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Research Article

In vitro and *In vivo* Anti-inflammatory Activities of *Mesua ferrea Linn*

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ABSTRACT

Objective*: Mesuaferrea* L is a medicinal plant belongs to the family Clusiace, it is extensively used in folk medicine for treatment of chronic inflammatory diseases.The present study was aimed to evaluate *in vitro* and *in vivo* anti-inflammatory activity of *M. ferrea* L. Methods: The *in vitro* anti-inflammatory activities such as nitric oxide, PGE_2, pro-inflammatory cytokines (TNF-α and IL-1β) were studied in RAW 264.7 cells and *in vivo* studies were carried out on carrageenan -induced inflammation in Wistar rats. The sequentially extracted *M. ferreaL* bark extracts (MFBHE, MFBEE, and MFBME) exhibited inhibitory effects on pro-inflammatory mediators such as nitric oxide, prostaglandin E_2, tumour necrosis factor-αandinterleukin-1βproduction in concentration dependent manner in LPS induced RAW 264.7 cells andCarrageenan induced paw oedema in Wistar rats. Conclusion: The result of the present study indicated that *M. ferrea L* ethyl acetate bark extract exhibited significant *in vitro*and *in vivo*anti-inflammatory activity.

Keywords: Anti-inflammatory agents; ProstaglandinE_2; Nitric oxide;pro-inflammatory cytokines; Carrageenan.

INTRODUCTION

Inflammation is a vital response of living organisms against foreign challenges which leads to the restoration of tissue functions[1]. Up-regulation of chronic inflammation leads to pathogenesis of several inflammatory diseases such as atherosclerosis, arthritis, asthma, neuro degenerative disease and auto immune ailments[2]. Pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) up-regulates the inflammation by activating both arachidonic acid dependent and independent pathway. Based on previous studies targeting of both arachidonic acid dependent and independent pathways are useful for attenuation and treatment of various inflammatory diseases[3]. Both steroidal and non-steroidal anti-inflammatory drugs have potent anti-inflammatory activity, long term administration of these drugs can leads to various side effects such as mucosal irritation, increased risk of vascular diseases and nephritic failures[4], Therefore, naturally occurring anti-inflammatory agents with a high therapeutic index,less side-effectsand highselectivity are required as substitutes for steroidal and non-steroidal anti-inflammatory drugs.

Menghini et.al[5] reported that, *in vitro* studies showed that natural formula (5-1000µg/ml) was able to significantly inhibit ROS and PGE2 production and same concentration to inhibited both TNFα and IL-6 gene expression. *In vivo studies*, to young and aged female rats the natural formula at 5mg/rat for 21 days, significantly reduced the inflammatory PGE2 and NFκ-B activity.

It is found that 40% of all medicines derived from natural sources and out of them 25% are from plant sources.Plant-derived compounds with high therapeutic value are used to cure both acute and chronic inflammatory diseases. Ayurvedic and traditional medicines are most acceptable practices for curing various inflammatory diseases[6].Pycnogenol is a phenolic compound purified from the bark of *Pinus maritime Mill* belongs to the family pinaceae, it is an effective anti-inflammatory compound which is highly capable of reducing the synthesis of IL-1β in LPS stimulated RAW 264.7 cells.

Mesua ferrea L belongs to the family Calophyllaceae, native of Sri Lanka and also widely distributed in North East region of India. From the review studies on *M.ferrea*L it is reported that, i*n vivo* carrageenan-induced rat paw oedema is appreciably repressed by ethanolic extracts of *M.ferrea*L flowers at 200 and 400 mg/kg b.w.[7]. No further work has been carried out on the mechanism of anti-inflammatory activity of *M. ferrea* L bark extracts.

Hence, the aim of the present study wasto evaluate anti-inflammatory activityof *M. ferrea*Lbark extracts by determining inhibitory effect on LPS induced NO, PGE_2,

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TNF-α and IL-1β in LPS induced RAW 264.7 cells and *in vivo* studies using carrageenan induced paw edema model.

MATERIALS AND METHODS

Materials

Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100µg/ml Streptomycin and 100 U/ml penicillin (In vitrogen), DMSO,3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Griess reagent, L-nitro arginine methyl ester (L-NAME), Lipopolysaccharide (LPS), sodium nitrite ($NaNo_3$), PGE_2 ELISA kits (Cayman Chemical, Ann Arbor, MI, USA), TNF-α and IL-1β ELISA assay kits (eBioscience, USA). Nimesulide was obtained from Cayman Chemical (Ann Arbor, MI). Carrageenan (Sigma Aldrich), Diclofenac sodium (Sigma Aldrich). All reagents used are of analytical grade.

