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Full Length Research Paper

Assessment of the *in-vivo* and *in-vitro* antioxidant potential of *Strychnos potatorum* Linn. seeds in Freund's adjuvant induced arthritic rats

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The Indian system of medicine provides *Strychnos potatorum* Linn. seeds for the treatment of various ailments like jaundice, bronchitis, diabetes, renal and vesicle calculi, conjunctivitis, chronic diarrhoea, dysentery etc. The present study involves the effect of crude powder (SPP) and aqueous extract (SPE) of *S. potatorum* Linn seeds on *in vivo* antioxidant defense system against Freund's complete adjuvant (FCA) induced arthritis in rats at the dose of 200 mg/kg/p.o for 14 and 28 days and *in vitro* antioxidant activity against Ferrous thiocyanate (FTC) and Thiobarbiturate (TBA) induced lipid peroxidation. The antioxidant defense system parameters studied in plasma and tissues of arthritic rats were found to be altered significantly. These alterations observed in arthritic rats may be due to the increased oxidative stress in the tissues, which were significantly reduced in SPP and SPE treated arthritic rats. The results reveal that, the drug treatment decreased the LPO levels in plasma and tissues, either by interception of the formation or by scavenging the active oxygen species. The raise in enzymic and nonenzymic antioxidants level may be due to the sparing effect of antioxidant defense system as the drugs SPP and SPE have scavenged the free radicals by various antioxidant phytochemicals like steroids, triterpenes and polyphenolics present in it either by individual or by combined action. In *in vitro* total antioxidant analysis by FTC and TBA methods, SPE was found to show higher antioxidant activity when compared with SPP.

Key words: *Strychnos potatorum,* enzymic and non-enzymic antioxidants, free radical scavenging, lipid peroxidation.

INTRODUCTION

Adjuvant induced arthritis in rats develops chronic swelling in multiple joints with the influence of inflammatory cells, erosion of joint cartilage and bone destruction. It has close similarities to human rheumatoid disease. It is well established now that free radicals/reactive oxygen species play an important role in inflammation (Satoh, 1978). The pathogenesis of rheumatoid arthritis is associated predominantly with the formation of free radicals and proinflammatory cytokines at the site of inflammation. The inflammatory process develops in the tissue of the synovium; primary sources of reactive oxidative species (ROS) in RA are leukocytes, which are recruited to accumulate within the synovium. ROS can be produced by activated macrophages in the synovial membrane and by activated neutrophils in the synovial cavity (Bauerova et al., 2009).

Strychnos potatorum Linn (Family: Loganiaceae) is a moderate sized tree found in southern and central parts of India, Sri Lanka and Burma (Kirtikar and Basu, 1933). The seeds are used in Indian system of medicine either monotherapy or in combination with other plant drugs for

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hepatopathy, nephropathy, gonorrhoea, leucorrhoea, gastropathy, bronchitis, chronic diarrhoea, dysentery, renal and vesicle calculi, diabetes, burning sensation, conjunctivitis, scleritis, ulcers and other eye diseases (Kirtikar and Basu, 2000). The seeds are reported to have many potential therapeutic activities in traditional systems of medicine in India (ISM) may be due to the presence of phytoconstituents like Diaboline (Singh et al., 1975), Steroids and triterpenes (Singh and Kapoor, 1975) and mannogalactans (Venkatrao et al., 1991). The seeds are reported to have various pharmacological activities like antidiabetic (Mathuram et al., 1981), antihypercholesterolemic activity (Venkatrao et al., 1991), diuretic (Biswas et al., 2001), antidiarrhoeal (Swathi et al., 2002), hepatoprotective (Sanmuga priya and Venkataraman, 2006) and antiulcer (Sanmuga priya and Venkataraman, 2007).

The other species of the same genus, *Strychnos nuxvomica* was used in the treatment of rheumatoid arthritis and it was proved to be effective (Yin et al., 2003). The whole seed powder and the aqueous extract of *S. potatorum* Linn seeds were found to show antiarthritic activity against Freund's adjuvant induced arthritic rat model. The study elaborated the normalization of altered hematological and biochemical parameters in FCA induced arthritis (Sanmuga et al., 2010). The present study deals with the effect of the aqueous extract (SPE, 200 mg/kg/p.o) and whole seed powder (SPP, 200 mg/kg/p.o) of *S. potatorum* on the antioxidant defense system in adjuvant induced arthritic rats.

