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## Evaluation of wound healing activity of *Annona squamosa* and cow urine ark on contaminated wounds of dogs

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#### Abstract

The wound healing activity of *Annona squamosa* and cow urine ark was evaluated on contaminated wounds of dogs. The granulation tissue score and contraction of wound area were studied by Visual Analog Score card. Clinically, the granulation tissue score and contraction of wound area was similar in povidone iodine and aqueous extract ointment of *Annona squamosa* treated group followed by fresh leaves paste of *Annona squamosa* treated group. However, the granulation of tissue and contraction of wound area were very less observed in cow urine ark treated group.

The mean per cent healing of different treatment groups was 47.28, 49.25, 25.75 and 36.69 per cent for povidone iodine, 10 per cent ointment of *Annona squamosa*, cow urine ark and fresh leaves paste of *Annona squamosa* respectively on day 7 of treatment. The result showed non-significant difference between group 1 and 2 which were treated with povidone iodine and 10 per cent ointment of *Annona squamosa* respectively. However the mean per cent healing of cow urine ark treated wounds was significantly lower than other treatment groups.

**Keywords:** *Annona squamosa*, cow urine ark, granulation tissue score, contraction of wound area, wound healing

#### Introduction

In various indigenous and traditional sources of medicine, plants have been extensively used for treatment. Various parts of plants such as leaves, fruits, bark, root and even the seeds are being used for preparation of medicine. *Annona squamosa* commonly known as "sitaphal" is also been extensively used as traditional medicine in various culture. The genus name, 'Annona' is from the Latin word 'anon', meaning 'yearly produce', referring to the production of fruits of the various species in this genus. The leaves of the plants have been used as insecticide, anthelmintic, styptic and externally used as suppurant (Saha, 2011) [6]. The previous phytochemical investigations made on the plant have proved that they possess a wide variety of compounds like acetogenins which were responsible for anti-feedant, anti-malarial, cytotoxic and the immunosuppressive activities. Diterpenes isolated from the *Annona squamosa* possess the anti-HIV principle and the anti-platelet aggregation activity. The partially purified flavonoids were reported from the same source as the responsible agent for the anti-microbial and other pesticidal activities. Some lignans and other hydroxyl ketones were also found to be present in this plant. The number of alkaloids that were reported from this plant belongs to different categories such as aporphine and benzoquinazoline. The above provided evidences suggested that the plant is known for its various medicinal values (Gajalakshmi *et al.*, 2011) [1].

Cows were regarded as wealth and were the backbone of the economy of ancient Indians. Cattle husbandry was well developed during the Rigvedic period (1500–1000 BC) and the cow (Kamadhenu) was adored and considered the 'best wealth' of mankind. In Sushrut Samhita, several medicinal properties of cow's urine have been mentioned and cow urine was known to cause weight loss and to cure leprosy, cardiac and kidney problems, indigestion, stomach ache, edema etc. This kind of alternative treatment is termed as 'panchgavya therapy' or 'cowpathy' (Kaviratna and Sharma, 1996) [3]. Cow urine based preparations possess powerful antimicrobial, antiviral, antiallergic and antioxidant activities (Shivkumar *et al.*, 2011) [10].

Thus, the present research work was planned to evaluate the wound healing activity of *Annona squamosa* and cow urine ark on contaminated wounds of dogs.

**Material and Methods****Location and place of work**

The proposed work was conducted in the Department of Veterinary Pharmacology and Toxicology in collaboration with Department of Veterinary Microbiology and Teaching Veterinary Clinical Complex (T.V.C.C), College of Veterinary Science and Animal Husbandry, N.D.V.S.U., Jabalpur (M.P).

**Selection of animals**

The dogs presented with contaminated wound at TVCC, College of Veterinary Science and Animal Husbandry, Jabalpur were selected for the present study.

