

The Scientific Signs of Using *Salvadora persica* as Antibiotic

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الخلاصة

بينت الدراسة الحالية امكانية استخدام مسواك عود الاراك كمضاد حيوي في علاج الأرناب المصابة تجريبيا بمجوعتين، فالمجموعة الأولى أصيبت إصابة فموية بـ *Salmonella enteritidis* بتركيز 10^7 وحدة تكوين المستعمرة / مل، اذ ظهرت أعراض جهازية للإصابة متمثلة بالاسهال بعد ١٢ ساعة. في حين المجموعة الثانية أصيبت إصابة جلدية بـ *Pseudomonas aeruginosa* بتركيز 10^7 وحدة تكوين المستعمرة / مل، فظهرت اعراض جلدية متمثلة بالخراج بعد ٤٨ ساعة.

أظهرت الدراسة ان استخدام مسواك عود الاراك اظهر فعالية جيدة في علاج حالات الاسهال وخلال فترة ١٨ ساعة من التجريع مقارنة مع استخدام المضادات الحيوية (Rifampicin, Amoxycilin) والتي أعطت نفس النتائج وخلال فترة ٢٤ ساعة وبنفس الوقت أظهرت الدراسة فعاليته في علاج الإصابة الجلدية وخلال فترة ٣٦ ساعة مقارنة مع استخدام المضادات الحيوية (Cephalexin and Penicillin, Streptomycin)، والتي أعطت النتائج نفسها وخلال فترة ٧٢ ساعة كما في المجموعة الثانية من الإصابة.

Abstract

The present study showed the possibility of using a *salvadora persica* as antimicrobial agent in the treatment of experimentally infected rabbits in two groups, the first group includes rabbits infection occurred after induction of *Salmonella enteritidis* orally at a concentration of 10^7 cfu / ml, diarrhea appeared after 12 hr.

The second group of rabbits infected cutaneously after induction of *Pseudomonas aeruginosa* in burned area on the thigh at a concentration of 10^7 cfu / ml, the abscess after 48 hr.

The study showed that *Salvadora persica* has a good activity in the treatment of diarrhea after 18 hr of administration compared with the antibiotics (Rifampicin, Amoxicillin) which gives the same results with in 24 hr., in the same time, that *Salvadora persica* showed a good activity in the treatment of the infected burned skin 36 hr, compared with the antibiotics (cephalexin and pencillin, streptomycin) which gives the same results with in 72 hr. in the second group of infected rabbits.

Introduction

Salvadora is a genus of tree or shrub in the plant family Salvadoraceae. There are three species, including *Salvadora australis*, *Salvadora oleiodes* and *Salvadora persica*.

It grows as small tree up to seven meters high, but it's branches commonly hang down to the ground, making it look more like a shrub than a tree, *Salvadora* is native to the hottest, driest areas of the middle east (1), (2), it is widely used in middle eastern and eastern and African cultures, it has been shown that extract of *Salvadora persica* posses various biological properties including significant antibacterial and antifungal effects (3), (4) and (5)

It contains a number of medically beneficial properties including abrasives antiseptics, astringent detergents, enzyme inhibitors and fluoride (6).

Also, it is predominant in Muslim areas but its use predates the inception of Islam, its use has spread from the Middle East to South and South East Asia.

Prophet Mohammad (PBUH) taught us the complete way of life, and using miswak was highly recommended by him, also said "siwak cleans the mouth and pleas the Lord", and "If I had not found it hard for my followers or the people, I would ordered them to clean their teeth with siwak before every prayer" (7), (8) and (9).

It has been reported that some anionic components naturally occurring in plant species exert antimicrobial activities against various bacteria (10).

Salmonella infections are zoonotic, can be transmitted by human to animals and vice versa. Infection via food is also possible, it causes diarrhea, people at risk are infants, small children, the elderly, HIV patients and those with suppressed immunity can become seriously ill (13), (14) and (15).

However, investigations of vacuum cleaner bags have shown that households can act as a reservoir of the bacterium, this is more likely if the households has contact with an infection source, for example members working with cattle or in a veterinary clinic (16).

