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STUDIES ON ANTI-INFLAMMATORY EFFECT OF *MESUA FERREA* LINN. IN ACUTE AND CHRONIC INFLAMMATION OF EXPERIMENTAL ANIMALS

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Keywords:

Mesua ferrea, Inflammation, Carrageenan, Cotton pellet granuloma and formalin induced paw edema

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ABSTRACT: *Mesua ferrea* Linn. (*M. ferrea*) is an endemic medicinal plant used for the treatment of inflammation, rheumatism, bronchitis, fever, headache, asthma and skin diseases. The aim of this study was to investigate the anti-inflammatory activities of 80% ethanol extract from stem bark of *Mesua ferrea* Linn. using animal models. Female Wistar albino rats weighing (150-200g) were used for the study. Anti-inflammatory activity test was done on rats at two different doses (100 and 200mg/kg) by using carrageenan induced paw edema, cotton pellet induced granuloma and formalin induced paw edema. The results showed that the 80% ethanol extract of stem bark (SBE) 200mg/kg and indomethacin (10mg/kg) exhibited significant reduction of edema in carrageenan induced rat paw edema 39.3 ($p < 0.05$) and 38.8% ($p < 0.01$) respectively at the end of 4 h. In cotton pellet granuloma test, SBE (200mg/kg) and indomethacin significantly reduced the wet (42.5 and 40.6% ($p < 0.01$)) and dry (46.7 and 42.8% ($p < 0.01$)) weight of the cotton pellet respectively, while in formalin induced paw edema significant reduction in persistent edema from 4th day to 10th day of the Investigation was observed. SBE 200mg/kg exhibited significant anti-inflammatory effect *i.e.*, 40.3% ($p < 0.05$) when compared to indomethacin 33.3% ($p < 0.05$). The results obtained in this study indicated that the 80% ethanol extract of *Mesua ferrea* Linn. possess potent anti-inflammatory activity in both acute and chronic models.

INTRODUCTION: Inflammation is an immune response to infection and tissue injury has been implicated in the pathogenesis of arthritis, Cancer as well as cardiovascular disease. The main purpose of inflammation is to eliminate the injurious agent to remove damaged tissue components, so that the body can begin to heal [1, 2].

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The inflammatory response involved in changes of blood flow, increase in permeability of blood vessels, migration of fluid, proteins, and leukocytes from the circulation to the site of tissue injury [3]. Inflammation may be acute or chronic, occurs in two different phases. Acute inflammation response starts from few minutes to hours or one or two days, the first line of host defense against foreign invaders and danger molecules. The classical symptoms of acute inflammation are heat, redness, pain, swelling and loss of function [4]. If the resolution of inflammation fails for any reason, the acute inflammation turns into a chronic stage [5]. Chronic inflammation response for longer duration

is characterized by proliferation of fibroblasts, infiltration of mononuclear cells, collagen fibers and formation of connective tissue [6]. Some of the drugs are widely used to suppress the inflammatory disorders such as painkillers, non-steroidal anti-inflammatory drugs (NSAIDs), disease modifying anti-rheumatic drugs (DMARDS) and corticosteroids (steroids) but produce undesirable adverse effects [7]. Therefore, development of new and powerful drugs is still needed.

Mesua ferrea Linn. (Family Clusiaceae) is an endemic medicinal plant, found in eastern Himalayas, Assam, Bengal, Eastern and Western Ghats, Andaman Islands of India [8] and used for the treatment of various inflammatory diseases like fever, cold, asthma, sores, scabies, wounds, rheumatism, gastritis, bronchitis and renal disorders [9, 10]. Phytochemical studies revealed the presence of phenyl coumarins, xanthones, flavonoids, terpenoides and steroids [8, 10]. However, our previous *in vitro* studies reported that 80% ethanol extract of stem bark of *M. ferrea* Linn. has potential anti-inflammatory activity [11]. Therefore, the present study was undertaken to explore possible anti-inflammatory potential to justify the traditional uses of this plant.

