



Review Article

JOURNAL OF APPLIED PHARMACEUTICAL RESEARCH | JOAPR

www.japtronline.com

ISSN: 2348 – 0335

A REVIEW OF WOUND HEALING ACTIVITY ON DIFFERENT WOUND MODELS

Renuka Verma*, Pushpa Prasad Gupta, Trilochan Satapathy, Amit Roy

Article Information

Received: 19th June 2018

Revised: 16th October 2018

Accepted: 23rd December 2018

Keywords

Wound healing, *in-vitro*, *ex-vivo*, *in-vivo*, proliferation.

ABSTRACT

Wound is an injury of living tissue or break in the epithelial integrity of the upper layer of skin. This may lead to disturbance of skin anatomical structure and their function. The normal wounds start to heal immediately after an injury. Normal healing process involved: inflammatory phase, proliferation phase and maturation phase. Now a day wound healing is a challenging clinical problem. So, necessity of effective wound management is required. The various screening models play important role to understand the basic process of tissue repair and treatment of wounds. Wide varieties of *in-vitro*, *ex-vivo* and *in-vivo* models have been developed for evaluation of wound healing activity. The *in-vitro* models includes Chick chorioallantoic membrane assay, Fibroblast assay, Collagen assay, Scratch assay, Endothelial cell *in vitro* tube formation assay, Keratinocytes assay. *Ex-vivo* models includes organotypic culture, human *ex vivo* skin culture, porcine model, Human organotypic skin explanted culture and the *in-vivo* models includes Excision wound model, Incision wound Model, Burn wound model, Dead space wound model. The aim of this review article is detailed study of different types of *in-vitro*, *ex-vivo* and *in-vivo* models for evaluation of wound healing activity.

INTRODUCTION

A wound can be defined as an injury of living tissue or break in the epithelial integrity of the upper layer of skin. This may cause to disturbance of skin anatomical structure and their function.

Mechanism of wound healing

The normal wounds are starts to heal instantly after an injury, but in some cases wounds failed to heal in an appropriate and organized manner. These types of wounds required to continue

management. The normal wound healing process are- Inflammatory phase, Proliferative phase and Maturation phase [3]. The inflammatory phase arises between few minutes to up to 24 minutes after injury. Homeostasis is a first step of wound healing. In this phase many extrinsic and intrinsic coagulation factors will activated within minutes of injury, this may leads to degranulation and releasing chemotactic factors (chemokine's) and growth factors (GFs) which may help to the formation of clots [4]. The second steps of the inflammatory

*Department of Pharmacology, Columbia Institute of Pharmacy, Tekari, Near Vidhan Sabha, Raipur (C.G) India

*For Correspondence: renu8326@gmail.com

©2019 The authors

This is an Open Access article distributed under the terms of the Creative Commons Attribution (CC BY NC), which permits unrestricted use, distribution, and reproduction in any medium, as long as the original authors and source are cited. No permission is required from the authors or the publishers. (<https://creativecommons.org/licenses/by-nc/4.0/>)

phase are neutrophils, is the first cells to appear at the injury site, for cleanse waste and bacteria to provide a good environment for wound healing. The last step of inflammatory phase is macrophage, the most important cells act as the key regulatory cells for repair. Macrophages accumulate and facilitate phagocytosis of bacteria and damage tissue. Macrophages initiate two important aspects of healing such as angiogenesis and fibroplasia. They are essential for providing the metabolic needs of the healing process [5, 6]. The proliferative phase starts at 3 day of wounding and last up to 2–4 weeks. In stage of macrophage the Fibroblast-stimulating

factor are activated. They released fibroblasts. Fibroblasts act as the key cells of the proliferative phase, When activation of fibroblast they produced Collagen and proteoglycans and subsequently effects on remodeling of the granulation tissue. Granulation tissue increase wound tensile strength and reduces size of the wounded-epithelialization is important for wound healing [7]. The last stage of wound healing is maturation or remodeling phase. Maturation phase completed around 2 year. It depends on continuous collagen synthesis. The contraction rate and regular increase in tensile strength are seen in this phase [8, 9].

