



A COMPARATIVE STUDY TO ACCESS HEALING OF THE DONOR AREA BY CONVENTIONAL PARAFFIN GAUZE DRESSING VERSUS CENTELLA ASIATICA HERBAL SILK DRESSING.

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ABSTRACT

Objective:-This study aims to compare conventional paraffin gauze dressing with Centella Asiatica Herbal Silk Dressing (Fibroheal™) in the healing of wounds of donor area.

Methods:-A blinded, prospective, randomized trial was conducted on 30 adult inpatients at Level 1 Trauma Centre under the Division of Trauma Surgery & Critical Care from November 2013 to September 2014, who had wounds requiring skin grafting. After grafting, each donor site wound was half covered with Fibroheal sheets and half with paraffin gauze dressing. Donor site assessment was performed on the basis of 6 parameters on weekly basis till 4 weeks by a single observer blinded to the randomization. After performing a thorough assessment the wound was washed with saline and 1 Fibroheal sheet (10x10cm) was placed on site 1 while site 2 was dressed with paraffin gauze followed by secondary dressing.

Results: - Size of the wound was compared for both the sites every 7th day. Significant reduction in size was noted and P-value(<.01) was calculated using Wilcoxon Signed Rank Test. Mc Nemar test was used to analyse presence of infection and pus discharge on the basis of wound culture. Tenderness, erythema and oedema was measured as present and absent. Pain was measured using VAS Scale every 7th day. Centella Asiatica Herbal Silk dressing provided less pain due to its smothering effect as compared to paraffin gauze which adheres to the wound. Henceforth the low adherent nature of Centella Asiatica Herbal Silk dressing is definitely beneficial for the wound in terms of pain and healing.

Conclusion: - Centella Asiatica Herbal Silk dressing significantly improved the quantitative and qualitative appearance of donor site as compared to paraffin gauze dressing. This dressing has promising future for wounds due to its versatility and low adherence to wound. The study was uneventful.

KEYWORDS : Wound healing, Fibroin, Tissue Engineering, Skin Graft

Introduction:

Silk isolated from the silkworm (*Bombyx mori*) cocoon, has been used as biomedical suture material for so many decades due to its excellent biocompatibility and mechanical strength. Silk proteins are produced within specialized glands of the silkworm and spun into fibres (1,2).Silk is a fibrous protein characterized by highly repetitive primary sequence of amino acids that leads to highly homogenous secondary structure predominantly in the form of β -structure making silk mechanically strong. Beta structure coupled with its biocompatibility makes silk a very prospective material for tissue engineering specifically in the area of bio-medical applications (3,4,5).

The silk that we are discussing about has at least two major fibroin proteins, light and heavy chains, with molecular weight around 25 and 325 kDa respectively (6, 7). These core fibers are encased in a sericin coat, a family of glue-like proteins that holds two fibroin fibers together to form the composite fibers of the cocoon case to protect the growing worm. Silkworm cocoon silk production, known as sericulture, produces high yields since the larvae can be maintained in high densities (8, 9, 10).The core sequence in the fibroin heavy chain from *B. mori* include alanine-glycine repeats with serine or tyrosine. Silk studies in vitro have demonstrated that

proteases such as chymotrypsin will cleave the less-crystalline regions of the protein to peptides which are then capable of further metabolism by the cells (7,10).Furthermore, protease cocktails and chymotrypsin (known to be produced by macrophages) are also capable of degrading silk. There is also clear evidence in the literature that silk, as a protein, is susceptible to proteolytic degradation in vivo and over longer time periods it gets absorbed (6, 7, 10).

The emergence of tissue engineering has enhanced the requirement for a diverse portfolio of compatible biomaterials to support the development of tissues in vitro and subsequent test in vivo. The biomaterial in the form of matrix plays a key role in communicating to cells seeded within or on the matrix, in order to develop biologically viable functional tissue. In other words, the matrix needs to support cell attachment, spreading, growth and differentiation. In addition, ideal matrix will be capable of degrading into biocompatible fragments in the prevailing environment. The rate of degradation of silk is sufficiently slower than rate of new tissue growth and development with no abnormal reactions or responses (11, 12, 13, 14).

