

Margaritaria discoidea (Euphorbiaceae) stem bark extract attenuates allergy and Freund's adjuvant-induced arthritis in rodents

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ABSTRACT

Background: Various parts of *Margaritaria discoidea* find use in traditional medicine in the treatment of pain and oedema. This study evaluated the anti-allergic, anti-inflammatory and anti-arthritis effects of a 70% (v/v) aqueous ethanol extract of the stem bark of *Margaritaria discoidea*, MDE in rodents. **Materials and Methods:** Systemic anaphylaxis was induced by the injection of compound 48/80 into mice and their survival rate was monitored to evaluate the anti-allergic action of the extract. The effect of MDE assessed on the maximal and total oedema responses in the mouse carrageenan-induced paw oedema was used to evaluate the anti-inflammatory action of the extract while the Freund's adjuvant-induced arthritis model was employed to study the anti-arthritis effects of MDE. **Results:** MDE dose-dependently increased the time for compound 48/80-induced mortality in mice. MDE suppressed the mean maximal swelling and the total paw swellings induced over 6 h in the carrageenan-induced paw oedema when administered either prophylactically or therapeutically. MDE caused a reduction in serum levels of TNF α and IL-6 and significantly suppressed Freund's adjuvant-induced arthritis. **Conclusion:** *Margaritaria discoidea* suppresses allergy and exhibits anti-inflammatory activity in mice. In addition it attenuates Freund's adjuvant-induced arthritis through a reduction in serum levels of TNF α and IL-6 in rats.

Key words: Allergy, anaphylaxis, anti-inflammatory, arthritis, *Margaritaria discoidea*

INTRODUCTION

Inflammation underlies virtually all human and animal diseases and consequently attracts the focus of global scientific research. It is the response of living tissues to injury and involves a complex array of activation of protein tyrosine kinases and subsequent release of autocooids. There is extravasation of fluid, cell migration, tissue damage and repair.^[1] Allergy an immediate hypersensitivity reaction is a component of the inflammatory response. An important manifestation of the allergic response is mediated by mast cells and basophils. These immune cells express on their surface the receptor with high affinity for immunoglobulin E (IgE) called the Fc ϵ RI.^[2] Cross-linking

of surface bound IgE results in an immediate explosive release of preformed mediators, including histamine, the more gradual elaboration of pro-inflammatory lipid mediators such as arachidonic acid and its metabolites and finally the *de novo* synthesis of cytokines and chemokines.^[3] Clinical manifestations of the allergic response can range from seasonal allergic rhinitis to the life-threatening anaphylaxis.^[4-7] Further, there are reports that mast cells accumulate at sites of chronic inflammation and this provides an irrefutable evidence that their role is not only restricted to the initiation of immune responses; examples include the gut in inflammatory bowel disease or helminthic infection, the asthmatic airway, sclerodermatous skin, and lung in interstitial pulmonary fibrosis.^[8-11]

Though significant progress has been recorded in medical research during the past decades, treatment of inflammatory diseases still remain one of the world's major health problems.^[12-14] The conventional steroidal and non steroidal anti-inflammatory drugs (NSAIDs)

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used to ameliorate this phenomenon are bedevilled with life-threatening side effects. Consequently, plants meet the prescription for the search for new regimens with reduced or no side effects. One such plant is *Margaritaria discoidea* (Baill.) Webster syn. *Phyllanthus discoideus* (Baill.) Mull-Arg of the Euphorbiaceae family. It abounds in tropical Africa and is very common in Senegal and Western Cameroon.^[15] It grows to a height of about 30m. In West and Central Africa the stringy and fibrous bark is commonly used as a purgative and anthelmintic.^[16,17] Among the uses of the plant in traditional medicine, Irvine (1961) reports that in Congo (Brazzaville) the bark decoction is also used for quick relieve of stomachache and kidney complaints and to facilitate parturition while the powdered bark-extract is applied to swellings and inflammation and toothache in Malawi and Sierra Leone respectively.^[18] Phytochemical screening of the bark of *M. discoidea* has been reported to yield about 10% tannins^[15,16] while a number of alkaloids have been isolated notably phylochrysin and securinine with the former exhibiting a central nervous stimulant account in part for the plant's stimulatory properties.^[15-17]

Recent pharmacological screening of the plant by Adedapo *et al.*, (2009) demonstrated anti-inflammatory and analgesic activities of the aqueous extracts of the stem bark in rats^[19] while Dickson *et al.*, (2010) also reported of antibacterial, antioxidant and anti-inflammatory effects of the leaves and stem bark in the carrageenan-induced paw oedema in chicks.^[20] However, literature reviews indicated that no studies specifically on the anti-allergic and anti-arthritis actions of the stem bark of *M. discoidea* have so far been undertaken.

Taking this in view and as a part of our on-going research on anti-inflammatory actions of medicinal plants, we evaluated the effect of a 70% aqueous ethanol extract of the stem bark of *M. discoidea* on experimental immediate allergic reactions and Freund's adjuvant-induced arthritis in the present study.

