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Original article

Comparative clinical evaluation of leech therapy in the treatment of knee osteoarthritis

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Abstract

Introduction: Historically physicians have used leech therapy for various ailments. *Hirudo medicinalis* is commonly used in western countries for medicinal purpose and in other parts of the world, different species are used. In India, *Hirudinaria granulosa* species is used traditionally for this purpose. Although this treatment approach has been used for many centuries, there is little scientific data on its effectiveness. The aim of this study was to validate the efficacy of leech therapy in knee osteoarthritis.

Materials and methods: This study was a randomized, parallel group, controlled trial with the approval of Institutional ethics committee. The total of 60 patients, 30 in each group, completed the study. The outcome measures included; Visual Analogue Scale (VAS), Knee injury and Osteoarthritis Outcome Score (KOOS), range of motion, 15-m walking time and knee circumference were used to assess clinical efficacy. The test group received leech therapy along with a Unani formulation. The other group (control) received the Unani formulation only.

Results and discussion: The test group demonstrated highly significant improvements in evaluated parameters when compared with baseline values. Statistically significant differences were observed in KOOS total score and its sub scores ($P < 0.0001$), VAS ($P < 0.0001$) at the 4th week when compared with the control group. The reduction in pain, other symptoms and physical function, were observed even 4 weeks after the treatment ($P < 0.0001$).

Conclusion: The leech therapy seems to be an effective treatment for reducing symptoms of knee osteoarthritis and improving physical function with no major adverse effects.

Keywords: Leech therapy; Knee joint; Osteoarthritis; Clinical study; Unani medicine; *Qurse mafasil*

Introduction

Leech therapy is a treatment modality where leeches are applied to a particular area of body in order to treat certain ailments. Leech application has been used throughout history from ancient times [1], and currently it is used in modern medicine particularly in plastic or reconstructive surgery [2]. The term 'leech' has two distinct meanings according to old English (the Anglo-Saxon language); one is physician and other is blood-sucking worm [3].

Bloodletting is an ancient therapy, which has been practiced since Stone Age. Ancient healers believed that many ailments were caused due to inappropriate, excessive collections of blood and its constituents [3]. Hippocrates (the Father of medicine) believed that the veins were the site of pathologic humours [4]. Therefore, ancient physicians used bloodletting more frequently, when treating many illnesses. As an alternative to instrumental bloodletting, the leech offered some advantages; leeching is slower, less painful; many practitioners favoured leech therapy as it has more quantitatively dependable extraction of blood [5].

They are widely used in plastic surgery to treat venous congestion of skin grafts [6,7], also used in breast reconstruction, replanted digits, ears, lips and nasal tips in reconstruction surgery

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and periorbital hematomas [8,9]. In Unani system of medicine, leech therapy has been used for several conditions such as skin diseases, arthritis, sciatica, pleurisy, pneumonia, pericardial pain, myocardial pain, hepatic pain. Famous Unani scholars like Avicenna, Rhazes, Galen, Al Bhagdadi, practiced leech therapy and mentioned this important treatment modality in their books [10–14]. This therapy reduces inflammation; hence, it relieves pain, stiffness, inflammation and improves the joint function in inflammatory disease like knee osteoarthritis [15,16].

Osteoarthritis (OA) is a chronic degenerative disorder of multifactorial aetiology characterized by loss of articular cartilage, hypertrophy of bone at the margins, subchondral sclerosis and range of biochemical and morphological alterations of the synovial membrane and joint capsule [17]. The Subcommittee on Osteoarthritis of the American College of Rheumatology Diagnostic and Therapeutic Criteria Committee defined OA as "A heterogeneous group of conditions that lead to joint symptoms and signs which are associated with defective integrity of articular cartilage, in addition to related changes in the underlying bone at the joint margins". Clinically, the condition is characterized by joint pain, tenderness, limitation of movement, crepitus, occasional effusion, and variable degrees of local inflammation [18].

In modern medicine, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are the mainstay of treatment of OA. Nevertheless, these NSAIDs have many adverse effects like gastric ulceration, gastro-intestinal bleeding and perforations [19]. Considering the large number of people suffering from OA, limitations in the conventional medical management and the known adverse effects associated with NSAID and glucocorticoids use, indicate a real need for safe and effective treatment of arthritis patients [20], for which alternative medicine is the best answer, because they have been used successfully on human beings without any reported major adverse effects over centuries. These challenges drive us to explore alternative modes of treatment having the least or no side effects for this painful condition.

