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Research paper

Investigation of wet cupping therapy's effect on oxidative stress based on biochemical parameters

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ABSTRACT

Introduction: Wet cupping therapy is one of the main applications which has been used in the Unani medicine system. Current literature explaining how this traditional treatment method works is still limited. The aim of this study was to investigate whether wet cupping therapy could affect reactive oxygen species and antioxidant levels.

Methods: This was a single arm pre and posttest intervention study conducted in Karabuk University Teaching and Research Hospital (KUTRH) between 1st January and 1st May 2018. Twenty-four participants were included. Wet Cupping Therapy was applied to all individuals once every month for three months. Venous blood samples were collected before the first (Venous1) and after the last application (Venous 2) and cupping blood samples were collected during the first and last applications. Malondialdehyde (MAD) and total oxidant status (TOS) which both indicate oxidation; glutathione (GSH), superoxide dismutase (SOD), total antioxidant status (TAS), catalase (CAT) activities that indicate antioxidant effect, and protein levels were measured on the obtained serums.

Results: The highest MDA and TOS levels and the lowest TAS, GSH, SOD and CAT levels were found in initial cupping blood. Significant improvement was observed in second cupping blood for these parameters when compared to initial values. We also found that MDA and TOS levels, as well as TAS, GSH, SOD and CAT levels, all changed favorably in the Venous 2 blood sample compared to the Venous 1 ($p < 0.001$).

Conclusion: Wet cupping therapy seems a promising method for increasing antioxidant levels and curbing oxidative stress.

1. Introduction

By the beginning of the current millennium, traditional and complementary therapies resumed the popularity that seemed to have vanished during the last century [1,2]. The strategy by World Health Organization (WHO) to integrate traditional medicine within national health care systems may therefore accelerate the use of traditional medicines in the next decade [3,4]. Cupping therapy, rooted in ancient Egypt, is one of the oldest and most common traditional and complementary methods that has been used as a treatment for thousands of years in various cultures and regions. The oldest evidence of cupping was found in Ebers Papyrus, which was one of the oldest medical textbooks dating back to 1550 BC and found in the tomb of Tutankhamun (King Tut). This art was inherited by the Greeks and Romans as observed in the applications of Hippocrates [460-377 BC] and Galen [131-200 AD] [5]. It is also known that cupping therapy was used traditionally in China, India, Africa, Europe and across all Muslim lands. Furthermore, since it has been considered as one of the main instruments in Prophetic Medicine [6], cupping therapy is not only traditional, but also a religious means of remedy in Muslim communities. Thus, healthy individuals receive cupping therapy in certain dates in a year for general wellbeing in several Muslim countries including Turkey. Cupping therapy has two basic categories; dry and wet cupping. Dry cupping is based on mobilizing noxious substances by

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promoting circulation of affected areas [7]. To achieve this, the practitioner places the cups and applies a negative pressure with different types of vacuum devices on the skin without disrupting its integrity. Wet cupping differs since it utilizes approximately 1.5 mm deep and 1.5 mm wide incisions on the skin incised by a special lancet in order to remove the capillary blood in the disturbed region [5]. While Chinese tradition prefers dry cupping, wet cupping, which is known as hijama, is common in Muslim countries [6,7].

Reactive oxygen species (ROS) are continuously produced as a normal product of aerobic metabolism in all living tissues; however their production could reach large quantities under pathological conditions. Oxidative stress is the disruption of the balance between ROS production and cellular antioxidant defense system in favor of the oxidants [8,9]. Oxidative stress plays a role in the etiology of several diseases including cardiovascular diseases, neurodegenerative disorders, cancer and aging [10–12]. The main source of free radicals in living organisms is the autooxidation of flavin thiols, the activity of the electron transport chain, oxidases, cyclooxygenases, peroxidases, etc. [13]. The environmental factors behind oxidative stress include xenobiotics, organic solvents, pesticides, tobacco smoke, anesthesia, drugs and radiation [13].

Previous studies in the literature have revealed the effectiveness of cupping therapy in diseases such as hypertension, migraine, constipation, joint and back pains [14–17]. However, these studies, particularly those conducted on wet cupping, are scientifically limited, due to small sample size and they have not attempted to explain the mechanism regarding how this method works. Therefore additional studies are needed to investigate the possible effects and the underlying mechanisms of wet cupping therapy (WCT).