MATERIALS AND METHOD:

Chemicals: Carrageenan was purchased from Sigma Chemical (St. Louis, MO, USA). Formalin (S.d. Fine Chemicals Ltd., Mumbai, India). Indomethacin was procured from Himedia. All other chemicals used in experiments were of analytical grade.

Plant Collection and Extraction: Stem bark of *M. ferrea* was collected from the Agumbe reserve forest area of Western Ghats, Shimoga, Karnataka state, India, with the permission of forest department (Megaravalli, Karnataka). The air dried stem bark was coarsely powdered and subjected to soxhlation using 80% ethanol solvent for 48 h, the filtrate was concentrated under reduced pressure at 40 °C and stored in a refrigerator. The percentage yield of 80% ethanol extract was 12.3%.

Animals: Female Wistar albino rats weighing (150–200g) were procured from central animal house (S.S. Hospital, Davanagere) used for the study. They were kept in polypropylene cages and maintained at a temperature of 25 ± 1 °C. Animals were acclimatized for one week, fed on rodent diet and had free access to drinking water. However, they were fasted for at least 12 h prior to experimentation. All experiments were conducted as per the guidelines of the Animal Ethics Committee CPCSEA (Reg. No. NCP/ IAEC/ CL/ 12/ 12/ 2010- 2011).

Acute Toxicity: The acute oral toxicity study was carried out as per OECD-423 guidelines [12]. The animals were divided into three groups of six animals each. The normal group received distilled water (10ml/kg, p.o.) and treated groups received single oral doses of 1000 and 2000mg/kg body weight of SBE. Behavioural parameters and mortality were observed at 1, 4 and 24 h after the treatment. The rats were further observed for up to 14 days for any signs of delayed toxicity and mortality.

Carrageenan Induced Rat Paw Edema: The acute anti-inflammatory effect was evaluated by carrageenan induced rat paw edema according to the procedure as described by Winter *et al.*, [13]. The animals were divided into five groups. Each group has six animals. Group I served as normal (without treatment), Group II received carrageenan (negative control), Group III treated with standard drug Indomethacin (10 mg/kg b.w.) (Positive control) and Group IV and V were treated with (SBE) 80% ethanol extract (100 and 200mg/kg b.w.) respectively. Edema was induced by injection of 1% suspension of carrageenan in 0.9% sterile saline solution into the left plantar region of all the rats except group I. Paw volume was measured using Digital Vernier caliper at an interval of 0, 1h, 2h, 3h and 4h after injection [14]. The inhibition of inflammation was calculated by using the formula: Percentage of inhibition = [Vc – Vt / Vc] X 100. Where Vc represents mean edema in control and Vt mean edema in group treated with standard/extract.

Cotton Pellet Induced Granuloma: The sub-acute anti-inflammatory effect was evaluated by cotton pellet induced granuloma according to the method of Winter and Porter [15]. Four groups of six rats in each group were included in the study. After shaving off fur, the animals were anaesthetized. Sterile pre-weighed cotton pellets (10mg) were implanted in the axilla region of each rat through a

single needle incision. SBE (100 and 200mg/kg b.w.), Indomethacin (10mg/kg b.w.) or vehicle alone was administered orally for seven consecutive days, from the day of cotton pellet implantation. On the eighth day the animals were anaesthetized, the cotton pellets were removed and made free from extraneous tissues. The pellets were dried at 60 °C for 24 h. Mean weight of granuloma tissue formed around each pellet was evaluated. The percent inhibition in the weight of the cotton pellets was calculated by: Percentage of inhibition = [Wc – Wd / Wc] X 100, where Wc = Difference in pellet weight of the control group. Wd = Difference in pellet weight of the standard drug/extract treated group.

