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RESEARCH ARTICLE

Toxicity study of *Vernonia cinerea*

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Abstract

The methanol extract of *Vernonia cinerea* Less (Asteraceae), which exhibited antimicrobial activity, was tested for toxicity. In an acute toxicity study using mice, the median lethal dose (LD_{50}) of the extract was greater than 2000 mg/kg, and we found no pathological changes in macroscopic examination by necropsy of mice treated with extract. As well as the oral acute toxicity study, the brine shrimp lethality test was also done. Brine shrimp test LC_{50} values were 3.87 mg/mL (6 h) and 2.72 mg/mL (24 h), exhibiting no significant toxicity result. In conclusion, the methanol extract of *V. cinerea* did not produce toxic effects in mice and brine shrimp.

Keywords : Artemia salina*; oral acute toxicity;* Vernonia cinerea*; brine shrimp lethality test*

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Introduction

Vernonia cinerea Less (Asteraceae), an annual herb, is reported to have many medicinal properties (Iwalewa et al., 2003). *V. cinerea* has many therapeutic uses in different traditional medicines of the world, including use for treatment of a number of disorders such as malaria fever, worms, pain, inflammation, infections, diuresis, cancer, abortion, and various gastrointestinal disorders (Jain & Puri, 1984; John, 1984; Singh & Ali, 1989; Bhattarai, 1991; Bajpai et al., 1995; Grainger, 1996). Every part of the plant can be used medicinally (Chopra et al., 1985; Latha et al., 1998). An extract of the plant is used to relieve cold and menstruation-related problems (Verma et al., 1993). The plant has also been used as a tonic, stomachic, and astringent (Misra et al., 1984). The flowers are used to treat conjunctivitis, fever, and rheumatism (Rastogi & Mehrotra, 1991). The leaf extracts of the plant are reported to be diuretic and antidiuretic (Adeboye et al., 1997), anti-inflammatory (Mazumder et al., 2003), analgesic, and antipyretic (Iwalewa et al., 2003). Our previous study showed that the methanol extract of *V. cinerea* exhibited a good antibacterial and antiyeast activity (Yoga Latha et al., 2005).

Although medicinal plants may produce several biological activities in humans, generally very little is known about their toxicity (Mukinda & Syce, 2007), and the same applies for *V. cinerea*. Safety should be the principal criterion in the selection of medicinal plants for use in healthcare systems (Tomlinson & Akerele, 1998). Hence, formal toxicological evaluation of a plant is necessary as well as documented ethnomedicinal information. Therefore, the present study carried out basic toxicological tests and established the safety of the methanol extract of *V. cinerea* (whole plant part, including roots), focusing on its acute toxicity in mice and the brine shrimp lethality assay.

Materials and methods

Plant material

The whole plant part including roots of *V. cinerea* was obtained from the University Science of Malaysia campus, Penang, Malaysia in April 2004, and was authenticated by Associate Professor Dr. Shaida Fariza Sulaimana, a taxonomist at the University Science of Malaysia, Penang, Malaysia. A dried sample (with voucher number

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VC0404) was deposited at the herbarium of the School of Biological Sciences.

Preparation of the crude extract

Approximately 200 g of dried plant material was boiled in a Soxhlet apparatus with 200 mL of methanol [high performance liquid chromatography (HPLC) grade] for 48 h. The entire extract was further concentrated to dryness using a rotary evaporator, yielding 18 g extract (Nisha et al., 2008).

Animals

Mice (males and females) weighing 25 and 35 g from the Animal House of the University of Science Malaysia were used. Ten mice were kept in each matte plastic cage, measuring 17 × 27 × 14 cm. The cages with the mice were placed in a room with controlled cycles of 12 h of light and 12 h of darkness; the light went on at 7 a.m. Water and food were provided to animals *ad libitum*. Experiments were conducted in accordance with the internationally accepted principles for laboratory animal use and care (EEC Directive of 1986; 86/609/EEC).

