Afr. J. Med. Med. Sci. (2019) **48**, 307-317

Formulation and *in vivo* anti-inflammatory properties of diclofenac multiple emulsions prepared using *Vitellaria paradoxa* fat (Shea Butter)

OR Issa[1], TO Ajala[1], OA Odeku[1*], AA Oyagbemi[2] and OO Oridupa[2]

Department of Pharmaceutics and Industrial Pharmacy[1], Faculty of Pharmacy and Department of Veterinary Physiology, Biochemistry and Pharmacology[2] Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria

Abstract

Background: In the present study, diclofenac multiple emulsion was formulated using shea butter (*Vitellaria paradoxa*) as the oil phase and the anti-inflammatory properties of the multiple emulsion assessed in Wistar rats.

Methods: The multiple emulsions were prepared using the double emulsification technique and the properties (mean globule size, viscosity and creaming index) of the emulsions were assessed. The *in-vivo* anti-inflammatory activity of the multiple emulsion was assessed after topical application using two models of inflammation induction, namely formalin-induced paw lick and egg albumin-induced paw oedema.

Results: Stable diclofenac multiple emulsion was obtained with shea butter as the oil phase and surfactant mixtures, Tween 80: Span 80 ratio of 1:1 with water content of 20 %; and Tween 80: Span 80 ratio of 1:1.5 with water content of 25 %. The ranking of inhibition of inflammation after topical application of the formulation was shea butter emulsion < 2 % diclofenac emulsion < standard diclofenac gel < 1 % diclofenac emulsion < shea butter < < No treatment. This indicates that the formulation of shea butter as multiple emulsion significantly ($p < 0.05$) increased its anti-inflammatory properties while diclofenac multiple emulsion gave a dose dependent activity. In addition, the serum myeloperoxidase activity was significantly lower in treated animals compared to untreated animals.

Conclusion: Stable diclofenac multiple emulsions possessing anti-inflammatory activity was successfully developed using shea butter as carrier.

Keywords: *Shea butter, Vitellaria paradoxa, diclofenac, multiple emulsion, anti-inflammatory properties.*

Correspondence: Professor Oluwatoyin A. Odeku, Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, University of Ibadan, Nigeria. Email: o.odeku@ui.edu.ng; pejuodeku@yahoo.com.

Résumé

Contexte: Dans la présente étude, des émulsions multiples de diclofénac ont été formulées en utilisant du beurre de karité (*Vitellaria paradoxa*) comme phase huileuse et les propriétés anti-inflammatoires de l'émulsion multiple évaluée chez le rat Wistar .

Méthodes: Les émulsions multiples ont été préparées en utilisant la technique de la double émulsification et les propriétés (taille moyenne des globules, viscosité et indice de crémage) des émulsions ont été évaluées. L'activité anti-inflammatoire *in vivo* de l'émulsion multiple a été évaluée après application topique à l'aide de deux modèles d'induction d'inflammation, à savoir le léchage de la patte induit par le formol et la patte d'œdème induit par l'albumine d'œuf.

Résultats : Une émulsion multiple stable de diclofénac a été obtenue avec du beurre de karité en tant que phase huileuse et mélanges tensioactifs, rapport Tween 80 : Span 80 de 1:1 avec une teneur en eau de 20%; et rapport Tween 80 : Span 80 de 1:1,5 avec une teneur en eau de 25%. Le classement de l'inhibition de l'inflammation après l'application topique de la formulation était émulsion de beurre de karité <2% émulsion diclofénac < gel diclofénac de norme <1% émulsion diclofénac < beurre de karité << Pas de traitement. Cela indique que la formulation de beurre de karité en émulsion multiple a significativement augmenté ($p < 0,05$) ses propriétés anti-inflammatoires, tandis que l' émulsion multiple de diclofénac a donné une activité dépendante de la dose. De plus, l'activité de la myéloperoxydase dans le sérum était significativement plus faible chez les animaux traités que chez les animaux non traités.

Conclusion: Des émulsions multiples stables au diclofénac possédant une activité anti-inflammatoire ont été développées avec succès en utilisant du beurre de karité comme support.

Mots clés : *beurre de karité, Vitellaria paradoxa, diclofénac , émulsion multiple, propriétés anti-inflammatoires*

Introduction

The non-steroidal anti-inflammatory drugs (NSAIDs) have been a main-stay in the management of pain occasioned by inflammatory arthritis.

Whitehill [17]. Preliminary formulation studies were carried out to optimise the composition of the multiple emulsion, temperature, stirring speed, stirring time and phase-volume ratio. The composition of the multiple emulsions is presented in Table 1.

using a heavy-duty laboratory mixer (Model L2R, Silverson Machines Limited, Chesham Bucks, England) for five minutes. The primary emulsion obtained was then re-emulsified in melted shea butter containing a low HLB surfactant, Span 80, also maintained at 50-60 °C, making up the remaining 50

Table 1: Composition and stability of Shea butter multiple emulsion

Code	Tween 80 conc (%v/v)	Span 80 conc (%v/v)	Water content (%v/v)	Shea butter content (%v/v)	Stirring speed (rpm)	Type of instability	Instability observed (days)
D1	2.5	0.5	22.5	74.5	600	Phase separation solidification	3
D2	2.5	1.0	22.5	74.0	600	Phase separation and solidification	3
D3	2.5	2.5	22.5	72.5	600	Solidification	6
D4	2.5	5.0	22.5	70.0	600	Solidification	6
D5	2.5	7.5	22.5	67.5	600	Solidification	6
D6	5.0	2.5	20	72.5	600	Solidification	6
D7	5.0	5.0	20	70.0	600	Stable	-
D8	5.0	7.5	20	67.5	600	Stable	-
D9	7.5	0.5	17.5	74.5	600	Solidification	6
D10	7.5	2.5	17.5	72.5	600	Phase separation	0
D11	5.0	5.0	25	65.0	600	Solidification	20
D12	5.0	5.0	30	60.0	600	Phase separation	2
D13	5.0	5.0	40	50.0	600	Phase separation	0
D14	5.0	7.5	25	62.5	600	Stable	-
D15	5.0	7.5	30	57.5	600	Phase separation	1
D16	5.0	7.5	40	47.5	600	Phase separation	0
D17	5.0	2.0	20.5	72.5	600	Stable	-
D18	5.0	7.5	25	72.5	800	Stable	-
D19	5.0	7.5	25	72.5	1000	Stable	-

Preparation of Vitellaria paradoxa multiple emulsion
Fifty percent of the total emulsion volume was first prepared as the oil-in-water primary emulsion by emulsifying equal volume of melted shea butter with distilled water containing a high HLB surfactant, Tween 80. The oil, water and surfactant were maintained at 60 °C using an electric hot water bath (OC-4743-E, Gallenkamp, England). The emulsification was done under high stirring intensity

% of the multiple emulsion. This was done at low shear rate using a magnetic stirrer (SHC-1 Maple Scientific Instruments, Staffordshire, England) maintained at 600 rpm for five minutes, to avoid rupturing of the primary emulsion globules.

