RESEARCH ARTICLE

The positive effects of wet cupping therapy on thiol disulfide balance

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Abstract

Objective: To explore the oxidant and antioxidant effects of wet cupping therapy on thiol disulfide balance.

Method: The cross-sectional study was conducted at Alanya Training and Research Hospital, Turkey, from March 15 to September 15, 2021, and comprised volunteers who received two sessions of wet cupping therapy with an interval of four weeks. Blood samples were taken before the session and then again after the session. Total thiol, native thiol and disulfide were measured in the blood samples using the colorimetric method. Data was analyzed using SPSS 26.

Results: Of the 50 subjects with mean age 47.10±15.16 years, 25(50%) were males and 25(50%) were females. There was a statistically significant difference between baseline and post-intervention levels of total thiol, disulfide, reduced thiol ratio, oxidized thiol and thiol oxidation reduction ($p<0.001$).

Conclusion: Wet cupping therapy could remove oxidants, reduce oxidative stress and produce antioxidant effects.

Keywords: Cupping therapy, Antioxidant activity, Oxidative stress, Traditional medicine, Thiols, Disulfide. (JPMA 76: 16; 2026)

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Introduction

Many traditional and complementary medicine methods are widely used for treatment purposes around the world. One of the most well-known and frequently used methods is cupping therapy,[1,2] which is used in many parts of the world. Cupping therapy is applied in two different ways. First, to increase blood circulation, dry cupping is applied using the local vacuum application process. Next, after the dry cupping application, the cups are removed, and small scratches of 1-1.5mm are made on the skin surface within the boundaries of the epidermis. These scratches are then vacuumed again for 5-10 minutes. The process of waiting for the treatment and hygienically removing the accumulated waste materials is called wet cupping therapy (WCT), or 'AL-hijamah'. Cupping therapy is widely used to treat many diseases in contemporary traditional and complementary medicine practices. However, only a few controlled experimental studies have been conducted on the use of WCT to treat clinical diagnoses and conditions, and more evidence-based controlled studies are needed.[1-5]

The physiological functioning of the human body involves the continual formation of free radicals and oxidative stress. The antioxidant system actively works to neutralize the free radicals. However, if the delicate balance between the oxidant and antioxidant systems is disrupted in favour of oxidative stress, there is a subsequent increase in reactive oxygen species (ROS). It is well-established that oxidative stress, induced by ROS, is linked to the development of chronic diseases and malignancies.[6-8] Thiols, characterized by the presence of a sulfhydryl group (-SH), are organic compounds. Thiol bonds, recognized for their protective role against oxidative stress, undergo a reversible transformation into disulfide (-SS-) bonds when exposed to ROS. This transformation is both reversible and quantifiable. Studies have found alterations in the thiol-disulfide homeostasis in various chronic diseases[9,10] but, to our knowledge, no study has so far focussed on the impact of WCT on the thiol-disulfide balance.

The current study was planned to fill the gap in literature by exploring the oxidant and antioxidant effects of WCT on the thiol-disulfide balance in humans.

Subjects and Methods

The cross-sectional study was conducted at Alanya Training and Research Hospital, Turkey, from March 15 to September 15, 2021. After approval form the ethics review committee of Alanya Alaaddin Keykubat University, Turkey, the sample size was calculated in line with literature which suggests a minimum of 30 participants in studies that use parametric tests.[11,12]

Sampling technique: Sample selection was made using a convenience sampling method. Because the sample size required a minimum of 30 to be parametric, the sample size was determined in accordance with the literature, taking into account the budget and measurement kit resources used in the method.[11,12] The first 50 individuals who met the inclusion criteria, did not meet the exclusion criteria, and agreed to participate voluntarily were selected as the

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study group. The sample size was determined in accordance with similar study in the literature.[11] Those excluded were individuals with haemoglobin (Hb) <9.5g/dL, fasting blood glucose (FBG) >250mg/dL, glycated haemoglobin (HbA1c) >10 and international normalized ratio (INR) <1.2.

