

## EVALUATION OF WOUND HEALING ACTIVITY OF METHANOLIC EXTRACT OF *BALANITES AEGYPTIACA* L. LEAVES

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### **ABSTRACT**

*Balanites aegyptiaca* L. belongs to the family *Zygophyllaceae*. It is mainly found on deep sandy loam and free access to water. It is a folkloric medicine for a variety of ailments including purgative, jaundice, syphilis, epilepsy, anthelmintic and antimalarial etc. Excision wound model was assessed for wound healing activity. The rats were depilated on back by cutting away 500 mm<sup>2</sup> full thickness of skin on ethanol sterilized dorsal thoracic region of rats. Group I served as control and received simple ointment base topically. Group II served as standard and treated externally with Povidone iodine ointment. Group III treated with methanolic extract of the *Balanites aegyptiaca* Ointment. The ointment was topically applied daily till the complete epithelialization starting from the day of operation. The parameters studied were wound closure and time of epithelialization. The wounds were traced on mm<sup>2</sup> graph paper on the days of 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup> and 16<sup>th</sup> and thereafter on alternate days until healing were complete. The present work indicates that the methanolic extract of *Balanites aegyptiaca* L. leaves extract shows significant wound healing activity. Acute toxicity studies were conducted for the methanolic extract of *Balanites aegyptiaca* leaves. The maximum tolerated dose was found to be 2000 mg/kg b.w when the extract was administered orally.

**Keywords:** *Balanites aegyptiaca* L., *Zygophyllaceae*; Povidone iodine ointment; Wound healing.

### **INTRODUCTION**

In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have some or other side effects, whereas plants still hold their own unique place, by the way of having no side effects. *Balanites aegyptiaca* L. (*Zygophyllaceae*) is a small evergreen thorny tree found in drier parts of India[1]. It also known as 'desert date'. Synonyms of the plant include *Agialida senegalensis* van Tiegh, *Agialida tombuctensis* van Tiegh, *Balanites ziziphoides* Milbr. Et Schlechter. It is multibranched, spiny shrub or tree can grow to 6-10 meters in height, is highly resistant to stresses such as sand storms and heat waves, and grows with minimal available moisture, widely distributed in dry land areas of Africa and South Asia. This tree is native to Africa. Within India, it is found in Rajasthan, Gujarat, Madhya Pradesh, and Deccan[2]. The tree has thick, tough glossy leaves, spiny branches, double route system, and produces date like fruits. The tree begins to flower and fruit at 5 to 7 years of age. It is distributed by cultivation and naturalization. It contains proteins, lipids, carbohydrates, alkaloids, saponins, flavonoids, and organic acid. In folk medicine, *Balanites aegyptiaca* is used to treat skin disease, tape worm, jaundice, wounds, malaria, syphilis, epilepsy, dysentery, constipation, diarrhea, hemorrhoid, stomach aches, asthma, and fever[3]. No wound healing activity has been reported on the leaves. Hence a systematic pharmacological investigation of methanolic extract of leaves of *Balanites aegyptiaca* was selected based on the traditional use.

### **MATERIALS AND METHODS**

#### **Collection and authentication of plant material**

The leaves of *Balanites aegyptiaca* L. were collected from local area of Vangapally village of Nalgonda District, Andhra Pradesh in November 2011 and were authenticated by Dr. S. Srinivas Rao, Dept of Botany, S.L.N.S Degree and PG College, Bhongir, Andhra Pradesh, India.

#### **Preparation of the leaves extract**

The leaves were washed with fresh water to remove adhering dirt and foreign particles and are washed with absolute ethanol to avoid the microbial growth, and were dried under the shade. The dried leaves were crushed and grinded to get powder and weighed. The powdered material of leaves of *Balanites aegyptiaca* L. was refluxed successively with methanol in a Soxhlet extractor for 72 hrs. The solution so obtained was transferred to china dish and then allowed

for drying. The extract so obtained was thoroughly washed with Ethyl acetate so as to remove the chlorophyll and was dried kept in a desiccator for further use.

#### **Ointment preparation for topical application**

An alcohol free extract of *Balanites aegyptiaca* leaf was used for the preparation of the ointment for topical application. A 0.2 % (W/W) of extract ointment was formulated using soft paraffin base.

#### **Experimental animals**

Albino rats (Wistar) weighing 150-200 g either sex were used in this study. They were housed in polypropylene cages under standard laboratory conditions (12-h light/ 12-h dark cycle, 21 ± 2 °C, and relative humidity 55 %). The animals were given standard rodent pellets and tap water *ad libitum*. The rats were acclimatized to laboratory condition for 7 days before commencement of experiment. Ethical clearance for handling the animals is obtained from the Institutional animal ethical committee prior to the beginning of the project work from Institutional Animal Ethical Committee (IAEC) of SASTRA University, Thanjavur, and Tamilnadu. The experiments were conducted as per the guidelines of CPCSEA, Chennai, India. (Approval no: 86 / SASTRA / IAEC / RPP).