MATERIALS AND METHODS

Preparation of the extract

The air-dried seeds were coarsely powdered and subjected to hot water decoction for 2 h at 100°C. It was then filtered and the filtrate was evaporated to dryness. A grey colored semisolid mass was obtained which was dried under vacuum and kept in a dessicator. The percentage yield of the extract (SPE) was 22.5% (w/w) from the starting crude material. The seed powder (SPP) as such was used for the treatment. For the experimental study, both drugs (SPP and SPE) were triturated with distilled water and administered immediately. Preliminary qualitative analysis of SPP and SPE was carried out and reported by us in a previous study. As per the standard literature sources, the detailed screening tests were performed for analysis of various phytochemicals from the selected samples by preliminary chemical tests and HPTLC fingerprinting (Sanmuga and Venkataraman, 2010).

Animals

Wistar albino rats (140 ± 20 g) procured from TANUVAS (Tamilnadu University for Veterinary and Animal Sciences) was used in the study. The animals were kept in polypropylene cages and maintained at a temperature of 22 ± 2°C. They were fed with standard pelleted feed (TANUVAS) and water *ad libitum*. The study has got the approval from the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Induction of arthritis

Arthritis was induced in rats by the intradermal injection of 0.1 ml of Complete Freund's Adjuvant (CFA) in the left hind paw (Newbould, 1963). The adjuvant contained heat killed *Mycobacterium tuberculosis* ($H_{37}R_v$ strain, Tuberculosis Research Centre, ICMR, Chennai) in sterile paraffin oil (10 mg/ml) was used for the study.

Experimental setup

The animals were divided into nine groups of six animals each. Group I served as control, Group II served as arthritic control, Group III was treated with Diclofenac sodium, the standard anti - arthritic drug, Groups IV and VII were treated with SPP and SPE for 28 days, whereas Group V and VIII received SPP and SPE drug treatment respectively for 28 days from the induction day. Groups VI and IX received SPP and SPE respectively for 14 days from the 14^{th} day of induction. The body weight changes were observed on every week. On the 29^{th} day, at the end of experiment, all animals were sacrificed by cervical decapitation and whole blood was collected in plain tube and immediately used for haematological studies. Serum was separated by centrifuging the blood collected in EDTA. The tissues liver, kidney and spleen were immediately dissected out and washed with cold saline and weighed amount of tissues were homogenized in appropriate ice-cold buffer in Teflon homogenizer. The serum and tissue samples were subjected to biochemical analysis like lipid peroxidation tissue (Ohkawa et al., 1979) and plasma (Yagi, 1976) enzymic antioxidants like superoxide dismutase (Marklund and Marklund, 1974), catalase (Sinha, 1972), glutathione peroxidase (Rotruck et al., 1973), non-enzymic antioxidants like reduced glutathione (Moron et al., 1979), vitamin C (Omaye et al., 1979) and vitamin E (Desai, 1984).

In vitro antioxidant activity

Ferric thiocyanate method (FTC)

The methods of Mitsuda et al. (1967) and Osawa and Namiki (1981) slightly modified by Kikuzaki and Nakatani (1993) were used. A mixture of 4 mg weight sample in 4 ml absolute ethanol, 4.1 ml of 2.52% linoleic acid in absolute ethanol, 8 ml of 0.05 M phosphate buffer (pH 7.0) and 3.9 ml of water was placed in a test tube with a screw cap and then placed in an oven at 40°C in the dark. To 0.1 ml of this solution was added 9.7 ml of 75% ethanol and 0.1 ml 30% ammonium thiocyanate. Precisely 3 min after the addition of 0.1 ml of 0.02 M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance was measured at 500 nm for every 24 h until the absorbance of the control reached maximum. The control and the standard were subjected to the same procedures as the sample except that for the control, only the solvent was added, and for the standard, 4 mg sample was replaced with 4 mg of Vitamins C and E.

