MODULATORY ROLE OF PHONOPHORESIS THERAPY IN ERYTHROCYTE ANTIOXIDANT ENZYME ACTIVITIES ON FREUND’S ADJUVANT INDUCED ARTHRITIC RATS

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Abstract
The aim of the present study was to investigate the erythrocyte levels of lipid peroxidation (MDA), and antioxidant enzyme activities in Freund’s complete adjuvant (FCA) induced arthritis in rats. Group I serves as normal. Group II to IV served as arthritis animals induced by FCA. Group III and IV treated with ultrasound and phonophoresis (Application of ultrasound along with Plumbago zeylanica root extract gel) respectively. Group II served as arthritic animals (Control). The phonophoresis has been conducted through Ultrasound device, one session per day for 3 weeks. Erythrocyte levels of malondialdehyde (MDA), the activities of antioxidant enzymes, as well erythrocyte sedimentation rates (ESR) were estimated in phonophoresis treated rats and Freund’s complete adjuvant (FCA) induced arthritis rats. Erythrocyte MDA concentrations were significantly higher in arthritis rats than those with phonophoresis treated and control rats. Erythrocyte SOD, GPx and CAT activities were found to be significantly lower in arthritis rats as compared with control rats. Ultrasound and phonophoresis (Application of ultrasound along with Plumbago zeylanica root extract gel) treated groups restored in the altered level of parameters were observed. Phonophoresis treated groups significantly restored the antioxidant activity as compared with ultrasound treated rats. These results indicate that application of phonophoresis to arthritic rats enhanced the antioxidant activity.

Keywords: Arthritis, Erythrocytes, Freund’s complete adjuvant, Plumbago zeylanica, Phonophoresis, Ultrasound.

1. INTRODUCTION
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 epidemiological ratio of arthritis in female and male is 3:1 and the prevalence is 1% of the world population. Arthritis is a common inflammatory joint disease characterized by inflammation of the synovial membrane, pain and restricted joint movement (Pearson CM, 1956). 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 (Buch and Emery, 2002). 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 (Katz and Piliero 1969). Presently many non steroidal, steroidal and immunosuppressive drugs are used to control inflammatory symptoms and pain; they are associated with certain undesirable side effects. With these difficulties, the field of arthritis research has progressed exponentially towards herbal therapies that have been considered safe and effective in all elevating chronic pain associated with arthritis Rao et al., (1999).

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 (Bare et al., 1996 and Goraj-Szczypiorowska et al., 2007). 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 1952). 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 Kassan et al., (1996). 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 anthoxanths, all of which are known to have anti-inflammatory effects. Some of these polyphenols, which have been tested for the treatment of arthritis (Khanna, 2007). The medicinal value of chosen plant Plumbago zeylanica root belonging to the family of Plumbaginaceae. Therefore, the present study was to investigate the antioxidant activity of ultrasound and phonophoresis therapy in Freund’s adjuvant induced arthritic rats.

2. MATERIALS AND METHODS

2.1 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.

2.2 Animals

Male rats were obtained from the Sri Venkateshwaras 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.

2.3 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.

2.4 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.

2.5 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.

2.6 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 (arthritic rats). The gel based plant extract of phonophoresis 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.

\[
\text{Percentage of inhibition} = \frac{V_c - V_t}{V_c} \times 100
\]

Where \(V_c\) is the edema volume of the control group and \(V_t\) is the edema volume of the treated group.

2.7 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.

2.8 Isolation of erythrocyte and its membrane

Blood collected with 3.7% trisodium citrate, as anticoagulant (0.1ml), was used for erythrocyte isolation. Plasma was separated by centrifugation at 2000 x g for 20 minutes. The packed cells were washed thrice with physiological saline and the plasma free red cells were used for the analysis. Erythrocyte membrane was isolated according to the method of Dodge et al., (1963).

2.9 Biochemical estimations

MDA released from endogenous lipoperoxides, reflecting the lipid peroxidation process, were assayed (Beuge and Aust, 1978). The activity of enzymatic antioxidants as superoxide dismutase, glutathione peroxidase and catalase activity were assayed by the method of Kakkar et al., (1984), Beers R and Sizer (1952) and Rotruck et al., (1973). ESR sedimentation rate by the method of Ochei and Kolhatkar, 2000).
Table-1: Effect of Ultrasound and Phonophoresis on Biochemical markers in Freund’s adjuvant induced arthritis in experimental rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAD (nmole/ mg Hb)</td>
<td>14.54±0.98</td>
<td>20.90±1.42*</td>
<td>17.27±1.17*</td>
<td>16.36±1.11*</td>
</tr>
<tr>
<td>SOD (U/mg Hb)</td>
<td>10.22±0.69</td>
<td>7.81±0.53*</td>
<td>8.97±0.59***</td>
<td>9.47±0.64**</td>
</tr>
<tr>
<td>CAT (U/mg Hb)</td>
<td>11.70±0.79</td>
<td>7.80±0.53*</td>
<td>9.10±0.61***</td>
<td>10.40±0.70*</td>
</tr>
<tr>
<td>GPX (U/mg Hb)</td>
<td>9.46±0.64</td>
<td>7.46±0.50*</td>
<td>8.7±0.57**</td>
<td>8.8±0.59**</td>
</tr>
<tr>
<td>ESR (mm)</td>
<td>15.30±1.04</td>
<td>22.30±1.51*</td>
<td>19.12±1.38*</td>
<td>18.4±1.31*</td>
</tr>
</tbody>
</table>

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

2.10 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.

