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Research Article

EVALUATION OF WOUND HEALING POTENTIAL OF METHANOL EXTRACT OF FLOWER OF *BUTEA MONOSPERMA* (LAM)

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ABSTRACT

The study aims to evaluate the wound healing potential of methanol extract of flower of *Butea monosperma* (Lam) belongs to the family Fabaceae. In this study the flower powder was extracted with methanol, it was used further for study. Thus prepared extracts were subjected to preliminary phytochemical analysis. The wound healing activity of the methanolic extract of flower of the *Butea monosperma* were evaluated in excision wound healing models using Albino wistar rats. The wound healing activity was assessed by the period of epithelialization and area of wound contraction in excision wound model. The methanolic extract of the flower of *Butea monosperma* (lam) showed the significant wound healing activity on excision wound model. Hence the present study supports the traditional claim of *Butea monosperma* as a wound healing drug in the Indian System of Medicine.

Keywords: *Butea monosperma*, Excision wound, MEBM- Methanolic extract of *Butea monosperma*

INTRODUCTION

Butea monosperma (Lam.) Taub., commonly called the flame of the forest, is considered as one of the most beautiful trees of India due to its gorgeous canopy of scarlet flowers which looks like a flame. *Butea monosperma* is also known as Palash. It belongs to the family Fabaceae. It is a well known traditionally used medicinal plant[1]. *Butea monosperma* (Lam) is growing in Burma, India and Sri Lanka. The flowers are tonic, astringent, aprodiasic and diuretic. The decoction of the bark is traditionally used in cold, cough, fever, various forms of haemorrhages, in menstrual disorders and in the preparation of tonics and elixirs. The stem bark is reported to possess antitumour, antiulcer, antifungal and antidiarrhoeal activities [2, 3, 4]. It is also reported that the powder of the stem bark is used to apply on injury caused due to an axe, the juice of the stem is applied on goiter of human beings and the paste of the stem bark is applied in case of body swellings [5]. The roots are reported in the treatment of filariasis, night blindness, helmenthiasis, piles, ulcers, and tumors [6]. It is reported that the ethanolic extract of seeds of *Butea monosperma,* on oral administration showed antifertility activity in mice and in rats [7]. Palsonin an active principle isolated from *Butea monosperma* seeds and its piperzaine salt exhibited good anthelmintic activity *in vitro* on *Ascaris lumbricoides* and *in vivo* on *Taxicara canis* [6]. The petroleum ether extract and triterpene isolated from flowers of *Butea monosperma* exhibited anti convulsant activity [8, 9]. It has been reported that the methanolic extract of stem bark of *Butea monosperma* showed anti inflammatory and analgesic activity [10]. The *in vitro* and *in vivo* anti inflammatory activity of *Butea monosperma* stem bark extract and the anti inflammatory activity of flavonoid fraction isolated from the stem bark of *Butea monosperma* were reported [11, 12]. It is reported the efficacy of *Butea monosperma* on dermal wound healing in rats [13]. Since a little information is available about the wound healing potential of *Butea monosperma* flower, it was considered worthwhile to study the wound healing potential of methanolic flower extract of *Butea monosperma* (Lam) on wistar rats.

Wounds are physical injuries that result in an opening or break of the skin. Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin. Healing is a complex and intricate process initiated in response to an injury that restores the function and integrity of damaged tissues. Wound healing involves continues cell–cell and cell–matrix interactions that allow the process to proceed in three overlapping phases viz. inflammation (0–3 days), cellular proliferation (3–12 days) and remodeling (3-6 months) [14, 15, 16]. Healing requires the collaborative efforts of many different tissues and cell lineages [17]. It involves platelet aggregation and blood clotting, formation of fibrin, an inflammatory response to injury, alteration in the ground substances, angiogenesis and re-epithelialization. Healing is not complete until the disrupted surfaces are firmly knit by collagen [18].

The basic principle of optimal wound healing is to minimize tissue damage and provide adequate tissue perfusion and oxygenation, proper nutrition and moist wound healing environment to restore the anatomical continuity and function of the affected part [19]. Cutaneous wound repair is accompanied by an ordered and definable sequence of biological events starting with wound closure and progressing to the repair and remodeling of damaged tissue [20]. Presence of zinc helps in wound healing and is good for protein metabolism. It is beneficial for the development of the reproductive system. Zinc, helps maintain collagen, which keeps the skin smooth, supple, and firm [21].

