



Thymoquinone as a Novel Antibiotic and Chemotherapeutic Agent: a Natural Therapeutic Approach on Staphylococcus aureus, Bacillus anthracis, and Four NCI-60 Cancer Cell Lines

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Abstract

Recently, alternative medicine has received more attention in mainstream science as the pharmacology of many ancient medicinal herbs is understood. Various naturally occurring compounds have shown to work effectively as therapeutics including the antimicrobial drug penicillin and paclitaxel, a widely-used chemotherapeutic. Thymoquinone (TQ), a phytochemical compound found in the seeds of the plant *Nigella sativa*, has shown potential as a novel therapeutic agent. The seeds have been used as a folk remedy for various ailments for thousands of years. The activity of TQ was tested on two pathogenic bacteria: *Staphylococcus aureus*, and *Bacillus anthracis*, and four cancer cell lines: Colo205, SK-Mel-28, PC3, and A549. TQ was applied to the bacteria and cancer cells at seven different concentrations and concentration-response curves were generated. The minimum bactericidal concentration was determined by plating of TQ-treated bacterial cultures on LB-agar plates followed by overnight growth. The minimum bactericidal and inhibitory concentration of TQ to *Staphylococcus aureus* was 31 µg/mL (188.8 µM), and the minimum inhibitory concentration of TQ for *Bacillus anthracis* was 3 µg/mL (18.3 µM). The minimum bactericidal concentration of TQ on *B. anthracis* was not determined. TQ promoted the growth of cancer cell lines at low concentrations, but was found to be cytotoxic at concentrations ranging from 50-100 µg/mL (304.5-609.0 µM). While the growth-promoting effects of TQ on cancer cell lines at low concentrations is worrisome for its future development as a chemotherapeutic, TQ deserves further exploration as an effective alternative to customary medicines for chemotherapy and diseases caused by pathogenic bacteria.

Introduction

Nigella sativa, a plant native to Asia, the Middle East, and Africa, has been used for centuries as a natural approach to promote health and fight various diseases¹. The major product from this plant is the seeds, which are used as a spice and food preservative. The major bioactive constituent of this seed is thymoquinone (TQ) (figure 1), a phytochemical compound that has been reported to exhibit antimicrobial effects on Gram-positive and Gram-negative bacteria, though it has shown more activity against Gram-positive bacteria².

One Gram-positive bacterium of concern is *Staphylococcus*

aureus, which causes a variety of pus-forming infections in humans. It causes superficial skin lesions, more serious infections such as pneumonia, meningitis, urinary tract infections, and deep-seated infections³. *S. aureus* causes food poisoning by releasing enterotoxins into food and toxic shock syndrome by release of superantigens into the blood stream. Another Gram-positive bacterium of note is *Bacillus anthracis*, a large, spore-forming bacterium that causes anthrax⁴. Anthrax is a disease of domesticated and wild animals, particularly herbivorous animals, which can be fatal. The bacterium forms endospores that are very long lived in the environment⁵. If spores are ingested, inhaled, or come into contact with a skin lesion on a host, they reactivate and multiply rapidly. Because of the need for new therapeutics in treating these infections, we tested TQ for its growth inhibitory and bactericidal properties against these species of bacteria.

In addition to its effects on bacteria, TQ has shown activity against several cancers^{1,7-12}. Cancer has a high mortality rate in the United States, and various types of treatment include chemotherapy, radiation therapy, and surgery; however, these treatments can have adverse side effects. Chemotherapy causes the immune system to weaken, and makes a patient more susceptible to other infections and diseases⁶. Many forms of chemotherapy also damage healthy cells⁷. Surgery carries serious risks and can have a long recovery time, and radiation therapy can lead to toxic side-effects. TQ has shown anti-proliferative effects on cell lines derived from cancers of the colon, ovary, breast, larynx, lung, myeloblastic leukemia, and osteosarcoma⁸⁻¹³. TQ has shown a degree of selectivity towards cancer cells, since normal cells such as human pancreatic ductal epithelial cells and mouse keratinocytes are resistant to the apoptotic effects of TQ^{14,15}. Taken together, these studies suggest that TQ could be useful in intervening in the inflammatory cascade, which may cause the inhibition of cancer progression and therefore improve a patient's morbidity and mortality rates.

