# Long-Term after-Effects of Wet Cupping Therapy on Some Inflammatory Mediators and Antioxidant parameters in Jordanian Healthy Adult Men

Nafe M. Al-Tawarah, PhD*

## ABSTRACT

**Background and Aim: This work was performed to evaluate the long-term after-effect of Wet Cupping Therapy (WCT) on the healthy volunteers regarding some inflammatory markers, which include IL-6, IL-10, C3, C4, IgA, IgM and IgG. Moreover, this study aimed to investigate the effectiveness of WCT in enhancing the antioxidant levels such as GPX, GR, SOD and GST in healthy individuals after receiving this intervention.**

**Method: Venous blood samples were collected from 29 volunteers out of 31 healthy adult men engaged during the study (two excluded). A sensitive sandwich ELISA kit evaluated the inflammatory markers. The antioxidant parameters were spectrophotometrically measured.**

**Results: Analysis of results revealed a significant chronic drop in all pre-and post- inflammatory markers with some variations including a more obvious drop. Additionally, there was an increase in the serum level of antioxidants after receiving WCT.**

**Conclusion: WCT dramatically induces a significant drop in many inflammatory markers; pre-and post-inflammatory factors. Moreover, this intervention induces the efficiency of antioxidant parameters known as a defensive system against various reactive oxygen species (ROS).**

**Keywords: Wet cupping, Inflammatory markers, Antioxidants, IL-6, IL-10**

## INTRODUCTION

In most civilizations; Chinese, Greek, Egyptians and Arabs, cupping therapy has been used for many thousands of years as alternative medicine for treatment of many ailments[1]. There are different categories of cupping therapies; however, wet cupping (WCT) and dry cupping are the two main types of cupping therapy[2]. While Chinese tradition performs dry cupping, WC or even called AL-hijamah and bleeding cupping method, is commonly used in Muslim countries thought to promote well-being[3,4]. WC approach includes the same primary step of dry cupping; creation of a suction over targeted skin area, but later with furthermore step by scarifying the epidermal layer to allow bloodletting[5]. Several clinical studies proved the effectiveness of WC in treatment of some diseases including carpal tunnel syndrome, iron overload[6], asthma[7], acne, headache, migraine[2,8,9] and rheumatoid arthritis[10].

Many studies in healthy people and patients, characterize WCT as well-excretory route that removes wastes and some noxious substances such as inflammatory mediator, ferritin and autoantibodies from the body[11]. Furthermore, studies also revealed some general effects associated with WCT including; enhancement of immune system[12], modulation of the immunity and get rid of some substance that may aid ease of the pain[13], and stimulation of autonomic nervous system[14]. Although many studies have been performed on WC, but information about the physiological, biochemical and pathophysiological comprehensive effects as well as mechanism of action still not enough[15].

Among different pre-inflammatory cytokines, Interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are considered to be the main defensive immune mediators of the body[16-18]. Both mediators are stimulated by macrophages, NK-cells and T-cells during inflammatory responses in which they play a fundamental role in regulating body immune responses[19]. TNF-α effectively causes stimulation of the inflammatory responses which in turn help in controlling the infection, while IL-6 causes activation of lymphocytes, as well as production of antibodies[20]. From another hand, Interleukin-10 (IL-10) is considered as important anti-inflammatory cytokine, produced by activated immune cells, its action is to limit immune response[21] as well as to stimulate the proliferation and differentiation of B cells that can in turn express high levels of IgM, IgG and IgA antibodies[22].

In fact, serum Immunoglobulins level reflects the humeral immunity level, in which deficiency in such Ig types defines immunodeficiency, while excess may be observed in some pathological conditions[23]. For that, in many tests IgG blood level could be indicative to assess humoral immunity level[24]. Moreover, as a part of immune response, complement system with its proteolytic cascade plays a critical role against invading pathogen and so enhances innate immunity[25]. C3, a member from the complement system, is considered as the core of the complement components. It plays a major role for activation of complement system and its level increases during acute and chronic inflammation[26].

Toxic molecules known as reactive oxygen species (ROS) continuously and normally are generated in various types of living tissues as by-products during aerobic respiration. Our cells are occupied with efficient antioxidant system, which removes all types of produced ROS, and terminate their harmful effects on different cell biomolecules[27,28]. Oxidative stress occurs in body cells in which the rate of generation of ROS exceeds the capacity of antioxidant defensive mechanism[29].

* Human physiology
Department of Medical Laboratory Sciences
Faculty of Science, Mutah University
Jordan
E-mail: Nafitawa77@gmail.com; nafitawa77@mutah.edu.jo

Several studies reported that oxidative stress is the etiological mechanism which initiate several diseases[30-35].

