Anti-Arthritic Potential of Ethyl Acetate Extract of *Stereospermum colais* in Animal Model

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ABSTRACT

BACKGROUND

Stereospermum colais also known as yellow snake tree is widely utilised to alleviate rheumatic pain and inflammation in the conventional medicinal system. Lapachol has been reported to be anti-inflammatory by inhibiting the production of nitric oxide (NO) and tumour necrosis factor (TNF)-alpha by means of modulating the metabolism of arachidonic acid, activation of NF-aB, suppression of nitric oxide synthase (iNOS) expression and expression of cyclooxygenase-2 (COX-2). It has also been analysed for anticancer and antioxidant activity, renal disorders, endometriosis and cardiac dysfunction. The present study investigates the anti–arthritic activity of the ethyl acetate extracts of *Stereospermum colais*.

METHODS

The fruit rind of *Garcinia indica* was used to prepare extract and was quantified using liquid chromatography–mass spectrometry (LC-MS) / MS. Ethyl acetate extract showed increased content of the phytochemical constituent necessary for the treatment of arthritic pain. So, the ethyl acetate extract of bark of *S. colais* was evaluated for anti-arthritic activity by complete Freund's adjuvant (CFA). Arthritis index, body weight changes, and the biochemical analysis parameters were measured. Histopathological evaluation along with TNF-alpha and interleukin (IL)-6 assays were also studied.

RESULTS

The ethyl acetate extract showed significant reduction in arthritis index (P < 0.01), paw swelling (P < 0.01) and arthritic score (P < 0.01), thereby demonstrating anti-inflammatory potential. A good improvement in the biochemical parameters in extract treated animals indicates good protection against the inflammation.

CONCLUSIONS

The results show that *Stereospermum colais* can be used as a potential anti arthritic drug.

KEY WORDS

Rheumatoid Arthritis, Complete Freund's Adjuvant, *Stereospermum colais*, Ethyl Acetate, Anti-Arthritis, Meloxicam

BACKGROUND

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune condition that mainly affects the joints and manifests as pain, rigidity and synovitis, resulting in cartilage and bone erosion by invading the fibrovascular tissue.[1] The disease affects about 1 % of the world population with an annual estimated incidence of approximately 40 in 100,000 globally.[2] Generally women are mostly affected twice the times as men. The onset of disease is between 45 and 65 years of age and occurs at all groups.[3]

The major pathological changes that occur in rheumatoid arthritis includes loss of articular cartilage, inflammation of synovial membrane and bone damage, which ultimately lead to deformation of articular surface.[4] Dense cellular infiltrates primarily comprising macrophages, T cells and B cells, characterize the inflamed rheumatoid synovium. T cells play a vital role in maintaining the RA inflammation, and more T-helper (Th) cells are found in the synovial infiltrate.[5] But the excessive amounts of interleukin (IL)-1, IL-6, and TNF in the synovium indicates that the activated monocyte / macrophage lineage cells also have an equally important role in the inflammatory process. Biological activities are imputed to TNF like IL-1 and IL-6 induction, neutrophil activation, enhancement of leukocyte migration and expression of adhesion molecules by endothelial cells and leukocytes.[6] Cytological, morphological and molecular assessment of arthritic joints is the most rudimentary method of analysis in preclinical RA studies. Evaluation of rheumatoid arthritis using animal models is very important to facilitate the understanding of the pathophysiology of RA. It also helps to classify possible therapeutic targets, and is the beginning point for the in-vivo implementation of new therapeutic agent recognition.[7]

