

# Thymoquinone Inhibits Murine Leukemia WEHI-3 Cells *In Vivo* and *In Vitro*

Landa Zeenelabdin Ali Salim, Rozana Othman , Mahmood Ameen Abdulla, Karim Al-Jashamy, Hapipah Mohd Ali, Pouya Hassandarvish, Firouzeh Dehghan, Mohamed Yousif Ibrahim, Fatima Abd Elmutaal Ahmed Omer, Syam Mohan

Published: December 22, 2014 • DOI: 10.1371/journal.pone.0115340

## Abstract

### Background

Thymoquinone is an active ingredient isolated from *Nigella sativa* (Black Seed). This study aimed to evaluate the *in vitro* and *in vivo* anti-leukemic effects of thymoquinone on WEHI-3 cells.

### Methodology/Principal Findings

The cytotoxic effect of thymoquinone was assessed using an MTT assay, while the inhibitory effect of thymoquinone on murine WEHI-3 cell growth was due to the induction of apoptosis, as evidenced by chromatin condensation dye, Hoechst 33342 and acridine orange/propidium iodide fluorescent staining. In addition, Annexin V staining for early apoptosis was performed using flowcytometric analysis. Apoptosis was found to be associated with the cell cycle arrest at the S phase. Expression of Bax, Bcl2 and HSP 70 proteins were observed by western blotting. The effects of thymoquinone on BALB/c mice injected with WEHI-3 cells were indicated by the decrease in the body, spleen and liver weights of the animal, as compared to the control.

### Conclusion

Thymoquinone promoted natural killer cell activities. This compound showed high toxicity against WEHI-3 cell line which was confirmed by an increase of the early apoptosis, followed by up-regulation of the anti-apoptotic protein, Bcl2, and down-regulation of the apoptotic protein, Bax. On the other hand, high reduction of the spleen and liver weight, and significant histopathology study of spleen and liver confirmed that thymoquinone inhibited WEHI-3 growth in the BALB/c mice. Results from this study highlight the potential of thymoquinone to be developed as an anti-leukemic agent.

## Figures

<p><b>Citation:</b> Ali Salim LZ, Othman R, Abdulla MA, Al-Jashamy K, Mohd Ali H, et al. (2014) Thymoquinone Inhibits Murine Leukemia WEHI-3 Cells <i>In Vivo</i> and <i>In Vitro</i>. PLoS ONE 9(12): e115340. doi:10.1371/journal.pone.0115340</p>
<p><b>Editor:</b> Zhengqi Wang, Emory University, United States of America</p>
<p><b>Received:</b> July 17, 2014; <b>Accepted:</b> November 21, 2014; <b>Published:</b> December 22, 2014</p>
<p><b>Copyright:</b> © 2014 Ali Salim et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.</p>
<p><b>Data Availability:</b> The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper.</p>
<p><b>Funding:</b> This research is supported by High Impact Research Grant UM.C/HIR/MOHE/SC/09 (HIR grant F000009-21001) from the Ministry of Higher Education Malaysia, and PPP grant (PG141-2012B) and UMRG grant (RP002/2012B) from the University of Malaya. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.</p>
<p><b>Competing interests:</b> The authors have declared that no competing interests exist.</p>

## Introduction

A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. *Nigella Sativa* is an annual herbaceous flowering plant [1], found in southern Europe, northern Africa, and Asia. It is an amazing herb with a rich historical and religious backgrounds [2]. This plant is cultivated for its seeds and is classified to be fit for human consumption. It is a bushy, self-branching plant with white or pale to dark blue flowers. Many studies have been recently carried out related to the anti-cancer activities of *N. sativa* and some of its active compounds, such as thymoquinone and alpha-hederin [3]. Acute and chronic toxicity studies have recently confirmed the safety of *N. sativa* oil and its most active component, thymoquinone (2-isopropyl-5-methyl-1,4-benzoquinone) [4]. Thymoquinone has a variety of beneficial properties including anti-oxidative and anti-inflammatory activities [5], and has been successfully used in treating allergic diseases in humans [6]. The effects of thymoquinone have been demonstrated in animal models mainly when given orally [7]. Since 1960, thymoquinone had been investigated for its anti-oxidative, anti-inflammatory and anti-cancer activities in both *in vitro* and *in vivo* models [8], [9]. Its anti-oxidative/anti-inflammatory effect has been reported in various disease models, including encephalomyelitis, diabetes, asthma, carcinogenesis and gastric ulcer [10]. Moreover, thymoquinone could act as a free radical and superoxide radical scavenger, as well as preserving the activity of various anti-oxidant enzymes such as catalase, glutathione peroxidase and glutathione-S-transferase. The anti-cancer effects of thymoquinone are mediated through different modes of action, including anti-proliferation, apoptosis induction, cell cycle arrest, reactive oxygen species (ROS) generation and anti-metastasis. In addition, thymoquinone was found to exhibit anti-cancer activity through the modulation of multiple molecular targets, including p53, p73, PTEN, STAT3, PPAR- $\gamma$ , activation of caspases and generation of ROS [11], [12]. The anti-tumor effects of thymoquinone have also been investigated in tumor xenograft mice models for colon, prostate, pancreatic and lung cancers [13], [14].

Cancer is a major public health problem in the United States and many other parts of the world. One in 4 deaths in the United States is due to cancer [15]. It is estimated that the population of cancer survivors will increase to nearly 18 million (8.8 million males and 9.2 million females). Death rates for leukemia, in particular,