

## Study on the Effect of Wet Cupping Therapy on Oxidative Stress Index and Total Antioxidant Capacity Levels: A Pilot Study

### Islak Kupa Tedavisinin Oksidatif Stres İndeksi ve Total Anti Oksidan Kapasite Düzeylerine Etkisinin Araştırılması: Pilot Bir Çalışma

Hasan Esat YÜCEL<sup>1</sup>  Kenan GÜÇLÜ<sup>2</sup>  Cahit UÇAR<sup>3</sup>  Selcen DURAN<sup>4</sup>   
Naime Meriç KONAR<sup>5</sup> 

#### ÖZ

**Amaç:** Islak kupa tedavisi bilinen en eski tedavi yöntemlerinden biridir. Son zamanlarda oksidanları uzaklaştırarak, oksidatif stresi azalttığına dair bazı çalışmalar yapılmıştır. Ancak bu çalışmalar sınırlı sayıdadır. Amacımız ıslak kupa tedavisinin oksidan ve anti-oksidan sistem üzerindeki etkinliğini araştırmaktır.

**Araçlar ve Yöntem:** Çalışmamıza 26 hasta dahil edildi. Islak kupa tedavisinde hemen önce venöz kan örnekleme yapıldı. Kupa tedavisiyle birlikte kupaya dolan kandan, işlemden hemen sonra ve 14. günde yine venöz kandan örnekleme yapıldı. Tüm örneklerden total oksidan düzey, total anti-oksidan düzey ve oksidatif stres indeksi düzeyleri çalışılarak karşılaştırılması yapıldı.

**Bulgular:** Kupa kanındaki total oksidan düzey, total anti-oksidan düzey ve oksidatif stres indeksi seviyeleri, tüm venöz kan parametrelerinden yüksek bulundu. Uygulama sonrası, total oksidan ve total anti-oksidan düzeylerde belirgin bir değişiklik olmazken, oksidatif stres indeksi önemli bir şekilde azalmıştır.

**Sonuç:** Islak kupa tedavisi, oksidatif doku hasarının en önemli göstergesi olan oksidatif stres indexini azaltır. Bu nedenle oksidatif doku hasarıyla ilişkili hastalıkların tedavisinde yer alabilir.

**Anahtar Kelimeler:** ıslak kupa tedavisi; oksidatif stres indeksi; total oksidan düzey; total anti-oksidan düzey

#### ABSTRACT

**Purpose:** Wet cupping treatment is one of the oldest known methods of treatment. Recently, some studies have shown that wet cupping therapy removes oxidants and reduces oxidative stress. However, these studies are limited. The aim of this study is to investigate the effectiveness of wet cupping treatment on oxidant-anti oxidant system.

**Materials And Methods:** Our study consisted of 26 patients. Right before the wet cupping treatment, venous blood samples were collected. During the procedure, samples were collected from the cup blood. Right after the wet cupping treatment venous blood samples were collected again. On the 14th day, the participants were called and venous blood samples were collected. In all of these samples, total oxidant level (TOS), oxidative stress index (OSI) and total antioxidant level (TAS) levels were studied and compared.

**Results:** Total anti-oxidant level, total oxidant level and oxidative stress index levels in cup blood were higher than all venous blood samples. While there was no significant change in TAS and TOS levels after the application, oxidative stress index levels decreased significantly.

**Conclusion:** Wet cupping therapy reduces the oxidative stress index, which is the most important indicator of oxidative tissue damage. Therefore, it may be involved in the treatment of diseases associated with oculatory tissue damage.

**Keywords:** oxidative stress index; total oxidant level; total anti-oxidant level; wet cupping treatment

Received: 05.12.2020 Accepted: 24.03.2021

<sup>1</sup> Ahi Evran University, Faculty of Medicine, Department of Internal Medicine, Kırşehir, Turkey.

<sup>2</sup> Research and Training Hospital, Department of Biochemistry, Kırşehir, Turkey.

<sup>3</sup> Ahi Evran University, Faculty of Medicine, Department of Internal Medicine, Kırşehir, Turkey.

<sup>4</sup> Ahi Evran University, Faculty of Medicine, Department of Neurology, Kırşehir, Turkey.

<sup>5</sup> Ahi Evran University, Faculty of Medicine, Biostatistics and Medical Science Department, Kırşehir, Turkey.

