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### Phytochemical screening and *in-vivo* evaluation of anti-inflammatory and anti-arthritic potential of *Sterculia foetida* L. bark

MD Imad Uddin<sup>1\*</sup>, Asma Butool S<sup>2</sup>, Saadia Zubedi S<sup>2</sup>, Selvi SK<sup>2</sup>, Manasa P<sup>2</sup>.

<sup>1</sup>Dept. of Pharmacology, Pulla Reddy Institute of Pharmacy, Annaram, Jinnaram, SangaReddy, Telangana-502313.

<sup>2</sup>Dept. of pharmacology, MAK College of Pharmacy, Moinabad, Telangana-500075.

#### ORIGINAL RESEARCH ARTICLE

#### ABSTRACT

**Background:** In the study, we investigated phytochemical composition, anti-inflammatory activity and anti-arthritic potential of *Sterculia foetida* L. bark extract for the first time.

**Material and Methods:** Phytochemical evaluation of bark extract was done by standard procedures. Anti-inflammatory activity was tested against carrageenan induced rat paw edema. Acute non-immunological Arthritis was induced by injecting formaldehyde on 1<sup>st</sup> and 3<sup>rd</sup> day in right hind paw of female Wistar rats; paw width and height were measured on alternate days up to 10<sup>th</sup> day. Animals were sacrificed on 10<sup>th</sup> day and haematological parameters, Erythrocyte Sedimentation Rate (ESR), vascular permeability were measured.

**Results:** Phytochemical evaluation revealed the presence of alkaloids, cardiac glycosides, carbohydrates, tannins, flavonoids, saponins and phytosterols. In carrageenan induced rat paw edema model methanolic extract of *S. Foetida* bark (MESF) 1000 mg/kg showed highly significant anti-edematogenic effect at 3<sup>rd</sup> hr with % oedema inhibition of 50%. In Acute non-immunological Arthritis model, MESF 500 and 1000 mg/kg showed maximum inhibition of paw edema, width and height on 6<sup>th</sup> day of study. Results of haematological parameters, ESR and vascular permeability provide further support to anti-arthritic activity of MESF.

**Conclusion:** Hence our results confirmed Anti-Arthritic potential of *S. Foetida* bark and supports the traditional use of bark in rheumatism.

**Keywords:** Rheumatoid arthritis, *Sterculia foetida*, Formaldehyde, Carrageenan, edema, Vascular permeability.

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\*Author for correspondence

E-mail: [imadpharma111@gmail.com](mailto:imadpharma111@gmail.com)

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#### INTRODUCTION

Rheumatoid arthritis (RA) is a progressive, chronic, multisystem, autoimmune disorder with unidentified cause distressing about 1.3 million people worldwide manifested by discomfort, edema and painfulness of synovial joints (Lipsky, 2005). Treatment of RA is of pivotal concern the existing treatment involves use of NSAIDs, corticosteroids, immunosuppressant's, DMARD and also newer biological agents such as TNF- $\alpha$  and monoclonal antibodies (Smolen et al., 2007; Simon, 2005). Although long term use of NSAIDs causes gastrointestinal disorders such as bleeding and ulceration, cardiovascular disorders such as myocardial infarction. Some NSAIDs were withdrawn from the market because of increased risk of heart attacks and some were distributed in black box indicating risk of stroke (Pennings et al., 1997).

DMARDs and biological agents have increased risk of immunosuppression. Therefore an alternative treatment for RA is still a major concern of research however use of herbal drugs reduces the risk of adverse effects.

*S. foetida* L. (Malvaceae) is a tropical, soft wooded tree that can grow up to 115 feet tall commonly known as "Java olives" (English), "jangli badam" (Hindi) found in various parts of India (Sharma and Sanjappa, 1993). The striking characteristic of *S. foetida* is its seeds holds anti-bacterial (Kudle et al., 2013), anti-haemolytic (Schmid and Patterson, 1988) properties. Seed oil holds chemosterilant, anti-fertility action (Sheehan and Vavich, 1965). *S. foetida* gum was used as an excipient as natural polymer in preparation of ophthalmic mini tablets (Paresh et al.,

2010). The leaves hold mitogenic activity (Scarpelli, 1974), anti-fungal (Schmid and Patterson, 1988), anti-microbial and also used in poultry for pink egg formation also act as anti-feedant (Usharani and RajasekharReddy, 2009), anti-oxidant (Manivannan et al., 2011) and CNS depressant (Mujumdar et al., 2000). Many claims are still undetermined and are of keen interest (Rupesh and Nitin, 2012). Likewise Peng Fei et al., in 2009 isolated 46 compounds which includes 33 flavonoids, 4 coumarins, 6 organic acids and 3 steroids among which 4 compounds namely "hypolaetin 3'-methyl ether 8-O-B-D- glucuronide 6"-methyl ester", "hypolaetin 8-O-B-D-glucuronide 6"-ethyl ester", chrysoeriol 7-O-B-D-glucuronide", "luteolin 7-O-B-D-glucuronide" showed obvious anti-inflammatory activities against croton oil-induced rat ear edema at 20 mg/kg (Pengfei et al., 2009). Moreover leaves of *S. Foetida* contains Taraxer-14-en-3-beta-ol as active constituent which was claimed to have anti-inflammatory property (Naik et al., 2004). Furthermore, it was also divulged that methanolic extract of bark of *S. foetida* showed anti-inflammatory potential against in-vitro model (Imad uddin et al., 2015). The main rationale of present work, was to develop the drugs with fewer side effects and to overcome the plight of synthetic drugs.

## MATERIALS AND METHODS

### Collection of plant material

Fresh *S. foetida* bark was collected from local areas of Moinabad, India during the month of December 2016. Taxonomically plant was authenticated by Scientist, In-Charge, Botanical Survey of India, Deccan Regional Centre, Hyderabad Unit, Telangana and was deposited with a specimen No. MAKCP/2016/010.

