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Comparison of Wound Healing Properties of Herbal Ointments with Povidone iodine on Basis of Histological Changes

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ABSTRACT

Present study was conducted on 24 dogs, aged between 1 to 8 years, irrespective of sex, breed. The animals were randomly divided in to four groups. In group I, animals were treated with standard dressing material Povidone iodine (5.0%). In animals of group II, III, IV herbal ointments (10% w/w) of hydroalcoholic extract of Marigold leaves, Guava leaves and mixture of both Marigold and Guava leaves extracts respectively were used for dressings of wounds for 7 consecutive days. In animals of group IV, marked re-epithelialization and moderate inflammatory cells, neovascularization and thicker but scattered collagen fibrils were seen on day 7 and mature collagen fibres were observed with least number of inflammatory cells on day 14 as compared to other three groups. Therefore, it can be concluded that group IV (mixture of Marigold and Guava leaves hydroalcoholic extract) revealed better wound healing properties as compared to group I (Povidone iodine 5.0%), group II (Marigold leaves hydroalcoholic extract) and group III (Guava leaves hydroalcoholic extract).

Keywords: Wound healing, Marigold leaves, Guava leaves, Dogs

Wounds or traumatic lesions can be classified as open wounds or external wounds and closed or internal wounds. The open wounds comprises of incised, lacerated, penetrated, gun-shot, abrasion, avulsion, perforated, punctured, aseptic, contaminated, infected, granulating and ulcerating wounds.

Marigold (*Tagetes erecta*), besides being an ornamental plant, have various medicinal properties. It is having nematocidal, fungicidal, antibacterial, insecticidal and wound healing properties (Dixit *et al*., 2013). *Psidium guajava* is a common plant grown all over India for its delicious fruits "Guava". Its leaves have been used to enhance external wound healing in traditional medicine. Many pharmacological studies have demonstrated the ability of this plant to exhibit antioxidant, anticough, antidiabetic, anti-inflammatory, wound healing and antimicrobial properties (Gutierrez *et al*., 2008).

Formulations containing two or more than two herbs are called Polyherbal formulation. Popularity of Polyherbal formulation is due to their high effectiveness in a vast number of diseases. They have wide therapeutic range (effective at low dose and safe at high dose), fewer side effects, eco-friendly, cheaper and readily available (Tayde and Patil, 2015). Therefore, present study was planned to compare the wound healing properties of Povidone iodine 5.0%, hydroalcoholic extract of Marigold leaves (10% w/w), hydroalcoholic extract of Guava leaves (10% w/w) and a 1:1 mixture of hydroalcoholic extract of combination of both leaves of Marigold (10% w/w) and Guava (10% w/w) on the basis of histological changes.

MATERIALS AND METHODS

The study was carried out in Department of Veterinary Surgery and Radiology, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur (M.P). The study was conducted

on 24 dogs, aged between 1-8 years, irrespective of sex, breed. These animals were presented for treatment of wound and were randomly divided into four groups of six animals each.

Animal ethical approval

Present study was conducted after approval from Institutional Animal Ethical Committee (No. 81/IAEC/Vety/2017; dated: 08/08/2017).

Preparation of extract

The leaves were collected from plants *Tegetes erecta* and *Psidium guajava* in the local area. Fresh leaves of plant were cleaned in water and air dried, powder was made with grinder or mixer. The powder (75 g) was packed into thimble and put in a Soxhlet apparatus and extracted with 500 ml of hydroethanolic solvent (350 ml ethanol 95% and 150 ml water) at 65 ^{o}C to 70 ^{o}C for 24 h (Chatterjee *et al*., 2011). The extracts were dried under vacuum and extractability of the dried mass in Marigold leaves and Guava leaves was 5.5 gm, 8.9 gm respectively.

Preparation of ointment

For preparation of ointment (10% w/w), 5g of dry powder of each Marigold and Guava hydroethanolic extract was mixed in 45g white petroleum gelly base individually. Mixed ointment (10% w/w) was prepared with 2.5g of dry powder extract of Marigold, 2.5g of dry powder extract of Guava and 45g white petroleum jelly and mixed properly.