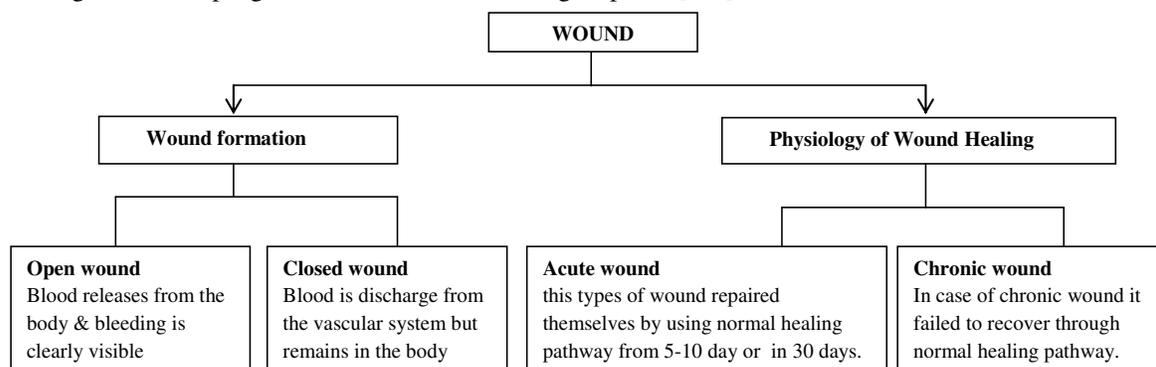


Fig. 1- Types of wound

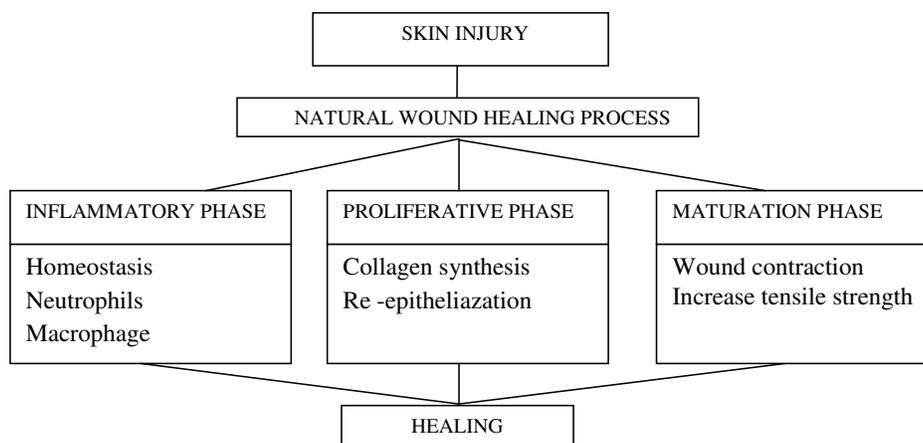


Fig. 2 Natural pathway for wound healing

DIFFERENT MODELS FOR WOUND HEALING

In-vitro Studies

The term *in-vitro* is derived from Latin phrase which means “the technique which performed outside the living animal”. In this type of study the tissue are removed from animal body and preserved for few days to several months in the suitable growth medium. The *in-vitro* assay is useful for determination of antimicrobial effects and healing enhancing agents [10].

Chick chorioallantoic membrane assay: The wound healing agents produce angiogenesis. It stimulates new vessels

formation in CAM model. The angiogenesis process is essential for wound healing. They provide the re-establishment of normal blood flow and tissue repaired [11]. In this model collect nine day-old fertilized chick eggs and small window (1.0 cm²) is made on the surface of shell. Hole is created for air space then air is sucked out through rubber bulb. The window is open and methylcellulose is fixed with test sample placed at the joint of two large vessels. The window is resealed by tape and eggs are incubated at 37°C in a well-humidified chamber for 72 h. The eggs are open and observe new vessel

formation. Compared eggs disk containing drug or without drug. FGF (Fibroblast Growth Factor) are used as a standard [12].

Fibroblast assay: Fibroblast assay are used for the determination of the total protein of viable cells present by staining with an appropriate dye such as sulforhodamine B (SRB) or neutral red.

