

Determination of toxicological effect of latex of *Calotropis procera* (Ait.) R.Br. in Freshwater snails

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Abstract

Calotropis procera (Ait.) R.Br. commonly known, as “Arka” is a popular medicinal plant found throughout the tropics of Asia and Africa. It has been widely used in the Indian traditional system for the treatment of a variety of disease conditions due to the presence of various cardenolides, triterpenoids, anthocyanins and hydrocarbons in it. In this paper we have evaluated the toxicity of latex of *C. procera* against harmful snails *Lymnaea acuminata* and *Indoplanorbis exustus*. The toxicity of the extracts of latex was both time and dose dependent. Aqueous extract of latex of *C. procera* was less toxic compared to the extracts of ethanol and carbon tetra chloride. The 96h LC₅₀ of aqueous extract of latex was 68.49 mg/l and 76.40 mg/l whereas same of ethanolic extract was 40.91mg/l and 35.80mg/l against the snails *Lymnaea acuminata* and *Indoplanorbis exustus* respectively. The CCl₄ extract of latex was more toxic to the snails, *Lymnaea acuminata* and *Indoplanorbis exustus* and 96h LC₅₀ was found to be 14.25mg/l and 22.88 mg/l respectively.

Environmental pollution caused by synthetic pesticides has diverted researches towards herbal pesticides. Synthetic pesticides has been implicated in causing environmental problems such as, ground and surface water contamination, negative effects on non target organism, accidental poisoning of human being and development of pesticide resistance¹³. Switch over to botanical pesticides made us turn to a locally available plant *Calotropis procera*. It is a medicinal plant and belongs to the family Asclepiadaceae.

A xerophytic shrub widely found in West Africa, Madagascar, the Arabian Peninsula, Southern Asia, and Indochina to Malaysia¹⁹. It occurs almost throughout India. The three species i.e. *Calotropis procera*, *Calotropis gigantea* and *Calotropis acia* occur in India. *C. procera* is also known as sodon apple, calotrope, French cotton, small crown flower (English), *algodon de seda, bomba* (Spanish) *cotton-france, arbre de soie, and bois canon* (French)^{6,14,16,18}. Latex and floss are the main products obtained from it.

In Southeast Asia, especially India and Thailand, *Indoplanorbis exustus* and *Lymnaea acuminata* snail harbor *Metacercaria* and *cercariae* of several flukes such as the bovine blood fluke, liver fluke¹, schistosome spindale⁷ and intestinal echinostomiasis^{3,4,22}. Human infections are acquired by consumption of the raw snails infected with *Metacercariae* or *cercariae*. The people in northeastern India and Thailand are fond of eating raw food, including fish, prawns and snails. Thus, these diseases are prevalent in India and Thailand. Control measures for these diseases may be achieved by destroying the intermediate host. However, controlling the intermediate host is difficult because these snails have high reproduction potential¹⁰ and can survive in different habitats¹⁵. Many plant species have been tested as molluscicides all over the world, as indicated by Kloos and Mc-Cullough⁹, Kuo¹² and Jurberg *et.al.*,⁸. *Calotropis procera* is a plant with great potential as medicinal plant. The present study aims to evaluate the molluscicidal activity of the plant *Calotropis procera* against the snail *Lymnaea acuminata* and *Indoplanorbis exustus*.

Plant Material :

The latex of *C. procera* was collected from the twigs of plants growing in nearest areas of Ramgarhtal in the district Gorakhpur Uttar Pradesh, India and identified by the herbarium of the Botany department, DDU Gorakhpur University, Gorakhpur, Uttar Pradesh, India.

Preparation of Aqueous Extract :

Ten ml. of fresh latex of plant *C. procera*

was mixed with 100 ml of distilled water in an electric macerator. The extracts were passed through Whatman filter paper and the filtrate was used for subsequent experiments.

Preparation of Ethanolic and CCl₄ Extracts :

The latex was dried under shade at ambient temperature and ground to small granules. The granules of dry latex (DL) were soxhlated successively with ethanol and carbon tetrachloride (CCl₄). The solvents were evaporated under vacuum to yield the dry extracts which were stored at 4°C temperatures.