The current study aimed to assess the capacity of WCT in decreasing the ROS levels and increasing the antioxidant levels in healthy individuals who received prophylactic WCT application.

2. Material and methods

2.1. Experimental design

This was a single arm pre and posttest interventional study that was conducted in Karabuk University Teaching and Research Hospital (KUTRH). Karabuk University is a government university with medical school. This study has a repeated measures design, to compare these measures at 95% confidence level ($\alpha = 0.05$) with 90% power ($\beta = 0.10$) when the effect size was expected to be 0.95, the minimum sample size was calculated as 18. Inclusion criteria of this study were being adult, being healthy and attending Traditional and Complementary Medicine Center of KUTRH for WCT to preserve well-being. Exclusion criteria were as follows: History of any chronic disease, being on any ongoing medical treatment, and at least one contra-indication to WCT that was detected in subject's history or in the routine blood examinations prior to enrollment (Hgb <9.5; INR >1.2; history of hemophobia). Thirty consecutive healthy individuals who attended the center between 1st January and 1st May 2018 were enrolled. Six of the participants dropped out during the study period: Four refused to receive further WCT applications after the first session and the remaining two subjects were lost to follow up after the second session.

2.2. Ethics approval and informed consent

All participants were included in the study after their informed consent was obtained. Ethical approval was obtained from Karabuk University Clinical Trials Ethics Committee (No:1/5; 09.01.2018).All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was successfully registered in the ClinicalTrials.gov Protocol Registration and Results System (Registration ID: NCT03503903).

2.3. Wet cupping technique

The remaining 24 participants received three WCT sessions once every month for three months (On 0, 30, and 60 days). WCT was conducted using disposable vacuum cups on 5 acupuncture points: DU 14 (Dazhui) point on the posterior median line, in the depression below the processus spinosus of the 7th cervical vertebra; UB 42 (Pohu) bilateral points on the back, 3.0 cun lateral to the lower border of the spinous process of the 3rd thoracic vertebra interscapulum region; and UB 46 (Geguan) bilateral points on the back, 3.0 cun lateral to the lower border of the spinous process of the 7th thoracic vertebra. Each WCT procedure was performed by triple S technique and in 3 phases:

2.3.1. Primary sucking

After disinfection of the selected regions with povidone iodine, the cup was placed on the selected site and the air was pressured out of the cup with manual suction. The cups were placed on the skin and kept on the skin for a period of 3–5 minutes.

2.3.2. Scarification

2–3 mm deep and 3–5 mm long superficial incisions were made on the skin with a No. 11 sterile surgical blade.

2.3.3. Secondary sucking and blood-letting

The cups were located on the skin in the manner described above again and left at the application site until filled with the blood from the capillary.

At the end of the WCT procedure, the cups filled with blood were removed and destroyed in medical waste. A sterile sponge dressing was applied.

2.4. Samples

In the study, venous blood samples (Venous 1) were collected from each patient before the first WCT and about 8 cc blood sample (WCB 1) was collected from the cup during the application. No samples were obtained in the second application. During the third and the last WCT cupping blood samples (WCB2) and a few hours after the application venous blood samples (Venous 2) were collected.

2.5. Biochemistry

Collected blood samples were centrifuged at 600 *g* and +4 °C for 15 min to obtain serum samples. In the serum, catalase (CAT) and superoxide dismutase (SOD) activities, glutathione (GSH), malondialdehyde (MDA), total antioxidant status (TAS), total oxidant status (TOS) and protein levels were studied.

MDA analysis was conducted with the method described by Ohkawa et al. [18]. MDA level was measured with a spectrophotometer (T80 UV / VIS Spectrometer, PG Instruments Ltd., Leicestershire, UK) at 535 nm. The results were expressed in nmol/g protein.

The GSH level was measured using the method described by Ellman [19]. GSH level was determined with the measurement of the color density at 410 nm with a spectrophotometer. The results were expressed in nmol/g protein.

SOD activity was measured with the method reported by Sun et al. [20]. The absorbance at 560 nm of the resulting formazan was used to calculate the SOD activity. SOD activity was expressed in U/g protein.

