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A comparative biochemical study on the antioxidant status in peripheral and cupping blood samples of smokers and non-smokers

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ABSTRACT

Introduction: Smoking-induced oxidative stress causes the oxidation of biomolecules and results in smoking-related disorders. On other hand, Interest in complementary and alternative medicine has recently grown among patients with life-threatening diseases. This study was conducted to investigate the interactions of wet cupping and smoking on enzymatic and non-enzymatic antioxidant system.

Material and methods: 40 healthy, non-athlete men were assigned into non-, mild, and heavy smoker groups. They were subjected to two sampling times with one week interval. Then the levels of antioxidant enzyme activities, oxidative damage biomarkers, nicotine, carboxyhemoglobin, and vitamins C and E were evaluated in peripheral and cupping blood samples.

Results: We found increase in antioxidant enzyme activities in smokers with respect to non-smokers. Smoking decreased the levels of malondialdehyde and reactive carbonyl derivatives, and increased the level of 8-hydroxy-2'-deoxyguanosine, nicotine and carboxyhemoglobin in both peripheral and cupping blood samples. In both sampling times, antioxidant enzyme activities and oxidative damage biomarkers in peripheral blood were significantly higher and lower, respectively, as compared with cupping blood. At second sampling, antioxidant enzyme activities were higher and oxidative damage biomarkers were lower with respect to first sampling as they evaluated in both kinds of blood samples. Nicotine and carboxyhemoglobin in cupping blood were lower than peripheral blood, and at second sampling were higher than first sampling.

Conclusions: Smoking at any levels, elevated antioxidant enzyme activities in peripheral and/or cupping blood that may raised from oxidative damage caused by smoking. However, wet cupping was able to decrease the oxidative damage biomarkers. © 2013 Trade Science Inc. - INDIA

KEYWORDS

Smoking;
Wet cupping;
Antioxidant enzymes;
Oxidative damage;
Vitamin.

INTRODUCTION

Cigarette smoking is considered as a risk factor for

several diseases worldwide^[1,2] such as cardiovascular disorders, various forms of cancer, pulmonary dysfunctions^[3,4], and development of atherosclerosis^[5]. Ciga-

rette smoke contains a large number of free radical species and other oxidants^[6-8] that can result in oxidative stress^[1,9]. Smoking-induced oxidative stress cause the oxidation of lipids, induction of DNA single-strand breakage, inactivation of certain proteins, and the disruption of biological membranes consequently^[1,4,6]. Oxidative damage of lipids, proteins, and DNA lead to production of their oxidative damage biomarkers, malondialdehyde (MDA), 8-hydroxy-2'-deoxyguanosine (8-OHdG), and reactive carbonyl derivatives (RCD), respectively^[10-12]. Oxidative damage is normally eliminated and minimized by biological antioxidant systems including enzymatic and non-enzymatic elements^[9]. Important antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX)^[1,6]. Vitamin C in the aqueous and vitamin E in lipid media are the major non-enzymatic antioxidants^[13,14].

SOD is the major ROS detoxifier in the cell and catalyzes the formation of H_2O_2 and oxygen from superoxide radical anion^[15]. CAT detoxifies H_2O_2 by conversion to O_2 and H_2O ^[16]. GPX protects aerobic cells against lipid peroxidation, and as an erythrocyte enzyme, specifically reduces H_2O_2 by GSH^[17,18]. Vitamin E protects the polyunsaturated fatty acids of cell membranes from free-radical damage and knows as radical scavenger^[19]. Vitamin C is one of the most important biomolecules, which acts as antioxidant and radical detoxifier and terminate the radical chain reactions within cells^[20].

Nicotine (NIC) is one of the major toxic components in cigarette smoking^[21]. It is oxidized primarily into cotinine in the liver, generates ROS in tissues, and induces oxidative tissue injury^[22]. It is an active pharmacological agent responsible for lung-related disorders and cardiovascular diseases^[23]. It has been implicated to use in certain neurodegenerative diseases such as Alzheimer and Parkinson and oxidative stress has been implicated in the progression of such diseases^[24].

In addition, cigarette smoking increases the levels of carbon monoxide (CO) in the blood and forming carboxyhaemoglobin (COHb)^[25]. COHb results from the displacement of oxygen from hemoglobin by CO^[26]. Increased COHb elevated hemoglobin-O₂ affinity^[27,28], causing inadequate oxygenation of the blood circulating through the lungs; and increased erythrocyte oxidant damage. These lead to erythrocytosis and increased

production of erythropoietin^[28].