Formalin Induced Rat Paw Edema: The chronic anti-inflammatory effect was evaluated by formalin induced paw edema according to the method as described by Singh *et al*., [16]. The animals were divided into five groups. Group I served as normal (without treatment), Group II received formalin (negative control), Group III treated with standard drug Indomethacin 10mg/kg b. w. (positive control) and Group IV and V were treated with SBE 100 and 200mg/kg b. w. respectively. Formalin (0.1ml 2% v/v) was injected into the left hind paw of all the rats on the first and third day of the experiment except group I animals.

Each group was administered orally to the animals once daily for ten consecutive days starting from the first day of formalin injection. The paw volume was measured daily using a Vernier caliper. The percentage inhibition of edema in the test drug treated group was calculated by using the formula: Percentage of inhibition = [Vc – Vt / Vc] X 100, Where Vc and Vt are average edema volume of control and test, respectively.

Biochemical Estimations: At the end of the experimental period, the animals were sacrificed by cervical decapitation. Blood was withdrawn from each animal by retro orbital vein puncture. Blood was collected immediately into tubes containing EDTA for analysis of hematological parameters, RBC and WBC counts according to the method of Chesbrough and Mc Arthur [21] in an improved Neubauer chamber. Estimation of erythrocyte sedimentation rate (ESR) was followed by the method of Westergren [22] and separation of serum was done by centrifugation of blood samples at 3000 rpm for 10 min. The serum was carefully removed and subjected to biochemical parameters such as protein level in the serum was analyzed by the method of Lowry *et al.,*[17] and CRP in serum was determined by using Immunoturbidimetric kit (Diasys Diagnostics, Holzheim, Germany). The paw/granuloma tissue was measured by assaying Myeloperoxidase (MPO) activity as described by Bradley *et al.,* [18], Nitric oxide (NO) was determined as described by Green *et al.*, [19], while lipid peroxide (LP) activity was estimated by the method of Wright *et al.,* [20].

Statistical Analysis: Results are expressed as mean ± SEM. The statistical analysis was carried out using one way ANOVA followed by Tukey's t-test. The differences in values at $p < 0.05$ or $p < 0.01$ were considered as statistically significant. Statistical analysis was performed by ezANOVA 0.98 version.

RESULTS:

Carrageenan Induced Rat Paw Edema: In carrageenan induced animal models, SBE (100 and 200mg/kg) and indomethacin (10mg/kg) significantly reduced inhibition of paw edema by 20.9, 39.3 ($p < 0.05$) and 38.8% ($p < 0.01$) respectively, compared to control values. Results are shown in **Table 2**.

TABLE 1: ANTI-INFLAMMATORY ACTIVITY OF *M. FERREA* LINN. IN CARRAGEENAN INDUCED RAT PAW EDEMA

Groups	Paw volume after drug/ extract administration (mm)					
	0 min	30 min	1 h	2h	3h	4h
Normal	3.64±0.29	3.64±0.29	3.64±0.29	3.64±0.29	3.64±0.29	3.64±0.29
Control	3.68±0.10	5.62±0.25	6.26±0.5	5.88±0.58	5.69±0.13	5.63±0.12
Indomethacin	3.66±0.09	5.12±0.36	5.94±0.12	5.83±0.13	5.41±0.35**	4.89±0.37**
SBE 100mg/kg	3.69±0.11	4.80±0.61	5.80±0.15	5.52±0.24	5.19±0.12	4.86±0.11
SBE 200mg/kg	3.74±0.06	5.49±0.16	5.83±0.30	5.71±0.10	5.44±0.20	5.24±0.26*

Values are expressed as mean ± SEM (n = 6), *p<0.05; **p<0.01 denotes significance with respect to the control group using one way ANOVA followed by Tukey's test.

