IMPACT: International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS)
ISSN(E): 2321-8851; ISSN(P): 2347-4580
Vol. 4, Issue 2, Feb 2016, 93-104

EFFECT OF CUPPING TREATMENT ON SOME BIOCHEMICAL VARIABLES OF THI-QAR PROVINCE

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ABSTRACT

The aim of this study was to investigate the effect of of cupping treatment on serum glucose , serum oxidation-antioxidants status. Using the lipid peroxidation marker, malondialdehyde (MDA) and preventative antioxidant Ceruloplasmin (Cp) and Albumin (Alb), in serum of volunteer

Blood samples were obtained from (100) volunteer (before and after cupping treatment), as well as (35) healthy subjects as a control group. They divided into two groups as the following:

Control Group: - Included Thirty fivehealthy subjects aged (20-70 years).

Pre Cupping Group: - Included one hundred volunteer before cupping aged (20-70 years).

Post Cupping Group: - Included one hundred volunteer after cupping aged (20-70 years).

Results: The results showA significant decreased can be observed in post cupping group when comparison with pre cupping group ($p \leq 0.05$), while a non-significant different in the level of serum glucose can be observed in post cupping group in comparison with control group ($p \leq 0.05$). Serum MDA also shown A significant decrease can be observed in post cupping group in comparison with pre cupping group ($p \leq 0.05$), while a non-significant differs in the level of serum MDA can be observed in post cupping group in comparison with control group ($p \leq 0.05$). As well pre cupping group shown that significant increase ($p \leq 0.05$) in serum MDA when comparison with post cupping and control groups. While serum Cp levels shown that significant increase ($p \leq 0.05$) in serum CP can be observed in pre and post cupping groups when compared to with control group, as well as post cupping group show significant decrease ($p \leq 0.05$)in serum CP in comparison with pre cupping group. As well as serum Alb show that significant increase ($p \leq 0.05$) in serum Alb can be observed in pre and post cupping groups when comparison with control group, as well as post cupping group shows a significant decrease ($p \leq 0.05$)in serum Alb in comparison with pre cupping group.

KEYWORDS: Cupping, Volunteer, Oxidant-Antioxidants Status

INTRODUCTION

Cupping is one of the oldest and most effective methods of releasing toxins from the body's tissues and organs, Cupping therapy is a simple procedure in which negative pressure is applied to the skin through sucking cups (dry cupping therapy) **(1).** Cupping therapy for treating hypertension, neck pain, headache, chronic hepatitis, ophthalmic diseases, skin diseases and infectious diseases **(2**). Blood diseases such as hemophilia, hypertension, rheumatic conditions, pain relief, inflammatory conditions, mental and physical relaxation **(3),** migraine headache **(4)** polycythemia, hemochromatosis **(5),**

hyperlipidemia **(6),** menopause syndrome **(7)** pain of the knee, liver diseases, renal and uretric colic and other diseases **(8).** The main purpose of this therapy is to precipitate the circulation of blood and to remove blood-stasis and waste from the body. Local damage of the skin and capillary ves-sels may act as a nociceptive stimulus **(9).** Cupping is thought to remove noxious materials from skin microcirculation and interstitial compartment **(10).** Wet cupping has been claimed to drain excess fluids and toxins, loosen adhesions and lift connective tissue, bring blood flow to skin and muscles, and to stimulate the periph-eral nervous system **(11).** Also, cupping is said to reduce pain and high blood pressure as well as modulate neurohormones and the immune system **(9).** Cupping therapy is also used to improve subcutaneous blood flow and to stimulate the autonomic nervous system **(9).**

There are 2 types of Cupping (hijama) is:Dry cuppingthis is the process of using a vacuum on different areas of the body in order to gather the blood in that area without incisions (small, light scratches using a razor) **(12).**Wet cuppingThis is the process of using a vacuum at different points on the body but with incisions in order to remove 'harmful' blood which lies just beneath the surface of the skin (It is recommended that wet cupping is only administered by a cupping therapist) **(12).**

According to the findings of the literature there have not been many side effects associated with cupping. There was one case of panniculitis **(9).** Other side effects for wet cupping are an increased risk of infections Hepatitis B and C, HPV and HIV as in the ancient medicine one horn was used for many patients. Recent studies however showed that no infections were apparent when sterile methods were used **(13).** Cupping practice today is commonly using sterile methods thus reducing the likelihood of unwanted side effects. Farhadi *et al.* (2009) reported that the adverse effect with wet cupping was fainting (vaso-vagal syncope) but it was seen only in the younger patients. Other general side effects are circular ecchymosis lesions as cupping breaks the superficial blood vessels in the papillary dermis **(9).** The immediate effects of cupping are ‘erythema, swelling, bruising, bleeding and bullae formation’ due to the cutting of the skin for bloodletting. Some individuals had scars (6 %) whereas some had hyper pigmented lesions (4%). These effects were only short term and completely healed within a maximum of 3 weeks. Some individuals reported a slight discomfort which was technique based rather than the effect of cupping **(14).**

