International Journal of Multidisciplinary Research and Development

International Journal of Multidisciplinary Research and Development

Volume: 2, Issue: 10, 155-158
Oct 2015
www.allsubjectjournal.com
e-ISSN: 2349-4182
p-ISSN: 2349-5979
Impact Factor: 5.742

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Impact of Phonophoresis Therapy in Hematological Changes on Freund's Adjuvant Induced Arthritic Rats

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Abstract
Haematological profiles of blood can provide important information about the internal environment of the organism. In the present investigation the effect of ultrasound and phonophoresis on the blood components is determined in Freund's adjuvant induced arthritic rats. The decrease in RBC count, Hb and ESR observed in the present investigation could be described are retarded haemopoisis, destruction and shrinkage of RBC. MCV, MCH and MCHC showed significant decrease in the present investigation, due to destruction of RBC (size and shape) and decrease in Hb synthesis and hemoglobin content. These symptoms imply the microcytic - hypo chromic anemia. A decreased percentage of neutrophils in peripheral blood observed in animals with Freund's adjuvant induced arthritic rats may suggest, neutrophils involves in phagocytosis during Freund's adjuvant intoxication, during which some of the neutrophils might have ruptured. 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 and improving heamatological parameters. Thus, the increase in the Hb and RBC count brought about by ultrasound and phonophoresis treatment further support its anti-arthritic effect. The potential phonophoresis therapy might be due to various ingredients in *Plumbago zeylanica* extract acting synergistically and working in concert for overall antiarthritic activity.

Keywords: Hematology, Ultrasound, Phonophoresis, *Plumbago zeylanica*, Arthritis

Introduction
Hematology is defined as the branch of biology, which deals with the morphology of blood and blood forming organs. Blood is a specialized bodily fluid that delivers necessary substances to the body's cells such as nutrients, oxygen and transports of waste products away from those of same cells. Blood is the most important body fluid that governs vital functions of the body like respiration, circulation, excretion, osmotic balance and the transport of metabolic substance. Circulation of the blood within the cardiovascular system is essential for transportation of gases, nutrients, minerals, metabolic products and hormones between different organs1. Blood parameters are probably the more rapid and detectable variations under stress and are fuel in assessing the health condition 2. The importance of haematological parameters in clinical biochemistry, population genetics and medical anthropology is well established. Recent speculations have proved that they may be used as valuable indicators of disease or stress in animals 3.

Arthritis is a common inflammatory joint disease characterized by inflammation of the synovial membrane, pain and restricted joint movement 4. Rheumatoid arthritis (RA), 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, which is responsible for the deformity and disability. The consequent morbidity and mortality has a substantial socio-economic impact 5. Adjuvant induced arthritis in rats is a chronic inflammatory disease characterized by infiltration of synovial membrane in association with destruction of joints resembles RA in humans 6. Physiotherapy has great potential to play a vital role in ortho, sports, neuro as well as cardio and also the prevention of injury and developing a particular skill for an athlete in the specialized field. Proper diagnosis, choosing the appropriate modalities and applying the perfect methods are the pillars of the successful treatment 7, 8. So choosing the appropriate modality is the key to produce good results. 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.

Phonophoresis, the use of ultrasound to enhance the percutaneous absorption of drugs, was first reported by Fellinger and Schrnid 9. They demonstrated successful treatment of polyarthritis of the hand by driving hydrocortisone ointment into the inflamed area with ultrasound. The term ultrasound refers to sound waves with frequencies beyond the human audible range of 20 kHz 10. 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 11. The medicinal value of chosen plant *Plumbago zeylanica* root belonging to the family of Plumbaginaceae. Therefore, the present study was to investigate the hematological profile of ultrasound and phonophoresis therapy in Freund's adjuvant induced arthritic rats.

Materials and Methods

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.

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.

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.

Freund's Complete Adjuvant induced Arthritic Model

Adult Wistar male rat with an initial body weight of 180 to 220g 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)). Application of ultrasound and phonophoresis based ointment treated to group III and Group IV rats respectively were started on the first day and continued for 21 days. Group II rats served as control rats (arthritis rats). The gel based plant extract of phonphoresis has been conducted through Ultrasound device, one session per day for 3 weeks. The rats were holding on comfortable position, then clean and hydrate the body part under treatment. The ultrasound device treated on paw edema sites. Adjust the US frequency to 1.5MHz, with intensity 1.5 W/cm2 and the time of treatment was 5 min. For group III, the rats were applied the gel based ointment to the selected area once daily.

The degree of inflammation was measured by a mercury displacement method. The edema formation and the percentage of inhibition were calculated as follows.

$$\text{Percentage of inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where Vc is the edema volume of the control group and Vt is the edema volume of the treated group.

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.

