and separated into two parts. One part was homogenized in phosphate buffer saline (PBS) 50 mM pH (7.4) for estimation of protein content, SOD, CAT enzymes activities and GSH level, the second was homogenized in potassium phosphate buffer 10 mM pH (7.4) for estimation of MDA level and GPx activity. The crude tissue homogenate was centrifuged at 10,000 rpm, for 15 minutes in cold centrifuge, and the resultant supernatant was used for biochemical analysis.

Biochemical analysis

Measurement of the Serum biomarkers

The analysis of serum Blood urea nitrogen (BUN) and serum creatinine (Cr) levels were estimated by using diagnostic kits (Pars Azmoon kit, IRI) on an automatic analyzer (Abbott, model Alcyon 300, USA). Levels of the inflammatory mediators (TNF-α, IL-6 and IL-1b) in the serum were evaluated using specific ELISA kits for mice according to the manufacturer's instructions (eBioscience, Bender MedSystems, Vienna, Austria)

Measurement of the tissue biomarkers

Protein estimation

Protein content in tissue homogenate was measured according to the method of Bradford et al. (26).

Spectrophotometric determination of tissues MDA and GSH levels

MDA

Lipid peroxidation was assessed in the homogenates of the whole kidney. The formation of MDA, an end product of fatty acid peroxidation was measured spectrophotometrically at 532 nm by using a thiobarbituric acid reactive substance (TBARS) essentially by the method of Genet *et al*. (27).

GSH

GSH in the tissues was estimated by the method of Ellman (28). This method was based on the formation of 2-nitro-5-thiobenzoic acid (a yellow colour compound) when 5, 5'-dithio-bis (2-nitrobenzoic acid) (DTNB) was added to compounds containing sulphydryl groups. The absorbance can be measured at 420 nm.

Measurements of enzymes

Assay of SOD

The activity of SOD was assayed by the method of Kakkar *et al*. (29). The assay is based on the inhibition of the formation of NADH-phenazine methosulphate-nitroblue tetrazolium formazon. The reaction was initiated by the addition of NADH. After incubation for 90 s adding glacial acetic acid stopped the reaction. The color developed at the end of the reaction was extracted into n-butanol layer and measured. The enzyme concentration required to inhibit the chromogen produced by 50% in one min under standard conditions was taken as one unit.

Assay of CAT

The activity of CAT in the tissues was determined by the method of Sinha (30). Dichromate in acetic acid was converted to perchromic acid and then to chromic acetate, when heated in the presence of H_2O_2. The chromic acetate formed was measured at 620 nm. The catalase preparation was allowed to split H_2O_2 for various periods of time. The reaction was stopped at different time intervals by the addition of dichromate-acetic acid mixture and the remaining H_2O_2 as chromic acetate was determined colourimetrically. The specific activity was expressed as mol of H_2O_2 consumed/min/mg of protein for tissues.

Assay of GPx

GPx activity was estimated by the method of Rotruck *et al*. (31). A known amount of enzyme preparation was allowed to react with H_2O_2 in the presence of GSH. GPx utilize GSH for the decomposition of H_2O_2. After a specific time period, the remaining GSH content was measured. The specific activity was expressed as moles of GSH consumed/min/mg protein for tissue.

PCR analysis

Expression of pro-inflammatory genes, TNF-a, IL-1b, and IL-6 in renal tissues was examined using reverse transcriptase-PCR (RT-PCR). Total RNA was isolated from renal tissues using Trizol according to the manufacturer's instructions (Madison, WI, USA). Four micrograms of total RNA were reverse transcribed into cDNA using the PrimeScript RT Master Mix as instructed. PCR primers (Invitrogen, USA) for all analyzed genes are shown in Table 1. PCR was conducted at 95°C for 30 sec, followed by 40 cycles at 95°C for 5 sec, 60°C for 34 sec and 95°C for 15 sec. The amount of mRNA for each gene was normalized by β-actin.

Statistical analysis

Data were analyzed using ANOVA-one way by InStat 3.0 program followed by Tukey-Kramer *post-hoc* test for multiple comparisons. Kolmogorov Smirnov tests showed that these data were normally distributed. The evaluation was made by the comparison of groups. The results were presented as means ± SEM and $p<0.05$ was considered significant.