Diclofenac (1 and 2 %w/v) was incorporated into the multiple emulsion by mixing half of the required quantity of diclofenac into the oily phase of the primary emulsion and the other half into the

Table 2: Composition and stability of diclofenac multiple emulsion

Code	Conc. of diclofenac sodium (%v/v)	Tween 80 conc. (%v/v)	Span 80 conc. (%v/v)	Water content (%v/v)	Shea butter content (%v/v)	Type of instability	Instability observed (days)
D20	1	5	5	20	69	Stable	-
D22	2	5	5	20	68	Solidification	4
D23	1	5	7.5	25	61.5	Stable	-
D24	2	5	7.5	25	60.5	Solidification	4

Diclofenac, belonging to the phenylacetic acid class of NSAIDs, has been widely used for the treatment of arthritis but its gastrointestinal (GI) side effects had limited its potential for long term therapy [1]. Diclofenac is also poorly water-soluble especially in acidic medium (about 15 μg/ml) and unstable in aqueous solutions resulting in its poor oral bioavailability [2, 3]. The shortcomings of diclofenac when used orally have necessitated the need for alternative routes of administration with a view to improve its usefulness in the treatment of arthritis for long term therapy.

Topical preparation has proved to be a formulation of choice for both the prescribers and patients. Prescribers can give long term effective drug management of arthritis without the concern of creating other complications and the patients are able to comply with the dosage regimen due to the ease of use, non-invasiveness and minimal side effects, thus optimising therapy. A randomized controlled clinical trial has demonstrated that adverse drug reactions (ADRs) from topical NSAIDs were lower compared to the orally ingested dosage forms [4].

Multiple emulsions are a class of emulsions in which both oil-in-water and water-in-oil emulsions exist simultaneously within a single system and are stabilised by hydrophilic and lipophilic surfactants, respectively [5]. In contrast to macroemulsions, multiple emulsions consist of oil (O) dispersed in water (W) and the emulsion formed is further dispersed in oil making O/W/O or water in W/O/W. This creates the internal, middle and external phases which present an improved opportunity to enclose the active drug. Thus, the active drug contained in the innermost phase is partitioned between several phases (oil, water and emulsifier) which provide an additional reservoir for drug partitioning that would act as depot for gradual release of drugs over a given period [6 - 8] and enhance dermal absorption [9]. The embedded drug in multiple emulsions is released to elicit its therapeutic activity by different mechanisms. The drug moves from the internal phase to the external through the middle layer by diffusion, carrier mediated transport, micelle transport, thinning of oil membrane, rupture of oil phase or solubilisation of internal phase in oil membrane. Diffusion is the most common of all the mechanisms where unionized drug moieties which are hydrophobic in nature diffuse through the semi-permeable liquid membrane which is the oil layer [10]. The drug release rate and effectiveness of such agent are affected by factors such as droplet size, pH, phase volume ratios, viscosity and the nature of entrapped material. Multiple emulsions are promising drug delivery system due to their thermodynamic stability, macroscopic homogeneity, ease of preparation and small droplet size [6].

Shea butter obtained from the kernels of the African Shea tree – *Vitellaria paradoxa* C.F Gaertn (family Sapotaceae) formerly known as *Butyrospermum paradoxum* C.F Gaertn and *Butyrospermum parkii* G. Don [11], which is indigenous to the Savannah belt of Africa; extending from Nigeria and Mali in the West, to Ethiopia and Uganda in the East. Shea butter is an off-white or ivory-coloured fat, which is solid at room temperature but readily softens at body temperature when applied to the skin [12]. Shea butter has a remarkable composition of unsaponifiable fats in comparison with other oils and this portion is responsible for keeping the skin young by stimulating the tissue and helping the skin make its own collagen, making shea butter invaluable in cosmetic industries [13]. Shea butter, due to its high yield and fatty content, has been used traditionally in West Africa as cooking oil. It is used for medicinal purposes such as rheumatism, nasal inflammation, nasal congestion, cough, leprosy and in minor bone dislocation. Shea butter is also used for soothing and accelerating healing after circumcisions and prevention of stretch marks in pregnant women [14]. Studies have shown that Shea butter possesses anti-inflammatory [16], moisturising and skin healing properties [14] and is useful as a vehicle in the delivery of sulphur [15]. Hence, shea butter has the unique potential of being both an active ingredient and excipient. Thus, in the present study, diclofenac multiple emulsions have been formulated for topical application using shea butter as a carrier and the *in vivo* anti-inflammatory activity of the formulations evaluated in Wistar rats in comparison to a marketed brand of diclofenac sodium gel.

Materials and methods

The materials used were diclofenac sodium powder (Caesar and Loretz GmBH, Hilden, Germany), Tween 80 and Span 80 (Sinopharm Chemical Reagent Company Limited, China) and 1 % diclofenac sodium gel (Olfen® gel, Merckle, Ulm, Germany). Shea butter was obtained from local shea butter producer (Alheri Women Co-operative/ Global Shea Alliance, Bosso LGA, Niger state, Nigeria). All other reagents used were of analytical grade.

Preliminary formulation studies

Multiple emulsions were prepared using the double emulsification technique described by Florence and

$$\% \, Inhibition = \frac{(Ct-Co)\ control-(Ct-Co)\ treated]}{(Ct-Co)control} \times 100 \quad (2)$$

where C_t is the mean paw size at time t and C_o is the initial mean paw size.

