EFFECT OF VITEX NEGUNDO LEAF EXTRACT ON THE FREE RADICALS SCAVENGERS IN COMPLETE FREUND'S ADJUVANT INDUCED ARTHRITIC RATS

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ABSTRACT

The effect of the oral administration of Vitex negundo leaf extract on the levels of enzymic and non-enzymic antioxidants were studied in the adjuvant induced arthritic (AIA) rats. The levels of antioxidant enzymes such as SOD, CAT, GPx, G6PD, GSH and Vit-C were estimated in various groups of the experimental rats. It was observed that the antioxidant enzyme levels in the AIA were significantly low when compared to normal rats. A significant decrease in enzymic antioxidant – SOD, CAT, GPx, G6PD and non-enzymic antioxidant – GSH, Vit-C were observed in the liver of AIA rats compared to the normal rats. These results suggest that the leaf extract of Vitex negundo possesses antioxidant activity.

KEY WORDS

Antioxidant, Free radicals, Vitex negundo

INTRODUCTION

Rheumatoid arthritis is a chronic inflammatory disease of joints that results in joint pain, swelling and destruction. It affects an estimated 1% of the adult population throughout the world. Progression of the disease results in joint destruction deformity and significant disability (1). Adjuvant induced by intradermal injection of Complete Freund's Adjuvant (CFA) is a chronic generalized reaction and is an accepted experimental model of rheumatoid arthritis (2).

Oxygen derived free radicals and their products are known to play an important role in the pathogenesis of chronic inflammatory disorders (3). These activated, oxygen intermediates together with secondarily formed radicals, like the hydroxyl radicals (OH•) are able to destroy membrane lipids, proteins, DNA, hyaluronic acid and cartilage (4).

The importance of oxygen free radicals and related activated oxygen intermediates in the pathogenesis of rheumatoid

arthritis has been identified with increasing incidence (5). Polymorphonuclear leucocytes and macrophages are stimulated which results in the production of inflammatory mediators including large amount of superoxide and hydrogen peroxide (6). Free radicals and in particular, superoxide radicals cause cellular disruption due to peroxidation of membrane lipids (7).

Hence the study of antioxidant status during a free radical challenge can be used as an index of protection against the development of degenerative inflammatory processes in experimental condition for therapeutic measures. *Vitex negundo* belongs to the family verbenaceae is a large aromatic shrub or a small tree of about 3M in height. It is claimed to possess anti-inflammatory, anti-spasmodic and anti-asthmatic properties (8). The present study aims to investigate the antioxidant property of the leaf extract of *Vitex negundo* in AIA.

MATERIALS AND METHODS

Plant collection and preparation of plant extract : *Vitex negundo* leaves were collected from local gardens. They were authenticated by the Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamil nadu. The fresh leaves were collected washed with distilled water and shade dried. The dried plant material was powdered and extracted

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with ethyl acetate by immersing for 72 hours (9).

Selection of animals : Female albino rats, an average weight of 90-100 gms were obtained from Mannuthy, Thirussur. (The animal used for experiments were accepted by ethical clearance committee). The female rats were housed in large spacious cage and were allowed to consume adequate amount of diet for 10 days. (Acclimatization period)

Diet used : The rats were fed with "Manna Health Mix", Cooked rice and the commercial pelleted animal feed marketed by M/S. Hindustan lever Ltd, Bangalore, India, under the trade name "Gold Mohur Feed" was used. 100g of this diet contained protein 21%, lipid 5%, crude fibre 4%, ash 8% calcium 1%, phosphorus 0.6%, nitrogen free extract 55% and provided with metabolic energy at 3600 Kcal/Kg and water was given *ad libitum*.

Induction of arthritis : Rats were induced with 0.1 ml of Freund's Complete Adjuvant intradermally containing 10mg/ml of heat killed mycobacterium tuberculosis (10, 11).

Experimental set-up : Rats were divided into four groups comprising of six animals in each groups, as follows

- Group I - Served as control
- Group II - Control rats treated with the extract of *Vitex negundo*
- Group III - Freund's Complete Adjuvant induced rats (0.1 ml)
- Group IV - Arthritis induced rats were administered with extract of *Vitex negundo* (50mg/kg body weight) for 30 days by intubation starting 10 days of adjuvant infection.

