International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2015): 6.391

Assessment of *Lantana camara* L. Leaf Extract in Wound Healing in Induced Rabbits Skin Injuries

Farah D. Salim[1], Kadhim M. Ibrahim[2], Waleed H. Yousif[3]

[1, 2, 3]Al- Nahrain University, College of Science, Department of Biotechnology, Baghdad, Iraq

Abstract: ***The present study was conducted to evaluate wound healing potentials of Lantana camara. Two different solvent extracts (ethanol and aqueous) were prepared from the leaves of L. camara by estimated using soxhlet and maceration. The total flavonoids compounds concentration were estimated using high performance liquid chromatography (HPLC). The highest concentration of flavonoids were observed in ethanol extract of L. camara than aqueous extract. Acute inflammation was induced in laboratory animals (rabbits) by injected 0.1mL of 2.5% formalin into the wound incision. aqueous and ethanol extracts of L. camara had anti-inflammatory and healing effect in compared with the Vaseline on rabbit skin. Utilizing histopathological study to notice healing of the wound. Explicate the histological examination that healing of the skin was obvious by vanishing of odema and decrease in scar size, enhancement of fibroblast proliferation, collagen regeneration, Keratinization and Epithelialization as compared with the treatment groups during using L. camara. Extracts of L. camara have the ability of inhibition of inflammation induced by formalin and appeared to be the most active component for healing the wound in rabbits as preclinical study.***

Keywords: *Lantana camara*, phytochemical analysis, excision wound, wound healing activity

1. Introduction

Wound healing process involves different steps including coagulation, inflammation, formation of granulation tissue, matrix formation, remodeling of connective tissue, collagenization and acquisition of wound strength (1). According to Puratchikody *et al.* (2006) (2) the four phases of normal wound healing involve hemostasis, inflammation, proliferation, and remodeling. Healing will be completed only after the disrupted surface was firmly knit by collagen. Kumarasamyraja *et al.*(3) showed that nearly six million people suffer from chronic wounds in some third world countries because of the expensive modern drugs that might have side effects. This problem became a focus to researchers in order to solve the wound infection by using plant as an alternative solution. Plant products may have the potential to heal wound because they promote the repair mechanisms in natural way. *Lantana camara* Linn. is a flowering ornamental plant belongs to the family Verbenaceae. The plant *L. camara* common names are mynah in Iraq and wild or red sage in America. Generally the studies on lantana are indicate the presence of several chemical compounds in different parts, including flavonoids, alkaloids, phenols, tannins, saponin, steroids, proteins, tri-terpenoids, catechins, isocatechins ,glycosides and reduced sugar. It is used traditionally to treat various diseases like cancers, chicken pox, asthma, ulcers, swellings, eczema, tumors, measles, high blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism and malaria (4). However, there is no exactly proved information to explain whether the *L. camara* has wound healing activity or not. Therefore, this study was planned to estimate the effects of *L. camara* leaf extract on wound healing in rabbits.

2. Material and Methods

2.1 Plant Collection

The fresh *Lantana camara* L. leaves were collected from gardens of Al- Nahrain University, air dried at room temperature, and ground into powder by using coffee grinder.

2.2 Preparation of extracts

2.2.1 Aqueous extract

A quantity of 50g of *Lantana camara* L. leaves powder was boiled in distilled water for 15-20 minutes at 80°C, and filtered using cloth mesh. Then concentrated at 40°C by rotary evaporator and kept in a freezer until use (5).

2.2.2 Alcohol extract

A quantity of 50g of *Lantana camara* L. leaves powder was mixed with 250ml of 96% ethanol by soxhlet apparatus for 6 hrs. At 70°C, then the solvent was removed under reduced pressure by a rotary evaporator at 40°C. The crude solid extracts were kept in a deep freezer until use (6).

The tested preparations of sample crude extracts at doses 100, 50, or 25mg.ml^{-1} were mixed with Vaseline in a ratio of (3:1 *v*⁄v) and applied topically daily for 10 days (7).

2.3 Identification of total flavonoids by high performance liquid chromatography (HPLC)

The ethanol and aqueous extracts taken to drought and the deposit taken up for HPLC analysis (8). For standard compounds were dissolved at a concentration of 10mg/ml in distilled water (2:1 v/v). The standard compounds included stock solution of rutin, quercetin, apegenin, leuteolin, kaempferol, pinobanksin, pinocembrin, pinobanksin-3-acetate, chrysin, galangen, tecrochrysin, genistein, curcumin, and merietin. Optimum conditions: Column: DB-C18

(50×2.0 mm, 3µm particle size), Flow rate: 1.2ml/min, Mobile phase: phosphate buffer: methanol (60: 40 v/v).