Pseudomonas aeruginosa is a bacterium which can cause disease in animals and human, it is found in soil, water and flora of the skin, most man – made environments throughout the world, it uses a wide range of organic material for food, in animals, the versatility enables the organism to infect damaged tissues or people with reduced immunity, the symptoms of such infections are generalized inflammation and sepsis, a form of dermatitis associated with poor hygiene and low maintenance of hot tubs (17), (18).

Pseudomonas is also a common cause of post operative infection in radial keratotomy surgery patients.

The organism is also associated with the skin lesion ecthyma gangrenosum(19).

The aim of study

The present study reveals moderate efficiency of *Salvadora persica* in treating infections caused by *Salmonella enteritidis* and *Pseudomonas aeruginosa*.

Materials and methods

The study consist of two line:

- A. The systemic infection.
- B. The cutaneous infection.

A- The systemic infection.

This study consisted of:

1. In vivo experimental study.
2. In vitro experimental study.

1. In vivo experimental study.

I. **Bacterial strains:** strains of *Salmonella enteritidis* had been taken from college of science/ department of biology/ university of Mosul, that originated from Germany (field of animals) and diagnosed using the biochemical tests, strains were subcultured in nutrient broth, incubated at 37C° under aerobic condition for 24 hr., then stored at 4 C° in refrigerator until used (20).

II. **Preparation of the extract of *Salvadora persica*:** The sticks of *Salvadora persica* were obtained from private market, extract was prepared depending on the method as follows:

- 1- Grinding by electrical machine and smoothing.
- 2- Dissolved 10 gm /100 ml distil water.
- 3- Mixing by using magnetic sterrier.
- 4- Keep the extract for 24 hr. in the refrigerator.
- 5- Then the extract was filtered through a 0.45 mm membrane.
- 6- Finally the extract will be used as a powder which filled in a capsule 250 mg (21).

III. Technique of the infection: 9 rabbits (private breed) were used in this line of study, their ages ranged between 5-8 months.

The induction of *Salmonella enteritidis* infection was orally at a concentration of 10^7 cfu / ml, diarrhea appeared after 12 hr.

The 9 infected rabbits were divided into 3 groups(3 rabbits/ group).

- The first group (3 rabbits) were treated with extract of *Salvadora persica* as capsule 250 mg twice daily for (4) days.
- The second group (3 rabbits) were treated with antibiotics (Rifampicin 300 mg and Amoxycillin 500 mg) as the first group.
- The third group (3 rabbits) were treated with saline.

2. In vitro experimental study (Antibacterial activity):

This study depended upon disc diffusion method on the surface of nutrient agar which were flooded with 0.1 ml of the inoculum of *Salmonella enteritidis* (10^7 cfu/ml), then plates were desiccated 5-10 min, for adequate drying at room temperature, then disc impregnated with the extract of *Salvadora persica* at a concentration of 1 mg/ml of sterile distill water were placed on the surface of inoculated, antibiotics (Rifampicin, Amoxycillin) were used as positive control, while the disc impregnated with saline was used as a negative control and incubated at 37°C and the results were recorded after 24 h by the appearance of inhibition zone was recorded as a positive results (5).

B- The cutaneous infection:

Also this study includes:

1. In vivo experimental study.
2. In vitro experimental study.

1. In vivo experimental study.

I. Bacterial strains: strains of *Pseudomonas aeruginosa* had been taken from Al-Zahrawii teaching hospital in Mosul, which were isolated from swabs collected from burned patients, strains were subcultured from a starter culture incubated at 37°C under aerobic condition for 24 hr. in nutrient broth and stored in refrigerator at 4°C until used (20).

II. Technique of the infection: 9 rabbits (private breed) were used in this study, their ages ranged between 4-5 months, the thigh of rabbit was shaved and clipped of for all the rabbits, these area were burned using heated scalple, burned area were infected with an inoculum of *Pseudomonas aeruginosa* at concentration of 10^7 cfu/ml subcutaneously and these rabbits were left for the following 2 days to ensure the occurrence of infection, (Image 1).



Image (1): site of Wound showing the abscess.

- The 9 infected rabbits were divided into 3 groups (3 rabbits / group).
- 3 rabbits of the first group were treated topically with the prepared extract of *Salvadora persica* as capsule 250 mg with vaseline twice daily morning and evening for 5 days.
 - Rabbits of the second group (3 rabbits) were treated topically with antibiotics (cephalexin 500 mg and penicillin, streptomycin 250 mg) as a group of positive control.
 - The third group (3 rabbits) were treated topically with saline as a negative control group.

