Complementary and alternative medicine has grown in the past decades to be a major field of medicine with many of its systems having been incorporated into university-level degrees worldwide. Wet cupping (Al-hijamah) is a therapeutic approach that is believed to combat illness and promote well-being. Some may claim that wet cupping differs from Al-hijamah, and that the latter has been used even in old civilizations. According to Baghdadi et al., although the latter is more comprehensive, the term wet cupping refers to both Al-hijamah and Chinese wet cupping. Wet cupping therapy (WCT) is a combination of dry cupping therapy and bloodletting. A practitioner places glass cups on precise acupoints on the patient's skin to produce a therapeutic outcome. A thorough review of research articles on wet cupping with relevance to proteomics field that are indexed by Google Scholar, PubMed, and/or Science Direct databases was performed. Eight original research articles were summarized in this paper. Overall, wet cupping did not have a significant effect on C-reactive protein, Hsp-27, sister chromatid exchanges, and cell replication index. In contrast, wet cupping was found to produce higher oxygen saturation, eliminate lactate from subcutaneous tissues, remove blood containing higher levels of malondialdehyde and nitric oxide, and produce higher activity of myeloperoxidase. The proteomic effects of wet cupping therapy have not been adequately investigated. Thus, future studies on wet cupping that use systemic and sound protocols to avoid bias should be conducted.
relieves pain, reduces LDL and LDL/HDL ratio values, and improves the quality of life of patients with chronic illnesses. Previous studies showed that the combination of wet cupping and iron chelation therapy has positive effects, and it may even be better than phlebotomy. It was reported that treatment of rheumatoid arthritis using WCT may help enhance the health of the general body clinically and minimize severity of pain with no significant side effects. According to Baghdadi et al, mechanism of action of WCT on rheumatoid arthritis could be attributed to reducing in levels of serum ferritin, autoantibodies, and inflammatory mediators which will lead eventually to improve the natural immunity against pathogens. In fact, mechanism of action of WCT still unknown as there are several methods of WCT and each method has its own mode of action which could illustrate the effect of WCT. For example, neural, hematological, psychological, and antioxidant effects were reported in case of studying the effects of WCT on chronic neck pain. Wet cupping therapy will lead to lowering the blood pressure of tissues at the pain area and evacuation of the inflammatory agents and toxins will happen, this will lead eventually to improvement in the lymph and blood flow. Proteins are the functional units of the human body. Genomics and proteomics, at least in principle, hold the promise of an unbiased and systematic discovery route. Clinical proteomics has become more popular, because it has much to tell about diseases at the cellular functional level as opposed to the molecular genetic level—the traditional tool of diagnosis, treatment, and monitoring of chronic diseases. Studies on this therapeutic approach are limited and using of this technique in several developing countries has yielded positive effects, whereas it has yielded negative effects on use in Western countries. Unfortunately, this has made the use of wet cupping extremely controversial, although it was found to be safe based on long-term clinical use. Previous studies on wet cupping are limited, and several of them are influenced by cultural, social, and religious factors that cause a risk of bias. Randomized clinical trials in this field also showed a variable risk of bias across all domains and have methodological limitations.

In this review, we focused on the proteomic analysis on the relevant effects of wet cupping to obtain useful information regarding possible research targets. This work may contribute to the elucidation of the actual measurable effect of bloodletting therapy in the future and can provide researchers with a sound scientific perspective when investigating the technique of wet cupping.

**Strategy of searching history.** The implemented strategy was to explore the relevant publications indexed by Google Scholar, PubMed, and/or Science Direct databases. Keywords, such as wet cupping, hijamah, and al-hijamah, were used to search for publications between the years 2012 and 2017. Approximately 104 articles related to the keyword used were produced at the initial search. But, only 8 articles were obtained after searching within the scope of the current review and excluding the books, duplicates, abstracts, conference proceedings, case report, and review articles (Figure 1).