2.4 Experimental Animals

Nine of healthy mature rabbits 2.5-3Kg weight were used. They were maintained at a temperature of 20- 25°C, with free access to food (standard pellets) and water throughout the experimental work. The rabbits were kept two days for acclimatization. As the experiment was designed to assess the histopathological effects, the animals were divided into three groups, as follows:

1) First group (A): Animal skin was wounded to left side of the dorsal surface with no treatment (negative control), whereas the right side of the dorsal surface was treated with Vaseline (positive control).
2) Second group (B): Animal skin was wounded and treated with ethanol extract of *L. camara* leaf at different concentrations (100mg/ml, 50mg/ml, or 25mg/ml).
3) Third group (C): Animal skin was wounded and treated with aqueous extract of *L. camara* leaf at different concentrations (100, 50, or 25mg.ml^{-1}).

2.4.1 Animal Treatment

Both sides of the animal were cleaned with sterile distilled water and then shaved with sharp blade. After removal of hair from both sides, all animals were wounded by making incisions at both sides of dorsal surface (1cm) below the scapula, which was 3cm long and 1cm deep (9). Both sides of the dorsal surface contained three incisions for treatment with different extracts. After half an hour, a syringe was used to inject 0.1 ml of 2.5% formalin into the wound incision daily for two days to induce an acute inflammation (10).

2.4.2 Measuring wound healing activity

Wounded area was measured by digital photographs using the image analysis program. The evaluated surface area was used to calculate the percentage of wound contraction (11; 12).

$$\%\ \text{of wound contraction} = \frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$

2.5 Statistical analysis

Analysis of variance (ANOVA) was performed to test whether group variance was significant or not, according to Al-Mohammed *et al.*, (13). The level of significance was considerable at p≤ 0.05.

3. Results and Discussion

3.1 High Performance Liquid chromatography (HPLC) Analysis

The ethanol and aqueous extracts of *L. camara* showed the following curve with different peaks area representing the presence of different flavonoids compounds. Flavonoid compounds were identified in extracts (Figure 1). The total concentration of these compounds recorded in aqueous extract of *L. camara* leaves were contain on 318.048, 421.774, 618.077, 114.957, 554.613 µg.mL^{-1} for quercetin, apegenin, leuteolin, kaempferol and rutin respectively. While, the ethanol extract of *L. camara* was contain on1104.031, 124.629, 324.885, 60.606, 2045.031 µg.mL^{-1} for quercetin, apegenin, leuteolin, kaempferol and rutin respectively. According to HPLC results, in previous study, flavonoids were identified by Venkatachalam *et al.* (14) as (apegenin, kaempferol and rutin).

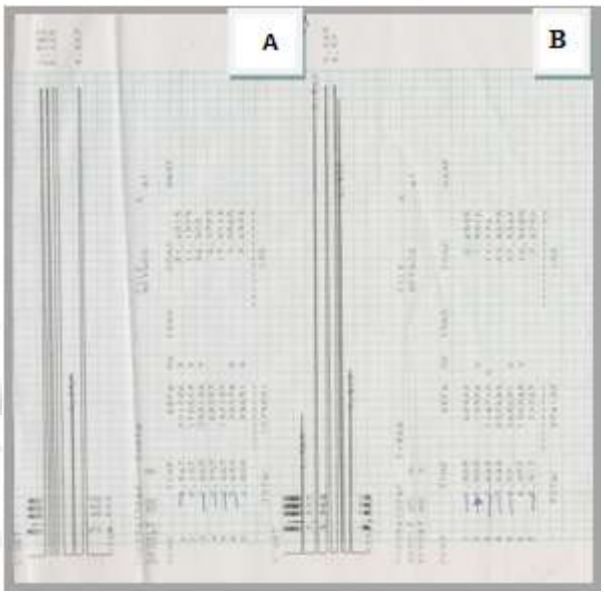

Figure 1: HPLC chromatogram of *Lantana camara* L. leaves (A) ethanol extract (B) aqueous extract

3.2 Effect of different extracts on wounds healing

Our researches on excision wound healing type exposes that all treatment groups illustrated reduced wound area (p≤ 0.05) from day to day. Wound healing activity has been observed at eighth day of treatment compared to control skin, in table (1) treated wound showed a rate of wound closure with small scar, length of incision became smaller which means skin breaking stretch, and inflammation edema size disappeared.

