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COMPARATIVE EVALUATION OF ANTIOXIDANT STATUS AND INFLAMMATORY MARKERS IN THE LEAVES OF *Pterospermum canescens* Roxb. AND *Pterospermum heyneanum* Wall. AGAINST FREUND'S COMPLETE ADJUVANT INDUCED ARTHRITIS IN ALBINO RATS

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease predominantly affecting the joints and periarticular tissue. RA still remains a formidable disease, being capable of producing severe crippling deformities, functional disabilities and cartilage destruction, caused by number of proinflammatory molecules released by macrophages including reactive oxygen species and ecosanoids such as prostaglandins, leukotrienes and cytokines. Only management of the disease is done by using anti-inflammatory agents like NSAIDs, disease modifying antirheumatoid drugs, corticosteroids and by biological agents, but yet a complete cure for this disease is still lacking. The present research was undertaken to evaluate and compare the ethanolic extract of leaves of *Pterospermum canescens* (EEPC) and *Pterospermum heyneaneum* (EEPH) in relation to antioxidant status and inflammatory markers against Freund's Complete Adjuvant (FCA) induced arthritis in rats. The treatment was started from the 12th day of FCA induction. On 28th day the blood sample was withdraw by retro orbital puncture to determine the pro- inflammatory mediator (TNF-α) and the synovial fluid was collected to measure the level of inflammatory markers (total leukocytes, lymphocytes, monocytes/ macrophages). Articular tissue samples were taken and homogenized to measure antioxidant enzymes. From the present study EEPC and EEPH showed dose dependent significant reduction in lipid peroxidation, restored the endogeneous antioxidant enzymes , decreased inflammatory markers as compared to the arthritic control respectively.

Key words: *Pterospermum canescens*, *Pterospermum heyneanum*, Lipid peroxidation, Antioxidant enzymes, Inflammatory markers, Freund's complete adjuvant-induced arthritis.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic and systemic inflammation that affects the synovial membrane, articular cartilages, and bones. Rheumatoid arthritis occurs in 0.5- 1.0% of the adult population worldwide and it is associated with an increased mortality rate, mainly due to cardio vascular complications caused by inflammatory process (Comar *et al.*, 2013). RA starts in synovium, the membrane produces sac surrounding the joint. This sac containing synovial fluid which lubricate the joints, along with that supplies nutrients and oxygen to cartilage which coats the end of bones. Cartilage is made of collagen which gives support and flexibility to the joints. In rheumatoid arthritis, destructive molecules produced by an abnormal immune system response which is responsible for continuous inflammation of the synovium. Collagen is gradually destroyed, narrowing the joint space and finally damaging the bone. In a progressive rheumatoid arthritis, destruction of the

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cartilage accelerates. Further pannus formation occurs due to the accumulation of fluid and immune system cells in the synovium. The pannus produces more enzymes which destroy nearby cartilage, worsening the area and attracting more inflammatory white cells (Jaya Sankar Reddy *et al.*, 2014).

The pathophysiology of arthritis involves an intense hyperplasia of the articular cartilage with participation of T cells, B cells, macrophages, fibroblasts, and proinflammatory cytokines, particularly interleukin-1(IL-1), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α). In addition to the cytokines, reactive oxygen species (ROS) also play an important role in rheumatoid arthritis. The overproduction of proinflammatory cytokines stimulates neutrophils and activate macrophages to secrete ROS in the synovial fluid, which act as mediators of tissue injury. Cytokines released into the synovium may also reach the systemic circulation and act in other tissues. In accordance, the oxidative stress biomarkers are increased in both articular inflammation sites and plasma of patients with rheumatoid arthritis. In addition to affecting the articular cartilage, rheumatoid arthritis also evokes marked inflammatory responses and immunological alterations in other organs, such as lungs, vascular tissue, liver and muscles. Metabolic alterations are equally prominent, such as for example the muscle wasting condition known as rheumatoid cachexia, mediated by TNF-α and IL-1 and occurs in approximately two-third of all patients with rheumatoid arthritis (Comar *et al.*, 2013). Considering all the above factors leading to rheumatoid arthritis, the present work was planned to investigate EEPC and EEPH in relation to the antioxidant status and the inflammatory markers against FCA induced arthritis in albino rats.