In fibroblast assay, fibroblasts are activate and secreted collagens and subsequently effect on remodeling of the granulation tissue on woundarea. In this model the Cells are incubate in an atmosphere of CO₂, air at 37°C in a tissue culture incubator, and suspended at viable cells in the growth media (DMEM). The suspended cells are fertilized with FCS (fetal calf serum) and allowed to equilibrium for 3 d. individually test and standard drug is introduced in medium on 3th day. In 9th day media is changed and wash with phosphate buffered saline solution. Cells are divided into two parts. One part used for hydroxyproline content assay and the second one is DNA. The cells capability is determining by trepan blue [13].

Keratinocytes assay: Keratinocytes provide covering of epidermal layer and prevents fluid loss and delays bacterial attacks. Keratinocytes cell is incubated with serum-free keratinocyte growth medium (KGM) at 37°C temp with CO₂. Vascular permeability factor (VPF) cells are subculture in 24-well plates. After overnight culture the media is replaced with fresh KGM. At intervals the medium is collected and extract cell lyser with lysis buffer samples are centrifuge and store (70°C) for immunoassay [14, 15].

Collagen assay: The presences of TGF-3 (Transforming growth factor type-3) in cells or cell fragments play some intrinsic role in inflammation and tissue repair. In this assay the NRK 49 F cells are seed in Dulbecco's modified Eagle's medium (DMEM) /calf serum. After the cell sreach confluence, the medium is change to minimal essential medium (MEM) (containing glutamine, Hepes buffer, and plasma-derived serum), and growth factors is add HCl containing bovine serum albumin. After 16h the media is changed to ascorbate and other supplements, but without plasma. Growth factors are re-added. After 15 min, proline are added and incubate continue for 3h at 37°C. Collagen is determined on the Poole medium from triplicate wells using bacterial collagenase [16,17]

Scratch assay: This method is based on formation of “scratch” on cell monolayer. The cells on the edge of the newly formed gap are move toward the opening to closing the “scratch” until new cell–cell contacts are recognized again [18]. The fibroblast L929 (1-104 cells) and keratinocytes HaCaT (2-104 cells) are seeded in 24-well cell culture plate. Linear scratch is prepared in confluent cell monolayer by using pipette slope. Remaining cell is washing out with simple DME medium. The standard drug is used as positive control. The cellular gap in the cell monolayer is measured at different time intervals [19, 20].

Endothelial cell in-vitro tube formation assay: Fibrin media are prepared by using of thrombin to fibrinogen solution. After polymerization, the thrombin is inactivated by incubating in HMEC medium. EC (endothelial cell) are seed confluent on the surface of each medium. After 16h of attachment the EC are stimulate with HMEC media supplement with TNF- α in presence or absence of VEGF (vascular endothelial growth factor) or bGF (fibrinogen). In final steps the gels are fixed with formaldehyde, paraffin-embed. The EC growth into fibrin mediums are analyzed by using microscope [21].

Ex-vivo studies

The word *ex-vivo* is derived from Latin word means “out of the living”.The study is carried out with an organ or tissue isolated from an entire animal. *Ex-vivo* models are positively used to evaluate epithelization rate after treatment and complete molecular information about wound healing [22].

Human organotypic skin explanted culture (hOSEC): hOSEC model are used to evaluate cell migration of skin and keratinocyte cytotoxicity, disappearance of keratinocyte and RNA are evaluated by this model. Methyl green-pyro nine (MGP) stain are used for staining of tissue. Collect skin from informed patients suffering from breast or abdomen surgery. The tissue is cut (1.0 cm²) and cultured in culture medium at 37°C temperature with CO₂ and humidified air. The Dulbecco's Modified Eagle Medium (DMEM) with Fetal Bovine Serum, antibiotic (penicillin, streptomycin and amphotericin B) and L-glutamine containing medium are used. After culture, the tissue are collect and fixed with formalin and inserting with paraffin and histology or immunohistochemistry methods are applied for determination of cell migration [23, 24].