### Extraction of plant material

The bark was pared from the stems and cut into small pieces, further it was dried under shade for 13 days and minced in grinder to procure coarse powder. Initially the powder was defatted by Petroleum Ether (40-60 °C) and then successive extraction was done by Chloroform (61.15 °C), Ethyl acetate (56 °C), Methanol (64.7 °C) and Water (100 °C) by Soxhlet extractor (Nikhal et al., 2010). Obtained extracts were dried and preserved in air tight containers for future use.

### Phytochemical screening

Phytochemical evaluation was done by using standard procedures as described by Khandelwal and varundasethi, 2009, Sofowara, 1993, and Trease and Evans, 1989.

### Animals

Experiments were performed in accordance with the direction of IAEC, on healthy female Wistar rats (130-150 gm) and female Swiss albino mice (18-28g) obtained from animal house of Sanzyme PVT. LTD.

Hyderabad, Telangana (India). All the animals were kept under standard husbandry conditions (Temp. 22-28°C; relative humidity 65 ±10%) for 12 hr dark and 12 hr light cycle respectively in standard propylene cages.

### Chemicals and drugs

Formaldehyde and Acetone (S.D. Fine Chemicals Limited, Hyderabad), Carrageenan (Hi-media laboratories, Mumbai) Evans blue (Oxford laboratories Pvt Ltd, Mumbai), Tri sodium citrate and Sodium sulphate (Accord labs, Hyderabad). Diclofenac sodium (Zuche Pharmaceuticals Pvt. Ltd, New Delhi). All other chemicals of laboratory grade were obtained from The Scientific syndicate, Hyderabad.

### Acute oral toxicity test

Acute oral toxicity study of MESF was performed on female Swiss albino mice (18-28g) according to OECD guideline No. 423. Animals were divided in to two groups with 3 animals in each, all animals were fasted for 4hrs and to first and second group, saline (1 ml/kg) and MESF 2000 mg/kg was administered orally, respectively. After oral administration all animals were monitored for first 4hrs for autonomic and neurobehavioral indications, death and then for 14 days for weight loss and mortality. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step. All observations are systematically recorded with individual records being maintained for each animal. Additional observations will be necessary if the animals continue to display signs of toxicity. (OECD, 2001)

### Carrageenan induced hind paw oedema in rats

Female Wistar rats were divided into 5 groups with 4 in each. Normal control group (Group I) and Negative control group (Group II) received Normal Saline (NS) 1 ml/kg. Positive control group (Group III) and treated groups (Group IV & Group V) received Diclofenac Sodium (DS) 10 mg/kg, MESF 500 mg/kg and MESF 1000 mg/kg, respectively. DS, extracts and NS were all administered orally to overnight starved animals. 0.1ml of Carrageenan solution (1.0% w/v NS) was injected into sub-plantar region of the right hind paw after 1hr of drug administration to all groups except normal control group. Paw volume was measured by plethysmometer before injection (0 hr) and at 0.5, 1, 2, and 3 hr after carrageenan injection (Araico et al., 2009).

The percentage inhibition of paw edema volume was calculated by the equation, Percentage (%) inhibition =  $(V_c - V_t / V_c) \times 100$ ; where,  $V_t$  = mean paw volume of treated group and  $V_c$  = mean paw volume of control group.

## Formaldehyde induced acute non-immunological Arthritis in rats

Female Wistar rats were divided in to 5 groups with 4 in each. Group I (Normal control), Group II (Negative control) received tween 80, (2% v/v, orally), group III received DS (10 mg/kg BW/rat/day, orally), group IV and V received MESF (500 and 1000 mg/kg BW/rat/day, orally), respectively for 10 days. Arthritis was induced in sub plantar (S.P) region of right hind paw by injecting 0.2ml formaldehyde (2.5% v/v) after 30 min of drug administration in all except group 1 animals and was repeated on 3<sup>rd</sup> day. Arthritis was assessed by measuring the mean increase in paw volume, paw height and Paw width on 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup>, and 10<sup>th</sup> day (Manjusha et al., 2014).

### Parameters assessed

#### Paw volume, height and width

Right hind paw volumes were measured by using mercury plethysmometer (Fereidoni et al., 2000). Whereas right hind paw height and width was measured by using Vernier calliper (Anderson et al., 2004). All these assessments were done just before formaldehyde injection on day 0 and after formaldehyde injection on alternate days i.e., 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup> and 10<sup>th</sup> day.

#### Haematological parameters and ESR assessment

On 10<sup>th</sup> day the blood was withdrawn from each animal through retro orbital plexus. For ESR measurement 1 part of sodium citrate solution (3.8%) was mixed with 4 parts of blood. Mixed blood was taken into Westergren's pipette up to 0 mark and time was noted. ESR reading as mm/hr was taken at the end of 1 hour (Drabkin and Austin, 1932 and Wintrobe et al., 1974). For other haematological valuation blood was taken into EDTA tubes and parameters like RBC, Hemoglobin, MCV, PCV, MCH, MCHC, WBC and differential leucocytes i.e., neutrophils, basophils, eosinophils, lymphocytes and monocytes were assessed (Huxtable 1990 and Raghuramulu et al., 1983).

