Evaluation Of Anti-Arthritic Activity Of Ethanolic Extract Of Sida-Cardifolia

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Abstract: Rheumatoid arthritis is a chronic, systemic inflammatory disorder that may affect many tissues and organs.Sida cardifolia.L is used in folk medicine for the treatment of inflammation.A 50% of ethanolic extract of Sida cardifolia.L tested on rats showed potent anti-antioxidant and anti-inflammatory activity when compared with standard drug deprenyl.

INTRODUCTION:

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks synovial joints. The process produces an inflammatory response of the synovium (synovitis) secondary to hyperplasia of synovial cells, excess synovial fluid, and the development of pannus in the synovium. The pathology of the disease process often leads to the destruction of articular cartilage and ankylosis of the joints.

1) ACUTE TOXICITY STUDIES:

Experimental animals:

Inbreed male healthy Swiss albino mices (20-30 g) were obtained from National Institute of Nutrition, Hyderabad. The animals were housed in siri pharma. The mices were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. Standard pellet feed (Hindustan Lever Limited., Bangalore) and drinking water was provided *ad libitum* throughout experimentation period. Rats were acclimatized to laboratory conditions one week prior to initiation of experiments. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee). Registered No: 1521/PO/a/11/CPCSEA.

Acute oral toxicity study

The procedure was followed by using OECD 425 (Acute Toxic Class Method). The acute toxic class method is a step wise procedure with four mice of a single sex per step. Depending on the mortality or moribund status of the mices and the average two to three steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use number of mices while allowing for acceptable data based scientific conclusion. The method used to defined doses (2000, 1000, 500, 50, 5 mg/kg body weight) the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemicals which cause acute toxicity.

Experimental procedure:

Male albino mice weighing 20-30 gm were used for the study. The starting dose level of *Sida cardifolia.L* was 100, 600, 1200, 1800 and 2000 mg/kg body weight p.o. Dose volume was administered to overnight fasted mice with were *ad libitum*. Food was withheld for a further 3-4 hours after administration of *Sida cordifolia.L* and observed for signs for toxicity. The body weights of the mices before and after administration were noted that changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system and motor activity and behavior pattern were observed and also sign of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity also noted.

MATERIALS AND METHODS:-

Drugs & Chemicals:

- Freund's Adjuvant injection Diethyl ether
- Indomethacin

Distilled water

- Carboxy methyl cellulose
- Formaldehyde

- **Ethanol**
- **surgical spirit**

Instruments Used:

- Weighing balance
- Centrifuge
- Digital balance
- Plethysmograph
- Auto analyzer

SCREENING METHODS FOR ANTI ARTHRITIC ACTIVITY:

ACUTE SYSTEMIC ANAPHYLAXIS IN RATS

PURPOSE AND RATIONALE

Rats are immunized with ovalbumin and *Bordetella pertussis* suspension as adjuvant. After 11 days the animals are challenged by intravenous injection of ovalbumin. The shock symptoms can by inhibited by corticosteroids and intravenous disodium cromoglycate.

ANTI-ANAPHYLACTIC ACTIVITY (SCHULTZ-DALE REACTION)

PURPOSE AND RATIONALE

Guinea pigs are sensitized against egg albumin. Challenge after 3 weeks causes in isolated organs release of mediators, e.g. histamine, which induce contraction in isolated ileum.

PASSIVE CUTANEOUS ANAPHYLAXIS

PURPOSE AND RATIONALE

Passive cutaneous anaphylaxis is a immune reaction of the immediate type. By passive immunization of rats in the skin with rat anti-ovalbumin serum and a challenge 2 days later

with ovalbumin at the same skin area antigen-antibody complexes are formed in the mast cells inducing release of mediators. This results in vasodilatation, increase in permeability of the vessel walls and leakage of plasma. To make the allergic reaction visible, Evan's blue dye is administered along with the antigen. Evan's blue dye is attached to the albumin fraction of plasma, producing a blue spot. This blue spot indicates that an anaphylactic reaction has taken place in the skin.

ARTHUS TYPE IMMEDIATE HYPERSENSITIVITY

PURPOSE AND RATIONALE

The immune complex induced Arthus reaction comprises inflammatory factors that have been implicated in the acute responses in joints of rheumatic patients. Complement and polymorphonuclear neutrophiles are activated via precipitating antigen-antibody complexes leading to an inflammatory focus characterized by edema, hemorrhage and vasculitis. Arthus reaction of the immediate type becomes maximal 2–8 h after challenge.

