Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (3):283-290
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Assessment the effect of saffron ethanolic extract (*Crocus sativus* L.) on oxidative damages in aged male rat liver

Saeed Samarghandian[1*], Majid Asadi-Samani[2], Tahereh Farkhondeh[3] and Mahmoud Bahmani[4]

[1]*Department of Basic Medical sciences, Neyshabur University of Medical Sciences, Neyshabur, Iran*
[2]*Student Research Committee, Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran*
[3]*Department of Immunogenetics, BuAli Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran*
[4]*Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran*

ABSTRACT

Using antioxidant nutrients may be a good diet strategy for the prevention of oxidative stress associated to age-related disease. We assessed the changes in activities of antioxidant enzymes (superoxide dismutase-SOD glutathione-S-transferase-GST, catalase-CAT) and the levels of lipid per-oxidation (Malondialdehyde -MDA) in liver and serum nitric oxide (NO) of rats 2, 10 and 20 months old, and to determine the effect of saffron on the status of selected oxidative stress. The aged rats were given intraperitoneal injection of saffron extract daily for 4-week. Data were analyzed using ANOVA-one way followed by Tukey-Kramer post-hoc test for multiple comparisons. The results demonstrated that aging caused significant increase in the levels of serum NO and MDA with a reduction in the activities of SOD, GST and CAT in liver. This study showed that saffron ameliorated increased serum NO and MDA levels and decreased GST activity in liver of 20 months old rats. Therefore, saffron can be effective to protect susceptible aged liver.

Keywords: Saffron extract, Oxidative damage, Liver, Aging, Rat.

INTRODUCTION

Age-related diseases have increased with increase in the proportion of older population in the world [1]. Aging is defined as progressive loss of biological function accompanied by decline in survival which all species suffer with advancing age. The elderly population shows the effects of aging on the liver and also the generalized longevity of the organism's ability control the biological aspects. Although the age has not a significant influence on liver function tests, liver diseases presentation may be subtler in the older adults than that of younger patients [2].

It is obvious that aging is a complex process of an array of intertwined molecular pathways [3]. This complex process is controlled by several factors, such as changes in lifestyle, diet and physical activity, as well as drug treatments and medication. It is postulated that finding the cellular and molecular mechanisms of aging development would bring a suitable treatment to the disorders of aging [4]. With increasing age, hepatic failure may be induced via increase in the production of reactive oxygen species (ROS) or reduction in the antioxidant capacity of the body

[5, 6]. Enzymatic and non-enzymatic antioxidants during aging constitute defense system to clear up ROS in liver tissue [7, 8]. A group of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione-*S*- transferase (GST), act as superoxide anion and H_2O_2 scavengers to prevent ROS-induced damage, which may lead to the changes of some biomarkers [9-11]. Malondialdehyde (MDA) production, a major biomarker of the occurrence of lipid peroxidation, initiated by excessive ROS [12- 15]. An increase in the hepatic MDA concentration cause tissue damage, and failure of the antioxidant defense system to prevent the formation of excessive free radicals [16, 17]. Considering that age related disease may be delayed by prevention of lipid peroxidation, supplementation with antioxidants has been reported to be beneficial to retard or reverse this aging process [18, 19]. Medicinal herbs and their active ingredients contain bioactive substances that act through antioxidants activities [20-40]. Recently, numerous traditional medicines have been found to possess potential anti-aging activities by scavenging ROS and detoxifying potent genotoxic oxidants, and have attracted considerable interest as potential candidates for the development of novel anti-aging therapies [6]. Saffron, the dried stigmas of the flowers of saffron (Crocus sativus L., Iridaceae), is widely used in human applications and has commercial value for a long time. Greece, along with India, Iran, Spain, Azerbaijan and Morocco, is one of the principal world saffron producers [41]. It has been reported that saffron and its ingredients have hypolipidemic, anti-inflammatory, antioxidant and anticancer effects, moreover, this is applicable for the treatment of asthma [42-47].

To our knowledge, the literature lacks the information about the capacity of saffron extract to prevent free radical formation and lipid peroxidation in the liver of ageing the rats. Therefore, this study was designed to investigate the effect of saffron ethanolic extract on pro-oxidant and antioxidant status in aged rat livers. For this reason, the activities of antioxidant enzymes (SOD, GST and CAT), MDA levels in the livers and serum nitric oxide (NO) content of aged rats were compared with respective age-matched controls and also young rats.

MATERIALS AND METHODS

Chemicals

All purified enzymes, coenzymes, substrates, standards and buffers and kits were obtained from Sigma Chemicals Company, USA. (St. Louis, USA).