CAT activity was measured with the method reported by Aebi et al. [21]. The separation of H_2O_2 to water and oxygen by the catalase enzyme in the serum leads to a decrease in absorbance at 240 nm. The decrease observed in the absorbance was recorded for 1 min to measure the enzyme activity. CAT activity was expressed in K/g protein.

The TOS level was studies with Erel's method [22] with the total oxidant status kit (Rel Assay Diagnostics, Gaziantep Turkey). To determine the absorbance in the resulting complex at 530 nm, the measurements were conducted with an ELISA reader set at 25 °C and it was directly proportional to the amount of oxidant in the sample. The results were expressed in μmol H_2O_2 equiv./l.

TAS level was studied based on the method developed by Erel [23]. TAS was measured with a Rel Assay brand kit (Rel Assay Diagnostics, Gaziantep Turkey). TAS levels were determined by reading the absorbance at 660 nm with an ELISA reader set at 25 °C after the incubation. Results were expressed in mmol trolox equiv./l.

2.6. Statistical analysis

Since the biochemical data did not exhibit normal distribution, the Friedman test was used for comparisons and the Conover method was used for the paired comparisons. The data were summarized using the median, minimum and maximum values. The significance level was accepted as 0.05 in all tests.

3. Results

Twenty four healthy individuals completed the study. The participants of the study were all males whose ages ranged from 18 to 51 years while the mean age was 40.5 + 7.5. Demographic features of the participants are expressed in Table 1.

Biochemical findings obtained with the blood samples are presented in Table 2. It was determined that there were significant differences between cupping blood and venous blood samples based on MDA and GSH levels. Comparison of the blood samples demonstrated that the highest MDA value was determined in WCB 1 [467.5 (335–1538)]. There was a significant decrease in Venous 2 MDA values [372 (298–449)] when compared to the Venous 1 [395.5 (335–472)] ($p < 0.001$). Furthermore, the comparison of the cupping blood samples that the WCB 2 MDA value [350 (278–442)] was significantly lower when compared to WCB 1 [467.5 (335–1538)] ($p < 0.001$).The lowest GSH value was observed in WCB 1 [827 (641–872)] blood samples. There was a significant increase in GSH in Venous 2 [955 (833–1417)] when compared to the Venous 1 [840 (795–994)] samples ($p < 0.001$). There were also significant increases in WCB 2 GSH levels [875 (814–1103)] when compared to WCB 1 [827 (641–872)] ($p < 0.001$) (Fig. 1, Table 2).

It was demonstrated that there was a significant difference between the cupping and venous blood based on SOD and CAT enzyme activities. The lowest SOD and CAT activities were found in WCB 1 samples. Venous 2 SOD [34.9 (23.6–52.4)] and CAT [31.74 (3.04–65.67)] activities demonstrated significant increases when compared to Venous 1 SOD [24.85(8.2–37.8)] and CAT [9.72 (0.16–32.06)] activities ($p < 0.001$ in both). Furthermore, SOD [28.1 (8.4–45.4)] and CAT [23.1 (1.28–40.03)] activities in WCB 2 were significantly lower than SOD [19.65–33.4)] and CAT [4.57 (0.16–24.49)] activities in WCB 1($p < 0.001$ in both) (Fig. 2, Table 2).

Table 1
Demographic features of the participants.

Variable	Participants (N = 24)
Age (years)	40.5 ± 7.5
Gender (N, %)	Male (24, 100%)
Education (N, %)	Primary (2, 8%)
	Secondary (18, 75%)
	University (4, 17%)
Married/Single (N, %)	Married (23, 96%)
	Single (1, 4%)
Smoking (N, %)	Smoker (13, %54)
	Non-smoker (11, %46)

The lowest serum TAS level was determined in WCB 1, while the highest TOS level was found in WCB 1. It was also determined that Venous 2 TAS level [1.51 (1.06–1.97)] was significantly higher when compared to Venous 1 [1.23 (0.96–1.7)] ($p < 0.001$). Furthermore, WCB 2 TAS level [1.37 (1.04–1.7)] was significantly higher when compared to WCB 1 [1.09 (0.9–1.42)] ($p < 0.001$). Venous 2 TOS levels [2.83 (1.41–7.83)] were significantly lower when compared to Venous 1 [6.63 (4.46–24.67)] ($p < 0.001$). In addition, it was found that WCB 2 TOS levels [4.89 (1.74–10.87)] were significantly lower when compared to WCB 1 [12.23 (6.09–30.87)] ($p < 0.001$) (Fig. 3, Table 2).

