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Evaluation of the rodenticidal effect of *Conium maculatum* plant extract under laboratory conditions

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Abstract

Background: Rodent control increasingly demands environmentally safe alternatives to conventional chemical rodenticides. *Conium maculatum* L., a plant known for its potent alkaloid content, has been proposed as a potential source of natural rodenticides. This study aimed to evaluate the behavioural and toxicological effects of ethanol and aqueous extracts of *C. maculatum* on free-living house mice (*Mus musculus*) under controlled laboratory conditions. The assessment focused on clinical signs, changes in motor activity, and lethality dynamics to determine extract efficacy and onset of toxicity.

Results: Mice fed with diets containing 95% (v/v) ethanol extract exhibited delayed but sustained toxicity, with lethality observed within 48 h. In contrast, diets formulated with the aqueous extract induced rapid and acute effects: clinical symptoms appeared within 6 h, and deaths occurred within 24 h. Both extracts triggered pronounced neurotoxic manifestations, including reduced locomotion, tremors, and immobility. Comparative analysis indicated that the extraction method substantially influences the onset, intensity, and progression of toxic effects. No sub-lethal recovery was observed in ethanol-treated mice, whereas some transient behavioural disturbances were noted in the aqueous extract group prior to mortality.

Conclusion: The findings demonstrate that *C. maculatum* extracts exert potent neurotoxic and lethal effects on *M. musculus*, with the extraction method critically modulating toxicity patterns. These results support the potential use of plant-derived toxins as environmentally friendly alternatives to chemical rodenticides. Further studies are warranted to optimize dosage, assess long-term ecological safety, and explore application strategies for sustainable rodent management.

Keywords: *Mus musculus*; *Conium maculatum*; ethanolic extract; aqueous extract; toxic effects.

1. Background

Of the four species of the genus *Conium* L. (*Apiaceae* Lindl.) distributed in the Caucasus, only one species is known from Azerbaijan: *Conium maculatum* L. (spotted hemlock) (Əsgərov, 2006). This species is a biennial plant, 100–200 cm in height, with a somewhat lignified stem covered with fine hairs and marked by characteristic purple spots. During the first year it forms a basal rosette of leaves; in the second year, a thickened, rigid, non-hollow stem develops. The leaves are triangular, compound, and 2–4 times pinnately divided. The leaves on the lower part of the stem are larger and petiolate (50–60 cm). The middle and upper leaves are smaller than the basal leaves and also differ in the shape of their terminal segments. The flowers are white, very

small, and arranged in compound umbels. Umbels bear 10–20 rays; flowering occurs in June - July, and fruiting in July – August. The fruit is a small ovoid schizocarp, 3.0–3.5 mm long, consisting of two mericarps (i.e., two-seeded). The chemical constituents of *C. maculatum* are predominantly basic compounds. Such substances are referred to as alkaloids and are considered plant-derived secondary metabolites. They are nitrogen-containing organic (often heterocyclic) compounds that can form corresponding salts upon reaction with plant-derived acids. The basic properties of these compounds arise from the presence of nitrogen in their molecular structure. Accordingly, alkaloids in their free-base form are generally readily soluble in organic solvents, whereas in aqueous media they often occur as water-soluble salts.

Throughout their life cycle, many plant species produce highly toxic compounds that protect them from being consumed by animals. Such plants have historically been used by humans for various purposes and continue to be utilised. In folk medicine, *C. maculatum* has been used against breast tumours. The plant has an unpleasant, pungent odour, which is attributed to its volatile alkaloids. These compounds are volatile liquids in their pure form, possess a strong smell, and are classified as oxygen-free alkaloids. When chopped plant material is dried, the odour becomes even more pronounced because volatilisation of alkaloids accelerates in open containers; under warm conditions, this process may take 10–15 days.

Coniine and N-methylconiine obtained from *C. maculatum* are oxygen-free alkaloids and, in their pure form, are volatile liquids. By contrast, alkaloids isolated from the plant in crystalline or amorphous form are indicative of oxygenated structures and are typically odourless bioactive compounds. At present, *C. maculatum*-derived preparations have been reported for use in haemophilia and in the treatment of malignant tumours (Qasimov et al., 2006).

