

Original Article

Evaluation of Aqueous Creams Containing Ethanolic Extract of *Curcuma longa* (Turmeric) as Bioactive Ingredient for the Management of Wounds

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Abstract

Background: Wounds are considered health indispositions with detrimental socio-economic influences on the afflicted and their kin. *Curcuma longa* or turmeric has been used in the treatment of wounds. Employing the extracts of turmeric as a

bioactive ingredient in an aqueous cream could enhance its value in wound treatment.

Aim: This study aimed to formulate aqueous creams containing concentrations of ethanolic extract of turmeric (EET) as bioactive ingredients, evaluate their stability and wound healing activities in male

Wistar rats using hydroxyproline (HXP) as a biochemical marker.

Materials and methods: Solutions of 1.5, 3.0 and 5.0 % w/v of crude EET were prepared and also incorporated as bioactive ingredients in aqueous creams. The stability of the creams was evaluated and their wound healing effects were studied using distilled water, dimethyl sulphoxide (DMSO) and cholesterol as controls in male Wistar rats.

Results: The creams were stable in colour, pH, viscosity, etc. and exhibited wound healing activities. The animals treated with the crude 1.5 % w/v EET and its cream demonstrated the highest tissue HXP level showing significant percentage differences in tissue HXP levels from the control groups ($p < 0.05$).

Conclusion: The crude EET has been confirmed to possess wound healing properties with an optimal effective concentration for wound healing in male Wistar rats determined as 1.5 % w/v which when incorporated as a bioactive ingredient in an aqueous cream retained its efficacy in wound healing and could therefore be beneficial in the treatment of body injuries.

Keywords: Wound healing; Aqueous cream; *Curcuma longa*; Turmeric; Bioactive ingredient; Hydroxyproline; Wistar rats

Introduction

An injury or wound is a harm to the superficial cover of the body which interferes with the other soft skin. It is a universal term that discusses the hurt triggered by accidents, falls, hits, weapons, surgery, sutures or stitches, etc. Injuries range from minor to life-threatening types which could occur at work or play,

indoors or outdoors, driving a car or walking across the street. Wounds are damages that disrupt the skin or other body tissues and includes cuts, scrapes, scratches and punctured skin [1,2]. The existence of wounds brings about the socio-economic burden to the victim and their relations [3]. Body injuries may be caused by physical, chemical, thermal, microbial or immunological manipulation to the body tissue [4,5] and may be described based on their degree, the time-lapse between its initiation and restoration, the advance of restoration, the existing disease state in the victim, the accompanying risk of mortality and the effect on the worth of life of the victim [6,7]. When a body is broken or torn by any sharp object, it is considered as an open wound. On the other hand, when an unsharpened object or forceful distress causes a bump on the body, the result is described as a closed wound. The types of hurtful damages considered as a burn could be triggered by fire, heat, radiation, chemicals, electricity or sunlight [4,5,8]. Wound healing takes some time to have the original flesh fully reestablished as the procedure to achieve that is somewhat complicated with some transforming stages of cellular and biochemical reactions which must take place consistently and at the due period to harness the reestablishment of the natural flesh. The actualization of these implicates continuous cell-cell as well as cell-matrix interactions giving room for the process to advance in sundry conforming sectors and ways encompassing inflammation, wound contraction, re-epithelialization, tissue re-modelling and development of granulation tissue with angiogenesis. The extent of progress a damaged part of the body could be repaired is time- dependent. In other words, if the expected degree of restoration fails to take place at the right time frame, it is possible that the expected healing may fail to be attained and of

course, may lead to such injuries that take a long time to heal [9-12]. The paramount stage of restoring an injury attempts to control bleeding [13,14]. The processes that bring about the constriction of the blood vessels, platelet migration and realization of coagulated fibrin reinstates haemostasis soon after vascular damage, giving room for an extracellular setup for cell migration. Following these, mediators of wound healing draw inflammatory cells to the area of injury permitting the successive phase of inflammation [15]. This additional stage intersects with the previous stage comprising haemostasis and clotting and is commenced in a few hours following the injury. This point is predominantly distinctive from the accumulation of leukocytes and macrophages [14,16]. At this time macrophages migrate into the distressed area, discharge growth factors like a platelet, easing the improvement of fresh connective tissue or granular tissue [14]. Macrophages likewise aid in the resolution of inflammation and ignite tissue regeneration, supporting the modification from the inflammatory to reparative steps of proliferation and remodelling, taking place in the time frame of about 21 days from the day the injury occurred. In the third stage of wound healing, proliferation is illustrated by the development of granulation tissue, re-epithelialization of the surface of the wound and receding of the margins of the injury [13,16]. Granulation tissue encompasses macrophages, fibroblasts and immature collagen. These are all agreed to encourage the formation of granulation tissue. Concurrently, blood vessels will motivate capillary growth. Fibroblasts about the surface of the injury ignite the production of collagen, one of the key constituents of the extracellular matrix. The last phase of restoration of a wound may well linger to an extent of time and involve the reorganization of

