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Studies on Zinc and Copper Ion in Relation to Wound Healing in Male and Female West African Dwarf Goats

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Summary: Wound healing remains a challenging clinical problem for which precise and efficient management is essential in order to curtail morbidity and mortality. Wound healing has been shown to depend upon the availability of appropriate trace elements like copper and zinc which serve as enzyme cofactors and structural components in tissue repair. This study aims at evaluating the distribution of zinc and copper found in the hair as well as skin during epidermal wound healing. Adult and healthy West African dwarf (WAD) goats of both sexes fed with concentrate, grass, cassava peel and water *ad libitum* were used. The animals were housed for three weeks before commencement of the experiments. Epidermal wounds were created on the trunks of all the goats using cardboard template of 1cm². Progressive changes in wound contraction were monitored grossly by placing clean and sterile venier calliper on the wound margin. Hair and skin elemental (copper and zinc) analyses were done using atomic absorption spectroscopy (AAS). Significant increases in Cu level were observed in the female hair compared with that of males. There were significant increases in the Zn levels of the females' hair compared with the males. The wound healed faster in female goat compared with the males. The ratio of copper to zinc is clinically more important than the concentration of either of these trace metals. The pattern of distribution between zinc and copper concentration in the skin and hair of the male and female goats observed in this study could be added factor responsible for early wound healing in female. Therefore, our findings suggest that the distribution in the Cu and Zinc level in skin and hair of both male and female goats could also be a factor for wound healing in the animals.

Keywords*:* Wound healing, Copper, Zinc, Hair, Skin.

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INTRODUCTION

The skin is the largest organ that makes up about10% of the whole body which is largely exposed to physical and mechanical assaults daily. Loss of a large portion of the skin integrity arising as a result of injury or illness usually leads to disabilities or death (Adam *et al*., 1999; Robson *et al*., 2001). In normal skin, the epidermis and dermis exist in steady state of equilibrium, forming a protective barrier against the external environment. This can range from a simple break in the epithelial integrity of the skin or it can be deeper, extending into subcutaneous tissue with damage to other structures such as tendons, muscles, vessels, nerves, parenchymal organs and even bone (Alonso *et al.*, 1996).

Wounds can be grouped according to various criteria (Robson *et al.*, 2001) which can be clinically categorized as acute and chronic with reference to their time frame of healing (Bischoff *et al.*, 1999). Other criteria considered during wound classification include aetiology, degree of contamination, morphological characteristics and communication with hollow or solid organs (Komarcevic *et al.*, 2000). Wounds may also be referred to as closed, where the underlying tissue has been traumatized but the skin has not been severed; or as open, where the skin layer has been damaged with the underlying tissue exposed (Attinger *et al.*, 2006). Once the protective barrier is broken, the normal physiological process of wound healing sets in immediately (Nguyen *et al.*, 2009).

Wound healing is a dynamic and highly regulated process consisting of cellular, humoral and molecular mechanisms (Reinke and Sorg, 2012). The healing process consists of a sequence of overlapping events including inflammatory responses, regeneration of the epidermis, shrinkage of the wound and finally connective tissue formation and remodelling (Choucair and Phillips, 1997; Leaper and Harding, 1998).

Several factors are responsible for wound healing many of which if interfered with might lead to improper or impaired tissue repair (Guo and DiPietro, 2010). Skin wound healing depends upon the availability of appropriate trace elements serving as enzyme cofactors and structural components in tissue repair (Lansdown *et al.*, 1999).

Trace elements refer to "elements that occur in natural and perturbed environments in small amounts and that, when present in sufficient bioavailable concentrations are toxic to living organisms.'' Although these elements account for only 0.02% of the total body weight, they play significant roles, such as, being active centers of enzymes or as trace bioactive substances (Wada, 2004). Some of the trace elements control important biological processes by facilitating the binding of molecules to their receptor sites on cell membrane, alternating the structures or ionic nature of membranes to prevent or thus allowing or obstructing the entry of specific molecules into a cell thus inducing gene expression leading to protein formation involved in life processes (Nielsen, 1990).

Copper, as a trace element, plays a very important role in body metabolism as it aids the functionality of many critical enzymes (Harris, 2001). Copper has a selected biochemical function in haemoglobin (Hb) synthesis, connective tissue metabolism and bone development (Turnlund, 1998). Copper metal in contact with skin is purported to exert anti-inflammatory properties, but the extent to which copper penetrates the layers of the skin is a matter of debate (Hostynek and Maibach, 2003). Scientists think that introducing copper into wound dressings would not only reduce the risk of contamination, but also stimulate healing. Releasing copper from the dressings directly onto the wound promotes skin regeneration (Wilkinson and Hawke, 2000).

