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Effect of *Bauhinia variegata* on Complete Freund's Adjuvant Induced Arthritis in Rats

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Abstract: Effect of ethanol extract of *Bauhinia variegata* (EBV) was evaluated for antiarthritic activity on complete Freund's adjuvant (CFA) induced arthritis in rat. The EBV was administered orally at the dose level of 250 mg kg^{-1} for 15 days. The paw volume was measured at 3rd, 5th, 10th and 15th days. At the end of these 15 day, the animals were sacrificed and various biochemical parameters such as serum aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total cholesterol and triglycerides were estimated. Antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and lipid peroxide (LPO) in liver and kidney of normal, arthritic control and EBV treated rats were studied. Oral administration of EBV effectively inhibits rat paw edema volume. EBV significantly ($p<0.01$) altered the biochemical parameters which got affected in arthritic rats. There was a significant alteration in LPO, SOD, catalase and GPx levels when compared to arthritic control rats. Our finding showed a significant antiarthritic effect of EBV against CFA induced arthritis in rats.

Key words: *Bauhinia variegata*, complete Freund's adjuvant induced-arthritis, biochemical parameters, antioxidants

INTRODUCTION

Rheumatoid arthritis is a chronic progressive autoimmune disorder characterised by symmetric erosive synovitis (Recklies *et al*., 2000). The exact etiology of rheumatoid arthritis remains unknown. It has assumed that either a foreign agent or some alteration in control of cellular responses is involved in the chronic persistent synovial inflammation (Krune and Simon, 1986). This disease affects about 1% of the general population worldwide (Haris, 1989). Conventional medicine, including treatment with steroids, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and biological agents such as tumour necrosis factor alpha and interleukin-1 beta antagonists has only limited success against rheumatoid arthritis. Such therapies are helpful in controlling the symptoms of acute rheumatoid arthritis, but their effects on chronic, prolonged rheumatoid arthritis are unsatisfactory, associated with various side effects (Chandrasekaran *et al*., 2002). Research indicates that in the United States, 60-90% patients suffering from arthritis, particularly rheumatoid arthritis, used complementary and alternative medicine, predominantly herbal therapies (Cai *et al*., 2005). Nowadays, increasing efforts are being directed towards traditional herbal medicines and plant derived foods for the development of drugs with long acting anti-inflammatory activity.

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Complete Freund's adjuvant-induced arthritis has been used as a model of sub - chronic or chronic inflammation in rats and is of considerable relevance after the study of pathophysiological and pharmacological control of inflammatory processes, as well as the evaluation of analgesic potential or anti-inflammatory effects of drugs (Butler *et al*., 1992; Besson and Guilbaud, 1988). One of the reasons for the wide utilization of this model is due to the strong correlation between the efficiency of therapeutic agents in this model and in rheumatoid arthritis in humans.

Anti-inflammatory drugs, presently available for the treatment of joint inflammation of various kinds, have undesirable side effects such as causing peptic ulcers. Therefore plant remedies have become increasingly popular and are often preferred to synthetically derived pharmaceuticals. *Bauhinia variegata* Linn (Caesalpiniaceae) grows as a medium sized, deciduous tree found throughout India and is commonly called Shemmandarai in Tamil (Nadkarni, 1996). It is traditionally used in bronchitis, leprosy, tumours and ulcer (Kirtikar and Basu, 1993) and its extracts have been found to have antibacterial, antifungal, anti-inflammatory, anticancer and antiulcer activity (Ali *et al*., 1999; Reddy *et al*., 2003; Rajkapoor *et al*., 2003, 2006a, b, c. Phytochemical studies revealed the presence of 5,7-dimethoxy and dihydroxy flavonone-4'-O-α-L-rhamnopyronosyl-β-D-glucopyranosides, 5- hydroxyl 7, 3', 4', 5' -tetra-methoxy flavone 5-O-β-D-xylopyronosyl (1->2) α-L-rhamnopyroanoside, lupeol, β-sitosterol, quercertin, flavanone and dihydrodibenzoxepin (Reddy *et al*., 2003; Duret and Paris, 1977; Gupta *et al*., 1979, 1980; Yadava and Reddy, 2001, 2003). The present study is aimed to evaluate the antiarthritic activity of *Bauhinia variegata* on complete Freund's adjuvant induced arthritis in rats.

