Research & Reviews

ISSN: 2319-9865

Research and Reviews: Journal of Medical and Health Sciences

Wound Healing and Indigenous Drugs: Role as Antioxidants: A Review

B Somashekar Shetty*

*Department of Biochemistry, American University of Antigua, West Indies and Melaka Manipal Medical College, Manipal Campus – 576104, Karnataka, INDIA

Review Article

Received: 14/02/2013
Revised: 13/03/2012
Accepted: 20/03/2013

***For Correspondence**

Associate Professor in Biochemistry American University of Antigua, College of Medicine, Coolidge, Saint John's Antigua, West Indies P.O.W-1451.

Keywords: Wound healing, Indigenous drugs, antioxidants, Immune system

ABSTRACT

Traditional medicine, especially herbal medicine has recently been receiving heightened interest all over the world. Man from the very beginning has been aware of the health problems and has been taking care of health through diet and drugs for which plants were used extensively. Molecular oxygen plays a central role in the pathogenesis and therapy of chronic wounds. When reactive oxygen species are overproduced, oxidative stress results in detrimental cytotoxic effects causing delayed wound healing. Therefore, elimination of reactive oxygen species (ROS) could be an important strategy to improve healing of chronic wounds. Currently therapeutic strategies targeting reactive oxygen species (ROS) by antioxidants are being introduced into the treatment of chronic wounds. Natural compounds from medicinal plants having antioxidant and immunomodulatory activities are used as therapeutic agents. Indian medicinal plants with these activities have been identified and their antioxidant and immunomodulatory effects have been reviewed.

INTRODUCTION

Wound may be defined as interruptions of cellular and anatomic continuity of living tissue. Wound may arise due to physical, chemical or microbial agents. Every wound is unique and each deserves individual care. Thus the process of wound healing has been one of the earliest medical problems. It is not wound closure that is important, but it is the quality of the scar and restoration of functional competence that is important. Healing is thus essentially a survival mechanism, and represents an attempt to maintain normal anatomical structure and function. Several scientific studies revealed that many of the plant products are useful in the early and proper maturation of granulation tissue and also enhance the deposition of collagen.

Background on Indian Medicinal Plants

Medicinal plants are an important element of indigenous medicinal systems in most of the countries including India. India is perhaps the world's largest producer of medicinal herbs and is rightly called the "Botanical Garden of the World". The revival of interest in natural drugs especially those derived from plants started in the last decade mainly because of the widespread belief that "Green Medicines" are healthier and safer than the synthetic ones. In recent years, the use of such information in medicinal plants research has received renewed interest in the media and in some segments of the scientific community. In the last decade of the 20th century, the 'Western' use of such information has come under increasing scrutiny and the national and indigenous rights on these resources have become acknowledged by most academic and industrial researchers. Development of new herbal drugs is a field of intense activity in recent years and hence the need for such basic scientific investigations on medicinal plants used in indigenous medicinal systems becomes ever more evident[1].

ISSN: 2319-9865

Screening of the traditional medicinal plants are expected to provide drugs for antibiotic resistant infectious diseases, new epidemics, various types of cancers, wound healing, aging related ailments and AIDS. Efforts are also underway to genetically engineer the plants to acquire the life saving medicinal properties. Recent WHO studies indicate that over 30% of the world's plant species have been used for medicinal purposes. The products relating to about 20,000 higher plant species are being marketed world over. About 120 chemical compounds of plant origin have been developed into modern pharmaceuticals [1].

Immune System and Indian Medicinal Plants

Reactive oxygen and nitrogen species are generated during the functioning of the immune system, such as in phagocytosis. Their overproduction they can affect the components of the immune system by inducing oxidative damage. Natural compounds from medicinal plants having antioxidant and immunomodulatory activities have potential as therapeutic agents in this regard. Indian medicinal plants with these activities have been identified and their immunomodulatory and antioxidant effects have been reviewed.
Oxidative stress may influence the immune system either by hyperexcitation to cause autoimmune disorders or suppress it, resulting in higher susceptibility to infections. Excited mental status are now known to enhance the generation of excessive free radicals leading to several disorders. Ayurvedic rasayana drugs may play a major role in handling these problems. However, their full potential still remains to be explored.

The modulation of immune response by using medicinal plant products as a therapeutic measure has become a subject of active scientific investigation. The basic concept has, however, existed in the ancient Vedic scripture, the Ayurveda and has been practiced in Indian traditional medicine that evolved in India thousands of years ago, probably represented the first record of scientific medicine in the history of world. Indian medicinal plants are rich source of substances that are claimed to induce paraimmunity, the non-specific immunomodulation of essential granulocytes, macrophages, natural killer cells and complement functions[2].

The immune system plays an integral role in successful wound healing. In addition to contributing to host defences and inflammation, immune cells are critical regulators of wound healing through the secretion of cytokines, lymphokines and growth factors[3].

Therapeutic agents derived from the plant and animals were used by ancient man whenever he was confronted with illness and disease. Over the ages the observed knowledge of these medicinal substances and their toxic potential were passed on by oral tradition and eventually annotated in herbals and texts of Materia Medica. Study of traditional medicine on sound scientific method is desirable and relevant in any field of drug development. It is more so in the area of wound pharmacology. Such investigations can provide useful materials that can complement technological advances and surgical skills. Further, such investigations can improve our understanding of wound biology and provide therapeutic tools that may help in successful management of abnormal healing. So, an investigation into traditional/folk medicine from this angle is necessary.

Indian medicinal plants with immunomodulatory and antioxidant activities of many Indian medicinal plants have been studied by various workers. Following are some of the examples, namely *Aloe vera, Andrographis paniculata, Asparagus racemosus, Azadirachta indica, Curcuma longa, Emblica officianalis, Glycyrrhiza glabra, Piper betel, Terminalia chebula, Tinospora cordifolia, Allium sativum, Allium cepa* etc[4]..

Investigation of the endogenous status of free radical scavengers during cutaneous wound healing were studied in immunocompromised rats. Antioxidants and lipid peroxidation (interms of malondealdehyde-MDA) have been monitored in the wound tissue of immunosuppressed rats at different intervals following cutaneous injury. A significant increase in MDA content and decrease in glutathione, catalase, glutathione peroxidase and vitamin C content was observed in the skin of immunocompromised rats as compared to control subjects [5].

OXYGEN FREE RADICALS AND WOUND HEALING

Our present day understanding of wound healing has been greatly enhanced over the past 25 years by research elucidating the role of oxygen in this process. Oxygen gradients have been identified across the healing wound, confirming the well perfused periphery of the wound as compared to its relatively anoxic central part [6,7,8,9].. Studies modulating the oxygen tension of the wound demonstrate the beneficial effect of increasing the amount of oxygen delivered to the wound and the adverse effect of hypoxia [10,11,12]. Despite the beneficial effects of oxygen on collagen synthesis and rates of epithelization, it is also somewhat paradoxically apparent that hypoxic tissue gradients and anaerobic metabolism are important in the initiation of collagen synthesis and angiogenesis[13]. The role of oxygen in preventing wound infection has similarly been established[14]. All oxygen-dependent mechanisms for intracellular killing by leukocytes result in the generation of highly reactive oxygen metabolites[15]. These oxygen metabolites, collectively termed oxygen radicals, have also been implicated in a variety of ischemic and inflammatory diseases. It is only recently that the effects of these radicals on wound healing have been investigated and clarified. Despite the importance of oxygen in promoting wound healing and preventing wound infection, it is now apparent that oxygen metabolites may in many situations be deleterious to wound healing[7]. or may result in tissue injury [16,17,18].