Plant

The saffron used in this study was collected from a private garden and identified by the herbal medicinal specialist at Ferdowsi University.

Plant extract preparation

In the maceration method, 10 g of stigmas were macerated in 500 ml ethanol (80 v/v) for three days. The mixture was subsequently filtered and concentrated under reduced pressure at 40°C. The extract yield was 51% w/w.

Study design

Male Wistar rats of different ages namely 2, 10, and 20 months (n = 10 for each group) were obtained from the Center for Experimental Medical Research of Mashhad University. The animals were kept at a constant temperature of 25^{o}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. Old rats (10 and 20 months old) were divided into four subgroups as one untreated (vehicle) and three saffron-treated groups. The aged rats (10 and 20 months old) were given intraperitoneal injection of saffron extract (5, 10, 20 mg/kg/day) daily for 4-week. Control animals received an equal volume of vehicle.

Biochemical analysis

After 30 days of saffron extract treatment, animals were anesthetized with diethyl ether and blood was collected from retro orbital sinus. Serum was separated by centrifugation at 3000 rpm for 10 min for NO assay [48]. Rat livers were quickly removed, weighed, and washed in 0.9% NaCl and these samples were frozen in liquid nitrogen and kept at -80^{o}C. The homogenates were prepared; the pellet obtained at 12,000 g containing supernatant fractions was used for total protein, MDA, CAT, SOD and GST assays [49]. Total protein was estimated in subcellular fractions by the method of Bradford (1976) using bovine serum albumin (BSA) as standard [50]. Lipid peroxidation was assessed in the homogenates of the whole liver. 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 [51].

CAT activity was assayed by H_2O_2 consumption, following Aebi (1984) [52] method and modified by Pieper et al (1995) [53]. SOD activity was determined by the method of Marklund and Marklund (1979) using inhibition of pyrogallol autoxidation at pH 8 [54]. GST activity was measured using cumene hydroperoxide and 1-chloro-2, 4-dinitrobenzene as substrates respectively [55]. NO level can be determined spectrophotometrically by measuring the accumulation of stable degradation products, nitrite and nitrate. The serum nitrite level was determined by the Griess reaction [56].

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

The body weights of the aging animals increased significantly ($p<0.001$) in 10 and 20 months when compared with two-month old control rats. There was a significant decrease in body weight with saffron extract (20 mg/kg) treatment in 20 month old groups when compared with matched control ($p<0.01$). The protein content in the whole homogenates, supernatant, and pellet fractions did not show significant changes with aging and saffron extract treatment. Liver weight increased with age but was proportionally less than bodyweight and the ratio of LW to BW declined. The ratio increased after treatment with saffron extract. Results are shown in Table 1.

Table 1 Body weight, liver weight and protein concentration of 2, 10, and 20 months of control (C) and saffron extract-(SE)-treated aging rats

Age/treatment	Body wt. (g)	Liver wt. (g)	LW/BW (%)	Protein content supernatant (mg/g)
2 months				
C	119 ± 22	7.2 ± 1.0	6.89 ± 0.10	95 ± 6.2
10 months				
C	398 ± 27***	11.1 ± 1.2**	2.78 ± 0.44***	84 ± 4.3
5mg/kg S	386 ± 32	13.3 ± 1.6	3.44 ± 0.50	86 ± 5.1
10mg/kg S	350 ± 20	15.9 ± 1.4	4.54 ± 0.70	89 ± 3.7
20mg/kg S	280 ± 22	17.2 ± 1.7^{+}	6.14 ± 0.77^{++}	92 ± 4.6
20 months				
C	591 ± 31***	14.51 ± 1.3**	2.45 ± 0.41***	77 ± 4.1
5mg/kg S	586 ± 25	16.4 ± 1.2	2.79 ± 0.48	80 ± 6.3
10mg/kg S	550 ± 34	18.5 ± 1.0	3.36 ± 0.29	82 ± 5.5
20mg/kg S	410 ± 40^{++}	20.2 ± 1.3^{+}	4.92 ± 0.32^{+}	89 ± 4.9

*Each value is a mean ±SEM of five or more separate values from two to three experiments. The comparisons of experimental values are with the control values. Statistical significance: +:p<0.05, ++:p<0.01 comparing age-matched controls versus saffron extract treatment and **:p<0.01, ***: p<0.001 versus 2 months*

Lipid peroxidation was measured as the formation of MDA level in whole homogenates, supernatant, and pellet fractions of aging rat liver from control and saffron extract-treated aging animals. With aging there was a significant increase ($p<0.001$) in MDA levels in 10 and 20 month old groups as compared to the two-month old control animals. For saffron extract (10 and 20 mg/kg) treatment in 20 months old animals, the MDA levels decreased significantly ($p<0.05$and $p<0.001$) when compared with age-matched controls. There was a significant change in MDA level in the 10 month old group after saffron extract (20 mg/kg) treatment when compared with age-matched controls ($p<0.05$) (Figure. 1a).

