

Co-formulation and characterisation of gentamicin-loaded alkyl acrylate cross polymer hydrogel infused with ethanol extract of *Tetracarpidium conophorum* impregnated on gauze sponge for wound dressing

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Abstract

Background: This study aims to develop a hydrogel containing gentamicin and ethanol extract of *Tetracarpidium conophorum* (EETC) impregnated gauze to be used as potential wound dressing to promote wound healing via inhibition of growth of microorganisms by gentamicin, and removal of products of inflammation via high radical-scavenging capacity to facilitate wound healing.

Method: A modification of free radical initial polymerisation of the alkyl acrylate polymer was utilised to formulate the gentamicin hydrogel with triethanolamine as the cross linker. The hydrogel membranes were evaluated via Fourier transform infrared and the formulations were assessed for swelling index, skin irritancy and in-vitro release of the gentamicin where kinetics were applied to study drug-release kinetics.

Results: In-vivo wound healing tests and histopathology were performed with Carbopol[®] Ultrez 21 exhibiting higher swelling index (97.6–99.6%) due to the availability of more alkyl acrylate polymer chains for cross linking with triethanolamine (TEA), ensuring adequate moisture entrapment in the wound area, thus reducing exudate build up. The presence of EETC enhanced the healing effect in-vivo with $65.7\% \pm 0.21$ – $73.5\% \pm 0.10$ wound size reduction compared to the control drug which exhibited $63.46\% \pm 0.37$ within fourteen days. Re-epithelisation rates were 45–50% and the number of inflammatory cells was 90–300 cell/mm² of field compared to 314 cell/mm² for the control drug.

Conclusion: GH5 hydrogel gauze was found to be most effective in promoting wound healing, exhibiting the highest swelling index, excellent in-vitro drug release and drug content. Transcutol 0.1%w/w, which was present in GH5, increased permeation of gentamicin and EETC. The presence of 0.1% w/w EETC in the hydrogel gauze facilitated the re-epithelisation by $93.7\% \pm 0.7$ and reconstruction of skin tissues on the full-thickness wounds with the thickness of the central region being 2.6–2.9 mm from the epidermis to the dermis thus potentially very useful as a wound dressing.

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Introduction

Wound dressings are applied to a wound to promote healing and protect the wound from further harm. Wound dressings are designed to help healing by optimising the local wound environment.¹ Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of absorbing large amounts of water or biological fluids and are frequently utilised in the formation of wound dressings.² Due to their high water content, porosity and soft consistency, they closely simulate natural living tissue, more than any other class of synthetic biomaterials. Hydrogels may be chemically stable or they may degrade and eventually disintegrate and dissolve.² They are prepared from materials such as gelatin, polysaccharides, cross-linked polyacrylamide polymers, polyelectrolyte complexes, and polymers or copolymers derived from methacrylate esters. They are insoluble in water and are available in dry or hydrated sheets or as a hydrated gel in drug delivery systems designed for single use³ and are useful in the treatment of wound infections.

Wound infection further increases the local tissue damage, is a common complication, while systemic inflammatory and immunological responses might lead to a higher predisposition to

life-threatening sepsis and multi-organ failure.⁴ Therefore, the need for the appropriate design of effective wound dressings and topical preparations heavily relies on an understanding of the healing process, as well as knowledge of the various factors that affect wound healing. The treatment for wounds must provide the ideal environment for healing and prevent infections.

Reactive oxygen species (ROS) is produced as a by-product of neutrophil activity in high amounts at the site of the wound as a defence mechanism against invading bacteria. At high concentrations, ROS initiates severe tissue damage, leading to neoplastic transformation, hampering the healing process by damaging cellular membranes, DNA, proteins and lipids.⁵ Because of these adverse effects, the overall role of antioxidants appears to be significant in the successful treatment and management of wounds.

Antioxidants reduce these adverse effects by removing products of inflammation.⁶ They counter the excess proteases and ROS often formed by neutrophil accumulation in the injured site and protect protease inhibitors from oxidative damage.⁷ The most likely mechanism of antioxidant protection is direct interaction of the extracts (or compounds) and the hydrogen peroxide rather than

the alteration of the cell membranes and limitation of damage. Compounds with high radical-scavenging capacity have been shown to facilitate wound healing.^{6,8,9}

