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EVALUATION OF *C. ALBICANS* INDUCED WOUND HEALING ACTIVITY OF METHANOLIC LEAF EXTRACT OF *ANDROGRAPHIS PANICULATA*

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ABSTRACT

To establish the wound healing activity of methanolic extract of *A. paniculata* two model were performed to evaluate the wound healing i.e., incision and excision model. In incision model the parameter which was carried out was breaking strength of wound skin. In excision model percentage wound concentration and period of epithelization was established for the extract. Reference stand drug was Framycetin sulphate cream ointment for comparison with other group. From the observation in both the model. Methanolic extract was found to have greater wound healing activity it terms of breaking strength in incision model and percentage wound concentration, period of epithelization was highest in excision model compared with group. In conclusion methanolic extract of *A. paniculata* better healing ablity.

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INTRODUCTION

A large number of plants are used by folklore tradition in India for treatment of cuts, wound and burns. Various research data revealed that plants may worked as healing and regeneration of the tissue by multiple mechanisms. There are several reports stating that the extract of several plants, used for wound healing properties (Stephen *et al.*, 2010; Pirbalouti *et al.*, 2010; Subhashin & Arunachalam, 2011; Dewangan *et al.*, 2012).Trauma to skin is a concern in human life; indeed, open wound areas provide appropriate, humid and nutritious environments for colonization by various pathogens and loss of skin might result due to the chronic infection. It has been estimated that 1 to 2 % of the people in developing countries suffer from chronic skin wounds and infection, which is a known major cause of morbidity and mortality (Siddiqui and Bernstein, 2010).

C. albicans is one of the normal flora pathogen in skin and mucous membranes (Agarwal *et al.*, 2010; Mandal and Mandal, 2011) and is something specifically watched in immunosuppressive patients, but it is still known as significant in patients death in some of cases (Murray *et al.*, 2008; Young and Naught 2011). Pharmaceutical plants have many uses in medicine, nutrition and industry. Due to synthetic drug-induced infections, in topical and even oral applications, biological chemical, such as officinal herbs (for examples, *A. paniculata* have been considered as propriate alternatives. *A. paniclata* belongs to the Acanthaceae family and rich source for natural antioxidants including catalase, a superoxide dismutase and glutathione-S-transferase (Ojha *et al.*, 2009). To the best of our knowledge, there are limited studies reporting the beneficial/ protective impacts of *A. paniculata* extract on candiaisis under controlled conditions; therefore, the present study was carried to evaluate topical effect of *A. paniculata* extract on skin wound infected with *C. albicans*, in rat.

MATERIALS AND METHODS

Collection of plant materials and identification

The leaves of *Andrographis paniculata* were collected from in and around Coimbatore district, Tamilnadu, India. The collected leaves were identified by Botanical Survey of India, Coimbatore, Tamilnadu with the authentication number BSI/SRC/5/123/2013-14/Tech 1934. The collected plant leaves of *A. paniculata* were washed twice with tap water and rinsed with distilled water to remove or dust particles attached with leaves and the plant leaves subjected to dry in shade. Followed by this step, the dried plant leaves were then subjected to cold percolation method to obtain *A. paniculata* leaves powder.

Preparation of plant extract

About 10 g of air dried powder was taken in 100 mL of methanol. Plugged with cotton wool and then kept on a rotary shaker at 220 rpm for 24 hrs. Then the supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume and stored at 4 ^{o}C in air tight container.

Phytochemical analysis

The methanolic extract of *A. panicualata* was screened for the presence of secondary metabolites using the procedures of Harborne, (1998) and Kokate *et al*., (2003).

Experimental animals

Ten-week-old male Wister Albino strain rat weighing 210 ± 6.64 g were used for the study. The rats were procured from the Small Animal's breeding center of Kerala Agricultural University, Mannuthy, Thrissur. The rats were grouped and housed in polyacrylic cages with not more than six animals per cage and maintained at temperature of 25 ± 2 °C; relative humidity of 55 ± 5 %, 14 /10 h, dark/ light cycle, with free access to feed and water (ad libitium). The rats were acclimatized to laboratory conditions for 10 days before commencement of the experiment. All studies will be conducted in accordance with Committee for the Purpose of Control and Supervision on Experiments on Animals (659/02/a/CPCSEA), "CPCSEA Guidelines for Laboratory Animal Facility".

