Velmurugan C. *et al.* / *International Journal of Phytopharmacology*, 4(3), 2013, 152-157.

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

GOSSYPIUM HERBACEUM HASTEN WOUND HEALING IN DEXAMETHASONE DELAYED WOUND HEALING MODEL IN RATS

C. Velmurugan[*1], Anurag Bhargava[2], S Vijaya Kumar, P.R. Logesh kumar[4], T. Thiyagarajan[4] and S. Vetriselvan[5]

[1]Department of Pharmacology, Sri Krishna Chaithanya College of Pharmacy, Madanapalle, Andhra Pradesh-517325, India.
[2]Department of Pharmacognosy, CH. Devi lal College of Pharmacy, Jagadhri, Haryana-135003, India.
[3]Department of Pharmacognosy, Sri Krishna Chaithanya College of Pharmacy, Madanapalle, Andhra Pradesh-517325, India.
[4]Department of Pharmaceutical Chemistry, Sri Krishna Chaithanya College of Pharmacy, Madanapalle, Andhra Pradesh-517325, India.
[5]Department of Pharmacology, Masterskill University, Malasiya.

ABSTRACT

The wound healing activity of ethanol and ethyl ether fractions of leaves of *Gossypium herbaceum* (EFGH and EEFGH) was investigated by dexamethasone delayed wound healing model in rats. The dexamethasone is an example of diabetic and Immunosuppressed delayed wound healing model. The plant *Gossypium* were shows decreased glucose level against dexamethasone. In excision wound model wound contraction area is increased and decrease the epithelization period, scar area and significantly increase percentage of wound healing with *Gossypium* treated groups. In incision model combination of extract plus dexamethasone and individual fractions significantly increases the breaking strength. Hydroxyproline content significantly increased with treated groups compare to dexamethasone group. The result suggest, *Gossypium* have good anti-diabetic and immune-stimulant effect which will helpful for fast healing of wounds in infectious and disease condition like diabetes.

Key words: Excision wound, Incision wound, *Gossypium herbaceum*, Dexamethasone, Diabetes, Immunosuppressant.

INTRODUCTION

Wound infections are most common in developing countries, such as Sub-Saharan African and South Asian countries, than in developed countries. Current estimates indicate that nearly 6 million people suffer from chronic wounds worldwide (Kumar B *et al*., 2007). Wounds are major case of physical disabilities (Baddui *et al*., 2011) . A wound which is disturbed state of tissue caused by physical, chemical, microbial (or) immunological insults (or) typically associated with loss function. According to the wound healing society wounds are physical injuries that results in an opening (or) break of the skin that cause disturbance in the normal skin anatomy and function (Strodtbeck F, 2001). Wound healing is an interaction of complex cascade of cellular and bio chemical actions healing to the restoration of structural and functional integrity with regain of strength of injured tissues. Involves continuous cell – cell interaction and cell matrix interactions that allow the process to proceed in different overlapping phases and process including inflammation , wound contraction , Re epithelialization tissue, re modeling , & formation of granulation tissue with angiogenesis (Martin P, 1991). Many Ayurvedic plants have a very important role in the process of wound healing. Plants are more potent healers because they promote the repair mechanisms in the natural way (Chitra shenoy MB *et al*., 2009). Plant based

Corresponding Author

Velmurugan C
Email: velu0906@gmail.com

therapy not only accelerate healing process and also maintains the aesthetics (Galal EE *et al*., 1965). The plant base materials are used first aid – antiseptic coagulants and wound wash.