The present study has been designed to increase our knowledge about the long-term after-effect (two week) of WCT on the healthy volunteers. Many inflammatory markers were selected during the study to assess the immunity level including IL-6, IL-10, C3, C4, IgA, IgM and IgG. Additionally, the effect of WCT on antioxidant levels in healthy individuals was investigated. The initial antioxidant values were determined in venous blood prior to WCT and compared to the venous blood samples post-WCT.

## MATERIAL AND METHODS

**Experimental Design:** The study was approved by the Ethics Committee of Scientific Research (ECSR) at Zarqa University (1/3/2021) and written informed consent was obtained from all participants. All procedures were performed in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Twenty-nine healthy men were engaged in this study during the period of 21-22 January 2021. In fact, selection of male gender, but not female, related to some traditional conditions that make it difficult to perform cupping therapy in women, also to avoid gender-related variations during the study. Cupping procedure was performed with professional and registered experts in cupping centre, Al-Zarqa University, in which all volunteers were from the university staff. Before performing cupping, two main points have been clarified. First is to make sure that all volunteers with no previous chronic medical history like elevated blood pressure, diabetes, cancer...etc. This step was done by a registered physician presented during WCT approach. The second, participant should have met all inclusion criteria as mentioned later. Ethically, during present study we were very interesting to make sure that every volunteer must be convinced that his information and samples will be confidential, and will be used only for this study.

**Inclusion Criteria:** Only those volunteers met the inclusion criteria eligible for this study. Inclusion criteria including healthy adults, between (20-50) years old, weighed between 50-80 kg, normal body temperature (37±0.5˚C), and blood pressure (130/85±10 mmHg for systolic and diastolic, respectively). In addition, participants during this research have no previous history of any chronic disease such as diabetes mellitus (DM), infectious disease, malignancy, immune disorders, or allergy, coagulation disorders and neurology disorder[36], in which the total number of participant were 29 man.

**Exclusion Criteria:** Any participant out of inclusion criteria have has been excluded from this study, and be informed. Moreover, any participant had chronic illness mentioned in inclusion criteria or any other blood-borne disease or taking anti-platelet or anti-coagulant agents were excluded. Moreover, unwillingness of any participant in this study was excluded immediately. Two volunteers have been excluded from the test, as it was discovered later that they were suffering from some diseases.

**Wet Cupping Approach:** Cupping approach was performed in the cupping centre, Zarqa University by a certified and expert therapist. WC was performed using three cups, two in the posterior torso-bilateral area of the thoracic spine and one in the middle as illustrated in Figure 1. Previous areas were selected as it is selected and recommended traditionally by most WC therapists in normal healthy people. To perform WC, disposable sterile cups with about 5 cm diameters and disposable lancets were used.

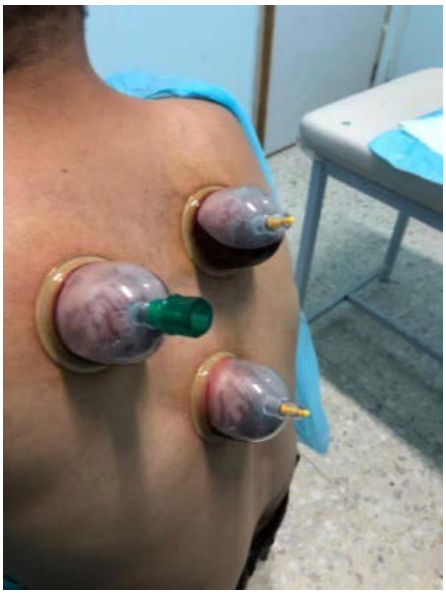

**Figure 1:** Cupping site. Three cups were placed on the dorsal thoracic inverted-triangle. Two of them in the posterior torso-bilateral area of the thoracic spine and one in the middle.

About 10 ml of venous blood samples were collected just before performing WC, and then placed into plane tubes, specific for serum preparation. Cups were placed on the selected areas, and the air was evacuated from the cups with a manual vacuum (cupping) pump. The cups were left attach to the skin for 3-5 min and then removed, after which skin and subcutaneous tissue swelled. The swollen areas were wiped with sterile gauze after disinfection with povidone-iodine. Disposable lancet was used to make a superficial incision with 1-2mm depth. In some instance, secondary suction was applied in the scarified area in the same way as described above. This allows blood leakage from the capillary vessels of the skin and subcutaneous tissue and filled 5-10 ml per cup. Finally, WC area was dressed and cover with sterile pads. During the WC approach, physician and nurse were standing with emergency kits and monitoring the procedure for any adverse reaction, in which no adverse reaction noted for all participants. For long-term after effect, two weeks later from cupping day, another 10 ml from venous blood were collected from all volunteers, in which blood also placed in a plane tubes specific for serum preparation. Approximately 10 min after each venous blood collection, before and after WC, serum was separated immediately from both blood samples by centrifugation (2000×g, 10 min at room temperature) and stored at - 80°C. In addition to the experimental group (n=31), blood samples were collected from four volunteers who have not been exposed to WC and considered as the control group.