MATERIALS AND METHODS

Plant Material and Extraction

Stems of *Bauhinia variegata* were collected in and around Salem District in the month of November 2005 and authenticated by Dr. G. Murthy, Botanical Survey of India, Coimbatore, Tamilnadu, India. A voucher specimen (DEC-46) has been kept in our laboratory for future reference. The stems were dried in the shade and pulverized. The powder was treated with petroleum ether for dewaxing as well as to remove chlorophyll. The powder was then packed into Soxhlet apparatus and subjected to hot continuous percolation using ethanol (95% v/v) as solvent. The extract was concentrated under vacuum and dried in a vacuum desiccator (yield 4.5 % w/w) and then suspended in 5% gum acacia for the pharmacological studies.

Animals

Male Wistar rats (100-125 g) were procured from Tamilnadu Veterinary College, Chennai, India. They were housed in standard microlon boxes with standard laboratory diet and water *ad libitum*. The experimental protocol was approved by the Institutional animal ethics committee (IAEC), Vel's College of Pharmacy, Chennai, India.

Experimental Induction of Arthritis

Rats were divided into three group, each group containing 6 rats. The treatment schedule of animals belonging to different group are shown below.

Group 1: Control animals received normal saline (2 mL kg^{-1})
Group 2: Complete freund's adjuvant (0.1 mL rat^{-1})
Group 3: Complete freund's adjuvant (0.1 mL rat^{-1}) and *Bauhinia variegata* (250 mg kg^{-1}, p.o.)

On day 0 arthritis was induced in animals belonging to group 2 and 3 by injecting 0.1 mL of complete Freund's adjuvant (Sigma Chemical Co, USA) below the plantar aponeurosis of the right foot

paw of the rats (Newbould, 1963). The next day onwards the test drug was administered to animals at the doses of (250 mg kg^{-1}, p.o.) up to 15 days and measured the paw volume at day 3, 5, 10 and 15. At the end of the experimental period the animals were fasted overnight and then sacrificed by cervical decapitation. Blood was collected and serum separated out. The liver and kidney were immediately removed and suspended in ice cold saline.

Biochemical Estimation

Serum was analysed for the following biochemical parameters: serum aspartate transaminase (AST) (Reitman and Frankel, 1957), alanine aminotransferase (ALT) (Reitman and Frankel, 1957), alkaline phosphatase (Kind and King, 1954), total protein (Lowry *et al*., 1951), cholesterol (Wybenga and Pileggi, 1970) and triglyceride (Hawk *et al*., 1954). A 10% homogenate of the tissue was used for the assay of lipid peroxidation (LPO) (Devasagayam and Tarachand, 1987), superoxide dismutase (SOD) (Marklund and Marklund, 1997) catalase (Sinha, 1972) and Glutathione Peroxidase (GPx) (Rotruck *et al*., 1973).

Statistical Analysis

The results are expressed as mean±SD and the statistical significance was analysed by ANOVA followed by Tukey multiple comparison test for determination of paw edema volume and antioxidant status in liver and kidney of control and experimental animals. Where as biochemical parameters were analysed by ANOVA followed by Dunnette test.

RESULTS AND DISCUSSION

Table 1 showed the time course of edema and inhibition rate after the administration of CFA and EBV. The hind paw developed edema in footpad. Edema value of the injected footpad significantly ($p<0.001$) increased and reached a peak at 15 days. Administration of EBV at a dose of 250 mg kg^{-1} body weight significantly ($p<0.001$) inhibited the development of the swelling induced by CFA. The dose of EBV (250 mg kg^{-1}) exhibited anti-inflammatory activity, which was maintained until the experiment was terminated on day 15.

As a result of arthritis induced by CFA, the level of serum aspartate transaminase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were increased (Table 2). It also showed the lipid profiles following the administration of EBV at 250 mg kg^{-1} in rats. There was a significant difference in the concentration of total cholesterol and triglyceride was found.

Table 1: Effect of EBV on CFA induced paw edema in rats

		Paw volume (mL)			
Drug treatments	Dose (mg kg^{-1})	3rd day	5th day	10th day	15th day
Normal	-	0.21±0.01	0.2 ±0.01	0.26±0.01	0.24±0.10
CFA	0.1 mL	0.56±0.02[a]	0.64±0.01[a]	0.72±0.02[a]	0.74±0.03[a]
B. variegata	250	0.29±0.02[b]	0.29±0.03[b]	0.27±0.01[b]	0.18±0.01[b]

CFA- Complete Freund's adjuvant, N = 6, [a]$p<0.001$ vs Normal; [b]$p<0.001$ vs CFA, Data were analysed by one way ANOVA followed by Tukey multiple comparison test

Table 2: Effect of EBV on biochemical parameters against CFA induced arthritis in rats

