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Effects of Coconut oil and fusidic acid extract in alternative traumatic wound healing in RATS model

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ABSTRACT

The goal of this study was to improve the appropriate and economic treatment of wounds, agency for improving injury healing processes using an animal model with the use of indigenous material such as VCO oil, we used 24 rats, they divided four groups each group (6) rats, first group (G1) treatment by coconut oil, (G2) treatment by fucine ointment, (G3) treatment by coconut oil as the first group and treated with fucine ointment and Control negative (G4) control negative, Every animal treated daily as study design in groups of 10 days. VCO group and VCO – fucine treated group wounds cured much faster than other groups, as the time for complete epithelization(10days) decreases and different skin components increase. Pepsinsoluble collagen and high level of elastin showed a significant increase in VCO–fucine groups treated the wound, indicating a higher collagen cross-linking, beside the quick effect of VCO in the re-epithelialization process and increase close examination (contraction) of the wound, Conclusion: VCO's potential advantage can be due to the combined influence of multiple small biologically active components with the power activity of fucine.

Keywords: wound healing, Growth tissue, Coconut oil, fucine

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INTRODUCTION

Wound cure is a complex process in which after the injury the skin or other body tissue repairs. In ordinary skin, Epidermis (surface layer) and dermis form a defensive barrier to external conditions. If the barrier is damaged and the biochemical cascade orchestrated. Wound cure is achieved in four precisely and highly programmed phases: weathering, inflammation, proliferation and reshaping, as a normal biological procedure in the human body. All four phases must be completed in the correct sequence and time frame for a wound to heal successfully (Santos et al., 1980). In conjunction with general efforts to manage the underlying causes of chronic not healing wounds, dressings can play a major additional role (Sharma et al., 1990). Wound dressing has undergone an evolutionary process from simple wound surrounded and cloaked natural materials, materials that concentrate on the management of moisture and, more recently, materials that provide active ingredients or interact with cell or specific chemicals in the local wound environment. The aim of treating wounds is to treat the wound with minimal pain, discomfort and scarring in the shortest possible time (MacKay & Miller, 2003), Adequate nutrition and the use of antimicrobial topics and systemic agents. Although some topically utilized antimicrobial agents can kill cells like bacteria, during healing i.e. immature and nonadherent keratinocytes) cell proliferation, followed by a delayed capping of the injury may also be impaired (Daeschlein et al., 2007). Plant products have been shown to facilitate wound cure because they consist of various antioxidant and anti-inflammatory principles (Santos et al., 1980). The Ayurvedic method of medicine has long used coconut oils, including wound healing and microbial infections, in various skin disorders.

MATERIAL AND METHODS

Animal:

The research included twenty-four (24) white rats, then the animals' weight ranged (400±600 g) (male adult) from 9 months to 2 years (March-April) bred in our facility and the adult male were housed in groups in stainless-steel cages, then the rats were fed with pellets and clean water was given daily in glass bottles, besides were kept in a well-aired environment with a temperature between 10±18 C and they were exposed to sunlight and kept in a room, within days, the animals were prepared for beginning the experiment, through shaved with a standard electric shaving machine to obtain smooth and hairless skin, then burning was done in the Posterior near to the tail of the animals, so animals were kept under standard laboratory conditions and veterinary supervision with no restrictions on water and food**.** The second steps animals were locally anaesthetized by intramuscular injection of 3 mg/kg Lidocaine burn injury site. Every deep partial-thickness (DPT) of the animal brings up the desired stamp to redness. Among burn areas, approximately 2 cm of preserved skin has been retained.

Coconut oil Extraction

Thirty coconuts (*Cocosnucifera* L) were obtained from the market, the exocarp and mesocarp were removed. then the endocarp (meat) was blended using a blender. The coconut water obtained from the coconuts was added to the blended coconut and mixed. After which, the blended coconut particles (chaffs) were sieved and squeezed out of the mixture, leaving the liquid remnant which is a mixture of the coconut milk and coconut water. The liquid remnant is heated to evaporate the water present thereby leaving the oil which comes settle on top of the coconut cake that is being formed. The coconut oil was then carefully filtered in a clean sterile bottle and stored in glass case maintained at room temperature. (Ahmad *et al*., 2015).

Experimental Design:

To treated The animals were divided into four groups as follows.

1st group **(G1)** had a 3-time daily application of coconut oil (0.5mg) only.

2nd group **(G2)** was treated with**(** fusidic acid) Fucidin(R) 20 mg/g ointment only.