Corresponding Author: Hasan Esat Yücel, Ahi Evran University, Faculty of Medicine, Department of Internal Medicine, Kırşehir, Turkey.  
e-mail: drh.esat@hotmail.com

**How to cite:** Yücel HE, Güçlü K, Uçar C, Duran S, Konar NM. Study on the effect of wet cupping therapy on oxidative stress index and total antioxidant capacity levels: a pilot study. Ahi Evran Med J. 2021;5(2):134-139. DOI: 10.46332/aemj.836358

## INTRODUCTION

Wet cupping therapy is an ancient medical treatment method dating back to thousands of years. Chinese, Koreans, Egyptians and Arabs use this practice as a form of traditional treatment. In recent years, wet cupping has started to be used as complementary treatment in cases of chronic diseases or in cases that do not respond to conventional treatment in many parts of the world such as USA, UK, Germany, Norway and Denmark.<sup>1-3</sup> There are many different kinds of applications of wet cupping treatment. These can be counted as follows; dry cupping, wet cupping (Al-Hijama), moving cupping, empty cupping, needle cupping, water cupping, hot cupping/moxa cupping and herbal cupping. However, wet and dry cupping treatments are frequently applied in practice. In both practices, the cups are placed on the skin creating negative pressure, additionally, epidermal incisions are applied to the skin and blood is taken out during the wet cup treatment<sup>4,5</sup> Its application is easy, and it's highly cost effective. Wet cupping therapy is an ancient treatment method which has been an integral part of Traditional Anatolian Medicine. It was applied in Anatolia since ancient times not only for treatment, but also for health protection in certain periods observing lunar phases. According to this areas where cupping is applied are; painful areas on the skin, especially between the shoulders, head, under the chin, lower extremities, dorsal areas, reflex areas and acupuncture points.<sup>6-8</sup> Some hypotheses have been proposed regarding the mechanism of action of cupping treatment. In traditional Chinese medicine, diseases are thought to be caused by blockages in the life energy source (Qi). It is assumed that this energy is restored to the body in a balanced and regular manner with cupping treatment.<sup>9,10</sup> Cao et al., on the other hand, thought that the cupping treatment applied to acupuncture points had a therapeutic effect by creating hyperemia and hemostasis on the skin.<sup>11</sup> El Sayed et al. have put forward the Taibah theory. According to this theory, the balance of the human body depends on the harmony of the systems and organs with each other. Diseases occur when this physiological balance is disrupted. With wet cupping treatment, it is accepted that this balance is restored by providing the elimination of potentially harmful substances that cause diseases. According to this theory, when cupping treatment is applied, the pathological substances in the skin,

tissues, blood and intercellular distance, depending on the percutaneous pressure applied to the skin, are simultaneously and significantly cleared by the effect of negative pressure. Thus, filtration takes place from the dermis layer and capillary endothelium. Similar to renal glomerular filtration, the skin carries out excretory function through capillary vessels.<sup>12,13</sup> Researchers have recently become interested in this treatment method and it is quite effective in diseases such as; migraine, herpes zoster, musculoskeletal disorders, fibromyalgia, fibrositis, lumbar disc lesion, cervical spondylosis, rheumatoid arthritis and painful conditions like chronic osteoarthritis.<sup>14,15</sup> In addition, some studies have shown that wet cupping therapy removes oxidants and reduces oxidative stress.<sup>16,17</sup> As is known, there is a balance between oxidant and antioxidant systems in physiological conditions.<sup>18,19</sup> If this balance is disrupted in favor of oxidants, tissue damage, known as oxidative stress, occurs.<sup>20</sup> Oxidative tissue damage is caused by the reactions of oxidant molecules on the cell building blocks of proteins, lipids, carbohydrates and nucleic acids<sup>21-23</sup> and plays a role in the pathogenesis of many diseases.<sup>24-28</sup> We planned to investigate the effect of wet cupping treatment, oxidative stress index (OSI) and the total antioxidant level (TAS) on these patients.

