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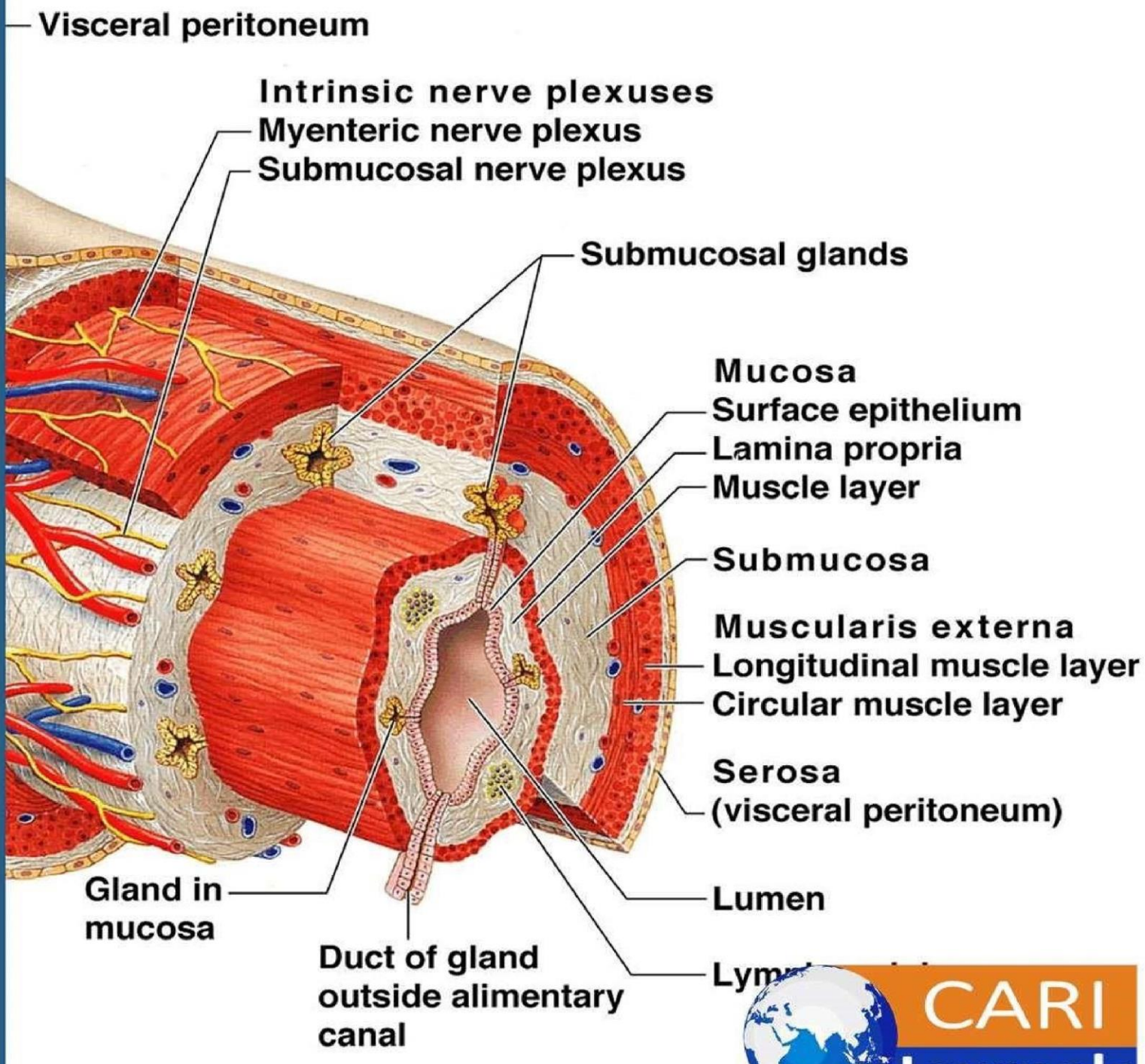

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Therapeutic Effects of Aqueous Extracts of *Acmella Oleracea* on Cutaneous Wounds

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ABSTRACT

Purpose: Wounds are injuries which arise when the structural continuity of a tissue is physically disrupted. It has been established scientifically that plants produce metabolites which potentially accelerate wound healing. Approximately 80% of human population globally, according to WHO, use phytoextracts that have not been scientifically accredited as medicines. This research focused on establishing *in vivo* the efficacy of lyophilized aqueous extracts of *A. oleracea* in enhancing cutaneous wound healing in *Mus musculus*, male albino mice.