Based on the long tradition of using the seeds of *N. sativa* in alternative medicine, various groups have been working on TQ to study its biological effects. In this work, the efficacy of TQ was tested on pathogenic bacteria and cancer cell lines to see if it would limit their growth in vitro. Based on previous work with TQ, the compound should be able to inhibit the growth of the tested bacteria and cancer cell lines at micromolar concentrations. This study expands on previous work by examining the effectiveness of TQ against additional cell-based models of cancer and bacterial species.



Materials and Methods

Thymoquinone was purchased from Sigma (St. Louis, MO) and dissolved at 100 mg/mL in DMSO. PC3, Colo205, A549 and SK-Mel-28 cancer cells lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and grown in Dulbecco's modified Eagle's Medium (DMEM) supplemented with fetal bovine serum to 10 % volume, 50- μ g/mL gentamicin (all from Life Technologies, Grand Island, NY), and 10-mM HEPES, pH 7.3 (Quality Biological, Gaithersburg, MD) at 37 °C and 5 % CO₂ in a humidified atmosphere. *Staphylococcus aureus* Cowan I was purchased from the ATCC. PC3 (prostate adenocarcinoma), Colo205 (colorectal adenocarcinoma), A549 (lung carcinoma), and SK-Mel-28 (malignant melanoma) cancer cell lines were harvested from T-75 flasks, counted, and plated in sextuplet in 96-well plates at 3 x 10⁴ cells/well in 150 μ L DMEM and allowed to grow overnight. The next day, serially diluted TQ in 50 μ L DMEM was added at seven concentrations ranging from 0 μ g/mL to 250 μ g/mL (0 to 1522 μ M) (final concentration) and the cells were incubated for 48 hours. For the last hour of the experiment, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), was added at 0.5 mg/mL and the media was aspirated following this incubation. The MTT was dissolved in 91 % isopropanol containing 0.038 M hydrochloric acid and 0.5 % SDS. The absorbance of the wells were measured at 470 nm and 590 nm using a SpectraMax Pro 190 spectrophotometer and the difference of absorbances was used to determine viable cell density, which was compared to a no-treatment control to determine percent cell density. Frozen glycerol stocks of *Staphylococcus aureus* Cowan I and *Bacillus anthracis* Ames 33 (cured of pXO1 and pXO2) were streaked on Luria-Bertani (LB) agar plates and grown overnight at 37 °C. The following day, single colonies of each strain were inoculated into 3mL of LB broth and grown overnight in a shaker-incubator at 37 °C and 225 rpm. The next day, LB broth containing serially diluted TQ at concentrations of 1-1000 μ g/mL (6.090-6090 μ M) was prepared and the optical density of the culture at 600 nm (OD₆₀₀) of the overnight culture was measured. To determine the minimum inhibitory concentration, the TQ LB broth was inoculated with *S. aureus* or *B. anthracis* at an OD₆₀₀ of 0.05 and allowed to grow overnight. The following day, the OD₆₀₀ of the TQ-treated cultures was measured and recorded. To determine the minimal bactericidal concentration, 200 μ L of the highest concentration to show growth with TQ and the two lowest concentrations to show no growth were plated on LB agar and grown overnight.

Results

Four cancer cell lines, PC3, SK-Mel-28, A549, and Colo205, were treated with thymoquinone (TQ) to determine the compound's ability to limit their growth. Cancer cell lines treated with low doses of TQ showed greater proliferation than the no-treatment condition; however, at higher concentrations, TQ was able to effectively kill the cells (figure 2). The EC₅₀ for TQ on all four lines was between 50 and 100 μ g/mL (304.5-609.0 μ M). Colo205 showed no enhanced growth at low doses of TQ while the cell densities of the other three lines were 1.5 to 3 times higher than

the no-treatment control.

TQ was tested for its ability to either inhibit the growth of, or kill, two bacterial species, *S. aureus*, and *B. anthracis*. TQ was able to inhibit the growth of both bacteria, but did not show bactericidal activity against *B. anthracis* at the concentrations tested (figures 3 & 4). *S. aureus* had both a minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 31 μ g/mL (188.8 μ M) of TQ. *B. anthracis* had a MIC of 3 μ g/mL (18.3 μ M) TQ.

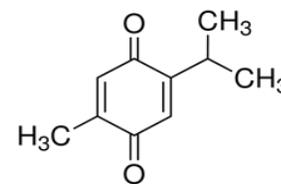


Figure 1. Chemical structure of Thymoquinone.

