doi.org/10.36721/PJPS.2021.34.3.REG.1023-1029.1

REPORT

Effect of topical *Curcuma longa* extract gel on incision wound angiogenesis in Balb/C mice

Afiat Berbudi[1,2]*, Muhammad Tito Anugerah Ramadhan[3] and Nur Atik[4]

[1]Department of Biomedical Sciences, Parasitology Division, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia
[2]Parasitology Laboratory, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia
[3]Undergraduate Program, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia
[4]Department of Biomedical Sciences, Cell Biology Division, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia

Abstract: Wound prevalence is still high, both nationally and globally, having a negative impact on patients' physical, psychological and financial wellbeing. The wound healing process involves hemostasis, inflammation, proliferation and remodeling, with angiogenesis being one aspect of the proliferation phase. *Curcuma longa* has long been used for the therapeutic effects of one of its components, curcumin, which has been shown to have a positive effect on the wound healing process. The aim of this study was to determine the effect of *Curcuma longa* extract gel (*Curcuma longa*) administration on angiogenesis in wound healing. This study used a laboratory experimental mouse model. Twenty-five Balb/C male mice aged 8 to 10 weeks were divided into five different intervention groups (positive control, negative control, 1%, 3%, 10% *Curcuma longa* extract gel administration). An incision wound was made on the back of the mice and the mice were treated for 7 days. The total blood vessels in each mouse were then observed in three random visual fields under the light microscope. There was a significant mean difference (p=0.017) in the total blood vessels observed between the five groups, with significantly more blood vessels in the mice treated with 1% *Curcuma longa* extract gel than in the negative control group. The topical administration of 1% *Curcuma longa* extract gel has a positive effect on angiogenesis in a mouse model of wound healing.

Keywords: Angiogenesis, *Curcuma longa* extract gel, incision wound.

INTRODUCTION

Wounds are defined as loss of epithelial integrity of the skin, and are classified into two types, namely acute and chronic wounds, based on the duration and nature of the healing process (Bonifant and Holloway, 2019). The healing process in acute wounds occurs in a predictable and regular time frame, whereas the healing process fails to follow the phase that should restore the structural and functional integrity of the tissue in chronic wounds (Dhivya *et al*., 2015).

Wounds are of ten experienced by everyone. Data from the WHO World Report on Child Injury Prevention states that around 10 million children in the world need hospital treatment due to non-fatal injuries, with most of them experiencing various disability complications as a result of injuries with lifelong consequences (Peden and Oyegbite, 2008). Wounds can cause physical and psychological discomfort, ultimately affecting daily activities and the quality of life of patients (Ousey *et al*., 2015). In addition, modern medical care of wounds also has an impact on the financial conditions of the sufferer due to the high cost of healthcare (Mohammadi *et al*., 2016). Modern medical care also requires extensive attention and treatment and sometimes cannot fully restore the function of the injured skin tissue (Mohammadi *et al*., 2016).

The burden of disease caused by wounds is not evenly spread but is largely borne by low-income countries. Data from the WHO Global Burden of Disease in 2004 showed that more than 95% of deaths caused by wounds occurred in low-middle income countries. Furthermore, accessibility to good quality health services in such countries may be difficult (Peden and Oyegbite, 2008). Therefore, an alternative treatment for wound healing that is relatively inexpensive and easily accessible is needed to reduce the negative impact of injury.

Indonesia is a tropical country with many plants which have been used as traditional medicine, such as turmeric (*Curcuma longa*) (Usman and Salikunna, 2015). Based on Indonesia 2014 Horticultural Production Statistics data, turmeric is the second largest contributor to the total production of national biopharmaceutical plants (18.82%) (Indonesian Ministry of Agriculture, 2015). Indeed, turmeric has been used in terms of treatment for at least 2500 years in Asia. Curcumin, a polyphenol, is an important component of turmeric which gives it its distinctive yellow color (Kocaadam and Sanlier, 2017). Previous studies have reported that curcumin has an anti-

**Corresponding author:* e-mail: a.berbudi@unpad.ac.id

aging, anti-oxidant, anti-inflammatory, anti-psoriatic, anti-microbial, anti-infection anti-mutagenic and anti-neoplastic effects (Akbik *et al*., 2014; Gupta *et al*., 2013; Hussain *et al*., 2017; Kocaadam and Şanlier, 2017; Mohammadi *et al*., 2016). These properties give curcumin the potential to be used in the treatment of various diseases, including wound healing (Akbik *et al*., 2014; Alibolandi *et al*., 2017; Chereddy *et al*., 2013; Mohanty and Sahoo, 2017; Sharma *et al*., 2018).

