Macedonian Pharmaceutical Bulletin, 72 (Suppl 1) 651 - 652 (2026)
Online ISSN: 1857-8969
DOI: 10.33320/maced.pharm.bull.2026.72.03.313
Short communication

Assessing the cytotoxicity of *Cetraria islandica* via Brine Shrimp Bioassay

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Introduction

Natural products and medicinal plants represent an important source of biologically active compounds with potential pharmacological applications. In recent years, increasing scientific attention has been focused on the evaluation of herbal extracts for their therapeutic effects. *Cetraria islandica* (Lichen islandicus) commonly known as Iceland moss is traditionally used in herbal medicine due to its high content of polysaccharides, phenolic compounds, and lichen acids associated with various therapeutic effects, including antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory activities (Molnár and Farkas, 2010). Despite its widespread traditional use, limited data are available regarding its cytotoxic activity and biological safety profile.

The *Brine Shrimp Lethality Assay* (BSLA) is a rapid, inexpensive, and reliable preliminary bioassay commonly used for screening the cytotoxicity of natural products and plant extracts. The assay is based on the lethality of *Artemia salina* larvae after exposure to different concentrations of tested samples and is considered a useful predictor of general biological activity (Meyer et al., 1982; Sleet & Brendel, 1983).

The aim of this study was to evaluate the cytotoxic activity of *Lichen islandicus* ethanolic extract using BSLA and to determine the LC_{50} values after different exposure periods.

Materials and methods

Plant material and preparation of the extract

Dried and pulverized *Lichen islandicus* plant material (Alkaloid, Skopje) was used for extract preparation. Extraction was performed using 0.5 g of plant material with 50 mL of 50% ethanol (v/v). The obtained mixture was treated in an ultrasonic bath operating at a frequency of 50/60 Hz and power of 720 W for 15 minutes. After sonication, the extract was filtered through filter paper and adjusted to a final volume of 50 mL using 50% ethanol, obtaining a stock solution with a final concentration of 10 mg/mL.

Brine shrimp lethality assay (BLSA)

The cytotoxic activity of the prepared extract was investigated using the Brine Shrimp Lethality Assay (Meyer et al., 1982), a widely used preliminary

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method for screening bioactive compounds. *Artemia salina* larvae were cultured in artificial seawater containing sodium chloride (38 mg/mL), maintained at pH 9, under continuous aeration and controlled temperature conditions of 25-28 °C (Sleet and Brendel, 1983).

A series of test concentrations (5, 3, 1, 0.5, 0.1, and 0.01 mg/mL) was prepared by serial dilution of the stock solution using sodium chloride solution as the solvent. Each experimental unit consisted of 5 mL of test solution and 10mL *Artemia salina* larvae. Positive control ($K_2Cr_2O_7$) and negative control (sodium chloride solution) were included and processed under identical conditions to ensure experimental validity. Larval mortality was recorded after 1, 2, 4, 6, and 24 hours of exposure, and percentage mortality was calculated for each concentration. LC_{50} values were determined through probit regression analysis. Toxicity classification was performed according to Meyer's and Clarkson's toxicity scales (Meyer et al., 1982; Clarkson et al., 2004).

Results and discussion

The obtained results demonstrated time-dependent cytotoxic activity of the *Lichen islandicus* extract. After 1 hour of exposure, the extract exhibited an LC_{50} value of 1045.58 µg/mL and was classified as non-toxic according to both Meyer and Clarkson toxicity scales (Meyer et al., 1982; Clarkson et al., 2004). However, prolonged exposure resulted in a progressive decrease of LC_{50} values, indicating increased cytotoxicity.

After 2 hours, the LC_{50} value decreased to 666.80 µg/mL, classifying the extract as toxic according to Meyer scale and low toxic according to Clarkson scale (Meyer et al., 1982; Clarkson et al., 2004). At 4 and 6 hours of exposure, identical LC_{50} values of 471.30 µg/mL were obtained, corresponding to moderate toxicity according to Clarkson classification (Clarkson et al., 2004). The highest cytotoxic effect was observed after 24 hours, with an LC_{50} value of 325.02 µg/mL, indicating moderate toxicity.

The results demonstrate a time-dependent increase in cytotoxic activity of *Cetraria islandica* extract, as evidenced by a progressive decrease in LC_{50} values with prolonged exposure. Similar biological activity has been reported in previous studies, where *C. islandica* extracts exhibited moderate to strong antiproliferative effects *in vitro* (Meyer et al., 1982; Moshi et al., 2004). This activity is primarily associated with lichen secondary metabolites, including fumarprotocetraric acid, protolichesterinic acid, and usnic acid, which have been widely reported to possess cytotoxic and other pharmacological properties (Molnár and Farkas, 2010). These compounds are considered the main contributors to the observed bioactivity of lichen extracts.

Conclusion

The ethanolic extract of *Cetraria islandica* exhibited time-dependent cytotoxic activity in the *Brine Shrimp Lethality Assay*. A progressive decrease in LC_{50} values was observed with increasing exposure time, indicating enhanced cytotoxic effect over time. Further pharmacological and toxicological studies are required to better evaluate the biological activity and safety profile of this medicinal species.

References

Clarkson, C. et al., 2004. Toxicity classification of medicinal plant extracts. J. Ethnopharmacol. 94(1–2), 205–213. https://doi.org/10.1016/j.jep.2004.04.010

Meyer, B.N. et al., 1982. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 45(5), 31–34. https://doi.org/10.1055/s-2007-971236

Molnár, K., Farkas, E., 2010. Current results on biological activities of lichen secondary metabolites: a review. Z. Naturforsch. C 65(3–4), 157–173. https://doi.org/10.1515/znc-2010-3-401

Moshi, M.J. et al., 2004. Brine shrimp lethality of medicinal plants. Pharm. Biol. 42(8), 682–685. https://doi.org/10.1080/13880200490893649

Sleet, R.B., Brendel, K., 1983. Artemia salina methodology for toxicity testing. Aquaculture 35(1–4), 103–110. https://doi.org/10.1016/0044-8486(83)90106-6