

ORAL AND INTRAPERITONEAL LD₅₀ OF THYMOQUINONE, AN ACTIVE PRINCIPLE OF *NIGELLA SATIVA*, IN MICE AND RATS

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Background: Thymoquinone is the major active principle of *Nigella sativa* (*N. sativa*) and constitutes about 30% of its volatile oil or ether extract. *N. sativa* oil and seed are commonly used as a natural remedy for many ailments. Using modern scientific techniques, a number of pharmacological actions of *N. sativa* have been investigated including immunostimulant, anti-inflammatory, anticancer, antioxidant, antihistaminic, antiasthmatic, hypoglycemic, antimicrobial and antiparasitic. There are only few reports regarding the toxicity of thymoquinone. **Methods:** The present study was carried out to determine LD₅₀ of thymoquinone both in mice and rats, orally as well as intraperitoneally, by the method of Miller and Tainter. Autopsy and histopathology of liver, kidney, heart and lungs were also determined. **Results:** The LD₅₀ in mice after intraperitoneal injection was determined to be 104.7 mg/kg (89.7–119.7, 95% confidence interval) and after oral ingestion was 870.9 mg/kg (647.1–1094.8, 95% confidence interval). Whereas, LD₅₀ in rats after intraperitoneal injection was determined to be 57.5 mg/kg (45.6–69.4, 95% confidence intervals) and after oral ingestion was 794.3 mg/kg (469.8–1118.8, 95% confidence intervals). The LD₅₀ values presented here after intraperitoneal injection and oral gavages are 10–15 times and 100–150 times greater than doses of thymoquinone reported for its anti-inflammatory, anti-oxidant and anti-cancer effects. **Conclusion:** Thymoquinone is a relatively safe compound, particularly when given orally to experimental animals.

Keywords: *Nigella sativa*, Thymoquinone, LD₅₀, mice, rats, oral, intraperitoneal

INTRODUCTION

The *Nigella sativa* (*N. sativa*) seeds, commonly called *Habbah Al-Sauda* in the Arabic language, are frequently used in Saudi Arabia and other Middle East countries as a natural remedy for many ailments.¹ Using modern scientific techniques a number of pharmacological actions of *N. sativa* have been investigated including immune stimulant¹, anti-inflammatory²⁻⁴, anticancer^{5,6}, antioxidant⁷⁻⁹, antiasthmatic¹⁰, hypoglycemic¹¹⁻¹³, choleric¹⁴, antimicrobial¹⁵⁻¹⁸ and antiparasitic¹⁹. Some active principles have been isolated from *N. sativa*, including thymoquinone (TQ), hydrothymoquinone, polythymoquinone, nigellidine, nigellimine-N-oxide, thymol, carvacrol and alpha-hedrin.²⁰⁻²⁴

Thymoquinone is the major active principle of *N. sativa* and constitutes about 30% of its volatile oil or ether extract. Many of the pharmacodynamic effects reported above for *N. sativa* are due to thymoquinone.^{2,3,6-9,14,25} There are only few reports in literature on the toxicity of thymoquinone and because of the wide spread use of *N. sativa*, it is necessary to study the toxicity of its constituents in the laboratory animals.

El-Dakhkhani was the first to report LD₅₀ of thymoquinone as 10 mg/kg, when injected intraperitoneally in rats.¹⁴ Later, another study reported that doses of 4, 8, 12.5, 25 and 50 mg/kg intraperitoneally in mice did not alter the biochemical parameters, including serum alanine transaminase, aspartate transaminase and lactate dehydrogenase.²⁵ In the same study the LD₅₀ of thymoquinone was reported to be 90.3 mg/kg (77.9–104.7, 95% confidence intervals) when given intraperitoneally in mice. Similarly, in many other studies

conducted to determine anti-inflammatory, anticancer, antioxidant and cytoprotective effects of thymoquinone the investigators have used doses between 5–12.5 mg/kg, injected intraperitoneally in mice and rats without significant deleterious effects.^{2,3,6-9,14,25} Moreover, much higher LD₅₀ of thymoquinone, i.e., 2.4 g/kg (1.5–3.8, 95% confidence intervals) has been reported after oral administration in mice.²⁶

Differences between El-Dakhkhani's and other studies regarding the toxicity of thymoquinone drew our attention to determine LD₅₀ of thymoquinone both in mice and rats, given orally as well as intraperitoneally, in order to determine whether these variations were due to differences in species and/or the route of administration.

