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Mediterranean Journal of Biosciences

Mediterranean Journal of Biosciences **2016**, 1(4), 147-152

In vivo antibacterial and wound healing activities of *acacia ehrenbergiana*

Samah Awad AbduRahim [1,*], NaserEldin Bilal Mohammed [1] and Aisha Zoheir Almagboul [2]

[1] Department of Microbiology, Faculty of Medical Laboratory Sciences, University of Khartoum

[2] Medicinal and Aromatic Plants Research and Traditional Medicine Institute, National Centre for Research, Khartoum, Sudan

Abstract: A prospective study was carried out to investigate *in vivo* antibacterial and wound healing activities of methanol extract of *Acacia ehrenbergiana* in albino rats using excision model. The rate of wound healing was assessed by measuring wound contraction. *In vivo* antibacterial activity was determined by viable count of *Pseudomonas aeruginosa* (ATCC 27853).The results revealed that poly ethylene glycol (PEG) ointment containing 3% *A.ehrenbergiana* had *in vivo* antibacterial but less wound healing activity when compared with the same concentration of tetracycline.

Key words: *Acacia ehrenbergiana,* wound healing activity, albino rat, antibacterial activity

Introduction

Wound healing disorders present a serious clinical problem and are likely to increase since they are associated with diabetes, hypertension and obesity. Thus, several animal models have been established to serve as experimental basis to determine the mechanisms underlying and controlling undisturbed healing process [1]. The simplest form of skin wound healing occurs when uninfected incisions and other clean wounds without loss of tissue are closed promptly by surgical sutures and this is referred to as healing by primary intention. When the healing of an open wound with loss of tissue occurs by the formation of more substantial amounts of granulation tissue which grows from the base of the wound to fill the defect, this is called healing by secondary intention [2]. The first phase of wound healing is hemostasis in which the wound fills with the blood clot. The inflammatory phase follows with exudation of fluids, deposition of further fibrin and migration of neutrophil polymorphs, monocytes and lymphocytes. Then, the proliferative phase which is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization, wound contraction and lastly remodeling [3].Excised wound is aided by contraction of the surface area at sites where the skin is mobile and loosely attached to underlying tissue [3]. Myofibroblasts, which are similar to smooth muscle cells, are responsible for contraction [4].

The basic mechanisms of healing by primary and secondary intention are similar. However, there is a greater connective tissue response in healing by secondary intention and the whole process is much slower. Treatment of infection accelerates wound healing because infection impairs the healing process by delaying epithelialization which is important for granulation [2].Herbal products have been used in treatment and healing of wounds over the years. The phyto-medicines for wound healing are not only cheap and affordable but also purportedly safe since hyper sensitive reactions are rarely encountered with the use of these agents [5]. Thus, these products need to be identified and formulated for treatment and management of wounds.

Acacia ehrenbergiana Hayne (Salam) ; see Figure 1; is belonging to the family of Memosaceae. Trees are multi stemmed and spreading from the base 2-7 m. It is found in Saudi Arabia, Northern and east Africa and is used in folklore in the production of gum and some medicines. It contains Gallic acid, methyl gallate , rutin, , myricetin, quercetin ,myricetin 3- *O*-(3'''-*O* -galloyl)-β-D-rutinoside and catechin [6]. Methanol extract from stem bark of *A. ehrenbergiana* exhibited broad spectrum antibacterial activity *in vitro* [7], this activity was confirmed *in vivo* in the present work in the septic wounds of albino rats.

**Corresponding author: Samah Awad AbduRahim*
Email address: samahawad90@yahoo.com
DOI: http://dx.doi.org/

Figure 1. *Acacia ehrenbergiana*

Material and methods

Plant collection

The stem bark of *A. ehrenbergiana* was collected from Marawi (Northern Sudan) in 2012 and authenticated in Medicinal and Aromatic plants and Traditional Medicine Research Institute (MAPTMRI). Voucher specimens were deposited at the herbarium of the institute. The plant was shade dried and kept until extraction.

Preparation of extract

Each of the coarsely powdered plant material was exhaustively extracted for 20 hours with chloroform in Soxhlet apparatus. The methanol extract was filtered and evaporated under reduced pressure using Rota-vap and dissolved in 20 ml of methanol (con. 100mg/ml), then kept in refrigerator until used.