Treatment design

The animals were randomly divided into four equal groups. First, the wound was flushed with normal saline to decrease microbial load in all groups. In group I, open wound was dressed with povidone iodine (5.0%) for 7 consecutive days. In group II, open wound was dressed with hydroalcoholic extract of *Tegetes erecta* leaves (10% w/w) for 7 consecutive days. In group III, open wound was dressed with hydroalcoholic extract of *Psidium guajava* (10% w/w) for 7 consecutive days. In group IV, open wound was dressed with a 1:1 mixture of hydroalcoholic extract of *Tegetes erecta* (10% w/w) and *Psidium gujava* plant leaves (10% w/w) for 7 consecutive days. Inj. Amoxycillin sulbactum @ 10mg/kg b.w., i.m. was administered twice daily for 5 days in all groups.

Histological studies

A tissue of 3 mm in size was harvested from the wound by punch biopsy on 0, 7th and 14th day. The tissue was preserved and fixed in 10% formal saline and processed, sectioned and stained with haematoxiline and eosine (H & E) to study the histological changes. Van Gieson's stain was used for demonstration of collagen fibres as per the standard procedure (Lillie, 1954).

RESULTS AND DISCUSSION

The highest occurrence of wounds was recorded in animals of age group 3 - 5 years (45.83%), followed by 1 - 2 years (41.60%) and 6 - 8 years (12.57%). The wounds were found to be more common in male animals (66.66%) than female (33.33%) animals. The wounds were found to be more common in non-descript dogs (62.5%) followed by Spitz (25.0%), German shepherd (8.33%) and St. Bernard (4.16%). Most of the dogs were having bite wounds (33.33%) followed by lacerated wounds (25.00%), chronic infected wounds (20.83%), maggoted wounds (8.33%) and punctured wounds (12.5%). The maximum number of wound in present study was 2-3 old (62.5%), followed by 4-5 days (16.6%), 0-1 day (16.6%) and 6 days and above (4.1%) in dogs.

Phytochemical analysis

Phytochemical screening of hydro alcoholic extract of Marigold leaves was positive for sterol, phenol, tannin, glycosides and flavonoids. However, hydro alcoholic extract of Guava leaves was positive for saponin, sterol, phenol, tannin, glycoside and flavonoids (Table 1). Similarly, Fernandes *et al*. (2010) and Arya *et al.* (2012) conducted phytochemical screening of hydroalcoholic extract of Guava leaves and Rajvanshi *et al*. (2017) performed phytochemical screening of hydroalcoholic extract of Marigols leaves.

The qualitative analysis revealed the presence of different phytonutrients in leaves namely terpenoids, alkaloids, flavonoids, quinones carbohydrates, tannins and coumarins. These compounds have valuable antifungal, antibacterial

and anti-inflammatory properties (Hassanshshian *et al*., 2014). Wound healing properties of plant leaves is probably due to tannin fractions (Rao, 2015).

Table 1: Phytochemical analysis of hydroalcoholic extracts of Marigold and Guava leaves

Chemical test	Hydroalcoholic extract of Marigold leaves	Hydroalcoholic extract of Guava leaves
Saponin test (Froth test)	Negative	Positive
Sterol test (Salkowaski test)	Positive	Positive
Phenols and Tannin (Ferric chloride test)	Positive	Positive
Alkaloid test (Wagners reagent)	Positive	Negative
Glycoside test	Positive	Positive
Flavonoid test	Positive	Positive

Histological studies

In animals of group I, the breach in the continuity of the epidermis was seen along with necrosed tissue, migration of epithelial cells in the form of epithelial spurs and heavy infiltration of inflammatory cells on day 0 (Fig. 1a). Scarce or loosely arranged scattered collagen fibres and neovascularization was observed. Inflammatory cells were less in number on day 7 (Fig. 2a). The inflammatory cells were seen along with neovascularization and collagen fibrils were thin and scarce on day 14 (Fig. 3a). Similarly, Barbalho *et al*. (2012) and Singh *et al*. (2016) observed that there was hyperplasia of the epidermis and granulation tissue was visible between the divided dermis in and mild collagen fibrils observed up to day 14 in Povidone iodine treated animals.

