Pathophysiology 26 (2019) 323–330

Contents lists available at ScienceDirect

Pathophysiology

journal homepage: www.elsevier.com/locate/pathophys

Anti-inflammatory efficacy of methanolic extract of *Muntingia calabura* L. leaves in Carrageenan induced paw edema model

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ARTICLE INFO

Article history:
Received 5 March 2019
Received in revised form 6 August 2019
Accepted 8 August 2019

Keywords:
Muntingia calabura
Acute toxicity
Anti-inflammatory
Paw edema

ABSTRACT

The plant *Muntingia calabura* L. is a well-known herb which gained attention due to its pharmacological value. The necessity of this plant in human ailments is illustrious in old medical practices. *Muntingia calabura* L. leaves were therapeutically used for ulcer, fever, headache etc. The study was designed to assess the acute toxicity and anti-inflammatory potential of methanolic extract of *Muntingia calabura* L. (MEMC) in *in vivo* models. Two different doses (550, 2000 mg/kg body weight) of MEMC were taken to evaluate the acute toxicity response. The drugs were given orally to wistar rats and were monitored for behavioral changes and mortality for 14 days period. The blood parameter analysis, serum analysis of liver and kidney injury markers and histopathological evaluation of kidney, heart and liver were carried out. The Carrageenan induced paw edema model was performed to inspect the anti-inflammatory response of MEMC. The level of CRP in serum and the histological alterations in the paw tissue were evaluated. There were no evident symptoms of toxicity observed in animals treated with MEMC at the dose of 2000 mg/kg body weight. The Carrageenan induced paw edema model study established the anti-inflammatory potential of MEMC. The MEMC, which is innoxious, can act as a potential anti-inflammatory drug.

1. Introduction

Inflammation is the accomplishment of a protective response which usually occurs when our body encounters with microbial attack, tissue injury, cancer, cell death etc [1,2]. It offers a beneficial role by eliminating the harmful stimuli and by healing the impaired tissue. The advancement of inflammation is mostly by the combined effect of innate and adaptive immune response [3,4]. Immune cells like macrophages, dendritic cells, lymphocytes, neutrophils etc displayed an influential role in the development of inflammatory response [5]. Various inflammatory mediators (pro-inflammatory mediators and anti-inflammatory mediators) were released upon different inflammatory responses [6]. Interferons, interleukins, tumor necrosis factor, prostaglandins, leukotrienes etc are some of the mediators of inflammatory response. Free radical overcrowdling, activation of complex enzymes, release of inflammatory and pro-inflammatory mediators etc may lead to the advancement of acute inflammatory responses. Cyclooxygenase enzyme (COX) plays a major role in the process of inflammation by synthesizing prostaglandins, prostacyclins and thromboxanes which elicits inflammation, pain and aggregation of platelets [7]. Steroidal and non-steroidal anti-inflammatory drugs are the extensively used drugs for various inflammatory disorders until now. The adverse effects offered by the long term usage of these drugs are gastric lesions, renal failure [8] and gastro intestinal detriments [9,10]. So, there is a deemed necessity to develop safe, effective non-toxic anti-inflammatory drugs. Ayurveda, Siddha, and Unani systems of medicine is still utilizing a large number of medicinal plants for the treatment of human diseases since ancient times. Plants can synthesize a vast range of phytochemicals (secondary metabolites) with varied therapeutic values [11]. *Muntingia calabura* of Elaeocarpaceae family is a common roadside tree which is locally called Jamaican cherry tree. In Peruvian folklore medicine, the leaves, flowers and barks are thought to possess various therapeutic uses like antiseptic activity, for the relief from cold, headache and gastric ulcer, reduces swelling of prostate gland, antispasmodic activity etc. The boiled barks of *Muntingia calabura* are used for reducing the swelling of lower extremities [12,13]. The phytochemical studies conducted in our laboratory revealed the presence of various phyto constituents like alkaloids, phenolics, flavonoids, steroids, terpenoids etc in ethyl acetate, methanol and aqueous extracts of *Muntingia calabura* (data not shown here). Phytochemical screening studies established the fact that phenolics and flavonoids are its principal components. So the study was

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https://doi.org/10.1016/j.pathophys.2019.08.002
0928-4680/

aimed to evaluate the efficacy of MEMC in acute inflammatory condition. A well acknowledged method for studying the acute inflammation is the Carrageenan induced paw edema model. Some plants offer resistance to diseases and at the same time it exerts some toxicity too. In order to avoid this, an attempt was made to investigate the toxicity level of MEMC.