Preparation of bacterial suspensions

A loopful of isolated colonies of *Pseudomonas aeruginosa* was inoculated into 4 ml peptone water and incubated at 37ºC for 4 hours. The turbidity of actively growing bacterial suspension was adjusted to match the turbidity standard of 0.5 MC Farland units [8].

Preparation of the control sample

A loopful of isolated colonies of *Pseudomonas aeruginosa* ATCC 27853, was inoculated into 2 ml of normal saline, the turbidity of suspension was adjusted to match the turbidity standard of 0.5 MC Farland unites (10^8 colony forming unite/ml).

In vivo testing of extracts for antimicrobial activity

The wound evaluation model was used to assess the *in vivo* antimicrobial activity of the selected plant extract [9].

Ointment preparation

Polyethylene glycol (PEG) was used as a water soluble base to prepare ointments of 3% of *Acacia ehrenbergiana* extract in PEG using (1:1) mixture of 400: 4000 PEGs, the mixtures were stirred gently by infusion in water bath till they are homogenously distributed and then cooled with continuous stirring.

Experimental animals

Swiss Wistar Albino rats of either sex, weighing 60-95g were used. Animals were supplied by the National Experimental Animal House (NEAH), Medicinal and Aromatic Research Institute (MAPRI), National Center for Research (NCR), Ministry of Science and Technology (MOST), Sudan. Rats were housed in a ventilated animal house before and after surgery. They had access to standard diet prepared in (NEAH) supplemented with water ad libitum. The holding room was illuminated with 12 hours, light/dark cycles. Room temperature was between 30-35 C^o with 45% to 55% humidity.

In vivo wound healing activity of plant extracts

Full thickness wounds were made in the skin of the tested animals according to the model [9]. Hair of the lower back and right flank of animals was fully shaved. The animals were held in standard crouching position, and the mobile skin of flank was gently stretched and held by fingers. A metal circular object measuring 1 cm in diameter was placed on stretched skin and an outline of the object was traced on the skin using a fine tipped pen. The wound was made by excising the skin within the border of the object to level of loose subcutaneous tissue, using sterile forceps and scalpel blade. The artificial wounds were circular with a diameter of 1 cm and the corresponding infection was introduced using *Pseudomonas aeruginosa* (ATCC 27853).

The first day of experiment was regarded as day Zero. Animals were divided into three groups, each containing five animals: Group 1 (wounded + Infection); infected control group, animal wounds were artificially infected with standardized *Pseudomonas aeruginosa* suspension (10^8 C.F.U. /ml), spread 0.5 ml of the suspension in every wound. Group 2 (wounded + infection + 3% Tetracycline ointment); wound of these animals were artificially infected using the same method as used in group 1, and treated topically with 3% tetracycline ointment every 12 hours as standard healing agent starting from first day. Group 3 (wounded + infection + MeOH extract of *Acacia ehrenbergiana* 3% in PEG; wounded animals were artificially infected using the same method as used in group 1, and treated topically with Polyethylene glycol (water washable base PEG) ointment which contained 3% of the *Acacia ehrenbergiana* stem park methanol extract every 12 hours starting from the first day.

Evaluation method of wound healing percentage

In order to determine the rate of wound healing, every 24 hours, each animal was held in the standard

crouching position and two diameters of the wound circle (horizontal and vertical) were measured using a transparent ruler. The area of the wound in day zero was considered as 100% and the wound areas on subsequent days were compared with the wound on day zero. Healing percentage in a certain day was the difference between the initial wound (in day zero) and healing wound on that certain day [10].

Evaluation method of in vivo antimicrobial activity

In order to determine *in vivo* antimicrobial activity, viable count of bacteria was carried out. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique [11].

Data analysis

Analysis of variance ANOVA was applied to the data to determine the differences between the three groups ($p<0.05$).