Acute toxicity study of V. cinerea methanol crude extract

All mice of both sexes were fasted overnight before treatment and were given food 1 h after treatment. We expected that *V. cinerea* would be relatively safe because of its application in traditional medicine. Thus, a single high dose, as recommended by the Organization for Economic Co-operation and Development (OECD) guidelines (2000), of 2000 mg/kg of crude extract dissolved in water was administered by gavage to 10 male and 10 female mice, and water was given to 10 male and 10 female mice as a control group. After a single administration, signs of possible toxicity were observed every hour for the first 6 h and every day for 14 days. Surviving animals were weighed daily and observed for any signs or symptoms of toxicity and for mortality for up to 14 days, as described previously (Lee et al., 2002; Ryu et al., 2004). Visual observations included changes in the skin and fur, eyes, and mucous membranes, and also respiratory, circulatory, autonomic, and central nervous systems, as well as somatomotor activity and behavioral pattern.

After this observation period of 14 days, seven mice of both sexes in each group were sacrificed to measure organ/body weight indices. Liver, lungs, spleen, and kidneys were removed from the remaining three mice of both sexes from each group after euthanizing and killing them by cervical dislocation. Tissues were fixed in 10% buffered formalin. After fixation, the tissues were dehydrated in a graded series of alcohol, cleared in xylene, and embedded in paraffin wax. Multiple 5 μm sections from each block were mounted on slides and stained with hematoxylin and eosin.

Toxicity testing against brine shrimp

Hatching shrimp

Brine shrimp eggs, *Artemia salina* L. (Artemiidae), were hatched in artificial seawater prepared by dissolving 38 g of sea salt in 1 L of distilled water. After a 24-h incubation period at room temperature (22–29°C), the larvae were attracted to one side of the vessel with a light source and collected with a pipette. The larvae were separated from the eggs by aliquoting them three times in small beakers containing seawater.

Brine shrimp assay

The bioactivity of the extract was monitored by the brine shrimp lethality test (Meyer et al., 1982). Samples were dissolved in dimethylsulfoxide (DMSO) and diluted with artificial seawater. Seawater (2 mL) was placed in all bijoux bottles. A two-fold dilution was carried out to obtain concentrations from 100 to 0.195 mg/mL. The last bottle was filled with sea salt-water and DMSO only, serving as a drug-free or negative control. A suspension (0.1 mL) containing about 10–15 larvae was added to each bottle and incubated for 24 h. The bottles were then examined, and the number of dead larvae in each bottle was counted after 6 and 24 h. The total number of shrimp in each bottle was counted and recorded. The mean percentage mortality was plotted against the logarithm of concentration, and the concentration that could kill 50% of the larvae (LC_{50}) was determined from the graph (Finney, 1971; Geran et al., 1972).

Calculations and statistics

The results are expressed as mean ± standard deviation (SD). The acute toxicity study data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test for comparing the control and the various groups, using SPSS Version 12.0. The mean results of brine shrimp mortality against logarithm of concentration were plotted using the Microsoft Excel computer program, which also presents regression equations. The regression equations were used to calculate the LC_{50} value. Extracts giving LC_{50} values greater than 1.0 mg/mL were considered to be non-toxic (Simionatto et al., 2005; Sasidharan et al., 2008). Statistical significance was assumed at the 0.05 level.

Results and discussion

Results for the toxicity of the crude extract of *V. cinerea* on mice and brine shrimp are shown in

