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A STUDY ON EFFECT OF HERBAL PHONOPHORESIS ON PAW VOLUME IN FREUND'S ADJUVANT INDUCED ARTHRITIS IN EXPERIMENTAL RATS

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ABSTRACT

Background: Arthritis is a musculoskeletal system disorder following mechanical and biological events that destabilize normal coupling between degradation and synthesis withinarticular cartilage. Rheumatoid arthritis (RA) is one of the commonest autoimmune diseases, is a chronic, progressive, systemic inflammatory disorder affecting the synovial joints and typically producing symmetrical arthritis that leads to joint destruction (Figure 1), which is responsible for the deformity and disability. **Objective:** To evaluate the effect of ultrasound with herbal phonophoresis on paw volume in freund's adjuvant induced arthritis in experimental rats. **Intervention:** Adult Wistar male rat with an initial body weight of 180 to 200g were taken, and divided into four groups each containing six animals. Group I served as normal rats. On day zero, group II to IV rats were injected into the sub plantar region of the left hind paw with 0.1ml of Freund's complete adjuvant. This consists of Mycobacterium butyricum suspended in heavy paraffin oil by thorough grinding with motor and pestle to give a concentration of 5mg/ml (This dose confirmed in our lab followed by different concentrations (1 to 10mg/ml)). **Design:** Comparative Experimental Design. **Results:** Within group analysis revealed significant improvement in either groups ($p<0.05$) after 1week of intervention. However when both groups were compared, there was no statistical significant difference found. **Conclusion:** this study conclused that there was significant effect on ultrasound with herbal phonophoresis on paw volume in freund's adjuvant induced arthritis in experimental rats.

KEYWORDS: Phonophoresis, Arthritis, Oxidative stress, Freund's complete adjuvant.

INTRODUCTION

Arthritis is a musculoskeletal system disorder following mechanical and biological events that destabilize normal coupling between degradation and synthesis within articular cartilage. Arthritis can affect individuals of any age but is more predominant in the age range of 25 and 50 years with a peak in the age range of 40-50years (Kaur *et al*., 2013). India (and South Asia more generally) is an important region in which to pursue humoral constructions of arthritis and joint disorders. Many Indians suffer from joint pain and rheumatic problems: osteoarthritis is widespread and rheumatoid arthritis, the far less prevalent but more incapacitating form of the disease, affects an estimated ten million Indians, 80% of which are women (Times of India, 1999).

Rheumatoid arthritis (RA) is one of the commonest autoimmune diseases, is a chronic, progressive, systemic inflammatory disorder affecting the synovial joints and typically producing symmetrical arthritis that leads to joint destruction (Figure 1), which is responsible for the deformity and disability. The consequent morbidity and mortality has a substantial socio-economic impact (Buch and Emery, 2002).

Rheumatoid arthritis (RA) is considered the most common chronic inflammatory autoimmune disease, occurring in 1 to 2% of the worldwide population (Firestein *et al*., 2005). The epidemiological ratio of arthritis in female and male is 3:1 and the prevalence is 1% of the world population. The peak age of onset is between 30 and 50 Annually, the incidence of RA is 30 per 100,000 people are reported every year in both developed and developing countries. Due to the fact that women are affected more than men, the prevalence of RA in women over the age of 65 is around 5% (Spector, 2010). Lower incidences of rheumatoid arthritis are reported every year in East Asia. The prevalence of arthritis is approximately in the West (Lipsky, 2005). The prevalence of RA in India subcontinent is 1.5-2 percent of population. The true picture of annual incidence rate of rheumatoid arthritis in India has not been well documented.

Today's medicine is based on traditional medicine. Traditional medicines exist in every continent of the globe and in every cultural area of the world. Over 80% of the world's population relies on traditional plant-based medicines for their primary healthcare needs. According to WHO traditional system of medicine refers to "the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable, or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness" (Sharma, 2003). In many developing countries, traditional and herbal medicines are very much indispensable.

Physical therapy or physiotherapy is the health care profession primarily concerned with the remediation of impairments and disabilities and the promotion of mobility, functional ability, quality of life and movement potential through examination, evaluation, diagnosis and physical intervention. Many drugs are poorly absorbed through the skin by passive diffusion alone. The use of topical agents often requires vehicle formulations or chemical penetration enhancers that are potential irritants or sensitizers. Ionotophoresis and herbal phonophoresis are methods of driving topically applied substances across tissues by utilization of electric current or ultrasound, respectively.