The Survey of literature reveals that the medicinal plant of *Gossypium herbaceum* Linn. known as Karpasa and also called Karpasaaki, Samudranta, Tundikeri, Cavya, Picu belongs to the family Malvaceae is used in Ayurveda to treat various diseases. In Ayurveda the properties of Karpasa are Katu (Pungent), Kashaya (Astringent) in rasa; Laghu (Light), Tikshna (Penetrating) guna; Ushna (Hot) virya and Katu (Pungent) vipaka. It is used in Anartava (Amenorrhoea), Kashtartava (Dysmenorrhoea) and Prasutipashchatavikara (Purpueral disorders) (Chunekar KC, 1982; Sharma PC *et al*., 2001). Roots are thermogenic, emollient, abortifacient, emmenogougue, diuretic, haematopurative (Khare CP, 2007) and root bark is anticancerous(Jain SK, 1991). *Gossypium herbaceum* contains a Gossypol. Gossypol is a male contraceptive. It also assists menstrual flow and effectively inhibits egg implantation (Choudhry RR *et al*., 1980; Krishna Reddy M *et al*., 1984). Gossypol and its derivatives have been shown to have significant antimicrobial activity as well as wound healing effect (Reddy UM *et al*., 1981). However, the primary wound healing activity of *Gossypium herbaceum leaves* has been scientifically documented and it has proved that leaves of GH would involve in wound healing of normal rats in different model (Velmurugan C *et al*., 2012). As we know several factors delay (or) reduce the wound healing process including bacterial infection, necrotic tissue, & interference with blood supply, lymphatic blockage, immunosuppressant & diabetes mellitus, generally if the above factors could be altered by any agent, an increased healing rate could be achieved (Chitra P *et al*., 1998). Hence, the present study is undertaken for the investigation of the leaves of *Gossypium herbaceum* in dexamethasone delayed wound healing activity in Wistar albino rats.

MATERIAL AND METHODS

Preparation of extracts

The collected leaves were shade dried completely. The dried leaf was then coarsely powdered and was sieved (sieve # 60) to get uniform powdered. The powdered materials was loaded in Soxhlet's extractor and defatted with N-hexane. The marc was dried and extracted with solvent ethyl ether and 80% ethanol in a Soxhlet's apparatus. Final compound was concentrated by vacuum drying. The traces of the solvents were removed by keeping the dried extracts in to desiccators.

Preliminary phytochemical screening

The fractions of leaves of *Gossypium herbaceum* was screened for the presence of various phytoconstituents like alkaloids, flavonoids, saponin, tannin and glycosides etc. (Kokate CK, 1986)

Experimental Animals

All the experiments were carried out using Swiss Albino mice (25-30 g) and Wister rats (150-200 g). The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2oC and relative humidity of 30–70%. A 12:12 light: day cycle was followed. All animals were allowed free access to water and fed. Ethical clearance was obtained from Institutional Animal Ethical Committee (IAEC) of Sri K.V College of pharmacy, Chickballapur, Karnataka. No: SKVCP/IAEC/PGCOL/11-12/08

Acute oral toxicity studies

The acute toxicity study was carried out with fractions of GH as per OECD 425 Guidelines. Mortality in each group within 24 h was recorded. The animals were observed for a further 14 days for any signs for delayed toxicity.

PHARMACOLOGICAL ACTIVITY

Experimental study design

Wistar albino rats weighed about 150-200g were divided into six groups of six rats each

Group I: Control, administer tween -80 (1% V/V)

Group II: Receive ethanol fraction of *Gossypium herbaceum* (EFGH) 200 mg/kg orally.

Group III: Receive ethyl ether fraction of *Gossypium herbaceum* (EEFGH) 200 mg/kg orally 200 mg/kg orally

Group IV: Receive Dexamethasone 4mg/kg/sc

Group V: Dexamethasone 4mg/kg/sc + EFGH 200 mg/kg orally

Group VI: Dexamethasone 4mg/kg/sc + EEFGH 200 mg/kg orally

Dosing schedule

Tween 80 and fractions of *Gossypium herbaceum* were administered orally daily from day 0^{th} to 15^{th} post-operative day. Dexamethasone was given subcutaneously on alternative days (from day 0^{th} to 15^{th} postoperative day)

Excision wound

All the animals were anesthetized by using Thiopentone sodium [25 mg/kg, ip]. An impression was making on the dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear on the anaesthetized rat. The particular skin area was shaved prior to the experiment. The skin of impressed area was excised to the full thickness to obtain a wound area of about 500 mm^2. Haemostasis was achieved by blotting the wound with cotton swab soaked in normal saline.