MATERIALS AND METHODS

Collection of plant material

The fresh stem bark of *M. discoidea* as identified by a local herbalist was harvested in January 2012 at Kente in the Ashanti Region, Ghana. The identity was confirmed as the stem bark of *M. discoidea* (Baill.) Webster syn. *Phyllanthus discoideus* (Baill.) Mull-Arg (Euphorbiaceae) by anatomical observation and direct comparison with the authentic specimens, stored in the herbarium in the Department of Pharmacognosy, KNUST, Kumasi and a voucher specimen (FP/EU/01/2012) deposited in the same department. The plant was chopped into pieces and air dried for 7 days.

Preparation of plant extract

Dried stem bark of 2.0 kg of the plant was ground using a heavy duty blender (37BL85 (240CB6), Waring Commercial, USA) and extracted for 5 days with 70% *v/v* ethanol (4L) by cold maceration. The ethanol filtrate was concentrated under reduced pressure at 45°C by a vacuum rotary evaporator (R-210, BUCHI, Switzerland) and further dried in an oven (Gallenkamp OMT, Sanyo, Japan) to yield a solid mass of weight 200 g. The dried extract freshly dissolved in saline (0.9% *w/v*) was designated MDE and administered to the animals.

Animals

C57BL/6 and ICR mice (25-30 g) of both sexes and male Sprague-Dawley rats (180-200 g) were purchased from Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana. The animals were kept in the Animal House of the Department of Pharmacology, College of Health Sciences, KNUST, Kumasi, Ghana and allowed to acclimatize to the laboratory conditions (temperature 23 ± 2°C with a 12 h light-dark cycle) for 14 days. Animals had free access to commercial pellet diet (GAFCO, Ghana) and water *ad libitum* but starved overnight prior to use. Each animal was used only once as all animals were euthanised at the end of each experiment. The animals were humanely handled throughout the experiment in accordance with internationally accepted principles for laboratory animal use and care (EEC Directive of 1986: 86/609 EEC). Additionally all animal experiments were approved by the Ethics Committee of the Department of Pharmacology, KNUST.

Chemicals and reagents

Compound 48/80 (C2313), λ-Carrageenan, Dexamethasone and Aspirin were purchased from Sigma-Aldrich (St Louis, USA). Diclofenac and Liquid Paraffin were purchased from Troge (Germany) and Actavis Ltd (UK) respectively. Rat TNFα Quantikine (Cat.# RTA00) and rat IL-6 (Cat.# SEA079Ra) ELISA kits were purchased from R and D Systems, (USA) and Usbn Life Science Inc. (Wuhan, China) respectively.

Microorganism

Heat-killed *Mycobacterium tuberculosis* [strains C, DT and PN (mixed)] was a kind donation from the Ministry of Agriculture, Fisheries and Food, U.K.

Preparation of the adjuvant

Heat-killed *Mycobacterium tuberculosis* [strains C, DT and PN (mixed)] of 20 mg was finely ground in a mortar. Enough liquid paraffin was added and triturated to 5 mg ml⁻¹ suspension herein called Complete Freund's Adjuvant, CFA. Sterile liquid paraffin is referred to as Incomplete Freund's adjuvant, IFA.

Compound 48/80-induced systemic anaphylaxis

C57BL/6 mice (25-30 g) were given an i.p. injection of 10 mg kg⁻¹ of the mast cell degranulator Compound 48/80 to induce systemic anaphylaxis as previously described by Kim *et al.*, (2005).^[21] Either vehicle or MDE 30, 100 and 300 mg kg⁻¹ was given orally 1 h before administration of compound 48/80. Mortality was monitored for 1 h after induction of anaphylaxis.

Carrageenan-induced paw oedema

Pedal oedema was induced by injection of a 1% carrageenan suspension in normal saline (50 µl, s.c.) into the sub plantar tissue of the right hind paw of ICR mice (25-30 g) as earlier described by Winter *et al* (1962).^[22] Oedema was monitored with an electronic calliper (Z22855, Milomex Ltd, Bedfordshire, UK) at 1 h intervals over 6 h as the mean percentage increase in paw thickness. Total oedema induced during the 6 h was measured as area under the time course curves (AUC). Drug effects were evaluated by comparing the maximal and total oedema responses attained during 6 h in drug-treated groups with the corresponding values attained in drug vehicle-treated inflamed control groups. In the preventive (prophylactic) protocol, drug- vehicle, MDE 30, 100 and 300 mg kg⁻¹, and aspirin 100 mg kg⁻¹, was given orally 1 h before the induction of the oedema while in the curative (therapeutic) protocol, treatments were done 1 h post oedema induction.