One of the major drivers behind the development of certain synthetic drugs has been the investigation of structure–activity relationships of pharmacologically active alkaloids. The extract of *C. maculatum* and its alkaloid fractions exhibit notable pharmacological activity at low doses; however, at higher doses (above 20 mg/kg) they have been reported to exert toxic and teratogenic effects (Mansoor et al., 2018). The anticancer activity of *C. maculatum* extract was investigated in vitro under laboratory conditions by Mondal and colleagues in 2014, who presented evidence suggesting inhibition of cancer cell growth and spread (Mondal et al., 2014).

As is well known, most poisonous plant species are more widely distributed in the tropical belts of the Earth's Northern and Southern Hemispheres. In the flora of Azerbaijan, *Conium maculatum* L. was collected from the mid-montane belt in the north-western part of the Lesser Caucasus, in an area near a stream close to Poladlı village, Gadabay District. A second specimen was collected in the north-eastern part of the Greater Caucasus from Digah village, Quba District; a herbarium voucher was prepared and the material was identified at the Department of Systematics and Phylogeny of Higher Plants, Institute of Botany.

The primary aim of this study was to test the extracts obtained from the plant on *Mus musculus*, a representative of the order Rodentia. This species is among the most widely distributed mammals in the world and occurs throughout tropical and temperate zones, with the exception of tropical Africa (Gabr, et.al., 2004; Berry, 1970; Meehan, 1984; Long, 2003). One of the key reasons for the ecological success of house mice is their high adaptive capacity (Robert, 1994). Mice may ingest substances that are extremely bitter to humans, and even when food is harmful they do not vomit (Rentokil, 2001). If their diet contains approximately 15% water, they can survive without drinking water (Southern, 1954). Among their sensory modalities, olfactory, auditory, and tactile cues predominate (Naomi et al., 2004). Approximately 5% of rodent species are considered hazardous to humans (Hansen et al., 2016; Sharma et al., 2019; Witmer et al.,

2014). House mice (*Mus musculus*) pose a threat to native plant and animal species on islands and may cause damage wherever they become established (Gary et al., 2014). Rodents are responsible for losses in agriculture, horticulture, forestry, and public goods, and they contribute to the transmission of certain diseases in humans and animals (Krushna et al., 2025). They can destroy virtually any cultivated crop worldwide (Jokić et al., 2017). A variety of methods have been employed to control house mice (Row et al., 1964), among which chemical agents are the most widely used. Plant-derived products represent an attractive alternative to chemical control because they degrade rapidly, are more cost-effective, exhibit high biological activity, and are distinguished by their biodegradability, leaving no persistent residues in soil or water. Studies have shown that numerous plant species possess pesticidal activity (Fellows, 1979). In particular, the use of toxic plants is more widespread in developing countries, as these locally grown plants are more economically advantageous and serve as an alternative to synthetic chemical pesticides (El-Gengaihi et al., 1997; Abou-Hashem, 2012).

2. Materials and methods

2.1. Ethanol extraction of plant material

The plant material used in this study *C. maculatum* L. - was collected from the Gadabay District, located in the mid- montane belt of the Lesser Caucasus. The aerial parts of the plant were finely chopped, dried, and 500 g of the material was placed in a glass container. Subsequently, 5 L of 95% ethanol was added, and the mixture was left to macerate for 14 days. The resulting extract was then filtered through filter paper. The obtained plant extract was stored in a refrigerator at +2°C to +5°C for 8 years and was subsequently used for bioassays. During this storage period, no visible changes in the physical appearance of the extract were observed. However, potential changes in the chemical composition of the extract were not assessed using analytical methods prior to testing.

2.2. Extraction of plant material with distilled water

The root parts of *Conium maculatum* were cut into 1–2 cm pieces and air-dried under shaded outdoor conditions. A total of 300 g of the dried, chopped plant material was placed in a flask, and 3 L of distilled water was added. Aqueous extraction was performed by heating the mixture at 60°C for 3 h. After cooling, the extract was filtered, and the resulting aqueous extract was tested two days later.

2.3. Preparation of food samples containing ethanoic and aqueous plant extracts

To feed *Mus musculus* with the plant extracts, a formulated bait was prepared. Briefly, 3 g of flour, 1 mL of plant extract, and 1 mL of vegetable oil were added to a Petri dish, after which the mixture was homogenised and shaped into small pellets. Each pellet weighed 5 g. The prepared bait was used for feeding the mice.

