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PRELIMINARY SCREENING FOR ANTIBACTERIAL ACTIVITY OF *COFFEA ARABICA* BEANS (ROASTED AND UNROASTED) AGAINST DIFFERENT PATHOGENS

Research article

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ABSTRACT

Arabic Coffee (*Coffea arabica* L.) is one of the most famous beverages in the world. A Yemeni cultivar of *Coffea arabica* was screened for its antibacterial potential against 10 different referenced bacterial strains, using two methods; disc diffusion and cup-plate diffusion assays. The results revealed that ethanol extract (70% v/v) did not show any antibacterial activity using disc diffusion method, whereas weak antibacterial activity, varying between 6.5 to 8.5 mm inhibition zones, was observed with the un-roasted (green) beans against some bacterial strains, using cup-plate diffusion method, while the roasted coffee showed no antibacterial activity. The study concludes that roasting processes and long storage could reduce the potential antibacterial activity and recommend further future investigation using other different solvents since ethanol was not the best solvent to extract the presumable antibacterial molecules from *Coffea arabica* beans.

Keywords: *Coffea arabica*, antibacterial, disc-diffusion test, agar cup-plate method, pathogens

INTRODUCTION

Since more than 2000 years ago, Hippocrates said: "Let food be thy medicine and medicine be thy food" **(Smith, 2004).** Natural food and beverages are essential in the promotion of health and disease prevention. However, in this era, modern lifestyle has changed the food habits and led to numerous nutritional deficiency diseases **(Pandey *et al.*, 2010).**

Coffee is one of the most famous (non-alcoholic) drinks in the world. Coffee trees belong to the family *Rubiaceae* and there are more than 100 species of *Coffea* L.; however, only two species are commercially cultivated, which are *Coffea arabica* (Arabica coffee) and *Coffea canephora* (Robusta coffee) **(Mishraand Slater, 2012).** Historically, Arabic coffee is an ancient plant product, believed that it is first exploited in Yemen since 575 AD; However, it could be known earlier in southwestern Ethiopia highlands since around 1500 years ago **(Anthony *et al.*, 2002).** It was found that *Coffea arabica* (*C. arabica*) contains many phytochemical compounds, such as alkaloids, particularly Caffeine which is the most important alkaloids present in high quantity in *C.arabica*, phenolic compounds, terpenoids that gives coffee its distinctive aroma, carotenoids and enzymes **(Patay *et al.*, 2016)** and many more. The reported biological activities of *C.arabica* include many bioactive properties such as antioxidant **(Mussatto *et al.*, 2011),** anti-tumor **(Huang *et al.*, 1988),** anti-cancer **(Ross, 2005),** hepatoprotective **(Lima *et al.*, 2013),** antibacterial **(Almeida *et al.*, 2006),** wound healing **(Affonso *et al.*, 2016)** and it is also used as stimulant on the central nervous system so it could probably reduce the incidences of Parkinson's disease **(Patay *et al.*, 2016).** The purpose of this study was to investigate the antibacterial properties of *C.arabica* beans against different pathogens, and also to compare between the green beans of coffee and the roasted beans, to see if this process affects on its potential antibacterial efficacy.

MATERIALS AND METHODS

Plant material and extraction

Arabic coffee beans (*C.arabica*) were purchased from a coffee store in Alrass town, Saudi Arabia. A Yemeni

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cultivar was chosen and authenticated. The Yemeni coffee beans were divided into two parts, green coffee beans (unroasted) and a roasted coffee beans (average roasting). 100 grams from each part was ground into fine powder and macerated in 500 mL of ethanol (70% v/v), for up to 3 days in a well tighten container inside an incubator at 40°C, with frequent shaking. Then, the macerates were filtered with muslin cloth and Whatman filter paper (No.1), the filtrates were evaporated to get a semi-solid crude, which was put in an incubator at 45°C for up to 3 days to getdryextracts. The ethanolic extracts (roasted and unroasted coffee beans) were reconstituted in 10% Dimethylsulfoxide (DMSO) to get 500 mg/mL of concentration, which was kept in a well tighten glass container s in a refrigerator until used.

Microorganisms

Ten referenced bacterial strains representing different Gram-positive and Gram-negative pathogens were used in this study (Table 1). Bacterial strains were sub-cultured in nutrient broth for 18 hours at 37°C to reach the exponential phase. Then, adjusted to 0.5 McFarland's standard to get bacterial density equivalent to around 1.0 × 10^8 CFU/mlL (CFU: Colony forming unit), which was directly used in the antibacterial activity test.

