

Histopathological study on the antifungal activity of *Salix* plant extract on some dermatophytes

دراسة مرضية نسيجية لفعالية مستخلص نبات الـ *Salix* المضاد للفطريات على بعض الامراض الجلدية الفطرية

Kawkab, S.A.

*Al-Samarrae, K

**B. Rabee

College of Medicine/ Al-Nahrain University

*Biotechnology Research Center/ Al-Nahrain University

** Ministry of Minerals and Industry

Abstract

The study was designed to determine the activity of methanolic *Salix* extract as an antifungal agent. Rabbits were divided into three groups. The first group represents the control (6) while the second and the third groups were infected with fungal inoculums of the rabbit skin. After development of skin lesion, the infected area of the second group was treated with *Salix* extract until healing. The histopathological study revealed recovery from infection in the infected animals, which manifested by well-developed epithelial tissue similar to the control group.

المستخلص

تمت دراسة فعالية مستخلص نبات *Salix* methanolic كمضاد للفطريات . قسمت الارانب الى ثلاث مجاميع ، الاولى تمثلت بمجموعة السيطرة (6) بينما المجموعة الثانية والثالثة عرضت للاصابة بالفطريات الجلدية ، عند تطور الاصابة للمجموعة الثانية عرضت الى مستخلص نبات الـ *Salix* الى ان شفيت . الدراسة النسيجية اظهرت تحسن من الاصابة في المجاميع المصابة مقارنة بنسيج مجموعة السيطرة .

Introduction

A large number of drugs plant were used traditionally to treat various ailments all over the world including the Arabian countries [1].

Several spices of herbs have been known to posses antimicrobial activities. Many projects described the inhibitory properties of different active compounds including volatile oils,

alkaloids, phenolics, & others with their effect on a variety of microorganism [2].

Recently, the search for antibiotics from natural origin has markedly increased. However several plants have been screened for their fungitoxicity [3].

Salix is one of these plants which is considered to be the natural origin of the modern aspirin [4]. Previous studies indicated the presence of tannins and glycosides including salicine and its derivatives in this plant. Many flavonoids have been isolated from *Salix* spp [5]. It is used as antipyretic,

Materials and method

1. Methanolic plant extract was prepared by maceration of the leaves (50)gm with 70% methanol for 24 hours, and then evaporated to dryness by rotary evaporator [7].

2. Animals: 6 rabbits were divided into three groups. The first group represents the control (no treatment), while the rabbit of the second and the third group were infected with fungal inoculums on the skin of the rabbits. Skin lesions were developed after seven days. The infected areas were treated with *Salix* extract (until

antiphlogestic, analgesic and for pain caused by inflammation [2].

Dermatophytosis are a group of superficial fungal infection caused by mold fungi of the genera *Trichophyton*, *Microsporum* & *Epidermatophyton*, which share the ability to invade stratum corneum & keratinized structures such as hair and nails derived from epidermis [6].

This study is an attempt of histological study on the effect of *Salix* extract as an antifungal agent on skin infection caused by epidermatophyton.

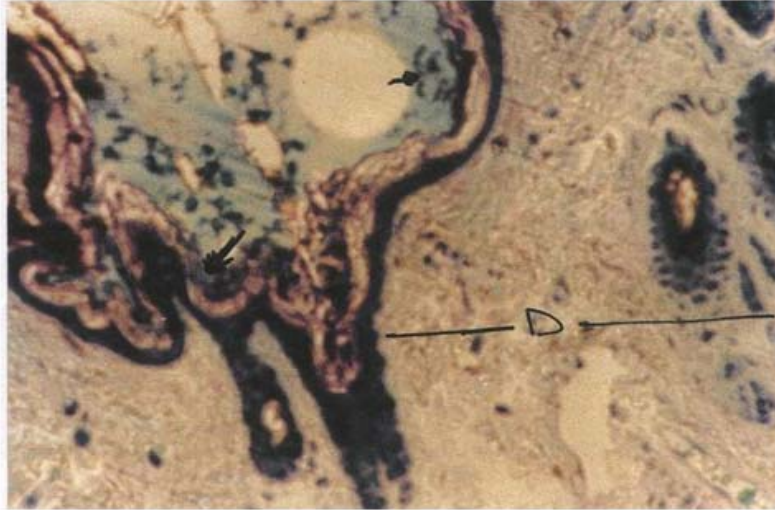
healing) for histopathological study in comparison with that of infected but not treated.

3. Tissue preparation: tissue specimens were fixed using immersion fixation for 4 hours in 2.5% phosphate buffered gluteraldehyde (PH.4) then post fixed with 1% osmium tetroxide for 1 hour and processed for plastic embedding. Semi-thick sections were sectioned for light microscope study, stained with 1% methylen blue basic fuchsine [8].