On the other hand, interest in use of complementary and alternative medicine (CAM) has recently grown in many countries around the World^[29] in particular among patients with life-threatening diseases such as cancer^[30], asthma and arthritis^[31], epilepsy, Parkinson's disease (PD), and multiple sclerosis (MS)^[32]. Considerable improvements have been reported in signs and medical manifestations of such radical related diseases. Accordingly, it is our conception that CAM may affect or alter the level of free radicals and try to evaluate it in cupping. Cupping, as a CAM method, today is used mainly in Asia as an alternative therapy for a variety of illnesses^[33].

Cupping involves the application of a vacuum to closed system cup on the skin^[34]. Two styles of cupping therapy are used today, dry cupping and wet cupping. Dry cupping simply pulls the local underlying tissue up into the suctioning cup^[35]. Wet cupping includes some superficial scarification of the skin before applying the cupping glasses^[36].

This study was conducted to investigate the interactions of wet cupping and smoking on enzymatic and non-enzymatic antioxidant system.

MATERIALS AND METHODS

This study was performed on forty healthy volunteer men aged between 20 and 45 years. All volunteers were tested and an expert physician confirmed their healthiness. They gave their informed consent to perform wet cupping on them. Based on data, they all were non-athlete and we assigned them into three groups including; non-smokers (group 1; N = 13), moderate smokers (group 2; volunteers who consumed 5-7 cigarettes per day; N = 13), and sever smokers (group 3; volunteers who consumed 15-20 cigarettes per day N = 14). All volunteers were considered for wet cupping on Jun. to Dec. 2010. Blood sampling was done at Iranian Institute of Hejamat Research and related institutions. Second sampling was done on the same volunteers seven days after first sampling.

In each case, a blood sample was taken from capital vein. In addition, the position on the vertebrae T2-T5 was disinfected with betadine. A glass cup was applied to the skin and a partial vacuum created inside the

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cup via electrical suction. The cup clung on the skin and left for a period of 5 min. Then superficial incisions were made to the skin with sterile surgical blades. The cup placed back on the skin, until it is filled with blood from the capillary vessels^[37]. Then the cup was removed and 5 ml of collected cupping blood used for biochemical measurements.

After each sampling, heparinized whole blood samples were immediately centrifuged at 3000 rpm in 4 °C for 10 minutes. The supernatant was aspirated and collected as serum and stored at -70 °C. To separate erythrocytes, the buffy coat was drained and the sediment was washed four times with 3 ml of 0.9% sodium chloride solution, and was centrifuged under the same conditions. Aliquots of the washed erythrocytes were lysed by freezing (-20°C) for 24 h and then they were used for measurements.

Antioxidant enzymes assay

The SOD activity in plasma was measured according to the method of Misra and Fridovich^[38] on the basis of their ability to inhibit free radical chain oxidation in which O₂ was a chain-propagating radical and the autooxidation of epinephrine was included. Human erythrocyte SOD was used as a standard and the activity was expressed in Unit/g.Hb.

CAT activity was determined by monitoring the disappearance of H₂O₂ at 240 nm. CAT activity was expressed as unit/mg.Hb. One unit of enzyme was the amount necessary to decompose 1 μmol of H₂O₂ per min at 25°C^[39].

GPX activity was measured by the method of Paglia et al. [40] Heparinized whole blood (0.05 ml) was diluted with 1 ml diluting agent, incubated for 5 min, and then 1 ml of double strength Drabkin's reagent was added and mixed well. GPX catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of GSH reductase and nicotinamide adenine dinucleotide phosphate (NADPH), the oxidized GSH was immediately converted to the reduced form with the concomitant oxidation of NADPH to NADP⁺. The decrease in absorbance at 340 nm was measured by a spectrophotometer.

The assay of oxidative damage biomarkers

MDA concentration in plasma was measured by the high-performance liquid chromatography technique

(model 4225; Unicam, LCD/Analytical Inc.) in which the MDA–thiobarbituric acid (TBA) adduct was separated^[41]. Briefly, plasma lipoperoxides were hydrolyzed by boiling in diluted phosphoric acid. MDA was reacted with TBA to yield the MDA–TBA adduct. The protein-free extract was fractionated on a C18 column of octadecyl silicagel to separate the MDA–TBA adduct by elution with methanol/phosphate buffer and quantified by a spectrophotometer at 532 nm (model: crystal 200; Unicam LCD/Analytical Inc.).