Most research has focused on the inflammatory mediators within the synovial membrane, rather than those released into the intra-articular space. FCA induced arthritis is a well established RA disease model and use of this model has gone a long way in aiding and understanding the time course of the pathology in clinical RA. This model closely mimics the pathology of human RA, including histopathological changes, cell infiltration, hypersensitivity and swelling of joints which are affected (Barton N *et al.*, 2007).

The drugs most frequently used in therapy are the NSAID's and the disease modifying antirheumatoid drugs (DMARDs). These drugs reduce the symptoms of rheumatoid disease but do not retard the progress of the disease. Some immunosuppressants (e.g azathioprine, cyclosporine) are also used as are the glucocorticoids. Newer agents, with more specific action against the disease processes of rheumatoid, are the anticytokine drugs (Rang and Dale, 2003). All the available drugs for the treatments have numerous and severe side effects including gastric ulcers and redistribution of body fat. Therefore, agents of natural origin with more potent activity and relatively less side effects are to be explored (Karnati *et al*., 2013). Hence the thrust was towards herbal remedies.

Nature has provided a complete store-house of remedies to cure all aliments of mankind. The genus *Pterospermum canescens*; synonym *Pterospermum suberifolium* and *Pterospermum heyneanum*; synonym *Pterospermum xylocarpum* (Family: Sterculiaceae) represents about 40 species in the world of which 12 species are reported from India and 8 species has been reported from Tamilnadu state. Both *Pterospermum canescens* and *Pterospermum heyneanum* were selected for the present study due to their wide application in traditional system of Indian medicine, paste prepared from the leaves of *Pterospermum canescens* is used in headache (Anonymous, 1969), the leaf paste is applied on the affected portion in the treatment of fracture and inflammation (Silja *et al*., 2008), Leaves of *Pterospermum xylocarpum* have been reported to be used in the treatment of leucorrhoea (Kirtikar and Basu, 1984).

An ethnomedicinal plant species *Pterospermum canescens* is distributed in most of the districts of Tamilnadu. The flavonoids present in the leaves of *Pterospermum canescens* are kaempferol, Kaempferol-3-β-D-galactoside, quercetin, quercetin-3-o-arabinoside and quercetin-3-o-rhamnoside (Gunasegaran *et al*., 1979). The plant has been reported to contain β-amyrin, belutin, lupeol, scopoletin and β-sitosterol (Jaiganesh *et al*., 2011). *Pteorspermum heyneanum* is a woody tree merely distributed in the tropical regions, found in the hills of Andhra Pradesh and Tamil Nadu (Narendra *et al*., 2013). The fresh flowers as well as the leaves of *Pteorspermum heyneanum* have been reported to contain Kampferol-3-o-β-D-galactoside as the major pigment and quercetin-3-o-β-D galactoside as the minor (Gunasegaran *et al*., 1979). Leaves of *Pteorspermum heyneanum* is reported to contain lupeol acetate, lupanone, taraxerone, friedelin, taraxerol and β-sitosterol (Anjaneyulu, *et al*., 1988).

Due to lack of scientific evidence, the aim of the present study was to evaluate the antioxidant status and the level of inflammatory markers in the leaves of *Pterospermum canescens* & *Pterospermum heyneanum* against FCA induced arthritis.

MATERIALS AND METHODS

Collection of plant material and authentication

Leaves of *Pterospermum canescens* and *Pterospermum heyneanum* were collected from Narthamalai - Pudukottai district and from kollimalai - Namakkal district of Tamilnadu respectively during the Month of February 2011. The plants were taxonomically identified and authenticated by Dr. P.Jayaraman, Ph.D., Director- Institute of Herbal Botany Plant Anatomy Research Centre, West Tambaram, Chennai with Reg

No.: PARC/2012/1399 & PARC/2012/1400 respectively.