Methodology: *Acmella oleracea* plants identified and confirmed by taxonomists were collected from their undisturbed natural ecosystem, dried under the shade and ground to powder using a mill. Extraction of phytoextracts was done by infusion which involved the addition of 500g of the powder to 4 liters of distilled water and maintaining the mixture at 60^0C for 6 hours. The mixture was filtered, concentrated, lyophilized and stored in lightproof plastic bags at -10^0C for bioassays. The extracts were assayed for their activity in the excision wound repair paradigm. Mineral ion constitution of the extracts was analyzed using TXFR technique. The presence of phytochemical composition was established using standard procedures. The animals were put into five groups, each consisting of five mice. Group A, a negative control, was given physiological saline. Group B, a positive control, was treated with a standard drug Flucloxacillin at a therapeutic dose of 40 mg/kg and groups C, D and E with test phytoextracts at therapeutic doses 50, 95 and 300mg/kg body weight respectively. This was done systemically via oral routes for 21 days. Statistical analyses were done by ANOVA followed by Tukey HSD post hoc test. $P \leq 0.05$ was considered statistically significant.

Findings: Lyophilized extracts significantly accelerated wound contraction in the first five days post wounding with respect to the negative physiological saline control doses. Results from TXFR revealed the presence of mineral elements like magnesium that enhance wound healing. Phytochemical analysis results revealed the presence of the metabolites flavonoids, tannins, phenols, alkaloids and saponins in varying concentrations.

Unique Contribution to Theory, Practice and Policy: In conclusion, the extracts demonstrated wound healing potential which would be attributed to the presence phytochemicals and mineral ions in the phytoextracts. This research therefore recommends the sustained use of these plant extracts for wound healing purposes.

Key words: *Cutaneous Wound Healing, Excision Wound, Wound Contraction, Acmella Oleracea*

INTRODUCTION

Wounds are physical injuries which occur when tissues such as muscles, skins & bones are severed as a result of accidents, animals and microbial attacks. Millions of people suffer from chronic wounds globally (Kumar *et al*., 2007). Wounds come with different effects on the patients' lives, ranging from severe pain and psychological turmoil to loss of vital body organs, workdays, increased dependence and fatalities. A portion of allopathic medicines used to treat ailments, wounds included, present a challenge of allergenicity and high cost of treatment. It is therefore essential that scientists work towards the establishment of safe agents that promote exponential wound healing (Myers *et al*., 1980). Plants are potential sources of wound therapeutic agents (Kalyon *et a*l., 2009). Reports by World Health Organization (WHO 2002-2005) reveal that about 80% of global human population currently uses herbal concoctions as medicines for treatment of diseases, wounds included. Herbal remedies form a pivotal backbone of modern pharmaceutical industry.

The need for establishment of better therapeutic alternatives (Sai and Babu, 1998) warranted scientific confirmation of the efficacy of the selected phytomedical products like *Acmella oleracea* in enhancing wound healing. This investigation aimed at determining the effectiveness of ethno-medical extracts from *Acmella oleracea* plants in accelerating cutaneous wound regeneration by comparing wound contraction with a physiological saline negative control and a Flucloxacillin positive control.

Laboratory analyses of phytomedicines have indicated that some plants are potential sources of therapeutic agents on wounds (Kalyon *et al*., 2009). *In vivo* tests have given positive results on wound healing. The following examples are a testimony:

Extracts from *Lantana camara* leaves have been found to promote wound contraction (Kurian, 1995) though potentially toxic. *Aloe vera* gel accelerated open wound healing in type 2 diabetic experimental rats when orally administered (Atiba *et al*., 2010, 2011). *Panax ginseng* has angiogenic activities confirmed *in vivo* (Liang *et al*., 2005). *Chromoleana odorata* aqueous leaf extract enhances hemostatic activity (Akah, 1990) as well as stimulating granulation and epithelialization. *Centella asiatica* extracts have promoted fast wound healing (Shukla *et al*., 1999). *Curcuma longa* has enhanced wound healing in rats (Rao *et al*., 2003)

Although some plants have been tested and found to have agents that enhance wound healing, apparently science is silent on the effectiveness phytoagents from *Acmella oleracea* in enhancing cutaneous wound healing, leaving a gap that needed to be addressed.