**Measurement of Targeted Inflammatory Mediators**: All serum samples were stored in deep-freezing - 82C. Serum samples were placed in a refrigerator for 1hour, then to the room temperature. Inflammatory mediator was measured by sandwich Enzyme-linked immunosorbent assays (EILISA) and Beckman Coulter AU Systems.

**Measurement of IL-6, IL-10 and TNF-α Concentration in Serum Samples**: Concentration of inflammatory mediators IL-6, IL-10 and TNF-α were measured using ELISA sandwich kit specific for human, in which the procedure has been performed according to the kit manufacture's protocol (Abcam, Cambridge, United Kingdom). Absorbance per well was measured at 450 nm with an ELISA reader, in which concentrations (pg/ml) were measured according to the standard curve. The results are depicted in Table 1.

**Table 1:** Inflammatory parameters and cytokines in study population groups before and after (chronic) WC

| Cytokines | Before | after | p-value |
|---|---|---|---|
| IL6 (pg/mL) | 48.45±43.06 | 20.875±12.33 | p≤ 0.05 * |
| TNF-alpha (pg/mL) | 73.32863±4.71484 | 68.94479±3.63269 | p≤ 0.05 * |
| IL10 (pg/mL) | 18.35±2.832334 | 15.30036±3.35221 | p≤ 0.05 * |
| C3 (mg/L) | 162±10.862 | 146.3333±12.37344 | p≤ 0.001 ** |
| C-reactive protein (mg/L) | 4.4±0.821347 | 3.9±0.751363 | P=0.0863 ns |
| C4 (mg/L) | 29.43333±11.15766 | 24.56667±9.25869 | P= 0.1513 ns |
| IGA (g/d) | 3.596667±1.096099 | 3.063333±0.86031 | P= 0.1209 ns |
| IGG (g/d) | 15.07±1.197943 | 13.8±0.755866 | p≤ 0.001 ** |
| IGM (g/d) | 1.203333±0.40464 | 1.133333±0.338575 | P=0.3399 ns |

Data presented as mean ± (SD) between the two study groups. The p-value = before compared to after. *p<0.05, **pp<0.01.

**Measurement of IgA, IgG, IgM, C3 and C4 Concentration in Serum Samples**: Concentration of inflammatory mediator's IgA, IgG, IgM, C3 and C4 were measured using Beckman Coulter AU Systems (at Beckman Coulter, Brea, California).

**Measurement of GPX, GR, SOD and GST levels :** The antioxidant enzymes; catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), Glutathione-S-transferase (GST) and glutathione reductase (GR) activities was evaluated.

**Catalase (CAT) Activity :** The activities of catalase were measured using the method described by Aebi[37]. The principle of the assay is based on the determination of the rate of hydrogen peroxide decomposition by measuring the absorbance changes per minute. The assays mixture contained 0.1M phosphate buffer pH 6.5, 1mM EDTA, 50 µl $H_2O_2$ and 20 µl of sample. The rate of reduction of $H_2O_2$ to $H_2O$ was followed at 240 nm. One unit of catalase activity means the decomposition of 1 µmol hydrogen peroxide in 1 minute at 37 C°. CAT activity was expressed as U/mg protein[38,39].

**Superoxide Dismutase (SOD) Activity :** SOD activity were measured using the method described by Khan[40]. The assay system was consisting of 50 mM sodium carbonate, 400µl of 0.1 mM EDTA, 800µl of 25µM NBT (Nitro blue tetrazolium chloride). 800µl of 1mM hydroxylamine hydrochloride and 100µl of sample in a cuvette. Then incubated for 15 minutes under tungsten lamp. The absorbance was measured at 560 nm. Whereas the specific activity of each is estimated in U/min/mg of protein.

**Glutathione Peroxidase (GPx) Activity:** The activity of glutathione peroxidase were measured using the method modified by Hunaiti et al.[41]. The assay mixture was contained 20 µl of 0.1M GSH, 10 µl of the sample, 100 µl of 1 U/ml glutathione reductase, 10 µl of 7mM t-butylhydroperoxide, 760 µl of 0.2M Tris-HCL buffer, and 100µl of 2mM NADPH (Nicotinamide adinine dinucleotide phosphate). Reaction was initiated by the addition of $H_2O_2$, and the change in absorbance at 340 nm was monitored by a spectrophotometer. Activity was the rate of NADPH oxidation from reduced form to oxidized form was followed. One unit of enzyme is defined as 1µmole of NADPH oxidized/minute, and the enzyme activity expressed as mU/mg protein.