The Siddha system is one of the oldest systems of medicine in India. The term 'Siddha' means achievement, and the Siddhars were saintly figures who achieved results in medicine through the practice of yoga. Eighteen Siddhars seem to have contributed towards the development of this medical system. The Siddha system is practiced in Tamil-speaking parts of India, and its literature is in Tamil (so called Tamilian medicine). The system is also called Agasthyar system in the name of its famous exponent sage Agasthya. This system of medicine developed within the Dravidian culture, which is also of the pre-Vedic period. The Siddha system is largely therapeutic in nature. According to this system the human body is the replica of the universe and so are the food and drugs irrespective of their origin. The Siddhars investigated and studied the causes of many diseases and the effect of many locally available plants and minerals on these diseases. About 500 medicinal plants are used in Siddha (Tiwari *et al*., 2004). Agents derived from plants that can modulate the expression of pro-inflammatory signals clearly have potential against arthritis. These include flavonoids, terpenes, quinones, catechins, alkaloids, anthocyanins and anthoxanthins, all of which are known to have anti-inflammatory effects. Some of these polyphenols, which have been tested for the treatment of arthritis (Khanna *et al*., 2007).

MATERIALS AND METHODS

Animals

Male rats were obtained from the Sri Venkateshwara Enterprises, Bangalore 560 021, India. The animals were housed in polypropylene cages. The cages were lined with paddy husk which was replaced every day. Rats were fed with pelleted food and water was provided through plastic bottles. All the rats used in the experiments were marked by tail marking growth of the animals was monitored regularly and rats showing poor growth rate were discarded from the experiments.

Chemicals

Complete Freund's adjuvant was obtained from Sigma Aldrich (Saint Louis, Missouri, USA) and Trichloro acetic acid, Ethylenediamine tetra acetic acid (EDTA), Glutathione and Thiobarbutric acid were purchased from Sisco Research Laboratories Pvt. Ltd., India. All other chemicals and solvents used were of analytical grade available commercially.

Collection of plant

The root of Plumbago zeylanica were collected from Thanjavur, December 2010, Tamil Nadu, South India. The collected leaves were identified and authenticated by a Botanist Dr. M. Jegadeesan, Prof. and Head, Department of Environmental and Herbal Sciences, Tamil University, Thanjavur, Tamil Nadu. A Voucher specimen (TUH: 194) has been deposited at Tamil University Herbarium. The plants were cut into small pieces and shade dried and powdered finely then used for extraction.

Preparation of plant extract

The collected plant materials were washed, sliced and completely dried in a hot-air oven at 37 °C. The dried materials was ground into make a fine powder and used for extraction. Three hundred grams (300g) of the powered plants were extracted with ethanol (70%) using "Soxhlet Apparatus" for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used.

Preparation of Gel base ointment

0.5g of Plumbago zeylanica root extract was weighed, dispersed in gel with mild stirring and allowed to swell for 5 minutes to obtain 0.5% gel.

Collection of blood sample

At the end of the experimental period, the animals were anaesthetized using chloroform vapour prior to dissection. Blood samples were collected from the tail vein into a micro centrifuge tube containing 50mM ethylenediamine tetra acetic acid (EDTA) for the determinations of hematological profile.

Hematological estimations

Haemoglobin was estimated by Cyanmethaemoglobin method 12. RBC and WBC counted by the method of

Ochei and Kolhatkar 13. ESR sedimentation rate measured by the method of Ochei and Kolhatkar 13. PCV counted by the method of Ochei and Kolhatka, 13. Differential leukocyte and Total leukocyte were counted by the method of Srikumar et al 13.

Rheumatoid factor

The latex turbidimetry method was used in the present study using RF turbilatex kit of SPINREACT Company. Calibration was carried out for linear range up to 100 IU/ml the reading of RF factor of all the groups obtained was compared with the control animals.

Values were expressed as IU/ml.

Statistical Analysis

Table-1.1: Effect of ultrasound and herbal phonophoresis on paw volume in Freund's adjuvant induced arthritis in experimental rats.

Days	Group I	Group II	Group III	Group IV
Day 4	-	5.13±0.07	4.56±0.07 *	4.21±0.05*
Day 8	-	5.23±0.09	3.54±0.04 *	3.42±0.09*
Day 15	-	5.58±0.10	2.84±0.12 *	2.56±0.18*
Day 21	-	5.69±0.11	1.78±0.08 *	1.05±0.14*
% inhibition of paw swelling on 21st day	-	-	60.96	75.05

Values were expressed as mean ± SD for six rats in each group.
*Significantly different from Group II *p< 0.05

Table 1.2: Inflammatory markers of Freund's adjuvant induced arthritis in experimental rats.

