

## Evaluations of Wound Healing Activity of Herbal Gel Containing the Flower Extract of *Butea monosperma* Lam.

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### ABSTRACT

The back ground and aim: In spite of tremendous advances in the chemical drug industry, the availability of substances capable of stimulating the process of wound repair is still limited. The present work was aim to investigate wound healing efficiency of Herbal gel containing ethanolic and aqueous extract of flower of *Butea monosperma* (Lam.) on excision wound model and incision wound model.

Materials and methods: Excision wound measuring about 500 mm<sup>2</sup> was created on the albino rats placed in four groups (n=6) and the gel applied topically on the wounded area which was measured at interval of 3 days until epithelization and complete wound closure. Blank gel and Soframycin Framycetin sulphate cream (FSC) 1% w/w served as the control and standard treatment respectively. Treatment group received topical application of aqueous extract and ethanolic extract (10% w/w) gel.

Results: Topical application of aqueous extract gel on excision wound in rats caused a significant (P<0.01) higher rate of wound healing (98.43%) and reduced epithelization period. In incision wound model, aqueous extract gel significantly (P<0.01) increased the breaking strength as compared to control (429.33±3.42) than ethanolic extract (383.66±2.53). The results proposed that treatment with aqueous extract gel of *B. monosperma* flowers had beneficial influence on the various phases of wound healing like wound contraction and resulting in faster healing than ethanolic extract.

Conclusion- The observations and results obtained in this study indicated that the aqueous extract gel of *B. monosperma* flowers significantly stimulated wound contraction. These findings could justify, role of this plant material as folkloric accounts in the management of wound healing.

**Keywords:** *Butea monosperma*, Herbal formulation, wound healing activity.

### INTRODUCTION

A wound may be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissue<sup>1</sup>. Healing of a wound is an important biological process involving tissue repairs and regeneration. It involves the activity of an intricate network of blood cells, cytokines and growth factors which ultimately leads to the restoration to the normal condition of the injured skin or tissue<sup>2,3</sup>.

The aim of wound care is to promote wound healing in the shortest time possible, with minimal pain, discomfort, and scarring to the patient and must occur in a physiologic environment conducive to tissue repair and regeneration. In folklore medicines, medicinal plants have been used widely in facilitating wound healing with high degree of successes. This has inspired many researches which are aimed at validating the claims and discovering mechanisms which are possibly explains the potential of these herbs on wound repair processes. The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention.

*Butea monosperma* Lam. (Fabaceae) is a medium sized deciduous tree also known as 'Flame of Forest'. It is used

extensively in Ayurveda, Unani and Homeopathic medicine and has become a cynosure of modern medicine. The flowers are tonic, astringent, aprodiasic, anticonulsant, and diuretic. The stem bark is reported to possess antitumour, antiulcer, antifungal and antidiarrhoeal activities. The roots are reported in the treatment of filariasis, night blindness, helmenthiasis, piles, ulcers, and tumors. Palsonin an active principle isolated from seeds and its piperzaine salt exhibited good anthelmintic activity. The gum is astringent to the bowels, used in treatment of dysentery, stomatitis and cures excessive perspiration<sup>5,6,7</sup>. The present study was designed to test the *in vivo* wound healing activity of the ethanol and aqueous extracts of flowers of *B. Monosperma* (Lam.).

### MATERIALS AND METHODS

Plant material: Flowers of *Butea monosperma* were collected from the local areas of Mahabubnagar district, Andhra Pradesh, India and the plant material was taxonomically identified and authenticated at Department of Botany, Govt. M.V.S .Degree & PG College, Mahabubnagar, Andhra Pradesh.