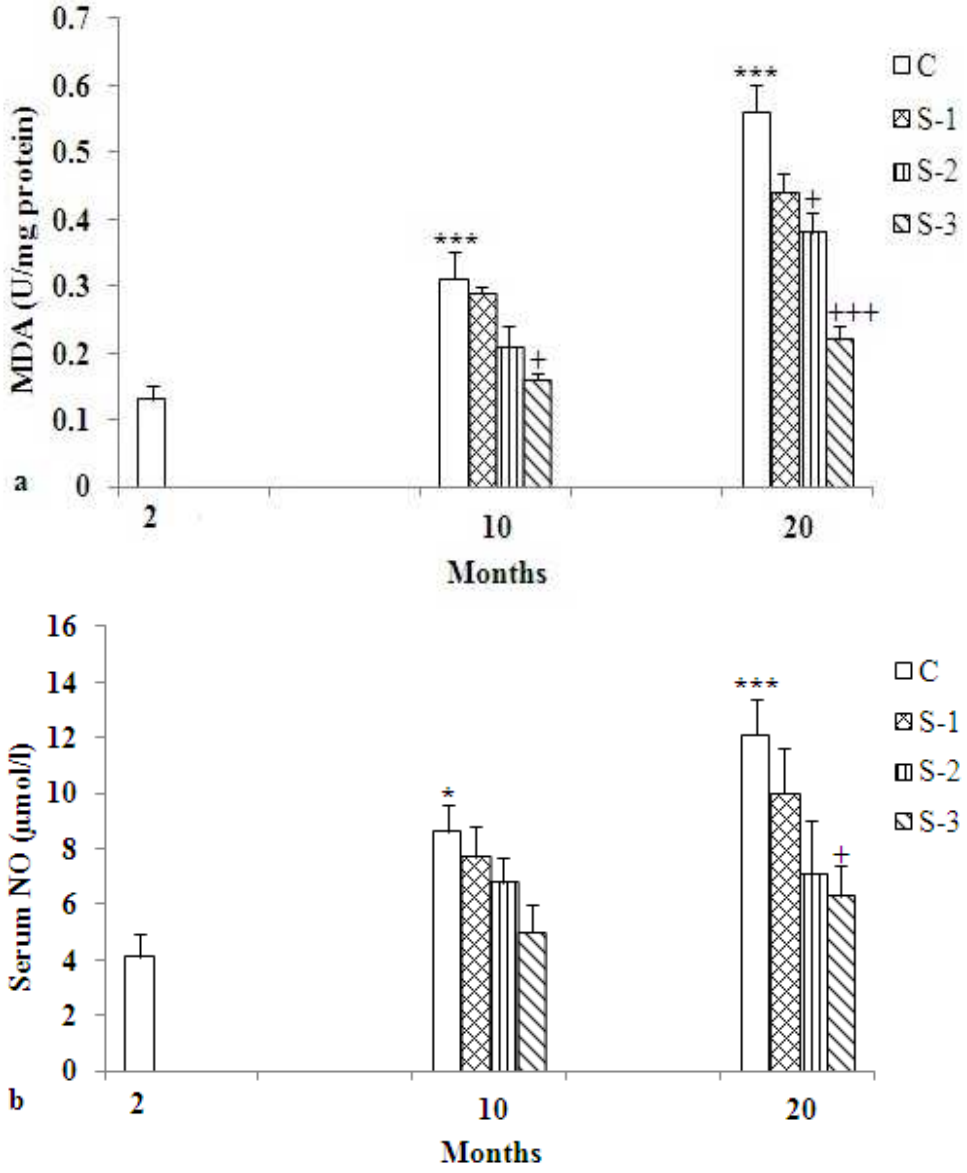

Figure 1. Changes in the level of (a) lipid preoxidation (MDA) in the liver homogenates and (b) serum nitric oxide (NO) in control (2, 10 and 20-month) rats (C), and saffron extract (S-1, S-2, S-3) treated aging rats (n = 10 per group). Values are presented as mean ± SEM. Statistical significance +; $p<0.05$, +++; $p<0.001$comparing age-matched controls versus saffron treatment and *; $p<0.05$, *; $p<0.001$**

Our data showed that aging resulted in a significant increase of serum NO as compared to the 2 month control group ($p<0.05$ and $p<0.001$). Compared with age-matched controls, treatment of saffron (20 mg/kg) in the 20 month old rats led to a significant decrease in serum NO level ($p<0.05$) (Figure. 1b).