Results

1. First group: animals infected with *Epidermophyton* . all infected animals showed pathological changes with adherence of fungus to keratinocytes,

through the stratum granulosum of the epidermis. The roofs and contents of the vesicles usually contain fungal hyphae. The epidermis exhibited atrophy of rete



Fig(1) : Plastic section of skin in animal infected with the fungus(400x)

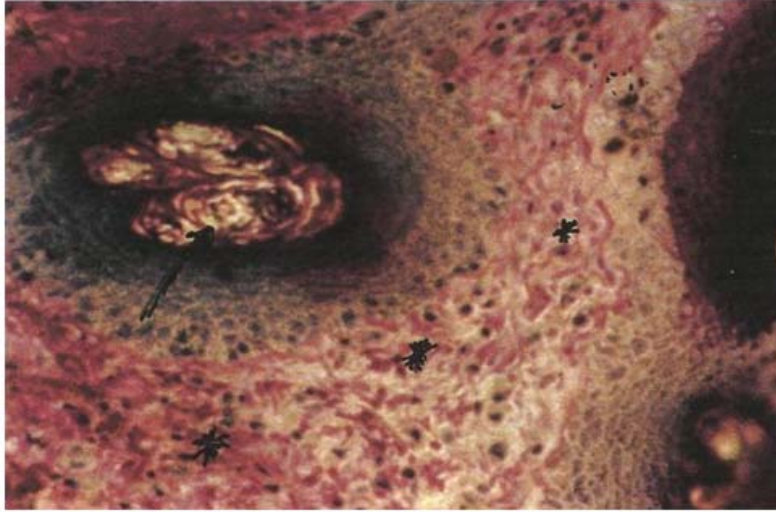
Note —→ : Fungal hyphae
 —→ : Damaged epidermis
 D : Dermis

other features are acute lesions characterized by vesicles which extend

ridge Fig.(1).

The underlying dermis manifested abundant fungal hyphae. The skin appendages especially hair follicles

appeared altered. The hair bulb and hair papilla were converted into keratinized layer Fig.(2).



Fig(2) : skin of animal infected with dermatophyte. (400x)

Note → the penetration of fungal hyphae and damaged hair follicle

Secondary to the cellular damage, there is an acute inflammation reaction in the vicinity. This manifested markedly with

degenerated epidermis and dermis with an exudates containing moderate number of lymphocytes Fig.(3).

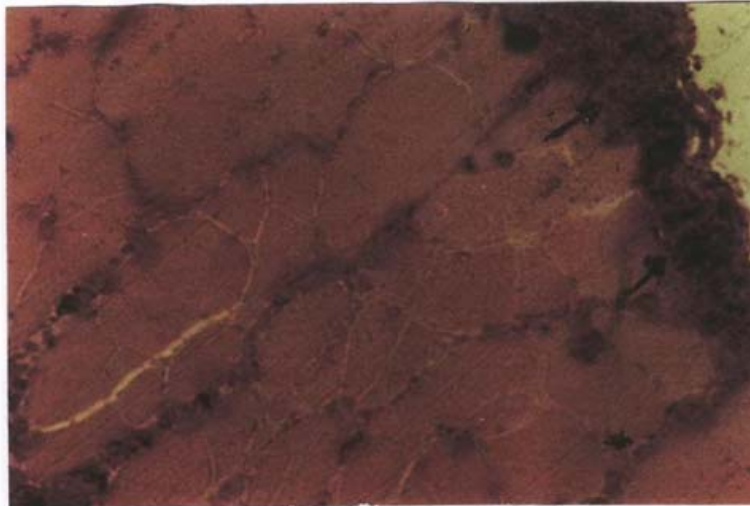


Fig (3): Dermis of infected animal. (400x).

Note the degenerated dermis layer →

Lymphocyte infiltration in the muscular layer

2. Second group: infected animals treated with *Salix* extract has shown recovery from infection which

manifested by well developed epithelial tissue similar to the control group Fig.(4, 5).