Evaluation of serum myeloperoxidase activity

The myeloperoxidase (MPO) activity was determined using the method of Xia and Zweier [24]. Blood collected from the rats were analysed biochemically for the neutrophil infiltration marker-myeloperoxidase. The blood was collected into plain sample bottles and then centrifuged to separate the serum using a centrifuge (Model 80-2, GB Medical Ltd, Hampshire, England,) at 4000 rpm for 30 minutes. The serum was carefully decanted using a Pasteur pipette into labelled plain tubes capped and stored in a freezer for the assay. The reagent (O-dianisidine mixture) for the myeloperoxidase assay was prepared with 16.7 mg O-dianisidine, 100 ml of 0.05 M potassium phosphate buffer and 50 µL of diluted hydrogen peroxide. Two millilitres of O-dianisidine mixture was placed into a cuvette and into it was added 70 µL of the serum sample. The cuvette was then immediately placed into its compartment in the spectrophotometer (Gumpton Medical and Scientific England, Model S23A) and the absorbance read at 450 nm at 30 and 60 seconds. One unit of MPO activity was defined as that degrading one micromole of peroxide per minute at 25 p C and was calculated using equation:

$$MPO\ activity\left(\mu\frac{mol}{L}\right) = \frac{Absorbance}{1.13} \times 100 \quad (3)$$

Statistical analysis

Data are presented as mean ± standard error of mean (SEM) except for data of MPO activity, which were expressed as mean ± standard deviation (SD). The differences between groups were analysed using one-way analysis of variance (ANOVA) test while the data for formalin-induced paw-lick test was analysed using t-test. The level of significance was taken as $p \leq 0.05$.

Results and discussion

Preliminary formulation studies

Instability is a common occurrence in emulsion systems because of its heterogeneous nature. This makes optimisation of formulation parameters an important aspect to obtain products that are sufficiently stable over time. The preliminary formulation studies to optimize the surfactant concentration, phase-volume ratio, and secondary emulsification speed, period that the formulations retained its semi-solid consistency and physical form after storage at room temperature (27±2 °C) are shown in Table 1. Shea butter was successfully used in the formulation of multiple emulsions, which were generally creamy-white to white in colour, free flowing, easily spreadable and smooth in texture. Stable diclofenac multiple emulsions were obtained with Tween 80 to Span 80 ratios of 1:1 (5:5 %) and 1:1.5 (5:7.5 %). Increasing the water content from 20 to 25 % reduced the stability of the formulation with surfactant ratio of 1:1 (formulations D7 and D11) but did not affect the stability of formulation containing surfactant ratio 1:1.5 (D8 and D14). Increasing the secondary emulsification speed from 600 to 1000 rpm had no significant effect on physical form of the emulsions (formulations D17 to D19). Thus, two multiple emulsion formulations consisting of Tween 80 to Span 80 ratio of 1:1, water content of 20 % (D7) and Tween 80 to Span 80 ratio of 1: 1.5, water content of 25 % (D14), which exhibited better stability were selected for the incorporation of 1 % (D20 and D23) and 2 % diclofenac (D22 and D24) as shown in Table 2.

Properties of diclofenac multiple emulsion

The properties of diclofenac multiple emulsion shown in Table 2 indicate that incorporation of drugs generally led to a decrease in the viscosity of the multiple emulsion although the viscosity increased with time. Formulations containing 1 %w/v diclofenac exhibited good consistency over the period of study whereas formulations containing 2 %w/v diclofenac showed a significant ($p<0.005$) increase in viscosity to a semi-solid consistency after 4 days. This indicates that increasing the drug content in the emulsion system led to an increase in the viscosity of the formulations. This is because the more solid materials are added, the more viscous the product. Viscosity has been described as the ability of a material to produce internal resistance to friction when one layer of molecules is involved in motion relative to the next, due to attractions between such molecules [25]. Low viscosity formulations have been shown to release the drug faster probably due to faster diffusion of drug through the vehicle with lower viscosity as a result of less resistance to the movement of the molecules of active ingredient [26]. The viscosity of the multiple emulsions presented

oily phase of the secondary emulsion to obtain an even distribution of the drug within the emulsion system. The composition of diclofenac multiple emulsions are presented in Table 2.

Viscosity measurements

The viscosity of the emulsion was measured using a Brookfield viscometer (DV-2+ Pro, Brookfield Engineering Laboratories Inc., Middleboro, USA) at 100 rpm using spindle size 5. The viscosity of the emulsion was conducted at different time intervals (0, 1, 7, 14 and 30 days).

Phase separation/ solidification

The emulsion was observed visually for signs of phase separation and solidification at different time intervals and the observations were recorded.

Determination of globule size

The globule sizes of the multiple emulsions were determined using a light microscope (Barska Monocular Compound Microscope AY11240, Barska Technology, Pomona CA, USA). A quantity of the multiple emulsion was stained with crystal violet and mounted on the slide to view under the microscope. The diameter for 100 globules was determined at different time intervals and the mean globule size was calculated.

Determination of creaming index

The creaming index was determined using the method described by Odeku *et al* [18]. Briefly, a one in ten dilution of the multiple emulsion was made by diluting 5 ml of the emulsion to 50 ml with distilled water to facilitate discernible differences in the rate and extent of creaming of the emulsions. The diluted emulsions were kept for observation in 50 ml plain bottles and the height of creaming was determined. The creaming index was then calculated from equation 1[19]:

$$\text{Creaming index (\%)} = \frac{\text{Height of cream layer (cm)}}{\text{Total height of emulsion (cm)}} \times 100 \quad (1)$$

In-vivo *anti-inflammatory studies*

The *in vivo* anti-inflammatory activity of the prepared formulations was carried out on 60 healthy albino Wistar rats of both sexes (99 ± 12 g). The animals were procured from the Faculty of Veterinary Medicine Experimental animal house at the University of Ibadan, Ibadan, Nigeria. The animals were allowed free access to food and water and allowed to acclimatize for 7 days before commencement of the experiment.

