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Efficacy of phonophoresis therapy in plasma antioxidant status on Freund's adjuvant induced arthritic rats

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Abstract

Antioxidant profiles of plasma can provide important information about the defense mechanism of an organism. In the present investigation the effect of ultrasound and phonophoresis on the plasma antioxidant is determined in Freund's adjuvant induced arthritic rats. The increased MDA content and decreased in superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and non enzymatic antioxidant, such as glutathione (GSH), vitamin C and vitamin E in arthritic rats were observed in the present investigation, due to increased free radical in arthritis rats. The result of the present experiment indicates that phonophoresis therapy possesses significant antioxidant activity as compared with ultrasound application. The possible mode of action of antioxidant activity of phonophoresis therapy appears to be enhanced membrane permeability and inhibit the free radical formation and thereby improving antioxidant parameters. Thus, the increase in superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and non enzymatic antioxidant, such as glutathione (GSH) brought about by ultrasound and phonophoresis treatment further support its antioxidant effect. The potential phonophoresis therapy might be due to various ingredients in *Plumbago zeylanica* extract acting synergistically and working in concert for overall antioxidant activity.

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

Introduction

Arthritis, the joint inflammation, refers to a group of diseases that cause pain, swelling, stiffness and loss of motion in the joints. Rheumatoid Arthritis (RA) is a chronic, systemic disease, in which various joints in the body are inflamed, leading to swelling, pain, stiffness, and the possible loss of function. Specifically it is targeting synovial membrane and extra-articular tissues Inflammatory infiltrates accumulate and persist in synovial membrane, and the clinical presentation of the syndrome is dominated by destruction of joint architecture [1]. It is an autoimmune disease in which the body's immune system attacks itself. Rheumatoid arthritis affects approximately 1- 2% of the total world's population. Annual incidence rate of rheumatoid arthritis is reported as between 0.5% to 1% of total population in both developed and developing countries. Lower incidences of rheumatoid arthritis are reported every year in East Asia. The prevalence of RA in Indian subcontinent is 1.5 to 2 percent of population. The prevalence of arthritis is more in the West and the true picture of annual incidence rate of rheumatoid arthritis in India has not been well documented. Rheumatoid Arthritis affects around 1 in 50 people and is more common among women than men [2].

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. Physiotherapy has a large part to play in the overall management of arthritis, and physiotherapists are trained to understand and treat the physical aspects of the human body. The most common types of treatment used by the therapist to manage arthritis are infra-red radiation (heat lamps), wax baths and hot packs for superficial treatments, microwave diathermy and shortwave diathermy for deep heating treatment. In addition, arthritis is also managed through electrical stimulation, ultrasonic sound waves, ice therapy and traction depending on the impairments noted in these patients. A balanced program of rest and exercise, and careful attention to optimize normal posture among these patients is an important part of pain management, joint protection and maintenance of joint function.

Among several choices of treatments available to manage arthritis, the application of ultrasound therapy in significant and several literatures are supporting the effectiveness of this modality.

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This physical modality offer methods for enhancing the percutaneous absorption of selected drugs. Likewise, there are numerous methods of administering drugs to the body, both passive and active. Active methods include the use of penetration enhancers and assisted drug delivery ('Phonophoresis' and 'Iontophoresis'). 'Iontophoresis' and 'Phonophoresis' are methods of driving topically applied substances across tissues by utilization of electric current or ultrasound, respectively. A recent review of the literature on phonophoresis reports that 75% of the studies reviewed reported positive effects of ultrasound on local subcutaneous drug diffusion, with some systemic effects being reported [3]. Herbal Based Phonophoresis possesses potentially useful for antioxidant activity. Hence in this research is to study the antioxidant activity of ethanolic extract of *Plumbago zeylanica* root gel based ointment and phonophoresis in Freund's adjuvant induced arthritic rat.

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 Thiobarbituric 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 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.

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 (arthritis 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 plasma samples

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 heparinized centrifuge tube and allowed to standing at room temperature for 30 minutes and then refrigerated for another 30 minute. The resultant clear part was centrifuged at 3000rpm for 10minutes, and then the plasma (supernatant) was isolated and stored at refrigerated for analysis.