**Glutathione-S-Transferase (GST) Activity:** The activity of glutathione S-transferase was measured in the blood samples according to the modified method used in Jakoby and Habig[42]. The assay mixture was containing 880µl of the GST- buffer, 50µl of GSH, 50µl of CDNB (1-chloro-2, 4-dinitrobenzene) and 20µl of the sample; whereas the specific activity was expressed as U/mg protein.

**Glutathione Reductase (GR) Activity:** The activity of glutathione reductase was measured in the hemolysate according to the method modified by Hunaiti et al.[41]. The assay mixture was containing 100µl of the oxidized glutathione, 10µl of sample, and 840µl of 50mM potassium phosphate buffer, pH 7.5, and 50µl of 2mM NADPH. The rate of NADPH oxidation was measured using spectrophotometer at 340nm. The specific activity was expressed as U/mg of protein.

**Statistical Analysis:** Collected data, before and after cupping approach, were considered as WC variables, in which, One-way analysis of variance (ANOVA) followed by Tukey test, Graph Pad were run. Harvested results were expressed as mean ± standard error of mean (SEM), in which $p < 0.05$ was considered as significant result[43,44].

## RESULTS AND DISCUSSION

Wet cupping, known in Arabic "Al-hijamah", is a traditional and ancient therapy mostly popular in Middle East and Muslim countries, as well as linked to religious facts related to the Prophet Mohammed[2]. This intervention is a form of complementary medicine that has long been efficient for the treatment of a variety of diseases, as well as improving the general physiological status in healthy people[45]. There are many therapeutic benefits of WC arise from excretory clearance of the blood, lymph and interstitial fluid from various toxic metabolites and causative pathological substances, thus creates a favorable balance between vital parameters in different body compartments[4,46]. Several studies showed potential activity of WCT for treatment of many health and medical conditions such as hypertension, diabetes, hyperlipidemia and kidney diseases, as well as relieve pain intensity[2,6-8,10,19,47-49]. The questionable points arise from these potential therapeutic activities of WC are to understand the physiological and immunological changes underling WC. Therefore, the present work was performed to study long-term antioxidant efficiency and immune reflex for some inflammatory mediators, after two weeks, in healthy volunteers underlying one session of WCT.

In the current study, the effect of wet cupping on selected inflammatory mediators (IL-6, IL-10 and TNF-α cytokines) was evaluated in 29 healthy individuals. Our study showed that compared with normal control group and experimental group before cupping, a single session of cupping had statistically significant reduction in the selected parameters at two weeks after WC procedure (Table 1). Our result was consistent with two theories out of four theories that explained anti-inflammatory effect of WC through the blood detoxification and/or activation of immune system mechanisms[15,20]. Moreover, our findings are in agreement with the study of Ekrami et al.[20] who reported a significant decrease in the two inflammatory markers IL-6 and TNF-α in healthy martial arts athletes after the wet cupping intervention.

Numerous studies were conducted to evaluate the effect of WC on various parameters in healthy individuals. Khalil et al.[50] reported that there was a significant elevation in the total WBC and 50% haemolytic