Preparation of the Extract

The leaves of both the plants were collected, the dust particles were removed and shade dried. The dried leaves were grinded mechanically to obtain a coarse powder; all the powders of both the plants were passed through sieve number 40 and stored in a clean, dry air tight container. Separately, both the powders were first defatted with petroleum ether (40-60^{o}C) and then extracted with ethanol by continuous hot percolation method using soxhlet apparatus. The solvent was removed using rotary evaporator. This method was continued until getting a desired amount of extracts from both the plants (Trease & Evans, 2008).

Preliminary phytochemical screening: The preliminary phytochemical screening of EEPC and EEPH was carried out according to kokate and Tiwari *et al* (Kokate,2010; Tiwari, 2011).

Animals: Female Wistar rats of body weight (150-200g) were used for the present study. The animals were maintained under standard environmental conditions and were fed with standard pellet diet and water *ad libitum.* The study was approved by Institutional Animal Ethical Committee (Reg. No. 409/01/a/CPCSEA).

Acute oral toxicity studies

According to Organization of Economic Co-operation and Development guidelines, (OECD, 2001) acute toxic class method was followed to arrive at the maximum safety dose of the drug extracts.

Treatment Protocol

The animals were divided into 9 groups of six animals in each as follows and the doses were fixed based on acute toxicity studies. Ethanol extract of leaves of *Pterospermum canescens* (EEPC) and ethanol extract of leaves of *Pterospermum heyneanum* (EEPH) were made into suspension with distilled water using 1% w/v Na-CMC and administered to the animals per orally.

Group I – Vehicle control 1% w/v Na-CMC, 10ml/Kg/p.o (Non-arthritic)

Group II - Arthritic control 1% w/v Na-CMC, 10ml/Kg/p.o

Group III - Arthritic animals treated with indomethacin 10 mg/kg bodyweight p.o suspended in distilled water using 1% w/v Na-CMC (Mali *et al*, 2011).

Group IV- Arthritic animals treated with EEPC 100 mg/kg body weight p.o

Group V- Arthritic animals treated with EEPC 250 mg/kg body weight p.o

Group VI- Arthritic animals treated with EEPC 500 mg/kg body weight p.o

Group VII- Arthritic animals treated with EEPH 100 mg/kg body weight p.o

Group VIII- Arthritic animals treated with EEPH 250 mg/kg body weight p.o

Group IX- Arthritic animals treated with EEPH 500 mg/kg body weight p.o

Assay of Plasma tumor necrosis factor-α (TNF-α): Using ELISA commercial kit, Plasma TNF-α concentration was determined. At the end of the experiment, samples of blood (0.5ml) were withdrawn by retro orbital puncture under anesthesia in a poly- ethylene tubes with 25µl of heparin solution (4000 IU). The plasma samples subjected to centrifugation for 10 min at 3000 g and 4°C were frozen at –80°C until assay. In brief, 100 µl of standards, samples and controls were added to each well of the coated micro plate. After 3 hours of incubation at 24°C the microplate was decanted and the liquid discarded. Then, 100µl of biotinylated anti-TNF-α antibody was added to each well. Incubation at 24°C for 45 min was carried out and further elimination of the liquid from the wells were done, 100µl of Streptavidin–horseradish peroxidase conjugate was added. After incubation for a further 45 min and washing of the wells, 100µl of chromogen were added. The absorbance of each well was read at 450nm spectrophotometrically. TNF-α values were expressed as pg/ml (Campo, *et al*., 2003).

***Determination of Leukocyte Concentration in Synovial Fluid*:** On the 28th day of the investigational period, Leukocyte concentration in the synovial fluid was determined. The animals were anesthetized and skin overlying the anterior aspect of the left knee was incised and a 26-gauge hypodermic needle was inserted into the synovial cavity. This arrangement facilitated the infusion of 250 µL 0.9 % w/v saline into the synovial cavity over a period of 2 min. A second hypodermic needle (26 gauge), which served as an outflow cannula, was inserted into the synovial cavity approximately 3 mm from the infusion needle. Five min after infusion of the saline, 200 µL of fluid was withdrawn from the synovial cavity over a period of 2 min. The concentration of total leukocytes, lymphocytes and monocytes / macrophages were determined in the synovial fluid using Coulter counter (Lewis, *et al*., 2006).