Hirudo medicinalis, described by Linnaeus in 1758, has long been believed the one and only European medicinal leech [21]. It is being presently approved by US Food & Drug Authority for use in medical procedures [22]. It is the most frequently used species of leech that is not native to the Indian subcontinent [23]. Nowadays modern scientists are trying to validate its beneficial effects by using acceptable scientific parameters. In India, *Hirudinaria granulosa* species is used traditionally for the therapeutic purposes [24]. *H. granulosa*, abundant in the states of Madras, Kerala, Madhya Pradesh, Uttar Pradesh and the Punjab, was the species commonly cultured for this purpose [25]. Since, the use of *H. granulosa* is far back, but the scientific data is very much less. Hence, this study has been carried out to validate the efficacy of leech therapy in knee osteoarthritis.

Materials and methods

This study was a randomized, parallel group, controlled trial carried out at Majeedia Hospital, Jamia Hamdard University, New Delhi, in accordance with the principles stated in the Declaration of Helsinki (2004). The protocol was approved by Institutional ethics committee for clinical trials in Unani drugs of Jamia Hamdard {DM/JH/FM/08 (ii)} at Jamia Hamdard. Patients were recruited Unani medical OPDs in Majeedia Hospital, New Delhi. Each participant was informed about the trial in accordance with Declaration of Helsinki. They were further given a description of anticipated risks and discomforts. Then, informed written consent was obtained from each participant in the prescribed format prior to performance of the study related procedures (i.e. physical examination, laboratory screening and other investigational procedure) and before administration of any study related medication. The study included individuals > 40 yrs, either sex, fulfilling the following criteria.

Inclusion criteria were as follows: diagnosed with OA of the knee of at least 6 months duration fulfilling American College of Rheumatology criteria, knee pain Visual Analogue Scale (VAS) – pain after walking 1524 cm (50 feet) in a flat surface >30 mm and <90 mm, Kellgren–Lawrence radiographic grading scale of osteoarthritis of the tibiofemoral joint grades 1–3, patients who were willing to discontinue all NSAIDs or other analgesic medication taken for any conditions, patients who had given their written informed consent and agreed to follow the protocol voluntarily were included.

Exclusion criteria were as follows: patients who are pregnant or lactating and the patients with anaemia (<10 g% Hb), bleeding disorders, Diabetes mellitus, history of surgery of the knee joints, tidal lavage or arthroscopy of either knee within the past 12 months, hypersensitivity/allergy to food &/drug or leech, history of alcohol or drug abuse, excessive smoking (more than 10 cigarettes/day), established/diagnosed neurological or psychiatric disorders and patients who were on steroid drug therapy, anti-coagulants (coumadin, heparin, aspirin > 325 mg per day), intra-articular corticosteroid injection of either knee within 3 months prior to baseline screening, NSAIDs, oral analgesics, muscle relaxants, or low-dose antidepressants were excluded.

Patients with acute medical or surgical conditions such as cardiac, renal, hepatic diseases, concomitant skin diseases at the application site and not willing to be randomized were also excluded from the study.

Allocation of patients to study group

The patients who qualified for the study were randomized by using stratified block randomization. The total of 60 patients were randomly allocated to test and control groups containing 30 in each. Stratification was done according to severity of illness (evaluated by VAS; more than 60 as more severe and less than 60 as less severe). Thus, either group had equal distribution in severity. These blocks were covered in an envelope and numbered in a sequential order containing two categories (more severe and less severe). When a patient was included in the study, one envelope (according to the patient's severity) which had the lowest sequential number was opened and assigned the patient to the respective group. The protocol therapy was an invasive and there was no placebo treatment possible. Therefore, patients could not be blinded to the therapy. At first visit, patients were screened by history, physical examination and baseline investigations.

Interventions

The test group received leech therapy (2 leeches one in medial side and other one in lateral side of the knee joint round the patella of both knees, every week for 4 weeks i.e. at day 0, 7, 14, 21 and 28) along with a Unani formulation – *Qurse mafasil Jadeed* (*QMJ*) 500 mg twice daily for 28 days. While, control group received the same Unani formulation alone in same dose for 28 days.

Qurs-e-Mafasil Jadeed (*QMJ*) is a Unani formulation mentioned in Qarabadeen-e-Majeedi (Pharmacopoeia), which is a part of National Formulary of Unani Medicine, Government of India. *QMJ* contains *Surinjan talkh* – Golden collyrium (*Colchicum luteum*) 25 g, *Chob zard* – Turmeric (*Curcuma longa*) 25 g, *Gond Keekar* – gum Arabic (*Acacia arabica*) 5 g.

Identification

The leech was identified as *H. granulosa* by Prof. Waseem Ahmad, Department of Zoology, Aligarh Muslim University, Aligarh, India.