ISSN: 2319-9865

Increasing evidence implicates excessive reactive oxygen species (ROS) generation and ROS derived degradation products in the pathogenesis of many skin diseases. Several attempts have been made to identify prognostic biomarkers of wound healing in skin but they are of limited success[19]. Oxygen radicals are produced by the univalent reduction of molecular oxygen, which is catalyzed principally by two enzyme systems, xanthine oxidase and NADPH dehydrogenase. Xanthine oxidase is principally generated in ischemic tissues, whereas NADPH dehydrogenase is a normal component of the neutrophil intracellular killing mechanism. Oxygen radicals are generated principally in ischemic or inflamed tissues. Cells have the ability to protect themselves from these radicals by a variety of enzyme systems. Nevertheless, the production of these highly reactive oxygen metabolites can initiate chain reactions that produce further oxidants capable of cellular injury. Oxygen radicals cause tissue injury by lipid peroxidation of membranes at both the cellular and the organelle level by degrading intracellular matrix and by oxidation of important protein and enzyme systems [19].

Inflammation and ischemia are both components of wound healing. One can speculate a possible role of oxygen radicals in wound healing based on their production by neutrophils involved in the inflammatory response and by reperfusion of ischemic tissues, which occurs during angiogenesis that is always seen at the ischemic wound edge. Oxygen radicals might be involved in promoting the cascade of inflammatory events that could be both beneficial and detrimental to wound healing. Oxygen radicals have been shown to nonenzymatically hydroxylate proline and lysine residues, thereby increasing collagen synthesis[20]. This increased collagen deposition and scar formation have been implicated in pathologic disease states such as retrolental fibroplasia, Peyronie's disease, pulmonary alveolitis[21]. and venous stasis ulceration[22]. The role of oxygen derived free radicals in normal wound healing can be indirectly demonstrated by examining the effects of oxygen radical scavengers on normal wound healing. Shandall and colleagues [23].examined colonic healing in rabbits by performing histology, breaking strength of granulation tissue and hydoxyproline content of tissue. Control animals received intravenous normal saline before ischemia was induced. Experimental groups received either aprotinin, a proteinase inhibitor that blocks the production of oxygen radicals by polymorphonuclear leukocytes (PMNs), superoxide dismutase (SOD) or allopurinol. This study demonstrated that colonic healing after anastomosis was improved by aprotinin as measured by the multiple parameters mentioned. Animals treated with SOD demonstrated histologic protection against tissue injury and decreased glutathione oxidation, confirming the decreased oxygen radical production. Allopurinol failed to demonstrate a significant improvement in healing as measured by wound tensile strength, hydroxyproline production, or histologic damage. These findings led the authors to conclude that oxygen radical production by PMNs represented an allopurinol insensitive route and that PMNs play a significant role in reperfusion injuries. The importance of oxygen in colonic anastomotic healing had been demonstrated by these authors. SOD was also found to improve survival and to limit ischemic injury caused by free radicals in an ischemic bowel anastomosis model in rats. Survival rates in rats treated with intravenous SOD undergoing bowel transection and anastomoses in a segment of bowel rendered ischemic by vessel ligation were not statistically significant[23]. SOD in a burn wound model, failed to improve epithelization and wound contraction rates. Further evidence of the direct involvement of oxygen radicals in wound healing is supported by Hogstrom[24]. In this unique set of experiments, wound strength was measured in various tissues within first 48hours of wounding. This time frame is important clinically because collagen deposition has yet to occur to any significant degree and suture material alone is primarily responsible for wound strength. The ability of the wound margin to hold these sutures has not previously been investigated[7].

Antineutrophil serum (ANS) effectively reduced the circulating PMN counts by more than 95% in the ANS treated animals, whereas the lymphocyte count was reduced by approximately 70% in those animals treated with preimmune serum (PIS) [25]. Animals pretreated with ANS failed to demonstrate the early decrease in wound tensile strength as compared with those animals treated with PIS, implicating the role of neutrophil in this early wound margin decrease tensile strength. With the neutrophil implicated, the role of oxygen free radicals were investigated by pretreating with SOD and catalase followed by 24hour continuous infusions. Control animals received saline infusions. The combination of these oxygen radical scavengers prevented approximately 50% of the decrease in breaking strength that occurred in saline treated animals[7].These findings suggest that oxygen free radicals were partly responsible for the early decrease in wound tensile strength. Pretreatment for 5 days with allopurinol prior to ilioanastomosis prevented approximately 30% of the decrease in breaking strength. Because oxygen radical scavengers and allopurinol failed to completely explain the decrease in tissue strength, the authors next investigated leukocyte proteinases as possible mechanisms. Hogstrom concludes that most cases of early wound dehiscence are caused by the inability of the wound margins to withstand the strains imposed upon them by the suture materials and result from a decrease in wound margin strength occurring after closure of the wound. The experiments described propose a significant role of the neutrophil and specifically for neutral proteinases and oxygen free radicals produced by the neutrophil. To date, these are the studies most clearly implicating the oxygen radical directly involved in wound healing.

CELL PROLIFERATION AND REACTIVE OXYGEN SPECIES

Proliferation of vascular cells is a key feature in vascular biology, wound healing and pathophysiological processes such as atherosclerosis. In atherosclerotic intima, cell proliferation co-localizes with oxidized LDL that indicate a local oxidative stress. During wound healing of confluent cell layer, cell proliferation associated with healing also induced enhanced extracellular ROS generation and LDL oxidation. Proliferation associated extracellular ROS generation is mediated through mitogenic signalling pathways. Data obtained with inhibitors of oxidases suggest that proliferation associated extracellular ROS are not generated by a single ROS generating system

ISSN: 2319-9865

and are not essential for cell proliferation. In conclusion, proliferating vascular cells generate high levels of extracellular ROS generating systems by mitogenic signalling. This constitutes a link between proliferative events and oxidative stress, LDL oxidation in atherosclerotic lesions [26].

Antioxidant vitamins, plant products such as carotenoids and polyphenolic flavonoids are potent antioxidant and wound healing substances in nature.

As free radical scavengers, flavonoids inhibit lipid peroxidation, promote vascular relaxation to prevent prolonged wound healing[27]. A sufficient supply of antioxidant in diet might help to prevent or delay the occurrence of pathological changes associated with oxidative stress.

PLANTS AS WOUND HEALING AGENTS

Research on wound healing is a developing area in modern biomedical sciences. Scientists who are trying to develop newer drugs from natural sources are looking toward the Ayurveda, the Indian traditional system of Medicine. Several drugs of plant and animal origin are described in the Ayurveda for their wound healing properties under the term *vranaropaka.* Most of these drugs are derived from plant origin. Some of these plants have been screened scientifically for the evaluation of their wound healing activity in different pharmacological models and patients, but the potential of most remains unexplored. In a few cases, active chemical constituents were identified. This part presents a limited review of plants used to study the wound healing activity.

