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The Potential Antibacterial Role of *Terfezia Claveryi* Extract Against Immune-Inflammatory Disorder and Oxidative Damage Induced by *Pseudomonas Aeruginosa* in Rat Corneas

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Abstract

Pseudomonas aeruginosa is a pathogenic gram-negative bacteria causing life-threatening infections. In the current study, the antimicrobial activity of an aqueous extract of the fruiting bodies of Terfezia claveryi truffle against P aeruginosa was investigated in vitro and in vivo. In in vitro study, the antimicrobial of the extract was tested against three clinical strains of P aeruginosa (ATCC 14028, ATCC 27853 and ATCC 9027) at a concentration of 200mg/ml. In vivo study. The suspension of P. aeruginosa ATCC 27853, (5 µl), containing 1.0 × 10^8 CFU, was topically applied onto the wounded rat corneas. The truffle extract was administered to infected rats as eye drops (1%) 24h post infection. In vitro study revealed that the extract showed an inhibitory effect on the growth of testing pathogen strains. The largest zone of inhibition (ZOI) was recorded against P. aeruginosa ATCC 27853 and confirmed by scanning electron microscope. In vivo study, the extract effectively ameliorated the biomarkers of cornea tissue damage including, malondialdehyde (MDA), glutathione peroxidase (GPx), C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), interferon-γ (IFN-γ) and 8- Hydroxydeoxyguanosine (8-OH-dG) in infected rats compared with infected untreated ones.In conclusion, the current results may suggest the use of T claveryi aqueous extract to treat bacterial infection induced cornea inflammatory damage.

Keywords: *Terfezia claveryi, truffle, Pseudomonas aeruginosa, cornea, C-reactive protein*

1. Introduction

Pseudomonas aeruginosa is an opportunistic human gram-negative bacteria, capable of colonizing and infecting virtually any tissue. The pathogen has the ability to disseminate from epithelial infected sites to the bloodstream, causing life-threatening infections (**1**). This pathogen can infect various types of tissues, including eye. Although, the eye has several defense mechanisms against bacterial colonization on the ocular surface **(2),** predisposing factors such as corneal injury, allergic hypersensitivity reactions, corneal abnormalities, overuse of contact lenses, complications after the laser in situ keratomileusis and systemic diseases, may alter these protective mechanisms of the ocular surface and permit bacteria to invade the cornea through epithelial eye cells **(3 -4)**. Bacterial keratitis is one of the most visually threatening ocular infectious pathologies due to its potential complications (**5)**. Most complications of bacterial keratitis are structural alterations of the cornea, development of secondary glaucoma and cataract or even complete loss of vision **(5).** These consequences are largely caused by the factors released by bacteria in addition to the host's inflammatory response. The pathogen

produces many factors that promote adherence to host cells, disrupt defense mechanisms and elicit host inflammatory immune response, leading to host tissue damage. It has been reported that enzymes liberated from proliferating bacteria, corneal epithelial cells and inflammatory cells are the causative factors of progressive corneal ulceration (**6- 8).**

