CONTRACTION RATE AND RE-EPITHELIALIZATION TIME OF FULL THICKNESS EXCISION WOUNDS OF RATS TREATED WITH DIFFERENT CONCENTRATIONS OF HYDROGEN PEROXIDE

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ABSTRACT
The aim of this study was to validate and hence evaluate the effects of different concentrations of hydrogen peroxide on the healing of excision wounds in rats. Following wound creation, the animals in their respective groups were treated as follows; group A-0.9% normal saline (control), group B-1% hydrogen peroxide, group C-3% hydrogen peroxide and group D-5% hydrogen peroxide. Post-wounding, rate of wound contraction and re-epithelialisation time were used to assess the wound healing rate. Group C animals achieved 100% wound contraction on day 21 while 68.75%, 79.33% and 79.00% were obtained on the same day in groups A, B and D respectively. The result of this study has validated 3% hydrogen peroxide as the optimum concentration necessary for wound dressing since it provided the environment necessary for faster rate of wound contraction and healing with no visible side effect in this study.
Keywords: excision wounds, hydrogen peroxide, rat, re-epithelialisation, contraction

INTRODUCTION
A wound is a disruption in the normal continuity of tissues and metabolic function of body structures (Alam*et al*., 2011). Wound healing is the restoration of the disrupted anatomical stability and functional status of damaged tissues (Room and Ukrainsky, 2005). The process of wound healing according to Drouso*et al*. (2003) begins with wound cleansing. This has been made possible clinically by employing various methods and agents that promote healing (Drouso*et al*., 2003). The agents popularly used in wound cleansing since the nineteenth century is antiseptics. Commonly used antiseptics include povidone iodine, chlorhexidine, methylated spirit, chlorine compounds, silver compounds, hydrogen peroxide and a host of others (Zhu *et al*., 2017).
Hydrogen peroxide according to Bruno and Raynard (1994) is the most widely used among all the commercially prepared antiseptics. Zhu *et al*. (2017) proposed that hydrogen peroxide is a typical antiseptic which kills pathogens through oxidative burst and local oxygen production. In recent times, there have been arrears of controversy on the use of hydrogen peroxide as an antiseptic (Drosou*et al*., 2003). Kwakman*et al*. (2011) reported that since hydrogen peroxide is dangerous to new cells, it should not be recommended to clean every wound. Kwakman*et al*. (2011) stated that hydrogen peroxide damages cells that are needed for the wound to heal and hinder them from getting to the areas where the healing needs to take place. Hydrogen peroxide at various concentrations has been claimed by some authors to have both antibacterial and good wound healing effect. A cream containing 1% hydrogen peroxide has been reported by (Neithammer*et al*., 2009) to be effective and safe topical treatment of human *Impetigo contagiosa*caused by*staphyloccusaureus.* Thereport also said that 1% hydrogen peroxide cream showed excellent antibacterial effect and eliminated most of the bacteria from the skin. No side effect such as skin irritation or allergic reaction in human patients was noted with use of 1% hydrogen peroxide cream. A cream containing hydrogen peroxide at 1.5-3% concentration has been reported to increase circulation in ischemic ulcer and surrounding skin in a guinea pig model (Tandara and Mustoe, 2004).
Animal and human studies have also shown hydrogen peroxide to have a negative effect on wound healing at 3% concentration. Hydrogen peroxide was reported not to retard re-epithlialization in a rat model after irrigation but showed minimal bactericidal effect in-vitro (Tidhar*et al*.,2017). Kwakman*et al*. (2011) also found acceleration of re-epthlializationin wounds of rat treated with 3% hydrogen peroxide. In view of the above reports, and with the use of hydrogen peroxide as the most popular antiseptics in wound management, surprisingly few experimental studies have been conducted to examine its efficacy on wound healing process and as a wound antiseptic.
Additionally, we have observed that use of commercially available hydrogen peroxide (6 % concentration) on open wounds without dilution causing severe charring. In view of the above reports, the benefits and demerits of low and high concentrations of hydrogen peroxide on wound healing

needs to be fully investigated. The basic objective of this study was to ascertain the appropriate concentration of hydrogen peroxide which when used on open wounds will facilitate wound proliferation thereby enhance wound healing.

ANIMALS

Adult wistar rats (n=32) weighing between 170-184 g were used for this study. They were fed on commercial growers mash (Vital feeds®) and water was provided *ad libitum*. These rats were acclimatized for 2 weeks in the animal house at the Department of Veterinary Surgery, University of Nigeria, Nsukka.