MATERIALS AND METHODS

Thymoquinone was obtained from Aldrich, SIGMA, USA and the olive oil was purchased from the local market (product of Italy and packed for Sasso via Benvenuto Cellini 75, Tavarnelle Val di Pesa (FI), Italy).

Stock solution of thymoquinone (200 mg/ml) was prepared in olive oil. Dilutions of thymoquinone in olive oil were made to permit the administration of increasing doses in suitable volume, up to a maximum of 0.5 ml orally and intraperitoneally in mice and 1.0 ml orally and intraperitoneally in rats.

White albino mice (male and female weighing between 25–50 gram) and albino Wistar rats, male and female weighing between 200–300 gram, were obtained from College of Veterinary Medicine, King Faisal University, Al-Hassa, Saudi Arabia. They were kept in large airy cages in groups of 5 animals per cage with free access to food and water.

An approximate LD₅₀ was initially determined in a pilot study by a so called 'staircase method' using a small number of animals (2 each dose) and increasing doses of thymoquinone. Five doses were then chosen for the determination of oral and intraperitoneal LD₅₀ in both the mice and rats (Tables-1 and 2) and given to five groups of albino mice and rats (10 in each group). One group of mice and another group of rats (6 in each) were given 0.5 ml and 1.0 ml of olive oil orally, respectively (oral controls). Similarly, one group of mice and another group of rats were given 0.5 ml and 1.0 ml olive oil intraperitoneally, respectively (intraperitoneal controls). The animals were observed for first 2 hours and then at 6th and 24th hour for any toxic symptoms. After 24 hours, the number of deceased mice and rats was counted in each group. The percentage of animals that had died at each dose level was transformed to probits²⁷ and then LD₅₀ determined by the method of Miller and Tainter²⁸.

Autopsy was carried out in four mice and rats from the control groups, after sacrificing them, and in the dead mice and rats from the test groups (oral as well as intraperitoneal). Their livers, kidneys, hearts and lungs were preserved in glutaraldehyde to identify any histopathological changes.

RESULTS

The LD₅₀ of thymoquinone in mice was found to be 104.7 mg/kg (89.7–119.7, 95% confidence interval) and 870.9 mg/kg (647.1–1094.8, 95% confidence interval) after intraperitoneal injection oral gavages, respectively. Results of intraperitoneal and oral administration of thymoquinone in mice are given in Tables-1a and 1b, respectively.

The LD₅₀ of thymoquinone in rats was determined to be 57.5 mg/kg (45.6–69.4, 95% confidence intervals) and 794.3 mg/kg (469.8–1118.8, 95% confidence intervals) after intraperitoneal injection oral gavages, respectively. Results for intraperitoneal and oral administration of thymoquinone in rats are given in Tables-2a and 2b, respectively.

In the animals receiving intraperitoneal injection, the abdominal muscle contractions and ataxia was observed, which persisted for few hours. At the 6th hour they were drowsy and less responsive. The severity of these effects was related to the level of dose. However, at 24th hour most of the survivors had recovered from these symptoms. The animals receiving oral thymoquinone gradually became more and more drowsy and dyspnoeic before death or they recovered after 24 hours.

On autopsy, both in the mice and rats receiving lethal doses of thymoquinone, the vital visceral organs (heart, lungs, liver and kidneys) and the peritoneum were found to be congested, without any apparent sign of damage or necrosis. The cause of death was possibly hypotension or shock. In the control animals, the visceral organs and the peritoneum were normal.

Histopathologically, no significant difference could be noted between the test and the control animals.

