

# Effect of *Lawsonia inermis* extract on the pathological changes of skin infection by *Streptococcus pyogenes* in lab. Mice

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## Abstract

In this study, the antibacterial effects of aqueous and alcoholic extracts of *Lawsonia inermis* leaves against *Streptococcus pyogenes* were investigated in vitro by using agar well diffusion method and in vivo using laboratory mice by treating it with prepared ointment from these extracts & compared the effects with Gentamicin. Results showed that both extracts demonstrated antimicrobial activity against the tested organism but the efficiency of the extracts was significantly affected by the solvent used in the extraction as well as the concentration of extract. Alcoholic extracts had the highest antibacterial activity it exhibited an inhibition zone 18.2-28.2 mm in comparison with 26.2 mm for gentamicin. A prepared ointment from alcoholic extract in a concentration 5% was tested for treatment of the experimentally infected skin of mice by *S. pyogenes* using scratching. The recovery period was 11 days in compared with 10 days for gentamicin. Biopsy was taken from the infected area to detect the pathological effects occurred by tested bacteria, also biopsy was again taken after the treatment with the prepared ointment to evaluate the anti-inflammatory, antibacterial and wound healing activity of *Lawsonia inermis*. Histopathological study showed after 3 days from skin infection with *Streptococcus pyogenes* hyperemia, swelling, congestion and pus formation also abscessation. While in treated animals with henna extracts gross skin lesions disappear after 11 days from infection and on day 10 showed increased well organized bands of collagen, more fibroblasts and few inflammatory cells when compared with the controls which showed inflammatory cells, scanty collagen fibres and fibroblasts.

## Introduction

*Streptococcus pyogenes* is a major human and animal bacterial pathogen that causes a wide range of infections from common and mostly uncomplicated cases of pharyngitis and impetigo, to severe invasive infections (1). In addition to infections of the upper respiratory tract and the skin, also acute rheumatic fever and acute glomerulonephritis. Recently, infection with *S. pyogenes* has an important cause of toxic shock syndrome (TSS), as well as common skin infections include cellulitis, erysipelas, impetigo, folliculitis, and furuncles and carbuncles (2). *S. pyogenes* is susceptible to penicillin but some people and animals develop a serious resistance condition also many strains have developed resistance to macrolides, tetracyclines and clindamycin (3). Failure of treatment with penicillin is generally attributed to other local commensal organisms producing  $\beta$ -lactamase. Certain

Experimental studies of infection showed a reduction in the efficacy of penicillin when large numbers of organisms are present (3), moreover this drug has many adverse effects such as nausea, diarrhea, urticaria and superinfection (including candidiasis), and up to 20% of all patients have penicillin allergy (4). Thus medicinal plants extract are used as alternative antibacterial compounds with high activity, low toxicity, cheaper, and more effective and Henna (*Lawsonia inermis*) is one of good examples for these plants. Henna or Hina (*Lawsonia inermis*, syn. *L. alba*) is a flowering plant, 2-6m in height. It is the sole species in the genus *Lawsonia* in the family *Lythraceae* (5). Henna produces a burgundy dye molecule, *lawsone* (6). This molecule has an affinity for bonding with protein, and thus has been used to dye skin, hair, fingernails, leather, silk and wool. The dye molecule, *lawsone*, is primarily

concentrated in the leaves. In some parts of the world, plants and herbs are still the prime medicines used in medical treatment (7). *L. inermis* is widely grown in various tropical regions in Asia, America and Africa. In Arabic, the word "henna" refers to *L. inermis* (8). Its core chemical components are the main one 2-hydroxynaphthoquinone (lawsone) about 0.5-1.5% of henna, mannite, tannic acid, mucilage and gallic acid. The main uses of henna are as a cooling agent, astringent, anti-fungal and anti-bacterial herb for the skin and hair (9). Also it used as an antihemorrhagic, intestinal antineoplastic, cardio-inhibitory, hypotensive, and a sedative. It has been used both internally

and locally in jaundice, leprosy, smallpox, and affections of the skin. Henna has exhibited antifertility activity in animals and may induce menstruation. As a cooling agent it is used for burning of skin. It also has great dandruff fighting ability. Alcoholic extract of the leaves showed mild anti-bacterial activity against *Staphylococcus aureus* and *E. coli*'. Some experimental and clinical studies have reported antibacterial and antifungal effectiveness and wound healing activity of this product (10). The purpose of this study was to evaluate the antibacterial properties of henna extracts in vitro and the anti-inflammatory and wound healing activity in vivo.