2. Materials and methods

2.1. Chemicals

The chemicals used in the study were of analytical grade and in good quality. Indomethacin purchased from Merck (Bangalore, India) and Carrageenan from Sigma Chemicals (St. Louis, MO, USA) were used for performing the *in vivo* experiments.

2.2. Plant materials

Muntingia calabura L. leaf samples were collected during March-April 2018 from kottayam district, Kerala, India (9.5947087 °N 76.4855729 °E). The plant material was authenticated by a taxonomist and the voucher specimen (SBSBRL.28) is maintained in the institute.

2.3. Preparation of MEMC

40 g of the plant leaf powder was weighed and extracted with 400 ml of methanol (99%) in soxhlet apparatus. The solvent was evaporated using a rotary evaporator with reduced pressure to obtain the dried MEMC. The dried MEMC were kept safe in refrigerator until further use.

2.4. Toxicity study of MEMC

The acute toxicity study was performed prior to anti-inflammatory experiments to determine whether the MEMC exerts any signs of toxicity on animal models or not. Acute toxicity study of MEMC was carried out as per Organization for Economic Cooperation and Development (OECD) guideline 423. LD_{50} was also calculated based on Acute Oral Toxicity Statistical Program (Version: 1.0).

2.4.1. Animals

For the toxicity and anti-inflammatory study, healthy female Wistar rats were used. Rats weighing 120–150 g were procured from small animal breeding station (SABS), Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala. The experimental procedures approved by the Institutional Animal Ethics Committee at School of Biosciences, Mahatma Gandhi University, Kottayam (IAEC NO. 22122017-4) in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines were followed.

2.4.2. Experimental design

Animals were divided into three groups with six rats each. Group I served as the normal control group which received only the distilled water instead of the drug. MEMC at a dose of 550 mg/kg body weight (b.wt) was orally administered to group II rats. Group III rats also orally received MEMC but at a dose of 2000 mg/kg b.wt. Group II and group III were treated as the test groups. The rats were fasted for 1–2 h prior to drug (MEMC) administration but provided a free access to water. After monitoring the body weight of each rat, a single dose of MEMC was orally administered as gavages. The treated rats were not supplied with food or water for 3 h. The MEMC administered animals were closely monitored till the entire experimental period of 14 days for any toxicity signs (convulsions, tremors, hyperactivity, salivation, lethargy, diarrhea, sleep, coma and mortality) [14,15]. On the day 15, all the rats were sacrificed after an overnight fasting and blood samples were collected. The organs of the rat (kidney, liver and heart) were weighed and kept preserved in 10% formalin for further studies.

2.4.3. Relative organ weight

Organs like liver, kidney and heart were taken out after the experimental period and weighed (as gram weight) separately. The relative organ weight was determined by the method followed by Pichika et al. [16].

2.4.4. Hematological evaluation

Blood samples collected (in EDTA tubes) were analyzed for white blood cell count (WBC), hemoglobin content (Hb) and erythrocyte sedimentation rate (ESR).

2.4.5. Tests for liver function

The serum seperated from blood samples were used for the estimation of liver function markers. Aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total bilirubin, total protein, albumin, globulin and A/G ratio levels were analyzed using standard diagnostic kits purchased from Span Diagnostics Limited, Surat, India.

2.4.6. Tests for renal function

The renal function markers such as Creatinine, blood urea nitrogen (BUN), blood urea and uric acid levels were analyzed using standard diagnostic kits procured from Span Diagnostics Limited, Surat, India.

2.4.7. Histopathological evaluation

Liver and kidney tissues of the sacrificed animals were lacerated into small pieces and were fixed in buffered formalin (10%). Those lacerated tissues were again processed via an automated tissue processor and sectioned (using rotary microtome) to obtain the tissues at a thickness of 5 μM. It was then dried overnight at 37^0C in an oven [17]. The sections were further stained using hematoxylin and eosin (H&E) and carefully observed under microscope to inspect the signs of toxicity.

2.5. Anti-inflammatory studies on MEMC

The anti-inflammatory effect of MEMC was determined using Carrageenan induced paw edema model.

2.5.1. Carrageenan- induced paw edema

Carrageenan- induced paw edema was performed based on the method followed by Winter et al [18].

2.5.2. Experimental design

A total of 30 rats were divided into 5 groups (6 rats in each group). Group I treated as the control group received only the saline. Group II was the Carrageenan (1%) alone treated group. Group III was the group treated with both Carrageenan and Indomethacin (3 mg/kg b.wt). Carrageenan and MEMC-100 (100 mg/kg b.wt) treated was the IV^{th} group and the group V rats received Carrageenan and MEMC-200 (200 mg/kg b.wt). MEMC and Indomethacin were orally administered 1 h prior to Carrageenan injection.