The animal experiments were conducted in compliance with the guidelines stated in Principle of Laboratory animal care [20]. The protocols were approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/ App/2015/052). The animals were randomized into six different groups as follows:

Group A - negative control (no treatment)
Group B - positive control (brand of 1 %w/w diclofenac gel)
Group C - shea butter alone
Group D - shea butter multiple emulsion
Group E - 1 %w/w diclofenac multiple emulsion
Group F - 2 %w/w diclofenac multiple emulsion

Formalin-induced paw-lick test

The analgesic property of the formulations was determined using the formalin-induced paw-licking test [21]. Briefly, the formulation was gently rubbed into the plantar surface of the left fore-paw for about 30 seconds and 50 µl of 2 % formalin was injected into the sub-plantar surface after 30 minutes. The total paw-lick time by the rats immediately after formalin injection was recorded for both the early phase (0-5 minutes) and late phase (15-30 minutes) inflammation; the early phase shows the initial acute neurogenic nociceptive response while the late phase shows the chronic inflammatory response [21].

Egg albumin-induced paw oedema

The anti-inflammatory activity of the formulations was determined using the egg albumin induced paw oedema method [22]. The rats were pre-treated by gently rubbing the microemulsion into the plantar surface of the right fore-paw for about 30 seconds. Thirty minutes after pre-treatment, the rats were injected with 0.2 ml of undiluted fresh egg albumin into the sub-plantar surface of the rat paw. The paw size (cm) was determined by measuring the circumference of the oedematous paw with a thread wrapped around the paw, which is then placed on a metre rule to determine the diameter. The measurement, which represented the inflamed paw size of the rats [22], was done immediately before egg albumin injection, immediately after injection and at 30 minutes intervals post inflammation induction for a period of 120 minutes. The percentage inhibition was calculated using the equation [23]:

the globule size of the multiple emulsions. Thus, the emulsion can be said to exhibit reasonable stability.

Creaming is a form of instability that is common in emulsion systems, although a creamed emulsion is not necessarily bad provided it can be re-dispersed with moderate agitation; it is preferable for the emulsion to exhibit low degree of creaming. The result of the percentage creaming in Figure 3 showed that the creaming index of the formulations ranged between 7 to 24 %. The cream was easily redispersible with moderate agitation.

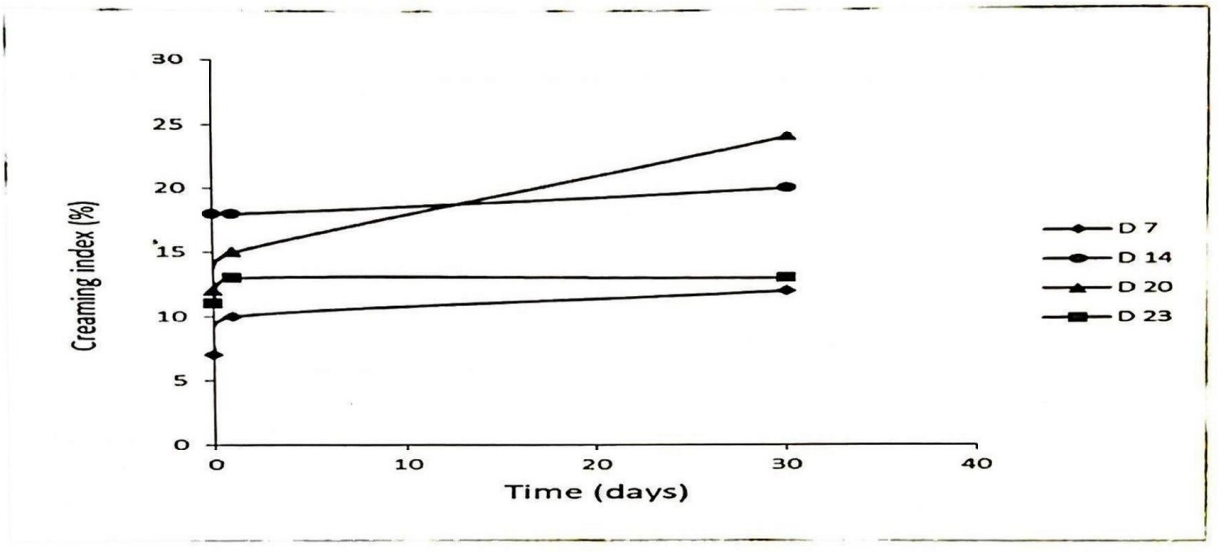

Fig. 3: Creaming index of unmedicated (D 7 and D 14) and medicated (D 20 and D 23) shea butter emulsion containing different oil:surfactant:water ratio over time

Stability studies over a period of twelve months indicated that there were no significant changes in the viscosity, mean globule size and creaming index of the emulsion (data not shown). It appeared that the changes that occurred in the multiple emulsions, i.e. either phase separation or solidification occurred within the first one week after preparation (Table 1). This indicates the relative stability of the multiple emulsions once the appropriate ratio of the oil: surfactant: water is used in the formulation.

In vivo *anti-inflammatory properties of diclofenac multiple emulsion*

The formalin induced paw-link test in mice has been used as a valid and reliable model of inflammation and nociception. Dilute formalin injected into the dorsal surface of the right hindpaw of rats, serves as a harmful stimulus causing an immediate and intense increase in the spontaneous activity of C-fiber afferent and evoke a distinctive and measurable behaviour indicative of pain confirmed by the amount of time the animals spend licking the injected paw [28-30]. Formalin test is a highly specific method which involves a biphasic response identified as: the initial phase (first 5 minutes) as a result of direct stimulation of the paw which gives a neurogenic nociceptive response that is centrally mediated; and the second phase otherwise known as inflammatory response (15 to 30 min after formalin injection) as a result of the peripheral release of pro-inflammatory mediators such as bradykinnin, histamine, serotonin and prostaglandins [21, 31, 32]. Centrally acting analgesics such as morphine, codeine, nefopam and orphenadrine can inhibit both phases; while peripherally acting drugs, such as NSAIDs such as indomethacin and naproxen and the corticosteroids, inhibits only the late phase [33].,

The *in vivo* anti-inflammatory property of the diclofenac multiple emulsions was compared with a known brand of diclofenac sodium topical gel containing 1 % diclofenac sodium and the results of the formalin-induced paw lick test are shown in Table 3. The result showed that the number of paw licking was higher at the late than the early phase. The ranking of the paw licking at the early phase of inflammation was 1 % diclofenac emulsion < standard diclofenac gel < shea butter emulsion < 2 % diclofenac emulsion = No treatment < shea butter. This shows that the 1 % diclofenac multiple emulsion gave higher activity than the standard diclofenac gel, while shea butter emulsion was more active than unformulated shea butter. However, during the late phase, the ranking was shea butter emulsion < 2 %