Tetracarpidium conophorum (African walnut plant), is a perennial climbing shrub 10 to 20 feet long, found growing wild in forest zones of sub-Saharan Africa, including Nigeria. Studies have shown that the African walnut possesses some beneficial properties like antibacterial,¹⁰ antioxidant and immune-stimulating activities.¹¹ It is commonly used in Nigerian folkloric medicine for the treatment of bacterial infections and ailments caused by oxidative stress.¹² The antioxidant properties of this plant, which have shown high radical-scavenging capacity, will be used to facilitate wound healing.⁶

This study aims to formulate a gentamicin and *T. conophorum* hydrogel-impregnated gauze to be used as wound dressing to promote wound healing via inhibition of growth of microorganisms by gentamicin, and removal of products of inflammation via varying mechanisms by *T. conophorum* extract, the combination thus promoting the healing cascade. Utilisation of antioxidants in tandem with antibiotics as a pharmacotherapeutic option in wound healing is currently not available, thus the novelty of the current research. The research design (Figure 1) entailed a modification of free radical initial polymerisation of the alkyl acrylate polymer to formulate the gentamicin hydrogel containing *T. conophorum* followed by quantitative in-vivo wound-healing monitoring.

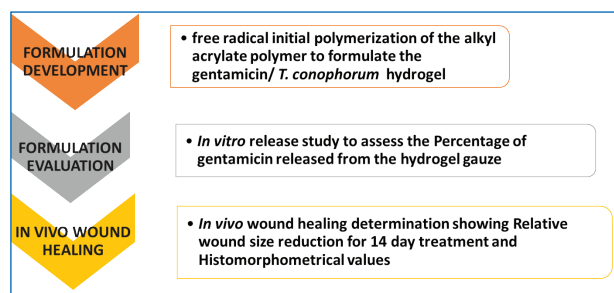


Figure 1. Flow chart showing the research process

Materials

Chemicals and reagents

Gentamicin sulphate (BP grade) was obtained as a gift from Drugfield Pharmaceuticals Limited (Ogun State, Nigeria); Carbopol® Ultrez 21 was obtained as a gift from Metchem Limited (Mumbai India); Carbopol® 940 (Lubrizol corporation USA); Propylene glycol,

Triethanolamine (Merck Germany); Transcutol® was obtained as a gift from Gattefosse (Cedex, France); O-phthalaldehyde OPA from Fluka (Steinheim Germany); N-acetyl cysteine (NaC) sodium hydroxide from Sigma Aldrich (St. Louis, USA); Sterile gauze (12 ply 10 cmx 10 cm) from Select® (Jacksonville, Florida). All other chemicals and reagents were of analytical grade.

Methods

Preparation of EETC (Ethanol extract of *Tetracarpidium conophorum*)

The plant was collected from farms in Nkwere Local Government Area, Imo state, Nigeria, and identified by Mr Oyebanji OO of the Department of Botany, University of Lagos, Lagos, Nigeria. A voucher specimen assigned reference number LUH6972 was deposited in the institutional herbarium for reference.

The ethanolic extract of the leaves was obtained using the method detailed by Ilomuanya et al.¹¹ The plants were air dried for 14 days and the leaves were separated, and then ground using a grinding machine. Two-hundred grams of finely ground leaves of *T. conophorum* was weighed, using a weighing balance, and placed in a container. Two litres of ethanol was added and it was allowed to macerate for 24 hours; it was then filtered. Ethanol was used three times in this process to extract the filtrate which was allowed to air dry to an appreciable volume after which it was centrifuged at 3 000 rpm to collect ethanolic extract of *T. conophorum* (EETC). This was then freeze dried and milled. The contents were introduced into a glass vial and kept in a desiccator at 18 °C.

Preparation of gentamicin-loaded acrylate copolymer-based hydrogels

0.1%w/w of gentamicin sulphate was dissolved in aliquots of purified water and propylene glycol was titrated in drops into the mixture. The permeation enhancers were incorporated into the aqueous phase of the formulation. At room temperature the gel phase was prepared by dispersing the alkyl acrylate cross-polymers Carbopol® 940 or Carbopol® Ultrez 21 in purified water using a mechanical stirrer at 120 rpm. The pH was adjusted with the cross-linking agent triethanolamine (TEA) to pH of 5.5. Both the aqueous fraction and the gel fraction were then mixed at a constant stirring rate of 150 rpm. The final pH of the hydrogel was maintained at pH 5.5. The constituents of the varying formulations are shown in Table 1. The hydrogel was loaded on Select® gauze-coated petri dish and