Extraction of plant material: The Flowers were washed thoroughly, dried under the shade and pulverized. Equal

Table 1. Effect of topical application of EEBM gel and AEBM gel *B. monosperma* (Lam.) flower on healing of excision wound model

Group	0-day	3 <sup>rd</sup> day	6 <sup>th</sup> day	9 <sup>th</sup> day	12 <sup>th</sup> day	15 <sup>th</sup> day	18 <sup>th</sup> day	Period of epithelization
Control	514.00 ±1.46 <sup>ns</sup> (0.00)	481.16 ±3.71 <sup>ns</sup> (3.71)	414.66 ±4.64 <sup>ns</sup> (19.32)	354.83 ±3.12 <sup>ns</sup> (30.96)	270.33 ±2.40 <sup>ns</sup> (47.40)	190.66 ±2.62 <sup>ns</sup> (62.90)	98.66 ±1.99 <sup>ns</sup> (80.80)	23.16±0.79 <sup>ns</sup>
Standard	510.83 ±1.74 <sup>ns</sup> (0.00)	406.50 ±2.32 <sup>**</sup> (20.42)	307.33 ±1.74 <sup>**</sup> (39.83)	209.83 ±2.24 <sup>**</sup> (59.01)	101.33 ±2.61 <sup>**</sup> (80.16)	32.00 ±1.46 <sup>**</sup> (93.72)	05.00 ±0.36 <sup>**</sup> (99.02)	16.33±0.61 <sup>**</sup>
EEBM gel	509.66 ±1.45 <sup>ns</sup> (0.00)	440.83 ±3.91 <sup>**</sup> (13.50)	335.83 ±2.05 <sup>**</sup> (34.10)	247.66 ±2.33 <sup>**</sup> (51.40)	145.83 ±1.42 <sup>**</sup> (71.38)	73.83 ±1.19 <sup>**</sup> (85.51)	22.16 ±0.94 <sup>**</sup> (95.65)	19.33±0.84 <sup>**</sup>
AEBM gel	511.00 ±1.77 <sup>ns</sup> (0.00)	424.83 ±1.81 <sup>**</sup> (16.86)	320.16 ±3.42 <sup>**</sup> (37.34)	235.61 ±2.19 <sup>**</sup> (54.01)	125.00 ±1.71 <sup>**</sup> (75.53)	45.16 ±1.66 <sup>**</sup> (91.16)	8.00 ±0.57 <sup>**</sup> (98.43)	17.16±0.70 <sup>**</sup>

Data was expressed as mean ± S.E.M. and statistical analysis was carried out by One Way ANOVA followed by Dunnett's test, and  $P < 0.01^{**}$  when compared to control group and ns is non-significant.

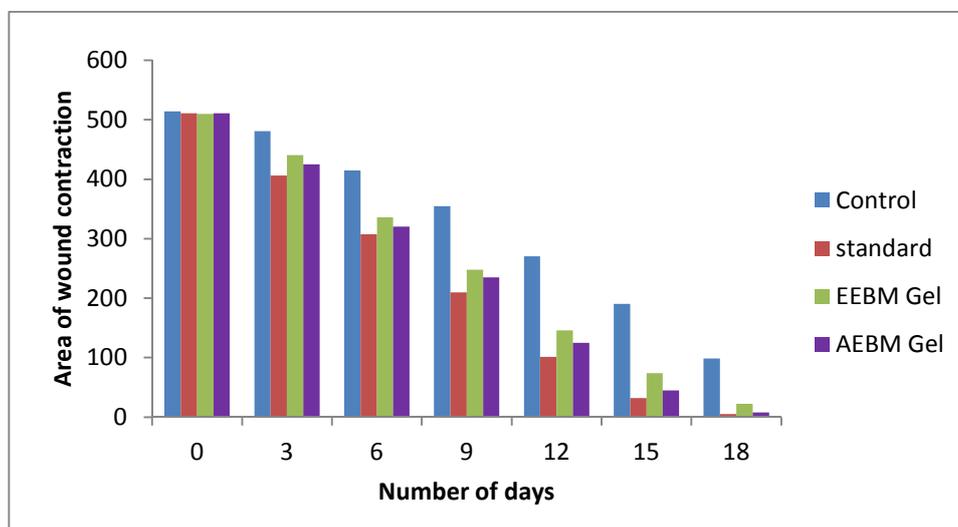


Figure 1. Effect of topical application of EEBM gel and AEBM gel of *B. monosperma* (Lam.) flower on healing of excision wound model

quantity of powder was passed through 40 mesh sieve and subjected to hot solvent using ethanol, at a temperature range of 40-60°C, in soxhlet apparatus. Finally the aqueous extract was prepared by maceration. The extracts were dried using rotary vacuum evaporator and stored in desiccators until further use.