The process of wound healing is a complex physiological response consisting of several stages that overlap and are related to each other, hemostasis, inflammation, proliferation and remodeling (Harper *et al*., 2014; Portou *et al*., 2015). The use of a dressing technique or an optimal wound healing agent is needed to protect wounds from bacterial infections, reduce free radicals, reduce inflammation and induce cell proliferation to reconstruct damaged tissue, ultimately promoting better wound healing (Kulac *et al*., 2013; Mohanty *et al*., 2012). One of the processes that occur in the proliferation phase is angiogenesis, that is, the formation of new blood vessels from existing blood vessels (Barbul *et al*., 2015). The function of angiogenesis in wound healing is to ensure adequate perfusion of the new tissue (Akbik *et al*., 2014; Thangapazham *et al*., 2013). Therefore, this laboratory experimental study was performed to investigate the effects of turmeric gel (*Curcuma longa*) on angiogenesis in skin tissue incision wounds in Balb/c mice.

MATERIALS AND METHODS

This study was conducted at the Animal Laboratory of Universitas Padjadjaran Education Hospital and was approved by the Research Ethics Commission of Universitas Padjadjaran, No. 853 / UN6.KEP / EC / 2018.

Research Subjects and Groups

The subjects were 8-10 week-old male mice (Mus musculus) Balb / C strain obtained from PT Biofarma Laboratory (Bandung), weighing 25-30g and in healthy condition, which was assessed from hair hygiene, active movement and no injury. Exclusion criteria included a decrease in body weight of mice of more than 10% of the initial body weight during the adaptation period and sick mice. Any mice that had infected or die during the experiment were classified as dropouts. Twenty-five mice were used in this study based on the resource equation method formula and randomly grouped into five experimental groups: Negative control group given blank gel, positive control group given povidone iodine and three treatment groups, each given a turmeric extract gel with a concentration of 1%, 3% and 10%.

Turmeric Extract Gel Preparation

Turmeric extract gel preparation was conducted at the Pharmacognosy and Natural Materials Pharmacy Laboratory, Faculty of Pharmacy, Universitas Padjadjaran and began with smoothing and sifting of the turmeric rhizome (*Curcuma longa*) to produce turmeric powder. Then, 100g of turmeric powder was macerated with 900mL of ethanol 96% v/v to produce the turmeric extract, which was mixed with 1% Carbopol, humectant and triethanolamine to produce the turmeric extract gel. Blank gel did not contain any active ingredients.

Incision wound induction

The mice were acclimatized for 7 days and weighed before the intervention. They were placed in standard mice cages, with ad libitum access to food and drink. After the adaptation period, the body weight was recorded again and elimination was conducted according to the exclusion criteria.

Hair on the back of each mouse was shaved and sterilized before injury. Anesthesia was given using a ketamine injection, before an incision wound was made on the back of the mouse by means of a straight incision using scissors, with the depth reaching the subcutaneous tissue. The intervention was given topically to the wound according to the experimental group, once a day for 7 days. All subjects were sacrificed on the 8th day by cervical dislocation.

Histology sample preparation

The skin wound tissue from each mouse was harvested and fixed in 10% BNF (Buffered Neutral Formalin) solution. After fixation, the tissues were drained, cut and arranged into a tissue mold, before dehydration and drying. The tissues were then placed in a mold and soaked with liquid paraffin. Paraffin was allowed to freeze on top of the cooling machine so that paraffin blocks produced could be cut using a microtome with a thickness of 3-4 µm. Tissue slices were placed on glass slides and put into a 60°C incubator, before staining with Hematoxylin-Eosin dye for histopathological examination.

Histopathological Analysis

The tissue sections were observed under a light microscope at 400x magnification. The number of blood vessels in three high power fields (HPF) randomly selected from the wound area were counted by two independent blinded observers to ensure the objectivity of the research. Data are presented as mean and standard deviation (mean +/- SD) of the number of blood vessels per HPF in each treatment group.