3. RESULTS AND DISCUSSION
Inflammation and tissue injury related oxidative stress has been implicated in the pathogenesis of rheumatoid arthritis. Free radicals are enormously produced at the site of inflammation and tissue injuries (Ostrakhovitch and Afanas’ev, 2001). Lipid peroxides that are generated at the site of inflammation of tissue injury diffuses into blood and can be estimated in serum or plasma, which in turn reflect the severity of the tissue damage. Susceptibility of erythrocytes to peroxide stress is increased in several diseased conditions (Gutteridge, 1995). The elevated plasma lipid peroxidation observed in the present study in Freund’s adjuvant (FA) induced arthritis can be related to excessive lipid peroxidation observed in erythrocytes and erythrocyte membranes, with consequent leakage into plasma or as a result of excessive generation and diffusion of lipid peroxides from the inflamed or injured joints of rheumatoid arthritis.

The purpose of the study is to screen and evaluate antioxidant activity 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 (Michele, 2005).

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 (Rober, 2003). Recent evidence-based guidelines conclude that the therapeutic US was effective in the treatment of calcific tendonitis of the shoulder (Philadelphia Panel Members, 2001).

In this study, RA is a chronic degenerative disease, oxidative stress (e.g. MDA) was higher in arthritis rats than control and ultrasound and phonophoresis treated rats. Furthermore, there was a close relationship between oxidative stress and inflammation in patients with RA. In addition, activities of some erythrocyte antioxidant enzymes (e.g.SOD, GSH-Px and CAT) in the arthritis group were lower than control and treated rats.

In RA, the polymorphonuclear leukocytes are activated, and ROS are generated in excessive amounts. These are reactive ephemeral molecules known to play an important role in the progression of various diseases. During chronic inflammation protective mechanisms increase to levels which cause damage to the tissue. Increased concentrations of ROS cause enormous MDA production, leading to toxic damage to tissues (Biemond et al., 1984 and Halliwell, 1994). MDA levels were found to be significantly (p < 0.05) elevated in arthritis rats compared to control and ultrasound and phonophoresis treated rats. Increased MDA levels in arthritis rats observed in this report and previously published reports (Shivanand Pandey et al., 2010, Gambhir et al., 1997 and Lunec et al., 1981) are indirect indicators of increased ROS production.

Increased white blood cell counts are a common feature of inflammatory reactions, especially those induced by microbial infection. So in arthritic group an increase in total leukocyte number was found. A significant reduction in total leukocyte number was found in case of ultrasound and phonophoresis therapy groups (Table 1). In our study, it was found that phonophoresis therapy leads to inhibition of leukocyte migration which may have beneficial effect for joint preservation. The activity may be due to presence of steroidal glycoside of plant extract.

Intracellularly localized Cu–Zn SOD and the selenium-dependent enzymes, GSH-Px and CAT, scavenge the ROS and primarily superoxide anions, thus acting as an antioxidant enzymes (Michiels et al, 1994, Mahajan and Tandon, 2004). Erythrocytes may be important in regulating oxidant reactions in the surrounding medium, thereby prevent- ing free-radical-mediated cytotoxicity (Winterbourn and Stern, 1987). However, the relationship between erythrocyte SOD and RA is not clear. Our result that the activity of erythrocyte SOD is unaltered is in agreement with most studies. (Gambhir et al., 1997, 1998).
Olivieri et al 1991 and Akyol et al., 2001). On the other hand, Banford et al., (1982) have reported a significant decrease in erythrocyte SOD activity in arthritis rats. Erythrocyte GSH-Px activities in arthritis rats show decreased (Taysi et al, 2002 and Ashour M et al 2000) levels. Our results of erythrocyte GSH-Px and CAT activities, similar to Rober et al., (2003) and Ijoeuma et al (2014) showed significantly lower levels in arthritis rats than in the other two groups. The statistically significant decreases in plasma GSH-Px and CAT activities between arthritis rats may be due to the inactivation of the enzymes by H2O2 and suggest that these enzymes may play an important role in the rheumatic process and increased oxidative stress. Ultrasound and phonophoresis treated rats shows significantly restored the antioxidant enzymes.

MDA levels were found to be significantly elevated in the arthritis rats compared to control and ultrasound and phonophoresis treated rats (p<0.05) (Table 2). This is in agreement with other studies in which higher MDA levels have been reported in patients with RA (Cimen et al., 2000, Taysi et al., 2002 and Akyol et al., 2001). It seems that increased oxidative stress, present in the inflamed joints, is due to activated neutrophils and is reflected as increased MDA in peripheral blood in inflammatory RA. Furthermore, decomposition of peroxidized lipid yields a wide variety of end-products, including MDA (Biemond et al 1984, Ozgunes et al 1994 and Gambhir et al 1997).

The results of our study indicate that the antioxidant defense system is compromised in arthritis rats, as evidenced by increased MDA concentrations (the most potent marker of oxidative stress) and decreased levels of antioxidant enzymes (SOD, GSH-Px, CAT). We have also demonstrated that ESR, a serologic marker of inflammation, shows a significant correlation to MDA levels in arthritis. MDA in RA patients may be useful in predicting disease activity.

In this study, we studied erythrocyte MDA, antioxidant enzyme levels in arthritis and compared these indices with those of control and ultrasound and phonophoresis treated rats. In conclusion, increased oxidative stress in arthritis rats probably depends on their inflammatory response. These results provide some evidence for the potential role of increased MDA and decreased antioxidant in arthritis rats as a result of its inflammatory character. Phonophoresis treated rats shows potential antioxidant activity than ultrasound treated rats. This might be due to various ingredients in Plumbago zeylanica extract acting synergistically and working in concert for overall antioxidant activity.

References


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