Table 1: Microorganisms used in the evaluation of antibacterial properties of *Coffeaarabica*beans.

Type of microorganisms	Scientific name	Strain code
Gram-positive Bacteria	*Bacillus cereus*	ATCC® 10876™
	Staphylococcus epidermidis	ATCC® 12228™
	Staphylococcus aureus	ATCC® 29213™
	Staphylococcus saprophyticus	ATCC® 43867™
	Streptococcus pneumonia	ATCC® 49619™.
Gram-negative Bacteria	*Escherichia coli*	ATCC® 25922™
	Proteus vulgaris	ATCC® 6380™
	Klebsiella pneumonia	ATCC® 27736™
	Pseudomonas aeruginosa	ATCC® 9027™
	Shigellaflexsneri	ATCC® 12022™

Testing for antibacterial activity

Disc diffusion test

Paper discs (6 mm in diameter) were cut from Whatman No.1 filter paper, put into well-tighten bottle and sterilized in an autoclave. About 20mL of the autoclaved Mueller Hinton agar were poured onto sterile disposable Petri dishes and left at room temperature until solidified. Petri dishes were then turned upside down and kept in the refrigerator for a while to get rid of any water droplets. 100µL of the previously adjusted bacterial suspensions were gently spread on the surface of the solidified Mueller-Hinton Petri-dishes, using sterile cotton swabs. Some sterile blank discs were saturated with the re-constituted extract at a concentration 500 mg/mL and put over the inoculated Petri dishes. Another group of sterile blank discs were saturated with 5 mg/mL chloramphenicol were also loaded, to serve as positive control. The solvent, 10% DMSO did not show any inhibition effect on bacteria. The seeded Petri dishes were incubatedat 37°C for 24 hrs. The susceptibility of the bacteria towards the extract was expressed as the mean zone of growth inhibition in millimeter (mm) **(Abdallah, 2016).**

Cup-plate diffusion test

Bijou Bottles containing 20mL of autoclaved Mueller-Hinton agar were poured on sterile Petri dishes (90 mm in diameter) and left to solidify at room temperature. Then, the previously adjusted bacterial stains were spread over the agar plates using sterile cotton swabs. 4 wells were punched on the agar surface of each Petri-dish using flamed cork borer (6 mm in diameter). 50µL from the reconstituted extracts (500 mg/mL) were taken with Eppendorf pipette and loaded into respective wells, also

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50µL of chloramphenicol (2.5 mg/mL) was loaded to a separate well and served as positive control. The pre-experimental testing showed that 10% DMSO has no effect on bacterial growth. The prepared plates were incubated for 24 hours at 37°C. After incubation, only clear zones of inhibition around the cups were measured in millimeters (mm) with a ruler and the mean of two zones were calculated **(Mekonnen *et al.*, 2016).**

Statistical analysis

Some quantitative data were expressed as a mean ± standard deviation; Paired-Samples T-test was employed to determine possible significant antibacterial differences between the ethanol extract of *C. arabica* and the antibiotic; chloramphenicol, at $P < 0.05$. The program used in tabulation and graphing was SPSS-Statistical Package, version 11.

RESULTS AND DISCUSSION

The antibacterial potential of the hydro-alcoholic (70%) ethanol extracts (Roasted and unroasted) of*C. arabica* beans, were determined by the disk diffusion and the cup-plate diffusion methods. Concentrations of 500 mg/mL were used for assaying the crude extracts against all test bacteria, which were 10 different ATCC bacterial strains (Table 1). The results of disc diffusion test showed no antibacterial activity (Table 2), while only weak or no antibacterial activity was recorded with the cup-plate diffusion method (Table 3). Moreover, as shown in (Table 3), the unroasted extract of *C. arabica* beans exhibited weak antibacterial activity, which was ranging between 6.5 to 8.5 mm inhibition zones. Whereas, the roasted extract of *C. arabica* beans showed approximately no antibacterial effects, which were ranging between 6 to 6.25 mm inhibition zones. These results were non-significant ($P<0.05$) when compared with the reference antibiotic, the chloramphenicol (Figure 1). Moreover, it was observed that, the cup-plate diffusion test was better in the antibacterial evaluation than the disc-diffusion test (Figure 2), it could be related to the quantity of the extract, in the disc diffusion method, the 6 mm blank disc absorb only 15µL of the extract solution, while in the cup-plate diffusion method the quantity of the extract solution that loaded in the 6mm cup was 50µL.