**Experimental material****Preparation of plant extract and ointment**

The fresh leaves of *Annona squamosa* were collected from Department of Botany, J.N.K.V.V Jabalpur. Leaves were dried in shade, powdered by using grinder and the powder was used for preparation of aqueous extract as per method described by Tandle (1984). For preparation of aqueous extract, the plant powder was soaked in distilled water and kept for 48 hours and then filtered. The filtrate was evaporated on boiling water bath and after complete evaporation of water the extract was used for preparing 10 per cent ointment with soft yellow paraffin base. After extraction, per cent extractability was calculated.

**Preparation of ointment**

For the preparation of 10 per cent ointment w/w of *Annona squamosa*, 10 g of aqueous extract of leaves of *Annona squamosa* was mixed with 90 g of yellow petroleum jelly with the help of ointment slab and spatula.

**Preparation of paste of fresh leaves of *Annona squamosa***

The fresh leaves of *Annona squamosa* were collected from Department of Botany, J.N.K.V.V Jabalpur. Dust and dirt were removed with fresh water and paste was made by grinding the leaves in grinder. Paste was stored in refrigerator at 4 degree celcius for 7 days.

**Cow urine**

Distillated Cow urine i.e. Cow urine ark from Patanjali Ind Ltd, Haridwar was used in the present research work.

**Design of experiment**

Evaluation of wound healing activity of *Annona squamosa* and cow urine was done in four groups of dogs consisting six in each group:

**Group-1**

The wound was flushed with distilled water to remove dust and dirt. The wound was dressed with povidone iodine (5 per cent) for 7 consecutive days. Inj. Meloxicam @ 0.3 mg/kg b.wt I/M was administered twice daily for 5 days.

**Group-2**

The wound was flushed with distilled water to remove dust and dirt. The wound was dressed with (10 per cent) ointment of aqueous extract of leaves of *Annona squamosa* leaves for 7 consecutive days. Inj. Meloxicam @ 0.3 mg/kg b.wt I/M was administered twice daily for 5 days.

**Group-3**

The wound was flushed with distilled water to remove dust and dirt. The wound was dressed with cow urine ark for 7 consecutive days. Inj. Meloxicam @ 0.3 mg/kg b.wt I/M was administered twice daily for 5 days.

**Group-4**

The wound was flushed with distilled water to remove dust and dirt. The wound was dressed with fresh leaves paste of *Annona squamosa* for 7 consecutive days. Inj. Meloxicam @ 0.3 mg/kg b.wt I/M was administered twice daily for 5 days.

**Parameters of study****Clinical observation**

Wounds were observed for gross appearance on day 0 (pre-treatment), day 3 and day 7 post treatment. The following parameters were studied:

- Granulation tissue score
- Contraction of wound area

All the above parameters were studied by using VAS (Visual Analog Score) card.

- Nil = -
- Mild = +
- Moderate = ++
- Severe = +++

(Singh, 2007)<sup>[11]</sup>.

**Percentage of wound healing**

The wound boundaries were marked with Indian ink and tracings were taken on cellophane paper before starting the treatment and subsequently on day 3 and 7 post treatment. These tracings were placed on a graph paper and percentage healing was calculated by the method described by Kumar and Tyagi (1972)<sup>[4]</sup>.

$$H \text{ (per cent)} = \frac{A - B}{A} \times 100$$

H (per cent) = Percentage of healing

A = Area of wound at the beginning of particular period

B = Area of wound at the end of particular period

**Statistical analysis**

Research data were analyzed using analysis of variance (ANOVA) statistical method as described by Snedecor and Cochran (1994)<sup>[12]</sup>.

**Results and Discussion****Evaluation of wound healing activity**

The wound healing activity of *Annona squamosa* and cow urine ark was evaluated on contaminated wounds of dogs. The study was under taken in 4 groups of dogs consisting 6 in each group. The wound healing activity was evaluated on the basis of clinical observation that is granulation tissue score, contraction of wound area and rate of healing. Singh (2007)<sup>[11]</sup>, and Sharma (2018)<sup>[8]</sup>, assessed the wound healing activity of different plant preparation on the basis of granulation tissue score, contraction of wound area and per cent wound healing activity.

