

## Effect of *Alternanthera brasiliana* (L) Kuntze on healing of dermal burn wound

Chandana C Barua<sup>1,\*</sup>, Archana Talukdar<sup>1</sup>, Shameem A Begum<sup>1</sup>, Bhaben Buragohain<sup>1</sup>, Jayanti Datta Roy<sup>1</sup>,  
Debesh C Pathak<sup>2</sup>, Dilip K Sarma<sup>3</sup>, Asheesh K Gupta<sup>†</sup> & Rumi S Bora<sup>4</sup>

Department of <sup>1</sup>Veterinary Pharmacology and Toxicology, <sup>2</sup>Veterinary Pathology, <sup>3</sup>Veterinary Microbiology and <sup>4</sup>Animal Production & Management, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati 781 022, India  
and

<sup>†</sup>Department of Biochemical Pharmacology, Defence Institute of Physiology and Allied Sciences, Delhi 110 054, India

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Wound healing activity of methanol extract of *Alternanthera brasiliana* [5% (w/w) ointment] was evaluated in experimental burn wound model in rats. Healing potential was assessed by the rate of wound contraction, estimation of anti-oxidants like catalase, superoxide dismutase, reduced glutathione, protein, vitamin C and hydroxyproline, along with histopathological examination on 8<sup>th</sup> day post wounding. The statistical data indicated that there was significant increase in wound contraction along with augmented level of antioxidants in granulation tissues in *A. brasiliana* treated group. Histopathological assessment of the granulation tissue revealed formation of epidermis with keratin layer and deposition of collagen fibers after treatment with the plant extract.

**Keywords:** *Alternanthera brasiliana*, Antioxidants, Collagen, Experimental burn, Wound healing.

*Alternanthera brasiliana* (L.) Kuntze (Amaranthaceae), a herbaceous plant, is used against inflammation, cough and diarrhoea in Brazilian popular medicine<sup>1</sup>. The extracts of *A. brasiliana* exhibited antinociceptive<sup>2</sup>, antimicrobial<sup>3</sup> and also anti-herpes simplex virus activity<sup>4</sup>. Aqueous or ethanol extracts of *A. brasiliana* were able to block human mitogen induced lymphocyte proliferation without any toxic effect<sup>1</sup>. Analgesic activity of methanol extract of leaves of this plant has been reported earlier<sup>5</sup>.

Despite the anecdotal evidence of the use of *Alternanthera brasiliana* as a traditional wound healing agent, the literature does not present any scientific data supporting the wound healing capability of this plant. In our previous study on the wound healing activity of *A. brasiliana*<sup>6</sup> in normal excision and incision wound model (*in vivo*) in Sprague Dawley rats and by Chorioallantoic membrane (CAM) model (*in vitro*) in 9 day old embryonated chicken eggs exhibited excellent wound healing activity in the methanol extract of *A. brasiliana* leaves. Hence, the present study was envisaged to investigate the healing efficacy and antioxidant activity of *A. brasiliana* methanol

leaf extract in slow healing wound model in experimental full-thickness burn wound in rats. Histopathological study and effect on antioxidant enzymes was also evaluated.

### Materials and Methods

*Plant material*—Leaves of *Alternanthera brasiliana* were collected from the medicinal garden of the Department of Pharmacology & Toxicology, College of Veterinary Science, Khanapara, India during the month of February-June, 2010. The plant was identified by taxonomist of NEIST, Jorhat, Assam and a voucher specimen was deposited in the Herbarium of Botanical Survey of India (Specimen No: AAU/CVSC/PHT/02).

*Preparation of methanol extract of A. brasiliana leaves*—Leaves of *A. brasiliana* were shade dried and powdered. About 250 g of powdered leaves was soaked in 1000 ml methanol for 72 h in a beaker and the mixture was stirred every 18 h using a sterile glass rod. Filtrate was obtained after passing through Whatman filter paper no 1 for three times and concentrated in Rotary evaporator (Equitron, Roteva) at 50°-60°C under reduced pressure. A dark brown methanol extract of *Alternanthera brasiliana* (MEAB) was obtained and stored in air tight container at 4°C for further use. Recovery was 6.12 % (w/w) in terms of dry leaves.

\*Correspondent author  
Telephone: 098640-13231  
Fax: 0361 2364941  
E- mail: chanacin@gmail.com

*Phytochemical screening*—MEAB was subjected to preliminary qualitative phytochemical investigations for presence of various phytoconstituents *viz.* steroids, alkaloids, phenolic compounds, tannins, flavonoids, glycosides, diterpenes, triterpenes and saponins<sup>7</sup>.