Results and Discussion

Results

Results were obtained by measuring the wound healing percentage of the three groups of rats (group 1: wound + infection, group 2: wound + infection + tetracycline 3% ointment, group 3: wound + infection + *Acacia ehrenbergiana* 3% ointment) as shown in Figure 2. Application of plant extract based ointment resulted in a diminishing level of total bacterial count in the infected wound. There was a major reduction from 10^8 CFU/ml to 10^2 CFU/ml in treated rats on day 4 when compared to control and tetracycline rats, which recorded 10^7 and 10^5 CFU/ml respectively. In all above mentioned groups, healing was completed in 11 days (Figures 2 to 8). Significant differences ($P< 0.05$) between groups were calculated three groups. Multiple comparisons showed significant differences when comparing the first and second groups with the third group. The mean of the first and second groups was higher than the mean of the third group (Table1).

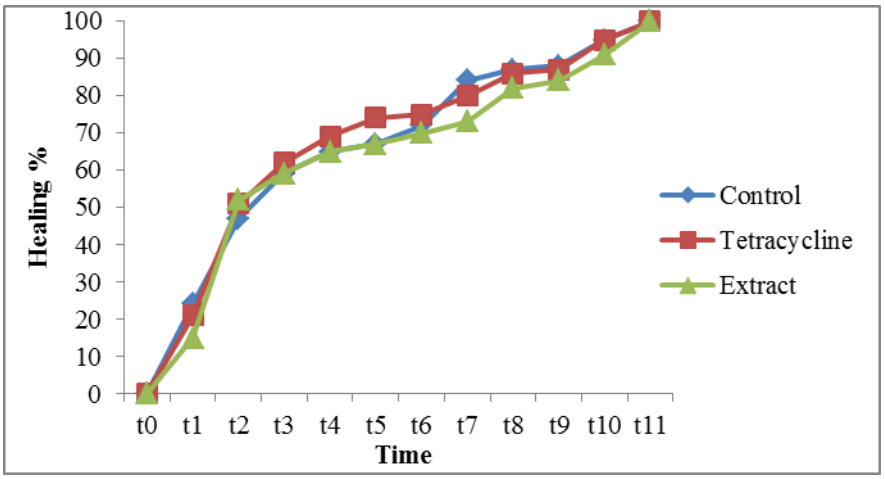

Figure 2. Percentage of wound healing activity of *Acacia ehrenbergiana*

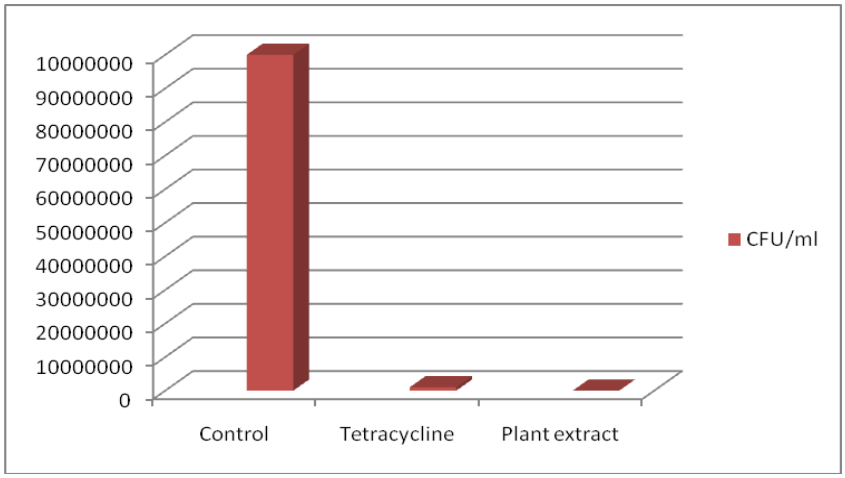

Figure 3. Viable count of *Pseudomonas aeruginosa* in the pus in day (4)