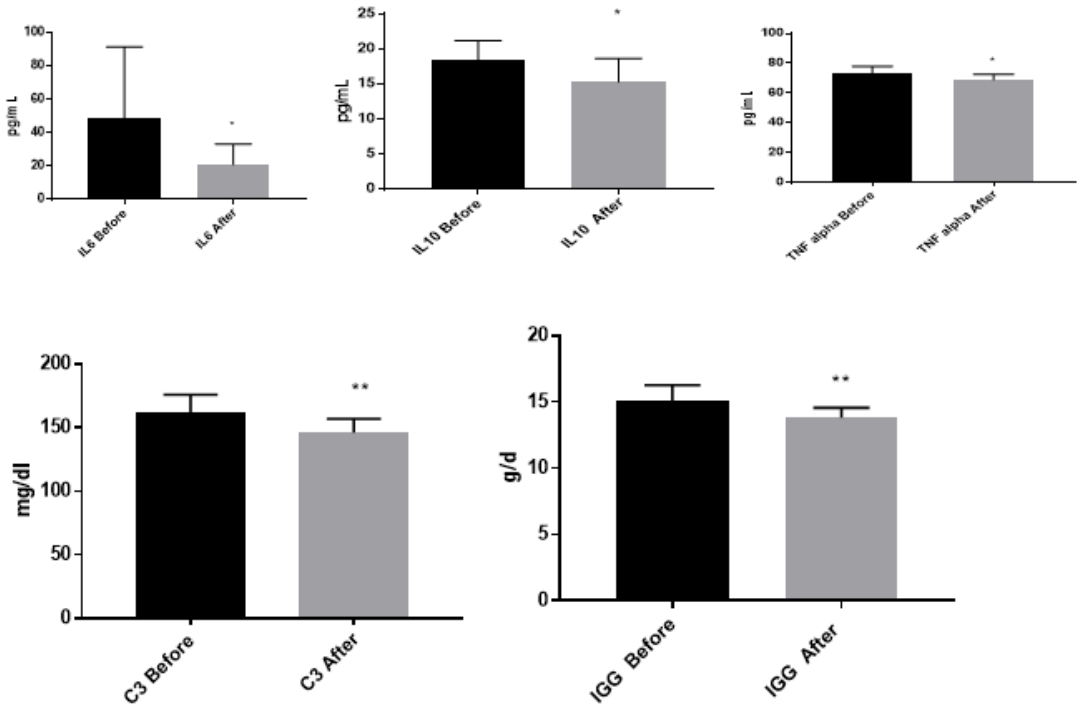


**Figure 2**: Mean ±SD levels for all inflammatory factors; IL6, IL10 and TNF alpha, C3 and IGG (pre- and long-after WC) *p < 0.05, ** p < 0.01

**Table 2:** Antioxidant parameters in the study population groups before and after (chronic) WC

| Antioxidant parameters | Before (pre-WCT) | after (post-WCT) | p-value |
|---|---|---|---|
| SOD | 0.006126±0.0031 | 0.015183±0.0233 | P = 0.0675 ns |
| GST | 0.132927±0.1049 | 0.24172±0.48363 | P = 0.1931 ns |
| GPX | 0.6839± 0.45 | 0.9013± 0.23 | P = 0.0478 * |
| GR | 0.2872±0.08850 | 0.3790±0.07211 | P= 0.0016 ** |

**Note:** Data presented as mean ± (SD) between the two study groups. The p-value = before compared to after. *p < 0.05, **p < 0.01 Superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione peroxidase (GPX) and glutathione reductase (GR)

complement activity (CH50) in experimental healthy men after one month of WC compared to the controls and experimental group before cupping. Authors indicated an obvious induction effect of WC on the complement part of the immune system, as well as modulation of the cellular part of the immune system[50] Furthermore, study on the modulation effect of WC on the immune system was demonstrated by Ahmed et al.[10]. Results in which there was marked improvement in the clinical conditions, laboratory markers (erythrocyte sedimentation rate, c-reactive protein and rheumatoid factor) innate (% of natural killer cells) and adaptive (soluble IL-2 receptor) immunological cellular parameters after one month of the combined wet cupping and conventional therapies in rheumatoid arthritis patients[10]. Niasari et al.[51] recommended the use of wet cupping as preventive measure against atherosclerosis and cardiovascular events because the authors found a substantial decline in serum LDL cholesterol level of healthy people after one week of a single wet cupping session[51]. Similar study in healthy young men reported an obvious improvement in serum lipid profile (elevation of HDL and reduction of LDL and triglycerides) after two consecutive months of Al-hijamah[52].

Regarding the IgG, IgM and IgA antibodies, our study reported a significant reduction in IgG but the decrease in IgM and IgA was insignificant (Table 1) and (Figure 2). This might be explained due to two facts. Firstly, IgG is the primary immunoglobulin found in blood circulation (represents 75% of serum antibodies) and extracellular fluid compared to IgA (mainly found in mucous secretions) and IgM (mainly produced by plasma B cells which resides in the spleen). Secondly, the basic principle of Al-hijamah is to clear the blood from toxic substances and to filter it when it passes through the superficial fenestrated skin capillaries upon application of negative suction pressure[11]. Therefore, the main antibody which is expected to be markedly removed is the IgG. However, our study detected a remarkable reduction in the level of the serum complement component C3 but no significant changes on serum complement component C4 and c-reactive protein (CRP) levels were observed (Table 1) and (Figure 2). In fact, measurement of CRP is useful for disease progression and inflammatory response[53]. This may explain the undetected effect of Al-hijamah on CRP protein, a result which was mentioned before by Hekmatpou et al.[5]. The effect of wet cupping on complement system components in healthy individuals has not been reported before. To investigate the long term effect of wet cupping on the complement part of the innate immunity, a study with frequent sessions of wet cupping on healthy individuals is recommended as measurement of serum C3 and C4 is useful in the diagnosis as well as monitoring of infectious and immune diseases.