**Group 1**

The wounds were flushed with distilled water and dressed with 5 per cent povidone iodine for 7 consecutive days.

**Clinical observation**

On clinical observation the granulation tissue formation was observed on day 3 which was increased on day 7 of treatment. However, the contraction of wound area was observed on day 3 and it was and further reduced on day 7 (Table 01).

**Rate of healing**

The size of wound area was measured by tracing wound boundaries on a cellophane paper which was recorded on day 0 (pre-treatment), day 3 and day 7 (post treatment). On the basis of wound area, the per cent healing activity of 5 per cent povidone iodine has been depicted in Table 02. The result indicated that 5 per cent povidone iodine exhibited 35.34 and 59.23 per cent healing activity on day 3 and day 7 respectively. The mean per cent healing activity of group 1 on different days was 47.28 with the standard error 3.78 (Table 02). The mean per cent healing of povidone iodine treated group showed non-significant difference from aqueous extract ointment of *Annona squamosa* Treated group (Plate 01).

The result of present study are in agreement with the findings of Hananeh *et al.* (2015)<sup>[2]</sup>. determine the wound healing activity of povidone iodine in contaminated wounds of dogs and excisional wounds of rats respectively.

**Group 2**

The wounds were flushed with distilled water and dressed with 10 per cent ointment of aqueous extract of *Annona squamosa* for 7 consecutive days.

**Clinical observation**

On clinical observation the granulation tissue formation was observed on day 3 which was increased on day 7 of treatment. However, the contraction of wound area was observed on day 3 and it was further reduced on day 7 (Table 01).

**Rate of healing**

The size of wound area was measured by tracing wound boundaries on a cellophane paper which was recorded on day 0 (pre-treatment), day 3 and day 7 (post treatment). On the basis of wound area, the per cent healing activity of 10 per cent ointment of aqueous extract of *Annona squamosa* has been depicted in Table 02. The result indicated that 10 per cent ointment of aqueous extract of *Annona squamosa* exhibited 34.52 and 62.01 per cent healing activity on day 3 and day 7 respectively. The mean per cent healing activity of group 2 on different days was 49.25 with the standard error 5.25 (Table 02). The mean per cent healing of aqueous extract ointment of *Annona squamosa* treated group showed non-significant difference from povidone iodine treated group but it was significantly higher than cow urine ark and fresh leaves paste of *Annona squamosa* treated group (Plate 02).

The findings of present research are in agreement with Shenoy *et al.* (2009)<sup>[9]</sup>. However, Ponrasu and Suguna (2014)<sup>[5]</sup>. evaluated wound healing efficacy of *Annona squamosa* in streptozotocin-induced diabetic rats.

**Group 3**

The wounds were flushed with distilled water and dressed with Cow urine ark for 7 consecutive days.

**Clinical observation**

On clinical observation the granulation tissue formation was not observed on day 3 but it appeared on day 7 of treatment. However, the contraction of wound area was observed on day 3 and it was same on day 7 also (Table 01).

**Rate of healing**

The size of wound area was measured by tracing wound boundaries on a cellophane paper which was recorded on day 0 (pre-treatment), day 3 and day 7 (post treatment). On the basis of wound area, the per cent healing activity of cow urine ark has been depicted in Table 02. The result indicated that cow urine ark exhibited 17.52 and 33.99 per cent healing activity on day 3 and day 7 respectively which was negligible in comparison to rest of the groups. The mean per cent healing activity of group 3 on different days was 25.75 with the standard error 3.10 (Table 02). The mean per cent healing of cow urine ark treated group was significantly lowest among all treated groups (Plate 03).