One of the widely used animal models to investigate the pathology of joint inflammation and evaluate the usefulness of preclinical therapeutic approaches is the collagen-induced arthritis (CIA).[8] It shares a lot of resemblances in pathological and immunological activities, such as systemic joint involvement, damage of peripheral joints, synovitis, cartilage along with bone erosions, and inflammatory cell infiltration of the synovium.[9] The options for treating the RA is limited as there are only few specific antirheumatic agents in current use. NSAIDs, glucocorticoids and disease-modifying antirheumatic drugs (DMARDs) are the major non-biologic pharmacological treatment groups for RA most of which are slow-acting, and some associated with significant toxicity hazards.[10] As a result of these limitations, a growing number of patients have begun to turn to care using natural products to relieve RA symptoms. Nevertheless, challenges in assessing the effectiveness of these items as well as limited knowledge on their mode of action are among the reasons for public and professional scepticism. The therapeutic approach to arthritis therefore allows the treatment to be cost-effective, long-lived, and with minimal or less side effects. The drugs should also be capable of suppressing the expression of inflammatory and proinflammatory cytokines, thereby preventing joint harm.[11]

Stereospermum colais (Bignoniaceae), widely known as Yellow snake tree in English is a bigger and straight stemmed deciduous tree, that grows around 18 - 30 m high and 2.8 m in girth seen in damp areas of India up to an elevation of about 1200 m.[12] It is used in ayurvedic medical system to treat vomiting, leprosy, fevers, mitigation, kapha, itching, diabetes, and clean contaminated wounds.[13] It also shows anti-diarrhoea activity, analgesic activity, antioxidant activity, wound healing activity, antibacterial, antifungal activity.[14-18] The juice of the leaves can be mixed with lime juice to treat maniacal cases. Decoction of the leaves has antipyretic properties and is used to treat chronic dyspepsia and also in the treatment of asthma and cough.[19] *Stereospermum colais* is also used as diuretic, lithotrophic, cardio tonic, anti-pyretic, expectorant, aphrodisiac, anti-inflammatory, antibacterial, febrifuge, anti-emetic, rheumatalgia, malarial fever, wound, asthma and cough. The complete parts of the tree can be used, including the root which is the main ingredient in few ayurvedic formulations. In the ayurvedic literature it is said to possess the properties of treating pain and inflammation. Therefore, the present study investigates the anti–arthritic activity of the ethyl acetate extracts of *Stereospermum colais*.

METHODS

Study Duration
The duration of the animal study was one month (April 2012). Total study duration was 2012 to 2013.

Plant Material
The plant bark of the stem of *S. colais* was collected in the month of September 2010 in Porur, Tamil Nadu, India. The botanical authentication and identification were performed by plant expert Dr. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Tambaram, Chennai. A voucher specimen No. PARC / 2010 / 2029 has been deposited for further reference. The bark materials were shade dried and coarsely powdered.

Preparation of Extract
The bark was shade dried, coarsely powdered and allowed to dry. For extraction, cold maceration method was used. Solvents of increasing polarity like n-hexane, chloroform, ethyl acetate and ethanol were utilised. After 72 hrs. of maceration process, the remaining content was filtered. Acetone extraction was performed separately by defatting with n-hexane solvent. Every time before extraction with the next higher polarity solvent, the marc was dried. The extracts were concentrated by distilling solvent after full extraction and then evaporated into dryness in the water bath. The percentage yield of ethyl acetate extract (64 % w / w) was calculated on the air-dried basis.

Phytochemical Screening
To classify the chemical constituents, the bark extracts obtained were subjected to preliminary phytochemical screening.[20]

Acute Toxicity Study
Acute oral toxicity study of bark extracts of *Stereospermum colais* was carried out according to the Organization for Economic Co-operation and Development guidelines 423.[21]

Animal Procurement and Maintenance

Male Wister rats (150 -250 g) were procured from Centre for Toxicology and Developmental Research (CEFT), SRU. The animals were maintained in animal house at a room temperature of 20 ± 2°C, with a relative humidity of 50 ± 5 % and alternating 12 hr dark-light. The animals had free access to standard laboratory feed and water ad libitum. The rats were acclimatised to the environment for 1week prior to experiment use. The study was approved by the standing institutional animal ethics committee (IAEC: No: XIII / SRU / 95 / 2008) of the Sri Ramachandra University.