Thiobarbituric acid (TBA) method

The method of Ottolenghi (1981) was followed. 2 ml of 20% trichloroacetic acid and 2 ml of TBA aqueous solution were added to 1 ml of sample solution prepared as in FTC procedure, and incubated in a similar manner. The mixture was placed in boiling water bath for 10 min.

After cooling, it was centrifuged, at 3000 rpm for 20 min and the

Table 1. Effects of SPP and SPE on lipid peroxidation of plasma and tissues of control and adjuvant induced arthritic rats.

Groups	Plasma	Liver	Kidney	Spleen
I	3.29 ± 0.05	1.58 ± 0.07	1.45 ± 0.08	2.21 ± 0.05
II	4.89 ± 0.08^{a*}	1.15 ± 0.06 ^{a*}	2.66 ± 0.08 ^{a*}	4.28 ± 0.07 ^{a*}
III	4.65 ± 0.06$^{a*b@}$	1.22 ± 0.05 $^{a*b@}$	2.16 ± 0.08 $^{a*b\#}$	4.05 ± 0.08 $^{a*b\#}$
IV	3.25 ± 0.11^{b*}	1.60 ± 0.08 ^{b*}	1.52 ± 0.05 ^{b*}	2.19 ± 0.06 ^{b*}
V	4.25 ± 0.12^{a*b*}	1.40 ± 0.05 $^{a@b\#}$	1.82 ± 0.07 $^{a@b*}$	3.15 ± 0.05 ^{a*b*}
VI	4.35 ± 0.09$^{a*b*c@}$	1.45 ± 0.06 $^{a@b\#}$	1.85 ± 0.09 $^{a\#b*}$	3.25 ± 0.08 ^{a*b*}
VII	3.24 ± 0.04 ^{b*}	1.62 ± 0.09 ^{b*}	1.52 ± 0.08 ^{b*}	2.18 ± 0.05 ^{b*}
VIII	4.15 ± 0.08 ^{a*b*}	1.40 ± 0.07 $^{a@b\#}$	1.79 ± 0.07 $^{a@b*}$	3.04 ± 0.04 ^{a*b*}
IX	4.28 ± 0.07 $^{a*b*c@}$	1.44 ± 0.08 $^{a@b\#}$	1.83 ± 0.08 $^{a@b*}$	3.15 ± 0.08 ^{a*b*}

Values are Mean ± S.E.M, n=6 animals in each group. Units: Plasma (mg/dl), tissues (n moles of MDA liberated/min/mg protein). Comparisons were made between: a- Group I vs II, III, IV, V, VI, VII, VIII and IX, b- Group II vs III, IV, V, VI, VII, VIII and IX, c- Group III vs V, VI, VIII and IX. Symbols represent statistical significance: * - $p < 0.001$, # - $p < 0.01$, @ - $p < 0.05$.

absorbance of the supernatant was measured at 532 nm. Antioxidant activity was based on the absorbance on the final day. The inhibition of lipid peroxidation in percentage was calculated by the following equation:

Percent inhibition = $[Ao-A_1/Ao] \times 100$

Where Ao is the absorbance of control and A_1 is the absorbance of sample.

Statistical analysis

The data represents mean ± S.E.M. Results were analysed statistically by one-way ANOVA followed by Tukey's multiple comparison using SPSS software student's version. The difference was considered significant when $P<0.05$.

RESULTS

Serum and tissue lipid peroxidation

In the present study, the plasma and tissue lipid peroxide levels were estimated (Table 1), which showed significant increase in lipid peroxide levels of plasma, kidney and spleen whereas decrease in liver lipid peroxide level. In SPP, SPE and standard drug treated groups the altered lipid peroxide levels were significantly normalized. Those groups treated with SPP and SPE alone without induction of arthritis did not show any significant change or abnormalities when compared to the normal control group.

Tissue enzymic and non-enzymic antioxidant levels

The alterations observed in enzymic and non-enzymic antioxidant levels of various tissues in adjuvant induced arthritic rat model are presented in Tables 2, 3 and 4. It was observed from the results that except SOD, all other enzymic and non-enzymic antioxidant levels were decreased in all the tissues of adjuvant treated rats when compared with the normal control. The tissue superoxide dismutase level was significantly increased in arthritic control rats.