Myeolperoxidase Analysis: Myeloperoxidase activity was analysed as an index of neutrophil infiltration in the articular tissue since it is closely correlated with the number of neutrophils present in the tissue. The tissue samples were separated from rat joints and were first homogenized in a solution containing 20 mM potassium phosphate buffer, pH 7.0, to 1:10 (w/v) and then centrifuged for 30 min at 20,000 g and 4º C. The supernatant of each sample was removed and the resulting pellet was added to a buffer solution consisting of 0.5 %

w/v hexadecyltrimethylammonium bromide dissolved in 50 mM potassium phosphate buffer, pH 6, containing 50 µL of protease and phosphate inhibitor. Samples were then sonicated for 1 min and centrifuged for 30 min at 20,000 g and 4º C. An aliquot of the supernatant was allowed to react with a solution of o-dianisidine dihydrochloride (0.167 mg/mL) and 0.0005 % v/v hydrogen peroxide. The rate of change in absorbance was measured spectrophotometrically at 405 nm (Shimadzu UV-Vis 1700). Myeloperoxidase activity has been defined as the concentration of enzyme degrading 1 µmol of peroxide/min at 37º C and was expressed as U/g of protein (Mullane *et al.,* 1985).

Measurement of TBARS: The malonaldehyde is measured as thiobarbituric acid-reactive substance (TBARS). TBARS was measured as a marker of lipid peroxidation in the articular cartilage. The supernatant of the homogenized tissue (0.4 mL) was added to 1.5 mL of 8.1 % w/v sodium dodecyl sulphate, 1.5 mL of 20 % w/v acetate buffer (pH 3.5) and 1.5 mL of 0.8 % w/v TBA (Thiobarbituric acid) solution. At 95°C the mixture was heated for 1 h. After cooling, 5 mL of n-butanol-pyridine (14:1) was added for extraction and the absorbance of n-butanol-pyridine layer was read at 532 nm (Shimadzu UV-Vis 1700) for determination of TBA reactive substance (Jung-Hwan *et al*., 2006).

Determination of Glutathione (GSH): The supernatant of the homogenized articular tissue (0.4 mL) was added into dark polyethylene tube containing 1.6 mL of 0.4 M Tris – EDTA buffer, pH 8.9. After vortex-mixing, 40 µL of 10 mM dithiobisnitrobenzoic acid in methanol was added. The samples were vortex-mixed again and the absorbance at 412 nm was read after 5 min (Shimadzu UV-Vis 1700). The unknown values of the samples were drawn from a standard curve plotted by assaying different known concentrations of glutathione (GSH). The amount of GSH was expressed as µmol/ g of protein (Campo *et al*., 2003).

Measurement of Glutathione Peroxidase (GPx): The reagents H_2O_2, 1 mM GSH (glutathione), 0.2 mM NADPH and the supernatant of homogenized articular tissue (0.4 mL) were all added to 0.1 M tris-HCl Buffer solution (pH 7.2) and reacted for 5 min at 25° C. NADPH consumed by the reduction of the oxidized form of glutathione was determined by measuring the absorbance at 340 nm (Shimadzu UV-Vis 1700) and glutathione peroxidase (GPx) activity was calculated. Enzyme activity is quoted as the units of NADPH oxidized nmol/1 mg protein/min (Jung-Hwan et *al*., 2006).

Measurement of Superoxide Dismutase (SOD): Total SOD activity was measured by determining the ability to inhibit the auto-oxidation of pyrogallol. The rate of auto-oxidation was determined by measuring the increase in absorbance at 420 nm. Reaction mixture containing 0.2 mM pyrogallol in 50 mM tris-cacodylic acid buffer (pH 8.5) and 1 mM diethylene triamine penta acetic acid was incubated at 25° C for 90s. One unit of SOD activity is defined as the amount of the enzyme required to inhibit the rate of pyrogallol auto-oxidation by 50 % (Arulmozhi *et al,* 2011).