PROCEDURE

Ovalbumin suspension

700 mg ovalbumin are suspended in 100 ml paraffin oil. 4.38 ml pertussis vaccines are suspended in 70 ml 0.9% NaCl-solution. Both suspensions are mixed to form an emulsion. Wistar or Sprague-Dawley rats of either sex weighing220–280 g can be used. Seven days prior to start of the experiment rats are sensitized by i.m. administration of 0.5 ml of the ovalbumin suspension. They are housed in groups of eight with standard food and water ad libitum. Twenty-four hours and one hour prior to induction of the Arthus reaction, test compounds are administered to groups of 8 animals. The rats are challenged by injection of 0.1 ml of 0.04% solution of highly purified ovalbumin in the left hind paw. Swelling of the paw occurs which reaches a maximum after a few hours. The footpad thickness can be measured by calipers. One group of sensitized animals treated with solvent alone serves as positive control; one group of non sensitized animals treated with solvent alone serves as negative control. Standard doses are 30 mg/kg cortisone or 10 mg/kg prednisolone p.o.

DELAYED TYPE HYPERSENSITIVITY

PURPOSE AND RATIONALE

Delayed type hypersensitivity is a reaction of cell mediated immunity and becomes visible only after 16–24 h. The same methods as for testing immediate type hypersensitivity can be used.

3) ANTI -ARTHRITIC ACTIVITY:

Evaluation of the anti-arthritic activity in rats by Complete Freund's Adjuvant method

Animals:

Inbreed healthy Wister albino male rats of weighing 150-200gm were used in the study. Every experimental animal was clinically examined pre-operatively for any disease. The animals were kept under observation in laboratory and allowed to acclimatize for 1 week before experiment. Animals kept in separate spacious clean cages under controlled room temperature (24+2 ^{0}C) & relative humidity 60-70%; in a 12:12 hrs light dark cycle. They were fed with a standard chow diet and filter water. Before the experiment the rats were divided into five groups.

Compound (drugs to be administered) preparation:

- Extract: The extract was weighed according to rat body weight and suspended in 1% CMC solution.
- Extract Dose Selection: Based on Acute toxicity Studies, 250 and 500mg/kg b.w doses of EEMC were selected and administered against Freund's adjuvant induced Arthritis. Starting point of study is animal selection and randomly dividing them into 4 groups (by considering animal body weights).
- CFA: It contains 5 mg mycobacterium tuberculosis (Difco) was suspended in heavy paraffin oil (Merck) by thoroughly grinding with mortar and pestle to give a concentration of 5 mg/m
- Indomethacin: Indomethacin (10mg/kg b.w) was weighed according to rat body weight and dissolved in 1% CMC solution

Adjuvant induced arthritis:

Wistar albino rats (150-200 g) were taken and divided into five groups, each group contains 6 animals. The animals were fasted over night before the experiment. On first day, they were injected into the sub plantar region of the left hind paw with 0.05 ml of complete Freund's adjuvant. This consists of 5 mg mycobacterium tuberculosis (Difco) being suspended in heavy paraffin oil (Merck) by thoroughly grinding with mortar and pestle to give a concentration of 5 mg/ml dosing with the test compounds or the standard was started on the same day and continued for12 days. Paw volumes of both sides and body weights were recorded on the day of injection, whereby paw volume was measured plethysmographically with equipment as described in the paw edema tests. On 5th day, the volume of the injected paw is measured again, indicating the primary lesion and the influence of therapeutic agents on this phase. The severity of the induced adjuvant disease is followed by measurement of the non injected paw (secondary lesions) with a plethysmometer. From day 13 to 21, the animals were not dosed with the test compound or the standard. On 21stday, the body weight is determined again and the severity of the secondary lesions is evaluated visually and graded according to the following scheme in Table no.1.