#### Vascular permeability

Evans Blue Dye (EBD) 50 mg/kg was administered via jugular vein into anaesthetized animals. After 4hr of EBD administration, rats were sacrificed by high dose of anaesthetic ether, anterior and posterior synovial capsules and fat pad were dissected from each knee joint and cut into smaller pieces followed by mixing them with acetone & 1% sodium sulphate in the ratio of 7:3. Samples were shaken gently and continuously for 24 hr at room temperature by using magnetic stirrer (Model no. 3v05016, Sisco, Delhi, India) to extract dye. Later it was centrifuged for 10 min at 2000 rpm and 3 ml of the supernatant was separated

for measurement of absorbance at 620 nm using UV-spectrophotometer (Model no. G6860A cary 60 uv-vis, Agilent technologies, Hyderabad, India). The amount of dye recovered was calculated by extrapolating with standard curve prepared with different concentrations of EBD solution (Franchis et al., 2004) Percentage inhibition was calculated by using the formula:

Percentage (%) inhibition =  $(v_c - v_t / v_c) \times 100$ ; Where  $v_t$  = % inhibition of treated group;  $v_c$  = % inhibition of control group

### Statistical analysis

The results were expressed as Mean  $\pm$  SEM. Statistical comparison was made between treated groups and control group. Statistical difference between two means was determined by one-way ANOVA followed by Dunnett's multiple comparison test using Graph pad prism 6.01 software. Only those mean values showing statistical difference  $p < 0.0001$ ,  $p < 0.001$ ,  $p < 0.01$ ,  $p < 0.05$  were considered as statistical significant and  $p > 0.05$  was considered as non-significant.

## RESULTS

### Phytochemical screening

Percentage yield was determined by using formula, % Extract =  $\text{Weight of extract in grams} / \text{Weight of sample in grams} \times 100$ . Percentage yield of Petroleum Ether extract, Chloroform Extract, Ethyl Acetate Extract, Methanolic Extract and Water Extract was found to be 0.48%, 4.08%, 0.32%, 19.5% and 8.15% respectively. Preliminary phytochemical assessment of all extracts was found to contain alkaloids, cardiac glycosides, carbohydrates, saponins, tannins, flavonoids, and phytosterol while phenols, proteins and amino acids were not detected (Table 1).

### Acute oral toxicity test

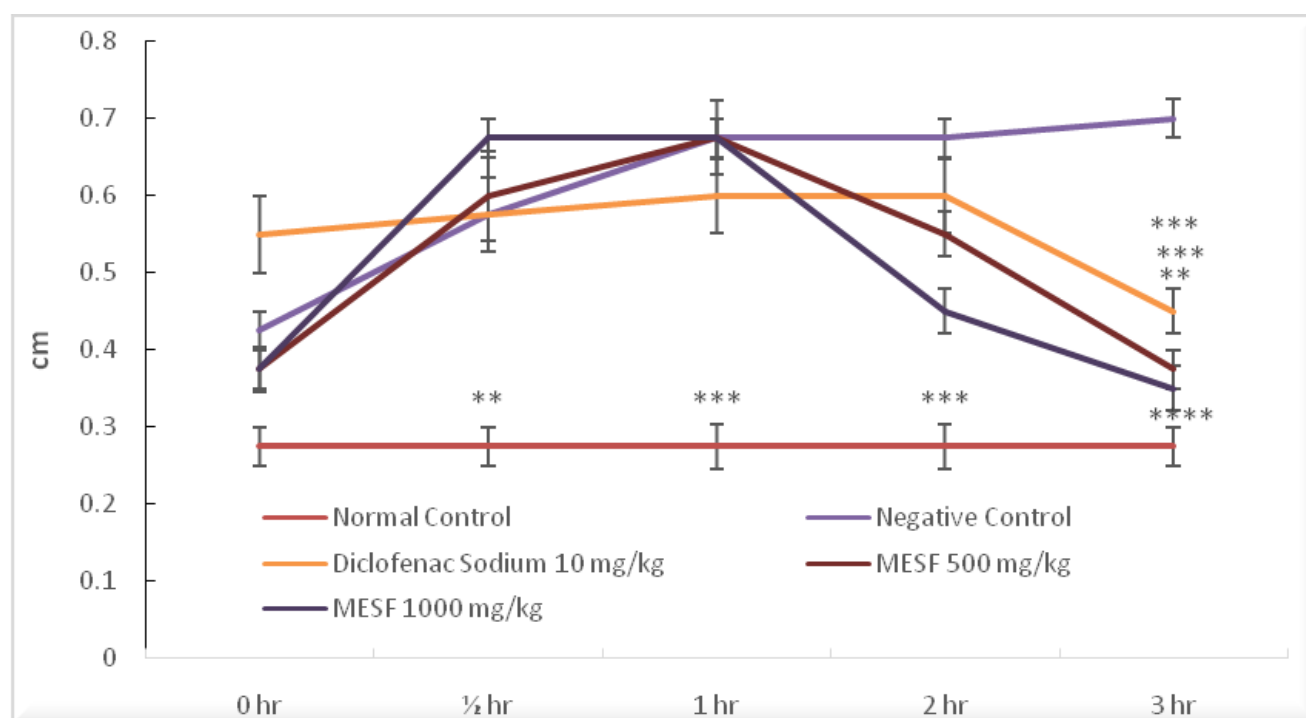
In accordance with OECD guidelines-423, there were no perceptible changes in the autonomic and behavioural patterns of animals on oral administration of MESF with a maximum dose of 2000mg/kg. Therefore LD50 of MESF was calculated as greater than 5000mg/kg. A well below dose i.e. 1/10th (500 mg/kg) and 1/5<sup>th</sup> (1000mg/kg) dose of LD50 value was selected for screening of anti-inflammatory and anti-arthritic potential.

### Carrageenan induced hind paw oedema in rats

Increase in joint diameter was measured in terms of oedema at different intervals like 0 hr, ½ hr, 1<sup>st</sup> hr, 2<sup>nd</sup> hr and 3<sup>rd</sup> hr. MESF (500 and 1000 mg/kg) inhibited significantly ( $p < 0.001$ ) increase in edema only at 3<sup>rd</sup> hr. DS also decreased edema ( $p < 0.01$ ) during 2<sup>nd</sup> hr only. %of edema inhibition at 3<sup>rd</sup> hr of DS, MESF 500 and 1000 mg/kg was found to be 35.7%, 46.4 % and 50% respectively (Figure 1a).

Table 1: Phytochemical screening of *S. Foetida*.