Changes in the activities of CAT, SOD and GST in cytosolic fractions in the liver of 2, 10, and 20 month old control and saffron-treated aging rats are summarized in Figure. 2.

The activity of CAT and SOD levels was found to be significantly lower in the 20 month old rats versus to the two-month old control ($p<0.001$and $p<0.05$, respectively), however, CAT and SOD activity showed a non-significant decrease in the 10 month old rats compared with two-month old controls. Supplementation with saffron extract had no significant change in the activity of CAT and SOD levels in the aged animals.

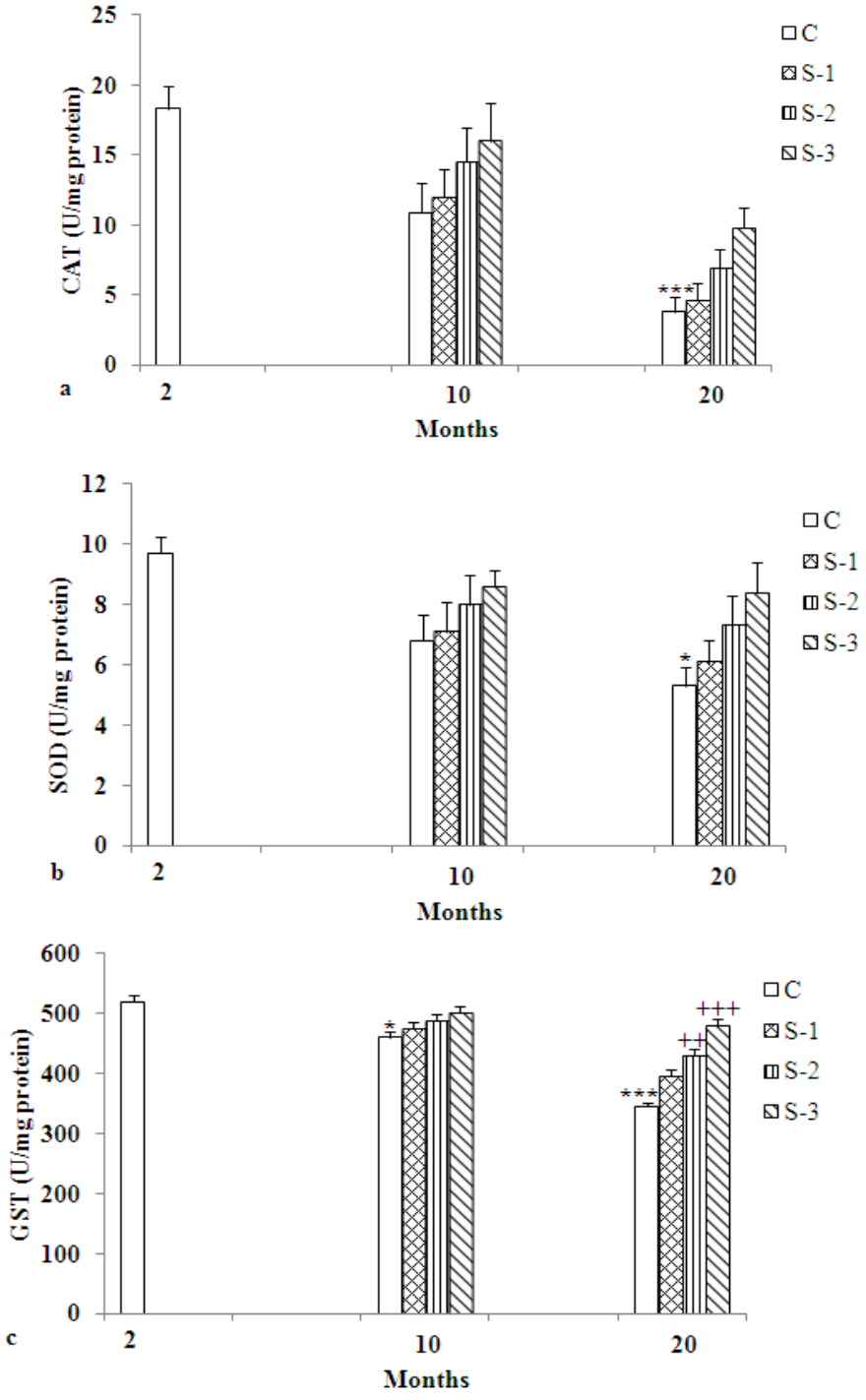

Figure 2. Changes in the activities of antioxidant enzymes (a) catalase (CAT), (b) superoxide dismutase (SOD), (c) glutathione S-transferase (GST) in liver supernatant fraction of in control (2, 10 and 20-month) rats (C), and saffron extract (S-1, S-2, S-3) treated aging rats (n = 10 per group). Values are presented as mean ± SEM. Statistical significance for the difference between the data of young control vs other groups: *; $p<0.05$, *; $p<0.001$. Statistical significance ++; $p<0.01$, +++; $p<0.001$comparing age-matched controls versus saffron extract treatment**

In the10 and 20 month old control groups, there was a significant (p<0.01 and p<0.001, respectively) decrease in GST activity when compared with two-month old control rats. When compared with the respective age control group, an increase in the GST activity was seen in the saffron extract (10 and 20 mg/kg)-treated 20 month animals (p<0.05 and p<0.001).There was no significant change in GST activity in saffron extract-treated 10 month old groups when compared with age-matched control rats.

DISCUSSION

Oxidative stress and aging induce molecular oxidative damage, which in turn cause cellular dysfunction and impairment of organ function thus they involve in the organism life span [57, 58]. In the present study, we found the association between increasing level of oxidation and aging. In author's knowledge for the first time, we showed the level of endogenous MDA and enzymatic antioxidant activities in liver homogenate as well as serum NO level alter with aging. Our study confirmed that CAT, SOD and GST activity in the liver were significantly higher, while MDA and NO levels were lower in the two-month-old rats compared with the aging rats.

Furthermore, our data showed that increased endogenous MDA, NO and decreased GST ameliorate after saffron extract treatment in the old animals. Therefore, in the current study, we investigated anti-aging and protective potential of saffron extract treatment on the activities of antioxidant enzymes (SOD, GST and CAT), and MDA level in the liver as well as serum NO level of the aging rats.

