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

المستخلص
تمت دراسة فعالية مستخلص نبات Salix كمضاد للفطريات. قسمت الأرانب إلى ثلاث مجاميع، الأولى تمثلت بمجموعة السيطرة (6) بينما المجموعة الثانية والثالثة عرضت للإصابة بالفطريات الجلدية، عند تطور الإصابة للمجموعة الثانية عُرضت إلى مستخلص نبات Salix لان شفيت. الدراسة النسيجية أظهرت تحسن من الإصابة 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, 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 & Epidermaphyton, 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.

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 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 tetraoxide for 1 hour and processed for plastic embedding. Semi-thick sections were sectioned for light microscope study, stained with 1% methylene 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

![Image](image_url)

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

Note: - Fungal hyphae
- Damaged epidermis
- Dermis

other features are acute lesions characterized by vesicles which extend

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).
Second 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).

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).
Stratum germinativum of epidermis include a lymphocytic infiltration to the dermo-epidermal junction associated with vasodilatation at the basal layer Fig.(5).

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. Lymphocyter also was observed. Fig.(6, 7).
Discussion
Our findings 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 dermis 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 antimicrobial agents [15].

**References**

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15. السامرائي، خلود وهيب . باسمة ربيع الخفاجي (2002) تأثير مستخلص نباتي سم القرآف Salvia والميرامية Withania Somnifera في تنشيط نمو الفطرين Microspoum و Scopulariopsis المجلة العراقية للاحياء المجهرية ، المجلد 14 العدد (1).