Bacterial eradication by the host immune response involves production of toxic reactive oxygen species (ROS) and inflammatory cytokines (interleukin (IL) -1, IL-6 and tumor necrosis factor (TNF) -α) that synergize to elicit inflammatory events leading to ulceration during bacterial keratitis (**9-10).** Also, previous study has been reported that human endothelial cells are the bacterial target in the course of *P. aeruginosa* bacterial infection (**11**). *P. aeruginosa* can be selectively internalized by endothelial cells via a phagocytic-like process (**11**).These cells respond to intracellular bacteria by producing ROS and nitric oxide (NO) intermediates (**12)**. This cellular response induces a state of oxidative stress resulted from the over production of such species and decrease in antioxidant defense systems (**13)**. Thus endothelial cells do not resist the excessive free radical production induced by the bacterial infection and die (**12, 14)**.Desert truffles are edible economically important fungi that are distributed naturally from North Africa to the Middle East. They are well known for their nutritional values (**15),** as they are rich sources of proteins, amino acids, fatty acids, minerals and carbohydrates **(15-17**). Besides, they represent unlimited sources of therapeutic compounds with anti-inflammatory, antioxidant, antimicrobial, antimutagenic, anticarcinogenic, anti-malarial, anti-tuberculoid and hepatoprotective properties (**18-24).** *Terfezia* species are edible desert dark brown truffles (**25).** These truffle species have therapeutic potential effects in the treatment of several ailments. They possessed antiviral and antibacterial activities, which may have potential impacts for the treatment of eye and skin diseases (**18, 26)**. Some authors reported that aqueous and methanol extracts of *T. claveryi* (Family, Terfeziaceae) have antimicrobial activity against *P. aeruginosa* and *Staphylococcus aureus* **(18, 27).** *T. claveryi* was also found to exhibit hepatoprotective activity and a higher oxidative inhibition against lipid peroxidation **(23, 28**). It was found that the antioxidant capacity and the therapeutic value of different truffles including *T. claveryi* may be related to their chemical constituents, such as vitamin C, carotenoids and many phenolic compounds, which have strong antioxidant potential action with high ability to scavenge oxygen radicals and reduce lipid peroxidation (**22, 24, 26**). The objective of the current study is to explore the antimicrobial activity of the aqueous extract of *T. claveryi* fruiting bodies against the pathogenic bacteria, *P. aeruginosa* in vitro and in vivo. In vitro study, the antimicrobial of the extract was conducted against three clinical strains of *P. aeruginosa* (*P. aeruginosa* ATCC 14028, *P. aeruginosa* ATCC 27853 and *P. aeruginosa* ATCC 9027). In vivo study investigated the potential role of truffle extract against oxidative stress, immune-inflammatory tissue damage and deoxyribonucleic acid (DNA) oxidative damage induced in rat corneas in response to *P. aeruginosa* ATCC 27853 bacterial infection. The study may provide a better understanding of the damaging mechanisms that are induced in the cornea after *P. aeruginosa* challenge and also may provide targets for alternative treatment for this disease.

2. Materials and methods

Chemicals

All chemicals used were of high analytical grade, the product of Sigma and Merck companies. Kits used for the quantitative determination of different parameters were purchased from Biogamma, Stanbio, West Germany.

Desert truffle:

T. claveryi fruiting bodies *(*dark brown red in color, rounded and small in size) were purchased from the local market during summer.

Pathogenic bacteria:

Three strains of *P aeruginosa* bacteria (*P aeruginosa* ATCC 14028, *P. aeruginosa* ATCC 27853 and *P. aeruginosa* ATCC 9027) were obtained from the Laboratory of Eyes Hospital in Saudi Arabia and were confirmed at Medical Microbiology and Parasitology Department. They were maintained at a temperature of 37°C on the Mueller-Hinton Agar medium (Oxoid, CM 41).

Preparation of Truffle aqueous extract:

For preparation of truffle aqueous extract , about 500 g of *T. claveryi* fruiting bodies were washed and homogenized in a distilled water (1:3 w/v), using a household blender. The homogenate was refrigerated overnight, filtered through cheesecloth and then centrifuged at 4000 r.p.m. for 15 min. The supernatant was then dried using a rotary evaporator **(27).**

***In vitro* study**

Determination of susceptibility *P. aeruginosa* to *T. claveryi* truffle aqueous extract

Suspensions of different strains of the tested bacteria were made in a sterile normal saline. The agar–well diffusion method was employed for the determination of antibacterial activity of the truffle extract (**29**). Suspensions of the different strains of the tested bacterial species , 10^8 colony forming unit / ml (CFU / ml) each, were cultured on Mueller Hinton Agar medium, then a well of 5 mm was made in the agar medium using sterile cork borer and placed onto the incubated plates. Truffle extract was dissolved in sterile distilled water (200mg/ml) and added into labeled medium plates. Control without truffle extract was also included in the experiment. These plates were then kept at a low temperature (4°C) for 2 h. to allow maximum diffusion of samples. The plates were then incubated at 37°C for 24 h. The sensitivity of different strains of *P. aeruginosa* to truffle extract was determined by measuring the diameters of inhibition zones (ZOI mm) produced after incubation. The experiment was carried out in triplicate for each *P aeruginosa* strain and the average zone of inhibition was calculated.