collagen fibres to produce new skin [13,16]. This might progress less than a quarter of its eventual strength within three weeks from the day of the injury and oftentimes, it may be difficult to achieve restoration of the original skin [14].

Numerous plant-based formulations have severally been engaged in the handling of wounds and have similarly been used to support blood clotting, fight infection and fast-track the curing of wounds. These invaluable benefits resulting from plant extracts are a pointer to the great need to screen as much plant extracts as are available for their phytoconstituents and screening them for their wound healing activities [17-19]. Phytoconstituents derived from plants include the alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins and phenolic compounds [20,21]. The medicinal value of plant extracts is influenced by the bioactive phytochemical constituents providing positive physiological activity in the human body [21,22]. The *in vitro* assays using these plant extracts are convenient, rapid and reasonably cheap. Small animals offer a multitude of model varieties for several human wound conditions [17].

Curcuma longa, ordinarily known as turmeric is of the family, Zingiberaceae. The dried pulverized rhizome of it serves as a spice or colourant in foods. Turmeric has a long history of medicinal use since over 4000 years [23-25] and has been useful in Ayurvedic medicine in the handling of inflammatory health disorders. The following constituents have been identified in *Curcuma longa*: the three curcuminoids: curcumin (diferuloylmethane - responsible for its yellow colour), demethoxycurcumin and bisdemethoxycurcumin, volatile oils (turmerone, atlantone and zingiberone),

sugars, proteins and resins. The multiple pharmacological activities, antioxidant and antimicrobial benefits of turmeric have been accredited to curcumin [26]. Turmeric paste has been used to restore wounded bodies or to guard against contamination. In certain parts of Bangladesh, turmeric has been the commonest application to the postpartum excised umbilical cord [27] and has also been found useful in the healing of peptic ulcers [28]. In old Hindu medicine, turmeric is widely used for the management of sprains and swelling caused by injuries. Traditionally, Indian medicine uses turmeric powder for the treatment of biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis [29]. In China, *Curcuma longa* has been in use for ailments related to intestinal discomforts [30] and has also demonstrated anti-inflammatory, anticarcinogenic, anti-ageing, antioxidant activity, as well as efficacy in wound healing [31]. The contemporary works on the wound healing properties of curcumin indicated that it can improve granulation tissue formation, collagen deposition, tissue remodeling and wound contraction [32].

Remarkably, curcumin is a hormetic agent (hormetin), being stimulatory at low doses (i.e. antioxidant effects) and inhibitory at high doses (i.e. induction of autophagy and cell death). This prompts the biphasic dose-response of curcumin on cells, with low doses having stronger effects than high doses. By this, it has been documented that curcumin modulates wound healing *in vitro* in a biphasic dose-response manner, being stimulatory at low doses and inhibitory at higher doses [31]. Curcumin has also been reported to expedite fibrinolysis and cellular migration in the healing of injury through the modification of urokinase plasminogen activator

expression [33]. Up-to-date studies have shown that compared to its oral administration, topical application of curcumin has more noticeable effects on wound healing due to the greater accessibility of the drug at the wound site [34].

This study was undertaken to appraise the stability and wound healing dimensions of ethanolic extract of turmeric (EET) as a bioactive ingredient in an aqueous topical cream formulated for the management of wounds using male Wistar rats as model and hydroxyproline (HXP) as a biochemical marker. The extent of wound healing activity of the EET as a bioactive ingredient in the topical creams will be based on the level of wound contraction, however, especially on the level of tissue HXP detectable in the portion of the healed wound in male Wistar rats. HXP is a non-essential amino acid derivative formed during post-translational protein modification through hydroxylation of the amino acid, proline by the enzyme prolyl hydroxylase which requires vitamin C as a co-factor. HXP is a major component of the protein, collagen and plays a key role in the stability of the collagen triple helix [35].