Acute dermal toxicity - fixed dose procedure

The acute dermal toxicity study was carried out in adult male albino rats by "fix dose" method of OECD (Organization for Economic Co-operation and Development) Guideline No.434. Methanol extract of the *A. paniculata* leaf was applied topically at dose level 2000 mg/kg. OECD (Organization for Economic Co-operation and Development) Guideline No.434.They were continuously observed for 4 hrs and there after 14 days to detect any changes in the behavior in relation to the posture, mood and motor activity. All animals were observed twice daily for mortality during the period of study. Based on the acute toxicity results, 100, 200 and 400 mg kg^{-1} b.w would be chosen as low, mid and high dose for the evaluation of sub-acute toxicity.

Evaluation of *C. albicans* induced wound healing activity of *A. paniculata*

Linear incision wound model

The rats were anaesthetized prior to and during creation of the wound. The dorsal fur of the animals was shaved with an electric clipper. A longitudinal paravertebral incision, 1.5 cm in length was made through the skin and cutaneous muscle on the back (Ehrlich and Hunt, 1968). After the incision, surgical sutures were applied to the parted skin at intervals of one centimeter. The wounds were left undressed. The sutures were removed on the 8th post wound day and the treatment was continued. The skin-breaking strength was measured on the 10th day by the method of (Lee, 1968).

Excision wound model

Animals were anaesthetized prior to and during creation of the wounds. The rats were inflicted with excision wounds as described by (Morton and Malon, 1972). The dorsal furs of the animals were shaved with an electric clipper and the anticipated area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of circular area 500 mm^2 and 0.2 cm depth was created along the markings using toothed forceps, a surgical blade and pointed scissors. The entire wound was left opened (Diwan and Tilloo, 1982). The wound closure rate was assessed by tracing the wound on days 2, 4, 6, 8, 10, 12 and 14 post-wounding using transparency papers and a permanent marker. The wound areas recorded were measured using a graph paper. Number of days required for falling of eschar without any residual raw wound gave the period of epithelialization.

Experimental design

After one week of acclimatization period, the animals were divided into five groups with six animals in each.

Group I: Received no treatment and served as control

Group II: Rats received application of methanolic extract of *A. paniculata* 100 mg kg^{-1} b.w which considered as low dose.

Group III: Rats received application of methanolic extract of *A. paniculata* 200 mg kg^{-1} b.w which considered as moderate dose.

Group IV: Rats received application of methanolic extract of *A. paniculata* 400 mg kg^{-1} b.w which considered as high dose.

Group V: Rats received application of standard drug Framycetin sulphate cream ointment (1% w/w).

Under light chloroform anaesthesia an impression of 500 sq mm was made on the shaved back of the rats as described in Morton and Malone (1972). The skin of the impressed area was excised carefully. Animals are kept in separate cages. The day on which wound was made consider as day '0' (Zero). Drugs were topically applied once a day till complete of epithelization, starting from day of excision. The progressive changes in wound area were monitored metrically by tracing the wound and sebsequently by 2^{nd}, 4^{th}, 6^{th}, 8^{th} 10^{th} 12^{th} 14^{th} 16^{th} 18^{th} and 20^{th} days post wounding number of days required for falling of sub without any residual raw wound, gave the period of epitheliazation.

Collagen estimation (hydroxyproline content)

Hydroxyproline present in the acid hydrolysate of granulation tissue oxidized by sodium peroxide in the presence of copper sulfate, when complexed with para-dimethyl amino benzaldehyde, develops a pink colour that was measured at 540 nm using colorimetry.

Determination of wound breaking strength

The anesthetized animal was secured to the table, and a line was drawn on either side of the wound 3 mm away from the line. This line was gripped using forceps one at each end opposed to each other. One of the forceps was supported firmly, whereas the other was connected to a freely suspended light weight metal plate. Weight was added slowly and the gradual increase in weight, pulling apart the wounded edges. As the wound just opened up, addition of weight was stopped and the weights added was noted as a measure of breaking strength in grams. Three readings were recorded for a given incision wound, and the procedure was repeated on the contra lateral wound. The mean reading for the group was taken as an individual value of breaking strength. The mean value gives the breaking strength for a given group (Lee, 1968).

Determination of percentage of wound contraction (Bhat *et al*., 2007)

The percentage of wound contraction was calculated was the following formula

$$N^{th}\ \text{day}\ -100 = \frac{\text{Wound area on the } N^{th}\ \text{day}}{\text{Wound area on the } 1^{st}\ \text{day}} \times 100.$$

Collection of samples

After the experimental regimen, the animals were sacrificed by cervical dislocation under mild chloroform anesthesia. Blood was collected in EDTA and centrifuge tubes by an incision made in the jugular veins and serum was separated by centrifugation at 2000 rpm for 20 min and utilized for various biochemical assays. The skin tissue were excised immediately and thoroughly washed with ice cold physiological saline and blotted dry. A part of the tissues such was removed and fixed in 10 % formalin for histopathological study.

