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Selection of appropriate HLB values for a stable honey-shea butter emulsion and its efficacy in treating chemical burn wound.

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ABSTRACT

Background & Aim: Since ancient times till today, honey and shea butter are used in wound treatment. This present study aims to determine an effective hydrophile-lipophile balance (HLB) value that will produce a stable W/O honey-shea butter emulsion and to evaluate its healing potential on chemical burn wound in mice.

Experimental: Using various ratios of span 20/tween 20, 6 formulations of honey-shea butter emulsion were prepared and subjected to accelerated stability study as per ICH guideline. The most stable preparation was further evaluated for its wound healing ability. The animals were sacrificed via chloroform inhalation after treatment for 7 days from wound created by dropping 0.2 ml, hydrochloric acid for 15 sec. The burn area was harvested and subjected to histopathological study.

Results: Most stable emulsion was those prepare with ratio 5:5 of span 20/ tween 20. Haematoxylin-eosin and Verhoeff-Van Gieson stain revealed that groups treated with this emulsion had normal fibroblast, fibrocyte, epidermal cells, inflammatory cells, gland cells, collagen and elastic fibres; outperforming honey, shea butter and silver sulfadiazine groups. The present formulation gave a percent wound contraction of 36, while those of honey, shea butter, silver sulfadiazine were 29, 25 and 28, respectively. The occlusive effect of emulsion and the synergistic effect achieved by combining honey and shea butter could also be responsible for its superior wound healing activity.

Recommended applications/industries: Our study concluded that honey-shea butter prepared with 5:5 span 20/tween 20 was relatively stable and possesses greater wound healing activity compared to commercial preparation and can safely be utilize as an effective natural therapy for burn wound.

1. Introduction

Emulsion is composed of two phases which are thermodynamically not stable and will most likely separate into the oil and aqueous components on standing (Baccarin and Lemos, 2017). Processes like coalescence flocculation, phase inversion, creaming and sedimentation are responsible for such separation. Hence, surfactants are needed to stabilize the emulsifying process (Juttulapa *et al.*, 2013).

Hydrophile-lipophile balance (HLB) is a primary method for choosing an emulsifier. A stable emulsion is best prepared with surfactants or combinations of surfactantswith HLB value close to the required HLB value ($RHLB$) of the oil phase used (Loden and

Andersson, 2008). Spans and Tweens are mild non-ionic surfactants that are stable in mildalkalis, acids and electrolytes with no reaction on ionic ingredients (Loden and Andersson, 2008).

By combining Spans and Tweens, variety of O/W and W/O emulsion systems can be prepared. They both offer many benefits including increased stability and formulation flexibility (Loden and Andersson, 2008). The skin is the largest organ of the human body, with critical structure shielding its internal tissues from mechanical damage, microbial infection, ultraviolet radiations and extreme temperature (Baccarin and Lemos, 2017).This makes it injury prone with significant impact to both individual patients and the economy (Mohammed *et al.*, 2018).

During acute wound, haemostasis occurs to stop bleeding through activation of platelets to form a fibrin clot. This is followed by inflammatory phase to activate an elaborate immune response which destroys pathogens entering the wound, and then prepare the tissue for the restoration of anatomical integrity (while in chronic wound, the inflammatory phase fails to resolve leading to poor and delay healing) (Serap *et al.*, 2018). The proliferative or angiogenesis stage involves the formation of granulation tissue, neovascularization and re-epithelialization to form new blood vessels (Subrahmanyam, 2007). Finally, is the remodelling phase where granulation tissue is replaced with a scar and the epidermis is freed from immune cells, which either dies by apoptosis or relocate to the dermis (Raham *et al.*, 2017).

Chemical burns also called caustic burns happens when one comes in contact with corrosive substances acids, alkalis, bleach, or caustic chemicals. They can result from an accident or assault (Yaghoobi *et al.*, 2013). Chemical burns can be either superficial or first degree, affecting only the epidermis. It could be partial thickness or second degree extending to the dermis. It could also be full thickness or third degree, which goes through the skin and damage tissue underneath (Davrieux *et al.*, 2010). Infections accompanying burn is the major cause of mortality in burn patients, hence it is recommended that burn wound be managed within a short possible time (Nitish *et al.*, 2016; Vijay *et al.*, 2017). With many therapies for wound healing readily available, they are only moderately effective possessing certain critical limitation such as addition of antimicrobial agents which makes them cytotoxic on prolong usage; also some commercial products loses their moisturizing ability with time making them to adhere to wound surface and damage newly formed epithelium- hence the need for a more effective treatment (Verma *et al.*, 2012).