Materials and methods

Materials

The following materials were used for the studies as procured and include hydroxyproline assay kit (Elabscience, China), dimethyl sulphoxide (DMSO) (Sigma-Aldrich, USA), cholesterol (Molychem, India), emulsifying wax, liquid paraffin and soft paraffin (Kerax, UK).

Methods

Collection and extraction of the sample of turmeric

The sample of rhizomes of turmeric used was identified by a Taxonomist, assigned an e-herbarium identification no. EH/P/069; EH/C/016 and deposited in the Ecoland Herbarium, University of Port Harcourt.

The rhizomes were air-dried and pulverized. A 100.0 g of the powder was extracted in a Soxhlet apparatus at 78°C using ethanol. The ethanol was evaporated in a rotary evaporator to obtain the ethanolic extract of turmeric (EET).

Adaptation of animals for studies

Forty-five adult male Wistar rats weighing 200-250 g were sourced from the animal house of the Faculty of Pharmaceutical Sciences, University of Port Harcourt and kept in separate cages to acclimatize for two

weeks with free access to standard feed and water all through and in standard conditions of temperature (25-29°C), relative humidity (55-66 %) and natural dark/light cycle. Each rat was anaesthetized by 50 mg/kg ketamine intramuscularly. Animal maintenance was generally conducted in firm observance of the ethical requirements of the University of Port Harcourt. All the investigations were conducted in line with the procedures for ethical conduct in the care and use of non-human animals in research [36]. The experimental protocols for the studies involving animals were verified and approved by the Research Ethics Committee of the University of Port Harcourt with approval reference no. UPH/CEREMAD/REC/MM71/044.

Preliminary verification of the wound healing potential and the optimum effective concentration of the EET for wound healing in male Wistar rats.

Table 1: Verification of the wound healing potential and determination of the optimum effective concentration of EET for wound healing in male Wistar rats

Ingredient	Batch								
	A	B	C	D	E	F	G	H	I
	Concentration (%)								
	Solution of EET			Aqueous cream of EET			Controls		
EET	1.5	3.0	5.0	1.5	3.0	5.0	-	-	-
DMSO	-	-	-	5.0	5.0	5.0	5.0	-	-
Cholesterol	-	-	-	10.0	10.0	10.0	-	10.0	-
Glycerol	-	-	-	5.0	5.0	5.0	-	-	-
Emulsifying ointment	-	-	-	20.0	20.0	20.0	-	-	-
Water, q. s.	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Formulation of the aqueous creams

Aqueous topical creams containing 1.5, 3.0 and 5.0 % w/w respectively of EET were prepared based on the formula in Table 1 for batches D-F respectively. In preparing the respective batches of creams, a separate mixture of DMSO, glycerol and emulsifying ointment was prepared and another separate mixture of EET, cholesterol and water was prepared. Both mixtures were separately raised to a similar temperature and later added together in a glass beaker and homogenized to obtain an aqueous cream which was made up to the required weight using distilled water at the same temperature. It was stirred and kept to cool to room temperature.

Evaluation of the stability of creams

The physical appearance and colour of the cool cream were considered. The consistency was evaluated by rubbing a little portion of it amid the fore and first fingers. Its homogeneity was measured in terms of creaming and phase separation. An amount of the cream was introduced in a wide-mouthed plastic bottle in triplicate and stored at -5°, ambient temperature and 40°C individually. They were examined daily for any changes in colour, appearance, consistency and homogeneity for 14 days.

The pH of the cream was assessed with a pH meter (Ultrameter II, 6PFC E; Myron L, UK). The cream was left at ambient temperature while its pH was studied every other day for 14 days [40]. It was also considered for its spreadability, a procedure determined by putting a noted quantity of it between two slides while a 100.0 g weight was positioned on it for 10 s and recording the distance shifted by the slides.

Viscosity

The viscosity of the creams was evaluated in alternate days for 14 days using a Brookfield viscometer (Brookfield DV2TLVTJO, USA) fitted with spindle no. 62 at ambient temperature.