**Inclusion criteria.** Studies conducted on humans or animals from January 2012 to July 2017 to evaluate the proteomic effect of WCT. Only full text articles were included.

**Exclusion criteria.** Articles published before January 2012 or after July 2017. Books, conference proceedings, and reviews, papers that were published in languages other than English with no available translation were excluded.

Studies that evaluate the effects of cupping treatment on mystical elements, such as Chi, and those that explain the efficacy of cupping through its effects on immeasurable or arbitrary factors were not considered in this review.

Based on the database search, a total of 8 articles on wet cupping therapy with relevance to the field of proteomics were published within the last 5 years. A list of the primary objectives, major findings, and study limitations of all publications cited in this work is presented in Table 1. In general, most studies were primarily conducted using human participants. Only one was found to have utilized rodents. The quantity of blood drawn and the period of suction after scarification varied among the studies. Most human WCT practitioners preferred the trapezius muscle region. Overall, the articles included in the study originated from various institutions worldwide, which are located in 5 different countries (Iran, Germany, Jordan, and Turkey).

**Proteomic effects of wet cupping therapy on human.** In Iran, a randomized control clinical trial was conducted to compare the efficacy of wet cupping and venesection on the arterial oxygen (O₂) saturation level of smokers. Male smokers (n=110) with chronic obstructive pulmonary disease were included and divided into 2 groups. One group received wet cupping treatment,
whereas the other groups were subjected to venesection. Hemoglobin and hematocrit levels were evaluated, and arterial O₂ saturation was measured using a pulse oximeter 6 and 12 hours after treatment. No significant change in the mean arterial O₂ saturation level was observed 6 or 12 hours after venesection. On the other hand, wet cupping resulted in a trend of enhanced arterial O₂ after 12 hours of treatment. A study was conducted to evaluate the effect of wet cupping therapy on sister chromatid exchanges (SCE), cell replication index (CRI), and some components of the immune system. Forty-four healthy men were recruited and divided into 2 groups based on age. Wet cupping was performed on the 32 participants, whereas the remaining participants were included in the control group. The procedure involved the trapezius muscle between the 2 scapula regions, and an average of 20-25 ml of blood was blood-letting. Measurements were taken prior to treatment and one week and one month after the use of venous blood. No significant changes on SCE, CRI, red blood cell count, or hemoglobin levels were observed. However, granulocyte levels significantly increased one month after treatment, with a clear enhancement of the complement activity in older individuals.

Researchers attempted to evaluate the metabolic changes in the tissues under the cupping glass. Volunteers with chronic neck pain were recruited (n=6) for the study, in addition to 6 participants who were included in the control group. The method used was dry cupping. However, the results could be extrapolated to other cupping techniques that used vacuum directly to the trapezius muscle, such as wet cupping. A microdialysis system was implanted subcutaneously on both sides (right and left) of the neck to measure lactate, pyruvate, glucose, and glycerin levels. Cupping was performed for 15 min, and the procedure could lead to an increase in the lactate/pyruvate ratio after 160 minutes of intervention, indicating a prolonged anaerobic metabolism in the surrounding tissue.

In 2012, a pilot study was conducted to assess the effectiveness of a single WCT on chronic non-specific neck pain (CNP) as well as mechanical sensory thresholds. In this study, 50 patients were randomly distributed into 2 groups (25 patients in the treatment group and 25 were kept as control group). Treatment group was subjected to a single cupping treatment and several parameters were measured before and 3 days after treatment and compared with the control group. These parameters include pain at rest (PR), disability index (NDI), mechanical detection (MD), pain related

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**Figure 1** - Flow-chart showing the strategy for searching relevant articles.
Proteomic effects of wet cupping ... Almaiman

Table 1 - Summary of studies investigated the beneficial proteomic effects of cupping therapy.