4. Discussion

In the current study, the effect of WCT on ROS and antioxidant levels in healthy individuals was investigated. The initial ROS and antioxidant values were determined in venous blood and compared to the cupping blood samples. Also, after three WCT applications conducted during a two-month period, the initial plasma values were compared with the final values. The follow-up work makes the present study a unique research on WCT.

The main study findings can be summarized as follows: The highest MDA and TOS levels and the lowest TAS, GSH, SOD and CAT levels were found in WCB1 blood samples. The high MDA and TOS levels and low TAS, GSH, SOD and CAT levels in Venous 1 blood samples improved in the Venous 2 blood samples. Furthermore, it was observed that TAS, GSH, SOD and CAT levels were significantly lower whereas MDA and TOS levels were higher in WCB1 when compared to Venous 1 and these figures significantly improved in WCB2 blood samples when compared to WCB1. Therefore, it was concluded that even just after the first cupping treatment, most of the oxidant compounds in the blood were removed significantly.

Initial plasma TOS and MDA levels that reflected oxidative stress were significantly higher when compared to post-application levels, while TAS, SOD, CAT and GSH levels that reflected the antioxidant capacity were significantly lower in the initial venous samples. Lipid peroxidation was monitored with the measurement of MDA levels, one of the final products of increased free radical attacks to the cellular membrane. Lipid peroxidation modifies the functional properties of cellular membranes, impairs their permeability and leads to the inactivation of membrane-bound receptors and enzymes. MDA could also deactivate membrane carriers by forming intramolecular and intermolecular crosslinks [24]. GSH is a non-enzymatic antioxidant and plays an active role in endogenous antioxidant defense, neutralizes free radicals and reduces hydrogen peroxide [25]. It was found in the present study that the highest MDA and the lowest GSH levels were in WCB1, and these values improved significantly in WCB2. Also, in pretreatment Venous 1 samples, MDA levels were high and GSH levels were low, however post-treatment Venous 2 samples exhibited a significant improvement.

The organism possesses an enzymatic defense system against oxidants such as the SOD and CAT. The present study findings demonstrated that the lowest levels of these enzyme activities were observed in WCB1 blood samples, where the oxidants were at the highest levels, and this was one of the most significant findings of the current study. In WCB2, significant improvements were observed in activities of the above-mentioned enzymes consistent with the decrease in oxidant levels. Furthermore, while oxidant levels were elevated in Venous 1 blood collected prior to the cupping treatment, the enzyme activities increased, and oxidant compounds decreased as the levels of these enzymes were reduced in Venous 2 blood samples collected after the treatment. These findings demonstrated that WCT led to the removal of oxidants and a significant increase in antioxidant enzyme levels, which are important in physical defense.

Oxidative stress is induced by the deterioration of the oxidant/antioxidant balance in favor of the oxidants. In case of oxidative stress, the increase in free radical species plays a role in the etiology of several

Table 2
Blood samples oxidant–antioxidant parameters.

	N	Venous 1 Median (min.-max.)	WCB 1 Median (min.-max.)	WCB 2 Median (min.-max.)	Venous 2 Median (min.-max.)	p
MDA	24	395,5 (335–472)[a]	467,5 (335–1538)[b]	350 (278–442)[c]	372 (298–449)[c]	<0,001
GSH	24	840 (795–994)[a]	827 (641–872)[b]	875 (814–1103)[c]	955 (833–1417)[d]	<0,001
TAS	24	1,23 (0,96–1,7)[a]	1,09 (0,9–1,42)[b]	1,37 (1,04–1,7)[c]	1,51 (1,06–1,97)[d]	<0,001
TOS	24	6,63 (4,46–24,67)[a]	12,23 (6,09–30,87)[b]	4,89 (1,74–10,87)[c]	2,83 (1,41–7,83)[d]	<0,001
SOD	24	24,85 (8,2–37,8)[a]	19,65 (6,3–33,4)[b]	28,1 (8,4–45,4)[c]	34,9 (23,6–52,4)[d]	<0,001
CAT	24	9,72 (0,16–32,06)[a]	4,57 (0,16–24,49)[b]	23,1 (1,28–40,03)[c]	31,74 (3,04–65,67)[d]	<0,001