8-OHdG levels were measured essentially as described previously^[42]. Briefly, an automated column switching LCEC method for 8-OHdG was based on the unique selectivity of the integral porous carbon column for purines.

Samples were injected onto a C8 column and the band containing 8-OHdG was then quantitatively trapped on a carbon column. The selectivity of the carbon column for 8-OHdG allowed elimination of interfering peaks by washing the column with a second mobile phase and then eluting 8-OHdG to an analytical C18 column with an identical mobile phase containing adenosine to displace 8-OHdG. Detection with series colorimetric electrodes provides qualitative certainty for 8-OHdG peak by response ratios. RCD were detected by their reactivity with DNPH to form protein hydrazones and their amount evaluated at 370 nm and expressed in nmol/mg. protein^[43].

NIC levels in blood samples were determined by reversed phase high performance liquid chromatographic (RP-HPLC) method The analytical column, an MZ Kromasil C₈, 250 × 4 mm, 5 μm, was operated at ambient temperature with backpressure values of 290 kg/cm². The mobile phase consisted of A: 0.05 M ammonium acetate and phase B: CH₃OH at a volume ratio 60 : 40, delivered at a flow rate of 1.4 mL/min. Detection was performed with a variable wavelength UV-visible detector at 262 nm, resulting in a detection limit of 0.2 ng per 20 μL injection and a quantitation limit of 1.0 ng, while linearity held up to 20 ng/μL for nicotine^[44].

COHb percent in Blood was performed as follow. Heparinized fresh blood samples were mixed with various amounts of carbon monoxide and analyzed in the IL 282 CO-Oximeter and in a Hewlett-Packard Model 7620A Research Gas Chromatograph by a modification of the technique of Collison, Rodkey, and O'Neal^[45].

Vitamins assay

The evaluation of vitamin C was performed based on the method of Jacques-Silva et al. Plasma was precipitated with one volume of a cold 5% trichloroacetic acid solution and by centrifuged. An aliquot of 300 mL of the supernatants was mixed with 2,4-dinitrophenylhydrazine (4.5 mg/mL), CuSO_4 (0.075 mg/mL) and trichloroacetic acid 13.3% (final volume 1 mL) and incubated for 3 hours at 37 °C. Then, 1 mL of H_2SO_4 65% (v/v) was added to the medium. The level of vitamin C was calculated using a standard curve and expressed as mg vitamin C/ml of plasma^[46].

Determination of plasma vitamin E was performed by HPLC (model 4225; Unicam, LCD/Analytical). To precipitate proteins, aliquots (200 µl) of freshly plasma samples were mixed with 200 µl of HPLC-grade water and 400 µl of ethanol. For the vitamin extraction, 800 µl of hexane were added, and mixed for 30 min. Then, 600 µl of upper layers (hexane) were collected, dried in Speed-Vac system, and dissolved in 150 µl of mobile phase with 0.5% (m/v) BHT for the vitamin stabilization. An aliquot of 20 µl of this solution was injected into the HPLC system^[47].

Statistical analysis. All data were expressed as mean ± SEM (standard error of mean). The results were analyzed by one-way analysis of variance (ANOVA) followed by Paired-Samples T Test to evaluate the significance of the difference between groups using Statistical Package for Social Science (SPSS 16.0 for windows). A significant change was accepted at $p < 0.05$.

RESULTS

Clinical characteristics of cases are presented in TABLE 1. All subjects were at the same range of age with mean of 32.7 to 33.2 years. Hematocrit and blood pressure of studied groups were within normal ranges. There were no significant differences between smoking groups for hematocrit (Hct) and blood pressure as they were evaluated at first and/or second sampling times. In addition, second sampling did not alter these parameters significantly with respect to counterpart groups in first sampling.

TABLE 2 demonstrates antioxidant enzyme activi-

ties in peripheral and cupping blood samples for three studied groups at first and second samplings. In both kinds of blood, antioxidant enzyme activities increased in group 2 and 3 respectively. The increases in activity of GPX and CAT for group 3 were significant with respect to group 1 as they were assayed in cupping or peripheral blood samples. In each studied groups, the activities of three enzymes in peripheral blood were significantly higher than cupping blood at first or even at second sampling. In both kinds of blood, the activities of all three enzymes in each group in second sampling were significantly higher than their counterpart group at first sampling.

