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**Evaluation of Anti-oxidant Potential of *Cleome gynandra* L. Leaf Extract  
On The Components of the Lymphoid Organs in Adjuvant  
Induced Arthritis in Rats**

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**Abstract:** In the present study, anti-oxidant effect of ethanolic extract of *Cleome gynandra* leaves on the components of immune system in adjuvant induced experimental rats was evaluated. Freund's Complete Adjuvant (FCA) was used to induce arthritis in experimental rats. Levels of Lipid peroxides (LPO) and activities of Superoxide dismutase (SOD), catalase, Glutathione peroxidase (GPx) and reduced glutathione (GSH) were evaluated in the lymphocytes, spleen and thymus of control and experimental rats. Further, the levels of ROS such as, hydroxy radicals, superoxide radicals and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were also measured in spleen, thymus and lymphocytes in experimental rats. Paw volume was also measured in the experimental animals. A significant increase in the levels of LPO, ROS and decreased activities of antioxidant enzymes in arthritic rats were observed. The above changes were reverted to near normal in *Cleome gynandra* leaf extract treated rats. The biologically active compounds such as, terpenes, flavonoids, saponins, steroids, phenolic compounds, alkaloids, carotenes, vitamins, minerals etc., which are present in *C. gynandra* leaves might be responsible for its anti-oxidant properties. The results demonstrated that anti-oxidant and anti-arthritic potent of *C. gynandra* leaves.

**Key words:** Anti-oxidant, *Cleome gynandra*, free radical, reactive oxygen species, anti-arthritic, lymphoid organs

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## INTRODUCTION

Free radicals are highly reactive and unstable entities capable of damaging cellular components and they have been regarded as a fundamental cause of many human degenerative diseases. Free radicals, causing tissue oxidative stress, have been implicated in several human diseases such as inflammation and rheumatoid arthritis, ageing, cancer, arthritis and Parkinson's disease (Carreras *et al.*, 2004; Cho *et al.*, 2006). There is accumulating evidence that RA has characteristics of a free radical produced disease (Greenwald and Moy, 1980). Adjuvant Induced Arthritis (AIA) is thought to occur through cell-mediated autoimmunity by structural mimicry between mycobacteria and cartilage proteoglycans in rats. Activated macrophages and lymphocytes by adjuvant inoculation or their products, monokines may be involved in abnormal lipid and protein metabolism (Van Eden *et al.*, 1985).

The spleen is involved in most diseases that affect the other hematologic and lymphatic organs such as the bone marrow and lymph nodes. Pathologists are often called upon to evaluate a spleen for evidence of lymphoma, an important staging technique in the evaluation of lymphomas.

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The anti-oxidants have displayed a wide range of pharmacological activity, such as anti-cancer, anti-inflammatory and anti-ageing actions (Mayne 2003; Pinnell, 2003). The anti-oxidant potential of some natural compounds, such as vitamins and minerals, polyphenols and other non-nutrient compounds of plants, inhibiting the generation of reactive oxygen species or the scavenging of free radicals, was believed to be beneficial for human health (Braca *et al.*, 2002; Badami *et al.*, 2003).

Although the number of drugs used in the treatment of RA has been increased over the past 10-20 years, there is still an urgent need for more effective drugs with reduced side effects. Medicinal plants have become a major component of human health care as they no or less side effects. Surveys conducted in Australia and US indicate that almost 48.5 and 34% of respondents had used at least one form of unconventional therapy including herbal medicine (Edeas, 2000).

*Cleome gynandra* L. (Cat's whiskers) is a common weed, which grows in most tropical countries. The leaves and seeds of the plant have long been in use as indigenous medicine for treatment of headaches and stomach aches. Sap from leaves has been used as an analgesic particularly for headache, epileptic fits and earache. A decoction or infusion of boiled leaves and/or roots has been administered to facilitate childbirth. Bruised leaves, which are rubefacient and vesicant, are also used to treat neuralgia, rheumatism and other localized pains (Chewya and Mnzava, 1997; Kumar and Sadique, 1987). Recently we have reported the effect of *Cleome gynandra* leaf extract on hematological alterations on adjuvant induced arthritic rats (Narendhirakannan *et al.*, 2005a,b).

In the present study, an attempt has been made to evaluate anti-oxidant potential of *Cleome gynandra* L. leaf extract on the components of the lymphoid organs in adjuvant induced arthritis in rats.

## **MATERIALS AND METHODS**

### **Chemicals**

Freund's Complete Adjuvant (FCA) was procured from Difco Laboratories Detroit, MI, USA. Histopaque (1077) were obtained from Sigma chemicals, St. Louis, MO, USA. Thiobarbitric acid, glutathione (reduced) and 2,4-dithio nitrobenzoic acid (DTNB) from E. Merk were used.