Collection of plant material

Mesua ferrea L is commonly used in traditional medicinal practices like Ayurveda to cure various diseases such as arthritis, allergy, asthma, leprosy, cough, fever, wounds and rheumatism. It was collected from Uppa Village, Chintapalli Mandal, Visakhapatnam District, A.P, India, and authenticated by Dr. S.B. Padal, Associate Professor, Department of Botany, Andhra University. A voucher specimen (AU BDH 21910) was deposited in theDepartment Botany, Andhra University for future reference.

Preparation of plant extracts for preliminary anti-inflammatory activities

M. ferrea L. bark was properly washed with distilled water, shade dried and coarse powdered. Powder weighing 350 grams was first defatted with petroleum ether and then extracted with hexane, ethyl acetate and methanol by a hot percolation method using a Soxhlet's apparatus. The successive plant extracts were concentrated in a rotavapour according to the boiling temperatures of the solvents to dryness to obtain organic solvent crude extracts. The obtained hexane (6.46g), ethyl acetate (20.06g) and methanol (15.2g) extracts were evaluated for *in vitro, in vivo* anti-inflammatory activities.

In vitro anti-inflammatory assays

RAW 264.7 cell culture

RAW 264.7, a mouse peritoneal macrophage cell line was obtained from the American type culture collection (ATCC); Manassas,VA, USA and cultured with Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum (FBS), 100µg/ml Streptomycin and 100 U/ml penicillin (Invitrogen), maintained at 37^0C in a 5% CO_2 incubator.

The *M. ferrea* L bark extracts were solubilised with 2% dimethylsulfoxide (DMSO) resulting in concentration 1mg/ml in DMEM culture medium. These preparations were filtered by 0.2µm filters and these extracts were used for evaluation of anti-inflammatory activities. The DMSO concentration has never increased 0.1%.

Cell viability by MTT assay

In order to study the cytotoxic effect of *M.ferrea* L bark extracts and ethyl acetatecolumn fractions, RAW 264.7 cells were cultured at a density of 1 x 10^5 cells/well in 24 well plates (B.D. Bioscience) using fresh DMEM medium and were treated with different concentration of 10, 25, 50, 100, 200, 500µg/ml *M. ferrea* L bark extracts i.e (MFBHE, MFBEE, MFBME) and 10, 50, 100µg/ml column fractionswith or without LPS for 24 hours.The absorbance was measured by using a micro plate reader at 570nm. The control group consists of untreated cells were considered as 100% viable cells. Results are expressed as percentage of viable cells when compared with the control group.

All the experiments were performed in triplicates.

$$\%\ cell\ viablity = \left(\frac{O.D.of\ control - O.D.of\ test}{O.D.of\ control}\right) \times 100$$

Measurement nitric oxide levels in LPS-induced RAW 264.7 cells by Griess assay

The nitrite concentration in the culture medium was measured by the Griess reaction method[8]. RAW 264.7 cells (2 x 10^5 cells/well) were seeded in 24 well culture plate and pre-treated with or without different concentrations of both (50, 100µg/ml)*M.ferrea*L barkextracts (MFBHE, MFBEE, MFBME), *Mesua ferrea* bark ethyl acetate column fractions (10, 50, 100µg/ml) and 100µM of L-NAME, an inhibitor of NO was used as a positive control. After 2 hours of treatment, stimulation with LPS was carried out at 1µg/ml for 24 hours maintained at 37^0C in a 5% CO_2 incubator. After 24 hours of incubation, 100µl of the culture supernatant was transferred into 96 well plate and the same quantity of Griess reagent (1% Sulfanilamide and 0.1% N-1-(napthyl) ethylene diamine-HCl in 2.5% H_3PO_4) was added, then the plate was incubated for 15 minutes at the room temperature and the absorbance was measured at 540nm with the microplate reader (Bio-Rad). Cells without any treatment served as basal control, but LPS treated cells acts as a control. All the experiments were performed in triplicates. The amount of NO was calculated using sodium nitrite standard curve and IC_{50} values were determined graphically (n=3). All the experiments were performed in triplicates.

$$\%\ Inhibition\ of\ nitric\ oxide = \frac{O.D.of\ control - O.D.of\ Test}{O.D.of\ control} x100$$

Measurement of PGE_2 levels in LPS-induced RAW 264.7 cell culture supernatants by ELISA

