Supplementary material

1-Preparation of *Nigella Sativa* extract:

1.1 Materials:

- *Nigella sativa*, L. seed extract; NAWAH Scientific, Cairo, Egypt
- Microcrystalline cellulose (Avicel PH 101), FMC Corporation, Philadelphia, USA
- Silicon dioxide (Aerosil 200), Davison Chemical Division, Baltimore
- Carbopol 934P (CP); Goodrich Chemical Company, Ohio, USA
- N-hexane (HPLCgrade); Leda, Schar-lau chemie (European Union)

1.2 Method:

- The *Nigella Sativa*, L. (Ranunculaceae), seed oil extract was prepared by NAWAH Scientific, Cairo, Egypt. Briefly, the *Nigella Sativa*, L. seed oil was extracted by n-hexane. The seeds were crushed and the ratio of the seeds: solvent was 9:1. The solvent was removed by lyophilisation by Lyophilizer (freeze drier); Novalyphe-NL 500, Savant, Holbrook, NY, at −45°C under a vacuum of 7×10^{-2} mBAR Ma et al., (2019).

2-High Performance Liquid Chromatography (HPLC):

HPLC Quantification of Thymoquinone in *Nigella Sativa* extract.

Analysis Report

HPLC analysis

a) Device specification:

Waters 2690 Alliance HPLC system figure equipped with a Waters 996 photodiode array detector.

b) Standard preparation:

Thymoquinone 98% was sourced from Sigma-Aldrich, Germany, and stock solution of 1 mg/mL in Ethanol 6serial dilutions were prepared in concentrations of 10 µg/mL, 250 µg/mL, 50 µg/mL, 100 µg/mL, 150 µg/mL, and 200 µg/mL. Each of the dilutions was filtered using 0.22 µm syringe filter then 10 µL was injected.

c) Sample preparation:

0.767 g of seed oil in 5ml ethanol and filtered using 0.22 µm syringe filter then 10 µL was injected.

d) HPLC analysis conditions:

• Column C18 Xterra: 4.6 × 100 mm, 5 µm

• Mobile phase: 20mMPhosphate Buffer (PH2.6): Acetonitrile

• Mode of elution: Gradient

• Flow rate: 1.3 mL/min

• Temperature: Ambient

• Wavelength: 254 nm

Results:

Concentration of thymoquinone is 0.702 mg/g of seed oil

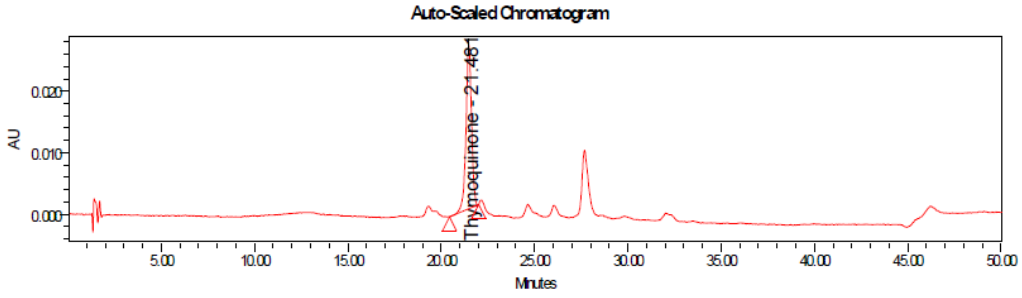

Figure (1): Auto-scaled chromatogram of thymoquinone

3-Preparation of buccal tablets:

3.1 Equipment:

- Electric balance (AND Co, Ltd, Japan)
- Tablet single punch press machine (Royal Artist, Bombay, India)
- Tablet hardness tester (Copley, UK)
- Tablet micrometer (Locally manufactured)

3.2 Method:

The objective was to prepare *Nigella Sativa* buccal tablets having good mucoadhesion force, sustain the mucoadhesion with the mucosa for 8 hours (the release period) and having maximum release extent after 8 hours with a suitable release rate.

The buccal tablets were prepared by firstly mixing different amounts (250 and 500 mg) of *Nigella Sativa* extract with different amounts of Avicel to form a wet mass. Aerosil was added to the wet mass to coat it and transfer it to dry powder and mixed for 2 minutes, followed by Carbopol in different amounts and the powder was mixed for 2 minutes. The ratios between Avicel: Aerosil and between Avicel: Carbopol were kept constant for all tablets at the level of 10:1. The buccal tablets were directly compressed using a single-punch tablet machine equipped with 11 mm (for 250 mg extract) and 13 mm (for placebo and 500 mg extract) round punches. The force of compression was adjusted so that hardness of all the prepared tablets ranged from 4–8 kg.

Formula	Avicel (mg)	Aerosil (mg)	Carbopol (mg)	*Nigella Sativa* extract (mg)	Total tablet weight (mg)
F1	500	50	50	250	600
F2	750	75	75	500	900
F3 (placebo)	750	75	75	0	900

The study used the dose by factor method to estimate the human equivalent dose (HED) of a drug, based on the no observed adverse effect levels (NOAEL) from preclinical animal studies. This method applies a body surface area exponent of 0.67 to account for metabolic differences between species, with the HED calculated using the equation:

$$HED(mg/kg) = \text{Animal NOAEL (mg/kg)} \times \left(\frac{\text{Weight of animal (kg)}}{\text{Weight of human (kg)}}\right)^{(1-0.67)}$$

Using the NOAEL of 10 mg/kg/day for mice, as reported by Ong et al. (2016), and the weight values from Nair & Jacob (2016), the HED for a 60 kg human was calculated to be 0.712 mg/kg. For a 60 kg human, this translates to a total dose of 42.72 mg. This value

was then divided by a safety factor of 10, resulting in an initial dose of 4.272 mg for human trials.

For the thymoquinone dose in tablets, a 10 mg/kg tablet contains 0.351 mg of thymoquinone, meaning that 4 tablets of NS provide 1.4 mg. A 5 mg/kg tablet contains 0.175 mg of thymoquinone, with 4 tablets providing 0.70 mg.

References

Ma, C., Liu, C., Ahmed, A. F., Niu, Y., & Kang, W. (2019). Optimum extraction technology for the seed oil of Nigella sativa L. J. Food Qual. https://doi.org/10.1155/2019/2592731

Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm. 2016 Mar;7(2):27–31. doi: 10.4103/0976-0105.177703.

Ong YS, Saiful Yazan L, Ng WK, Noordin MM, Sapuan S, Foo JB, Tor YS. Acute and subacute toxicity profiles of thymoquinone-loaded nanostructured lipid carrier in BALB/c mice. Int J Nanomedicine. 2016 Nov 9;11:5905–5915. doi: 10.2147/IJN.S114205.