Statistical Analysis*:* Experimental results were expressed as mean ± SEM of six animals. Analysis of variance was performed by One-way ANOVA followed by Newmans – Keul multiple comparison test. P values less than 0.05 were regarded as significant.

RESULTS: ***Preliminary phytochemical screening:*** The preliminary phytochemical screening of EEPC and EEPH were carried out showing the presence of phenolics, flavonoids, triterpenoids, steroids, tannins and carbohydrates commonly in both the plants.

Effect of EEPC and EEPH on the levels of TNF-α (pg/ml): On 28th day the blood sample was collected by retro orbital puncture to measure the level of pro-inflammatory mediator TNF-α. Standard treated group produced a significant decrease ($p<0.001$) in TNF-α as compared to arthritic control. EEPC (100, 250 & 500 mg/kg), produced a significant decrease ($p<0.001$) in TNF-α as compared with arthritic control respectively, EEPH (250 & 500 mg/kg) produced a significant decrease ($p<0.001$) as compared with the arthritic control respectively. EEPH 100 mg/kg did not produce any significant decrease as compared to the arthritic control.

Effect of EEPC and EEPH on leukocyte infiltration: On the 28th day of the study, Synovial fluid was estimated for the inflammatory markers such as total number of leukocytes, lymphocytes, monocytes/macrophages. *Total leukocytes*: Standard treated group, treatment groups EEPC (100, 250 & 500 mg/kg) and EEPH (100, 250 & 500 mg/kg) showed a same significant decrease ($p<0.001$) in leukocyte level as compared with the arthritic treated group respectively. *Number of Lymphocytes:* Standard treated group showed a significant decrease ($p<0.001$) in number of lymphocytes as compared to the arthritic control. Both EEPC and EEPH 100mg/kg did not produce any significant change as compared with the arthritic control. Whereas EEPC (250 & 500 mg/kg) and EEPH (250 & 500 mg/kg) produced a significant decrease ($p<0.001$) as compared with the arthritic control respectively. *Number of Monocytes / Macrophages:* Standard treated group showed a significant decrease $p<0.05$ in the number of monocytes as compared to the arthritic control. Among the treated groups, only EEPC

500mg/kg showed a significant decrease ($p<0.05$) as compared with the arthritic control.

Analysis of Myeloperoxidase: Standard treated group and all the other treatment groups EEPC (100, 250 & 500 mg/kg) and EEPH (100, 250 & 500 mg/kg) produced a significant decrease ($p<0.001$) in myeloperoxidase level as compared with the arthritic control respectively.

Assessment of TBARS: Standard treated group, EEPC (100, 250 & 500 mg/kg) and EEPH (250 & 500 mg/kg) produced a significant decrease ($p<0.001$) in TBARS by inhibiting lipid peroxidation in the articular tissue as compared with the arthritic control respectively. EEPH 100mg/kg did not produce any significant decrease in TBARS.

Assay of GSH: GSH level of standard treated group was significantly restored ($p<0.001$) as compared to arthritic control. EEPC (100, 250 & 500 mg/kg) significantly restored the level of GSH ($p<0.05$, $p<0.001$, $p<0.001$) as compared to arthritic control respectively. EEPH (250 and 500 mg/kg) both restored the level of GSH significantly ($p<0.001$, $p<0.001$), but EPPH 100 mg/kg did not show any significant difference as compared to the arthritic control respectively.

Assay of GPx: The level of GPx was significantly restored ($p<0.001$) by the standard treated group as compared with the arthritic control. EEPC 100mg/kg and EEPH 100mg/kg treated group did not show any significant change as compared to the arthritic control. Whereas EEPC (250 and 500 mg/kg) and EPPH (250 and 500 mg/kg) significantly restored the level of GPx ($p<0.001$, $p<0.001$, $p<0.01$, $p<0.001$) respectively as compared to the arthritic control.