Sanganal *et al.* (2011)<sup>[7]</sup>. observed that the application of cow urine on excision wounds of albino rats for 14 days causes significant decrease in wound area.

**Group 4**

The wounds were flushed with distilled water and dressed with fresh leaves paste of *Annona squamosa* for 7 consecutive days.

**Clinical observation**

On clinical observation the granulation tissue formation was observed on day 3 and it was almost same on day 7 of treatment also. However, the contraction of wound area was observed on day 3 and it was further reduced on day 7 of treatment (Table 01).

**Rate of healing**

The size of wound area was measured by tracing wound boundaries on a cellophane paper which was recorded on day 0 (pre-treatment), day 3 and day 7 (post treatment). On the basis of wound area, the per cent healing activity of fresh leaves paste of *Annona squamosa* has been depicted in Table 02. The result indicated that fresh leaves paste of *Annona squamosa* exhibited 28.36 and 45.02 per cent healing activity on day 3 and day 7 respectively. The mean per cent healing activity of group 4 on different days was 36.69 with the standard error 2.84 (Table 02). The mean per cent healing of fresh leaves paste of *Annona squamosa* treated group showed significant difference from group 1 and group 2 but the difference was not significant from cow urine ark treated group (Plate 04).

In the present study fresh leaves paste of *Annona squamosa* was evaluated for its wound healing activity. However, most of the previous research indicated use of ointment prepared with different solvent extracts of leaves or seeds of *Annona squamosa*.

**Table 1:** Clinical observation of wound healing activity of *Annona squamosa* and cow urine ark

Group	Treatment	Granulation tissue			Contraction of wound area		
		Day 0	Day 3	Day 7	Day 0	Day 3	Day 7
G-1	Povidone iodine + Inj meloxicam	-	+	++	-	+	++
G-2	10 % Ointment of aqueous extract of <i>Annona squamosa</i> + Inj meloxicam	-	+	++	-	+	+++
G-3	Cow urine ark + Inj meloxicam	-	-	+	-	+	+
G-4	Fresh leaves paste of <i>Annona squamosa</i> + Inj meloxicam	-	+	+	-	+	++

Visual Analog Score: - Nil, + Mild, ++ Moderate and +++ Severe

**Table 2:** Percent wound healing activity of *Annona squamosa* and cow urine ark on contaminated wounds of dogs

Group	Treatment	Per cent healing activity		Mean Per cent healing activity
		Day 3	Day 7	
G-1	Povidone iodine + Inj meloxicam	35.34	59.23	47.28 <sup>ab</sup> ± 3.78
G-2	10 per cent Ointment of <i>Annona squamosa</i> + Inj meloxicam	34.52	62.01	49.25 <sup>a</sup> ± 5.25
G-3	Cow urine ark + Inj meloxicam	17.52	33.99	25.75 <sup>c</sup> ± 3.10
G-4	Fresh leaves paste of <i>Annona squamosa</i> + Inj meloxicam	28.36	45.02	36.69 <sup>bc</sup> ± 2.84

\*Similar superscripts indicates non-significant difference between treatment ( $p \leq 0.05$ )

**Note:** Each group consist 6 animals.



**Plate 1:** Wound healing efficacy of povidone iodine (a) day 0 (b) day 3 (c) day 5 (d) day 7 (e) day 10



**Plate 2:** Wound healing efficacy of aqueous extract ointment of *Annona squamosa* (a) day 0 (b) day 3 (c) day 5 (d) day 7 (e) day 12



**Plate 3:** Wound healing efficacy of cow urine ark (a) day 0 (b) day 3 (c) day 5 (d) day 7



**Plate 4:** Wound healing efficacy of fresh leaves paste of *Annona squamosa* (a) day 0 (b) day 3 (c) day 5 (d) day 7

### Conclusion

The mean per cent healing activity of aqueous extract ointment of *Annona squamosa* was maximum followed by povidone iodine, fresh leaves paste of *Annona squamosa* and cow urine ark.

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