Results

Effect of the hydro-ethanolic extract on body weight, kidney weight and renal functional in aging

Table 1 shows the changes in body weight (before and after experiment), kidney weight, food intake, and water intake during the study period. The untreated aged rats exhibited a significant increase in body weight during experimental period. However, this increase in body weight during experimental period slightly grew by the treatment of the saffron hydro-ethanolic extract in a dose dependently manner. The kidney weight in the middle and aged rats (10 and 20 months rats) was 2 and 2.57 times higher than that in the young rats respectively, but was diminished dramatically in the aged rats (20 months old) to which different concentrations of the saffron hydro-ethanolic extract were treated ($p<0.5$). While, the food and water intakes were not changed by the saffron hydro-ethanolic extract treatment (Tab. 1). Regarding the renal functional index, the serum Cr and BUN levels in the untreated aged rats (20 months old) was higher compared to the young rats ($p<0.01$), although this higher level was reduced in a dose manner by the treatment of the different saffron extract. So that, the aged rats treated the saffron hydro-ethanolic extract (20 mg/kg) showed reduction in the BUN level compared with the untreated aged rats ($p<0.05$) (Tab. 1).

Table 1. Physiological and biochemical analysis
10M, non-treated 10 months aged rats; 10M + S-5, saffron (5 mg/kg)-treated 10 M aged rats; 10M + S-10, saffron (10 mg/kg)-treated 10 M aged rats; 10M + S-20, saffron (20 mg/kg)-treated 10 M aged rats; 20M, non-treated 20 months aged rats; 20M + S-5, saffron (5 mg/kg)-treated 20 M aged rats; 20M + S-10, saffron (10 mg/kg)-treated 20 M aged rats; 20M + S-20, saffron (20 mg/kg)-treated 20 M aged rats. Data are expressed as mean ± SEM. Significance:

		Aged Rats							
Parameters	Young Rats (2 M)	10M	10 M + S-5	10 M + S-10	10 M + S-20	20M	20 M + S-5	20 M + S-10	20 M + S-20
B.W.									
Before exp.	161.3 ±14	422.6±48	417.9±43	430.1±44	428.6±52	663.7±59	671.8 ±43	654.9±37	668.8 ±40
After exp.	223.7±21	456.9±54	433.4±38	453.8± 42	451.5±50	668.3±55	680.8±53	666.5±58	688.9±52
Kidney Weight (g/body weight)	0.33±0.02	0.67±0.03 ***	0.61±0.02 ***	0.58±0.02 ***	0.56±0.03 **	0.85±0.07 ***	0.72±0.06 ***	0.68±0.04 ***	0.65±0.03 ***, +
Food Intake (g/day)	21.3 ±2.7	29.9±4.6	30.5± 4.9	31.2± 5.1	32.4±6.3	34.4±5.0	35.2± 5.8	36.1± 6.1	36.7± 6.3
Water Intake (ml/day)	42.8± 6.7	58.9± 4.9	56.4± 6.2	51.7± 3.8	49.6± 5.2	50.4± 4.0	48.9±7.9	47.4± 6.1	45.8± 4.1
Serum Cr(mg/dl)	0.50±0.1	0.8±0.1	0.71±0.08	0.73±0.09	0.69±0.08	1.5±0.2	1.45±0.28 **	1.39±0.27 **	1.30±0.2 * *
Serum BUN (mg/dl)	19.3±3.5	26.7±2.5	25.9± 2.9	24.7± 3.3	23.9± 2.4	36.7±3.9 *	30.3± 3.2	26.6± 3.1	22.2± 3.8 +

** < 0:05, ** < 0:01, ** < 0:001 vs. Yang rats; + <0.05 vs non-treated 20 aged rats.*

Effect of the saffron hydro-ethanolic extract on lipid peroxidation in aging

Lipid peroxidation was measured as the formation of MDA in whole homogenates of aged rat kidney from control (untreated) and the saffron hydro-ethanolic extract -treated aged animals. With aging there was a significant increase in the MDA level at the 10 (p<0.05) and 20 month old rats (p<0.001) as compared to the young rats. The saffron hydro-ethanolic extract treatment (10 and 20 mg/kg) decreased significantly the level of MDA (p<0.01; p<0.001 respectively) in the kidney of the 20 month old rats (aged rats) when compared with the untreated aged rats. In contrast, the saffron hydro-ethanolic extract treatment did not alter the aging-induced increase in the MDA level of 10 month old rats (middle-aged rats). There was not a significant change in the MDA level at the saffron (10 and 20 mg/kg) treated aged rats (20 months old) when compared with the young rats (Fig. 1a).

