



Trends in
Medical Research

ISSN 1819-3587



Academic
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Efficacy of the Desert Truffle *Terfezia claveryi* to Cure Trachoma Disease with Special Emphasis on its Antibacterial Bioactivity

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ABSTRACT

The aim of the present study was to apply natural drops of truffle (*Terfezia claveryi*) to patients diagnosed with trachoma for curing the disease and inhibiting the chlamydial bacteria and explore the potential for the development of new treatment modalities compared to currently used antibiotics. Results showed that the truffle juice was very effective against trachoma in the third phase of the disease. There was clear reduction of symptoms in patients with cortisone compounds in the form of drops plus truffle juice drops. There were also complete healing of the conjunctiva and healing of the keratitis. Partial curing was observed when cortisone plus cream of chloramphenicol was added included healing of trachoma without any change of fibrosis in the conjunctiva. In the treatment of trachoma with cortisone drops and cream plus truffle juice, complete of healing was observed but continued swelling in the capillaries with no fibrosis occurred. It was clearly noticed that in all of the treatments, there was a noticeable reduction in fibrosis of the affected site in the eye. It was also concluded that truffle juice seems to prevent fibrosis of trachoma by a large extent. On the other hand, the use of chemical antibiotic or cortisone-based compounds produced several side effects. Though these chemicals reduced the toxins caused by trachoma thereby reducing the enlargement of the cells. We can therefore clearly recommend adding truffle juice to the traditional treatment for trachoma at all stages.

Key words: Desert, truffle, *Terfezia claveryi*, trachoma disease, *Chlamydia trachomatis*, antibacterial activity

INTRODUCTION

Trachoma disease is caused by *Chlamydia trachomatis* (Thygeson, 1939; Burton and Mabey, 2009) of the bacterial phylum Chlamydiae. It is an obligate intracellular parasite and remains a major cause of avoidable blindness among underprivileged populations in many developing countries, where people live in overcrowded conditions with limited access to water and health care (Janakat *et al.*, 2005). The disease is a chronic contagious inflammation from which most inhabitants of the Arab world, Mediterranean region suffer (Janakat *et al.*, 2005; Al-Rahmah, 2001; Bokhary, 1987). Symptoms are internally scarred eyelids, followed by eyelids turning inwards and if untreated this condition leads to the formation of irreversible corneal opacities and blindness (Potroz and Cho, 2015). It is estimated that about 150 million people have active trachoma and nearly six million people are blind due to complications associated with repeated infection

(Evans and Solomon, 2011; Thylefors *et al.*, 1987). The distribution of trachoma is worldwide. In the Middle East and Sub-Sahara African, Indian Subcontinent, Southern Asia and China the blinding trachoma is widespread. Sporadic distribution of the disease occurs in Latin America, Australia and the Pacific Islands (Personal communication WHO, Water Sanitation and Health unit-WSH) staff. The WHO along with an alliance of interested parties has adopted the "SAFE" strategy to combat trachoma (Solomon *et al.*, 2006). The four components of the protocol include: surgery, antibiotic treatment (Tetracycline or azithromycin eye ointment), facial cleanliness and the fourth strategy is environmental changes (Ejere *et al.*, 2012; Mariotti, 2004). However, the increasing resistance of many bacteria and the side effects to the currently used antibiotics are documented (Skiest *et al.*, 2007; Aldebasi *et al.*, 2013; Burton *et al.*, 2005). To substitute synthetic antibiotic use due to resistance by the bacterium and repeated use of cortisone compounds, truffles are widely appreciated to treat several diseases. These truffles have been reported to possess anti-inflammatory, immunosuppressing and anti-carcinogenic properties (Al-Laith, 2010; Stanikunaite *et al.*, 2007; Janakat *et al.*, 2004). Truffles are mycorrhizal fungi. Among the well known edible desert truffle only two species of the dark brown color belonging to the genus *Terfezia* and the other of the white color belonging to the genus *Tirmania* (Al-Rahmah, 2001). The aim of the present study was to apply the juice of truffle (*Terfezia claveryi*) to patients diagnosed with trachoma and explore the potential for the development of new treatment modalities compared to currently used synthetic antibiotics.

MATERIALS AND METHODS

Sample collection: Truffles samples were purchased in Riyadh city from importers dealing in truffles imported from Yemen in March, 2015. A typical *T. claveryi* fruiting bodies (Variety Zubiedi) were collected. Samples were put in clean polyethylene bags and were labeled carefully with pertinent information i.e., habitats (wild), date of collection, etc. The samples were then brought to the Laboratory of Microbiology in the College of Applied Medical Sciences at Shaqra University. In the laboratory samples of 2 kg were washed carefully with running water, blotted on kitchen sucking papers and cut into small pieces. Samples were then dried using hot oven.