Although, each method has its own advantages. In general, studies on *C. arabica* in Arab countries are scant and no data found regarding the antibacterial activity of the Yemeni cultivar of *C. arabica*. However, the results of the current investigation are in contradiction with the previous studies on the antibacterial potential of *C. arabica* from other different localities; **Daglia *et al.*, (1994)** cited that the aqueous extract of the roasted beans of *C. arabica* has an antibacterial activity. **Duangjai *et al.*, (2016)** stated that the aqueous extract of *C. arabica* showed varied antibacterial activities against *Staphylococccus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Although, **Wijaya *et al.*, (2016)** metioned that beans of Robusta coffee ethanolic extract recorded antibacterial activity greater than the Arabica coffee extract. In our study, the ethanol extract of the roasted beans of *C. arabica* did not show any antibacterial effects compared with the green (unroasted) beans, which may be due to lost or denaturation of some bioactive molecules during roasting. Our results could be supported by the findings of **(Patay *et al.*, 2016)** who reported that, in Coffee, some phytochemical contents such as alkaloids (Caffeine) is reduced during the roasting process. Accordingly, the weak activity showed from the current study is related to two main reasons; firstly, the nature of the antibacterial molecules present in *C. arabica* are tending to be more polar, as most of the previous studies tested mostly the aqueous extracts. Secondly, the antibacterial nature of *C. arabica* affected greatly by long storage. The author tends to the second interpretation, **Selmar *et al.*, (2008)** metioned that Storage of Coffee beans for a prolonged time might be the cause of the decrease in potential aroma precursors. Yet, the compound(s) responsible of the claimed antibacterial efficacy of *C. arabica* remains unknown; **Almeida *et al.*, (2012)** considered that the antibacterial efficacy of *C. arabica* is attributed to the presence of Caffeine. **Runti (2015)** claimed that the decaffeinated aqueous extract showed good antibacterial activity and this activity is independent from caffeine content. In conclusion, since the ethanolic extract of the un-roasted beans of *C. arabica* showed weak antibacterial activity against different pathogens, so the antibacterial molecules are presumably present in low quantity, meaning that 70% ethanol is not the best solvent for this plant product. It was reported that the extraction parameters affected the yield of the bioactive constituents of the plant products **(Baldosano *et al.*, 2015).** Accordingly, a further future study using different solvents is recommended in order to isolate these antibacterial molecules, if present. On the other side, based on the findings of the current study, the roasting process could decrease the potential antibacterial activity.

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Table 2: The antibacterial activity of the ethanol extracts of *Coffea arabica* beans using disc diffusion test

Tested compounds	Mean zone of inhibition (mm)									
	Gram-positive bacteria					Gram-negative bacteria				
	Sa	Se	Ss	Sp	Bc	Ec	Pa	Pv	Kp	Sf
Roasted (500 mg/mL)	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Unroasted(500 mg/mL)	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Chloramphenicol (5 mg/mL)	36	35	33	29	36	30	22	28	27	29

*6.0= No inhibition (the disc diameter), Sa=*Staphylococcus aureus* ATCC29213, Se=*Staphylococcus epidermidis* ATCC 12228, Ss=*Staphylococcus saprophyticus* ATCC 43867, Sp=*Streptococcus pneumonia* ATCC 49619, Bc=*Bacillus cereus* ATCC 10876, Ec=*Escherichia coli* ATCC 25922, Pa=*Pseudomonas aeruginosa* ATCC 9027Pv=*Proteus vulgaris* ATCC 6380, Kp=*Klebsiella pneumonia* ATCC 27736, Sf=*Shigella flexsneri* ATCC 12022.