**Formulation of Gel:** The gel was composed of carbapol 5%; propylene glycol 1%, triethanolamine 0.8 ml, extract 10%, methyl paraben and propyl paraben added as a preservative and distilled water in a quantity sufficient to prepare 100 gm gel<sup>8</sup>. Water required for these formulations was divided into two parts. In one part the exact amount of extract was dissolved and in other part, carbapol was soaked overnight and to this solution propylene glycol and propyl paraben were added. Both these solutions were mixed in a beaker and triethanolamine was added to the mixture dropwise to obtain the proper gel consistency. Two formulations were prepared, one containing ethanolic extract (EEBM gel) and aqueous extract (AEBM gel) of *Butea monosperma*.

**Animal and grouping:** Albino wistar rats weighing 150-180g were selected for the study and were acclimatized to standard laboratory condition with temperature 25±2°C and fed with standard animal pellet feed and water *ad libitum*.. The protocol was approved by institutional animal ethical committee (IAEC).

Wounds were induced by dividing them into following groups: Group-I (control) received application of blank gel. Group-II (standard) received application of standard drug (Framycetin cream). Group-III received application of EEBM gel and Group-IV received application of AEBM gel.

**Experimental Methods:** Excision and Incision wound models were used to evaluate wound healing activity.

**Excision wound model:** The rats were anaesthetized with slight inhalation of diethyl ether. A circular wound of about 500 sq. mm. was made on depilated ethanol sterilized dorsal thoracic region of the rats. The areas of the wound were measured immediately by placing transparent polythene graph paper over the wound and then tracing the area of wound on it. This was taken as the initial wound

