

Effect of emulgel containing *Newbouldia laevis* stem bark extract on croton oil-induced hemorrhoids in mice

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ABSTRACT: Traditionally, different parts of the plant *Newbouldia laevis* ranging from the stem, leaves, flower, roots, barks and stem barks are widely used in Africa for the management of numerous disease conditions including hemorrhoids/pile. The aim of this study is to formulate and evaluate emulgel formulations containing the ethanol extract of *Newbouldia laevis* stem bark for management of recto-anal inflammation. Stem bark of the plant was cleaned, crushed and macerated in absolute ethanol for 24 h at room temperature, the mixture was filtered and concentrated to obtain the dried extract which was used to prepare emulgel formulations at different concentrations (0.50, 0.75, 1.00 %w/w). The emulgels were evaluated for physical properties such as color, odor, feel, homogeneity, grittiness, phase separation; chemical evaluations such as pH, density, spreadability, extrudability and particle size of the formulations were analyzed. Anti-hemorrhoidal activity of the emulgels on croton-induced hemorrhoids in laboratory mice was also evaluated. Results show that all the emulgels had acceptable organoleptic and physicochemical properties. Histopathological analysis revealed dose-dependent reduction of inflamed tissues in croton-induced rectal inflammation. This study shows the development of a standardized dosage form; emulgel, containing the ethanol extract of *Newbouldia laevis* with propensity to ameliorate inflammation in the recto-anal region.

KEYWORDS: *Newbouldia laevis*; emulgel; inflammation; anti-hemorrhoidal, histopathology.

1. INTRODUCTION

Anorectal inflammation refers to those conditions that affects the anus and/or rectum; most common conditions include hemorrhoids, anal fissures, anal warts, anal fistulas among others. These conditions are almost routinely encountered in general medicine practice however; they are only managed to get temporary pain relief or control [1].

Hemorrhoids are abnormal conditions of the vascular plexuses due to rectal and presented as congestion, engorgement and bleeding [2]. Even though the etiology of hemorrhoid is unknown, some conditions like poor bathroom habit, low fiber diets, sedentary life style, smoking, alcohol, pregnancy, aging, persistent diarrhea or constipation, genetic factor and anal sex can increase the potential of developing this disease [3]. The prevalence of hemorrhoids is not well documented, however, its prevalence worldwide from hospital visits is estimated at about 4.4. % [4]. In Africa, earlier reports revealed hemorrhoids represented 38.5 % of anorecto-sigmoid diseases reported in Gabon and in Mali, 30.4 % of anorectal consultations were reported [5]. Although its prevalence is low compared to prevalence of other diseases, hemorrhoids is a health challenge that impacts the quality of life of those affected and diverse remedies are being sort after to bring succor those affected including the use of herbal remedies.

Herbal medicines have been used for treatment of diseases since the beginning of time, they play a major role in medicine as they serve as alternative sources of drugs. Herbal medicine is receiving significant attention in global health systems and has formed an integral part of the healthcare system particularly in developing and under-developed countries. It is estimated that about 80 % of the population in developing countries still relay on herbal remedies for their primary health care [6]. Preference for herbal medicines/remedies in such climes like Africa, is mainly due to high poverty rate which limits access to and affordability of available conventional drugs. Herbal medicines, are relatively cheap, easily affordable and accessible in addition to being readily accepted due to its purported claim of relative natural safety.

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Furthermore, with increased health awareness worldwide, herbal medicines are receiving wide acceptance even in developed countries because they are thought to promote healthier living [7]. Medicinal plants contain many constituents responsible for their diverse activity and efficacy which range from anti-infective, anti-inflammatory, antiviral, to anti-parasitic among others.