### **Plant Materials**

Fresh *C. gynandra* leaves were collected from a rural region of Dindigul district, Tamilnadu, India and the plant was identified by Prof. V. Kaviyaran, Centre for Advanced Studies in Botany, University of Madras, Chennai, India. The leaves were dried under shade. The voucher specimen of the plant is being retained in the Department Herbarium.

### **Preparation of Cleome Gynandra Leaf Extract**

The dry leaves were chopped and subjected to extraction with 95% ethanol by Soxhlet apparatus for 15 h. Nearly 85% of the solvent was recovered by distillation over a boiling water bath at atmospheric pressure and the remaining under reduced pressure (temperature at 40-50°C). The yield was 3.2/100 g. The extract was stored in a refrigerator until further use.

### **Test Animals**

Male albino rats of Wistar strain weighing around 160-180 g were procured from Tamilnadu Veterinary and Animal Science University, Chennai for the present study. The animals were housed in solid-bottomed polypropylene cages and acclimatized to animal house conditions. The rats were fed with commercial rat diet (Hindustan Lever Limited, Mumbai, India) and water *ad libitum*. The experiments were designed and conducted in accordance with the ethical norms approved by Ministry

of Social Justices and Empowerment, Government of India and Institutional Animal Ethical Committee Guidelines (IAEC). Once arthritis developed, food was served on the bottom of the cages as severely arthritic rats have difficulty in feeding from the cage top.

### **Induction of Arthritis**

Arthritis was induced by a single intradermal injection of 0.1 mL of Freund's Complete Adjuvant (FCA) containing 10 mg mL<sup>-1</sup> dry heat-killed *Mycobacterium tuberculosis* per milliliter sterile paraffin oil into a foot pad of the left hind paw of male rats (Mizushima *et al.*, 1972). A glass syringe (1 mL) with the locking hubs and a 26 G needle was used for injection. The rats were anesthetized with ether inhalation prior to and during adjuvant injection, as the very viscous nature of the adjuvant exerts difficulty while injecting. The swelling in hind paws were periodically examined in each paw from the ankle using plethysmography (Winter *et al.*, 1962).

### **Experimental Design**

Animals were divided into four groups of six animals in each group as follows:

Group I: Control rats

Group II: Control rats administered with extract of *C. gynandra* leaf (150 mg kg<sup>-1</sup> body weight/rat/day for 30 days).

Group III: Adjuvant induced arthritic rats

Group IV: Arthritis induced rats administered with extract of *C. gynandra* leaf (150 mg kg<sup>-1</sup> body weight/rat/day for 30 days by incubations starting 10 days after adjuvant injection).

### **Measurement of Paw Volume**

The paw volume was measured using plethysmography. During the experimental period, scoring of paw was restricted to 2-3 times a week to avoid excessive handling of the animals as this can reduce the severity or incidence of arthritis after adjuvant injection and the paw oedema was calculated.

### **Experimental Procedure and Preparation of Tissue Homogenate**

At the end of the experimental period the rats were fasted overnight and sacrificed by cervical decapitation. Blood was collected with heparin and processed for lymphocyte isolation (Hudson and Hay, 1991). Spleen and thymus were removed, washed with ice-cold saline and homogenized in Tris-HCl buffer (0.1 M, pH 7.4). The homogenized tissues were passed over cotton mesh and the eluent was layered over the density gradient and processed similarly as that of blood.

### **Biochemical Assays**

Lipid peroxides in tissue homogenate were estimated by the method of Devasagayam and Tarachand (1987). Lymphocytic lipid peroxide level was estimated by the method of Yagi (1976). The assay of superoxide dismutase was carried out by the method of Marklund and Marklund (1974). The procedure adapted by Rotruck *et al.* (1973) was used for the estimation of glutathione peroxidase. Glutathione was determined by the method of Moron *et al.* (1979). Catalase was assayed by the method of Takahara *et al.* (1960). Estimation of protein was followed by the method of Lowry *et al.* (1951).

Reactive oxygen species namely hydroxyl radical (Gutteridge, 1981), superoxide radical (Nishikimi *et al.*, 1972) and hydrogen peroxide (Wolff, 1994) were measured in spleen cells, thymic cells and lymphocytes, respectively.

### Statistical Analysis

All the grouped data were statistically evaluated with SPSS/10 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. p-values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as Mean±SD for six animals in each group.