Additionally, the current study assessed the capacity of WCT procedure in increasing the levels of various antioxidants such as superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione peroxidase (GPX) and glutathione reductase (GR) in healthy men who received this application. The biochemical findings obtained with the blood samples are illustrated in Table 2. It was demonstrated that there were significant differences between venous blood samples pre

and post WCT based on GPX and GR levels. Our results showed a significant increase in GPX and GR after the wet cupping (P = 0.0478 and P = 0.0016, respectively). However, the antioxidant efficacy of WC was insignificantly higher regarding SOD and GST. These findings generally demonstrated that WCT led to elevation in antioxidant enzyme levels, which are important in physical defense against endogenous reactive oxygen species (ROS).

In the first study which evaluated the effect of WCT on oxidant-antioxidant system, Tagil and his co-authors reported that WC removes oxidants and decreases oxidative stress[30]. However, the authors measured the differences in the level of serum nitric oxide, malondialdehyde (MAD), superoxide dismutase and myeloperoxidase activities between the venous and cupping blood samples and they did not mention any long-term effects of WC as they did not evaluated post-WCT venous blood samples[30]. The second study that mentioned a promising effect of WC in enhancing antioxidant activity was performed by Ersoy et al.[54]. Our results are in agreement with the results of this study in which Ersoy et al.[54] reported a significant increase in the antioxidant parameters: glutathione (GSH), superoxide dismutase (SOD), total antioxidant status (TAS) and catalase (CAT) after three monthly applications of WC on healthy male individuals[54]. Additionally, the authors found a significant decrease in the oxidative stress parameters MAD and total oxidant status (TOS)[54]. This study is considered as the first report which evaluated the long-term effect of WC on the antioxidant capacity in healthy men. Actually, very limited studies investigated the antioxidants effect of WC on healthy people by comparing pre and post WC levels in venous blood samples. To the best of our knowledge this is the second study in the current literature which evaluated the long-term effect of WC therapy on curbing oxidative stress and revealed a significant improvement in antioxidant parameters.

## CONCLUSION

**In healthy adult-male volunteers, WCT dramatically induce a significant drop in many inflammatory selected inflammatory mediators such as IL-6, IL-10 and TNF-α cytokines. Moreover, results illustrate that WC is promising intervention in enhancing the antioxidants level and increasing the efficiency of cells in curbing oxidative stress and limiting the toxic effects of various ROS on endogenous biomolecules. Therefore, this traditional application might be a prophylactic treatment for various ROS-induced pathologies such as tumors, cardiovascular and neurodegenerative disorders.**

---

**Authorship Contribution:** All authors share equal effort contribution towards (1) substantial contributions to conception and design, acquisition, analysis and interpretation of data; (2) drafting the article and revising it critically for important intellectual content; and (3) final approval of the manuscript version to be published. Yes.

**Potential Conflict of Interest**: None

**Competing Interest**: None

**Acknowledgments:** The authors want to thankful generally Al-Zarqa University and specifically all experts in cupping center, for there technical support during this study.