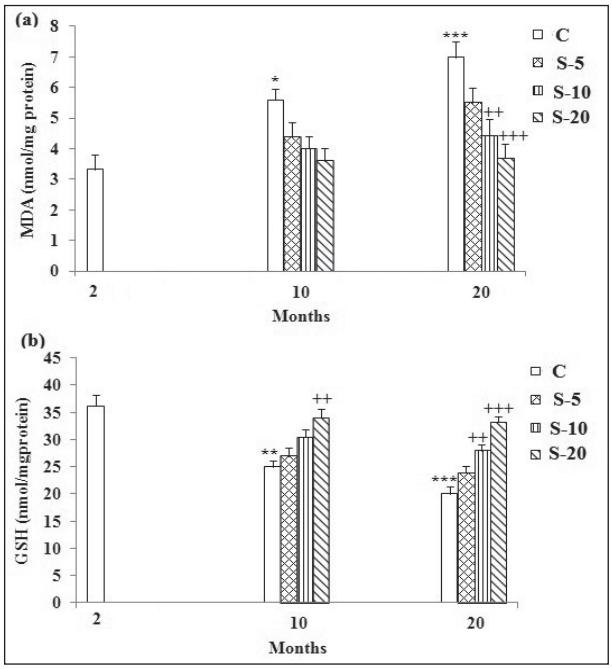

Figure 1. Changes in the levels of (a) MDA and (b) GSH in the kidney homogenates in untreated (2, 10 and 20-month) rats (C), and saffron hydro-ethanolic extract (S-5, S-10, S-20) treated aged rats (n = 10 per group). Values are presented as mean ± SEM. Statistical significance for the difference between the data of the young rats vs other groups: *; p<0.05, **; p<0.01,***; p<0.001. Statistical significance ++; p<0.01, +++; p<0.001 the untreated aged rats versus saffron hydro-ethanolic extract treated aged rats.

Effect of the saffron hydro-ethanolic extract on GSH content in aging

GSH was reduced with aging but increased with treatment of the saffron hydro-ethanolic extract in the kidney of the aged animals. The levels of GSH decreased in the kidney of the 10 and 20 month old rats (middle and aged rats) as compared to the young rats (p<0.01; p<0.001 respectively). The saffron hydro-ethanolic extract (20 mg/kg)-treated aged rats had a significantly decreased the GSH level of kidney as compared with the untreated aged rats (p<0.01; p<0.001 respectively). Furthermore, the saffron hydro-ethanolic extract treatment (10 mg/kg) increased significantly the level of GSH (p<0.01) in the kidney of the 20 month old rats (aged rats) when compared with the untreated aged rats. Significant difference was not observed between the saffron hydro-ethanolic extract (20 mg/kg) treated aged rats and the young rats (Fig.1b).

Effect of the saffron hydro-ethanolic extract on enzymatic antioxidant activities in aging