2.8 Biochemical estimations

Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust [4]. Reduced glutathione was estimated by method of Moron *et al.* [5]. The level of ascorbic acid was estimated by the method of Omaye *et al.* [6]. α -tocopherol was estimated by the method of Baker *et al.* [7]. Copper-zinc superoxide dismutase activity was determined by the procedure of Kakkar *et al.* [8]. The activity of catalase and glutathione peroxidase was assayed by the method of Beers and Sizer [9] and Rotruck *et al.* [10].

2.9 Statistical Analysis

Values of the dependent variables were expressed as mean \pm SD for each group and to study the effect of experimental interventions between the groups, one way analysis of variance (ANOVA) was used. When significant F ratio was found, Turkey's test for multiple comparisons was used to find out the effect of treatment between each group separately. P value of <0.01, 0.001 are considered as significant. Analysis was carried out using SPSS (Statistical Packages for Social Studies) version 11 was used.

3. Results and conclusion

3.1 Effect of plant extract gel and Phonophoresis on Plasma Antioxidant status in Freund's adjuvant induced arthritis in experimental rats

Oxidative stress results from an imbalance between the generations of oxygen derived radicals and the organism's antioxidant potential. Various studies have shown that arthritis is associated with increased formation of free radicals and decrease in antioxidant potential. Due to these events, the balance normally present in cells between radical formation and protection against them is disturbed. This leads to oxidative damage of cell components such as proteins, lipids,

and nucleic acids. In the present study was investigating the oxidative stress and antioxidant defence markers in Freund's adjuvant induced arthritic rats. The content of lipid peroxidation product as MDA was increased and decreased activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and non enzymatic antioxidant, such as glutathione (GSH), vitamin C and vitamin E in arthritic rats were observed (Table 1). Application of plant extract gel and phonophoresis technique to Freund's adjuvant induced arthritic rats demonstrated that strengthen endogenous antioxidant defense by its ability to reduce the free radicals formed as a result of inflammation.

Table 1: Plasma Antioxidant status in Freund's adjuvant induced arthritis in experimental rats

| Plasma | Group I | Group II | Group III | Group IV |
|-------------------------|------------|-------------------------|-------------|-------------|
| MDA (nmol MDA formed/l) | 11.81±0.80 | 20.00±1.36 [#] | 15.63±0.92* | 13.63±0.92* |
| GSH (mg/dl) | 23.18±1.57 | 14.36±1.11 [#] | 19.63±1.26* | 20±1.36* |
| CAT (U/ml) | 9.75±0.66 | 5.45±0.39 [#] | 7.15±0.48* | 7.80±0.53* |
| GPx (U/ml) | 9.2±0.62 | 6.26±0.42 [#] | 7.46±0.50** | 7.86±0.53* |
| SOD (U/ml) | 4.69±0.31 | 3.55±0.24 [#] | 4.25±0.28** | 4.38±0.29* |
| Vit C (mg/dl) | 8.13±0.55 | 5.52±0.37 [#] | 6.87±0.44* | 7.54±0.51* |
| Vit E (mg/dl) | 7.2±0.48 | 4.5±0.30 [#] | 5.7±0.38* | 6.6±0.44* |

Values were expressed as mean ± SD for six rats in each group.

* Significantly different from Group II **p< 0.001; ** p< 0.01

[#] Significantly different from Group I

Rheumatoid arthritis is a major cause of morbidity as it affects the joints, causing stiffness and loss of mobility. The cause of rheumatoid arthritis is mainly joint inflammation initiated by oxidative stress. Involvement of oxygen free radicals (OFR) in the pathophysiology of inflammation in a number of organs and tissues has been reported in literature [11, 12]. Evidence of OFR generation in patients with RA has been observed by measuring the product of lipid peroxidation malondialdehyde. Antioxidant status was assessed by measuring superoxide dismutase and glutathione reductase, In view of the recent animal studies strongly suggesting anti-inflammatory role of antioxidants like superoxide dismutase [13] and vitamin E [14] in experimentally induced arthritis, antioxidant therapy strategies have been proposed for the prevention and treatment of RA [15, 16].

