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Research Article

CLINICAL WOUND HEALING ACTIVITY OF *ALOE VERA* GEL IN DOGS

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ABSTRACT

Aloe vera is a very important and effective plant with multiple health applications and barely any part of human or animal body remain uninfluenced by its healing medicinal use. It acts as a natural fighter against all classes of infection. Considering the major property of *Aloe vera* plant and their easy availability, *Aloe vera* plant leaves were selected to assess their dermal safety in rats and clinical wound healing activity in dogs. In the acute dermal toxicity, no evidence of erythema and oedema were noted in any of the wistar rats exposed to 5% *Aloe vera* gel formulation. Even no sign of clinical and behaviour abnormalities was noted in the exposed rats. The clinical evaluation of 5% *Aloe vera* gel formulation on wound was carried in the dogs reported at Veterinary clinical complex. Twelve dogs with wound were divided into two groups, one applied with povidone iodine solution and other with *Aloe vera* gel. There were no significant changes observed in haematological, biochemical and in wound contraction in both the groups. On the basis of results, it is concluded that, *Aloe vera* gel was found safe for dermal application and accelerates the rate of percent wound contraction in cases of dogs.

Keywords: Acute dermal toxicity, *Aloe vera* gel, Wounds, Wound healing

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India's pet ownership statistics show that 60% of the population owns at least one pet, with dogs being the most popular choice. Number of pet dogs in 2023 was over 31 million, expected to reach 43 million by 2026. Although, due to their playful nature or jumping over the fences, fighting or being dragged across a rough surface, biting dogs often get wound (Elad, 2024).

Wound is considered as an interruption of cell, anatomical and functional continuity of a living tissue caused by physical, chemical, thermal, microbial or immunological harm to the body tissue. Clinical management of wound is one of the commonly encountered conditions among Veterinarians in their routine practice. Wound caused by various reasons such as accidental injuries, maggots, dog bites, etc. These wounds, based on the extent and cause can be detrimental to the dog to the extent of being life threatening and hence, faster recovery is of paramount importance. Wound if left untreated may lead to further complications such as secondary bacterial infections, pus formations, abscess development and maggot infestation, (Zinat *et al.*, 2020). In recent years, new herbal medications are developed by using medicinal plants as a primary source of drug development. India is making progress in the health care sector by advancing the traditional medical system of AYUSH (Ayurveda, Unani, Siddha and Homoeopathy) through international networks (Shakya, 2016). Plant based therapy not only accelerate wound healing process but also maintain the aesthetics. More than 70% of wound healing pharmaceutical products are plant based, 20% are mineral based and remaining containing animal products as their base material (Kumarasamyraja, 2012). There are more than 5000 species of plantmaterials shows promising pharmacological action. Among them Aloe vera has a long history as a medicinal plant and considered to be a more potent in wound healing (Saleem *et al.*, 2022).

MATERIALS AND METHODS

Fresh *Aloe vera* leaves were collected from surrounding areas of Vasantrao Naik Marathwada Krishi Vidyapith (VNMKV), Parbhani and were cleaned with fresh tap water and scraped off to collect pulp. This pulp was thoroughly mixed in an electric grinder and the 5% *Aloe vera* gel was prepared by using carbopol and 98% tri-ethanol amine.

Preparation of *Aloe vera* gel formulation:

Aloe vera gel formulation was prepared by using the 20 gm of carbopol (@ of 2% concentration), 900 ml of distilled water and 5 ml of tri-ethanolamine (@ 0.5% concentration).

In the procedure 20 gm of carbopol was mixed in the 900 ml of distilled water and kept the solution for 8-10 hrs for proper dissociation of carbopol in the distilled water. After 10 hrs, poured 5 ml of tri-ethanol amine drop by drop in the prepared mixture with continuously stirring and then added the 5% concentration of *Aloe vera* pulp in the

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prepared mixture and the volume of the prepared mixture was adjusted to 1000 ml by adding the required distilled water.