STATISTICAL ANALYSIS

The Shapiro-Wilk normality test and Levene homogeneity test were performed on the data using IBM® SPSS® version 25 statistical software. ANOVA test was used as the data were normally distributed and homogeneous. A confidence interval (CI) of 95% was applied and results were considered statistically significant if the value of $p<0.05$. Post hoc Tukey test was used to determine

whether there were significant differences in angiogenesis in wound healing in Balb / c mice with the topical administration of turmeric extract gel (*Curcuma longa*).

RESULTS

None of the 25 experimental mice were excluded and no wound complications occurred during the experiment. The mean number of blood vessels per HPF in the experimental groups are presented in fig. 1.

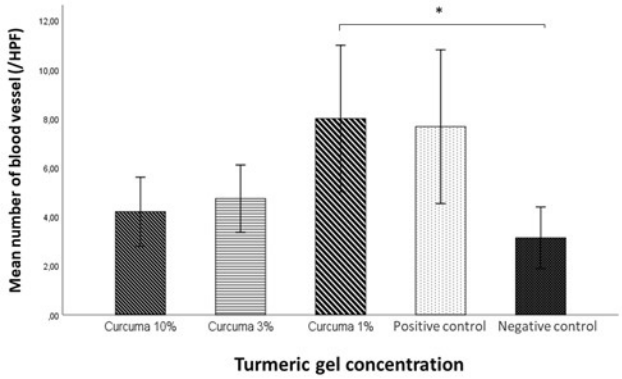

Fig. 1: The mean number of blood vessels per field of view. Interventions were given in the form of turmeric extract gel (concentration of 1%, 3% and 10%) / blank gel / povidone iodine once a day for 7 days and the number of blood vessels was calculated by observing three random HPF using a light microscope with 400x magnification. Data are presented as the mean number of blood vessels per HPF ± standard deviation in each trial group (n=25). ANOVA followed by post hoc Tukey showed a significant difference between the 1% extraction treatment group and negative controls (*$p<0.05$).

The mean number of blood vessels per HPF in all treatment groups given turmeric extract gel tended to be higher compared to the negative control group, with the highest number of blood vessels per HPF observed in treatment group III (1% turmeric extract). The positive control group (povidone iodine) had the second highest average value after treatment group III, followed by treatment group II (3%), treatment I (10%) and negative control (blank) (fig. 1). The microscopic structure of skin tissue after 7 days treatment among the groups were presented in fig. 2.

According to ANOVA, there were significant differences in the mean number of blood vessels per HPF between the experimental groups, with the post hoc analysis revealing that there was a significant difference in the number of blood vessels per HPF between treatment group III (1%) and the negative control group ($p=0.038$).

DISCUSSION

Turmeric has long been used in traditional medicine and the rhizome contains various substances including proteins, fats, minerals, carbohydrates, essential oils and curcuminoids (Kocaadam and Şanlier, 2017). The curcuminoids contained in turmeric rhizomes include curcumin, demethoxycurcumin (curcumin II) and bisdemethoxicurcumin (curcumin III) (Cooksey, 2017; Kocaadam and Şanlier, 2017). Curcumin has been shown to possess anti-cancer, anti-aging, anti-inflammatory and wound healing effects (Kianvash *et al*., 2017; Mohanty and Sahoo, 2017) mediated through the regulation of various transcription factors, growth factors, cytokines, protein kinases and other enzymes (Kocaadam and Şanlier, 2017; Thangapazham *et al*., 2016). Administration of oral and topical curcumin is reported to be effective in normal wound healing but has no effect on diabetic wounds (Berbudi *et al*., 2018; Thangapazham *et al*., 2013).