## Materials and methods

### Plant samples and extraction procedure:

*L. inermis* were collected from private gardens in Al Diwania City. The leaves were left to dry at room temperature for 24 hours. The dried leaves were ground to a powder and were kept in dry containers. The alcoholic extract was prepared by mixing 50 gm of henna powder with 500 mL of 70% ethanol and mixed by hot plate magnetic stirrer for 24 hours. Further extraction of the residue was repeated until a clear supernatant liquid was obtained. The extracts were sterilized by 0.22 µm Millipore filter. The solvent was dried and concentrated using vacuum Rotary evaporator at 50°C, The evaporation was done until a solid state of extracted liquid was obtained. Extract from this method was then weighed and stored in closed container at 4°C until further use. Aqueous extract was prepared in the same way except that distilled water was used instead of alcohol. Then different concentration (25, 50, 75, 100 mg/ml) were prepared from these extracts by dissolving the dry extract in sterile distilled water (W/V).

### Phytochemical screening :

The phytochemical analysis of the plant were determined for Tannins, glycosides, saponins, alkaloids, coumarins, flavonoides and resins as described in (11, 12).

Bacterial suspension S: *pyogenes* was obtained from department of Microbiology in Vet. Med College in Al Qadisiya uni. culturing and biochemical tests were used for identification of bacteria, then kept in slant agar stock (13). Suspension were prepared that contain  $1 \times 10^8$  CFU which used for induce infection at dose 0.1 ml.

In vitro antibacterial assay: The antibacterial activity of the extracts was determined using the agar well diffusion method (14). The prepared culture media were inoculated with strain of bacteria before poured into plates (1 mL of an overnight culture of bacterial isolate equivalent to  $10^7$ - $10^8$  cfu /mL was used to seed culture media maintained at 45°C). Equidistant wells on the surface of the agar were done in the seeded plates. The wells were filled with 0.1 mL of each the different extract concentration (25, 50, 75, 100 mg/ml) as well as the standard antibiotic solution (Gentamycin) and sterile distilled water, were added under aseptic conditions. Multiple plates were done for each of the extract (three replications). The plates were then maintained at room temperature for 1h allowing for diffusion of the solution. All plates were then incubated at 37°C for 24 hours and the zones of inhibition were calculated by measuring the diameter of the inhibition zone around the well (in

mm) including the well diameter. The readings were taken in three different fixed directions and the average values were tabulated.

#### In vivo antibacterial assay:

Animals: White albino Balb\C male mice (20-30 g) were supplied by the animal house at the Vet. Med. College of Al Qadisiya University. The mice were housed in standard metal cages were fed a stock diet .Water was supplied ad libitum. These mice were kept at room temperature 22°C before and during the experiments. 20 mice were divided into 4 equal groups:

G1: five mice infected with *Streptococcus pyogenes* in dorsal skin as an infected control group.

G2: five mice infected with *Streptococcus pyogenes* in skin then treated with henna ointment.

G3: five mice infected with *Streptococcus pyogenes* in skin then treated with vasaline only.

G4: five mice infected with *Streptococcus pyogenes* in skin then treated with antibiotic (Gentamycin 0.3%) locally.

Skin infection was done after scratching of the dorsal area of skin and contaminated it by cotton swabe socked in bacterial suspension.

(This prepared previously). Swabs were taken from skin infection and cultured in suitable media. Then skin infection was treated by ointment previously mentioned. Skin samples were taken after 12 days from infection and preserved in 10% of buffered formalin for histopathological study.

## Results

The results of the antimicrobial assay of the *L.inermis* extract indicated that the plant exhibited antimicrobial activity against the tested microorganisms' at all different concentrations but this activity varied according to the type of extract and its concentration.

1-Results of active chemical ingrediants in Henna:The preliminary Phytochemical screening of *L.inermis* extract showed the presence of bioactive components like glycoside, tannins, saponins, coumarins, resins and flavonoids (Table 1).