Changes in the activities of SOD, CAT and GPx in the kidney of the 2, 10, and 20 month old rats and the saffron hydro-ethanolic extract -treated aged-rats are summarized in Figure 2. In the10 and 20 month old rats a significant (p<0.05 and p<0.01) decrease was seen in the SOD activity when compared with the young rats. Treatment of the saffron hydro-ethanolic extract (20 mg/kg) to the aged rats increased the SOD activity in the aged rats, however, there was not a significant change in the 10 month old rats when compared with the untreated aged rats. However, the saffron hydro-ethanolic extract treatment (20 mg/kg) restored the antioxidant enzyme activity in the aged rats nearly to that of the young rats (Fig. 2a). The activity of CAT was found to be significantly lower in the aged rats versus the young rats (p<0.001), but; the CAT activity showed a non-significant decrease in the 10 month old rats (middle-aged rats) compared with the young rats. Supplementation with the saffron hydro-ethanolic extract (20 mg/kg) for 4-week increased the CAT activity (p<0.01) in the kidneys of the aged rats, so that; there was non-significant in the CAT activity in the saffron hydro-ethanolic extract (20 mg/kg)-treated aged rats versus the young rats (Fig. 2b). In the 10 and 20 month old control groups, there was a significant (p<0.05; p<0.001 respectively) decrease in the GPx activity when compared with the young rats. When compared with the respective age control group, an increased the GPx activity in the saffron hydro-ethanolic extract (10 and 20 mg/kg)-aged treated animals was seen. There was no significant change in the GPx activity in the saffron hydro-ethanolic extract (20 mg/kg)-aged treatment groups when

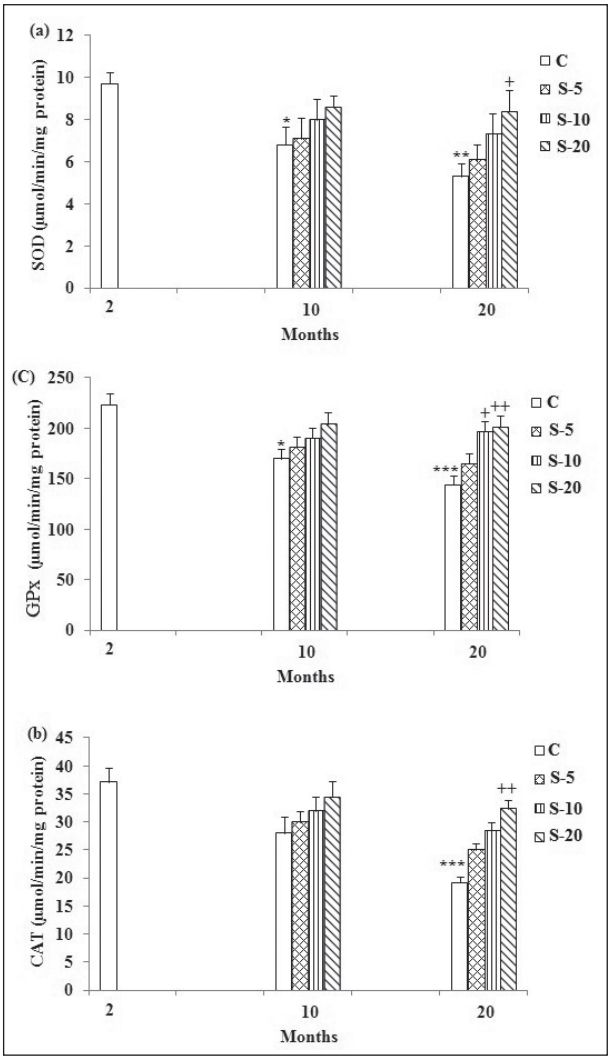

Figure 2. Changes in the activities of antioxidant enzymes (a) SOD, (b) CAT, (c) GPx in kidney of in untreated (2, 10 and 20-month) rats (C), and saffron hydro-ethanolic extract (S-5, S-10, S-20) treated aged rats (n = 10 per group). Values are presented as mean ± SEM. Statistical significance for the difference between the data of the young control vs other groups: *; p<0.05, **; p<0.01, ***; p<0.001. Statistical significance +; p<0.05, ++; p<0.01 comparing untreated aged rats versus saffron treated aged rats.

compared with young rats. The saffron hydro-ethanolic extract treatment had no effect on decreased the GPx activity in the 10 month old rats (Fig. 2c).

Effect of the saffron hydro-ethanolic extract on inflammatory genes in aging

The results demonstrated that the aged rats had a significantly higher mRNA expression of TNF-a (Fig. 3a), IL-6 (Fig. 3b) and IL-1b (Fig. 3c), in kidney tissues than the young rats ($P < 0.01$). Saffron extract treatment (20 mg/kg) significantly reduced the expression of these pro-inflammatory factors in kidneys in the treated aged rats versus to the untreated aged rats. ($p<0.001$, $p<0.01$, and $P < 0.05$, respectively). These findings illustrated that the saffron extract inhibited local inflammatory response in aging, which might relieve inflammatory detriment in the kidney during aging.