Data were summarized with median, minimum and maximum values. WCB 1;Wet Cupping Blood 1, WCB 2; Wet Cupping Blood 1, MDA; Malondialdehyde, GSH; reduced glutathione, SOD; superoxide dismutase, CAT; catalase, TAS; total antioxidant status, TOS; total oxidant status. The groups with different superscripts represent the statistical significance ($p < 0.05$).

diseases including cancer as well as aging [12]. Antioxidant defense systems in the organism control the free radicals [26]. However, these antioxidant defense systems may not be sufficient to remove those harmful effects. Thus, ROS increase in inflicted tissue and organs. Considering the various organs and systems that have the potential to be damaged by ROS, remedies or applications which might exert an antioxidant effect without significant adverse reactions are of great importance. When evaluated from this point of view, the findings revealed in the current study may contribute to acceptance of WCT as a promising treatment method in the near future.

Theories on the way that WCT produces beneficial effects in treatment have not reached a concrete conclusion. While ancient medicine emphasized the detoxification effects of WCT, during the time of Hippocrates, who developed the humoral theory, bloodletting was claimed to have balancing properties. According to this theory, diseases that occur in any of the four humors (blood, phlegm, yellow bile and black bile) exhibit a controlling nature. Hence, letting the excess liquid through any means available would restore the balance. In his famous "Canon of medicine," Avicenna expanded the debate on the mechanisms of WCT. According to Avicenna, humors in inflicted body parts accounted for the pain and inflammation, which should be sucked to the surface and evacuated from there to alleviate the ailment. Among the scholars of his time, who considered WCT as a method to purge hazardous material from the body, Avicenna was no exception [5].

Ibn Shareef, who was an outstanding physician in Ottoman Empire, defined WCT as "a through purge" in his famous book "*Yadigar*" and claimed that it was superior to other forms of purge since it allows for the clearance of all excess humors in the body [27]. Current literature also underlines the detoxifying effect of WCT including several theories proposed to explain the mechanisms involved in WCT. Within the context of the *Taibah* theory, Salah M. El Sayed et al. [3] suggested that culprits of several pathologies may be removed from blood, lymph and intercellular area via WCT. In this method, skin, which is a natural excretory member of the body, was used more efficiently through a surgical intervention. This procedure also allows both capillary ends to drain more effectively to fix and maintain physiological equilibrium as well as promoting immunity. We suggest that the removal of ROS and the increase in the antioxidant capacity demonstrated in our study might be explained by the theories like *Taibah* which emphasize the detoxifying effect of WCT.

On the other hand the detected effects of WCT on ROS and antioxidants, observed in our study, may further illuminate the mechanisms of various clinical benefits observed in different interventional studies with WCT. In conclusion, antioxidant effects of WCT could account for its suggested effects in both traditional and clinical literature.

In a study which was conducted on healthy volunteers effect of blood donation on oxidative stress was investigated. Blood samples were withdrawn from participants before and 24 h after the blood donation and serum nitric oxide (NOx), MDA, SOD and myeloperoxidase (MPO) levels were measured. The levels of SOD and NOx increased significantly after blood donation while changes on the MDA and MPO were not statistically significant. It was concluded in the study that blood donation removes oxidants and decreases oxidative stress [28]. Although WCT is called "bloodletting" in some documents, this misuse may ensue in incomprehensibility, as the same term is also attributable to withdrawal of venous blood. Nonetheless, the latter is quite different from WCT. In the study that we cited, venous bloods were withdrawn; a procedure cannot be directly compared with WCT. As studies of this kind are scarce, we couldn't find a study similar to ours. However, evaluating the results of two studies, it seems that oxidative substances may be removed more effectively by WCT. But we could not confirm this assumption without comparing two methods in two cohorts in the