Scanning Electron Microscope (SEM)

SEM was carried out on the most susceptible *P. aeruginosa* strain to truffle extract. Cells of *P. aeruginosa* ATCC 27853 in nutrient agar were treated with the extract for 24 h. Bacterial cells without plant extract were served as controls. The cells were collected by centrifugation and washed with a sterile phosphate buffered saline (PBS). The samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, at 4°C overnight, and post-fixed in 1% osmium tetroxide in the phosphate buffer for 1 h at room temperature before processing for SEM (XL20-Philips).

***In vivo* study**

Preparation of eye drops of *T. claveryi* aqueous extract:

The eye drops were prepared by dissolving the dried aqueous extract (1%) of *T. claveryi* in 25 mmol/l sterilized sodium phosphate buffer (pH 7.4), containing 2.527 g of sodium dihydrogen phosphate dihydrate, 1.36 g of disodium phosphate dodecahydrate, and 800 ml bidistilled water. The used buffer was sterilized by autoclaving at 140°C for 3 hours in a vacuum.

Animals and treatments

Animals

Forty healthy male albino rats (120–150 g) were supplied by Experimental Animal Center, King Fahad Medical Research Center, Jeddah, King Abdelaziz University. Animal utilization protocols were performed in accordance with the guidelines provided by the

Experimental Animal Laboratory and approved by the Animal Care and Use Committee of the College of Science, King Abdelaziz University. The rats were kept in special cages and maintained at a constant 12-h light/12-h dark cycle with air conditioning, a controlled temperature of 20 ± 2 °C, and relative humidity of 50–70%. The animals were fed a standard rat pellet chow with free access to tap water ad libitum for one week before the experiment for acclimation.

Infection of rats

It has been shown by the *in vitro* study that *P. aeruginosa* **(**ATCC 27853**)** is the most sensitive strain to *T claveryi* truffle extract, so it has been chosen for the *in vivo* experiment to constitute a corneal infection.

Twenty rats were lightly anesthetized with isoflurane with 1% isoflurane by inhalation (Aerrane; Anaquest, Madison, WI, USA) and placed beneath a stereoscopic microscope at 40 × magnification. The central corneas of the rat eyes were injured using sterile needles. The rat eyes were examined histologically to ensure that the wounds penetrated only the superficial corneal stroma. A bacterial suspension (5 µl) containing 1.0×10^8 CFU of *P. aeruginosa* ATCC 27853 was topically applied onto the wounded cornea. The rat eyes were examined outwardly 24 h post-infection (p.i.) to ensure that all rats were similarly infected. All animals were treated humanely and in accordance with the guidelines of the Association for Research in Vision and Ophthalmology (ARVO) on the usage and treatment experimental animals.

Experimental design

The rats were divided into four groups, each of 10 animals

G1: Normal, healthy animals; G2: Normal rats administered eye drops of truffle aqueous extract**;** G3: Animals infected with *P aeruginosa* ATCC 27853 (5 µl containing 1.0×10^8 CFU); G4: Animals infected with *P aeruginosa* ATCC 27853 and treated with eye drops of truffle aqueous extract.

After 24h of inoculation, the bacterial keratitis was confirmed by eye redness and inflammation, then topical therapy, with eye drops of truffle aqueous extract, was started. Animals from G 2- G4 were treated respectively by instillation of 1% eye drops, two drops were given in each eye, twice a day for one week. One week later, rats of each group were sacrificed under ether anesthesia by inhalation and corneas were collected and washed using the chilled saline solution. The corneas of animals in each group were homogenized in a PBS to yield 10% homogenates. The homogenates were centrifuged for 15 minutes at 10000 g. at 4°C and the supernatants were used for biochemical corneal tissue analysis.