Animals were treated daily with drugs as mentioned above under experimental design from 0th day to 15th post-wounding day. Wound area is measured on 15th day post wounding for determination of wound contraction and percentage wound contraction was calculated. Falling of scar leaving no raw wound behind was taken as end point of complete epithelization and the days required for this was taken as period of epithelization. The blood glucose level of all the animals was checked with digital rite chek glucometer on 0^{th} and 15^{th} day (Nayak BS *et al*., 2007).

Incision wound

All groups of rats made Para vertebral straight incision of 6 cm length was making through the entire thickness of the skin, on either side of the vertebral column with the help of a sharp scalpel under anesthetic condition. After complete homeostasis, the wound was closed by means of interrupted sutures placed at equidistance points about 1 cm apart. Animals were treated daily with drugs as mentioned above under experimental design from 0th day to 15th post-wounding day. The wound breaking strength was determined on 15th day by tensiometer. The blood glucose level of all the animals was checked with digital rite chek glucometer on 0^{th} and 15^{th} day (Baie SH *et al*., 2000; Sharma S *et al*., 2008).

Hydroxyproline estimation

Determination of the hydroxyproline content on the 15^{th} day, the animals from each group were euthanized using diethyl ether and used to determine hydroxyproline content. The protein content of the tissue was estimated using the techniques described by Neuman RE *et al*., 1950. The wound tissue was excised and its weight recorded. The tissue was dried in oven at 60 ◦C for 12 h and the dry weight was again noted. They were hydrolyzed in 6N HCl for 24 h at 110 ◦C in sealed glass tubes. The hydrolysate was neutralized to pH 7.0. The samples (200µl) were mixed with 1ml of 0.01M CuSO4 followed by the addition of 1ml of 2.5N NaOH and then 1ml of 6% H2O2. The solution was mixed and shaken occasionally for 5 min. All the tubes were incubated at 80 ◦C for 5min with frequent vigorous shaking. Upon cooling, 4ml of 3N H2SO4 was added with agitation. Finally, 2ml of 5% p-dimethylaminobenzaldehyde was added. The samples were incubated at 70 ◦C for 16 min, cooled by placing the tubes in water at 20 ◦C, and the absorbance was measured at 500 nm using a digital photo colorimeter (EI Products, India). The amount of Hydroxyproline in the samples was calculated using a standard curve prepared with pure L-Hydroxyproline at the same time.

Statistical analysis

Experimental data are expressed as mean±standard error of mean (SEM). Statistical analysis was performed by one-way ANOVA followed by Dunnett's method of multiple comparisons was employed using Graphpad Instat 3.0 software. Data were considered significant at $p < 0.05$.

RESULT

Preliminary phytochemical screening

The preliminary phytochemical analysis of fractions of *Gossypium herbaceum* shows presence of steroids, alkaloids, flavonoids, glycosides, tannin and carbohydrate (Table 1).

Table 1. Phytochemical screening of different fractions of *Gossypium herbaceum*

Fractions	Steroids	Alkaloids	Glycosides	Saponin	Flavonoid	Tannin	Carbohydrates
Ethyl ether	-		-	-	+	+	-
ethanol	-	+	+	-	+	+	-

Table 2. EFGH and EEFGH effect on wound contraction, scar area, period of epithelization and Percentage of wound healing in excision wound model