2.4. Experimental animals

Ten *Mus musculus* individuals (body mass: 18–25 g), captured using traps in private horticultural plots, were tested under laboratory conditions (Figure.1-11.). Prior to experimentation, females were housed separately from males for 21 days in laboratory cages (30 × 20 cm). During the pre-experimental period, the mice were provided with a mixed diet (bread, unsalted cheese, wheat, raw potato, and sausage), drinking water, an appropriate light regime, and normal room

temperature. Considering that potatoes contain approximately 79 - 80% water [Eric and Mario], mice housed in cages 2 and 4 were not provided with additional water. Female mice housed in cages 1, 3, and 5 were provided only with sausage and water. Despite being maintained under laboratory conditions, these animals exhibited high agility, normal behaviour, and favourable welfare indicators. In contrast, reduced welfare was observed in mice housed in cages 2 and 4 despite the same general level of husbandry. The study was conducted in accordance with animal welfare principles.

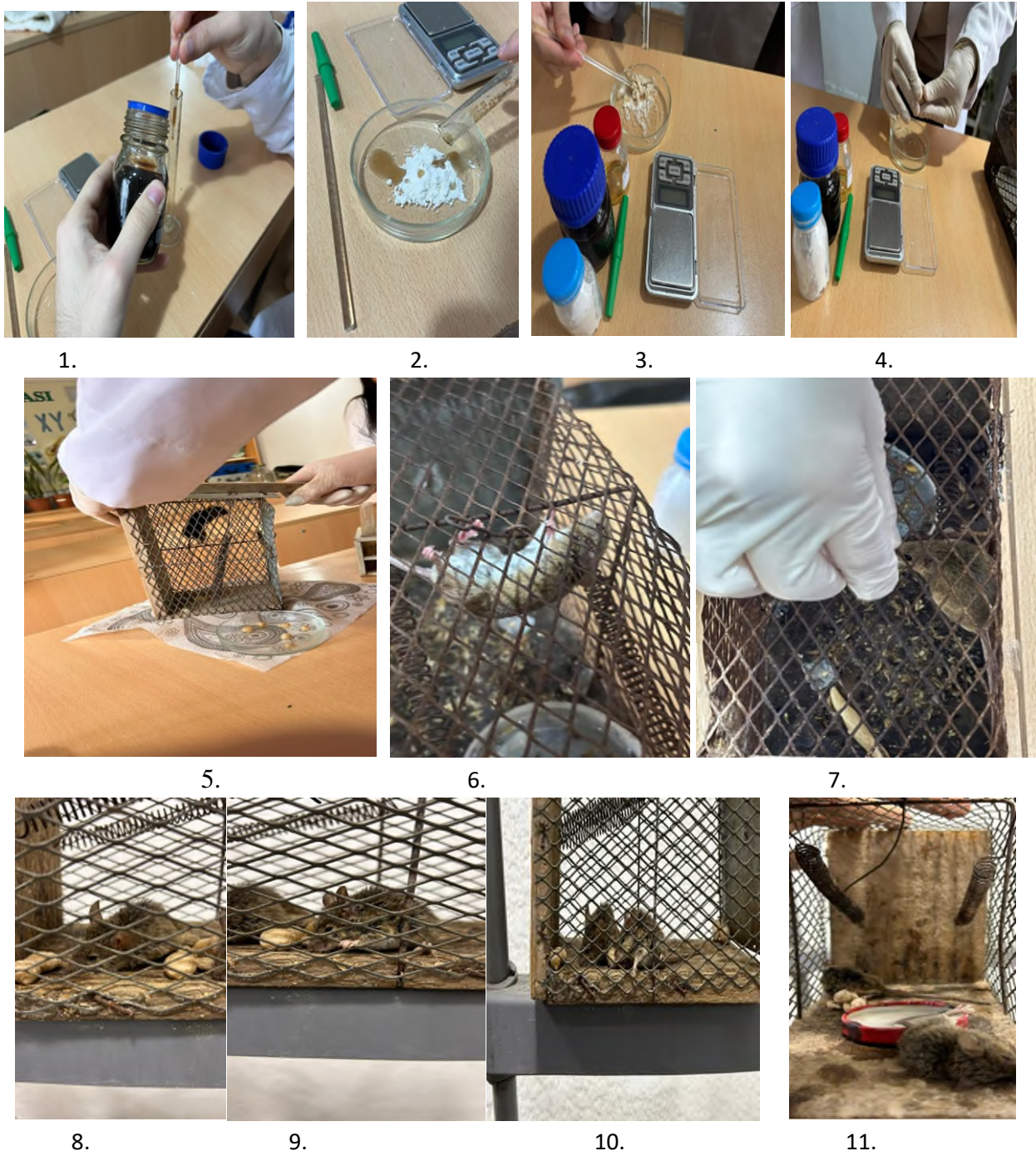


Figure 1-11. *Mus musculus* individuals used in the laboratory experiment. Figures 1-4 show the preparation of food. Figures 5-7 depict mice maintained under normal behavioral and welfare conditions. Figures 8-10 indicate a decline in welfare status, while figure 11 shows a lethal outcome observed during the experiment.

2.4.1. Experimental design

The study was conducted on 10 *Mus musculus* individuals, which were assigned to five experimental groups. Female mice were allocated to Groups I, II, and III, whereas male mice were allocated to Groups IV and V. Group I was maintained without experimental intervention and served as the negative control. Groups II and III were fed bait prepared with the 95% ethanol extract, while Groups IV and V were fed bait prepared with the aqueous extract. During extract-bait feeding, animals in all treated groups had access to drinking water. Specifically, Group II received 12 pellets prepared with the 95% ethanolic extract (5 g each), Group III received one pellet prepared with the 95% ethanolic extract (5 g), Group IV received six pellets prepared with the aqueous extract (5 g each), and Group V received one pellet prepared with the aqueous extract (5 g). Groups II and IV consisted of three mice per group (six animals in total), whereas Groups III and V consisted of one mouse per group (two animals in total); the remaining two animals were assigned to the control group (Group I). Marked behavioural changes were observed 24 h after feeding in the ethanolic-extract groups (II and III). The mice moved by weak crawling, and tremors were recorded. Deaths occurred by 48 h. In mice fed bait prepared with the aqueous extract (Groups IV and V), behavioural changes were observed after 6 h; the animals remained motionless in the corner of the cage and tremors were noted. Deaths occurred within 24 h.