This study was conducted to assess the effects of the topical application of a turmeric extract gel on angiogenesis during wound healing in Balb / c mice. The topical application of curcumin was chosen because of the greater accessibility to the wound site and it is more effective than oral administration (Mohanty and Sahoo, 2017). After seven days of treatment, the number of blood vessels observed in curcuma-treated mice was higher than the control group, with the highest number of blood vessels was observed in tissue sections from mice treated with 1% turmeric extract gel. The results of this study are in accordance with several previous studies which showed an increase in neovascularization in groups of animals given curcuma (Kant *et al*., 2015; Kianvash *et al*., 2017; Mohammadi *et al*., 2016; Sharma *et al*., 2018; Thangapazham *et al*., 2016). Study by Kant *et al*. (2015) using 60 diabetic mice showed an increase in microvesel density on days 3, 7 and 14 treatment ($p<0.05$) with immunohistochemical testing for CD31 in the curcumin-treated group compared to the control group (Kant *et al*., 2015). Furthermore, Kulac *et al*. conducted a study using Wistar-albino rats with burn wounds which were grouped into groups of days 4, 8 and 12 (post burn) and each group contained burn and burn + curcumin subgroups. After biochemical and histopathological analysis, the final results of the study showed that the rats given curcumin recovered faster with one indicator in the form of a higher level of angiogenesis than the control group ($p <0.05$) (Kulac *et al*., 2013). Similarly, Emirogru *et al*. (2017) in his study stated that curcumin can support neovascularization and small capillary formation (Emiroglu *et al*., 2017). Oil extracted from Curcuma longa also showed significant effects of proangiogenesis (Araújo *et al*., 2016).

The effect of turmeric extract gel on wound healing, especially in the aspect of angiogenesis, can be explained by first understanding the basic mechanism of angiogenesis. Angiogenesis involves various growth factors, cell-cell interactions, interactions with ECM (extra cellular matrix) proteins as well as tissue enzymes

(Kumar *et al*., 2013; Richarz *et al*., 2017). These factors can be categorized as proangiogenic or antiangiogenic factors according to their effect on angiogenesis (Meric-Bernstam and Pollock, 2015). VEGF (vascular endothelial growth factor) is one of the most important stimulators of angiogenesis (Meric-Bernstam and Pollock, 2015; Yar *et al*., 2017), stimulating endothelial cell migration and proliferation, initiating capillary sprouting, and inducing vasodilation through stimulation of the production of NO (nitric oxide) when it binds to VEGFR2 (VEGF receptor-2), ultimately promoting the formation of a new blood vessel lumen (Buijs *et al*., 2017; Kumar *et al*., 2013).

In addition to VEGF, TGF-β (transforming growth factor-β) also has a strong angiogenic effect (Sharma *et al*., 2012), increasing VEGF synthesis through the Akt and ERK pathway (Kant *et al*., 2015). During wound healing, angiogenesis is triggered by the formation of hemostasis plugs in conjunction with thrombocyte release of TGF-β, PDGF (platelet-derived growth factor) and FGF (fibroblast growth factor) (Harper *et al*., 2014). FGF stimulates endothelial cell proliferation, while PDGF helps to stabilize the new blood vessels formed (Kumar *et al*., 2013). VEGF is also released due to hypoxia, which also induces angiogenesis. Mixed metalloproteinase (MMP) enzyme is also activated by neutrophils in hypoxic tissue, supporting angiogenesis through VEGF release and remodeling from the extra cellular matrix (Harper *et al*., 2014). Wound hypoxia also increases the expression of HIF-1α (hypoxia inducible growth factor-1alpha), further increasing the synthesis of VEGF and SDF-1α (stromal cell-derived factor-1α), which promote homing of endothelial progenitor cells that participate in the formation of new blood vessels (Kant *et al*., 2015).

Gao *et al*. (2015) showed that curcumin can induce polarization of M2 macrophages as evidenced by an increase in classic M2 markers such as MMR (macrophage mannose receptor), Arg-1 (arginase-1) and PPAR-γ (peroxisome proliferator-activated receptor-γ) (Gao *et al*., 2015). M2 macrophages can then influence angiogenesis through increased production of angiogenic factors such as VEGF-A, FGF and TGF-β (Barbay *et al*., 2015). Sharma *et al*. revealed curcumin can induce angiogenesis through upregulation of MMP-2, membrane type 1-MMP, VEGF and TGF-β at protein and mRNA levels (Sharma *et al*., 2012). This was supported by Kant *et al*. (2015), who reported increased expression of VEGF, TGF-β1, HIF-1α, SDF-1α and HO-1 in the curcumin-treated group. HO-1 (heme oxygenase-1) plays a role in angiogenesis through its action in regulating the synthesis and activity of VEGF and SDF-1α (Kant *et al*., 2015). Through the induction of these angiogenic factors, curcumin stimulates vascular sprout formation (Thangapazham *et al*., 2016), as evidenced by a higher mean number of blood vessels in all groups treated with the turmeric extract gel in comparison with the negative control group in the present study. In addition, the group treated with 1% turmeric extract gel had significantly more blood vessels than the negative control group, with more blood vessels than the positive control group, although this did not reach significance. The administration of povidone iodine as the positive control can improve neovascularization via stimulation of TGF-β expression (Wang *et al*., 2017).