RAW 264.7 cells (2 x 10^5 cells/well) were seeded in 24 well plates and pre-treated with or without different concentrations (50, 100µg/ml) of *M.ferrea* L bark extracts (MFBHE, MFBEE, and MFBME). After 2 hours of treatment, stimulation with LPS was carried at 1µg/ml for 24 hours maintained at 37^0C in a 5% CO_2 incubator. For measurement of PGE_2 production, supernatants were collected by centrifugation at 2,500g for 15 minutes. 50µl of supernatant of culture medium was collected and PGE_2 levels were determined using PGE_2 ELISA kit (Cayman Chemical, Ann Arbor, MI, USA) as per the manufacturer's instructions. The IC_{50} values were determined graphically (n=3). All the experiments were performed in triplicates.

$$\%\ Inhibition\ of\ PGE2 = \frac{O.D.of\ control - O.D.of\ Test}{O.D.of\ control} x100$$

Figure 1

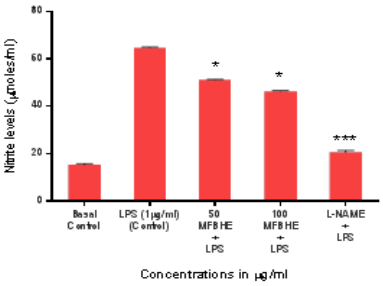
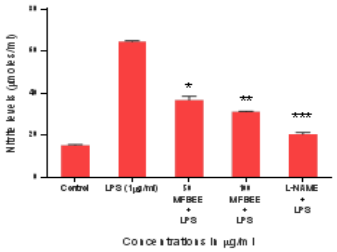


Figure 1a



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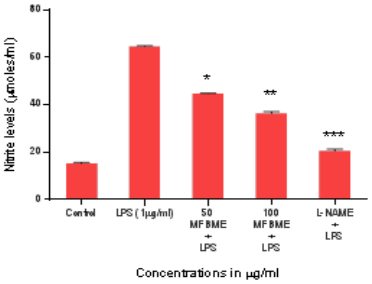


Figure 1c

Effect of *M. ferrea L* bark hexane, ethyl acetate, methanol extracts (Figure 1a:MFBHE, Figure 1b: MFBEE and Figure 1c:MFBME), at concentrations of 50 and 100µg/ml on LPS-induced nitric oxide production in RAW 264.7 cells. Values are mean of three replicates ± SEM. *p≤0.05, **p≤0.01, ***p≤0.001 represents a significant difference compared with LPS- treated control group

Figure 2

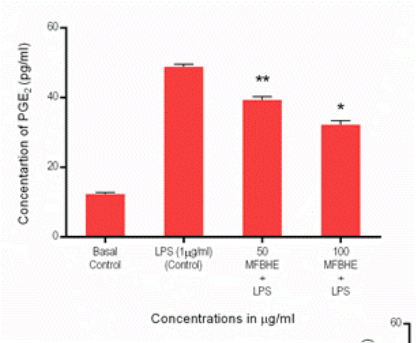


Figure 2a

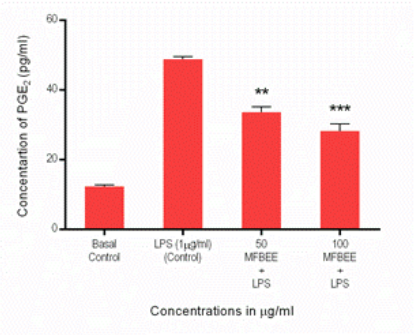


Figure 2b

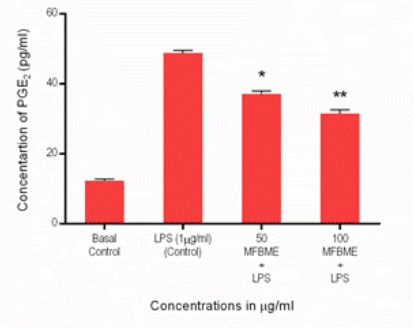


Figure 2c

Effect of *M. ferrea L* bark hexane, ethyl acetate, methanol extracts (Figure 2a:MFBHE, Figure 2b: MFBEE and Figure 2c:MFBME), at concentrations of 50 and 100µg/ml on LPS-induced PGE_2 production in RAW 264.7 cells. The PGE_2 production in culture supernatants was measured by ELISA method. Values are mean of three replicates ± SEM. *p≤0.05, **p≤0.01, ***p≤0.001 represents a significant difference compared with LPS- treated control group.