Effect of the saffron hydro-ethanolic extract on inflammatory secretion in aging

For moreover illustrate the effect of the saffron hydro-ethanolic extract on suppressing inflammatory response after aging phenomenon in kidney, we further determined the expression of pro-inflammatory cytokine TNF-α, IL-6 and IL-1b in the serum after renal aging. The result showed the untreated aged rats increased the expression of TNF-α (Fig. 4a), IL-6 (Fig.4b), and IL-1b (Fig. 4c) in the serum as compared with that of the young rats. However, treatment with saffron extract significantly decreased the level of TNF-α, IL-6 and IL-1b in the serum of the treated aged rats as compared with that of untreated aged rats. Taken together, our data indicated that treatment with of saffron extract could significantly suppress the pro-inflammatory cytokine secretion after aging.

Discussion

The present results also demonstrated that the saffron hydro-ethanolic extract supplementation significantly ameliorated the oxidant/antioxidant imbalance and inhibited the molecular inflammatory pathways induced by advancing age. In our previous research, the saffron hydro-ethanolic extract showed a therapeutic effect on age-related diseases, such as can-

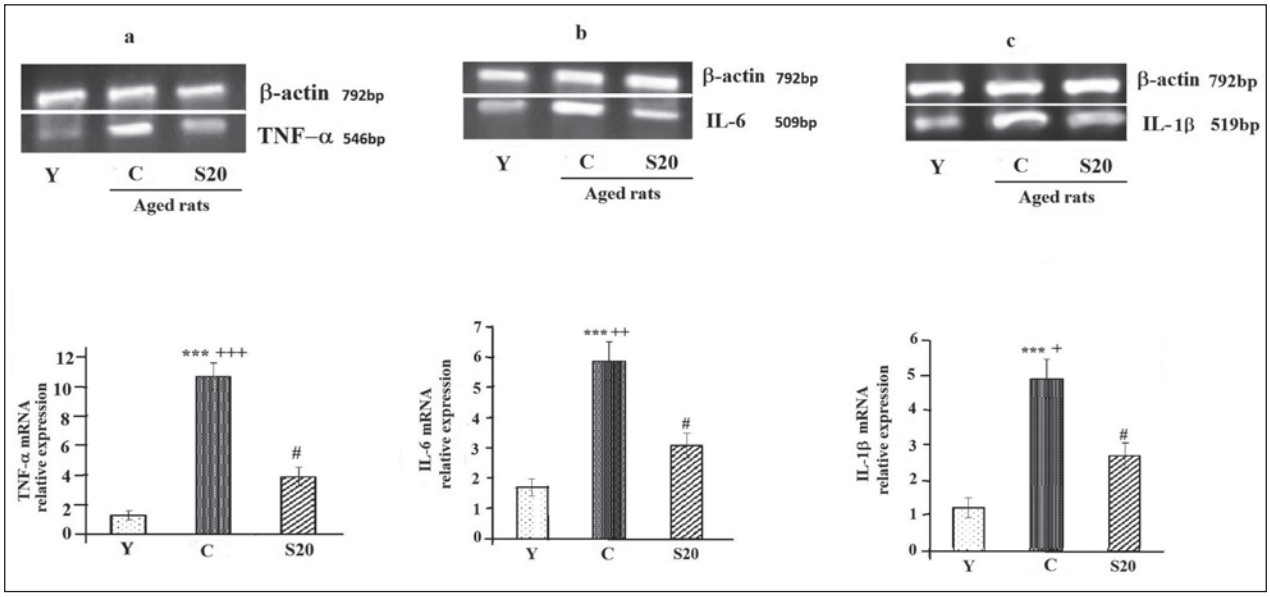

Figure 3. Renal expression of pro-inflammatory cytokines. RT-PCR was employed to assess the expression of pro-inflammatory cytokine in the kidney. We selectively analyzed the expression levels for tumor necrosis factor-α (TNF-α) (a), interleukin-6 (IL-6) (b), and IL-1β (c) in non-treated aged rats (C), and saffron hydro-ethanolic extract (S-20) treated aged rats. Data were represented as mean ± SEM (n = 10). *** $P < 0.001$ untreated aged rats (C) vs. young rats (Y); +++$P < 0.001$, ++ $p< 0.01$, + $p<0.05$ untreated aged rats (C) vs. S-20; # $P < 0.05$ S-20 vs. young rats (Y).