Table 1: Effect of different treatment on the wounds contraction in normal rabbits

Treatments		Wound contraction	
		4day	8 day
Negative control (Untreated)		13.61±3.19	50.24±6.20
Positive control (Vaseline)		23.19±4.50	69.03±3.31
Aqueous extract of *L.camara*	25 mg.mL^{-1}	18.56±6.73	65.63±3.96
	50 mg.mL^{-1}	24.10±6.38	70.87±1.45
	100 mg.mL^{-1}	31.30±4.89	82.58±0.6
Alcohol extract of *L.camara*	25 mg.mL^{-1}	14.8±4.60	70.89±4.40
	50 mg.mL^{-1}	24.50±6.63	73.86±2.67
	100 mg.mL^{-1}	34.13±4.52	87.84±1.18

* Values are mean of three replicates ± S.D

The results of the present study revealed that the highest wound healing activity was observed in ethanol extract of *L. camara* at concentration (100mg.mL^{-1}), followed by aqueous extract of *L. camara* at concentration (100mg.mL^{-1}).

The measurement on 8th day showed that the mean percentage wound closure area was found to be 50.24±6.20

(negative control group), 69.03±3.31(positive control group), 65.63±3.96, 70.87±1.45, and 82.58±2.60 (aqueous extract of *L. camara)* for concentrations 25, 50, 100 mg.mL^{-1}, respectively, and 70.89±4.40, 73.86±2.67, 87.84±1.18 (ethanol extract of *L. camara*) for concentrations (25, 50, 100 mg.mL^{-1}, respectively) (table 1).

Histological features of incision in animals skin are illustrated in figure (2 n), (Group A). The histopathological changes in the negative control are characterized by the presence of wound injury, and congestion of blood vessels and neutrophils aggregation led to form pus of dead neutrophils surrounded by capsule consisted of fibrous connective tissue, there was epithelialization of epidermis and also a granulation tissue characterized by fibroblast and collagen fiber. While the histopathological changes of the positive control (Vaseline treated) involved the presence of granulation tissue covered by few layers of epithelialization cells which represented the epidermis (figure 2 p). Vaseline (petroleum jelly) kept wounds clean and moist, and also provided an occlusive layer. It kept germs out, thus decreasing the risk of infection. Apart from that it hydrated the wound and stimulated the healing process (15).

In the groups B (figure a, b, c), the skin looks like normal consisted of epidermis and dermis. Healing occurred completely just with thin layer of epidermis epithelializetion with few granulation tissue. At concentration 50mg.mL^{-1} the healing was less than at 100mg.mL^{-1} of *L. camara*. Interrupted epithelializetion cover granulation tissue which consisted of congestion in blood vessels. While at 25mg.mL^{-1}, the healing is characterized by granulation tissue infiltrated with mononuclear cells (inflammatory cells).
Examination of figures (a, b, c) shows sections treated with aqueous extract of *L. camara* (group C) at 100mg.mL^{-1} reveals a clear epithelializetion interrupted in some areas. The granulation tissue infiltrated with mononuclear cells. At 50mg.mL^{-1} the healing also appeared clear, the epithelializetion started with one layer of epithelializetion and the granulation tissue infiltrated with eosinophil. While at 25mg.mL^{-1} a wide area of dermal tissue covered the granulation tissue. It was normal area infiltrated with inflammatory cells polymorphnuclear cells. The effect of extracts was clear and rapid after eight days of treatment, through reduced scar formation, exhibited increased fibroblast proliferation, collagen regeneration, angiogenesis, keratinization and epithelialization as compared to treatment groups. Levinson and Coworkers. (16) stated that a decrease of open skin wounds, leaves a extraordinarily little scar as the circumference ordinary skin moves centripetally to shut the wound. Enhanced healing was ascribed to generate collagen fibers, and angiogenesis.

During the process of wound healing, various inflammatory cells (neutrophils, macrophages, and fibroblasts) produce reactive oxygen species (ROS) and increase proteases, which lead to kill fibroblasts , other cells and less flexible for skin lipids, so oxidative stress occupies a central role in wound healing to defense against microorganisms preventing host cell damage by inhibiting ROS (17). The antioxidants such as flavonoids, tannins, vitamin C and vitamin E are identified to accelerate the wound healing action by mainly eliminating the oxidants, the alteration in homeostasis leading to oxidative stress (18).