In animals of group II, the intense inflammatory reaction was dominant. There was beginning of formation of the demarcation line under the necrosed tissue. The cellular inflammatory reactions were seen. The demarcation line consisted of polymorph nuclear leukocytes. Epidermis was thickened and mitotic activity was seen in the stratum basale on day 0 (Fig. 1b). Granulation tissue was seen on day 7 (Fig. 2b) with neovascularization and more dense collagen fibrils than group I. Thick collagen fibrils along with fibrobalst and fibrocytes were observed. Inflammatory cells were not observed on day 14 (Fig. 3b). Paul *et al*. (2017) reported similar findings of wound healing activity of Marigold and Turmeric paste in wounds of Bengal goat.

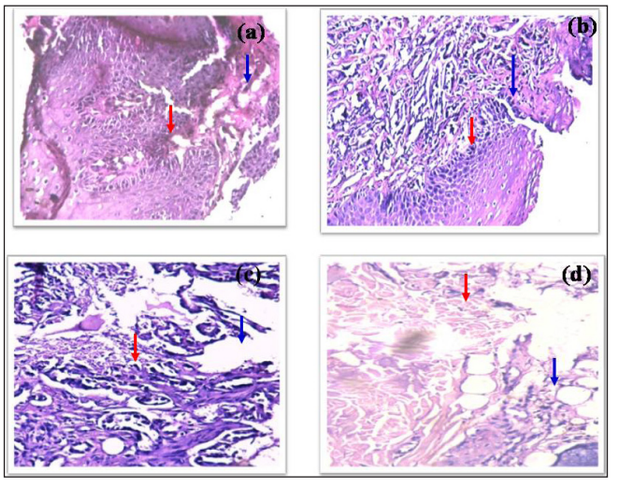

Fig. 1: Photomicrograph of skin tissue on day 0 (H&E × 100) showing breach in the continuity of the epithelium (→) and presence of inflammatory cells in (→) (a) Group I; (b) Group II; (c) Group III and (d) Group IV

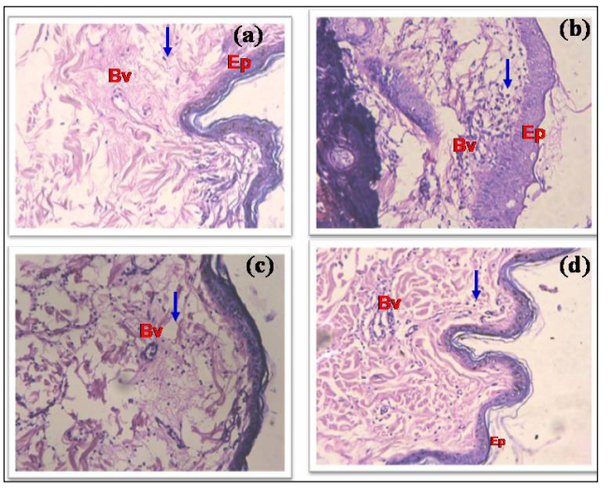

Fig. 2: Photomicrograph of skin tissue on day 7 (H&E × 100) showing few to numerous inflammatory cells (→), neovascularization (Bv) and epithelialization (Ep) in groups (a) Group I; (b) Group II; (c) Group III and (d) Group IV

In animals of group III, there was breach in the continuity of the cells and infiltration of inflammatory cells on day 0 (Fig. 1c) and less number of inflammatory cells with more fibrocytes or fibroblast were seen. Collagen fibrils were

thicker and densely placed than group II on day 7 (Fig. 2c). The collagen fibrils were moderate, thick arranged in bundles. Inflammatory cells were not evident. Fibrocytes were seen along with collagen fibres on day 14 (Fig. 3c, Fig. 4c). Similarly, mild to moderate collagen fibres and faster wound healing up to day 14 was reported by Fernandes *et al*. (2010) in Wistar rat wound model treated with Guava extract.