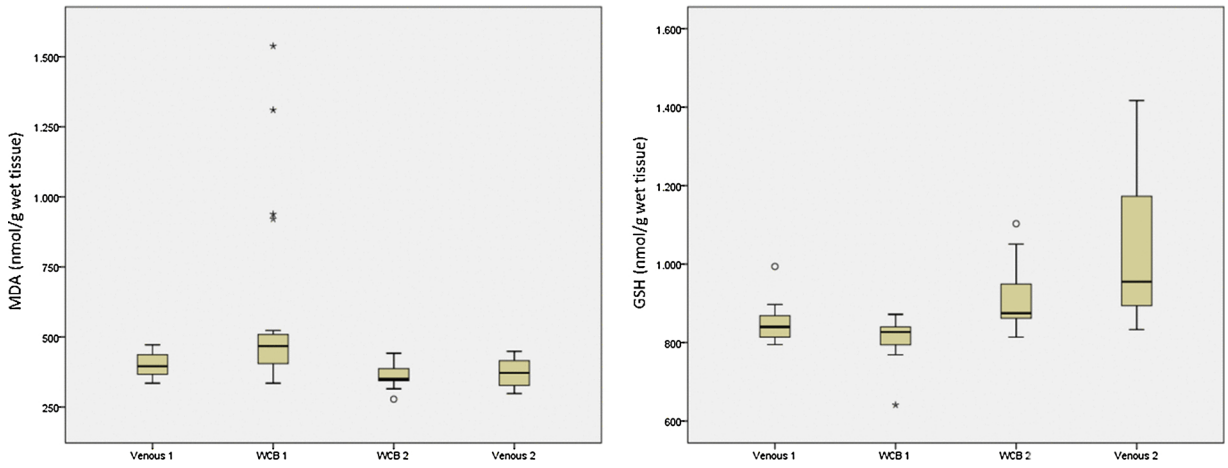

Fig. 1. MDA and GSH levels in blood samples. $p < 0.05$ is accepted as significant.
Abbreviations: MDA; malondialdehyde, GSH; glutatithon, WCB 1; wet cupping blood 1, WCB 2; wet cupping blood 2.

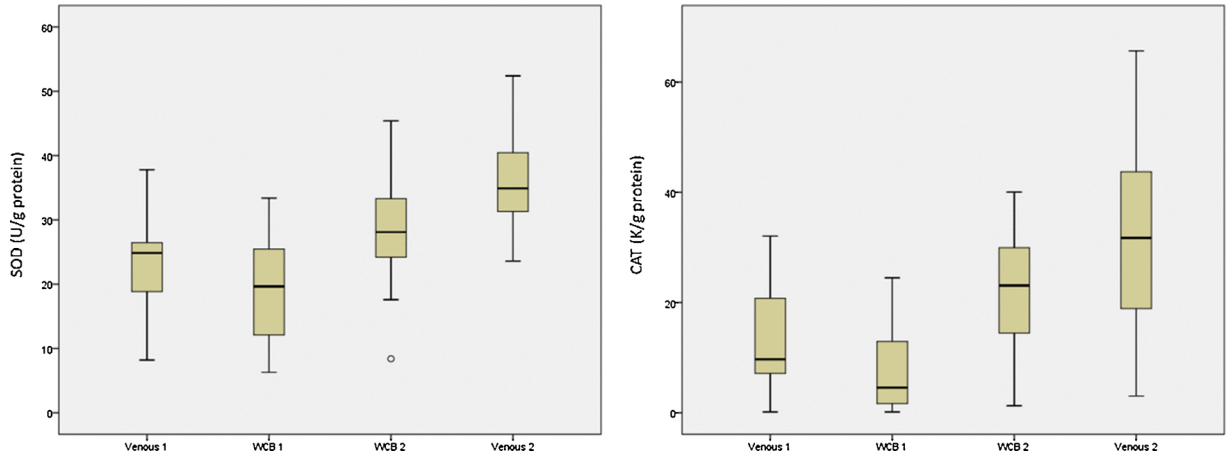

Fig. 2. SOD and CAT enzyme levels in blood samples. $p < 0.05$ is accepted as significant.
Abbreviations: SOD; superoxide dismutase, CAT; catalase, WCB 1; wet cupping blood 1, WCB 2; wet cupping blood 2.