This balance assures appropriate mechanical and physiological

compatibility during integration of the host and/or implanted tissue in vivo. Silk fibroin offers versatility in matrix scaffold design in the form of foams, films, fibres and meshes for a number of tissue engineering needs in which mechanical performance and biological interactions are major factors for success, including skin, bone, ligaments, tendons, blood vessels and cartilage (15, 16).

The utility of silk fibroin film in culturing various animal cells including human mouth epidermoid carcinoma in comparison to collagen matrix has been demonstrated. Films of both fibroin and collagen supported equivalent cell growth after 5 days in cell culture conditions. The ability of fibroin and sericin films, as well as a control collagen (cast bovine acid soluble) film, in supporting attachment, spreading and growth of the L-929 fibroblast cell line was examined. The results support the concept that extracted fibroin free of sericin is a suitable matrix for cell and tissue culture (18, 19, 20).

Trauma represents a modern day epidemic. Loss of skin cover following trauma & burns is associated with high morbidity and mortality. Aim of all surgical approaches is to establish skin coverage over the injured area and to obtain skin cover i.e. skin graft donor sites are created. Large number of wound dressing materials is available for donor area. Conventionally Paraffin gauze dressing is in practice to heal these donor sites (21, 22).

This study is aimed at comparing conventional paraffin gauze dressing with Centella Asiatica Herbal Silk dressing (available in market as Fibroheal™) for healing of donor area.

Method:

A blinded, prospective, randomized trial was conducted on 30 adult in-patients at Level 1 Trauma Centre under the Division of Trauma Surgery & Critical Care from November 2013 to September 2014, who had wounds requiring skin grafting. The inclusion criteria were wound size at least 10x10cm, patient currently not on any other interventional treatment, subject > 18years of age, subject willing to give written consent and complete the follow up evaluation required by the study protocol, subject who agrees to abstain from any other treatment of wound(s) during the course of study. The Exclusion criteria include current inflammatory disease, Infection/allergy on the tested area, Chronic wounds showing signs of copious slough and purulent discharge, subject on topical medication on wound for less than 1 year, cutaneous disease which may influence the study results, Subject on oral corticosteroids with dose>10mg/day, any underlying medical conditions like liver disease or H/o alcoholism, HIV or any other serious medical illness like diabetes mellitus, pregnant and lactating women.

After grafting, two sites were created on the donor site wound, site 1 covered with Fibroheal sheets and site 2 with paraffin gauze. Donor site assessment was performed on the basis of 6 parameters on a weekly basis for 4 weeks by a single observer blinded to the randomization. There were 30 patients (27 men and 3 women) in the study group. The median patient age was 35 years (above 18 years).

The parameters monitored in the study were, the size of wound which was measured with the help of a scale, infection which was assessed on the basis of pus cultures, presence of granulation tissue on the wound bed assessed by observation, Tenderness, Erythema and oedema done by palpating the site for its presence and absence. Pain which was assessed using VAS (visual analogue scale). Besides monitoring the parameters, the clinical photographs were taken each time on dressing change. After performing the thorough assessment the wound was washed thoroughly with saline and 1 Fibroheal sheet 10x10cm placed on site 1 and site 2 dressed with paraffin gauze followed by secondary dressing.

Results:

Centella Asiatica Herbal Silk Dressing significantly improved the quantitative and qualitative appearance of donor site as compared to paraffin gauze dressing. This dressing has promising future for wound due to its versatility and low adherence to wound. The study was uneventful. The sample size was 30, out of which majority of

them were male (the ratio is 27:3). The wound (donor) site was split into two parts:-site 1 with paraffin gauze dressing and site 2 with herbal silk dressing (Fibroheal). Pre-operative general examination included cardiovascular system, kidney, respiratory, neurological system, skin, gastro intestinal, musculoskeletal, endocrine system, ear, nose and throat and eyes. It also included lab investigations i.e., haemoglobin, hematocrit, white blood cell, neutrophils, platelet counts, creatinine, glucose and sodium. The initial wound assessment was done in the intra-operative day (week 0), wound measurement and digital photography was done for both the site 1 and site 2, followed by weekly assessment by independent paramedic.