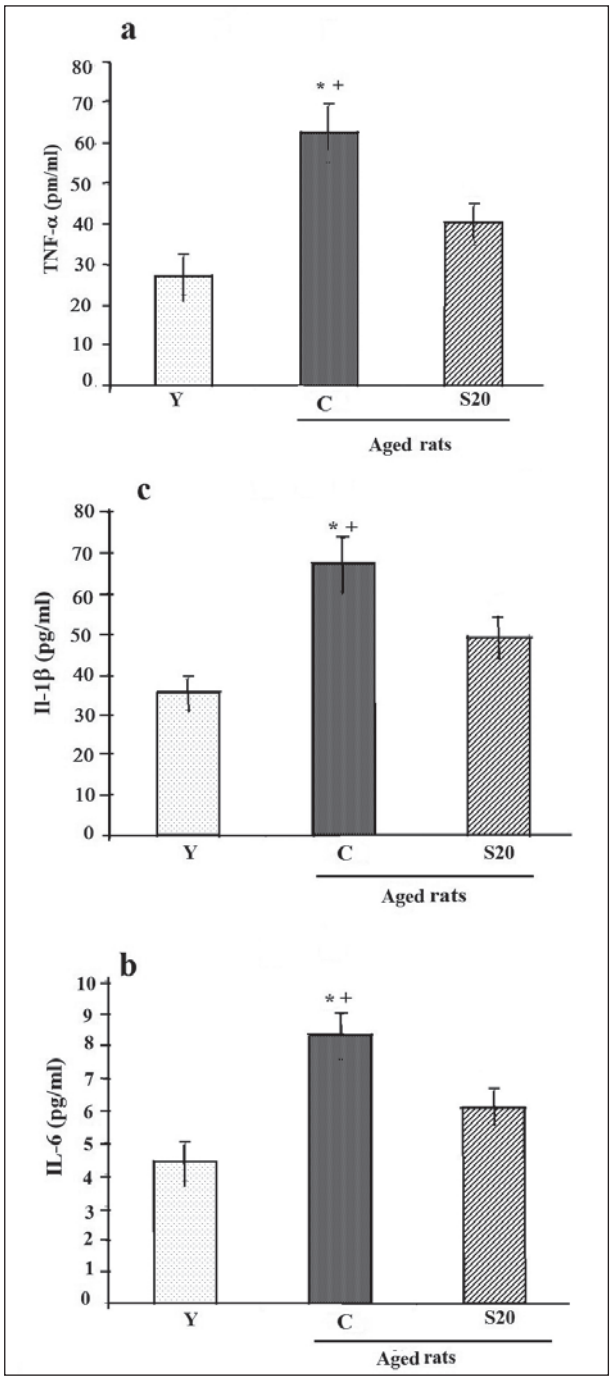

Figure 4. Secretion level of pro-inflammatory cytokines in serum. ELISA was employed to assess the secretion level of pro-inflammatory cytokine in serum. We selectively analyzed the secretion levels for tumor necrosis factor-α (TNF-α) (a), interleukin-6 (IL-6) (b), and IL-1β (c) in untreated aged rats (C), and saffron hydro-ethanolic extract (S-20) treated aged rats. Data were represented as mean ± SEM (n = 10). * P < 0.05 untreated aged rats (C) vs. young rats (Y); +P<0.05 untreated aged rats (C) vs. S-20.

cer and diabetes (19, 20, 32-34). Moreover, it has reported that the saffron hydro-ethanolic extract and its gradients could have powerful properties of anticancer, anti-inflammatory, and antioxidant (35, 36). Therefore, the saffron hydro-ethanolic extract is supposed to provide a novel therapeutic approach to the aging process. Thus, to consider the anti-aging and anti-oxidant effects of the saffron hydro-ethanolic extract during the aging process, the present research concentrated on its effects against renal injury linked to oxidative stress and inflammation using an animal model.