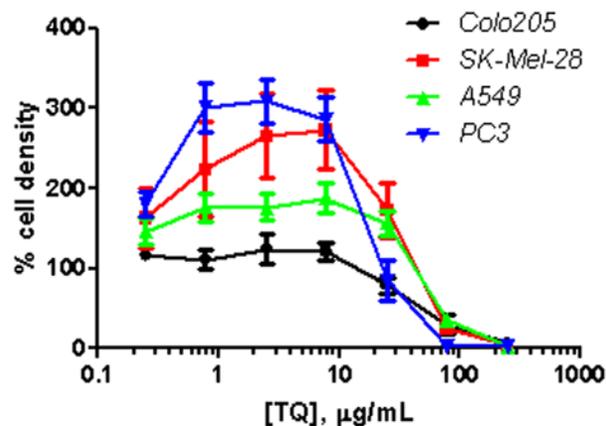


Figure 2. Cell density of Colo205 (circles), SK-Mel-28 (squares), A549 (upwards triangles), and PC3 (downwards triangles) cells treated with TQ. Cell lines were treated for 48 hours with varying concentrations of TQ and viable cell density was measured by staining with MTT. Data are reported as the mean of six replicates compared to a no-treatment control. Error bars are standard error.

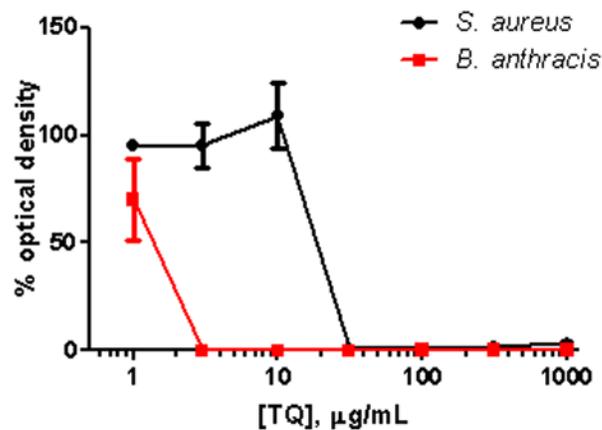


Figure 3. Effect of TQ on growth of *S. aureus* (circles) and *B. anthracis* (squares). The two bacteria were grown overnight in LB broth containing different concentrations of TQ. The optical density is reported at the mean percentage of a no-treatment control and error bars are the standard error of three replicates.