TABLE 2: PERCENTAGE REDUCTION ON PAW VOLUME AGAINST *M. FERREA* LINN. IN CARRAGEENAN INDUCED PAW EDEMA

Groups	Initial paw volume	Final paw volume	Difference	% inhibition in paw edema
Normal	3.64±0.29	3.64±0.29	------	
Control	3.68±0.10	5.63±0.12	1.96±0.07	------
Indomethacin	3.66±0.09	4.89±0.37	1.20±0.03**	38.8
SBE 100mg/kg	3.69±0.11	4.86±0.11	1.55±0.17	20.9
SBE 200mg/kg	3.74±0.06	5.24±0.26	1.19±0.41*	39.3

Values are expressed as mean ± SEM (n = 6), *p<0.05; **p<0.01 denotes significance with respect to the control group using one way ANOVA followed by Tukey's test.

As shown in **Table 3**, there was a non-significant difference in total protein and RBC levels in SBE (100 and 200mg/kg) and indomethacin (10mg/kg) treated rats when compared to control. CRP, ESR and WBC levels significantly ($p < 0.01$) reduced in SBE 200mg/kg and indomethacin treated group compared to control group. Furthermore the amount of NO, LP and MPO levels in paw tissues were significantly reduced in SBE (100 and 200mg/kg) and indomethacin ($p < 0.01$) when compared to control.

TABLE 3: EFFECT OF *M. FERREA* LINN. ON BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS OF CARRAGEENAN INDUCED RAT PAW EDEMA

Parameters	Normal	Control	Indomethacin (10mg/kg)	SBE 100mg/kg	SBE 200mg/kg
Total protein (g/dl)	7.21±0.44	6.43±0.12	6.11±0.17	5.66±0.21*	6.36±0.28
CRP (mg/ml)	0.42±0.04	0.90±0.07	0.64±0.04**	0.69±0.05*	0.56±0.05**
Nitric oxide (%)	---	---	44.3±5.61**	35.2±6.51**	54.6±7.52**
LP (nmol/mg)	6.87±0.89	10.53±0.23	3.83±0.53**	5.56±0.46**	3.77±0.28**
MPO (U/mg)	0.21±0.02	0.30±0.01	0.14±0.01**	0.18±0.01**	0.10±0.01**
ESR (mm/hr)	1.35±0.05	2.70±0.39	1.69±0.06*	1.76±0.04*	1.65±0.03**
RBC (millions/ µl)	6.03±0.20	6.84±0.32	7.31±0.24	7.72±0.10*	7.34±0.10
WBC(thousands/µl)	8.13±0.25	11.47±0.57	9.85±0.10**	10.45±0.53	9.33±0.37**
Neutrophils (%)	56.67±1.53	77.67±2.52	67.00±4.36*	70.00±1.73*	62.33±3.06**
Lymphocytes (%)	48.0±2.00	63.33±1.53	55.67±1.53**	58.0±1.00**	55.33±1.15**
Eosinophils (%)	1.50±0.58	3.25±0.50	2.00±0.82*	2.25±0.50*	1.75±0.58**

Values are expressed as mean ± SEM (n = 6), *p<0.05; **p<0.01, denotes significance with respect to control group using one way ANOVA followed by Tukey's test.

Cotton Pellet Granuloma: In cotton pellet granuloma model, SBE (SBE) of *M. ferrea* has inhibited the formation of granuloma tissues compared to control group. At the doses of 100 and 200mg/kg, SBE inhibited wet granuloma tissue formation by 19.3 and 42.5% ($p < 0.01$) respectively and dry granuloma tissue was found to be 19.6 ($p < 0.05$) and 46.7% ($p < 0.01$). The standard drug indomethacin (10mg/kg) showed 40.6 and 42.8% ($p < 0.01$) reduction in the weight of wet and dry granuloma tissues respectively. Results are shown in **Table 4**.

TABLE 4: EFFECT OF *M. FERREA* ON WET AND DRY WEIGHT CHANGES IN COTTON PELLET INDUCED CHRONIC INFLAMMATION IN RAT MODEL

Groups	Dose (mg/kg)	Weight of wet granuloma (mg)	% inhibition of granuloma	Weight of dry granuloma (mg)	% inhibition of granuloma
Control	---	169.25±5.85	---	57.25±5.56	---
Indomethacin	10	100.5±2.89**	40.6	32.75±2.22**	42.8
SBE	100	136.5±7.23**	19.3	46.0±3.92*	19.6
SBE	200	97.25±8.50**	42.5	30.50±2.38**	46.7

Values are expressed as mean ± SEM (n = 6), *p<0.05; **p<0.01 denotes significance with respect to the control group using one way ANOVA followed by Tukey's test.