Ancient physicians have mentioned the characteristics of medicinally usable leeches. Avicenna has stated the following characteristics for the identification of nonpoisonous leeches for medicinal purpose. They are

- The leeches which are thin and having tiny heads
- Emerald green leeches, which are predominantly green and having yellow stripes or leeches which resemble liver colour
- Brown leeches with round sides
- Leeches which look like little locusts and those resemble mouse tails

Avicenna has described poisonous (medicinally non-usable) leeches, which have large heads, antimonial, black or green colour, having soft hairs and those resembling eels (snakefish). They may produce swelling syncope, haemorrhage, fever, paralysis and malignant ulcers [12]. Though famous Unani physicians have divided leeches into poisonous and nonpoisonous varieties, it now scientifically known that no aquatic, sanguivorous leech is poisonous. The adverse effects of leeches, mentioned in Unani classics, may be due to improper use which can produce same conditions, and which had been attributed to the poisonous leeches by the Unani physicians [34].

Application procedure

Leeches were cleaned and put into water mixed with turmeric (in order to increase the activity and sterilize the leech). Application site was cleaned with water and rubbed until it becomes red. Then, two leeches, one in medial side and other in lateral side of the knee joint were applied. Fresh leeches were applied each time to prevent cross infection, even in same patient.

Leeches remained attached for 40–70 min. When it spontaneously detached from the sites of biting, bite wounds were cleaned with antiseptic solution and a tight bandage applied to prevent further bleeding or oozing. Patients were advised to avoid physical work for 4–6 h in order to prevent bleeding. After leech therapy, the leeches were destroyed by placing them in 70% alcohol solution for minimum 10 min and then they were treated as bio-hazardous waste, disposed of in an appropriate waste receptacle.

Outcome measures

Outcome measures were Visual Analogue Scale (VAS), Knee injury and Osteoarthritis Outcome Score (KOOS), active range of motion (AROM) and passive range of motion (PROM), 15-m walking time, knee circumference and Kellgren–Lawrence radiographic grading scale. Primary end point is at 4th week and the secondary end point was 8th week

A VAS is a measurement instrument that tries to measure a characteristic or attitude that is believed to range across a continuum of values and cannot easily or directly measured. Operationally a VAS is usually a horizontal line; 100 mm in length, on which higher score indicates more pain, The VAS score is determined by measuring in mm from the left hand end of the line to the point that the patient indicates. The two extremes of the scales are marked as 0 and 100, respectively. Zero represents no pain whereas 100 represent extreme pain. The subjects were given the scale and asked to mark the point that represents their perception of current state of pain [26].

KOOS is developed as an instrument to assess the patients' opinion about their knee and associated problems. KOOS is meant to be used over short and long time intervals; to assess changes from week to week induced by treatment (medication, operation, physical therapy) or over years due to the primary injury or posttraumatic OA. KOOS consists of five subscales; KOOS pain, other symptoms, Activities in Daily Living (ADL), function in sport and recreation (sport/rec.) and knee related Quality of Life (QOL). KOOS has been used in patients 14–78 years old. KOOS includes WOMAC Osteoarthritis Index LK 3.0 in its complete and original format. KOOS is patient-administered, the format is user friendly, and takes about 10 min to fill out [27,28].

ROM was measured before and after the treatment, by universal goniometer. The subjects were positioned prone with knee suitably stabilized and both active and passive range of motions were taken with the universal goniometer. The stationary arm was placed with the free end pointing towards the greater trochanter of the ipsilateral femur, and the moving arm pointing towards the medial malleolus of tibia. The final ranges were recorded [29].

Patients were asked to walk across 15-m distance at their natural speed; walking time was obtained with the help of stopwatch. Three readings were taken and the mean was calculated and recorded [30,31].

Knee circumference was measured at mid patellar level with a measuring tape positioning the leg slightly bent. Difference of knee joint circumference more than 1.0 cm represents a real clinical improvement (smallest real difference) [32,33].

Kellgren–Lawrence radiographic grading scale of osteoarthritis of the tibiofemoral joint was used; X-rays

of the affected joints were taken before and after the treatment to compare the radiological changes [34,35].

Laboratory investigations were performed before treatment (at baseline) and after treatment (after 28th day), which included full blood count, liver function test (LFT), kidney function test (KFT), bleeding time, clotting time and prothrombin time. Results were analyzed by using Friedman test within the group for repeated observations and Wilcoxon signed rank test for paired observations. Level of significance was taken at $P < 0.05$. The analysis of the observational data was performed and presented in the form of graphs and tables by using Graphpad instat 3 and Microsoft® Excel (2007) softwares.

Results

All 60 patients completed the study, 30 in each group. 38 patients were female while 22 patients were male. Mean age of participants was 51.53 ± 1.658 years in test group and 53.27 ± 1.981 years in control group. Mean BMI of participants in test group was $29.18 \pm 1.031\ kg/m^2$, while in control group it was $27.03 \pm 1.046\ kg/m^2$. Differences of baseline characters between two groups were not statistically significant (Table 1).