SOD Activity: Standard treated group significantly ($p<0.001$) limited the decline of SOD as compared with arthritic control. EEPC 100mg/kg, EEPH 100 and 250mg/kg did not produce any significant change in SOD levels as compared with the arthritic control. EEPC 250mg/kg, 500mg/kg and EEPH 500mg/kg restored the levels of SOD significantly ($p<0.01$, $p<0.001$, $p<0.001$) as compared with the arthritic control respectively.

Table 1. Effect of EEPC and EEPH on Inflammatory Markers in FCA Induced Arthritis

Parameters	Vehicle Control 1% w/v Na-CMC	Arthritic Control + 1% w/v Na-CMC	Indome-thacin 10 mg/kg	EEPC 100 mg/kg	EEPC 250 mg/kg	EEPC 500 mg/kg	EEPH 100 mg/kg	EEPH 250 mg/kg	EEPH 500 mg/kg
Number of Total leukocytes	1.33 ± 0.21	114 ± 2.78^{c}	48.83 ± 2.80***	95.00 ± 1.63***	80.67 ± 1.54***	74.33 ± 1.74***	100.5 ± 2.21***	92.17 ± 1.75***	82.67 ± 2.61***
Number of Lymphocytes	0.16± 0.16	6.00 ± 0.25^{c}	1.83 ± 0.30***	5.33 ± 0.21^{c}	4.33 ± 0.21***	3.00 ± 0.25***	5.50 ± 0.22^{c}	4.33 ± 0.33***	3.83 ± 0.16***
Number of monocytes/ Macrophages	0.16 ± 0.16	1.50 ± 0.22^{b}	0.33 ± 0.21*	1.17 ± 0.30^{a}	1.17 ± 0.16^{a}	0.33 ± 0.21*	1.33 ± 0.21^{a}	1.33 ± 0.21^{a}	0.66 ± 0.33
TNF alpha (pg/ml)	17.50 ± 1.82	51.17 ± 1.99^{c}	33.33 ± 1.40***	41.83 ± 1.10***	39.33 ± 0.66***	36.8 ± 0.80 ***	49.33 ± 2.06^{c}	42.17 ± 1.24***	38.67 ± 0.66***

Values are expressed as mean ± SEM, n=6, where, ^{a} $p<0.05$, ^{b} $p<0.01$ and ^{c} $p<0.001$ when compared to Vehicle control respectively; * $p<0.05$ and *** $p<0.001$ when compared to Arthritic control respectively.

Table 2. Effect of EEPC and EEPH on Lipid Peroxidation and Antioxidant enzymes in FCA Induced Arthritis

Parameters	Vehicle Control 1% w/v Na-CMC	Arthritic Control + 1% w/v Na-CMC	Indome-thacin 10 mg/kg	EEPC 100 mg/kg	EEPC 250 mg/kg	EEPC 500 mg/kg	EEPH 100 mg/kg	EEPH 250 mg/kg	EEPH 500 mg/kg
TBARS (nmol/mg of protein)	5.37 ± 0.26	14.42 ± 0.58^{c}	7.58 ± 0.40***	11.07± 0.32***	10.02 ± 0.35***	8.90 ± 0.19***	13.59 ± 0.83^{c}	10.67± 0.20***	9.80 ± 0.44***
GSH(µmol/g of protein)	6.39 ± 0.27	2.55 ± 0.12^{c}	5.85 ± 0.32***	3.41 ± 0.09*	4.29± 0.16***	4.59 ± 0.09***	2.68 ± 0.17^{c}	3.75 ± 0.12***	3.96 ± 0.26***
GPx (nmol/mg of protein)	40.5 ± 0.99	20 ± 0.57^{c}	33.17 ± 1.19***	22.67 ± 0.95^{c}	25.17 ± 0.70***	28 ± 0.57***	20.67 ± 0.61^{c}	24.67 ± 0.80**	25.17 ± 0.70***
SOD (U/mg of protein)	8.46 ± 0.28	3.26 ± 0.16^{c}	5.73 ± 0.11***	3.24 ± 0.16^{c}	4.01 ± 0.07**	4.71 ± 0.03***	3.35 ± 0.10^{c}	3.52 ± 0.09^{c}	4.31± 0.05***
Myeloper-oxidase (U/g of protein)	0.13 ± 0.01	1.82 ± 0.04^{c}	0.17± 0.02***	0.92 ± 0.02***	0.64 ± 0.07***	0.57 ± 0.03***	1.55± 0.05***	1.12± 0.04***	0.77 ± 0.05***

Values are expressed as mean ± SEM, n=6, where ^{c} p<0.001 when compared to Vehicle control; * p<0.05, ** at p<0.01 and *** p<0.001 when compared to Arthritic control respectively.