Liver is one of the important organs in detoxification. Its function declines gradually due to structure atrophy with age [13]. Previous studies have shown that carnosine plus vitamin E appeared to be useful in deceasing oxidative stress in aged liver, heart and brain tissues [59]. These results are fully in agreement with those obtained in the present work. These studies suggest that age-related degeneration of liver tissue in the rat may be due to a rise in free radical production in the mitochondria [60]. In this study, it was determined that MDA and NO levels were significantly lower in the saffron extract treated rats compared with the untreated aged groups. In addition, these results indicated that GST activity was higher in the saffron extract treated rats compared with the untreated aged groups. Importantly, mechanistic studies evaluating the role of oxidative stress in the human liver disease pathogenesis will require measurement of markers of oxidative stress, including the end products of lipid peroxidation (MDA) [61]. Researchers confirm that the reactive species oxygen (ROS) and nitrogen (RNS) are the prominent factor inducing aging –that associated with physiological disorders [62]. An impairment in mitochondrial function with age has been also reported, [63] which may be a major factor underlying the increase in the rate of ROS production in mitochondria. SOD and CAT constitute a mutually supportive team of defense against ROS. SOD is an important defense system to catalyze superoxide radicals into H_2O_2 and CAT decomposes hydrogen peroxide to water and oxygen; thus, these enzymes may contribute to the modulation of redox state of plasma [64]. GST belongs to a group of multigene and multifunctional detoxification enzymes, which defend cells against a wide variety of toxic insults from chemicals, metabolites, and oxidative stress [65]. The lower activities of CAT, SOD and GST in aged rats may be a consequence of inhibitory effects due to down-regulation phenomenon or excess ROS generation. Our observations confirmed that saffron extract may be effective to control of age related tissue damage by balancing the oxidative system in the liver. Similarly, previous studies have indicated that aqueous saffron extract and its active constituent, crocin and safranal, exhibited significant radical scavenging activity and thus antioxidant activity [66]. Furthermore, saffron may exert its chemopreventive effects by modulation of lipid peroxidation, antioxidants and detoxification systems [67]. Our data also confirmed the previous studies on pharmacological effects of plant medicine [68-83]. In summary, the results of this study showed that the saffron extract was found to be effective in enhancing the activity of the GST and decreasing the MDA level in the liver homogenates as well as decreasing in the level of serum NO. Thus saffron extract can be effective to protect susceptible aged liver from oxidative damage by balancing the oxidative system. This study is another confirmation for the use of antioxidant as a health beneficial food component during aging.