3rd group **(G3)** was treated with coconut oil (0.5mg) and Fucidin(R) 20 mg/g ointment.
4th group **(G4)** consider as control negative (distal water only).
The dressings were changed daily applied Fucidin & oils was cleaned with a saline solution. was only wiped with dry gauze. Treatment ointment& oils were reapplied and new gauze dressings were held in position with a self-adhering wrap-around bandage for the (10) days then all animal was sacrificed to study the healing effects of coconut oil and silver sulfadiazine as a well histopathological study of the skin healing.

Collagen and Elastin Estimation
A procedure based on the oxidation of hydroxyproline peroxide to pyrrol-2-carboxylic acid was used for the approximate collagen. It is then condensed to form a red chromogen using p-dimethylaminobenzaldehyde. To estimate elastin use successive extraction with 5 per cent TCA at 90°C for 15 minutes and once again with 01 N NaOH, the residue was removed from acetone-dried tissue by washing the residue with H 2 O and autoclaved with 0,1 N acetic acids at a 15-lb pressure for 2 h. Elastin was precipitated by 5% TCA and was estimated by the digestion method of micro-Kjeldahl(Chithra et al., 1998).

Histopathological Examination:
The tissues were fixed with a (10) per cent tampon formaldehyde solution, immediately after removal, to specimens of (1 x 1 x 1) cm dimensions including the spleen, liver, lung, heart and brain. The specimens were washed with tap water after 72 hours of fixation, and then processing took place regularly by upgrading the alcoholic concentration from 70% to 100% in every single hour to extract tissue water from it, by extracting xylol from water and by infiltrating the samples with a 58 °F semi-liquid paraffin wax, then by covering the tissue with the specimens Hematoxylin and Eosin(H&E) darkened all tissues and histopathological changes were seen under a light microscope **(Luna, 1968).**

Statistical analysis:

G	W.C
G1	61.05±0.01b
G2	59.03±0.05c
G3	65.01±0.01a
G4	32.01±0.03d

All the grouped data were statistically read by SPSS program, Version 17 software (2010).Testing methods including one way ANOVA for comparisons among groups followed by the least significant differences (LSD) test for comparison between two groups. P values of p <0.05 were considered to record statistical significance. All data were expressed as means ± standard error (SE) **(Leech *et al.*, 2011).**

RESULTS

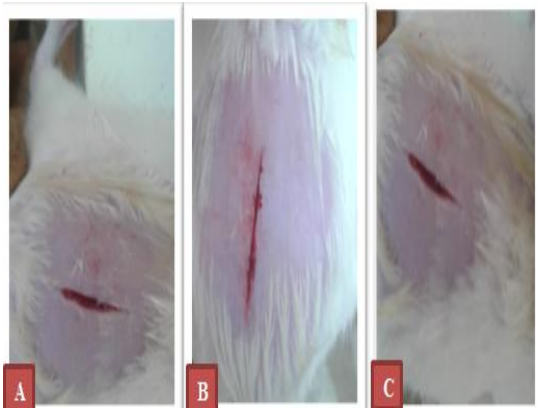
Table 1: Table 1. Influence of Fucidin and VCO on Collagen and Elastin during the healing at day 10.

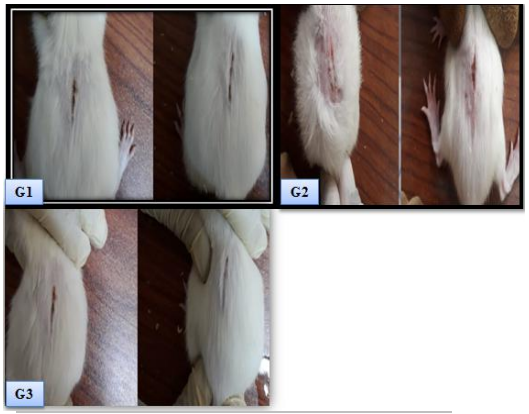
*.The mean difference is significant at P < 0.05.

Table 2: Wound contraction (WC) percentage for research groups during healing at day 10

* The mean difference is significant at P < 0.05.

Figure (1): show healing stages in different current study groups in the day **(1)** (A, B, C) and day **(3)** (G1, G2, G3) after treated.





G2	2.1±0.04c	0.20 ±0.071c
G3	4.08±0.002a	0.33 ±0.001a
G4	0.10±008d	0.02 ±0.001d

Figure (2): Show progress the wound healing at day **(7)** in groups of study after treatment.