***Aloe vera*-** This plant belongs to the family Liliaceae. The therapeutic effects of *Aloe vera* in preventing progressive dermal ischemia caused by burns, frost bite, electrical injury, distal dying flap and intra-arterial abuse were examined and the plant exhibited significant healing activity[28]. Comparative evaluation of *Aloe vera* was done in the management of burn wounds using Hartley guinea pigs. *Aloe vera* gel extracts were compared with silver sulfadiazine, salicylic acid cream and plain gauze occlusive dressing. It was found that *Aloe vera* gel extracts permit faster healing of burn wounds[29]. *Aloe vera* could exhibit the actions of both anti-inflammation and wound healing promotion when applied on a second degree burn wound[30]. *Aloe vera* treatment of wounds in diabetic rats may enhance the process of wound healing by influencing phases such as inflammation, fibroplasia, collagen synthesis and maturation and wound contraction. These effects may be due to the reported hypoglycemic effects of the aloe gel[31]. Use of an *Aloe vera* gel resulted in full healing after treatments such as antibiotics, surgical debridement and skin grafting had failed[32]. Topical and oral treatments with *Aloe vera* were found to have a positive influence on the synthesis of glycosaminoglycans and thereby beneficially modulate wound healing[33]. Glycoprotein fraction isolated from *Aloe vera* is involved in the wound-healing effect via cell proliferation and migration[34]. Antioxidant components in *Aloe vera* were examined for lipid peroxidation using rat liver microsomal and mitochondrial enzymes. Among the aloesin derivatives examined, isorabaichromone showed a potent antioxidative activity. As one of the most potent components, isorabaichromone together with feruloylaloesin and p-coumaroylaloesin showed potent superoxide anion scavenging activities. Electron spin resonance (ESR) using the spin trapping method suggested that the potent superoxide anion scavenging activity of isorabaichromone may have been due to its caffeoyl group. As *Aloe vera* has long been used to promote wound healing, the inhibitory effects of aloesin derivatives for cyclooxygenase (Cox)-2 and thromboxane (Tx) A 2 synthase were examined and the participation of p-coumaroyl and feruloyl ester groups in the aloesin skeleton was demonstrated. These findings may explain, at least in part, the wound healing effects of *Aloe vera*[35].

***Aristolochia bracteolata*-** (Family Aristolochiaceae. It is a perennial herb and twining plants found in the tropical and temperate regions of the world. Eight species are known to occur in India, of which *A.bracteolata, A.indica, A.tagala* are of medicinal importance. They generally contain alkaloids and reputed to be useful in the treatment of snakebites. The leaf paste is used by the tribals and the villagers in the Chittoor district of Andhra Pradesh for the rapid healing of cuts and wounds[36]. *A.bracteolata* leaf extract has got wound healing activity, supported by an enhancement in the levels of antioxidant enzymes in the granulation tissue [37].

***Anogeissus latifolia*-** (Family: Combretaceae) This is a deciduous tree, found in drier areas. Wound healing potential of ethanolic extract of *Anogeissus latifolia* bark for treatment of dermal wounds in rat was studied on excision and incision wound. The results obtained indicated that the plants accelerate the wound healing process by decreasing the surface area of the wound and increasing the tensile strength [38].

***Areca catechu*-** (Family: Leguminosae) The effects of *Areca catechu* (betel nut) extract and its two constituents namely arecholine and polyphenols in male Wistar rats were studied on three wound models (excision, incision and dead space wound) and it was found that except arecholine both polyphenol and the crude extract promoted wound healing by increasing wound breaking strength, percent wound contraction and hydroxyproline level in the granulation tissue[39].

***Argemone mexicana*-** (Family: Papavaraceae) Ethanolic crude extract of *Argemone mexicana* and its five different crude fractions (petroleum ether, solvent ether, ethyl acetate, butanol and butanone) were screened for wound healing activity in incision, excision and

ISSN: 2319-9865

dead space wound models in albino rats. Ethanol extract and its petroleum ether and butanol fractions exhibited significant wound healing activity [40].

***Azadirachta indica*-** (Family: Papillonaceae) Evaluation was carried out on Neem oil obtained from *Azadirachta indica* and neem ointments in paraffin bases, in experimentally created incised and gap wounds in bovine calves. The progress of healing was monitored and resulted in effective and potent healing [41].

***Butea monosperma*-** (Family: Leguminosae) The topical administration of an alcoholic bark extract of *Butea monosperma* increased cellular proliferation and collagen synthesis at the wound site, evidenced by increase in DNA, total protein and total collagen content of granulation tissues. The extract treated wounds were found to heal much faster as indicated by improved rates of epithelization and wound contraction which was also confirmed by histopathological examinations. In addition, the plant also showed antioxidant properties by its ability to reduce lipid peroxidation [42].

***Calendula officinalis*-** (Family: Araceae) Studies carried out on fractions isolated from the flowers of *Calendula officinalis* in combination with allantoin in Wistar albino rats by inducing skin wounds surgically showed that this combination markedly stimulates physiological regeneration and epithelization. The ethanol extract and gel from the stem bark of *Calendula officinalis* and *Stryphnodendron barbadetinam* were found to be effective, in the treatment of domestic sun burn cases in Brazil and in the treatment of patients with various ulcers and skin lesions[43]. The effect of tinctures of *Calendula officinalis* has compared with *Hypericum* species on wound healing in male albino rats. It was concluded that the tincture of *Hypericum* enhanced the wound breaking strength and reduced the period of epithelization more effectively as compared to tincture of Calendula [44].

***Cassia alata*-** (Family: Leguminaceae) Investigation on the wound healing activity of an ointment containing *Cassia alata* leaf extract in rabbits showed that the healing was better when the extract was formulated in a polyethylene glycol base[45].

***Celosia argentea*-** (Family: Amaranthaceae) This plant is used in traditional medicine for sores, ulcers and skin eruptions. Wound closure occurred earlier in the treated rats. Granulation tissue showed an increase in collagen and hexosamine content at a faster rate in the treated groups. The alcoholic extract promoted cell motility and proliferation of primary dermal fibroblasts but did not alter these responses in primary keratinocytes[46].

***Centella asiatica*-** (Family: Umbelliferae) This plant is reputed for its medicinal use in skin diseases. The effect of the triterpenoid fraction of *Centella asiatica* on macromolecules was studied in human skin fibroblast cultures. A statistical increase in the percentage of collagen helps to explain the action of the triterpenoid fraction in promoting wound healing[47]. An enzyme linked immunosorbent assay was used to determine the levels of secretion of type I and type III collagen in human fibroblast cultures with or without asiaticoside and madecassoside (both triterpenes) isolated from *Centella asiatica*. These triterpenes were shown to stimulate collagen secretion. Type I secreted collagen was increased by 25%-30% with asiaticoside and madecassoside. However, only madecassoside was able to increase significantly collagen III secretion[48]. Asiaticoside, isolated from *Centella asiatica*, promotes fibroblast proliferation and extracellular matrix formation in wound healing[49]. Oral and topical administration of the plant extract shown increased fibrocytic activity, new vessel formation and epidermal healing in chronic wound[50]. The *Centella asiatica* leaf extract promotes the wound healing activity [51]. in rats and also overcome the wound healing suppressing action of dexamethasone[51].