Evaluation of the wound healing potentials of the pure EET and its aqueous creams

Forty-five male Wistar rats were placed into 9 groups (A-I) (n=5). Each rat was anaesthetized with 50 mg/kg ketamine intramuscularly. Shaving, cleaning of the dorsal area of each rat and excision of a 1.5 cm × 1.0 cm full-thickness open wound was made [37]. Groups A-C were treated with topical application of solutions containing 1.5, 3.0 and 5.0% w/v of EET in water. Groups D-F were treated with topical aqueous cream containing 1.5, 3.0 and 5.0% w/w of EET respectively. Groups G-I were treated with 5.0% v/v of DMSO, 10.0% w/v of cholesterol and distilled water respectively serving as controls. DMSO and cholesterol were employed as controls to evaluate their effect in the evaluation of the wound healing influence of the creams in which they were constituent. Wounds were treated daily, measuring wound contraction each day before cleaning and application of the various wound treatment interventions. The entire treatment was carried out for 21 days. The percentage of wound contraction was calculated using equation 1 [38].

$$\text{Percentage of wound contraction} = \frac{\text{Initial wound size} - \text{specific day wound size}}{\text{Initial wound size}} \times 100 \dots\dots (1)$$

Tissue bioassay of hydroxyproline (HXP)

At the end of the wound treatment, the rats were sacrificed. Tissue bioassay was conducted using 100.0 mg of the tissues collected from the site of the healed wound of the respective rats. It was added to 1

ml of 6.0 M hydrochloric acid, boiled for 6 h and cooled. The pH was adjusted to 6.8 while the volume was made up to 10.0 ml using distilled water. Each sample was centrifuged and 1ml of its supernatant was used for the assay of HXP level using the HXP kits (Elabscience, China) and conducting the experiment based on the protocols outlined in the manufacturer's manual which is in line with the methods described by Bergman and Loxley [39] after the principle that the oxidation product produced by HXP under the action of an oxidant reacts with dimethylaminobenzaldehyde (DMAB; Ehrlich's reagent) showing a purplish red colour. The HXP was calculated by measuring the absorbance at 550 nm using a UV-VIS spectrophotometer (Jenway 6405, UK). The values were reported as $\mu\text{g/g}$ dry weight of tissue.

2.2.6 Statistical analysis

The figures were presented as a mean \pm standard deviation (SD). One-way analysis of variance (ANOVA) was performed followed by Fisher's Least Significant Difference (LSD) post hoc test to determine the level of significance.

Results and discussion

Evaluation of the aqueous creams

A homogeneous yellow smooth and steadily stable creams were produced. Medicinal constituents used in the healing of skin disorders are expected to access the target cells in the skin to exert its therapeutic influence. With this, topical management of skin ailments becomes attractive since local drug delivery offers a robust straight contact to the target cells with minor unwanted effects in contrast to systemic drug administration [41].

It is vital to look at an appropriate medicinal agent that boosts the peak distribution of the active component bringing about the effectiveness of the therapeutic agent as such agents are considered as applicable for the managing of open wounds. Such topical agents are expected to be smeared without difficulty as well as providing emollient features. The EET was conveyed as an aqueous cream using the techniques of oil-in-water emulsions [42] as shown in Table 1. The addition of emulsifying ointment in the formulation is to improve the firmness of the oil-in-water cream and bring about the hydrophilic part. This amid water and glycerol affords emollient and moisturizing influence and supports the decrease of dryness and irritation around the injured outer body cover and creates an occlusive barrier on the skin which obstruct the escape of moisture from the outer cover of the body. Moisturizing of the *stratum corneum* allows the opening up of the intra and intercellular channels for easy infiltration of EET into the cells and hurt tissues [43]. Both the DMSO and cholesterol incorporated in the design of the creams function as amphipathic surfactants which provide additional stability to the product particularly imparting hydrophobic and hydrophilic characteristics to the creams to stabilize the EET to retain its activity in the environment of other adjuvants. In addition to its actions as an amphipathic surfactant, DMSO also acts as a penetration enhancer [44,45] and would be predictable to increase the permeation of the EET into the body tissue, thereby improving the effectiveness of the formulation. Additional characteristics of the oil-in-water based cream consider ease of washability and high skin pore occlusion efficiency. Generally, occlusion of wounds has been acknowledged to vividly decrease inflammation and reducing pains, scarring and fast-tracking healing [46-48]. The stability of cream is