Diabetes Mellitus (DM)

Diabetes Mellitus means an increase in blood glucose level related to a group of etiology disorders resulted in difficulties in carbohydrates, proteins and fats metabolism. The result is absolute or estimated disorders in providing of insulin or lack of its functions **(15).**Generally Beta cells are responsible for providing insulin in response to any increase in blood serum glucose. Any disorder in functions of beta cells may be resulted in loosing of insulin. Regarding the low capacity of antioxidants enzyme beta cells are ready for being destructed by free radicals **(16).** Furthermore some of the safety cells including macrophages and B & T cells are able to produce free radicals which may cause further damages to beta cells **(17).** On the other hand, oxidative stress has a great role in diabetes pathogens **(18).**

Lipid Peroxidation (LPO)

lipid peroxidation (LPO) can be defined as the oxidative deterioration of lipid containing a number of double bonds between carbon **(19).** It has been suggested that an increase in the free radicals may cause neuronal degeneration through lipid peroxidation and a decrease in the glutathione peroxidase levels. The lipid peroxidation product,

malondialdehyde (MDA) is commonly used as a measure of the oxidative stress in cells. Lipid peroxidation, being a free radical reaction, it occurs when the hydroxyl radicals, possibly oxygen, react with the unsaturated lipids of the bio-membranes, resulting in the generation of lipid peroxide radicals (ROO•), lipid hydroperoxide (ROOH) and fragmentation products such as MDA **(20;21).**

Malondialdehyde, is a highly reactive three carbon dialdehyde produced as a byproduct of polyunsaturated fatty acid peroxidation **(22)** and also through arachidonic acid metabolism for the synthesis of prostaglandins **(23)** . MDA can combine with several functional groups on molecules including proteins, lipoproteins, RNA and DNA **(24).** MDA has been reported to be induced in various conditions and chronic disease states such as smoking, hepatitis C infection, and HIV seropositive children and diabetes **(25).**

Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols **(26).**

Ceruloplasmin (CP) is the major plasma copper binding protein but it is generally agreed that the main function of CP is to participate in iron homeostasis due to its ferroxidase activity through the conversion ferrous to ferric iron **(27).** It also inhibits the peroxidation of membrane lipids catalyzed by metal ions, such as iron and copper. The site of synthesis of this enzyme appears to be the liver **(28)** and plasma ceruloplasmin activities are dependent on liver copper reserves, with lowered activities reflecting a depletion of these reserves. CP is a serum ferroxidase that contains greater than 95% of the copper found in plasma **(29).** CP is an enzyme which has a role as an oxidant or antioxidant depending on the existence of Fe ions and similar material levels in the micro base, and it also scavenges superoxide anion radical ($O^{\bullet -2}$)**(30).**

Albumin is a highly soluble multidomain protein, without prosthetic groups or bulky appending carbohydrates, that is very stable and available at high purity and low cost **(31).** Albumin synthesized by the liver after loss of a 24-residue propeptide and immediately secreted into the bloodstream without being stored **(31).,** consisting of 585 amino acids with M.W. of approximately 66,248 Dalton . It is most abundant in human plasma. Usually, it constitutes about 55–60% of all plasma proteins and has a serum half-life of about 20 days **(31)**

PATIENTS *AND* METHOD

Design of Study

This study conducted at Bent alhuda Hospital in Thi-Qar, Biochemistry Laboratory in College of Science, at the period between 1/12/2014 to 25/5/2015. It included (135) cases, (35) control and (100) volunteer.

Table 1: Data of Patients and Controls Groups

Groups	NO.
volunteer	100
Controls	35

There were (135) male, control and Volunteer whose age are (20-70) years were included in this study. They have been divided into two groups as the following :-

Contrl Group:- included thirty-fife (35) healthy subjects aged (20-70) .

Volunteers Group: - included 0ne-handered (100) Persons who conducted the cupping process more than once aged (20-70) .

Collection of Blood Samples

About (5mL) of blood samples from volunteer of cupping treatment and controls were taken and allowed to clot at room temperature in empty disposable tubes centrifuge to separate it in the centrifuge at 3000 rotor per minute (rpm)for 10min,the serum samples were separated and stored at (-20ºC) for later measurement of biochemical parameters, unless used immediately.