2.5.3. C- reactive protein (CRP) level estimation

C-reactive protein is a special kind of protein released by the liver in response to inflammatory cytokine production. Serum (separated from the blood samples of sacrificed rats) was used for the estimation of CRP using DiaSys Diagnostic kit (Germany).

Table 1
Relative organ weights of rats received MEMC (Dose in mg/kg b.wt) for 14 days. Values are expressed as mean ± standard deviation (n = 6 for each group).

Experimental groups	Liver	Kidney	Heart
Normal	3.73 ± 0.01	0.91 ± 0.02	0.65 ± 0.03
MEMC-550	3.72 ± 0.01	0.91 ± 0.01	0.66 ± 0.02
MEMC-2000	3.73 ± 0.02	0.92 ± 0.01	0.64 ± 0.01

Table 2
Hematological parameters of rats receiving MEMC (Dose in mg/kg b.wt) for 14 days. Values are expressed as mean ± standard deviation (n = 6 for each group).

Experimental groups	ESR (mm/hr)	Hb (g/dL)	Leukocytes($x10^3/\mu L$)
Normal	0.034 ± 0.01	14.13 ± 1.20	6.20 ± 0.02
MEMC-550	0.032 ± 0.02	14.38 ± 0.90	6.19 ± 0.01
MEMC-2000	0.035 ± 0.01	14.67 ± 0.70	6.21 ± 0.01

2.5.4. Histopathological analysis

Paw tissue sections of size 5 μm were fixed in 10% buffered formalin. Paraffin-embedded paw tissue sections were prepared and stained with hematoxylin–eosin (H&E). The stained paw tissue sections were thoroughly examined under a light microscope to visualize the cellular inflammatory response [17].

2.6. Statistical analysis

GraphPad Prism© version 5.03 for Windows (GraphPad Software, USA) was used to carry out all the statistical analysis. The one-way analysis of variance (ANOVA) and Dunnett's multiple comparison tests were performed with a significant p value of $p \leq 0.05$.

3. Results

3.1. Toxicity study of MEMC

Animals received MEMC at a dose of 2000 mg/kg b.wt. did not show any kind of ill effects or mortality for an experimental period of 14 days. There observed no notable clinical signs. There were no evident changes in body weight and the food and water consumption rates when compared with the normal untreated rats.

3.1.1. Relative organ weight

The relative organ weight of the treated and the non treated (normal) rats were compared and found that there was no significant ($p > 0.05$) weight difference. The relative weight of liver, kidney and heart of normal rats were found 3.73 ± 0.01, 0.91 ± 0.02 and 0.65 ± 0.03 respectively. The weight of liver, kidney and heart of MEMC treated rats were seen almost proportional with that of the normal and that was represented in Table 1.

3.1.2. Hematological evaluation

The WBC count, Hb count and the ESR are the hematological parameters analyzed in the toxicity study of MEMC. There was no significant ($p > 0.05$) variations observed in MEMC treated groups when compared with normal rats. The WBC count, Hb count and the ESR of the treated rats (MEMC treated) were found approximately equivalent to the normal rats with ESR-0.034 ± 0.01 mm/hr, Hb-14.13 ± 1.20 g/dL, and WBC-6.20 ± 0.02 × $10^3/\mu L$ and displayed in Table 2.

3.1.3. Tests for liver function

The various liver function markers evaluated in the acute toxicity study were AST, ALP, ALT, total bilirubin, total protein, albumin, globulin and A/G ratio. The AST, ALP, ALT level in the blood samples of normal rat was seemed to be 132.80 ± 0.05U/L, 143.40 ± 0.40U/L and 46.87 ± 0.50U/L respectively and the MEMC treatment did not exert any notable changes from the normal. The normal untreated rats showed an A/G ratio of 1.24 ± 0.01, albumin level of 3.33 ± 0.02 g/dL, globulin level of 2.83 ± 0.01 g/dL and bilirubin of 0.77 ± 0.05 mg/dL. There was no significant difference ($p > 0.05$) observed between the normal rats and the rats administered with MEMC which was shown in Tables 3 and 4.

Table 3
Effect of MEMC (Dose in mg/kg b.wt) on liver biochemical parameters in acute oral toxicity study. Values are expressed as the mean ± standard deviation (n = 6 for each group).