**Acceptance Date:** 21 May 2022

## REFERENCES

1. Chirali IZ. Traditional Chinese Medicine: Cupping Therapy. Elsevier Health Sciences 1999.
2. Al Bedah AMN, Khalil MKM, Posadzki P, et al. Evaluation of wet cupping therapy: systematic review of randomized clinical trials. J Altern Complement Med 2016;22(10):768-77.
3. Mehta P, Dhapte V. Cupping therapy: A prudent remedy for a plethora of medical ailments. J Tradit Complement Med 2015;5(3):127-34.
4. El Sayed SM, Mahmoud HS, Nabo MMH. Methods of wet cupping therapy (Al-Hijamah): in light of modern medicine and prophetic medicine. Altern Integr Med 2013;1-16.
5. Hekmatpou D, Moeini L, Haji-Nadali S. The effectiveness of wet cupping vs. venesection on arterial O2 saturation level of cigarette smokers: A randomized controlled clinical trial. Pakistan J Med Sci 2013;29(6):1349.
6. El Sayed SM, Al-quliti A-S, Mahmoud HS, et al. Therapeutic benefits of Al-hijamah: in light of modern medicine and prophetic medicine. Am J Med Biol Res 2014;2(2):46-71.
7. Abd al-Jawad MEM, Mohamed SA, Elsayed BA, et al. Evaluation of wet cupping therapy (Hijama) as an adjuvant therapy in the management of bronchial asthma. Indian J Physiother Occup Ther 2011;5:122-6.
8. Ahmadi A, Schwebel DC, Rezaei M. The efficacy of wet-cupping in the treatment of tension and migraine headache. Am J Chin Med 2008;36(1):37-44.
9. Soliman Y, Hamed N, Khachemoune A. Cupping in dermatology: a critical review and update. Acta Dermatovenerol Alp Pannonica Adriat 2018;27(2):103-7.
10. Ahmed SM, Madbouly NH, Maklad SS, et al. Immunomodulatory effects of blood letting cupping therapy in patients with rheumatoid arthritis. Egypt J Immunol 2005;12(2):39-51.
11. Baghdadi H, Abdel-Aziz N, Ahmed NS, et al. Ameliorating role exerted by Al-Hijamah in autoimmune diseases: effect on serum autoantibodies and inflammatory mediators. Int J Health Sci (Qassim) 2015;9(2):207.
12. Zeng K, Wang J. Clinical application and research progress of cupping therapy. J Acupunct Tuina Sci 2016;14(4):300-4.
13. AlBedah A, Khalil M, Elolemy A, et al. The use of wet cupping for persistent nonspecific low back pain: randomized controlled clinical trial. J Altern Complement Med 2015;21(8):504-8.
14. Yoo SS, Tausk F. Cupping: East meets West. Int J Dermatol 2004;43(9):664-5.
15. Al-Bedah AMN, Elsubai IS, Qureshi NA, et al. The medical perspective of cupping therapy: Effects and mechanisms of action. J Tradit Complement Med 2019;9(2):90-7.
16. Dinarello CA. Historical insights into cytokines. Eur J Immunol 2007;37(S1):S34-S45.
17. Alqaraleh M, Kasabri V, Mashallah S. Evaluation of anticancer and anti-inflammatory properties of branched chain amino acids. J Biochem Cell Biol 2018;1(2):108.
18. Alqaraleh M, Kasabri V, Al-Majali I, et al. Branched chain amino Acids as in vitro and in vivo Anti-Oxidation Compounds. Res J Pharm Technol 2021;14(7):3899-904.
19. Tartibian B, Parse R, Baghaiee B. Effect of olive oil on IL-6, TNF-α and cortisol hormone levels in active girls after one session of an exhaustive exercise: a brief report. Tehran Univ Med J 2013;71(6).
20. Ekrami N, Ahmadian M, Nourshahi M, et al. Wet-cupping induces anti-inflammatory action in response to vigorous exercise among martial arts athletes: A pilot study. Complement Ther Med 2021;56:102611.
21. Asadullah K, Sterry W, Volk HD. Interleukin-10 therapy—review of a new approach. Pharmacol Rev 2003;55(2):241-69.
22. Kalampokis I, Yoshizaki A, Tedder TF. IL-10-producing regulatory B cells (B10 cells) in autoimmune disease. Arthritis Res Ther 2013;15(1):1-12.