critical to its efficacy and safety. The form and consistency of the preparations were factors considered in the evaluation of the stability of the creams since obvious instability may seem as an alteration in colour and/or consistency. Making an allowance for these sorts, there were no suggestions of coalescence, alteration in colour or inconsistency in the creams in the several stress conditions they were open to. Degeneration in certain creams could follow as changes in pH giving rise to undesirable

experiences like skin irritation [49]. The pH of the respective batches of creams exhibited a very minor decrease in value as the storage time increased (Figure 1) while their mean pH varied significantly with the increasing concentration of the EET ($p < 0.05$) (Figure 2) which, however, are within the normal range of pH for the skin. Variable skin pH values are being reported in the literature, all in the acidic range but with a broad range from pH 4.0 to 7.0 [50,51].

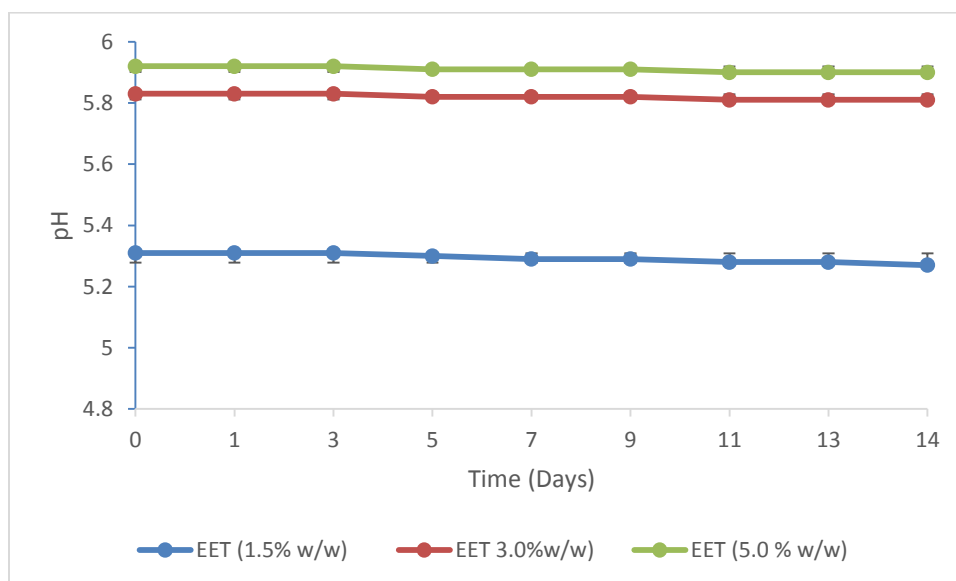


Figure 1: pH of creams containing various concentrations of EET following several days of storage

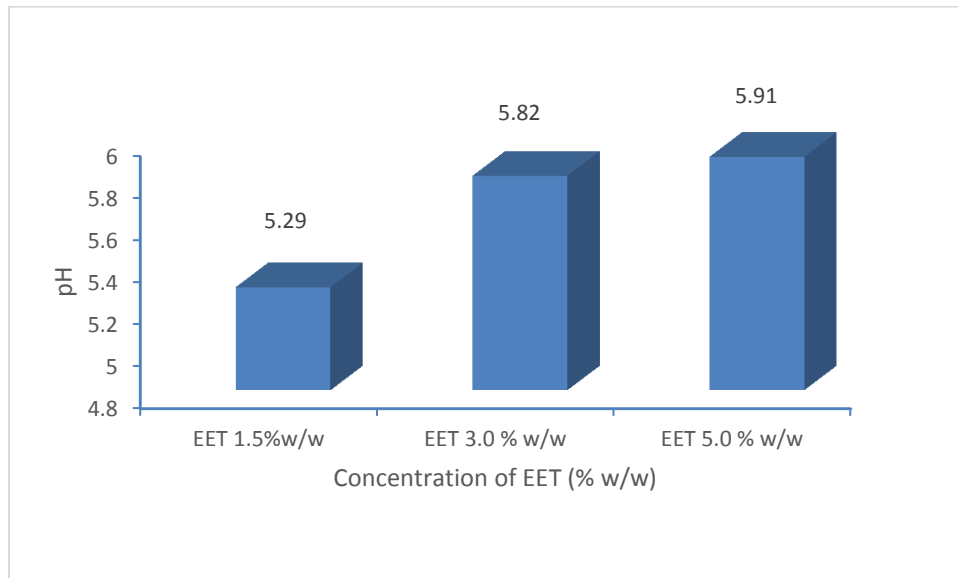


Figure 2: Mean pH of creams following storage and evaluation

The viscosities of the respective batches of creams exhibited a very minor decrease with storage (Figure 3). However, statistically incremented level of viscosity was observed with increasing concentration of EET in the creams ($p < 0.05$) (Figure 4).