Induction of Arthritis

Animals were injected with 0.1 ml of complete Freund's adjuvant intraperitoneally into each rat's right hind paw to cause arthritis.[22] The rats were categorised into groups of 6 animals: group I, the arthritic control group and group II, the non-arthritic control group. Group I received only CFA intraperitoneal injection, but group II (incomplete Freund's adjuvant group (IFA)) received 0.1 ml IFA intraperitoneal injection (sterile paraffin oil). Erythromycin ethylsuccinate-EAESC (200 mg / Kg and 400 mg / Kg) and standard meloxicam (0.1 ml / Kg) were injected to the rats in the different groups respectively. Water displacement plethysmograph was used to calculate paw volume for both the ipsilateral (injected paw) and the contralateral paw (non-injected paw) before administration of intraperitoneal CFA injection (day 0) and every week.[23] The oedema element of inflammation was determined by the difference in foot volumes on day 0 and day 28. Two sets of experiments were carried out, one is curative, and another is prophylactic. After the intraperitoneal administration of 0.1 ml CFA, there was onset of arthritis and drug was administered on day 9. The drugs were given on day 0 in a prophylactic regimen and CFA was administered intraperitoneally 24 hours later. The animals were grouped as:

- Group 1- Non-control / IFA (intraperitoneal injection of 0.1 ml of IFA)
- Group 2 - Arthritic control/ CFA (intraperitoneal injection of 0.1 ml CFA)
- Group 3 - EAESC 200 mg / Kg respectively from day 9 (curative) and from day 0 (prophylactic)
- Group 4 - EAESC 400 mg / Kg respectively from day 9 (curative) and from day 0 (prophylactic)
- Group 5 - Meloxicam (0.1 ml / Kg)

The ipsilateral and contralateral paw volume scores were independently standardised from the value at day 0 as a percentage change and then the average was taken for each treatment group. The extract and standard were suspended in 0.5 % CMC and the drugs were freshly prepared.

Arthritis Index

Every week the rats were evaluated for arthritis. The following grading method was used to analyse the physical symptoms of arthritis.[24] 0 = Paw is normal; 1 = Toe erythema; 2 = Swelling and erythema of paws; 3 = Ankle swelling; 4 = Whole leg swelling thus unable to bend it. The achievable score to the maximum is 16. By adding the four scores of individual paws the arthritis index of each rat is calculated. When at least one

non-injected paw was seen inflamed it shows that the animal is sensitized and considered to have arthritis.[25]

Measurement of Body Weight

For every rat the weight of the body was recorded prior and every week after inoculation of the adjuvant. The intake of food and weight gain is measured throughout the arthritic period. The change in body weight in arthritic rats is calculated by determining the body weight at each day and that of day 0. Percentage of body weight changes was calculated.

Measurement of Paw Volume Changes

Following adjuvant inoculation, the hind paw volumes before and after the adjuvant administration were determined using water displacement plethysmometry.[26] Variations in the volumes of hind paws were estimated from the measurements on day 0.

Biochemical Analysis

All the animals were anaesthetised, blood withdrawal was done for serum separation by retro-orbital puncture and collected in plain and EDTA containing tubes respectively at the end of experiment, on the 28th day. Biochemical examination like blood urea nitrogen (BUN), creatinine (CR), total protein (TP), alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), alkaline phosphate (ALP), and c-reactive protein (CRP) in blood and in liver superoxide dismutase (SOD), reduced glutathione (GSH), lipid peroxidation (LPO), glutathione peroxidise (GPX), and protein was conducted on the homogenised samples. All the analysis was done by using standard diagnostic kit (Transasia Bio-Medical Ltd., Mumbai) protocol provided by the vendor was followed for the all the assay.

TNF-Alpha and IL-6 Assays

After disease induction, animals were immolated on the 28th day and blood samples were withdrawn to distinguish adjuvant non-treated and SNP-treated arthritic rats. Enzyme-linked immunosorbent assay (ELISA) kits (M / s. Bender Medsystems GmbH, Vienna, Austria) were used to determine serum levels of TNF-alpha and IL-6. In the wells of the microtiter strips, antibodies specific to rat TNF-alpha and IL-6 were coated and samples including standards of established rat TNF-alpha and IL-6 were incubated, washed and pipetted into the wells. The colour intensity was measured at 450 nm with a correction wavelength of 630 nm.