The plant *Newbouldia laevis* (P. Beauv.) Seeman ex Bureau. is a fast-growing tropical evergreen shrub belonging to the family Bignoniaceae. It is a small shrubby tree that grows in the wooden Savanna region and West African forest and commonly found in Nigeria, Senegal, Cameroon, Gabon, Angola [8]. It is commonly called "fence African border tree" or "fertility tree" and in Nigeria it is locally called "adurukua" in Hausa, "ogirishi" in Igbo and "ewe akoko" in Yoruba language [8, 9, 10]. Different parts of the plant, such as the stem, leaves, flower, roots, barks and stem barks are widely used in African traditional remedy to treat certain conditions like inflammation, pains, tumors, rheumatic swellings, ulcers, skin disorders, diabetes, hypertension and sickle cell disease [10, 11]. In Nigeria, decoction of the bark is used as part of a remedy to treat, constipation, pile among others [12]. Other parts of the plant such as the leaf and fruits have been used in treating fever, as wound dressing and for stomach ache [13]. In Senegal, paste prepared from the fresh bark is used for treatment of rheumatism and painful knees arthritis [10].

Literature reveals some experimental data confirming the folklore beliefs of the plant *Newbouldia laevis* (*N. laevis*). An ethnobotanical survey revealed *N. laevis* as one of the effective plants used in the management of hemorrhoids in the South-Western parts of Nigeria [14]. The study by Odukoya *et al.*, [15] evaluated the effect of astringent herbal extracts (*Achyranthes aspera* Linn, *Adansonia digitata* Linn, *Dialium guineense* Willd, *Harungana madagascariensis*, *Kigelia africana*, *Newbouldia laevis* and *Spondias mombin*) in promoting tissue contraction and healing of mucous membranes. Their report showed that these herbs activated coagulation of proteins in the epidermis and/or perianal lining which relieved pain and discomfort associated with hemorrhoids. In another study, significant efficacy of some medicinal plant extracts including *Khaya senegalensis*, *Anogeissus leiocarpus*, *Parkia biglobosa*, *Newbouldia laevis* and *Prosopis africana* used in the North-Eastern part of Nigeria for the treatment of hemorrhoid induced in laboratory mice was reported. Their study showed that the extracts possess anti-hemorrhagic properties [16]. Another ethnobotanical survey showed that traditional practitioners use a decoction of the *Newbouldia laevis* barks and roots for sitz bath to relief symptoms of hemorrhoids and administer same orally for treatment of hemorrhoids [17].

Due to the fact that hemorrhoids affect the anal region, use of topical preparations are often sort to relief its symptoms [18]. Topical preparations are beneficial in the treatment of hemorrhoids because they are rapidly absorbed when applied to the lesion site giving quick relief in addition, they do not undergo first pass effect making them effective with limited side effects. In this study, we prepared emulgels; these are semisolid topical delivery systems consisting of the mixture of an emulsion and a gel with dual properties of the emulsion and the gel. They are easily spreadable, easily washable from surfaces, non-greasy, non-residue producing, possess emollient properties, cosmetically appealing, stable over a long period and are suitable for formulation and delivery of hydrophobic drug components [19, 20].

The aim of this study is to prepare emulgel formulations from the ethanol extract of the stem bark of *Newbouldia laevis*, evaluate their physicochemical properties and assess the anti-hemorrhagic property of the formulated emulgels in laboratory mice.

2. RESULTS

2.1. Physical Properties of Herbal Emulgels

The physical properties of the formulated emulgels are presented in Table 1. The color of the emulgels containing *Newbouldia laevis* extract were milky to light brown with characteristic odor while NE0 without the extract was white and odorless. All the emulgel formulations had glossy texture, were homogenous and non-gritty with a cooling sensation when applied to the back of the hand. There was no sign of phase separation upon visual assessment in all the formulations 24 h after preparation or after 3 and 6 months of storage at room temperature.

Table 1. Physical Properties of Herbal Emulgels

Batch	NE0	NE1	NE2	NE3
Color	White	Milky	Light Brown	Brown
Odor	odorless	Characteristic	Characteristic	Characteristic
Homogeneity	+++	+++	+++	+++
Texture	Smooth and Glossy	Smooth and Glossy	Smooth and Glossy	Smooth and Glossy
Grittiness	None	None	None	None
Feel	Cool	Cool	Cool	Cool
Phase separation (after 24 h)	-	-	-	-
Phase separation (after 3 M)	-	-	-	-
Phase separation (after 6 M)	-	-	-	-

Key: +++ = very homogenous, - = no phase separation, M = months.