The levels of oxidative damage biomarkers; MDA, RCD, and 8-OHdG in both peripheral and cupping blood at first and second sampling are presented in TABLE 3. In both sampling times, the levels of MDA and RCD fell down insignificantly from group 1 to 2 and then 3 in both peripheral and cupping blood samples. However, the level of 8-OHdG revealed increase pattern from group 1 to 2 and then 3, and this increase was significant in group 3 as compared with group 1. In each studied group, the level of these three indexes was significantly higher in cupping blood with respect to peripheral blood at first and/or second sampling. Comparison of second with first sampling showed that the levels of all three indexes in peripheral and/or cupping blood were lower than the same kind of blood at first sampling time. The decreases in MDA and RCD were significant for all three studied groups. There was only insignificant decrease in 8-OHdG level in cupping blood samples of each group at second sampling with respect to the same kind of blood sample collected from counterpart groups at first sampling.

The levels of NIC and COHb are presented in TABLE 3. At first and/or second sampling time the concentration of NIC increased significantly in groups 2 and 3, respectively. In both peripheral and cupping blood samples, NIC was lower in second sampling with respect to first sampling. At first sampling, NIC concentration in cupping blood samples of group 2 and 3 was significantly lower as compared with peripheral blood. But there was no significant difference between cupping and peripheral blood samples at second sampling.

In both sampling times, the level of COHb increased

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significantly from group 1 to 2 and then 3 in both peripheral and cupping blood samples. COHb level of group 1 in cupping blood was lower than peripheral blood, and it was lower in second sampling as compared with first sampling. In group 2 and 3, COHb level in peripheral blood were significantly higher than cupping blood at first and second sampling. In both kinds of blood, the level of COHb in group 2 and 3 in second sampling were significantly higher than their counterpart group at first sampling.

Vitamin C and E levels in peripheral and cupping blood samples at first and second sampling times are

presented in TABLE 4. In both kinds of blood samples and in each studied groups, we found no tangible differences in vitamin C and E levels as they were compared at first and/or second sampling times. In addition they varied within normal rang.

TABLE 1 : Clinical descriptive data of the studied groups. data are expressed as mean \pm SD.

Studied groups	blood pressure		Hct	Age (year)
	diastolic	systolic		
group 1 (N = 13)	9.21 \pm 0.38	13.54 \pm 0.84	51.29 \pm 2.39	32.76 \pm 8.55
group 2 (N = 13)	9.04 \pm 0.36	13.74 \pm 0.29	49.09 \pm 2.77	33.23 \pm 8.40
group 3 (N = 14)	9.17 \pm 0.30	13.55 \pm 0.47	49.94 \pm 2.57	32.71 \pm 8.37

TABLE 2 : The levels of antioxidant enzyme activities in peripheral and cupping blood samples at first and second sampling.

enzyme		first sampling		second sampling	
		peripheral	cupping	peripheral	cupping
SOD (U/g.Hg)	group 1	383.30 \pm 11.47*	366.30 \pm 11.77*	424.00 \pm 13.00*	406.00 \pm 12.84
	group 2	413.69 \pm 11.52*	394.23 \pm 13.08*	446.53 \pm 15.55*	425.76 \pm 15.26
	group 3	421.21 \pm 10.16* [®]	401.85 \pm 7.93*	463.53 \pm 47.76*	447.71 \pm 13.04
GPX (U/g.Hg)	group 1	110.53 \pm 7.34*	101.92 \pm 7.04*	121.00 \pm 7.70*	110.00 \pm 7.15
	group 2	134.00 \pm 6.76* [®]	122.61 \pm 6.63*	145.23 \pm 6.74* [®]	130.30 \pm 6.37
	group 3	144.57 \pm 5.38* [®]	132.85 \pm 5.34* [®]	158.07 \pm 5.73* [®]	141.50 \pm 5.39 [®]
CAT (U/g.Hg)	group 1	156.69 \pm 5.32*	151.61 \pm 5.29*	164.46 \pm 5.51*	158.53 \pm 5.76
	group 2	169.61 \pm 6.46*	162.92 \pm 6.90*	178.38 \pm 6.65*	172.84 \pm 6.69
	group 3	183.00 \pm 7.27* [®]	176.50 \pm 6.82* [®]	191.50 \pm 7.19* [®]	185.35 \pm 7.26 [®]

*Differed significantly as compared with cupping blood ($p < 0.05$). [®] Differed significantly with respect to group 1 ($p < 0.05$).