Acmella oleracea, family Asteraceae, is native to America and now widely spread in the continents of Africa, Asia and Australia. It is locally known as "Bisolola mare" by the Bukusu community in Kenya who have traditionally used it treat oral ulcers. It is a perennial herb whose

branching stems grow erect or prostrate. Its concoction is traditionally used to remedy toothache (Chung *et al.*, 2008).

MATERIALS AND METHODS

1 Selection and authentication of ethnopharmacological assay phytomaterials

Selection of the specimen plant was conducted on the basis of its ethnopharmacological uses obtained through interrogation of traditional administrators of the herbal medicines. It was identified by its vernacular name. Scientific identity of *Acmella oleracea* (Voucher Number BM 005) was done by a taxonomist from the University of EIdoret; Kenya. Authentication of the plant was conducted in accordance with the International Code of Botanical Nomenclature (ICBN) protocols by an expert from the National Museums of Kenya. The selected specimens were banked at the Kenyatta University's; Kenya, Herbarium for future reference.

2 Collecting, drying and powdering of selected medicinal plant materials

Ten kilograms of fresh leaves of whole plant of *Acmella oleracea* were collected from its natural habitat near Matunda (0^0 49'17.0"N 35^0 07'10.8E), in Likuyani sub-county, Kakamega County of Western Kenya. The collected plant materials were thoroughly rinsed in distilled water, dried under the shade for a period of four weeks and ground into powder using a mill. The powders was packed and closed in lightproof, dry plastic bag then stored at room temperature for further assays.

3 Phytoconstituents extraction

Extraction was done by infusion (Handa *et al*, 2008). 500g of the powder was mixed with 4 litres of distilled water in a plastic container. The container containing the mixture was then heated while stirring, in a 60°C electric thermostatic water bath for 360 minutes. The temperature was set at 60°C to protect thermo labile components that would lose their properties at higher temperatures. The content was cooled, strained using a clean piece of cotton cloth. Final filtrations were done using Whatman No. 1.0 filter papers. The concentration of the filtrate was then done at 60°C after which the contents were lyophilized to limit oxidative changes of valuable metabolites (Papageorgiou *et al*, 2008) during drying. The appearance of the lyophilized aqueous extracts of *Acmella oleracea* is as shown in the photo in plate2 below. The lyophilized extracts weighing 205.00g, were packed in airtight and lightproof plastic bags and stored at -10° C until used for bioassays at a later date.

4 Quantitative phytochemical analyses

Quantitative phytochemical analysis of *Acmella oleracea* was done using standard procedures.

5 Elemental content determinations in the phytoextracts

Determination done by TXRF

6 Data processing

Means and standard deviations of each set of aliquots were determined using Excel spreadsheet.

The calculated means and standard deviations were values of the final concentrations (Hagen, 2007).

7 Reconstitution of the phytoextracts for oral administration

Reconstitution of the lyophilized aqueous extracts was done shortly prior to dispensing. Preparation of the three therapeutic test phytoextracts doses of 50, 95 and 300mg/kg of body weight was done by mixing 600mg, 1140mg and 3600mg, respectively, each with 10 ml of a vehicle solution, namely physiological saline. Positive control standard drug, flucloxacillin, was reconstituted by emptying the 500mg capsule content into 10ml of physiological saline vehicle.

8 Experimental animals

Healthy white male albino Swiss mice, *Mus musculus*, aged 5 weeks, were obtained from the Department of Microbiology, Biochemistry and Biotechnology of Kenyatta University. Only male mice were used to avoid physiological variability linked to oestrous cycle of female mice. Acclimatization of the experimental mice in their cages was done for 7 days before commencement of the experimentation. The surgically operated mice were housed one per cage, with wood shaving beddings, to avoid interaction or injury by other animals. They were housed under standard laboratory provisions of light periodicity of 12 hours, ambient room temperature (14.3-25.3^{0}C) and fed on standard commercial pellets for rodents and watered *ad libitum*. The use of mice in experimentation commenced after ethical approval from Animal Care and Use Committee of Kenyatta University.

9 Experimental design

The five mice in each group were randomly assigned. Experimental mice were put into a total of five groups of five animals each. The excision wound was induced in each study animal as described in section 8 below.