Experimental groups	AST (U/L)	ALP (U/L)	ALT (U/L)
Normal	132.80 ± 0.05	143.40 ± 0.40	46.87 ± 0.50
MEMC- 550	132.40 ± 0.05	143.30 ± 0.20	46.73 ± 0.20
MEMC- 2000	132.50 ± 0.10	142.90 ± 0.10	47.10 ± 0.10

Table 4
Effect of MEMC (Dose in mg/kg b.wt) on biochemical parameters in acute oral toxicity study. Values are expressed as mean ± standard deviation (n = 6) for each group).

Experimental groups	A/G ratio	Albumin (g/dL)	Globulin (g/dL)	Bilirubin (mg/dL)
Normal	1.24 ± 0.01	3.33 ± 0.02	2.83 ± 0.01	0.77 ± 0.05
MEMC- 550	1.22 ± 0.02	3.31 ± 0.01	2.82 ± 0.01	0.76 ± 0.05
MEMC- 2000	1.24 ± 0.01	3.33 ± 0.01	2.81 ± 0.01	0.77 ± 0.05

3.1.4. Tests for renal function

Creatinine, blood urea nitrogen (BUN), blood urea and uric acid were evaluated for determining the status of kidney function during the period of toxicity study. The MEMC received rats even at its highest concentration (2000 mg/kg b.wt) depicted no significant change ($p > 0.05$) in the parameters analyzed when compared with the normal and was represented in Table 5. The MEMC-550 (exhibited urea level of 28.24 ± 0.02 mg/dL, Creatinine level of 0.83 ± 0.02 mg/dL, uric acid level of 2.22 ± 0.02 mg/dL, BUN level of 22.44 ± 0.03 mg/dL and total protein range of 7.81 ± 0.01 g/dL) and MEMC-2000 (exhibited urea level of 28.23 ± 0.03 mg/dL, Creatinine level of 0.85 ± 0.02 mg/dL, uric acid level of 2.24 ± 0.01 mg/dL, BUN level of 22.45 ± 0.03 mg/dL and total protein range of 7.83 ± 0.01 g/dL) did not cause any alterations in renal functioning and all the renal function markers analyzed were found to be proportionate with the normal.

3.1.5. Histopathological evaluation

Histopathological evaluation of liver, kidney and heart tissues of MEMC administered animals did not show any altered histopathology or toxicity signs. The liver appeared normal with a clear lumen of central vein which is devoid of any lesions or necrosis. There were no notable changes observed in the cardiac tissue sections of MEMC treated rats. There was no evidence of renal injury in the tissue sections of MEMC treated rats. The Bowman's capsule, glomeruli, proximal and distal tubules were found to be natural and was shown in Fig. 1.

3.2. Anti-inflammatory study

3.2.1. Carrageenan -induced paw edema

The Carrageenan induced edema formation was notably suppressed by the action of MEMC-100 (71.42% inhibition), MEMC-200 (76.18% inhibition). The rate of inhibition exerted by the standard drug Indomethacin was found to be 75.36% (Fig. 2). The MEMC

Table 5
Effect of MEMC (Dose in mg/kg b.wt) on renal parameters in acute oral toxicity study. Values are expressed as mean ± standard deviation (n = 6 for each group).

Experimental groups	Urea(mg/dL)	Creatinine (mg/dL)	Uric acid(mg/dL)	BUN (mg/dL)	Total protein (g/dL)
Normal	28.24 ± 0.03	0.84 ± 0.02	2.23 ± 0.02	22.46 ± 0.03	7.83 ± 0.02
MEMC- 550	28.24 ± 0.02	0.83 ± 0.02	2.22 ± 0.02	22.44 ± 0.03	7.81 ± 0.01
MEMC- 2000	28.23 ± 0.03	0.85 ± 0.02	2.24 ± 0.01	22.45 ± 0.03	7.83 ± 0.01