2.4.2. Toxicological evaluation

The observations indicated that the 95% ethanolic extract exerted high toxicity in the experimental animals. In Groups II and III, the pronounced reduction in motor activity and the occurrence of tremors 24 h after extract ingestion suggest central nervous system impairment. The recording of deaths within 48 h confirms a delayed yet strong lethal effect. In Groups IV and V, which were fed bait prepared with the aqueous extract, the toxic effect manifested over a shorter timeframe (6 h). The observed behavioural changes and deaths within 24 h demonstrate that the aqueous extract produced a more rapid, acute toxic response.

2.4.3. Time - dependent toxicological evaluation of the exposure–time relationship

The timing of the observed clinical signs and mortality indicates that the toxic effects of the extracts were time-dependent. Whereas the ethanolic extract required a longer period to reach maximal toxicity, the aqueous extract triggered an acute toxic reaction within a shorter time. This difference may be related to factors such as the storage duration of the ethanolic extract (8 years), differences in absorption rate, metabolic transformation, and effects on target organs.

3. Results

3.1. Clinical observations

The results of the experimental study indicate that both the 95% ethanolic extract and the aqueous extract obtained from *Conium maculatum* L. exerted pronounced toxic and lethal effects on *Mus musculus* under laboratory conditions. The clinical signs observed following oral exposure via bait - reduced motor activity, tremors, and immobility - support the conclusion that these plant extracts possess neurotoxic activity involving the central nervous system.

3.2. Toxicity indices and lethality profile of the ethanolic extract

Significant differences were identified between the extracts with respect to the onset of effects and lethality. In animals fed the 95% ethanolic extract, toxic signs appeared at a later stage (after

24 h), and deaths were recorded within 48 h. These findings suggest that the ethanolic extract produces a relatively delayed yet sustained and potent toxic effect. Possible explanations include the use of the extract after an eight-year storage period and/or gradual absorption and accumulation of lipophilic alkaloid fractions. Lipophilic alkaloids are compounds that dissolve readily in fats, cross biological membranes efficiently, and may produce cumulative toxicity in vivo.