Rat adjuvant-induced arthritis

Adjuvant arthritis was induced as previously described.^[23] Right hind paw of Sprague-Dawley rats (200-250 g) were injected intraplantar with 100 µl of Complete Freund's Adjuvant (CFA). Arthritic control group received intraplantar injection of CFA, while non-arthritic control group received only intraplantar injection of 100 µl sterile paraffin oil (Incomplete Freund's Adjuvant, IFA). Foot volume was measured by water displacement plethysmography^[24] for the ipsilateral (injected) and contra-lateral (non-injected) hind paws prior to intraplantar injection of CFA (day 0) and daily for 28 days. The oedema component of inflammation was quantified by measuring the difference in foot volume between day 0 and the various time points. Data for the paw volumes were individually normalized as percentage of change from their values at day 0 and then averaged for each treatment group. Total oedema induced during the acute phase (day 0-10) and polyarthritic phase (day 0-28) was measured as area under the time course curves (AUC). In the preventive (prophylactic) protocol, drug vehicle, MDE 30, 100 and 300 mg kg⁻¹ and diclofenac 6 mg kg⁻¹, was given orally 1 h before the induction of the oedema on day 0 and daily for 28 days while in the curative (therapeutic) protocol, treatments commenced on the 10th day post oedema induction till the 28th day. All drugs were freshly prepared on each day of drug administration.

Quantitative determination of serum cytokines (TNF α and IL-6) concentration by enzyme-linked immunosorbent assay

Adjuvant arthritis was induced as previously described.^[23] Drug-vehicle, dexamethasone 1 mg kg⁻¹ or MDE 30, 100, 300 mg kg⁻¹ was given orally 1 h before the induction of the oedema and daily for 12 days. Blood was collected from the rats by cardiac puncture and allowed to clot for 2 h at room temperature and then centrifuged at $\times 1000$ g for 20 min. Serum levels of TNF α and IL-6 were measured in duplicate with the appropriate ELISA kit following the manufacturer's instructions. Briefly, a monoclonal antibody specific for TNF α or IL-6 was precoated onto a microplate. The standard and test samples were then pipetted into the wells to allow binding of TNF α or IL-6 to the immobilized antibodies. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for TNF α or IL-6 was added to the wells. Following removal of unbound antibody-enzyme reagent through washing, a substrate solution was added to the wells and colour was developed. The enzyme-substrate reaction was terminated by the addition of a Stop solution and the colour change measured spectrophotometrically, measuring the OD in an Ultra Microplate Reader ELx 808IUI (Bio-Tek Instruments, Inc, USA) at a wavelength of 450 ± 2 nm. The concentration of TNF α or IL-6 in the serum was accordingly calculated from standard curves.

Statistical analysis

All data were reported as Mean values \pm standard error of mean (s.e.m), $n = 5$. Group means were compared using a one-way analysis of variance (ANOVA), followed by Neuman-Keul's range test where a difference existed. Differences were considered significant at $P \leq 0.05$. In case of the lethality rate, the Kaplan-Meier Survival plots were used and data analysed by the Log-rank (MantelCox) test. All graphs were plotted with GraphPad prism for Windows Version 5.00 (GraphPad, San Diego, CA).

RESULTS

Effect of *Margaritaria discoidea* extract, on Compound 48/80-induced systemic anaphylaxis

We investigated whether MDE exerts an inhibitory effect on anaphylaxis since allergen-induced degranulation of the mast cell is a component of allergy and an extreme form of the allergic reaction is anaphylaxis. To determine the effect of MDE on allergic reaction, an *in vivo* model of a systemic anaphylactic reaction was used in which compound 48/80 (10 mg kg⁻¹) was used as a model of induction for a systemic fatal allergic reaction. After the i.p. injection of compound 48/80, the mice were monitored for mortality

within 1 h. Injection of compound 48/80 into mice induced fatal shock in 100% of animals in 10 min. When MDE was orally administered (30, 100 and 300 mg kg⁻¹) the latent period for mortality with compound 48/80 was dose-dependently increased from 10 to 11.7, 13.2 and 24.2 min respectively with a corresponding significant increase in the median survival proportions [Figure 1].

Effect of *Margaritaria discoidea* extract, MDE on mouse carrageenan-induced paw oedema

Since our data showed an inhibitory effect on allergy we next examined the effect of MDE on acute inflammation. We injected 1% carrageenan into sub plantar tissue of the right hind paw of mice and evaluated drug effects by comparing the maximal and total oedema responses attained during 6 h in drug-treated groups with the corresponding values attained in control groups before and after the induction of the oedema.

When administered before (preventive) the induction of the carrageenan paw oedema, MDE (30, 100, 300 mg kg⁻¹) caused the mean maximal swelling attained during 6 h to be significantly ($P \leq 0.0005$) reduced respectively to 22.18 ± 5.43 , 26.64 ± 5.69 , and $19.40 \pm 4.05\%$ of the inflamed control response of $58.16 \pm 1.36\%$ [Figure 2a]. The total paw swellings induced over the 6 h (measured as the area under the time course curve, AUC) were also

dose-dependently and significantly ($P \leq 0.0005$) suppressed to 57.02 ± 7.68 , 53.41 ± 8.03 and $42.49 \pm 7.73\%$ of the inflamed control response respectively [Figure 2b]. After the induction of the carrageenan paw oedema MDE administered at 30 and 100 mg kg⁻¹ suppressed albeit insignificantly the mean maximal swelling to 55.45 ± 2.89 and $50.77 \pm 2.28\%$ respectively of the inflamed control response [Figure 2c]. However, at the highest dose of 300 mg kg⁻¹, the mean maximal swelling relative to the vehicle-treated groups was significantly ($P \leq 0.0005$) reduced to $45.56 \pm 2.77\%$ [Figure 2c]. Similarly, the total paw swellings induced over the 6 h were suppressed to 96.71 ± 5.04 , 91.06 ± 4.97 and $81.16 \pm 4.61\%$, respectively of the inflamed control responses [Figure 2d]. Aspirin (100 mg kg⁻¹) the reference drug used suppressed significantly all the parameters investigated [Figures 2a-d].