Measurement of pro-inflammatory cytokines (TNF-α and IL-1β) in RAW 264.7 cell culture by ELISA

The effect of different concentrations of *M. ferrea* L bark extracts on production of TNF-α and IL-1β were measured

Figure 3

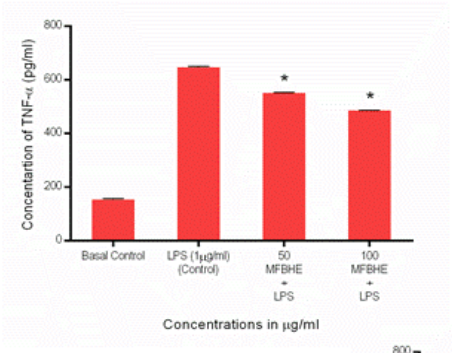


Figure 3a

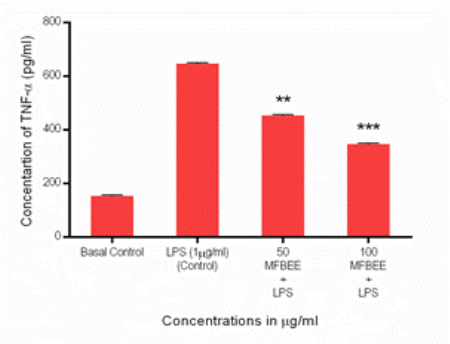


Figure 3b

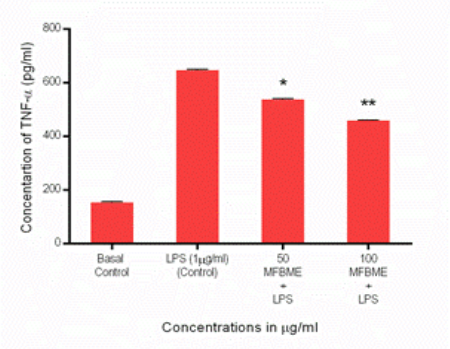


Figure 3c

Effect of *M. ferrea L* bark hexane, ethyl acetate, methanol extracts (Figure 3a:MFBHE, Figure 3b: MFBEE and Figure 3c:MFBME) at concentrations of 50 and 100µg/ml on LPS-induced TNF-α production in RAW 264.7 cells. The TNF-α production in culture supernatants was measured by ELISA method. Values are mean of three replicates ± SEM. *p≤0.05, **p≤0.01, ***p≤0.001 represents a significant difference compared with LPS-treated control group.

Figure 4

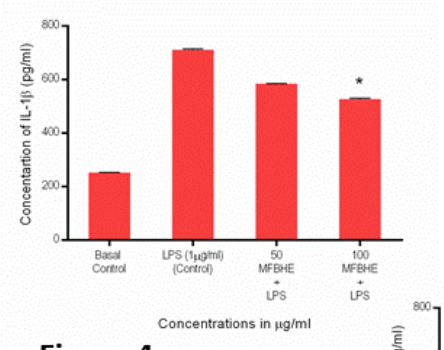


Figure 4a

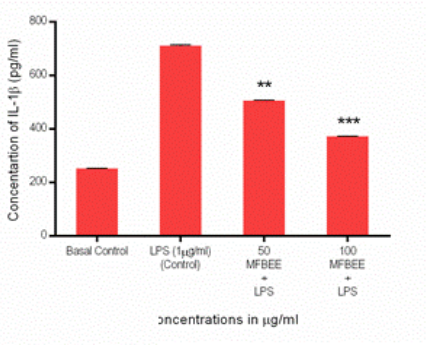


Figure 4b

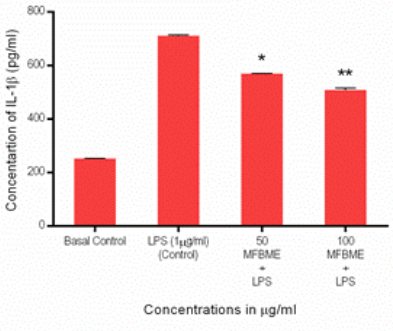


Figure 4c

Effect of *M. ferrea L* bark extracts (Figure 4a:MFBHE, Figure 4b: MFBEE and Figure 4c:MFBME) at concentrations of 50 and 100µg/ml on LPS-induced IL-1β production in RAW 264.7 cells. The IL-1β production in culture supernatants was measured by ELISA method. Values are mean of three replicates ± SEM. *p≤0.05, **p≤0.01, ***p≤0.001 represents a significant difference compared with LPS- treated control group.