3.3. Rapid and acute toxicity characteristics of the aqueous extract

In groups receiving the aqueous extract, the shorter time to onset of toxicity (approximately 6 h) and the occurrence of deaths within 24 h indicate a faster and more acute toxic mechanism. This difference may be explained by rapid absorption of hydrophilic constituents and their earlier activation within metabolic processes. The principal alkaloids reported in *C. maculatum* (coniine hydrochloride, γ -coniceine hydrochloride, and conhydrine hydrochloride) contain nitrogen atoms and therefore become protonated in acidic environments, forming salt forms. These substances dissolve rapidly in gastric contents and, after passing into the small intestine, are readily absorbed.

3.4. Time ratio of toxic effects

Based on a quantitative assessment, the time to onset of toxicity for the aqueous extract was approximately four-fold shorter than that for the ethanolic extract, while the time to recorded lethality was two-fold shorter (Fig. 1). A cumulative toxicity index was calculated as the ratio of the time to recorded lethality to the time to onset of toxicity. Accordingly, for the ethanolic extract, $K_{\text{EtOH}} = 48/24 = 2$, whereas for the aqueous extract, $K_{\text{H}_2\text{O}} = 24/6 = 4$.

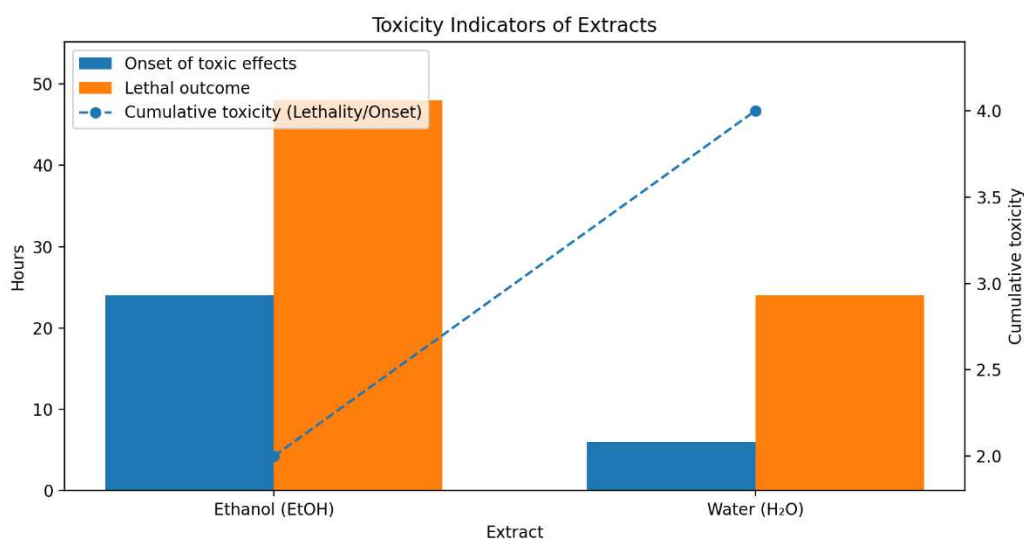


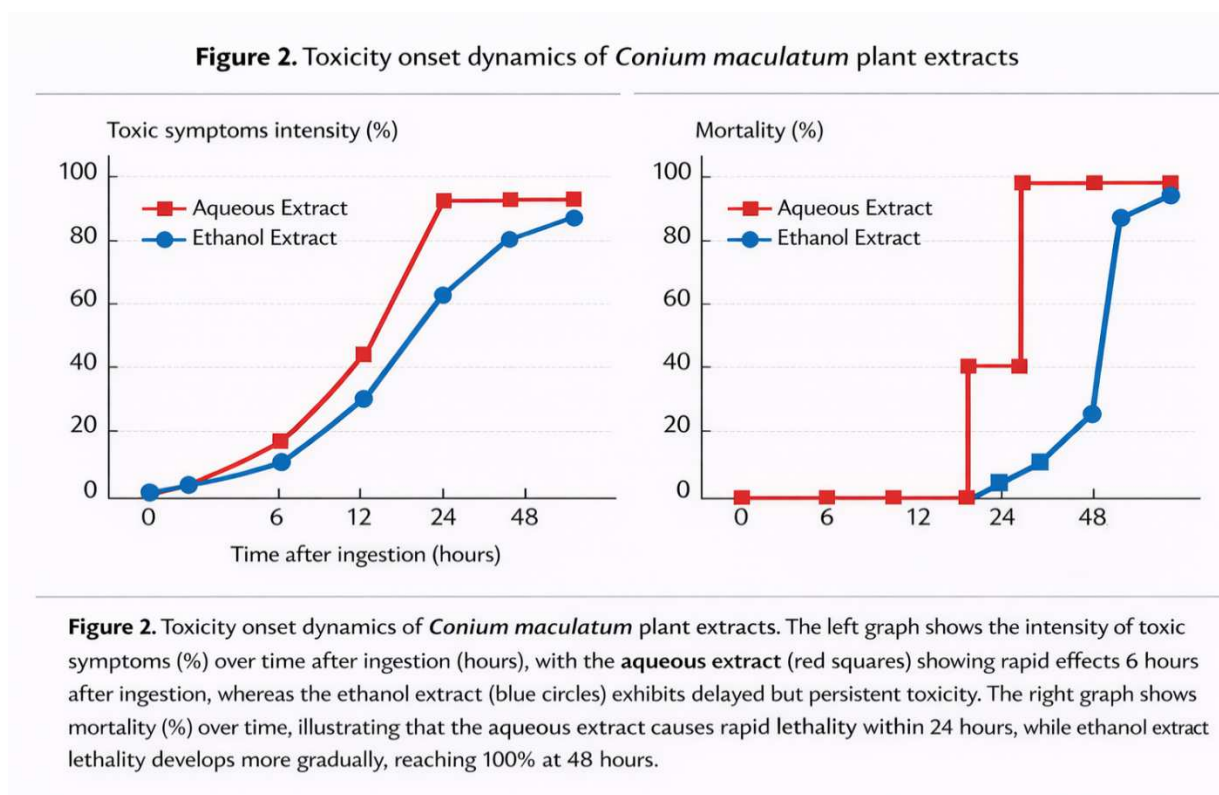
Fig. 1. Comparison of the onset and lethality times of *C. maculatum* extracts (ethanolic and aqueous).

4. Discussion

The findings of the present study demonstrate that the investigated plant extracts exerted marked toxic effects on *Mus musculus*. In particular, in Groups II and III, which received bait prepared with the 95% ethanolic extract, a pronounced reduction in motor activity, weak crawling movements, and tremors were observed within 24 h. The gradual progression of behavioural

alterations suggests a staged pattern of toxicity.