S No.	Phytochemical constituents	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Water
1	Alkaloids					
	1. Mayer's test	+	+	-	+	+
	2. Wagner's test	+	+	-	+	+
	3. Dragendorff's test	+	+	-	+	+
	4. Hager's test	+	+	-	+	+
2	Carbohydrates					
	1. Molisch test	+	+	+	+	+
	2. Benedict's test	-	-	-	+	+
	3. Fehling's test	-	-	+	+	+
3	Cardiac glycosides					
	1. Legals test	-	+	+	+	+
	2. Kellerkilani test	+	+	+	+	+
4	Saponins	+	+	+	+	+
	1. Foam test					
5	Tannins					
	1. Iodine test	+	+	+	+	+
	2. Gelatin test	-	-	+	-	-
	3. Lead acetate	+	+	+	+	+
	4. Acetic acid test	-	-	-	-	-
	5. Dil.kmno4 test	+	+	+	+	+
6	Phytosterol					
	1. Salkowiskis test	+	-	+	+	+
	2. Libermann buchard test	+	+	+	-	+
7	Phenol					
	1. Ferric chloride test	-	-	-	-	-
8	Proteins and aminoacids					
	1. Ninhydrin test	-	-	-	-	-
	2. Millons test	-	-	-	+	-
9	Flavanoids					
	1. Alkaline reagent test	-	-	+	+	+
	2. Lead acetate test	-	+	-	-	+
	3. Sulphuric acid test	+	+	+	-	+
Bioavailability key: + = present, - = absent						



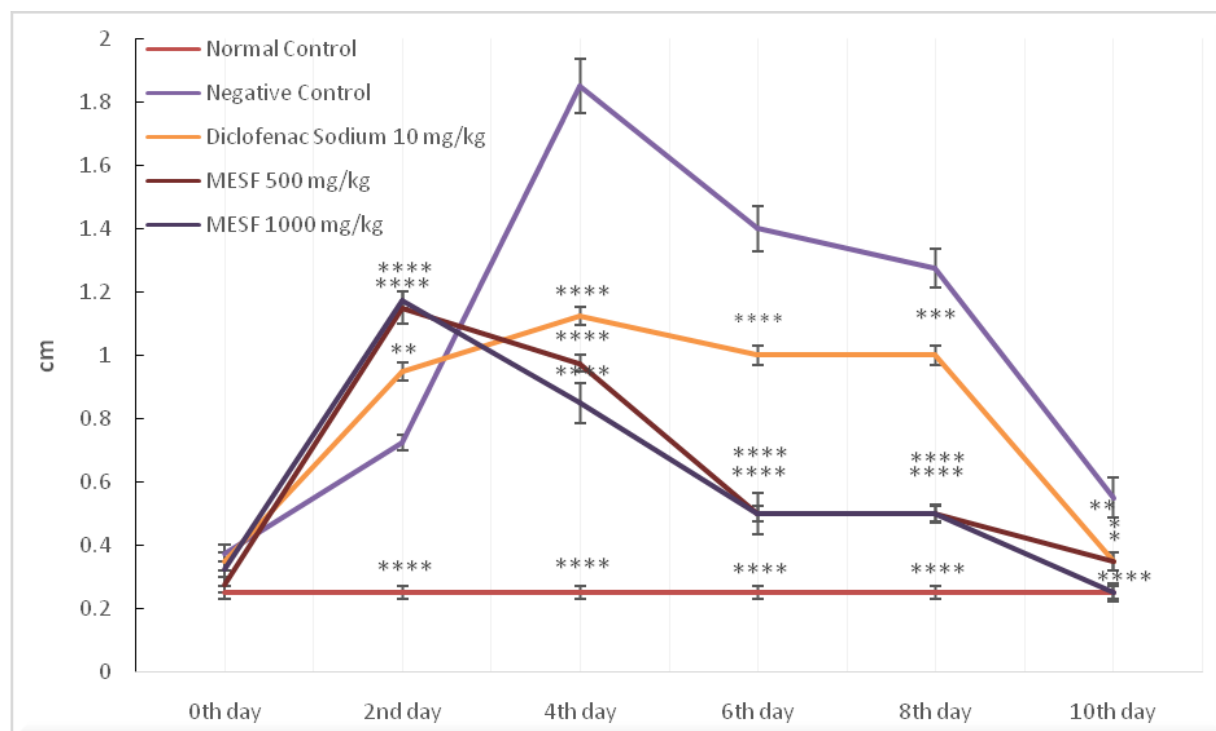
**Figure1a:** Effect of MESF and DS on carrageenan induced paw edema height in rats, All values are expressed as Mean  $\pm$  SEM, n=4, analysed by One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$  as compared to control group; ns= non-significant.