The seemingly consistency in the pH and viscosities of the creams following storage points to the consistency and stability of the batches of the topical aqueous creams.

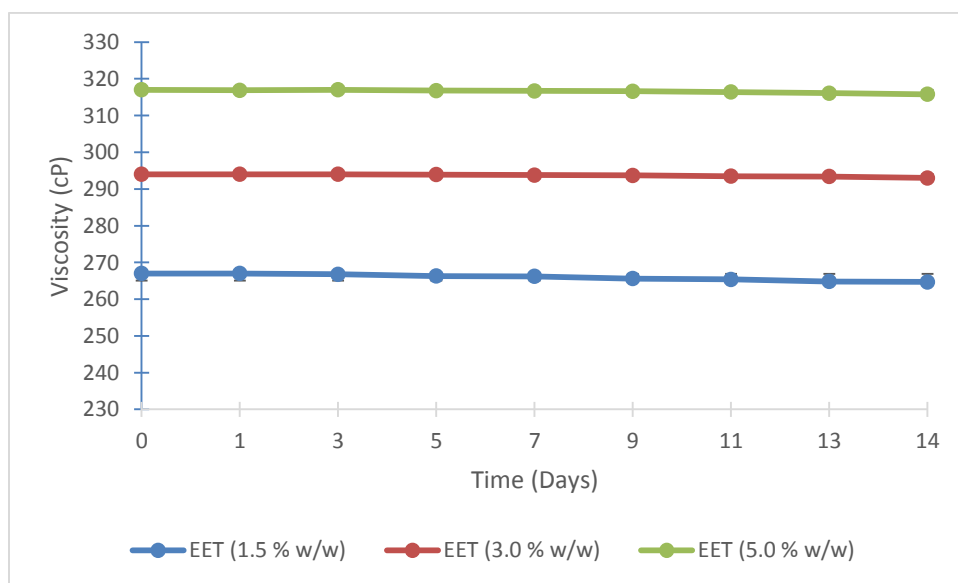


Figure 3: Viscosity of creams following storage and evaluation

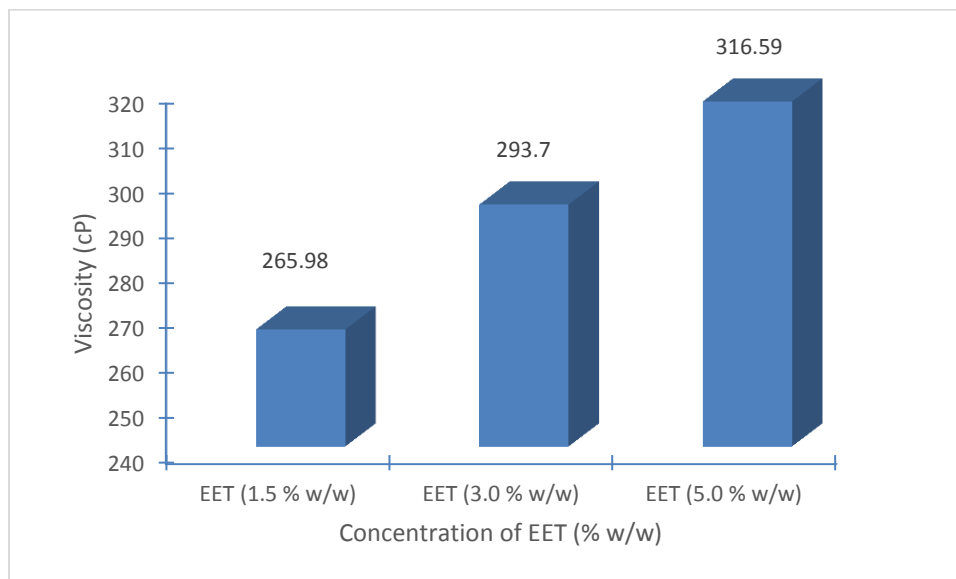


Figure 4: Mean viscosity of creams following storage and evaluation

Evaluation of the wound-healing effects of the wound treatment agents

Figure 5 is a graphical representation of the mean wound closure patterns following the days of treatment while Figure 6 depicts the day of 100% wound contractions for the respective wound treatment interventions employed.