Acknowledgements
The authors would like to thank Research Affairs of Neyshabur University of Medical Sciences for financially supporting this work.

REFERENCES

[1] VM Mendoza-Núñe; L Martínez-Maldonado; E Correa-Muñoz, *BMC GERIATR*, **2009**, 9, 9-40.

[2] A Floreani, *Dig Dis*, **2007**, 25, 138-143.
[3] H Xue; B Xian; D Dong; K Xia; S Zhu; Z Zhang, *Mol Syst Biol*, **2007**, 3, 147.
[4] BF Oliveira; JA Nogueira-Machado; MM Chaves, *Sci World J*, **2010**, 10, 1121-1128.
[5] S Samarghandian; M Azimi-Nezhad; A Borji; T Farkhondeh, *Prog Nutr*, **2016**, 18. In Press.
[6] S Samarghandian; M Azimi-Nezhad; F Samini, *Exp Anim*, **2015**, 64, 65-71.
[7] S Samarghandian; M Azimi-Nezhad; R Afshari ; T Farkhondeh ; F Karimnezhad, *J Biochem Mol Toxicol*, **2015**, 29, 249-253.
[8] P Sudheesh; TA Ajith; V Ramnath; KK Janardhanan, *Clin Nutr*, **2010**, 29, 406-412.
[9] HS Moghaddam ; S Samarghandian ; T Farkhondeh , *Toxicol Mech Methods*, **2015**, 25, 507-513.
[10] S Samarghandian; R Afshari ; T Farkhondeh , *Int J Clin Exp Med*, **2014**, 7, 1449-1453.
[11] S Samarghandian; MM Shabestari; F Jabbari; T Farkhondeh; F Bafandeh, *Interdiscip Toxicol*, **2015**, 8, 101–104.
[12] S Samarghandian; A Borji; R Afshari; MB Delkhosh; A gholami, *Toxicol Mech Methods*, **2013**, 23, 432-436.
[13] SK Farahmand; F Samini; M Samini; S Samarghandian, *Biogerontology*, **2013**, 14, 63-71.
[14] S Samarghandian; A Borji; MB Delkhosh; F Samini, *J Pharm Pharm Sci*, **2013**, 16, 352-362.
[15] S Samarghandian; A Borji; SH Tabasi, *Cardiovasc Hematol Disord Drug Targets*, **2013**, 13, 231-236.
[16] S Samarghandian; M Azimi-Nezhad; F Samini; T Farkhondeh, *Can J Physiol Pharmacol* , **2015**, 13:1-6.
[17] S Samarghandian; T Farkhondeh; F Samini; A Borji, *Biochem Res Int*, **2016**, 2016, 2645237.
[18] A Kumar; A Prakash; S Dogra, *J Asian Nat Prod Res*, **2011**, 13, 42-55.
[19] M Wang; Y Lei, *Chin J Integr Med*, **2011**, 17, 635-639.
[20] S Samarghandian; JT Afshari; S Davoodi, *J Med Plant Res*, **2010**, 4, 2551-2556.
[21] T Farkhondeh; S Samarghandian, *Pharmacognosy Res*, **2015**, In Press.
[22] T Farkhondeh; S Samarghandian; M Azimin-Nezhad; F Samini, *Int J Clin Exp Med*, **2015**, 8, 2465-2470.
[23] S Samarghandian; M Azimi-Nezhad; H Mehrad-Majd; SR Mirhafez; *Pharmacology*, **2015**, 96, 112-117.
[24] S Samarghandian; M Hasanzadeh; F Jabbari; T Farkhondeh, *Pharmacogn Mag*, **2016**, In Press.