During each assessment skin analysis was done for both site 1 and site 2 for the size, infection, presence of granulation, discharge, tenderness, erythema, oedema and subjective pain. Clinical photographs were taken along with the scale respectively. Depending on the wound, the dressing was initiated, out of which 80% does not require further dressing.

1) Size of wound:-

Measurement of wound size was done using measuring scale consecutively. Donor site wound was divided into two equal parts on the same thigh for each patient. Site 1 wound was covered with paraffin gauze and site 2 with Centella Asiatica herbal silk sheets. The measurement was done on every 7th day during which the dressing was changed for both the sites. After comparing site 1 & 2, reduction in the size of the wound showed significant difference (p value<.01) measured using Wilcoxon signed-rank test. Size of wound was calculated as zero once the wound was dry and pink (Fig 1 & Fig 2).

FIG:1 Case 1- Picture represents the weekly reduction in sizes



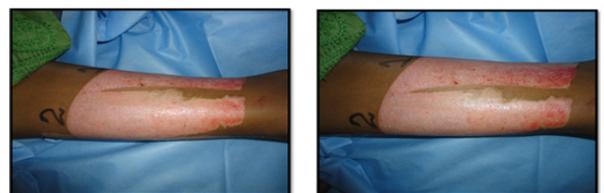
i. Day 0 (Intraoperative)

Site 1 (Paraffin) Site 2 (Centella Asiatica herbal silk dressing)



ii. Day 7

Observed difference in Tissue Granulation site 1 (Paraffin) still showed raw area and site 2(Centella Asiatica herbal silk dressing) is pink.



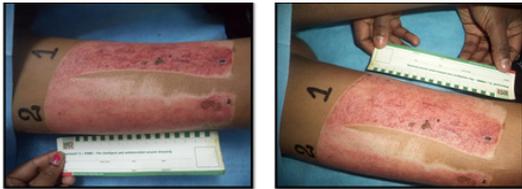
iii. Day 14

Site 2 (Centella Asiatica herbal silk dressing) has healed completely in comparison with site 1(paraffin)



iv. Day 21

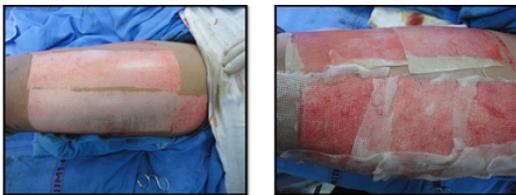
Site 2 (Centella Asiatica herbal silk dressing) Site 1 (Paraffin)



v. DAY 28

Site 2 (Centella Asiatica herbal silk dressing) Site 1 (Paraffin)

FIG 2: Case 2-Picture represents the weekly reduction in sizes



i. DAY 0 (Intraoperative)

Site 1 (Paraffin) Site 2 (Centella Asiatica herbal silk dressing)



ii. Day 7

Site 1 (Paraffin) Site 2 (Centella Asiatica herbal silk dressing)



iii. Day 14

Site 1 (Paraffin) showing raw area
Site 2 (Centella Asiatica herbal silk dressing) was dry and pink



iv. Day 21

Site 1 (Paraffin) showing raw area
Site 2 (Centella Asiatica herbal silk dressing) was dry and pink