GROUPING OF ANIMALS:

Group 1: Normal control (distilled water, p.o for 12 days)
Group 2: Positive Control (Freund's adjuvant 0.05 ml, Sub-plantar region)
Group 3: Freund's adjuvant + Standard (Indomethacin 10mg/kg p.o for 12 days)
Group 4: Freund's adjuvant + Low dose of EESC p.o for 12 days
Group 5: Freund's adjuvant + High dose of EESC p.o for 12 days

Table no.1: *Arthritis Index*

RESULTS:

PRELIMINARY PHYTOCHEMICAL STUDIES:

Sr.no	Physico chemical Tests	Absence / Presence (- / +)
1.	**TEST FOR CARBOHYDRATES**	
a)	Molisch's Test	+
b)	Fehling's Test	+
c)	Borfoed's Test	+
2.	**TEST FOR GLYCOSIDES**	
a)	Borntrager's Test	+
b)	Modified Borntrager's Test	+
c)	Legal's Test	+
d)	Killer Kellani Test	+
3.	**TEST FOR FLAVONOIDS**	
a)	Shinoda Test	+
b)	Alkaline reagent test	+
4.	**TEST FOR SAPONINS**	
a)	Foam Test	+
5.	**TEST FOR STEROIDS**	
a)	Liberman – Buchard Test	+
b)	Salkowski Test	+
c)	Sulphur test	+
Sr.no	**Physico chemical Tests**	**Absence / Presence (- / +)**
6.	**TEST FOR ALKALOIDS**	
a)	**Hager's Test**	**+**
b)	**Wager's Test**	**+**
7.	**TEST FOR FIXED OILS AND FATS**	
a.	**Spot Test**	
b)	**Saponification Test**	**+**
8.	**TEST FOR ACIDIC COMPOUNDS**	**+**
a)	**Extract + NaHCo3 Solution**	
b)	**Extract + Water, Warm, Litmus paper Turns to Blue colour**	**--**
		--
9.	**TEST FOR AMINOACIDS**	**--**
a)	**Ninhydrin Test**	
b)	**Biuret Test**	**+**
c)	**Xanthoproteic Test**	**+**
10.	**TEST FOR TANNINS**	
a)	**Extract + FeCl3**	
b)	**Lead acetate test**	**--**
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Note:
+:- Presence
--:- Absence

Table no: 2 *phytochemical studies of ethanolic extract of whole plant of Sida cordifolia.L (Malvaceae)*

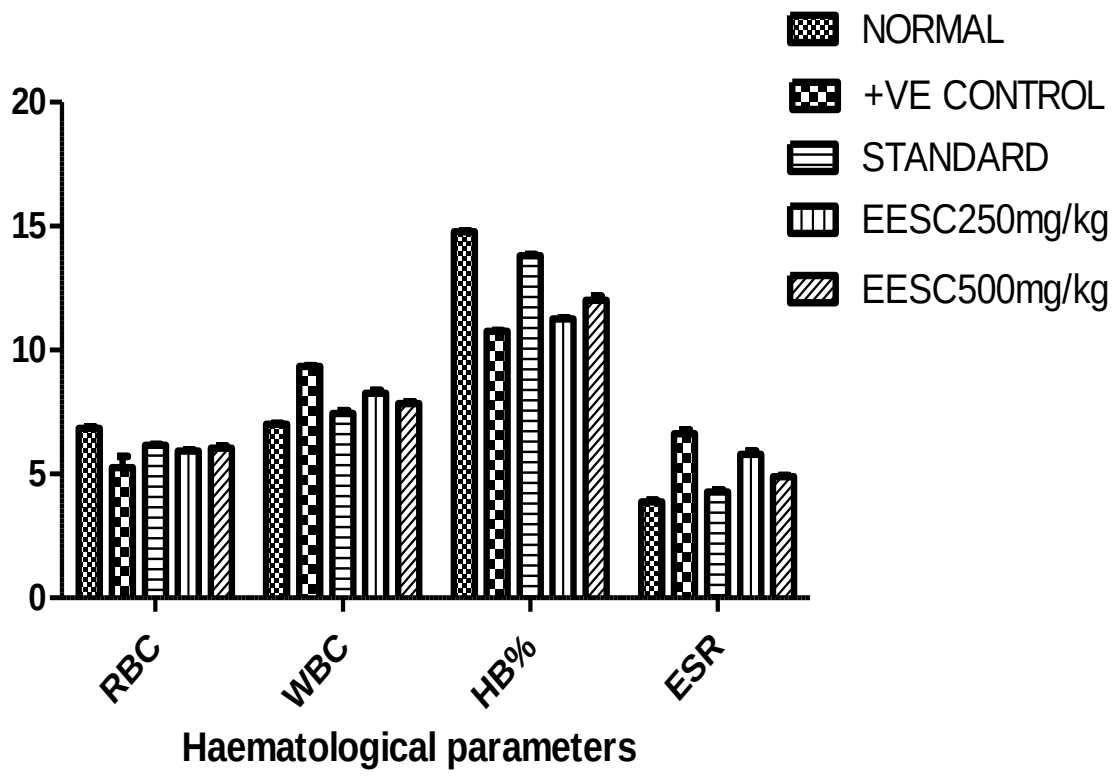
HAEMATOLOGICAL PARAMETER:

S.NO	TREATMENT	RBC (10^6 cells/mm^3)	WBC (10^3 cells/mm^3)	Hb (gm %)	ESR (mm/hr)
1.	-ve Control	6.84 ± 0.069	7.00 ± 0.035	14.77 ± 0.031	3.87 + 0.078
2.	+ve Control	5.26±0.455**	9.34±0.043**	10.75±0.038**	6.63±0.140**
3.	Standard	6.16+0.031**	7.44+0.120**	13.80+0.063**	4.27+0.081**
4.	EESC- 250mg/kg	5.92±0.051**	8.25±0.117**	11.25±0.056**	5.79±0.131**
5.	EESC- 500mg/kg	6.04±0.077**	7.85±0.065**	12.00±0.175**	4.89±0.050**

EESC: Ethanolic Extract of *Sida cordifolia.L*

Values are expressed in mean ± SEM, (n=6), when compared with control, *P<0.05, **P<0.01, ***P<0.001 one way ANOVA followed by Dunnet's t – Test.

Table no.3 *Haematological parameters evaluation of ethanolic extract of whole plant of Sida cordifolia.L (Malvaceae).*



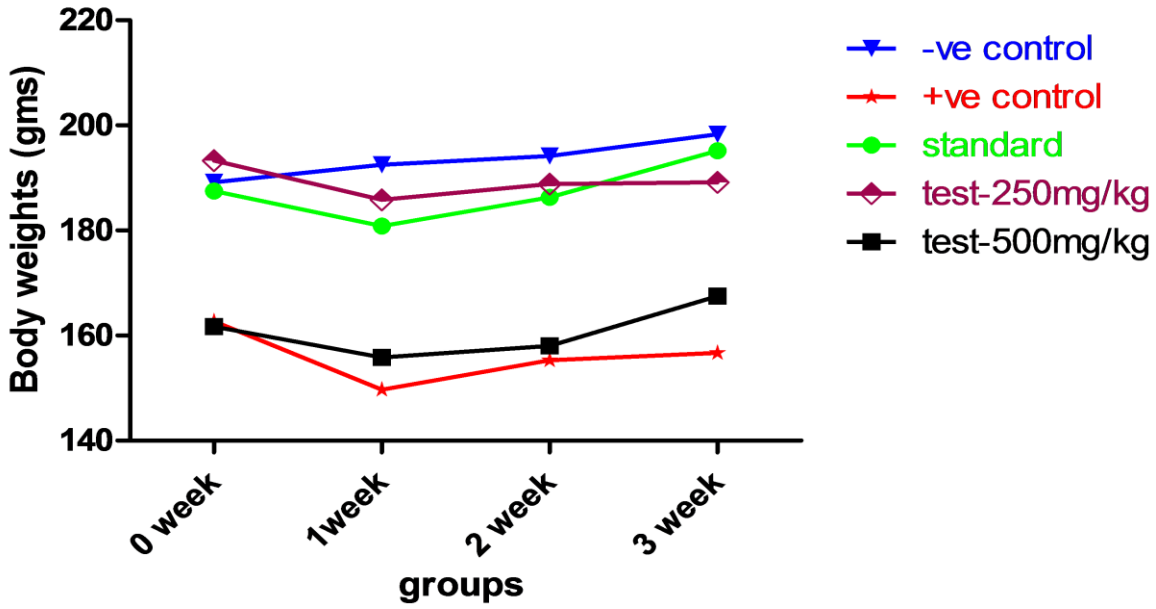
Graph no: 1 *Effect of ethanolic extract of whole plant of Sida cordifolia.L (Malvaceae) on Haematological parameters (RBC, WBC, HB% & ESR).*