Biochemical studies : The female albino rats were killed on 30th day. The liver was removed and washed with ice cold saline and weights were recorded.

Preparation of tissue homogenate : The liver was cut into small pieces and washed with ice cold saline, 10% homogenate of liver tissues were prepared using 0.01 M Tris-HCl buffer pH 7.4 at 4°C and centrifuged at 12,000g for 30 min (10, 12). The supernatant fluid was used for the assay of various antioxidant enzymes SOD activity – SOD activity in the tissues were determined by using the nitrite method of Das et al. (13), CAT activity were assayed by the method of Sinha (14), GPx activity were assayed by the method of Rotruck et al (15), G6PD activity were estimated by the method of Balinsky & Bernstein (16), GSH were estimated by the

method of Moron et al (17), Vit-C were estimated by the method Omeye et al (18), the level of protein were estimated by the method Lowry et al (19).

Statistical Evaluation : The results obtained in this study were expressed as mean±SD. The results were analysed by the application of one-way analysis of variance (ANOVA) (20).

RESULTS

The result of antioxidants in liver are depicted in the table 1. The changes in the enzymic antioxidant levels of SOD, CAT, GPx, G6PD showed a considerable decrease in the group III (rats treated with adjuvant). The values of adjuvant induced rats treated with the leaf extract were reverted to near normal when compared with group I (control rats). There was no significant difference found in the group II (control rats treated with leaf extract of *Vitex negundo*).

Similarly the non-enzymic antioxidant levels of GSH, Vit-C in control and experimental rats were depicted in table 2. The activities of enzymes were considerably decreased in group III (adjuvant induced arthritic rats) when compared with group I (control rats) group 'a'. The antioxidant defence system were reverted to near normal condition in group IV animals (control rats treated with leaf extract) when compared to group III animals - group 'c' in terms of the parameters investigated. No significant changes were observed in group IV animals when compared with group II animals.

DISCUSSION

The present study shows that the altered levels of free radicals, antioxidant enzymes were reverted to a considerable extent with the oral administration of the leaf extract in the arthritic rats, proving its antioxidant property.

The body has a effective mechanism to prevent and neutralize the free radical induced damage. This is accomplished by a set of endogenous antioxidant enzymes, such as SOD, CAT, GSH, etc. When the balance between ROS (reactive oxygen species) production and antioxidant defence is lost, 'oxidative stress' results, which through a series of events deregulates the cellular function leading to various pathological conditions (21). Any compound natural or synthetic with antioxidant properties might contribute towards the partial or total alleviation of this type of damage. A major systemic event that occurs in the rat following the induction of inflammation is marked alteration in the cellular defence mechanism.

Table 1 : Activities of enzymic antioxidants in liver tissue – SOD, CAT, in control and experimental rats

Parameter	Group I C	Group II C+E	Group III AI	Group IV AI+T
SOD	7.31±0.099	7.83±0.119*	2.51±0.166**	7.05±0.051**
Catalase	68.59±0.513	65.38±0.448**	15.23±0.175**	20.49±0.340**
Glutathione Peroxidase	12.19±0.186	12.91±0.230*	6.47±0.129**	12.46±0.164**
Glucose-6 Phosphate Dehydrogenase	3.12±0.055	2.62±0.134*	1.35±0.032**	3.06±0.129**

Values are mean ±SD

Statistical comparison was done between Group I – III, Group I – II and Group III – IV; * at 5%; ** at 1% and rest not significant.

Enzymes units are expressed as :

SOD – 50% inhibition of nitrite / min / mg protein; CAT - μ moles of H_2O_2 decomposed / min / mg protein; GPx - μ g of GSH / min / mg protein and G6PD - 0.01 O.D / min / mg protein

Many cellular defence mechanisms are directed against the toxic effects of these radicals in inflammatory process. SOD is a metallo protein and is the first enzyme involved in the antioxidant defence by lowering the steady state level of $O_2^{\cdot-}$. SOD which converts superoxide radicals to H_2O_2 is widely distributed to protect such cells against the toxic effects of superoxide anion (22). Increased delivery of NADPH from the stimulated HMP shunt during inflammation is proposed to lead to the activation of SOD in arthritic rat (23).