Test group had extremely significant improvement in KOOS pain score, symptoms score, activities in daily living, sport/recreational score, quality of life score and KOOS total score at 4th week and at 8th week. Visual Analogue Scale also showed a highly statistically significant improvement in the test group at 4th week and 8th week. Statistically significant improvement was noticed in AROM, PROM and Knee circumference in right and left knee joints at 4th week and 8th week. Walking time was also improved significantly. These parameters were compared with baseline values and the values are represented in Table 2 with their Standard Error of Mean (SEM).

In control group, statistically significant improvement was noticed at 4th week in KOOS pain score, KOOS symptoms score, KOOS ADL, KOOS sport/rec., KOOS QOL, KOOS total score, AROM, VAS, walking time, knee circumference (right) at 4th week when comparing with baseline findings. However, at 8th week statistically significant difference was not found in most of the parameters (Table 3).

There were statistically significant improvements found in KOOS pain score, KOOS symptoms score, KOOS ADL, KOOS sport/rec., KOOS QOL, KOOS total score, VAS at 4th week and 8th week in test group when compared with control group (Table 4).

Table 1
Baseline characteristics of study patients.

Variables	Test group (30)	Control group (30)
Age (years)	51.53 ± 1.658	53.27 ± 1.981
Male (*n*)	10	12
Female (*n*)	20	18
BMI (kg/m^2)	29.18 ± 1.031	27.03 ± 1.046
KOOS pain score	37.23 ± 1.502	39.73 ± 2.399
KOOS symptoms score	38.26 ± 2.492	42.74 ± 3.122
KOOS ADL score	33.43 ± 2.729	35.64 ± 2.341
KOOS sports/rec score	16.50 ± 1.611	18.83 ± 2.295
KOOS QOL score	26.53 ± 2.616	28.53 ± 3.109
KOOS total score	30.39 ± 1.629	33.09 ± 2.185
AROM right knee joint (°)	123.83 ± 2.585	124.57 ± 2.443
AROM left knee joint (°)	125.8 ± 2.343	125.03 ± 2.315
PROM right knee joint (°)	128.90 ± 2.367	129.17 ± 2.235
PROM left knee joint (°)	130.57 ± 2.190	129.6 ± 2.215
VAS	68.90 ± 1.691	66.83 ± 1.901
Walking time (s)	25.43 ± 0.728	23.97 ± 0.617
Knee circumference – right (cm)	33.93 ± 0.71	31.92 ± 0.75
Knee circumference – left (cm)	33.77 ± 0.686	31.3 ± 0.853
K–L grading scale	2.207 ± 0.125	2.0 ± 0.122

Values are expressed in means ± SEM.

Laboratory investigations

A highly significant ($P < 0.0001$) improvement in ESR (mean difference 9.1, 95% CI is 6.712–11.488) was noticed before

Table 2
Outcome measures for the test group at baseline (Bl), 2nd week (2 W), 4th week, and 8th week (8 W).

	Bl	2 W	4 W	8 W
KOOS pain score	37.23 ± 1.5	$69.17^{***} \pm 2.03$	$74.83^{***} \pm 2.28$	$64.8^{**} \pm 1.95$
KOOS symptoms score	38.26 ± 2.49	$61.53^{***} \pm 2.53$	$74.4^{***} \pm 2.48$	$62^{**} \pm 2.27$
KOOS ADL score	33.43 ± 2.73	$59.2^{*} \pm 2.19$	$74.23^{***} \pm 1.58$	$67.03^{***} \pm 1.87$
KOOS sports/rec score	16.5 ± 1.61	$46.33^{***} \pm 2.98$	$55.17^{***} \pm 2.9$	$42.33^{***} \pm 2.89$
KOOS QOL score	26.53 ± 2.62	$64.73^{***} \pm 2.83$	$73.37^{***} \pm 2.41$	$62.27^{***} \pm 2.29$
KOOS total score	30.39 ± 1.63	$60.19^{***} \pm 1.68$	$70.4^{***} \pm 1.59$	$59.69^{**} \pm 1.74$
AROM-right (°)	123.83 ± 2.59	$126.77^{**} \pm 2.5$	$127.63^{**} \pm 2.5$	$127.43^{**} \pm 2.51$
AROM-left (°)	125.8 ± 2.34	$129.03^{\dagger} \pm 2.22$	$129.67^{**} \pm 2.19$	$129.33^{*} \pm 2.2$
PROM-right (°)	128.9 ± 2.37	$132.73^{*} \pm 2.26$	$132.93^{*} \pm 2.23$	$132.67^{\dagger} \pm 2.23$
PROM-left (°)	130.57 ± 2.19	$133.53^{\dagger} \pm 2.09$	$134.53^{**} \pm 2.0$	$134.5^{**} \pm 1.97$
VAS	68.9 ± 1.69	$43.93^{***} \pm 1.75$	$27.87^{***} \pm 1.58$	$31.67^{***} \pm 1.97$
Walking time (s)	25.43 ± 0.73	$22.97^{*} \pm 0.63$	$22.75^{**} \pm 0.59$	$22.99^{*} \pm 0.62$
Knee circumference-right (cm)	33.93 ± 0.71	$33.02^{**} \pm 0.65$	$32.6^{**} \pm 0.61$	$32.59^{**} \pm 0.61$
Knee circumference-left (cm)	33.77 ± 0.69	$33.14^{\dagger} \pm 0.64$	$32.66^{*} \pm 0.6$	$32.54^{**} \pm 0.59$