In the present study, administration of the drugs (SPP and SPE) to arthritic rats caused a significant decrease in elevated SOD level, a significant increase in glutathione peroxidase, catalase, reduced glutathione, vitamins C and E levels. The drug treatments have significantly increased these antioxidant vitamin levels when compared with the arthritic control.

In vitro antioxidant activity

The total antioxidant activity of the drugs SPP and SPE were assessed by FTC and TBA methods and compared with vitamins E and C (Figures 1 and 2). In FTC method, the drugs and standards possessed low absorbance values when compared with the control. SPP showed the least increase in absorbance followed by vitamin E, vitamin C and SPE.

The absorbance values from TBA method showed total peroxide values produced by the oxidation of linoleic acid. The higher the absorbance values, the lower the level of antioxidant activity. The control had the highest absorbance value followed by SPP, vitamin E, vitamin C and SPE. The results were similar to that of FTC method.

DISCUSSION

Oxygen free radicals (OFR) have been implicated as mediators of tissue damage in rheumatoid arthritis. The

Table 2. Effects of SPP and SPE on liver enzymic and non enzymic antioxidants of control and adjuvant induced arthritic rats.

Groups	Superoxide dismutase	Catalase	Glutathione peroxidase	Reduced glutathione	Vitamin C	Vitamin E
I	2.50 ± 0.03	145.67 ± 3.42	16.83 ± 0.04	3.58 ± 0.02	1.40 ± 0.09	2.21 ± 0.05
II	4.24 ± 0.04 ^{a*}	100.33 ± 2.56 ^{a*}	10.82 ± 0.04 ^{a*}	2.32 ± 0.08 ^{a*}	0.74 ± 0.05 ^{a*}	1.49 ± 0.03 ^{a*}
III	3.98 ± 0.02 $^{a*b\#}$	118.03 ± 3.56 $^{a*b\#}$	14.22 ± 0.06 ^{a*b*}	2.86 ± 0.05 ^{a*b*}	0.94 ± 0.07 $^{a*b\#}$	1.75 ± 0.04 ^{a*b*}
IV	2.67 ± 0.03 ^{b*}	146.48 ± 4.02 ^{b*}	17.55 ± 0.02 ^{b*}	3.56 ± 0.02 ^{b*}	1.35 ± 0.04 ^{b*}	2.34 ± 0.06 ^{b*}
V	3.39 ± 0.05 ^{a*b*}	124.05 ± 2.86 ^{a*b*}	14.34 ± 0.08 ^{a*b*}	2.83 ± 0.04 ^{a*b*}	1.12 ± 0.06 ^{a*b*}	1.84 ± 0.05 ^{a*b*}
VI	3.42 ± 0.05 ^{a*b*}	118.00 ± 3.31 $^{a*b\#}$	14.58 ± 0.05 ^{a*b*}	2.92 ± 0.09 ^{a*b*}	1.09 ± 0.08 ^{a*b*}	1.79 ± 0.04 ^{a*b*}
VII	2.60 ± 0.05 ^{b*}	143.40 ± 3.65 ^{b*}	17.62 ± 0.08 ^{b*}	3.55 ± 0.06 ^{b*}	1.33 ± 0.05 ^{b*}	2.22 ± 0.08 ^{b*}
VIII	3.24 ± 0.07 ^{a*b*}	131.64 ± 3.45 ^{a*b*}	14.19 ± 0.06 ^{a*b*}	3.10 ± 0.05 ^{a*b*}	1.10 ± 0.06 ^{a*b*}	1.99 ± 0.09 ^{a*b*}
IX	3.31 ± 0.06 ^{a*b*}	126.69 ± 1.26 ^{a*b*}	14.28 ± 0.08 ^{a*b*}	3.02 ± 0.03 ^{a*b*}	1.02 ± 0.05 ^{a*b*}	1.81 ± 0.04 ^{a*b*}

Values are mean ± S.E.M, n=6 animals in each group. Units: Superoxide dismutase (units/mg protein), catalase (µmoles of H_2O_2 consumed/min/mg protein), glutathione peroxidase (µg of GSH utilized /min/mg protein), reduced glutathione (µg/mg protein), vitamin C (µg/mg protein), vitamin E (mg/g tissue). Comparisons were made between: a- Group I vs II, III, IV, V, VI, VII, VIII and IX, b- Group II vs III, IV, V, VI, VII, VIII and IX, c- Group III vs V, VI, VIII and IX. Symbols represent statistical significance:* - $p < 0.001$, # - $p < 0.01$, @ - $p < 0.05$.