BODY WEIGHTS

S.NO	Treatment and Dose	Body Weight (gms)			
		0 week	1st week	2nd week	3rd week
1.	-ve Control	189.17±8.002	192.50±7.610	194.17±6.379	198.33±5.110
2.	+ve Control	162.67±7.839	149.67±5.011	155.33±6.184	156.67±6.280
3.	Indomethacin (10mg/kg)	187.50±6.677	180.83±7.236	186.33±5.136	195.17±4.214
4.	EESC-250mg/kg	193.33±4.014	185.83±4.549	188.83±4.269	189.17±3.516
5.	EESC-500mg/kg	161.67±3.870	155.83±3.005	158.00±2.436	167.50±2.141

EESC: Ethanolic Extract of *Sida cordifolia.L*

Table no.4 *Effect of ethanolic extract of whole plant of Sida cordifolia.L (Malvaceae) on mean changes in body weight.*



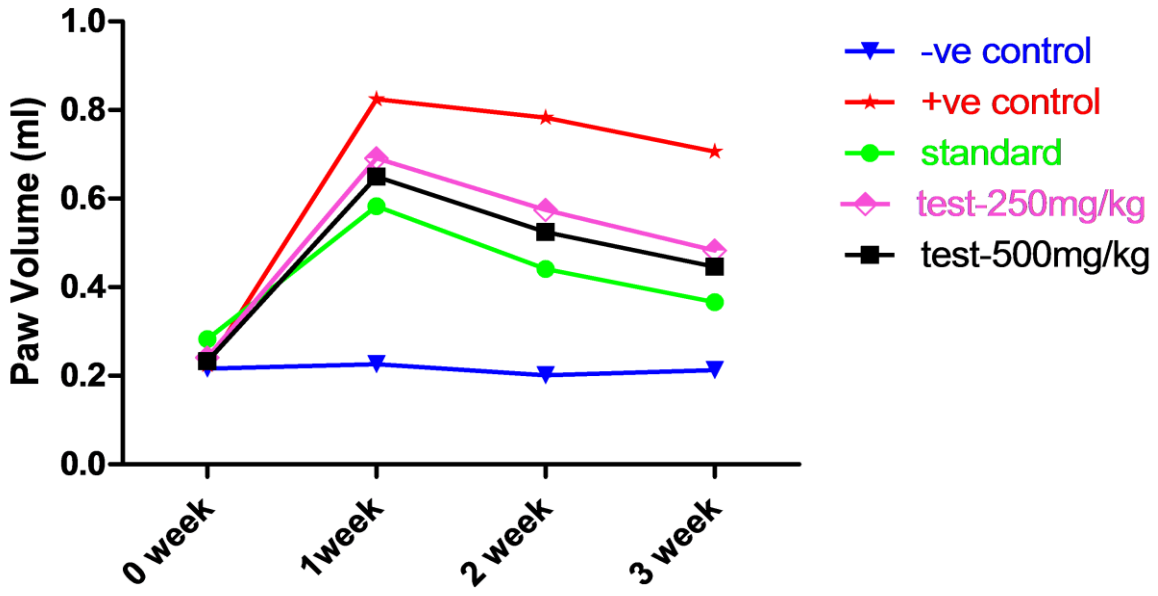
Graph no: 2 *Effect of ethanolic extract of whole plant of Sida cordifolia.L (Malvaceae) on mean changes in body weight.*

PAW VOLUME

S.NO	Treatment and Dose	Paw Volume				% Inhibition
		0 week	1st week	2nd week	3rd week	
1.	-ve Control	0.216±0.1430	0.226±0.0105	0.201±0.0130	0.213±0.0095	---
2.	+ve Control	0.225±0.1708	0.825±0.0381**	0.783±0.0247**	0.706±0.0147**	---
3.	Indomethacin (10mg/kg)	0.283±0.0210	0.583±0.0401**	0.441±0.0351**	0.366±0.0307**	55.63
4.	EESC-250mg/kg	0.241±0.0300	0.691±0.0271**	0.575±0.0381**	0.483±0.0333**	41.45
5.	EESC-500mg/kg	0.233±0.0166	0.65±0.0150**	0.525±0.0335**	0.446±0.0166**	45.93

EESC: Ethanolic Extract of *Sida cordifolia.L*
Values are expressed in mean ± SEM, (n=6), when compared with control, *P<0.05, **P<0.01, ***P<0.001 one way ANOVA followed by Dunnet's t – Test.