Inflammation and tissue injury related oxidative stress has been implicated in the pathogenesis of arthritis. Free radicals are enormously produced at the site of inflammation and tissue injuries. 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 [17]. In the present study mean level of MDA was increased significantly in arthritis rats as compared to control. Our findings are in accordance with the research of Shaabani *et al.*, [18], Walwadkar *et al.*, [19], Ansari and Jaiswal [20]. MDA is a decomposition product of lipid peroxidation of polyunsaturated fatty acids which is used as an index of oxidative damage [21] also reported markedly increased concentrations of MDA. Enhanced lipid peroxidation may occur as a result of imbalance between scavenging mechanisms and free radical generation process. Similar reports of elevated MDA levels have been reported in arthritis [22]. Treatment with the ultrasound and phonophoresis significantly decrease MDA content indicate the significant recovery from the arthritic progress. Among the ultrasound and phonophoresis therapy, phonophoresis possesses significant activity than ultrasound therapy.

Antioxidants are the compounds of exogenous or endogenous in nature which either prevent the generation of toxic oxidants

or intercept any that are generated and inactivate them and thereby block the propagation of chain reaction produced by these oxidants. These can be classified as enzymatic antioxidants, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione reductase, non-enzymatic antioxidants like (nutrient antioxidants) beta-carotene, alpha-tocopherol, ascorbic acid, bioflavonoids and (metabolic antioxidants) like glutathione, ceruloplasmin, albumin, bilirubin, ferritin, transferrin, uric acid and lactoferrin [23]. In recent years increasing experimental and clinical data has provided compelling evidences for the involvement of FR/ROS in large number of pathophysiological states [24] including rheumatoid arthritis (RA) [22, 25]. This has led to increased interest amongst the researchers globally to evaluate role of antioxidant therapy in RA.

Glutathione peroxidase prevents the accumulation of oxidized lipids in mitochondrial cell membranes and also detoxifies H₂O₂ by utilizing reduced glutathione as a cosubstrate [26]. Glutathione levels and glutathione peroxidase activity were decreased in plasma as compared to control. It has been reported that decrease in glutathione, a cosubstrate of glutathione peroxidase, results in a greater susceptibility to oxidative stress [27]. The altered activity of glutathione peroxidase in plasma observed in the present study can therefore be related to the availability of reduced glutathione in plasma.

Superoxide radicals play an important role as a chemical mediator on the inflammatory response of rheumatoid arthritis [28]. The decreased activity of plasma superoxide dismutase observed in the present study could therefore be due to the excess superoxide radicals that are generated and diffused from the inflammatory sites into plasma and inactivate the enzymes. The hydrogen peroxide formed due to the above reaction could not have been detoxified due to lowered activity of plasma glutathione peroxidase and catalase observed in the present study. It has been demonstrated that the hydrogen peroxide produced due to dismutation of superoxide could possibly be converted to hydroxyl radical by iron released from haemoglobin of lysed erythrocytes.

Superoxide dismutase and catalase, play an important role in the protection of erythrocytes from oxidative stress. Lowered activities of catalase and superoxide dismutase have been reported in arthritis [22]. Our results lend credibility to these observations. The elevated lipid peroxidation in plasma observed in the present study can therefore be related to compensatory changes in the antioxidant defence system of arthritis.

In the present study, we have observed a multidirectional changes of non enzymatic and enzymatic antioxidants as compared to control. Vitamin E, Vitamin C and reduced glutathione, a well known antioxidants, play an important role in protecting the lipids of lipoproteins and other biomembranes against peroxidative damage by intercepting oxidants before they can attack the tissues [29]. Lower concentration of vitamin E has been reported in the joint fluid of rheumatoid arthritis [30]. An inverse relationship between lipid peroxidation and non enzymatic antioxidants has been well documented [31]. Hence, the decrease in plasma non enzymatic antioxidants can be correlated to impairment in the antioxidant defence mechanism, due to excess utilization by the inflamed tissues to scavenge the excessive lipid peroxides that are generated at inflammatory sites, or to scavenge accumulated lipid peroxides in plasma. Treatment with the ultrasound and phonophoresis significantly increased the enzymatic and non enzymatic antioxidants indicate the significant recovery from the inflammatory progress. Among the ultrasound and phonophoresis therapy, phonophoresis possesses significant activity than ultrasound therapy.

The result of the present study indicates that phonophoresis therapy possesses significant antioxidant activity as compared with ultrasound application. The possible mode of action of antioxidant activity of phonophoresis therapy appears to be enhanced membrane permeability and inhibit the free radical formation and thereby improving antioxidant parameters. The potential phonophoresis therapy might be due to various ingredients in *Plumbago zeylanica* extract acting synergistically and working in concert for overall antioxidant activity.

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