2. in vitro experimental study.

Psudomonas aeruginosa was inoculated in nutrient broth, incubated for 24 hr. at 37C^o, Nutrient agar plates were seeded with 0.1 ml of liquid inoculum (10⁷ cfu/ml) prepared from the strains of *Psudomonas aeruginosa*, the first filter paper discs were impregnated with a extract of *Salvadora persica* at a concentration of 1 mg/ml of sterile distill water, antibiotics (cephalexin and penicillin, streptomycin) and saline were placed on the surface of nutrient agar plates, results were recorded after incubation 24 hr. of incubation at 37C^o.

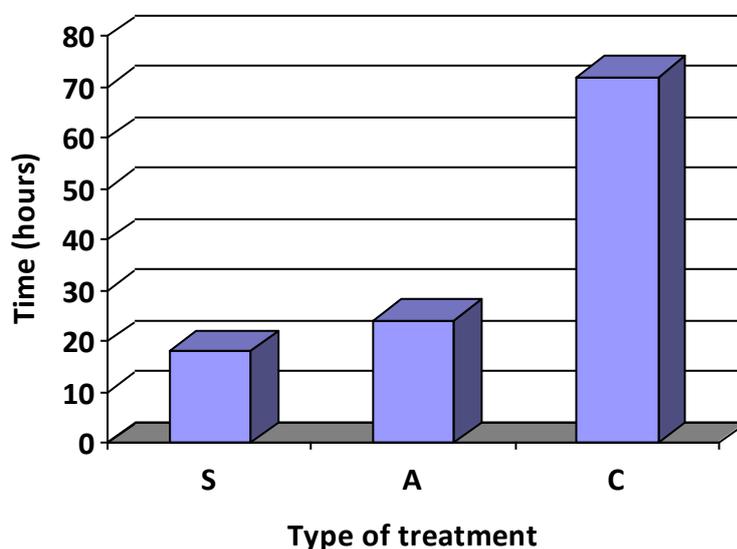
Results

The results of this study was to assess the antibacterial effect of *Salvadora persica* (siwak) as our study divided into two line, in each line of the study showed the results confirmed the curative properties of *Salvadora persica*.

In systemic infection, the first group treated with an aqueous extract of *Salvadora persica* played a role of antibiotic in treating this infection with in 18 hr. compared with the second group which was treated with Rifampicin and Amoxycillin 24 hr. after that taken feaceses of rabbits in the first group, culturing on nutrient agar to confirmed that cases were healed by using extract of *Salvadora persica*, that showing no growth of *Salmonella enteritidis* on these plates.

The Scientific Signs of Using *Salvadora persica* as Antibiotic.

The results reported in the figure 1 indicate that the first group treated with extract of *Salvadora persica* recovered after 18 hr. while the second group treated with Rifampicin and Amoxicillin recovered after 24 hr., and the third group treated with saline recovered after 72 hr.



S = (*Salvadora persica*).
A = (Rifampicin and Amoxicillin).
C = (Control).

Figure (1): Relation between time of recovery and type of treatment

Important differences in the rate of treatment of diarrhea were observed between diarrhea treated with saline and diarrhea treated with either *Salvadora persica* and antibiotics, extract of *Salvadora persica* gave the fastest rate of recovery compared with other groups, this was the results of invivo experimental study, the same results were obtained from the invitro experimental study, zone of inhibition against *Salmonella enteritidis* was seen the extract and this zone was larger than that for antibiotics but control disc (saline) showed no inhibitory effect by length (1.9 cm) (1.6 cm) (0 cm) respectively, (Image 2).