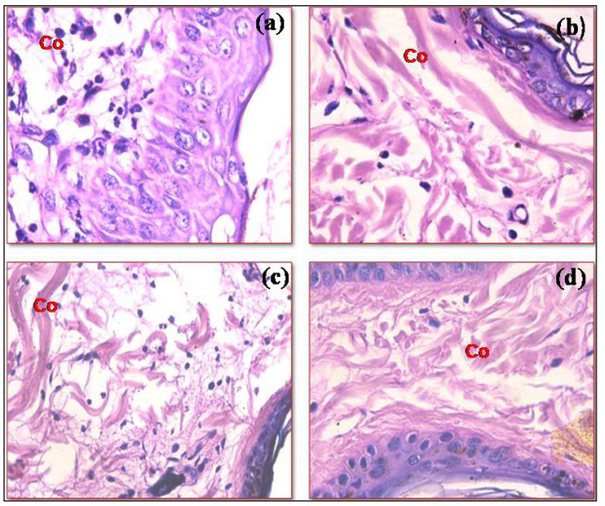

Fig. 3: Photomicrograph of skin tissue on day 14 (H&E × 400) showing loose to densely arranged collagen fibres (Co) in (a) Group I; (b) Group II; (c) Group III and (d) Group IV

In animals of group IV, there was breach in the continuity of the epidermis of skin with leukocytes infiltration on day 0 (Fig. 1d). Marked re-epithelialization and moderate inflammatory cells, neovascularization and thicker but scattered collagen fibrils were seen on day 7 (Fig. 2d). Mature collagen fibres were observed with least inflammatory cells. Fibrocytes and fibroblast were evident on day 14 (Fig. 3d, Fig. 4d). Nagar *et al*. (2016) carried out histological evaluation of *Cestrum noctrum* ointment in wound of Wistar albino rats and found prominently increased fibroblast cells, blood vessels and well organized collagen fibres on day 16. These findings were in accordance with the findings of Das (2013); Nilugal *et al.* (2014) in experimental Wistar rat wound model. Umachigi *et al.* (2009) also observed that polyherbal formulation was better than other groups and showed higher collagen fibres and fibroblast on day 16 in experimental animals. In group IV, histological study showed better result than other three groups. It may be due to the mixed herbal ointment having synergistic effect of different phytochemicals which decreased exudation and inflammation earlier and promoted wound healing.

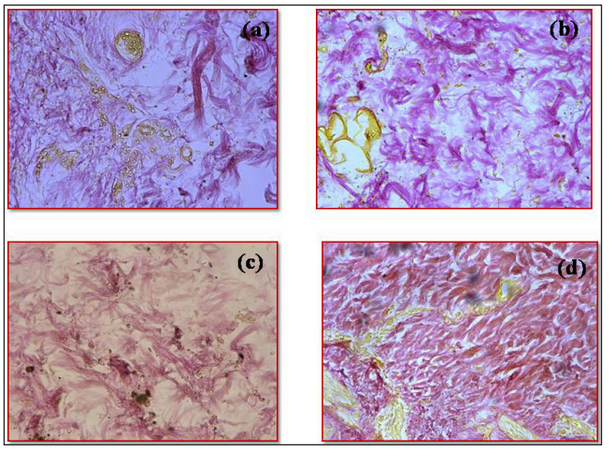

Fig. 4: Photomicrograph of granulation tissue on day 14 (Van Gieson's × 100) showing (a) in group I, thin collagen fibres (Co); (b) mild to moderate collagen fibres (Co); (c) moderate collagen fibres (Co) and (d) mature type of collagen fibres (Co)

CONCLUSION

Ointment of Marigold leaves extract (10% w/w), Guava leaves extract (10% w/w) and 1:1 mixture of Marigold and Guava leaves extract (10% w/w) were more effective for wound healing in clinical cases without any adverse effect as compared to Povidone iodine (5%). Further, the group IV (mixture of Marigold and Guava leaves hydroalcoholic extract) revealed the best wound healing properties as compared to group I (Povidone iodine 5.0%), group II (Marigold leaves hydroalcoholic extract) and group III (Guava leaves hydroalcoholic extra).

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