Table1: Phytochemical screening of *L.inermis* extract

	Glycosides	Tannins	Saponins	Coumarins	Resins	Flavonides
Henna	+	+	+	+	+	+

+ means presence of active chemical ingrediants

differences ( $P < 0.05$ ) between them. Water extract had less antibacterial effect on *S.pyogenes* than alcoholic extract, the inhibitory zones produced by it 21.2 ,20.6 ,21.6, 25.2 mm at the 25, 50, 75 and 100 mg/ml respectively ,the statistical analysis showed there's no signifecant differences between water extract and antibiotic. As illustrated in table 2 the effect of each extract was increased with high concentrations.

2-Effect of alcoholic and water extracts on growth of bacteria:Result presented in table 2 show that alcoholic extract of *L.inermis* leaves was more effective than the water extract which had little effects ,it exhibited an inhibition zone 18.2, 21.4, 22.8 and 28.2 mm in diameter in the concentration 25, 50, 75 and 100 mg/ml respectively .Moreover the inhibition zone at 100 mg/ml was larger than this produced by antibiotic, and there was a significant

Table 2: The antibacterial activity of aqueous and alcoholic extracts of *L.inermis* against the growth of *S.pyogenes*

Concentrations Mg/ml	Diameters of inhibition zone of alcoholic extract on bacteria(mm)	Diameters of inhibition zone of water extract on bacteria(mm)
25	18.2 ± 1.36	20.2 ± 1.24
50	21.4 ± 1.5	20.6 ± 1.17
75	22.8 ± 1.4	21.6 ± 0.93
100	28.2 ± 1.77	25.2 ± 1.32
Gentamycin0.3	26.8 ± 0.37	26.8 ± 0.37
Distel water	—	—
L.S.D	5.03	3.13

3-Pathological changes in laboratory animals:Animals of control group showed after 3days from skin infection with *Streptococcus pyogens* hypremia, swelling with abscess formation, congestion fig (2).Microscopically lesions showed infiltration of inflammatory cells especially neutrophils, edema and presence of abscessation in subepidermis tissue fig (3).After 10 days of skin infection with bacteria lesions showed highly infiltration with inflammatory cells, ulceration, sever abscess formation, and congestion fig (4).While in treated animals with henna extract, gross skin lesions appear as scar

tissue then disappear after 11 days from infection with same time of using antibiotic fig (5).Furthermore microscopically after 5 days in animals treated with extract was showed slight infiltration of inflammatory cells, regeneration of tissue and congestion, compared with control group.On day 10 showed in fig (6) increased well organized bands of collagen, more fibroblasts and few inflammatory cells when compared with the controls which showed inflammatory cells, scanty collagen fibres and fibroblasts.



Fig(1) Showed the effect of henna extracts on growth inhibition of *Streptococcus pyogenes*.  
1=distel water , 2=25mg/ml , 3=50mg/ml , 4=75mg/ml , 5= 100mg/ml , 6= gentamycin



Fig(2) Animal showed after 3days from skin infection with *Streptococcus pyogenes* swelling, congestion and pus formation.

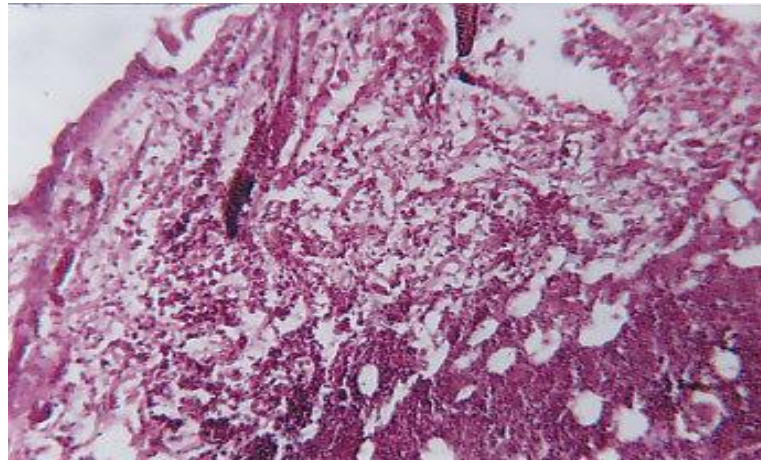


Fig (3) Histological section in skin of the animal at 3 days post inoculation with *Streptococcus pyogenes* showed inflammatory cells infiltration especially neutrophils and subcutaneous abscess with edema, congestion of blood vessel and aggregation of neutrophils in sub cuts .(H &E stain: 40X)