| Target | Methodology | Cohort | Major findings | Limitations | Ref. |
|--------|-------------|--------|----------------|-------------|------|
| To compare the efficacy of WCT and venesection on arterial O₂ saturation level of smokers up to 12 hours. | Vacuum was created and 50-75 ml of blood was drawn. It repeated 3 to 5 times, each lasting for 3 to 5 min. | 110 male smokers (55 TG and 55 CG) with chronic obstructive pulmonary disease | Wet cupping and venesection increased O₂ saturation level up and beyond 12 hrs after treatment. | Sample size did not allow for the observation of the effects on different cupping sites and manners of WCT. | 14 |
| To assess the effects of WCT on selected immunocytogenetic parameters for one month follow up. | Vacuum was created at the trapezius muscle before skin puncturing and drawing 20-25 ml of blood into plastic cups. | 44 healthy men | No effect on either sister chromatid exchanges or cell replication index. | Small sample size. No phlebotomy group was used as control. | 29 |
| To investigate the mechanisms of WCT by detecting metabolic changes in the tissue under the cups for 280 minutes after cupping. | A microdialysis system was implanted subcutaneously on both sides (right and left) of the neck before dry cupping to measure metabolic changes every 20 min during baseline measures and for 280 min after cupping. | 12 subjects (6 with neck pain and 6 CG) | Increased lactate/pyruvate ratio, indicating anaerobic metabolism in the surrounding tissue. Increased immediate PPT in some areas. | To avoid influencing the microdialysis measures, the local cupping effect on the neck was not measured. | 30 |
| To assess the effectiveness of a single WCT on CNP as well as mechanical sensory thresholds up to 3 days after cupping. | TG was subjected to a single cupping treatment and several parameters were measured before and 3 days after treatment and compared with CG. | 50 patients (25 TG and 25 CG) | No significant effects were observed for NDI, MD, PM, and VD. | Effects of repeated TCI at different time points for long-term are required. Investigation on mechanisms of action is paramount. | 24 |
| To investigate the effects of WCT on hs-CRP and Hsp-27 antibody titers after treatment for 6 and 12 weeks. | Vacuum was created on the skin overlying the trapezius muscle at the level of scapular spine before scarifying the skin and drawing blood into glass cups. | 126 metabolic syndrome patients | No effect on either hs-CRP or Hsp-27 levels. | WCT was performed only once coupled with a special regimen for ethical purposes. No phlebotomy group was used as control. | 31 |
| To investigate the effects of WCT on oxidative stress. | Five points of the posterior neck, bilateral perispinal areas of the neck and thoracic spine were selected for WCT; and 3 to 5 ml were drawn from each point to measure MPO, SOD, MDA, and nitric oxide. | 31 healthy subjects (15 females & 16 males) | A higher activity value for MPO. Lower activity of SOD. Higher levels of MDA and nitric oxide. | Post-treatment venous blood samples were not collected and the effects on oxidative stress could not be observed | 23 |
| To assess the effect of WCT on high blood pressure patients and the occurrence of possible side effects. | TG was subjected to WCT and conventional hypertension treatment. CG underwent only conventional hypertension treatment. Follow-up was done for 8 weeks. | 80 participants (40 TG & 40 CG) | Reduction of the systolic blood pressure was observed immediately and after 4 weeks. No statistically significant reduction was observed in blood pressure of TG compared to CG. | Small sample size. Effect of WCT alone, without any associated anti-hypertension therapy need to be done. | 20 |
| Animal study | WCT was done for one time only at the left and right paralumbar regions. After 24 hr, blood were collected from the skin subjected to WCT to determine Hsp-70 and ß-endorphin. | 16 male Wistar rats (8 TG + 8 CG) | WCT induced the expression of Hsp-70 and ß-endorphin. | The results need to be demonstrated clinically in human subjects. | 32 |

WCT - wet cupping therapy, Hsp-70 - heat shock protein 70, hs-CRP - high-sensitivity C-reactive protein, SOD - superoxide dismutase, MDA - malondialdehyde, MPO - myeloperoxidase, TG - treated group, CG - control group, TCI - traditional cupping interventions, CNP - chronic non-specific neck pain, PR - pain at rest, PM - pain related to movement, NDI - neck disability index, MD - mechanical detection, VD - vibration detection, PPT - pressure pain thresholds.