Preparation of extracts: Extraction was carried out according to the method adopted by Simao *et al.* (2015). In accordance with this method, 80 g of Desert Truffle *Terfezia claveryi* was crashed to a fine powder using mortar and extracted successively with petroleum ether and methanol using Soxhlet apparatus for about 4 h for petroleum ether and 8 h for methanol. Solvents were then evaporated under reduced pressure using Rotary evaporator apparatus and extracts were left to air till complete dry. The petroleum ether extract was dissolved in petroleum ether and the methanol extract was dissolved in methanol (Neggaz and Fortas, 2013).

Yield percentages were calculated as followed:

$$\text{Yield \%} = \frac{\text{Weight of extract}}{\text{Weight of plant}} \times 100$$

To prepare aqueous extract, 100 g of *Terfezia claveryi* powdered fruits were boiled in 500 mL distilled water in water bath at 70°C for 15 min and filtered. The filtrate was dried using a freeze drier apparatus (Simao *et al.*, 2015).

Bacterial strains: Four types of bacteria namely *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* were used for antibacterial experiments *in vitro*.

Table 1: Antibacterial activity of Saudi desert truffle (*Terfezia claveryi*) (Methanol extract) against different species of bacteria*

Tested organisms	Extract concentration (%)			
	5	10	20	40
<i>Staphylococcus aureus</i>	-	-	3±0.13	5±1.81
<i>Bacillus subtilis</i>	-	-	2±1.33	4±0.85
<i>Escherichia coli</i>	-	-	4±0.15	5±0.34
<i>Pseudomonas aeruginosa</i>	-	-	3±1.65	4±0.25

*: Mean measurement of inhibition zone (mm) ±SD, -: No inhibition zone was detected

These bacteria were isolated from clinical cases and provided by Khartoum educational hospital, Sudan. The bacteria were cultured on nutrient broth (Oxoid) at 37°C for 24 h.

Antibacterial activity: Each Truffle extract (water, methanol and petroleum ether was bioassayed against the four types of bacteria using the cup-plate agar diffusion method (Cheesbrough, 1994; Perez *et al.*, 1990) and the inhibition zones were measured. Means diameters ±SD of the inhibition zones were reported in Table 1.

Preparation of *Terfezia claveryi* juice: *Terfezia claveryi* juice was extracted from tubers of the desert truffle using the Filatov method (Potroz and Cho, 2015). The liquid was then dried and turned into powder in central laboratory of Al-Neelain University, Khartoum, Sudan. So that it could be preserved for a long time. When it was used, the powder was dissolved in distilled water to reach the same concentration as natural truffle juice. Truffle juice is brown in color and has pungent smell.

***In vivo* experiments using truffle juice on trachoma disease of the eye:** Our experiments were confined to the third stage of trachoma (Active trachoma along with clear eye inflammation). Experiments included 30 patients, 18 males and 12 females admitted to the eye hospital with clear diagnosis of third stage of trachoma. The protocol of the experiments as follows:

Treatment 1: Included 6 males at the age of 7-10 years old that were treated with cortisone compounds in the form of drops and cream every night for two weeks.

Treatment 2: Included 4 girls at the age of 7-10 years old that were treated with cortisone compounds in the form of drops and cream every night for two weeks.

Treatment 3: Included 6 male patients at the age of 7-10 years old who were treated with cortisone compounds in the form of drops plus truffle juice drops three times daily for two weeks.

Treatment 4: Included 4 female patients (girls) at the age of 7-10 years old who were treated with cortisone compounds in the form of drops plus truffle juice drops simultaneously three times daily for two weeks.

Treatment 5: Included 6 male patients (boys) at the age of 7-10 years old who were treated only with truffle juice drops five times daily for two weeks.

Treatment 6: Included 4 female patients (girls) at the age of 7-10 years old who were treated only with truffle juice drops five times daily for two weeks.

RESULTS AND DISCUSSION

Trachoma disease has three different stages of disease development. The first stage of trachoma is characterized by the appearance of fleshy growth in the lens of the eye that can be easily seen with magnifying lens only. The second stage is called active trachoma in which the lymphatic follicles appear to the naked eye. The third stage is also called active trachoma along with clear eye inflammation.

Results of an *in vitro* analysis showed that truffle juice is slightly effective or ineffective against different strains of bacteria tested (Table 1-3).

Data obtained from serial experiments on the use of truffle juice against trachoma patients revealed that using of the juice against trachoma of the eye was very effective in the third phase of the disease (Table 4). We can therefore clearly recommend adding truffle juice to the traditional treatment for trachoma at all stages. There was a clear reduction in all of the patients treated with cortisone compounds in the form of drops plus truffle juice, drops cured trachoma completely both in males and females (4 girls). At the end of the 14 days treatment, a smear was done from the conjunctiva and examined with the compound microscope (40 X), for all of the 10 cases were examined (6 males+4 females). There was complete healing of the conjunctiva and healing of the keratitis. Partial curing was observed when cortisone plus cream of chloramphenicol was added included healing of trachoma without any change of fibrosis in the conjunctiva and here only 3 cases (males) and 2 females were cured. Also, in the treatment of trachoma with cortisone drops

Table 2: Antibacterial activity of Saudi desert truffle (*Terfezia clavervyi*) (Petroleum ether extract) against different species of bacteria*