Effect of *Margaritaria discoidea* extract, MDE on rat adjuvant-induced arthritis

As the events of carrageenan-induced oedema and the primary phase of adjuvant-induced arthritis correspond to those in the early exudative phase of inflammation which is an important feature of the inflammatory pathology we investigated the effect of the extract on adjuvant arthritis, a model of chronic inflammation. Adjuvant arthritis was induced in the right hind paw of rats with an intraplantar

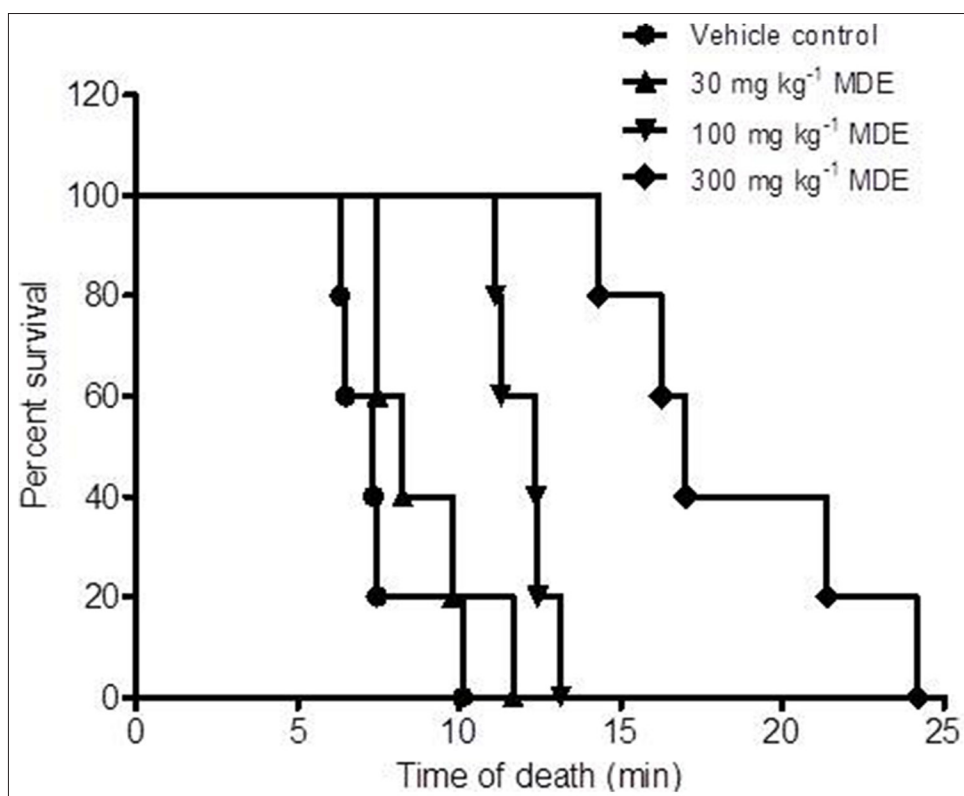


Figure 1: C57BL/6 mice (25-30 g) were pre-treated for 1 h with MDE 30-300 mg kg⁻¹. Compound 48/80 was injected (10 mg kg⁻¹, i.p.) and mortality monitored for 1 h after induction of anaphylaxis. Data was analysed using the Log-rank (Mantel Cox) test and the survival rates were significant *** $P \leq 0.0002$ showing a significant trend *** $P \leq 0.0002$ ($n=10$)