CAT is a hemo protein, localized in the peroxisomes or the microperoxisomes. This enzyme catalyses the decomposition of H_2O_2 to H_2O and O_2 and thus protecting the cell from oxidative damage by H_2O_2 and $OH^{\cdot}$. The main function of catalase is to detoxify H_2O_2 . Although catalase is significantly increased in rheumatoid arthritis its concentration is very low to expect considerable protection against H_2O_2 (24). GPx is a seleno enzyme, two third of which (in liver) is present in cytosol and one third in the mitochondria. It catalyses the reaction of hydroxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of H_2O_2 . Catalase and glutathione peroxidase activities were found to be decreased in the arthritic condition. GPx scavenges the highly reactive lipid hydroperoxide in the aqueous phase of cell membranes. The decreased activity of these enzymic

antioxidants may be due to the accumulation of H_2O_2 which inturn causes the inhibition of these enzymes (25). Tissue level of selenium correlate closely with GPx activities and deficiency of selenium result in pathological condition (26). In rheumatoid arthritis, the selenium level was reported to be decreased which could account for the decreased activity of GPx (27). Drug administration produced a significant increase in their activity which enables scavenging the free radical produced during arthritic condition.

G6PD is the first enzyme in HMP shunt that generates NADPH, which generates GSH from oxidized glutathione. The inhibition of G6PD causes decreased supply of reducing equivalents like NADPH for conversion of GSSG to GSH in presence of glutathione reductase (28). A significant reduction in the activity of G6PD was noted in AIA when compared to group I. on treatment with plant extract it was able to restore the activity of G6PD. There was no much difference in the values of group I & II (group 'b').

GSH is a predominant low molecular weight thiol in the cytoplasm, which protects the tissue against invivo toxicity of sulfhydryl – binding toxicants (29, 30). The level of GSH appears to be reflux mechanism to protect against extracellular free radicals in chronic arthritis (30, 31). Adjuvant arthritis

Table 2 : Activities of non-enzymic antioxidants in liver tissue – GSH and Vitamin-C in control and experimental rats

Parameter	Group I C	Group II C+E	Group III AI	Group IV AI+T
Reduced Glutathione (μ g/mg protein)	4.63±0.199	3.65±0.172**	3.03±0.150**	4.44±0.142**
Ascorbic acid (Vit-C) (μ g/mg protein)	5.86±0.170	6.22±0.122 ^{NS}	2.66±0.168**	6.85±0.127

Values are mean ± SD

Statistical comparison was done between Group I – III, Group I – II and Group III – IV or ** at 1% level ; rest not significant.

produced a significant decrease in the level of non-enzymic antioxidants. Glutathione is endogenously synthesized in the liver and is the first line of defence against peroxidation (32). Glutathione exist in the oxidized and reduced forms which are inter-convertable. The reduced GSH, in turn, keeps up the cellular level of the active form of Vit-C. GSH plays an important role in the protection of cells and tissue structure (33). The slow rate at which the level of GSH declines in the arthritis reflects its rate of utilization (34).

Ascorbic acid has multiplicity of antioxidant property and has been claimed to be most important antioxidant in human, because it disappears faster than other antioxidants when exposed to reactive oxygen species. In arthritic condition, the level was found to be significantly decreased which may be due to decreased level of GSH and of enzyme system (NADH-semidehydro ascorbate reductase) (35). During the oxidative stress, the ascorbic acid was found to be in oxidized form. The change in ascorbic acid level is in good agreement with the early decrease in Vit-E.

After the drug treatment, the non-enzymic antioxidant levels were significantly increased. This result obtained in the case of treated group, on the effect of the drug in raising the antioxidant level may be due to sparing effect of the antioxidant defense system as the drug has quenched the increased generation of free radical by the presence of flavonoids.

Natural antioxidants strengthen the endogenous anti-oxidant defence from ROS ravage and restore the optimal balance by neutralizing the reactive species. They are gaining immense importance by virtue of their critical role in disease prevention. In this context *Vitex negundo* can rightly be mentioned as a plant with antioxidant activity. Efforts are in progress to carry out the reaction of isolation and purification of flavonoids, which will pave way for the drug designing.

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