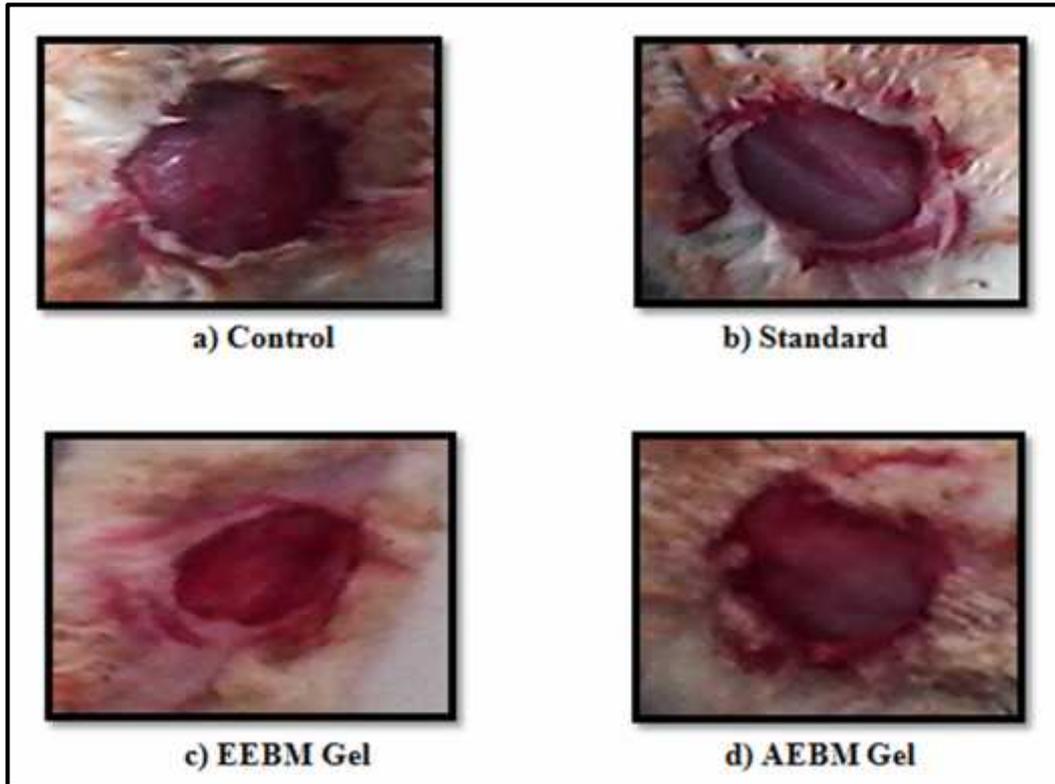


Figure2. Macroscopic observation of wound on 0 day in the respective groups

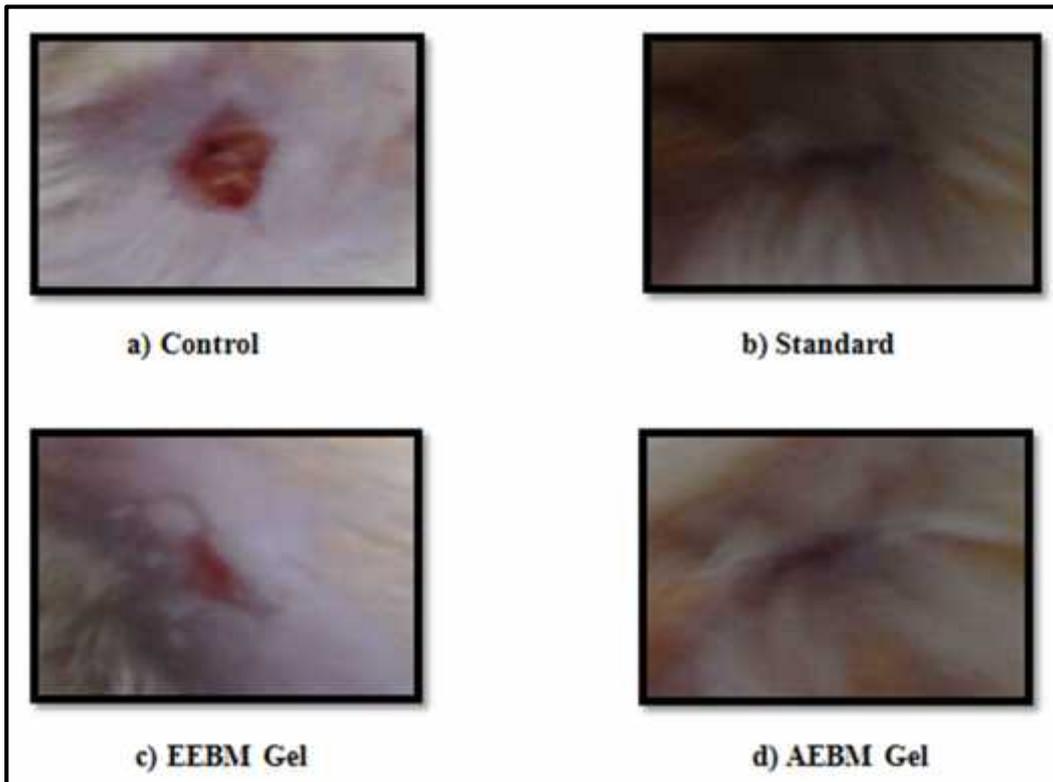


Figure3. Macroscopic observation of wound on 18<sup>th</sup> day in the respective groups

area reading. All the samples control (blank gel), standard (Framycetin sulphate cream 1%w/w), EEBM gel and AEBM gel were applied once daily for 18 days, starting from the day of wounding. The observations of percent wound closure were made on the 0, 3<sup>rd</sup>, 6<sup>th</sup>, 9<sup>th</sup>, 12<sup>th</sup>, 15<sup>th</sup>