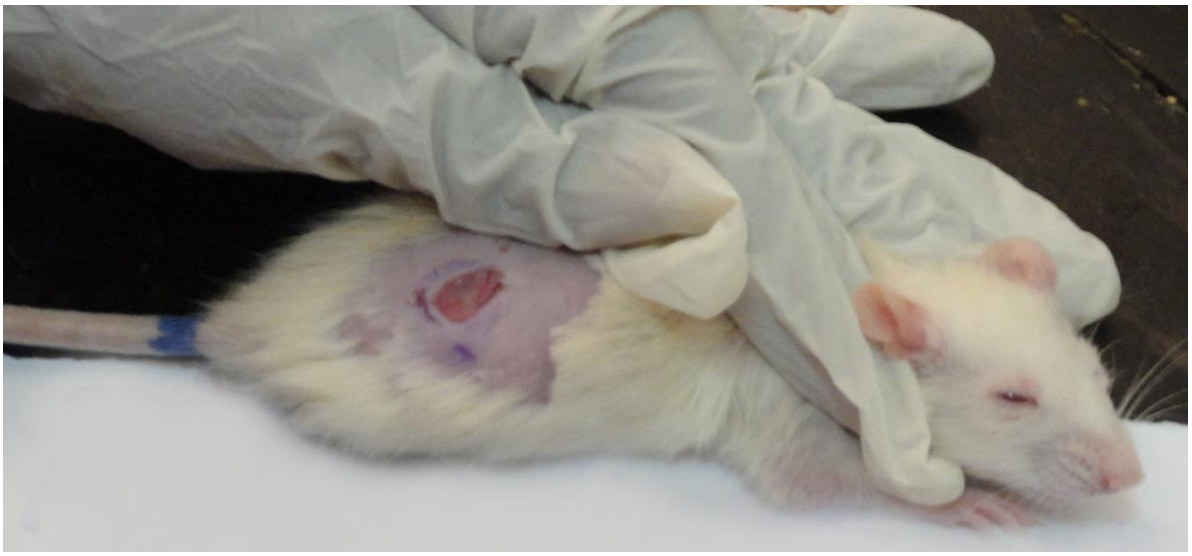

Figure 4. *In vivo* wound healihg activity trial, infected albino rat (day zero), the wound diameter is 1cm

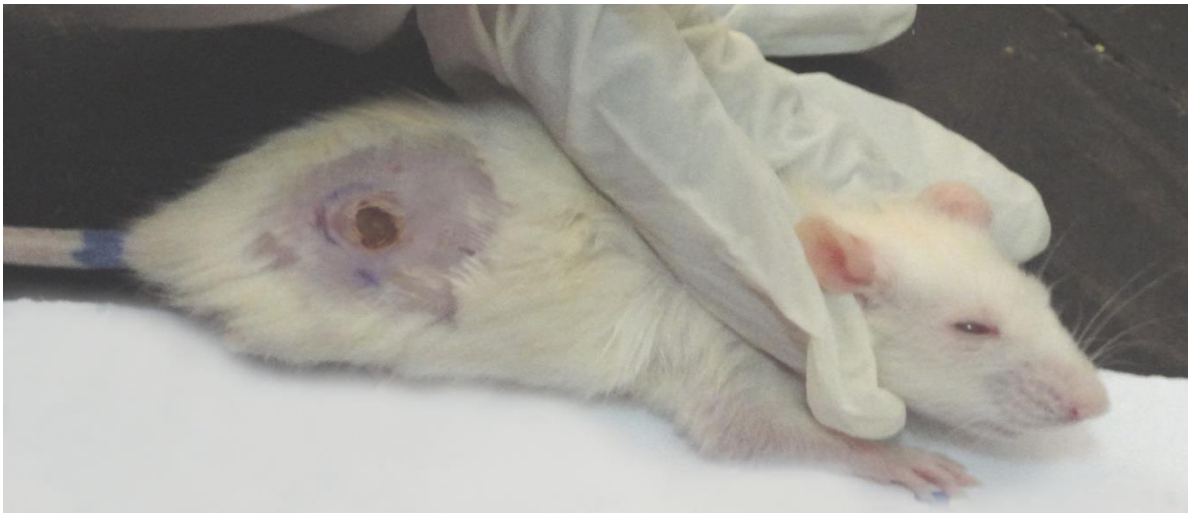

Figure 5. *In vivo* wound healihg activity trial, infected albino rat treated with 3% *Acacia ehrenbergiana* in PEG ointment (day 4), there is a reduction in wound diameter from 1cm to 0.35 cm