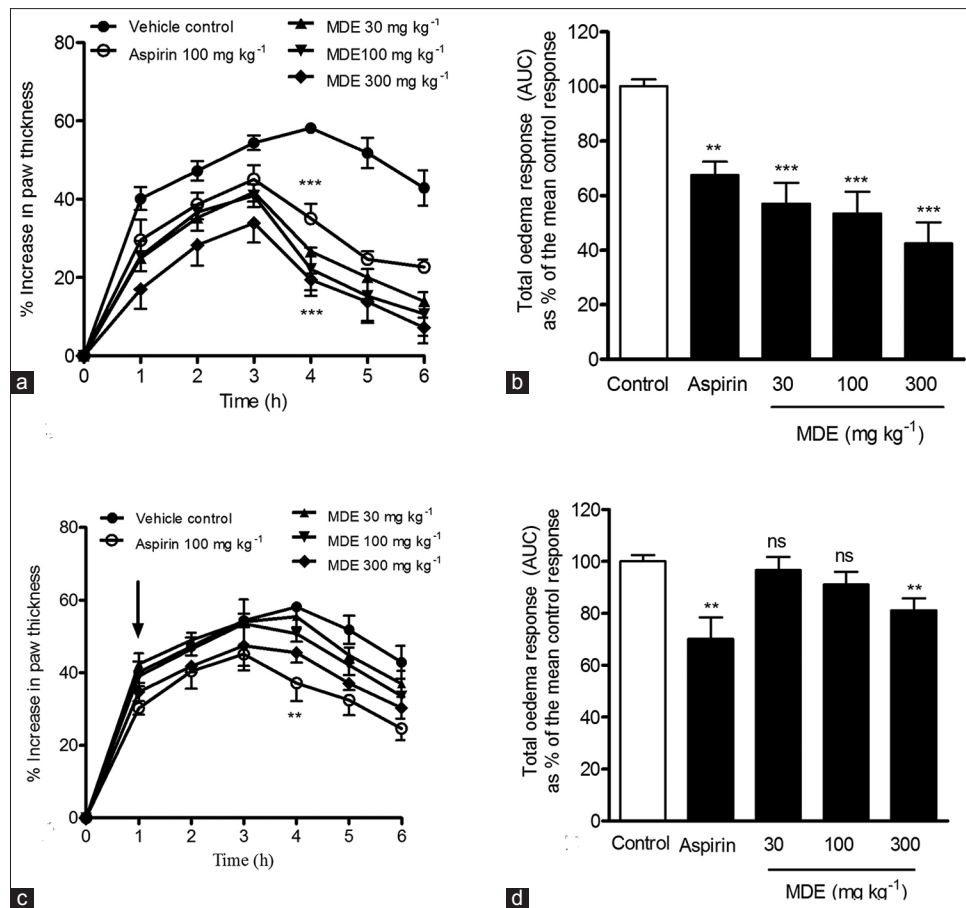


Figure 2: ICR mice (25-30 g) were injected with 50 µl of 1% carrageenan suspension into the right hind paw. Oedema was monitored at 1 h intervals over 6 h as percentage increase in paw thickness (a and c). Total oedema induced during the 6 h was calculated as area under the time course curves, AUC (b and d). In the preventive protocol (Top panel), drug vehicle, MDE 30-300 mg kg⁻¹, and aspirin 100 mg kg⁻¹ was given orally 1 h before the induction of the oedema while in the curative protocol (Bottom panel), treatments were done 1 h post oedema induction. Drug effects were evaluated by comparing the maximal and total oedema responses attained during 6 h in drug-treated groups with the drug vehicle-treated inflamed control groups. Data is presented as Mean ± s.e.m. (n=5). *** $P \leq 0.0005$, ** $P \leq 0.01$ when compared with control. Arrow indicates point of extract administration in the therapeutic protocol

injection of CFA. Drug effects were evaluated by comparing the maximal and total oedema responses attained during 28 days in drug-treated groups with the corresponding values attained in control groups before and after the induction of the oedema. All arthritic control rats showed acute inflammatory oedema at the ipsilateral (injected) paw [Figures 3a and e] followed by subsequent chronic polyarthritic phase in which the inflammation had spread to the contra-lateral (non-injected) paw [Figure 3c]. There was no significant change in the paw volume of the non-inflamed control groups that were injected with IFA throughout the study [Figure 3a].

In the preventive protocol, daily administration of the extract at 30 mg kg⁻¹ for 28 days did not cause any significant effect on the maximal oedema response. However, at 100 and 300 mg kg⁻¹ MDE, produced significant ($P \leq 0.001$) dose-dependent suppression of the mean maximal adjuvant-induced swelling to 93.05 ± 12.30 and $54.20 \pm 8.23\%$ of the mean inflamed control response

of $150.58 \pm 9.34\%$ in the injected limbs [Figure 3a]. The total adjuvant-induced response (AUC) over 28 days was also significantly and dose-dependently inhibited to 75.87 ± 3.54 , 70.85 ± 0.90 and $56.65 \pm 4.53\%$ respectively of the mean inflamed control response [Figure 3b]. The peak swelling attained in the acute phase of the adjuvant-induced response, (measured as the increase in the paw volume of the injected limb over the first 10 days after the injection of the CFA) was significantly ($P \leq 0.0005$) suppressed by the extract to 34.82 ± 1.41 , 32.97 ± 0.94 and $32.51 \pm 1.10\%$ respectively of the mean inflamed control response of $77.57 \pm 14.41\%$ [Figure 3a]. The total adjuvant-induced response (AUC) over the 10 days of the acute phase was also significantly and dose-dependently inhibited to 59.09 ± 1.03 , 57.97 ± 0.94 and $55.51 \pm 1.10\%$ respectively of the mean inflamed control response [Figure 3b].

On the contra-lateral hind limb, MDE in the same doses significantly ($P \leq 0.0001$) dose-dependently suppressed the mean maximal oedema response to 55.97 ± 4.41 ,

45.64 ± 2.24 and 22.24 ± 3.88% respectively of the mean control response of 97.67 ± 6.83% when administered prophylactically [Figure 3c] and significantly ($P \leq 0.001$) inhibited the total oedema response (AUC) over 28 days to 78.46 ± 3.49, 62.79 ± 4.19 and 45.71 ± 2.05% respectively of the mean inflamed control response [Figure 3d].