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to movement, quality of life (SF-36), neck, vibration detection (VD), and pressure pain thresholds (PPT). At the beginning, no significant differences were detected in any of the measured parameters between the treatment group and control group. Treated group showed significant effects in the level of pain at rest, quality of life and body pain compared to control group. Whereas, no significant effects were observed for disability index, mechanical detection, pain related to movement, and vibration detection. Significantly higher PPT at pain-areas were noticed among the treated group compared to control group (p<0.01). It was concluded that single application of WCT could exert significant effects on quality of life, body pain.

Based on the clinical data collected from 113 metabolic syndrome patients, the effects of wet cupping on the levels of high-sensitivity C-reactive protein (hs-CRP) and Hsp-27 antibody titers were investigated. High-sensitivity C-reactive protein and Hsp-27, which are known markers of cardiovascular diseases, were measured before treatment, and 6 and 12 weeks after wet cupping of the interscapular region using a cobas autoanalyzer and an in-house Enzyme-linked immunosorbent assay (ELISA) kits, respectively. Results showed that no remarkable effects were observed on the levels of both proteins. Thus, wet cupping did not reflect a significant effect on the markers of cardiovascular diseases.

To assess the effect of wet cupping therapy on oxidative stress, researchers recruited 31 healthy participants. The participants underwent wet cupping therapy and donated some venous blood concurrently. The levels of nitric oxide and malondialdehyde (MDA) in the serum blood samples were measured. The activities of superoxide dismutase (SOD) and myeloperoxidase (MPO) were also measured using a spectrophotometer. A higher MPO and lower SOD activity was observed based on the blood drawn through wet cupping. Moreover, compared to venous blood, wet cupping blood had higher levels of MDA and nitric oxide, indicating that the technique was beneficial in terms of oxidant removal from the body.

Another one study was carried out at King Abdulaziz University Hospital, Jeddah, Saudi Arabia, from May 2013 to February 2014 to evaluate the effect of WCT on high blood pressure patients and the occurrence of possible side effects. Eighty participants (40 participants were undergo WCT and conventional hypertension treatment and another 40 were subjected to hypertension treatment only) were included in this 8 weeks follow up study with age ranged between 19-65 years for both gender. The result of this study exhibited that WCT reflects reduction in the systolic blood pressure immediately and after 4 weeks. While, after 8 weeks no statistically significant difference was observed in blood pressure of treated group compared to control group.

**Proteomic effects of wet cupping therapy on animals.** A study was conducted in male rats to study the relationship between heat shock protein-70 (Hsp-70) and β-endorphin levels and pain threshold after wet cupping therapy. A total of 16 animals were used (8 for the control group and 8 for the treatment group), and all animals were treated with complete Freund’s adjuvant (CFA). The procedure was performed at the left and right paralumbar regions. After a 24-hour period, all rats were tested for pain threshold via the hot plate test. The animals were then sacrificed, and their paralumbar skin was excised to determine the Hsp-70 and β-endorphin levels using monoclonal antibodies. The Hsp-70 and β-endorphin expression was significantly higher in the treatment group, which had a higher pain threshold after WCT. This result indicated that pain reduction via WCT was mediated by the expression of these 2 proteins.

**Discussion.** Wet cupping has been practiced for centuries. It was used by famous physicians, such as Galen (131-200 AD), Paracelsus (1493-1541), and Ambroise Paré (1509-1590). It is a low-cost therapeutic technique with no major adverse effects when performed in sanitary conditions. According to Cao et al the majority of the published studies on cupping therapy between 1959 and 2008 have utilized the wet cupping technique, followed by retained cupping, moving cupping, and other kinds of cupping. However, the mechanisms of the effects of wet cupping have not been explored.