## MATERIALS and METHODS

There are patients who apply to Kırşehir Ahi Evran University Traditional and Complementary Medicine Clinic for complaints such as headache, neck pain, back pain, shoulder pain, low energy, weakness, fatigue, difficulty concentrating and decrease in the quality of life. medical histories of these patients were taken. It was determined that their complaints had been continuing for at least 1 year. There was no known chronic disease or continuous drug use. Physical examinations were performed and evaluated with laboratory results. Iron deficiency anemia (Hbg < 10 gr/dl), vit B12 - folate deficiency, vitamin D deficiency, sediment and crp elevation, which are signs of acute or chronic inflammation, were not detected. Kidney, liver and thyroid function tests were normal. It was found that these complaints of patients developed due to the stressors caused by the intense work pace and social life. Our study consisted of 26 patients. Of these, 50.0% (n = 13) were male and 50.0% (n = 13) were female. The study

was conducted on the patients who applied to the Traditional and Complementary Medicine Clinic of Kırşehir Education and Research Hospital between June and September 2019. The mean age of volunteers was  $(35,35 \pm 9,806)$  and the mean BMI was  $26,166 \pm 4,117$ . We excluded participants who used anti-aggregate drugs (acetylsalicylic acid, warfarin, clopidogrel), also who had chronic inflammatory diseases such as ankylosing spondylitis and rheumatoid arthritis. Participants with anemia (Hbg < 10 gr/dl), bleeding prone diseases such as hemophilia and also the participants at risk for infectious diseases such as AIDS, chronic hepatitis B and C were not included in our study. Approval was obtained from the ethics committee of Kırşehir Ahi Evran University with resolution number 2019-05/72 and informed consent form was obtained from the individuals who accepted to be included to the study.

Right before the wet cupping treatment, venous blood samples were collected. During the procedure, samples were collected from the cup blood. Right after the wet cupping treatment venous blood samples were collected again. On the 14th day, the participants were called and venous blood samples were collected. All these samples were placed into biochemistry tubes. After 30 minutes of clotting, the serums were separated by centrifugation at 3000 rpm for 10 minutes. Serums were stored at -80 degrees until the analysis of total oxidant capacity level, total anti-oxidant capacity level and oxidative stress index levels.

### Wet Cupping

Wet cupping was applied by three physicians certified by Ankara Yıldırım Beyazıt University Traditional and Complementary Medicine Center. Application areas were cleaned using anti-septic solutions. A total of 6 points were selected, including the projection of the 7th cervical vertebra and the 3rd thoracic vertebra on the skin and 4 in the thoracic areas. Cups with a diameter of 7 cm were placed (Figure 1). Negative vacuum was applied with the cup pump. Three minutes were waited. Then the cups were removed and superficial incisions were made with the 11th scalpel. The cups were re-placed and negative vacuum applied again and waited for approximately 5 minutes. The blood accumulated in the cup was wiped away by cotton. During the procedure, the doctor, nurse and emergency

equipment bag were prepared to prevent any unwanted effects such as hypotension and syncope attacks. There were no complications. Application areas were closed with sterile pads and the process was terminated.



Figure 1. Areas Selected for Wet Cupping Treatment

### Total Anti-Oxidant Capacity And Total Oxidant Status Level Measurement

**Total Anti-Oxidant Capacity (TAC) level and Total Oxidant Status (TOS) levels:** These levels were measured with the autoanalyzer (Cobas c501, Roche Diagnostic Corp., Mannheim, Germany) using the Rel brand colorimetric method commercial kits (Rel Assay Kit Diagnostics, Gaziantep, Türkiye) developed by Erel O. Trolox, a water-soluble analogue of vitamin E, was used as a calibrator for TAC measurement. The results were expressed as mmol Trolox equiv./lt. Hydrogen peroxide was used as a calibrator for TOS measurement. The results were expressed as  $\mu\text{mol H}_2\text{O}_2$  Equiv./L.<sup>29,30</sup>

**Oxidative Stress Index (OSI):** OSI which is an indicator of oxidative stress degree and is expressed as the ratio of TOS to TAC can be calculated using the following formula.

Results were expressed as “arbitrary unit” (AU).

$$\text{OSI (AU)} = [\text{TOS } (\mu\text{mol H}_2\text{O}_2 \text{ Equiv./L}) / \text{TAC (mmol Trolox Equiv./L)}] \times 100.$$

**Statistical Analysis**

Mean ± standard deviation and median, minimum, maximum values were given as basic descriptive statistics for quantitative data, while frequencies and percentages were reported for categorical variables. Shapiro-Wilk was applied for normality assumption. G-Power v. 3.1.9.2. was used for determining the sample size at  $\alpha=0.05$ ,  $d=0.65$ ,  $Power(1-\beta)=0.80$ . Repeated measures ANOVA was utilized for evaluating there peated measures of TAS, TOS and OSI values. Significancel evel was taken as  $p<0.05$  in all analyses. All analyses were performed vith Statistical Package for Social Sciences version 21.0 software for Windows (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp., USA).