Table 3. Effects of SPP and SPE on kidney enzymic and non enzymic antioxidants of control and adjuvant induced arthritic rats.

Groups	Superoxide dismutase	Catalase	Glutathione peroxidase	Reduced glutathione	Vitamin C	Vitamin E
I	2.19 ± 0.08	136.33 ± 2.19	15.17 ± 0.04	1.79 ± 0.09	1.48 ± 0.08	1.67 ± 0.05
II	3.32 ± 0.05 ^{a*}	99.50 ± 1.68 ^{a*}	8.11 ± 0.06^{a*}	1.04 ± 0.08 ^{a*}	0.68 ± 0.03 ^{a*}	1.06 ± 0.09 ^{a*}
III	2.84 ± 0.06 $^{a*b\#}$	105.39 ± 1.83 $^{a*b@}$	10.69 ± 0.04 $^{a*b\#}$	1.30 ± 0.01 $^{a*b\#}$	0.90 ± 0.06 $^{a*b\#}$	1.26 ± 0.02 $^{a*b@}$
IV	2.25 ± 0.05 ^{b*}	135.63 ± 3.68 ^{b*}	15.33 ± 0.06 ^{b*}	1.82 ± 0.02 ^{b*}	1.44 ± 0.07 ^{b*}	1.73 ± 0.05 ^{b*}
V	2.56 ± 0.04 $^{a\#b*}$	125.75 ± 3.24 $^{a@b*}$	12.66 ± 0.08 ^{a*b*}	1.46 ± 0.04 $^{a\#b*}$	1.18 ± 0.02 ^{a*b*}	1.48 ± 0.04 $^{b\#}$
VI	2.68 ± 0.03 ^{a*b*}	117.26 ± 2.47 ^{a*b*}	12.56 ± 0.05 ^{a*b*}	1.45 ± 0.09 $^{a\#b*}$	1.11 ± 0.06 ^{a*b*}	1.43 ± 0.01 $^{b\#}$
VII	2.23 ± 0.04 ^{b*}	138.38 ± 2.57 ^{b*}	15.26 ± 0.08 ^{b*}	1.75 ± 0.05 ^{b*}	1.38 ± 0.04 ^{b*}	1.75 ± 0.06 ^{b*}
VIII	2.48 ± 0.05 $^{a\#b*}$	126.56 ± 2.08 ^{a*b*}	13.46 ± 0.08 ^{a*b*}	1.56 ± 0.06 $^{a@b*}$	1.26 ± 0.06 $^{a\#b*}$	1.58 ± 0.08 $^{b\#}$
IX	2.58 ± 0.01 $^{a\#b*}$	121.83 ± 3.59 $^{a\#b*}$	12.83 ± 0.06 ^{a*b*}	1.47 ± 0.08 $^{a\#b*}$	1.16 ± 0.09 $^{a\#b*}$	1.47 ± 0.06 $^{b\#}$

Values are mean ± S.E.M, n=6 animals in each group. Units: Superoxide dismutase (units/mg protein), catalase (µmoles of H_2O_2 consumed/min/mg protein), glutathione peroxidase (µg of GSH utilized /min/mg protein), reduced glutathione (µg/mg protein), vitamin C (µg/mg protein), vitamin E (mg/g tissue). Comparisons were made between: a- Group I vs II, III, IV, V, VI, VII, VIII and IX, b- Group II vs III, IV, V, VI, VII, VIII and IX, c- Group III vs V, VI, VIII and IX. Symbols represent statistical significance: * - $p < 0.001$, Symbols represent statistical significance:* - $p < 0.001$, # - $p < 0.01$, @ - $p < 0.05$.

involvement of free radicals in various inflammatory conditions like synovitis and RA are well documented (Merry et al., 1989; Halliwell et al., 1988). There is evidence that the inflammatory cells such as neutrophils, lymphocytes and macrophages are present in synovial fluid and produce large amounts of superoxide and hydrogen peroxide radicals (Halley and Cheesemen, 1993). The increase in plasma and

Table 4. Effects of SPP and SPE on spleen enzymic and non enzymic antioxidants of control and adjuvant induced arthritic rats.