Biochemical corneal tissue analysis:

Lipid peroxidation was estimated by measuring the formed MDA (an end product of fatty acid peroxidation) by using thiobarbituric acid reactive substances (TBARS) method (**30).** This assay is based on the formation of red adduct in an acidic medium between thiobarbituric acid and MDA, the product of lipid peroxidation was measured at 532 nm. MDA concentration was calculated using the extinction coefficient value (ε) of MDA-thiobarbituric acid complex (1.56×10^5 /M/cm). Glutathione peroxidase **(GPx**) activity was quantified using the dithio-binitrobenzoic acid method (**31)**, based on the reaction between the remaining glutathione after the action of GPx and 5,59-dithio bis-(2-nitro benzoic acid) to form a complex that absorbs maximally at 412 nm. C-reactive protein **(CRP)** was measured with latex-enhanced immunonephelometry on a Behring BN II Nephelometer (Dade Behring). In this assay, polystyrene beads coated with rat monoclonal antibodies, bind to CRP that is present in the sample and form aggregates. The intensity of the scattered light is proportional to the size of the aggregates and thus reflects the concentration of CRP that is present in the sample. **The**

concentrations of IL-2 (h-interleukin-2-ELIZA; Roche Diagnostics GmbH, Sandhoferstr, Mannheim, Germany)**,** IFN-γ and **TNF-α** (DuoSet kits; R&D Systems, Minneapolis, MN, USA) were determined using commercially available ELISA assays following the instructions supplied by the manufacturer. For analysis of **8- Hydroxydeoxyguanosine (8-OH-dG)** formation, the DNA was extracted from the nuclear fraction with the DNA Extractor WB Kit (**32).** To the extracted nuclear DNA (~ 100 mg/100 ml of 0.1 mM ethylenediaminetetraacetic acid, EDTA), 1 µl of 2 M sodium acetate, 4 µl of nuclease P1 (5 mg/ml, Yamasa Co., Japan, YA7801) and 2 µl of acid phosphatase (47 mg/ml, suspension in 1.8M $(NH4)_2SO4$, Sigma, P-1435) were added and incubated at 37°C for 30 min. The 8-OH-dG content in the digested DNA was measured by High-performance liquid chromatography with electrochemical detection systems (HPLC-ECD systems), as described previously **(33).**

3. Statistical analysis

Data were analyzed statistically. Values for different treatment groups were compared with the values of individual controls. Results are presented as mean ± SD. Significant differences among values were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni's test post-ANOVA. Probability (P) values<0.05 were taken to indicate a significant difference between mean values.

4. Results

In vitro study

Antimicrobial activity of *T claveryi* extract

Table 1 shows the antibacterial activity of the aqueous extract from the fruiting bodies of *T claveryi* against three strains of *P. aeruginosa* (*P. aeruginosa* ATCC 14028, *P. aeruginosa* ATCC 27853 and *P. aeruginosa* ATCC 9027). From the table, it can be noticed that the *T. claveryi* extract showed a significant inhibitory activity on the three clinical *P. aeruginosa* strains, however *P. aeruginosa* ATCC 27853 was the most susceptible one to the plant extract as confirmed by the maximum inhibition zone around the well in the Petri dishes that contain the growth media (Figure 1)

Scanning Electron Microscope observation

The most sensitive *P aeruginosa* ATCC 27853 strain to the truffle extract was selected to study the potential effect of this extract on the surface morphology of the pathogen cells using SEM (Figure 2). The result showed that exposure of the pathogen cells to the truffle extract resulted in morphological disorganization, including severe alterations of the cell wall with the formation of invaginations or even destruction. Some cells appeared cocci and others appeared as short bacilli (Figure 2a), while the controls untreated cells showed normal morphology (Figure 2b).