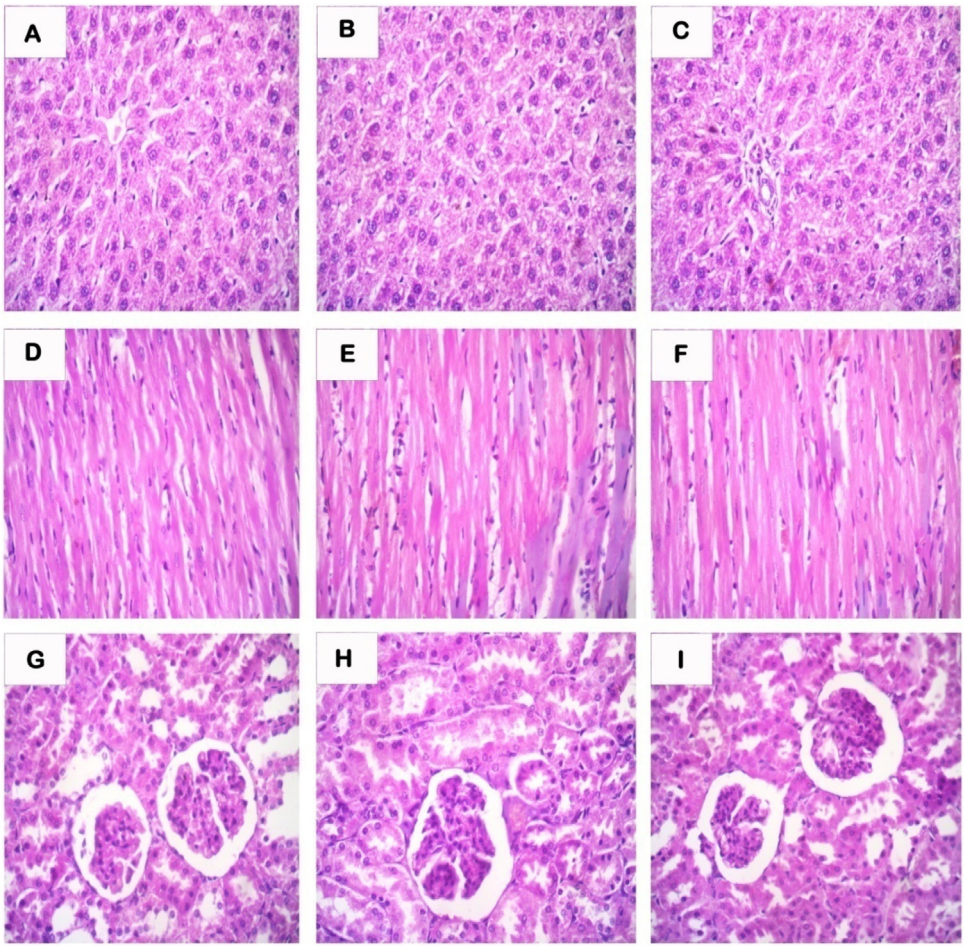

Fig. 1. Histopathology of liver (A,B,C), heart (D,E,F) and kidney (G,H,I) of control and MEMC treated rats in the acute toxicity study for 14 days. A, D, G: Normal rat; B,E,H: MEMC-550 mg/kg b.wt ; C,F,I: MEMC-2000 mg/kg b.wt.

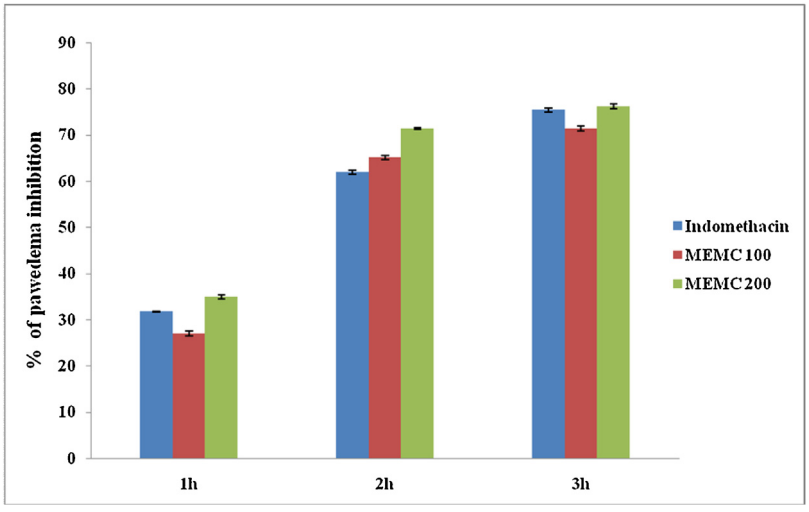

Fig. 2. Effect of MEMC on Carrageenan-induced hind paw edema in rats. All data are expressed as mean ± S.D. (n = 6).