Honey has been proven to be non-adherent to wound surface, provide moist environment, prevent cross infection by forming bacterial barrier and also prevent infecting barrier (Subrahmanyam, 2007). Antibacterial attribute of honey is linked to its osmotic effects, acidity, generation of hydrogen peroxide and phytochemical components.Its phytochemical components include flavonoids, carotenoids, ascorbic acid and phenolic acid which are effective in removing free radicals-saving cells from damage.

Shear butter is a complex fat that isrich in triglyceride and other fatty acids as well as, vitamin E, vitamin A, cathecin and cinnamic acid. It has been evaluated to have numerous health benefits such as shrinking of tumour and cancerous cells, reduction of inflammation and pain as well as promoting wound healing and wound-scars reduction (Loden and Andersson, 2008).

The healing benefits of honey and shea butter especially in skin wounds care have been established (Yaghoobi *et al.*, 2013; Davrieux *et al.*, 2010; Verma *et al.*, 2012). An amalgam of honey and shea butter is sure to produce synergistic benefits. However, the physical properties of honey and shea butter makes it difficult for direct mixing and application of both substances especially on an affected area due to liquefaction and leakage at higher temperatures, this restrict the body location on which they can be used. This study aims to develop a stable W/O honey and shea butter emulsion by selecting an appropriate HLB value that is effective, and also evaluate its healing potential on chemical burn wound in mice.

2. Materials and Methods

2.1. Materials

Span 20 and Tween 20 were obtained from Loba Chemie Pvt. Ltd India, Shea butter and Honey were purchased from Karmo, a local market in Abuja Federal Capital Territory of Nigeria, all other reagents used were of analytical grades.

2.2. Emulsion formulation

Quantities of Span 20® and Tween 20® that corresponds to the required HLB values as (Table 1)

were appropriately weighed out separately into 50 ml beakers. A 50g of shea butter was weighed into a 200 ml beaker and melted over water bath. The weighed portions of surfactants were added to the melted shea butter and heated to 70 °C. A 50 g of honey was weighed out and added dropwise into the shea buttersurfactant mixturewith continuous stirring using a magnetic stirrer until a homogenous mixture is formed. Another sample corresponding to the control (Sample F) was prepared without surfactants.

Table 1. Formula for emulsion preparation.

	Ratio	Span 20 (g)	Tween 20 (g)	Honey (g)	Shea butter (g)	Target HLB
A	5:5	5	5	50	50	12.7
B	3.5:6.5	3.5	6.5	50	50	13.9
C	6.5:3.5	6.5	3.5	50	50	11.4
D	10:0	10	0	50	50	8.6
E	0:10	0	10	50	50	16.7
F	Control	0	0	50	50	-

2.3. Physical studies of emulsion

2.3.1. Organoleptic Properties

The prepared emulsions were evaluated for its colour, odour, homogeneity and evidence of phase separation.

2.3.2. Measurement of pH

The pH of the preparedemulsion was determined using a calibrated digital pH meter (Mettler Toledo, model EL-20).

2.3.3. Spreadability

Two glass slides of standard dimensions (7 cm× 2.3 cm) were selected. About 0.2 g of the formulation was placed over one of the slides. The second slide was placed over the slide in such a way that the formulation was sandwiched between them across a length of 7 cm along the slide. A 20 g weight was placed on the upper slide so that the emulsion is between the two slides. After a minute, the weight was removed and the diameter of the spread area was measured and recorded (Tzu-Kai *et al.*, 2018).

2.3.4. Occlusion

A 25 ml distilled water was transferred into a beaker of 50 ml capacity. It was covered with 0.45 µm filter paper and sealed with a masking tape. A known amount of sample was spread on the filter papers and stored at ambient temperature, 75 % RH for 144 h. Beaker covered with filter paper without samples was considered as control. The occlusion factor (F) was determined as:

$$F = 100 * (A - B)/A$$

Where A is the water loss of control, and B is water loss with sample (Subrahmanyam, 2007).