Treatments	Dose (mg kg^{-1})	AST (U L^{-1})	ALT (U L^{-1})	ALP (U L^{-1})	Total protein (mg %)	Cholesterol (mg %)	Triglycerides (mg %)
Normal	-	54.5±1.65	51.25±1.65	177.5±3.52	6.27±0.34	61.75±1.80	67.0±1.36
CFA	0.1 mL	156.2±3.49[a]	61.80±1.90[a]	301.0±3.50[a]	7.46±0.49	68.80±1.15	78.4±1.60[a]
B. variegata	250	82.8±1.12[b]	35.00±0.8[b]	279.0±9.16	5.58±0.12[c]	52.00±3.37[b]	69.0±2.12[c]

CFA- Complete Freund's adjuvant; AST- Serum aspartate transaminase; ALT- Alanine aminotransferase; ALP- Alkaline phosphatase, N = 6; [a]$p<0.001$ Vs Normal; [b]$p<0.001$; [c]$p<0.01$ vs CFA, Data were analysed by one way ANOVA followed by Tukey multiple comparison test

In the adjuvant treated rats an increase in lipid peroxide was seen in liver and kidney. An arthritic rat treated with EBV was found to have altered values which were closer to that of controls. An increase in superoxide dismutase (SOD), GPx and decreased catalase level in liver and kidney has been observed in adjuvant induced arthritis (Table 3 and 4). After treatment with EBV, altered the antioxidant levels near to normal.

The adjuvant elicits arthritis predominantly in the joints of hind limbs, promoting significant reduction of motor activity and increased itching and scratching behaviors (Calvino *et al*., 1987). In addition, increased sensitivity of the affected paw to pressure or flexion and extension of the inflamed joints and weight loss are observed. The arthritis observed in rats is associated with a hyperalgesia phenomenon and spontaneous behaviors, such as protection of the affected paw, evidenced by curving and/or elevation of the paw, as well as a voidance of supporting the body on the paw (Clatworthy *et al*., 1995). The hyperalgesia is more evident during the acute inflammatory phase, when spontaneous behaviors, indicative of painful response are more pronounced. Increased paws diameter (posterior and anterior), due to inflammation and edema is also observed (Cain *et al*., 1997). The initial inflammatory response is developed within hours, but more critical clinical signals emerged form the 10th post-inoculation day and thereafter and the alterations remain detectable for several weeks (Clopaert *et al*., 1982). The results of the present study indicate that the EBV exhibits anti arthritic effects in rat with Freund's adjuvant-induced arthritis, either on its acute as well as its chronic phase.

Tissue damage was assessed by measuring the activities of enzyme in the serum and in the respective organ, since liver impairment is also a feature of adjuvant arthritis (Marylatha *et al*., 1998). The increase in aminotransferases is due to their release from the cells of the damaged organ (Rainsford, 1982). Subrata *et al*. (1994) reported a similar increase in aminotransferases was observed in arthritic animals. Alkaline phosphatase has been reported to be present mainly in the blood vessels, pia arachnoid and choroid plexus. Alkaline phosphatase activity has been reported to increase during the morphological and functional development of the tissues. Aminotransferases and alkaline phosphatase were significantly reduced in arthritic mice after the administration of EBV. This reducing effect may be related to their anti-inflammatory activity.

Table 3: Effect of EBV on antioxidant status in liver of control and experimental animals

Treatment	Dose (mg kg^{-1})	Lipid peroxidation	SOD	Catalase	GPx
Normal	-	141.80±4.48	1.91±0.02	46.56±1.4	5.15±0.65
CFA	0.1 mL	394.20±11.94[a]	4.74±0.03[a]	33.92±2.59[c]	16.50±1.50[a]
B. variegata	250	160.03±2.4[b]	2.46±0.10[b]	41.80±1.98[d]	8.67±0.86[b]

CFA- Complete Freund's adjuvant; SOD- Supeeroxide dismutase; GPx- Glutathione peroxide; N = 6; [a]p<0.001; [c]p<0.01 vs Normal; [b]p<0.001; [d]p<0.05 vs CFA, Data were analysed by one way ANOVA followed by Tukey multiple comparison test; LPO = μ moles of MDA/min/mg protein

Gpx = μ moles of GSH oxidised/min/mg protein
SOD = Units/min/mg protein
CAT = μ mole of H_2O_2 consumed/min/mg protein

Table 4: Effect of EBV on antioxidant status in kidney of control and experimental animals

Treatment	Dose (mg kg^{-1})	Lipid peroxidation	SOD	Catalase	GPx
Normal	-	83.62±2.90	2.13±0.02	36.12±1.04	2.62±0.28
CFA	0.1 mL	327.04±7.94[a]	3.49±0.46[c]	27.53±1.47[c]	17.81±0.36[a]
B. variegata	250	152.26±4.6[b]	2.33±0.10[d]	30.68±1.64	6.81±0.49[b]