BIOCHEMICAL PARAMETERS

Diabetes Mellitus (DM)

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts , under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red- violet quinoneimine dye as indicator **(32).**

Lipid Peroxidation Marker (Serum MDA)

Determinations of serum MDA level that consider as a lipid peroxidation marker were performed according to the method of **Muslih (33)**. MDA concentrations were calculated, using the molar extinction coefficient of MDA (ε_{MDA}) equal to 1.56 x105 mol-1. Cm-1 **(34)**. MDA formed from breakdown of polyunsaturated fatty acid, serves as a convenient index of peroxidation reaction.

Serum Antioxidants

Serum Cp concentration was measured by the method of Menden et al.,1977 **(35)** which using the extinction coefficient of Cp (ε Cp) equal to (0.68) to calculate it concentration. The bromocresol green (BCG) method, colorimetric method, is the simplest technique which have been developed to determine Alb concentration **(36)**.

Statistical Analysis

Statistical analysis was done using the software SPSS version 17.0; the results were expressed as mean ± standard deviation (mean ± SD). One way ANOVA-test was used to compare parameters in different studied groups. P-values ($P \leq 0.05$) were consideredstatistically significant.

RESULT AND DISCUSSIONS

Serum Glucose

Table (2) showeda significant decreased can be observed in post cupping group when comparison with pre cupping group ($p \leq 0.05$), while a non-significant different in the level of serum glucose can be observed in post cupping

group in comparison with control group ($p \leq 0.05$). As well pre cupping group shown that significant increase ($p \leq 0.05$) in serum glucose when comparison with post cupping and control groups.

Cupping appears to have a positive effect on diabetes which was reduced after cupping, The suction force generated by cupping working on output intestinal waste products from the Portal circulation in the liver which increases the metabolism within the liver are considerably less sugar, The suction force generated by cupping the output.Also working acids (Hexosamines) of muscle and fatty tissue under the skin, allowing the insulin receptors in conjunction receptors and increased his sensitivity, which reduces the sugar**(37),**Also (No) nitric oxide that secrete Striping with cupping to stimulate blood circulation in the pancreas and lead also to adjust the rate of insulin **(38).,** from the point of view of therapeutic disease, These results can be interpreted that there is an increase in the amount of free radicals in diabetes mellitus, today one of the important events is the role of free radicals in reducing of the defense system in diabetes. So cupping is able to enrich the Antioxidation and prevent the progress of the effects and control of any production free radicals and safety from cells such as macrophages and T, cells B (3,4,14,15). In another study by Shariat Zadeh *et al.*2000 under the title effects of cupping on oxidative stress, it was obvious that cupping may cause a reduction in various oxidative stress indexes **(39).**One of the factors is the high sugar shortage of blood perfusion, which causes non-members the ability to do its activity and therefore weak activity occurs (as it weakens the activity of the pancreas responsible for the sugar high vulnerability of blood supply. Body and respond to ischemia liberalization of glucose (sugar) to raise the activity of its members, but unfortunately Perhaps is not burning and the ability, but a lack of blood perfusion that weaken the members and this is what accounts for the healing of many diabetic patients after their implementation of cupping immediately(**39).**

The results of this study are in contrast with previous studies. It seems that the reason is the number of made cupping in this study. Totally we can say that cupping will cause improvement in blood factors of diabetes patients. But since there is no exact mechanism for it, it is proposed to evaluate cupping as a complementary method.

Blood sugar, were further reduced after blood cupping. Blood cupping enhances insulin sensitivity in healthy donors with normal glucose tolerance and normoferritinemia **.(40).**

This result was found to be consistent with the previous studies such as **(41),** and consistent with**(42).**

Lipid Peroxidation Status (Malondialdehyde)

The results of serum MDA concentration are shown in table (3), A significant decrease can be observed in post cupping group in comparison with pre cupping group ($p \leq 0.05$), while a non-significant differs in the level of serum MDA can be observed in post cupping group in comparison with control group ($p \leq 0.05$). As well pre cupping group shown that significant increase ($p \leq 0.05$) in serum MDA when comparison with post cupping and control groups.