Groups	Wound contraction in mm^2	Scar area in mm^2	Epithelization period in days	Percentage of wound contraction
Groups I	391 ± 2.59^{a}	108 ± 2.98^{b}	26.5 ± 0.34^{b}	2.56
Groups II	229 ± 1.46^{c}	43.66 ± 1.68^{d}	19.5 ± 0.42^{d}	42.93
Groups III	183.5 ± 2.26^{c}	34.16 ± 1.35^{d}	17.66 ± 0.61^{d}	54.27
Groups IV	401.3 ± 6.36^{a}	137.16 ± 1.35^{a}	29.5 ± 0.56^{a}	0
Groups V	292.83 ± 3.42^{b}	75.66 ± 1.45^{c}	22.5 ± 0.61^{c}	27.02
Groups VI	280 ± 3.73^{b}	67.5 ± 1.92^{c}	21 ± 0.96^{c}	30.22

Significant difference at P<0.01when compared to dexamethasone control and between groups. Values are Mean ± SEM from 6 animals in each group. Means bearing different superscript differ significantly.

Table 3. EFGH and EEFGH effect on Incision wound model

Groups	Tensil strength/Breaking strength
Groups I	219±3.4[b]
Groups II	289±2.5[c]
Groups III	336±3.2[c]
Groups IV	185±1.2[a]
Groups V	244±3.2[b]
Groups VI	256±2.7[b]

Significant difference at P<0.01 when compared to dexamethasone control and between groups. Values are Mean ± SEM from 6 animals in each group. Means bearing different superscript differ significantly.

Acute toxicity

The ethyl ether and ethanol fractions of *Gossypium herbaceum* had good margin of safety and did not shown any lethal effects on the animals up to the doses of 2000mg/kg. Hence the LD50 of both fractions of *Gossypium herbaceum* was considered as 2000mg/kg. Studies were carried out with 1/10 of the LD50 as effective dose 200mg/kg.

Excision wound

In excision wound model, ethanol, ethyl ether fractions and fractions plus dexamethasone groups revealed significantly increases the wound contraction area and decreases the scar area and epithelization period when compared to dexamethasone control. Group II and Group III significantly differ from Group I, IV, V and Group VI. Group I is no significant to Group IV in wound contraction area but significantly different from Group V and VI. Group II, III, V and VI shows significant variation in scar area and epithelization compare to Group I and IV (Table 2).

Incision wound

The group II, III, V and VI significantly increase the breaking strength or tensil strength when compare to Group IV. The Group I significantly differ from Group II, III, IV and no significant to Group V and VI (Table 3).

Hydroxyproline content

The measurement of hydroxyproline can be used as an index for collagen turnover (Neuman RE *et al.*, 1950). Increase in hydroxyproline content indicates increased collagen synthesis which in turn leads to enhanced wound healing. In our study, the hydroxyproline content was found to be significantly increased in Group-II&III ($p < 0.01$) as compared with the Group-IV, I, V & VI. (Figure1). The content was found to be increased in the Group- I, V & VI as well, but group I was not significant to Group IV.

Blood glucose level

The individual and combination of fractions of gossypium with dexamethasone shows significant decrease in blood glucose level in both models compare to dexamethasone induced control on 15th day. An ethyl ether fraction shows more significant reduction compare to other groups (Figure 2&3).

Figure 1. EFGH and EEFGH effect on hydroxyproline content

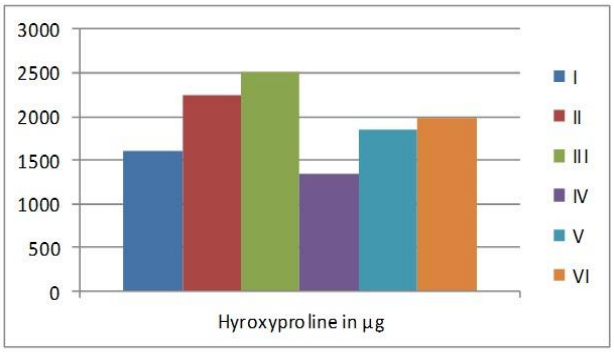


Figure 2. EFGH and EEFGH effect of blood glucose level on excision wound model rats at 0th and 15th day