Table 1: Acute oral toxicity study of *M. ferrea L* bark extracts

M. ferrea bark Extracts	LD_{50} Cut Off Dose along with $1/10^{th}$ and $1/5^{th}$ of Extracts			*ED_{50} Effective Dose
	LD_{50} Cut Off Dose	$1/10^{th}$ of LD_{50}	$1/5^{th}$ of LD_{50}	
MFBHE	2500mg/kg body weight	250mg/kg body weight	500mg/kg body weight	250mg/kg body weight
MFBEE	2500mg/kg body weight	250mg/kg body weight	500mg/kg body weight	250mg/kg body weight
MFBME	2500mg/kg body weight	250mg/kg body weight	500mg/kg body weight	250mg/kg body weight

* ED_{50}: The "median effective dose" is the dose that produces effect (all or nothing) in 50% of the subjects.

by ELISA assay kit (eBioscience, USA). 2 x 10^5 RAW 264.7 macrophages were seeded in 24 well plates for overnight. Cells were pre-treated with or without different concentrations (50, 100µg/ml) of*M.ferrea*L barkextracts (MFBHE, MFBEE, MFBME) for 2 hours, then incubated with LPS 1µg/ml for 24 hours at 37^0C. Positive controls were only treated with LPS, whereas in negative control well medium containing 0.1% DMSO was added. The cells were maintained at 37^0C, in humidified incubation under an atmosphere supplemented with 5% CO_2. All the experiments were performed in triplicates.

Supernatants were collected by centrifugation at 2,500g for 15 minutes and quantified by usingTNF-αand IL-1β enzyme linked immune sorbant assay kit from eBioscience according to manufacturer's instruction protocol. Cells without any treatment served as basal control, but LPS treatment alone acts as a control. All the experiments were performed in triplicates.

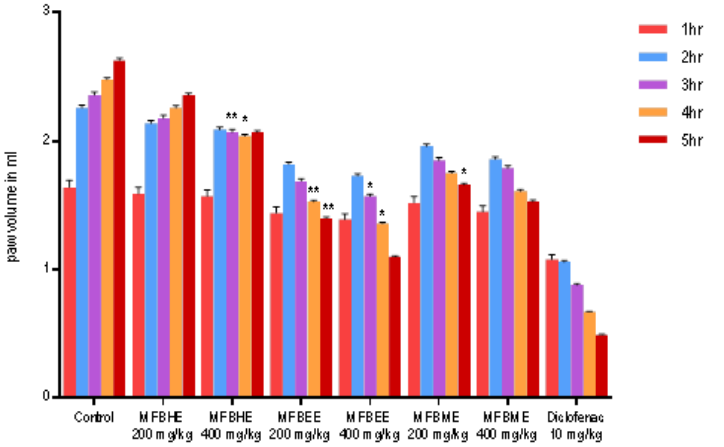
$$\%\ Inhibition\ of\ TNF\alpha\ \&\ IL1\beta\ = \frac{O.D.of\ control\ -O.D.of\ Test}{O.D.of\ control} x100$$

In vivo anti-inflammatory assays

Acute toxicity assay

The acute toxicity assay used to determine the lethal dose (LD_{50}) of bark extracts *M.ferrea*Lusing different doses according to the methods described by the Organization for Economic Co-operation and Development (OECD) guidelines. Healthy young mice of either sex weighing 25-30 grams were used for acute toxicity studies, each group contain 3 animals.The animal experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of GITAM University (IAEC no. 517/IAEC/2012) and Institutional Animal Ethical Committee of Indian Institute of Chemical Technology (IICT), Hyderabad, Telangana, India. The animals were kept fasting for overnight providing only water after administration of hexane, ethyl acetate and methanol extracts of *M. ferrea* L bark orally at doses range of 5 – 3000 mg/kg body weight. The animals were then allowed to take food and water and observed for 1-2 days for signs of acute toxicity. The number of deaths in this period was recorded. The determination of acute toxicity by adopting fixed dose as per the guidelines of CPCSEA and 1/10th, 1/5th of LD50, cutoff values of bark extracts were taken as screening doses for assessment of *in vivo* anti-inflammatory activity of *M. ferrea* L.

Figure 5



Inhibitory effect of *Mesua ferrea* bark extracts on carrageenan induced paw volume (ml) in Wistar rats. Values are mean of six replicates ± SEM (n=6).*p≤0.05, **p≤0.01, ***p≤0.001 represents a significant difference compared with the control group.

Assessment of anti-inflammatory effect of M. ferrea Lstem bark extracts on carrageenan -induced inflammation in Wistar rats

Carrageenan-induced paw oedema model developed by[9,10], is mostly used for the evaluation of anti-inflammatory activity of *M. ferrea* L stem bark extracts**.**Male Wistar albino rats weighing 150-200 g were obtained from M/s Mahavir Enterprises (Hyderabad, Telangana, India). The animals were fed with standard laboratory diet, which was purchased from M/s Rayan's Biotechnology Pvt. Ltd. (Hyderabad, Telangana, India). During the experiment the rats were allowed to have access towater and food ad *libitum*. Animal experiments were conducted according to Committee for the Purpose of Control and Supervision ofExperimental Animals (CPCSEA) guidelines.