The results of tissue HXP assay revealed that a statistically similar highest mean HXP level was obtained in the groups treated with 1.5% w/v EET (crude) and the topical aqueous cream containing 1.5% w/w EET respectively ($p > 0.05$). This value of $1.741 \pm 0.01 \mu\text{g/g}$ was significantly different from every other result obtained from wound treatment agents (Table 2). The statistical equality of the mean tissue HXP levels is an indication that in as much as 1.5% of EET (crude) serves as the mean optimal effective concentration for wound healing in male Wistar rats, obtaining a statistically similar result with its cream shows that its efficacy is retainable in aqueous topical cream, and also proves the

consistency and stability of the aqueous creams, especially the batch containing 1.5% w/w EET. The consistency and stability of the creams could be attributed to their content of DMSO and cholesterol which are considered as amphipathic surfactants, providing both hydrophobic and hydrophilic ends to stabilize the bioactive ingredient (EET), hence, its activities was retained in the cream. DMSO is also considered as a penetration enhancer [44,45]. In as much as the groups treated with the controls (distilled water, DMSO and cholesterol) with proven abilities to cause the production of collagen as exhibited by the mean tissue HXP (Table 2), the data displayed in Table 3 indicates that the influence of wound healing brought about by 1.5% EET (crude) and its content in a topical cream was independent of the effects of these controls with statistical differences in the mean tissue HXP of 63.782, 31.495 and 35.592% ($p < 0.05$) for distilled water, DMSO cholesterol respectively.

The results recorded in this study support the literature survey that turmeric has been useful in the reinstatement of injured bodies, safeguard against contamination (having it as a common practice in Bangladesh to apply it the postpartum excised umbilical cord [27], in the healing of peptic ulcers [28], diabetic wounds [29] with the ability for granulation tissue development, collagen deposition, tissue remodeling and wound contraction [32].

The mechanism of wound healing of the EET may be attributed to its various phytoconstituents [52,53] based on their anti-oxidant, anti-inflammatory and its

proven antimicrobial properties, especially imparted by its rich curcumin [26].

Conclusion

Ethanollic extract of turmeric (EET) has been confirmed to possess wound healing properties. Its optimal effective concentration for wound healing in male Wistar rats has been determined as 1.5% w/v. This concentration has also been found to retain effective wound healing properties in its aqueous cream, hence, it could be useful as a bioactive constituent in the development of an aqueous topical cream beneficial in the treatment of body injuries.

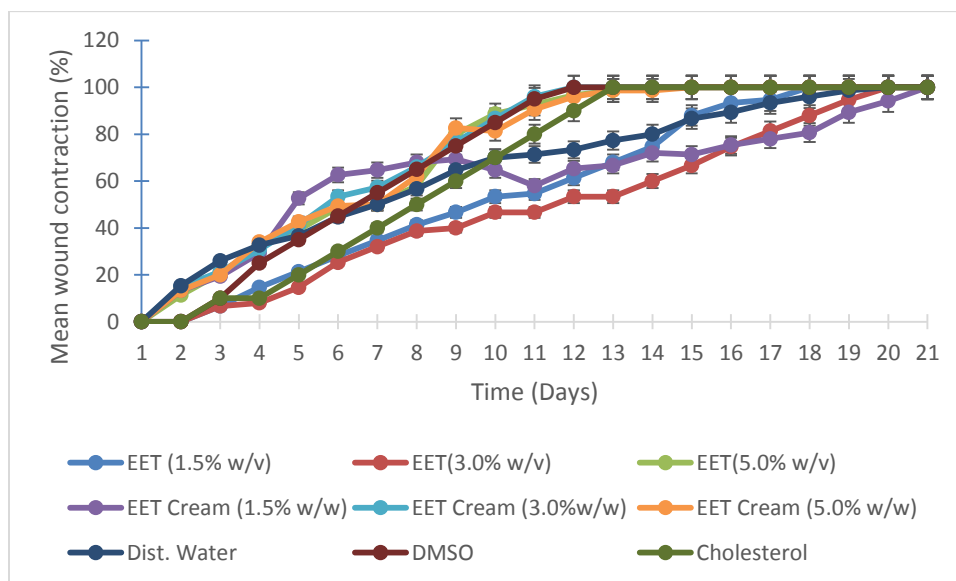


Figure 5: Mean wound contraction following the treatment with creams containing various concentrations of the EET