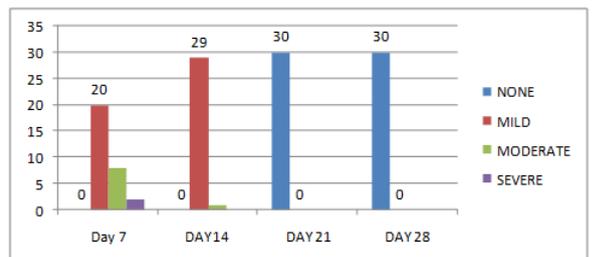
v. Day 28

Site 1 (Paraffin) showing raw area
Site 2 (Centella Asiatica herbal silk dressing) was dry and pink

- 2) Infection /pus discharge:-
Presence of infection and pus discharge was monitored on the basis of wound culture. The wound was observed for presence and absence of pus and infection. Statistical analysis was done using Mc Nemar test and there was no significance [p value < .001]
- 3) Tenderness:-
Tenderness was elicited on palpation as absent and present. Weekly monitoring was done during change of dressing. Out of 30 samples no tenderness was found.
- 4) Erythema and oedema:-
The presence of erythema and oedema was done by paramedical staffs who were not directly involved in the study. On observation it was noted that out of 30 samples, there was no significant Erythema and oedema seen.
- 5) Pain:-
Patient pain was measured using visual analogue scale every 7th day. Statistical analysis was done, using Wilcoxon signed-rank test. On 7th and 14th day the statistical analysis showed significant P value (<.001). Fibro heal dressing provide lesser pain due to its smothering effect as compared to paraffin gauze which adheres to the wound. Henceforth the low adherent nature of Fibroheal dressing is definitely beneficial for the wound in terms of pain and healing (Graph 1)

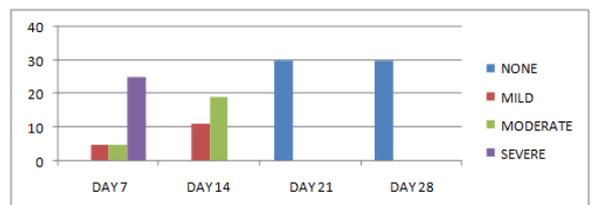
Graph comparing the severity of pain with Fibroheal dressing.

Graph 1: Severity of pain on Y Axis with number of days on X Axis.



Graph comparing the severity of pain with paraffin dressing.

Graph 2: Severity of pain on Y Axis with number of days on X Axis



- 6) Granulation
Vascularization was at a much higher level in site 1 (Centella Asiatica herbal silk dressing) compared to Site 2 (conventional Paraffin gauze). Tissue granulation was monitored in 30 samples, the epithelialisation period was observed on every seventh day. The appearance of the wound was not very relevant on the seventh day (1st dressing) for both site 1 and site

2 (Paraffin gauze). But a very significant improvement of the wound status was seen on the 14th day dressing (2nd dressing). Faster wound healing rate (pinkish and dryness) was observed on site 1 as compared to site 2.



FIG 3: Day 14

Site 1 (paraffin) has not healed in comparison with site 2 (Centella Asiatica herbal silk dressing)

Discussion

Silk isolated from the silkworm (*Bombyx mori*) cocoon, has been used as biomedical suture material for so many decades and speaks of its excellent biocompatibility and mechanical strength. Trauma represents a modern day epidemic. Loss of skin cover following trauma & burns is associated with high morbidity and mortality. The aim of all surgical approaches is to establish skin coverage over the injured area and to obtain skin cover i.e. skin graft donor sites are created.

The study we undertook is aimed at comparing conventional paraffin gauze dressing with Centella Asiatica Herbal dressing (developed by modification of silk protein as the main base material and Centella Asiatica Herb the adjunct to the main scaffolding substance, available in market as Fibroheal) for healing of donor area. The Fibroheal dressing used for the coverage of split-skin donor sites, gives ease of application, comfort, low risk of infection and faster healing as compare to paraffin gauze dressing. We monitored 6 parameters during the period i.e. Size of the wound, presence of infection, tenderness, Erythema, oedema, pain and granulation tissue. We conducted a literature review using the keywords "fibroin", "wound healing", and "antioxidants", and found that there have been very few clinical and empirical studies conducted on the effects of silk protein sericin in wound healing models.