The mechanism action of wet cupping therapy is not well known despite its common use. Wet cupping therapy might act through a lot of different mechanisms. We hypothesize that one of the mechanisms of action of wet cupping may be through oxidative balance. The cardinal findings of this study include that: (i) compared with the venous blood, wet cupping blood has higher activity of myeloperoxidase (MPO) (ii) lower activity of superoxide dismutase (SOD), (iii) and higher levels of Nitric oxide (NO_x) **(43).**

The therapeutic effects of wet cupping in various conditions might be due to the excretion of these oxidants from

the body**(43).**In conclusion, wet cupping removes oxidants and decreases oxidative stress**(43)** .

Serum Antioxidants

Serum Ceruloplasmin Concentration

Table 4 showeda significant increase ($p \leq 0.05$) in serum CP can be observed in pre and post cupping groups when compared to with control group, as well as post cupping group show significant decrease ($p \leq 0.05$)in serum CP in comparison withprecupping group**.**

Ceruloplasmin is a copper-containing enzyme. It oxidizes a variety of amines including epinephrine, melatonin, serotonin, para phenylene diamine **(44).** It also converts ferrous ion (Fe^{+2}) to ferric ions (Fe^{+3}) and hence is required for utilization of iron, hence also known as ferroxidase I **(45).** Ferroxidase ceruloplasmin (Cp), which is produced by the liver and secreted into the plasma, also plays an important role in the movement of iron. By oxidizing the ferrous [Fe (II)] form of iron to the ferric [Fe (III)] form, Cp promotes iron loading onto transferrin, which only binds the ferric form of the metal **(46).** In addition, Cp is an effective antioxidant, because of its ability to oxidize highly toxic ferrous iron to the relatively nontoxic ferric form and thus help prevent oxidative damage to proteins, lipids, and DNA **(47) .**

Recent studies support a role of Cp in regulating nitric oxide (NO) homeostasis.2 Isolated Cp was shown capable of catalytically consuming.

NO through NO oxidase activity, and plasma NO oxidase activity was decreased after Cp immunodepletion,**(48).**

Cp functions as an NO oxidase in vivo, suggesting that Cp elevations may lead to decreased NO bioavailability and endovascular dysfunction. **(48).**The reason that cupping works may be due to its physiological affect, as described above by either stimulating or relaxing the body by bleeding **(49).**

Ceruloplasmin serves as a ferroxidase that converts toxic ferrous iron to nontoxic ferric ion, which binds to transferrin **(50).** It acts as an antioxidant by removing the free ferrous ion which acts as a major producer of oxidants (superoxide and hydroxyl radicals) **(51).** In addition to this, ceruloplasmin also acts as an antioxidant by catalyzing the destruction of oxygen radicals , and can bind to and inhibit neutrophil myeloperoxidase oxidant activity **(31).**

Serum Albuminconcentration

Table (5) showed that there were significant increase ($p \leq 0.05$) in serum Alb can be observed in pre and post cupping groups when comparison with control group, as well as post cupping group shows a significant decrease ($p \leq 0.05$)in serum Alb in comparison withprecupping group**.**

This result is in agreement with the result of**[**Fatin *et al.*,2014] **(52),** decrease in albumin levels in cupping and after 2weeks from cupping has caused dropping their levels returned back near to its normal values, and agreement with Hussein, Al-M &Ahmoud, S 2011**(53).**

The reason of the significant difference between cupping and control samples isn't known yet. We just can confess that these are the first steps to search about cupping mechanism and further investigations seems more crucial. This is in agreement with Ahmed *et al*., 2005**(54).**

Table 2:- Serum Glucose Levels in All Studied Groups

Groups	No.	Glucose Conc. (Mg/Dl) Mean ± SD
Pre cupping	100	145.4[a]± 80.9
Post cupping	100	89.7[b]± 18.3
Control	35	89.3[b]± 11.5
L.S.D		11.264

Table 3:- Serum Malondialdehyde Levels in All Studied Groups

Groups	No.	MDA (µmol/L) Mean ± SD
Pre cupping	100	3.7 [a] ± 1.6
Post cupping	100	3.2 [b] ± 0.9
Control	35	3.3 [b] ± 1.4
L.S.D		0.276

Table 4:- Serum Ceruloplasmin Concentrations in All Studied Groups

Groups	*No.*	*CP (g/L) Mean ± SD*
Pre cupping	100	3.8 [a] ± 1.1
Post cupping	100	3.4 [b] ± 0.9
Control	35	2.3 [c] ±0.6
L.S.D		0.2187

Table 5:- Serum Albumin Concentrations in All Studied Groups

Groups	No.	Alb (G/Dl) Mean ± SD
Pre cupping	100	5.8 [a] ± 0.7
Post cupping	100	4.4 [b] ± 0.5
Control	35	4.1 [c] ± 0.5
L.S.D		0.1480

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