Table I. Composition of gentamicin-loaded acrylate copolymer hydrogel gauze %w/w

Ingredient	GH1	GH2	GH3	GH4	GH5	GH6	GH7	GH8
Gentamicin	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Carbopol® Ultrez 21	1.5	–	1.5	–	1.5	–	1.5	–
Carbopol® 940	–	1.5	–	1.5	–	1.5	–	1.5
Propylene glycol	10	10	15	15	10	10	15	15
Triethanolamine	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
EETC	–	–	0.1	0.1	0.1	0.1	–	–
Transcutol®	–	–	–	–	0.1	0.1	0.1	0.1
Purified water to	100	100	100	100	100	100	100	100

kept in an oven of $30\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ for 24 hours. The gentamicin-loaded hydrogel gauze was then air dried at room temperature for 24 hours. The hydrogel-loaded gauze (HLG) was removed from the petri dish aseptically, labelled and packaged. The HLG for wound dressing was 5 cm x 5 cm in area with an average thickness of $2.4\text{ mm} \pm 0.03$. The HLG was stored in a static dessicator prior to analysis.

Drug content determination

One gram of hydrogel was dissolved in 10 mls of water and centrifuged at 500 rpm for 45 minutes and filtered using a 0.5 μm millipore filter. Utilising a 1:50 dilution, the concentration of gentamicin was obtained using a UV/VIS spectrophotometer (UV/VIS 2600 Shimadzu Analytical and Measuring Instruments) after derivatisation utilising O-phthalaldehyde reagent by Kowalczuk's method.¹³ Pthalaldehyde reagent was formulated prior to use by dissolving 20 mg of O-phthalaldehyde in 0.1 ml of methanol, to 1.5 mls of 10% N-acetyl cysteine and diluting to 10 mls with 0.2 mol^{-1} solution of borate buffer pH10. Gentamicin, an amyloglycoside antibiotic, does not absorb UV light due to its weak chromophore, hence the need for derivitisation. The pthalaldehyde reagent, once prepared was stored in an amber-colour bottle and kept in a dark cupboard prior to use.

Determination of skin irritancy via patch test

0.5 g of the hydrogel was applied to the shaved surface of male wister rats weighing 300 g each. One hour after application the skin was visually examined for erythema and oedema.

Swelling index

3 x 3 cm pieces of the gauze hydrogel was dried at $30\text{ }^{\circ}\text{C}$ and stored under vacuum HG_A . The gauze was then soaked in phosphate buffer (PBS) saline at $37\text{ }^{\circ}\text{C}$ HG_B . The swelling ratio was determined as shown in Equation 1 below:

$$\% \text{ SWI} = \text{HG}_B / \text{HG}_A \times 100 \quad (\text{Equation 1})$$

HG_B and HG_A represent the weight of the gauze-loaded hydrogel samples dried at $30\text{ }^{\circ}\text{C}$ for 24 hours and soaked in PBS at $37\text{ }^{\circ}\text{C}$ respectively.

In-vitro release studies

The rate of release of gentamicin was determined for formulations H1, H3, H5, and H7. Utilising USP Disket[®] Dissolution apparatus, 3 x 3 cm pieces of the gauze hydrogel were introduced into the dissolution vessel. PBS (pH7.4) was used as the dissolution medium, 50 rpm at $37.1\text{ }^{\circ}\text{C} \pm 0.7$. Samples were withdrawn at predetermined time intervals and the concentration of gentamicin released was determined after derivitisation with O-phthalaldehyde reagent at 331 nm. Release kinetics and the mechanism of drug release was evaluated by fitting the release data into zero order, first order, Higuchi and Korsmeyer Peppas model.

In-vivo healing test

The study was approved by the College of Medicine, University of Lagos Health Research Ethics Committee (CMULHREC number: CMUL/HREC/04/17/117, 29.06.2017). Thirty (30) male Sprague Dawley rats weighing 350–400 g were purchased at the start of the

experiment from RawLabs[®] (Ibadan, Nigeria). The rats acclimatised in their new environment, seven days prior to commencing the experiment. The rats were anaesthetised by i.p. injection of 0.03 ml urethane/kg body weight of the rat. The dorsal hair of each animal was shaved with an electric razor. After creating two full-thickness wound areas ($1.5 \times 1.5\text{ cm}$) by excising the dorsum, 70% ethanol was used for sterilisation. Each wound was covered with sterile gauze (control), the hydrogel without drug, the hydrogel with drug, and the commercial wound-dressing product, respectively. All materials were fixed with an adhesive bandage. The rats were kept separately in individual cages. At the predetermined intervals, each wound size was measured using a digital camera. The relative wound size reduction was calculated as in Equation 2 below:

$$\text{Relative wound size reduction (\%)} = \left[\frac{A_0 - A_t}{A_0} \right] \times 100 \quad (\text{Equation 2})$$

where A_0 and A_t are the wound size at initial time and time "t", respectively. The wound size was surveyed using the Adobe[®] Acrobat[®] 7 Program.