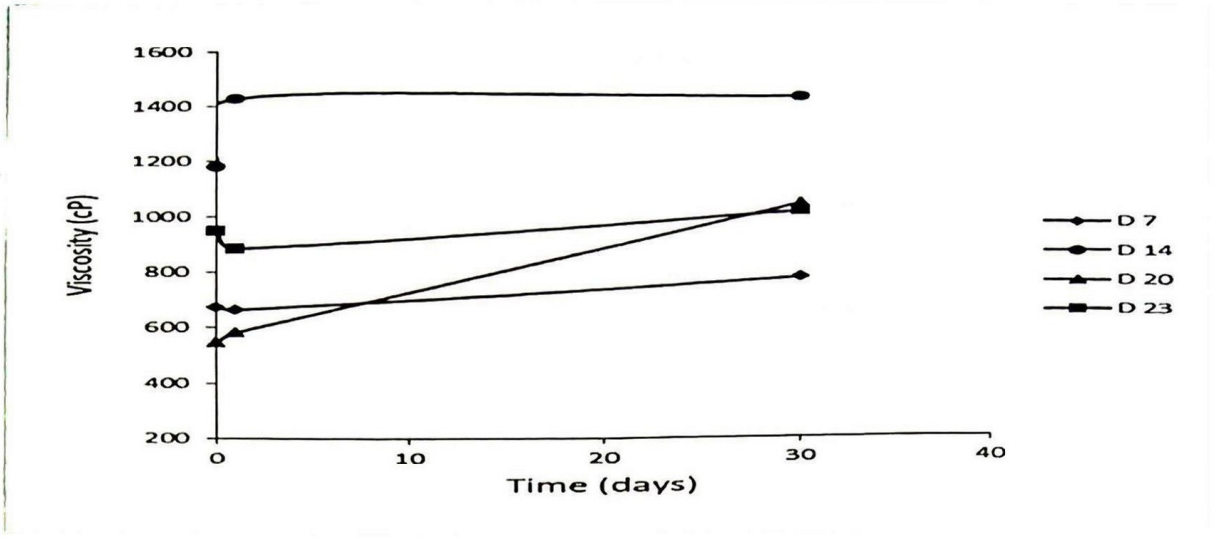

Fig.1: Viscosity changes of unmedicated (D 7 and D 14) and medicated (D 20 and D 23) shea butter multiple emulsion containing different oil:surfactant:water ratio over time.

in Figure 1 indicates that the viscosities of the formulations generally increased with storage.

Formulation with surfactant concentration of 7.5 % and water content of 25 % (D14) showed significantly ($p<0.05$) higher viscosity probably due to a higher concentration of surfactant and water available to facilitate emulsification of the oil. Formulations for topical application should possess adequate viscosity to prevent run-off from the skin surface after application. A major advantage of high viscosity formulations is the ability of such preparations to offer prolonged activity at the site of action because of adherence to the skin [27], which is also desirable since the frequency of application is reduced. Thus, the multiple emulsions possess adequate viscosity to facilitate the adherence of the formulation to the skin.

Increase in globule size due to coalescence has been shown to be a sign of instability and deterioration in disperse systems like multiple emulsions. The results of the mean globule size of the multiple emulsions shown in Figure 2 indicate that the mean globule size increased with time although the increase was generally not significant ($p > 0.05$). Generally, the addition of diclofenac did not appear to have significant ($p > 0.05$) effect on

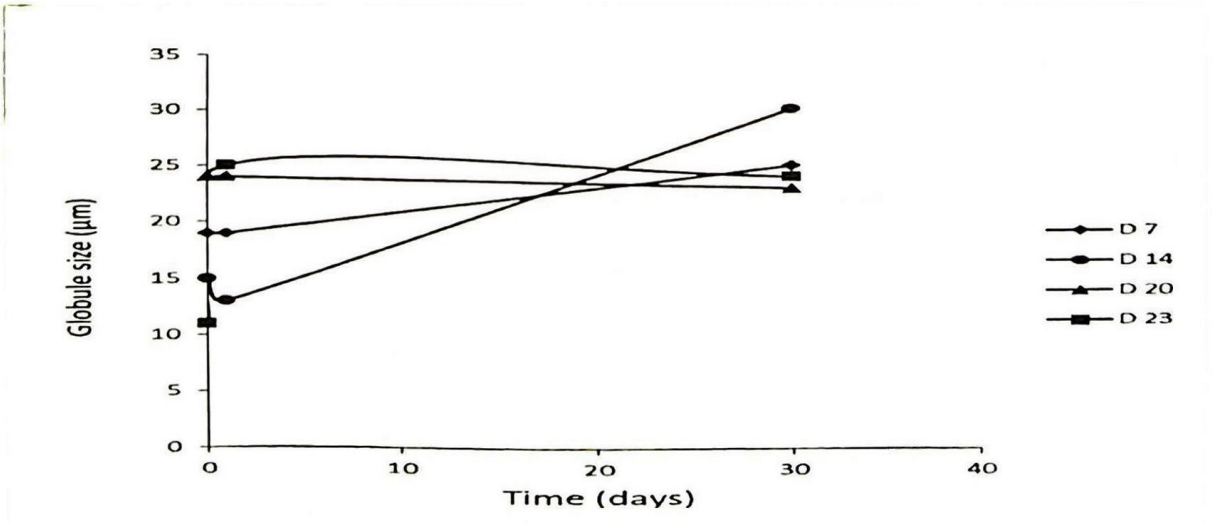

Fig. 2: Globule size of unmedicated (D 7 and D 14) and medicated (D 20 and D 23) shea butter multiple emulsion containing different oil:surfactant:water ratio over time

In addition, shea butter, its multiple emulsions and diclofenac multiple emulsion formulations produced inhibition at both early and late phases like diclofenac gel. This indicates that the formulations acted both centrally and peripherally, and enhanced effects were observed at the late phase. While the early phase is basically nociceptive (analgesic), the late phase establishes the anti-inflammatory properties of the formulations. Furthermore, NSAIDs such as diclofenac exerts its slower onset of action with the 2 % formulation exhibiting increased anti-inflammatory activity in the later phase of the experiment to give a similar inflammation inhibition comparable with the standard diclofenac gel. This adds credence to the effectiveness of diclofenac sodium multiple emulsion in inhibiting inflammation.