Acute dermal toxicity of *Aloe vera* gel in Wistar rats:

The acute dermal toxicity was evaluated in wistar rats of 250-300 gm weight. The Institutional Animal Ethical Committee (IAEC) approval was accorded with the resolution number IAEC/127/23 dated on 15/12/23. All the rats were maintained as per the CCSEA guidelines. In the safety evaluation the prepared *Aloe vera* gel was applied on the dorsal skin areas of rats and were monitor for the skin lesions, behavioural patterns, adverse clinical signs, mortality, body weight and feed intake up to 14 days. Evaluation of skin reaction is observed by erythema and edema formation by using Draize criteria (Yonezawa *et al.*, 2015). At the end of the study the rats were sacrificed for necropsy and histopathology of vital organs.

Table 1. Scoring pattern for erythema and edema according to Draize criteria

Parameter	No. of Rats	Observation period (hrs)		
		24 hrs	48 hrs	72 hrs
Erythema	Rat 1	0	0	0
	Rat 2	0	0	0
Edema	Rat 1	0	0	0
	Rat 2	0	0	0

Table 2. Mean values of wound contraction (%) in group A and group B

Groups	Days interval					F cal	CoV
	0^{th}	7^{th}	14^{th}	21^{st}	28^{th}		
Group A	$0^{d} \pm 0$	$37.71^{c} \pm 3.15$	$75.9^{b} \pm 4.53$	$93.09^{a} \pm 2.9$	$99.7^{a} \pm 0.21$	257.35	9.73
Group B	$0^{d} \pm 0$	$41.2^{c} \pm 3.33$	$79.7^{b} \pm 4.13$	$98.08^{a} \pm 0.8$	$100^{a} \pm 0$		

Treatments found to be significantly different at 1% and 5% level, CD (0.01) = 9.408, CD (0.05) = 7.057

Table 3. Haematological analysis at different intervals in group A and group B

Parameters	Groups	Days interval		F cal	CoV
		Day 0	Day 14		
Haemoglobin (gm/dl)	Group A	15.06 ± 0.53	15.14 ± 0.52	1.54	8.37
	Group B	13.96± 0.45	14.11 ± 0.47		
Packed cell volume (%)	Group A	43.88 ± 1.04	44.56 ± 1.11	1.002	7.06
	Group B	45.66 ± 1.44	46.86 ± 1.54		
Red Blood Cells ($\times 10^{6}$/cu.mm)	Group A	6.25 ± 0.26	6.35 ± 0.26	2.47	9.99
	Group B	6.89 ± 0.29	7.14 ± 0.25		
White Blood Cells ($\times 10^{3}$/cu.mm)	Group A	10.29 ± 0.81	10.31 ± 0.81	1.22	17.18
	Group B	8.95 ± 0.51	9.06 ± 0.49		

Table 4. Biochemical analysis at different intervals in the group A and group B

Parameters	Groups	Days interval		F cal	CoV
		Day 0	Day 14		
Aspartate aminotransferase (IU/L)	Group A	24.30 ± 2.72	23.65 ± 2.57	0.11	35.43
	Group B	26.43 ± 4.41	25.10 ± 4.45		
Alanine transaminase (IU/L)	Group A	36.33 ± 2.52	34.67 ± 2.44	2.43	21.48
	Group B	29.83 ± 3.27	26.83 ± 2.89		
Total Protein (mg/dl)	Group A	6.36 ± 0.21	6.21 ± 0.21	1.50	8.32
	Group B	6.11 ± 0.22	5.76 ± 0.19		
Blood Urea Nitrogen (mg/dl)	Group A	17.27 ± 1.13	17.16 ± 1.13	0.006	21.71
	Group B	17.45 ± 7.44	17.23 ± 1.83		
Serum Creatinine (gm/dl)	Group A	0.89 ± 0.10	0.84 ± 0.10	1.04	25.5
	Group B	0.91 ± 0.06	0.71 ± 0.06		

Clinical wound healing evaluation of *Aloe vera* gel in dogs:

The clinical efficacy of 5% *Aloe vera* gel was evaluated in the dogs presented at the Veterinary Clinical Complex (VCC), COVAS, Parbhani. A total of 12 clinical cases of wound in dogs were equally divided into group A and group B for the evaluation of the *Aloe vera* gel formulation. Group A received, the standard treatment, involving the topical application of (7.5% w/v) povidone iodine twice daily until complete healing. Group B received, topical application of *Aloe vera* gel formulation (5% w/v) twice daily till complete recovery. The Visual parameters (wound swelling, wound colour, exudation from wound, pain sensation and wound irritation) and

percent wound contraction were observed and scored on day 0, 7 and 14 of healing. In addition, hematological (Hb, PCV, TEC, TLC and DLC) and biochemical (AST, ALT, TP, BUN and Serum creatinine) parameters were analyzed on day 0 and day 14. Blood samples were collected from the cephalic vein of dogs using EDTA vials on both days for both treatment groups.

Statistical analysis:

Statistical analysis was carried out by using Web Agri Stat Package version 2.0 (WASP 2). All the parameters were analyzed by using completely randomized design (CRD) statistical method.

RESULTS AND DISCUSSION

Acute Dermal toxicity:

There were no adverse skin reactions (erythema and edema) was observed in rats applied with that *Aloe vera* gel during after 24, 48 or 72 hours (Table 1). Score for erythema and edema formation of skin was recorded zero in the rats according to Draize criteria, 1959 (Yonezawa *et al.*, 2015).

Based on observations, it was found that *Aloe vera* gel did not show any sign of dermal lesion on exposed skin area of rats @2000 mg/kg body weight. Even no any adverse behavioural patterns, clinical signs, mortality, body weight and feed intake were observed in the exposed rats. There were no histo-architectural alterations observed in collected organ/tissue in the exposed rats. Acute dermal toxicity study of *Aloe vera* gel formulation revealed that the 5% *Aloe vera* gel was safe to use topically.

Evaluation of clinical wound healing efficacy of *Aloe vera* gel in dogs

Visual observations of wounds:

Clinical wound healing efficacy of *Aloe vera* gel in dogs were evaluated by visual observations of wound swelling, pain, irritation, exudation, change in wound colour on weekly basis till complete wound healing in both group A and B. Quality of wound healing was measured as percent wound area contraction.

The percent wound contraction in group A at 0, 7th, 14th, 21st and 28th days were 0d, $37.71^{c} \pm 3.15$, $75.9^{b} \pm 4.53$, $93.09^{a} \pm 2.9$, $99.7^{a} \pm 0.21$, respectively. In group B it was 0d, $41.2^{c} \pm 3.33$, $79.7^{b} \pm 4.13$, $98.08^{a} \pm 0.8$, $100^{a} \pm 0$, respectively.

In present investigation, wounds in group B, showed non significantly faster wound contraction compared to group A from 7th to 28th day (Table 2). Results of visual wound parameters and percent wound contraction suggested that *Aloe vera* gel formulation shown better wound healing activity than povidone iodine in dogs. Similarly, Attah *et al.* (2016) and Kayode (2017) reported that 5% of *Aloe vera* gel shown greater wound contraction rate. Yadav (2012) reported that, the increased wound contraction in Aloe vera gel treatment may be due to active elements like vitamins, minerals, glycoproteins, growth factors and enzymes.

Haematological analysis

Mean haematological parameters such as haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC) and white blood cells (WBC) in group A and B were analysed on day 0 and 14 and recorded in (Table 3).

The haemoglobin count in group A was 15.06 ± 0.53 and 13.96 ± 0.45 in group B on 0 day and get increased to 15.14 ± 0.52 and 14.11 ± 0.47 on 14 days, respectively. The PCV, RBC and WBC count in group A and B were 43.88 ± 1.04, 45.66 ± 1.44; 6.25 ± 0.26, 6.89 ± 0.29 and 10.29 ± 0.81, 8.95 ± 0.51, respectively on 0 day of reporting at VCC campus, but there was increased in the PCV, RBC and WBC count were 44.56 ± 1.11, 46.86 ± 1.54; 6.35 ± 0.26, 7.14 ± 0.25 and 10.31 ± 0.81, 9.06 ± 0.49, respectively on 14 days of *Aloe vera* application.