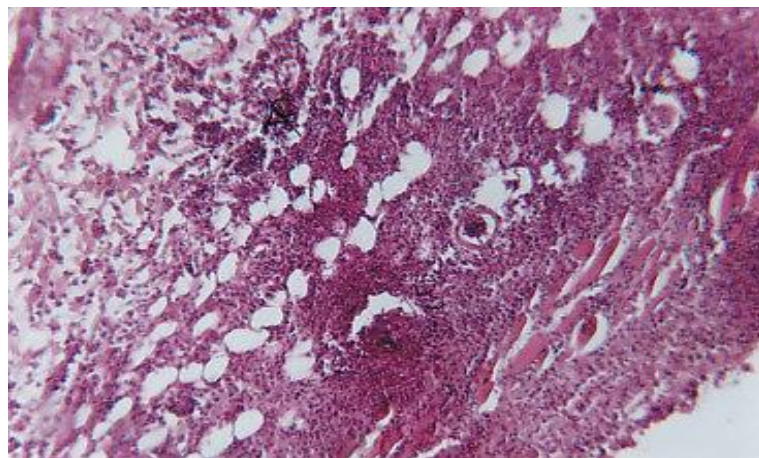
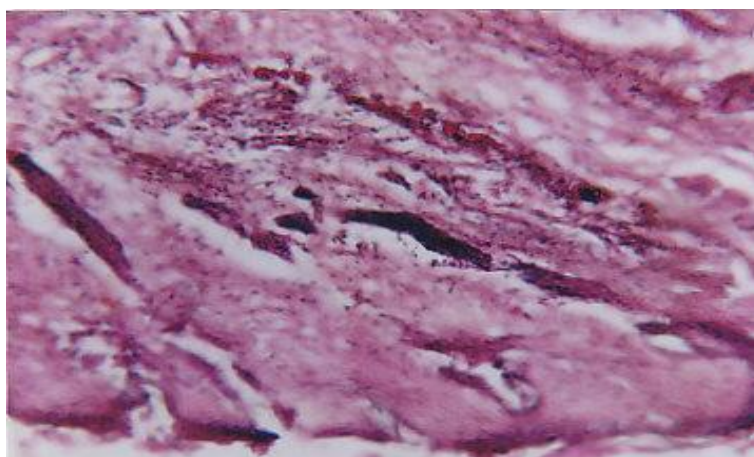


Fig (4) Histological section in skin of the animal at 10 days post inoculation with *Streptococcus pyogenes* showed highly neutrophils infiltration extend to the layers ,ulceration and congestion .(H &E stain: 40X)



Fig(5) healing of infected animal with *Streptococcus pyogenes* after 10 days from treating with henna extracts. Notice the tissue regenerating with presence of scar tissue.



Fig(6) Histological section in skin of the animal at 10 days post treated with henna extract showed slight infiltration of inflammatory cells, regeneration of tissue and congestion and more fibroblasts (H &E stain: 40X).

### Discussion

*S. pyogenes* is the causative agent of many important human and animals diseases ranging from mild superficial skin infections to life-threatening systemic diseases (4). Furthermore the present study showed that henna extracts were capable of inhibiting the growth *S. pyogenes* that are involved in causing wound infections, and this result was accepted with (7). Antibacterial activity is recorded

when the zone of inhibition is greater than inhibition zone of antibiotic. In this study alcoholic extract has high activity in inhibition of bacterial growth at concentration 100mg/ml which is near the effect of antibiotic so this result was mentioned in (5). Antimicrobial activity may be due to numerous free hydroxyls that have the capability to combine with the carbohydrates and proteins in the bacterial cell wall.

They may get attached to enzyme sites rendering them inactive (6). Also the Tannins which present in the leaves are astringent that either bind and precipitate or shrink proteins and various other organic compounds including amino acids (15). This study shows that water extract has slight antibacterial activity compared to alcoholic extract. This may be due to the lack of the solvent properties which plays an important role in antibacterial efficacy (9). Also the phytochemical ingredients which found in henna have antibacterial effect, and using of ethyl alcohol in concentration 80% in plants extracts help to obtain substances which dissolved in water and alcohol. The antipyretic, analgesic and anti-inflammatory effect of ethanolic extract of *L.inermis* was previously reported in rats (16). So many products have been developed for the protection of skin integrity. Topical henna is an extract of the lawsonia plant. Some experimental and clinical studies have reported antibacterial and antifungal effectiveness and wound healing activity of this product (17). Henna is an easily accessible, inexpensive antibacterial product. In the present study we find the antibacterial effect of henna extracts on Streptococcal skin infection. The extract-treated animals showed reduction in the wound area when compared with control. Histological studies of the tissue obtained on day 10 from the extract-treated group showed increased well organized bands of collagen, more fibroblasts and few inflammatory cells when