Figures 1–3 and Table 1. To establish the safety of the extract, 2000 mg/kg crude extract was administered to both male and female mice. No toxic symptoms or death was observed in any of the animals, and they lived up to 14 days. An autopsy at the end of the experimental period revealed no apparent changes in any organs (Table 1, Figure 1). Total body weight in both male and female extract-treated mice was similar to that of control mice. There were no significant differences in the organ-to-body weight indices of the heart, kidneys, liver, lungs, or spleen (Table 1). Gross examination at autopsy and histopathological evaluations of the various organs stained with hematoxylin and eosin revealed no significant differences (Figure 1). The overall feed consumption of animals receiving *V. cinerea* extract was generally similar to that of the controls (data not shown). Similarly, the feed consumption in the recovery group was similar to the respective control. Food intake exhibited the same pattern of evolution in each sex and group. Therefore, the acute minimum fatal dose of the extract of *V. cinerea* for mice was higher than 2000 mg/kg body weight, which is the single high dose recommended by OECD (2000) guidelines for testing oral acute toxicity. Thus, our test suggested that *V. cinerea* does not cause any apparent acute toxicity.

The extract of *V. cinerea* showed no significant toxicity against brine shrimp, with LC_{50} values of 3.87 mg/mL (6 h) and 2.72 mg/mL (24 h). With respect to the effect of the time of exposure in the brine shrimp assay, no significant changes in toxicity were detected at 6 or 24 h of exposure (Figures 2 and 3). The results on brine shrimp assay indicate that the extract had an LC_{50} value greater than 1.0 mg/mL, which is the recommended cut-off point for detecting cytotoxic activity (Simionatto et al., 2005). This suggests that this plant extract may not be toxic. A toxicity study was also reported by Kuo et al. (2003). They tested two novel sesquiterpene lactones, vernolide-A (**1**) and -B (**2**), isolated from the ethanol extract of stems of *V. cinerea*, against human KB (oral epidermoid carcinoma), DLD-1 (colon adenocarcinoma), NCI-661 (lung large cell carcinoma), and Hela (cervix epithelioid carcinoma) tumor cell lines. They reported that vernolide-A demonstrated potent cytotoxicity against human

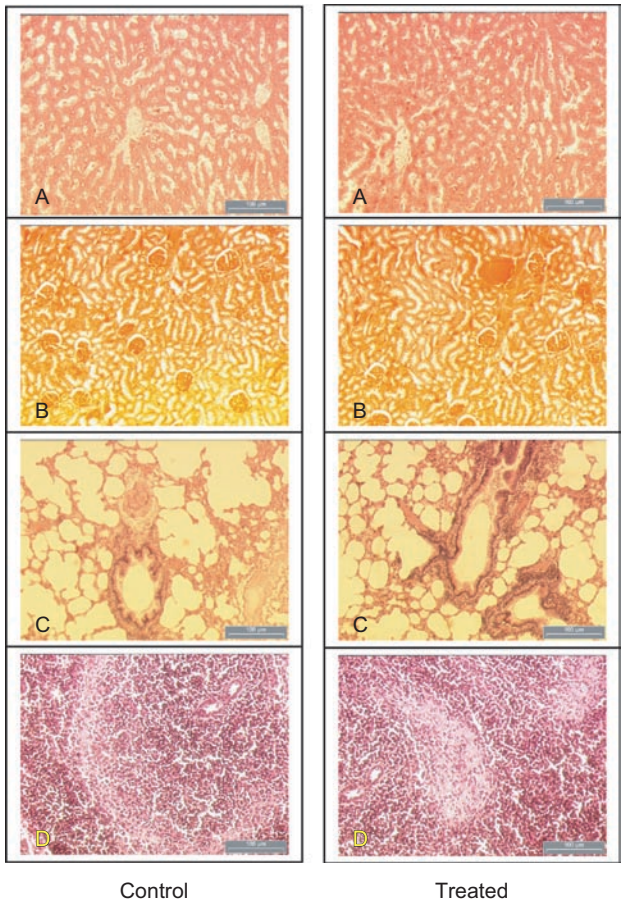

Figure 1. Histological examination: liver (A), kidney (B), lungs (C), and spleen (D).

Table 1. Effect of *Vernonia cinerea* crude extract[a] on organ-to-body weight index (%) in mice.