Histological process

Fifteen days postoperatively, after testing of the wound-healing sites was completed, the rats were euthanised. The wounded area of skin containing the dermis and hypodermis was then sampled and carefully trimmed with a cutter. All trimmed skins were fixed in 10% neutral buffered formalin. After paraffin embedding, 3- to 4- μm sections were prepared. Representative sections were stained with haematoxylin and eosin (H&E) and Masson's trichrome. The light microscopic examination on histological profiles of individual skin was performed.

Histomorphometry

The desquamated epithelium regions (mm), numbers of microvessels in granulation tissues (vessels/ mm^2 of field), numbers of infiltrated inflammatory cells in granulation tissues (cells/ mm^2 of field), percentages of collagen-occupied regions in granulation tissues ($\%/\text{mm}^2$ of field), and thicknesses of central regions of granulation tissues (mm from epidermis to dermis) were measured on the histological skin samples using a digital image analyser (DMI-300, DMI, South Korea), respectively. Re-epithelisation was calculated as in Equation 3 below.

Re-epithelisation (%) =

$$\frac{\text{Total wound length (mm)} - \text{Desquamated epithelium region (mm)}}{\text{Total wound length (mm)}} \times 100 \quad (\text{Equation 3})$$

Results and discussion

Hydrogel characterisation

The hydrogels prepared were all translucent, clear and with a slight odour. The consistency of the formulations varied depending on the type of polymer used. The hydrogel GH1 formed using Carbopol[®] Ultrez 21 showed the best consistency of all the formulations. The hydrogel-impregnated gauze had a sticky feeling as expected in hydrogels. The pH of all the formulations is shown in Table III with GH2 having the highest pH (5.82) of all the formulations. Most of the formulations had a pH slightly above the skin's pH of 5.5, except GH1

and GH7. The results show that the pH of the various formulations was below 6.0, with GH2 having the highest pH. The buffer used to adjust the pH of the formulation, was triethanolamine (0.4 ml) to ensure the efficiency and safety of the formulation for skin use.¹⁴ The skin irritancy test showed that the impregnated gauze is tolerable to the skin as expected of the polymer used.⁴ There was no sign of erythema and oedema throughout the observation period and shows the formulation can be used safely on the skin as shown in Table II.

The formulations with Carbopol® Ultrez 21 had a higher drug content above 70% than formulations containing Carbopol® 940. The formulation with the highest drug content was GH5 that contained both permeation enhancer and the EETC. The polymer matrix of the hydrogel holds and releases the gentamicin adequately in the Carbopol® Ultrez-loaded gauze.

The swelling index is a very important characteristic of hydrogels, because the relationship between the nature of the swelling medium and swelling of the polymer is fundamental.¹⁴ All the Carbopol® Ultrez 21-impregnated gauze had a higher swelling index than the hydrogels in phosphate buffer saline medium, with the swelling index as high as 99.6% in the GH5-impregnated gauze (PBS pH 7.4). Carbopol® Ultrez 21 polymer provides more efficient thickening than other Carbopol® polymers, such as Carbopol® 934, Carbopol® 940 and Carbopol® 980 polymers⁴, so the higher swelling index is expected. The hydrophobically -modified cross linked polyacrylate polymer efficiently imparts a uniform thickening and suspending property to the hydrogel formulation loaded unto the gauze.

In-vitro release study

The release study of the hydrogel-loaded gauze conducted was represented as the percentage of gentamicin which was released in 24 hours. GH5 and GH7 had the highest amount of gentamicin released with 90% and 87.99% released in eight hours, as shown in Figure 2. The increased number of alkyl acrylate polymer chains in Carbopol® Ultrez compared to Carbopol® 940 accounts for the increased swelling ability as well as better release of the gentamicin. This release data showed a rapid release of gentamicin from the formulations in the