TABLE 3 : The levels of oxidative damage biomarkers in peripheral and cupping blood samples at first and second sampling.

enzyme		first time		second time	
		peripheral	cupping	peripheral	cupping
MDA (μ mol/mg.pro)	group 1	35.38 \pm 3.11*	38.07 \pm 3.38*	32.92 \pm 2.92*	35.76 \pm 2.86
	group 2	29.23 \pm 2.58*	31.23 \pm 2.58	27.00 \pm 2.48*	30.00 \pm 2.55
	group 3	26.85 \pm 2.37*	29.14 \pm 2.61*	24.28 \pm 2.31*	26.92 \pm 2.38
RCD (nmol/mg.pro)	group 1	55.46 \pm 3.05*	58.46 \pm 3.38*	46.30 \pm 2.98*	49.69 \pm 3.40
	group 2	49.00 \pm 2.59*	51.61 \pm 2.61*	40.46 \pm 2.75*	44.15 \pm 2.72
	group 3	47.35 \pm 2.40*	50.00 \pm 2.74*	38.42 \pm 2.42*	41.42 \pm 2.49
8-OHdG (nmol/mg.pro)	group 1	7.70 \pm 0.14*	7.93 \pm 0.19	7.59 \pm 0.11*	7.90 \pm 0.17
	group 2	8.26 \pm 0.13*	8.44 \pm 0.14	8.09 \pm 0.13	8.27 \pm 0.13
	group 3	8.70 \pm 0.23 [®]	8.82 \pm 0.23 [®]	8.57 \pm 0.22* [®]	8.88 \pm 0.25 [®]
NIC (ng/ml)	group 1	0	0	0	0
	group 2	25.54 \pm 2.11* [®]	20.16 \pm 1.88 [®]	38.74 \pm 3.22 [®]	35.12 \pm 3.22 [®]
	group 3	68.32 \pm 5.24* [®] [©]	56.21 \pm 4.62 [®] [©]	89.15 \pm 7.55 [®] [©]	81.37 \pm 7.62 [®] [©]
COHb (%)	group 1	1.18 \pm 0.84	1.30 \pm 0.79	1.05 \pm 0.66	1.24 \pm 0.85
	group 2	3.15 \pm 0.66* [®]	2.73 \pm 0.19 [®]	6.17 \pm 0.26* [®]	5.83 \pm 0.67 [®]
	group 3	5.69 \pm 0.88* [®] [©]	5.15 \pm 0.75 [®] [©]	8.82 \pm 0.24* [®] [©]	8.14 \pm 0.45 [®] [©]

*Differed significantly as compared with cupping blood ($p < 0.05$). [®]Differed significantly with respect to group 1 ($p < 0.05$).

[©]Differed significantly with respect to group 2 ($p < 0.05$).

TABLE 4 : The levels of vitamin C and E activities in peripheral and cupping blood samples at first and second sampling.

vitamin		first sampling		second sampling	
		peripheral	cupping	peripheral	cupping
vitamin C (mg/dL.10 ⁻²)	group 1	45.58 ± 1.45	43.98 ± 1.46	45.92 ± 1.81	45.00 ± 1.37
	group 2	41.76 ± 1.06	42.42 ± 0.46	44.20 ± 1.48	47.70 ± 1.14
	group 3	42.98 ± 1.34	42.46 ± 2.28	46.04 ± 1.21	43.52 ± 1.82
vitamin E (mg/dL.10 ⁻²)	group 1	70.94 ± 1.12	71.74 ± 2.19	72.74 ± 3.84	71.30 ± 1.45
	group 2	70.36 ± 3.40	67.62 ± 2.26	72.66 ± 3.91	68.64 ± 2.50
	group 3	71.98 ± 4.46	71.80 ± 4.22	74.88 ± 2.08	72.64 ± 4.18

DISCUSSION

Cigarette smoking is a major lifestyle and risk factor influencing the health of human beings^[48]. However, the number of smokers continues to increase worldwide^[49]. In this investigation, we focused on oxidative status in different conditions of studied volunteers with respect to cupping effects. Many studies showed various results of smoking influence on antioxidative responses. In one hand, Some reports revealed low levels of antioxidants in smokers with respect to non-smokers^[4,7]. On the other hand, many studies documented higher activities of SOD, CAT, and GPX, raised from smoking^[50-54]. Bogdanska et al observed no significant differences in antioxidant enzyme activities between smokers and non-smokers^[55]. In agreement with these studies, we found significant rise in antioxidant enzyme activities in smokers with respect to non-smokers with potentiating effect of smoking habit. It is our conviction that smoking exerted induction on antioxidant enzymes synthesis to overcome ROS toxicity.