As shown in **Table 5**, there was a non-significant difference in total protein and RBC levels in SBE (200mg/kg) and indomethacin (10mg/kg) treated rats when compared to control group. The level of CRP, WBC and ESR were significantly ($p < 0.01$) reduced. However, the levels of NO, LP and MPO

were also significantly ($p < 0.01$) reduced in SBE (100 and 200mg/kg) and indomethacin (10mg/kg) treated groups when compared to control group.

TABLE 5: EFFECTS OF *M. FERREA* LINN. ON BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS OF COTTON PELLET INDUCED GRANULOMA IN RAT MODEL

Parameters	Control	Indomethacin (10mg/kg)	SBE 100mg/kg	SBE 200mg/kg
Total protein (g/dl)	6.67±0.15	6.27±0.21	5.90±0.30	6.53±0.35
CRP (mg/ml)	0.89±0.06	0.56±0.03**	0.71±0.04**	0.50±0.02**
Nitric oxide (%)	---	39.5±7.02**	30.0±7.05**	56.5±10.5**
LP (nmol/mg)	15.32±1.73	8.50±0.74**	10.37±0.46*	7.08±0.61**
MPO (U/mg)	0.12±0.01	0.08±0.01**	0.10±0.01*	0.06±0.01**
ESR (mm/hr)	2.80±0.45	1.60±0.55**	2.00±0.71	1.40±0.55**
RBC (millions/ µl)	6.37±0.21	7.17±0.31*	6.83±0.35	7.20±0.30*
WBC(thousands/µl)	8.03±0.32	9.73±0.42**	10.70±0.92**	9.57±0.15**
Neutrophils (%)	56.33±1.53	70.0±2.0**	68.0±1.0**	66.0±1.0**
Lymphocytes (%)	46.67+0.58	59.0+1.0**	53.67+4.16*	51.0+1.0**
Eosinophils (%)	0.67±0.58	1.33±0.58	2.00±1.00	1.67±0.58

Values are expressed as mean ± SEM (n = 6), *p<0.05; **p<0.01, denotes significance with respect to control group using one way ANOVA followed by Tukey's test.

The sections of granulation tissues (**Fig. 1**) when stained with haematoxylin and eosin showed increased fibroblasts, thick fibrous tissue and dense inflammatory infiltrate in the control group (a), whereas Indomethacin (b) and SBE 100 (c) and 200 mg/kg (d) treated groups revealed reduced number of fibroblasts, collagen tissue and sparse inflammatory cells.