***Cinnamomum zeylanicum*-** (Family: Lauraceae) The ethanol extract of the bark extract of *Cinnamomum zeylanicum* was evaluated for wound healing activity in Wistar rats. The extract significantly enhanced the wound breaking strength in the case of incision wound, the rate of wound contraction and the period of epithelization in the case of excision wound. The granulation tissue weight, its breaking strength and its hydroxyproline content was also increased by the extract in the dead space wound[52].

***Cedrus deodara*-** (Family: Pinaceae) The clinical efficacy of a herbal gel containing distilled extracts of *Cedrus deodara* and *Acorus calamus*, neem oil and eucalyptus oil was tested on dogs, suffering from wound lesions, scabies, pyoderma etc. and it was concluded that the gel is an efficacious broad spectrum and safe herbal product for skin problems[53]

***Curcuma aromatica*-** (Family: Zingiberaceae) The wound healing activity of the powdered rhizome of *Curcuma aromatica* and dry aqueous extract of *Piper betel* leaves, incorporated in an ointment with soft paraffin was studied in rabbits. The wound contraction was found to be better in the former[54]

***Curcuma longa*-** (Family: Zingiberaceae) The medicinal properties of *Curcuma longa* were reviewed for the use of this plant mainly in anti-inflammatory, wound healing, anticancer, antimicrobial, antiviral activities and the responsible constituents for the activities were found to be curcumin, curcuminoids and essential oil. Herbal treatment for septic wounds in diabetics using *Curcuma longa* powder was reported[55]. Curcuminoids from *Curcuma longa* protected normal human keratinocytes from hypoxanthine/xanthine oxidase injury. Since

ISSN: 2319-9865

curcuminoids synergistically inhibited nitroblue tetrazolium reduction, a decrease in superoxide radical formation leading to lower levels of cytotoxic hydrogen peroxide was proposed as an explanation for this protective effect[56]. Pretreatment with curcumin significantly enhanced the rate of wound contraction, decreased mean wound healing time, increased synthesis of collagen, hexosamine, DNA, and nitric oxide and improved fibroblast and vascular densities. This study demonstrates that curcumin pretreatment has a conducive effect on the irradiated wound and could be a substantial therapeutic strategy in initiating and supporting the cascade of tissue repair processes in irradiated wounds[57]. Wounds of animals treated with curcumin showed earlier re-epithelialization, improved neovascularization, increased migration of various cells including dermal myofibroblasts, fibroblasts, and macrophages into the wound bed, and a higher collagen content. Immunohistochemical localization showed an increase in transforming growth factor-beta1 in curcumin-treated wounds compared to controls. This could partly explains the reason for better healing in curcumin treated animals [58].

Davallina orientalis- (Family: Asclepidaceae) The effect of the crude methanol extract of the herb *Davallina orientalis* on bone healing using mice indicated increase in breaking strength of a fracture site[59].

Ixora coccinea- (Family: Rubiaceae) This is a medium sized hardy shrub, cultivated for ornamental purposes. The alcoholic extract of flowers of *Ixora coccinea* has promoted wound healing in dead space wound model in rats by increasing collagen deposition as well as better alignment and maturation [60].

Jatropha curcas- (Family Euphorbiceae) This plant is commonly known as Physic nut. The physic nut is a folk remedy for alopecia, burns, syphilis, dermatitis, inflammation, rash, rheumatism, ulcers, scabies and sores. The latex applied topically to bee and wasp stings and also used to dress sores and ulcers[61].The treatment with the fresh homogenized crude bark extract of the plant accelerated the wound healing process in rats by decreasing epithelialization period and increasing the wound breaking strength. [62].

Jatropha multifida- (Family: Euphorbiceae) The anti-complement constituents present in the latex of *Jatropha multifida* were found to be useful in traditional application of the latex in the treatment of infected wounds[63].

Hyptis suaveolens- (Family Labiaceae). This is an aromatic herb. The essential oil of *HS* showed antibacterial activity[64].The bark extract of the plant showed antiviral activity. When used along with *Centella asiatica*, it is active against Vibrio Cholerae, Salmonella Typhii and Staphylococcus Pyogenes[65,66]. Ethanolic extract of the plant exhibited hypoglycaemic action in rats and anticancer activity against P388 lymphocytic leukemia in mice. The animal treated with ethanolic leaf extract of the plant showed significant increase in the levels of SOD and catalase, the two powerful antioxidant enzymes of the body that are known to quench superoxide radicals [67].

Mimosa tenuiflora- (Family: Fabaceae) An active principle with skin regeneration properties was extracted from *Mimosa tenuiflora* bark using chloroform as solvent along with ethanol and water. The compound was found to be useful in burns, abrasions, eczemas and wounds[68].

Moringa oleifera- (Family: Moringaceae) This plant is also known as drumstick tree. The anti-inflammatory and wound healing properties of the root extracts of *Moringa oleifera* along with *Aegle marmelos* (Bilva) was studied. It was found that Bilva showed significant acute anti-inflammatory activity whilst *Moringa* showed significant increase in the tensile strength as well as lysyl oxidase activity[69]. The aqueous plant extract treatment showed significant increase in wound closure rate, skin-breaking strength, granuloma breaking strength, hydroxyproline content, granuloma dry weight and decrease in scar area in rats. The prohealing actions seem to be due to increased collagen deposition as well as better alignment and maturation[70].

Ocimum gratissium- (Family: Labiaceae) The wound healing effect of methanolic leaf extract of *Ocimum gratissium* was investigated in adult male Wistar rats. The results revealed that significant wound contraction in the excision wound model and ten day old granulation tissue histology showed denser inflammatory infiltrate as reflected by increased cellularity in the control group relative to that of the experimental group which though appeared adequate was not as dense as the control group[71]. Marked enhancement in the inflammatory and proliferative phases of wound healing in the rabbits treated with Ocimum oil, suggesting that the oil facilitated the healing process to a greater extent than the control and reference products[72].

Ocimum sanctum- (Family: Labiaceae) This is considered to be a sacred plant in India. It is also known as Holly basil. It is commonly called as Tulasi. Nair and Gunasegaran[73]. reported that the leaves contained ursolic acid, apigenin, luteolin, apigenin-7-O-glucuronide, 7-O-glucuronide, orientin and molludistin. Aqueous and alcoholic extracts of *O.sanctum* and their fractions were assessed for their *in vitro* free radical scavenging effect with respect to superoxide radical, hydroxyl radical, hydrogen peroxide and the extent of inhibition of lipid peroxidation. Their efficacy in preventing *in vitro* induced lipid peroxidation in red cell membranes was also investigated[741].The in vivo and in vitro free radical scavenging activity of the aqueous extract of the plant is mainly responsible for the wound healing accelerating property of the plant[75].