[25] S Samarghandian; JT Afshari; S Davoodi, *Pharmacogn Mag*, **2011**, 7, 46-52.
[26] MR Hajzadeh; Z Rajaei; S Shafiee; A Alavinejhad; S Samarghandian; M Ahmadi, *Pharmacology online*, **2011**, 1, 809-817.
[27] S Samarghandian; JT Afshari; S Davoodi, *Clinics (Sao Paulo)*, **2011**, 66, 1073-1079.
[28] S Samarghandian; MA Hadjzadeh; F Amin Nya; S Davoodi, *Pharmacogn Mag*, **2012**, 8, 65-72.
[29] M Bahmani; K Saki; M Asadbeygi; A Adineh; S Saberianpour; M Rafieian-Kopaei; F Bahmani; E Bahmani, *J Chem Pharm Res*, **2015**, 1, 646-653.
[30] B Baharvand-Ahmadi; M Bahmani; N Naghdi; K Saki; S Baharvand-Ahmadi; M Rafieian-Kopaei, *Der Pharma Lettre*, **2015**, 7, 189-196.
[31] B Baharvand-Ahmadi; M Bahmani; N Naghdi; K Saki; S Baharvand-Ahmadi; M Rafieian-Kopaei, *Der Pharma Lettre*, **2015**, 7, 160-165.
[32] M Bahmani; Z Eftekhari; M Jelodari; K Saki; R Abdollahi; M Majlesi; M Rafieian-Kopaei; S Rasouli, *J Chem Pharm Res*, **2015**, 2, 519-526.
[33] M Bahmani; K Saki; S Shahsavari; M Rafieian-Kopaei; R Sepahvand; A Adineh, *Asian Pac J Trop Biomed*, **2015**, 5, 858-864.
[34] M Bahmani; K Saki; M Gholami-Ahangaran; P Parsaei; A Mohsenzadegan; N Zia-Jahromi, *Middle East J Sci Res*, **2012**, 12, 260-263.
[35] B Delfan; H Kazemeini; M Bahmani, *J Evid Based Complementary Altern Med*, **2015**, 20, 173-179.
[36] M Bahmani; M Mirhoseini; H Shirzad; M Sedighi; N Shahinfard; M Rafieian-Kopaei, *J Evid Based Complementary Altern Med*, **2015**, 20, 228-238.
[37] B Baharvand-Ahmadi; M Bahmani; A Zargaran; Z Eftekhari; K Baharvand- S. Ahmadi; M Rafieian-Kopaei, *Der Pharma Lettre*, **2015**, 7, 172-173.
[38] B Baharvand-Ahmadi; M Bahmani; P Tajeddini; N Naghdi; M Rafieian-Kopaei, *J Nephropathol*, **2016**, 5, 44-50.
[39] M Bahmani; A Zargaran, *Eur J Integr Med*, **2015**, 7, 657-662.
[40] S Samarghandian; MAR Hadjzadeh; AS Davari; M Abachi, *Avicenna J Phytomed*, **2011**, 1, 36-42.
[41] S Samarghandian; MH Boskabady; S Davoodi, *Pharmacogn Mag*, **2010**, 6, 309-314.
[42] S Samarghandian; J Tavakkol Afshari; S Davoodi, *Appl Biochem Biotechnol*, **2011**, 164, 238-247.
[43] S Samarghandian; MM Shabestari; *Indian J Urol*, **2013**, 29, 177-183.
[44] S Samarghandian; A Borji; SK Farahmand; R Afshari; S Davoodi; *Biomed Res Int*, **2013**, 2013, 417928.
[45] S Samarghandian; ME Shoshtari; J Sargolzaei; H Hossinimoghadam; JA Farahzad, *Pharmacogn Mag*, **2014**, 10, S419-24.