## Formaldehyde induced acute non-immunological Arthritis in rats

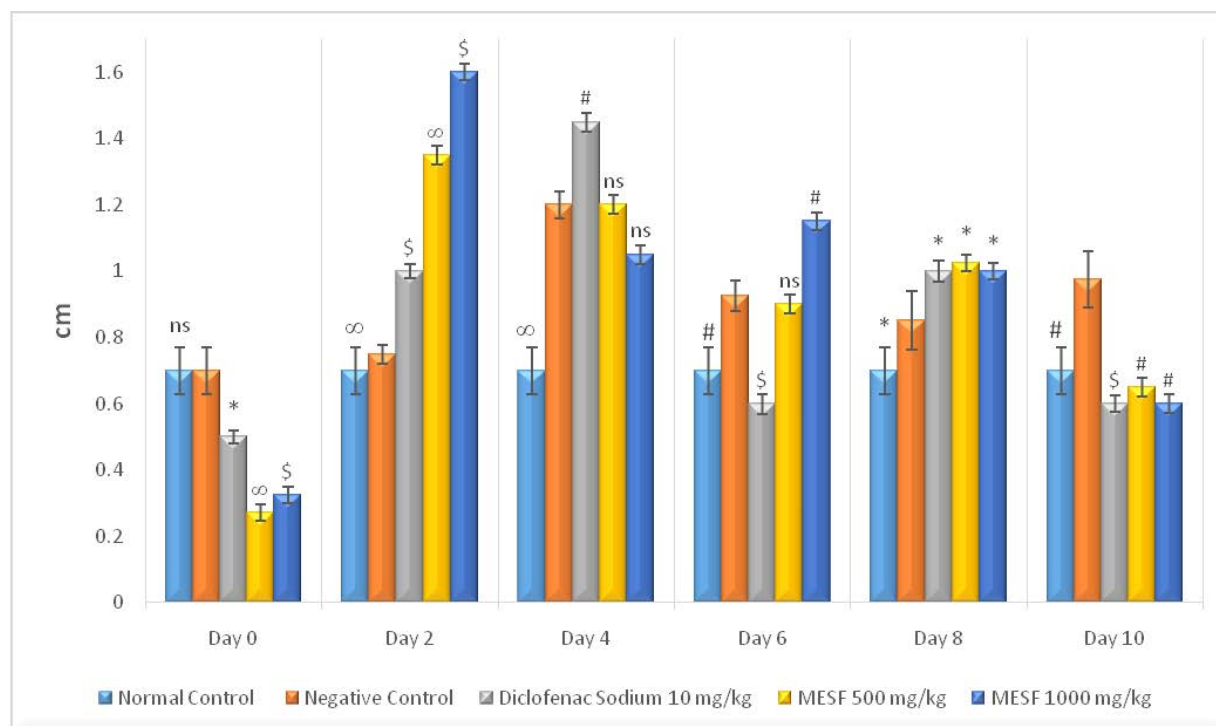
### Effect of MESF and DS on paw volume, width and height

Injection of formaldehyde in S.P region on 1<sup>st</sup> and 3<sup>rd</sup> day in right hind paw increased joint diameter which reached maximum on 4<sup>th</sup> day and thereafter decreased

up to 10<sup>th</sup> day. DS and MESF significantly inhibited paw edema and paw width from day 2 to day 10 and paw height only on day10, after formaldehyde injection. % of edema inhibition of DS, MESF 500 and 1000mg/kg was found to be 36.3%, 36.3% and 54.54% respectively (Figure1b, 1c &1d).

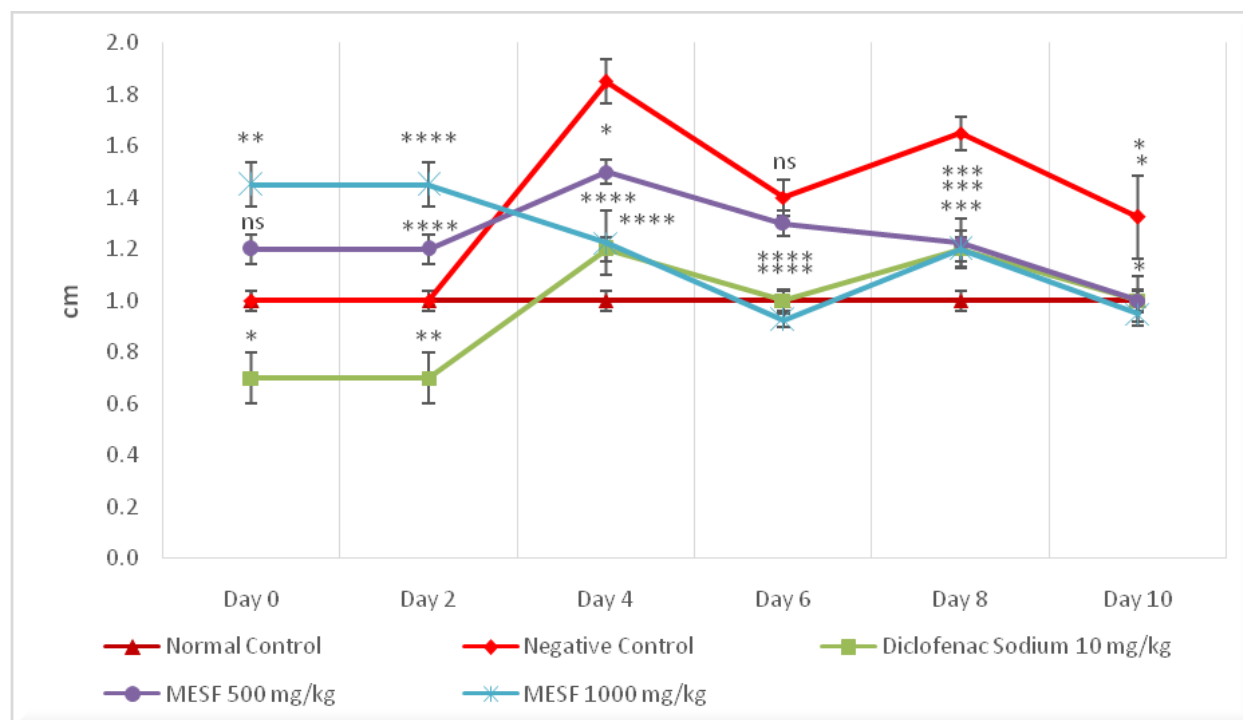


**Figure 1b:** Effect of MESF and DS on formaldehyde induced paw edema volume in rats, All values are expressed as Mean  $\pm$  SEM, n=4, analysed by One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test; \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001 as compared to control group; ns= non-significant.



**Figure 1c:** Effect of MESF on formaldehyde induced paw edema height in rats. All values are expressed as Mean  $\pm$  SEM, n=4, analysed by One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test; \* p<0.05, # p<0.01, \$ p<0.001, ∞ p<0.0001 as compared to control group; ns= non-significant.