The animals were divided intofive groups each group contain six animals (n=6). The first group was given normal saline by gastric intubation. The second, third and fourth groups (200 and 400 mg/kg body weight) received the hexane, ethyl acetate, methanol *Mferrea* L bark extracts (MFBHE, MFBEE, MFBME) for 10 days and the fifth group received Diclofenac sodium (positive control) as a standard (10 mg/kg body weight). The paw volume was measured plethysommetrically (UgoBasile, Italy) at 0h, 1h, 2h, 3h, 4h, and 5h after the injection of 0.05 ml of 1% carrageenan. The percentage of inhibition of paw volume of treated groups was calculated by comparing with a mean paw volume of the control group.

$$\%\ Inhibition\ = \frac{Control\ paw\ volume\ -\ Test\ paw\ volume}{Control\ paw\ volume} x100$$

All the results were expressed as the mean (n=3-6) ± standard error mean (SEM). The statistical analysis of experimental groups was carried out by one-way ANOVA followed by Dunnet's test using Graph pad Prism software version 6.0. The IC_{50} values were calculated using MS Excel. *p≤0.05, **p≤0.01, ***p≤0.001 represents a significant difference compared with the control group.

RESULTS

Effect of M. ferrea L bark extracts on cell viability of RAW 264.7 cells

The RAW 264.7 macrophage cells were treated with different concentrations (10, 25, 50, 100, 200 and 500µg/ml) of *M. ferrea* L bark extracts with or without the addition of LPS (1µg/ml) and incubated for 24 hours.The results showed that *M. ferrea* L bark extracts did not affect the cell viability at the range of 10-500µg/ml apart from the LPS presence up to a range of 90% of cell viability. Thereforethe dose range of (10-500µg/ml) of *M. ferrea* L bark extracts was used for *in vitro* anti-inflammatory studies.

Effect of M.ferrea L bark extracts on LPS-induced nitric oxide levels in RAW 264.7 cells

As shown in the figure1 the nitric oxide production was decreased in *M.ferrea* L *bark extracts* treated RAW cells. LPS-stimulated RAW 264.7 macrophages significantly increased nitrite level production (64.68±0.29µmol/ml) compared to untreated control RAW 264.7 cells (15.37±0.34 µmol/ml).However *M.ferrea* L bark extracts (MFBHE, MFBEE and MFBME) exhibitdose-dependent decrease in nitrite levels in the presence of LPS.The nitric oxide inhibitory activities of MFBHE, MFBEE and MFBME at 50 and 100µg/ml were found to be 20.85% &28.32%, 40.48% & 51.55% and 30.80% & 43.61% respectively, and L-NAME, an inhibitor of nitric oxide synthase was taken as a positive control for comparing the nitric oxide inhibitory activity of *M.ferrea*Lbark extracts and its percentage of inhibition at 100µm was found to be 68.15.The results have shown that MFBEE showed significant anti-inflammatory activity on nitric oxide production compared to MFBHE and MFBME.

Effect of M.ferrea L bark extracts on LPS-induced PGE_2 levels in RAW 264.7 cells

PGE_2 is an important proinflammatory mediator produced in COX-2 pathway, bacterial lipopolysaccharides (LPS) and proinflammatory cytokines Interlukine-1β, interlukine-6, tumor necrosis factor-alpha (TNF-α) induce COX-2 gene expressions in pathological conditions.Therefore, to evaluate the anti-inflammatory effect of *M. ferrea* L bark extracts (MFBHE, MFBEE and MFBME) on PGE_2production, the RAW 264.7 cells was pre-treated with *M. ferrea* L bark extracts of various concentrations for 2 hours before treatment with LPS for 24 hours.PGE_2 concentration in culture medium was measured by using ELISA.

As shown in the Figure2 *M. ferrea* L bark extracts (MFBHE, MFBEE and MFBME) exhibitedconcentration dependent decreasein PGE_2 levels in the presence of LPS. The IC_{50} values of MFBHE, MFBEE and MFBME were found to be 100.92µg/ml, 73.28µg/ml and 90.58µg/ml respectively. Among different extracts *M.ferrea*Lbark ethyl acetate extract exhibited the significant inhibitory(***p≤0.001)effecton PGE_2 production with less IC_{50} values.The results reveal that MFBEE showed significant anti-inflammatory activity on PGE_2 production compared to MFBHE and MFBME.