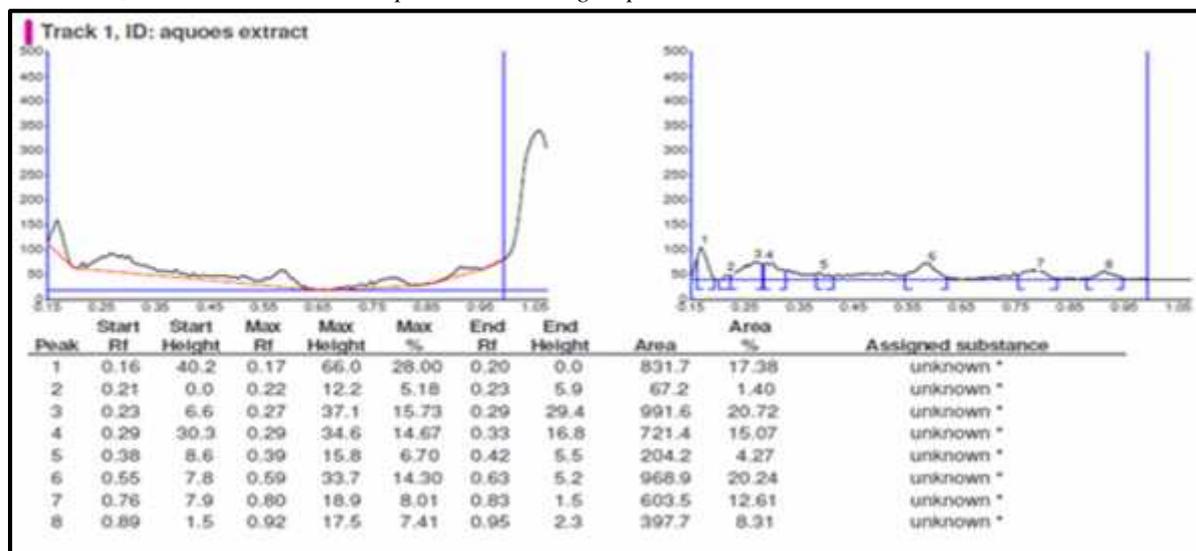
and 18<sup>th</sup> post wounding days. The percentage wound closure and period of epithelization were recorded<sup>9</sup>.

Incision wound model: The rats were anaesthetized with light ether. One para-vertebral straight incision of 6 cm was made on either side of the vertebral column with the help of scalpel blade. The wounds were closed with

Table 2. Effect of topical application of EEBM gel and AEBM gel of *B. monosperma* (Lam.) flower on healing of incision wound model

Groups	Breaking strength (g)
Control	273.16±4.65 <sup>ns</sup>
Standard	520.00±4.28 <sup>**</sup>
EEBM gel	383.66±2.53 <sup>**</sup>
AEBM gel	429.33±3.42 <sup>**</sup>

Data was expressed as mean ± S.E.M. and statistical analysis was carried out by One Way ANOVA followed by Dunnett's test, and  $P < 0.01^{**}$  when compared to control group.

Figure 4. HPTLC of Aqueous Extract of *Butea monosperma* with Rf values

interrupted sutures in 1 cm apart. All the samples control (blank gel), standard (Framycetin sulphate cream 1% w/w), EEBM gel and AEBM gel were applied topically applied once in a day. The sutures were removed on the 8<sup>th</sup> post wound day. The skin breaking strength of the wounds were measured on the 10<sup>th</sup> day by continuous constant water flow technique<sup>10</sup>. The breaking strength was expressed as minimum weight of water necessary to bring about gaping of the area.

### STATISTICAL ANALYSIS

Data was expressed as mean ± S.E.M. and statistical analysis was carried out by One Way ANOVA followed by Dunnett's test at  $P < 0.01$  significance level.

TLC and HPTLC of AEBM extract: The aqueous extract was subjected to thin layer chromatography and High performance Thin Layer Chromatography to find out number of constituents present in it. Different solvent systems were tried. The most suitable solvent systems was ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:79) by using 5% Methanolic sulphuric acid.

### RESULTS

Excision wound model: Excision wounds heal by contraction (wound closure) and epithelization. In excision wound model, the mean percentage closure of wound area was calculated on the 3, 6, 9, 12, 15 and 18 post wounding days. (Table 1) Application of EEBM gel and AEBM gel showed significant ( $P < 0.01$ ) wound healing activity. AEBM gel produced highest rate of wound healing

(99.43%), reducing the epithelization period to (17.16 ± 0.70) compared to the control. EEBM gel treated animals showed wound healing (95.65) which is lesser than AEBM gel and standard dug (99.02) Framycetin sulphate cream (2 % w/w).