Values are expressed in means ± SEM.
* Significant ($P < 0.05$).
** Very significant ($P < 0.01$).
*** Extremely significant ($P < 0.001$).
† Not significant ($P > 0.05$).

Table 3
Outcome measures for the control group at baseline (Bl), 2nd week (2 W), 4th week, and 8th week (8 W).

	Bl	2 W	4 W	8 W
KOOS pain score	39.73 ± 2.4	50.33*** ± 2.35	56.93*** ± 2.21	42.53† ± 2.57
KOOS symptoms score	42.74 ± 3.12	48.45** ± 3.29	52.14*** ± 3.45	42.976† ± 3.02
KOOS ADL score	35.64 ± 2.34	47.4** ± 2.45	54.02*** ± 2.3	38.14 † ± 2.5
KOOS sports/rec score	18.83 ± 2.3	27.33*** ± 2.51	31.33*** ± 2.58	18.94† ± 2.17
KOOS QOL score	28.53 ± 3.11	39.22* ± 2.93	39.22* ± 3.55	29.79† ± 3.04
KOOS total score	33.09 ± 2.19	42.55*** ± 2.18	46.74*** ± 2.03	34.39† ± 2.09
AROM-right (°)	124.57 ± 2.44	125.63** ± 2.34	125.6** ± 2.34	125.03† ± 2.36
AROM-left (°)	125.03 ± 2.32	126.33† ± 2.28	126.80† ± 2.24	125.90† ± 2.19
PROM-right (°)	129.17 ± 2.24	130.20† ± 2.26	130.37† ± 2.25	129.93† ± 2.29
PROM-left (°)	129.6 ± 2.22	130.3† ± 2.23	130.5† ± 2.16	130.7† ± 2.15
VAS	66.83 ± 1.9	57.30** ± 2.5	56.80** ± 2.57	62.20† ± 2.17
Walking time	23.97 ± 0.62	22.60* ± 0.61	22.85* ± 0.53	23.80† ± 0.59
Knee circumference-right (cm)	31.92 ± 0.75	31.73** ± 0.75	31.73** ± 0.75	31.73* ± 0.75
Knee circumference-left (cm)	31.3 ± 0.85	31.18† ± 0.86	31.12† ± 0.85	31.12† ± 0.85

Values are expressed in means ± SEM.
* Significant ($P < 0.05$).
** Very significant ($P < 0.01$).
*** Extremely significant ($P < 0.001$).
† Not significant ($P > 0.05$).

and after treatment in test group, ESR decreased by 30.1% (mean value and SEM at baseline 29.37 ± 1.667; after treatment 20.27 ± 1.205), while in control group, it was not significant (mean difference 3.37, 95% CI is −0.462 to 7.195; $P = 0.0643$). A significant improvement was found in the test group ($P < 0.05$), when compared ESR between the groups. Number of CRP positive patients was decreased from 7 to 3 in test group, whereas, there was no change in the number of

Table 4
Comparison of outcome measures between two groups at baseline, 4th week and 8th week.