Effect of M. ferrea L bark extracts on pro-inflammatory cytokines in RAW 264.7 cells

Effect of M. ferrea L *bark extracts on TNF-α production in RAW 264.7 cells*

As TNF-α is one of the crucial pro-inflammatory cytokine causing inflammation, the different *M. ferrea* bark extracts (MFBHE, MFBEE and MFBME) were evaluated on TNF-α production in RAW 264.7 cells as measured by ELISA[11].As shown in the Figure 3 the TNF-α production was decreased in plant *M. ferrea* L bark extracts treated cells. LPS-stimulated RAW 264.7 macrophages significantly increased TNF-α production (646.83±2.96pg/ml) compared to untreated control RAW 264.7 cells (153.70±3.42pg/ml). However *M. ferrea* L bark extracts (MFBHE, MFBEE and MFBME) exhibit a concentrationdependent decrease in TNF-α level in the presence of LPS. The TNF-α inhibitory activity of MFBHE, MFBEE and MFBME at 50 and 100µg/ml were found to be 8.73% & 15.44%, 29.90% &46.50% and 16.99% & 29.00% respectively. The IC_{50} values of MFBHE, MFBEE and MFBME were found to be 144.97µg/ml, 71.98µg/ml and 119.83µg/ml respectively. Among different extracts *M. ferrea* L bark ethyl acetate extract exhibited the significant inhibitory (***p≤0.001) effecton TNF-α production with less IC_{50} values. The

results revealed that MFBEE showed a significant anti-inflammatory activity on TNF-α production compared to MFBHE and MFBME.

Effect of M. ferrea L bark extracts on IL-1β production in RAW 264.7 cells

As Interlukin-1 is oneof the important acute inflammatory mediators, the effect of *M. ferrea* L bark extract was assessed in RAW 264.7 cells[12].As shown in the Figure 4 the IL-1β production was decreased in *M. ferrea* L bark extracts treated cells. LPS-stimulated RAW 264.7 macrophages significantly increased IL-1β production (48.75±0.77pg/ml) compared to untreated control RAW 264.7 cells (12.32±0.43pg/ml). However *M. ferrea* L bark extracts (MFBHE, MFBEE and MFBME) exhibit concentration-dependent decrease in IL-1β levels in the presence of LPS. The IL-1β inhibitory activities of MFBHE, MFBEE and MFBME at 50 and 100µg/ml were found to be 17.87%& 25.86%, 28.86% & 47.62% and 19.87% & 28.43 respectively.

The IC_{50} values of MFBHE, MFBEE and MFBME were found to be 130.53µg/ml, 72.33µg/ml and 114.54µg/ml respectively. Among different extracts *M. ferrea* L bark ethyl acetate extract exhibited the significant inhibitory (***$p \leq 0.001$) effect on the IL-1βproduction with less IC_{50} values.

The results reveal that MFBEE showed significant anti-inflammatory activity on the IL-1β production compared to MFBHE and MFBME.

In vivo anti-inflammatory assays

Acute toxicity studies on *M. ferrea* L extracts

An acute toxicity study was carried out according to OECD guidelines. In acute toxicity studies no mortality was observed in mice for all the doses of *M.ferrea* L bark extracts (MFBHE, MFBEE and MFBME) during the observation period of 24-48 hours. The lethal dose (LD_{50}) cutoff doses obtained for various extracts are shown in the Table 1.

Based on these LD_{50} values, two doses (200 and 400mg/kg body weight) of *M. ferrea* L bark extracts were selected as safe doses in *in vivo* anti-inflammatory models such as carrageenan-induced paw oedema.

Anti-inflammatory effect of M. ferrea L bark extracts on carrageenan-induced paw edema in Wistar rats[9].

The anti-inflammatory effects of *M. ferrea* L bark extracts (MFBHE, MFBEE and MFBME) in carrageenan-induced rat paw oedema model were shown in the Figure 5.

It was observed that doses of MFBEE-200 and 400 groups exhibited significant inhibition at both early and late phases, though the maximum inhibition has occurred in late phase i.e. 46.90% and 58.35% respectively and doses of MFBME-200 and 400 groups exhibited inhibition at both early and late phases, though the maximum inhibition has occurred in late phase i.e. 36.79% and 41.94% respectively, howeverdoses of MFBHE-200 and 400 groups exhibited moderateinhibition at both early and late phases, though the maximum inhibition has occurred in late phase i.e. 10.26% and 21.38% respectively. Both MFBEE-200 and MFBEE-400 groups exhibited anti-inflammatory activity in dose dependent manner and significantly (***$p \leq 0.001$) reduced mean paw oedema at late phase as compared with control. MFBEE exhibited maximum inhibitory activities than MFBHE and MFBME. MFBEE extracts anti-inflammatory activity is comparable with reference drug diclofenac (10 mg/kg) showed significant percent inhibition of 81.26% in early and late phases.These results suggest that the inhibitory effect of MFBEE on paw oedema formation is probably due to inhibiting synthesis of inflammatory mediators by inhibiting COX-2 and LOX enzymatic systems.