The effect of MDE on the acute phase of the arthritis is insignificant relative to the control response on both parameters (results not shown).

In the curative protocol, daily administration of MDE at 100 and 300 mg kg⁻¹ commencing the 10th day of

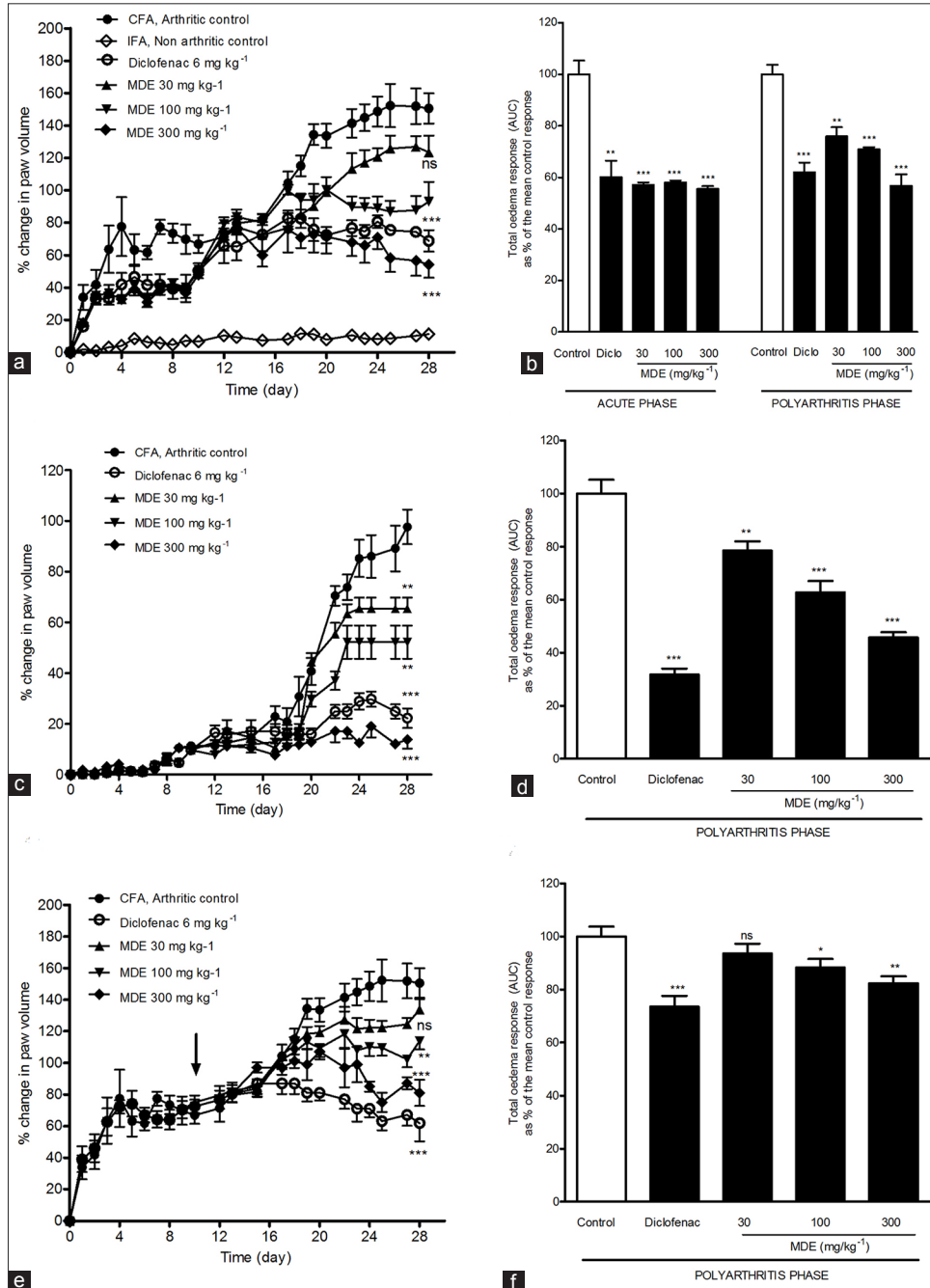


Figure 3: Sprague-Dawley rats (200-250 g) were injected with 100 µl of CFA or IFA into the right hind paw. Foot volume was measured daily for 28 days. The oedema component of inflammation was monitored as the percentage change in paw volume (a, c and e). Total oedema induced during the acute (days 0-10) and polyarthritis (days 0-28) phases was calculated as area under the time course curves, AUC (b, d and f). In the preventive protocol (Top and middle panels), drug vehicle and either diclofenac 6 mg kg⁻¹ or MDE 30-300 mg kg⁻¹, was given orally 1 h before the induction of the arthritis and daily for 28 days while in the curative protocol (Bottom panel), treatments were done from 10 days post arthritis induction. Drug effects were evaluated by comparing the maximal and total oedema responses attained during the 28 days in drug-treated groups with the drug vehicle-treated inflamed control groups. Data is presented as Mean ± s.e.m. (n=5). * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ when compared to vehicle-treated control group. ns means non significant. Arrow indicates point of extract administration in the therapeutic protocol