	Group I	Group II	Group III	Group IV
RF (IU/ml)	8.21±0.55	48.46±3.2#	24.22±2.43*	13.66±0.92**
TNF-α (pg /ml)	10.64±0.72	15.74±1.07#	11.42±0.77*	8.95±0.60**
IL-6 (pg /ml)	33.21±2.25	98.44±6.69#	72.94±5.59*	56.89±3418**
Homocystine (µg/ml)	7.86±0.53	13.45±0.91	12.04±0.83*	10.54±0.71**
Serum Cortisol (ng/ml)	8.24±0.56	11.89±0.80	10.64±0.69***	9.21±0.62*
N-acetyl-β-glucosaminidase (U /min/ml)	31.95±2.17	47.24±3.21#	42.45±2.87***	33.82±2.29*
β-glucuronidase (mU)	1.36±0.09	2.87±0.19#	2.56±0.19**	1.86±0.12*
Hexose (µg/ml)	205.7±5.2	335.89±7.67#	321.98±7.08**	248.62±5.82*
Hexosamine (µg/ml)	49.70±2.57	64.22±3.16#	59.58±2.85***	56.04±1.65*
Sialic acid (µg/ml)	84.39±2.06	104.39±4.26#	97.76±3.18**	95.21±2.88*
NO (µM/L)	27.36±1.86	58.41±3.97#	52.68±3.25***	34.96±2.37*

Values were expressed as mean ± SD for six rats in each group.
*Significantly different from Group II #*p< 0.001; ** p< 0.01; *** p< 0.05
Significantly different from Group I

Table 1.3: Hematological changes in Freund's adjuvant induced arthritis in experimental rats.

	Group I	Group II	Group III	Group IV
Hb (gm/dl)	15.34± 1.04	8.52±0.57#	10.79±0.73*	12.50±0.85*
RBC (Million/cu.mm)	10.20±0.69	7.00±1.08#	13.00±0.88*	11.80±0.80*
WBC (cu.mm)	3.1±0.21	4.6±0.31#	3.8±0.25*	3.3±0.29*
ESR (mm)	15.30±1.04	22.30±1.51#	19.12.4±1.38**	18.4±1.31*
PCV (%)	43±2.92	53±3.60#	47±3.19***	46±2.99**
MCH (pg/cell)	15.03±1.02	5.32±0..36#	8.30±0.56*	10.59±0.72*
MCHC (%)	35.67±2.42	16.07±1.03#	22.95±1.56*	27.17±1.84*
MCV (cubic micron)	42.15±2.86	33.12±2.38#	36.15±2.45*	38.98±2.65*

Values were expressed as mean ± SD for six rats in each group.
*Significantly different from Group II #*p< 0.001; ** p< 0.01; *** p< 0.05
Significantly different from Group I

Table 4.7: Tissues Antioxidant status in Freund's adjuvant induced arthritis in experimental rats.

Tissues	Group I	Group II	Group III	Group IV
MAD (nmole/mg protein)	4.15±0.28	6.62±0.45#	4.22±0.28*	4.37±0.29*
GSH (µg/mg protein)	5.16±0.35	2.58±0.17#	4.72±0.32*	4.87±0.33*
CAT (U/mg protein)	5.56±0.37	3.25±0.22#	5.20±0.35*	5.49±0.37*
GPX (U/mg protein)	8.13±0.55	5.35±0.38#	7.33±0.49*	7.86±0.53*

SOD (U/mg protein)	5.77±0.39	2.98±0.39[#]	5.27±0.12*	5.45±0.37*
Vit C (µg/mg protein)	5.63±0.38	3.18±0.25[#]	5.27±0.12*	5.45±0.37*
Vit E (µg/mg protein)	4.05±0.27	2.1±0.14[#]	3.6±0.24*	3.7±0.25*

Values were expressed as mean ± SD for six rats in each group.
*Significantly different from Group II [#]*p< 0.001
[#] Significantly different from Group I

CONCLUSION

The result of the present experiment indicates that phonophoresis therapy possesses significant antiarthritic activity as compared with ultrasound application. The possible mode of action of anti- arthritic activity of phonophoresis therapy appears to be enhanced membrane permeability and inhibit the inflammatory reactions by scavenging of pro-oxidant and improving anti-oxidant parameters. The potential phonophoresis therapy might be due to various ingredients in Plumbago zeylanica extract acting synergistically and working in concert for overall antiarthritic activity

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