Statistical Analysis

Statistical analysis is performed using SPSS. Data are expressed as mean ± SD and statistically assessed using one-way ANOVA followed by Tukey test; $p < 0.05$ was considered significant.

Results and Discussion

Hematological parameters, such as hematocrit, hemoglobin, and numbers of erythrocytes and white blood cells, can be used as indicators of toxicity and have a broad potential application in environmental and occupational monitoring 15, 16. Most chronic inflammatory rheumatic diseases are complicated by hematologic abnormalities, including anemia; disorders of leukocytes, platelets, and the coagulation system; and hematologic malignancies 17. Ultrasound (US), which is a deep tissue heating modality, can elevate tissue temperature. The physiologic response due to ultrasound therapy includes increased collagen tissue extensibility, pain threshold and enzymatic activity, along with changes in nerve conduction velocity and contractile activity of skeletal muscle 18. Recent

evidence-based guidelines conclude that the therapeutic US was effective in the treatment of calcific tendonitis of the shoulder 19.

The purpose of the study is to screen and evaluate hematological profile using phonophoresis technique. Evaluation of antioxidant activity of phonophoresis therapy (Application of ultrasound along with *Plumbago zeylanica* root extract gel) and ultrasound was studied on Complete Freund's Adjuvant (CFA) induced arthritis in Wistar strain albino rats. The choice of the animal strain has been found to be very important for the performance of this test. Wistar-strain rats have been proven to be very suitable in contrast to other sub strains.

Table 1: Hematological changes in Freund's adjuvant induced arthritis in experimental rats

Parameters	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	16.00±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
Significantly different from Group I
# *P < 0.001; **P < 0.01; ***P < 0.05

Decrease in RBC count and haemoglobin level represents the anemic condition in arthritic rats. It is proposed that the reduction in the Hb count during arthritis results from reduced erythropoietin levels, a decreased response of the bone marrow erythropoietin and premature destruction of red blood cells. The more important causes are the abnormal storage of iron in the reticulo endothelial system and synovial tissue and failure of bone marrow to respond to anemia. MCV, MCH and MCHC showed significant decrease in the present investigation (Table 1). due to destruction of RBC (size and shape) and decrease in Hb synthesis and hemoglobin content. These symptoms imply the microcytic hypo chromic anemia. Thus, the increase in the Hb and RBC count brought about by ultrasound and phonophoresis treatment further support its anti-arthritic effect.

In arthritic condition there is mild to moderate increase in the WBC count which plays a major role in body defense mechanism. WBC count increase is may be due to the release of interleukins, responsible for production of both granulocytes and macrophages colony stimulating factor 20. In the present study; CFA exhibited significant increase in the ESR which is attributed to the accelerated formation of endogenous protein such as fibrinogen and α, β globulin, and such a rise in the ESR indicates an active disease process. Increase in the erythrocyte sedimentation rate is an indication of active but obscure disease process which elevate in response to stress, inflammation and cell necrosis 21. In the present study**,** ultrasound and phonophoresis treatment restored the altered haematological profile by decreasing the ESR provides support for its antiarthritic effect. Treatment with the ultrasound and phonophoresis significantly decrease the ESR and the WBC count indicate the significant recovery from the arthritic progress

Table 2: Total and Differential count of leucocytes on Freund's adjuvant induced arthritis in experimental rats.

Groups	Total count (TLC) (Cu.mm/ml)	Differential count (DLC) (%)				
		Neutrophil	Eosinophil	Monocyte	Basophil	Lymphocyte
Group I	4628.33 ± 80.37	41.33 ± 2.04	1.43 ± 0.21	1.31 ± 0.12	1.12 ± 0.19	50.63 ± 1.82
Group II	5926.66 ± 182.56#	38.83±1.97 a	1.38 ± 0.19#	1.64 ± 0.18#	0.93 ± 0.16a	68.66 ± 2.13#
Group III	5596.63 ± 137.60**	35.65 ± 1.63 a	1.40 ± 0.20 a	1.28 ± 0.11*	0.61 ± 0.19 a	62.63 ± 1.85*
Group IV	5445.83 ± 123.40*	38.33 ± 1.92 a	1.46 ± 0.23 a	1.12 ± 0.08*	0.60 ± 0.22 a	58.83 ± 2.10*

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

The significant increase in total and differential leukocytes count (Table 2) has been suggested to be due to stimulated lymphopoiesis and/or enhanced release of lymphocytes from lymph myeloid tissue. Such lymphocyte response might be due to the presence of toxic substances may be associated with the pollutant induced tissue damage and severe disturbance of the non-specific immune system leading to increased production of leukocytes and the respective decrease in SPP and SPE treated groups showed its immunomodulation effect. The total and differential leukocytes count which significantly decreased in arthritic control group has been remarkably counteracted by ultrasound and phonophoresis restoring back to near normal thus justifying its significant role in arthritic conditions

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 and improving hematological 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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