	Male		Female	
Organ	Control	Crude extract	Control	Crude extract
Kidney	1.42 ± 0.01	1.40 ± 0.01	1.36 ± 0.01	1.35 ± 0.01
Liver	7.11 ± 0.06	7.12 ± 0.06	6.95 ± 0.01	6.62 ± 0.01
Lung	1.22 ± 0.01	1.29 ± 0.01	1.12 ± 0.01	1.10 ± 0.01
Spleen	0.51 ± 0.02	0.52 ± 0.02	0.46 ± 0.01	0.48 ± 0.01
Body weight (g)	31 ± 0.57	32 ± 0.56	30 ± 0.51	29 ± 0.49

Organ body index was calculated as (organ weight × 100)/body weight.

[a]Crude extract of *V. cinerea* was administered to mice at a dose of 2000 mg/kg. Values are mean ± SD ($n = 7$).

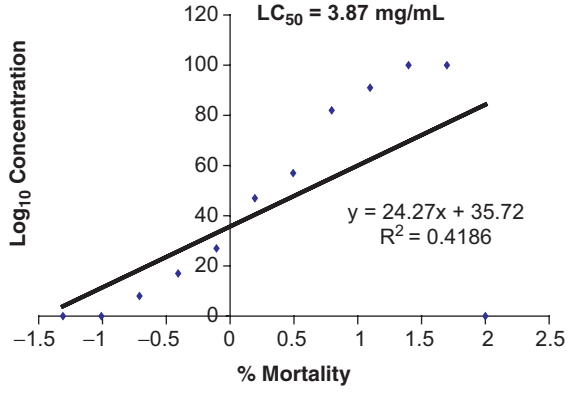

Figure 2. Toxic effects of *Vernonia cinerea* methanol extract after 6 h using brine shrimp lethality assay.

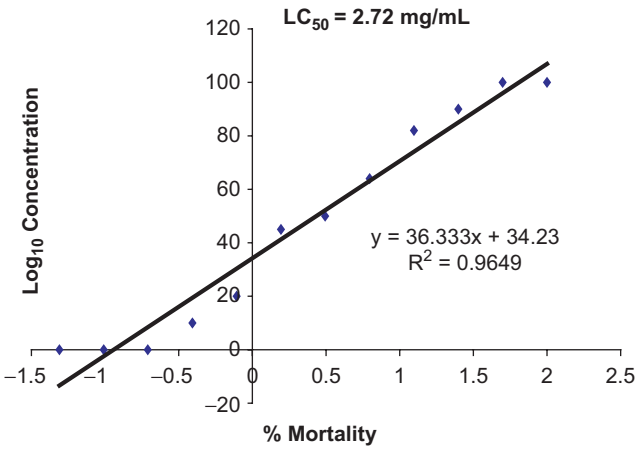

Figure 3. Toxic effects of *Vernonia cinerea* methanol extract after 24 h using brine shrimp lethality assay.

KB, DLD-1, NCI-661, and Hela tumor cell lines (ED_{50} = 0.02, 0.05, 0.53, 0.04 mg/mL for KB, DLD-1, NCI-661, and Hela, respectively); vernolide-B had marginal cytoxicity (ED_{50} = 3.78, 5.88, 6.42 mg/mL for KB, NCI-661, and Hela, respectively).

Thus, our test suggested that *V. cinerea* does not cause any apparent *in vivo* toxicity. The results of the current study concur with the use of this plant by traditional healers. A World Health Organization survey indicated that about 70–80% of the world's population rely on non-conventional medicine, mainly of herbal source, in their primary healthcare (Akerele, 1993). This is particularly the case in developing countries where the cost of consulting a Western style doctor and the price of medication are beyond the income of most people. Our present study showed that *V. cinerea* does not exhibit any apparent toxicity and may be used as an antimicrobial agent in known dosages, especially in rural communities where conventional drugs are unaffordable because of the high cost or are unavailable in developing countries. Studies of this type are needed before a phytotherapeutic agent can be generally recommended for use.

Declaration of interest: The authors report no conflicts of interest.

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