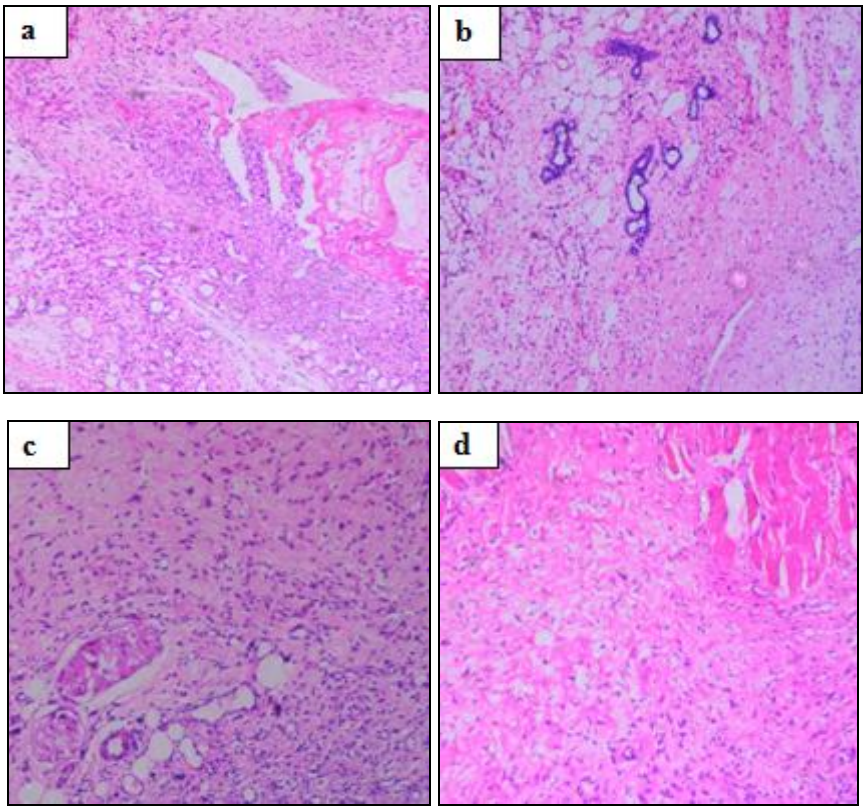

FIG. 1: MICROPHOTOGRAPH OF GRANULATION TISSUE (HAEMATOXYLIN AND EOSIN STAIN, ×100), (A) CONTROL GROUP; (B) INDOMETHACIN GROUP; (C) SBE 100mg/kg AND (D) SBE 200mg/kg

Formalin Induced Paw Edema: In formalin induced paw edema, a dose-dependent reduction in paw thickness was exhibited SBE of *M. ferrea*. The percentage of paw volume inhibition of SBE (100 and 200mg/kg) was found to be 20.9 and 40.3% ($p < 0.05$) in treated groups compared to control, whereas standard drug Indomethacin (10mg/kg) showed an inhibition of 33.3% ($p < 0.05$). As shown in **Table 7**.

TABLE 6: EFFECT OF *MESUA FERREA* LINN. ON FORMALIN INDUCED PAW EDEMA IN RAT MODEL

	Normal	Control	Indomethacin (10mg/kg)	SBE 100mg/kg	SBE 200mg/kg
0 Day	3.55±0.10	3.57±0.12	3.63±0.12	3.59±0.06	3.65±0.14
1 Day	3.55±0.10	5.81±0.15	5.78±0.08	5.15±0.78	5.59±0.14
2 Day	3.56±0.11	6.45±0.27	6.90±0.12**	6.17±0.64	5.34±0.30
3 Day	3.58±0.12	8.62±0.32	7.79±0.16**	8.05±0.07*	6.93±0.17*
4 Day	3.59±0.11	8.37±0.30	7.55±0.16**	7.81±0.15*	6.77±0.11*
5 Day	3.61±0.09	7.98±0.32	7.25±0.24**	7.61±0.18	6.54±0.21*
6 Day	3.64±0.09	7.63±0.41	6.91±0.19**	7.21±0.40	6.31±0.20
7 Day	3.67±0.10	7.39±0.39	6.58±0.23**	6.81±0.40	6.15±0.14*
8 Day	3.68±0.09	7.15±0.43	6.19±0.16*	6.52±0.38	6.05±0.18*
9 Day	3.70±0.09	6.94±0.45	5.98±0.10*	6.32±0.35	5.75±0.21*
10 Day	3.71±0.09	6.72±0.51	5.82+0.28*	6.08±0.43	5.54±0.24*

Values are expressed as mean ± SEM (n = 6), *p<0.05; **p<0.01, denotes significance with respect to the control group using one way ANOVA followed by Tukey's test.