2.3.5. Moisture sorption

Three desiccators labelled A, B and C were used. A known volume of water was transferred into a desiccator believed to have a 100 % RH, and labelled A. Another desiccator containing a solution prepared by adding 3 part of sodium chloride (NaCl) to 1 part of distilled water to give a 75 % RH was labelled B. While a third desiccator containing 3 part of potassium hydroxide (KOH) to 1 part of distilled water to achieve 8 % RH was labelled C.Anaccurately weighed1g of each sample were placed in the 3 desiccators already saturated with solutions. The desiccators were covered appropriately. Samples were weighed at 30 min intervals for 3 h to check for increase/decrease in weight.

2.3.6. Swelling Index

A phosphate buffer solution (pH 6.8) was prepared. A 10ml of the solution was transferred in to 6 different Petri dishes. Then, 1g of each formulation was weighed out on an aluminium foil, neatly wrapped and placed on the Petri dishes. The foil and its content were re-weighed after an hour intervalto check for any increase/decrease in weight (Subrahmanyam, 2007).

2.3.7. Globule size/Photomicrograph analysis

Globule size of the emulsions was evaluated with the aid of an optical light microscope (Olympus Light Microscope). It was fitted with a camera and a computer software (Motic MC 1000), for imaging and transmission to a monitor. Samples were prepared by evenly spreading a thin layer of the emulsion on a specimen slide, stained with crystal violet and covered with a slip. One hundred globules were measured from

each sample and the photomicrographs documented before and after the stability studies (Verma *et al.*, 2012).

2.3.8. Viscosity

Theviscosity of the emulsions at 4 °C, 25 °C and 40 °C was determined using a Biobase viscometer (NDJ-85). A concentric cylinder system was submerged in the emulsion and the force needed to overcome the resistance of the viscosity to the rotation is measured. The viscosity value was determined based onthe speed and geometry of the probe (Verma *et al.*, 2012).

2.4. Animal studies

Animals: Adult Wistar albino mice (15 males and 15 females) weighing between 40-44 g were shared into 5 groups of 6 maintained at animal facility centre of NIPRD. They were housed in standard polypropylene cages with saw dust as beddings, under ambient conditions. The animals were fed on standard rodent feed and had free access to clean drinking water ad libitum. The animals were handled according to the Institutional Animal Guidelines for Care and Use of Animals as recorded in the Standard Operating Procedure of the Department of Pharmacology and Toxicology, NIPRD (SOP No. 05:03:02) and the internationally accepted principles for laboratory animal use and care (NIH publication NO 85-23, revised, 2010).

Procedure: Using 10 mg/kg of ketamine, the animals were anaesthetized intraperitoneally. With the aid of a clipper, the dorsal skin of the animals was shaved. The shaved area was disinfected with 70 % alcohol. A 0.2 ml of 37 % hydrochloric acid was dropped for 15 seconds on the shaved area of the animal while under anaesthesia. Wounds were treated topically according to grouping (6 per group) with the control group treated with silver sulfadiazine cream. Wounds were treated daily for 7 days from the day of wound creation. Sacrifice of the animals was done on the tenth day via chloroform inhalation. The burn area was harvested and subjected to histopathological studies. The skins were removed and fixed, dehydrated, embedded and cut for light microscopy section. Three ons were made for each wound and stained with hematoxylin-eosin (basic stain for light microscopy) and Verhoeff-Van Gieson (for collagen and elastic fibre staining). Sections were evaluated by an independent observer.

Wound contraction rate was expressed as the percent change in the original area using the follow equation:
Original wound area- wound area after day 7 × 100/ Original wound area
Group H serve as test group treated with raw honey
Group S serve as test group treated with raw shea butter oil
Group HS serve as test group treated with honey-shea butter emulsion
Group SS serve as test group treated with silver sulfadiazine cream
Group UT serve as untreated (negative control) group

Skin irritancy test: The prepared emulsion was applied topically to the dorsal skin of 6 mice to evaluate their skin sensitization. The area of application was occluded with gauze and covered with non-sensitizing micro porous tape, and the development for erythema and oedema was monitored for 3 consecutive days (Mohammed *et al.*, 2018).