Groups	Superoxide dismutase	Catalase	Glutathione peroxidase	Reduced glutathione	Vitamin C	Vitamin E
I	1.50 ± 0.05	75.06 ± 3.51	4.87 ± 0.04	2.86 ± 0.08	1.86 ± 0.08	0.75 ± 0.11
II	1.95 ± 0.09 ^{a*}	50.62 ± 3.54 ^{a*}	3.98 ± 0.06 ^{a*}	1.48 ± 0.03 ^{a*}	1.19 ± 0.06 ^{a*}	0.30 ± 0.04 ^{a*}
III	1.80 ± 0.08 ^{a*b*}	58.48 ± 2.55 $^{a*b\#}$	4.21 ± 0.03 $^{a*b\#}$	1.91 ± 0.05 ^{a*b*}	1.38 ± 0.09 $^{a*b\#}$	0.47 ± 0.09 $^{a\#b\#}$
IV	1.46 ± 0.06 ^{b*}	77.45 ± 3.40 ^{b*}	4.81 ± 0.05 ^{b*}	2.80 ± 0.07 ^{b*}	1.82 ± 0.04 ^{b*}	0.79 ± 0.07 ^{b*}
V	1.64 ± 0.02 $^{a\#b*}$	67.68 ± 3.95 $^{a\#b*}$	4.56 ± 0.03 $^{a\#b*}$	2.07 ± 0.06 ^{a*b*}	1.62 ± 0.05 $^{a@b*}$	0.54 ± 0.04 $^{a\#}b^{\#}$
VI	1.70 ± 0.04 $^{a\#b*}$	65.98 ± 4.78 $^{a\#b*}$	4.50 ± 0.05 $^{a\#b*}$	1.91 ± 0.04 ^{a*b*}	1.54 ± 0.03 $^{a\#b*}$	0.49 ± 0.08 $^{a\#b\#}$
VII	1.51 ± 0.03 ^{b*}	75.36 ± 3.56 ^{b*}	4.82 ± 0.04 ^{b*}	2.83 ± 0.06 ^{b*}	1.83 ± 0.10 ^{b*}	0.76 ± 0.06 ^{b*}
VIII	1.61 ± 0.05 $^{a@b*}$	68.56 ± 2.98 $^{a\#b*}$	4.56 ± 0.06 $^{a\#b*}$	2.05 ± 0.05 ^{a*b*}	1.68 ± 0.05 $^{a@b*}$	0.61 ± 0.04 $^{a*}b^{\#}$
IX	1.68 ± 0.09 $^{a\#b*}$	65.32 ± 3.02 $^{a\#b*}$	4.48 ± 0.03 ^{a*b*}	1.99 ± 0.05 ^{a*b*}	1.58 ± 0.04 $^{a@b*}$	0.57 ± 0.07 $^{a@b\#}$

Values are mean ± S.E.M, n=6 animals in each group. Units: Superoxide dismutase (UNITS/mg protein), catalase (µmoles of H_2O_2 consumed/min/mg protein), glutathione peroxidase (µg of GSH utilized /min/mg protein), reduced glutathione (µg/mg protein), vitamin C (µg/mg protein), vitamin E (mg/g tissue). Comparisons were made between: a- Group I vs II, III, IV, V, VI, VII, VIII and IX, b- Group II vs III, IV, V, VI, VII, VIII and IX, c- Group III vs V, VI, VIII and IX.