To administer the therapeutics orally, 0.1 ml or 100 micro litres of the preparation was drawn into a syringe and a cannula fixed on it. Fleshy part at the dorsal side of the animal towards the head was held, the cannula inserted into the throat and content gently emptied into the animal. The dosage was administered two times per day for 21 days running. Group A, a negative control, was dosed with 0.1ml physiological saline. Group B, a positive control, was dosed with Flucloxacillin, standard drug for comparison with the wound healing potential of the specimen phytoextracts at the assay therapeutic dose of 40 mg/kg and test phytoextracts at therapeutic doses 50, 95 and 300mg/kg body weight to groups C, D and E respectively via oral routes.

10 Induction of excision wound and measurement of wound contraction to determine the efficacy of the extracts in enhancing cutaneous wound healing

Diethyl ether was employed in anaesthetizing of experimental animals. The inter-scapular dorsal thoracic regions of the pre-weighed mice were shaved and sterilized using 70% ethanol. One excision wound was surgically induced in the shaved region after impression by pinching a skin fold and cutting off approximately 2.0cm x 2.0cm full thickness of the skin from the depilated regions by means of a sterilized scalpel (Abu-Al-Basal, 2001). The panniculus carnosus was also removed during surgery. Wounds were sited in the inter-scapular dorsal thoracic regions purposely to hinder animals from reaching and disturbing the recovery process due to irritation or during grooming. Direct pressure was applied on the wound to enhance hemostasis.

The resultant wound areas were measured by placing a transparent, sodium hypochlorite solution sterilized, tracing paper on the wound and tracing its margin. The wound traces were then transferred from tracing papers using carbon papers onto graph papers from which the wound sizes in mm^2 were worked out. Subsequent measurement of wound sizes was conducted at a five day interval up to the 25^{th} day post wounding/until wounds closed. The mice were anesthetized using diethyl ether every moment wounds were measured. The contraction of wound size, expressed in percentage, was calculated as follows; $\frac{(IWS-SWS)}{IWS} \times 100\%$, where IWS is day one wound size in mm^2 and SWS subsequent day wound size in mm^2. The rate of wound contraction in % per day in the first five days post wounding was calculated using following expression, $\frac{(D1PWS-D5PWS)}{Time\ (days)}$ %, where D1PWS is day one % wound size and D5PWS day five % wound size.

10 Data management and analysis

Means and standard deviations were adopted as descriptive statistics measures in summarizing the results numerically in all tests. One-way analysis of variance (ANOVA) was adopted in establishing statistically significant differences between test groups and both normal and positive control groups at $\rho \leq 0.05$. Tukeys HSD post hoc test was used to establish the specific paired groups that were significantly different. Statistical analysis was done using Minitab version 17 software.

RESULTS

The impact of reconstituted phytoextracts on wound contraction in mice

The efficacy of reconstituted lyophilized aqueous extracts of *Acmella oleracea* in enhancing excision wound contraction after oral administration is presented in Table 1. The results of wound contraction, expressed as percentage, in the first five days post wound induction show that there was a significant acceleration of excision wound contraction after systemic administration of all the three test therapeutic doses of *Acmella oleracea via* oral route. This was in respect of normal control at $\rho \leq 0.05$. No significant effect on wound contraction was recorded

at 50 and 95mg/kg body weight dose with respect to positive control. There was a significant decline in the rate of wound contraction with respect to positive control mice at 300mg/kg body weight test therapeutic dose of aqueous extracts of *Acmella oleracea*. A comparison of the effects of the three test doses of *Acmella oleracea* indicated that the first and second test dose, that is, 50 and 95mg/kg body weight doses had similar but significantly higher effects on the rates of wound contraction than the third when systemically administered via oral route.

Table 1: The impact of dispensing reconstituted crude aqueous extracts of *Acmella oleracea* to mice via oral route on the percent contraction of wound