TABLE 7: PERCENTAGE REDUCTION ON PAW VOLUME BY *M. FERREA* LINN. IN FORMALIN INDUCED PAW EDEMA IN RAT MODEL

Groups	Initial paw volume (mm) (0^{th} day)	Final paw volume (mm) (10^{th} day)	Difference	% inhibition of paw edema
Normal	3.55±0.10	3.71±0.09	0.19±0.04	-----
Control	3.57±0.12	6.72±0.51	3.15±0.50	-----
Indomethacin	3.63±0.12	5.82+0.28	2.10±0.27*	33.3
SBE 100mg/kg	3.59±0.06	6.08±0.43	2.49±0.37	20.9
SBE 200mg/kg	3.65±0.14	5.54±0.24	1.88±0.34*	40.3

Values are expressed as mean ± SEM (n = 6), *p<0.05 denotes significance with respect to the control group using one way ANOVA followed by Tukey's test.

TABLE 8: EFFECTS OF *MESUA FERREA* LINN. ON BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS IN FORMALIN INDUCED RAT PAW EDEMA

Parameters	Normal	Control	Indomethacin (10mg/kg)	SBE 100mg/kg	SBE 200mg/kg
Total protein (g/dl)	7.03±0.47	6.43±0.40	5.03±0.55	6.00±0.50	5.40±0.56
CRP (mg/ml)	0.36±0.08	0.94±0.06	0.46±0.05**	0.71±0.04**	0.56±0.09**
Nitric oxide (%)	----	----	40.4±8.80**	33.4±3.94**	50.7±3.68**
LP (nmol/mg)	16.75±1.25	20.59±0.41	12.96±0.46**	13.75±0.75**	11.50±1.04**
MPO (U/mg)	0.15±0.01	0.23±0.01	0.11±0.01**	0.16±0.02**	0.08±0.01**
ESR (mm/hr)	1.20±0.45	2.40±0.55	1.40±0.55*	1.80±0.71	1.60±0.55*
RBC (millions/ µl)	5.63±0.15	4.87±0.35	5.43±0.15	5.17±0.15	5.53±0.06
WBC(thousands/µl)	7.44±0.52	11.37±0.71	7.50±0.15**	8.97±0.46**	7.69±0.18**
Neutrophils (%)	54.0 ±2.65	73.67±3.21	56.6±7.64**	65.0±3.0*	60.3±4.51*
Lymphocytes (%)	47.67±2.52	65.67±6.03	52.33±2.52*	58.0±2.0	56.33±1.53*
Eosinophils (%)	1.0±0.71	2.40±0.55	1.40±0..55*	2.0±0.71	1.60±0.55*

Values are expressed as mean ± SEM (n = 6), *p<0.05; **p<0.01, denotes significance with respect to the control group using one way ANOVA followed by Tukey's test.

Treated rats showed no significant decline in the protein and RBC level. On the other hand WBC and CRP levels were significantly ($p < 0.01$) decreased by SBE (100 and 200mg/kg) and indomethacin treated groups compared to control. ESR was significantly ($p < 0.05$) reduced by high dose of SBE (200mg/kg) and indomethacin.

The levels of NO, LP and MPO were significantly ($p < 0.01$) reduced in treated groups when compared to control (**Table 8**).

DISSCUSION: The present study establishes the anti-inflammatory activities of SBE of *Mesua ferrea* Linn. in the model used. Using acute toxicity assay, by the administration of SBE of *M. ferrea* in a single dose of 1000 and 2000mg/kg bodyweight, no mortality was recorded after 14 days of treatment. The control and extract treated groups of animals were normal and did not show any symptoms associated with toxicity. Therefore, two different doses 100 and 200mg/kg body weight were selected for pharmacological study.

In *in-vivo* anti-inflammatory tests, 80% ethanol extract of *M. ferrea* showed significant anti-inflammatory effect in the carrageenan induced paw edema (acute inflammation), cotton pellet induced granuloma test (Sub acute inflammation) and formalin induced paw edema (chronic inflammation).