2.2. Physicochemical Properties of Prepared Emulgels

Physicochemical properties of the herbal emulgels are presented in Table 2. The pH of the emulgels ranged between 5.43 and 6.59 with formulation NE0 prepared without the extract had the highest pH while the others prepared with the extract showed concentration-dependent decrease in pH. Density of the emulgels were similar across the batches with values between 1.05 and 1.08 g/mL while viscosity was also found to increase in a dose-dependent manner. Formulation NE3 containing the highest amount of extract showed the highest viscosity (4801.9 mPas) while those of NE2 and NE1 were lower although similar (4189.0 and 4189.3 mPas respectively). Extrudability value was found to be higher in formulation NE3 ($p < 0.05$) than in the others. Spreadability values of the emulgels were between (13.17 and 25.50) g.cm/sec with the non-medicated emulgel (NE0) having the least value while NE3 had the highest value.

Table 2. Physicochemical Properties of Herbal Emulgels

Batch	NE0	NE1	NE2	NE3
pH	6.59 ± 0.12	5.95 ± 0.06	5.61 ± 0.04	5.43 ± 0.03
Density (g/mL)	1.05 ± 0.03	1.08 ± 0.02	1.08 ± 0.01	1.07 ± 0.04
Viscosity (mPas)	4240.30	4189.30	4189.00	4801.90
Extrudability (g/sec)	0.037 ± 0.00	0.041 ± 0.00	0.036 ± 0.00	*0.044 ± 0.00
Spreadability (g.cm/sec)	13.17 ± 0.23	14.94 ± 0.66	14.32 ± 0.45	25.50 ± 0.50

*Highest extrudability value

2.3. Particle Size of Prepared Emulgels

Figure 1 below shows that formulations NE3 and NE2 exhibited smaller particle sizes than NE1. 50 % of the particles of the medicated emulgels (D50) were distributed between 175.15 and 7724.14 nm; formulation NE3 had 175.15 nm, NE2 had 198.18 nm and NE1 had 7724.14 nm. On the other hand, polydispersity index was found to be similar across the batches; 0.4, 0.4 and 0.5 respectively at ($p < 0.05$).

2.4. Skin Irritancy Test

There was no oedema or erythema, no redness, swelling seen on the skin of treated mice during the 72 h observation period (Table 3). Also, no dryness, flaking or discoloration of the skin was observed within the 14 days the animals were monitored. Behavioural pattern for treated animals were similar to control. There was no significant difference between the body weight of control and treated animals (Table 4).

Table 3. Score Irritation Parameters of Animal Skin in Irritancy Test after Topical Application of Herbal Emulgels

Batch/Time	24 h		48 h		72 h	
	E	O	E	O	E	O
NE0	0	0/3	0	0/3	0	0/3
NE1	0	0/3	0	0/3	0	0/3
NE2	0	0/3	0	0/3	0	0/3
NE3	0	0/3	0	0/3	0	0/3

E = Erythema; O = Oedema.

Results presented as scores and the ratio of number of animals with reaction to number of animals tested.

Table 4. Body Weights (g) of Animals in Skin Irritancy Test after Topical Application of Herbal Emulgels

Batch/Time	Day 0	Day 7	Day 14
Control	22.37 ± 0.30	23.97 ± 0.22	24.87 ± 0.40
NE0	22.17 ± 0.62	23.34 ± 0.81	24.30 ± 0.60
NE1	22.10 ± 0.40	23.33 ± 0.57	24.87 ± 0.18
NE2	21.97 ± 0.12	23.60 ± 0.47	24.73 ± 0.27
NE3	22.50 ± 0.65	24.33 ± 0.27	24.87 ± 0.30

Data expressed as Mean ± SEM (n=3). One way ANOVA p<0.05. No significant difference treatment versus control