Table 3: The antibacterial activity of the ethanol extract of *Coffea arabica* beans using cup-plate diffusion test

Tested compounds	Mean zone of inhibition±Std.Deviation									
	Gram-positive bacteria					Gram-negative bacteria				
	Sa	Se	Ss	Sp	Bc	Ec	Pa	Pv	Kp	Sf
Roasted(500 mg/mL)	6.25 ±0.35	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.25 ±0.35	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.25 ±0.35
Unroasted(500 mg/mL)	7.0 ±0.0	8.0 ±0.0	7.0 ±0.0	6. 5 ±0.0	8.0 ±0.0	7.25 ±0.35	9.0 ±0.7	6.5 ±0.0	7.5 ±0.7	8.5 ±0.7
Chloramphenicol (2.5mg/mL)	36.0	35.0	33.0	33.0	36.0	30.0	22.0	28.0	27.0	26.0

*6.0= No inhibition (the disc diameter), Sa=*Staphylococcus aureus* ATCC29213, Se=*Staphylococcus epidermidis* ATCC 12228, Ss=*Staphylococcus saprophyticus* ATCC 43867, Sp=*Streptococcus pneumonia* ATCC 49619, Bc=*Bacillus cereus* ATCC 10876, Ec=*Escherichia coli* ATCC 25922, Pa=*Pseudomonas aeruginosa* ATCC 9027Pv=*Proteus vulgaris* ATCC 6380, Kp=*Klebsiella pneumonia* ATCC 27736, Sf=*Shigella flexsneri* ATCC 12022.

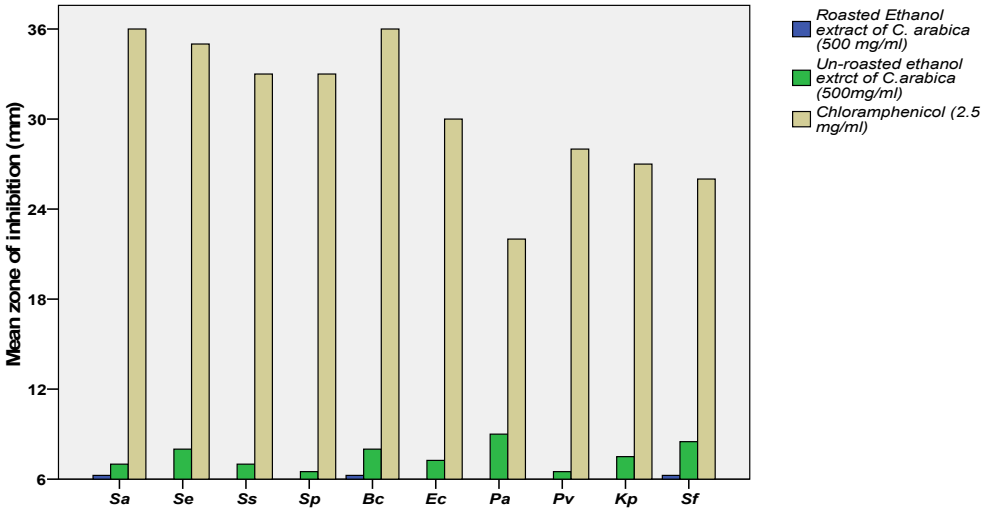

Figure 1: Weak antibacterial effects compared with the antibiotic, using cup-plate diffusion method

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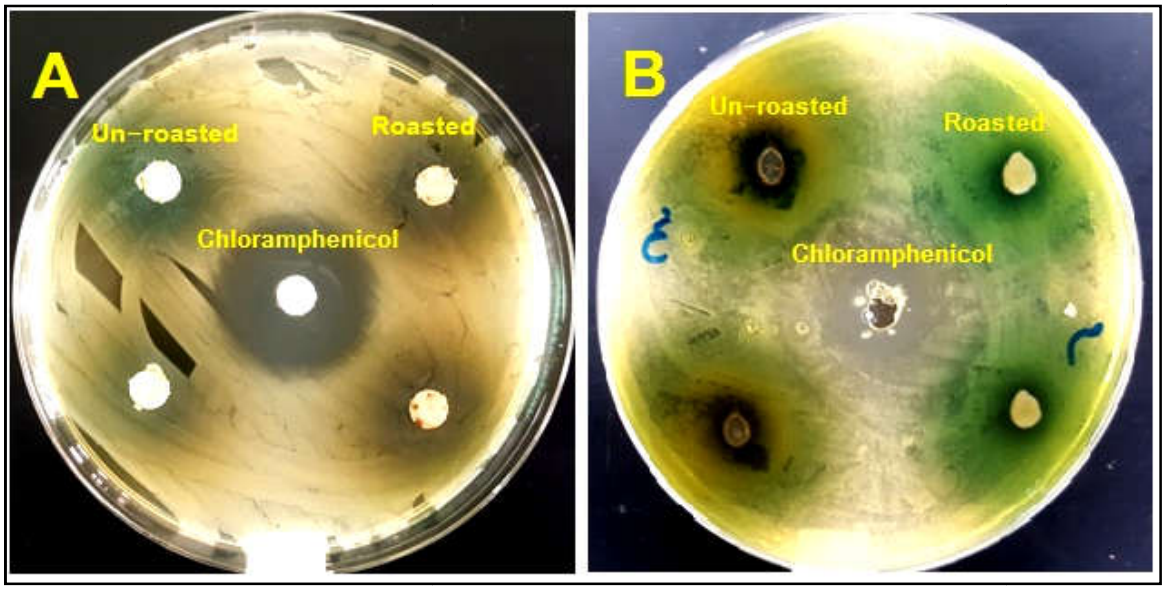