Cupping is a popular alternative therapy for a variety of ailments that is especially used in patients with pain syndromes^[56-58]. It is a treatment for chronic pain and a whole array of respiratory, gastroenterological, and gynaecological disorders^[58]. As we found, apart from sampling time, cupping blood samples had lower antioxidant enzyme activities with respect to peripheral blood samples in both smoker and non-smoker volunteers. This may probably raised from cupping blood intrinsic condition, diminished the promotion of enzyme generation even under effect of smoking.

In addition, we found higher activity of antioxidant enzyme activities in peripheral blood samples from volunteers subjected to wet cupping after seven days with

respect to first sampling time, and this increase was more remarkable in smoker blood samples as compared with non-smokers. Therefore, wet cupping had an increase effect on the observed smoking induction on antioxidant enzymes. It is probably that smoking exerts inflammatory stimulus and oxidative stress^[50]. Wet cupping diminishes these events by increasing the antioxidant enzyme activities.

Vitamin C as an effective free radical scavenger^[52] and Vitamin E as an important immune promoting agent^[59] are potent antioxidant in living systems. Many studies reported significantly decrease in levels of vitamin C^[60,61] and vitamin E^[62,63] in smokers with respect to non-smokers. Some studies suggested that decreased concentration of vitamin C in smokers is associated with metabolism of this vitamin^[64]. The lower plasma vitamin E in smokers may be due to increase in clearance or reduction in absorption of this vitamin^[63]. Christine A. et al reported no differences in vitamin E between smokers and non-smokers^[64]. Brown A.J. found no change in plasma vitamin C and E in response to acute smoking^[5]. Our evaluations were in consistent with this study. We found no significant differences for vitamin E and C between studied groups. It is likely that enzymatic defense provide sufficient protection against ROS.

Cigarette smoke induces oxidative stress in peripheral leukocytes^[65]. It is associated with significant elevation in levels of DNA damages^[66], which results in DNA adducts formation^[3]. Asami et al. revealed significantly increase in 8-OHdG levels in smoker leukocytes and lung tissues^[65,67]. Kiyosawa et al observed increase in 8-OHdG levels in a relatively short time after cigarette smoking^[68]. Byung Mu Lee reported higher levels of 8-OHdG in leukocyte DNA of smokers than non-smokers^[69]. It is in accordance with our results that showed significantly higher levels of 8-OHdG in smok-

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ers as compared with non-smokers.

MDA and RCD are the major biomarkers that used for determination of smoking-induced oxidative damage of lipids and proteins^[10,70]. Many studies suggested significantly higher plasma MDA^[10,71,72] and RCD^[73-75] in smokers compared to non-smokers. Some studies found no significant difference in MDA between smokers and non-smokers^[76,77]. In opposite with these studies, we observed lower levels of MDA and RCD in smokers as compared with non-smokers. It is likely that the elevation in antioxidant enzymes induced by smoking overcome the ROS toxicity resulting in lower concentration of these indexes in smokers.

Reduction of body iron stores, as a free radical, in peripheral tissues by bloodletting is well documented^[78-80]. Bloodletting could be used in the prevention and treatment of all free radical induced diseases^[78]. However, we have no evidence that bloodletting to reduce oxidative damage biomarkers resulted from ROS. Our evaluations on these parameters showed higher levels of these three damage indexes in cupping blood as compared with peripheral blood. It is our assumption that cupping blood can absorb and collect oxidative damage materials from peripheral blood. Accordingly, wet cupping through collection of oxidative damage biomarkers is an effective method to clean the body from these hazardous indexes, leading to protection against their harmful effects.

It is well documented that NIC and CO are two major components in cigarette smoking^[59]. CO bound to hemoglobin and increased the percentage of blood COHb^[81]. many studies reported increased levels of NIC^[21,82] and COHb^[83,25] in smokers blood as compared with non-smokers. Our data consistent with previous studies showed significantly higher levels of NIC and COHb among cigarette smokers and non-smokers.

In addition, our results revealed lower levels of NIC and COHb in cupping blood as compared with peripheral blood. We found that these indexes level increased significantly in peripheral blood between two cupping times. Therefore, wet cupping is not an effective procedure to diminish NIC and COHb levels in peripheral blood.

Based on the results of this study, wet cupping increased the induction level of smoking on antioxidant enzyme activities. In addition, oxidative damage mate-

rials in the smoker's blood can excrete from their bodies by subjecting to wet cupping. Therefore, Wet cupping can act as an effective procedure to lowering the oxidative effects of cigarette smoking.

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