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THE EFFECT OF SOME MEDICINAL PLANT EXTRACTS ON WOUND HEALING IN FARM ANIMALS.

(With 4 Fig.)

By

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تأثير خلاصة بعض النباتات الطبية على إلتئام الجروح فى حيوانات المزرعة

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استهدفت هذه الدراسة الوقوف على تأثير خلاصة بعض النباتات الطبية مثل زهر البابونج والصفصاف والزعرور وحب البركة على إلتئام الجروح المتقحى بالقصد الثانى فى حيوانات المزرعة .

ولدراسة تأثير هذه الخلاصات استخدام الفحص الاكلينيكي والفحص الهستوباثولوجي والهستوكيميائي وكذلك عزل الميكروبات من الجروح والتعرف عليها وتصنيفها .

وقد اتضح من النتائج ان الالتهام كان أفضل مع استخدام زهر البابونج كفسول ومرهم تلاها استخدام خلاصة نبات زهر البابونج كفسول والزعرور كمرهم فخلاصة نبات حبة البركة كفسول وخلاصة نبات الصفصاف كفسول والزعرور كمرهم وأخيراً عند استخدام خلاصة الصفصاف كفسول .

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SUMMARY

This work was undertaken to assess the role of extracts of *Matricaria chamomilla*, *Salix fragilis*, *Polygonum bistorta* and *Nigella sativa* on second intention healing of infected wounds in farm animals. The assessment of topical application of these extracts based on clinical assessment, histopathological studies, histochemical studies and microbial study. The result revealed that healing was the best with the use of *Matricaria chamomilla* lotion and ointment followed by *Matricaria chamomilla* lotion and *Polygonum bistorta* ointment, *Nigella sativa* lotion, *Salix fragilis* lotion and *Polygonum bistorta* ointment and lastly *Salix fragilis* lotion.

Keywords: Effect, medicinal plants, extracts, wound, healing, farm animals.

INTRODUCTION

Many of wounds that a veterinarian is asked treat have been traumatized or have undergone some degree of tissue loss or were induced on a movable area of the body. In these instances, first intention wound healing is not possible. These wounds can be treated however, so they heal by second or third intention. The increased universal interest in medicinal plants drew the attention of the authors to investigate the healing provoking effect of topical applications of *Matricaria chamomilla*, (Zahr babuing), *Salix ftragilis*, (sefsaf), *Polygonum bistorta* (Zaater) and *Nigella sativa* (Habbit El-baraka) on second intention wound healing in farm animals.

MATERIALS AND METTHODS

This work was undertaken on randomly selected three groups of calves, sheep and goats, each of thirty animals. The age of calves ranged from five to six months and in sheep and goats from nine to twelve months. The used animals were apparently and they were kept in an open yard with dust floor. The calves and sheep were kept on fattening ration while goats were on a maintenance one. Water was given ad-libiaum. Each group of animals was divided into sub-groups each of six where as the effect of some plants on wound healing was studied as follows:- Sub-group (1) *Matricaria chamomilla* lotion and ointment, subgroup (2) *Salix fragilis* lotion, sub-group (3) *Matricaria chamomilla* lotion and *Polygonum bistorta* ointment, sub-group

(4) *Salix fragilis* lotion and *Polygeoum bistorta* ointment and sub-group (5) *Nigella sativa* lotion.

For washing wounds all the used plants except *Nigella stiva* were soaked and boiled. The obtained infusion and decoction were kept in bottles in the refrigerator till the time of use (MAHMOUND 1980, Rouaha 1983, and Ashour 1985). Dried plants as flower heads in *Matricaria chamomilla*, leaves in *Polygonum bistorta*, and bark in *Salix fragilis* were put in 75% ethyl alcohol in Soxhlet apparatus to get the extract. Using vaseline base 20% ointments were prepared from the obtained extract. *Nigella sativa* was used as marketed in the form of oil preparation for both washing and dressing of wounds.

In each animal two standard wounds were inflicted on the two sides of the thigh using a standard frame. The length, width and depth of such wounds were 2X2X1 cm in calves and 2X2X1/2 cm in sheep and goats. The left side wounds were left as control and treated routinely where it was washed with Savlon and then swabbed with 2% Tincture of Iodine day after day till complete healing. All inflicted wounds were kept untreated for the first two days then each wound was treated according to its subgroup.

The wounds were clinically examined daily for colour exudation, granulation tissue formation, vascularization and general appearance. Two swabs were collected from the depth of the wounds of each animal under complete aseptic condition for microbial examination. Then they were directly transferred to nutrient broth and incubated at 37 °C for 24 hours then inoculated on different bacteriological media using the usual microbiological methods. The samples were taken at the 3rd, 7th and 14th day after wound infliction and additional swabs were taken if required.