**RESULTS**

TAS ( $1.895 \pm 0.3535$ ), TOS ( $38.959 \pm 22.483$ ) and OSI ( $2131.038 \pm 1447.449$ ) levels in the cup were significantly higher than the changes in venous blood at all times ( $p:0.008$ ) (Table-1).

**Table 1.** Comparison of cupped and venous blood with TAS, TOS and OSI levels at all times

Variable	Mean ± SD	p-value
TAS Cup blood	1.895 ± 0.3535	0.008
TAS Venous blood before cupping	1.782 ± 0.328	
TAS Venous blood after cupping	1.774 ± 0.324	
TAS Venous blood 14 day	1.801 ± 0.366	0.008
TOS Cup blood	38.959 ± 22.483	
TOS Venous blood before cupping	11.984 ± 3.283	
TOS Venous blood after cupping	10.538 ± 4.344	0.008
TOS Venous blood 14 day	9.272 ± 3.275	
OSİ Cup blood	2131.038 ± 1447.449	
OSİ Venous blood before cupping	701.154 ± 249.889	0.008
OSİ Venous blood after cupping	629.154 ± 278.258	
OSİ Venous blood 14 day	528.231 ± 175.861	

When the course of TAS and TOS levels in venous blood was treated before and after the cupping, no significant result was observed( $p:0.999$ )(Table -2).

**Table 2.** TAS, TOS and OSI levels before and immediately after treatment and on the 14th day

Variable	Mean ± SD	p-value
TAS Venous blood before cupping	1.782 ± 0.328	0.999
TAS Venous blood after cupping	1.774 ± 0.324	
TAS Venous blood 14 day	1.801 ± 0.366	
TOS Venous blood before cupping	11.984 ± 3.283	0.999
TOS Venous blood after cupping	10.538 ± 4.344	
TOS Venous blood 14 day	9.272 ± 3.275	
OSİ Venous blood before cupping	701.154 ± 249.889	0.999
OSİ Venous blood after cupping	629.154 ± 278.258	
OSİ Venous blood 14 day	528.231 ± 175.861	

However, when the OSI levels( $528.231 \pm 175.861$ ) were examined, it was seen that they significantly decreased on the 14th day after the cupping treatment. This result should be statistically significant( $p:0.032$ )(Table 2). When the changes in TAS, TOS and OSI levels in venous blood after cupping were examined, no significant finding was observed( $p:0.999$ )(Table-3).

**Table 3.** TAS, TOS and OSI levels immediately after cupping treatment and on day 14

Variable	Mean ± SD	p-value
TAS Venous blood after cupping	1.774 ± 0.324	0.999
TAS Venous blood 14 day	1.801 ± 0.366	
TOS Venous blood after cupping	10.538 ± 4.344	
TOS Venous blood 14 day	9.272 ± 3.275	0.999
OSİ Venous blood after cupping	629.154 ± 278.258	
OSİ Venous blood 14 day	528.231 ± 175.861	

## DISCUSSION

ormal TOS level in plasma is 4-6  $\mu\text{mol H}_2\text{O}_2$  Equiv./ L, TAS level is 1.2-1.5 mmol Troloxequiv./lt.<sup>29,30</sup> Average TOS value in the venous blood of our patients in the study before the wet cupping treatment is  $11.984 \pm 3.283 \mu\text{mol H}_2\text{O}_2$  Equiv./ L, Average TAS value was determined as  $1.782 \pm 0.328$ . The levels of both variables were higher than normal plasma levels, with a greater increase in the TOS value. Oxidative Stress Index (OSI) is expressed as a percentage of the ratio of TOS levels to TAS levels and OSI is regarded as the most important indicator of the oxidative stress level.<sup>29,30</sup> However, the TAS, TOS and OSI levels belonging to the blood in the cup were found to be higher than the levels in the venous blood before and after the cupping treatment. However, this may have been caused by the hemolysis of the cup after the incision under the influence of negative percutaneous pressure. Otherwise, we do not expect TAS levels to be high. Because, in a study conducted by Tagil et al., It was found that oxidants in cup blood were high, while anti-oxidants were low.<sup>12</sup> In Taibah theory, it was accepted that oxides and toxic substances in cup blood were high and they were removed from the organism by the cup method. It has been suggested to be provided.<sup>13</sup>