MATERIALS AND METHODS

Plant material

The flowers of *Butea monosperma* (palas) were collected in the month of November, 2011 from the local market of Ghaziabad, India. The flower were authenticated by the Dr. Roshini Nayar, National Bureau of Plant Genetic Resources, (Indian Council of Agricultural Research), Pusa campus, New Delhi-110 012 and the voucher specimen NHCP/NBPGR2012-04 is preserved in the pharmacognosy department of our institute for further reference. The flowers were dried under shade with occasional shifting and then powered with mechanical grinder and stored in an airtight container for further extraction purpose.

Preparation of extract

The powder material (flower, 100gm) was subjected to hot continuous soxhlet extraction with methanol (35°C-45°C) for 36 hours. The filtrate was collected and the mass were again subjected to extraction process for 24 hours. The filtrates were collected and were concentrated over water bath maintaining a temperature of 40°C. The concentrated mass was cooled and finally it was placed in the desiccators and was used for further studies. The percentage yield of the extract was calculated as 12.6%w/w. The preliminary phytochemical screening were carried out and it was found that the following constituents like tannins, flavanoids, alkaloids, sterols and terpenes were present.

Animals

Albino rats (Wistar) weighing 150-200g of either sex were used for the study. They were procured from the animal house of NIET, Greater Noida. The animals were acclimatized for one week under laboratory conditions. They were housed in polypropylene cages and maintained at 27 C±2°C less than 12 hrs dark/light cycles. They were fed with standard rat feed and water *ad libitum* was provided.

The litter in the cages was renewed thrice a week to ensure hygenicity and maximum comfort for animals. Ethical clearance for handling the animals was obtained from the Institutional Animals Ethical Committee prior to the beginning of the project work bearing the protocol number NIET\IAEC\2011\39.

Method

Excision wound model

The experimental animals were grouped into four containing 6 animals each and treated as follows:

Group I: Received control

Group II: Received Soframycin ointment

Group III: Received test ointment, which is made up by methanolic extract of flowers of *Butea monosperma* (5% w/w)

Group IV: Received test ointment, which is made up by methanolic extract of flowers of *Butea monosperma* (10%w/w)

A circular wound of about 500sq mm full thickness of a pre-determined area was made on the depilated back of the rat. The ointment was topically applied once a day, starting from the day of wound made, till complete epithelialisation. The parameters studied were wound closure and epithelialisation time. The wound were traced on mm^2 graph paper on days 3, 6, 9, 12, 15 and 18 and thereafter on alternate days until healing was complete. The percentage of wound closure was calculated. The period of epithelialisation was calculated as the number of days required for falling of the dead tissue remnants of the wound without any residual raw wound.

Statistical Analysis

The experimental results were expressed as multiple comparisons of Mean ± SEM were carried out by one way analysis of variance (ANOVA) followed by Dunnet Multiple Comparisons Test and the values of *(P<0.05) and **(P<0.01)were considered statistically significant.

RESULTS

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. The period of epithelialisation 22.29±0.12 was found to be high in control group which was untreated and take more days in healing while in standard group it was significantly reduced to 16.14±0.31**(P<0.01) of standard drug Soframycin. The period of epithelialisation of test ointment of (5%w/w) and (10%w/w) methanolic extract of flowers of *Butea monosperma* was found to be 19.05±0.69*(P<0.05) and 17.66±0.11**(P<0.01) respectively.

From the experiment data, the (10%w/w) B test ointment was more potent than (5%w/w) A test ointment as in B, the wound were traced on mm^2 graph paper on days 3, 6, 9, 12, 15 and on 18 day, the wound was (100%) completely heal, without any residual raw wound. In A test ointment was also showed healing effect but less effective than B test ointment. As the wound were traced on mm^2 graph paper on days 3, 6, 9, 12, 15 and on 18 day, the wound was 99.13% closed which was more effective than control group.