ISSN: 2319-9865

The ethanolic leaf extract of *O.sanctum* antagonized the action of dexamthasone by increasing the collagen synthesis, maturation and bundle formation[76]. Extract of *O.sanctum* also reduced the incidence of gastric ulcer formation in swimming endurance test in albino rats[77]. The two flavonoids of *O.sanctum*, orientin and vicenin provided protection against radiation induced lipid peroxidation in the mouse liver[78]. Anti-inflammatory activity of fixed oil of *O.sanctum* against carrageenan induced paw edema in rats has been reported by Singh S et al[79]. The antibacterial activity of *O.sanctum* leaf extract[80,81]. and essential oil from the leaves against a number of species of bacteria has been reported [82].

***Piper betle*-** (Family: Piperaceae) Treatment with the ethanolic extract of leaf of *Piper betle* at a dose of 150mg/kg body weight daily for 10days, after induction peptic ulcer by nonsteroidal anti inflammatory drugs (NSAID) in albino rats, produced significant healing effect. During the process of healing, antioxidant enzymes superoxide dismutase (SOD) and catalase activity increased and oxidized lipid and oxidatively modified proteins were reduced within 7-10days. The extract also showed significant *in vitro* free radical scavenging action. The results of their study suggested that free radical scavenging activity of the plant extract may be responsible for its healing action [83].

***Punica granatum*-** (Family: Punicaceae) The methanolic extract of dried pomegranate (fruit of the plant) peels showed the presence of phenolic compounds along with other constituents. This extract was formulated as a water soluble gel and was studied for its wound healing property against an excision wound on the skin of wistar rats. The activity was compared with that of a commercial topical antibacterial applicant. The amount of hydroxyproline was increased by two fold in the group treated with gel. Histopathological studies also supported the wound healing action upon application of the gel [84].

***Rhizophora mangle*-** (Family: Rhizophoraceae) The aqueous bark extract of *Rhizophora mangle* promoted wound healing in patients with open wounds from surgical intervention of pilonidal cyst or fistula. The faster healing of the wound could be explained by the antioxidant effect of plant polyphenols and their free radical chelating properties, and also by increase in wound contraction and proliferation of fibroblasts and capillaries [85].

***Thespesia populnea*-** (Family: Malvaceae) The aqueous extract of *Thespesia populnea* fruit showed significant wound healing activity in the excision wound and incision wound models in rats following topical and oral administration respectively [86].

***Tridax procumbans*-** (Family: Asteraceae) The aqueous extract of *Tridax procumbans* leaves showed significant increase in lysyl oxidase activity, protein content and breaking strength of granulation tissue in dead space wound[87].The effect of whole plant extract, aqueous extract, butanol fraction and petroleum ether fraction of *Tridax procumbens* were studied on dead space wound healing in albino rats. The effect of whole plant extract was found to be more active as compared to the others[88]. It has been stated that juice from the leaves of *Tridax procumbens* resembled dexamethasone in effects on wound contraction and granulation, but it significantly counteracted the effects of dexamethasone on the tensile strength and epithelization suggesting that this plant has prohealing effect [89].

***Terminalia arjuna*-** (Family: Combretaceae) This plant is commonly known as Arjuna bark, indigenous to India and Bangladesh. The oral and topical application of alcoholic extract of *Terminalia arjuna* promoted wound healing activity in excision and incision wound models[90]. The effect of topical application of phytoconstituents (fraction I, II and III) fractionated from a hydroalcohol extract of the bark of the plant, *Terminalia arjuna*, was assessed on the healing of rat dermal wounds using in vivo models. The results indicated a statistically significant increase in the tensile strength of the incision wounds and the percent epithelialization of excision wounds compared with control[91].

***Visnea mocanera*-** (Family: Theaceae) The effect of several extracts of *Visnea mocanera* was studied on the bleeding time and gastrointestinal transit in mice. This plant extracts were found to be effective in ethno-medicine for treatment in the healing of wounds[92].
***Vernonia scorpioides*-** (Family: Mimosaceae) The ethanolic extract of leaves from *Vernonia scorpioides* showed that they promote wound healing. The probable mechanism is due to proteins and wound exudates interconnected with the extract constituents favouring the local homeostasis and protecting the new tissue by forming an external cover that furnished mechanic protection[93].

***Wedelia calendulacea*-** (Family: Compositae) The effect of the aqueous extract of the leaves of *Wedelia calendulacea* on wound healing in open and sutured wound models were studied and found to be useful in wound healing[94].

DISCUSSION AND CONCLUSION

In recent years oxidative stress has been implicated in a variety of degenerative processes, diseases, and syndromes. These include acute and chronic inflammatory conditions including healing of wounds[95].The oxidants rich at the wound site were contributed mostly by macrophages and neutrophils[96]. Molecular oxygen plays a central role in the pathogenesis and therapy of chronic wounds. Overproduction of reactive oxygen species (ROS) results in oxidative stress thereby causing cytotoxicity and delayed wound healing.

ISSN: 2319-9865

Therefore, elimination of ROS could be an important strategy in healing of chronic wounds[97]. Oxygen free radicals play an important role in the failure of ischemic wound healing. Studies have shown that antioxidants improve the healing in ischemic skin wounds[98]. Low levels of antioxidants accompanied by raised levels of markers of free radical damage play a significant role in delaying wound healing in rats[99].Therefore estimation of antioxidants like SOD, catalase and glutathione in granulation tissues is also relevant because these antioxidants hasten the process of wound healing by destroying the free radicals[100].

Increasing evidence implicates excessive ROS generation and ROS derived degradation products in the pathogenesis of many skin diseases. The significant alteration in the antioxidant profile accompanied by the elevated levels of MDA, a marker of free radical damage, may be attributed to impaired wound healing in immunocompromised rats[101]. Numerous attempts have been made to identify prognostic biomarkers of wound healing in skin, these have met with limited success.

A recently coined term "Nutriceuticals" describes a variety of nonprescription products, which include mainly flavonoids to enhance health[102]. Antioxidant properties of natural products and medicinal herbs were evaluated mainly by assessing the direct scavenging action of *in vitro* generated free radicals and inhibition of lipid peroxidation and assessing indirectly the effect of that compound on endogenous antioxidant defences. Several antioxidants are ingredients of our daily diet and/or cellular components eg. β carotenes, vitamins A, C, E and flavonoids. The flavonoids are a heterogenous group of ubiquitous plant polyphenols that abound in the human diet and are endowed with the several biological activities including immunomodulating and antioxidant activities. Study of 12 different plant flavonoids has revealed that their *in vitro* antioxidant activity also works under *in vivo* conditions.

Flavonoids have been reported to protect against oxidative stress. A detailed review indicates the presence of flavonoids[103] ,which is responsible for the antioxidant and wound healing activities. Flavonoids are the plant pigments, which have the ability to inhibit specific enzymes and to scavenge free radicals which may ultimately help in the healing of wounds[104].

As free radical scavengers, flavonoids inhibit lipid peroxidation, promote vascular relaxation and help to prevent prolonged wound healing. A sufficient supply of antioxidant in the diet might help to prevent or delay the occurrence of pathological changes associated with oxidative stress.