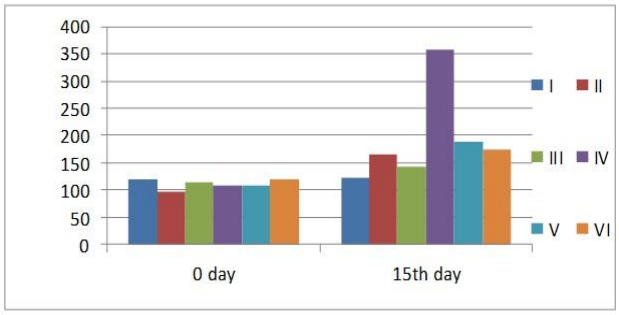
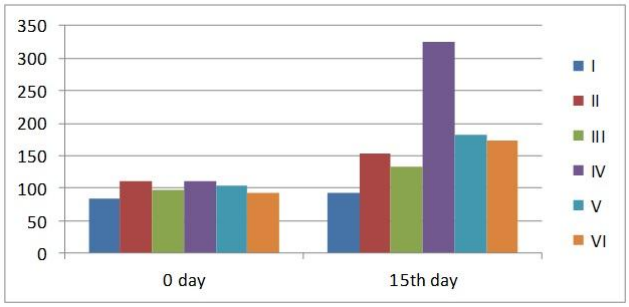


Figure 3. EFGH and EEFGH effect of blood glucose level on excision wound model rats at 0th and 15th day



DISCUSSION AND CONCLUSION

Wound healing is the interaction of a complex cascade of cellular and biochemical actions leading to the restoration of structural and functional integrity with regain of strength of injured tissues. It involves continuous cell-cell interaction and cell-matrix interactions that allow the process to proceed in different overlapping phases and processes including inflammation, wound contraction, reepithelialization, tissue remodelling, and formation of granulation tissue with angiogenesis.

The phases of wound healing normally progress in a predictable, timely manner, and if they do not, healing may progress inappropriately to either a chronic wound (Martin P, 1991). The dexamethasone model is a best model for delayed wound healing because it will suppress the immune system at the same time increase the blood sugar level. A well-known that immune cells regulating cytokines which is necessary for proliferative phase of wound healing and immune cells like neutrophil, monocyte, macrophage and lymphocyte involved in inflammatory phase of wound healing. Within a few hours after injury, inflammatory cells invade the wound tissue. Neutrophils arrive first within a few minutes, followed by monocytes and lymphocytes. They produce a wide variety of proteinases and reactive oxygen species as a defense against contaminating microorganisms, and they are involved in the phagocytosis of cell debris.

In addition to these defense functions, inflammatory cells are also an important source of growth factors and cytokines, which initiate the proliferative phase of wound repair. In addition to the importance of cell-cell and cellmatrix interactions, all stages of the repair process are controlled by a wide variety of different growth factors and cytokines. Multiple studies have demonstrated a beneficial effect of many of these growth factors such as platelet-derived growth factors (PDGFs), fibroblast growth factors (FGFs), and granulocyte-macrophage colony stimulating factor (GM-CSF) on the healing process, both in animal models and also in patients suffering from different types of wound healing disorders (Abraham JA *et al.*, 1996; Edmonds M *et al.*, 2000; Greenhalgh DG, 1996; Harding KG *et al.*, 2002; Nath C *et al.*, 1998).

Dexamethasone is a potent and highly selective glucocorticoid used in the treatment of inflammation. High exposure to glucocorticoids impairs insulin sensitivity, contributing to the generation of metabolic syndrome including insulin resistance and hypertension (Kabosova A *et al.*, 2003). The mechanism by which dexamethasone induces peripheral insulin resistance is by inhibiting GLUT-4 translocation (Dake QI *et al.*, 2004). The increased blood glucose level leads to delayed wound healing (Haber RS et al., 1992). Patients with diabetes often have difficult-to-heal wounds. The initial barrier to healing is an increased blood glucose level, which causes cell walls to become rigid, impairing blood flow through the critical small vessels at the wound surface and impeding red blood cell permeability and flow and impaired hemoglobin release of oxygen results in oxygen and nutrient deficits in the wound. A less than optimal immune function also contributes to poor wound healing in the patient with diabetes.