The graph shows the percentage of healing and period of epithelialisation of control, standard, A test ointment and B test ointment, which was prepared by methanolic extract of flowers of *Butea monosperma.*

Table 1: Effect of methanolic extract of flower of *Butea monosperma* on excision wound model

Groups	Area of wound healing contraction (in days)							Period of epithelialisation
	0-day	3-day	6-day	9-day	12-day	15-day	18-day	
Control	512.72±0.12 (0.00)	486.31±1.21 (5.15)	401.91±1.80 (21.61)	352.48±0.56 (31.25)	268.16±0.62 (47.69)	192.32±0.54 (62.49)	98.53±0.48 (80.78)	22.29±0.12
MEBM (5%w/w)	508.68±1.23* (0.00)	436.69±1.21* (14.20)	367.40±0.63* (27.77)	291.26±0.63* (42.74)	148.26±0.41* (70.85)	61.22±0.33* (87.96)	4.38±0.11* (99.13)	19.05±0.69*
MEBM (10%w/w)	508.81±1.41** (0.00)	424.42±1.12** (16.58)	352.14±0.34** (30.79)	285.17±0.33** (43.95)	132.11±0.28** (74.03)	12.90±0.23** (97.46)	0** (100)	17.66±0.11**
Standard Soframycin	516±1.46** (0.00)	402.30±0.71** (22.03)	324.59±1.21** (37.09)	242.12±0.42** (53.07)	128.36±0.37** (75.12)	8.32±0.34** (98.38)	0** (100)	16.14±0.31**

No. of animals N=6, P<0.05*, P<0.01**, when compared with control group. One way Anova

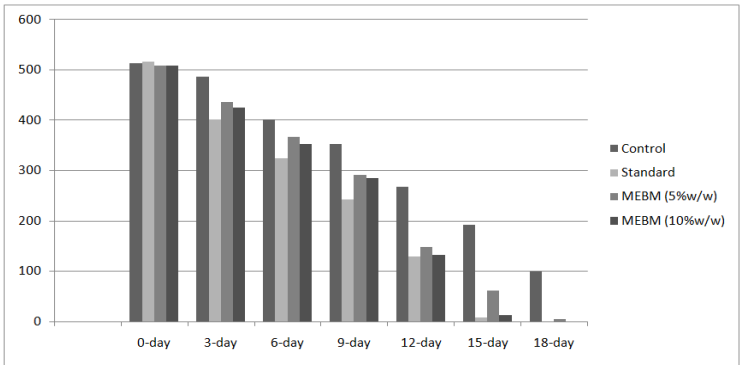

Fig. 1: Excision wound model-0, 3, 6, 9, 12 and 15 days of MEBM (10%w/w)