Carrageenan induced paw edema model is accepted as useful phlogistic tool for the investigation of acute inflammation. Edema was formed after the injection of carrageenan in left hind paw that is associated with two distinct phases. First phase is mediated by mast cell degranulation and histamine, serotonin and kinins release in the first hour after injection of carrageenan, while the second accelerating phase of swelling is due to the release of prostaglandins like substance in 2 to 3 h [23]. However in the present study, the anti-edematous effect of the 80% ethanol extract of *M. ferrea* was significantly maintained during the first and second phases of edema development.

Cotton pellet granuloma model is widely used to assess the transudative, exudative and proliferative components of chronic inflammation [24]. It has three phases in the inflammatory responses [25]. First phase (transudative phase) is imbibition of fluid containing low protein takes place at the site of cotton pellet implantation. Second phase starts after 2-3 days, exudation of fluid containing the protein. Whereas the third phase is the proliferative phase, synthesis of mucopolysaccharide, appearance of collagen and increase in the number of fibroblasts that occurs between 3 to 6 days after the implantation [26]. In the present study, the SBE (200mg/kg) has significantly reduced leukocyte migration during the process of inflammation and decrease of granuloma weight indicates the suppression of the proliferative phases, which was effectively inhibited by SBE of *M. ferrea*.

Formalin induced paw edema in rats is a useful model for assessment of anti-inflammatory agents against chronic inflammation [27]. Formalin produces a painful irritation when injected into the skin, to produce inflammation of the joint tissues through the release of toxic substances like histamine, serotonin, bradykinin, nitric oxide including prostaglandins in the synovium that leads to cartilage destruction [28, 29].

In the present investigation, SBE of *M. ferrea* Linn. inhibited the paw edema, induced by formaldehyde injection. Reduction of paw swelling was observed from 4th day onwards perhaps due to immunological protection rendered by SBE of *M. ferrea*. The above studies make it clear that *M. ferrea* has shown significant effect on various parameters. The control rats exhibited a reduced RBC count and an increased ESR.

All these indicated the anemic condition which is a common diagnostic feature in patients with chronic disorders. The treatment with stem bark of *M. ferrea* improved the RBC count and the ESR to a near normal level indicating the significant recovery from the inflammatory process. The migration of leukocytes into the inflamed area is also significantly suppressed by *M. ferrea* Linn. as seen from the significant decrease in total WBC count. The level of serum protein content is lowered in *M. ferrea* treated groups compared to normal group. C-reactive protein (CRP) is an acute-phase protein and significantly upregulated during infection, tissue damage and inflammatory disorders [30, 31].

In the present study the levels of CRP significantly decreased in *Mesua ferrea* treatment as compared to control group. Myeloperoxidase (MPO) is considered as a biomarker of inflammation, released by neutrophils azurophilic granules and is responsible for invoking tissue damage [32]. The excessive concentration of MPO in plasma and in tissues indicates a huge activity of neutrophils [33]. However, elevated concentrations of MPO in patients contribute to tissue damage resulting in significant risk for initialization and multiplication of acute and chronic inflammation diseases.

The present result suggested that MPO activity in paw tissue was significantly increased in arthritic control rats but, after the administration of *Mesua ferrea*, the activity of MPO was significantly reduced. Nitric oxide (NO) plays an important role in signaling molecule released in an acute and chronic inflammatory response and synthesized from L-arginine by nitric oxide synthase (NOS) and the inducible NO synthase [34]. However, increasing expression of iNOS enzyme generates excessive amount of NO leading to inflammation.

The elevated levels of nitrite and lipid peroxide were significantly decreased in *Mesua ferrea* treated group compared to control group. Above results suggested that *M. ferrea* Linn. has potential anti-inflammatory activity in acute and chronic models of Wistar albino rats.

CONCLUSION: These findings suggest that the 80% ethanol extract of stem bark of *Mesua ferrea* L. possesses anti-inflammatory activity. The results justify the use of the plant in the preparation of ethanomedicines used in the treatment of ailments associated with inflammation. Further investigations are required to isolate the active constituents responsible for anti-inflammatory activity, which would facilitate the use of *M. ferrea* in inflammation disorders.

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