Hematological assay

The whole blood sample was analyzed for the estimation of hematological parameters like RBC, Hb, WBC, PCV and platelets, and it was performed by using (SYSMEX Xs – 800 i) automatic hematology analyzer.

Biochemical assays

Biochemical analysis of untreated control (Group I) rats showed significantly ($p < 0.05$) decreased levels of AST, ALT, ALP, urea, creatinine and protein in serum when compared to high dose (400 mg/kg b.w) and standard drug post wound treated rats. Topical application of *A.paniculata* extracts different doses post wound treated rats gradually increased the levels compared to untreated control animals. Significant changes were not observed in the high dose treated rats (Group IV) when compared with the standard drug treated rats (Group V).

Histopathology study

A specimen sample of tissue was isolated from the healed skin of each group of rats for the histopathological examination (Marja *et al*., 1999). The cross-sectional full-thickness skin specimens from each group were collected at the end of the experiment to evaluate for the histopathological alterations. Samples were fixed in 10% formalin, processed and blocked with paraffin and then sectioned into 5 µm and stained with Hematoxylin and Eosin (HE), Van Gieson's (VG) and Toluidine Blue (TB) stains (Raquel Pulido *et al* ., 2000) Sections were analyzed and scored as mild (+), moderate (++) and severe (+++) for epidermal or dermal re-modeling. Re-epithelization or ulcus in epidermis; fibroblast proliferation, mononuclear and/or polymorphonuclear cells, neovascularization and collagen depositions in dermis were analyzed to score the epidermal or dermal re-modeling. Van Gieson's stained sections were checked for collagen deposition and toluidine blue stained sections checked for metachromatic staining of mast cells. At the end of the examination, all the wound healing processes were combined and staged for wound healing phases as inflammation, proliferation and re-modeling in all groups.

RESULTS AND DISCUSSION

The phytochemical analysis

The methanolic leaf extract of A. paniculata upon preliminary screening phytochemicals of secondary metabolites showed the presence of alkaloids, flavonoids, glycosides, steroids, tannins and Terpenoids (Table 1). The phytochemicals are non- nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. There are many phytochemicals in fruits and herbs and each works differently. Many plants and their extracts used against microbial infections due to the presence of secondary metabolites such as phenols (Kazmi et al., 1994) essential oils (Daferera, 2003), terpenoids (Taylor et al., 1995) alkaloids (Omulokoli et al., 1997) and flavonoids (Batista et al., 1994).The phytochemical analysis of methanolic leaf extracts of A. paniculata showed the presence of alkaloids, flavonoids, glycosides, steroids, tannins and terpenoids. Similar results were reported by Radha et al. (2011) in leaf extract of A. paniculata using various solvents. Sule et al. (2012) reported the bioassay guided isolation from DCM and MEOH extract and afforded 3-O-β-d-glucosyl-14-deoxyandrographiside, 14-deoxyandrographolide, and 14-deoxy-11, 12-didehydroandrographolide as antifungal compounds. The compounds such as 2, 6, 10-Trimethyl, 14-Ethylene-14-Pentadecne, 3, 7, 11, 15-Tetramethylhexadec-2-EN-1-OL, Neophytadiene, Hexadecanoic acid, Methyl ester, n-Hexadecanoic acid, Phytol, Andrographolide, Phthalic acid, mono-(2-ethylhexyl) ester, All-trans-Squalene, 13,15-Octaosadiyne, alpha- Tocopherol, 7-(2-Hydroxy-1-Methyl)-1,4A-Dimethyl-2, 3, 4A, 5, 6, 7, 8-Octahydro-2-M, Stigmasta-5, 23-Dien-3-OL, (3.BETA.) and Gamma-Sitosterol were found to be present in the GC-MS analysis of methanolic leaf extract of A. paniculata. Several workers have reported numerous compounds in different solvent fractions such as petroleum ether, ethanol, aqueous and methanolic extracts. These chemical compounds may be the reason for the wide spectrum of activity against fungal pathogens.

Table 1: Phytochemical analysis of methanolic leaf extract of *A. paniculata.*

Phytochemicals	Present/Absent
Alkaloids	+
Flavonoids	+
Glycosides	+
Steroids	+
Tannin	+
Terpenoids	+

+ (Presence) ; - (Absent).

Effects of topical application of *A. paniculata* extracts in incision wound model.