## RESULTS

Table 1 result shows that swelling and redness developed over a 24 h period in the foot injected with adjuvant and reached maximum intensity on day 4. This inflammatory reaction subsides slightly during the next 8 to 10 days and then increased at that time when disseminated arthritis appeared which was greater than primary phase swelling. Administrations of *C. gynandra* extract significantly reduce the paw swelling of the injected foot.

Table 2 elucidates the significant (p<0.05) decrease in the activities of superoxide dismutase in spleen, thymus and lymphocytes of arthritis induced rats (Group II) when compared to control rats (Group I). The *C. gynandra* treated rats (Group III) showed a significant increase in superoxide dismutase activity to near normalcy.

Table 3 exhibits that a marked decrease in the activity of catalase was observed in arthritis rats (Group II). Arthritic rats treated with *C. gynandra* leaf extract showed a significant (p<0.05) increase in the activity of this enzyme (Group III).

Lipid peroxide level was found significantly increased (p<0.05) in arthritis-induced rats when compared to control animals (Table 4). After the *C. gynandra* treatment, the level was found to be significantly reduced in Group III animals when compared to Group II animals.

A significant decline in the levels of GSH was found in arthritic rats (Group II). Arthritic rats (Group III) treated with *C. gynandra* leaf extract showed a marked increase (p<0.05) to near normal levels (Table 5).

Table 1: Measurement of paw volume in control and experimental rats

Days	0	5	10	15	20	25
Group I	3.91±0.15	3.92±0.15	3.91±0.16	3.92±0.18	3.94±0.17	3.95±0.17
Group II	3.84±0.16 <sup>a*</sup>	5.89±0.2 <sup>a*</sup>	7.21±0.22 <sup>a*</sup>	6.40±0.25 <sup>a*</sup>	8.32±0.27 <sup>a*</sup>	8.41±0.29 <sup>a*</sup>
Group III	3.90±0.13 <sup>b*</sup>	6.01±0.21 <sup>b*</sup>	7.05±0.23 <sup>b*</sup>	6.50±0.25 <sup>b*</sup>	6.80±0.26 <sup>b*</sup>	6.20±0.25 <sup>b*</sup>

Values are given as mean±SD for groups of six animals each. Values are statistically significant at \*p<0.05: Statistical significance was compared within the groups as follows: <sup>a</sup> Arthritic rats compared with normal rats: <sup>b</sup> *C. gynandra* leaf treated arthritic rats were compared with arthritic rats

Table 2: Activities of *Cleome gynandra* on superoxide dismutase in spleen, thymus and lymphocytes of control and experimental rats

Organs	Group I	Group II	Group III	Group IV
Spleen	2.31±0.05	1.40±0.03 <sup>a*</sup>	2.01±0.03 <sup>b</sup>	2.50±0.04
Thymus	2.58±0.06	1.50±0.04 <sup>a*</sup>	2.03±0.03 <sup>b**</sup>	2.52±0.05
Lymphocytes	21.09±1.25	14.70±1.03 <sup>a*</sup>	17.40±1.11 <sup>b*</sup>	20.04±1.21

Values are given as mean±SD for groups of six animals each. Values are statistically significant at \*p<0.05: Statistical significance was compared within the groups as follows: <sup>a</sup> Arthritic rats compared with normal rats: <sup>b</sup> *C. gynandra* leaf treated arthritic rats were compared with arthritic rats

Table 3: Activities of *Cleome gynandra* on catalase in spleen, thymus and lymphocytes of control and experimental animals

Organs	Group I	Group II	Group III	Group IV
Spleen	253.12±5.6	187.34±3.5 <sup>a*</sup>	234.31±5.1 <sup>b</sup>	250.54±4.9
Thymus	240.91±4.9	177.47±3.88 <sup>a*</sup>	225.70±5.12 <sup>b</sup>	241.78±5.1
Lymphocytes	13.20±1.6	8.10±1.1 <sup>a*</sup>	11.30±1.3 <sup>b</sup>	12.40±1.5

Values are given as mean±SD for groups of six animals each. Values are statistically significant at \*p<0.05: Statistical significance was compared within the groups as follows: <sup>a</sup> Arthritic rats compared with normal rats: <sup>b</sup> *C. gynandra* leaf treated arthritic rats were compared with arthritic rats

Table 4: Lipid peroxidation activity of *Cleome gynandra* in spleen, thymus and lymphocytes of control and experimental animals

Organs	Group I	Group II	Group III	Group IV
Spleen	3.01±0.07	6.03±0.09**	4.02±1.01 <sup>ab</sup>	2.95±0.06
Thymus	3.92±1.01	7.31±1.60**	5.38±1.30 <sup>ab</sup>	3.68±1.01
Lymphocytes	172.18±3.80	228.50±3.80**	191.35±2.90 <sup>ab</sup>	176.94±2.30