first few minutes of observation and thereafter a period of sustained release was observed. The rapid release effect ensures a rapid reduction of bacterial count in the wounded tissue. The presence of the EETC didn't negatively affect the release of gentamicin from the wound dressing. The Transcutol had a positive effect on the release of gentamicin from the Carbopol® Ultrez 21, hydrogel-impregnated gauze, as seen from the release data of GH5 and GH7. The same effect was not seen in the Carbopol® Ultrez 940 hydrogel-impregnated gauze as the release was retarded in GH6 and GH8. The Transcutol facilitated the release of gentamicin from the impregnated gauze matrix. This hydrogel-loaded gauze will be very effective as wound dressing as its prolonged release over 24 hours will prevent growth of bacteria over a long period of time and allow the wound to heal by shortening the inflammatory phase. The release kinetics of gentamicin from the hydrogel was studied in-vitro and the data was entered into various kinetic models. The mechanism of release predominantly observed was the Higuchi model thus relating that initial drug concentration in the hydrogel matrix is much higher than drug solubility, with drug diffusion taking place in one dimension with edge effect being negligible. This accounts for increased release through pores in the matrix hydrogel system. R² values obtained for this model were 0.919–0.987 in GH6 compared to the zero order kinetic model (0.546–0.665) and first order model (0.557–0.699) which have drug release either independent of initial concentration, or concentration dependent respectively, as shown in Table II.

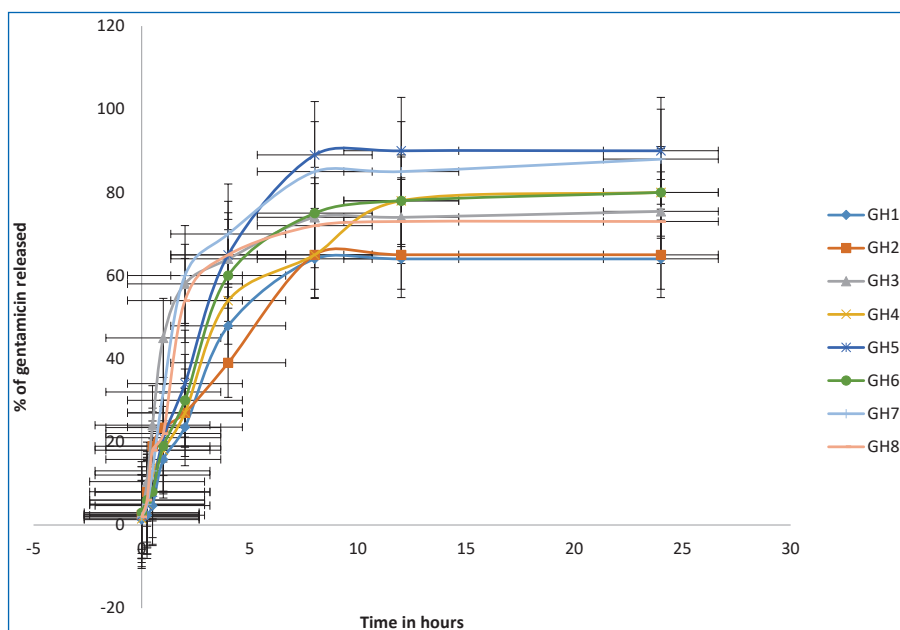


Figure 2. Percentage of gentamicin released from the hydrogel gauze against time in hours

Table II. Physicochemical characteristics and kinetic modelling of the hydrogel formulations

Gauze hydrogel	% drug content	pH	Swelling index	Kinetic modelling			Skin irritancy
				Zero order (K ₀)	First order (K _f)	Higuchi model (K _h)	
GH1	90.32 ± 1.89	5.53 ± 0.03	98.4 ± 1.23	0.546	0.652	0.932	Nil
GH2	65.66 ± 2.13	5.82 ± 0.04	68.2 ± 0.96	0.665	0.699	0.911	Nil
GH3	79.99 ± 0.91	5.59 ± 0.08	99.2 ± 2.12	0.639	0.587	0.972	Nil
GH4	60.00 ± 4.07	5.60 ± 0.13	72.3 ± 1.11	0.611	0.639	0.954	Nil
GH5	93.09 ± 3.11	5.71 ± 0.14	97.6 ± 0.93	0.601	0.627	0.954	Nil
GH6	83.41 ± 3.03	5.59 ± 0.07	73.5 ± 3.22	0.632	0.584	0.987	Nil
GH7	92.13 ± 1.11	5.55 ± 0.07	99.6 ± 1.11	0.568	0.562	0.919	Nil
GH8	65.00 ± 2.31	5.61 ± 0.07	72.9 ± 1.68	0.599	0.573	0.922	Nil

