Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(11)60033-6

In vivo toxicity study of *Lantana camara*

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ARTICLE INFO

Article history:
Received 14 February 2011
Received in revised form 25 February 2011
Accepted 11 March 2011
Available online 30 March 2011

Keywords:
Lantana camara
Artemia salina
Toxicity

ABSTRACT

Objective: To investigate the toxicity of methanol extract of various parts (Root, Stem, Leaf, Flower and Fruit) of *Lantana camara*(*L. Camara*) in *Artemia salina*. **Methods:** The methanol extracts of *L. camara* were tested for *in vivo* brine shrimp lethality assay. **Results:** All the tested extract exhibited very low toxicity on brine shrimp larva. The results showed that the root extract was the most toxic part of *L. camara* and may have potential as anticancer agent. **Conclusions:** Methanolic extract of *L. camara* is relatively safe on short-term exposure.

1. Introduction

Folk healers in Asia and South America have used lantana species including *Lantana camara*(*L. camara*) for centuries to treat various human ailments such as dermatological and gastrointestinal diseases, tetanus, malaria and tumors[1]. Traditional healers have used lantana species for centuries to treat various diseases. Different parts of *L. camara* is used for the treatment of various human ailments such as itches, cuts, ulcers, swellings, bilious fever, catarrh, eczema, tetanus, malaria, tumors and rheumatism[2]. Considering both the ethnobotanical and pharmacological applications of the plant, the aim of this study was to investigate the possible toxic effects of the leaf extract of *L. camara* agaist *Artemia salina*.

Estimation of toxicity of a natural product or other compounds usually passes through three levels of observations. First level includes the test of the alterations in morphology, cell growth and metabolism using normal human or animal cell lines. At the second level, lower animals such as fishes and *Artemia salina* are exploited for close monitoring of toxic changes in eggs or whole body. Brine shrimp *Artemia salina*, also known as sea monkey, is a marine invertebrate about 1 mm in size[3]. It is used as a "benchtop bioassay" for the discovery and purification of bioactive natural products and is an excellent choice for elementary toxicity investigations of consumer products. The shrimp lethality assay is based on the ability to kill laboratory-cultured *Artemia nauplii* (animal's eggs)[4].

2. Material and methods

2.1. Plant samples

Different parts of *L. camara* were collected from Amanjaya, Kedah, Malaysia, on February 2008. The identity of plant was confirmed by Dr. S. Sudhakaran, associate professor in faculty of applied sciences, AIMST University, Kedah, Malaysia. A voucher with number 11008 was deposited in the herbarium of Biology School, Universiti Sains Malaysia, Penang, Malaysia.

2.2. Extraction procedure

In the laboratory, the different parts of *L. camara* sample were washed with freshwater and brushed with a soft brush before drying. Clean plant material was transferred to oven (ECOCELL) in 50 ℃ temperature for 96 h for drying. Then they were powdered by electric blender. Approximately 100 g of different parts of *L. camara* powder was added to

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Foundation Project: Supported by a grant form Universiti Sains Malaysia University.

400 mL methanol and soaked for 4 days. Removal of the plant material from solvents was done by filtration through cheesecloth, and the filtrate was concentrated using a rotary evaporator.

2.3. Hatching shrimp

Brine shrimp eggs, *Artemia salina* were hatched in artificial seawater which was prepared by dissolving 38 g sodium chloride in 1 liter distilled water. After 36-h incubation at room temperature (22-29 ℃) under a light source, the larva (nauplii) were separated from shells and unhatched eggs by siphoning with a plastic tube.

2.4. Brine shrimp assay

Bioactivity of the *L. camara* extracts was measured by the brine shrimp lethality test[5]. Samples were dissolved in DMSO and diluted with artificial seawater (concentration 100 μg/mL). Two mL artificial seawater was placed in all the universal bottles. A two-fold dilution was carried out to obtain a concentration of 50-0.195 mg/mL. The last bottle was left with salt water and DMSO to serve as the drug free control. Vitamin C and chloramphenicol were used as reference drugs. Hundred microliters of suspension of nauplii containing about 10-15 larva was added into each bottle and incubated at room temperature. After 6 h and 24 h, the number of dead nauplii and the total number in each bottle was counted. The experiment was done in triplicate.

2.5. Data Analysis

To ensure that the mortality is attributed to bioactive compounds and not to starvation or DMSO effect, the dead larva in each treatment was compared with the dead larva in the control. The percentage of mortality (% M) was calculated as:

% M = percentage of survival in the control − percentage of survival in the treatment.

The best equation of fit curve was obtained by preliminarily comparison of R squares achieved by linear and nonlinear regression analysis by SPSS 16.0.0 program (SPSS Inc. TEAM EQX). Lethality concentration fifty(LC_{50}) values were determined at 70% confidence intervals. Following one way ANOVA, the results were evaluated by Spearman's rho to find out the potential correlation between observations at different times.

3. Results

The LC_{50} values of different extracts of *L. camara* and reference drugs with by 70% confidence intervals are shown in Table 1. The results showed that root extract was the most toxic part followed by leaves, flower, fruits and stem in descending order. Figure 1 summarized LC_{50} values in boxplot bars. Effects of exposure time on mortality percentage were depicted in Figure 2. After exposure to the extract for 6 h, LC_{50} of leaf was 6 0419.1 μg/mL(70% *CI*=7 281.2-113 557.0), and the profile had a sharp rise in mortality at lower concentrations and a slowly increase at higher doses, while after exposure for 24 h the profile showed a gradual increase of mortality at lower concentrations and become constant at 5 000 μg/mL.

Table 1

LC_{50} values for different parts of *L. camara* and reference drugs (Vitamin C and chloramphenicol) based on brine shrimp lethality assay.