Interestingly, previous studies have shown that curcumin can act as a stimulator or inhibitor of angiogenesis (Kant *et al*., 2015). There are studies that showed antiangiogenic effects of curcumin through suppression of HIF-1α and VEGF expression in pituitary adenomas (Kant *et al*., 2015). Curcumin was also stated to have antiangiogenic properties in fibrosarcoma cells (Sharma *et al*., 2012). Curcumin was known to suppress proliferation of human vascular endothelial cells in vitro. In addition, curcumin can also inhibit the response of FGF-2-induced angiogenesis in vivo (Pari *et al*., 2008). Curcumin can affect the whole process of angiogenesis through downregulation of transcription factors such as NF-κB and proangiogenesis factors such as VEGF, bFGF, and MMPs, which all of them are closely associated with the process of tumorigenesis (Wang and Chen, 2019). This suggests that curcumin can also be used as an anti-cancer agent related to its effect of suppressing tumor angiogenesis (Pari *et al*., 2008).

The concentration of curcuma extract used in this study was 1%, 3% and 10%. The curcuma dose may be one of the factors that can explain why curcuma has dual proangiogenesis and antiangiogenesis activities, where the effect of proangiogenesis was seen at lower doses, whereas the antiangiogenesis effect at higher doses, which was also seen in the results of this study (Wang and Chen, 2019).

The number of blood vessels in the mice treated with 3% and 10% turmeric extract gel was lower than those in mice treated with 1% turmeric extract gel, which may be due to the toxicity of curcumin on cells. Phan *et al*. (2001) showed that the increase in the curcumin concentration was toxic to fibroblast cells (Phan *et al*., 2001), possibly interfering with angiogenesis as the production of growth factors, such as VEGF, TGF-β and PDGF, by fibroblasts is inhibited (Newman *et al*., 2011). In addition, Jackson *et al*. (2013) also reported that curcumin at certain concentrations can inhibit endothelial cell DNA synthesis (Jackson *et al*., 2013).

The limitation of this study lies in that only the number of blood vessels per field of view was counted without assessing other indicators of angiogenesis, such as levels of proangiogenic proteins in the wound tissue. There may also have been counting errors, repeating counts in the same vessel in one field of view.