#### **Excision wound model**

Three groups of six animals in each group were anesthetized by open mask method with anesthetic ether[4]. The rats were depilated on back. One excision wound was inflicted by cutting away 500 mm<sup>2</sup> full thickness of skin on ethanol sterilized dorsal thoracic region of rats. The wound was left undressed to the open environment. This model was used to monitor wound contraction[5].

Group I: Served as control and received simple ointment base topically.

Group II: Served as standard and treated externally with 0.2 % w/w Povidone iodine ointment.

Group III: Treated with methanolic extract of the *Balanites aegyptiaca* Ointment.

The ointment was topically applied daily till the complete epithelialization starting from the day of operation. The parameters studied were wound closure and time of epithelialization. The wounds were traced on mm<sup>2</sup> graph paper on the days of 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup>

and 16<sup>th</sup> and thereafter on alternate days until healing were complete. The percentage of wound closure was calculated. The number of days required for falling of the scar without any residual of the raw wound gave the period of epithelialization[6].

#### Acute toxicity studies as per OECD Guideline 425

In the assessment and evaluation of the toxic characters of the substance, determination of acute oral toxicity is usually an initial step[7]. LD (medium lethal 50 dose), oral, is a statistically derived single dose of a substance that can be expected to cause death in 50 % of animals when administered by the oral route. The LD value 50 expressed in terms of test substance per unit weight of test animal (mg/kg). Up and down (UDP, stair case method) was performed for acute toxicity. In this method animals of a single sex, usually females, with the first animal receiving a dose just below the best estimate of the LD<sub>50</sub>. Depending on the outcome for the previous animal, the dose for the next is increased or decreased, usually by the factor of 3.2.

Healthy Wistar rats weighing between 150-180 g were used to carry out acute toxicity studies by the 'staircase' method. Methanolic extract of *Balanites aegyptiaca* in 0.5 % tween 80 was administered orally *Balanites aegyptiaca* in 0.5 % tween 80 was administered orally to three animals in each group. Animals were observed

individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days to check the mortality rate (**OECD 425, 2001**).

#### Statistical analysis

The results were subjected to statistical analysis by using ANOVA followed by Turkey Krammer multiple comparison test. The values are expressed as Mean ± SEM, n=6 in each group.

#### RESULTS

The preliminary phytochemical investigation of the plant extract showed the presence of glycosides, flavonoids, tannins and phenolic compounds, steroids and saponins. In excision wound model the potency of wound healing activity of the plant was found to be highly significant. Excision wound showed that there is almost complete healing on the 16<sup>th</sup> post wounding day with methanolic extract. The topical application of *Balanites aegyptiaca* ointment increased the percentage of wound contraction and this indicates rapid epithelialization. The administration of this extract *Balanites aegyptiaca* accelerated the progression of wound healing by 16<sup>th</sup> day i.e. (95.42+0.47 %) compared with control (86.65+ 0.84 %) in Table 1.

**Table 1: Wound healing activity of BAME on rats by Excision wound model**

Groups	4 <sup>th</sup> day	8 <sup>th</sup> day	12 <sup>th</sup> day	16 <sup>th</sup> day	Epithelialization period (in days)
Control Simple ointment base	32.36 + 1.32	38.38 + 1.34	46.66 + 1.2	86.65 + 0.84	21.33+ 0.42
Povidone iodine ointment Std (0.2%)	43.34 + 0.55**	59.66 + 1.4**	79.14+ 0.92**	97.02+ 0.44**	13.66 + 0.33
BAME (0.2 %)	31.23 + 0.75**	54.49 + 1.1**	72.46+ 0.12**	95.42+ 0.47**	14.33 + 0.84

n=6, values are in mean + SEM, \*\*Significant p<0.001

#### DISCUSSION

Wound may be defined as break in the epithelial integrity of the skin or loss of functional continuity of living tissue[9]. Wound may occur in the form of burns, cuts or tissue grafting. Each type has its own mechanism of action in restoring the original skin texture[8]. A wound healing involves different phases such as contraction, granulation, epithelialization, and collagenation[10,11]. Phytoconstituents such as tannins and flavonoids are responsible for wound healing activity by their astringent and antimicrobial property. Excision wounds heal by contraction, epithelialization and the percentage of wound closure rate includes by recording the changes in wound are at regular intervals like 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup> and 16<sup>th</sup> day after treating with methanolic extract.

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