2.5. Studies on Hemorrhoid-Induced Test

Macroscopic examination of the isolated tissues revealed oedema with bleeding and blood congestion in the recto-anal tissues of the negative control group. Gross pathological evaluations showed that application of the emulgel formulations containing the extracts of *N. laevis* ameliorated the damage caused by croton oil as the severity of oedema, redness and bleeding in the tissues was observed to be less intense (Figure 2). Further, microscopic analysis of the tissues showed deep mucosal ulceration, moderate hyperplasia of inflammatory cells, medium to high degree of necrosis of mucosal epithelium in the control animals (Table 5). Meanwhile, the sham group showed normal architecture of the tissues in the recto-anal region.

Table 5. Pathology of Anal Tissues Showing Effect of Emulgel of *Newbouldia laevis* on Croton Oil-Induced Haemorrhoids

Batch	Macroscopic parameters		Histology parameters		
	Weight of rectoanal tissue (g/100g bd wt)	Gross pathology	Inflammation	Necrosis	Ulceration
Sham	0.013 ± 0.002	0.17 ± 0.17	0.30 ± 0.15	0.20 ± 0.13	0.10 ± 0.10
NE0	0.026 ± 0.005	3.17 ± 0.31	3.00 ± 0.26	3.30 ± 0.15	2.00 ± 0.33
NE1	0.022 ± 0.005	2.33 ± 0.33	2.70 ± 0.21	2.50 ± 0.31	0.40 ± 0.16 ^c
NE2	0.021 ± 0.004	2.00 ± 0.26 ^a	2.40 ± 0.16	2.30 ± 0.27 ^a	0.30 ± 0.12 ^c
NE3	0.021 ± 0.004	1.33 ± 0.21 ^c	1.80 ± 0.20 ^a	1.50 ± 0.34 ^b	0.10 ± 0.10 ^c

Values are expressed as mean ± SEM; One-way ANOVA treatment versus negative control ^ap<0.05, ^bp<0.01, ^cp<0.001

3. DISCUSSION

The crude ethanol extract of the stem bark of *Newbouldia leavis* and not the individual constituents of the extract. This is based on the efficacy of the crude extract in its use in folklore medicine as already documented and included in the literature review section. The dried extract was in powdered form, dark brown in color with herbal characteristic odor.

After preparation of the emulgel, the color intensity of emulgels containing the extract was found to increase due to the increasing amount of extract incorporated in the formulations while the white color of NE0 is attributed to the color of the formed emulgel. Homogenous texture of the emulgels shows that there was uniform distribution of the ingredients into each other which could be attributed to the method and expertise employed during the preparation. This also implies that they can be smoothly applied into and over the anus without causing any pain. Cooling sensation is a characteristic feature of emulgels which could aid in reducing itching and irritation at the inflammation site. All the prepared emulgels were observed to be physically stable. Other studies have also reported similar findings [24, 25] while other reported differently especially with the color/appeal of the emulgel [26, 27].

The pH in the rectum is naturally considered neutral between 7 and 8 with minimal buffering capacity, which favors the absorption of drugs with pKa values a little above or below the physiologic range [28]. Our results show that formulations containing the extract show near optimum pH which is important because products with too low or high pH cause irritation of the rectum leading to loss of patient compliance [29]. Our findings are also similar to those of Isimi *et al.*, [30] who reported pH values between 5.12 and 6.67 for herbal suppositories. Density of the formulations containing the extract seemed dose-dependent although the difference was not significant ($p > 0.05$). Viscosity is an important factor for rectal formulations because it influences the rate of drug release, distribution of the formulation and its ability to be retained in the rectal region [26]. Increase in viscosity with increase in amount of extract could be attributed to the presence of some secondary metabolites which could increase the gelling power of the gelling agent used [31]. High viscosities as seen in NE3 is desirable as it could prevent seeping of the formulation from the anus which could cause discomfort to the patient. Extrudability denotes the ease with which the formulation would be ejected from the orifice of its package. This is important because a product that is too fast running or is difficult to extrude from its package could dissuade the patient from using the product appropriately [32]. Faster extrusion of NE3 may be more acceptable than the other formulations containing extract. Spreadability shows the potential of a formulation to be evenly spread, it is an essential evaluation for semisolid formulations because it helps to ensure that a standard dose of formulation is properly distributed onto the required surface which improves patient acceptability as well as efficacy of the formulation [33]. These values show the magnitude of spread of the emulgels in the presence of minimal shear. Formulation NE3 was found to have significantly wider spread at significantly shorter time ($p < 0.05$) than NE2 and NE1 and correlates with the results of extrudability already presented. A similar report showing higher spreadability values with increase in amount of extract has also been documented [24] but is different from the study of Basha *et al.*, [34] where some batches of herbal emulgels had poor extrudability.