Height, weight and blood pressure (BP) measurements were taken, and necessary advice and interventions were provided to those with low or high BP. Also, body mass index (BMI) was calculated for each individual. Routine blood tests included FBG, HbA1c, INR and haemogram for which venous blood samples were taken from the antecubital vein. The remaining blood (about 8cc) from the blood was placed in an ethylene di amine tetra acetic acid (EDTA) gel biochemistry tube. After centrifugation at 1500rpm for 10 minutes, the serum obtained was transferred to Eppendorf tubes using Pasteur pipettes. Blood collected in Eppendorf tubes was kept at -80 degrees Celsius till further analysis. Those having no negative results received the first WCT seance and were asked to come back for the second seance four weeks later.

The WCT cups were applied to seven predetermined areas on the back (Figure). Prior to cup placement, the cups were cleansed with antiseptic solutions. Subsequently, negative pressure was created in the selected areas using the cups, and after about 10 minutes, the cups were removed. Following the application of a scalpel for epidermal incisions, the cups were reapplied, and after about 10 minutes, the cups filled with blood were collected and disposed of in a medical waste bag. Hypericum perforatum oil was applied to the cupped areas and they were covered with a pad.1-After 4 weeks, the second WCT session was conducted in the same manner (Figure). For routine haemogram measurements and research-purpose oxidant-antioxidant parameter measurements, venous blood samples were taken by us twice, before the first WCT session and after the second WCT session, with the consent of all volunteers. The measurement of thiol-disulfide balance in the serum derived from venous blood samples was performed colorimetrically.

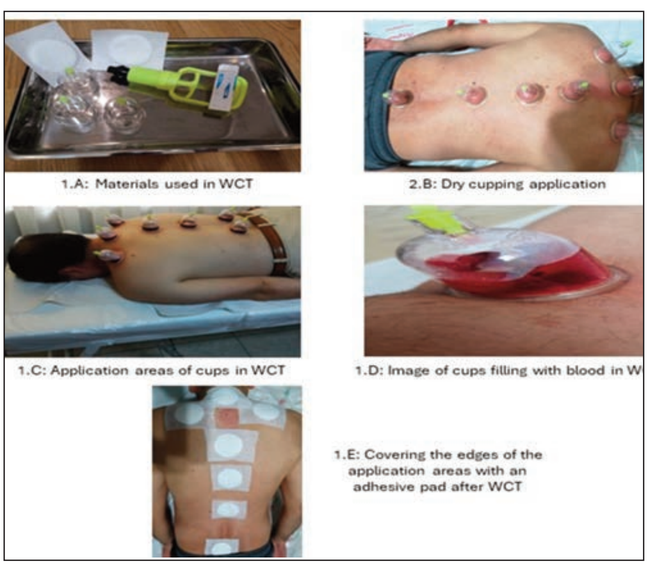

Figure: Wet cupping therapy (WCT) procedure.

The reducible disulfide bonds were initially reduced to free functional thiol groups. The presence of thiol groups, including both reduced and natural thiol groups resulting from the reaction with 5,5-dithiobis-2-nitrobenzoic acid (DTNB), was determined, and expressed as µmol/L. The dynamic amount of disulfide was calculated as half of the difference between total thiol and native thiol. Subsequently, the following ratios were computed: reduced thiol=(native thiol/total thiol)*100; oxidized thiol =(disulfide/total thiol)*100; and thiol oxidation reduction ratio=(native thiol/disulfide)*100. Disulfide levels were expressed as µmol/l, total thiol as µmol/l / albumin (g/l), native thiol as µmol/l / albumin (g/l), and disulfide as µmol/l / albumin (g/l).[9,10]

Data was analyzed using SPSS 21. Categorical variables were presented as frequencies and percentages, while continuous variables were expressed as mean ± standard deviation. Analysis of variance (ANOVA) test was used to explore significant differences. P<0.05 was considered statistically significant.