3. Results and discussion

3.1. Emulsion formulation

A limiting factor to most medicinal plants for treating ailment is their dosage form. Generally, emulsion is favoured over other topical semisolid formulation because it has a long resident time on the skin; it usually has higher viscosity; it has a good occlusion property that helps to moisturize flaky skin; better bio-adhesive properties; minimal skin irritation; it is not dependent on water solubility of active ingredients; easy to apply and better release properties (Juttulapa *et al.*, 2013). In this study, we used various HLB values to come up with astable honey-shea butter emulsion that will be suitable to treat chemical burn wound. The pH and viscosity of honey and shea butter used in this work were (3.95±0.06; 28.46±0.01 mPas) and (6.46±0.16; 89.39±0.02mPas), respectively, which is in agreement with previously reported data (Subrahmanyam, 2007; Verma *et al.*, 2012).

3.2. Physical properties of emulsion

3.2.1. Organoleptic properties

Six formulations (A-F) were prepared, and their colour ranges from yellow to brown with a characteristic's nutty shea butter smell at 4 ^{0}C, 25 ^{0}C

and 40 ^{0}C even after 3 months. Our formulations separate at all temperatures studied except for A which was stable at 25 ^{0}C and 4 ºC even after 3 months. They could not be washed off easily from skin surface, meaning their residence time will be longer and hence administration frequency will reduce. Grittiness was absent in all preparations.

3.2.2. pH determination

The pH of the emulsions prepared vary from 3.23 to 5.04 (Figure 1). Significant variations in pH could be a sign of chemical decomposition. Formulation A had a close pH range between 4.60 to 4.99 across the different temperatures, though slightly lower than that of a healthy skin (5.40-5.90) (Audu *et al.*, 2020). This pH however is acceptable and will favour the maintenance of acidic pH of the skin. Other authors have reported that a pH between 5 and 7 is ideal for a stable emulsion (Audu *et al.*, 2020).

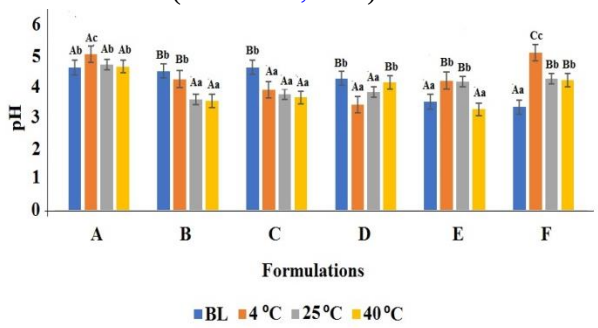

Fig 1. pH profile of Emulsions at different temperatures and storage time. Each bar denotes mean± standard deviation (n=6). Bars with different lower-case superscript are significantly different between different formulation at a studied temperature, while bars with different upper-case superscript are significantly different in a formulation at different temperatures (P<0.05).

3.2.3. Spreadability evaluation

Spreadability shows the area which a formulation covers when applied topically. It could tell how well a formulation will work and how it will be accepted by the end users. Poor spreadability will cause an uneven distribution of the formulation to the skin and subsequent poor delivery of the active ingredient, hence the expected result may not be achieved (Anna *et al.*, 2015). The larger the coefficient value the better the spreadability on the skin. Formulation A has the highest value with least variations even after 3 months across all storage conditions tested (Figure 2). This implies that with an application of small amount of shear stress it could spread easily .

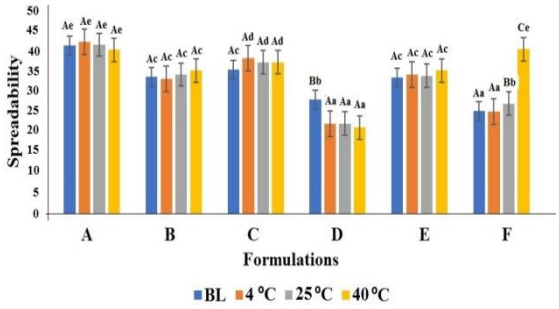

Fig 2. Spreadability of the eemulsions at different temperature and storage time. Each bar denotes mean± standard deviation (n=6). Bars with different lower-case superscript are significantly different between different formulation at a studied temperature, while bars with different upper-case superscript are significantly different in a formulation at different temperatures (P<0.05).