Several copper-dependent enzymes, mainly amine oxidase increase during wound healing (Rea *et al.,* 1998). These copper-dependent enzymes are important in the remodeling and healing of wounds. The direct role of copper in facilitating angiogenesis has been evident for two decades (Alessandri *et al.,* 1984).

Zinc stabilizes cell membranes, serves as an essential cofactor for several metallo-enzymes, participates in basal cell mitosis and differentiation (Landsdown *et al.,* 2007).The function of zinc in cells and tissues is dependent on metalloproteinase and these enzymes are associated with reproductive, neurological, immune, dermatological systems as well as gastrointestinal tract (GIT). Zinc is essential for normal spermatogenesis and maturation of sperm, genomic integrity of sperm, normal organogenesis, proper functioning of neurotransmitters, development of thymus, epithelialisation in wound healing, taste sensation, secretion of pancreas and gastric enzymes (Watson, 1998).

Zinc is also present in a number of zinc-dependent metallo-enzymes in the skin, including matrix metalloproteases (MMPs), superoxide dismutase (SOD), alkaline phosphatase, and RNA/DNA polymerases (Schwartz *et al.,* 2005). Many of the biochemical and molecular events in wound repair can be expedited by addition of supplementary zinc ion through up-regulation of metallothioneins (Lansdown, 2002) and zinc metallo-enzymes (Ravanti and Kahari, 2000). Zinc helps maintain the integrity of skin and mucosal membranes (Wintergerst *et al.,* 2007). Patients with chronic leg ulcers have abnormal zinc metabolism and low serum zinc levels (Lansdown *et al.,* 2007). However, the importance of copper and zinc during wound healing in the hair and skin has not being investigated especially in goats. There is also a dearth of information on the distribution of these elements as responsible for wound healing differences in male and female goats, hence the need for this study.

MATERIALS AND METHODS

Experimental animals

Ten adult West African Dwarf goats of equal sexes (between 1-1.5 years and weighing 10-12 kg), were put in stalls. The animals were housed in individual pens three weeks for stabilization before commencement of the experiment. Well-balanced diet consisting of concentrate, grass and cassava peels were fed to the animals and water provided *ad libitum*. The animals were dewormed with levamisole (10%) I/M at the dose rate of 10mg/kg body weight and also given penicillin-streptomycin pre-emptively to take care of possible bacterial infections.

Epidermal wound creation

Using a square stencil of dimension 1cm by 1cm, the portion of the epidermis to be surgically removed which is the right lateral side of the animal just ventral to the vertebrae column was marked using an ink marker. Three mg/kg of 2% lignocaine was used in caudal epidural block and local infiltration (inverted L-Block) to desensitize the skin in order to ensure complete desensitization of nerves that might escape epidural block and provide the required anaesthesia. Booster injections of up to one-half of the initial dose were administered as needed in order to ensure that the goats were pain-free during the skin excision procedure. Each marked portion was blocked individually before surgery was done.

Epidermal wounds were created on the trunk of all the goats. A sharp sterilized scalpel was used and bleeding reduced by the use of pressure gauze and shortening of surgery duration. The full thickness of the skin within the incision was then carefully stripped away by sharp dissection from its underlying muscle. All excisions were made using scalpel blade and forceps with particular care taken that wound edges were sharply defined (Olaifa, 2016)

Measurement of wound contraction

Each wound was measured (in centimetre^2) daily using the length of the mid-horizontal and mid-vertical sides of the wound with the aid of a vernier calliper. Error due to parallax was reduced by ensuring that wounds were measured under adequate illumination using the same blind observer all through the experiment. The length (L) and breadth (B) were then used to calculate the wound area in cm^2 (Olaifa, 2016)

Elemental analysis

Skin biopsies and hair shavings were taken on the day of wound creation and submitted to I.A.R&T laboratory Ibadan, to check for copper and zinc levels in the skin and hair of the trunk. This was done using Atomic absorption spectroscopy (AAS) which is a spectroanalytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state. The skin and hair were weighed, and 0.5g of each was put in the digestion tube. Five (5) ml perchloric: nitric acid mixture in the ratio 1:3 was dispensed into digestion tube containing each sample, put in microwave oven for 5 minutes, removed after and put in cupboard to cool. After cooling, 45mls of distilled water was dispensed, mixed together for homogeneity and the portion needed for analysis was decanted. Copper and zinc were read from the decant of both skin and hair samples in the atomic absorption spectrophotometer (AAS) from their individual fluorescent lamps (Siraj and Kitte, 2013).