Values are given as mean±SD for groups of six animals each. Values are statistically significant at \*p<0.05: Statistical significance was compared within the groups as follows: <sup>a</sup> Arthritic rats compared with normal rats: <sup>b</sup>C. *gynandra* leaf treated arthritic rats were compared with arthritic rats

Table 5: Effect of *Cleome gynandra* on GSH in control and experimental rats

Organs	Group I	Group II	Group III	Group IV
Spleen	3.01±0.12	1.82±0.09**	2.24±0.1 <sup>ab</sup>	2.99±0.11
Thymus	1.72±0.08	1.01±0.06**	1.4±0.08 <sup>ab</sup>	1.69±0.06
Lymphocytes	3.24±0.14	2.08±0.11**	2.86±0.05 <sup>ab</sup>	3.19±0.07

Values are given as mean±SD for groups of six animals each. Values are statistically significant at \*p<0.05: Statistical significance was compared within the groups as follows: <sup>a</sup> Arthritic rats compared with normal rats: <sup>b</sup>C. *gynandra* leaf treated arthritic rats were compared with arthritic rats

Table 6: Glutathione peroxidase activity of *Cleome gynandra* in spleen, thymus and lymphocytes of control and experimental animals

Organs	Group I	Group II	Group III	Group IV
Spleen	12.11±1.2	8.01±0.9 <sup>a</sup>	9.98±1.0 <sup>b</sup>	11.98±1.3
Thymus	9.25±1.0	6.3±0.2 <sup>a</sup>	8.12±1.1 <sup>b</sup>	9.11±1.3
Lymphocytes	0.61±0.03	0.38±0.01 <sup>a</sup>	0.49±0.02 <sup>b</sup>	0.58±0.03

Values are given as mean±SD for groups of six animals each. Values are statistically significant at \*p<0.05: Statistical significance was compared within the groups as follows: <sup>a</sup> Arthritic rats compared with normal rats: <sup>b</sup>C. *gynandra* leaf treated arthritic rats were compared with arthritic rats

Table 7: Levels of superoxide anion of control and experimental animals

Organs	Group I	Group II	Group III	Group IV
Spleen	34.86±2.3	70.36±4.2 <sup>aa</sup>	40.68±2.5 <sup>ab</sup>	32.91±2.1
Thymus	29.41±1.9	61.62±3.2 <sup>aa</sup>	36.78±2.1 <sup>ab</sup>	28.94±1.8
Lymphocytes	70.65±4.1	205.48±8.2 <sup>aa</sup>	112.78±4.3 <sup>ab</sup>	68.43±3.9

Values are given as mean±SD for groups of six animals each. Values are statistically significant at \*p<0.05: Statistical significance was compared within the groups as follows: <sup>a</sup> Arthritic rats compared with normal rats: <sup>b</sup>C. *gynandra* leaf treated arthritic rats were compared with arthritic rats

Table 8: Levels of hydrogen peroxide in spleen, thymus and lymphocytes of control and experimental rats

Organs	Group I	Group II	Group III	Group IV
Spleen	39.12±1.9	83.62±2.9 <sup>aa</sup>	48.65±2.1 <sup>ab</sup>	40.11±2.6
Thymus	35.42±2.1	78.61±3.8 <sup>aa</sup>	49.38±2.6 <sup>ab</sup>	36.18±2.2
Lymphocytes	79.54±3.8	161.73±5.2 <sup>aa</sup>	109.82±4.8 <sup>ab</sup>	78.12±3.2

Values are given as mean±SD for groups of six animals each. Values are statistically significant at \*p<0.05: Statistical significance was compared within the groups as follows: <sup>a</sup> Arthritic rats compared with normal rats: <sup>b</sup>C. *gynandra* leaf treated arthritic rats were compared with arthritic rats

Table 6 revealed that the levels of GPx were significantly decreased (p<0.05) in arthritis induced rats (Group II) than control rats (Group I). Treatment with the extract of *C. gynandra* increased the levels of GPx in Group III.

Table 7 and 8 represents the levels of ROS such as superoxide anion and hydrogen peroxide in control and experimental animals, respectively. ROS levels were found to be significantly increased in arthritic rats (Group II) when compared to that of control rats (Group I), which were found to be reverted back in *C. gynandra* treated rats (Group III).

Table 9 show the significant elevation in the levels of hydroxyl radicals in arthritic rats (Group II). A significant (p<0.05) decrease in the levels was noticed after the administration of *C. gynandra* leaf extract.