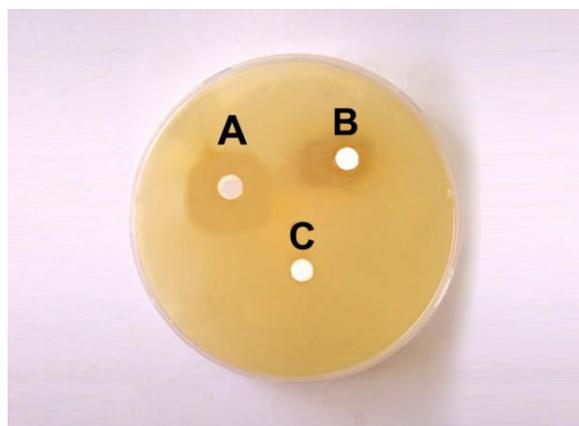


Image (2): zone of inhibition of *Salvadora persica* (A), Rifampicin and Amoxicillin (B), saline (c) against *Salmonella enteritidis*(C)

In the cutaneous infection, the results of our study were estimated the healing rate of skin infection by that infection healed with extract, the results showed (36) hr. compared with the second group which was treated with cephalixin and penicillin, streptomycin which showed complete healing within (72) hr., while the healing of the third group (control group) take (120) hr., then taken swabs from site of wound of rabbits in the first group, culturing on nutrient agar to assess that infections were healed by using extract of *Salvadora persica*, that showing no growth of causative agent on plates.

The results reported in the figure, (2) indicate that the wound treated with extract showed complete healing after 36 hr. (Image 3)

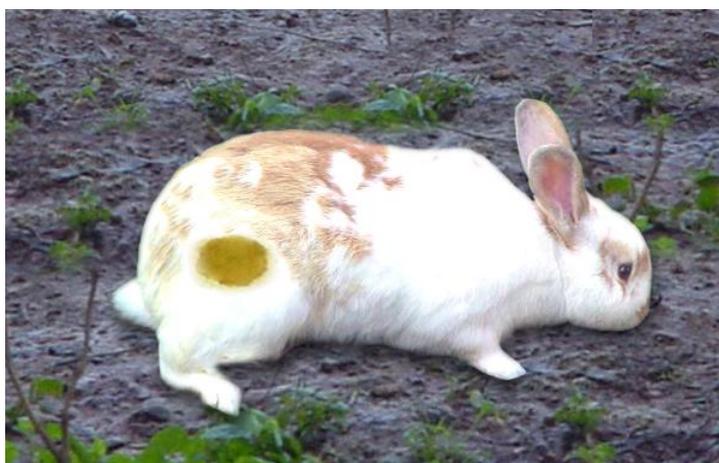


Image (3): Healing of wound by extract of *Salvadora persica*

Otherwise wound treated with cephalixin and penicillin, streptomycin after (72) hr. (Image 4).

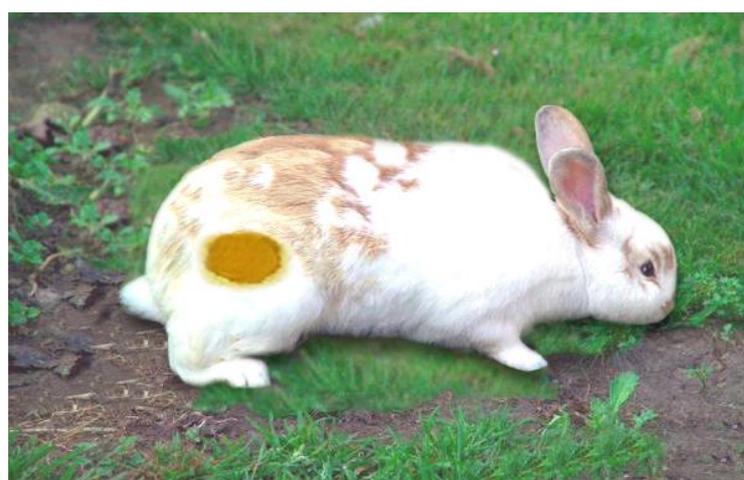


Image (4): Healing of wound by antibiotics (cephalexin and penicillin streptomycin)

Also wound treated with saline as a control group healed completely after (120) hr., (Image 5).