Porcine model: Porcine model are used to dose-dependent calculation of corneal epithelial wound healing. Freshly porcine eye are used. In experiments only those eyes used that completely show transparent cornea and no signs of injury in Excimerlaser operating microscope. Eye is gently rinsed with water and removes adhering blood. Basic salt solution at is added. Porcine eye dip in tissue-culture medium for 3 min then placed in sterile containers to prevent drying of the ocular surface. The process was carried out by semi sterile (laminar-flow) conditions. The 5.0 mm in diameter and 40 mm in depth are created on corneal epithelium. After laser treatment the eyes are fixed with custom-built support and maintain physical intraocular pressure. The transparent cavity are cannulated with needle and filled with tissue-culture medium. The ocular surface are cleansed by tissue-culture medium and incubated at 1 h. Test solution are applied. The size of each wound is measured by horizontally and vertically in two different positions under ultraviolet lights [25].

Human ex-vivo skin culture (HESC): HESC model are used to Detect Keratinocyte Differentiation, Keratinocytes Apoptosis and Proliferation rate. Skin is carefully removed with blade and disinfected with ethanol solution then fixed with sterilized cutting board. A thin layer of epidermis is cut and rinse with Dulbecco's phosphate-buffered saline solution with penicillin-streptomycin. It is located on the bottom of nylon mesh cell strainer. After fixing of skin the cell culture medium is added. The skin graft are cultured in liquid-air interphase and kept in cell culture. The cell culture is incubated at 37°C with CO₂. The medium are changed every day. The cultured skin grafts are collected at different time intervals and immediately fixed with formalin. Fixed tissues are embedded with paraffin and divided with 5 mm thickness. Hematoxylin & eosin (H&E) solutions are used as strainer to stain tissue section and observed under Nikon Eclipse 50i light microscope [26, 27].

Organotypic culture (OTC): Fibroblasts and liquid collagen mixture is add and incubated at 37°C for 24 h. keratinocytes cell are suspended with E-medium. Medium is altered every day till the sample is collected. The skin grafts were collected at 12th day of post lifting. The cultured skin scions are collected at day 0, 2, 4, 10, 12, 14, and fixed with formalin. The fixed tissues are embedded with paraffin and sectioned with 5 mm thickness. The tissue section is stain with hematoxylin & eosin (H&E) solutions and observed under light microscope [28, 29].

In-vivo studies

The term *in-vivo* is derived from Latin means “in the living”. It can be defined as the test is performed in living organism (mice, rat and rabbits etc.) pharmacological effect are same in human as in animals due to which non clinical studies in animal is required before administration to human. Following types of wounds are made in laboratory animals are studied [30].

Excision wound model: In this model the rate of contraction and epithelization are determined. Animals are anesthetized and shaven back region with shaving cream after that hollow is made on the dorsal thoracic region of the animals. Remove 300 mm² circular areas with the help of surgical blade and scissor. The depth of wound is not more than 2mm. Treatment start from the day of operation. Measurement of wound area, period of epithelisation, wound index, collagen, estimation of protein and hexosamine parameters are studied [31, 32].

Incision wound Model: In this model tensile strength are measured. Animal is anesthetized with ether or ketamine HCl at a specific dose. Back area is shaved. After shaving, 6 cm long and 2mm depth incision is prepared with the help of surgical blade (No. 9). Wound is sealed by surgical needle (No. 32) and surgical thread (No. 000). On 7th day sutures is removed. After 10th day of post wounding tensile strength is evaluated by tensiometer [33].

Burn wound model: Rate of contraction is determine by burn wound model. Animals are anesthetized with local anaesthetic agent. burn wound model are divided on the basis of degree of wound such as a).first degree wound, b) Second degree burn, c) third degree burn (full thickness) [34].

a) First degree wound:-First-degree burn are commonly occurs in direct contact of flame or intense sunlight in short period. In this type of burn only epidermis and Stratum conium are injured, and there is no damage in dermis layer. A special metal plate 2x2cm with holder is heated to 60°C and applied to the dorsal area of the animals for 30 s to induce partial thickness burn wound. b) Second degree burn: -This type of wound is clinically characterised by pain and erythema. The healing of wound is depends on the deepness of skin damage and infection. wound are made by placing the 90°C hot plate on the selected dorsal area of the animal for 10 s or hot molten wax is poured into shaven back area of animal until wax get

solidified. Wax is removed and marked with partial thickness circular burn [35]. c) Third degree burn is caused by hot water, fire and prolonged contact with electrical current. The wound area exhibits tightness and brightness as the elasticity of the skin is lost, causing abnormal shrinkage. In such cases, all structures of skin are damaged and result is coagulation and necrosis of the skin. Metal plate is heated at 100°C and applied at the dorsal area of the animal for 30 sec. The parameters for evaluation of burn wound are wound contraction and period of epithelisation [36,37].