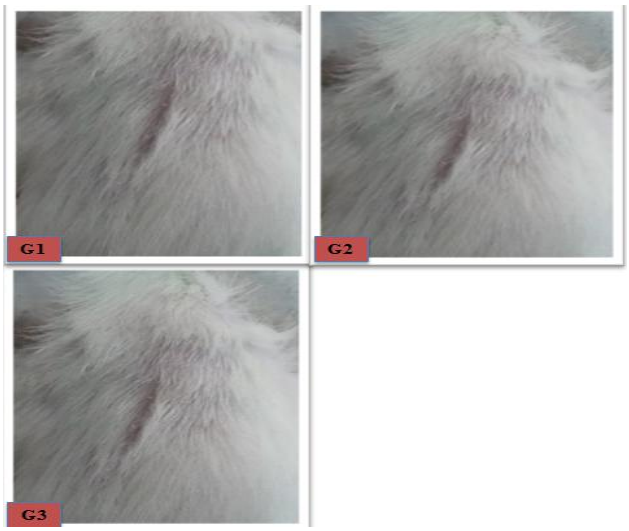
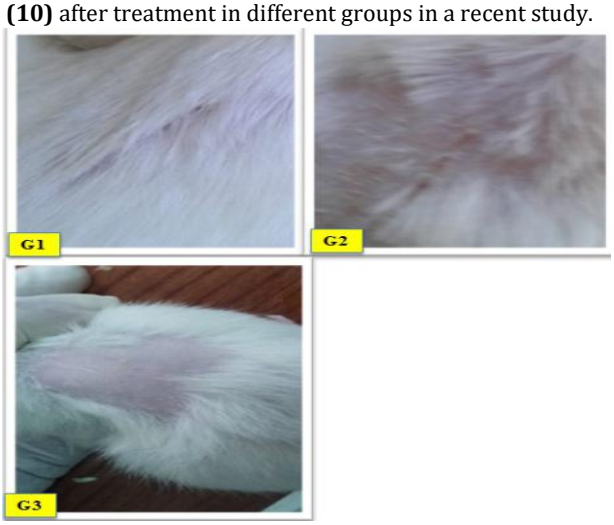


Figure (3): Show completely healing of wounds at day **(10)** after treatment in different groups in a recent study.



Histopathological study of skin after 10 days

Treatment by coconut oil G1

The epidermal was very thin and covered with a corrugated_layer of keratin, the dermal layer was condensed by collagen bundles of a different direction, these are braved by hair follicles, the hypodermis was formed by the presence of adipose tissue and skeletal muscle fibers which are arranged in horizontal and longitudinal pattern fig (4).

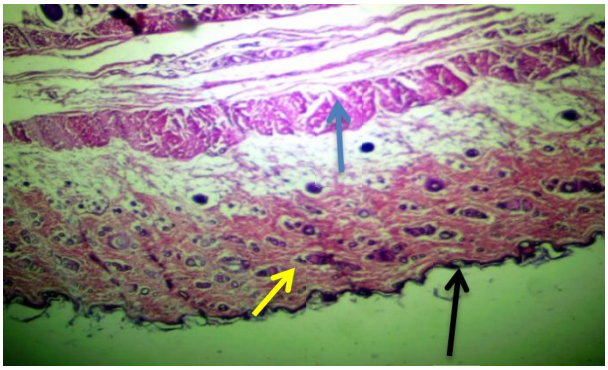


Figure (4): Corrugated layer of keratin of epidermis (arrow black), condensed layer keratin bundle (arrow yellow) of dermis hypodermis with skeletal muscle (arrow blue) (H&E stain X20).

Strands of keratin were appeared desquamated from the epidermis, also the site of hair shaft traversing the epidermis was noted and the dermis was infiltrated with a few numbers of lymphocytes and fibroblast fig(5).

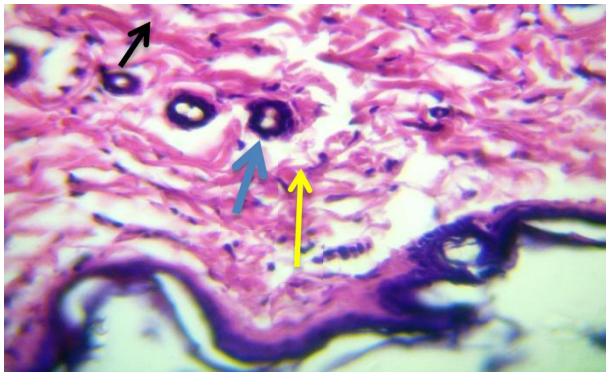


Figure (5): Desquamated keratin from hypodermis (arrow black)collagen bundles (arrow yellow), hair follicles (blue arrow) (H&E stain X40).