Figure 2: Representative photo showing a comparison between (A) disc diffusion and (B) cup-plate diffusion methods

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TREATMENT OF CONSTIPATION USING CUPPING AN ALTERNATIVE MEDICINE: A CASE STUDY

Case Study

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ABSTRACT

Unique kind of case study, which concerned about complained of abdominal pain and chronic constipation since last 5 years. Frequency of defecation was 4 to 5 days in a week with intervention. The pain considered being more severe after high carbohydrates diet and relief with the maximum dose of laxatives. No significant finding was observed on laboratory analysis. After the consent of patient glass cupping method was applied on abdominal pelvic nine-region scheme and Lumbosacral Region of the Spine. The procedure was performed on 17th lunar moon dates with wet cupping. After the therapy patient complain of lower abdominal pain and chronic constipation was relieved without any intervention and therapy.

Key words: cupping, constipation, symptoms, and therapy.

INTRODUCTION

Pain is the most common symptoms for seeking therapies. Severity of the pain leads towards other kind of treatment including conventional and alternative therapies, mind and bosy therapies and acupunctural massage **(Abbott *et al.*, 2011, Fleming *et al.*, 2007**).

The Arabic name of cupping is Al-Hijamah which means to reduce in size i.e. '*to* return the body back to its natural state'**(Al-Rubaye, 2012).**Cupping is a technique applied by the acupuncturist or other therapist for the treatment of pain on affected area. The procedure was performed with the help of glass or bamboo cup by creating suction on it (**Michalsen *et al.*, 2009**). Cupping is widely used for relieving pain in Asian and Eastern countries **(Yoo and Tausk, 2004).** 38 research studies proposed two types of cupping therapies **(Cao *et al.*, 2010a).** The included types are dry cupping and wet cupping **(Huang *et al.*, 2013)**. In dry cupping skin pulls with the help of cup without drawing blood however in wet cupping, the skin is lacerated with a blade so that blood is drawn into the cup. Tissue paper burned in the cup, which was then flipped over and applied to the skin. The vacuum sucked both tissue and blood into the cup. When the cup is again applied to the lacerated skin, the blood (about 10 mL or less) drawn out through the wound **(Al-Bedah *et al.*, 2016, Huang *et al.*, 2013)**. Major 550 studies proved that cupping have potential benefits for the relief of pain including cough, dyspnea, herpes zoster, pain related conditions, hypertension, stroke **(Cao *et al.*, 2010b; Kim *et al.*, 2011; Dal Kwon and Cho, 2007; Cao *et al.*, 2012; Kim *et al.*, 2010).**

Cupping helps for the diseases caused by dampness, such as low back pain, lumbago, sciatica, pain in arms and shoulders, pain in the legs, pain in the muscular part of the body, post-surgical pain stomachache, migraine, vomiting, sprains, etc. Depression, insomnia, anxiety, bed-wetting and other psychological problem, Lung disease like asthma, bronchitis, common cold and flu, high blood pressure and angina. Digestive system: constipation, diarrhea, irritable bowel syndrome (IBS),

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Thyroid disease, laziness, sleepiness, urinary incontinence and kidney pain, Skin disease like boils, urticaria, tinea, acne, eczema, leprosy. It also helps to decrease vision, increase hair growth, painful, suppressed or irregular menses. Metabolic: low energy, fatigue, anemia, atrophy of the tissues, cellulite, emaciation, and weight gain. It gives the feeling of deep pleasure and profound relaxation so can be done in healthy person for his general physical and mental well being **(Cao *et al.*, 2012; Cao *et al.*, 2010b; Cho *et al.*, 2012).**