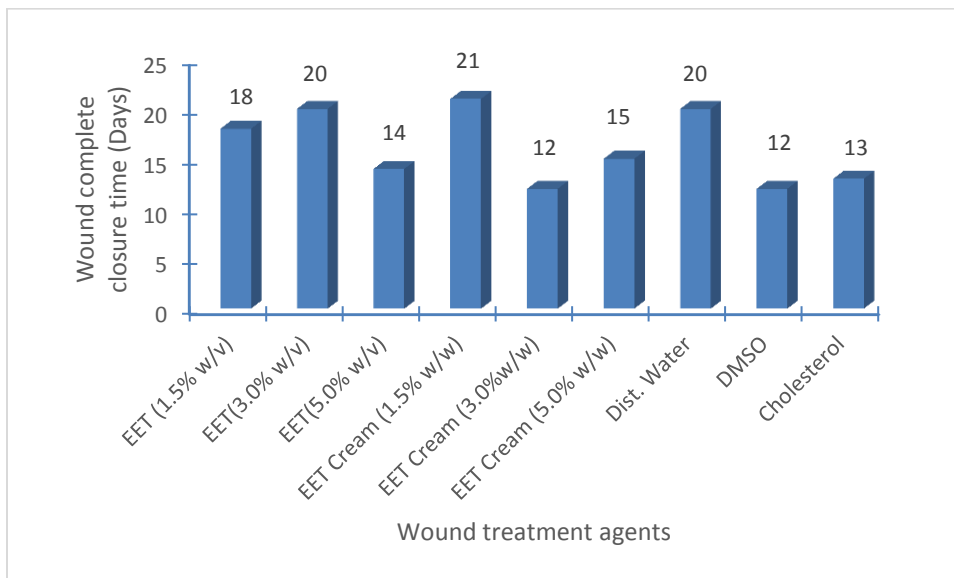


Figure 6: Time for the complete closure of wound by various treatment agents

Table 2: Mean tissue HXP levels obtained with various wound treatment agents

Wound Treatment Agent	Mean HXP ($\mu\text{g/g}$)
EET(1.5% w/v)	1.741 \pm 0.01
EET (3.0% w/v)	1.469 \pm 0.05
EET(5.0% w/v)	1.377 \pm 0.10
EET-Cream (1.5% w/w)	1.741 \pm 0.01
EET-Cream (3.0% w/w)	1.587 \pm 0.10
EET-Cream (5.0% w/w)	1.598 \pm 0.03
Dist. water	1.063 \pm 0.05
DMSO (5.0% v/v)	1.324 \pm 0.49
Cholesterol (10.0% w/v)	1.284 \pm 0.03

Table 3: Difference in the mean tissue HXP levels of treated groups compared to the control groups (distilled water, DMSO and cholesterol)

Wound treatment agent	Mean tissue HXP ($\mu\text{g/g}$)	Difference of HXP levels of treated groups to controls groups (%)			
		Dist. Water	DMSO	Cholesterol	Remark
EET(1.5% w/v)	1.741 \pm 0.01	63.782	31.495	35.592	$P<0.05$
EET (3.0% w/v)	1.469 \pm 0.05	38.194	10.951	14.408	$P<0.05$
EET(5.0% w/v)	1.377 \pm 0.10	29.539	4.000	7.243	$P<0.05$
EET-Cream (1.5% w/w)	1.741 \pm 0.01	63.782	31.495	35.592	$P<0.05$
EET-Cream (3.0% w/w)	1.587 \pm 0.10	49.294	20.695	23.598	$P<0.05$
EET-Cream (5.0% w/w)	1.598 \pm 0.03	50.329	19.864	24.455	$P<0.05$
Dist. water	1.063 \pm 0.05	-	- 19.713	- 17.212	-
DMSO (5.0% v/v)	1.324 \pm 0.49	24.553	-	3.115	-
Cholesterol (10.0% w/v)	1.284 \pm 0.03	20.790	-3.021	-	-

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