The lymphocytic infiltration in the dermis also present around the shaft of the hair fig(6).

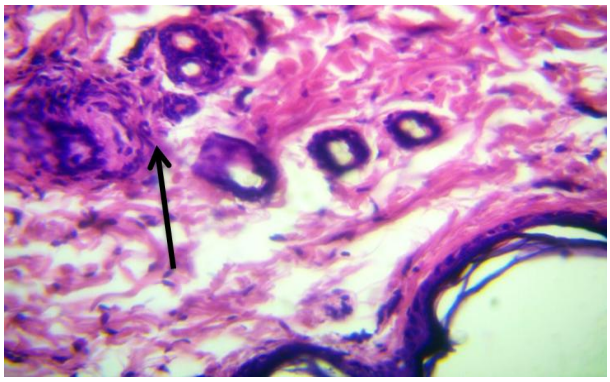


Figure (6): Lymphocytic diffusion (H&E stain X40)

Treatment by fucine G2

The epidermis was ill-defined for stratified epithelium, appeared as a homogenized layer, containing collagen fibres with the presence of hair follicles, increased deeply for the dermis and the hypodermis was continuing bundles of skeletal muscles fibre fig (7).

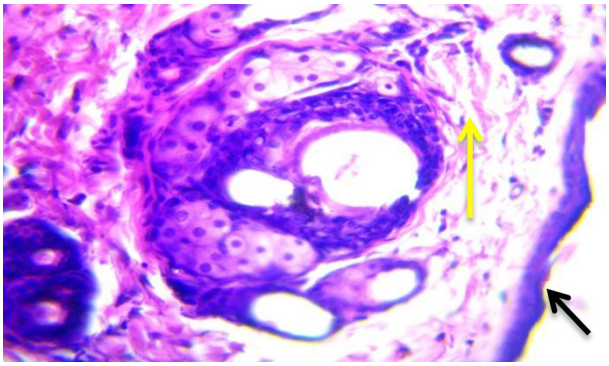
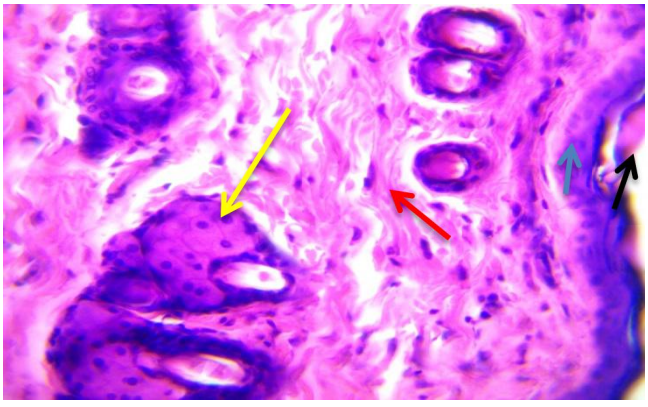


Figure (7):The epidermis with keratin (arrow black).the dermis (yellow arrow)hair follicles (blue arrow) (H&E stainX40)

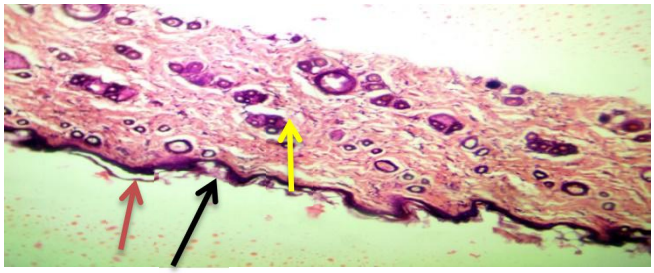
The epidermis had the discontinuous region of keratin from epithelium below this epithelium is the presence of lymphocytic infiltration with the presence of hair follicles, The hypodermis was embedded with hair follicles which were surrounded with follicular shaft cells and sebecouce gland fig (8).