Aging leads to renal frailer as revealed the significant increase of Cr and BUN levels in a dose-manner. In our study, we found that the saffron hydro-ethanolic extract administration could reduce the level of Cr and BUN compared with the untreated aged rats. Suggesting protective treatment of rats with the saffron hydro-ethanolic extract against renal damage during the aging, our study indicated saffron might be effective therapeutic choice to prevent renal injury during aging.

The aging process is characterized by disruption of the pro-oxidant-antioxidant balance, and degeneration of the physiological function (2, 5). In the present study, pro-oxidant antioxidant balance was assessed by measuring endogenous MDA level and enzymatic antioxidants in kidney homogenate of the old rats. Increased endogenous MDA and decreased GSH, SOD, GPx and CAT levels showed that the balance changed on the behalf of pro-oxidation in the kidney homogenates of the old rats.

Our study confirmed that renal SOD, GPx and CAT activity and GSH level were significantly higher in the young rats than in the middle and aged rats. Supplementation of the saffron hydro-ethanolic extract resulted in an improved renal GSH content in the middle and aged rats as well as the activities of antioxidant enzyme in the aged rats (20 month old). These changes were accompanied by a significant decrease in lipid preoxidation in the kidney of the aged rats. It is suggested that the active principles from the saffron hydro-ethanolic extract might act by modulating the antioxidative capability (18, 19, 37-39). Several studies showed that aging leads to a decrease in the activity of the antioxidant enzymes during the development in kidney (3, 8). The present study also indicated significant decrease in renal GSH content and the

activities of SOD, CAT and GPx accompanied by a significant increase in aldehydic products of lipid peroxidation, indicating an increased renal oxidative stress which may also occur with advancing age. The aldehydic products of lipid peroxidation including MDA are more cytotoxic and stable than ROS and react quickly with cellular constituents. Besides these negative effects, MDA are modulators of signal transduction pathways that disturb cellular activities (37). This in turn may contribute to the disruption of intracellular and membrane redox state of many cells (38). The improvement recorded after the saffron hydro-ethanolic extract treatment of aging rats might propose a protective effect of saffron against aging that might be mediated through neutralization of oxygen free radicals (15-17). A stimulating effect of the synthesis of GSH by saffron was observed in the present study. The GSH reacts with free radicals and is a crucial substrate for GPx and glutathione- S-transferase (GST) which take part in the cellular defense mechanisms against intermediate oxygen products (39, 41). The ameliorative effect of the saffron hydro-ethanolic extract on renal lipid peroxidation produced during aging may be related to the significant rise in renal GSH induced by the active components in saffron (43). It may be relevant that the ratio of GSH/GSSG plays a critical role in cellular homeostasis and membrane redox state (30). The saffron hydro-ethanolic extract induced an increase in renal GSH content which might enhance the GSH/GSSG ratio and decrease renal lipid peroxidation (16, 17, 43). Parallel to these events, renal SOD activity was increased in the aging rats supplemented with the saffron hydro-ethanolic extract as compared with young rats. SOD is responsible for removal of superoxide radicals; thus, it may contribute to the modulation of redox state of kidney cells. The interplay of these events may contribute to the favorable effects of saffron on kidney damage produced by the generation of free radicals. The SOD protects against oxygen free radicals by converting super oxide radicals into hydrogen peroxide. The ROS scavenging activity of SOD is effective only when its activity is followed by the actions of CAT and GPx, since hydrogen peroxide produced by SOD is further scavenged by CAT and GPx. The balance between these enzymes is important for the effective removal of oxygen radicals from intracellular organs (39-42). The saffron hydro-methanolic extract may also inhibit lipid peroxidation by inducing SOD, CAT and GPx (18). Our observations confirmed that the saffron hydro-ethanolic extract may be effective to control of age related tissue damage by decrease in free radicals generation and increase in antioxidant defenses. Previous study also confirmed that the saffron aqueous extract and its main ingredients (safranal and crocin) showed good antioxidant activity (42, 43). The saffron hydro-ethanolic extract administration regulates the expression of antioxidant, longevity related genes, and consequently oxidant contents in aging animals. In the biological systems saffron indicates its antioxidant impact through stabilizing the membranes, inhibiting ROS and reducing peroxidation of unsaturated membrane fatty acids. It also has been reported that saffron supplementation could reduce lipid peroxidation (44). It was shown that the radical scavenging activity of the saffron methanol extract and its constituents, crocin and safranal, is significant, probably because these donate hydrogen atoms for DPPH radical stabilization (45, 46). Furthermore, these data indicated that the saffron hydro-ethanolic extract treatment had no effect on the balance changed of pro-oxidation in the kidney homogenates of the 10 month old rats. However, this was observed that supplementation with high the saffron hydro-ethanolic extract concentration increased GSH content in the kidney homogenates of the 10 month old rats as compared with the untreated aged rats. Our data showed that, the balance changed of oxidative system was inconsiderable in the kidney homogenates of the 10 month old rats when compare to the young rats. In summary, aging generated higher oxidative stress in rat kidney, by decreasing GSH level and suppressing SOD, CAT and GST activities, while increasing the lipid peroxidation. The saffron hydro-ethanolic extract found effective in enhancing the GSH level and SOD, CAT and GPx activities and decreasing lipid peroxidation. Thus, the saffron hydro-ethanolic extract can be effective to protect susceptible aged kidney from oxidant/ antioxidant imbalance by increasing antioxidant defenses. There is another confirmation to use of antioxidant as a health beneficial food component during aging.