*Preparation of ointment (5%; w/w) of A. brasiliensis*—MEAB (5 g) was mixed with 95 g of soft white petroleum jelly (S.D. Fine Chemicals, India) to prepare 5% ointment (w/w). Himax (Indian Herbs Research & Supply Co. Ltd. Darra Shivrpur, Saharanpur) was used as standard drug.

*Experimental animals*—Healthy adult Sprague Dawley rats of either sex, approximately of same age, weighing between 180-200 g and adult Swiss albino mice of either sex weighing between 25-30 g were used. The animals were fed with standard pellet diet and water *ad libitum*. They were housed individually in polypropylene cages containing sterile paddy husk bedding under controlled conditions at 25±3°C, 50±5% RH and kept under 10/14 h light/dark cycles. The experiments were performed as per guidelines of the Institutional Animal Ethical Committee, College of Veterinary Science, Khanapara, Guwahati, India (No. 770/03/ac/CPCSEA/FVSc, AAU/IAEC/06/21) and conform to the national guidelines on the care and use of laboratory animals, India.

*Acute toxicity studies*—Acute toxicity study was carried out according to the Organization of Economic Corporation Development (OECD) guidelines No. 425. MEAB was administered orally in doses of 100, 200, 400, 800, 1000 and 2000 mg/kg to the group of mice (n=6) and the percentage mortality was recorded for a period of 24 h. During the first hour after the drug administration, the mice were observed for any gross behavioral change and the parameters observed were hyperactivity, grooming, convulsions, sedation and loss of righting reflex, respiration, salivation, urination and defecation<sup>8</sup>.

Based on the above toxicity study, direct limit test was done. Initially a particular dose, on the basis of the above study was administered to single female rat and the rat was observed for 48 h with close surveillance up to initial 4 h (same as in case of first rat) after 48 h (of the second administration), same dose was administered in 2 more female rats and observation was done similarly as for previous rats. The rats were observed for 14 days and no adverse effect was found. The weight of the animals was recorded on day 7 and 14.

*Burn wound model*<sup>9</sup>—Prior to wounding the rats were anaesthetized by intra-peritoneal injection of thiopentone sodium (25 mg/kg). As per standard protocol, the dorsal surface of the animals was shaved and the underlying skin was cleaned with 70% ethanol. Full-thickness burn wound was created by using an aluminum metal rod (diam 1.8 cm; area 250 mm square; melting point 660°C) heated to 85°C. The temperature of the metal rod was monitored with a fabricated digital computerized multimeter. The hot rod was exposed on the shaved area of the rat for 20 sec, resting on its own weight of 30 g. No additional pressure was applied on the hand-held metal rod. After 24 h, dead tissues were excised using sterile surgical blade.

*Study design*—The animals were randomly allocated into 3 groups (n=6). Group I served as control and the rats received topical application of the vehicle, *i.e.* soft white petroleum jelly twice daily for 7 days. In Group II, 5% (w/w) ointment of MEAB was applied topically twice daily for 7 days. The animals of group III received topical application of standard drug *i.e.* himax, twice daily for 7 days.

*Pro-healing parameters*—The outer margin of the excision wound was traced on transparent film immediately before performing a biopsy on day 8 post wounding to determine wound contraction. The area (mm<sup>2</sup>) within boundary was measured planimetrically. The excised tissue was used for analysis of various biochemical parameters *viz.* hydroxyproline<sup>10</sup>, total protein<sup>11</sup> as well as antioxidant enzyme levels.

*Antioxidant analysis*—A 10% homogenate of granulation tissue was used for estimation of reduced glutathione (GSH)<sup>12</sup>, superoxide dismutase (SOD)<sup>13</sup>, catalase (CAT)<sup>14</sup> and vitamin C<sup>15</sup> level.

*Histopathological study*—Histopathological evaluation of granulation tissue was done as per standard method<sup>16</sup>.

*Statistical analysis*—Results obtained from animal experiments were expressed as mean±SE. The data were analyzed by one-way ANOVA using the SPSS software (version 11.5). *P*<0.05 was considered as significant.

## Results

Phytochemical screening of MEAB showed the presence of alkaloids by Wagner's and Dragendroff's test, steroids by Salkowski's and Lieberman Burchardt's test and triterpenes by Salkowski's and Lieberman Burchardt's test.

Oral administration of MEAB up to 2000 mg/kg did not produce any toxic effect in mice. No mortality was observed and the extract was found to be safe at the given dose.