In-vivo wound-healing determination

All the rats utilised for the study survived throughout the postoperative process until euthanasia. There was no evidence of tissue necrosis observed on the surface of the inflicted wounds. At 48 hours postoperatively there was visible inflammation taking place in all the rats; the control drug and the formulated hydrogel gauze were all superior to the untreated control which showed a dense wound area with slight scabbing. The hydrogel gauze did not cause or increase the incidence of haemorrhaging. Relative wound size reduction, as in Figure 3, was highest in formulations GH5, GH6 and GH4 having 92.4%, 90.1% and 89.9% reduction respectively with GH1, GH2, GH3 and GH7 being comparable with the control drug. The presence of permeation transcutol enhanced the release of gentamicin into the wound surface with EETC present in the hydrogel, facilitating removal of products of inflammation via high radical-scavenging capacity through its antioxidant activity, thus facilitating wound healing. The hydrogel gauze formulation containing EETC ensured an even smoothness on the surface of the rat skin which shows that microvessels in the granulation tissue are reduced and can be compared to that of an intact skin.

Histopathology

At 15 days postoperatively the animals were euthanised and slides prepared and analysed. Representative images of the haematoxylin and eosin and histomorphometry results are shown in Figure 4.

In undamaged skin, the epidermis (surface layer) and dermis (deeper layer) form a protective barrier against the external environment. When the barrier is broken, an orchestrated cascade of biochemical events is set into motion to repair the damage.¹⁵ Regardless of the aetiology of the wound, the repair processes are similar. At the time of insult, multiple cellular and extracellular pathways are activated, in a tightly regulated and coordinated fashion, with the aim of restoring tissue integrity. Classically, this process of wound healing is divided into four distinct phases: haemostasis, inflammation which involves epithelisation, proliferation and tissue remodelling.

The re-epithelisation rates observed were highest for GH5 (93.7% \pm 0.7) compared to the control of normal skin and the conventional

production (47.79% \pm 0.46 and 72.98% \pm 0.32 respectively). The hydrogels all showed high re-epithelisation rates, with those containing EETC and Carbopol® Ultrez 21 being significantly higher than the other formulations. Thus the optimal formulations which facilitated re-epithelisation, contained permeation enhancer transcutol¹⁶ and antioxidants (GH5, GH6 and GH7). They showed the highest re-epithelisation rates as shown in Figure 4A. The length of the desquamated epithelium layer was minimally affected by the formulated hydrogels. Figure 4C shows that the formulated hydrogels were comparable to the conventional product, with GH5 and GH6 having half the value obtained with the conventional product (0.9 mm and 1.1 mm respectively). A reduction in the length of the desquamated epithelium layer ensures that the intrinsic pathway of the clotting cascade (contact activation pathway) involving the initiation of the proteolytic cleavage cascade has been fully activated.

The number of infiltrated inflammatory cells, however, was significantly higher in the control than any of the formulations. The gauze hydrogels showed comparable results with the conventional product, however the gauze hydrogels GH5, GH6 and GH7 had the lowest number of infiltrated inflammatory cells at the end of the 14-day period, thus showing that neovascularisation occurring in the vascular tissue was greatly reduced, as also seen in Figure 4. The length of desquamated epithelial region was also considerably reduced in the GH5, GH6 and GH7 gauze hydrogel formulation because the hydrogels facilitated the re-epithelisation and reconstruction of skin tissues on the full-thickness wounds better than did the conventional product. They contained more collagen tissues and less inflammatory cells compared with the conventional product. The gauze hydrogels containing transcutol and EETC gave more rapid regeneration of wounds compared to the other hydrogels. This was primarily because the permeation enhancer ensured rapid release of gentamicin through the polymeric material to the surface of the wound.¹⁶ EETC aided removal of inflammation products by countering the excess proteases and ROS, often formed by neutrophil accumulation in the injured site. This is also evident by the percentage of collagen in the granulation tissue which was higher for the hydrogel gauze formulations when compared with the control and conventional product, and higher still when the

hydrogel gauze contained a permeation enhancer and EETC. The presence of 0.1%w/w EETC in the hydrogel gauze facilitated the re-epithelisation by 93.7% \pm 0.7 and reconstruction of skin tissue on the full-thickness wounds with the thickness of the central region being 2.6–2.9 mm from the epidermis to the dermis. The most likely mechanism of EETC antioxidant protection is direct interaction of gentamicin and the extracts in the hydrogel gauze on the inflammatory response, which can be seen in Figures 4 and 5. EETC has been shown to have antibacterial, antineoplastic, antiviral, anti-inflammatory, antiallergic,