End point	Test group	Control group	Estimated mean difference (95% CI)	*P*-value
KOOS pain score				
Baseline	37.23 ± 1.5	39.73 ± 2.4	−2.5 (−8.07 to 3.07)	$P = 0.4710$
4th week	74.83 ± 2.28	56.93 ± 2.21	17.9 (21 to 11.22)	$P = 0.0002$
8th week	64.8 ± 1.95	42.53 ± 2.57	22.27 (15.11 to 29.42)	$P < 0.0001$
KOOS symp				
Baseline	38.27 ± 2.49	42.77 ± 3.12	−4.5 (−11.95 to 2.95)	$P = 0.2110$
4th week	74.4 ± 2.48	52.2 ± 3.43	22.2 (14 to 30.4)	$P < 0.0001$
8th week	62 ± 2.27	43 ± 3.03	19 (11.32 to 26.68)	$P < 0.0001$
KOOS ADL				
Baseline	33.43 ± 2.73	35.7 ± 2.34	−2.27 (−9.15 to 4.62)	$P = 0.6810$
4th week	74.23 ± 1.58	53. 97 ± 2.3	20.27 (15.41 to 25.12)	$P < 0.0001$
8th week	67.03 ± 1.87	38.1 ± 2.5	28.93 (22.9 to 34.97)	$P < 0.0001$
KOOS sports/rec				
Baseline	16.5 ± 1.61	18.83 ± 2.295	−2.23 (−7.98 to 3.32)	$P = 0.5130$
4th week	55.17 ± 2.9	31.33 ± 2.58	23.83 (15.23 to 32.43)	$P < 0.0001$
8th week	42.33 ± 2.89	18.5 ± 2.17	23.83 (16.47 to 31.2)	$P < 0.0001$
KOOS QOL				
Baseline	26.53 ± 2.62	28. 57 ± 3.11	−2.03 (−9.19 to 5.13)	$P = 0.6230$
4th week	73.37 ± 2.41	39.3 ± 3.54	34.07 (25.4 to 42.74)	$P < 0.0001$
8th week	62.27 ± 2.29	29.9 ± 3.03	32.37 (25.12 to 39.61)	$P < 0.0001$
KOOS total				
Baseline	30.4 ± 1.63	33.03 ± 2.18	−2.63 (−7.42 to 2.16)	$P = 0.3502$
4th week	70.33 ± 1.61	46.8 ± 2.02	23.53 (18.59 to 28.48)	$P < 0.0001$
8th week	59.73 ± 1.75	34.4 ± 2.1	25.33 (20.21 to 30.46)	$P < 0.0001$
VAS				
Baseline	68.9 ± 1.69	66.83 ± 1.9	2.07 (−3.31to 7.44)	$P = 0.5460$
4th week	27.87 ± 1.58	56.8 ± 2.57	−28.93 (−35.27 to −22.6)	$P < 0.0001$
8th week	31.67 ± 1.97	62.2 ± 2.17	−30.53 (−36.38 to −24.69)	$P < 0.0001$

Values are expressed in means ± SEM.

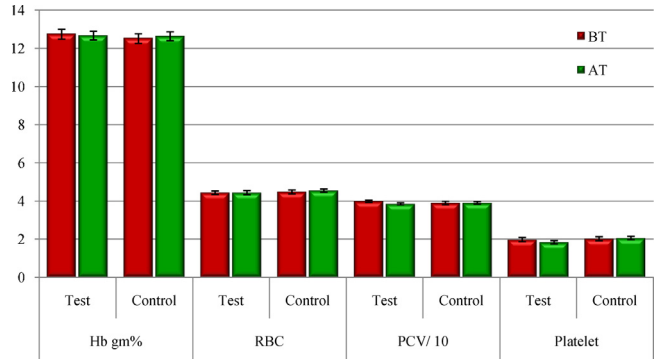

Fig. 1. Effect on haemoglobin (Hb), red blood cell (RBC) count, packed cell volume (PCV) and platelet count before treatment (BT) and after treatment (AT) for both groups.

CRP positive patients in control group. Though the degenerative changes are irreversible and were not aimed in the study, pre and post treatment X-ray were performed and it showed no significant change.

Laboratory investigations were performed before and after the treatment (after 28th day), which include haematology, liver function test (LFT), kidney function test (KFT), bleeding time, clotting time and prothrombin time. These laboratory parameters were taken to evaluate the safety of the treatment. Heamatological assessment such as Hb%, packed cell volume, RBC count and platelet count were not changed significantly when compared both, before and after the treatment (Fig. 1). Likewise, lymphocytes, neutrophils, basophiles, monocytes and total leucocytes count also did not change significantly in both groups.

There was no statistically significant change in Liver Function Test such as Serum Bilirubin, SGOT, SGPT and ALP in the subjects before and after the treatment and between the two groups (Fig. 2).

Bleeding time, clotting time and prothrombin time were also monitored for evaluation of safety and it was revealed that there was no any significant change in both the study groups (Fig. 3).

In addition, there was no statistically significant difference in kidney function tests such as urea, creatinine, protein, albumin, globulin and serum electrolytes, at the end of therapy. It was only for uric acid where a statistically significant difference was

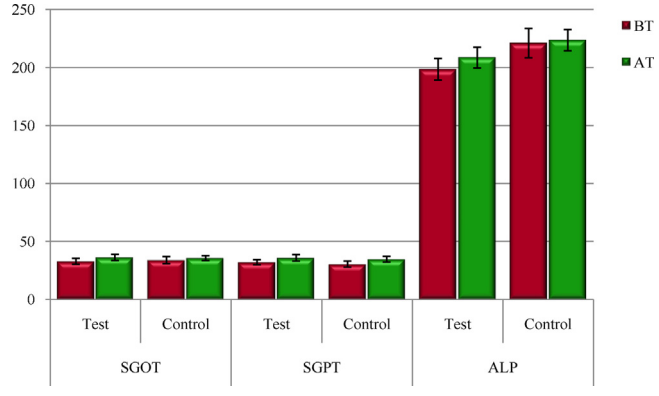

Fig. 2. Effect on serum glutamine oxalo-acetic transaminase (SGOT), serum glutamine pyruvic transaminase (SGPT) and serum alkaline phosphatase ALP before and after treatment.