Particle size measurements give an insight into the distribution and aggregation of particles in addition to describing stability of a formulation [35]. Formulation NE3 and NE2 had narrower size distribution while particles of NE1 were more broad distribution. However, polydispersity index (PI), which estimates the average heterogeneity of a particle mixture [36] was found to be low for all the formulations. PI values less than 0.7 are acceptable indicating minimal degree of non-uniformity of particles in the formulation and good dispersion homogeneity [37]. From the results, all the formulations show good dispersion however, based on the mean diameter values, NE3 and NE2 were observed to have particles with smaller size range. This is important because small particle sizes have been shown to improve drug delivery by enhancing absorption, permeation and stability of formulations. Similar reports were documented in the development of ointment containing herbal nanoparticles [38] and by Kotian *et al.*, [39] in the development of herbal topical gels for wound healing.

Although ethno-medical reports have stated the use of *Newbouldia laevis* for the treatment of hemorrhoids, this is however the first study to formulate the stem bark extract of *N. laevis*, evaluate its dermal toxicity effects and demonstrate the activity of the preparation of the plant extract on experimentally-induced hemorrhoids [40].

The skin irritation test is carried out to determine the potential risk that could be associated with use of the preparation when applied topically and also to provide the information required for the manufacturer's safety data and product registration with regulatory agencies [41]. Irritation or irritant contact dermatitis is a response displayed towards an external stimulus that may activate innate immunity, cellular changes and cause the appearance of severe eczematous lesions. While, skin sensitization, or atopic contact dermatitis is a delayed hypersensitivity response mediated by T-cells towards specific haptens. Parameters that can be used to determine skin irritation and sensitization, include skin scoring *via* macroscopic observation, white blood cell count, skin histology and skin-fold thickness determination. Hazardous chemicals that may cause skin disease are mainly determined by characterizing erythema and edema formation in the skin [42].

The results obtained from this study showed that the preparation has no irritant action. It has been proposed that skin irritation is a poor measure of danger indicators for allergenic reactions and not a key determinant of the relative potency of a skin sensitizing chemical and also irritancy alone does not represent a complete surrogate marker for the ability of a chemical to produce danger signals relevant to the induction of skin sensitization [43]. However, it is noted that topical formulations with irritant effects are generally avoided for topical treatment [44]. Thus, the data obtained in this study implies that the emulgel formulation of the extract of *N.laevis* is relatively safe for topical/cutaneous application.

Hemorrhoids are characterized by severe vasodilation at the anal region cumulating in inflammation of the surrounding tissues. Croton oil was used in this study to induce hemorrhoids because it causes inflammation as a result of release of some inflammatory metabolites [45]. Histopathology evaluations show that severity of tissue damage was attenuated from high and moderate inflammation and necrosis to slight

inflammation. No mucosal ulcers of the epithelium were observed in animals treated with the emulgels containing the extract. However, NE3 with the highest concentration of extract and higher efficacy gave better outcome with positive signs towards treatment of hemorrhoids compared to the other concentrations. Analgesic and anti-inflammatory drugs are part of the treatment protocol for hemorrhoids [46]. Previous studies have shown that *N. laevis* contain saponins, tannins, flavonoids, steroidal glycosides and alkaloids [47]. These compounds have been demonstrated to possess analgesic and anti-inflammatory and wound healing activities in *N. laevis* extracts [47,48,49]

On the whole, treatment with the herbal emulgel formulations showed protection against the effects of croton oil with a dose-dependent decrease in the abnormalities observed in the animals. The actions of the formulations may be attributed to the anti-inflammatory, analgesic and antioxidant effects of the plant constituents.