Dead space wound model: Determination of healing potential and collagen strength. Polypropylenetube (2.5×0.5 cm) is implant on each side of paravertebral lumbar region of the skin by subcutaneous route. Wounds are sutured with surgical suture. Treatment is start, After 10 day's treatment the granulation tissue is formed on the tube. Tissue is collected carefully and breaking strength of the granulation tissue is measured [38, 39].

CHALLENGES

In-vitro:-In-vitro models may be useful theoretically but are lacking the physical characteristics of the natural human skin. These models are unable for determining all the factors involved in wound healing [40].

Ex-vivo:-The major drawback of *ex-vivo* wound model are isolation of tissue, it is a critical steps of *ex-vivo* model requiring careful handling.

In-vivo: -The number of animal used in research purpose is increased in day by day. Every year millions of experimental animal are used all over the world. Many periods animal survive the pain, distress and death during experiments. The animal used for research is unethical and need to stop. Alternative models are used to overcome the drawback related with animal experiment and avoid the incorrect procedure [41, 42].

CONCLUSION

The study of wound healing agents has been an important field of research. This review provides effective knowledge about various models (*in-vitro*, *ex-vivo* and *in-vivo*) and alternative models to evaluate wound healing activity, which may help to wound care specialists to understand healing response.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest

REFERENCES

- [1] Rawat S, Singh R, Thakur P, Kaur S, Semwall A .Wound healing agents from medicinal plants: A review. *Asian Pacific Journal of Tropical Biomedicine*, 2 , 1910-1917 (2012)
- [2] Perelman S, Francis G, Rutledge T, Foote J, Martino F, Dranitsaris G. Sterile Versus Nonsterile Gloves for Repair of Uncomplicated Lacerations in the Emergency Department: A Randomized Controlled Trial. *Annals of Emergency Medicine*, 3, 362-370 (2004)
- [3] Velnar T, Bailey T , Smrkolj V, The Wound Healing Process: an Overview of the Cellular and Molecular Mechanisms. *The Journal of International Medical Research*, 37, 1528 -1542 (2009)
- [4] Wang P, Huang B, Horng H, Yeh C, Chen Y. Wound healing. *Journal of the Chinese Medical Association*, 81,94-101(2018)
- [5] Hanna R, DPM, Giacomelli J, DPM, MS, FACFAS. A Review of Wound Healing and Wound Dressing Products. *Journal of Foot and Ankle Surgery*, 36, 2-14 (1997)
- [6] Kumar V, Khan A, Nagarajan K. Animal Models for the Evaluation of Wound Healing Activity. *International Bulletin of Drug Research*, 3, 93-107 (2013)
- [7] Hunt T.K, Hopf H, Hussain Z. Physiology of Wound Healing. *Advances in Skin & Wound Care*, 13, 6-11(2000)
- [8] Reibman J, Meixler S , Lee T.C , Gold L.I , Cronstein B N , Haines K A . Transforming Growth Factor Beta 1, A Potent Chemoattractant for Human Neutrophils, Bypasses Classic Signal-Transduction Pathways. *Proceedings of the National Academy of Sciences of the United States of America*, 88, 6805-6809 (1991)
- [9] Fisher C , Gilbertson-Beadling S , Powers E.A , Petzold G , Poorman R, Mitchell M.A . Interstitial Collagenase is Required for Angiogenesis in Vitro. *Developmental Biology*, 162, 499-510 (1995)
- [10] Shay J.W, Wright W.E . The use of Telomerized Cells for Tissue Engineering. *Natural Biotechnology*, 18, 22-23 (2000)