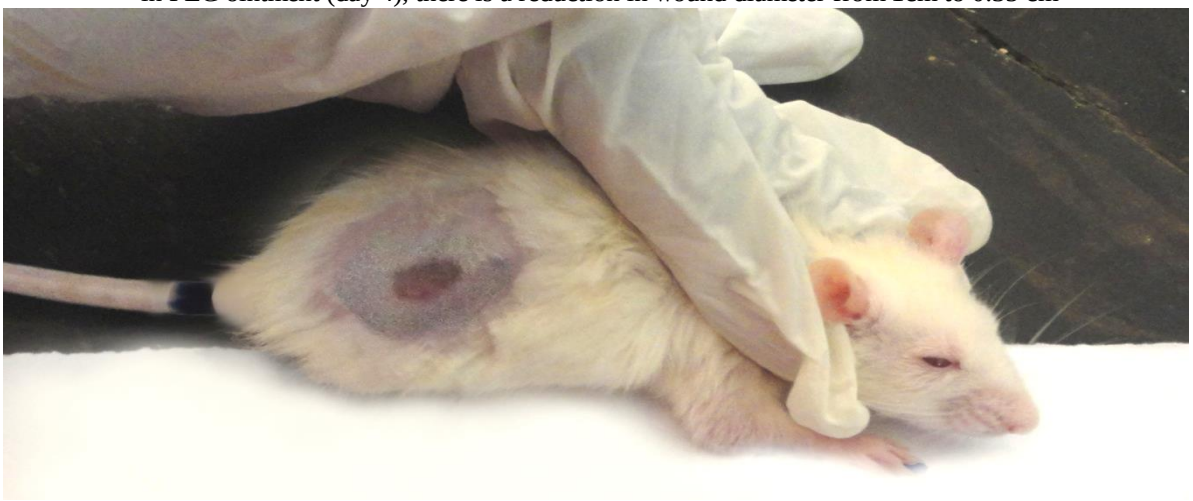

Figure 6. Infected albino rat treated with 3% tetracycline ointment (day 4), wound size is 0.31cm

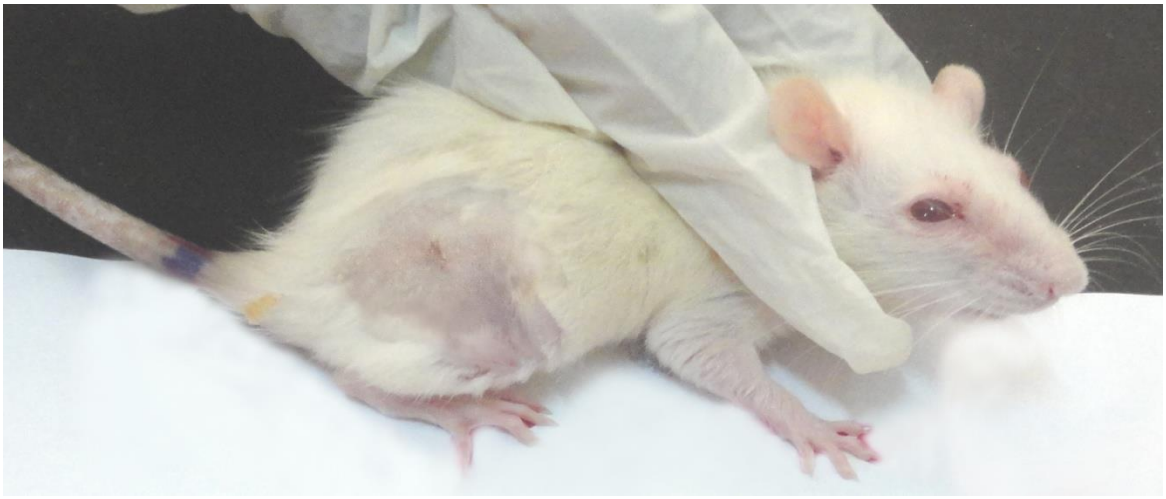

Figure 7. *In vivo* wound healihg activity trial, infected albino rat treated with 3% tetracycline ointment (day 11), the wound completely closed