Myeloperoxidase (MPO) is used as a marker in systemic inflammation since it is released into the extracellular fluid in the inflammatory process on

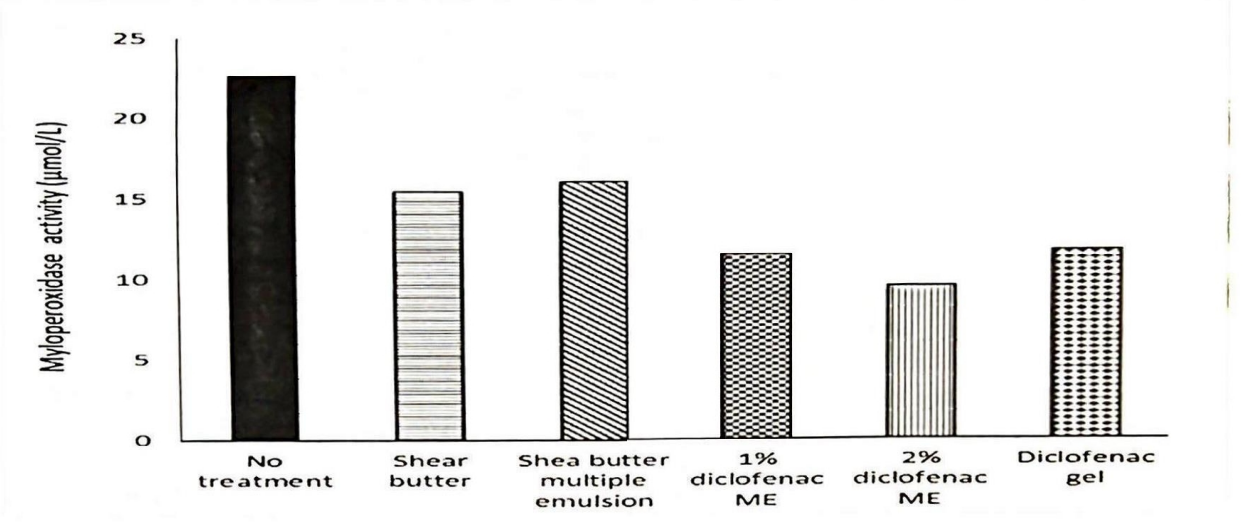

Fig. 4: Myeloperoxidase activity for formalin-induced paw lick test

action in a multimodal and novel mechanism of action indicating analgesic, antipyretic and anti-inflammatory properties [34]. Thus, shea butter elicits it anti-inflammatory effect in similar manner to diclofenac [35]. In addition, shea butter, which serves as the oil phase for encapsulation of the active drug also elicit anti-inflammatory activity leading to increased pharmacological activity.

Egg albumin-induced paw oedema is caused by the release of histamine and 5-hydroxytryptamine, which are mediators of inflammatory response [36]. The effect of formulations on egg albumin-induced paw oedema at varying times is presented in Table 5. The results showed that there was general reduction in paw size of the rats with time. There was a significant difference ($p < 0.05$) between paw size of all the treatment groups compared with the untreated group suggesting the effectiveness of all the formulations in inhibiting inflammation. Similar to the formalin-induced model, shea butter multiple emulsion facilitated higher reduction in the paw size than shea butter. The diclofenac multiple emulsion gave a activation of neutrophils and macrophages. This is achieved by MPO catalysing the conversion of chloride and hydrogen peroxide to hypochlorite which is secreted in inflammatory conditions [37]. Myeloperoxidase is characterised by powerful pro-oxidative and pro-inflammatory properties [38, 39] thereby making it a useful tool in assessing anti-inflammatory processes. The results (Figures 4 and 5) showed significant reduction in activity of the systemic inflammation marker, MPO, in treated groups compared to the untreated group for both models of inflammation induction, suggesting the effectiveness of the formulations in reducing systemic inflammation when applied topically.

Conclusion

Diclofenac sodium multiple emulsions were successfully formulated using shea butter as the oil phase. Stable emulsions were obtained with surfactant ratio of Tween 80: Span 80 of 1:1 and 1:1.5 and water content of 20 and 25 %, respectively. *In vivo* anti-inflammatory studies showed that shea butter and diclofenac multiple emulsions showed

Table 3: Effect of diclofenac-shea multiple emulsion on formalin-induced paw lick test

Group	Treatment	Number of licking	
		Early phase (0 – 5 min)	Late phase (15 – 30 min)
A	No treatment	72.2 ± 10.0	287.2 ± 29.0
B	Standard (1 % diclofenac gel)	54. 6 ± 14.0	159.4 ± 18.0
C	Shea butter	79.6 ± 13.0	187.2 ± 32.0
D	Shea butter multiple emulsion	65.0 ± 18.0	93.2 ± 18.0
E	1 % diclofenac multiple emulsion	49.4 ± 14.0	172.0 ± 39.0
F	2 % diclofenac multiple emulsion	72.2 ± 7.0	123.0 ± 28.0

Table 4: Effect of formulation on the percentage inhibition of inflammation in rats

Group	Treatment	Inhibition (%)	
		0-5 min	15-30 min
A	No treatment	0	0
B	Standard 1 % diclofenac sodium gel	24	45
C	Shea butter	10	35
D	Shea multiple emulsion	10	68
E	1 % diclofenac multiple emulsion	32	40
F	2 % diclofenac multiple emulsion	0	57

diclofenac emulsion < standard diclofenac gel < 1 % diclofenac emulsion < shea butter < < No treatment. The anti-inflammatory activity of diclofenac multiple emulsion was also found to be dose dependent and was comparable to that of diclofenac sodium gel. The higher concentration of diclofenac in the 2 % formulation was able to increase its anti-inflammatory activity in the late phase compared to the 1 % formulation suggesting prolonged action which could be as a result of the higher viscosity and long contact time.

significantly ($p< 0.05$) higher percent inhibition of inflammation and paw licking than plain shea butter indicating that formulation of shea butter as multiple emulsion significantly increased its anti-inflammatory properties. This is because Shea butter served as the oil phase which has been emulsified using a blend of hydrophilic (tween 80) and hydrophobic (span 80) emulgents. The emulsification produced small droplet sizes of the oil phase distributed within the emulsion system. These small globules offered improved spreadability and penetration into the skin to elicit its activity [9].