The results of wound healing effects of methanolic extract of *A. paniculata* showed significant wound healing activity in both excision and incision wound models. The effect of different concentration of methanolic extract of *A. paniculata* on incision wound model in rats was showed in the (Table 2) compares the breaking strength of the healed skin of different groups. In incision wound model, extract at high dose of methanolic extract of *A. paniculata* (400 mg kg^{-1} b.w) showed highly significant ($p<0.05$) changes in breaking strength (496.62 ± 0.30) when compared to control rats as shown in (Table 2). Minimum breaking strength was noticed (312.57 ± 1.33) in untreated control rats (Group I). A significant increase was observed in the skin breaking strength of the methanol extract-treated group at both low dose (100 mg kg^{-1} b.w.) and moderate dose (200 mg kg^{-1} b.w.) levels when compared with control group of animals.

Table 2: Effects of topical application of *A. paniculata* extract in incision wound model.

Groups	Breaking strength (g)	Granulation tissue wet Weight (mg)	Granulation dry weight (mg)	Hydroxyproline (mg gm^{-1} tissue)
Group I (Control - Untreated)	312.57 ± 1.33	91.2 ± 0.21	7.1 ± 0.41	37.7 ± 0.81
Group II (*A. paniculata* extracts - Low dose)	383.90 ± 0.17 *	131.4 ± 1.11*	21.1 ± 0.78*	79.7 ± 1.10*
Group III (*A. paniculata* extracts - Moderate dose)	438.21 ± 0.54*	139.8 ± 0.12*	24.1 ± 0.48*	86.1 ± 0.46*
Group IV (*A. paniculata* extracts - High dose)	496.62 ± 0.30 *	192.4 ± 0.41*	25.1 ± 0.71*	97.2 ± 1.48*
Group V (Standard FSC)	497.20 ± 1.28 *	194.0 ± 0.21*	26.7 ± 1.48*	97.7 ± 0.41*

Values are expressed as mean ± SD of six animals in each group Statistical comparison: Group II, III, IV and V vs Group I * - Significant at 5 % ($p < 0.05$), ns - not significant.

Methanolic extract of *A. paniculata* (100 mg kg^{-1} b.w, 200 mg kg^{-1} b.w and 400 mg kg^{-1} b.w) treated animals (group II, group III and group IV) showed a significant ($p<0.05$) increase in both wet granulation weight and dry granulation weight in a dose dependent manner when compared to control animals (group I). The animals treated with methanolic extract of *A. paniculata* (400 mg kg^{-1} b.w) indicated significantly high ($p<0.05$) levels of hydroxyproline (97.2 mg g^{-1}) when compared with control (Group I) rats (37.7 mg g^{-1}). Methanolic extract of *A. paniculata*-treated groups showed a significant dose-dependent increase in hydroxyproline content when compared to control animals. Significant changes ($p<0.05$) were not observed in the high dose treated rats (Group IV) when compared with the standard drug (Framycetin sulphate cream) treated rats (Group V) in all the parameters.

Wound healing process involves several steps, which involves coagulation, inflammation, formation of granulation tissue, matrix formation, remodeling of connective tissue, collagenization and acquisition of wound strength (Kaufman *et al*., 1988). The wound breaking strength is determined by the rate of collagen synthesis and in the initial stages wound will be having little breaking strength because the clot alone will be holding the edges together. Thereafter it increases rapidly as collagen deposition increases and cross-linkages are formed between the collagen fibers (Shanbhag *et al*., 2006; Ramachandra, 2012).

Collagen is a major protein of the extracellular matrix and is the component that ultimately contributes to wound strength (Hassan *et al*., 2011). The healing process depends, to a large extent, on the regulated biosynthesis and deposition of new collagens and their subsequent maturation (Puratchildy *et al*., 2006). Collagen, the major component which strengthens and supports extra cellular tissue is composed of the amino acid, hydroxyproline, which has been used as a biochemical marker for tissue collagen (Kumar *et al*., 2006). Highest breaking strength of the wounded skin was observed in the animals treated with methanolic extract of *A. paniculata* (400 mg/kg b.w). The increase in breaking strength of wounded skin indicates the promotion of collagen fibers and reveals that the disrupted surfaces are firmly knit by collagen. Hydroxyproline is an uncommon amino acid present in the collagen fibers of granulation tissues. Biochemical analysis revealed increased hydroxyproline content, which is a reflection of increased cellular proliferation and therefore increased collagen synthesis (Ricard and Ruggiero, 2005). The methanolic extract of *A. paniculata* demonstrated a significant increase in the hydroxyproline content of the granulation tissue indicating increased collagen synthesis and deposition.