***In vivo* study**

Biochemical results

The level of the oxidative tissue damage biomarker, MDA, as an index of membrane lipid peroxidation and the antioxidant enzyme, GPx, in rat corneas of different animal experimental groups are depicted in Figure 3. The results showed that infection of rat corneas with *P. aeruginosa* ATCC 27853 induced oxidative stress as observed by an increased level of MDA with a concomitant decreased in GPx in corneas of infected rats (G3) compared with normal group (G1) ($P \leq 0.001$). Administration of the aqueous extract of *T. claveryi* as eye drops, 24h post infection, markedly ameliorated the alteration in the oxidative stress and the antioxidant bio-markers in infected – treated animals (G4) with respect to infected untreated ones ($P \leq 0.001$).

The levels of inflammatory biomarkers, including, CRP, TNF-α, IL-2 and IFN-γ in rat corneas of different experimental groups are shown in Figure 4. These biomarkers were dramatically elevated in the corneas of infected rats versus normal ones (P□≤□0.001). Administration of truffle eye drops, significantly reduced the elevated levels of these indices in relation to infected untreated animals (P□≤□0.001).

Figure 5 illustrates the level of 8-OHdG as an index of oxidative DNA damage in corneas of different animal groups. The result showed that bacterial infection caused an elevated level of 8-OHdG in corneas of infected rat group versus normal ones (*P*□≤□0.001). Administration of truffle eye drops to infected rats pronouncedly reduced the elevated level of this biomarker compared with infected untreated animals (*P*□≤□0.001). No significant changes were found in the studied parameters on treating the normal rats with the used truffle eye drops (G2) compared with normal untreated group (G1).

Table (1): Effects of *T claveryi* aqueous extract on the growth rate of *P aeruginosa* ATCC 14028, *P. aeruginosa* ATCC 27853 and *P. aeruginosa* ATCC 9027 after 24 h.

Treatment	Inhibition zone (mm)
Control	00.0
Pseudomonas aeruginosa ATCC14028	21.00 ± 0.82*
Pseudomonas aeruginosa ATCC 27853	28. 00 ± 0.60
Pseudomonas aeruginosa ATCC 9027	19.00 ± 0.40*

Values are expressed as mean ± SD of triplicate independent experiments. *P□≤□0.001 compared with *P. aeruginosa* ATCC 27853 using ANOVA followed by Bonferroni as a post-ANOVA test

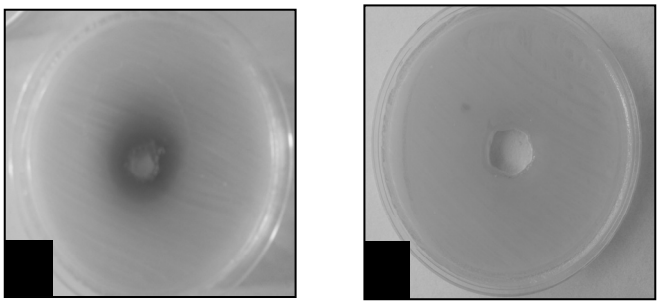


Figure 1: Effect of *T claveryi* aqueous extract on the growth of *P. aeruginosa* ATCC 27853, (a), inhibition zone of *P. aeruginosa* ATCC 27853 growth on Mueller Hinton Agar media and treatment with *T. claveryi* aqueous extract. Fig (b), control untreated *P. aeruginosa* ATCC 27853 with no inhibition zone.

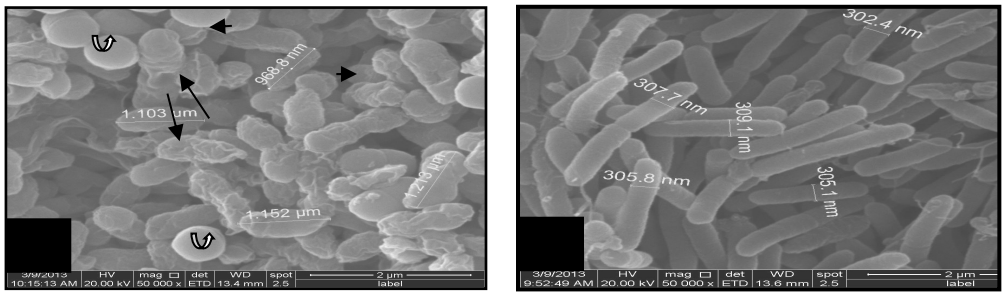


Figure 2: Scanning electron micrographs of *P. aeruginosa* ATCC 27853. (a) Bacterial cells treated with *T. claveryi* aqueous extract at 37°C for 24 h showing abnormal morphology including, membrane invagination (small arrows) or even destruction, some cells appeared cocci (curved arrows) and others appeared as short bacilli (large arrows). (b) Control untreated bacterial cells showing normal morphology.