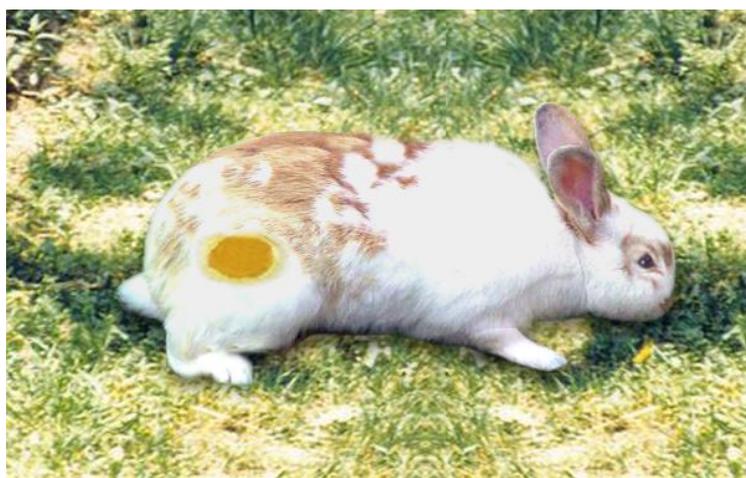
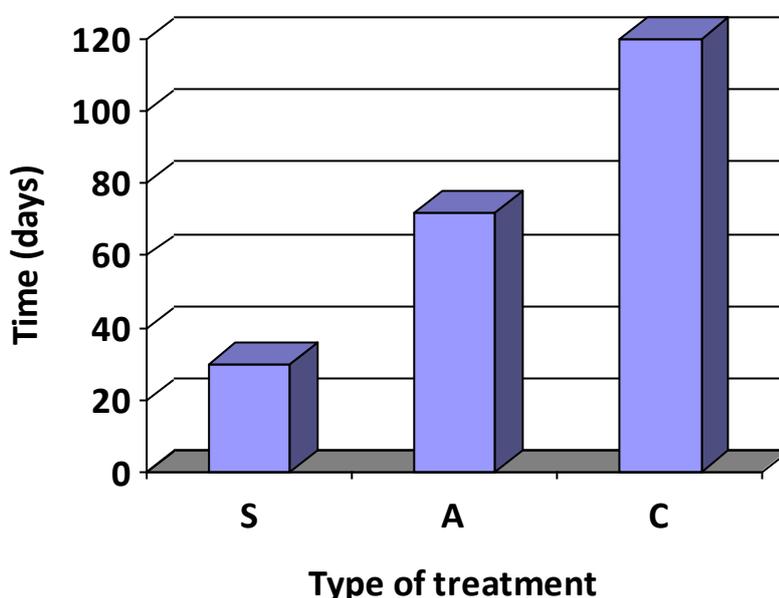


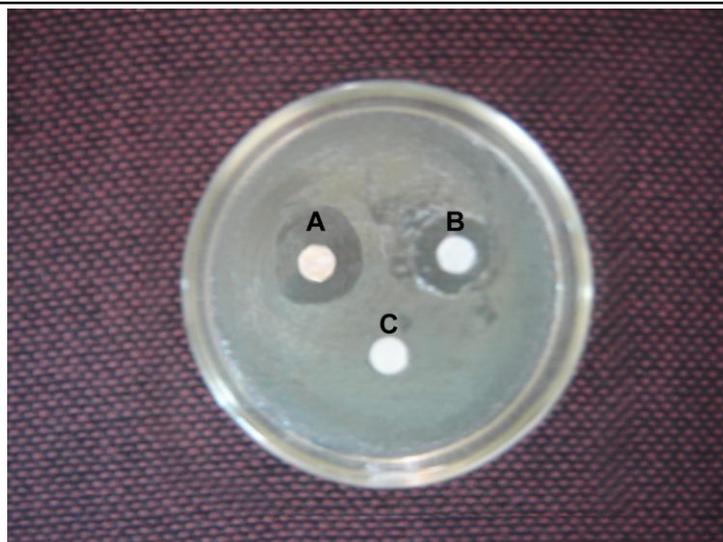
Image (5): Healing of wound by saline (control group)



S = *Salvadora persica*.
A = Cephalexin and Penicillin.
C = Control.

Figure (2): Relation between time of healing and type of treatment of wound

Significant difference in the rate of wound healing were observed between wound treated with saline and wound treated with either extract and antibiotics. Extract gave the fastest rate of healing compared with others, this was the results of invivo experimental study, the same results were obtained invitro experimental study as a zone of inhibition against the *Psaudomenas aeruginosa* for the extract was higher than that inhibition for antibiotics against *Psaudomenas aeruginosa*, but no zone of inhibition in control disc (saline) and the diameter of inhibition zone was (1.7 cm), (1.5 cm) and (0 cm) respectively, Image (6).