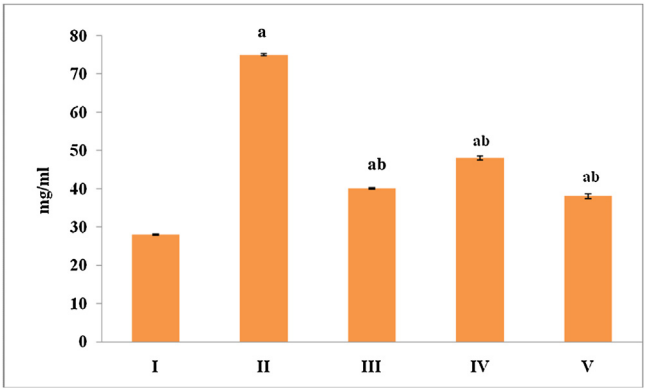

Fig. 3. Effect of MEMC on the CRP level in rats. All data are expressed as Mean ± SD (n = 6).
I-Normal control group; II- Carrageenan treated group; III- Carrageenan + Indomethacin treated group;IV-Carrageenan + MEMC (100 mg/kg b.wt) treated group; V- Carrageenan + MEMC (200 mg/kg b.wt) treated group. [a]Statistical difference with normal group at $P \leq 0.05$.[b]Statistical difference with Carrageenan treated group at $P \leq 0.05$.

was found effective in reducing the inflammation induced by Carrageenan.

3.2.2. *Estimation of C-reactive protein*

Blood samples collected from the sacrificed rats were analyzed for the CRP level. The Carrageenan alone treated group and the Indomethacin treated group exhibited a CRP level of 75.07 mg/ml and 40.07 mg/ml respectively. The CRP level of MEMC treated groups (MEMC-100-48.07 mg/ml and MEMC-200–38.05 mg/ml) demonstrated a diminished range. The results were shown in Fig. 3.

3.2.3. *Histology of paw tissue*

Inflammatory cell infiltration, proliferated epithelium, proliferated collagen, epidermal edema are the major manifestations of Carrageenan treatment. The subcutaneous layer, sub epidermal layer and the keratin layer were in normal architecture and found intact (Fig. 4a). The Carrageenan treatment caused keratin layer disruption, inflammatory cell infiltration, edema and sub epidermal edema induction (Fig. 4b). Mild epithelial hyper plasia (EHP) and Sub epidermal edema (SEE) were observed in the histology of paw tissue upon the treatment of Indomethacin (Fig. 4c). Keratin tissue regeneration, mild edema and mild inflammation were noticed in the paw tissue of MEMC (100 mg/kg b.wt) treated rats (Fig. 4d). MEMC (200 mg/kg b.wt) treatment exhibited more clear regeneration of keratin tissue. Mild inflammation (MI), Mild Sub epidermal edema(MSEE) were also noticed upon MEMC-200 treatment (Fig. 4e). MEMC was found capable of reducing the histological alterations offered by Carrageenan.

4. Discussion

Herbal medicines attained a global importance since prehistoric times. According to world health organization, 80% of the population of the remote area primarily depends upon phytomedicines for their health care needs since 60,000 years back [19]. Plant based medicines are used widely for various human ailments due to its effectiveness in disease control and safety value [20–22]. Bioactive compounds from plants are rarely evaluated for its safety and toxicity level before being used as human medicines [23]. Being the reservoir of bioactive compounds with health promoting effects, plants sometimes exerts some adverse effects too. Improper usage of herbal medicines put forth some fatal or rigorous side effects [24,25]. *In vivo* toxicity evaluation of herbal medicines is a key requisite before human administration [26,27]. Therefore the present study focused primarily on the toxicity evaluation of MEMC in experimental animal models. The study also aimed to reveal the anti-inflammatory efficacy of MEMC in *in vivo* models.

Muntingia calabura of Elaeocarpaceae family is the core species in the genus of Muntingia. The tree can perpetuate very easily and was used by the Peruvians for their health care needs. Various parts of this plant have been used by the people of different countries for so many ailments like mouth ulcers, headache, measles, stomach ache etc [28]. Leaves are used by the Peruvians for getting relief from gastric ulcer, to reduce swelling in the lower extremities etc [29]. This study was designed to provide a scientific evidence for the anti-inflammatory activity of MEMC.

The variations in the behavioral pattern and the mortality rate of rats are the indications of toxicity. Toxicity linked physiological changes were not observed in MEMC treated rats during the experimental period of acute toxicity study. The rats administered with the highest dose of MEMC (2000 mg/kg b.wt) were appeared to be normal without any change in their fur, skin and mucous membrane. Toxicity induced body changes like lethargy, convulsions, tremors, coma, mortality, etc were not observed in MEMC treated rats. The body weight of MEMC treated rats were also found normal.