Measurement of wound starts from operation theatre intra-operatively. Two sites were created on the donor area of thigh as site 1 (Fibroheal sheets) and site 2 (Paraffin gauze). The desired result in this study was to minimize pain, discomfort, scar formation and faster wound healing as compared to the conventional dressing used (paraffin gauze) (13). The size monitoring was done on every 7th day till the completion. Measurement was done using the same measuring scale each time along with digital recording. After the 7th day the wound covered with Fibroheal were visualized to have improved with epithelized edges, but the wound cover with gauze piece are left with raw area. The reduction in the size of the wound shows a very significant difference in site 1 as compared to site 2. Similar study on size reduction was also done by Biomedical Engineering, University of Rhode Island. Presence of infection and pus discharge was monitored on the basis of wound cultures. Out of the total 30 sample, statistical analysis was done using Wilcoxon signed-rank test resulting in very significant reduction in the wound sizes. A similar study was also done on the rate of inflammation and infection. Monitoring of inflammatory mediators induced by silk sericin was also done by Pornanong Aram Wit et al. They compared cream base-treated and normal saline-soaked dressing in full-thickness rat excision. Using wound size measurements and ELISA assays, sericin-treated wounds were shown to heal faster and had lower levels of inflammatory mediators, as compared with the cream base-treated and normal saline-soaked wounds (1).

Tenderness was elicited by the patient on the absence and presence by palpation. Weekly monitoring was done for all subjects. There was no significant tenderness noted in all the subjects, the most

significant result we observed with Fibroheal dressing was on wound size, granulation & pain (3). The presence of erythema and oedema was done by paramedical staff who were not directly involved in the study. On observation it was noted that out of 30 samples, there was no significant oedema seen and the wound bed was healthy (4).

Pain was measured in all the 30 subjects by using visual analogue scale. The low adherent nature of Fibroheal dressing provides a smothering effect which is definitely beneficial for the wound in terms of pain and healing. Konokpanont also studied effectiveness of silk protein in wound healing and trauma. Because of its good biocompatibility and infection-resistant nature, silk is a novel wound-coagulant material (19, 20). Silk Fibroin has been widely used as a biomaterial to fabricate bioactive wound dressings due to its appropriate physical, mechanical and biological properties, and the capability to promote wound healing. The rate of wound healing was faster in our study, 85% of the total subjects did not require any dressing and the wound was kept open. R.L. Moy, A. Lee, A. Zalka, also studied the rate of wound healing using non-adhesive wound dressing which applies to cover the wound surface as an absorbent pad that would absorb wound fluid while accelerate wound healing.

After the wound heals, the dressing must be removed, therefore, the non-adhesive wound dressing is required in order to reduce trauma and risk of repeated injury (2, 3, 4, 5, 6, 7, 8). The treatment groups showed significant increase in wound contraction and decrease wound healing time (9). The healing property appears to be due to increased collagen synthesis via activation of fibroblast (11, 22, 23, 24). The healing property of silk protein film appears to be due to epithelial proliferative action (11, 19, 22). Also the silk protein film is safe biomaterial without any skin sensitizing and skin irritating potential and with no effects on serum biochemical profile when applied dermally (10). Sericin has proliferative effects on some cell membranes, and also has various other biological activities.

Centella Asiatica appears to be a commonly ingested or topically applied herb for the purpose of wound healing, thus reducing bacterial contamination, reducing inflammation, and promoting resynthesis. Centella Asiatica appears to be recommended for the specific purpose of promoting resynthesis of tissue. Zainol MK in a scientific study proposed that Centella Asiatica Herb has anti-oxidative activity (12, 13) and provides nutritional support for wound healing (MacKay et al). The limitation we face was the Speedy recovery. It sometimes becomes a burden on the study, since the patient did not come for the follow up which negatively effect on the study. The patient length of stay was extended till week 2 as per the protocol of the study which in turn becomes bothersome in trauma setting. It has however, been found that cost of the dressing is high in comparison with paraffin dressing.