compared with the control which showed inflammatory cells, scanty collagen fibres and fibroblasts. Naphthoquinones are widely distributed in plants, fungi and some animals. Their biological activities have long been reported to include antibacterial effects on several species of both aerobic and anaerobic organisms (18). Furthermore several other biological activities for naphthoquinones have been described such as being anti-inflammatory (19), bactericidal (20). In general they are active against gram positive bacteria such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococcus faecium* and *Bacillus subtilis*, but are inactive against gram negative bacteria (21). Crude and ethanolic extract of *Lawsonia inermis* leaves showed dose dependent analgesic, antipyretic and anti-inflammatory effect in rats (16). Also our results showed after 5 days in treated animals there are slight infiltration of inflammatory cells, regeneration of tissue, congestion which investigated with (7). Whom mentioned that the effects of water and chloroform extracts of the leaves of henna plant against the primary invaders of burnt wounds, also investigated inhibition of the growth of such microorganisms suggests that henna may be valuable in the management of burnt wound infections (7). We concluded that henna has an in-vitro and in-vivo antibacterial activity against Streptococcal skin infection. These findings have also been mentioned in literatures (22).

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## تأثير مستخلص نبات الحناء على التغيرات النسيجية المرضية للإصابة الجلدية بجرثومة *Streptococcus pyogenes* في الفئران المختبرية

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### الخلاصة

تضمنت الدراسة الحالية تحضير المستخلص المائي و الكحولي لنبات الحناء *Lawsonia inermis* و دراسة تأثيره التثبيطي على نمو بكتريا *Streptococcus pyogenes* في الزجاج (In vitro) باستخدام طريقة الانتشار بحفر الأكار و في الجسم الحي (In vivo) باستخدام الفئران المختبرية حيث تم علاجها بمراهم محضرة من تلك المستخلصات و مقارنة تأثيرها بالمضاد الحياتي .بينت النتائج ان كلا المستخلصين يمتلكان تأثيرا مضادا للبكتريا المستخدمة ،و إن كفاءة المستخلصات اعتمدت بشكل كبير على نوع المذيب المستخدم في الاستخلاص (ماء أو ايثانول) و تركيز المستخلص .اظهر المستخلص الكحولي أعلى فاعلية مثبتة لنمو البكتريا المستخدمة مقارنة بالمستخلص المائي ،إذ أعطى نطاق تثبيط تراوحت أقطاره بين ١٨.٢ و ٢٨.٢ ملم .اختبر المرهم المحضر من المستخلص الكحولي لنبات الحناء بتركيز 5% في معالجة الفئران بعد إصابتها ببكتريا *Streptococcus pyogenes* بطريقة الخدش و قد اظهر كفاءة عالية في اختفاء العلامات السريرية بعد مرور 11 يوماً من العلاج وهي مدة قريبة على المدة التي حدث فيها الشفاء للمجموعة المعالجة بالمضاد الحياتي (10 أيام) .تم اخذ خزع نسيجية من المناطق المصابة لمعرفة التغيرات المرضية التي تحدثها بكتريا *S.pyogenes* كما أخذت خزع نسيجية من المناطق المصابة بعد معالجتها بمراهم المستخلصات النباتية لبيان مدى تأثير تلك المراهم في معالجة الأنسجة المصابة .أوضحت نتائج الفحص النسيجي لجلد الحيوانات المصابة بجرثومة *S.pyogenes* بعد ثلاثة أيام من الإصابة حصول فرط دم ،تورم، احتقان مع تكوين القيح ووجود الخراج ،في حين أن الحيوانات المعالجة بمرهم الحناء فقد اختفت فيها الآفات الجلدية العيانية بعد ١١ يوم من الإصابة .كما لوحظ زيادة واضحة في خيوط الكولاجين مع زيادة في أعداد الارومات الليفية ووجود عدد قليل من الخلايا الالتهابية مقارنة مع حيوانات السيطرة التي أظهرت ارتشاح هائل للخلايا الالتهابية وتكاثر الارومات الليفية.