Treatment	**Dose (mg/kg b.w)**	**Mouse Weight (g)**	**Mean wound size (WS) in mm^2 and % wound contraction (% WC)**					**Wound contraction rate**
			Day 1 (Initial wound size)	Day 5	Day 10	Day 15	Day 20	
PS		23.42±1.28^a	272.40±6.66	89.21±5.40	16.43±1.43	3.41±0.96	1.25±0.25	17.84±1.08
			0.00±0.00	67.25±1.80^c	93.97±0.73^c	98.75±0.35^c	99.54±0.12^b	13.45±0.36^c
Fluc	40	22.66±1.58^a	273.40±4.51	55.50±7.82	12.41±1.14	1.34±0.21	0.00±0.00	11.10±1.56
			0.00±0.00	79.70±2.95^a	95.46±0.43^b	99.51±0.08^b	100.00±0.00^a	15.94±0.59^a
Acmella oleracea	50	23.18±1.33^a	279.00±7.38	56.27±4.53	6.81±1.10	0.40±0.14	0.00±0.00	11.25±0.91
			0.00±0.00	79.83±1.84^a	97.56±1.10^a	99.88±0.45^a	100.00±0.00^a	15.97±0.37^a
	95	23.80±0.76^a	282.20±7.33	56.55±4.52	8.78±1.48	0.40±0.14	0.00±0.00	11.31±0.90
			0.00±0.00	79.96±1.16^a	96.89±0.47^a	99.86±0.05^a	100.00±0.00^a	15.99±0.23^a
	300	23.84±1.08^a	276.00±8.44	70.34±8.81	5.61±1.40	0.88±0.50	0.00±0.00	14.07±1.76
			0.00±0.00	74.51±3.28^b	97.97±0.43^a	99.68±0.08^a	100.00±0.00^a	14.90±0.09^b

Wound sizes in mm^2 and % wound contraction are presented as mean ± standard deviation (SD) of 5 mice per group. The first row for each dose is the wound size in mm^2 and the second row is the percent wound area reduction based on the initial wound size at the given time period. The last column indicates rate of wound contraction in mm^2 and % per day based on the first five days post wound induction.

DISCUSSION

Wounds often come with traumatizing clinical repercussions on the patients. Consequently, the victims seek medical intervention from modern medicament which pause challenges of allergenicity and prohibitively high cost of the drugs. These circumstances have driven many into using the readily available crude herbal medicines that have not been scientifically validated (WHO 2002-2005). There has, therefore, been need to establish better alternatives of medication (Sai and Babu, 1998). This investigation was conducted *in vivo* to determine the efficacy of ethno-pharmacological lyophilized crude aqueous extracts of *Acmella oleracea* used in enhancing cutaneous wound healing in western Kenya.

The wound contraction in mice given physiological saline proceeds under the natural mechanism of wound healing that sequentially progresses in four phases, the hemostasis,

inflammation, proliferation and remodeling phases. During hemostasis, which sets in automatically when tissues are severed, thrombocytes promote vasoconstriction by secreting vasoconstrictive agents. Vascular disruption and vasoconstriction limit blood supply to damaged tissues. This leads to temporary hypoxia which triggers wound healing although lengthy hypoxic conditions slow down the rate of wound healing (Bishop, 2008; Rodriguez *et al*., 2008). In the event of acute disruption of the tissue integrity, hypoxia induces the production of cytokines and growth factors from macrophages, keratinocytes and fibroblasts. The leakage of Adenosine diphosphate from severed tissues enhances the aggregation and adherence of the thrombocytes to the exposed collagen (Gachet, 2008). Thrombokinase secreted by thrombocytes stimulates the conversion of prothrombin to thrombin which triggers the formation of fibrin from fibrinogen leading to the formation of a haemostatic plug.

Inflammatory phase which is initiated by Platelet derived growth factor released by thrombocytes is characterized by the leakage of plasma and polymorphonucleocytes/neurophils from the blood vessels into the tissue (Wahl and Wahl, 1992). Polymorphonucleocytes are valuable in wound debridement by phagocytosis of microorganisms and products of tissue necrosis that would promote microbial growth thereby slowing down the rate of wound healing process. The macrophages are special cells which phagocytize microorganisms and secrete growth chemotactic agents like interleukin-l and Fibroblast growth factor among others that direct the proliferation phase. During this phase, collagen released by fibroblasts forms a skeleton that forms the basis of skin regrowth. Granulation that commences about four days post wounding involves the regeneration of dermal and sub-dermal components and wound contractions. Pericytes and endothelials are responsible for angiogenesis. Keratinocytes promote epithelialization which closes the wound surface. Wound contraction is promoted by special fibroblasts. Myofibroblasts attach to the skin edges and pull the epidermal layer inward leaving a smaller wound that is repaired by scar formation (Tomasek, 2002).