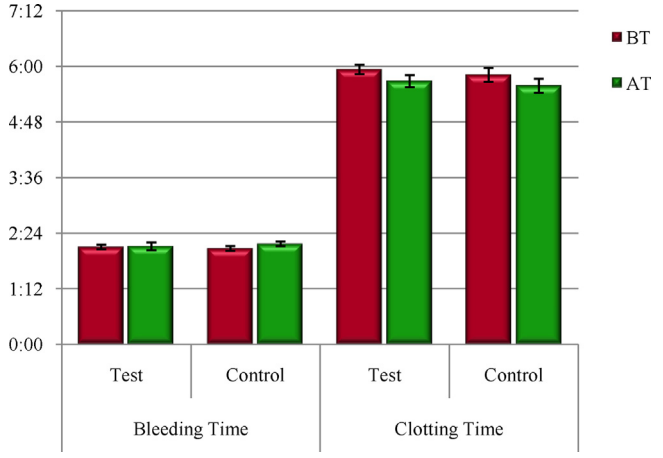

Fig. 3. Effect on bleeding time and clotting time before and after treatment for both groups.

obtained (Fig. 4). This may be due to the action of surinjan (*Colchium luteum*). Surinjan is well known for its action in Gout, characterized by a high level of uric acid in blood. Therefore this significant change in uric acid may be attributed to *Colchium*. Hence, the leech therapy as well as Unani formulation are well tolerated and free from toxicity.

Discussion

Osteoarthritis is a chronic, degenerative and progressive disease that results from complex interactions of multiple physical and biochemical factors [36]. It has social, economical and psychological impacts on the society in multiple ways. First the cost of treatment; second it usually affects working age group leading to economical burden in families [37]. The prevalence of OA is increasing due to lifestyle changes and an increasingly elderly population. Hence, this places a globally major burden on individuals, health care and social care systems. With the advancement of the medical science and health awareness schemes, the morbidity rate has declined but the prevalence rate is still high, due to lack of absolute treatment.

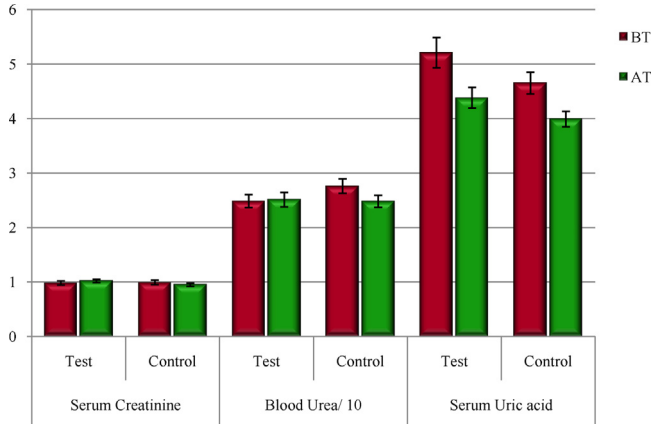

Fig. 4. Effect on serum creatinine, blood urea and serum uric acid before and after treatment for both groups.

Up to this date, there is no effective cure for this chronic disorder and whatever the treatment options available, they are not permanent and not completely free from adverse affects. In osteoarthritis, the main objectives of treatment are to hold-up the degenerative process and minimize symptoms; in addition to that, the therapy should be tolerable when used for a longer period without minimal or no adverse effects. Treatment for OA focuses on relieving symptoms and improving function and can include a combination of patient education, physical therapy, weight control and use of medications [38].

As per the Unani doctrine, derangement of the humour occurs due to the presence of morbid matters in the body and the blood circulation, which are responsible for the production of many diseases. Osteoarthritis is caused by derangement of humours, which are cold in nature like *Balghami* (phlegm), and *Saudavi* (black bile) humours, known as cold derangement of temperament. Leech therapy is applied for the purpose of elimination of morbid material from the body. Considering its vital role and successful use in Unani, the present study was designed and conducted to rationalize this idea scientifically.