Table no.5 *Effect of ethanolic extract of whole plant of Sida cordifolia.L (Malvaceae) on mean changes in paw volume.*



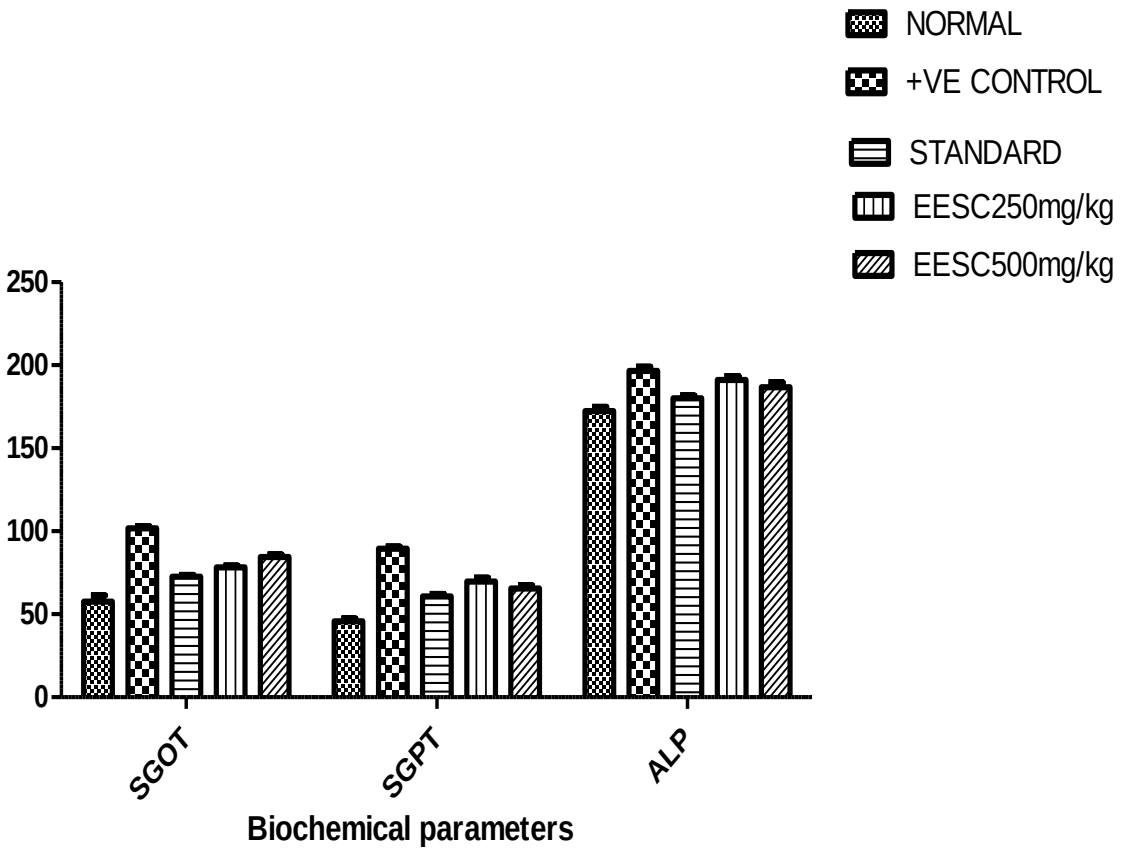
Graph no: 3 *Effect of ethanolic extract of whole plant of Sida cordifolia.L (Malvaceae) on mean changes in paw volume.*

BIOCHEMICAL PARAMETERS

S.NO	TREATMENT	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
1.	-ve Control	57.67± 3.721	45.67±1.706	172.33±2.616
2.	+ve Control	101.67±1.282**	89.50±1.176**	196.50±2.540**
3.	Standard	72.50±1.176**	60.83±1.424**	180.16±1.542*
4.	EESC- 250mg/kg	78.16±1.078**	69.67±2.261**	191.16±2.227**
5.	EESC- 500mg/kg	84.50±1.384**	65.50±1.821**	186.83±2.822**