inoculation caused a significant ($P \leq 0.001$) suppression of the adjuvant-induced swelling on the 28th day to 122.57 ± 4.78 and $112.03 \pm 5.96\%$ respectively of the mean inflamed control response of $150.58 \pm 9.34\%$ [Figure 3e]. The total adjuvant-induced swelling (AUC) produced during the 28 days was also significantly ($P \leq 0.05$) reduced to 88.28 ± 3.23 and $82.28 \pm 2.62\%$ respectively of the inflamed control response [Figure 3f]. At 30 mg kg^{-1} MDE did not cause any significant inhibition of the mean maximal or total oedema responses. The effect of MDE on the acute phase of the arthritis is insignificant relative to the control response on both parameters (results not shown). Diclofenac at 6 mg kg^{-1} significantly suppressed both parameters in all the studies performed [Figures 3a-3f].

Effect of *Margaritaria discoidea* extract, MDE on serum cytokine (TNF α and IL-6) levels

As the pro-inflammatory cytokine TNF α is a systemic marker of inflammation and since the exact cause of rheumatoid arthritis RA remains unknown, inhibiting the production and function of pro-inflammatory mediators are thought to be an effective method to treat RA. For this reason, we assayed the influence of our extract on the serum levels of TNF α and IL-6. Adjuvant-injected (CFA) rats showed an increase in the pro-inflammatory cytokines on day 12 of the study. While serum TNF α levels reached ($1042.0 \pm 31.7 \text{ pg/ml}$; Figure 4a) relative to healthy control animals (9.49 ± 2.8 , $P \leq 0.0001$), serum IL-6 levels peaked at ($214.1 \pm 14.0 \text{ pg/ml}$; Figure 4b) relative to healthy control animals (7.49 ± 2.3 , $P \leq 0.0001$). ELISA analysis showed that serum concentrations respectively of TNF α and IL-6 were significantly decreased in the MDE treated groups, as compared with the model control groups at all doses of the extract [Figures 4a and b].

DISCUSSION

In this report, we show that *Margaritaria discoidea* administered orally exerts inhibitory effect on systemic anaphylaxis a profound form of allergy and carrageenan-induced oedema which are acute inflammatory responses. We also report that *M. discoidea* suppresses adjuvant-induced arthritis, a chronic inflammatory response.

Compound 48/80 triggers the activation of signal transduction pathways in mast cells. This results in the release of preformed mediators notably histamine from granules, generation of newly synthesized mediators such as products of arachidonic acid metabolism and lastly increased expression of cytokines.^[3] All of these mediators contribute to acute anaphylaxis a fatal allergic reaction.^[4-7] The mechanism of action of compound 48/80 is to cause a perturbation in the cell membrane with the resultant increase in permeability of the lipid bilayer membrane resulting in the degranulation of the mast cell.^[25] A drug capable of suppressing or preventing this perturbation must exhibit a potent anti-allergic action. Our findings showed that prior treatment of mice with MDE caused a delay in the time of mortality of the animals that were injected with the compound 48/80. Thus MDE might stabilize the lipid bilayer membrane, thus suppressing the perturbation from being induced by compound 48/80 and hence regulating the *in vivo* degranulation of mast cells in mice.

We assessed the action of the extract on oedema induced by carrageenan since allergy and carrageenan-induced oedema both result in the release of autocoids from allergen-specific IgE-activated mast cells and these autocoids which include

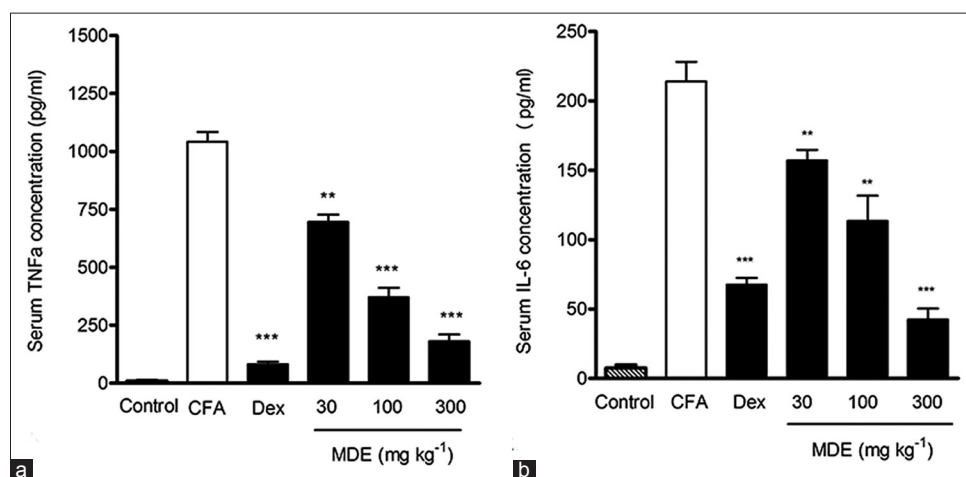


Figure 4: Sprague-Dawley rats (200-250 g) were injected with 100 μ l of CFA into the right hind paw. Drug-vehicle, dexamethasone 1 mg kg^{-1} , or MDE 30 , 100 , 300 mg kg^{-1} was given orally 1h before the induction of the oedema and daily for 12 days. Blood was collected by cardiac puncture and serum levels of TNF α and IL-6 were measured in duplicate by ELISA. Data is presented as Mean \pm s.e.m. ($n=5$). * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, compared to CFA-treated group