**Figure 1d:** Effect of MESF on formaldehyde induced paw width in rats. All values are expressed as Mean  $\pm$  SEM,  $n=4$ , analysed by One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test; \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ , \*\*\*\* $p<0.0001$  as compared to control group; ns= non-significant.

#### Effect of MESF and DS on Haematological parameters and ESR assessment

Treatment with DS and MESF (500 & 1000 mg/kg) showed a significant decrease in the levels of ESR, WBC, neutrophils, lymphocytes, monocytes,

eosinophils and basophils as compared to negative control group. Whereas haematological parameters like RBC, PCV, MCH, MCHC, and haemoglobin increased significantly in MESF (500 & 1000 mg/kg) and DS treated groups as compared to negative control group (Table 2 and Table 3).

**Table 2:** Effect of MESF and DS on RBC, Haemoglobin, MCV, PCV, MCH, MCHC and WBC.

Groups	PARAMETERS ASSESSED						
	RBC (millions/ $\text{cm}^3$ )	HAEMOGLOBIN (g/dl)	PCV (%)	MCV (%)	MCH (g/dl)	MCHC (g/dl)	WBC (thousands/ $\text{cm}^3$ )
Normal control	$8.500 \pm 0.14$ ****	$12.73 \pm 0.22$ ****	$38.5 \pm 0.27$ ****	$45.3 \pm 0.6$ ****	$14.93 \pm 0.28$ ****	$33. \pm 0.3$ ****	$10090 \pm 13.5$ ****
Negative control (2.5%, 0.2ml formaldehyde)	$6.900 \pm 0.0$	$11.10 \pm 0.0$	$36.00 \pm 0.0$	$46.70 \pm 0.0$	$13.20 \pm 0.0$	$28.4 \pm 0.0$	$16703 \pm 4.33$
Positive control (DS 10 mg/kg)	$8.700 \pm 0.0$ ****	$12.00 \pm 0.0$ ****	$40.0 \pm 0.57$ ****	$59.00 \pm 0.0$ ****	$17.80 \pm 0.0$ ****	$30.0 \pm 0.0$ ****	$11400 \pm 2.041$ ****
MESF (500mg/kg)	$9.450 \pm 0.0$ 2 ****	$12.60 \pm 0.0$ ****	$46.0 \pm 0.0$ ****	$49.33 \pm 0.6$ 7 ****	$13.30 \pm 0.0$ ns	$29.7 \pm 0.0$ ***	$14205 \pm 4.564$ ****
MESF (1000mg/kg)	$8.700 \pm 0.0$ ****	$12.00 \pm 0.00$ ****	$40.7 \pm 0.25$ ****	$62.2 \pm 0.37$ ****	$14.10 \pm 0.0$ 3 ****	$29.2 \pm 0.0$ *	$12799 \pm 3.146$ ****

All values are expressed as Mean  $\pm$  SEM,  $n=4$ , analysed by One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test; \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ , \*\*\*\* $p<0.0001$  as compared to control group; ns= non-significant.

#### Effect of MESF and DS on vascular permeability

For preparing standard graph of EBD stock solution [1000 $\mu\text{g}$  EBD in 20ml i.e. 50 $\mu\text{g}/\text{ml}$ ] and dilutions were made and absorbance of each concentration was observed at 620nm (Figure2). Oral administration of MESF extract evoked a mild dose related inhibition of vascular permeability on formaldehyde induced

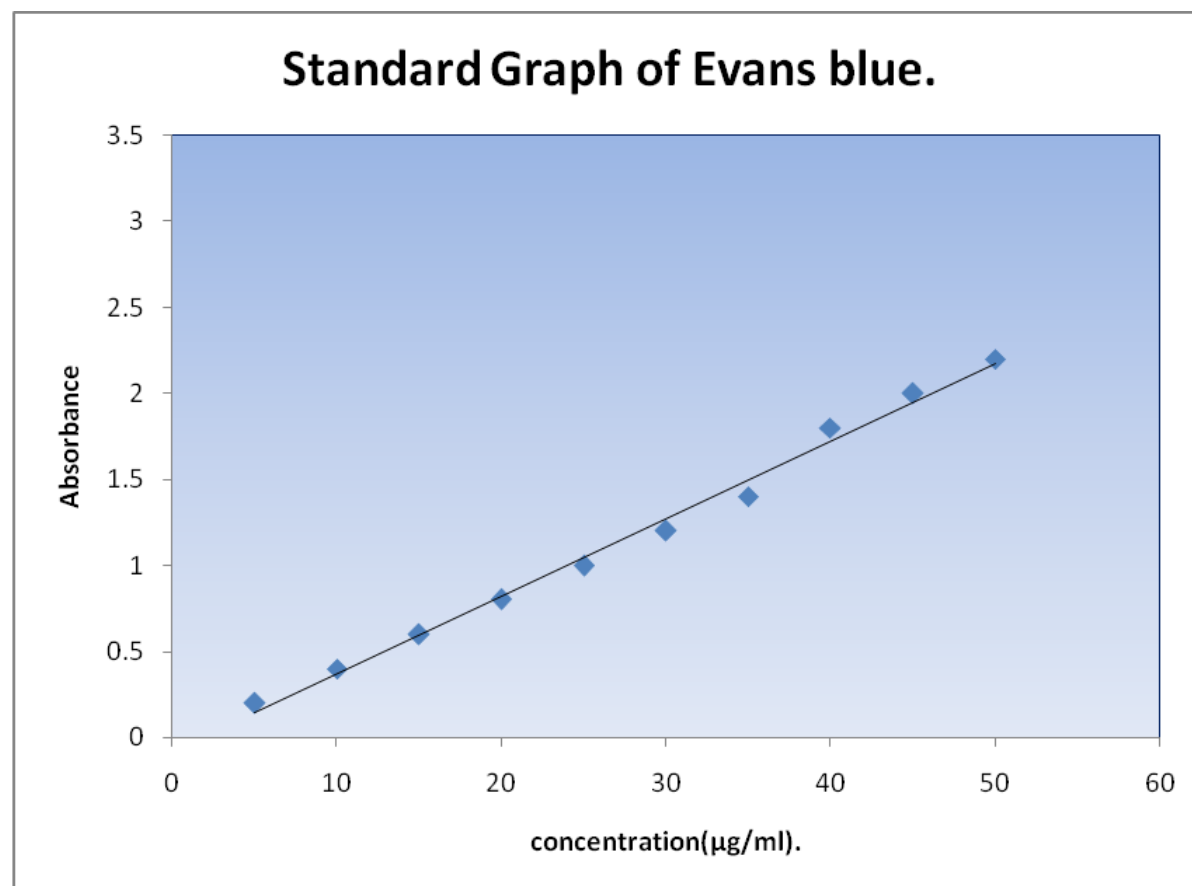
arthritis in rats. Negative control group has showed significant increased infiltration indicated by increased Evans blue extravasations which is due to increased endothelial gap of vascular components at knee joint of rats as compared to vehicle treated rats. Treated groups showed significant reduced infiltration i.e., DS 10 mg/kg ( $P<0.01$ ), MESF 500mg/kg ( $P<0.01$ ) and MESF1000 mg/kg ( $P<0.001$ ) when compared to