At the end of the first, second and third weeks histological samples from two animals of each sub-group were taken trimmed and labeled. Each specimen was separately fixed in Bouin's sol. and passed in several dilutions of alcohol and xylol, then embedded in paraffin and 6 microns sections were then cut (CARLETON, DRURY, WILLINGTON & COMMERON, 1967). These obtained sections were stained for general examination by Hematoxylin and Eosin (HARRIS, 1998), Crossman's stain for demonstration of collagen fiber and muscle cells (CROSSMAN, 1937). Alcian blue and periodic Acid Schiff (P.A.S) for demonstration of mucopolysaccharides (LINSON, 1954). Reticuline for demonstration of reticular fiber. Biopsies for histopathological examination were taken at 7th, 14th, and 21st day post wounding.

RESULTS

One week postwounding the animals of sub-group (1, 3, & control) showed bright red healthy granulation tissue starting from the wound edges to the center (Fig. 1). In sub-group (2, 4 & 5) there was very scanty granulation tissue of uneven surface and some wounds showed purulent discharge.

Histologically the wounds of sub-group (1, 3 & control) showed the least tissue destruction with very slight area of haemorrhage. The wounds contained young fibroblast cells with some vascular sprouts. In sub-group (3 & control) few round cells were found. Sub-groups (1 & 3) showed greatly positive P. A. A., Alcian blue and Reticulin (Fig. 20). In the meantime wounds of sub-group (2 & 4) showed negative results.

The microbial examination revealed that wounds in sub-groups (1 & 3) presented the least incidence of bacteria followed by control sub-group which did not show any fungal culture. The highest bacterial incidence was with sub-groups (2, 4 & 5).*

Two weeks postwounding all animals of sub-groups (1, 3 & control) showed contracted wounds with their groups almost full of healthy granulation tissue (Fig. 1). In sub-groups (4 & 5) granulation tissue filled the wound gaps partially. In sub-group (2) there was unhealthy overgranulation tissue with purulent discharge.

Histologically wounds of sub-groups (1, 3 & control) showed small and medium sized newly formed blood channels lined by endothelial cells. The wounds become cellular and going towards maturity (Fig. 3). Wounds of sub-groups (4 & 5) showed younger granulation tissue with very few infiltrating polymorphs. In subgroup (2) the surface of the wounds showed abundant round and polymorph cells. The wound base consisted of proliferated angioblasts and fibroblasts. Reticuline, P.A.S. and crosmann and Alcian blue gave moderate results in sub-group (1,3 & control), (Fig.4). In the meantime Alcian blue in sub-group (5), Reticuline and Crosmann in sub-groups (4 & 5) gave negative results.

Bacterial incidence was the same as after one week.

* The most prevalent isolated microorganism was *Staph. aureus*, while the other common isolates were *Strep. pyogenes*, *E. Coli*, *Pseudomonas Pycoaneous*, *C. pyogens*, *C. ovis*, *Candida albicans* and *Proteus vulgaris*.

After (19-21) days in sub-group (1), (20-22) days in sub-group (3), (22-24) days in control sub-group, (24-28) days in sub-group (5), (26-30) days in sub-group (4) and (28-36) days in sub-group (2); the wounds were hardly recognized and completely covered by epidermal layer.

In all sub-groups Reticuline, Alcian blue, P.A.S. and Crosman gave moderate positive results.

DISCUSSION

Categorizing wounds on injured animals is more than purely academic exercise. preoperative classification of a wound helps the surgeon to decide the most appropriate treatment. Second intention healing is the only acceptable choice for contaminated wounds to heal. However, the shape and the location of such wounds play a role in healing process. Hastings, Van winkle, Barker & Nichols, (1975) concluded that the square shape wound is one of the best models to study the process of second intention healing. In the present study it was easy to inflict and judge healing of such square wounds. Meanwhile, Fixnig the wound location in the work minimized the possible of the second point.

Concerning the assessment of wound healing it seemed a very difficult job because it is a very complicated series of biological steps. However, clinical assessment (Eloy & cornillac, 1992), histological investigation (Hauptmann, Schafer, fritz & Hauptmann, 1992) and Tolstykh, Arutyunyan & Manedov, 1990) and histochemical investigation (Luomanen & Virtanen, 1993 andkeyser, 1993) would be very helpful tools to achieve these purpose. it is worthy to ment in the present study the microbial status of the wounds was also investigated to reflect the efficacy of the used dressing (Greezer, Bitz & Hegglin, 1992).

Regarding the use of medicinal in treatment of open infected wounds, Green (1992) mentioned that their use in develping countries depends on the traditional ethnomedical beliefs. In the meantime Okuyama, Gao & Yamazaki, (1992) stated that plants to be used for wound dressing should have antiinflammatory, astringent, analgesic, antiseptic and vulnerary actions. The histopathological results obtained in the present study were parallel with the clinical findings. it was clear that plants which promoted healing clinically enhanced the formation of new blood capillaries growing into the wound bringing fibroblasts with them. In the same time there were blood capillaries to nourish the skin edges. Moreover, with the used plants, there were no mitotic divisions indicating malignancy (Luomanen & Virtanen, 1993).