Granulation tissue is formed in the final part of the proliferative phase is primarily composed of fibroblasts, collagen, edema, and new small blood vessels (Singh *et al*., 2005). The increase in dry granulation tissue weight in the test treated animals suggests higher protein content. Since granulation tissue from incision wounds treated with the methanolic extract of *A. paniculata* showed greater breaking strength, it may be inferred that it not only increases collagen synthesis per cell, but also aids in cross linking of the protein. Significant increase was also observed in skin breaking strength and hydroxyproline content which was a reflection of increased collagen levels by increased cross linking of collagen fibres. In addition, increase in dry granulation tissue weight indicated the presence of higher protein content (Manjunatha, 2006). The breakdown of collagen liberates free hydroxyl proline and its peptides and elevated level of hydroxyl proline is the index of increased collagen turnover and that was further supported by histological evidence and gain in granuloma breaking strength.

Effect of methanolic extract of *A. paniculata* leaf on wound area, percentage of wound contraction and epithelialization period of incision wound model in rats

In incision wound model, the mean closure of wound area was calculated on the 5, 10, 15 and 20th post-wounding days as shown in (Table 3) (Fig. 1). The methanolic extract of *A. paniculata* at all the doses (100 mg kg^{-1} b.w, 200 mg kg^{-1} b.w and 400 mg kg^{-1} b.w. respectively) showed significant ($p<0.05$) decrease in mean scar area (41.24 ± 1.15, 24.14 ± 0.71 and 6.46 ± 0.14 respectively) as compared to control (136.14 ± 0.81) on day 20. The open area of the incised wound had decreased significantly ($p<0.05$) in methanolic extract of *A. paniculata* (400 mg kg^{-1} b.w) treated rats (group IV) was observed on day 10 and even more on day 20 as compared to control rats (group I). The percentage of wound contraction rate includes by recording the changes in wound area at fixed intervals of time, viz 5th, 10th, 15th and 20th day after treatment. Maximum wound contraction (99.87 %) was observed on 20th day with the standard drug treated rats (Group V) but the untreated control rats (Group I) showed the percentage contraction of only 72.77 %. Between 5 to 20 days of post wound treatment, the wound contractions of methanolic extract of *A. paniculata* treated groups (100, 200 and 400 mg kg^{-1} b.w) were found to be significantly ($p<0.05$) higher than the untreated control rats (group I) in a dose dependent manner. The mean period of epithelialization of the control group was 27.14 ± 1.21days. It was significantly ($p<0.05$) reduced to 20.15 ± 1.26 days in methanolic extract of *A. paniculata* treated rats (400 mg kg^{-1} b.w). The mean period of epithelialization in standard drug treated rats (Group V) was 19.09 ± 0.43 days which was significantly ($p<0.05$) reduced when compared with that of the untreated control rats (Group I). Meanwhile, the methanolic extract of *A. paniculata* showed a non significant activity when compared to the standard drug treated rats (Group V). Wound contraction is the process of mobilizing healthy skin surrounding the wound to cover the denuded area. This centripetal movement of wound margin is believed to be due to the activity of my fibroblast (Gabbaiani *et al*., 1976). Since methanolic extract of *A. paniculata* enhanced wound contraction, it would have either enhanced contractile property of my fibroblasts or increased the number of my fibroblasts recruited into the wound area. In incision wound model, methanolic extract of *A.* paniculata fasten the period of epithelization significantly by 20th day. It infers that methanolic extract of *A.paniculata* may be able to promote epithelization either by proliferation of epithelial cells or by increasing the viability of epithelial cells.

Table 3: Effect of methanolic extract of *A. paniculata* leaf on wound area, percentage of wound contraction and epithelialization period of incision wound model in rats.

Wound area (mm^2) mean ± SE and percentage of wound contraction					
Groups	**5th day**	**10th day**	**15th day**	**20th day**	**Epithelization period**
Group I (Control-Untreated)	391.41 ± 1.34 (21.71)	357.84 ± 0.75 (28.43)	209.12 ± 1.35 (58.17)	136.14 ± 0.81 (72.77)	27.14 ± 1.21
Group II (*A.paniculata* extract Low dose)	300.21 ± 0.61* (39.95)	231.74 ± 0.87* (53.65)	143.43 ± 1.75* (71.37)	41.24 ± 1.15* (91.75)	24.35 ± 0.89*
Group III (*A.paniculata* extract Moderate dose)	239.46 ± 1.21* (52.10)	176.74 ± 1.35* (64.65)	78.14 ± 1.26* (84.37)	24.14 ± 0.71* (97.17)	22.47 ± 0.46*
Group IV (*A.paniculata* extract High dose)	212.14 ± 1.46* (60.77)	158.11 ± 0.21* (64.37)	35.71 ± 0.91* (92.85)	6.46 ± 0.14* (99.50)	20.15 ± 1.26*
Group V (Standard FSC)	206.41 ± 1.12* (58.71)	146.34 ± 1.11* (70.73)	34.43 ± 0.91* (93.11)	4.11 ± 0.02* (99.87)	19.09 ± 0.43*

Values are expressed as mean ± SD of six animals in each group. Values in parenthesis represent wound closure (%) calculated relative to the wound diameter on day 0 Statistical comparison: Group II, III, IV and V vs Group I * - Significant at 5 % ($p < 0.05$), ns - not significant.