3.2.4. Viscosity determination

Viscosity affects the release of drug from a semisolid preparation. Consistency of a dosage form and drug content release relies primarily upon viscosity. It has been reported elsewhere that while viscosity increases, the rate of drug release decreases (Alexandra *et al.*, 2020). This however implies that formulation A will have a faster release time since its viscosity across different temperatures were lower and less variable when compared to others (Figure 3).

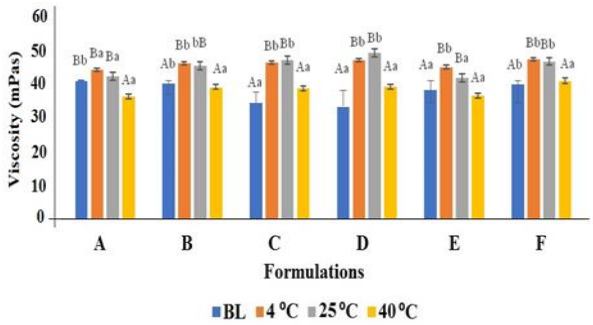

Fig 3. Viscosity profile of the emulsions at different temperatures and storage time. Each bar denotes mean± standard deviation (n=6). Bars with different lower-case superscript are significantly different between different formulation at a studied temperature, while bars with different upper-case superscript are significantly different in a formulation at different temperatures (P<0.05).

3.2.5. Occlusion properties

The occlusive properties of O/W emulsions may depend on the type and amount of oils used and on the volume of the oily phase. When a product is occlusive, it means it could form barrier on the skin and hence affect skin hydration by restoring skin lipids, maintain skin smoothness and elasticity(Alexandra *et al.*, 2020). Our findings show that the control formulation had the highest occlusion value after 144 hours (Figure 4). The result also shows that the HLB values of each formulation did not really have any significant effect on the occlusion property since some formulations with higher HLB value still had poor occlusion.

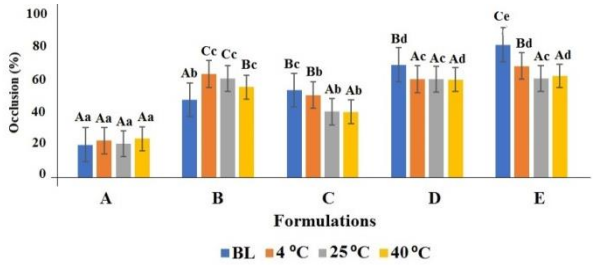

Fig 4. The occlusion characteristic of the emulsions at different temperatures and storage time. Each bar denotes mean± standard deviation (n=6). Bars with different lower-case superscript are significantly different between different formulation at a studied temperature, while bars with different upper-case superscript are significantly different in a formulation at different temperatures (P<0.05).

3.2.6. Swelling index study

Swelling index of the formulations were seen to be proportional to temperature (Figure 5). There was a gradual increase in swelling as temperature increases indicating that the associative internal forces within the formulations are weaker (Yaman *et al.*, 2010).

3.2.7. Moisture sorption

Water activity for all formulations ranges from 0.11-0.95 at 8 %, 75 % and 100 % RH (Figure 6a-c). Studies show that product with water activity greater than 0.8 will grow mould easily (Nitish *et al.*, 2016). Hence, formulation C may not be stable when kept in an environment of 100 % RH. Temperature affects the mobility of water molecules and the dynamic equilibrium between the vapour and adsorbed gases. If water activity is kept constant, an increase in temperature will cause a reduction in water content as can be seen in present work.

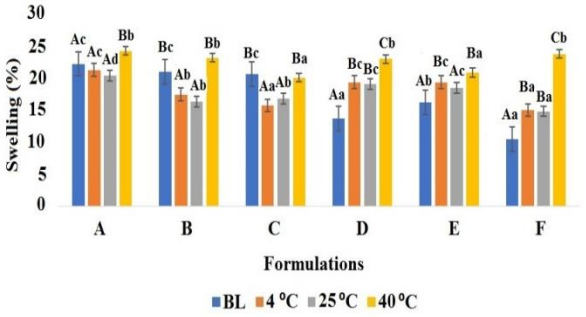

Fig 5. The swelling of characteristics of the emulsions at different temperature and storage time. Each bar denotes mean± standard deviation (n=6). Bars with different lower-case superscript are significantly different between different formulation at a studied temperature, while bars with different upper-case superscript are significantly different in a formulation at different temperatures (P<0.05).