In our study, we did not observe an effective decrease in TOS levels, because wet cupping therapy is a microsurgical procedure. Acute inflammatory cascade may be activated with incision and cause oxidation in the organism for a short time. This situation may have prevented the significant decrease in TOS and OSI levels, especially immediately after the cup. However, our study also has some limitations: the small number of participants, the application of a single-session cup, and our evaluation of short-term results. In a study by Ersoy et al., A total of 3 sessions of cups were applied to the patients once a month, and it was shown that TOS levels decreased and TAL levels increased.<sup>31</sup>

What is important here is the balance between TOS and TAS rather than the numerical changes. The most important indicator of this balance is OSI. As the OSI index increases, the balance shifts in favor of the oxidants and the oxidative tissue damage occurs. As the OSI index decreases, the opposite situation occurs. Of course, there are

many parameters that will affect OSI index levels during this time. Nutritional status, physical activity, short-term infection, drug use such as pain killers, supplement support, smoking and alcohol intake can affect the dynamic process of oxidant-antioxidant balance positively or negatively. Despite all these external factors, short-term follow-up and single-session application, OSI significantly decreased. However, we recommend conducting longer-term studies with larger participants. There is a need to investigate the effectiveness of wet cupping therapy on TOS, TAS and OSI levels, which may contribute significantly to elucidating the mechanism of action.

## Conflict of Interest

There is no conflict of interest to declare among the researchers.

## Acknowledgements

We would like to thank Assoc. Prof. Dr. Aydın Güçlü from Kırşehir Training and Research Hospital, Department of Internal Medicine for his advice on the software used for data analysis.

## Authors' Contributions

Concept/Design: HEY, KG. Data Collection and/or Processing: HEY, CU, SD. Data analysis and interpretation: NMK, HEY. Literature Search: HEY, KG, CU, SD. Drafting manuscript: HEY, KG, NMK. Critical revision of manuscript: SD, CU. Supervision: AG.

## REFERENCES

1. Ullah K, Younis A, Wali M. An investigation into the effect of Cupping Therapy as a treatment for Anterior Knee Pain and its potential role in Health Promotion. *Internet J. Altern Med.* 2007;4(1):1-9.
2. Michalsen A, Bock S, Lüdtker R, et al. Effects of Traditional Cupping Therapy in Patients With Carpal Tunnel Syndrome: A Randomized Controlled Trial. *J. Pain.* 2009;10(6):601-608.
3. Eisenberg DM, Davis RB, Ettner SL, et al. Trends in Alternative Medicine Use in the United States, 1990-1997: Results of a Follow-up National Survey. *JAMA* 1998;280(18):1569-1575.
4. Christopoulou-Aletra H, Papavramidou N. Cupping: an alternative surgical procedure used by Hippocratic physicians. *J Altern Complement Med.* 2008;14(8):899-902
5. Kim T, Kang JW, Kim KH, et al. Cupping for Treating Neck Pain in Video Display Terminal (VDT) Users: A Randomized Controlled Pilot Trial. *J Occup. Health.* 2012;54(6):416-426.