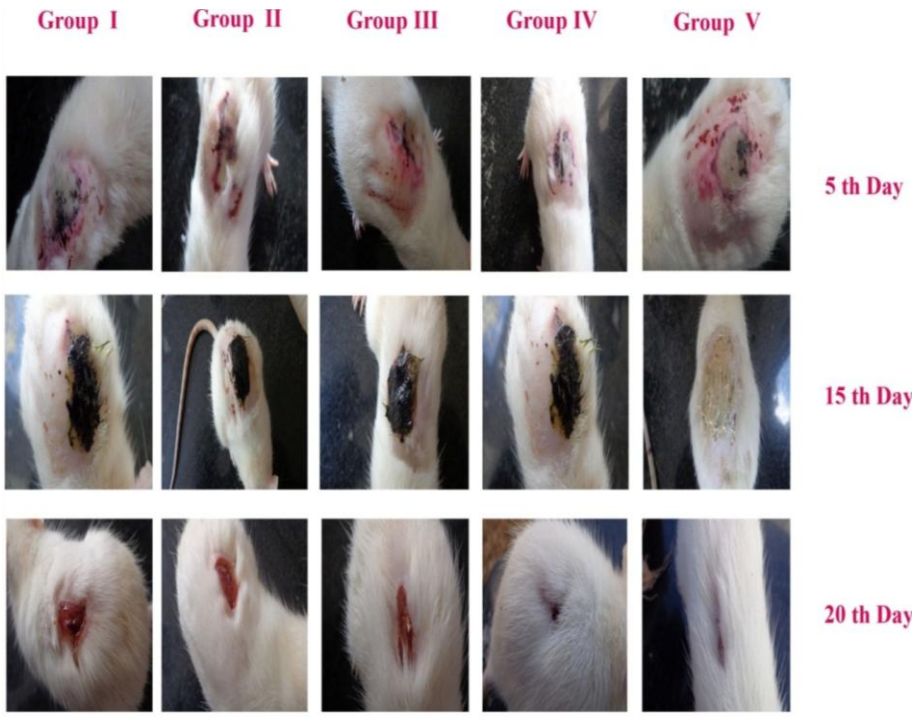

Fig 1: The Photographic representation of wound healing area on different days of experimental rats shown in the Figure. High dose and standard drug treated rats showed faster healing which was comparable with low and moderate doses treated groups.

Effect of methanolic extract of *A. paniculata* on percentage of wound contraction and epithelialization period of excision wound model in rats

In the excision wound model, the progressive wound contraction was noted with the different doses of methanolic extract of *A. paniculata* and the standard drug as shown in (Table 4). In excision wound model, the percentage wound contraction was significantly ($p<0.05$) increased by the curative effect of all the doses (100 mg kg^{-1} b.w, 200 mg kg^{-1} b.w and 400 mg kg^{-1} b.w.) by decreasing the time of epithelization period (22.45 ± 0.73,18.48 ± 0.12 and 17.88 ± 1.38 days respectively) as compared to untreated control rats (Group I). Significant changes were not observed in the high dose treated rats (Group IV) when compared with the standard drug treated rats (Group V). It was also observed that epithelization period of treated and standard group were less in comparison to untreated rats (Group I). The wound contraction of standard drug and methanolic extract of *A. paniculata* treated groups was found to be significant ($p<0.05$) in comparison to untreated rats (Group I). The standard drug also facilitated the rate of wound contraction significantly from 2nd day to 14th day. The percent rate of wound contraction in rats, treated with methanolic extract of *A. paniculata* (400 mg kg^{-1} b.w) and standard drug treated rats (Group V) was showed increase in wound contraction from 28.17 % on day 2 to 99.04 % on day 14 and 16.29 to 99.56 % from day 2 to day 14, respectively.

Table 4: Effect of methanolic extract of *A. paniculata* on percentage of wound contraction and epithelialization period of excision wound model in rats.