In all the experiments there was no significant changes found in extract alone administered rats (Group IV).

Table 9: Levels of hydroxyl radicals in control and adjuvant induced arthritic rats

Organs	Group I	Group II	Group III	Group IV
Spleen	6.01±0.9	15.48±1.4 <sup>ab</sup>	10.54±1.2 <sup>ab</sup>	6.18±0.8
Thymus	5.18±0.8	12.36±1.0 <sup>ab</sup>	7.92±0.9 <sup>ab</sup>	4.99±0.7
Lymphocytes	16.38±1.6	37.28±2.5 <sup>ab</sup>	21.35±1.9 <sup>ab</sup>	17.03±2.0

Values are given as mean±SD for groups of six animals each. Values are statistically significant at \*p<0.05: Statistical significance was compared within the groups as follows:<sup>a</sup> Arthritic rats compared with normal rats: <sup>b</sup>C. *gynandra* leaf treated arthritic rats were compared with arthritic rats

## DISCUSSION

Adjuvant arthritis induced by intra dermal injection of Freund's complete adjuvant, is a chronic, generalized reaction and is an accepted experimental model of rheumatoid arthritis (Pearson, 1964). It has been suggested that the pathogenesis of AIA, involves the formation of immune complex which allow the dissemination of mycobacterial antigen throughout the body and ultimately gets deposited at various sites and initiates joint lesions (Mackenzie *et al.*, 1978).

In the investigation of adjuvant arthritis in rats, the arthritic rats showed a soft tissue swelling that was noticeable around ankle joints and was believed due to edema of tissues such as ligaments and joint capsules. Initial reduction of edema and soft tissue thickening at the deposit site is probably due to the effect of the adjuvant, whereas the late occurring disseminated arthritis and flare in the injected foot is presumably immunological events (Ward and Cloud 1965; DiRosa *et al.*, 1971).

Phospholipids, with their unsaturated fatty acid side chains, are major constituents of all biological membranes and are therefore, potential targets for oxygen radical attack. Oxygen free radicals are potent lipid peroxidation-inducing agents that cause the depletion of unsaturated fatty acids of the cell membrane, thus inducing loss of cell integrity and functional alteration of cell receptors and enzymes (Bowles *et al.*, 1991). In many diseases, especially rheumatoid arthritis, membrane damage often occurs in some organ or tissue, which provokes and accelerates the disorder structurally and functionally (Yagi, 1987). The lack of antioxidant defense leads to an increase in lipid peroxidation and subsequent deleterious effects (Scott *et al.*, 1989). In the present study, the increased level of lipid peroxides in spleen, thymus and lymphocytes may be due to poor antioxidant defense systems. The lymphoid organs spleen and thymus are affected during arthritic conditions (Spector, 1964).

Reduced glutathione (GSH) comprises more than 90% of the non-protein low molecular weight reduced thiols in cells and is most important intracellular mechanism protecting normal cells from oxidative injury by acting both as a free radical scavenger and a substrate in the GSH redox cycle (Meister and Anderson, 1983). It is known that GSH is a component of the first line of antioxidant defense against oxidative process. Further more, immune activation and particularly the activation of the TNF system seem to be important in the development of GSH abnormalities (Roederer *et al.*, 1991). In the present study it is observed that the levels of reduced GSH decrease in spleen, thymus and lymphocytes. As adequate concentrations of GSH are required for a variety of immune functions, it has been suggested that oxidative stress and deficiency of thiol compounds may play an important pathogenic role in the development of immune deficiency (Stall *et al.*, 1992; Aukrust *et al.*, 1995). The results of this study may help to further understand the potential mechanisms underlying arthritis.

Glutathione peroxidase (GPx) is localized in the cytoplasm and mitochondria, which catalyses the degradation of various peroxides by oxidizing glutathione with the formation of its conjugates. GPx has more affinity than catalase for H<sub>2</sub>O<sub>2</sub>. GPx is essential for the conversion of glutathione to oxidized glutathione during which H<sub>2</sub>O<sub>2</sub> is converted to water (Alessio and Blasi, 1997). The observed decrease in the activity of GPx in spleen, thymus and lymphocytes in arthritic rats indicate the weak free radical defense system against oxidative stress and may help understanding the pathogenesis associated with arthritis (Vijayalakshmi *et al.*, 1997).