Natural antioxidants from plants strengthen the endogenous antioxidant defenses from ROS ravage and restore the optimal balance by neutralizing the reactive species. They are gaining immense importance by virtue of their critical role in disease prevention.

In my study of wound healing and indigenous drugs, I have evaluated the wound healing efficacy and antioxidant properties of 10 different plants namely *Aristolochia bractolata* (Aristolochiacae), *Calotropis procera* (Asclepiadaceae), *Centella asiatica* (Umbelliferae), *Glycyrrhiza glabra* (Leguminaceae), *Hyptis suovilens* (Labiaceae), *Jatropha curcas* (Euphorbiceae), *Ocimum sanctum* (Labiaceae), *Phyllanthus niruri* (Euphorbiceae), *Tinospora cordifolia* (Menispermaceae) *Withania somnifera* (Solanaceae). Among the 10 indigenous plants used for screening studies, the crude extract of *Ocimum sanctum* exhibited significant prohealing activities, which was superior to remaining plant extracts investigated.

The aim of this paper was to establish the effect of oxygen free radicals, antioxidant properties of medicinal plants and the role of this property of the plants in wound healing. The antioxidant effects of plant extract have been observed on tissue regeneration, epithelialization, wound breaking strength and scar formation using experimental animal models with some of these plants. Wound healing is a clinical challenge especially where resources are limited. It therefore behoves the wound fraternity to examine all options available with which wound management may be bettered for the benefit of all.

ACKNOWLEDGMENT

The authors are thankful to Melaka Manipal Medical College (MMMC) and Manipal University, India for all support during the study. Sincere thanks to Professor S.L.Udupa, Professor A.L.Udupa and the Dean Dr. K. Ramnarayan, MMMC of Manipal University for their cooperation and valuable suggestions in many stages of the work.

REFERENCES

1. Heinrich M, Ankli A, Frei B, Weimann C, Sticher O. Medicinal plants in Mexico; Healers consensus and cultural importance. Soc Sci Med. 1998;47:1859-71.
2. Ganguly T, Saini KB. Inhibition of cellular immune responses by Tylophora indica in experimental models. Phytomedicine. 2001;8: 348-55.
3. Park JE, Barbul A. Understanding the role of immune regulation in wound healing. Am J Surg. 2004;187(5A): 11S-16S.

ISSN: 2319-9865

4. Devasagayam TP. Immune system and antioxidants especially those derived from Indian medicinal plants. Indian J Exp Biol. 2002;40: 639-55.
5. Gupta A, Singh RL, Raghubir R. Antioxidant status during cutaneous wound healing in immunocompromised rats. Mol Cell Biochem. 2002;241(1-2): 1-7.
6. Hunt TK, Zederfeldt B, Goldstick TK. Oxygen and healing. Am J Surg. 1969;118:521-25.
7. White MJ, Heckler FR. Oxygen free radicals and wound healing. Clin Plast Surg. 1990;17(3): 473-8.
8. Niinikoski, Hunt TK, Dunphy JE. Oxygen supply in healing tissue. Am J Surg. 1972;123: 247-52.
9. Remensnyder. Oxygen gradients in healing wounds. Am J Pathol. 1968;52: 301-23.
10. I Kivisaari J, Vihersaari T, Renvall S. Energy metabolism of experimental wounds at various oxygen environments. Ann Surg. 1975;18: 823-28.
11. Stephen FO, Hunt TK. Effect of changes in inspired oxygen and carbon dioxide tensions on wound tensile strength- An experimental study. Ann surg. 1971;173:515-19.
12. Vihersaari T. Effect of changes in inspired oxygen tension on wound metabolism. Ann Surg. 1974;179: 889-95.
13. Knighton DR, Hunt TK. Oxygen tension regulates the expression of angiogenesis factor by macrophages. Science. 1983;221(4617): 1283-85.
14. Knighton DR, Halliday B, Hunt TK. Oxygen as an antibiotic- The effects of inspired oxygen on infection. Arch Surg. 1984;119(2): 199-204.
15. Babior BM. Oxygen dependent microbial killing by phagocytes. New Eng J Med. 978;298: 721-5.
16. Gardner TJ. Reduction of myocardial ischemic injury with oxygen derived free radical scavengers. Surgery. 1983;94: 423-27.
17. Parks DA. Role of oxygen-derived free radicals in digestive tract diseases. Surgery. 1983;94: 415-22.
18. Taylor AE. The effects of oxygen radicals on pulmonary oedema formation. Surgery. 1983;94: 433-38.
19. Moseley R, Hilton JR, Waddington RJ, Thomas DW. Comparison of oxidative stress biomarkers profiles between acute and chronic wound environments. Wound Repair Regen. 2004;12(4): 419-29.
20. Trelstad RL. Nonenzymatic hydroxylations of proline and lysine reduced oxygen derivatives. Nature. 1981;289: 310-12.
21. McCormick JR. Suppresision by superoxide dismutase of immune complex induced pulmonary alveolitis and dermal inflammation. Am J Pathol. 1981;102: 55-61.
22. Angel MF, Ramasastry SS, Swartz WM. The causes of skin ulcerations associated with venus insufficiency- Unifying hypothesis. Plast Reconstr Surg. 1987;79(2): 289-97.
23. Shandall AA, Willams GT, Hallet MB, Young HL. Colonic healing: A role for polymorphonuclear leucocytes and oxygen radical production. Br J Surg. 1986;73(3):225-28.
24. Hogstorm H. Mechanisms and prevention of decrease in wound margin strength in intestinal anastomoses and leaparotomy wounds. Acta Chilr Scand (Suppl). 1987; 539: 1-63.
25. Hogstorm H. Neutrophil induced decrease in wound margin strength after intestinal anastomoses influence on collagen and mechanism of granulaocyte action. Eur Surg Re.s 1988;20: 260-66.
26. Duval C, Cantero AV, Auge N. Proliferation and wound healing of vascular cells trigger the generation of extracellular reactive oxygen species and LDL oxidation. Free Radic Biol Med. 2003;15; 35(12):1589-98.
27. Hirano R, Sasamoto W, Matsumato A, Itakura H, Igarashi O, Kondo K. Antioxidant ability of various flavonoids against DPPH radicals and LDL oxidation. J Nut Sci Vitaminol. 2001;47(5): 357-62.
28. Heggers JP, Pelay RP, Robson MC. Beneficial effects of *Aloe* in wound healing. Phytothe res. 1993;7(supple): 548s-52s.
29. Rodriguez, Bigas M, Cruz, Suarez A. Comparitive evaluation of *Aloe vera* in the management of burn wounds in guinea pigs. Plast Reconstr Surg. 1998;81(3): 386- 89.
30. Somboonwong J, Thanamittramanee S, Jariyaponqskul A, Putumraj S. Therapeutic effects of *Aloe vera* on cutaneous microcirculation and wound healing in second degree burn model in rats. J Med Assoc Thai. 2000 ;83(4):417-25.
31. Chithra P, Sajithlal GB, Chandrakasan G. Influence of *aloe vera* on the healing of dermal wounds in diabetic rats. J Ethanophormacol. 1998; 59(3):195-201.
32. Avijgan M. Phytotherapy: an alternative treatment for non-healing ulcers. J Wound Care. 2004;13(4):157-8.
33. Chithra P, Sajithlal GB, Chandrakasan G. Influence of *Aloe vera* on the glycosaminoglycans in the matrix of healing dermal wounds in rats. J Ethanophormacol. 1998;59(3):179-86.
34. Choi SW, Son BW, Son YS, Park YI. The wound-healing effect of a glycoprotein fraction isolated from *aloe vera*. Br J Dermatol. 2001;145(4):535-45.
35. Yagi A, Kabash A, Okamura N, Haraguchi H. Antioxidant, free radical scavenging and anti-inflammatory effects of aloesin derivatives in *Aloe vera*. Planta Med. 2002;68(11):957-60.
36. Vedavathy S, Mrudula V, Sudhakar A. Tribal medicine of Chittoor district, Andhra Pradesh India. Published by Herbal Folkore Research Center Tirupati, 1997;31-35.
37. Shrwaikar A, Somashekar AP, Udupa AL, Udupa SL, Somashekar S. Wound healing studies of *Aristolochia bracteolata* Lam. with supportive action of antioxidant enzymes. Phytomedicine. 2003;10:558-62.