Results

Of the 50 subjects with mean age 47.10±15.16 years, 25(50%) were males, 25(50%) were females, 40(80%) were married, 28(56%) were primary school graduates, 12(24%) were high school graduates, 10(20%) were university graduates, 43(86%) were non-smokers, 11(22%) were obese, 21(42%) were overweight, 2(4%) were underweight, and 16(32%) were of normal weight. Biochemical

Table-1: Routine laboratory parameters before the procedure.

Parameters	Mean±SD
Hb g/dL	13.93±1.40
HCT %	42.04±3.95
RBC 10^6 cells per microlitre of blood	4.87±0.56
WBC Cells per microlitre of blood	7.48±3.11
MPV fL	10.53±0.93
Neuth Cells per microlitre of blood	4.49±2.85
Lymph Cells per microlitre of blood	2.52±1.52
Glucose mg/dL	106.12±43.07
Urea mg/dL	27.33±9.72
Creatinine mg/dL	0.80±0.13
CRP mg/L	0.27±0.60
AST U/L	24.15±36.54
ALT U/L	23.08±27.24

Hb: Haemoglobin, HCT: Haematocrit, RBC: Red blood cells, WBC: white blood cells, MPV: Mean platelet volume, CRP: C-reactive protien, AST: Aspartate transaminase, ALT: Alanine aminotransferase.

Table-2: Thiol-disulfide balance before and after wet cupping therapy.

Parameters	Group	Mean±SD	SEM	*p*-value
Total Thiol	Before Procedure	415.42±35.82	5.07	<0.001*
	After Procedure	365.87±29.95	4.24	
Native Thiol	Before Procedure	232.34±48.14	6.81	0.268
	After Procedure	244.78±62.56	8.85	
Disulfide	Before Procedure	91.57±24.83	3.51	<0.001*
	After Procedure	60.57±22.66	3.20	
Reduced Thiol Ratio	Before Procedure	55.96±10.59	1.50	<0.001*
	After Procedure	66.40±13.33	1.89	
Oxidized Thiol	Before Procedure	22.01±5.30	0.75	<0.001*
	After Procedure	16.80±6.67	0.94	
Thiol Oxidation	Before Procedure	284.88±138.52	19.59	<0.001*
Reduction Ratio	After Procedure	533.03±403.16	57.01	

*Statistically significant; SD: Standard deviation, SEM: Standard error of mean.

parameters of the participants at baseline showed no abnormality (Table 1).

There was a statistically significant difference between baseline and post-intervention levels of total thiol, disulfide, reduced thiol ratio, oxidized thiol and thiol oxidation reduction ($p<0.001$) (Table 2).

Discussion

Oxidative stress occurs when there is an imbalance between ROS production and the cellular antioxidant defense system, favouring oxidants.[8-10] Consequently, ROS levels rise in the affected tissues and organs. Given the potential damage ROS can inflict on various organs and systems, interventions or medications that offer antioxidant effects without notable adverse reactions become crucial. Oxidative stress arises from a disturbance in the oxidant/antioxidant equilibrium, tilting it in favour of oxidants. This imbalance contributes to the aetiology of numerous diseases, including cancer.[8-10]

According to Avicenna, harmful substances in body fluids responsible for pain and inflammation must be absorbed into the surface and evacuated from there to relieve discomfort. Therefore, WCT can be applied as a method of clearing hazardous substances from the body.[13] Salah M. El Sayed et al suggested that many disease-causing toxic substances can be removed from the blood, lymph and inter cellular space by WCT, and the skin, which is a natural excretory organ of the body, could be used more effectively with surgical interventions.[14]