negative control group. % inhibition of joint infiltration of DS, MESF- 500 and 1000mg/kg was found to be 59.5%, 62.7% and 72.3% respectively [Table 3].

**Table 3:** Effect of MESF and DS on Differential leukocytes count, vascular permeability and ESR value.

Groups	Differential leukocytes count					Concentration of Evans blue- $\mu\text{g/ml}$ (% inhibition of joint infiltration)	E.S.R. value
	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils		
Normal control	25.00 $\pm$ 0.57****	67.50 $\pm$ 1.25**	3.00 $\pm$ 0.40**	3.25 $\pm$ 0.47**	1.25 $\pm$ 0.25 <sup>ns</sup>	4.500 $\pm$ 0.287*** (80.8%)	0.100 $\pm$ 0.0****
Negative control (2.5%, 0.2ml formaldehyde)	36.00 $\pm$ 0.0	62.00 $\pm$ 0.0	4.00 $\pm$ 0.0	2.00 $\pm$ 0.0	1.00 $\pm$ 0.0	23.500 $\pm$ 5.485	0.400 $\pm$ 0.0
Positive control (DS 10 mg/kg)	34.00 $\pm$ 0.0***	57.75 $\pm$ 1.31*	2.00 $\pm$ 0.0****	0.75 $\pm$ 0.25**	0.0 $\pm$ 0.0****	9.500 $\pm$ 0.288** (59.5%)	0.300 $\pm$ 0.0****
MESF (500mg/kg)	31.00 $\pm$ 0.0****	56.50 $\pm$ 0.86**	3.00 $\pm$ 0.0**	1.00 $\pm$ 0.0*	0.0 $\pm$ 0.0****	8.750 $\pm$ 0.433** (62.7%)	0.200 $\pm$ 0.0****
MESF (1000mg/kg)	32.50 $\pm$ 0.28****	55.00 $\pm$ 0.57***	3.00 $\pm$ 0.0*	1.00 $\pm$ 0.0*	0.0 $\pm$ 0.0****	6.500 $\pm$ 0.000*** (72.3%)	0.225 $\pm$ 0.025****

All values are expressed as Mean  $\pm$  SEM, n=4, analysed by One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test; \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001 as compared to control group; ns= non-significant.



**Figure 2:** Standard graph of Evans blue.

## DISCUSSION

Phytochemical screening divulged the constituents present in various extracts of *S. foetida*. Among them constituents with anti-arthritis interest are flavonoids, cardiac glycosides, saponins, tannins and phytosterols. Their potential in treatment of arthritis was evidenced in different studies as follows. Flavonoids anti-

arthritic action was evidenced in a study conducted by Rovinsky et al., in 2009, in this study use of detralex (flavonoid) alone and in combination with methotrexate decreased adjuvant induced arthritis complications in rats (Rovinsky et al., 2009). Cardiac glycosides, Digoxin anti-arthritis action was reported in collagen induced arthritis (CIA) in mice by suppressing pathogen Th 17 production (Lee et al., 2015). Moreover, many studies evidenced the role of

saponins in the treatment of RA. Xiao-Bo Sun et al., confirmed anti-arthritic activity of total saponins extracted from *clematis henryi* oliv in CIA in rats (Xiao-Bo Sun et al., 2016). Anti-arthritic activity of tannins was established by use of hydrolysable tannins rich pomegranate extract in CIA in mice (Meenakshi et al., 2008). Furthermore, anti-arthritic potential of phytosterols was also reported by Bigoniya in adjuvant induced arthritis model (Bigoniya et al., 2013).

The acute oral toxicity testing was done to obtain information on the biologic activity of a chemical and gain insight into its mechanism of action. LD50 value was determined, it defined as the statistically derived dose that, when administered in an acute toxicity test, is expected to cause death in 50% of the treated animals in a given period, is currently the basis for toxicological classification of chemicals (Chan and Hayes, 1994).