38. Ig Govindarajan R, Vijayakumar M, Rao CV, Shirwaikar A, Mehrotra S, Pushpangadan P. Healing potentials of *Anogeissus latifolia* for dermal wound in rats. Acta Pharma. 2004;54(4): 331-38.
39. Padmaja PN, Bairy KL, Kulkarni DR. Prohealing effect of beetle nut and its polyphenols. Fitoterapia. 1994;65(4): 298-300.
40. Vaidya AD, Pandita N, Godse C, Meshram M. *Argemone mexicana* and epidemic dropsy. Phytopharmacology and putative sequelae and therapy. J Assoc Physicians India 2001;49: 1041-42.
41. Bhardwaj RL, Sharma DN. Evaluation of Neem oil as potent wound healer. Indian Vet Med J. 1997;21(3): 187-190.
42. Sumitra M, Manikandan P, Suguna L. Efficacy of *Butea monosperma* on dermal wound healing in rats. Int J Biochem Cell Biol. 2005;37(3): 566-73.
43. Klouchek, Popova E. Influence of the physiological regeneration and epithelization using fractions isolated from *Calendula officinalis*. Acta Physiol Pharmacol Bulg. 1982;8(4): 63-7.
44. Rao CM, Ramesh KV, Bairy KL, Kulkarni DR. A simple method to quantify maturation of wound collagen. Indian J Exp Biol. 1991;29: 156-8.
45. Palanichamy S. Wound healing activity of *Cassia alata*. Fitoterapia. 1991;62(2):153- 56.
46. Priya KS, Armugam G, Rathinam B, Wells A, Babu M. *Celosia argentea Linn* leaf extract improves wound healing in a rat burn wound model. Wound Repair Regen. 2004;12(6): 618- 25.
47. Tenni R. Effect of triterpinoid fraction of *Centella asiatica* on macromolecules of the connective matrix in human skin fibroblast cultures. Ital J Biochem. 1998;37(2): 69-77.
48. Bonte F. Asiaticoside and Madecassoside on type I and III collagen synthesis by cultured human fibroblasts. Annales Pharamceutiques Fraciass 1995; 53(1): 38-42.
49. Lu L, Ying K, Wei S, Fang Y, Liu Y. Asiaticoside induction for cell cycle progession, proliferation and collagen synthesis in human dermal fibroblasts. Int J Dermat. 2004;43(11): 801-07.
50. Biswas TK, Mukherjee B. Plant medicines of Indian origin for wound healing activity: A review. Int J Lower Extremity wounds. 2003;2:25-39.
51. Shetty BS, Udupa SL, Udupa AL, Somayaji SN. Effect of *Centella asiatica* L on normal and dexamethasone suppressed wound healing in Wistar albino rats. Int J Lower Extremity wounds. 2006;5(3):137-143.
52. Kamath JV, Rana AC, Chowdhury AR. Pro-healing effect of *Cinnamomum zeylanicum* bark. Phytother Res. 2003;17(8): 970-72.
53. Uthapp D, Sharma BN. Clinical efficacy of a herbal gel on skin disorders in dogs. Indian Vet Med J. 1996;20(3): 231-33.
54. Santharam Gand S, Nagarajan. Wound healing activity of *Curcuma aromatica* and *Piper betel.* Fitoterapia. 1990;61(5): 263-65.
55. 55. Sidhu GS, Singh AK, Thaloor D. Enhancement of wound healing by curcumin in animals. Wound Repair Regen. 1998;6(2): 167-77.
56. Bonte F, Noel-Hudson MS, Wepierre J, Meybeck A. Protective effect of curcuminoids on epidermal skin cells under free oxygen radical stress. Planta Med. 1997;63(3): 265-66.
57. Jagetia GC, Rajanikant GK. Role of curcumin, a naturally occurring phenolic compound of turmeric in accelerating the repair of excision wound, in mice whole-body exposed to various doses of gamma-radiation. Surg Res. 2004;120(1):127-38.
58. Sidhu GS, Mani H, Gaddipati JP, Singh AK. Curcumin enhances wound healing in streptozotocin induced diabetic rats and genetically diabetic mice. Wound Repair Regen. 1999;7(5):362-74.
59. Chow SP, Yeung HW, Law LK. The effect of *Davallina orientalis* on bone healing - a preliminary report. Am J Chin Med. 1982;10(1): 101-6.
60. Nayak BS, Udupa SL. Effect of *Ixora coccinea* flowers on dead space wound healing in rats. Fitoterapia. 1999;70: 233-36.
61. Watt JM, Breyer Brandwijk MG. The medicinal and poisonous plants of Southern and Eastern Africa, 2nd Ed, E and S, Edinburgh and London, 1962.
62. Shetty S, Udupa SL, Udupa AL, Vollala VR. Wound healing activities of Bark Extract of *Jatropha curcas* Linn in albino rats. Saudi Med J. 2006;27(10):1473-6.
63. Kosasi S. Inhibitory activity of *Jatropha multifida* latex on classical complement pathway activity in human serum mediated by a calcium binding proanthocyanidine. J Ethnopharamacol. 1989;27(1): 81-9.
64. Pandey DK, Asthana A, Tripathi NN, Dixit SN. Volatile plant products vis-a-vis potato pathogenic bacteria. Indian Perfume.1981;5(3,4) :10-14.
65. Jain SR, Kar A. The antibacterial activity of some essential oils and their combinations. Planta Med. 1971;20: 118-23.
66. Jain SR, Jain PR, Jain MR. Antibacterial evaluation of some indigenous volatile oils. Planta Med. 1974;26: 196-199.
67. Shirwaikar A, Shenoy R, Udupa AL, Udupa SL, Shetty S. Wound healing property of ethanolic extract of leaves of *Hyptis suaveolens* with supportive role of antioxidant enzymes. Indian J Exp Biol. 2003;41:238-41.
68. Jiang YL. Triterpenoid glycosides from the bark of *Mimosa tenuiflora*. Phytochemistry. 1991;30(7): 2357-60.
69. Rathi BS, Bodhankar SL, Baheti AM. Evaluation of aqueous leaves extract of *Moringa oleifera* Linn for wound healing in albino rats. Indian J Exp Biol. 2006;44(11):898-901.
70. Udupa AL, Udupa SL, Kulkarni DR. Studies on the anti-inflammatory and wound healing properties of *Moringa oleifera* and *Aegle marmelos.* Fitoterapia. 1994;65(2): 119-23.