tissue lipid peroxide levels in the arthritic control rats indicate that, the tissues are subjected to increased oxidative stress. The increased level in plasma but decreased level in liver lipid peroxide content may be due to the increased removal of lipid peroxides from liver into blood in arthritic animals. It was also proposed that the suppression of liver lipid peroxidation in the adjuvant treated rats is caused by a damage of the ascorbic acid-Fe^{2+} dependent mechanism, which is responsible for lipid peroxidation in the liver (Roback, 1978). The results of the present study indicate that, the drugs SPP and SPE decreased the lipid peroxide content in plasma and tissues (kidney and spleen) either by interception of the formation or by scavenging the active oxygen species. The liver lipid peroxide level in the drug treated group was considerably increased; showing that the removal of lipid peroxide from liver to blood was reduced. Superoxide dismutase can convert superoxide anion radical to hydrogen peroxide (Free, 1980). Also, this increase in enzyme activity may be due to protection against the extracellular oxygen free radicals (Marklund et al., 1987). In the present study, administration of the drugs (SPP and SPE) to arthritic rats caused a significant decrease in elevated SOD level. Catalase cleaves the hydrogen peroxide into water and oxygen. Glutathione peroxidase is also a detoxifying enzyme, changing the peroxides to water (Lawrence and Burk, 1976). The decrease in GPX and catalase activities was observed in various tissues of arthritic rats, which may be due to the degradation of the enzyme by free radicals during detoxification process (Karatas et al., 2003). It was also proposed that, increased accumulation of H_2O_2 causes inhibition of GPX and catalase (Rister and Banchner, 1976). SPP and SPE administration produced a significant ($P<0.001$) increase in their activity, which enables scavenging of the free radicals produced during arthritic condition. The increase in superoxide radical activity may cause increased dismutation of superoxide anion radicals into hydrogen peroxide, but the hydrogen peroxide could not have been detoxified by the decreased levels of GPX and catalase. Thus, the hydrogen peroxide may possibly be converted into reactive oxygen species, which may be involved in the increased lipid peroxidation in adjuvant induced arthritic rats. All these abnormal alterations that occurred were found to be significantly normalized by reducing the SOD activity and enhancing the GPX and catalase activities by treating the arthritic rats with SPP and SPE.

Reduced glutathione is intracellular thiol rich tripeptide, which plays a major role in the protection of cells and tissue structure from OFR (Meister, 1983). In the present study, tissue glutathione, vitamins C and E levels were decreased in arthritic control rats, which were significantly increased after treatment with SPP and SPE. These results show that, chronic inflammation affects the antioxidant vitamin levels in RA. The decreased activity of vitamin C may be

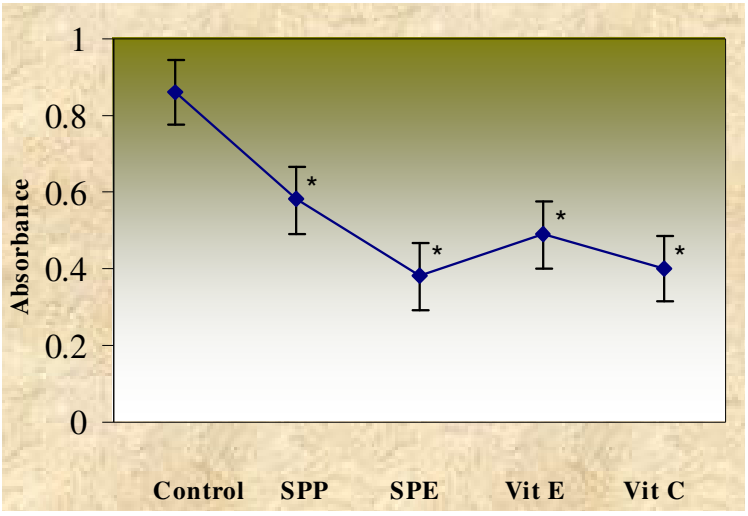

Figure 1. Effect of SPP and SPE on *in vitro* antioxidant activity by FTC method. Data represents mean ± S.E.M of 6 values,* p<0.001.