The administration of lyophilized crude aqua extracts of *Acmella oleracea* at of 50, 95 and 300mg/kg of body weight test therapeutic doses, systemically via oral route, significantly accelerated the rate of wound contraction, in percentage, with respect to the negative control in the first five days post-wounding. The findings of this research are in agreement with the findings of Oladejo *et al*., (2003) who reported that plant extracts, from *Ageratum conyzoides*, significantly enhanced the contraction of cutaneous wounds. What then is the driving force behind these phenomenal effects of the specimen plant extracts on wound healing process? The answer to this question lies in the analysis of lyophilized crude aqueous phytoextracts of the study plants which revealed the presence of bioactive secondary metabolites like alkaloids, flavonoids, tannins and saponins that could have a correlation with elevated rates of wound contraction and epithelialization *in vivo*.

Flavonoids in the extracts (Priya and Shyamala, 1999) posses' antioxidant and free radical scavenging properties that are valuable in enhancing wound healing. Their antibacterial property is also valuable in keeping the bacterial load at wound site low to enhance the healing process.

Saponins are bioactive phytochemicals that have been found to be proangiogenic. Hong *et al*., (2009) confirmed the angiogenic activity of total saponins extracted from *Panax notoginseng*. They demonstrated that the extracts stimulate proliferation using umbilical vein endothelial cells *in vitro*. Saponins also stimulate Vascular Endothelial Growth Factor (VEGF) that is involved in pro-angiogenic events.

Tannins which are polyphenolic metabolites have been found to have efficacy in the treatment of heat burns (Okoli *et al.,* 2007). Tannins promote wound healing via various mechanisms. Wounds are prone to microbial attack especially by *Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* which slow down the wound healing process (Rojas, 2002) by for example releasing enzymes that breakdown collagen. Tannin has antimicrobial properties; for example, they act by breaking down the cell walls of bacteria. Tannins promote the expression of vascular endothelial growth factor (VEGF) hence improved angiogenesis and wound contraction.

Results from research have indicated that alkaloids promote wound healing by promoting increased epithelial cell formation hence enhanced re-epithelialization. The alkaloids are also proangiogenic. Efficiency in angiogenesis proportionally leads to increased blood flow, hence increased oxygen and nutrient supply, to the damaged tissues which in turn increases wound contraction rate. The bioactivity of alkaloids in wound healing process could be attributed to increased angiogenesis and deposition of collagen in the granulation tissue (Paladini *et al*., 1996). Studies have shown that alkaloids may be antimicrobial against gram positive and gram negative bacteria like *Pseudomonas aeruginosa* which is resistant to antibiotics and invades skin burns causing sepsis. This makes the treatment of infected wounds difficult (Kumar *et al*., 2010). Studies have revealed that alkaloid, which constitutes a class of compounds like quinolone, indolizidine, isoquinoline, polyamine and agelasine, possess antimicrobial properties. For example, pergularinine and tylophorinidine, alkaloids in the class indolizidine, exert their antimicrobial activity by inhibiting the activity of dihydrofolate reductase enzyme and nucleic acid synthesis (Rao and Venkatachalam, 2000). The enzyme dihydrofolate reductase is involved in the conversion of dihydrofolate to coenzyme tetrahydrofolate which is an essential requirement in the synthesis purines and thymidylate. Isoquinolines like protoberberinine and benzophenanthridine act by intercalating DNA, inhibiting enzymes like gyrase, topoisomerase (IV), and RNA polymerase as well as cell division (Yi *et al*., 2007). Quinolones such as dictamnine exert their antimicrobial activity by inhibiting type II topoisomerase enzymes and consequently inhibiting DNA replication (Heeb *et al*., 2011).

Normoglycemia was recorded during this study. In hyperglycemic diabetics, chronic wounds like diabetic foot ulcers are associated with hypoxia (Tandara and Mustoe, 2004) which may be due to inadequate perfusion and angiogenesis. Hypoxia increases the concentration of oxygen radicals thereby delaying the wound healing process (Mathieu *et al*., 2006). Hyperglycemia may lead to the damage of eyes, kidneys, nerves and heart. The condition is associated with stiffer blood vessels which slows blood circulation causing reduced tissue oxygenation and reduced leukocyte migration to the wound hence infection.