There are different types of cupping which include wet cupping, flash cupping, medicinal cupping, retained cupping, moving cupping, and needle cupping. At the beginning of cupping therapy process, physicians are using different kinds of cups such as glass, bamboo, and earthen cup to fix it on specific area on skin of patient, then hyperemia and/or hemostasis process will arise.

Practitioners claim that wet cupping helps eliminate causative pathologic substances (CPSs) through pressure-dependent and size-dependent non-specific filtration. Unlike bloodletting from a venous source, wet cupping allows blood to be drawn from capillary tubes, which may include some lymph fluids that modify its concentration and eliminate waste materials. However, it was recently hypothesized that the technique is beneficial because it promotes the elimination of oxidants from the blood. Overall, the possible mechanisms of wet
cupping may be classified as neurological/psychological and hematological/immunological. One published theory about the possible mechanism of lowering blood pressure of hijamah named the “Taibah Theory”, stated that hijamah helps to remove the interstitial fluids, extra intravascular fluid, and destructive or harmful substances from the body. This theory also postulates that hijamah lead to elimination of free radicals and vasoactive fluids. In addition, stimulation of production of nitric oxide which will help to keep the balance between antioxidants and free radicals.19

Usually, WCT could be achieved by gender-steps procedures which include skin segregation, decontamination, cupping, puncturing, cupping and sterilization. In general, during WCT procedures, fluids in the tissues will filtered from the capillary walls to lymphatic capillaries and return back to vascular system. There is a continuous exchange between the intravascular fluid and post capillary venules through the interstitial fluid of the end products of metabolism and free radicals formed inside the cells.37

Type of disease and pathological stage will determine the site of occurrence of chemical and biological substances, bacteria, toxins, pro-inflammatory cells, and inflammatory cells which could be detected through plasma and/or blood samples. Many factors are playing a vital role to determine the contents of the interstitial fluids. These factors include permeability and surface area of capillaries, and lymph transport. In addition, physical and chemical properties of the interstitial fluid itself would have an effect on its components.37

The effects of cupping on glycolysis or any mitochondrial energy production mechanisms have not been verified. In the present review, wet cupping was not observed to have a positive effect on hs-CRP, Hsp-27, sister chromatid exchanges, and cell replication index. In contrast, the procedure was found to produce higher O2 saturation, eliminate lactate from subcutaneous tissues, and remove blood that contained higher levels of MDA and nitric oxide. In rodents, WCT resulted in the increased expression of both Hsp-70 and β-endorphin at the local site of cupping.32

The insignificant effect of cupping may be due to the wide observation gap between treatment and measurement, which in some studies exceeded a month. Moreover, several researchers attempted to observe the effect of WCT after a single treatment.27 In the past, cupping had a favorable outcome after a series of treatments.38 However, due to limited data, whether or not wet cupping has therapeutic effects cannot be verified. Both the short-term and the long-term effects of the intervention should be verified before drawing any conclusions, and the amount of blood that is collected during the procedure should be considered.

Studies on the wet cupping technique are significantly limited and lack comprehensiveness. Furthermore, although the Chinese government recently made an effort to promote the national standardization of cupping therapy.35,38 Wet cupping is yet to be fully standardized, which made the results of various studies on the technique practically incomparable due to differences in terms of the treatment method and protocol. One of the limitations of the current study is that this study explored only the published research from 2012 to 2017 from few websites, so few researches were obtained in this review, accordingly the general conclusion will not be strong enough to take it as an evidence for the future research. Also, this study focused on the general evaluation of the effect of wet cupping therapy on proteomic analysis in different diseases which should be carried out for each disease separately to enable us evaluating the actual changes which could be occurred in that specific disease. A comprehensive study using better and more comprehensive protocols combined with molecular genetics when investigating the therapeutic effects of wet cupping on specific ailment is highly recommended.

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