23. Dispenzieri A, Gertz MA, Therneau TM, et al. Retrospective cohort study of 148 patients with polyclonal gammopathy. Mayo Clin Proc 2001;76(5):476-87.
24. Agarwal S, Cunningham-Rundles C. Thymoma and immunodeficiency (Good syndrome): a report of 2 unusual cases and review of the literature. Ann Allergy Asthma Immunol 2007;98(2):185-90.
25. Markiewski MM, Lambris JD. The role of complement in inflammatory diseases from behind the scenes into the spotlight. Am J Pathol 2007;171(3):715-27.
26. Kasperska-Zajac A, Grzanka A, Machura E, et al. Increased serum complement C3 and C4 concentrations and their relation to severity of chronic spontaneous urticaria and CRP concentration. J Inflamm 2013;10(1):1-5.
27. Huang Z, Chen Y, Zhang Y. Mitochondrial reactive oxygen species cause major oxidative mitochondrial DNA damages and repair pathways. J Biosci 2020;45(1):1-17.
28. Yousef I, Oran S, Alqaraleh M, et al. Evaluation of Cytotoxic, Antioxidant and Antibacterial Activities of Origanum dayi, Salvia palaestina and Bongardia chrysogonum Plants Growing Wild in Jordan. Tropical J Nat Prod Res 2021.
29. Gonçalves RV, Costa A, Grzeskowiak L. Oxidative Stress and Tissue Repair: Mechanism, Biomarkers, and Therapeutics. Oxid Med Cell Longev 2021.
30. Tagil SM, Celik HT, Ciftci S, et al. Wet-cupping removes oxidants and decreases oxidative stress. Complement Ther Med 2014;22(6):1032-6.
31. Lin MT, Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 2006;443(7113):787-95.
32. Nathan C, Cunningham-Bussel A. Beyond oxidative stress: an immunologist's guide to reactive oxygen species. Nat Rev Immunol 2013;13(5):349-61.
33. Mei Y, Thompson MD, Cohen RA, et al. Autophagy and oxidative stress in cardiovascular diseases. Biochim Biophys Acta (BBA)-Molecular Basis Dis 2015;1852(2):243-51.
34. Tarawneh M, Al-Jaafreh AM, Al-Dal'in H, et al. Roasted date and barley beans as an alternative's coffee drink: micronutrient and caffeine composition, antibacterial and antioxidant activities. Syst Rev Pharm 2021;12(1):1079-83.
35. Khwaldeh A, Shraideh Z, Badran D, et al. Ameliorative Effect of Turmeric and Cocoa Extract against Acute Second Hand Exposure of Tobacco Smoking on Hepatocytes and Enterocytes in Albino Rats: Ultrastructural Study. Biomed Pharmacol J 2021;14(1):199-206.
36. Alqaraleh M, Kasabri V, Farha RA, et al. Branched amino acids as potential biomarkers in metabolic syndrome patients and as hypolipidemic compounds. EurAsian J Biosci 2019;13(2):2233-41.
37. Aebi H. Catalase in vitro. In: Methods in Enzymology. Elsevier 1984;105:121-6.
38. Tarawneh KA, Al-Tawarah N, Abdel-Ghani AH, et al. Characterization of verotoxigenic Escherichia coli (VTEC) isolates from faeces of small ruminants and environmental samples in Southern Jordan. J Basic Microbiol 2009;49(3):310-7.
39. Qaralleh H, Khleifat KM, Al-Limoun MO, et al. Antibacterial and synergistic effect of biosynthesized silver nanoparticles using the fungi Tritirachium oryzae W5H with essential oil of Centaurea damascena to enhance conventional antibiotics activity. Adv Nat Sci Nanosci Nanotechnol 2019;10(2):025016.
40. Khan MS, Mudassar S, Hameed M, et al. Effect of Age on Antioxidant Enzymes in Women Folk. JMS Ski 2012;15(2):119-22.
41. Hunaiti A, Soud M, Khalil A. Lead concentration and the level of glutathione, glutathione S-transferase, reductase and peroxidase in the blood of some occupational workers from Irbid City, Jordan. Sci Total Environ 1995;170(1):95-100.
42. Jakoby WB, Habig WH. Glutathione transferases. Enz Bas Det 1980;2:63-94.
43. Khleifat K, Abboud M, Al-Shamayleh W, et al. Effect of chlorination treatment on gram negative bacterial composition of recycled wastewater. Pak J Biol Sci 2006;9:1660-8.
44. Al-Asoufi A, Khlaifat A, Al Tarawneh A, et al. Bacterial Quality of Urinary Tract Infections in Diabetic and Non Diabetics of the Population of Ma'an Province, Jordan. Pak J Biol Sci 2017;20(4):179-88.
45. Qureshi NA, Ali GI, Abushanab TS, et al. History of cupping (Hijama): a narrative review of literature. J Integr Med 2017;15(3):172-81.
46. Bilal M, Khan RA, Afroz S. Partial evaluation of technique used in cupping therapy. J Basic Appl Sci 2011;7(1).
47. Arslan M, Yeşilçam N, Aydin D, et al. Wet cupping therapy restores sympathovagal imbalances in cardiac rhythm. J Altern Complement Med 2014;20(4):318-21.
48. Cao H, Li X, Liu J. An updated review of the efficacy of cupping therapy. PLoS One 2012;7(2):e31793.
49. Aleyeidi NA, Aseri KS, Matbouli SM, et al. Effects of wet-cupping on blood pressure in hypertensive patients: a randomized controlled trial. J Integr Med 2015;13(6):391-9.
50. Khalil AM, Al-Qaoud KM, Shaqqour HM. Investigation of selected immunocytogenetic effects of wet cupping in healthy men. Spat DD 2013;3(2):51-7.
51. Niasari M, Kosari F, Ahmadi A. The effect of wet cupping on serum lipid concentrations of clinically healthy young men: a randomized controlled trial. J Altern Complement Med 2007;13(1):79-82.
52. Refaat B, El-Shemi AG, Ebid AA, et al. Islamic wet cupping and risk factors of cardiovascular diseases: effects on blood pressure, metabolic profile and serum electrolytes in healthy young adult men. Altern Integ Med 2014;3(1):151.
53. Ismailov RM, Shevchuk NA, Khusanov H. Mathematical model describing erythrocyte sedimentation rate. Implications for blood viscosity changes in traumatic shock and crush syndrome. Biomed Eng Online 2005;4(1):1-16.
54. 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.