Toxic effect of chemical or drugs ingested may alter the body weight of the experimental animals. Any unusual gain or loss in weight may be due to the noxious effect of the tested plant material. Food and water consumption, body weight etc were found to be normal during the entire experimental period. MEMC, even at its higher dose did not amend the relative organ weight of vital organs such as liver, kidney and heart when compared with the normal group. A distinct indication of toxicity is the relative weight of organs and not the absolute weight of organs [30]. Hematological parameters document both physiological as well as the pathological status of experimental animals and it represents a warning signal of drug induced toxicity [31]. Hematological evaluation of rats administered with MEMC acknowledged the noxious nature of MEMC.

Some biomolecules of herbal origin causes organ damage or dysfunction as an indication of toxicity [32,33]. The elevated level of liver or kidney injury marker enzyme in blood is a clear evidence for toxicity. The blood analysis of MEMC treated rats did not shows a significant ($p > 0.05$) increase in renal or liver marker enzyme status. The two major organs predominantly involved in the process of detoxification are liver and kidney, which is more susceptible to drug induced toxicity. The liver function marker enzymes (AST, ALT, and ALP) were released into the blood stream upon tissue destruction and its level is proportional with the extent of hepatic damage [34]. The level of AST, ALT, and ALP were analyzed to document the possible hepatotoxicity of MEMC. There was no notable variation observed in the enzyme level when compared with the normal. The evaluation of non enzymatic liver markers (total protein, albumin, globulin, bilirubin and A/G ratio) also illustrated the non toxic nature of MEMC. Histopathological observations from the liver tissue sections of MEMC treated rats revealed a fact that MEMC did not exert any kind of hepatic damage. Kidney damage was assessed by measuring the level of creatinine, urea, uric acid and BUN in the blood. There was no significant variation observed in the kidney function marker enzymes in blood samples of MEMC treated rats. The MEMC treated and the non treated animals were compared for histopathological observations and were found that MEMC was safe for renal functioning. There was no noticeable histological alternations in the cardiac tissues of MEMC treated rats which proven the fact that MEMC is safer for heart.

The process of inflammation induced by Carrageenan is a biphasic event [35]. The histamine and serotonin were released during the initial phase of inflammation [36]. The release of prostaglandin,