TXRF analysis of the extracts indicated the presence of micronutrients such as magnesium, iron and copper which have been found to accelerate wound regeneration. Mg^{2+} is vital in the activation of the enzyme that controls collagen synthesis. Iron is essential in the hydroxylation of lysine and proline that play key role in collagen formation. Cu^{2+} is required for cross-linking of collagen and as a cofactor for cytosolic antioxidant superoxide dismutase and cytochrome oxidase enzymes (Shepherd, 2003). Cytochrome oxidase catalyzes the reduction of reactive molecular oxygen (O_2) to water. Cytosolic superoxide dismutase catalyses the disintegration of superoxide anion into oxygen and hydrogen peroxide. The above nutrients are therefore important antioxidant agents whose deficiency could prolong wound healing process. Although all the test phytoextracts therapeutic doses recorded a significantly accelerated rate of wound contraction with respect to the negative control mice, *Acmella oleracea* at 300mg/kg of body weight dose generally performed lower than 50mg and 95mg of body weight therapeutic doses. This could be probably due the depressant effects of higher concentration of anti-inflammatory agents such as steroidal alkaloids that impede fibroblast proliferation and collagenation (Feeser *et al*., 2009). As anti-inflammatory agents, studies have shown that flavonoids and tannins reduce inflammation by inhibiting pro-inflammatory enzymes lipooxygenase and cyclo-oxygenases (Lee *et al*., 2003). Lipo-oxygenases are involved in the synthesis of inflammatory and allergic reaction mediators, leukotriene, from arachidonic acid (Skrzypczak-Jankun *et al*., 2011). Leukotrienes are phagocyte chemoattractants of the innate immune system to inflammation sites. Cyclo-oxygenases are involved in the metabolism of arachidonic acid to produce prostaglandins and thromboxane A_2. Prostaglandins enhance inflammation, pain and swelling (De Wet, 2011). Thromboxane A_2 activates platelets whenever a break occurs in the endothelium (Hassal *et al*., 1983). Pathological consequences can arise if the activation of is not controlled.

Effective immune system is very important in enhancing wound healing process because it fights off the microbial attacks, thereby keeping the pathogen load at the wound site low. Heavy microbial invasion on wounds promotes tissue necrosis hence increasing the wound healing period. Selenium optimizes immune responses, in both innate and acquired immunity, through its anti-oxidant properties and its deficiency impairs antibody generation (Maggini *et al*., 2007). Zinc (Zn) also enhances both innate and acquired immunities, supports T-helper 1(Th1) immune response and positively contributes towards the maintenance of skin and mucosal integrity

(Maggini *et al*., 2007). Optimum copper (Cu) supply supports Th1 response while imbalance in the supply of Cu modulates the immune response. Iron (Fe) is an essential constituent of the enzymes that are required for the functioning of immune cells and takes part in the regulation of cytokine synthesis and its action (Maggini *et al*., 2007).

CONCLUSIONS

Policy makers have not had peace of mind because of the safety of the widely consumed non-validated ethno-medical products as scientists search for safe phytoagents that enhance wound healing. In a nutshell, this study aimed at determining *in vivo* the efficacy of the assay aqua phytomedical extracts in accelerating cutaneous wound regeneration on the basis of wound contraction and their safety on the basis of acute and sub-acute toxicity with a focus on mortality, moribund status and effects on hematological and biochemical parameters in experimental mice. Determination of the presence of phytochemicals and mineral elements that probably have a correlation with wound healing in the extracts was also a point of focus. On basis of the results in experimental mice in this research, it can be concluded that Lyophilized crude phytomedical aqueous extracts of *Acmella oleracea* administered systemically via oral route significantly increased the rate of wound contraction in the first five days post-wounding with respect to the negative control. The extracts are therefore effective in enhancing cutaneous wound healing. The crude extracts contain bioactive secondary metabolites namely saponins, tannins, phenols, flavonoids and alkaloids which may be linked to the observed acceleration of wound contraction. On the basis of TXFR analysis, the extracts from the plant contain several mineral ions such as zinc, iron, magnesium and copper that enhance wound healing.

RECOMMENDATIONS

For practice/policy purpose, this study recommends the continued utilization of lyophilized crude extracts of *Acmella oleracea* in enhancing cutaneous wound healing. These wildly growing plants should also be domesticated and eventually commercialized. For further studies**,** the study suggests that assays on the efficacy of topical application of *Acmella oleracea* extracts on cutaneous wound healing needs to be conducted. Also there's a need to work towards isolation, purification and packaging of bioactive constituents of the phytoextracts.

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