Numerous tests are employed to gauge the levels of antioxidant and oxidant molecules, providing insights into oxidative stress within a living organism. Thiols represent a category of organic compounds characterized by a sulfhydryl (-SH) group composed of a hydrogen and a sulfur atom bonded to a carbon atom. Renowned for their reducing properties, thiols function as antioxidant molecules. ROS generated in the organism transfer surplus electrons to thiols, leading to their oxidation and the formation of disulfide bonds. Notably, these disulfide bonds are reversible, capable of transforming back into thiols, depending on the organism's antioxidant-oxidant balance. Consequently, thiol-disulfide homeostasis is dynamic, playing a pivotal role in antioxidant protection, detoxification, signal transduction, apoptosis, regulation of enzymatic activation, transcription factors, and cellular signaling mechanisms.[8-10] In instances of diseases triggered by oxidative stress, it is anticipated that the dynamic thiol-disulfide homeostasis of the organism would be affected. Oxidative stress has the potential to induce diseases by causing abnormal structural changes in proteins, membrane lipids, deoxy ribo nucleic acid (DNA) and ribo nucleic acid (RNA). Recognizing alterations in thiol-disulfide homeostasis resulting from oxidative stress holds significance for understanding various abnormal biochemical processes. Hence, there is a need for a new, easily calculable, and relatively inexpensive method to investigate thiol-disulfide homeostasis as an oxidative stress parameter.[8-10]

In the current study, the effect of WCT on total thiol, native thiol and disulfide levels was investigated. Tagil et al. conducted a study in 2014 on 31 volunteers, and reported that WCT removed oxidants and reduced oxidative stress.[15] Blood collected after WCT in a study was compared with venous blood collected before WCT. Myelo peroxidase (MPO), malon dialdehyde (MDA) and nitric oxide (NOx) values were found to be higher in WCT blood than in venous blood. Only superoxide dismutase (SOD) value was found to be higher in venous blood.[16] In another study, MDA), total oxidant status (TOS), total antioxidant status (TAS), glutathione (GSH), SOD and catalase (CAT) serum values were found to be different compared to pre-application values ($p<0.05$), indicating that WCT increased antioxidant levels and reduced oxidative stress.[17]

A study conducted on healthy volunteers investigated blood donation on oxidative stress. Serum NOx, MDA, SOD and MPO levels were measured. While SOD and NOx levels increased significantly after blood donation, changes in MDA and MPO were not statistically significant. The study concluded that blood donation removed oxidants and reduced oxidative stress.[16]

Yucel et al. in 2021 showed that WCT did not cause any change in TAS and TOS values, but caused a significant decrease in oxidative stress index (OSI).[18]

The current clinical research, which evaluated the effects of WCT on the oxidant-antioxidant balance through the thiol-disulphide balance, revealed pioneering and highly original data. It is very important to demonstrate the possible concrete effects and mechanisms of traditional and complementary medicine practices through clinical studies. The current results showed that WCT, one of the most frequently applied traditional and complementary medicine practices, increased antioxidant capacity through the thiol-disulfide balance, and had significant effects on preventing oxidative damage.

The current study has limitations, such as the absence of a control group and the lack of dietary restrictions between sessions. For example, the consumption of foods with known antioxidant properties was not restricted. The delay in publishing our study can be attributed to the low international recognition of cupping therapy, a traditional and complementary medicine practice, particularly in Western and European countries. Because such research articles are not widely available in our country and the West, they are often under-reviewed. Therefore, the delay in publishing our original research is one of the limitations of this study. The sample size was calculated in accordance with the literature, taking into account the budget and measurement kit resources used. However, one of the limitations of the study is the inability to work with a larger sample size.

Conclusion

WCT could make a positive contribution to the protection of public health and the treatment of diseases by removing oxidants, reducing oxidative stress and having an antioxidant effect.

Disclaimer: None.

Conflict of Interest: None.

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Author Contribution:

HBS: Design, performed study, data collection, performed laboratory studies, discussed the results, strategy, supervision, directed, managed the study and final approval.

IS: Design, performed study, data collection, discussed the results, strategy and final approval.