In contrast, in Groups IV and V fed bait prepared with the aqueous extract, toxic signs emerged at an earlier stage – approximately 6 h after food intake. The rapid development of immobility and tremors in these groups indicates that toxicity was expressed over a shorter timeframe. The difference in onset time between the extracts may be attributable to solvent-dependent extraction of bioactive constituents, resulting in distinct chemical profiles and bioavailability. In addition, the fact that the ethanolic extract had been prepared from plant material eight years earlier may also be considered a factor influencing the temporal dynamics of the observed effects. Overall, the behavioural changes and recorded mortality in both treatment conditions indicate that the examined *C. maculatum* extracts possess toxic and lethal activity toward *Mus musculus*. The time-course dynamics of toxicity of *C. maculatum* extracts are presented in Figure 2.



The graphs indicate that the aqueous extract induced toxic symptoms more rapidly and resulted in lethality within 24 h, whereas the ethanolic extract produced a delayed but sustained toxic effect, with lethality reaching its maximum level within 48 h.

Overall, the results show that both ethanolic and aqueous extracts derived from *C. maculatum* exerted strong toxic and lethal effects on *Mus musculus*. In particular, the observed reduction in motor activity, tremors, immobility, and passive cornering behaviour clearly indicate a central nervous system-directed action of the plant extracts. Such behavioural alterations have been widely described in rodents following exposure to neurotoxic agents (Witmer et al., 2014; Sharma et al., 2019; Sabine et al., 2016). The findings are consistent with the alkaloid-rich chemical profile of *C. maculatum*. Its principal alkaloids, coniine and γ -coniceine, are known to block neuromuscular transmission and may lead to peripheral paralysis (Mansoor et al., 2018). This mechanism plausibly explains the weakness, crawling-like movements, and terminal immobilisation observed in our experiments. Similar clinical signs have also been reported in previous experimental studies using *M. musculus* and other rodent models exposed to plant-derived neurotoxins (Gary et al., 2014; Hansen et al., 2016). The relatively late onset of effects of the ethanolic extract (after 24 h) and the recording of deaths within 48 h indicate a delayed yet sustained toxic action. This pattern may be explained by the