Conclusion

Silk isolated from the silkworm (*Bombyx mori*) cocoon, has been used as biomedical suture material for so many decades now which speaks of its excellent biocompatibility and mechanical strength. This dressing has promising future for wounds due to its versatility and cost effectiveness. In our study we have monitored five parameters which include Presence of infection and pus discharge, size of wound, pain, Erythema and edema, tenderness. There was significant p value (< .001) for pain (Wilcoxon signed-rank test). Fibroheal (silk protein+ Centella Asiatica Herb) dressing significantly improved the quantitative and qualitative appearance of donor site as compared to paraffin gauze dressings. Based on the positive result on appearance of the site, Fibroheal dressing will also prove to be a beneficial for burn patients. Further another study can be initiated for the role of Fibroheal as dressing material for various other wounds.

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References

1. Aramwit, Pornanong, Kanokpanont S, Eknamkul WD, and Srichana T. "Monitoring of Inflammatory Mediators Induced by Silk Sericin." *Journal of Bioscience and Bioengineering* 107, no.5 (May 2009):556–61. doi:10.1016/j.jbiosc.2008.12.012.
2. Kanokpanont, Sorada, Damrongsakkul s, Ratanavaraporn j, and Aramwit P. "Physico Chemical Properties and Efficacy of Silk Fibroin Fabric Coated with Different Waxes as Wound Dressing." *International Journal of Biological Macromolecules* 55 (April 2013): 88–97. doi:10.1016/j.jbiomac.2013.01.003
3. Moy RL, Lee A, Zalka A. "Commonly used suture materials in skin surgery" *American Family Physician*, 44 (1991), pp. 2123–2128
4. Baoyong L, Jian Z, Denglong C, Min L. "Evaluation of a new type of wound dressing made from recombinant spider silk protein using rat models" *Burns: Journal of the International Society for Burn Injuries*, 36(Sep 2010), 891–896
5. Kundu SC. "Silk biomaterials for tissue engineering and regenerative medicine" *Elsevier/Woodhead Publishing, Woodhead Publishing is an imprint of Elsevier.* (2014) pp 555.
6. Minoura N, Aiba S, Gotoh Y, Tsukada M and Imai Y, "Attachment and Growth of Cultured Fibroblast Cells on Silk Protein Matrices," *Journal of Biomedical Materials Research*, Vol. 29, No. 10, 1995, pp. 1215–1221.
7. Yeo J H, Lee KG, Kim HC, Oh HYL, Kim AJ, Kim SY *Biological and Pharmaceutical Bulletin*, 23 (2000), pp. 1220–1223
8. Zhou Y, Yang X, Liu X, Mao J, Gu S, W. Xu *International Journal of Biological Macromolecules*, 53 (2013), pp. 88–92
9. Duque, Nascimento PA, Pinto NCC, Mendes RF, Silva JM, Aragao DMO et al. "In Vivo Wound Healing Activity of Gels Containing Cecropia Pachystachya Leaves." *The Journal of Pharmacy and Pharmacology* 68, no. 1 (January 2016): 128–38. doi:10.1111/jphp.12496.
10. Omer A, Tezcaner A, Bilgili H, Devci MS, Gecit MR, and Keskin D. "Evaluation of Sericin/collagen Membranes as Prospective Wound Dressing Biomaterial." *Journal of Bioscience and Bioengineering* 112, no. 3 (September 2011): 279–88. doi:10.1016/j.jbiosc.2011.05.014.
11. Lin, Hung K, Yang YY, Yang CM, Huang M, Chuan Liu K, Shen Lin H, and Yu Chao P. "Antioxidant Activity of Herbaceous Plant Extracts Protect against Hydrogen Peroxide-Induced DNA Damage in Human Lymphocytes." *BMC Research Notes* 6 (2013):490. doi: 10.1186/1756-0500-6-490.