When blood glucose levels are persistently elevated, chemotaxis and phagocytosis are compromised. Chemotaxis is the process by which white cells are attracted to the site of an infection; phagocytosis is the ingestion of bacteria by white cells. Both processes are important in wound infection control. Diabetic infections take longer to heal because of delayed macrophage introduction and diminished leukocyte migration, which causes a prolonged inflammatory phase in the wound healing cascade (Kane DP, 2001).

Healing is a physiological process and does not normally require much help but still wounds cause discomfort and are prone to infection and other complications. Therefore, use of agents expediting healing is indicated. Further, some diseases like diabetes, immunosuppressed conditions, ischemia and conditions like malnourishment, ageing, local infection and wounds lead to delay in healing. Such conditions specially require the use of agents, which can facilitate healing. The plant *Gossypium herbaceum* has been, therefore, studied for its wound healing activity in dexamethasone induced delayed wound healing in rats. Our result shows significant increased wound contraction, breaking strength and decreased epithelization period with different fractions of GH. Collagen, the major protein of the extracellular matrix, is the component that ultimately liberates free hydroxyproline and its peptides. Therefore, measurement of hydroxyproline can be used as an index for collagen turnover (Neuman RE *et al.*, 1950). Increase in hydroxyproline content indicates increased collagen synthesis corresponding to enhanced wound healing. So once again *Gossypium herbaceum* confirmed the rapid wound healing property with increased Hydroxyproline content. The phytochemical test revealed presence of flavanoids and tannins in both fractions may be responsible for fasten wound healing by decreasing the glucose level or increases the growth factor involved in wound healing or both.

From this study we concluded plant *Gossypium herbaceum* speedup the inflammatory and proliferative phases of wound healing and its more valuable plant to treat chronic wound or wounds in diabetic and immunosuppressed patient.

REFRENCES

Abraham JA, Klagsbrun M. Modulation of wound repair by members of the fibroblast growth factor family In, The Molecular and Cellular Biology of Wound Repair (2nd ed.), edited by Clark RAF. New York: Plenum, 1996, p. 195–248.

Baddui, Prakesh ,Nagori. Role of medicinal plants in wound healing, *Research Journal of medicinal plants*, 5(4), 2011, 392-40.

Baie SH, Sheikh KA. The wound healing properties of channa striatuscertrimide cream-wound contraction and glycosaminoglycan measurement. *Journal of Ethnopharmacology*, 73, 2000, 15-30.

Chitra P, Sajithalal GB, Chandrakasan G. Influence Aloe Vera, on collagen turnover in healing of dermal wounds in rates. *Indian journal of Exp. Biol*, 36, 1998, 896-901.

Chitra shenoy MB, Patil Ravikumar. Preliminary phytochemical investigation and wound healing activity of Allium.Cepalin (Liliaceae). *International journal of pharmacy and pharmaceutical sciences*, 2(2), 2009, 1-5.

Choudhry RR, Haq M, Gupta U. Review of plants Screened for antifertility activity – III. *Bulk Medico Ethnobot Res*, 1(4), 1980, 542-545.

Chunekar KC. Bhavprakash Nighantu. Varanasi: Chukhambha Bharati Acadamy; 1982, p.374.

Dake QI, Thomas P, Ding AN. *Diabetes* triggers a PARP1 mediated death pathway in the heart through participation of FoxO1. *Diabetes*, 53, 2004, 1790–97.

Edmonds M, Bates M, Doxford M, Gough A, Foster A, New treatments in ulcer healing and wound infection. *Diabetes Metab Res Rev*, 16(1), 2000, 51–54.