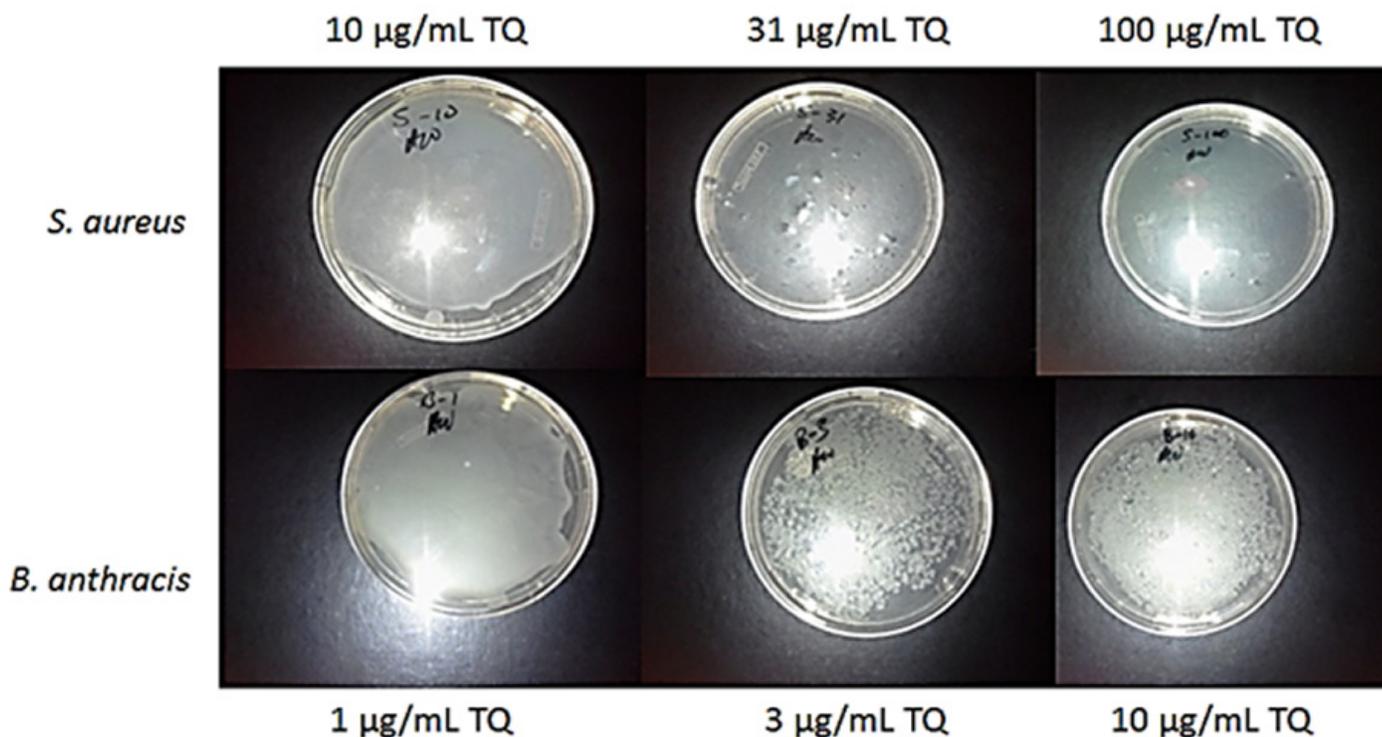


Figure 4. Determination of the MBC of TQ on *S. aureus* and *B. anthracis*. The overnight culture with the highest concentration of TQ to show growth along with the two lowest concentrations to show no growth for each species were plated on LB agar plates and grown overnight to determine if the effect was inhibitory or bactericidal.

Discussion

This work explored the ability of thymoquinone (TQ), a phytochemical compound from the plant *Nigella sativa* to inhibit the growth of bacteria and cancer cell lines. TQ showed growth inhibitory effects against *S. aureus* and *B. anthracis* at concentrations of 31 µg/mL (188.8 µM) and 3 µg/mL (18.3 µM), respectively. TQ was also bactericidal against *S. aureus* at 31 µg/mL (188.8 µM). Halawani found 3 µg/mL (18.3 µM) to be the MIC for *S. aureus* in his study¹⁸. However, they used a different method of treating *S. aureus* with TQ. Halawani did not show TQ's inhibitory effects against *B. anthracis*¹⁸. Perhaps, if specimens of *B. anthracis* treated with higher concentrations were plated, an MBC could have been found. TQ successfully inhibited growth of both the pathogenic bacteria. There are novel applications using TQ against both the *S. aureus* and *B. anthracis* bacteria. The use of TQ against neoplasms also merits further investigation. In our work, TQ was growth-stimulating at low concentrations against cancer cell lines and only started inhibiting growth at concentrations in the 50-100 µg/mL (304.5-609.0 µM) range. Previous work has found that normal cells are resistant to the apoptotic effects of TQ¹⁶. It would be essential to conduct further experiments using our methods against normal cells to conclude that TQ has no cytotoxic effects against normal cells. Such an experiment would further explore TQ as a potential chemotherapeutic. TQ also showed the ability to inhibit the tumor growth and block angiogenesis with almost no toxic side effects¹⁷.

Based on the data in our work, and from the works of others, it can be concluded that the black seeds of the plant *Nigella sativa* (which contains the phytochemical compound thymoquinone) have potential applications in the alternative medicine field and thymoquinone itself, merits further research as a bioactive natural product. Given its anti-proliferative effects against bacteria and cancer cell lines in vitro, it has the potential of being used as a chemotherapeutic or antibiotic. Previous work has found that TQ is much more effective against Gram-positive bacteria than Gram-negative bacteria, and in our work, the compound worked best against *S. aureus*, since it was both growth inhibitory and bactericidal. In the context of alternative medical treatment, TQ can be administered systemically by consumption of the seeds or dishes containing them, or local infection can be treated by compounding a cream or balm containing the seeds. It could also be presumed, based on historical evidence and the results of this work that TQ can act as a preservative in foods containing seeds from *N. sativa*. Development of purified TQ as a pharmaceutical is outside the scope of this work, as the biological characteristics such as serum half-life and bioavailability have not yet been studied, but its use in alternative and complimentary therapy is supported by the results.



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Acknowledgements

This work was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Allergy and Infectious Diseases.