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Short communication

Time-dependent cytotoxicity assessment of *Plantago major* extract in the Brine Shrimp model

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Introduction

Plantago major, commonly known as broad leaf plantain, is a plant in the Plantaginaceae family widely used for its beneficial healing properties such as antimicrobial, antidiabetic, antispasmodic and many other effects (Zhakipbekov et al., 2023). The health significance of *Plantago major* lies in the phytochemical components of the herb. In order to ensure human safety, the plant must be examined through *in vivo* studies under distinct circumstances (time, concentration, pH factor, extract etc.) (Zdralovic et al., 2019). The *Artemia salina* (brine shrimp) lethality bioassay is a strongly supported in the literature *in vivo* method used to determine the acute toxicity of plant-derived compounds (Rahamooz-Haghighi et al., 2021). Toxicity is traditionally classified using criteria set by Meyer and Clarkson, which categorize extracts based on their lethal concentration values (LC_{50}) (Clarkson et al., 2004; Meyer, et al., 1982).

Material and methods

Sample preparation

The extraction was prepared by using 0.5 g of dried and pulverized *Plantago major* plant material (Alkaloid, Skopje), which were extracted with 50 mL 50% ethanol (v/v). The resulting ethanolic extract was placed in an ultrasonic bath operating at a frequency of 50/60 Hz and a power of 720 W for a time of 15 minutes. Afterwards the extract was filtered through filter paper, and the filtrate was filled to volume of 50 mL with 50% ethanol to obtain a final concentration of 10 mg/mL.

Brine shrimp lethality assay (BSLA)

The cytotoxic potential of *Plantago major* extract was evaluated using the *Brine Shrimp Lethality Assay* (BSLA). *Artemia salina* larvae were cultured in artificial seawater prepared by dissolving sodium chloride (NaCl) in water at a concentration of 38 mg/mL, adjusted to pH 9, under continuous aeration and a stable temperature between 25°C and 28°C. From the prepared stock solution with a concentration of 10 mg/mL, working test solutions were diluted using sodium chloride as the solvent throughout the procedure. A concentration range of 5, 3, 1, 0.5, 0.1, and 0.01 mg/mL was prepared for the bioassay. Each analysis contained 5 mL of solution with the appropriate concentration and 10 shrimp. Concurrently, a positive control using potassium dichromate and a negative control using

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sodium chloride solvent were prepared and evaluated across the same concentration range.

Determination of toxicity

The mortality rates of the larvae were recorded at 1, 2, 4, 6, and 24 hours after exposure. The percentage mortality for each concentration was calculated based on the number of dead shrimp relative to the initial population. The final lethal concentration values were calculated using probit regression analysis. The calculated LC_{50} values were interpreted according to the Meyer (Meyer et al., 1982) and Clarkson toxicity scales (Clarkson et al., 2004). According to both standards, samples are classified as toxic if the LC_{50} value is less than 1000 µg/mL. The Clarkson scale further categorizes extracts as high (0–100 µg/mL), moderate (100–500 µg/mL), or low toxicity (500–1000 µg/mL) (Clarkson et al., 2004).

Results and discussion

The toxicological profiles obtained from the *Artemia salina* bioassay revealed a distinct, time-dependent increase in the lethality of the *Plantago major* extract across the 24-hour testing period. After 1 hour of exposure, the calculated LC_{50} value was found to be 1761.20 µg/mL. According to both the Meyer and Clarkson evaluation models, the extract was completely non-toxic at this stage. This baseline profile was maintained at the 2-hour interval, where the extract remained non-toxic with an LC_{50} value of 1244.08 µg/mL. At the 4-hour mark, the LC_{50} value decreased to 835.89 µg/mL. Under the Meyer criteria, this value is considered toxic, while according to the Clarkson scale, it is classified as low toxic. A similar toxicological trend was maintained at the 6-hour exposure interval, which yielded a calculated LC_{50} value of 756.53 µg/mL. This value denotes continued toxicity and low toxicity according to the Meyer and the Clarkson model correspondingly. The maximum lethal effect of the extract was recorded at the final 24-hour observation endpoint. The LC_{50} value dropped significantly to 185.75 µg/mL. At this concentration, the *Plantago major* extract is categorized as toxic under the Meyer criteria and advances to a moderately toxic classification (100–500 µg/ml) on the Clarkson scale (Meyer, et al.1982; Clarkson et al., 2004). This pronounced decrease in LC_{50} values over time emphasizes that the bioactive components within the *P. major* extract require an extended exposure window to exert their full cytotoxic potential on *Artemia salina*. The present findings partially agree with previous studies on *P. major* toxicity. Rahamooz-Haghighi et al., reported no detectable toxicity of *P. major* extracts toward *Artemia salina* at concentrations up to 1000 µg/mL after 24 h, whereas the current study yielded a lower LC_{50} value of 185.75 µg/mL. These differences may be attributed to variations in extraction methods, plant material, and phytochemical composition (Rahamooz-Haghighi et al., 2021).

Conclusion

Plantago major extract demonstrates a time-dependent toxicological profile against *Artemia salina*. The results provide basic toxicological data suggesting that while it is safe at acute intervals, its concentration and exposure duration must be carefully managed in biological applications.

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