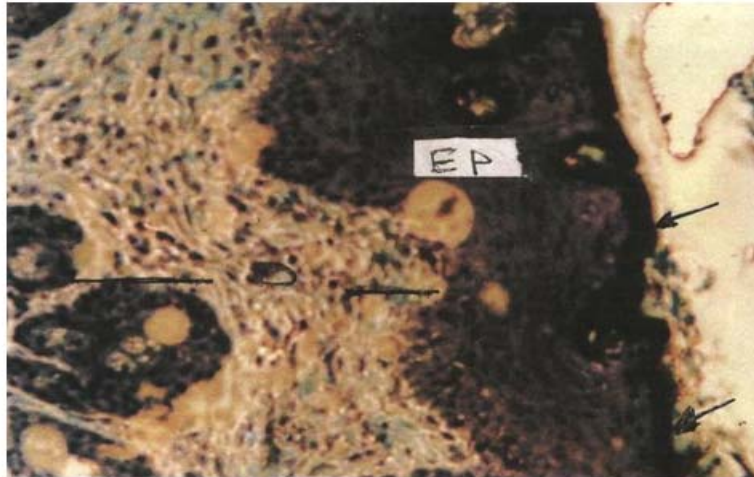


Fig (4): Skin in control animal group (400x)

Ep: Epidermal layer

D: Dermis

→ : Keratinized layer

Stratum germinativum of epidermis include a lymphocytic infiltration to the dermo-epidermal junction associated

with vasodilatation at the basal layer Fig.(5).

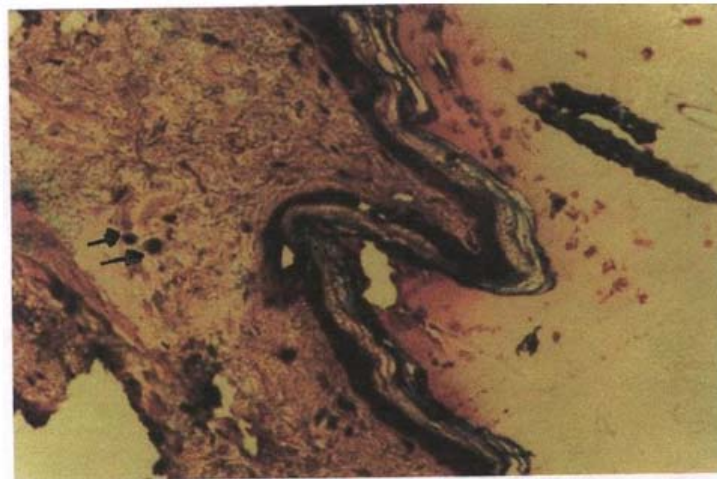


Fig (5): infected skin, treated with *Salix* extract (400x)

Note the appearance of inflammatory cells →

Portions of fibroblasts and macrophages were frequently observed among the collagen fibers. Regeneration & healing of the skin is also characterized by the

presence of muscles and increase in the number of blood vessels. Lymphocytic also was observed. Fig.(6 , 7).

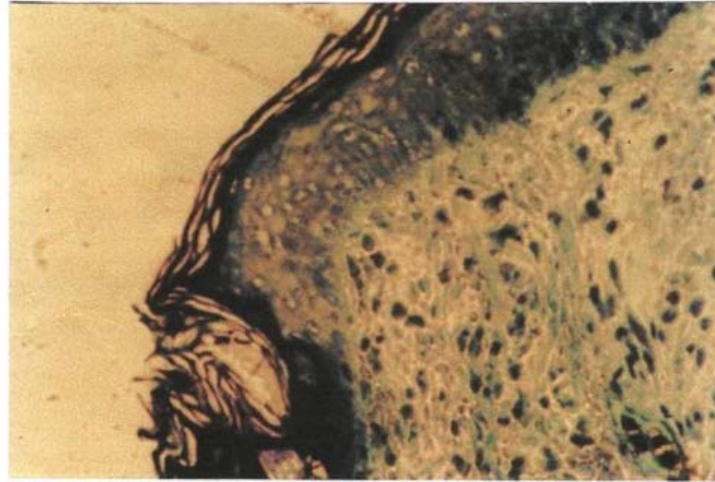


Fig (6): section with normal skin, healing after treatment with *Salix* extract. (400x)

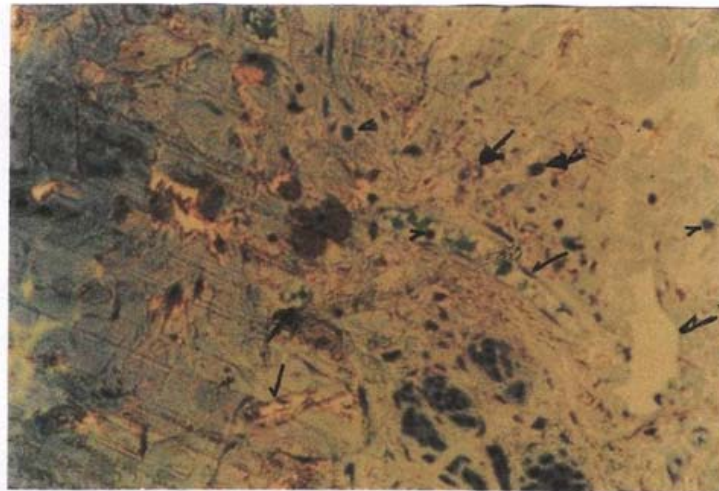


Fig (7): Section of healed skin after treatment with *Salix* extract (400x)

Note of Blood vessels

And presence of lymphocyte ←→

Discussion

Our finding support the model proposed by [9] who suggested that dermatophytes could lead to local or

systemic responses that result in an eczematous reaction of the epidermis.