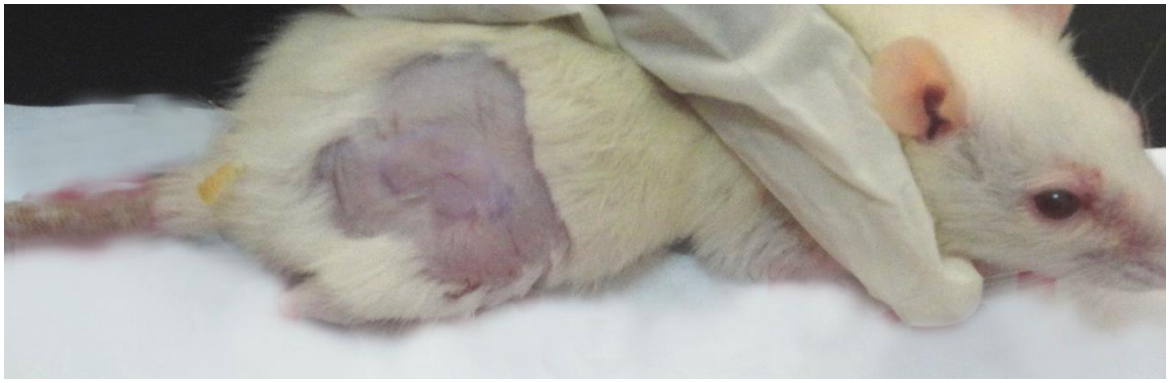

Figure 8. *In vivo* wound healihg activity trial, infected albino rat treated with 3% *Acacia ehrenbergiana in* PEG ointment (day 11), the wound comletely closed

Discussion

Topical application of drugs is effective on wounds , it can improve both antimicrobial activity and wound healing rate because of its large availability at the infected wound site. The ability of microorganisms in the wound bed to create massive damage depends on the virulence capacity of the organism, the amount of inoculum present in the wound site along with the host immune response. *Pseudomonas aeruginosa*is is one of the most common pathogen present in wound [12].Sensitivity tests measure antimicrobial activity against bacteria under laboratory conditions (*in vitro* activity), not in the Patient (*in vivo* activity). It cannot be assured, therefore, that an antimicrobial which kills or prevents an organism from growing *in vitro* will be a successful treatment *in vivo* [8]. Thus, *in vivo* study for the antibacterial activity of the most potent plant extract was carried out in septic wounds of Wistar rats which resemble humans in many features. In this present study, tetracycline was used as a reference drug. The rate of infection is directly related to the number of organisms inoculated. Inoculation of 10^8 CFU /ml of bacteria resulted in 100% of wound producing pus without mortality while at 10^{10} CFU /ml, all the tested animals died with an overwhelming infection and at 10^4 CFU / ml approximately 50% of the wounds showed no sign of infection. So, in the present study we inoculated the excision of Wistar rats with 10^8 CFU / ml of pathogens and a good local infection was established on post-operative days without mortality [13]. Significant reduction of bacterial count in the rats treated with poly ethylene glycol containing 3% *Acacia ehrenbergiana* ointment was observed in day 4 from 10^8 CFU to 10^2 CFU/ ml which further confirmed the *in vitro* antibacterial activity of *Acacia ehrenbergiana*. Regarding the wound healing activity, the percentage of healing with poly ethylene glycol containing *Acacia ehrenbergiana* 3% ointment was significantly lower ($p<0.05$) than tetracycline group. These results showed that PEG ointment containing 3% *Acacia ehrenbergiana* had *in vivo* higher antibacterial activity but lower wound healing activity when compared with the same concentration of tetracycline. To my knowledge, no literature was encountered in correspondence of wound healing activity of this plant.

Conclusions

In vivo antibacterial and wound healing activities of methanol extract of *Acacia ehrenbergiana* were investigated on infected wounds in Wistar albino rats. Poly ethylene glycol containing 3% *Acacia ehrenbergiana* had less wound healing activity than 3% tetracycline ointment. Significant reduction of bacterial amount in rats treated with poly ethylene glycol containing 3% of Acacia *ehrenbergiana* ointment was observed at day 4 and confirmed the *in vitro* antibacterial activity of *Acacia ehrenbergiana*.

Aknowledgments

The authors thank MAPTMRI for supporting the study through a project grant. We acknowledge the assistance extended by Dr. Raga Osman and Dr Um elkhair Mokhtar.

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