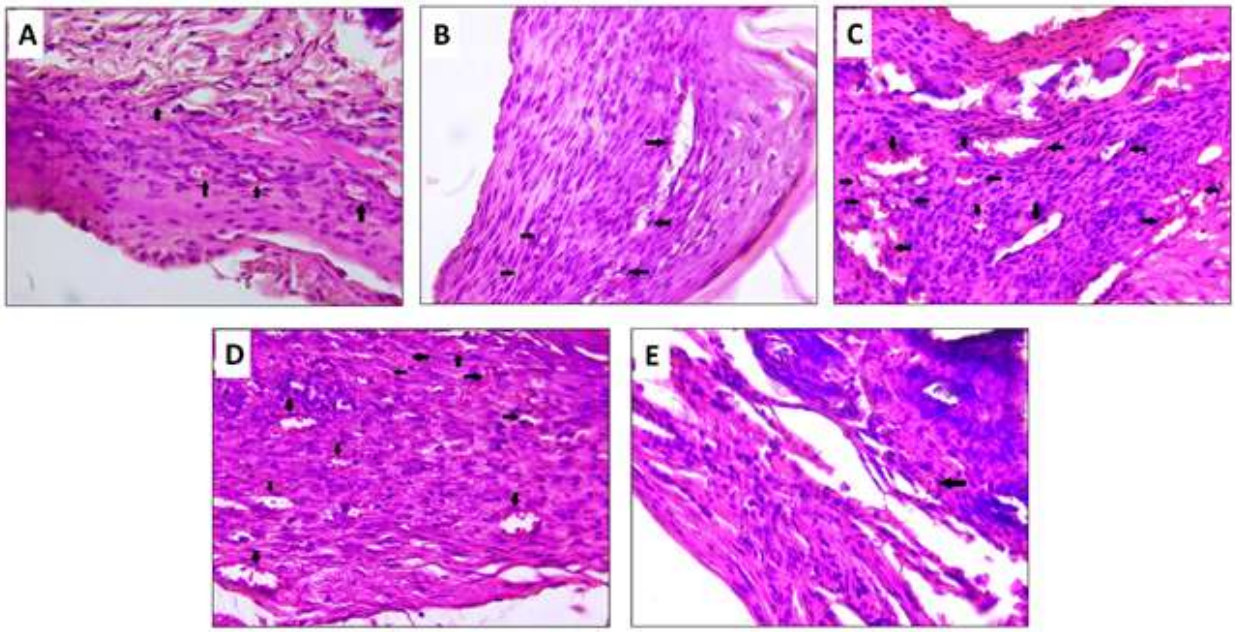

Fig. 2: Seven days of topical *C. longa* extract administration impacts the vessel number of incised skins. Microscopic of incised skin tissue that treated by 10% *C. longa* extract (A), 3% *C. longa* extract (B), 1% *C. longa* extract (C), povidone iodine (positive control) (D) as well as blank gel (negative control) (E). The microscopic vessels were pointed by black arrows. Histopathology of mice skin tissue were observed under microscope (400x total magnification).

CONCLUSION

This study showed that the topical application of *Curcuma longa* extract gel can promote angiogenesis in a mouse model of wound healing, with statistically more blood vessels in mice treated with 1% turmeric extract gel than the negative control group. Levels of proangiogenic proteins in the wounds should also be assessed to confirm the effects of turmeric extract gel in promoting angiogenesis. Furthermore, the optimal concentration of turmeric extract gel required for wound healing should be established due to the possible toxic effects at high concentrations. Future research is also required to identify the active components in the turmeric rhizome extract responsible for promoting angiogenesis.

REFERENCES

Akbik D, Ghadiri M, Chrzanowski W and Rohanizadeh R (2014). Curcumin as a wound healing agent. *Life Sci.,* **116**(1): 1-7.

Alibolandi M, Mohammadi M, Taghdisi SM, Abnous K, Ramezani M (2017). Synthesis and preparation of biodegradable hybrid dextran hydrogel incorporated with biodegradable curcumin nanomicelles for full thickness wound healing. *Int. J. Pharm.,* **532**(1): 466-477.

Araújo LA, Araújo RGM, Gomes FO, Lemes SR, Almeida LM, Maia LJQ, Gonçalves PJ, Mrué F, Silva-Junior NJ, Paulo R and DE Melo-Reis 2016. Physicochemical / photophysical characterization and angiogenic properties of *Curcuma longa* essential oil. *An. Acad. Bras. Cienc.,* **88**(3 Suppl.): 1889-1897.

Barbay V, Houssari M, Mekki M, Banquet S, Edwards-le F, Thuillez C, Richard V and Brakenhielm E (2015). Role of M2-like macrophage recruitment during angiogenic growth factor therapy. *Angiogenesis,* 18.

Barbul A, Efron DT and Kavalukas SL (2015). Wound healing, *In*: Brunicardi FC (Ed.), Schwartz's Principles of Surgery. Mc Graw Hill Education, pp.241-272.

Berbudi A, Hasibah UH, Faried A, Putri AC and Hamda ME (2018). Administration of *Curcuma longa* extract topically does not accelerate skin excision wound closure in alloxan-induced diabetic mice model. *Biomed. Pharmacol. J.,* **11**(1): 437-444.

Bonifant H and Holloway S (2019). A review of the effects of ageing on skin integrity and wound healing. *Br. J. Community Nurs.,* 24(Sup3): S28-S33.