Figure(8): skin demonstrating discontinuation of keratin and appeared desquamated from the epidermis fig(yellow arrow).lymphocytic infiltrated (red arrow) sebaceous gland around hair follicles (blue arrow)

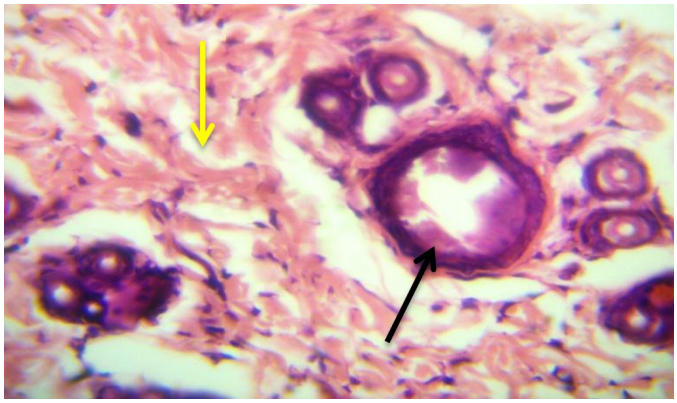
Treatment by (fucin ointment and coconut oil) G3

The epidermis appeared and irregular in its pattern with a strand of keratin separated from this layer .the epidermis was interdigitated with dermis .the dermis was enriched with bundles of collagen fibres and the section of hair follicles were present individual fig(9).



Figure(9): Epidermis with keratin (yellow arrow)dermal papillae (red arrow).hair follicles (blue arrow) H&E stain X20

The hair follicles surrounded by collagen bundles with fibroblasts fig (10).



Figure(10): Dermis with hair follicles (black arrow).surrounded fibroblasts and collagen bundles (yellow arrow) H&E stain X40.

DISCUSSION

In this study, rat burn assay was employed to study the delayed healing process in that form a problem in most species of animals and to give highlights to the effect of VCO extract to the healing process alone and the Synergistic effect of the VCO and fucine on this wounds. The analysis showed that coconut oil (VCO) has a significant positive effect on wound repair, though promote and accelerate the reepithelialization process in wound healing of the wound in rats (Nevin et al., 2010), and its effect on the cell viability through the fibroblast cell line **(**Zunairah et al., 2017), also, our study found the total collagen and elastin content of the granulation tissue from both VCO- fucine treated animal groups were found to be higher significantly as compared with the G1and G2 (table1) on day 10. The principal component of the extracellular matrix and the main granular protein Collagen, so the collagen 's role in the healing process starts during the injury and lasts for several weeks to months after the injury is closed(Shoshan, 1981), on the other hand, the total DNA of the granulated tissue was increased by VCO use, this rise in DNA indicates the hyperplasia of the cells. Untreated tests compared, the increasing of granulation tissue elastin amount in the present study as compared with the other treated groups in (table1) on day 10, which indicate that the ability of VCO and fucine to enhance the healing process and elastin is also a very significant commodity like collagen as a cross-linked protein is found in binary tissues such as skin and big blood vessels (Percival, 1997).

Moreover, many searchers described the ability of VCO to acts as antibacterial to promote the healing and prevent any contamination and the dabble effect show if mixed with a broad-spectrum antibacterial like fucine ointment, as many natural products with antibacterial activity can solve chemical and antibiotic resistance problems, they have the option (Sharifi-Rad et al., 2018), Caprylic acid and capric acid-functional components of coconut oil are 10%, lauric acid (LA) is 48%, and mertic acids are 17%, a potency antibacterial functional food was identified as Medium Chain Fatty Acids (MCFA) (Zentek et al., 2013).

VCO and fucine ointment enhanced wound healing of wounds However, mixture VCO with fucine ointment was proven to be significantly better than coconut oil and fucine ointment alone. The retained antioxidants and vitamins and anti-viral activities of VCO may be attributed to this (DebMandal et al., 2011; Mansor et al., 2012), which helps not only to eliminate bacteria but also to enhance the growth of wound tissue. On all days, (Qiu et al., 2007; Teoh et al., 2009) which support our findings. Histological research showed that the reepithelialization cycle was assisted by VCO, could be explained by the intact epidermis and dermal-epidermal interdigitation that were observed in the VCO group. This can describe the importance of the VCO in re-epithelializing, a crucial phase in the treatment of wounds (Serarslan et al., 2007).

A close examination (contraction) of the wound treatment of animals treated and the control, standard and experimental groups showed that on day 10 (Table 2), the presence of significant effect between groups, group treated with VCO and fucine gave a high level (65.01±0.01a) close wound contraction compared with others groups these results depict the ability of VCO to accelerated contraction of wounds (Esimone et al., 2005)

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