The superoxide radical is the first product of molecular oxygen reduction. In addition to its natural toxicity, it is an important source of hydroperoxides and other reactive free radicals (Okabe *et al.*, 1996). It takes the action of body defense and plays an important role in inflammation pathogenesis. During the abnormal pathogenesis, many inflammatory factors, such as cytokines prostaglandin E<sub>2</sub>, lysosomal enzymes and free radicals, take part in the genesis and development of inflammation (Palmlblad, 1984). SOD may play an important role in protecting cells against ROS (Yamaguchi, 1991). Present result shows that the activities of SOD was found to be decreased in arthritic animals which may be due to the increased generation of free radicals such as superoxide anions and hydroxyl radicals (Imadaya *et al.*, 1988).

Another antioxidant defense enzyme, catalase, also protects aerobic cells against oxygen toxicity and lipid peroxidation. Catalase is the enzyme which catalyses the disproportion of H<sub>2</sub>O<sub>2</sub> (Yamaguchi, 1991). H<sub>2</sub>O<sub>2</sub> generated by SOD is removed enzymatically by catalase, which is particularly efficient at high concentration of H<sub>2</sub>O<sub>2</sub>• catalase detoxifies H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O and O<sub>2</sub> (Imadaya *et al.*, 1988).

The decreased activity of these enzymatic antioxidants in arthritis may be due to supersaturation of enzyme SOD with a high concentration of reactive oxygen species formed. Due to increased lipid peroxidation, the levels of free radicals overcome the saturation level. The high concentrations of free radicals inhibit the activity of antioxidants and hence the activities of these enzymes appear to be reduced. The decrease in SOD activity leads to declined production of hydrogen peroxide. Since hydrogen peroxide is the substrate for the enzyme catalase and Gpx, their levels were also found decreased.

Free radicals such as superoxide radicals (O<sub>2</sub>•), hydroxyl radicals (HO•) are known to produce metabolically in living organisms. In addition, some non-radical derivatives of oxygen molecules such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hypochlorous acid (HOCl) can be generated in foods and biological systems. All of these ROS participate in the chain reaction of free radicals, thus tests of the ability of a substance to scavenge radical species may be relevant in the evaluation of antioxidant activity (Halliwell, 1990; Halliwell *et al.*, 1995).

## CONCLUSION

Many foods contain natural antioxidants, including enzymes such as superoxide dismutase, glutathione peroxidase and catalase, which are usually inactivated during food processing and non-enzymic antioxidants such as carotenoids, β-carotene, lutein, lycopene, tocopherols and other phenolic compounds. Two or more antioxidants can act together synergistically (Lakatos and Szentmihalyi, 1999; Pryor, 2000). On *C. gynandra* treatment, the activities of SOD, catalase and GPx are brought to near normal levels, which may be attributed to the free radical scavenging activity of flavonoids present in the drug (Sanz *et al.*, 1994). Recently, we have reported the presence of many biologically active phytochemicals such as triterpenes, tannins, anthroquinones, flavonoids, saponins, steroids, resins, lectins, glycosides, sugars, phenolic compounds, alkaloids etc., in the extract of *C. gynandra* and these compounds might be responsible for the anti arthritic properties observed in the present study (Narendhirakannan *et al.*, 2005a,b; 2006). Other reports also show *C. gynandra* presence of β-carotene, total phenolics, ascorbic acid etc. (Mathooko and Imungi, 1994; Chweya and Mnzava, 1997).

Further studies are in progress to isolate the active principle(s), which are responsible for the anti-inflammatory/antiarthritic properties of *C. gynandra*.

## REFERENCES

- Alessio, H.M. and E.R. Blasi, 1997. Physical activity as a natural antioxidant booster and its effect on healthy life style. Res. Q. Excer. Sport., 68: 292-302.