12. Yao, Chun-Hsu, Jen-Yu Yeh, Yueh-Sheng Chen, Ming-Hsien Li, and Chiung-Hua Huang. "Wound-Healing Effect of Electro spun Gelatin Nanofibres Containing Centella Asiatica Extract in a Rat Model." *Journal of Tissue Engineering and Regenerative Medicine*, February 25, 2015. doi:10.1002/term.1992.
13. Reimers K, Liebsch C, Radtke C, Kuhbier JW, and Vogt PM. "Silks as Scaffolds for Skin Reconstruction." *Biotechnology and Bioengineering* 112, no. 11 (November 2015): 2201–5. doi:10.1002/bit.25654.
14. Lin, Hung K, Yang YY, Yang CM, Huang MY, Lo HF, Liu KC, Lin HS, and Chao PY. "Antioxidant Activity of Herbaceous Plant Extracts Protect against Hydrogen Peroxide-Induced DNA Damage in Human Lymphocytes." *BMC Research Notes* 6 (2013):490. Doi: 10.1186/1756-0500-6-490.
15. Minoura N, Aiba S, Gotoh Y, Tsukada M, and Imai Y. "Attachment and Growth of Cultured Fibroblast Cells on Silk Protein Matrices." *Journal of Biomedical Materials Research* 29, no. 10 (October 1995): 1215–21. doi:10.1002/jbm.820291008.
16. Teramoto, Hidetoshi, Kameda T, and Tamada Y. "Preparation of Gel Film from Bombyx Mori Silk Sericin and Its Characterization as a Wound Dressing." *Bioscience, Biotechnology, and Biochemistry* 72, no. 12 (December 2008): 3189–96. doi:10.1271/bbb.80375.
17. Reddy RM and Prasad, "Silk-the prospective and compatible bio-material for advanced functional applications", *Trends Applied. Sci. Res.* 6(2011) 89-95.
18. Chevillard M, Deleage G and Couble P. "Amino acid sequence and putative conformational characteristic of the P25 silk protein of Bombyx mori. *Sericologia*. 1986b, 26: 435–49.
19. Min BM, Lee G, Kim SH, Nam YS, Lee TS, Park WH, 2004. "Electrospinning of silk fibroin nanofibers and its effect on the adhesion and spreading of normal human keratinocytes and fibroblasts in vitro." *Biomaterials*. 25: 1289–97.
20. Padol AR, Jayakumar K, Shridhar NB, Narayana Swamy HD, Narayana Swamy M, Mohan K. "Safety evaluation of silk protein film (a novel wound healing agent) in terms of acute dermal toxicity, acute dermal irritation and skin sensitization" vol.18 (2011) pp 17-2
21. Altman, Diaz, Jakuba, Calabro, Horan RL, Chen Lu, Richmond, J and Kaplan, D L. 2003 et al. "Silk-based biomaterials. *Biomaterials* 24(3): 401–16. (doi:10.1016/S0142-9612(02)00353-8) [CrossRef], [PubMed], [Web of Science *], [CSA]
22. Basal, G, Altioek, D and Bayraktar, 2010. Antibacterial properties of silk fibroin/chitosan blend films loaded with plant extract. *Fiber Polym*, 11(1): 21–7. (doi:10.1007/s12221-010-0021-0) [CrossRef], [Web of Science *]
23. Byun EB, Sung NY, Kim JH, Choi JJ, Matsui T, Byun MW, Lee JW. 2010. Enhancement of anti-tumour activity of gamma-irradiated silk fibroin via immunomodulatory effects. *Chem Biol Interact.* 186(1):90–5. (doi:10.1016/j.cbi.2010.03.032) [Cross Ref], [PubMed], [Web of Science *]
24. Chevillard M, Couble P and Prudhomme JC. 1986a. Complete nucleotide sequence of the gene encoding the Bombyx mori silk protein P25 and predicted amino acid sequence of the protein. *Nucleic Acids Res.* 14:6341–2. doi:10.1093 /nar /14.15.6341) [CrossRef], [PubMed], [Web of Science *], [CSA]