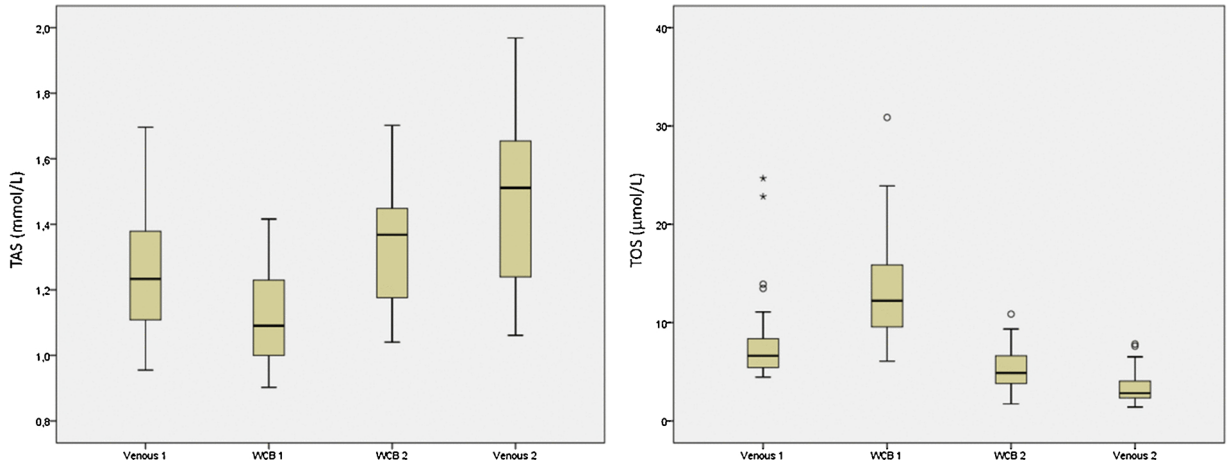

Fig. 3. TAS and TOS levels in blood samples. $p < 0.05$ is accepted as significant.
Abbreviations: TAS; total antioxidant status, TOS; total oxidant status, WCB 1; wet cupping blood 1, WCB 2; wet cupping blood 2.

same study.

To the best of our knowledge there is only one study in the current literature that investigated the antioxidant effects of WCT in healthy individuals. Tagil et al reported that WCT removed oxidants and decreased oxidative stress based on their findings obtained with venous blood samples and blood that was drawn by WCT [29]. However; they did not assess the post-WCT levels of oxidative stress parameters in venous blood to conclude in an antioxidant and free radical scavenging effect. This is the first study which evaluated oxidative stress and antioxidant parameters prior to and post WCT applications in venous and cupping blood samples and revealed significant improvements. Thus, the present study serves to confirm a permanent antioxidant effect by WCT on the human body.

4.1. Limitations and strengths

The main limitation of this study is the small sample size. Besides, there were no female subjects. This was not anticipated, as we enrolled consecutive patients who applied for WCT to Traditional and Complementary Medicine Center of KUTRH and asked them if they were eager to participate in the study. As been stated before, there is a public tradition of receiving WCT in certain dates in a year for general wellbeing in Turkey, like many Muslim countries. However, men are more inclined to follow this tradition while women rather apply for WCT for different disorders such as headache, fibromyalgia etc. Among the very few female applicants, none volunteered to take part in the study during this time period. So, it would not be appropriate to extrapolate our results to general population. Additionally, it was not double-blinded as there was no control arm. No doubt placebo or sham intervention controlled randomized trials provide the strongest evidence for scientific theories and are accepted to be the gold standard. However, implementation of a convincing sham cupping method remains to be a challenge. This is even harder in WCT. To imitate dry cupping, some authors suggested using a sham cupping device which applies little or no negative pressure due to a hole on its surface [30]. Even this method is open to argument as the difference between two procedures is so comprehensible for a population that was familiar to this traditional procedure. As for WCT, any intervention including bloodletting from the disturbed area will be more or less the therapy in question. Therefore we opted a self-controlled study design rather than including a sham control group. We hope that this study encourages further research using this intervention.

Strengths of this study are its novelty as venous blood values as well as WCT blood before and after intervention were not examined before, and the promising results it has yielded.

5. Conclusion

In the present study, we concluded that WCT was capable of significant removal of free radicals that lead to oxidative damages in the body and led to an increase in antioxidant capacity in a study group. These results suggests that WCT may exert prophylactic and therapeutic

effects on several pathologies induced by free radicals, a finding rather interesting and a good starting point for studies in larger scales.

Authors

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Data statement

The datasets used and/or analyzed during the current study and informed consent form are available from the corresponding author on reasonable request.

Declaration of Competing Interest

The authors declare no conflict of interest in this study.

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