Significant wound healing activity was observed with the 5% (w/w) ointment of MEAB on the experimental burn wound model. On day 8, the rate of reduction in the wound area of the extract treated animals was 92.13% which was significantly higher compared to the control (48.53%) animals. The MEAB treated animals showed better wound contraction as compared to himax treated (76.99%) group (Table 1).

The protein content increased significantly in rats treated with 5% (w/w) MEAB in comparison to control animals. There was significant increase in hydroxyproline content too in test group than the control group. Likewise, level of antioxidant enzymes viz. GSH, SOD and CAT level in granulation tissue increased significantly in rats in comparison to control animals. There was significant increase in Vitamin C level also in the treated group than the control group (Table 2).

Histopathological changes of the granulation tissues in animals in the control, MEAB and standard drug treated groups have been shown in Figure 1. The control group showed necrotic debris in the subcutaneous tissue (Fig. 1A), whereas, granulation tissue in extract treated group showed collagen fiber deposition (Fig. 1B). Formation of epidermis with

keratin layer in the MEAB treated group on day 8 (Fig. 1C) indicated better wound healing activity in *A. brasiliiana* treated animals. In contrast, himax treated group showed congestion, development of epidermis and keratin layer (Fig. 1D). The histological studies showed an overall early recovery and regeneration in MEAB treated group as compared to the control group. Furthermore, Vangiesons staining showed uniform and regularly arranged collagen fibers in the granulation tissues of MEAB treated rats (Fig. 1B).

### Discussion

The results of the present study demonstrated that methanol extract of *A. brasiliiana* possessed a definite prohealing action in burn healing as there was significant increase in the rate of wound contraction, augmented antioxidant levels and total protein content in the granulation tissue supported by histopathological study.

Angiogenesis is a critical component of wound healing. Delayed or aberrant revascularization at the wound sites contributes to the etiology of chronic wounds<sup>17</sup>. Healing efficacy of MEAB was reported by us in normal excision and incision wound along with angiogenesis in CAM model<sup>6</sup>. But its role in various impaired and slow healing wounds viz. diabetic, immunocompromised and burn wound was evaluated to further establish its prohealing activity.

Histopathological observation showed better proliferation of collagen, a major protein of extra cellular matrix that ultimately contributes to wound strength<sup>18</sup> in the extract treated group compared to the control group. Significant increase in the hydroxyproline content of the granulation tissue of the animals treated with *A. brasiliiana* indicates enhanced collagen maturation.

As levels of different enzymatic and non-enzymatic antioxidants increased significantly after *A. brasiliiana* extract treatment, the findings suggested decreased

Table 1—Wound healing activity of *A. brasiliiana* (5% w/w) on day 8 post wounding  
[Values are mean± SE of n= 6]

Groups	Area of wound (mm <sup>2</sup> )	Wound contraction (%)
Control	128.67 ± 5.99	48.53
MEAB	19.67 ± 2.03*	92.13
Standard (Himax)	57.53 ± 2.38*	76.990

\*Significant at  $P < 0.05$

Table 2—Effect of topical *A. brasiliiana* (5% w/w) on dermal burn wound  
[Values are mean± SE of 6 animals]

Parameter	Control	MEAB	Standard (Himax)
Reduced glutathione (µg/mg protein)	0.93 ± 0.06	1.22 ± 0.03*	1.14 ± 0.06*
Superoxide dismutase (U/mg protein)	8.2 ± 0.23	13.2 ± 0.24*	10.9 ± 0.58*
Catalase (U/mg protein)	4.24 ± 0.16	8.52 ± 0.19*	6.71 ± 0.18*
Vitamin C (µg/mg protein)	1.55 ± 0.04	1.96 ± 0.06*	2.81 ± 0.05*
Protein (mg/g tissue)	85.83±2.54	127.83±2.66*	111.83±2.75*
Hydroxyproline (mg/g dry wound tissue)	31.67±1.20	52.17±1.38*	42.08±1.16*