Aging is also associated with an increase in pro-inflammatory cytokines. To discover the precise mech-

anism of the saffron hydro-ethanolic extract against renal injury during the aging process, we examined its impact on the molecular inflammatory pathways. The unusual inflammatory response upon the renal injury during aging contributes to generation of excessive cytokines and chemokines, which in turn, ignite elaborated the molecular inflammatory pathways (47, 48). Several research have shown significant increases of pro-inflammatory mediators in the renal injury and diseases (49), suggesting that the severity of organ damage and dysfunction is positively associated with the level of pro-inflammatory mediators. Our results showed that aging led to elevations of TNF-α, IL-6, and IL-1b in the injured renal tissues and serum. However, this effect was significantly reduced by the saffron hydro-ethanolic extract administration. It proposed that the saffron hydro-ethanolic extract supplementation reduced renal injury associated with aging involving in suppressing the molecular inflammatory pathways.

This study focused on the effects of oxidative stress-related inflammation in the kidneys of the old rats treated with the saffron hydro-ethanolic extract. The present study showed that the administration of the saffron hydro-ethanolic extract to the aged rats had a favorable influence on the prevention of renal failure development, at least in part by ameliorating the signaling pathways of oxidants -induced inflammation.

Whereas the saffron hydro-ethanolic extract inhibited the molecular mechanisms of oxidants -related inflammatory cytokine expression in the kidney. Although the detailed mechanism of saffron hydro-ethanolic extract was not clarified in the present study, these findings provide therapeutic evidence for the beneficial effects of the saffron hydro-ethanolic extract on ameliorating the development of age-related renal damage. Further studies need to focus on addressing that the pathways involved in anti-inflammation by the saffron extract upon aging would be necessary. In summary, old rats showed increased renal damage associated with the activation of signal pathway oxidants -derived pro-inflammatory transcription factors and pro-inflammatory genes (TNF-a, IL-6, IL-1b). On the other hand, these unfavorable outcomes were reversed by the saffron hydro-ethanolic extract treatment in old rats. Saffron extract treatment of old rats improved the overall renal function, such as serum urea nitrogen.

In conclusion, we demonstrated that treatment of the saffron hydro-ethanolic extract in rats provided protection against renal injury during the aging process. This protection manifested as amelioration of serum changes to the kidney, oxidant/ antioxidant imbalance and suppression of molecular inflammatory pathways. Our findings suggest that the saffron hydro-ethanolic extract may be a novel practical strategy to prevent renal injury during aging process. Therefore, saffron and its ingredients are expected to bring a new therapeutic pathway to a range of conditions accompanied by oxidative stress and molecular inflammatory pathways in aging. On the other hand, the molecular protective pathway for the effects of saffron extract against the oxidative stress and inflammation involved in the aging process has not been examined.

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