histamine mediate the initial phase of the inflammatory response. The development of carrageenan oedema is reported to present a biphasic response with the first phase occurring within an hour. The preformed mediators such as cytoplasmic enzymes, histamine, and serotonin are released from mast cells during the first phase^[26] and these are capable of enhancing vascular permeability, contraction of non-vascular smooth muscles, dilating precapillary sphincters and postcapillary venules.^[27] The second phase of the carrageenan-induced oedema response extends beyond an hour and is mediated by arachidonic acid metabolites including prostaglandins, leukotrienes and thromboxanes. A dose-dependent suppression of the oedema by MDE when it was given pre-emptively is in agreement with an earlier report on the inhibitory effect of the 70 % (v/v) aqueous ethanol extract of the stem bark of *M. discoidea* on acute inflammation using the carrageenan-induced oedema model in rats^[19] and also in chicks.^[20]

Events in carrageenan-induced oedema are very similar to those occurring during the primary phase of adjuvant-induced arthritis and as these events correspond to those in the early exudative phase of inflammation^[28,29] we investigated the effect of MDE on Freund's adjuvant-induced arthritis in rats. Rat adjuvant arthritis and human rheumatoid arthritis share common pathological features which include joint swelling associated with cellular and pannus invasion of the joint space, release of lysosomal constituents into the joint space, and bone resorption.^[30] The *M. discoidea* extract suppressed the swelling associated with both the acute and polyarthritic phases of adjuvant-induced arthritis when used prophylactically. Aside reducing the general swelling, the extract prevented the systemic spread of the arthritis to the non-injected limb suggesting an inhibitory effect of the extract on the progression of the arthritis. Kaibara *et al* (1983) could demonstrate that cyclosporine, an immunosuppressive drug prevented the onset of collagen-induced arthritis in rats however when used after the disease had been established; it exacerbated the condition.^[31] Again as shown by Larson, *et al* (1990) limonide, an experimental drug developed against heterologous collagen-induced arthritis exhibited similar paradoxical effects.^[32] These earlier findings strongly suggest that demonstrated anti-inflammatory effect of a drug given prior to the induction of inflammation does not necessarily guarantee the same effect when given after the induction of the inflammation. Therefore on both the carrageenan-induced paw oedema and rat adjuvant-induced arthritis models, the ability of *M. discoidea* extract to exert anti-inflammatory effects both prophylactically and therapeutically support the presence of compounds most likely exerting inhibitory effects through interference with the pathophysiological

processes underlying inflammation.

It is established that tumor necrosis factor alpha (TNF α), interleukin 1 β (IL-1 β), and prostaglandin E2 (PGE2) are among the inflammatory mediators which are produced by the invading tumor-like synovium and are primary factors of joint inflammation and articular destruction as presented in RA.^[33,34] TNF α is a multifunctional cytokine that regulates among other functions the production of other pro-inflammatory cytokines, such as interleukin-6 (IL-6) and interleukin-1 (IL-1), to mediate and/or amplify their effects in peripheral organs.^[35] During the acute inflammatory process, overproduction of TNF α is crucial to the induction of inflammatory genes and the recruitment and activation of host immune cells.^[36,37] As the proinflammatory cytokine TNF α is a systemic marker of inflammation^[38] and since the exact cause of RA remains unknown, inhibiting the production and function of proinflammatory mediators are thought to be an effective method to treat RA. In this regard, Jin *et al.*, (2010) report that drugs such as rituximab, etanercept, and tocilizumab, have been successfully approved in the clinical treatment of RA because they target specifically the TNF α receptor.^[39] It is significant therefore that MDE at the doses used suppressed significantly the serum levels of both TNF α and IL-6 during the acute phase of adjuvant arthritis in the rat suggesting this as one mechanism by which the extract down regulates inflammation.

The stem bark of *M. discoidea* reportedly contains tannins^[15,16] which exert anti-phlogistic activity^[40,41] through potent inhibition of cyclo-oxygenase-1. The remarkable inhibitory effect exerted by the total crude extract respectively on both acute and chronic models of inflammation suggests that a lot more of the constituents of the total crude extract which reportedly include alkaloids most likely played roles in the anti-inflammatory action of the plant since some alkaloids example pseudo-akuammigine have been established to exhibit anti-inflammatory actions.^[42]

CONCLUSION

We conclude that the 70 % (v/v) aqueous ethanol stem bark extract of *Margaritaria discoidea* suppresses the *in vivo* degranulation of mast cells and demonstrate anti-allergic action. In addition, it exhibits anti-inflammatory activity and attenuates Freund's adjuvant-induced arthritis through reducing the serum concentrations of TNF α and IL-6. The demonstrated suppression of components of the inflammatory response supports the traditional use of the plant in managing inflammatory disorders.

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