DISCUSSION

Though *Mesua ferrea* L. has been used as traditional medicine for treatment of inflammatory related diseases such as arthritis, leprosy and cancer. There are only few studies reported on anti-inflammatory activities of *Mesua ferrea*. No further work has been carried out on the mechanism of action for *Mesua ferrea* bark extracts, sub fractions and its isolated compounds in particularly inhibitory effects on lipopolysaccharide-induced nitric oxide, PGE_2, TNF-α, IL-1β in RAW 264.7 cells.Hence the present study has been undertaken to evaluate the anti-inflammatory effects of *Mesua ferrea* bark extracts by determination of inhibitory effects on LPS-induced nitric oxide, PGE_2, TNF-α, IL-1β in RAW 264.7 cells. Macrophages play an important role in inflammation through the production of several pro-inflammatory mediators and cytokines such as NO, PGE_2, TNF-α, and IL-1β. Excessive productions of these pro-inflammatory mediators have been associated with inflammatory diseases including atherosclerosis, rheumatoid arthritis, cancer and septic shock. Therefore, inhibition of these inflammatory mediators has been considered as a novel strategy for developing anti-inflammatory compounds. In our present study *Mesua ferrea* ethyl acetate bark extract (MFBEE) dose dependently decreases the LPS-induces nitric oxide levels in RAW 264.7 cells.

PGE_2 is prominent prostaglandin produced in inflammatory site by COX-2 synthase. Abnormal levels of PGE_2 results in a wide range of inflammatory diseases such as cardiac diseases, autoimmune diseases and arthritis[13].In the present study *Mesua ferrea* bark ethyl acetate extract (MFBEE) dose dependently supress the LPS-induced PGE_2 levels in RAW 264.7 cells. Nobiletin is a polymethoxy flavonoid isolated from citrus fruits which inhibits the PGE_2 in RAW 264.7 cells[14,15].

TNF-α and IL-1β are major proinflammatory cytokines promotes various chronic inflammatory diseases and autoimmune diseases. Increased concentrations of both IL-1β and TNF-α involved in progression of inflammatory diseases by inducing synthesis of prostaglandins and acute phase proteins. Our results revealed that *Mesua ferrea* bark ethyl acetate extract (MFBEE) inhibits the synthesis of pro-inflammatory cytokines concentration dependently in LPS-induced RAW 264.7 cells. Pycnogenol is a phenolic compound purified from the bark of *Pinus maritime Mill* and is an effective anti-inflammatory compound which is highly capable of reducing the synthesis of IL-1β and TNF-α appearance of IL-1β mRNA in LPS stimulated RAW 264.7 cells[16]. From *in vivo* anti-inflammatory studies *M. ferrea* L bark extracts did not show toxic effects

up to 250 and 500mg/kg body weight. *M. ferrea* L bark extracts, especially MFBEE shows inhibitory activities at the late phase of carrageenan-induced paw oedema.

Our results revealed that *Mesua ferrea* bark ethyl acetate extract (MFBEE) inhibits the synthesis of pro-inflammatory cytokines concentration dependently in LPS-induced RAW 264.7 cells. From *in vivo* anti-inflammatory studies *M. ferrea* L bark extracts did not show toxic effects up to 250 and 500mg/kg body weight. *M. ferrea* L bark extracts, especially MFBEE shows inhibitory activities at the late phase of carrageenan-induced paw oedema.

All these *in vitro* results have been correlated with *in vivo* results of carrageenan-induced paw oedema inhibition implying the consistency in anti-inflammatory activities of *M. ferrea* L bark ethyl acetate extract. The potential anti-inflammatory activity of *M. ferrea* L was found to be due to the presence of phyto compounds such as flavonoids, terpenoids, steroids (glycosides, cardiac glycosides) and quinones. *M. ferrea* L bark extracts are a potential candidate for development of anti-inflammatory compounds.

CONCLUSION

The present study deals with the evaluation of anti-inflammatory activities of *Mesua ferrea* bark hexane, ethyl acetate and methanol extracts by *in vitro* and *in vivo* methods. All these *in vitro* results have been correlated with *in vivo* results of carrageenan-induced paw edema inhibition implying the consistency in inflammatory activities of *Mesua ferrea* bark ethyl acetate extract. The potential anti-inflammatory and antioxidant activities of *Mesua ferrea* were found to be due to the presence of secondary metabolites such as flavonoids, terpenoids, steroids (glycosides, cardiac glycosides) and quinones. *Mesua ferrea* bark extracts are a potential candidate for development of anti-inflammatory agents useful for curing various inflammatory diseases.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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