[46] S Samarghandian; A Borji, *Pharmacogn Res*, **2014**, 6, 99-107.
[47] S Samarghandian; M Azimi-Nezhad; F Samini, *Biomed Res Int*, **2014**, 2014, 920857.
[48] LC Green; DA Wagner; J Glogowski; PL Skipper; JS Wishnok; SR Tannenbaum, *Anal Biochem*, **1982**, 126, 131–138.
[49] MR Siddiqui; A Taha; K Moorthy ; ME Hussain; SF Basir; NZ Baquer, *J Biosci*, **2005**, 30, 483-90.
[50] MM Bradford, *Anal Biochem*, **1976**, 72, 248-54.
[51] H Ohkawa; N Ohishi; K Yagi, *Anal Biochem*, **1979**, 95, 351-351.
[52] H Aebi, *Methods Enzymol*, **1984**, 105, 12-16.
[53] GM Pieper; M Jordan; LA Dondlinger; MB Adams; AM Roza, *Diabetes*, **1995**, 44, 884-889.
[54] S Marklund; G Marklund, *Eur J Biochem*, **1979,** 47, 469-474.
[55] PK Smith; RI Krohn; GT Hermanson; AK Mallia; FH Gartner; MD Provenzano, Analytical Biochemistry, **1985**, 150, 76-85.
[56] S Hortelano; B Dewez; AM Genaro; MJ Díaz-Guerra; L Boscá, *Hepatology*, **1995**, 21, 776-786.
[57] DW Killilea; H Atamna; C Liao; BN Ames, *Antioxid Redox Signal*, **2003**, 5, 507-516.
[58] JA Knight, *Adv Clin Chem*, **2000**, 35, 1-62.
[59] J Coban; I Bingül; K Yesil-Mizrak; S Dogru-Abbasoglu ; S Oztezcan ; M Uysal , *Curr Aging Sci*, **2013**, 6, 199-205.
[60] M Sawada; JC Carlson, *Mech Ageing Dev*, **1987**, 41, 125-137.
[61] R Vuppalanchi; R Juluri ; LN Bell; M Ghabril ; L Kamendulis ; JE Klaunig; *Am J Med Sci*, **2011**, 342, 314-317.
[62] H Van Remmen; A Richardson, *Exp Gerontol*, **2001**, 36, 957-968.
[63] M Nishikimi; NA Rao; K Yagl, *Biochem Biophys Res Commun*, **1972**, 46, 844-853.
[64] SP Singh; AJ Janecki; SK Srivastava, *J Biol Chem*, **2002**, 277, 4232-4239.
[65] AN Assimopoulou; Z Sinakos; VP Papageorgiou, *Phytother Res*, **2005**, 19, 997-1000.
[66] M Rahbani; D Mohajeri; A Rezaie; Y Doustar; M Nazeri, *Afr J Pharm Pharacol*, **2011**, 5, 2166-2173.
[67] K Premkumar; SK Abraham; ST Santhiya; A Ramesh, *Phytother Res*, **2003**, 17, 614-617.
[68] S Samarghandian; H Ohata; N Yamauchi; T Shibasaki; *Neuroscience*, **2003**, 116, 519-524.
[69] MRA Hajzadeh; S Samarghandian; AS Davari; M Abachi, *J Med Plants Res*, **2012**, 6, 1567-1575.
[70] F Samini; S Samarghandian; A Borji; G Mohammadi; M bakaian, *Pharmacol Biochem Behav*, **2013**, 110, 238-244.
[71] S Samarghandian ; F Samini; MD Taghavi, *Afr J Pharm Pharacol*, **2014**, 8, 9-15.
[72] S Samarghandian; MA Nezhad; G Mohammadi, *Anticancer Agents Med Chem*, **2014**, 14, 901-909.
[73] S Samarghandian; AJ Tavakkol; H Shidaee, *J Med Plants Res*, **2013**, 7, 2940-2951.
[74] S Samarghandian; MA Hadjzadeh; JT Afshari; MHosseini, *BMC Complement Altern Med*, **2014**, 14, 192.
[75] H Nasri; M Bahmani; N Shahinfard; AM Nafchi; S Saberianpour; MR Kopaei; *Jundishapur J Microb*, **2015**, 8, e25580.
[76] M Bahmani; A. Sarrafchi; H Shirzad; N Shahinfard; M Rafieian-Kopaei; S. Shahsavari; B Baharvand-Ahmadi; M Taherikalani; S Ghafourian, *J chem pharm sci*, **2015**, 8, 683-692.
[77] M Bahmani; N Shahinfard; M Rafieian-Kopaei; K Saki; S Shahsavari; M Taherikalani; S Ghafourian; B Baharvand-Ahmadi, *J chem pharm sci*, **2015**, 8, 672-682.
[78] B Delfan; B Baharvand-Ahmadi; M Bahmani; N Mohseni; K Saki; M Rafieian-Kopaei; S Shahsavari; N Naghdi; M Taherikalani; S Ghafourian, *J chem pharm sci*, **2015**, 8, 693-699.
[79] B Delfan; M Bahmani; H Golshahi; K Saki; M Rafieian-Kopaei; B Baharvand-Ahmadi, *J chem pharm sci*, **2015**, 8, 667-671.
[80] M Bahmani; H Shirzad; S Rafieian; M Rafieian-Kopaei, *J Evid Based Complementary Altern Med*, **2015**, 20, 292-301.
[81] S Samarghandian; R Afshari; A Sadati, *Scientific World J*, **2014**, 2014, 251378.
[82] Bahmani M, Sarrafchi A, Shirzad H, Rafieian-Kopaei M. *Curr Pharm Des*. **2016**;22(3):277-85.
[83] Sarrafchi A, Bahmani M, Shirzad H, Rafieian-Kopaei M. *Curr Pharm Des*. **2015**;22(2):238-46.