Part of *L. camara* and reference drugs	LC_{50} after 24 h (μg/mL)	70% *CI* after 24 h
Root	940.7	128.8 − 1 752.5
Stem	3 966.0	1 402.9 − 6 529.0
Leaf	3 251.8	2 142.1 − 4 361.5
Flower	5 536.6	2 326.5 − 8 746.7
Fruit	3 964.7	3 057.8 − 4 871.6
Vitamin C	221.7	175.2 − 268.2
Chloramphenicol	748.1	495.9 − 1 000.3

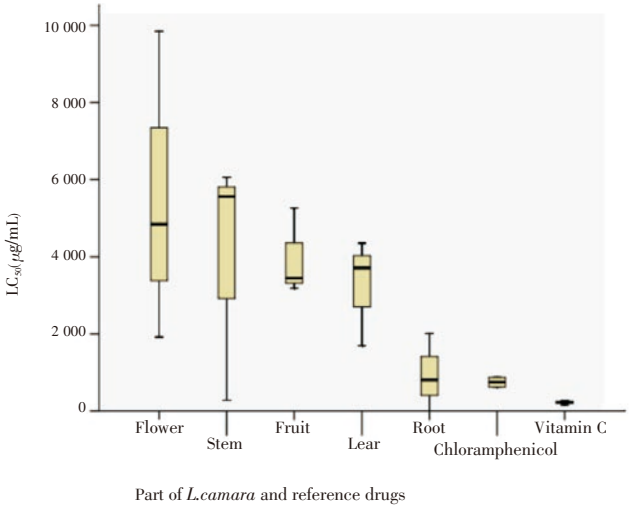

Figure 1. LC_{50} boxplot for different parts of *L. camara*, vitamin C and chloramphenicol tested by brine shrimp lethality assay.

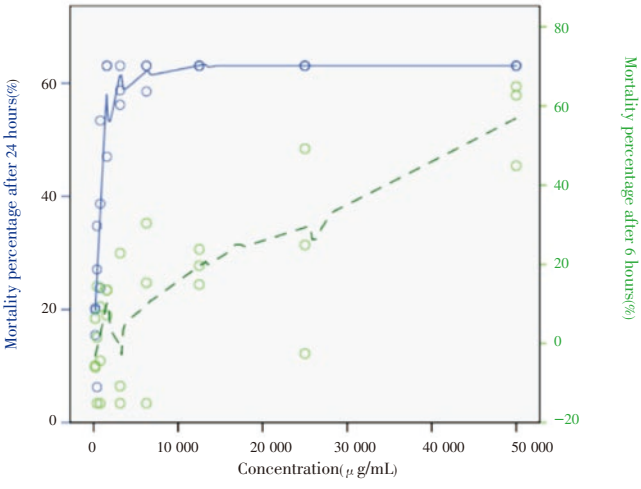

Figure 2. Mortality percentages after treatment of leaf extract of *L. camara* at different concentrations.

4. Discussion

The brine shrimp lethality test is used to evaluate different pharmacological activities of natural remedies, taking into account the basic premise that pharmacology is simply toxicology at a lower dose. Hence in this study we used the test to study the toxicity of various part of *L. camara* extract. Although the toxicities of root extract, vitamin C and chloramphenicol are higher than other extracts in the experiment, based on one way ANOVA analysis, the differences in LC_{50} among various parts of *L. camara* and also two reference drug are not significant ($P>0.05$). But if 100 μg/mL is regarded as an approximate border line for toxicity[6] all the tested compounds and extract exhibit very low toxicity on brine shrimp larva.

Some other toxicological researches using brine shrimp bioassay confirm that the lethality of *L. camara* extracts of various parts are considerably lower than studied plants. Adoum[7] reported that *Sclerocarya birrea*, *Momordica charantia*, *Boerhaavia diffusa* and *Nauclea aculeata* extracts have exhibited potent activity at LC_{50} values <60 μg/mL. Moshi[8] found that the intermediate and polar extracts of Terminalia sericea roots were toxic to brine shrimps with LC_{50} values ranging from 3.5-26.5 μg/mL, while that of cyclophosphamide, a standard anticancer drug, was 10.6-25.2 μg/mL.

The range of LC_{50} value for leaf extract is more than 2 000 μg/mL (confidence interval, 2 142.1-4 361.5 μg/mL) that is much higher than vitamin C (less than 300 μg/mL) and chloramphenicol (equal or less than 1 000 μg/mL). This is a privilege for leaf extract over mentioned reference drugs regarding antioxidant and antimicrobial activities. Spearman's rho statistical test shows a weak bivariate correlation between mortality percentage after 6 h and 24 h of treating brine shrimp larva with leaf extract (Correlation coefficient = 0.432). Figure 2 also illustrates that while the relation between increasing leaf extract concentration and mortality percentage is more linear in the acute lethality observation (after 6 h treatment), it's completely logarithmic in the lag lethality observation (after 24 h treatment). It indicates that the lethality effect of leaf extract is time dependent, increasing significantly after 24 h exposure. At this time nauplii are mainly in instar II/III[4].

From a pharmacological point, a good relationship has been found with the brine shrimp lethality test to detect antitumoral compounds in terrestrial plant extracts[5, 9, 10]. Then, root extract as the most toxic part of *L. camara* is more potential to anticancer phytochemicals. However, further toxicity studies are needed to determine the effects of this extract on chronic oral toxicity, animal foetus, pregnant animals, and their reproductive capacity, and to complete the safety profile of this extract since the *L. camara* possesses various useful biological activities as described in introduction.

Conflict of interest statement

We delcare that we have no conflict of interest.

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