6. Yoo SS, Tausk F. Cupping: East meets West. *Int J Dermatol*. 2004;43(9):664-665.
7. Ersoy S, İnci H, Sunay D, et al. Wet Cupping Therapy Improves Health Related Quality of Life: A Self-Controlled Interventional Study. *Ankara Med J*. 2019;(1):270-277.
8. Karabulut AR. *Tıbb-ı Nebevî Ansiklopedisi* 1-2, 6. baskı. Ankara: Kozan Ofset; 2006.
9. Zhang SJ, Liu JP, He KQ. Treatment of acute gouty arthritis by bloodletting cupping plus herbal medicine. *J Tradit Chin Med*. 2010;30(1):18-20.
10. Ahmedi M, Siddiqui MR. The value of wet cupping as a therapy in modern medicine An Islamic Perspective. *Webmed Central Alternative Medicine*. 2014;5(12):WMC004785.
11. Cao H, Li X, Liu J. An updated review of the efficacy of cupping therapy. *PloS one* 2012;7(2):e31793.
12. El Sayed SM, Mahmoud HS, Nabo MMH. Medical and scientific bases of Wet Cupping Therapy (Al-Hijamah): In Light of Modern Medicine and Prophetic Medicine. *AlternInteg Med*. 2013;2:1-16.
13. El Sayed SM, Al-Quliti A-S, Salah Mahmoud H, et al. Therapeutic Benefits of Al-hijamah: in Light of Modern Medicine and Prophetic Medicine. *American Journal of Medical and Biological Research*. 2014;2(2):46-71.
14. Kaki A, Sawsan R, Samiha M, et al. Wet Cupping Reduces Pain and Improves Health-related Quality of Life Among Patients with Migraine: A Prospective Observational Study. *Oman Med J*. 2019;34(2):105-109.
15. Kavadar G, Eroğlu Demir S, Aytekin E, Akbal Y. Use of traditional and complementary medicine for musculoskeletal diseases. *Turk J Med Sci*. 2019-18;49(3):809-814.
16. El-Shanshory M, Hablas NM, Shebl Y, et al. Al-hijamah (wet cupping therapy of prophetic medicine) significantly and safely reduces iron overload and oxidative stress in thalassemic children: a novel pilot study. *J Blood Med*. 2018; 9:241-251.
17. Tagil SM, Celik HT, Ciftci S, et al. Wet-cupping removes oxidants and decreases oxidative stress. *Complementary Ther Med*. 2014;22(6):1032-1036.
18. Urso ML, Clarkson, PM. Oxidative stress, exercise, and antioxidant supplementation, *Toxicology*. 2003;189(1-2):41-54.
19. Clarkson PM, Thompson HS. Antioxidants: What role do they play in physical activity and health?. *Am J Clin Nutr*. 2000;72(2):637-646.
20. Yeum KJ, Russell MR, Krinsky IN, Adlini G. Biomarkers of antioxidant capacity in hydrophilic and lipophilic compartments of human plasma. *Arch Biochem Biophys*. 2004;430(1):97-103.
21. Kisaoglu A, Borekci B, Yapca OE, Bilen H, Suleyman H. Tissue Damage and Oxidant/Antioxidant Balance. *EAJM*. 2013;45(1):47-49.
22. Goulart M, Batoreu MC, Rodrigues AS, Lares A, Rueff J. Lipoperoxidation products and thiol antioxidants in chromium exposed workers. *Mutagenesis*. 2005;20(5):311-315.
23. Yuliani S, Mustofa, Partadiredja G. The neuroprotective effects of an ethanolic turmeric (*Curcuma longa* L.) extract against trimethyltin-induced oxidative stress in rats. *Nutr Neurosci*. 2018;7:1-8.
24. Dai DF, Chiao YA, Marcinek DJ, Szeto HH, Rabinovitch PS. Mitochondrial oxidative stress in aging and healthspan. *Longevity Healthspan*. 2014;3(1):6.
25. Mei Y, Thompson MD, Cohen RA, Tong XY. Autophagy and oxidative stress in cardiovascular diseases. *Biochim Biophys Acta*. 2015;1852(2):243-251.
26. Erdamar H, Demirci H, Yaman H, et al. The effect of hypothyroidism, hyperthyroidism, and their treatment on parameters of oxidative stress and antioxidant status. *Clin Chem Lab Med*. 2008;46(7):1004-1010.
27. Weinberg F, Chandel NS. Reactive oxygen species-dependent signaling regulates cancer. *Cell Mol Life Sci*. 2009;66(23):3663-3673.
28. Kamat PK, Kalani A, Kyles P, Tyagi SC, Tyagi N. Autophagy of mitochondria: a promising therapeutic target for neurodegenerative disease. *Cell Biochem Biophys*. 2014;70(2):707-719.
29. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin Biochem*. 2004;37(4):277-285.
30. Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem*. 2005;38(12):1103-1111.
31. Ersoy S, Altinoz E, Benli AR, et al. Investigation of wet cupping therapy's effect on oxidative stress based on biochemical parameters. *Eur J Integr Med*. 2019;30:100946.