\*Significant at  $P < 0.05$

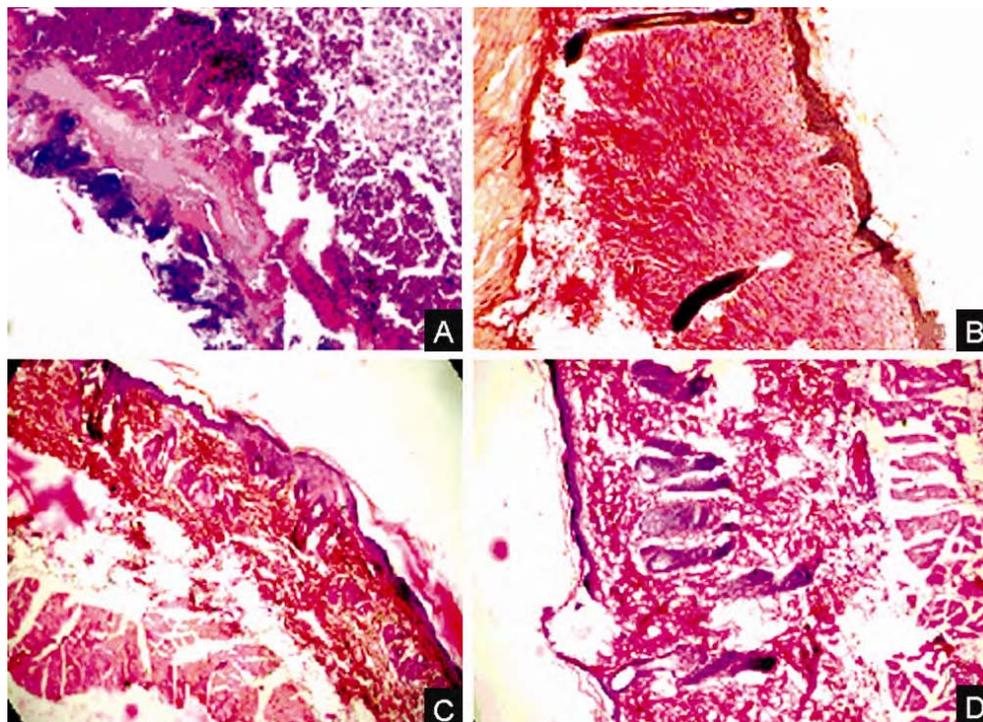


Fig. 1—Histopathological changes in 8 days old burn wound treated with methanolic extract [5% (w/w) ointment] of *Alternanthera brasiliiana* (A)— Control group showing necrotic debris in the subcutaneous tissue (H&E stain, 100x); (B)— *Alternanthera brasiliiana* treated group showing collagen deposition (Van Gieson's stain, 100x); (C)— *Alternanthera brasiliiana* treated group showing formation of epidermis with keratin layer (H&E stain, 100x); and (D)— Himax (standard) treated group showing congestion, development of epidermis and keratin layer (H&E stain, 100x).

oxidative injury of the wound, which could be due to increased quenching or scavenging of oxygen free radicals by the elevated levels of antioxidants. Reactive oxygen species (ROS) are produced in response to cutaneous injury. They impede the healing process by causing damage to cellular membranes, DNA, proteins and lipids as well<sup>19</sup>. Elimination of ROS could be an important strategy in healing of chronic wounds<sup>20</sup>. Preventive antioxidants, such as superoxide dismutase (SOD), glutathione peroxidase and catalase (CAT) are the first line of defence against ROS<sup>22</sup>. It seems reasonable to presume that their action is concerted, as SOD catalyses O<sub>2</sub>-dismutation producing H<sub>2</sub>O<sub>2</sub>, whereas CAT or peroxidases remove it<sup>21</sup>. SOD-1 is a key enzyme in dismutation of potentially toxic superoxide radicals into hydrogen peroxide and dioxygen<sup>22</sup>. A significant increase in the CAT activity observed in the extract treated group suggested that H<sub>2</sub>O<sub>2</sub> accumulated due to increased activity of SOD might be properly neutralized by CAT. Major endogenous thiol antioxidant in biological system is reduced glutathione (GSH), which serves as a coenzyme

necessary for GPx to eliminate the lipid hydro peroxide<sup>22</sup>. The GSH system plays important role in many of the processes involved in wound healing, *viz.* opposing the oxidative stress associated with inflammation and infection<sup>23</sup>. It also participates in many of the processes associated with proliferation of the cells to form new tissue<sup>23</sup>. Beyond its function in collagen formation, ascorbic acid is known to increase absorption of inorganic iron. It plays an essential role in the metabolism of folic acid, some amino acids and hormones as well as acts as an antioxidant. In recent years, research has been increasingly focused on antioxidant activity of ascorbic acid, as “oxidative stress” may be a causal factor in the etiology of diverse and important disorders<sup>24</sup>. The increased ascorbic acid in the extract treated group might be responsible for protecting the cells from oxidative stress leading to better wound healing.

In conclusion, it could be interpreted that topical application of *Alternanthera brasiliiana* exhibited significant pro-healing activity in experimental burn wound as evidenced by augmented endogenous

antioxidants and increased angiogenesis. These results validated the traditional use of *Alternanthera brasiliana* in various types of wounds as herbal remedy. Further pharmacodynamic investigations are required to identify the active fractions responsible for its wound healing activity for formulation of a plant based herbal medicine.

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