Increased epidermal turnover could be a part of eczematous reaction through the process of shading the dermatophyte and repairing epidermal damage.

Salix extract assists the naturalization and removal of organisms from the area by its antifungal activity against dermatophytes, as well as stimulates increased in epidermal turnover. Thus, the damaged area would tend to revert to normal and presumably healing takes place slowly, returning the keratinized epidermis to normal structure [3].

The fibroblast tend to arranged collagenous fibers in the derim layer invading into muscles and large amount on inflammatory cells (lymphocytes, neutrophils, plasma cells) infiltrating that assist the healing process [10,11].

As well as, *Salix* extract improve the tissue blood supply and have anti

inflammatory effect to promote epidermal repair in the skin.

From this study we can conclude that *Salix* extract exerts a marked antifungal activity against dermatophytes.

The activity of *Salix* extract may be attributed to the flavonoids or glycoside extracted by methanol [7, 12, 13]. Methanolic extract and flavonoid of *Salix* was active against wood fungi [12].

Three general mode of action for plant extracts were recognized according to [14]

1. Inhibition of microbial cell wall formation or biosynthesis of some protein.
2. Disruption of DNA metabolism.
3. Alteration of normal function of the cellular membrane.

Many researches pointed that tannins, flavonoid and glycosides are major groups of plant products considered as ant microbial agents [15].

References

1. Evans, W(1988). Pharmacognosy . 14th ed. UK.
2. Robbers, J.E ; M. K. speed; e; & V.E.Tyler (1966). Pharmacognosy & pharmacobiotechnology Lea&Febiger book p.337.
3. Al-Samarrae, K; kawkab, S.A. and B. Rabee (2005). Histological study on the anntiungal activity of *Salvia* (hexane extract) on some dermatophytes, Journal of science, collage of science, Al-

- Nahrain university (accepted for publication in 2006)
4. PDR for herbal medicine 2003 Medical Economics Company.ed.(internet).
5. Meier, B., Sticher, O., sulcunen, T.R.(1988). Pharmaceutical aspects of the use of willows in herbal remedies. *Planta medica*, 54(6) :559-560.
6. Zurita, I.D., and Hay, R.J.(1987). Adherance of dermato phyte microconidia and arthro conidia in vitro.*J. invest .Ddermatol*. 189: 529-534.
7. الخفاجي ، باسمة ربيع (2000) تأثير مستخلصات نبات سم الفراخ والميرمية والصفصاف على نمو بعض الفطريات الجلدية – رسالة ماجستير- الجامعة المستنصرية – كلية العلوم .
8. القيسي، كوكب سليم (1988) . دراسة بالمجهر الالكتروني للخلايا الظهارية وفعاليتها الافرازية في غدة التوتة للفئران بعد تثبيط هرمون النمو . رسالة ماجستير – كلية العلوم – جامعة بغداد.
9. Jones, H.E. ; Reinhardt, J.H. ; and Rinaldi, M.G.(1974). Acquired immunity yo dermatophytes. *Arch Dermatol* 109 : 840-848.
10. Kamide, R.(1997). Histopathological diagnosis by skin biopsy. *Nippon-Rinsho*.55:(9): 2259-2261.
11. Ergun, T. ;Gurbuz, O. ;Harvell, J.; Jorizzo, and white, W.(1998). The histopathology of : a chronologic study of skin hyperreactivity in Beheers disease. *Int.J.Dermatol* .37 (12) :929-933.
12. Maltervd, K.M.(1985) flavonoide from the wood of *Salix copera* as inhibitors of wood destroying fungi. *J. Nat. Prod*. 48:559-563.
13. Al-Janabi, A.A. (2004). Effects of *Calendula officinalis* extracts on the growth of some pathogenic microorganisms. MSc.thesis (collage of sciences). Al-Nahrain University, Iraq
14. Hugo, W.B. and A.D Russell, 1989, pharmaceutical microbiology. 4th ed .
15. السامرائي، خلود وهيب . باسمة ربيع الخفاجي (2002) تأثير مستخلص نباتي سم الفراخ *Salivia Withania Somnifera* والميرامية *officinalis* في تنشيط نمو الفطرين *Microspoum* و *Scopulariopsis* المجلة العراقية للاحياء المجهرية ، المجلد 14 العدد (1) .