Animal Collection :

Adult *Lymnaea acuminata* (2.25±2c.m length) and *Indoplanorbis exustus* (0.85±0.037cm) were collected locally and used as experimental animals. The animals were allowed to acclimatize for 72 hours. Toxicity experiments were performed by the method of Singh and Agrawal²¹. Ten experimental animals were kept in a glass aquarium, containing 3 liters of dechlorinated water at 24°C. The experimental animals were exposed continuously for 96 hours to different concentrations of plant extract (Table-1). The pH of water was 7.1-7.3 and dissolved oxygen was 6.5-7.2mg/l, six aquaria were set up for each concentration. Control animals were given an equal amount of de-chlorinated water. Mortality was recorded at every 24 hours (24, 48, 72 and 96 hours) during overall exposure period. Dead animals were removed on each observation to avoid contamination in aquarium water. Snail mortality was established by the contraction of the body within the shell and no response to the needle probe was taken as

evidence of death. LC₅₀ value, upper and lower confidence limit (UCL and LCL) and slope values were calculated according to the method of the POLO computer program of Russell *et.al.*,²⁰.

Tables-2 and 3 indicate that the toxicity of the ethanolic extract of latex of *C. procera* against *L. acuminata* and *I. exustus* was time and concentration dependent. Table 2 shows that the LC₅₀ of 24 hours of aqueous, ethanolic and CCl₄ extract of latex of *C. procera* against *L. acuminata* were 1067.94 mg/l, 171.43 mg/l and 150.90 mg/l respectively. The LC₅₀ of 96 hours LC₅₀ of aqueous, ethanolic and CCl₄ extract of latex of *C. procera* against *L. acuminata* was 68.49 mg/l, 40.91mg/l and 14.25mg/l respectively. Similarly Table-3 shows the LC₅₀ of 24 hours of aqueous, ethanolic and CCl₄ extract of latex of *C. procera* against *I. exustus* was 1006.98 mg/l, 198.98 mg/l and 172.92mg/l respectively. The LC₅₀ of 96 hours of aqueous, ethanolic, and carbon tetrachloride extract of latex of *C. procera* and *I. exustus* is time and dose dependent. It also indicates that CCl₄ extract of latex of *C. procera* was more toxic against both the snails than ethanolic and aqueous extract.

All parts of plant *Calotropis procera* yield latex. The latex also contains the cardiac glycosides, calotropin, uscharin, calotoxin, calactin and uscharidin. Calotropagenin is the common aglycone of all the glycosides. Calotropin and uscharin show Digitalin like action on the heart. A non toxic proteolytic enzyme, calotropain (2-3 percent), has been isolated from the latex. The latex of this plant also contains some poisonous constituents².

Our results on the toxicity of latex of *C. procera* show that it is highly effective against *I. exustus* and *L. acuminata*. The study also reveals that the toxic components of latex of *C. procera* show more solubility both in ethanolic and carbon tetrachloride. Carbon tetrachloride extract of latex of *C. procera* shows more toxicity compared to ethanolic extract indicating that the solubility of active molluscicidal component is more in CCl₄.

The extracts of latex of *C. procera* plant exhibit excellent molluscicidal activity against *I. exustus* was 76.40mg/l, 35.80 against *Lymnaea acuminata* and *Indoplanorbis exustus* and 22.88mg/l respectively. The results indicate the toxicity of aqueous, ethanolic and carbon tetrachloride extract of latex of *C. procera* against *L. acuminata* and *I. exustus*. These plants are easily available. Hence they can be effectively used to control the snail *L. acuminata* and *I. exustus* that are vectors of various flukes.

Table-1. Concentration of latex of *Calotropis procera* used for toxicity determination against *Lymnaea accuminata* and *Indoplanorbis exustus*.

Against <i>Lymnaea</i>	Concentration
<i>acuminata</i>	used (mg/l)
Aqueous	25, 50, 100, 150
Ethanolic	10, 25, 50, 70
Carbon tetrachloride	5, 10, 20, 30
Against <i>Indoplanorbis exustus</i>	
Aqueous	25, 50, 100, 200
Ethanolic	10, 25, 50, 75
Carbon tetrachloride	5, 10, 20, 35

Table-2. Toxicity of the aqueous, ethanolic and carbon tetrachloride (CCl₄) extract of latex of plant *Calotropis procera* against *Lymnaea acuminata*

Period	LC ₅₀ w/v mg/l aqueous extract	LC ₅₀ w/v mg/l ethanolic extract	LC ₅₀ w/v mg/l Carbon tetrachloride extract
24 h	1067.94	171.43	150.90
48 h	393.01	134.07	42.56
72 h	135.43	75.92	22.73

Table-3. toxicity of queous, ethanolic and carbon tetrachloride (CCl₄) extract of latex of plant *Calotropis procera* against *Indoplanorbis exustus*

Period	LC ₅₀ w/v mg/l aqueous extract	LC ₅₀ w/v mg/l ethanolic extract	LC ₅₀ w/v mg/l Carbon tetrachloride extract
24 h	1006.98	198.98	172.92
48 h	248.67	104.28	112.83
72 h	128.13	59.50	36.45
96 h	76.40	35.80	22.88

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