WOUND HEALING STUDIES

Thirty two rats were anaesthetized by injecting intramuscularly 10 and 50 mg/kg bw of xylazine hydrochloride (Xylazine 20®, Kepro Holland) and ketamine Hydrochloride (Mark pharmaceuticals® Nigeria) respectively. Under general anaesthesia, dorsum of the rats were shaved and with mild antiseptic solution (0.5% chlorhexidine). Full thickness circular excision wound of 2cm in diameter was made on the scrubbed area of each rat and carefully dissected out. The animals were left untreated for 24 hours to allow for bacterial contamination of the wounds. Post wounding, the rats were randomly assigned into 3 groups of 8 animals per group and treated as follows: group A was treated with normal saline while groups B, C and D were treated with 1%, 3% and 5% hydrogen peroxide respectively. The animals were housed individually in clean and dust-free cages in the Department of Veterinary Surgery, University of Nigeria, Nsukka to avoid wound biting and/or licking and treated daily with their respective extract concentrations or drug until complete healing occurred.

ASSESSMENT OF WOUND HEALING

Wound healing was assessed using the percentage wound contraction rate as described by Chah*et al.* (2006) while wound re-epithelialisation was studied as described by Okoli*et al.* (2009).

RESULTS

Rate of wound contraction

Assessment of the effect of the different concentrations of H_2O_2 on the rate of wound contraction showed that increase in the percentage wound contraction was first noticed on day 4 post surgery in groups B and C rats. Day 7 post surgery, group C animals significantly ($P<0.05$) had a higher rate of wound contraction (21.50 %) than those in groups A (8.40%), B (9.33%) and D (5.00%).By day 21, 100% wound contraction was recorded in group C animals while 68.75%, 79.33%, 79.00% and were obtained in groups A, B and D animals respectively. The rate of wound contraction of group C animals remained significantly higher ($P<0.05$) when compared with those of groups A, B and D (Table 1).

Table 1: Percentage rate of wound contraction in rats post wounding.

	Groups			
Days	A(N/saline)	B (1% H_2O_2)	C (3% H_2O_2)	D (5% H_2O_2)
1	-6.86 ± 1.32^{ab}	-5.25 ± 1.93^{ab}	-4.13 ± 0.79^{a}	-7.63 ± 1.35^{b}
4	-3.00 ± 0.79^{b}	1.75 ± 3.22^{ab}	5.13 ± 1.14^{a}	-2.13 ± 0.64^{b}
7	8.40 ± 1.81^{b}	9.33 ± 4.85^{b}	21.50 ± 4.19^{a}	5.00 ± 1.21^{b}
14	48.00 ± 6.55^{b}	57.67 ± 0.67^{b}	71.50 ± 3.23^{a}	55.75 ± 2.25^{b}
21	68.75 ± 4.59^{c}	79.33 ± 2.6^{b}	100.00 ± 0.00^{a}	79.00 ± 2.35^{a}

Different superscripts[a, b,c] in a row indicate significant difference between mean at $P<0.05$

Wound Epithelialization

In this study, group C animals treated with 3% concentration of H_2O_2 had higher rate of wound epithelialization followed by groups A, D and B animals. Wound epithelialization occurred on days 45, 28, 19 and 32 in groups A, B. C and D animals respectively.

Table 2: Wound epithelialization time of rats post wounding.

Groups	**A (n/saline)**	**B (1%)**	**C (3%)**	**D (5%)**
Days of wound epithelialization	44.75 ± 3.20^{b}	28.60 ± 0.29^{ab}	19.00 ± 0.71^{a}	32.00 ± 2.04^{b}

Different superscripts in a ^{a,b} row indicate significant difference between mean at $P<0.05$.

DISCUSSION

The effects of different concentrations of hydrogen peroxide on healing of excision wound were evaluated in this study. Grossly, the effect on contraction and rate of wound epithelialization were studied. Wound contraction and epithelialization play a significant role in healing of excision wounds. According to Eichler*et al*. (2006) contraction of wound begins approximately a week after wounding when fibroblasts have differentiated into myofibroblasts. Wound contraction as documented by Sussman*et al*. (2005) peaks at 5-15 days post wounding. In this study a progressive decrease in wound size was observed on day 7 in all the groups and this finding was in agreement with the works of other researchers as documented above.

The fastest rate of wound contraction observed in 3% H_2O_2 treated group could be attributed to its content of antimicrobial properties. It kills microbes by interrupting cellular respiration of the microbes and as well prevents wound contamination by microorganisms with minimal tissue damage around the wound (Edward and Harding, 2004).

In conclusion, the result of this study validates 3% H_2O_2 as the optimum concentration of hydrogen peroxide necessary for wound dressing as it provides faster rate of wound healing with no visible side effects while 5% concentration should not be used in wound dressing due to its damaging effects on the tissue and blood vessels thus delaying wound healing. Also, 1% hydrogen peroxide and normal saline should not be used as wound dressing agents for excision wounds probably due to their poor antimicrobial property.

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