In this randomized, controlled trial, patients who were in the test group experienced clinically significant improvement in most of the evaluated parameters. They had decreased perception in pain and other symptoms of osteoarthritis. Moreover, they experienced improved functional ability and day to day performance. In osteoarthritis, pain is the first and foremost symptom for which patient usually visits a doctor. In this study, extremely significant improvement was found in KOOS pain score and VAS at 2nd, 4th and 8th in test group, while in control group extremely significant improvement occurred at 2nd and 4th only. This shows pain relief more in test group and this effect was persisted minimum of 4 weeks after the treatment. The same kind of observations were recorded in KOOS symptoms score, KOOS ADL score, KOOS sports/recreational activity score, KOOS QOL score and KOOS total score. Pain and other symptoms of OA interconnected with each other. Whenever pain is relieved, then it leads to relieve other symptoms like stiffness, swelling, tenderness, etc. In other words it improves the functional ability.

The observed effects may be explained through different mechanisms. At first, leech injects some painkilling substances, which anaesthetize the area, and causes reduction in pain. Therefore, patient may not feel the pain of leech bite and this effect lasts for hours or more. A local anaesthetic is a polypeptide chain that initially affects the 'A' nerve fibres and the larger 'B' nerve fibres. There is also a long-term anaesthetic effect from leech saliva that specially affects the 'C' nerve fibres and the small 'B' nerve fibres that control the sympathetic nervous system. This anaesthetic effect can last several months and provide long term relief from sympathetic mediated pain [39].

Secondly, certain leech enzymes have anti-inflammatory properties. Proteinase inhibitors are mainly responsible for its anti-inflammatory action in the host, such as bdellin (inhibitors of trypsin, plasmin, and acrosin), tryptase inhibitor, eglin (inhibitors of alpha chymotrypsin, subtilisin and chymasin and the granulocyte proteinases elastase and cathepsin G), inhibitor of carboxypeptidase and inhibitor of complement component C1s [40]. Thus, it reduces pain and inflammation in knee joint. The various bioactive substances in leech saliva may also exert substantial effects in periarticular tissue and adjacent structures. Thirdly, physical effects of bloodletting may cause relief due to elimination or dilution of various pain and inflammatory mediators like cytokines which cause pain. Fourthly, placebo effects might be responsible for the symptomatic benefit. The principal limitation of this study is that the placebo effects of this invasive treatment cannot be ruled out. Currently, a sham leech treatment is not available and blinding the treatment is not feasible. The improvement in other outcome measures like morning stiffness, physical function is attributed mainly to the reduction in pain, as it is the chief symptom, which produces other complications.

Control group has statistically significant pain reduction during first four weeks, which could be attributed to *C. luteum* and *C. longa*, for its reported analgesic and anti-inflammatory activities [41–43]. However, the effect of this drug does not persist as long as the effects of leech therapy.

Test group showed statistically significant improvement in active and passive range of motion before and after treatment. Similarly, walking time also improved significantly in test group. These improvements could be due to decrease of pain and inflammation.

Knee joint circumference of more than 1.0 cm represents a real clinical improvement (smallest real difference) [33,32]. Knee circumference was measured and it showed extremely significant improvement in test group in both knee joints. This could be attributed to the subsidence of knee swelling and was more impressive at 4th week and persisted until 8th week. Improvement of knee swelling in test group clearly shows the anti-inflammatory effect of leech therapy, which reduces the inflammation, thus reducing knee swelling.

In test group, number of CRP positive patients was decreased and significant improvement was observed in ESR, when comparing both groups at the end of the treatment. This observation may be attributed to the anti-inflammatory activity of leech enzymes. Pre and post treatment X-ray were performed and showed no significant change. It is important to mention that the main limitation of this study was that modern diagnostic methods for evaluation of the osteoarhritic changes could not be used because of the lack of financial support.

As far as safety of leech therapy was concerned, haematological and biochemical parameters were evaluated before and after the therapy. During the whole therapy period, no significant change was seen in haematological parameters (haemoglobin, total leucocytes count, red blood cell count, platelet count), bleeding time, clotting time and prothrombin time.

Leech therapy was found to be safe and well tolerated, but in 16.67% patients, mild itching for a couple of days was noticed due to local irritation. Theoretically, leech therapy carries an infection risk because of the bacteria, which is found in leech gut *Aeromonus hydrophila*. However, there was no case reported in this study. It may be due to highly selective patients

(non-immuno compromised) and strict adherence to good hygienic practice.

Conclusion

There was statistically significant improvement observed in reduction of pain, other symptoms, and physical functions during treatment and even after 4 weeks of treatment. Therefore, the leech therapy seems to be an effective treatment for reducing symptoms of knee osteoarthritis and restoring the physical functions, moreover the therapy was found to be safe and well tolerated.

Authors contribution

Complete research was done by all the authors.

Financial support

None.

Conflicts of interest

None.

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