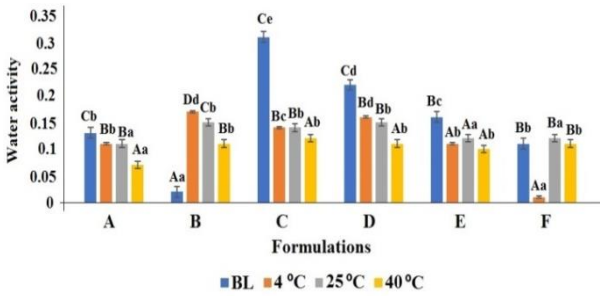

Fig 6a. Moisture sorption at 8 % relative humidity at different temperatures and storage time. Each bar denotes mean± standard deviation (n=6). Bars with different lower-case superscript are significantly different between different formulation at a studied temperature, while bars with different upper-case superscript are significantly different in a formulation at different temperatures (P<0.05).

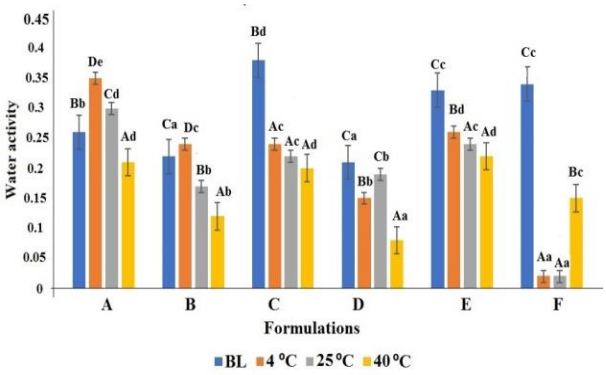

Fig 6b. Moisture sorption at 75 % relative humidity at different temperatures and storage time. Each bar denotes mean± standard deviation (n=6). Bars with different lower-case superscript are significantly different between different formulation at a studied temperature, while bars with different upper-case superscript are significantly different in a formulation at different temperatures (P<0.05).

3.2.8. Globule size analysis

Emulsions are generally not monodispersed systems since they contain a distribution of drop sizes, so that small globules can enter the void space among larger globules. The overall emulsion surface area relies largely on the square of the globule size, while the internal phase volume depends on the cube of the globule size (Alexandra *et al.*, 2020). The surface-to-volume ratio varies inversely to the size. Hence, it can be said that emulsions with small droplet size exhibit a higher surface area per unit volume than emulsions with big drop size. Also, the drop size distribution of an emulsion affects its overall stability (Alexandra *et al.*, 2020). The present results showed that the globule sizes were evenly distributed for all of the formulations with A having the least variations in globule size (6.997 µm at baseline, 6.844 µm at 4 °C, 7.022 µm at 25 °C and 8.546 µm at 40 °C after 3 months) (Table 2), this will confer more stability to it.

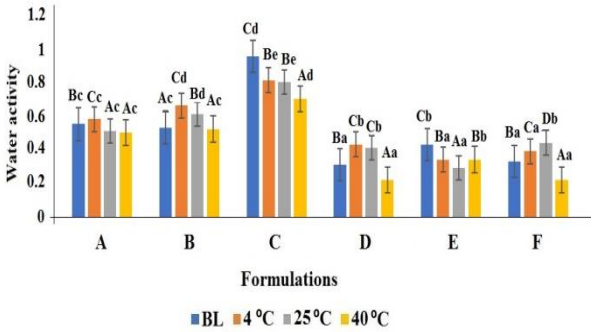

Fig 6c. Moisture sorption at 100 % relative humidity at different temperatures and storage time. Each bar denotes mean± standard deviation (n=6). Bars with different lower-case superscript are significantly different between different formulation at a studied temperature, while bars with different upper-case superscript are significantly different in a formulation at different temperatures (P<0.05).

Table 2. Mean globule size photomicrograph of formulation A, B, C, D, E and F at different temperatures and storage time.