- Aukrust, P., A.M. Svaral, F. Muller, B. Lunden, R. Berge and S.S. Froland, 1995. Decreased levels of total and reduced glutathione in CD<sup>4+</sup> lymphocytes in common variable immuno deficiency are associated with activation of the tumour necrosis factor system: Possible immunopathogenic role of oxidative stress. *Blood*, 86: 1383-1391.
- Badami, S., M.K. Gupta and B. Suresh, 2003. Antioxidant activity of the ethanolic extract of *Striga orobanchioides*. *J. Ethnopharmacol.*, 85: 227-230.
- Bowles, D.K., C.E. Torgan and S. Ebner, 1991. Effect of acute, submaximal exercise on skeletal muscle vit. E. *Free Rad. Res. Comm.*, 14: 139-143.
- Braca, A., C. Sortino, M. Politi, I. Morelli and J. Mendez, 2002. Anti-oxidant activity of flavonoids from *Licania licaniaeflora*. *J. Ethnopharmacol.*, 79: 379-381.
- Carreras, M.C., M.C. Franco, J.G. Peralta and J.J. Poderoso, 2004. Nitric oxide, complex I and the modulation of mitochondrial reactive species in biology and the modulation of mitochondrial reactive species in biology and disease. *Mol. Aspects Med.*, 25: 125-139.
- Cho, J.Y., S.C. Park, T.W. Kim, K.S. Kim and J.C. Song *et al.*, 2006. Radical scavenging and anti-inflammatory activity of extracts from *Opuntia humifusa* Raf. *J. Pharm. Pharmacol.*, 58: 113-119.
- Chweya, J.A. and N.A. Mnzava, 1997. Cat's whiskers (*Cleome gynandra* L.) Promoting the conservation and use of underutilized and neglected crops. 11. Institute of Plant Genetics and Crop Plant Research, Gatersleben/ International Plant Genetic Resources Institute, Rome, Italy, pp: 18.
- Devasagayam, T.P and U. Tarachund, 1987. Decreased lipid peroxidation in the rat kidney during gestation. *Biochem. Biophys. Res. Comm.*, 145: 469-474.
- DiRosa, M., J.P. Giroud and D.A. Willoughby, 1971. Studies of the mediators of acute inflammatory response induced in rats in different sites by carrageenan and turpene. *J. Path. Bact.*, 55: 817-819.
- Edeas, M., 2000. In: Proceedings of the 2nd International Conference on Superoxide Dismutase: Recent Advances, Clinical and Nutritional Applications, 18-19 May 2000, Institut Pasteur, Paris.
- Greenwald, R.A and W.W. Moy, 1980. Effect of oxygen derivative free radicals on hyaluronic acid. *Arth. Rheum.*, 23: 455-463.
- Gutteridge, J.M.C., 1981. Thiobarbituric acid- reactivity following iron-dependent free-radical damage to amino acids and carbohydrates. *FEBS Lett.*, 128: 343-346.
- Halliwell, B., 1990. How to characterize biological antioxidant biological antioxidant. *Free Rad. Res. Comm*, 9: 1-32.
- Halliwell, B., M.A. Murcia, S. Chirico and O.I. Aruoma, 1995. Free radical and antioxidants in food and in vivo: what they do and how they work. *Critical Rev. Food Sci. Nat.*, 35: 7-20.
- Hudson, L. and F.C. Hay, 1991. *Practical Immunology*. Blackwell Scientific Publications, Oxford, pp: 21-22.
- Imadaya, A., K.H. Terasawa, M. Okamoto and K. Toriizuka, 1988. Erythrocyte antioxidant enzymes are reduced inpatients with Rheumatoid arthritis. *J. Rheumatol.*, 15: 11-37.
- Kumar, P.S. and J. Sadique, 1987. The biochemical mode of action of *Gynandropsis gynandra* in inflammation. *Fitoter*, 58: 379-386.
- Lakatos, B. and K. Szentmihalyi, 1999. The antioxidant effect and clinical application of vitamin E. *Lege. Artis. Medicine*, 9: 716-725.
- Lowry, O.H., N.J. Rosenbrough, A.I. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.
- Mackenzie, A.R., C.R. Pick, P.R. Sibley and B.P. White, 1978. Suppression of rat adjuvant by cyclophosphamide pretreatment: Evidence for an antibody mediated component in the pathogenesis of the disease. *Clin. Exp. Immunol.*, 32: 86-89.