71. Orafidiya LO, Agbani EO, Abereoje OA, Awe T, Abudu A. An investigation into the wound-healing properties of essential oil of *Ocimum gratissimum* linn. J Wound Care. 2003;12(9):331-4.
72. Osuagwu FC, Oladejo OW, Imosemi IO. Wound healing activities of methanolic extract of *Ocimum gratissimum* leaf in wistar rats- A preliminary study. Afr J Med Sci. 2004;33(1): 23-26.
73. Nair AGR, Gunasegaran R. Chemical investigation of some south Indian plants. Indian J Chem. 1982;21B: 979-84.
74. Geetha RK, Vasudevan DM. Inhibition of lipid peroxidation by botanical extracts of *Ocimum sanctum in vivo* and *in vitro* studies. Life Sci. 2004;76(1): 21-28.
75. Shetty S, Udupa S, Udupa L, Somayaji SN. Wound healing activity of *Ocimum sanctum* Linn with supportive role of antioxidant enzymes. Indian J Physiol Pharmacol. 2006; 50(2):163-68.
76. Udupa SL, Shetty S, Udupa AL, Somayaji SN. Effect of *Ocimum sanctum Linn*. On normal and dexamethasone suppressed wound healing. Indian J Exp Biol. 2006;44:49-54.
77. Mandal S, De K, Chaudhuri SB, Chowdhury MK, Sahana CC. Chemical and pharmacological studies of *Ocimum sanctum Linn* on stress adaptation. Indian J Pharmacol. 1990;22: 57-8.
78. Uma Devi P, Ganasoundari A, Vrinda B. Radiation protection by the *ocimum* flavonoids orientin and vicenin: mechanism of action. Radiat Res. 2000;154(4): 455- 60.
79. Singh S. Comparative evaluation of anti-inflammatory potential of fixed oil of different species of *Ocimum* and its possible mechanism of action. Indian J Exp Biol. 1998;36(10): 1028-31.
80. Joshi CG, Magnar NG. Antibiotic activity of some Indian medicinal plants. J Sci Indian Res. 1952;11B: 261-64.
81. Gupta KC, Vishwanathan R. A short note on antitubercular substance from *Ocimum sanctum.* Antibiot Chemothe. 1955: 22-25.
82. Grover GS, Rao JT. Investigations on the antimicrobial efficiency of essential oils from *Ocimum sanctum* and *Ocimum gratissimum*. Perfum Kosmet. 1977;58: 326-29.
83. Majumdar B, Ray Chaudhuri SG, Ray A, Bandyopadhyay SK. Effect of ethanol extract of *Piper betle Linn* leaf on healing of NSAID-induced experimental ulcer- a novel role of free radical scavenging action. Indian J Exp Biol. 2003;41(4): 311-15.
84. Murthy KN, Reddy VK, Veigas JM, Murthy UD. Study on wound healing activity of *Punica granatum* peel. J Med Food. 2004;7(2): 256-59.
85. Fernandez O. Efficacy of *Rhizophora mangle* aqueous bark extract in the healing of open surgical wounds. Fitoterapia. 2002;73: 564-68.
86. Nagappa AN, Cherian B. Wound healing activity of the aqueous extract of *Thespesia populnea* fruit. Fitoterapia. 2001;72: 503-06.
87. Chaudhari M, Mengi S. Evaluation of phytoconstituents of *Terminalia arjuna* for wound healing activity in rats. Phytother Res. 2006;20(9):799-805.
88. Udupa SL, Udupa AL, Kulkarni DR. Influence of *Tridax procumbans* on lysyl oxidase activity and wound healing. Planta Med. 1991;57(4): 325-27.
89. Udupa AL, Kulkarni DR, Udupa SL. Effect of *Tridax procumbans* extracts on wound healing. Int J of pharmacognosy. 1995;33(1): 37-40.
90. Diwan PV, Kulkarni DR. Antiinflammatory activity of nandrolone phenylpropionate. Indian J Exp Biol. 1983;21: 569-71.
91. Rane MM, Mengi SA. Comparative effect of oral administration and topical application of alcoholic extract of *Termanalia arjuna* bark on incision and excision wound in rats. Fitoterapia. 2003;74: 553-58.
92. Hernandez-Perez M, Sanchez-Mateo CC, Darias V et al. Effects of *Visnea mocanera* extracts on the bleeding time, gastrointestinal transit and diuresis in rodents. J Ethnopharmacol. 1995;46(2): 95-100.
93. Leite SN. Wound healing activity and systemic effects of *Vernonia scorpioides* extract in guinea pigs. Fitoterapia. 2002;73: 496-500.
94. Hegde DA, Kosa RL, Chansouria JPN. Study of the effect of *Wedelia Calendulacia* on wound healing in rats. Phytotherapy research. 1994;8(7): 439-40.
95. Maier CM, Chan PH. Role of superoxide dismutase in oxidative damage and neurodegenerative disorders. Neuroscientist. 2002;8(4):323-34.
96. Khanna S, Roy S, Venojarvim. Dermal wound healing properties of redox active grape seed proanthocyanidines. Free Radic Biol Med. 2002;33(8): 1089-96.
97. Dissemond J, Goos M, Wagner SN. The role of oxidative stress in the pathogenesis and therapy of chronic wounds. Hautarzt. 2002;53:718- 23.
98. Senel O, Cetinkale O, Ozbay G, Ahcioglu F, Bulan R. Oxygen free radicals impair healing in ischemic rat skin. Ann Plast Surg. 1997;39:516-23.
99. Rasik AM, Shukla A. Antioxidant status in delayed healing type of wounds. Int J Exp Pathol. 2000;81(40): 257-63.
100. Halliwell B, Grootveld M, Gutteridge JM. Methods for the measurements of hydroxyl radicals in biomedical systems; Deoxyribose degradation and aromatic hydroxylation. Methods of Biochem Anal. 1988;33: 59-90.
101. Gupta A, Singh RL, Raghubir R. Antioxidant status during cutaneous wound healing in immunocompromised rats. Mol Cell Biochem. 2002;241(1-2). 1-7.
102. Ginugliano D. Dietary antioxidants for cardiovascular prevention. Nutr Metab Cardiovasc Dis. 2000;10(1): 38-44.

ISSN: 2319-9865

103. Shimoi K, Masuda S, Shen B, Furugori M, Kinae N. Radioprotective effects of antioxidative plant flavonoids in mice. Mutat res. 1996;350(1):153-161.
104. Havsteen BH. The biochemistry and medical significance of the flavonoids. Pharmacol Ther. 2002;96(2-3): 67-202.