Statistical Analysis

Statistical analysis was performed using SPSS software for windows (version 16.0). Results for reaction time and paw oedema (Mean ± SEM) were analyzed using One way ANOVA followed by Tuckey's *post Hoc* test to identify significance between groups. Result for pain score were analyzed using Kruskal Wallis non-parametric test. The differences were considered significant at $P < 0.05$.

RESULTS

On days 20 and 25 post creation of wounds, the healing rate of the wound in the female was significantly higher than that of the male goat (Fig. 1). The wounds in the male did not heal completely until after day 30.

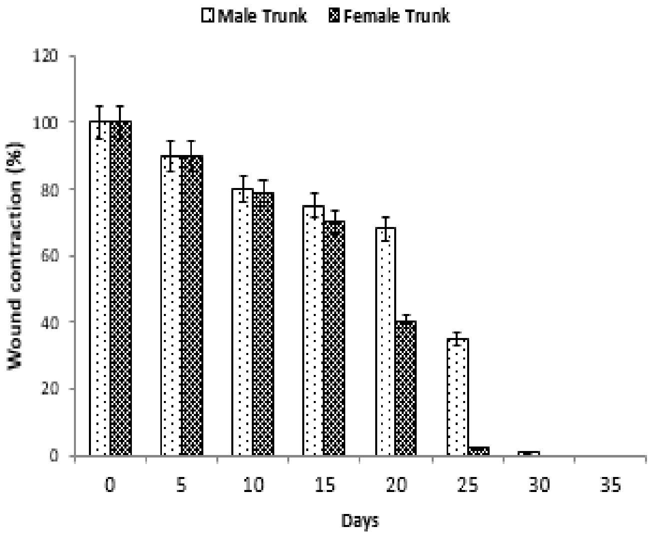

Fig 1: Wound healing in the male and female goats

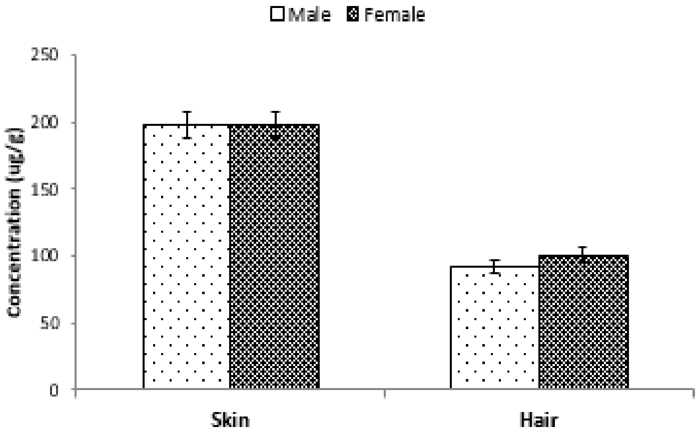

Fig 2: Average concentration of Cu on the skin of male and female West African Dwarf goats in µg/g (ppb)

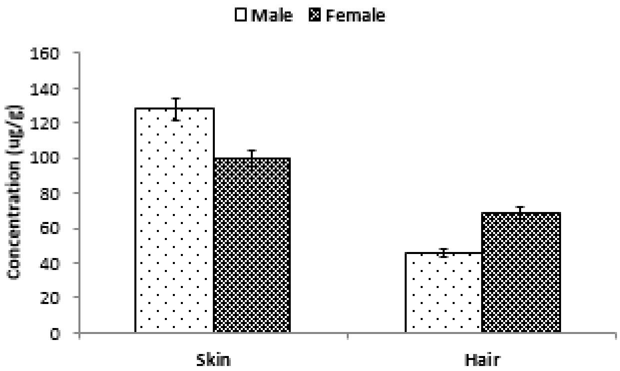

Fig 3: Average concentration of Zn on the skin of male and female West African Dwarf goats in µg/g (ppb)

Evaluation of the rate of wound healing continued until day 35 when all the wounds in the goats were healed.