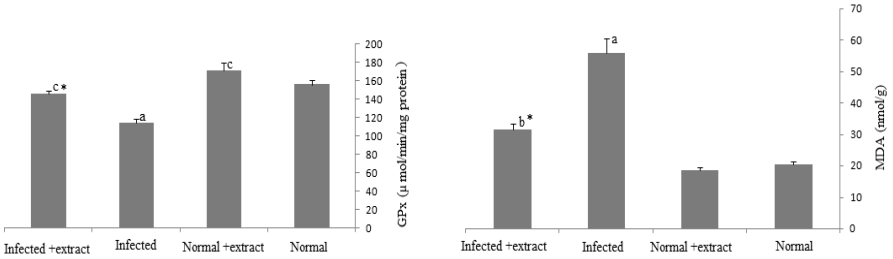
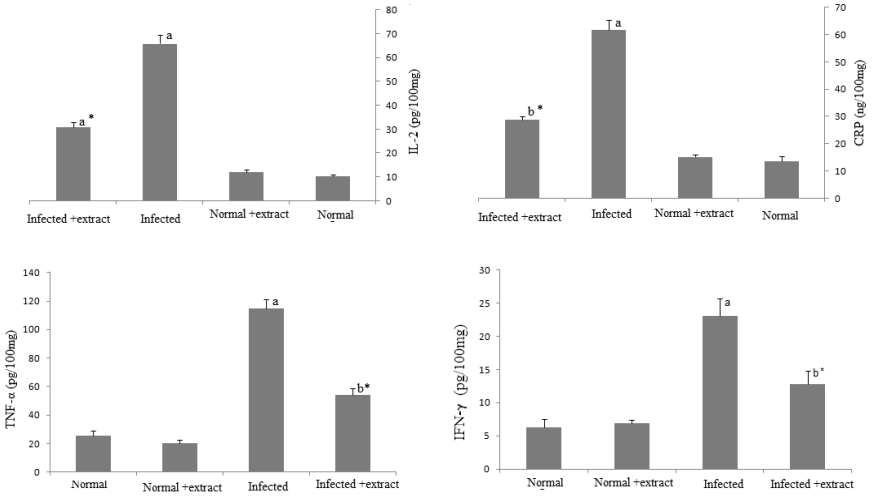


Figure 3: The Effect of *T. claveryi* aqueous extract (as eye drops) treatment on malondialdehyde (MDA) and antioxidant enzyme, GPx in corneas of rats infected with *P. aeruginosa* ATCC 27853. Values are expressed as mean ± SD of 10 rats. $^{a}P \leq 0.001$, $^{b}P \leq 0.01$, $^{c}P \leq 0.05$ compared with the normal group, *P ≤ 0.001, compared with the infected group using ANOVA followed by



Bonferroni as a post-ANOVA test

Figure 4:The Effect of *T. claveryi* aqueous extract (as eye drops) treatment on the inflammatory biomarkers, CRP, TNF-α , IL-2 and IFN-γ in corneas of rats infected with *P. aeruginosa* ATCC 27853. Values are expressed as mean ± SD of 10 rats. $^{a}P \leq 0.001$, $^{b}P \leq 0.01$ compared with the normal group, *P ≤ 0.001, compared with the infected group using ANOVA followed by Bonferroni as a post-ANOVA test