DISCUSSION

FCA induced arthritis is a chronic immune mediated joint inflammation which mimics human pathological state. FCA comprises of heat killed mycobacterium tuberculi suspended in paraffin oil. FCA arthritis follows a biphasic time course consisting of an acute inflammatory condition which peaks at 3 to 5 day and a chronic systemic reaction that shows a relaxing remitting course persisting for several weeks. Several mediators like PGE2 and cytokines (TNF-α, IL-1, IL-6 and GM-CSF) are mediators of joint inflammation in rheumatoid arthritis (Divya Gauba, *et al*, 2014). In FCA induced model, the affected articulations are infiltrated by blood-derived cells, mainly leukocytes, macrophages and dendritic cell. In response to activation, these cells generate free radicals. Which are released in large amounts in the surrounding tissue and damage the tissues (Divya singh *et al*., 2014)

Lipid peroxidation is considered as a critical mechanism of the injury that occurs during RA. The evidence supporting these biochemical changes is based on the analysis of a large number of intermediate products. An indicative method, extensively used, for evaluating lipid peroxidation is analysis of tissue malonaldehyde. The large amount of malonaldehyde found in the arthritic group at day 28 is consistent with the occurrence of damage mediated by free radicals. Treatment with EEPC (100,250 and 500mg/kg) and EEPH (250mg/kg and 500mg/kg) produced a significant decrease in MDA level & attenuation of cartilage injury dose dependently. The production of oxygen free radicals that occurs with the development of arthritis in the articular cartilage leads to decrease GSH, GPx and SOD levels as a consequence of their consumption during oxidative stress and cellular lysis. This decrease contributes to increased cellular damage by favoring attack by free radicals. EEPC (100,250 and 500mg/kg), EEPH (250 and 500mg/kg) blunted the depletion of GSH. EEPC (250 and 500mg/kg), EEPH (250 and 500mg/kg) restored the level of GPx. SOD levels were restored by (250 and 500mg/kg) and by EEPH (500mg/kg), probably by competing in scavenging for free radicals, which further helps to preserve the integrity of cellular membranes in the injured cartilage.

The myeloperoxidase results demonstrated that a strong decrease in infiltration of polymorphonuclear cells occurred in the articular tissue of joints. These decreases confirm the protective effects of EEPC and EEPH dose dependently. Decrease in leukocytes induced by EEPC and EEPH might be due to the inhibition of lipid peroxidation and the consequent decrease in the chemotaxis and reduction of peroxides.

Several areas of investigation have indirectly implicated TNF-α as a contributor to cellular damage in FCA induced arthritis. The high levels of this cytokine can be interpreted as a progression of cartilage cell injury. The antioxidant activity of EEPC and EEPH might have lowered plasma TNF-α concentration and consequently alleviated articular cell damage dose dependently (Campo, *et al*., 2006).

CONCLUSION

The present investigation demonstrates that both EEPC and EEPH dose dependently reduced the lipid

peroxidation, restored the levels of antioxidant enzymes, decreased the level of inflammatory markers and also decreased the level of pro-inflammatory mediators (TNF-α) against FCA induced arthritis in rats. Comparing EEPC and EEPH, EEPC showed to be more effective than EEPH in all parameters dose dependently. The anti-arthritic potential may be due to the presence of phenolics, flavonoids, triterpenoids and steroids commonly present in both plants, but with an important variation in the quantity of phytochemical constituents. Further studies are required to assess the individual quantity of phytochemical constituent responsible for these activities in each plant.

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