Histopathological Examination

The ankle joint tissues from control group and arthritic and treated rats were excised and fixed in 10 percent buffered formalin. It was then decalcified using 10 percent EDTA solution, embedded in paraffin, sectioned and stained with haematoxylin and eosin (H & E stain), for examination under a light microscope. Mononuclear inflammation, vascular proliferation, oedema, synovial hyperplasia and vasculitis causing vessel wall fibrinoid necrosis in periarticular and subcutaneous adipose tissue were the test parameters. A

pathologist, blind to the specimens, conducted the pathological assessment randomly.

Statistical Analysis
Data were presented as the mean ± standard error of deviation. The oedema volume measured in the various treatment groups was compared and contrasted by the two-way ANOVA and Dunnet multiple comparison tests with group I and group II. In order to determine the variations between the effects of different doses, significance tests have also been calculated.

RESULTS

Preliminary Phytochemical Screening
The preliminary phytochemical and biochemical screening of the bark ethyl acetate extract of *Stereospermum colais* reveals the presence of proteins, amino acids, saponins, flavonoids, phenolic compounds, tannins, terpenoids and steroids.

Acute Toxicity Study
There was no evidence of mortality for up to 24 hours and behavioural changes toxicity signs were observed in all animals. EAESC extract was safe up to a 2000 mg / Kg body weight of animals. This research was therefore performed at doses of 200 and 400 mg / Kg body weight of Wistar rats.

Body Weight Changes in Experimental Animals
There were significant body weight changes observed in the arthritic control group animals when compared to the normal and extracted animals in both prophylactic phase and curative phase present in Table 1.

Effect on Paw Volume
In the present analysis, the phases of adjuvant arthritis investigated were the acute and chronic phases corresponding to days 0 - 10 and 10 - 18 respectively after adjuvant inoculation. An important (P < 0.05) effect of drug therapy has been demonstrated by the two-way ANOVA (treatment X-time). The total oedema generated by each treatment is expressed as the AUC of the time course curves in arbitrary units. At the ipsilateral (injected paw) around 4 - 6 days, all arthritis control animals showed acute inflammatory oedema followed by subsequent polyarthritis process beginning around day 10 - 12. On day 12, with systemic inflammation, the improvement of inflammatory oedema in the non-injected paw was evident. The paw volume of the non-inflamed control group injected IFA did not alter significantly during the 28-day trial. In comparison to standard meloxicam, EAESC 200 mg / Kg and 400 mg / Kg have significantly modified the course of time (P < 0.05) and significantly reduced oedema in the ipsilateral paw (200 mg / Kg) by percentage inhibition. This meloxicam and EAESC group completely presented the spread of the arthritis from the injected to the non-injected paws of the treatment animals. EAESC 400 mg / Kg did not exhibit significant anti-arthritic activity. EAESC 200 mg / Kg and 400

mg / Kg did not show any significant effect compared to standard data present in Table 2.

Effect on Arthritic Score
For the arthritic groups with erythema and increased hind paw swelling, especially for the EAESC 200 mg / Kg body weight group and for the anti-arthritic groups 400 mg / Kg and meloxicam (0.1 ml / Kg) lower scores were observed as present in Table 3, the results of the arthritic score for curative and prophylactic phase was significant (P < 0.05).

Effect on Biochemical Parameters
The results for BUN showed significant (P < 0.05) increase in 400 mg / Kg dose, than 200 mg / Kg dose and standard in prophylactic phase when compared to normal control present in Table 4. The results for creatinine showed significant (P < 0.05) increase in 400 mg / Kg dose and standard, than 200 mg / Kg dose in prophylactic phase. In the curative phase there is a significant (P < 0.05) increase in both the dose and standard when compared to normal control present in Table 4. Results for total protein showed significant (P < 0.05) decrease in 200 mg / Kg and 400 mg / Kg dose, than standard in prophylactic phase and curative phase when compared to normal control present in Table 4.