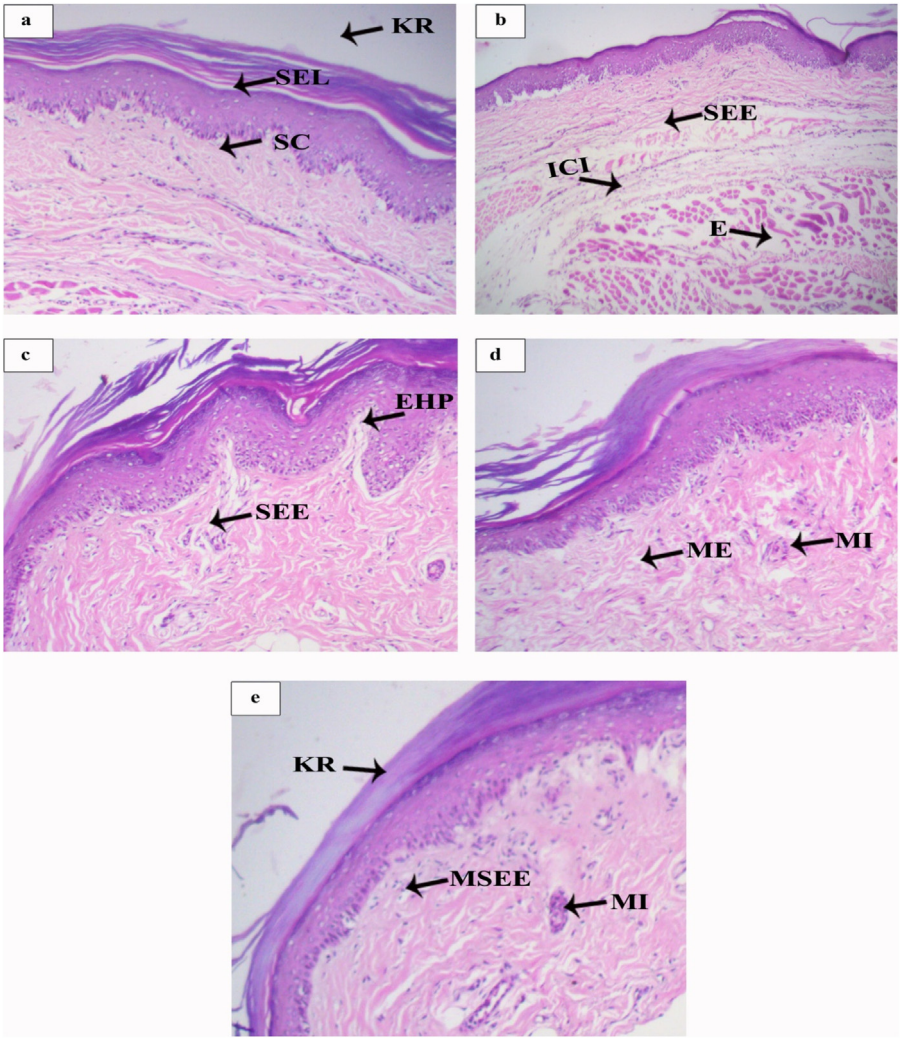

Fig. 4. Histology of rat paw tissue (H&E stain 40×) in Carrageenan-induced paw edema.a: Normal; b: Carrageenan; c:Carrageenan + Indomethacin; d: Carrageenan + MEMC-100 mg/kg b.wt; e:Carrageenan + MEMC-200 mg/kg b.wt. a) Cross section of normal paw tissue shows keratin (KR), Sub epidermal layer(SEL),Sub cutaneous layer (SC). b) Cross section of Carrageenan induced rat paw tissue shows massive influx of inflammatory cell infiltration (ICI), Sub epidermal edema (SEE) and Edema (E). c)Cross section of tissue of Carrageenan + Indomethacin shows sub epidermal edema (SEE), Mild epithelial hyper plasia (EHP). d) Cross section of the paw tissue of Carrageenan + MEMC (100 mg/kg b.wt) shows Mild edema (ME) and Mild inflammation (MI). e) Cross section of paw tissue of Carrageenan + MEMC (200 mg/kg b.wt) shows keratin (KR), Mild inflammation (MI), Mild Sub epidermal edema (MSEE).

lysozyme and bradykinin was mediated during the second phase of swelling. Steroidal and non-steroidal agents can exert its role during the second phase of edema formation [37]. The inhibition of edema by MEMC was clearly observed during the second phase of inflammation and that could be due to the anti-inflammatory effect of MEMC by the inhibition of prostaglandin secretion. The other possible mechanism of action of MEMC was by the suppression of histamine, serotonin and bradykinin, which are involved in the first and second phase of Carrageenan induced edema formation [38]. MEMC was found more effective in reducing the Carrageenan induced paw edema than the standard drug Indomethacin.

CRP is considered as one of the key regulators of inflammation. During acute and chronic inflammation, the level of CRP gets elevated [37]. Thus in this study the level of CRP was monitored to document the extent of inflammation. The level of CRP was found to be reduced in the MEMC treated groups and that could be due to the activity of anti-inflammatory components present in MEMC. MEMC was seen more effective than the Indomethacin. So MEMC have the potential for the replacing the currently used anti inflammatory drugs (NSAIDs).

Histopathological observation of rat's paw tissue showed marked reduction in inflammation and edema in MEMC treated group than the Carrageenan alone treated group. MEMC treated groups showed a distinct improvement from edema, inflammatory cell infiltration and other inflammatory responses aroused in association with inflammation. The anti-inflammatory effect exerted by MEMC can effectively resolve the histological alterations aroused as a result of inflammation than Indomethacin. A notable reduction in hyper keratinization also proved the anti-inflammatory nature of MEMC. The histological alterations developed in association with inflammation can be resolved by the trenchant anti-inflammatory potential of MEMC.

The possible mechanism of action of MEMC is thought to be due to the inhibition of the primary inflammatory mediators. The secondary inflammatory mediators were released upon the activation of primary mediators. Upon the inhibition of primary mediators via the action of MEMC, secondary mediators were not released and further inflammatory responses were blocked. Thus MEMC can be used for the effective reduction of inflammatory responses generated in association with various diseases.

5. Conclusion

The present study concluded that the MEMC did not exert any kind of toxicity upto a dose of 2000 mg/kg b.wt of experimental animals. The anti-inflammatory potential of MEMC was clearly understood from the Carrageenan induced paw edema model study. The reduced level of CRP in the blood samples of MEMC treated animals documented the anti-inflammatory efficacy of MEMC. The results obtained from this study offer a supportive data for the use of *M.calabura* in therapeutic purposes. Further studies are needed for the better understanding of the mechanism of action of MEMC for resolving the various health issues of humankind.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent

This article does not contain any studies with human performed by any of the authors.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

Acknowledgements

The authors are grateful to School of Biosciences, Mahatma Gandhi University, Kottayam, India for providing the excellent research facilities. Financial assistance in the form of JRF, from DST-PURSE-PII programme, Government of India, to Jisha ninan is gratefully acknowledged.

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