gradual absorption and subsequent accumulation of lipophilic alkaloid fractions. In rodents, sequestration of lipophilic compounds in adipose tissue followed by redistribution over time may lead to delayed behavioural manifestations as the nervous system becomes affected (Long, 2003; Witmer et al., 2014). In addition, the eight-year storage of the ethanolic extract increases the likelihood that certain alkaloids remained chemically stable and retained biological activity. By contrast, the more rapid onset of toxicity with the aqueous extract (approximately 6 h) and lethality within 24 h may be associated with the fast absorption of hydrophilic alkaloids. Protonated alkaloid salts dissolve readily in the gastrointestinal tract and can enter systemic circulation quickly, thereby producing earlier behavioural changes. Similar response patterns have been reported in mice consuming rodenticidal compounds in previous studies (Jokić et al., 2017).

An interesting observation was that all 12 bait pellets prepared with the ethanolic extract were consumed by the mice. When the bait pellets (soft dough containing the extract) were introduced into the cage, both mice initially ate one pellet and then stopped feeding. However, after approximately two hours, all pellets – by then partially dried – were found to have been eaten. This behaviour may reflect a weak association between taste and toxicity in house mice. As reported previously, *M. musculus* can ingest even highly bitter substances, and because the emetic reflex is poorly developed, they may not discontinue consumption of toxic food (Rentokil, 2001; Southern, 1954). These findings support the potential of plant-derived products as alternative tools for rodent control. Given concerns such as resistance to chemical rodenticides and adverse effects on the environment and non-target organisms, phytogenic toxins may be considered more selective and environmentally attractive options (Row et al., 1964; Hansen et al., 2016; Ibrahim, 2001).

In conclusion, this study demonstrates that *Conium maculatum* L. extracts exert strong neurotoxic effects on *Mus musculus*, accompanied by marked behavioural alterations. Differences between the ethanolic and aqueous extracts in onset time and lethality indicate that the extraction method substantially influences toxicokinetics/toxicodynamic profiles. These results provide a scientific basis for future research on plant-derived toxins in behavioural ecotoxicology and rodent management.

Conclusions

This study evaluated the rodenticidal effect of *Conium maculatum* extracts on *Mus musculus* under laboratory conditions. Both 95% ethanolic and aqueous extracts produced significant neurotoxic and lethal effects, with the aqueous extract exhibiting a faster onset of toxicity than the ethanolic extract. Observed behavioral changes – including reduced motor activity, tremors, and immobility – indicate central nervous system involvement. These results highlight that the extraction solvent significantly affects the onset and progression of toxic effects, likely due to differences in alkaloid solubility, absorption, and bioavailability. The observed bait consumption behavior suggests that *M. musculus* may not readily avoid toxic plant material, supporting the potential application of plant-derived extracts as alternative rodenticidal agents. Overall, this study provides a scientific basis for the use of *C. maculatum* extracts in rodent management and offers additional insights into the role of plant-derived neurotoxins in behavioral ecotoxicology.

Abbreviations

°C degrees Celsius
cm centimeters
DNA Deoxyribonucleic acid
EtOH -- Ethanol
g grams
h hours

K_EtOH cumulative toxicity index for ethanol extract
K_H₂O cumulative toxicity index for aqueous extract
L liters
L. Linnaeus (taxonomic author designation)
mg/kg milligrams per kilogram
mL milliliters
Ph.D. Doctor of Philosophy
pp. pages
ROS Reactive Oxygen Species
Vol. Volume

Declarations

Ethics approval and consent to participate

The authors declare that this work was conducted in accordance with the guidelines of the Research Ethics Committee of the Veterinary Scientific Research Institute. All experimental procedures involving animals were reviewed and approved by the Institutional Animal Ethics Committee of the authors institution and were carried out in accordance with international standards and ethical guidelines for animal welfare. Ethical approval number: EC-CM-MM-2025-01.

Consent for publication

Not applicable.

Availability of data and material

No datasets were generated or analysed during the current study.

Competing interests

The authors declare no competing interests.

Funding

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Author contributions

HM, conceived the study, collected the plant material, carried out the experimental work, analyzed and interpreted the data, and prepared the first draft of the manuscript. EA, contributed to the scientific supervision of the study and to the revision of the manuscript. RB, contributed to the design of the research and the interpretation of the results. All authors read and approved the final manuscript.

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