CFA- Complete Freund's adjuvant; SOD- Supeeroxide dismutase; GPx- Glutathione peroxide; N = 6; [a]p<0.001; [c]p<0.01 vs Normal; [b]p<0.001; [d]p<0.05 vs CFA; Data were analysed by one way ANOVA followed by Tukey multiple comparison test

LPO = μ moles of MDA/min/mg protein
Gpx = μ moles of GSH oxidised/min/mg protein
SOD = Units/min/mg protein
CAT = μ mole of H_2O_2 consumed/min/mg protein

The pathogenesis of rheumatoid arthritis is multifactorial and recent research has implicated oxygen free radicals as mediators of cartilage damage. Oxygen free radicals such as superoxide and hydrogen peroxide are produced by polymorphonuclear leukocytes when they ingest bacteria or immune complexes (Gutteridge, 1986). In rheumatoid arthritis, it has been suggested that OH radical or a similar oxidizing species, contribute to membrane damage, alteration in the protein structure, conformation and antigenicity, production of autoantibodies, hyaluronic degradation and destruction of antioxidants within the synovial joints (Gutteridge, 1986). Many cellular defense mechanisms are afforded against the toxic effect of these radicals in inflammation including serum sulfydryl groups, ceruloplasmin, albumin and blood glutathione (Fahim *et al*., 1995).

The increased lipid peroxide level noticed in arthritic rats in our study (group 2) may be due to its release from neutrophils and monocytes during inflammation (Greenwald and Moy, 1980). At the onset of inflammation, there is a rapid fall in the total iron content of blood plasma followed by an increased deposition of iron proteins in the synovial fluid. The drop in plasma iron correlates closely with the activity of the inflammatory process. In the synovial fluid of inflamed joints, the iron released during necrosis, might catalyze the formation of OH (hydroxy) radicals from H_2O_2, thus contributing increased lipid peroxidation in arthritis. From the literature reviewed, it is apparent that RA is exposed to oxidative stress and is prone to lipid peroxidation (Heliovaara *et al*., 1994). In the present study, the concentration of lipid peroxidation was significantly altered in arthritic rats after the administration of EBV, when compared with arthritic control rats.

Many cellular defence mechanisms are directed against the toxic effects of these radicals in inflammatory process. SOD which converts super oxide radicals to H_2O_2 is widely distributed in cells having oxidative metabolism and is believed to protect such cells against the toxic effects of super oxide anion. Increased delivery of NADPH from the stimulated HMP shunt during inflammation is proposed to lead to the activation of SOD in arthritic rats. Increased production of NADPH from HMP shunt during arthritis may cause an increase in SOD activity (Marklund *et al*., 1987). This increase in enzyme activity appears to be protective against the intracellular oxygen free radicals (Kasama *et al*., 1988). Administration of EBV to arthritic rats caused a significant decrease in elevated SOD activity.

Catalase in the erythrocytes functions to protect Hb against oxidation. Liver has been reported to be a major site of lipid peroxide metabolism. The main function of catalase is to detoxify H_2O_2 although catalase is significantly decreased in rheumatoid arthritis. Its concentration is very low to expect considerable protection against H_2O_2 in arthritic rats (Blake *et al*., 1981). Treatment of arthritic rats with EBV significantly increased the catalase enzyme levels. Superoxide anions are thought to be involved in inflammatory reactions as they are produced by phagocytic cells Babior *et al*. (1973). These cells are reported to produce hydroxyl radical (Salin and McCord, 1975) and singlet oxygen (Allen *et al*., 1972). Glutathione peroxidase is localized in the cytoplasm and mitochondria, which catalyses the degradation of various peroxides by oxidizing glutathione with the formation of its conjugates. The increased activity of glutathione peroxidase in liver and kidney of arthritic rats, as shown by the present results, indicate the free radical defense system against oxidative stress and may help to explain the pathogenesis associated with arthritis. After EBV treatment, GPx level were significantly decreased.

After EBV treatment, the alterations produced in arthritic rats with respect to lipid peroxidation and antioxidant concentrations were modulated nearly to normal levels. This antiperoxidative action observed in EBV treated arthritic rats might be due to the presence of compound like flavanoids in EBV. These compound have been shown to scavenge free radicals, including hydroxyl and superoxide anions and reduce the levels of lipid peroxidation in stress induced animals (Jovanovic and Simic, 2000).

In the present study, we thus conclude that oral administration of EBV has been shown to modulate the above biochemical changes observed in the adjuvant-induced arthritic animals. Thus the

modulating effect observed in EBV treated arthritic animals could be mediated through flavonoids, in EBV. A further research has begun to investigate the exact mechanism of action of EBV against arthritis.

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