Galal EE, Gawad MA. Antidiabetic activity of Egyptian onion Allium cepa extract. *J Egypt Med Assoc*, 48, 1965, 14-45.

Greenhalgh DG. The role of growth factors in wound healing. *J Trauma*, 41, 1996, 159–167.

Haber RS, Weinstein SP. *Role of glucose transporters in glucocorticoid-induced insulin resistance. GLUT4 isoform in rat skeletal muscle is not decreased by dexamethasone. Diabetes*, 41, 1992, 728–735.

Harding KG, Morris HL, Patel GK. Science, medicine and the future: healing chronic wounds. *Br Med J*, 324, 2002, 160–163.

Jain SK. Dictionary of Indian Folk Medicine and Ethnobotany. New Delhi: Deep publication, 1991, p.96.

Kabosova A, Kramerov AA, Aoki AM, Murphy G, Zieske JD, Ljubimov AV. Human diabetic corneas preserve wound healing, basement membrane, integrin and MMP-10 differences from normal corneas in organ culture. *Exp Eye Res*, 77, 2003, 211– 217.

Kane DP. Surgical repair in advanced wound caring. In: Krasner DL, Rodeheaver GT, Sibbald RG. Chronic Wound Care: A Clinical Source Book for Healthcare Professionals. 3rd ed. Wayne, PA: HMP Communications; 2001, 404.

Khare CP, Indian Medicinal Plants. New Delhi: Springer (India) Private Limited; 2007, p.293

Kokate CK. In: Practical Pharmacognosy, Preliminary Phytochemical Screening, first ed., Vallabh Prakashan, New Delhi, 1986, 111.

Krishna Reddy M, Ravi A, Kokate CK, Chari N. Effect of some drug combinations on menstrous cycle in albino rats. *East Pharma*, 27(321), 1984, 139-140.

Kumar B, Vijayakumar M, Govindarajan R, Pushpangadan, P. Ethnopharmacological approaches to wound Healing Exploring medicinal plants of India. *J. Ethnopharmacol*, 114, 2007, 103–113.

Martin P. Wound healing – Aiming for perfect skin regeneration. *science.* 276 (5309), 1991, 75-81.

Nath C, Gulati SC. Role of cytokines in healing chronic skin wounds, *Acta Haematol*, 99, 1998, 175–179.

Nayak BS, Anderson M, Periara LM, Pinto, Evaluation of wound healing potency of Catharanthus roseus leaf extract in rats. *Fitoterapia*, 78(7-8), 2007, 540-544.

Nayak SB, Pinto Pereira LM. *Catharanthus roseus* flower extract has wound healing activity in Sprague Dawley rats. *BMC Complementary and Alternative Medicine*, 6, 2006, 41–46.

Neuman RE, Logan MA. The determination of hydroxyproline. *Journal of Biological Chemistry*, 184, 1950, 299–306.

Reddy UM, Reddy MM, Reddy SM. Antibacterial activity of leaf extracts of *Gossypium herbaceum*. *Geobios*, 8(6), 1981, 277-278.

Sharma PC, Yelne MB, Dennis TJ. Database on Medicinal Plants Used in Ayurveda, New Delhi: Documentation and Publication Division, CCRAS, 2, 2001, 331.

Sharma S, Sikarwar MS. Wound healing activity of ethanolic extract of leaves of Eclipta alba, *Pharmacognosy Magazine*, 4(13), 2008, 108-111.

Strodtbeck F. Physiology of wound healing, new born infant nurse. *Rev.I*, 2001, p 43-45.

Velmurugan C, Venkatesh S, Sandhya K, Bhagya Lakshmi S, Ramsila Vardhan R, Sravanthi B. Wound healing activity of methanolic extract of leaves of *Gossypium herbaceum*. *Central European Journal of Experimental Biology*, 1 (1), 2012, 7-10.