The histochemical results supported both the clinical and histopathological results, as the positive periodic Acid Schiff (p.A.S) and Alcian blue reactions reflected formed mucopolysaccharides which would interact with collagen formation increasing the wound tensile strength. This finding was more clear with *Matricaria chamomilla* lotion and ointment and *Matricaria chamomilla* lotion and *polygnum bistorta* ointment than in other used plants. Furthermore, there is evidence that mucopolysaccharides stabilize collagen fibers and possibly control their ultimate and characteristic size, a finding that was proved by using the specific stain of collagen (Crossman stain) Moreover, the Reticuline results reflected the reasonable amount of formed reticular fibers. In an attempt to define precisely the role of mucopolysaccharides in wound healing, (Hartmann, Jonsson & Zederfeldt, 1992) proved that depolymerization of mucopolysaccharides delayed the formation of collagen thus they concluded that mucopolysaccharides are essential for collagen formation.

On the other hand, the microbial studies proved that the antimicrobial effect in vivo varied from one treatment to another. the anti-microbial potency of the used treatments were graded from best to worst in this order: *Matricaria chamomilla*, *Matricaria chamomilla* and *polygnum bistorta*, savlon and 2% Tincture of Iodine, *Nigella sativa*, *Salix, fragilis* and *polygnum bistorta* and lastly *salix fragilis*.

In conclusion, the obtained results proved that the use of extracts of *Matricaria chamomilla* and *polygnum bistorta* gave encouraging results in the treatment of infected wounds. these plants have antiseptic vulnerary and desiccating effects besides they are non expensive, available anywhere, easily prepared and has no harmful side-effects.

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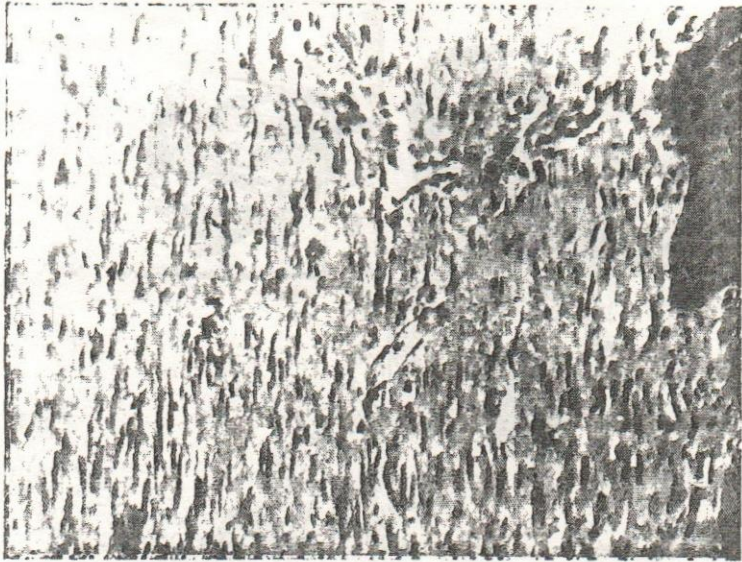


Fig.(3) H. & E X 250

Second week old wound treated with *Matricaria chamomilla* lotion and *Polygonum bistorta* ointment showing mature granulation tissue and the wound was partially covered by epidermal layer.



Fig.(4) Crossman' stain X100

Second week old wound treated with *Matricaria chamomilla* lotion and ointment showing moderate positive result.

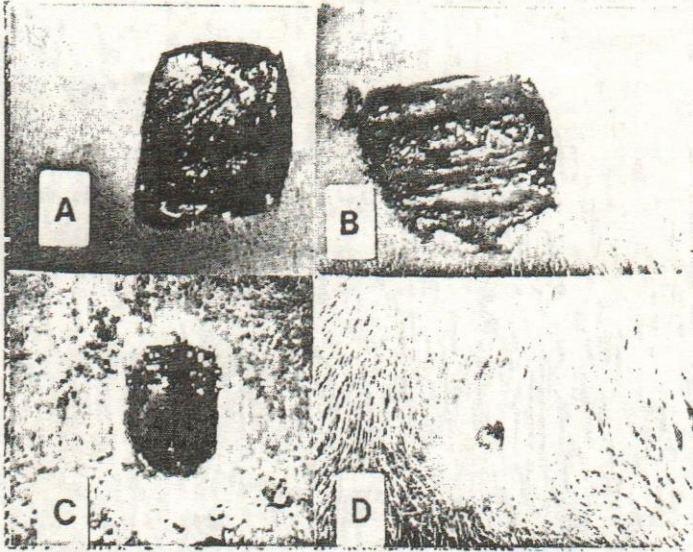


Fig.(1) Showing the effect of dressing with *Matricaria chamomilla* lotion and ointment on healing of infected wounds:

A- First day.

B- Frist week.

C- Second week.

D- Third week.



Fig.(2) Alcian blue X 250

Seven days old wound treated with *Matricaria chamomilla* lotion and *Polygonum bistorta* ointment showing positive results for acidmucopolysaccharides.