- [11] Subalakshmi M, Saranya A, Uma maheswari M, Jarina A, Kavimani S, Murali R .An Overview of the Current Methodologies used for the Evaluation of Drugs Having Wound Healing Activity. *International Journal of Experimental Pharmacology*,4, 127-131 (2014)
- [12] Shukla A, rasik A, jain G, Shankar R, kulshrestha D, dhawan B. in Vitro and in Vivo Wound Healing Activity of Asiaticoside Isolated from Centella Asiatica. *Journal of ethnopharmacology*, 65, 1-11 (1999)
- [13] Roberts A, Sporn M, Assoian R, Smith J, Roche N, Wakefield L. Transforming Growth Factor Type and Rapid Induction of Fibrosis and Angiogenesis in Vivo and Stimulation of Collagen Formation in Vitro. *Proceeding of the National Academy of Sciences of the United States of America*,83, 4167-4171 (2000)
- [14] Brown L.F, Yeo K, Berse B, Yeo T, Donald R, Senger D, Dvorak F, Vandewater D. Expression of Vascular Permeability Factor (Vascular Endothelial Growth Factor) by Epidermal Keratinocytes during Wound Healing. *Journal of Experimental Medicine*, 176, 1375-1379 (1992)
- [15] Cherreddy k, Coco R, Memvanga P, Ucakar B, Rieux A.D, Vandermeulen G, Preat V. Combined Effect of PLGA and Curcumin on Wound Healing Activity. *Journal of Controlled Release*,171, 208-215 (2013)
- [16] Liang C, Park A, Guan J. In Vitro Scratch Assay: a Convenient and Inexpensive Method for Analysis of Cell Migration in Vitro. *Journal of Natural Protocols*, 2, 29-33 (2007)
- [17] Montoya A, Daza A, Muñoz D, Ríos K, Taylor V, Cedeño D. Development of a Novel Formulation with Hypericin to Treat Cutaneous Leishmaniasis Based on Photodynamic Therapy in invitro and in Vivo Studies. *Antimicrobial Agents and Chemotherapy*, 59, 5804-5813 (2015)
- [18] Talekar Y.P , Apte K.G, Paygude S.V, Tondare P.R, Parab P.B. Studies on Wound Healing Potential of Polyherbal Formulation Using in Vitro and in Vivo Assays. *Journal of Ayurveda and Integrative Medicine*, 8, 73-81(2017)
- [19] Demirci S, Dogan A, DemirciS, ahin F. In vitro Wound Healing Activity of Methanol Extract of Verbascum Speciosum. *International journal of Applied Research in Natural Product*, 7, 37-44 (2014)
- [20] Koolwijk P, Vanerck M.G, DeVree W.J, Vermeer M.A, Weich H.A, Hanemaaijer R, Hinsbergh V. Cooperative Effect of TNF Alpha, bFGF, and VEGF on the Formation of Tubular Structures of Human Microvascular Endothelial Cells in a Fibrin Matrix. Role of Urokinase Activity. *The Journal of Cell Biology*,132, 1177-1188 (1996)
- [21] Shrivastava A, Mishra A, Alia S.S, Ahmad A, Abuzinadah M.F, Khana N.A. In Vivo Models for Assesment of Wound Healing Potential: A Systematic Review. *Wound Medicine*, 20, 43-53 (2018)
- [22] Keese C, Wegener J, Walker S, Gjaever I. Electrical Wound-Healing Assay for Cells In Vitro. *Proceeding of the National Academy of Science of the United States of America*, 101, 1554–1559 (2004)
- [23] Andrade T.A, Aguiar A.F, Guedes F.A, Leite M.N, Caetano G.F, Coelho E.B, Das P.K, Frade M.A, Ex Vivo Model of Human Skin (hOSEC) as Alternative to Animal use for Cosmetic Tests. *Procedia Engineering*, 110, 67-73 (2015).
- [24] Frade M, Andrade T, Aguiar A, Guedes F, Leite M, Passos W, Coelho E, Das P. Prolonged Viability of Human Organotypic Skin Explant in Culture Method (hOSEC). *Anias Brasileiros Dermatologia*, 90, 347-50 (2015)
- [25] Flueckiger F, Kodjikian L, Halberstadt M, Boehnke M, Garweg J. an ex-vivo, Whole-globe Porcine Model of Corneoepithelial Wound Healing Tested Using Immunomodulatory Drugs. *Journal of Ocular Pharmacology and Therapeutics*, 21, 365-375 (2005)
- [26] Greenwald P, Gottlieb J, Mass P. Full-Thickness Skin Wound Explants in Tissue Culture: a Mechanical Evaluation of Healing. *Plastic and Reconstruction Surgery*, 90, 289-294 (1992)
- [27] Burd A, Longaker T, Adzick N.S, Campion C, Harrison M, Siebert J.W, Ehrlich H.P. Fetal Wound Healing: an in vitro Explant Model. *Journal of Pediatric Surgery*, 25, 898-901 (1990)
- [28] Kirkpatrick CJ, Krump -Konvalinkova V, Unger R E, Bittinger F, Otto M, Peters K. Tissue Response and Biomaterial Integration: the Efficacy of in vitro Methods. *Biomolecular Engineering*, 19, 211-217 (2002)
- [29] Xu W, Hong S, Jia S, Zhao Y, Galiano R, Mustoe T. Application of a Partial-Thickness Human ex vivo Skin Culture Model in Cutaneous Wound Healing Study. *Laboratory Investigation*, 92, 584-599 (2012)
- [30] Krishnaveni B, Neeharika V, Venkatesh S, Padmavathy R, Reddy B.M. Wound Healing Activity of Carallia Brachiata