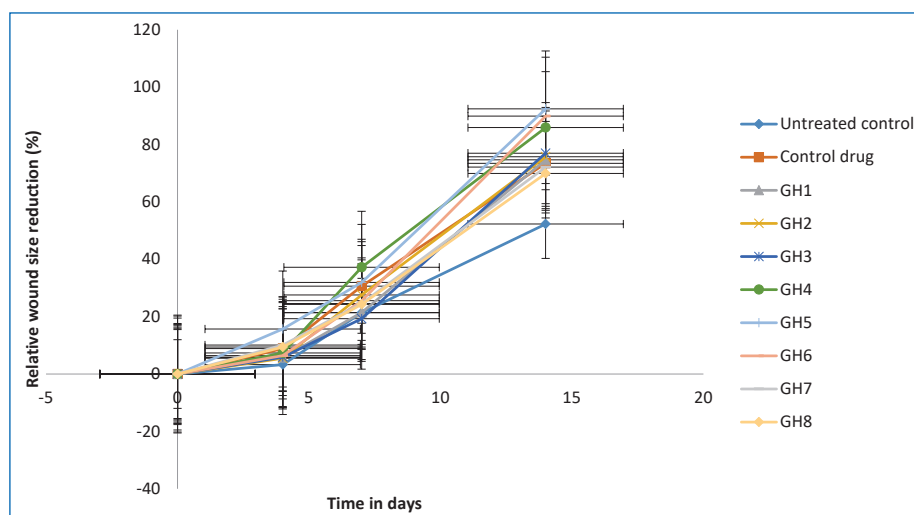


Figure 3. Relative wound size reduction after 14 days of treatment

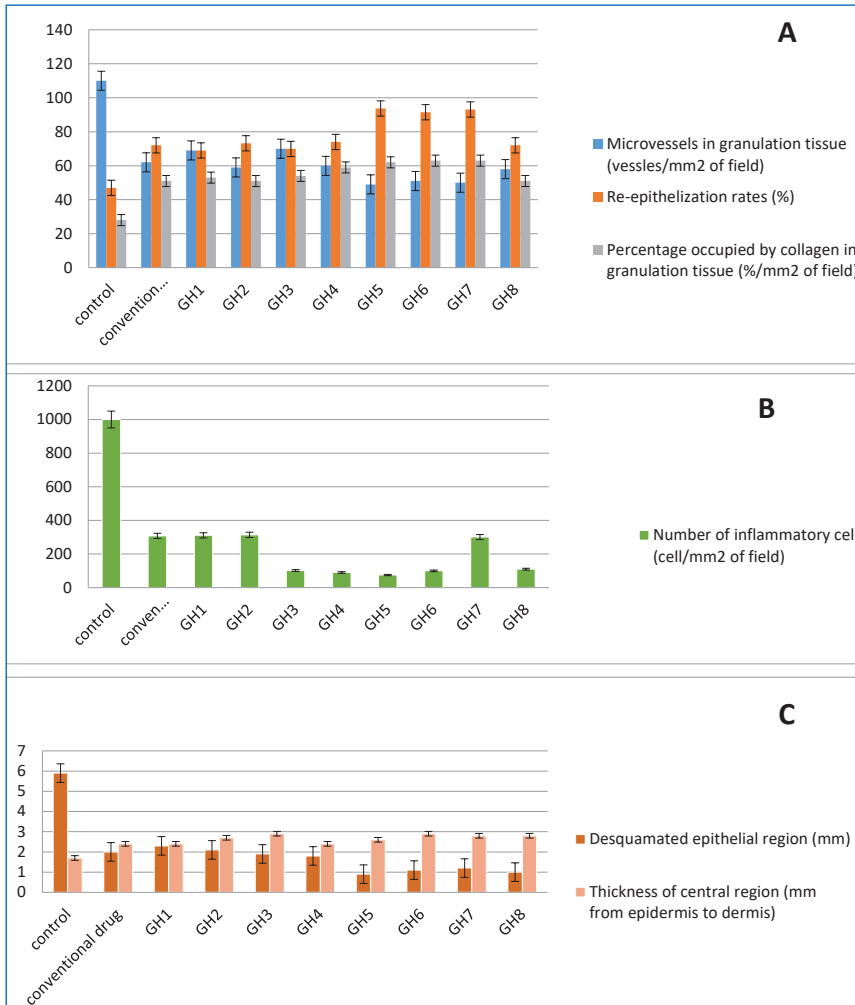


Figure 4. Histomorphometrical values showing: A) Microvessels in granulation tissue (vessels/mm² of field); Re-epithelisation rates (%), Percentage occupied by collagen in granulation tissue (%/mm² of field); B) Number of inflammatory cells (cell/mm² of field); and C) Thickness of central region (mm from epidermis to dermis); Desquamated epithelial region (mm). All data are P < 0.05 compared with normal skin and control drug.

antithrombotic, and vasodilatory activities as a result of its flavonoid content. The potent antioxidant activities of flavonoids have been suggested to be responsible for many of the above actions as oxidative damage is implicated in most disease processes, especially in the wound-healing process.