Tested organism	Extract concentration (%)			
	5	10	20	40
<i>Staphylococcus aureus</i>	-	-	-	-
<i>Bacillus subtilis</i>	-	-	-	-
<i>Escherichia coli</i>	-	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-

*: Mean measurement of inhibition zone (mm), -: No inhibition zone was detected

Table 3: Antibacterial activity of Saudi desert truffle (*Terfezia clavervyi*) (aqueous extract) against different species of bacteria*

Tested organism	Extract concentration (%)			
	5	10	20	40
<i>Staphylococcus aureus</i>	-	-	-	-
<i>Bacillus subtilis</i>	-	-	-	-
<i>Escherichia coli</i>	-	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-

*: Mean measurement of inhibition zone (mm), -: No inhibition zone was detected

Table 4: *In vivo* experiments using truffle juice on trachoma disease of the eye

Treatment	Dose(drops) /frequency	No. of patients	Age (years)	Ends of treatment (days)	Sex	No. of cured persons or recovered cases
Cortisone compound	Every night for two weeks	6	7-10	14	Males	6 (with partial curing and partial fibrosis occurred)
Cortisone compound	Every night for two weeks	4	7-10	14	Girls	4 (with partial curing and partial fibrosis occurred)
Cortisone compound +truffle juice	Three times daily for two weeks	6	7-10	14	Males	6 (with continued swelling in the capillaries with no fibrosis)
Cortisone compound juice	Five times daily for two weeks	4	7-10	14	Girls	6 (with continued swelling +truffle in the capillaries with no fibrosis)
Truffle juice only	Five times daily for two weeks	6	7-10	14	Males	6 (no fibrosis present)
Truffle juice only	Five times daily for two weeks	4	7-10	14	Girls	4 (no fibrosis present)

and cream plus truffle juice, a complete healing in all the cases but continued swelling in the capillaries with no fibrosis occurred (Table 4). In cortisone drops and cream healing, recovery of all cases in both males and females was observed and partial fibrosis in the conjunctiva was present. It was clearly noticed that in all of the treatments there was a noticeable reduction in fibrosis at the affected site of the eye. It is also suggested that truffle juice seems to prevent fibrosis of trachoma to a large extent, the formation of the cells that cause fibrosis. On the other hand, the use of chemical antibiotic or cortisone-based compounds may be limited and has side effects. Definitely these chemicals affected and reduced the toxins caused by trachoma and hence reducing the enlargement of the cells. At the same time, they prevent unnatural growth in the surface cells of the conjunctiva and increases nourishment to the cells by widening the capillaries in the conjunctiva. Because most of the complications of trachoma result from the fibrosis process, so truffle juice prevents such complications.

Experiments on antibacterial activity of truffles showed that there was no effect with aqueous and petroleum ether extracts against the tested four types of bacteria (Table 1-3). No inhibition zone was detected even when different concentrations are used. However, in methanol extracts no inhibition zones were detected i.e., 5 and 10% concentration. In the higher concentrations, 20 or 40% the activity of the extract varied with regard to the bacterial type. In case *Escherichia coli*, the inhibition zone was 4 and 5 in the concentrations of 20 and 40%, respectively (Table 1). The lowest inhibition zones were obtained in the case of *Bacillus subtilis* due to its chemical contents.

Truffles constitute an important source of protein. Aqueous extracts of the truffle *Terfezia claveryi* contains a potent antimicrobial agent that is protein in nature and may be used in the treatment of eye infections caused by *Pseudomonas aeruginosa* (Janakat *et al.*, 2005). The composition of the chemical constituents of the truffle *Terfezia claveryi*: 77% water and 23% other substances. These substances include 60% hydrocarbons, 7% fats, 4% fibers, 18% proteins and the remaining 11% are in the form of ashes (Ahmed *et al.*, 1981; Al-Qarawi and Mridha, 2012; Bokhary *et al.*, 1987). Seventeen amino acids have already been recognized in the proteins present in the truffles (Ahmed *et al.*, 1981; Daniel, 2006).

Present findings are in close agreement with those of Burton *et al.* (2005) and Burton and Mabey (2009) who reported the efficacy of truffle juice against trachoma disease. Other workers reported that lower doses (50 mg mL⁻¹) of the truffle water extract may not be as effective as higher doses in curing the third phase of this disease (Solomon *et al.*, 2006; Al-Qarawi and Mridha, 2012). Furthermore, some truffle fruits extracts are known to produce one or more mycotoxins or antibiotics (Janakat *et al.*, 2004; Evans and Solomon, 2011). Our result supports the traditional use of truffle for the treatment of trachoma disease in different Arab countries (Ahmed *et al.*, 1981; Al-Rahmah, 2001; Al-Laith, 2010; Aldebasi *et al.*, 2013). More investigations are needed to know the mechanism of truffle juice in disease suppression in all phases of the disease and its clinical role. Also intensive work is needed to isolate and characterize the bioactive compounds of the truffle fungus for pharmacological testing.

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