Table 5: Effect of formulations on egg albumin-induced paw oedema at various time intervals (mean ± SEM, n=5)

Groups	Treatment	Paw size (cm)			
		30 min	60 min	90 min	120 min
A	No treatment	0.64 ± 0.11	0.72 ± 0.11	0.50 ± 0.15	0.36 ± 0.09
B	Standard diclofenac sodium gel	0.64 ± 0.18	0.46 ± 0.10	0.32 ± 0.09	0.18 ± 0.06
C	Shea butter	0.40 ± 0.08	0.38 ± 0.04	0.36 ± 0.06	0.12 ± 0.08
D	Shea multiple emulsion	0.56 ± 0.04	0.52 ± 0.04	0.60 ± 0.05	0.24 ± 0.09
E	1 % diclofenac sodium multiple emulsion	0.66 ± 0.00	0.48 ± 0.08	0.44 ± 0.07	0.34 ± 0.07
F	2 % diclofenac sodium multiple emulsion	0.66 ± 0.11	0.52 ± 0.10	0.32 ± 0.09	0.18 ± 0.06

The results of the percent inhibition of inflammation for the formulations presented in Table 4 showed that shea butter multiple emulsions gave

Shea butter exhibited remarkable anti-inflammatory action in the late inflammatory phase thus supporting previous claim of its anti-inflammatory activity [12].

Butyrospermum parkii as a vehicle in sulphur ointment formulations. West Afr J Pharm. 2013; 23: 58-65.

16. Toshihiro A, Nobuo K, Takashi K, Ken Y, Harukuni T, Eliot TM, Aranya M and Jiradej M. Anti-inflammatory and chemopreventive effects of triterpene cinnamates and acetates from shea fat. J Oleo Sci. 2010; 59 (6): 273-280.
17. Florence AT and Whitehill D. The formulation and stability of multiple emulsions. Int J Pharm 1982; 11: 277-279.
18. Odeku OA, Itiola OA and Akinlosotu OD. A preliminary evaluation of khaya gum as an emulsifying agent. West Afr J Pharm 1997; 11: 30-37.
19. Onukwo GC and Adikwu MV. Stability of veegum/mucuna gum emulsions. STP Pharma Sci 1997; 74: 320-325.
20. National Center for Biotechnology Information. PubChem Compound Database. https://pubchem.ncbi.nlm.nih.gov/compound/5018304 (accessed Dec 5, 2015)
21. Onasanwo SA, Saba AB, Oridupa OA, Oyagbemi AA and Owoyele BV. Anti-nociceptive and anti-inflammatory properties of the ethanolic extract of *Lagenaria breviflora* whole fruit in rat and mice. Nig J Physiol Sci. 2011; 26: 071-076.
22. Olajide AO, Awe SO, Makinde JM, Ekhelar AI, Olusola A, Morebise O and Okpako DT. Studies on the anti-inflammatory, anti-pyretic and analgesic properties of Alstonia boonei stem bark. J Ethnopharmacol. 2000; 71: 179-186.
23. Oyemitan IA, Kolawole F, Oyedeji AO. Acute toxicity, anti-nociceptive and anti-inflammatory activity of the essential oil of fresh fruits of Piper guineense Schum and Thonn (Piperaceae) in rodents. J Med Plant Res 2014; 8 (40): 1191-1197.
24. Xia Y and Zweier JL. Measurement of myeloperoxidase activity in leukocyte-containing tissues. Analytical Biochem 1997; 245: 93-96.
25. Rawlins EA. eds, Emulsions. In: Bentley's Textbook of Pharmaceutics. Eighth edition, Bailliere Tindall, W.B Saunders UK. 2004. pp 256-268.
26. Felton LA. Ed. Remington essentials of pharmaceutics, Pharmaceutical Press, 1, London, UK. 2012. pp 571.
27. Adeyeye M, Jain A, Ghorab M and Reilly W. Viscoelastic evaluation of topical creams containing microcrystalline cellulose/sodium carboxymethyl cellulose as stabilizer. AAPS PharmSciTech. 2002; 3 (2): 16-25.
28. Hunskaar, S. and Hole K. (1987) The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 30 (1):103–114.
29. Heapy CG, Jamieson A, Russell NJW. Afferent C-fiber and A-delta activity in models of inflammation. Br J Pharmacol 1987; 90:164.
30. Karthikeyan M, Deepa MK. Anti-inflammatory activity of *Premna corymbosa* (Burm.f.) Rottl. & Willd. leaves extracts in Wistar albino rats. Asian Pac J Trop Med. 2011; 4 (7):510–513.
31. Ribeiro, R.V., Silva, R.M, Lima, J.C. and Martins, D.T. (2010) Antiinflammatory, antinociceptive and antipyretic effects of hydroethanolic extract from *Macrosiphonia velame* (A. St.-Hil.) M. Arg. in animal models. Braz J Pharm Sci 46 (3):515 -523.
32. Linardi A, Costa SK, DeSilva GR and Antunes E. Involvement of kinins, mast cells and sensory neurons in the plasma exudation and paw oedema induced by *Staphylococcal entrotoxin* B in the mouse. Eur J Pharmacol. 2002; 399:235-242.
33. Liao, C.R., Kao, C.P., Peng, W.H., Chang, YS, Lai, S.C. and Ho, Y.L. (2012) Analgesic and Anti-Inflammatory Activities of Methanol Extract of Ficus pumila L. in Mice. Evidence-Based Complementary and Alternative Medicine 2012:1-9.
34. Gan T.J. Diclofenac: an update on its mechanism of action and safety profile. Curr Med Res Opin.2010 26 (7): 1715-1731.
35. Nandini V Rina C Rakha H D, Hemant K G. Anti-Inflammatory Effects of Shea Butter through Inhibition of Inos, Cox-2, and Cytokines via the Nf-Kb Pathway in Lps-Activated J774 Macrophage Cells. J Comple Integr Med 2012; 9 (1): 1–11.
36. Nwafor PA, Jacks TW, Ekanem AU. Analgesic and anti-inflammatory effects of methanolic extract of *Pausinystalia mecroceras* stem bark in rodents. J Pharmacol 2007; 3: 86–90.
37. Loria V, Dato I, Graziani F and Biasucci LM. Myeloperoxidase: a new biomarker of inflammation in ischaemic heart disease and acute coronary syndromes. Mediators of Inflamm. 2008; Article 135625. http://dx.doi.org/10.1155/2008/135625.
38. Mullane KM, Kraemer R and Smith B. myeloperoxidase activity as a quantitative assessment of neutrophil infilteration into *ischemic myocardium.* J Pharmacol Methods. 1985; 14 (3): 157-167.
39. Holvoet P. Oxidative modification of low-density lipoproteins in artherothrombosis. Acta Cardiologica. 1998; 53, 5: 253-260.