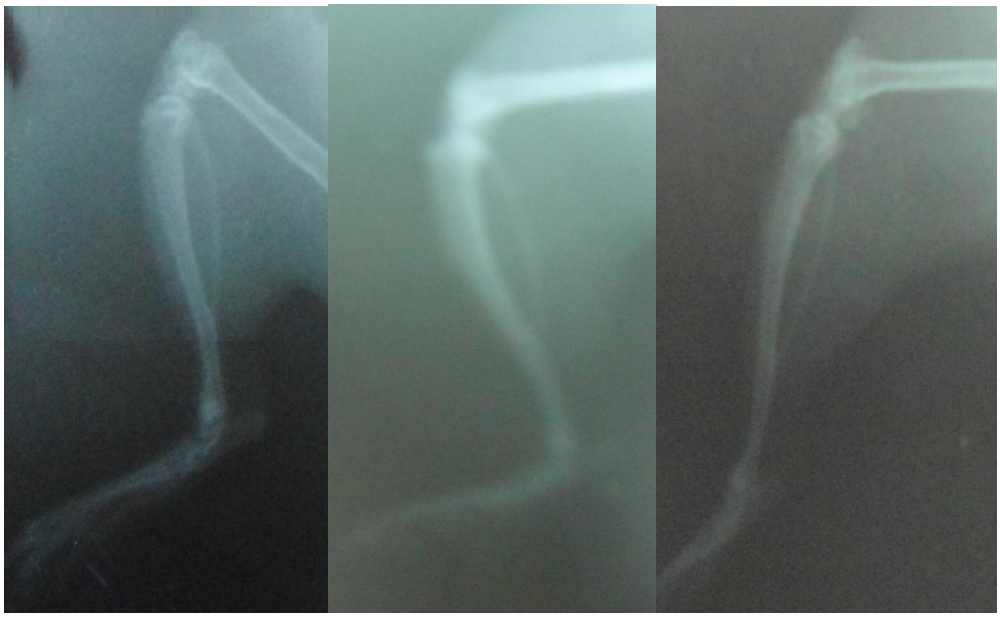
EESC: Ethanolic Extract of *Sida cordifolia.L*

Table no.6: *Biochemical parameters evaluation of ethanolic extract of whole plant of Sida cordifolia.L (Malvaceae).*



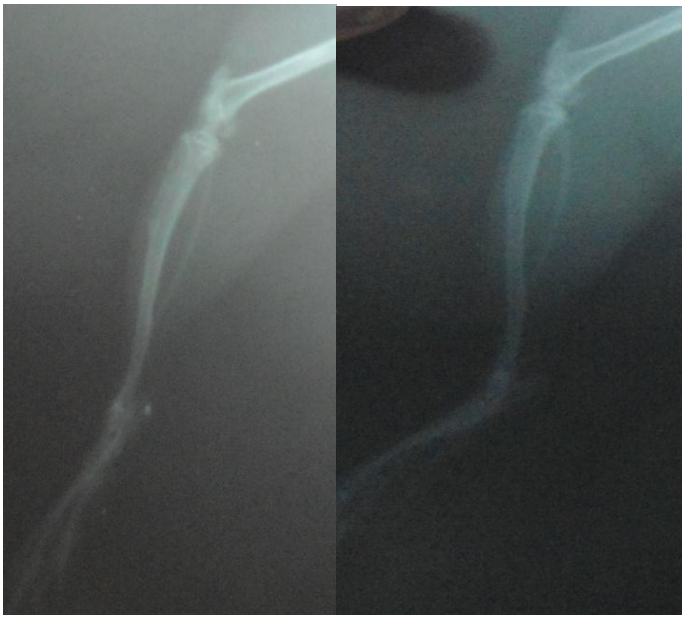
Graph no: 4 Effect of ethanolic extract of whole plant of *Sida cordifolia.L* (Malvaceae) on Biochemical parameters (SGOT, SGPT & ALP Levels).