The results demonstrated that extracts affected differently in wound healing activity, but ethanol extract of *L. camara* was the best in this regard. Such potent activity seemed to be correlated with the total polyphenolic compounds, because the strong wound healing activity occurred in extracts that are rich in phenolic and flavonoids compounds. It may due to the constituents possess very potent antioxidant and antimicrobial activities. Furthermore, the results are in agreement with Mekala *et al*. (19) who indicated that histological examination showed enhanced fibroblast proliferation and collagen regeneration in the ethanol extract of *L. camara* leaf treated wound tissue of diabetic rats.

It has been reported that flavonoids compounds are mostly pharmacological active constituents of various samples and they are well known that these products are powerful anti-oxidants. For this reason *L. camara* leaves is considered as being natural source of wound healing compounds. Wound healing activity of various samples may played an important role in their anti-microbial activity.

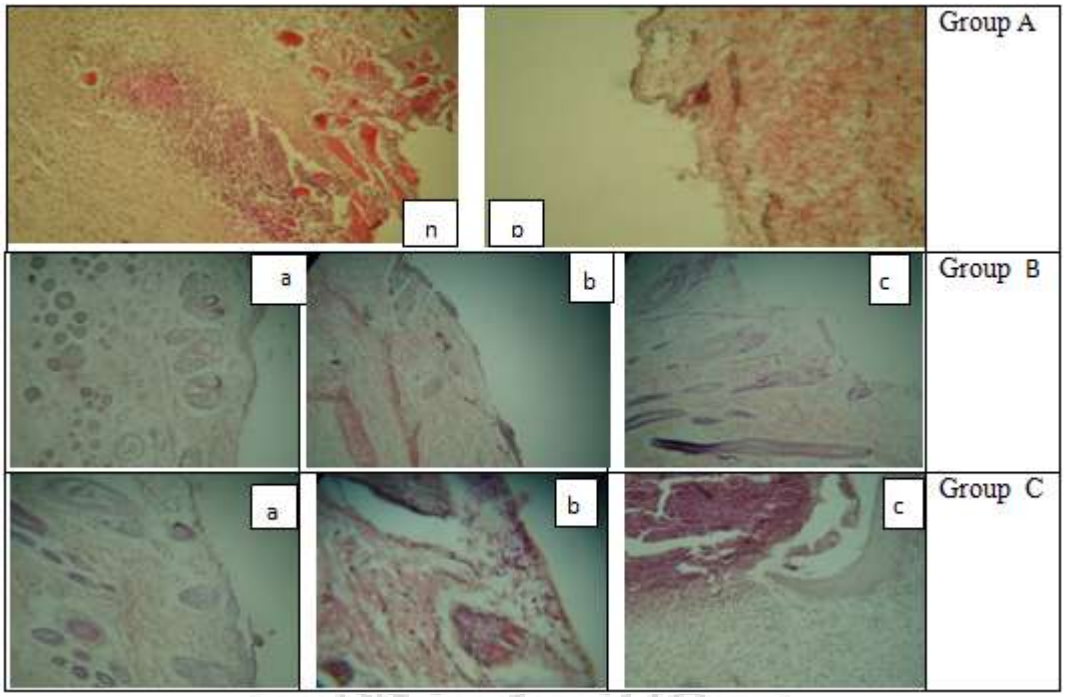

Figure 2: Longitudinal section showing the rabbit skin treated with *L. camara* aqueous and ethanol extract at (a) 100, (b) 50, (c) 25 mg.mL^{-1}.

4. Conclusion

In rabbits, Ethanol extracts of *L. camara* has a therapeutic effect on induced wound during eight days. Further studies are required to isolate the compound responsible for the wound healing activity of *Lantana camara* and to evaluate the mechanism of wound healing activity.

References

[1] **Suresh**, J., Rao, P. and Reddy, M. (2002). Wound healing effects of *Heliotropium indicum*, *Plumbago zeylanicum* and *Acalypha indica* in rats. J. Ethnopharmacol., 79: 249–251.

[2] **Puratchikody**, A., Nithya, C. and Nagalakshmi, G. (2006). Wound healing activity of *Cyperus rotundus* Linn. Indian J. Pharm. Sci., 68:97-101.