The results for ALT showed significant (P < 0.05) increase in 200 mg / Kg dose, then standard in prophylactic phase and curative phase when compared to normal control present in Table 4. Results for AST showed significant (*P < 0.05) increase in 200 mg / Kg dose and standard, than 400 mg / Kg dose in prophylactic phase when compared to normal control present in Table 4.

The results for ALP showed significant (P < 0.05) increase in 200 mg / Kg dose, than 400 mg / Kg dose and standard in prophylactic phase when compared to normal control present in Table 4. Results for CRP showed significant (*P < 0.05) increase in 200 mg / Kg dose and 400 mg / Kg, than 400 mg / Kg dose and standard in prophylactic phase and in curative phase significant (P < 0.05) increase in 200 mg / Kg dose only when compared to normal control present in Table 4.

The results for SOD showed significant (P < 0.05) increase in 200 mg / Kg dose and 400 mg / Kg, than standard in prophylactic phase and in curative phase significant (P < 0.05) increase in 400 mg / Kg dose and standard when compared to normal control present in Table 5. Results for GSH showed significant (P < 0.05) increase in 200 mg / Kg, 400 mg / Kg, and standard in prophylactic phase and in curative phase significant (P < 0.05) increase standard when compared to normal control present in Table 5. The results for LPO showed significant (P < 0.05) increase in 400 mg / Kg, and standard than 200 mg / Kg dose in prophylactic phase and in curative phase significant (P < 0.05) increase in 200 mg / Kg, 400 mg / Kg and standard when compared to normal control present in Table 5. Results for GPx showed significant (*P < 0.05) increase in 200 mg / Kg, than standard and 200 mg / Kg dose in curative phase when compared to normal control present in Table 5. Results for protein showed significant (*P < 0.05) increase in 200 and 400 mg / Kg, than standard in prophylactic phase and 200 mg / Kg curative phase when compared to normal control present in Table 5.

TNF-Alpha and IL-6 Assays

Quantitative real-time polymerase chain reaction (PCR) analysis of TNF-alpha and IL-6 mRNA expression in c-MIR-treated and control group synovial tissues showed that the expression of TNF-alpha and IL-6 increased prophylactically in a dose-dependent manner and is reversed in the curative phase of EAESC relative to the norm shown in Figure 1.

Histopathological Changes

To assess the general histopathological alterations, the H&E stained left tibiotarsal joints of rats from all the groups were examined. The left tibio-tarsal joints of all control group rats showed normal histology of the joint with no signs of arthritis as shown in Figure 2.

Complete Freund's adjuvant injected rats' tibiotarsal joints showed moderate to extreme proliferative synovitis with severe multifocal mononuclear cell infiltration marked by hyperplastic synovial membrane. In all rats in this group, pannus invasion into the synovial spaces, subchondral and cortical bone associated with moderate to severe multifocal cartilage deterioration and bone erosion was observed. In both the prophylactic and curative stages, meloxicam treated rats exhibited a normal synovium and articular cartilage with mild Mononuclear cell infiltration into the synovial stroma.

In the arthritic rats treated with a dose of 200 mg / Kg body weight (both prophylactic and curative phases), very mild hyperplastic synovium with few stromal inflammatory

infiltrates, normal articular cartilage and normal bone architecture was observed. These changes were considerably smaller than those found in adjuvant-injected rats and were equivalent to those treated with meloxicam.