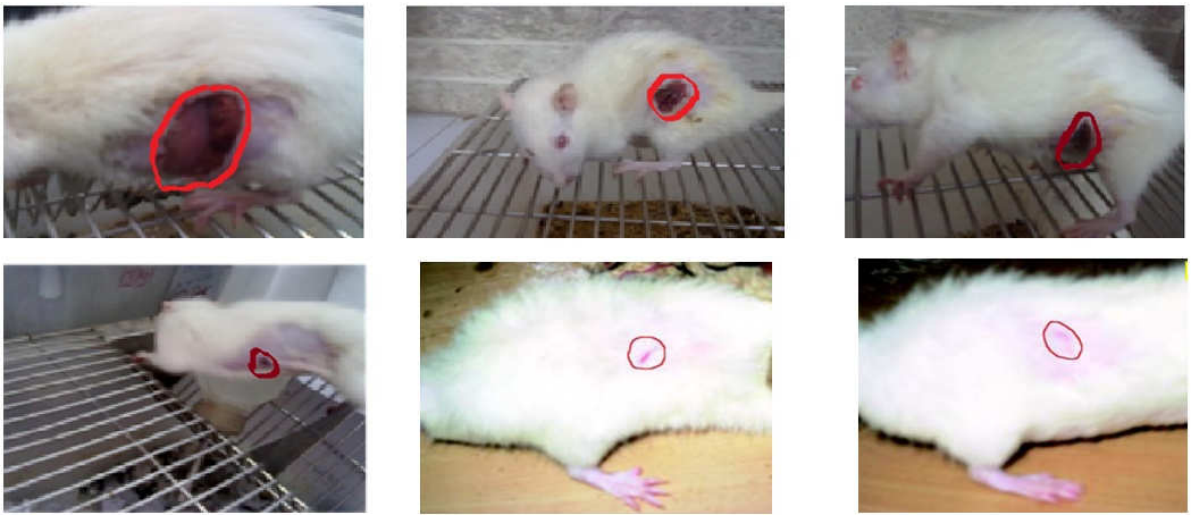

Fig. 2: Area of wound healing contraction

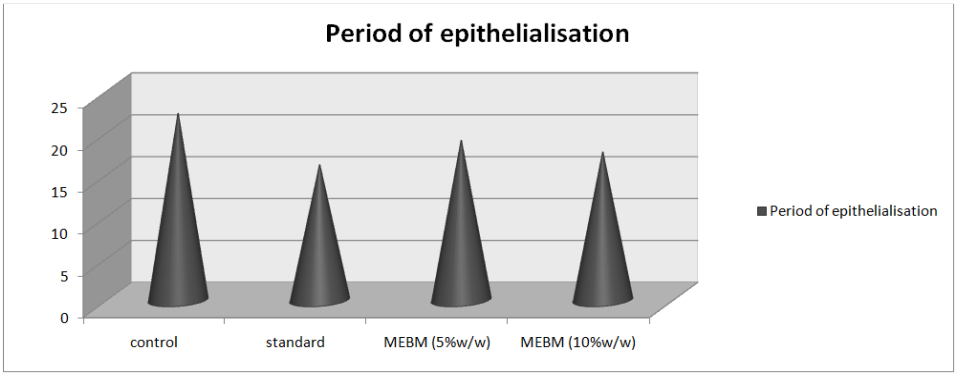

Fig. 3: Period of epithelialisation

DISCUSSION

The results of the present investigations revealed that the methanolic extract of the flower of *Butea monosperma* possess significant wound healing activity in excision wound models. 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. Therefore, a systematic approach should be made to find out the efficacy of plants against wounds so as to exploit them as herbal wound healing agents.

Experimental assessment of the wound healing activity of methanolic extract showed increased rate of wound contraction and epithelialization in treated animals. Topical application of the methanolic extract on excision wounds accelerated wound contraction and reduced epithelialization period in rats. Wound healing involves regeneration of specialized cells by proliferation of surviving cells and connective tissue response characterized by the formation of granulation tissue[22]. It is also characterized by haemostasis, reepithelialization and remodeling of the extracellular matrix. Epithelialization, which is the process of epithelial renewal after injury, involves the proliferation and migration of epithelial cells towards the centre of the wound while wound contraction is largely due to the action of myofibroblasts[23, 24]. Thus, the effect of ethanolic extract and the acetone fraction on wound contraction and epithelialization suggest it may enhance epithelial cells migration and proliferation, as well as the formation, migration and action of myofibroblasts. On chronic oral administration, ethanolic extract and the acetone fraction enhanced the granuloma tissue formation in dead space wounds. Granuloma tissue formed on an inert foreign body in a dead space comprises an accumulation of modified macrophages[22], histological giant cells and undifferentiated connective tissue consisting largely of collagen[22, 24, 25]. Increase in granuloma tissue in dead space wound is associated with enhanced collagen maturation and increased protein content as well as angiogenesis [26, 27, 28]. These processes are indicators of new tissues generation and suggest that methanolic extract may stimulate mechanisms associated with tissue regeneration. Closely related to this is the effect of growth factors secreted by macrophages on wounds. Macrophages secrete peptide growth factors that exert pro-healing effect by stimulating regeneration, fibroblast proliferation and activation and angiogenesis[22]. It is, therefore, likely that in addition to enhancing wound contraction and epithelialization, the methanolic extract may also stimulate processes associated with tissue regeneration.

CONCLUSION

The methanolic extract of *Butea monosperma* flower showed wound healing property. We propose that the additive and synergistic activity of phytochemicals such as flavonoids, steroids, tannins and alkaloids present in the methanolic extract of *Butea monosperma* were responsible for its potent wound healing property. The present investigation offers scientific evidence to the folkloric accounts of the use of flower in treating cuts and wounds.

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