For performing cupping,a dermatome is an area of skin that is mainly supplied by a single spinal nerve. Each of these nerves relays sensation (including pain) from a particular region of skin to the brain. These dermatomes and peripheral nerve areas embryologically have same origin as some visceral and musculoskeletel areas. Therefore, performing Cupping on these dermatomes causes effects on these visceras and areas (through nerve or vascular connection) **(Awad, 2008).** On the authority of Abu-Huraira (May Allah be pleased with them) who reported that Prophet (SallallaahuÁlayhiWasallam) said, Whoever performs cupping (hijama) on the 17th, 19th or 21st day (of the Islamic month) then it is a cure for every disease (**AL-Shamma and Abdil Razzaq, 2009**). Cupping has no major side effects aside from minimal discomfort due to the method of application and skin cuts, they might feel faint **(Hanan and Eman, 2013; Farhadi *et al.*, 2009**).

After each session, patient usually report feeling deeply relaxed, and their specific target cupping areas feel light and agile **(Hawker *et al.*, 2011).**

The patient should be fasting for more than four hours but should be mentally prepared. It can be done in fasting and in the state of ahram. This procedure should not done just after bath, vomiting, blood donation, dehydrated, very old or week person or person on blood thinning medicine. It is contraindicated in chronic liver or kidney disease, and in women during first trimester of pregnancy and menstruation **(Jiang *et al.*, 2005).**

Many researchers have investigated and demonstrated the benefits of cupping for blood disorders, pain relief, inflammatory conditions, mental and physical relaxation, varicose veins and deep tissue massage and quotes up to 50% improvement in fertility levels **(Sahraeian *et al.*, 2014**). It help with the procedure of IVF and support pregnancy **(Abduljabbar *et al.*, 2016).**

Case study

48 years females presented with complain of abdominal bloating, discomfort, and feeling of heaviness, low backache, with distended abdomen. She is suffering from this chronic constipation for last 5 years after the delivery of her last daughter, the frequency of defecation was once in four or five days with intervention. The condition worsens on eating high carbohydrate diet such as rice and presents with GERD. She is not having any history of fever, rigors; her medical history was also unremarkable. She is living with her husband and 3 children in separate society home and dependent on him for her living. She was a nondrinker and nonsmoker. Her discomfort eases by taking omeprazole 20 mg and laxatives like Syrup lactulose 4 tablespoon stat.

On examination patient was mild distressed with distended abdomen. Her vital were: blood pressure, 110/70 mm Hg; temperature, 98.9°F regular pulse, 82 beats/min; respiration rate, 19 breaths/min; and oxygen saturation, 92% while inhaling normal air. Marked distention was observed on per abdominal examination with reduce bowel sounds and discomfort on palpating deep in all abdomen. On rectal examination few hard stools mass observed in vault. Her complete blood count, routine urine analysis was also insignificant, the Anti HCV and HbsAg was also negative. Plain Abdominal radiograph shown gaseous dilatation of colon stasis in sigmoid and descended colon.

RESULTS

The procedures were involved by applying glass cups to the skin to help alleviate pain, expel toxins, and restore proper circulation to the body. The cups are positioned on a variety of specific trigger points on the body, and are typically left for ten - fifteen minutes. Each glass cup acts as a vacuum through the use of heat or a suction pump.

The patient was explained of procedure two days before she came in fasting on 17th of lunar moon date n was wet cupped on 1, 55,11,12,13, in first session and 28, 29, 31 on 21st of same month. She responded very effectively her abdominal distension, bloating and constipation was relieved, and she had defecated without any oral medication. She was contented and wishes to continue with further sessions in order to seek complete management of her disease.

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DISCUSSION

The studies further authenticate the treatment and procedure of capping specifically on abdominal distention and chronic constipation and acknowledge extensive classification of cupping. Cupping extensively used for improving the quality of the life **(Lee and Warden, 2011)**and proven the effect on senile habitual constipation **(Jiang *et al.*, 2005). C**upping therapy are used with the combination of other drug for improving the effects of the therapy and more better effects on patients **(Xianhua, 2005**).

CONCLUSION

Cupping is the oldest method for relieving the pain and other associated symptoms and helps for complicated chronic diseases like constipation and improves quality of life.

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