Buijs N, Oosterink JE, Jessup M, Schierbeek H, Stolz DB, Houdijk AP, Geller DA and Van Leeuwen PA (2017). A new key player in VEGF-dependent angiogenesis in human hepatocellular carcinoma: Dimethylarginine dimethylaminohydrolase 1. *Angiogenesis,* **20**(4): 557-565.

Chereddy KK, Coco R, Memvanga PB, Ucakar B, Des Rieux A, Vandermeulen G and Preat V (2013). Combined effect of plga and curcumin on wound healing activity. *J. Control. Release,* **171**(2): 208-215.

Cooksey CJ (2017). Turmeric: Old Spice, New Spice. *Biotech. Histochem.,* **92**(5): 309-314.

Dhivya S, Padma VV and Santhini E (2015). Wound dressings - A review. Biomed. 5(4):22

Emiroglu G, Ozergin Coskun Z, Kalkan Y, Celebi Erdivanli O, Tumkaya L, Terzi S, Ozgur A, Demirci M and Dursun E (2017). The effects of curcumin on wound healing in a rat model of nasal mucosal trauma. *Evidence-based Complement. Altern. Med.,* 2017: 9452392

Gao S, Zhou J, Liu N, Wang L, Gao Q, Wu Yan, Zhao Q, Liu P, Wang S, Liu Y, Guo N, Shen Y, Wu Yue, Yuan Z (2015). Curcumin induces M2 macrophage polarization by secretion IL-4 and/or IL-13. *J. Mol. Cell. Cardiol.,* **85**: 131-139.

Gupta SC, Sung B, Kim JH, Prasad S, Li S and Aggarwal BB (2013). Multitargeting by Turmeric, the Golden Spice: From Kitchen to Clinic. *Mol. Nutr. Food Res.,* **57**(9) 1510-1528.

Harper D, Young A and McNaught CE (2014). The physiology of wound healing. surg. (United Kingdom) **32**(9): 445-450.

Hussain Z, Thu HE, Amjad MW, Hussain F, Ahmed TA and Khan S (2017). Exploring recent developments to improve antioxidant, anti-iflammatory and antimicrobial eficacy of curcumin: A review of new trends and future perspectives. *Mater. Sci. Eng. C.,* **77**: 1316-1326.

Jackson SJT, Murphy LL, Venema RC, Singletary KW and Young AJ (2013). Curcumin binds tubulin, induces mitotic catastrophe, and impedes normal endothelial cell proliferation. *Food Chem. Toxicol.,* **60**: 431-438.

Kant V, Gopal A, Kumar Dhirendra, Pathak NN, Ram M, Jangir BL, Tandan SK and Kumar Dinesh (2015). Curcumin-induced angiogenesis hastens wound healing in diabetic rats. *J. Surg. Res.,* **193**(2): 978-988.

Kementerian Pertanian Direktorat Jenderal Hortikultura, (2015). Statistik Produksi Hortikultura Tahun 2014.

Kianvash N, Bahador A, Pourhajibagher M, Ghafari H, Nikoui V, Rezayat SM, Dehpour AR and Partoazar A (2017). Evaluation of propylene glycol nanoliposomes containing curcumin on burn wound model in rat: biocompatibility, wound healing and anti-bacterial effects. *Drug Deliv. Transl. Res.,* **7**(5): 654-663.

Kocaadam B and Sanlier N (2017). Curcumin: An active component of turmeric (*Curcuma longa*) and Its Effects on Health. *Crit. Rev. Food Sci. Nutr.,* **57**(13): 2889-2895.

Kulac M, Aktas C, Tulubas F, Uygur R, Kanter M, Erboga M, Ceber M, Topcu B and Ozen OA (2013). The effects of topical treatment with curcumin on burn wound healing in rats. *J. Mol. Histol.,* **44**(1): 83-90.

Kumar V, Abbas AK and Aster JC (Eds.), (2013). Inflammation and Repair, *In*: Robbins Basic Pathology. ELsevier Saunders, Philadelphia, pp.29-74.

Lazarus G, Cooper D, Knighton D, Margolis D, Percoraro R, Rodeheaver G and Robson M (1994). Definitions and guidelines for assessment of wounds and evaluation of healing. *Wound Repair Regen.,* **2**(3): 165-170.

Meric-Bernstam F and Pollock RE (2015). Oncology, *In*: Brunicardi, FC (Ed.), Schwartz's Principles of Surgery. Mc Graw Hill Education, pp.273-316.