Incision wound Model: Incision wounds heal by granulation and collagenation. EEBM gel and AEBM gel treated animal showed increase in tissue breaking strength (383.66±2.53) and (429.33±3.42) respectively when compared to control (273.16±4.65). The standard drug showed significant mean breaking strength in animals (520.00±4.28).

HPTLC of Aqueous Extract of *Butea monosperma*  
Chromatographic Conditions

Stationary Phase : HPTLC plates silica gel 60 F 254

Plate size (X x Y) : 20.0 x 10.0 cm

Mobile Phase : Ethyl acetate: formic acid: Glacial acetic acid: water (100:11:11:26)

Detection : UV 264 nm

### DISCUSSION

Wound healing is a natural process of regenerating dermal and epidermal tissues. Whenever there is a wound, a set of overlapping events takes place to repair the damage<sup>11</sup>. These processes have been categorized into phases which include the inflammatory, proliferative, and the remodeling phases<sup>12</sup>. Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin. It is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissues as closely as possible to its

normal state. Wound contracture is a process that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. In the maturational phase, the final phase of wound healing, the wound undergoes contraction resulting in a smaller amount of apparent scar tissue. Granulation tissue formed in the final part of the proliferative phase is primarily composed of fibroblasts, collagen, edema and new small blood vessels<sup>13</sup>.

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. Plants with ethno pharmaceutical importance are being exploited because of their healing properties<sup>14</sup>. The application of medicinal concoctions from plants to treat skin lesions, in particular, burns and wounds, has had a long tradition. Herbal gel or ointments containing different medicinal plants have been reported to be very beneficial in wound care, promoting the rate of wound healing with minimal pain, discomfort and scarring to the patient<sup>15</sup>.

The results of the present investigations revealed that in excision wound model significant wound healing activity was observed in the animals treated with AEBM gel than EEBM gel of *B. monosperma*. Significant decrease in the period of epithelialization and increase in wound contraction rate were observed in these groups of animals which is comparable with standard. In both extract treated animals, epithelialization was completed on 17th and 19th post wounding day respectively. While in control animals, the rate of wound contraction was slow and the complete epithelialization of the excision wound was extended up to 23th post wound day.

Breaking strength was measured to confirm the wound healing activity of EEBM and AEBM gel in incision wound model. The increase in breaking strength of treated wounds may be due to increase in collagen concentration and stabilization of fibres. The result showed that treatment with AEBM gel have more beneficial influence on the various phases of wound healing like fibroplasias, collagen synthesis and wound contraction resulting in faster healing.

In the present study preliminary phytochemical investigation was showed presence of different chemical constituents like flavonoids, flavones, flavonoidal glycosides, sterols, and triterpenoids. The wound-healing property of *B. monosperma* may be attributed to the phytoconstituents present in the plant and the faster process of wound healing could be a function of either the individual or the additive effects of the phytoconstituents. The process of wound healing is promoted by several natural products which are composed of active principles like triterpenes, alkaloids, flavonoids and biomolecules<sup>16</sup>. As, aqueous extract showed significant wound healing activity, it was subjected to thin layer chromatography and High performance thin layer chromatography for detecting the presence of Flavonoids. The Rf values 0.32, 0.63 and 0.80 of aqueous extract by TLC were found to be comparable with Rf 0.33, 0.63 and 0.83 value obtained by HPTLC, hence presence of flavonoids were confirmed.

## CONCLUSION

The present study has demonstrated that the aqueous extracts of *B. monosperma* flower has properties that render it capable of promoting accelerated wound healing activity. The present investigation offers scientific evidence to the folkloric accounts of the use of flower extract of *B. monosperma* in treating cuts and wounds. However, it needs further evaluation in clinical settings before consideration for the treatment of wounds. Also, studies with purified constituents are needed to understand the complete mechanism of wound healing activity of *B. monosperma*.

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