Table-1: Results of the lethal doses of thymoquinone for the determination of the LD₅₀ after oral ingestion and intraperitoneal injection in mice (n=10)

a. Intraperitoneal injection in mice

Group No.	Dose (mg/kg)	Log dose	% Deaths	*Corrected %	Probits
1	50	1.7	0	2.5	3.04
2	75	1.88	20	20	4.16
3	100	2	50	50	5
4	125	2.1	70	70	5.52
5	150	2.18	90	90	6.28

b. Oral ingestion in mice

Group No.	Dose (mg/kg)	Log dose	% Dead	*Corrected %	Probits
1	250	2.4	0	2.5	3.04
2	500	2.7	30	30	4.48
3	1000	3	50	50	5
4	1500	3.18	80	80	5.84
5	2000	3.3	90	90	6.28

*Corrected % Formula: For 0% dead: $100(0.25/n)^{27}$

Table-2: Results of the lethal doses of thymoquinone for the determination of LD₅₀ after oral ingestion and intraperitoneal injection in rats (n=10)

a. Intraperitoneal injection in rats

Group No.	Dose (mg/kg)	Log dose	% Dead	*Corrected %	Probits
1	25	1.4	0	2.5	3.04
2	50	1.7	40	40	4.75
3	75	1.88	70	70	5.52
4	100	2	90	90	6.28
5	150	2.18	100	100	6.96

b. Oral ingestion in rats

Group No.	Dose (mg/kg)	Log dose	% Dead	*Corrected %	Probits
1	100	2	0	2.5	3.04
2	500	2.7	10	10	3.72
3	1000	3	50	50	5
4	1500	3.18	70	70	5.52
5	2000	3.3	100	97.5	6.96

*Corrected % Formula: For 0 and 100,²⁷ For 0% dead: $100(0.25/n)$
For 100% dead: $100(n-0.25/n)$

DISCUSSION

The aim of the present study was to determine the LD₅₀ of thymoquinone given intraperitoneally and orally in mice and rats, because of wide differences in the reported results from 10 mg/kg intraperitoneally in rats¹⁴ to 2.4 g/kg orally in mice²⁶.

LD₅₀ of thymoquinone after intraperitoneal injection in mice determined in the present study was very close to another similar study, reporting 90.3 mg/kg (77.9–104.7, 95% confidence intervals).²⁵ However, in comparison with our results much higher LD₅₀, i.e., 2.4 g/kg (1.5–3.8, 95% confidence intervals) has been reported in literature after thymoquinone was given orally to mice.²⁶ This difference might be due to the differences in the vehicle and the method used for

the estimation of LD₅₀. Because thymoquinone is insoluble in water, it was dissolved in volatile oil and LD₅₀ estimated by the method of Miller and Tainter in our study, while it was mixed in corn oil and the method of Lichenfield and Wilcoxon was used in the other study.²⁶

In rats, after intraperitoneal injection, much lower LD₅₀ of thymoquinone (10 mg/kg) have been reported by El-Dakhkhany *et al*¹⁴ as compared to our results. They dissolved thymoquinone in propylene glycol and used Gaddam's method for the determination of LD₅₀. We could not find any reference in literature for the comparison of our results of LD₅₀ of thymoquinone after oral gavages in rats.

Our LD₅₀ values after intraperitoneal injection are 10–15 times higher and after oral ingestion 100–150 times greater (both in the mice and rats) than the doses of thymoquinone used in various other studies that demonstrate its anti-inflammatory, anti-cancer, anti-oxidant and cytoprotective effects,^{2,3,6-9,14,25} and show the safety of the use of this compound in the experimental animals.

CONCLUSION

The LD₅₀ of thymoquinone when given orally or intraperitoneally to rats was a bit lower than the value determined for mice. However, the LD₅₀ of thymoquinone given orally to both the mice and rats was ten times greater than when given intraperitoneally. Our results present thymoquinone as a relatively safe compound in the experimental animals, particularly when given orally.

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