The copper analysis (fig 2) shows that there was an insignificant increase in copper concentration in the skin of the male than the female. While the skin of the male goats had a mean copper distribution of 197.81 µg/g (ppb), the female had 197.26 µg/g(ppb). However, there was less copper on the hair of the male goats than the female goats. The hair of the male goats had a mean copper concentration of 91.35 µg/g (ppb), while the female had 100.62 µg/g (ppb). The result of the zinc analysis (fig 3) shows that there was more zinc concentration in the skin of the male than the female. While the skin of the male goats had a mean zinc distribution of 127.78 µg/g (ppb), the female had 99.82 µg/g (ppb). However, there was less zinc on the hair of the male goats than the female goats. The hair of the male goats had a mean zinc distribution of 45.26 µg/g (ppb) while the female had 69.01µg/g (ppb).

DISCUSSION

In the skin, oestrogens and androgens are involved in proliferation and differentiation of epithelial cells as well as activities of fibroblasts and skin immune cells all play important roles in wound healing (Strudwick *et al.*, 2006). The faster rate of wound healing in the females than the males agrees with earlier works in humans (Jorgensen *et al*., 2002; Ashcroft and Ashworth 2003) which might be due to the involvement of sex hormone in the animals. There is striking evidence from animal studies dating back to 1962 that oestrogens play a crucial role in cutaneous wound healing. Repair is significantly delayed in its absence, an event characterized by profound leukocyte recruitment during the initial stages of injury and tissue destruction (Jorgensen and Schmidt, 1962; Calvin *et al.,* 1998; Ashcroft *et al.,* 1999). Oestrogen could also modulate the local wound-healing response and downregulate inflammation through oestrogen receptor-mediated inhibition of macrophage migration inhibitory factor (MIF). In the absence of oestrogen, local increase in MIF expression occurs unchecked, resulting in excessive inflammation and delayed healing (Ashcroft *et al.,* 2003).

There was no appreciable difference in the average concentration of copper in the skin of both male and female WAD goats; while the average copper concentration in the female hair was elevated than in the male hair which may probably be a mechanism by which the wounds healed faster in the female. Some researchers have also measured copper and zinc in the skin and hair of animal (Onwuka *et al.,* 2000; Filistowicz *et al.,* 2012). The healing promoting activities of copper has also been reported when applied together with other treatments (Pereira and Felcman, 1998; Somayaji *et al.,* 1995). Copper metal in contact with skin is purported to exert anti-inflammatory properties; however, the extent to which copper penetrates the layers of the skin is a matter of debate (Hostynek and Maibach 2003). Several copper-dependent enzymes, mainly amine oxidases, are known to be increased during wound healing (Rea *et al.,* 1998). These copper-dependent enzymes are important in the remodelling and healing of wounds. For example, lysyl oxidase catalyses the formation of aldehyde cross-links and acts primarily on collagen and elastin during wound healing (Kobayashi *et al.,* 1994).

However, average zinc concentration was elevated in the skin of the males than the females while it was higher in the hair of the female than the male. Although this pattern of difference in zinc distribution on the skin and hair of male and female could not be easily explained except by natural consistency. Meanwhile, the importance of zinc to wound healing has earlier been highlighted (McCarthy *et al.,* 1992). Zinc is the only metal that appears in all enzyme classes (Broadley *et al.,* 2007) and it is involved in numerous aspects of cellular metabolism (Classen *et al.,* 2011). It has been estimated that 6 % of body zinc is located in the skin (King *et al*., 2000).

Many of the biochemical and molecular events in wound repair can be expedited by addition of supplementary zinc ion through up-regulation of Metallothioneins (Lansdown, 2002) and zinc metalloenzymes (Ravanti and Kahari, 2000). Zinc acts as a co-factor for enzymes involved in wound healing, most notable lysyl oxidase, which catalyzes the cross-linkage of collagen (Levenson and Demetriou, 1992). The importance of cross- linking for the mechanical strength of wounds was demonstrated by topical administration of ß-aminopropionitril, which irreversibly inhibits lysyl oxidase (Ågren and Franzen, 1990). Besides, any defect in the expression of zinc finger transcription factors in mRNA coding of growth factors is consistent with impaired wound healing (Sum *et al.,* 2005). The pattern of distribution between zinc and copper concentration in the skin and hair of the male and female goats observed in this study could be an added factor responsible for early wound healing in female aside the oestrogen factor which has been sufficiently established in both human and animals. The ratio and distribution of copper to zinc is clinically more important than the concentration of either of these trace metals (Osredkar and Suster, 2011). Therefore, imbalance in zinc and copper ratio in the hair and skin as a result of deficiency could alter wound healing. So, normalising the ratio below toxic level in wound therapy either through topical or oral route will be beneficial in accelerating wound healing in animals and humans. More studies are on-going in determining the probable mechanism responsible for these observations.

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