Groups	Epithelization Period (days)	Excision Wound model % of Wound contraction in different days						
		2	4	6	8	10	12	14
Group I (Contra- Untreat)	28.18± 1.56	12.69± 0.41	34.71± 0.73	41.28± 0.72	46.24± 0.41	52.34± 0.74	62.74± 0.76	66.74± 0.75
Group II (*A.paniculata* extract Low dose)	22.45± 0.73*	24.12± 1.26*	49.46± 0.86*	54.12± 0.76*	74.21± 0.96*	84.46± 0.71*	94.28± 0.78*	96.28± 0.42*
Group III (*A.paniculata* extract Moderate dose)	18.48± 0.12*	26.48± 0.76*	57.41± 0.73*	64.81± 0.53*	91.75± 0.24*	94.21± 0.66*	96.21± 0.63*	98.12± 0.48*
Group IV (*A.paniculata* extract High dose)	17.88± 1.38*	28.17± 0.48*	61.41± 0.76*	73.21± 0.71*	92.41± 0.76*	96.07± 0.73*	97.28± 0.48*	99.04± 0.35*
Group V (Standard FSC)	16.29± 0.49*	31.17± 0.14*	64.18± 0.41*	74.14± 0.34*	94.21± 0.92*	97.74± 0.41*	98.15± 0.47*	99.56± 0.76*

Values are expressed as mean ± SD of six animals in each group Statistical comparison: Group II, III, IV and V vs Group I * - Significant at 5 % ($p < 0.05$), ns - not significant.

Effect of *A. paniculata* extract on different biochemical parameters in rats

Biochemical analysis of untreated control (Group I) rats showed significantly ($p<0.05$) decreased levels of (table 5) AST, ALT, ALP, urea, creatinine and protein in serum when compared to high dose (400 mg kg-1 b.w) and standard drug post wound treated rats. Topical application of A. paniculata extracts different doses post wound treated rats gradually increased the levels compared to untreated control animals. Significant changes were not observed in the high dose treated rats (Group IV) when compared with the standard drug treated rats (Group V).

AST is more selectively a liver paranchymal enzyme than AST (Pingale, 2009). ALT is a sensitive indicator of acute liver damage and elevation of this enzyme in non hepatic disease is unusual. Normally AST ans ALP are present in high concentration in liver. Due to hepatocyte necrosis or abnormal membrane permeablity, these enzymes are releassed from the cells and their levels in the blood increases (Xu et al., 2013).

Wound healing is a complex phenomenon involving a number of well constructive processes. The processes include regeneration of parenchymal cells migration and proliferation of both parenchymal and connective tissue cells. Further processing includes synthesis of extra cellular matrix protein, remodeling of connective tissues parenchymal components collagenization and acquisition of wound strength. In this study, excision wound model were used to establish the healing potentials of methanolic extract of *A.paniculata*, were performed. From the results, it is evident that the methanolic extract possesses a definite potential healing action. It showed significant percentage closure by enhanced epithelialization which may be due to individual or combined action of phytochemical constituents like flavanoids, alkaloids, saponins and tannins present in it.Few studies are supported that phytochemical constituents like flavanoids, alkaloids, saponins and tannins are known to promote the wound-healing process (Tuschiya *et al*., 1996; Mukherjee, 2002; Madhura *et al*., 2002; Ansel, 2006). From the experimental results it was clear that methanolic extract at 400 mg/kg b.w had better activity on wound healing compared to other doses. Biochemical analysis showed that all functions of rats were normal, post wound healing and this indicates the methanol extract was non-toxic when applied topically and it was confirmed that it won't interfere with normal body function.

Table 5: Effect of *A. paniculata* extract on different biochemical parameters in rats.

Groups	AST	ALT	ALP	Urea	Creatinine	Protein
Group I (Control- Untreated)	21.12±2.05	19.81±2.02	145.41±5.21	12.75±1.04	0.66±0.05	5.75.±0.25
Group II (*A.paniculata* extract Low dose)	20.83±2.05	18.41±2.05	144.72±5.56	12.88±1.05	0.71±0.05	5.99±0.35
Group III (*A.paniculata* extract Moderate dose)	23.71±2.07	20.31±2.07	148±6.00	13.00±2.22	0.81±0.03	6.15±0.45
Group IV (*A.paniculata* extract High dose)	31.31±2.04*	28.71±2.07*	191.72±7.48*	18.34±2.45*	1.45±0.15*	6.85±0.23*
Group V (Standard FSC)	31.21±2.07*	29.89±2.07*	198.49±7.91*	20.13±2.55*	1.47±0.05*	6.90±0.30*

Values are expressed as mean ± SD of six animals in each group Statistical comparison: Group II, III, IV and V vs Group I * - Significant at 5 % ($p < 0.05$), ns - not significant. Units: AST, ALT - µ moles of pyruvate liberated / L, ALP - µ moles of phenol liberated / L, Urea, Creatinine - mg / dl, Protein-g / dl

Effect of *A. paniculata* extract on different hematological parameters in rats

The levels of hemoglobin, PCV, RBC and platelets were found to be significantly ($p<0.05$) increased, whereas, WBC levels were significantly decreased in (Group IV and Group V) post wound treated animals when compared with (Group I) untreated control animals. Topical application of methanolic extract significantly altered the hematological parameters. Significant variations were not observed in Group IV post wound treated rats compared to standard drug (FSC) treated rats. High dose extract significantly reduces infection and compared to low, moderate doses which made high dose of *A. paniculata* with its better wound closure time as a better would healing activity (Table 6). Administration of ethanolic extract of *Plumbago indica* with its better wound closure time as well as reducing WBC counts even when compared to commercial wound healing agent like Burnol, aminacrine hydrochloride, cetrimide IP (Jeba Kumar *et al*., 2013).