- Bark. *Indian Journal of Pharmaceutical Sciences*, 71, 576-578 (2009)
- [31] Agarwal P, Singh A, Gaurav K, Goel S, Khanna H, Goel R.K . Evaluation of Wound Healing Activity of Extracts of Plantain Banana (*Musa sapientum* var. *paradisica*) in Rats. *Indian Journal of Experimental Biology*, 47, 32-40 (2009)
- [32] Patil D, Kulkarni R., Shahapurkar A, Hatappakki C. Natural Cumin Seeds for Wound Healing Activity in Albino Rats. *International Journal of Biological Chemistry*, 3,148-152 (2009)
- [33] Murthy S, m. K. goel G, Purohit V, Sharma H, Goel R. K. Evaluation of in vivo Wound Healing Activity of *Bacopa Monniera* on Different Wound Model in Rats. *Biomed Research International Volume*, 201, 1-9 (2013)
- [34] Singh D, Nag M, patel S, Pradhan M, shrivastava S, daharwal S. Treatment Strategies in Burn Wounds: an Overview. *Research journal of Pharmacology and pharmacodynamics*,5, 341-35 (2013)
- [35] Gurung S, Skalko-Basnet N. Wound Healing Properties of *Carica Papaya* Latex: in vivo Evaluation in Mice Burn Model. *Journal of Ethnopharmacology*, 121, 338-341 (2009)
- [36] Srivastava P, Durgaprasad S. Burn Wound Healing Property of *Cocos Nucifera*: an appraisal. *Indian Journal of Pharmacology*, 40, 144–146 (2008)
- [37] Azeez S, Amudhan S, Adiga S, Rao N, Udupa LA. Wound Healing Profile of *Areca Catechu* Extracts on Different Wound Models in Wistar Rats. *Kuwait Medical Journal*, 39, 48-52 (2007)
- [38] Asif A, Kakub G, Mehmood S, Khunum R, Gulfray M. Wound Healing Activity of Root Extracts of *Berberis Lyceum Royle* in Rats. *Phytotherapy Research*, 21,589-591 (2007)
- [39] Tajik H, Jalali F.S.S. Influence of Aqueous Extract of *Yarrow* on Healing Process of Experimental Burn Wound in Rabbit: Clinical and Microbiological Study. *Journal of Animal and Veterinary Advances*, 6,1464-1468 (2007)
- [40] Eissa A.E, Zaki M.M, Saeid S, Abdelsalam M, Ali H.M, Moustafa A.A. In Vitro Evaluation of the Efficacy of Hemodialysate (*Solcoseryl*) as a Wound Healing Agent in Nile Tilapia (*Oreochromis niloticus*). *International Journal of Veterinary Science and Medicine*, 1, 57-64 (2013)
- [41] Rollin, B.E. Toxicology and New Social Ethics for Animals. *Toxicologic Pathology*, 31,128-131 (2003)
- [42] Doke S.K, Dhawale S.C. Alternatives to Animal Testing: A Review. *Saudi Pharmaceutical Journal*, 23, 223-229 (2015)