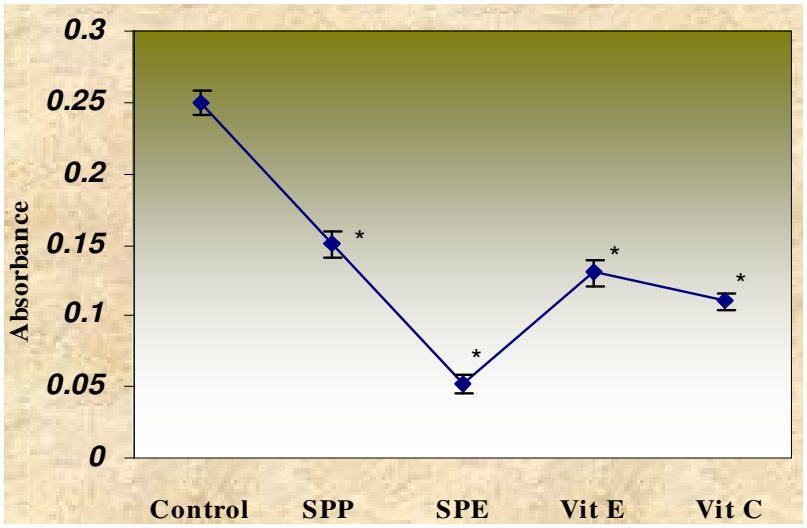

Figure 2. Effect of SPP and SPE on *in vitro* antioxidant activity by TBA method. Data represents Mean ± S.E.M of 6 values, * p<0.001.

due to decreased level of reduced glutathione and the enzyme system NADH- semidehydro ascorbate reductase (Frer et al., 1989). Tocopherol (vitamin E) is a lipid soluble molecule that functions as the most important lipid peroxidation chain breaking antioxidant and as a cell membrane stabilizer (Sen, 1995). Tocopherols delay lipid peroxidation by scavenging intermediate radicals such as lipid peroxyls. In FTC method, the drugs and standards possessed low absorbance values when compared with the control. SPP showed the least increase in absorbance followed by vitamin E, vitamin C and SPE. The absorbance values from TBA method showed total peroxide values produced by the oxidation of linoleic acid. The higher the absorbance values, the lower the level of antioxidant activity. The control had the highest absorbance value followed by SPP, vitamin E, vitamin C and SPE.

The results were similar to that of FTC method. During the oxidation process, peroxide is gradually decomposed to lower molecular compounds that are measured by FTC and TBA methods (Aruna et al., 1993). FTC method is used to measure the amount of peroxide at primary stage of linoleic acid peroxidation. From the results, it is clear that SPE possessed greater antioxidant activity than the standards, vitamins E and C. The absorbance values from TBA method showed total peroxide values produced

by the oxidation of linoleic acid. The higher the absorbance values, the lower the level of antioxidant activity. The control had the highest absorbance value followed by SPP, vitamin E, vitamin C and SPE. The results were similar to that of FTC method.

Based on the results, SPE possessed the highest antioxidant activity than the standards. The secondary stage of linoleic acid oxidation is the formation of malondialdehyde. The thiobarbituric acid reaction with MDA is generally considered to be an indicator of the secondary breakdown products of oxidized polyunsaturated fatty acids (Ismail et al., 2000). The highest antioxidant activity of SPE may be due to the presence of antioxidant phytochemicals like polyphenolics, steroids and triterpenes (Sanmuga priya and Venkataraman, 2010). In the preliminary pharmacognostical and phytochemical evaluation of SPP and SPE in our previous study, revealed the presence of phenolics, steroids, triterpenes/volatile oils, saponins, alkaloids and volatile oils. HPTLC fingerprinting of specified fractions like alkaloids, steroids/triterpenes and polysaccharide fractions were reported (Sanmuga priya and Venkataraman, 2010) earlier.

Conclusion

The results obtained in the treated groups shows the effect of drugs in raising the non enzymic antioxidant levels which may be due to the sparing effect of antioxidant defense system, as the drugs SPP and SPE might have scavenged the free radicals by the presence of its various antioxidant phytochemicals like steroids, triterpenes and polyphenolics either by individual action or by combined action. The present *in vivo* and *in vitro* study on SPP and SPE on the antioxidant status of FCA induced arthritic rats, have generated data in favour of SPP and SPE as potential herbal antioxidants, which may be useful to arrest the progression and complications of RA in humans.

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