The anti-inflammatory activity of MESF on carrageenan induced paw edema was probably due to their inhibitory effect on enzymes involved in the production of the chemical mediators of inflammation and metabolism of arachidonic acid. Carrageenan induced rat paw edema model is a well-recognized acute inflammatory model, which is commonly applied to assess the anti-inflammatory activity of plant extracts or chemical compounds. In this model, inhibition of inflammation was evaluated by measuring anti-edematogenic effect of testing compound (Thomazzi et al., 2010). Sub-plantar injection of carrageenan induced edema which is bi-phasic event. Usually edema formation starts at 1<sup>st</sup> hr and continues up to 5<sup>th</sup> hr (Khan et al., 2009) and reaches maximum at 3<sup>rd</sup> hr. So 3<sup>rd</sup> hr is taken as the point of time where maximum edema is produced in the injected animals (Lino et al., 1997). In initial phase, non-phagocytic edema was formed lasting up to 1hr or 1.5 hr due to the action of released inflammatory mediators like bradykinin, serotonin and histamine on vascular permeability (Zhu et al., 1997). While in the second phase of carrageenan induced edema prostaglandins (Yang et al., 1996) protease, lysosomes (Vinegar et al., 1969) nitric oxide are released, which reaches to their peak levels up to 3<sup>rd</sup> hr after sub-plantar injection. This second phase edema may be due to induction of cox-2 enzyme at the site of injection which causes the amplification of PGs. Macrophages also play a role at the site of injection by inducing the release of interleukin-1 from insulted tissue (Fujiwara Nand Kobayashi, 2005). This released interleukin-1 causes the accumulation of polymorphic nuclear cells (PMNs). When these PMNs are activated they causes the release of lysosomal enzymes and active oxygen species in the form of superoxide, which induces edema by causing the destruction of connective tissue (Nikolaus, 1997). MESF 500 mg/kg and MESF 1000 mg/kg inhibit paw edema in 3<sup>rd</sup> hr of study predominantly due to inhibition of COX-2 enzyme

which is responsible for amplification of prostaglandins followed by induction of edema. When compared to standard drug MESF showed dose dependent protection and it was found to be equivalent to DS in inhibiting the development of edema. This inhibition of edema was also evidenced in Analgesic and anti-inflammatory studies of cyclopeptide alkaloid fraction of leaves of *Zizyphus nummularia* (ManojGoyal et al., 2013).

Formaldehyde induced acute non-immunological arthritis in rat paw edema is a well-recognized chronic inflammatory model, which is commonly applied to assess the anti-arthritic activity. Induction of formaldehyde causes the paw to swell and becomes red. The formalin induced arthritis has two distinctive phases - early phase and late phase. The early and late phases of formalin have obvious different properties (Guo et al., 2008). The early (acute) phase is due to direct stimulation of nociceptors (also called neurogenic phase) and the late phase is mainly inflammatory in origin (also called inflammatory phase). The early phase reflects centrally mediated pain while the pain in the late phase is due to release of inflammatory mediators (Kale et al., 2007). The development of edema in the paw of the rat after injection of formaldehyde is due to the release of histamine, serotonin and the prostaglandin like substances at the site of injection (Kore et al., 2011). Formaldehyde induces arthritis by denaturing protein at the site of administration, which produces immunological reaction against the degraded product (Telang et al., 1999). Swelling around the ankle joint and paw of arthritic rat is considered to be due to the edema of particular tissue such as ligament and capsule (Buadonpri et al., 2009). The paw edema was evaluated by measuring paw width, paw height and paw volume increase in these parameters after the induction indicates the release of inflammatory mediators resulting in arthritis. MESF 500mg/kg and 1000 mg/kg significantly decreases the paw width, paw height and paw volume indicating the anti-arthritic potential of MESF.

Hemopoiesis is the key function to determine the functional abnormalities. In arthritis normal hemopoiesis is disturbed, it is accompanied with decrease in RBC leading to anemia and abnormal increase in WBC and differential leucocytes leading to leucocytosis and also increased ESR (Martí-Carvajal et al., 2014). ESR is defined as the rate in which RBC sediment in a period of 1 hr. It is expressed as mm/hr, shown by the height of the column of clear plasma at the end of one hour. In this test, a tall, thin tube holds a sample of blood. The speed at which the RBC fall to the bottom of the tube was measured. Inflammation can cause abnormal proteins to appear in our blood. These proteins cause RBC to clump together. This makes them fall more quickly. ESR higher than normal is associated with autoimmune



disorders, such as RA (Jens and Henrik, 1995). Treating with MESF 500 mg/kg and 1000mg/kg increased RBC count and decreased WBC count and ESR indicating its anti-arthritis potential.

Vascular permeability, is often in the form of capillary permeability or microvascular permeability, characterizes the capacity of a blood vessel wall to allow for the flow of small molecules (drugs, nutrients, water, ions) or even whole cells (lymphocytes on their way to the site of inflammation) in and out of the vessel (Dovark, 2003). The swelling of joint knees, causes increased blood vascular permeability (Ramesh et al., 2013). Evans blue extravasations method is used to assess for plasma protein extravasations in the rat knee joint, because Evans blue has high binding affinity to plasma proteins. Normally, large plasma proteins and bound EBD cannot pass through the endothelial gaps and therefore gets restricted in the vascular component (Nagy et al., 2003). Endothelial cells undergo activation, expressing adhesion molecules and presenting chemokine's, leading to enlargement of endothelial gaps as a result plasma protein and EBD complex can escape to the interstitial tissues. The measurement of the amount of EBD in the synovial capsule can provide us an index of the relative vascular permeability. The decreased extravasations reported for MESF-500mg/kg and 1000mg/kg and diclofenac-10mg/kg was due to decreased endothelial gaps which are caused by decreased expression of adhesion molecules.

## CONCLUSION

Use of either crude extract or isolated lead compounds from medicinal plants provided remedy for many human ailments with negligible toxic effects as compared to chemically synthesized drugs. In response to this supposition, this study was piloted to identify phytochemical composition of *S. Foetida* bark extract. Results of pharmacological screening concluded anti-arthritis potential of MESF may be due to prevention of cartilage destruction, decreasing vascular permeability and protection of synovial membrane. As MESF is equipotent with diclofenac sodium, action produced may be due to inhibition of COX-2 mediated PG production. Conclusion of present study postulates further research to isolate and characterise lead compounds as potent anti-inflammatory and anti-arthritis agents from *S. Foetida* bark.

## CONFLICT OF INTEREST

None declared.

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