4. CONCLUSION

In this study, herbal emulgel formulations containing various concentrations of the dried ethanol extract of *Newbouldia laevis* has been successfully prepared. The formulations showed optimum physicochemical properties. Histopathological evaluation showed dose-dependent efficacy of the extract with the formulation containing 1 %w/w of the extract producing the best positive sign towards treatment of hemorrhoids. The outcome of this study supports the ethnobotanical claim of its use in the treatment of hemorrhoids/pile and portrays the potential of developing a novel formulation for the treatment of inflammation related to hemorrhoids.

5. MATERIALS AND METHODS

5.1. Materials

Methyl paraben (sigma Aldrich Germany), Propyl paraben (Aldrich Chemicals, Inc. USA), Carbomer (Otto chemie Pvt Ltd Mumbai), Tween 80 and Span 20 (Loba chemicals Ltd, Mumbai), Liquid paraffin and Glycerol (Loba chemie, India), Ethanol extract of the stem bark of *Newbouldia laevis* plant prepared in National Institute for Pharmaceutical Research and Development (NIPRD), Abuja laboratory.

5.2. Animals

Swiss albino mice (25 - 32 g; aged 10 -11 weeks) of either sex were used for the experiment. The mice were obtained from the Animal Facility Centre of the Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria and subsequently maintained under ambient conditions (temperature $24 \pm 2^\circ\text{C}$; 12h light/dark cycle). Animals were fed with standard rodent pellets and provided with drinking water ad-libitum. The protocol of the study was approved by the Animal Care and Ethics Committee of the Department of Pharmacology and Toxicology, NIPRD, Idu, Abuja (NIPRD/05:03:05-35 dated September 6, 2022).

5.3. Methods

5.3.1. Collection, Identification of Plant Material and Extraction

The stem bark of *Newbouldia laevis* was collected in the botanical garden of NIPRD, Abuja, Nigeria and deposited at the NIPRD herbarium. It was authenticated and identified by Mr. Akeem Lateef and allocated a voucher number (NIPRD/H/7325). The dried stem bark was cleaned, dried reduced to coarse powder using a mechanical grinder. The powder was packaged and stored in a desiccator until further use.

5.3.2. Preparation of *Newbouldia laevis* stem bark extract

The method of Isimi et al., [50] was adopted for the extraction process. The powdered stem bark was macerated in absolute ethanol in the ratio 1: 20 (powdered bark:solvent volume) in for 24 h at room temperature with occasional stirring. Afterwards, the marc was collected by sieving using a muslin cloth and the filtrate was concentrated to dryness over a water bath (Karl Kolb, Germany) at 70°C . The resulting extract was pulverized, stored in an air tight container and placed in a desiccator until further use.

5.3.3. Preparation of Herbal Emulgel

The composition of ingredients used for preparation of *Newbouldia laevis* herbal emulgel is displayed as Table 6. Different formulations were prepared using altered concentrations of the extracts according to the

method of Khan *et al.*, [20] with some modifications. Gel base was prepared by dissolving appropriate quantity of carbomer in 50 mL of water with the aid of stirring over a magnetic stirrer. The oil phase of the emulsion was prepared by mixing Span 20 in light liquid paraffin and then heating to 80 °C. Tween 80 was mixed with water in a beaker, the preservatives (methyl paraben and propyl paraben) were dissolved in glycerol in another beaker, these two aqueous preparations were mixed together to make the aqueous phase. Appropriate amount of the extract was then dispersed into the aqueous phase and heated to 80 °C. The oily and aqueous phases were maintained at 80 °C; the oily phase was dispersed into the aqueous phase with continuous stirring until a stable emulsion was formed. The emulsion was mixed with the gel in the ratio 1:1 and stirred until the emulgel was formed. This was packaged in suitable containers and kept at room temperature until further use.