Group	Body Weight Changes (in %)							
	Prophylactic Phase				Curative Phase			
	1W	2W	3W	4W	1W	2W	3W	4W
Normal	4.22	5.10	7.33	8.80	4.22	5.10	7.33	8.80
Arthritic control	2.21	10.67	7.1	6.16	2.21	10.67	7.1	6.16
EAESC 200 mg / Kg	5.98	7.3	7.11	4.33	2.85	7.27	5.89	3.92
EAESC 400 mg / Kg	3.15	8.45	4.86	1.91	2.45	7.78	4.35	3.30
Meloxicam 0.1 ml / Kg	3.23	4.11	4.21	4.54	3.90	4.22	3.33	3.12
Table 1. Effect of EAESC Bark on Animal Body Weight Changes								

Data are expressed as mean ± SEM of 6 animals in each group. P < 0.05, P < 0.01 Compared with that of arthritic control

	Arthritic Index Score	
Group	Prophylactic Phase	Curative Phase
Normal	0.00 ± 0.00	0.00 ± 0.00
Arthritic control	4.80 ± 0.12	4.80 ± 0.12
EAESC 200 mg / Kg	4.32 ± 0.14	4.50 ± 0.15
EAESC 400 mg / Kg	3.80 ± 0.17*	4.00 ± 0.16*
Meloxicam 0.1 ml / Kg	3.70 ± 0.14**	3.70 ± 0.14**
Table 3. Effect of EAESC Bark on Arthritic Index Score		

*P < 0.05, **P < 0.01 compared with arthritic control.

Group	Paw Volume (in ml)									
	Prophylactic Phase					Curative Phase				
	0W`	1 W	2 W	3 W	4 W	0W	1 W	2 W	3 W	4 W
Normal	1.28 ± 0.03	1.34 ± 0.03	1.29 ± 0.03	1.32 ± 0.03	1.33 ± 0.02	1.28 ± 0.03	1.34 ± 0.03	1.29 ± 0.03	1.32 ± 0.03	1.33 ± 0.02
Arthritic control	1.27 ± 0.04	1.90 ± 0.11*	2.10 ± 0.06*	1.98 ± 0.09*	1.96 ± 0.09*	1.27 ± 0.04	1.90 ± 0.11*	2.10 ± 0.06*	1.98 ± 0.09*	1.96 ± 0.09*
EAESC 200 mg / Kg	1.21 ± 0.02	1.7221 ± 0.06**	1.81 ± 0.06**	1.70 ± 0.06**	1.65 ± 0.06**	1.3221 ± 0.03	1.6821 ± 0.03	1.93 ± 0.05**	1.74 ± 0.03**	1.70 ± 0.03**
EAESC 400 mg / Kg	1.91 ± 0.02	1.60 ± 0.06**	1.68 ± 0.06**	1.63 ± 0.06**	1.58 ± 0.06**	1.32 ± 0.03	1.64 ± 0.04**	1.92 ± 0.03**	1.66 ± 0.03**	1.61 ± 0.03**
Meloxicam 0.1 ml / Kg	1.20 ± 0.04	1.57 ± 0.06**	1.65 ± 0.05**	1.57 ± 0.05**	1.48 ± 0.05**	1.23 ± 0.03	1.60 ± 0.06**	1.88 ± 0.06**	1.65 ± 0.04**	1.56 ± 0.07**
Table 2. Effect of EAESC Bark on Paw Volume on CFA Induced Arthritic in Rats										

*P < 0.05, **P < 0.01 compared with arthritic control.