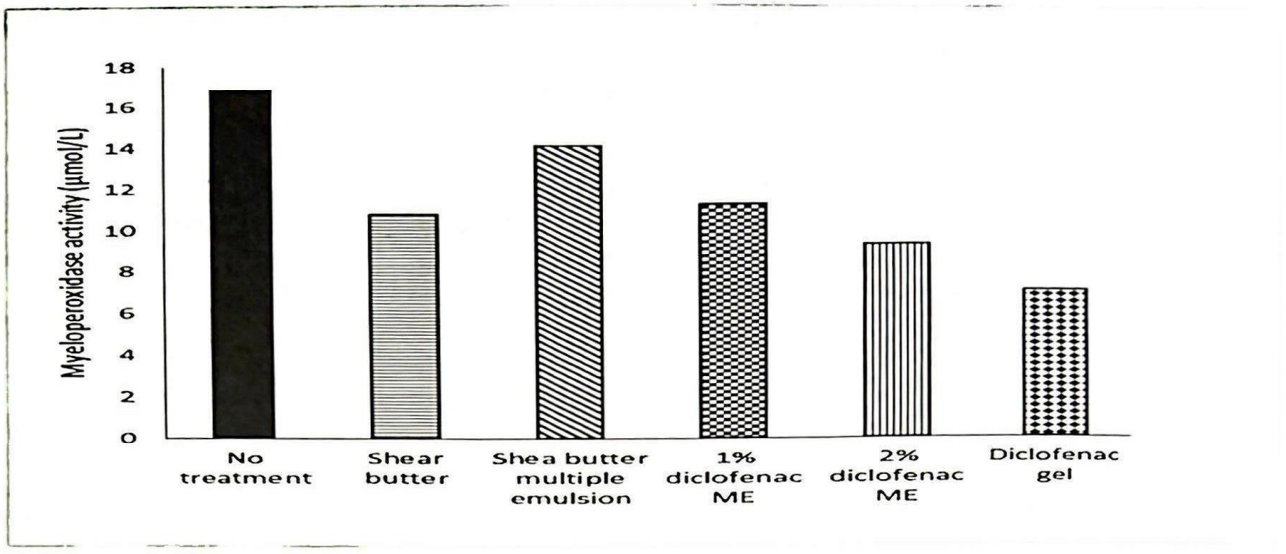

Fig. 5: Myeloperoxidase activity for egg albumin-induced paw oedema

topical anti-inflammatory properties comparable with diclofenac gel. The type and quantity of surfactant employed in the formulation of diclofenac multiple emulsions need to be carefully chosen to enable fast onset of action, high penetration and sustained anti-inflammatory properties.

References

1. Wang M and Fang L. Percutaneous absorption of diclofenac acid and its salts from Emulgel. Asian J Pharm Sci 2008; 3 (3): 131-141.
2. Pose VB, Santana PL, Perez-Marcos MB, Vila-Jato JL, Torres-Labandeira JJ. Dissolution behaviour of diclofenac sodium and hydroxypropyl- ß-cyclodextrin inclusion complexes. In Labandeira JJT, Vila-Jat JL (eds) Proceedings of the Ninth International symposium on cyclodextrins. Springer, Dordrecht. DOI https://doi.org/10.1007/978-94-011-4681-4105
3. Llinas A, Burley J.C, Box K.L. Glen R.C, Goodman J.M. Diclofenac Solubility Independent Determination of the Intrinsic Solubility of Three Crystal Forms. J. Med. Chem 2007; 50 (5): 979–983.
4. Mason L, Moore RA and Edwards JE. Topical NSAIDs for chronic musculoskeletal pain: Systematic review and meta-analysis. BMC Musculoskel Disorder 2004; 5: 28-32.
5. Rajesh K, Murugesan SK and Nanjaian M. Multiple Emulsions: A Review. Int J Recent Adv Pharm Res 2012; 2 (1): 9-19.
6. Sinha VR and Kumar A. Multiple emulsions - an overview of formulation, characterization, stability and applications. Indian J Pharm Sci 2002; 64 (3): 191-199.
7. Hitesh KD, Sukhbir LK, Bharat P, Kuldeep K and Sonia A. Formulation and evaluation of novel sustained release multiple emulsion containing chemotherapeutic agents. Int J Pharm Tech Res 2012; 4 (2): 866-872.
8. Sahu D, Kushwaha S and Agarwal P. Recent advancement, technology and applications of multiple emulsions. Innov J of Health Sci 2013; 1 (1): 19-23.
9. Bhatia N, Pandit S, Agrawal S, Gupta D. A Review on Multiple Emulsions. International J of Pharm Erud 2013: 3 (2): 22-30.
10. Vyas SP and Khar RK. Multiple emulsions: Novel carrier systems. CBS Publishers and Distributors. First Edition, 2004; 303-328.
11. Alfred T. Fats and Fatty Oils. Ullmann's Encyclopedia of Industrial Chemistry. Weinheim: Wiley VCH. 2002.
12. Oyedele AO. The skin tolerance of sheafat employed as excipient in topical preparations. Nig J Nat Prod and Med 2002; 6: 26-29.
13. Antonio M, Alvarez R, Rodriguez MLG. Lipids in pharmaceutical and cosmetic preparations. Grasas y Aceite 2000; 51 (1-2): 74-96.
14. Malachi OI. Effects of topical and dietary use of shear butter on animals. Amer J Life Sci 2014; 2 (5): 303-307.
15. Femi-Oyewo MN, Ajala TO and Mabadeje A. The evaluation of Shea butter from