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Short communication

Evaluation of cytotoxic activity of *Nigella sativa* using Brine Shrimp Lethality Assay

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Introduction

Medicinal plants and herbal products are widely investigated due to their potential pharmacological and biological activities (Ahmad et al., 2013). *Nigella sativa* L. (Ranunculaceae), commonly known as black seed or black cumin, is an annual flowering plant native to Southwest Asia and widely cultivated in the Mediterranean region, the Middle East, and North Africa. The seeds of *N. sativa* have been extensively used in traditional medicine for centuries for the management of various disorders, including respiratory, gastrointestinal, metabolic, and inflammatory conditions (Ahmad et al., 2013). The pharmacological potential of *N. sativa* is largely attributed to its diverse phytochemical composition, including thymoquinone, thymohydroquinone and dithymoquinone as well as alkaloids, flavonoids, phenolic compounds, and essential fatty acids. Previous studies have reported antioxidant, antimicrobial, anti-inflammatory, immunemodulatory, and anti-cancer properties associated with *N. sativa* seeds and seed oil (Ahmad et al., 2013).

The Brine Shrimp Lethality Assay (BSLA) is a simple, rapid, and cost-effective preliminary bioassay commonly used for screening the cytotoxicity of natural products and herbal extracts (Meyer et al., 1982). The assay is based on the lethality of *Artemia salina* larvae exposed to different concentrations of tested samples and may provide useful information regarding their cytotoxic activity.

The aim of this study was to evaluate the cytotoxic activity of *N. sativa* oil using BSLA and to determine LC_{50} values after different exposure periods.

Materials and methods

The stock solution of commercial *Nigella sativa* oil was prepared by dissolving 500 mg (550 μL) of the oil in dimethyl sulfoxide (DMSO) to obtain a final concentration of 10 mg/mL. The resulting solution was subjected to ultrasonic treatment in a bath operating at 50/60 Hz and 720 W for 15 minutes to ensure proper homogenization, followed by filtration.

The cytotoxic activity was assessed using the Brine Shrimp Lethality Assay (BSLA). Artemia salina larvae were cultured in artificial seawater consisting of a sodium chloride solution (38 mg/mL), maintained at pH 9, with continuous

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aeration and an incubation temperature ranging from 25 to 28 °C. Test concentrations (5, 3, 1, 0.5, 0.1, and 0.01 mg/mL) were prepared by serial dilution of the stock solution using sodium chloride solution as the diluent. Potassium dichromate ($K_2Cr_2O_7$) served as the positive control, while sodium chloride solution was used as the negative control, both prepared under identical experimental conditions.

Larval mortality was recorded after 1, 2, 4, 6, and 24 hours of exposure. The percentage of mortality was calculated for each concentration, and LC_{50} values were estimated using probit regression analysis. Toxicity classification was performed according to the Meyer and Clarkson toxicity criteria (Clarkson et al., 2004).

Results and discussion

The extract demonstrated a clear time-dependent increase in cytotoxicity, reflected by a progressive reduction in LC_{50} values with longer exposure. At 1 hour, the LC_{50} was 693.38 µg/mL. This value decreased substantially to 353.96 µg/mL at 2 hours, 180.56 µg/mL at 4 hours, and 127.87 µg/mL at 6 hours. The strongest cytotoxic effect was observed after 24 hours, where the LC_{50} reached 27.76 µg/mL.

Based on Meyer's toxicity criteria, which consider extracts with LC_{50} values below 1000 µg/mL as toxic, the *N. sativa* extract was classified as toxic at all exposure times. However, Clarkson's scale provided a more detailed interpretation of toxicity progression. According to this classification, the extract showed low toxicity at 1 hour (500–1000 µg/mL), moderate toxicity during the 2-, 4-, and 6-hour intervals (100–500 µg/mL), and high toxicity at 24 hours (below 100 µg/mL).

The results of this study indicate that the cytotoxic effect of the *Nigella sativa* extract is strongly dependent on exposure time. The marked decrease in LC_{50} from 693.38 µg/mL at 1 hour to 27.76 µg/mL at 24 hours suggests that prolonged exposure enhances the activity of the bioactive compounds against *Artemia salina* larvae. This pattern is consistent with previous studies in which extended exposure periods allow greater interaction between phytochemicals and biological targets, leading to increased toxicity. The pronounced cytotoxicity observed at 24 hours (LC_{50} = 27.76 µg/mL) indicates a high level of biological activity.

The strong cytotoxicity observed at 24 hours is likely associated with key phytochemical constituents of *N. sativa*, particularly thymoquinone, along with other bioactive compounds such as terpenes, flavonoids, and alkaloids. Thymoquinone is well known for its antiproliferative, pro-apoptotic, and cytotoxic effects in both invertebrate models and mammalian cancer cell systems (Ahmad et al., 2013). Overall, the shift of the extract into the "high toxicity" category under Clarkson's classification underscores its notable biological activity. These findings support further investigation involving isolation and characterization of the active compounds, as well as additional testing in mammalian cancer models to evaluate their potential therapeutic applications as anticancer agents.

Conclusion

Nigella sativa extract showed pronounced time-dependent cytotoxic activity, evidenced by decreasing LC_{50} values with longer exposure, indicating the presence of bioactive compounds. These findings highlight the potential pharmacological relevance of *Nigella sativa*, particularly in relation to its cytotoxic and bioactive properties. However, further comprehensive pharmacological and toxicological investigations are required to more precisely characterize its active constituents, evaluate its mechanism of action, and assess its safety profile, as well as to explore its possible therapeutic applications.

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