The study found non-significant increase in haemoglobin values in both the groups on 14 days. However, Ani (2018) found that after oral administration of 500 mg/kg *Aloe vera* gel in anaemic rats, haemoglobin values was increased and they confirmed that, it might be due to presence of antioxidants, vitamins and iron in *Aloe vera* and their resultant role in haemopoietic process. There were non-significant increases in packed cell volumes after 14 days was observed in both the groups. Ekanade (2015), found a similar significant increase in PCV and RBC in rats administered with *Aloe vera* extract for 24 hours. They concluded that, this increased PCV count attributed to stimulation of haematopoiesis. Study found no significant increase in total erythrocyte count in both the groups on 14 days, similar observations were noted by Iji (2010), Udefa (2018) and Nku (2018). Similar non significantly increased total leukocyte count were observed in *Aloe vera* treatment group by Perveen (2023) and Nku (2018) indicates the activation of defence mechanisms and immune system in infection and wound cases.

Biochemical analysis

Biochemical parameters include aspartate amino-transferase, alanine transaminase, total protein, blood urea nitrogen and serum creatinine in group A and B were evaluated on day 0 and day 14 and mentioned in Table 3.

In group A and B the aspartate aminotransferase, alanine transaminase, total protein, blood urea nitrogen

and serum creatinine values were 24.30 ± 2.72, 26.43 ± 4.41; 36.33 ± 2.52, 29.83 ± 3.27; 6.36 ± 0.21, 6.11 ± 0.22; 17.27 ± 1.13, 17.45 ± 7.44; and 0.89 ± 0.10, 0.91 ± 0.06 on 0 day, respectively, however these values were get reduced to 23.65 ± 2.57, 25.10 ± 4.45; 34.67 ± 2.44, 26.83 ± 2.89; 6.21 ± 0.21, 5.76 ± 0.19; 17.16 ± 1.13, 17.23 ± 1.83; and 0.84 ± 0.10, 0.71 ± 0.06, respectively on 14 days.

There was non-significant decrease in aspartate aminotransferase levels was observed in both the groups on 14 days of *Aloe vera* gel application. Madhav (2011) and Sharma (2013) also reported the similar observations in the diabetic rats treated with *Aloe vera* gel. There were also non-significantly decreased alanine transaminase values in both the groups on day 14 values. Similarly, Iji *et al*. (2010) found a significant reduction in alanine transaminase value in diabetic rats after oral administration of *Aloe vera* gel at 100, 250 and 500 mg/kg concentrations for four weeks. Even, there were non-significant decrease in total protein values were recorded by Chatterjee (2012), after administration of aqueous leaf extract of *Aloe vera* gel in gentamicin-induced nephrotoxic rats. In both the groups there was non-significant changes in blood urea nitrogen on 0 day and 14 dayswere observed, Iji (2010) and Chatterjee (2012) also noticed the similarly observations. Even, there were non-significant decrease in serum creatinine values were observed which was confirmed by Iftikhar (2015), who found similarly non significant decrease in serum creatinine values treated with *Aloe vera* gel at 200, 400 and 600 mg/kg in diclofenac induced nephrotoxicity in rabbits at different for 15 days.

CONCLUSION

Based on the observations of the present study, it can be concluded that the prepared 5% *Aloe vera* gel formulation found to be safe in rats exposed to acute dermal toxicity. In clinical cases of dogs, the wound healing activity (in terms of wound contraction) was higher in the *Aloe vera* gel applied group as compared to the routine treatment with povidone group. Overall, Aloe vera gel formulation did not exhibit any adverse reactions and might be considered as a safer and possibly the alternative for treating wounds.

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