	A (µm)	B (µm)	C (µm)	D (µm)	E (µm)	F (µm)
BL	6.9±1.5	6.1±6.0	5.2±3.3	6.7±3.2	8.9±0.4	4.9±1.0
4 °C	6.8±0.3	2.3±2.5	1.9±1.9	5.6±1.7	8.0±0.7	3.6±1.3
25 °C	7.0±0.9	9.1±5.1	5.9±0.9	4.3±4.2	12.1±4.0	5.9±1.1
40 °C	8.5±2.9	11.1±2.2	9.2±3.5	11.6±1.6	17.2±6.0	12.1±0.8

Values are presented as mean± standard deviations (n=100).

3.2.9. Histological study

It is noteworthy to mention that the potency of restoring the histological integrity of chemical burn wound tissue with honey-shea butter emulsion showed compatibility with group treated with sulfadiazine after day 7. Fibrocytes and fibroblast in those groups were normal as shown in table 3. Fibroblast and fibrocyte are known to deplete pathogens through the release of extracellular traps lysosomal peptides - producing cytokines, chemokines and growth factors; while secreting extracellular matrix proteins and glycosaminoglycan; hence promoting wound closure through α-SMA-mediated contraction; and enhance angiogenesis by transformation into other mesenchymal cell types (Nur *et al.*, 2016).

Table 3. Haematoxylin-eosin (HE) and Verhoeff-Van Gieson (VVG) results after 7 days.

	FB	FC	LH	K	E	G	AD	CL	EF
H	+	++	+	+	++	+	-	+++	++
S	++	++	+	-	+++	++	++	+++	+
HS	+++	+++	+++	-	+++	+++	++	+++	+++
SS	+++	+++	+	_	++	++	++	+++	++
UT	+	+	++	-	+	_	+	+++	++

FB=fibroblast, FC=fibrocyte, LH=inflammatory cell, K=keratin, EP=epidermal cell, GL=gland cell, AD=adipocyte, CL=collagen, EL=elastic fibre. H=honey, S=shea butter, H-S=honey-shea butter emulsion, SS=silver sulfadiazine, UT=untreated. +=Slight, ++=Moderate, +++=Normal
Values are presented as mean of n=6.

Inflammatory cells were normal in group treated with H-S emulsion (Figure 7). Inflammatory phase in wound healing is characterized by the presence of neutrophils and macrophages at injury site (Nur *et al.*, 2016). Neutrophils aggregate at the wound site at an early stage of inflammatory response and direct adaptive immune response by presenting antigen to T cells and promote T cell differentiation towards Th17, which subsequently affects wound healing (Hong *et al.*, 2017). On the other hand, macrophages when depleted,

it will lead to impaired or delayed wound healing (Nur *et al.*, 2016). Based on their functions during wound healing, wound macrophages are classified as pro-inflammatory (producing inflammatory mediators like ROS and NO), pro-wound healing (responsible for tissue repair and neovascularization) and pro-resolving macrophages (inhibit inflammation and immune cells) (Rodrigo and Steven, 2020). All 3 participates for proper healing to take place.