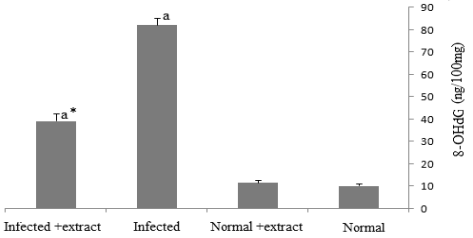


Figure 5: The Effect of *T. claveryi* aqueous extract (as eye drops) treatment on 8-OHdG as a biomarker of oxidative DNA damage in corneas of rats infected with *P aeruginosa* ATCC 27853. Values are expressed as mean ± SD of 10 rats. $^{a}P \leq 0.001$, compared with the normal group, *P ≤ 0.001, compared with the infected group using ANOVA followed by Bonferroni as a post-ANOVA test

5. Discussion

The present study evaluated the antimicrobial activity of the aqueous extract of *T. claveryi* fruiting bodies against three *P. aeruginosa* strains (*P. aeruginosa* ATCC 14028, *P. aeruginosa* ATCC 27853 and *P. aeruginosa* ATCC 9027) based on the formation of the zone of inhibition (ZOI) around the 5 mm well on the Petri dishes filled with the nutrient media and smeared with either of the three pathogen strains. The results showed that the plant extract markedly inhibited the growth of the three tested pathogen strains. The largest ZOI (28 ± 0.6 mm in diameter) was recorded against *P. aeruginosa* ATCC 27853 indicating that this bacterial strain was more sensitive to the plant extract than the other two tested strains. Similar antibacterial activity of *Terfezia* truffle extracts has been reported previously (18, 26-27). Baur (34) stated that the pathogen is resistant if the ZOI is less than 8.00 mm and mediate if greater than 11.00 mm. According to this author, the three tested strains of *P. aeruginosa* were considered sensitive to the aqueous extract of *T. claveryi* fruiting bodies. The scanning electron microscope was performed on the most sensitive bacterial strain (*P. aeruginosa,* ATCC 27853) to determine the major alterations in the microstructure of the bacterial cells under the effect of truffle extract. The results showed that the treatment of this bacterial strain with truffle extract caused morphological changes as shown by retraction or damage of the cell membranes. Some cells showed spherical appearance (cocci), but others appeared as short rods (bacilli) compared to the normal untreated ones which showed normal morphology. This observation may indicate that the used fungal extract kills bacterial cells by destroying their membranes. This result is supported by previous report, which concluded that the cell wall of the bacteria is the target for antimicrobial agents (35). Bacterial keratitis is one of the most visually threatening ocular infectious pathologies because of its potential complications (36). *P. aeruginosa* is a gram-negative pathogen implicated in bacterial keratitis that may lead to corneal damage and perforation (5). The pathogens can induce oxidative stress, inflammatory reactions and tissue injury by promoting both innate and acquired host immune responses (37). The current study revealed that the challenge of rat eyes with *P. aeruginosa* resulted in oxidative damage in corneas as evidenced by an increase in MDA (an index of membrane lipid peroxidation) and a decrease in the antioxidant enzyme GPx. This result is supported by some authors who suggested that the changes in the oxidant-antioxidant status may play an important role in the *P. aeruginosa* -induced tissue injury (38). Previous report stated that the lipopolysaccharide (LPS) elaborated by this organism as well as both innate and acquired host immune responses to infection, are considered as the key factors in tissue damage induced by this pathogen (37). The author demonstrated that the release of host-derived mediators are responsible for the influx of phagocytes to the infected tissue. These phagocytes exert their antimicrobial actions by releasing toxic metabolites, including ROS which can cause cell injury through lipid peroxidation. Administration of *T. claveryi* aqueous extract as eye drops to infected rats, 24h post bacterial infection, effectively decreased the induced corneal MDA and up-modulated the activity of GPx. This result may suggest that the used truffle extract has strong antimicrobial and/or antioxidant potential actions. This suggestion is in line with previous studies illustrated that *T. claveryi* aqueous extract has antimicrobial activity against *P. aeruginosa* and *S. aureus* (18, 24, 27). Some authors reported that the antioxidant capacity of different truffles including *T. claveryi* may be related to the various chemical constituents of the truffles such as vitamin C, carotenoids and many phenolic compounds, which have strong antioxidant potential action with high ability to scavenge peroxy radicals, prevent plasma membrane protrusion and reduce lipid peroxidation (22, 24). Previous investigations revealed that the *P. aeruginosa* LPS is an extremely potent toxin and has a deleterious impact by stimulating inflammatory cells to release biologically active substances, including