[3] **Kumarasamayraja,** D., Jeganathan, N. S. and Manavalan, R. (2012). A Review on medicinal plants with potential wound healing activity. Int. J. Pharm. Sci., 2 (4): 105-111.

[4] **Sanjeeb**, K., Gaurav, K., Loganathan, K. and Kokati, V. R. (2012). A review on medicinal properties of *Lantana camara* Linn. Res. J. Pharm. Tech., 5 (6): 711-715.

[5] **Harborne**, J. B. (1973). Phytochemical Methods. Science paper backs, Chapman Hall, London.

[6] **Mekala**, S., Kumar, N., Nishmitha, L., Amuthan, A., Vulli, V. and Bhogireddy, N. (2014). Evaluation of wound healing activity of ethanolic extract of *Lantana camara* in streptozotocin induced diabetic rats. Int. J. Pharm. Sci., 6 (1): 631-633.

[7] Farag, M. M.(2015). Isolation and individuation dermatophytes and resistant some extracts of plants. M.Sc. Thesis. College of Science, University of Al. Mustansiriyah, Iraq.

[8] **Suarez,** B., Palacios, N., Fraga, N. and Rodriguez, R. (2005). Liquid Chromatographic method for quantifying polyphenols in ciders by direct injection. J. of Chromatography, 1066: 105-106.

[9] **Radenahmad,** N., Saleh, F., Sayoh, I., Sawangjaroen, K., Subhadhirasakul, P., Boonyoung, P., Rundorn, W. and Mitranun, W. (2012). Young coconut juice can accelerate the healing process of cutaneous wounds. BMC Complementary and Alternative Med., 12:252.

[10] **Graham**, M., Pitcher Parent, A. and Terence, J. (1995). Noxious thermal and chemical stimulation induce increases in 3H-Phorbol 12,13=Dibutyrate binding in spinal cord dorsal horn as well as persistent pain and hyperalgesia. The J. Neuroscience, 5 (5): 3263-3272.

[11] **Guo-Bing**, S., Bing, W., Qiong, W., Tong-Chao, W., Chang-Li, W., Xue-Hui, S., Wen-Tao, Z., Ming, Y., Qing-Chun, Z, Yu-Feng, C. and Wei, Z. (2014). Evaluation of wound healing activity and antiflammatory activity of aqueous extracts from *Acorus calamus* L. Pak. J. Pharm. Sci., 27 (1): 91-95.

[12] **Sadaf**, F., Saleem, R., Ahmed, M. and Ahmed, S.I. (2006). Healing potential of cream containing extract of *Sphaeranthus indicus* on dermal wounds in guinea pigs. J. Ethnopharmacol., 107: 161-163.

[13] **Al-Mohammed**, N. T., Al-Rawi, K. M., Younis, M. A. and Al-Morani, W. K. (1986). Principles of Statistics. Al-Mosil University Press, Iraq (in Arabic).

[14] **Venkatachalam,** T., Kishor, V. K., Kalal, P. S., Avinash, O. M. and Senthil, N. K. (2011). Physicochemical and preliminary phytochemical studies on the *lantana camara* L. fruits. Int. J. Pharm. Pharmace. Sci., 3 (1): 52-54.

[15] **Levinson**, H., Moyer, K.E., Saggers, G. C. and Ehrlich, H.P. (2004). Calmodulin-myosin light chain kinase inhibition changes fibroblast-populated collagen lattice contraction, cell migration, focal adhesion formation, and wound contraction. Wound Repair Regen, 12:505-510.

[16] **Barku,** V. Y., Boye, A. and Quansah, N. (2013). Antioxidant and wound healing studies on the extracts

of *Corchorus olitorious* leaf. World Essays J. 1 (3): 67-73.

[17] **Grdisa,** M. (2010). Mechanism of wound healing in annelids. Int. Sci. J., 7: 192-197.

[18] **Mekala**, S., Kumar, N., Nishmitha, L., Amuthan, A., Vulli, V. and Bhogireddy, N. (2014). Evaluation of wound healing activity of ethanolic extract of *Lantana camara* in streptozotocin induced diabetic rats. Int. J. Pharm. Sci., 6 (1): 631-633.

Author Profile

Farah D. Salim received the B.Sc. and M.Sc. degrees in Biotechnology, College of Science, Al-Nahrain University in 2006 and 2010.