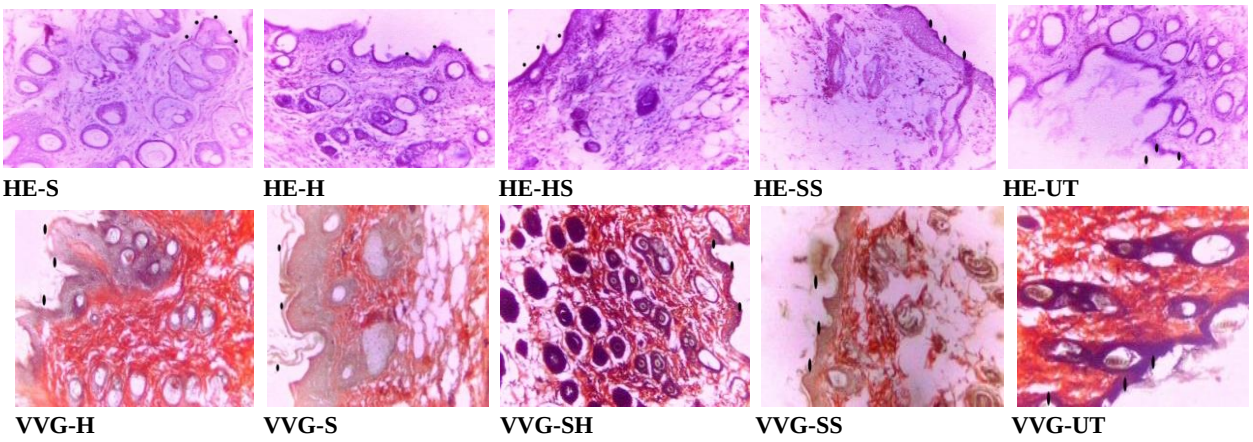

Fig 7. Hematoxylin-eosin (HE) and Verhoeff-Van Gieson (VVG) image after 7 days. (n=6). H=honey, S=shea butter, H-S=honey-shea butter emulsion, SS=silver sulfadiazine, UT=untreated. The black dots indicate the wound areas in all pictures.

We noticed a similar pattern of moderate deposition of adipocytes in groups treated with shea butter alone, H-S emulsion and sulfadiazine. Studies have shown that when dermal adipocytes are depleted prior to skin injury, macrophages recruitment to the wound site are reduced while revascularization and re-epithelialization of the wound bed are delayed (Lauren *et al.*, 2016). Epidermal layers were normal in groups treated with S and HS.

There was normal hyperplasia of glands in group treated with our emulsion, unlike those treated with sulfadiazine, honey or shea butter which only exhibit slight hyperplasia. The regeneration of a fully functional skin involves restoration of skin components such as the sweat glands (Samantha *et al.*, 2018). Sweat glands are vital to the skin, as they secrete sweats, excrete waste, maintain body temperature, and secrete lactate which in turn inhibit bacteria growth (Laure *et al.*, 2013).

Only group treated with honey shows the presence of keratin-a fibrous structural protein that gives mechanical stability to cells of the epithelium (Kamila *et al.*, 2021). They are implicated in wound healing by scaffolding (Kamila *et al.*, 2021).

VVG analysis of the wound tissue to determine its elasticity and collagenicity shows normal deposition of collagen for all groups, while for elastic fibre, only our emulsion shows normal deposition. The primary function of collagen is to serve as a scaffold in connective tissue while elastic fibres are rubber-like substance which contribute to resilience and elasticity in tissues (Annalisa, 2020) Elastic fibres are intertwined with collagen fibres in the extracellular matrix (Kamila *et al.*, 2021).

3.2.10. Wound contraction

Results for wound contraction are presented in Figure 8, with our emulsion outperforming all other groups achieving wound closure of 36 %. The occlusive ability of our emulsion could be a determining factor; also, the synergistic effect achieved by combining honey and shea butter could also be responsible.

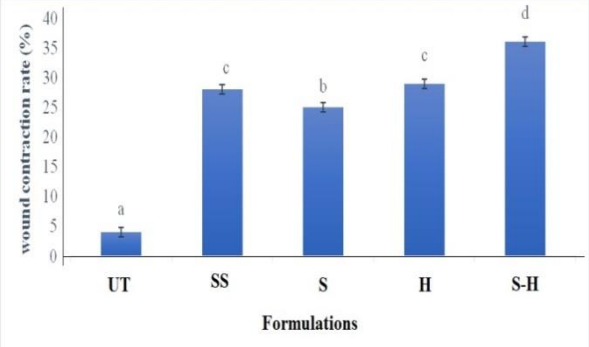

Fig 8. Percent wound contraction of mice treated with UT, SS, S, H and H-S after 7 days. H=honey, S=shea butter, H-S=honey-shea butter emulsion, SS=silver

sulfadiazine, UT=untreated. Each bar denotes mean± standard deviation (n=6). Bars with different lower-case superscript are significantly different at ($P<0.05$).

3.2.11. Skin irritancy test

Generally, there was no sign of irritation or erythema from our formulation, which will mean better compliance from patients.

4. Conclusion

We successfully achieved a stable honey-shea butter emulsion by combining surfactants with combined HLB value close to the required HLB value ($RHLB$) of the oil phase used. Looking at the quality parameters investigated, we can however conclude that formulation A was relatively the most stable. Secondly, the histological, skin irritancy and wound contraction data generated proves that combining honey and shea butter is more effective in treating chemical burn wound than using silver sulfadiazine, honey or shea butter alone.

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