Mohammadi MR, Rabbani S, Bahrami SH, Joghataei MT and Moayer F (2016). Antibacterial performance and in vivo diabetic wound healing of curcumin loaded gum tragacanth/poly(ε-caprolactone) Electrospun Nanofibers. *Mater. Sci. Eng. C.,* **69**: 1183-1191.

Mohanty C, Das M and Sahoo SK (2012). Sustained wound healing activity of curcumin loaded oleic acid based polymeric bandage in a rat model. *Mol. Pharm.,* **9**(10): 2801-2811.

Mohanty C and Sahoo SK (2017). Curcumin and its topical formulations for wound healing applications. *Drug Discov. Today*, 22(10):1582-1592.

Newman AC, Nakatsu MN, Chou W, Gershon PD and Hughes CCW (2011). The requirement for fibroblasts in angiogenesis: Fibroblast-derived matrix proteins are essential for endothelial cell lumen formation. *Mol. Biol. Cell,* **22**(20): 3791-3800.

Ousey K, Edward KL and Lui S (2015). Identifying and exploring physical and psychological morbidity and patient and family caregiver resilience following acute wound development and/or wound blistering post orthopaedic surgery: A systematic review. *Int. Wound J.,* **12**(1): 63-69.

Pari L, Tewas D and Eckel J (2008). Role of curcumin in health and disease. *Arch. Physiol. Biochem.,* **114**(2): 127-149.

Peden M and Oyegbite K (2008). World Report on Child Injury Prevention. Geneva: World Health Organization.

Phan T, See P, Lee S and Chan S (2001). Protective effects of curcumin against oxidative damage on skin cells *in vitro*: Its implication for wound healing. *J. Trauma.,* **51**(5): 927-931.

Portou MJ, Baker D, Abraham D and Tsui J (2015). The innate immune system, toll-like receptors and dermal wound healing: A review. Vascul. Pharmacol., 71:31-6.

Richarz NA, Boada A and Carrascosa JM (2017). Angiogenesis in dermatology insights of molecular mechanisms and latest developments. *Actas Dermosifiliogr.,* **108**(6): 1-9.

Sharma AV, Ganguly K, Paul S, Maulik N and Swarnakar S (2012). Curcumin heals indomethacin-induced gastric ulceration by stimulation of angiogenesis and restitution of collagen fibers via VEGF and MMP-2 mediated signaling. antioxid. *Redox Signal.* **16**(4): 351-362.

Sharma M, Sahu K, Singh SP and Jain B (2018). Wound healing activity of curcumin conjugated to hyaluronic acid: *in vitro* and *in vivo* evaluation. Artif. *Cells, Nanomedicine Biotechnol.,* **46**(5):1009-1017

Thangapazham RL, Sharad S and Maheshwari RK (2016). Phytochemicals in wound healing. *Adv. Wound*

Care, **5**(5): 230-241.

Thangapazham RL, Sharad S and Maheshwari RK (2013). Skin regenerative potentials of curcumin. *BioFactors,* **39**(1): 141-149.

Usman AR and Salikunna NA (2015). Pengaruh lendir Bekicot (*Achatina fulica*) terhadap waktu penutupan luka sayat (*Vulnus scissum*) pada Mencit (Musmusculus). *Med. Tadulako, J. Ilm. Kedokt.,* **2**(1): 31-39.

Wang L, Qin W, Zhou Y, Chen B, Zhao X, Zhao H, Mi Emma, Mi Ella, Wang Q and Ning J (2017). Transforming growth factor β plays an important role in enhancing wound healing by topical application of Povidone-iodine. *Sci. Rep.,* **7**(1): 1-8.

Wang T and Chen J (2019). Effects of Curcumin on vessel formation insight into the pro- and antiangiogenesis of curcumin. *Evidence-Based Complement. Altern. Med.,* pp.1-9.

Yar M, Shahzadi L, Mehmood A, Raheem MI, Roman S, Chaudhry AA, ur Rehman I, Ian Douglas CW and MacNeil S (2017). Deoxy-sugar releasing biodegradable hydrogels promote angiogenesis and stimulate wound healing. *Mater. Today Commun.,* **13**: 295-305.