Parameters	Normal Control	Arthritic Control	Prophylactic Phase			Curative Phase		
			200 mg / Kg	400 mg / Kg	Meloxicam 0.1 ml / Kg	200 mg / Kg	400 mg / Kg	Meloxicam 0.1 ml / Kg
BUN	23.46 ± 0.63	28.83 ± 0.53**	26.36 ± 0.7**	26.07 ± 0.31*	26.31 ± 0.77**	27.63 ± 0.55**	26.56 ± 0.50**	26.3 ± 0.43**
Creatinine	1.23 ± 0.04	1.89 ± 0.27**	1.77 ± 0.04**	1.73 ± 0.03*	1.69 ± 0.04*	1.72 ± 0.03*	1.71 ± 0.02*	1.69 ± 0.04*
Total Protein	6.32 ± 0.41	9.76 ± 0.32**	8.17 ± 0.55*	8.07 ± 0.32*	7.88 ± 0.45	8.06 ± 0.48*	7.89 ± 0.33*	7.64 ± 0.18
ALT	22.41 ± 0.44	29.88 ± 3.32**	28.37 ± 0.68*	27.45 ± 0.71	27.06 ± 0.44	27.85 ± 0.33*	27.39 ± 0.91	27.18 ± 0.22
AST	79.12 ± 0.26	88.24 ± 0.95**	87.14 ± 0.82**	86.15 ± 0.23**	84.30 ± 1.37*	86.73 ± 1.23**	84.95 ± 1.72**	84.24 ± 1.798*
ALP	215.02 ± 7.65	475.02 ± 6.87**	259.59 ± 10.80*	252.34 ± 13.46	233.2 ± 13.97	268.1 ± 8.11**	249.66 ± 7.32	241.68 ± 15.02
CRP	3.12 ± 0.18	5.02 ± 0.30**	3.69 ± 0.01*	3.69 ± 0.02*	3.62 ± 0.05	3.67 ± 0.04*	3.66 ± 0.03	3.59 ± 0.04
Table 4. Effect on Biochemical Parameters in Blood								

Values are mean ± SEM, N = 6. One-way ANOVA followed by Dunnett's test in comparison with normal control group, *P < 0.05, **P < 0.01

Parameters	Normal Control	Arthritic Control	Prophylactic Phase			Curative Phase		
			200 mg / Kg	400 mg / Kg	Meloxicam 0.1 ml / Kg	200 mg/ Kg	400 mg/ Kg	Meloxicam 0.1 ml / Kg
SOD	0.35 ± 0.02	0.11 ± 0.006**	0.26 ± 0.005*	0.25 ± 0.005*	0.24 ± 0.006	0.26 ± 0.006	0.26 ± 0.005*	0.25 ± 0.02*
GSH	3.13 ± 0.008	1.03 ± 0.15**	2.41 ± 0.29*	2.4 ± 0.07*	2.4 ± 0.09*	2.49 ± 0.13	2.48 ± 0.04	2.46 ± 0.05*
LPO	0.11 ± 0.008	0.24 ± 0.04**	0.23 ± 0.02**	0.22 ± 0.02*	0.22 ± 0.03*	0.22 ± 0.02*	0.21 ± 0.01*	0.21 ± 0.005*
GPX	8.55 ± 0.32	17.93 ± 0.65**	11.28 ± 0.66**	10.12 ± 0.06	10.02 ± 0.39	10.66 ± 0.59*	10.51 ± 0.32	10.26 ± 0.53
PROTEIN	116.76 ± 3.51	164.37 ± 5.94**	155.94 ± 16.53*	152.58 ± 13.60*	151.01 ± 1.20	152.09 ± 6.52*	151.04 ± 2.41	150.73 ± 0.47
Table 5. Effect on Antioxidant Parameters in Liver								

Values are mean ± SEM, N = 6.

In rats treated with 400 mg / Kg body weight, the degree of synovial proliferation, mononuclear cells (MNC) infiltration and pannus formation was moderate compared to that of adjuvant-injected rats. These results indicate that the pathological progression of adjuvant induced arthritis is suppressed by a 28-day EAESC therapy at a dosage of 200 mg / Kg / body weight.

DISCUSSION

Rheumatoid arthritis (RA) is a chronic inflammatory disorder of probable autoimmune origin, characterized by symmetric synovial joint inflammation that can lead to cartilage and bone damage and progressive loss of function.[27] Nonsteroidal anti-inflammatory medications, glucocorticoids, and disease-modifying anti-rheumatic drugs are the treatment choice for RA. These drugs, when used by patients show a lot of unnecessary side effects which created the need to look for new, efficient and safe therapeutic agents. Interestingly, a number of herbs and other natural products provide a vast resource for the treatment of arthritis.[28] CFA induced arthritic model is considered as a chronic, immunological, cellular and proliferative arthritis similar to clinical RA.