The gentamicin-loaded alkyl acrylate cross polymer hydrogel infused with ethanolic extract of *T. conophorum* impregnated on gauze sponge for wound dressing was non-toxic and biocompatible. The use of the cross linking agent in gel formation did not grossly affect the rate of wound healing. The gel, once loaded onto the gauze sponge, had the desired drug content as well as swelling index. It had better healing compared to the conventional product and the influence of co-formulation of an antioxidant and a permeation enhancer in the gel, which was loaded on a gauze sponge, greatly enhanced healing with the hydrogel base, preventing excessive exudate retention on the wound surface.

Conclusion

GH5 hydrogel gauze was found to be the most effective in promoting wound healing, with the highest swelling index, excellent in-vitro drug release and drug content. Transcutol 0.1%w/w, which was present in GH5, had a very positive effect on the healing activity of the impregnated gauze via increased permeation of the gentamicin and

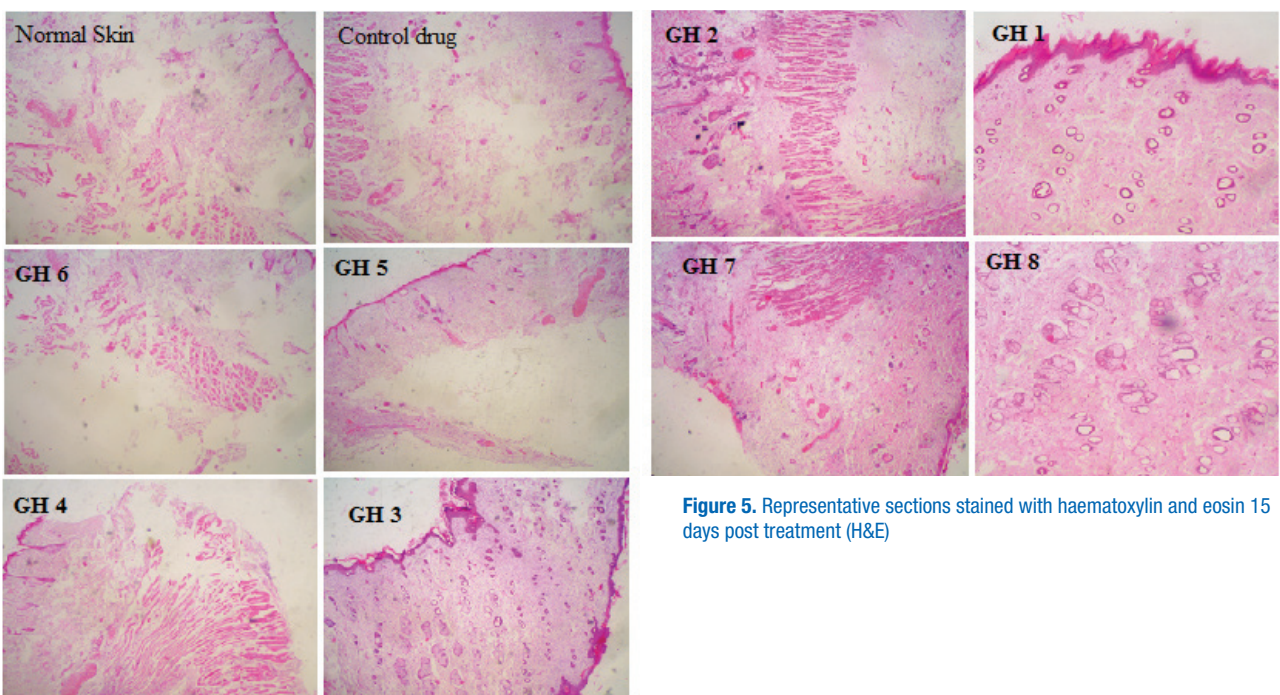


Figure 5. Representative sections stained with haematoxylin and eosin 15 days post treatment (H&E)

EETC to exhibit their activity. The presence of 0.1%w/w EETC in the hydrogel gauze facilitated the re-epithelisation by $93.7\% \pm 0.7$ and reconstruction of skin tissue on the full-thickness wounds with the thickness of central region being 2.6–2.9 mm from epidermis to the dermis; thus will be very useful as a potential wound dressing.

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