Table 6. Composition for Formulation of Herbal Emulgels

Ingredient/Batch	NE0	NE1	NE2	NE3
Carbomer (g)	1	1	1	1
<i>Newbouldia laevis</i> extract (g)	0	0.5	0.75	1
Liquid paraffin (mL)	10	10	10	10
Span 20 (mL)	2	2	2	2
Methyl paraben (g)	0.13	0.13	0.13	0.13
Propyl paraben (g)	0.07	0.07	0.07	0.07
Tween 80 (mL)	2	2	2	2
Glycerol (mL)	10	10	10	10
Water to (mL)	100	100	100	100

Key: NE0 = Herbal emulgel containing 0 %w/v extract, NE1 = herbal emulgel containing 0.5 %w/v extract, NE2 = herbal gel containing 0.75 %w/v extract, NE3 = herbal gel containing 1 %w/v extract

5.4. Evaluation of Herbal Emulgel

5.4.1. Physical evaluation

Physical evaluations such as appearance, color, odor, homogeneity, feel on hand, grittiness, phase separation was assessed by visual inspection.

5.4.2. Physicochemical evaluation

The following chemical evaluation were carried out;

pH measurements

The pH of the formulated undiluted emulgels were measured using the digital pH meter (Mettler Toledo).

Determination of Density

A syringe (2 mL capacity) was weighed on the balance, it was filled with the prepared emulgel and weighed again. The weight of the emulgel was obtained from the difference between the weight of the filled syringe and the empty syringe. Density of the emulgel was calculated as g/mL [32].

Determination of Viscosity

The viscosity of each batch of emulgel was determined using a Brookfield viscometer; AMETEK, Germany (Spindle L3) at 30 rpm at room temperature. An appropriate amount of emulgel formulation was placed in a beaker, the spindle was dipped into the formulation such that the groove was completely covered and the rpm was set. Viscosity measurements were taken after every minute and recorded.

Determination of Extrudability

Extrudability is determined by measuring the force required to push out the formulation from the orifice of a tube. The method as reported by Shah [51] was adopted with some modifications. The weight of an empty syringe (2 mL) was noted, it was filled with the emulgel and weighed again. Extrusion from the orifice of the syringe was regulated at 1 press/sec and the time taken for the emulgel to be completely extruded was noted. This determination was done in triplicates for each batch, the average was obtained and extrudability was computed as an expression of the weight of emulgel in the syringe per time taken to be extruded (g/sec).

Determination of Spreadability

Spreadability of the emulgels was determined based on the principle of “drag” and “slip” according to the method of Aremu *et al.*, [32] with some modifications. The emulgel; 125 mg (M) was placed on a slide and covered with another so as to sandwich the emulgel between both slides. A standard weight of 50 g was placed on top of the covered slide, the emulgel was allowed to spread maximally for 5 min and the extent of spread was measured (cm) in four (4) different directions (L) without removing the upper slide. In addition, the time taken to separate the upper slide from the lower slide (T) was recorded; spreadability was calculated in g.cm/sec using the equation below;

$$M \times L / T \quad (Eq. 1)$$

Particle Size Determination

The photon correlation particle size analyzer (BK-DLS802, Biobase Shandong Co. Ltd) was used to determine the particle size of the prepared formulations. All measurements were performed at room temperature and at 90° to the incident beam. Size distribution was taken from the median diameters D10, D50 and D90.

5.4.3. Skin Irritancy Test

Adult female nulliparous mice were used for this test. The hairs on the back of each mouse were carefully shaved 24 h prior to the test. The samples NE0, NE1, NE2 and NE3 (0.5 mL each) were applied on the skin of the animals (n = 3) and the area covered with a porous non-adhesive gauze, held in place by non-irritant tape for 24 h. A sham group was included to serve as control. The mice were housed in separate cages to avoid ingestion of the test substance. The mice were observed immediately after application of creams for behavioral, mucous membrane or other signs of toxicity. At the end of the exposure period, residues of the test samples were carefully removed with cotton wool. The animals were then returned to group housing for continued observation of signs of skin sensitivity and irritation using the Draize criteria [52] as reported by More *et al.*, [53] which is illustrated in Table 7. The treatment site was observed at 24, 48 and 72 h for behavioural, skin or fur changes and body weight of the animals was taken at day 0, 7 and 14 [54].