Table 6: Effect of *A. paniculata* extract on different hematological parameters in rats.

Groups	Hb (g %)	PCV (%)	WBC (10^6 µL)	RBC (10^{12} µL)	Platelets (10^9 µL)
Group I (Control- Untreated)	10.22±0.33	30.68±2.56	10.93±0.33	5.12±0.15	5.46±0.26
Group II (*A.paniculata* extract Low dose)	10.6±0.22	31.08±2.56	8.20±0.45	5.23±0.19	5.73±0.25
Group III (*A.paniculata* extract Moderate dose)	11.00±0.43	33.00±2.65	8.12±0.47	5.55±0.25	5.88±0.15
Group IV (*A.paniculata* extract High dose)	13.4±0.46*	36.12±2.34*	6.55±0.44*	6.66±0.34*	6.89±0.14*
Group V (Standard FSC)	13.6±0.45*	36.18±2.33*	6.34±0.48*	6.78±0.33*	6.99±0.15*

Values are expressed as mean ± SD of six animals in each group Statistical comparison: Group II, III, IV and V vs Group I * - Significant at 5 % ($p < 0.05$), ns - not significant.

Histopathological view of the granulation tissue of various treatment groups in dead space model

The cross sectional full thickness skin specimens from each group were collected at the end of the experiment to evaluate for the histopathological analysis. The tissues were examined by light microscope for epidermal remodeling shown in the (Fig.2). Histology of granulation tissue of control and low dose treated rats showed mononuclear inflammatory cells, fibroblast proliferation and few proliferating vasculature in granulation tissue, while the granulation tissue of rats treated with high and standard drug showed reduce inflammatory cells, fibroblast proliferation with few inflammatory cells.

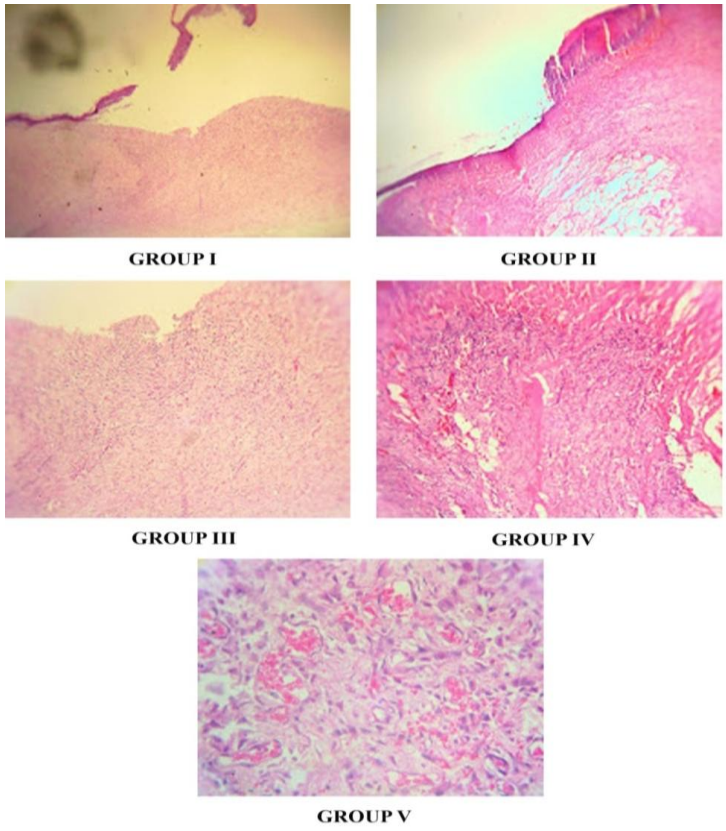

Fig.2. Histopathological view of the granulation tissue of various treatment groups in dead space model.

CONCLUSION

The current study revealed that treated with *A.paniculata* extracts as topical application of wounds significantly enhanced the wound-healing process. The wound healing study results manifest the potent wound healing of methanolic extract of *A. paniculata* as evident from the wound contraction, increased tensile strength and increased biochemical parameters in healing tissue, thus validate the ethnotherapeutic claim.

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