RADIOLOGICAL STUDIES



NORMAL CONTROL ARTHRITIS CONTROL STANDARD

(INDOMETHACIN)



EESC 250mg/kg EESC500mg/kg

Fig no: 5 *Effects of EESC on CFA induced arthritis in rats (X- Ray photo graphs)*

DISCUSSION

Rheumatoid arthritis (RA) is a chronic, systemicinflammatory disorder that may affect many tissues and organs, but principally attacks synovial joints. The process produces an inflammatory response of the synovium (synovitis) secondary to hyperplasia of synovial cells, excess synovial fluid, and the development of pannus in the synovium. The pathology of the disease process often leads to the destruction of articular cartilage and ankylosis of the joints. RA can also produce diffuse inflammation in the lungs, pericardium, pleura, sclera and also nodular lesions, most common in subcutaneous tissue. Although the cause of RA is unknown, autoimmunity plays a pivotal role in both its chronicity and progression, and RA is considered as a systemic autoimmune disease In this present work, it was planned to verify the therapeutic usefulness of locally available plants. Literature survey revealed that the plant *Sida cardifolia.L* apart from other medicinal uses was used as an ethnic folklore medicine for and arthritis. Hence, the alcoholic extract was prepared with whole plant of *Sida cardifolia.L* have been selected to study their anti-arthritic activity in experimental animals, rats. It is well known that Freund's adjuvant induced paw edema in rats is one of the most suitable test procedures to screen anti-arthritic and anti-inflammatory agents as it closely resembles human arthritis. Complete Freund's adjuvant (CFA) arthritis is a pathology that can be produced in rats by inoculation, into the tail base, with *Mycobacterium tuberculosis* suspended in oil and which has pathological and biochemical features that resemble human rheumatic disease. Apart from the primary inflammatory response which develops within hours at the site of inoculation and consist of oedematous swellings involving multiple joints, particularly in the hind paws. This is followed by the development of inflammed periarticular swellings in the limbs and of erythematous nodules and segmental radial swellings and ridgings encircling the tail adjacent to the intervertebral disks of the caudal vertebrae. In the paws the arthritis typically persists during the first 2 weeks after its appearance and may then, in some subjects, follow an undulating course during several months. Severely affected animals eventually develop chronic deforming joint lesions. Paw inflammation in rats after injected with Freund's adjuvant could be divided into three phases: (i) an early acute phase with paw swelling in the injected paw increasing with no detectable swelling in the un injected paw; (ii) an intermediate phase where paw swelling in the injected paw was significant but had subsided to approximately 80% above normal, and swelling in the un injected paw was detectable, but not significant; (iii) a late systemic phase where paw swelling in the injected paw suppressed swelling during the acute response, and swelling in the noninjected paw had increased by a significant 60-100% above normal. Studies using FA arthritis model shows that evaluation of bone changes in the hind paw have shown generalized osteopenia in addition to local demineralization. RA is characterized by both peri-articular and generalized osteoporosis. There are now extensive bodies of literature on the use of biochemical markers of bone turnover in the clinical development of drugs that affect bone metabolism. Biochemical markers of bone turnover fall into two categories namely, markers of bone formation and markers of bone resorption (degradation). A number of markers are used as bone turnover indices. Reports have shown that in arthritic disease, the bone formation was broadly reduced whereas bone resorption was specifically increased. FA increases then N-acetyltransferase (NAT) activity in blood and liver that not only shows the impact on the serum biochemical parameters like SGOT, SGPT and ALP but also on haematological parameters such as RBC, WBC, Hb% and ESR. Indomethacin is a nonselective inhibitor of cyclooxygenase (COX) 1 and 2, enzymes that participate

in prostaglandin synthesis from arachidonic acid. Prostaglandins are hormone-like molecules normally found in the body, where they have a wide variety of effects, some of which lead to pain, fever, and inflammation.

CONCLUSION:

Preliminary phytochemical investigations on the Ethanolic extract of Sida cardifolia were noted the presence of carbohydrates, flavonoids, saponins steroids, alkaloids and glycosides. No mortality or behavioral abnormality recorded in mice during experiments at the highest dose level of 5000 mg/kg tested for LD_{50} studies. The high dose of Ethanolic extract of Sida cardifolia exhibited a significant anti-arthritic activity by reducing serum biochemical parameters like ALP, SGOT, SGPT levels and reduced the haematological parameters like ESR and WBC and increases the RBC and Hb levels in FA induced arthritis models in rats. Radiological and histological studies revealed that near a normal structure of paw and knee joint respectively with the high dose of the extract in FA induced arthritis. Phytochemical constituents like flavonoids, saponins, glycosides and alkaloids were already reported for their anti-arthritic activity and these constituents were present in Ethanolic extract of Sida cardifolia. Hence these chemical constituents can be accounted for the observed anti-arthritic activities.

ACKNOWLEDGMENT

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