- Marklund, S. and G. Marklund, 1974. Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem.*, 47: 469-474.
- Mathooko, F.M. and J.K. Imungi, 1994. Ascorbic acid changes in three indigenous Kenyan leafy vegetables during traditional cooking. *Ecol. Food Nutr.*, 32: 239-245.
- Mayne, S.T., 2003. Antioxidant nutrients and chronic disease: Use of biomarkers of exposure and oxidative stress status in epidemiologic research. *J. Nutr.*, 133: 933S-940S.
- Meister, A., M.E. Anderson, 1983. Glutathione. *Ann. Rev. Biochem.*, 52: 711-760.
- Mizushima, Y., W. Tsukada and T. Akimoto, 1972. A modification of rat adjuvant arthritis for testing anti-rheumatic drugs. *J. Pharm. Pharmacol.*, 24: 781-785.
- Moron, M.S., J.W. Depierre and B. Mannervik, 1979. Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. *Biochim. Biophys. Acta.*, 582: 67-78.
- Narendhirakannan, R.T., M. Kandaswamy and S. Subramanian, 2005a. Anti-Inflammatory Activity of *Cleome gynandra* L. on Hematological and Cellular Constituents in Adjuvant-Induced Arthritic Rats. *J. Med. Food*, 8: 93-99.
- Narendhirakannan, R.T., S. Subramanian and M. Kandaswamy, 2005b. Free radical scavenging activity of *Cleome gynandra* L. leaves on adjuvant induced arthritis in rats. *Mol. Cell. Biochem.*, 276: 71-80.
- Narendhirakannan, R.T., S. Subramanian and M. Kandaswamy, 2006. Evaluation of anti-inflammatory activity of *Cleome gynandra* L. leaf extract on acute and chronic inflammatory arthritis studied in rats. *J. Pharmacol. Toxicol.*, (In Press).
- Nishikimi, M., N.A. Rao and K. Yagi, 1972. Occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. *Biochem. Biophys. Res. Commun.*, 46: 849-854.
- Okabe, T., K. Hamaguchi, T. Inafuku and M. Hara, 1996. Aging and superoxide dismutase activity in cerebrospinal fluid. *J. Neurol. Sci.*, 141: 100-104.
- Palmblad, J., 1984. The role of granulocytes in inflammation. *Scand J. Rheumatol.*, 13:163-72.
- Pearson, C.M., 1964. Experimental models in rheumatoid disease. *Arth. Rheum.*, 7: 80-86.
- Pinnell, S.R., 2003. Cutaneous photodamage, oxidative stress and topical antioxidant protection. *J. Am. Acad. Dermatol.*, 48: 1-19.
- Pryor, W.A., 2000. Vitamin E and heart disease: Basic science to clinical intervention trials. *Free Rad. Biol. Med.*, 28: 141-164.
- Roederer, M., F.T.G. Stad, H. Osada, L.A. Herzenberg and L.A. Herzenberg, 1991. CD<sup>4+</sup> and CD<sup>8+</sup> T cells with high intracellular glutathione levels are selectively lost at the HIV infection progress. *Int. Immunol.*, 3: 933-937.
- Rotruck, J.T., A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman and W.G. Hoekstra, 1973. Selenium, biochemical role as a component of glutathione peroxidase purification and assay. *Science*, 179: 588-590.
- Sanz, M.J., M.L. Fernandez, M. Cyuero and M.C. Terencio, 1994. Influence of a series of natural flavonoids on free radical generating systems and oxidative stress. *Xenobiotics*, 24: 689-699.
- Scott, M.D., J.N. Eaton, A.K. Frans, T. Daniel, Y. Chiu and B.H. Lubin, 1989. Enhancement of erythrocyte SOD activity effects on cellular oxidant defence. *Blood*, 74: 2542-2549.
- Spector, W.G., 1964. The acute inflammatory response. *Ann. NY. Acad. Sci.*, 116: 747-752.
- Stall, F.J., S.W. Ela, N. Roederer, M.T. Anderson, L.A. Herzenberg and L.A. Herzenberg, 1992. Glutathione deficiency and human immunodeficiency virus infection. *Lancet*, 339: 909-912.
- Takahara, S., B.H. Hamilton, J.V. Nell, Y. Ogura and E.T. Nishimura, 1960. Hypocatalasemia, a new genetic carrier states. *J. Clin. Invest.*, 29: 610-619.

- Van Eden, W., J. Holoshitz, Z. Nevo, A. Frenkel, A. Klajman and I. Cohen, 1985. Arthritis induced by a T-lymphocyte clone that responds to *Mycobacterium tuberculosis* and to cartilage proteoglycans. *Proc. Natl. Acad. Sci., USA.*, 82: 5117-5120.
- Vijayalakshmi, T., V. Muthulakshmi and P. Sachdanandam, 1997. Effect of milk extract of *Semecarpus anacardium* nut on glycohydrolases and lysosomal stability in adjuvant arthritis in rats. *J. Ethnopharmacol.*, 58: 1-8.
- Ward, J.R. and S. Cloud, 1965. Comparative effect of antirheumatic drugs on adjuvant induced polyarthritis in rats. *J. Pharmacol. Exp. Ther.*, 152: 116-121.
- Winter, C.A., E.A. Risley and G.W. Nuss, 1962. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med.*, 111: 544-552.
- Wolff, S.P., 1994. Ferrous oxidation in presence of ferric ion indicator xylenol orange for measurement of hydroperoxides. *Methods Enzymol.*, 123: 180-189.
- Yagi, K., 1976. Simple fluorimetric assay for lipoperoxide in blood plasma. *Biochem. Med.*, 15: 212-216.
- Yagi, K., 1987. Lipid peroxides and human diseases. *Chem. Phys. Lipids*, 45: 337-351.
- Yamaguchi, S., 1991. The role of SOD an antioxidant. *J. Natl. Cancer., Inst.*, 28: 221-232.