Image(6): Zone of inhibition of extract (A), cephalixin and penicillin, streptomycin (B), saline (C) against of *Psaudomenas aeruginosa*

Discussion

The selection of miswak from the *Salvadora persica* tree for the present study was based on a number of factors, that based on this happens either by denaturing the cell proteins or by modifying and / or damaging the physical and chemical properties of the cell membrane, the antibacterial activity of *Salvadora persica* extract may be due to the presence of tannin-like substances which are capable of protecting certain plants against bacterial infection, tannins have been shown to form irreversible complexes with proline-rich proteins causing inhibition of bacterial adherence, alternatively, the tannins may interact with surface bound lipoteichoic acids as in the case of salivary acidic glycoproteins resulting in bacterial aggregation (8), (13),

The antimicrobial and cleaning effects of *Salvadora persica* have been attributed to various chemicals detectable in the extracts.

These effects are believed to be due to it's high content of sodium chloride and potassium chloride as well as salvadourea and salvadorine, saponins, tannins, vitamin C, silica and resin, in addition to cyanogenic glycoside and benzyliothiocyanate (11), (12).

In addition, antibacterial found in *Salvadora persica* may interfere with bacteria and prevent their attachment, fluoride is another compound which may affect the glycolytic enzymes of bacteria associated with either their acid or intracellular polysaccharide production.

Benzyliothiocyanate (BIT) which is a compound isolated from *Salvadora persica* acts by inhibiting bacterial growth and it's acid production (11).

Salvadora persica as a successful philosophy of modren health policies need to be promoted to health policy makers as to be implemented in all public health programmes, accordingly the ability of

The Scientific Signs of Using *Salvadora persica* as Antibiotic.

Salvadora persica to eliminate their host pathogens from such systems is discussed, i.e. to remove the load of pathogenic bacteria from the host (22).

Therefore *Salvadora persica* can save our money and health away of antibiotic, as best cosmetic tool (12).

The selection of miswak from the *Salvadora persica* tree in all the studies was based on a number of factors, it is most common, it's taste is not unpleasant and it is cheap, furthermore, it manifests many pharmacological properties (13).

The results of another study regarding the effect of *Salvadora persica* extract on healthy and periodontally involved dentin, and on healthy dentin, the chemical analysis of *Salvadora persica* has shown it contains carbohydrates, trimethylamine, salvadorin, chlorides, fluoride in large amounts, silica, sulphur, vitamin C and small quantities of tannins, saponins, flavonoids and sterols, it would be interesting to find out the effect of these individual constituents of *Salvadora persica* (8) (13).

Studies opened a new area for research on the systemic effect of miswak (sewak) after discovering its great positive effect on the immune system (23).

Another study had opened a great field of science and researches by his last research "Miswak medicine theory" or sewak puncture medicine which led him to what is so called Beyond sewak: world of science and research, Miswak also is contributing in the fight against desertification, there by affecting our environment and global climate(24).

The yields obtained from *Salvadora persica* aqueous extracts showed that it contains chloride (Cl^-), sulphate (SO_4^{2-}), thiocyanate (SCN^-), Nitrate (NO_3^-), (SCN^-) acts as substrate for lactoperoxidase to generate by hypothiocyanite (OSCN^-) in the presence of hydrogen peroxide, (OSCN^-) has been demonstrated to react with sulphhydryl group in bacterial enzymes which in turn leads to bacterial death, (SO_4^{2-}) and (SCN^-) in *Salvadora persica* aqueous extracts released during hydrolysis of glucosinolates by myrosinase enzyme in the plant tissue.

It has been reported that glucosinolates hydrolysed to isothiocyanate, glucose and sulphate ions at neutral conditions (PH = 7), and in acidic medium (PH = 3-4) to nitrites, sulphur, sulphate and glucose Isothiocyanates in the presence of the enzyme myrosinase may decompose into their respective alcohol derivatives and thiocyanate, Nitrate (NO_3^-) in *Salvadora persica* aqueous extracts may be released from the residual nitrate ions taken up by the *Salvadora persica* tree or from the oxidation of ammonia and other nitrogen compounds, nitrate, nitrite and nitrosoamines have been shown that miswak extracts have antimicrobial activity against *Streptococcus faecalis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* which may be due to its content of nitrate (12).

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