There are two phases of this model: An acute phase and chronic phase. The acute phase starts from day 0 followed by a chronic inflammatory phase from day 15. The chronic phase is marked by inflammatory oedema in the contralateral non-injected paw.[29] The leucocytes migrate into the affected area and cause acute oedema by the release of cytokines like IL-1β, prostaglandins, TNF-alpha, histamine and serotonin. The chronic phase is a consequence of cell-mediated immunity and causes the degenerative changes in cartilage and bone.

The animals induced arthritis showed high arthritic index score and increased swelling on the paw in both prophylactic and curative phase. The treatment with EAESE showed reduction in arthritic index and paw swelling which showed the extract possess anti–arthritic activity. Inhibition of paw swelling was associated with inhibition of both TNF-alpha, IL-1β, and IL-6 cytokine production and cell infiltration, as analysed in the subcutaneous tissues of the paw.[30]

Change in body weight in the animals induced with CFA is a response to evaluate the drug and the duration of inflammation.[31] The inflammation resulted from the arthritis is more in the CFA induced animals than normal animals. There is a protection seen in the reduction of body weight to EAESE treated animals which shows the EAESE possess anti-inflammatory activity. CFA damages the liver architecture and release the enzymes into the circulation thus liver enzymes were elevated in the adjuvant inducted group.[32] The liver enzymes were not elevated in the EAESE treated group which shows the extract possess protection on liver damage. Literature evidence shows that *S. colais* possess protective effect on liver damage thus correlating our observation.

Several studies have confirmed that the environment that drives cartilage and bone degradation is pro-inflammatory cytokines, particularly tumour necrosis factor TNF-α, interleukin (IL)-1, IL-6, IL-17 and mediators generated via downstream pathways in the arthritic joints.[33] The arthritic group elevated the cytokines level and the same was reversed in the EAESC treated rats which showed the extract exhibits the reduction of inflammation by (TNF)-α via reactive oxygen species (ROS) levels reduction.

The main role of reactive oxygen species is deterioration of lipids through a metabolic process known as lipid peroxidation. This may significantly affect cell membrane structure and function. It has been involved in cancer, atherosclerosis, degenerative disorders, and inflammatory arthritis pathogenesis.[34] The arthritic group showed reduction in lipid peroxidation (LPO) due to destruction of cell membrane leading to over production of ROS. The antioxidant property is evident in this study as the EAESC treated group of rats showed protection in reduction in LPO when compared to arthritic control.

Superoxide dismutase (SOD), catalase and glutathione peroxidase are the enzymatic antioxidant defence mechanisms, that protect the cells against O2 toxicity and lipid peroxidation.[35] Our study findings indicate that SOD and glutathione peroxidase activities have been found to decrease in arthritic animals, which may be due to the huge and rapid increase of free radicals. Superoxide anions are thought to be involved in inflammatory reactions because phagocytic cells produce them and are known to generate radical hydroxyl and singlet oxygen.[36] Glutathione peroxidase is found in the cytoplasm and mitochondria, which, with the formation of its conjugates, catalyses the degradation of different peroxides by oxidizing glutathione.[37]

The treatment with EAESC for arthritis showed progress and effectiveness in relation to histopathological studies. Therapy with EAESC decreased the cellular invasion, oedema formation and inflammation in rats with induced arthritis. The overall results of the study indicate that EAESC can prevent arthritis and decrease inflammation and joint destruction in animals with induced rheumatoid arthritis.

CONCLUSIONS

Ethyl acetate extract of bark of *Stereospermum colais* (200 mg / Kg and 400 mg / Kg body weight) showed anti-arthritic properties similar to those observed for non-steroidal anti-inflammatory drug. It justifies the use of the stem bark of this plant by traditional medicinal practitioner in the treatment of rheumatism and inflammatory diseases. Phytochemical analysis of EAESC of stem bark showed the presence of flavonoids and phenol. Thus, it is proved that the aforesaid pharmacological action of EAESC is largely due to the presence of one or more bioactive compounds such a flavonoids and phenols.

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