Table 7. Draize Dermal Irritation Scoring System (DDISS).

Parameter	Score
No erythema/oedema	0
Very slight erythema/oedema	1
Well-defined erythema/ Slight oedema (edges of area well defined by definite raising)	2
Moderate erythema/oedema (raised approximately 1 mm)	3
Severe erythema (beet-redness), eschar formation/oedema (raised more than 1 mm and extending beyond the area of exposure)	4
Total possible score for primary irritation	8

5.4.4. Studies on Croton Oil-induced Hemorrhoids

The method reported by Isimi *et al.*, [30] and Ayun *et al.*, [55] were adapted for use in mice. Thirty mice of both sexes were weighed and randomly placed into five groups (n = 6). After an overnight fast, hemorrhoid was induced in animals in groups 2 - 5 by administration of a hemorrhoid inducing agent. The inducing agent consists of deionized water, pyridine, diethyl ether, and 6 % croton oil in diethyl ether prepared in the ratio of 1:4:5:10. Each mouse was given 0.02 mL inserted into the rectum (5 mm from the anal opening) by means of a flexible cannula attached to a micro syringe. After a duration of 7 h the animals were treated as follows:

- Group 1: Normal animals, no treatment given (Sham group)
- Group 2: NE0
- Group 3: NE1
- Group 4: NE2
- Group 5: NE3

Treatment was administered by rectal application of 0.1 mL of emulgel in a single daily dose for 5 days. On day 7 after hemorrhoid induction, animals were euthanized by an overdose of ketamine/xylazine (50/10

mg/mL) and the distal recto-anal tissue (2 cm) was dissected out. This was observed macroscopically and scored [55] for damage of rectoanal tissues, weighed and then placed in normal saline for histological analysis [56].

$$\text{Rectoanal coefficient} = \frac{\text{weight of rectoanal tissue (mg)}}{\text{body weight (g)}} \text{ (Eq. 2)}$$

5.4.5. Histological evaluation on Croton Oil-induced Hemorrhoids

The freshly collected tissues were placed on a filter paper to blot off fluid. The tissues were then placed in formal saline (10 %) for fixation. These were dehydrated using graded concentrations of ethanol and cleared with xylene. The tissues were infiltrated then embedded in paraffin wax. Thereafter, sections of the tissues (4-6 µm) were obtained using a rotary microtome and stained with hematoxylin-eosin. The sections were then observed using a light microscope. The plates were examined under a light microscope and histopathological assessment is done by assessing mucosal and submucosal inflammation, necrosis and ulceration [55, 57].

Macroscopic parameters evaluated were oedema with mucosal bleeding or blood congestion 0, normal; 1, minimal; 2, slight; 3, moderate; and 4, intense NAD: 0 (no abnormality detected); minimal: 1 (very small number of changes); mild: 2 (lesion is easily identified but limited severity); moderate: 3 (lesion is predominant); severe: 4 (the degree of changes is great enough in intensity or extent to expect significant tissue or organ dysfunction). Score results were taken based on observations from 10 fields of histology slides. The scores for changes were allocated as normal = 0, minimal = 1, mild = 2, moderate = 3, intense = 4: (minimal/very minute changes >10% = Score 1; Lesions <25 % = Score 2; Lesions 25-50 % = Score 3; Lesions >50 % = Score 4).

5.5. Statistical Analysis

All of the measurements were performed at least in duplicates or triplicates. Data were presented as the mean ± standard deviation and mean ± standard error of mean as appropriate. Data was analyzed using by One-way ANOVA using GraphPad Prism. The level of significance was set at $p < 0.05$.

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