proinflammatory cytokines such as TNF-α, IL-6, IL-2, IFN-γ, which have the major role in corneal ulceration during bacterial keratitis (8, 39). In accordance with these investigations, the current study showed that infection of rats with the current pathogen, caused marked production of immune-inflammatory mediators, including CRP, TNF-α, IL-2 and IFN-γ in rat corneas. There is no other published data until present on the effect of bacterial keratitis, on CRP, however previous authors have reported the elevated level of this inflammatory marker in corneal epithelial cells in response to inflammation induced by benzalkonium chloride (40). Administration of *T. claveryi* aqueous extract to *P. aeruginosa* infected rats successfully down-regulated the induced inflammatory biomarkers in eye corneas, indicating the anti-inflammatory potential action of the active components of this extract. This finding may indicate that the constituents of the used truffle extract have a direct antimicrobial potential impact against bacterial infection and / or have immunomodulatory and anti-inflammatory potential actions by their suppressing effect on the activities of immuno-inflammatory cells. There were no previous published data on the anti-inflammatory impact of the used truffle species in relation to the present measured inflammatory biomarkers, however the anti-inflammatory effect of another truffle namely *Elaphomyces granulatus*. (Elaphomycetaceae, Ascomycota) was proved against cyclooxygenase-2 (COX-2) enzyme which has an important role in the inflammatory process (21). The primary outcome of *P. aeruginosa* - induced toxicity in host cells was thought to be apoptosis (41). It was found that the production of inflammatory cytokines and the formation of ROS by bacterial various virulence factors, can induce host cell apoptosis through promoting genotoxicity (42), resulting in tissue damage. The nucleotide pool is an important target of ROS, and guanine is particularly susceptible to oxidation because of its low redox potential (43). 8-OH-dG is the most common form of the oxidative DNA adduct. Measurement of the level of 8-OH-dG is used as a biomarker of oxidative DNA damage (44). The current study showed that infection of rat corneas with *P. aeruginosa* induced oxidative DNA damage as evidenced by elevated level of 8-OHdG in the corneas. Wu et al. (45) suggested that the damaged DNA by *P. aeruginosa* infection may induce cell cycle arrest and ultimately cell death. Intensive cell death leads to tissue damage, organ failure, and eventual death. Administration of *T. claveryi* aqueous extract as eye drops to *P. aeruginosa* infected rats, pronouncedly decreased the level of 8-OHdG in their corneas, indicating the anti-genotoxic effect of the used truffle extract. Similar anti-genotoxic effect was obtained by fresh *Tuber aestivum* black truffle (19). The beneficial effect of the used truffle extract may be related to the antioxidant and anti-inflammatory impacts of its active constituents such as phenolic compounds (24), which have the major role in reducing ROS and inflammatory mediators responsible for oxidative DNA damage. The protective effect of the phenolic compounds on oxidative DNA damage was previously documented (46). Evaluation of the adverse impact of the natural products, accepted as remedies, is important in implementing safety measures for public health. The present work proved that the used aqueous extract of *T. claveryi* as eye drops at a dose of 1%, has no deleterious impacts on rat corneas as observed by non-significant changes in the studied parameters on treating the normal rats with the eye drops of T truffles aqueous extract versus normal untreated group.

6. Conclusion

In conclusion, the present study, demonstrated that aqueous extract of *T. claveryi* fruiting bodies possesses potential antibacterial beneficial impact against the tested strains of *P. aeruginosa.* The used extract also could ameliorate the oxidative stress, inflammatory tissue damage and DNA oxidative damage induced in rat corneas in response to *P. aeruginosa* ATCC 27853 infection. These results may support the use of *T. claveryi* fruiting bodies

aqueous extract as eye drops to treat inflammations and other ailments of external parts of the eye such as the cornea.

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