

# Impact of *Calotropis procera* on Behaviour of Leeches in Freshwater Environment

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**Abstract:** The extract of *Calotropis procera* (Common Name- Rui) were tested in vitro for behavioural impact on fresh water leech (*Poecilobdella viridis*). The behavioural activity of leeches was determined by exposing 8 adult leeches of near about same weight and size (2 in each container) to tap water with 1 ml of plant extract for 1 hr at room temperature, 1 ml of plant extract for 2 hr at room temperature, 1 ml of plant extract for 3 hr at room temperature, 1 ml of plant extract for 4 hr at room temperature. After this exposure the leeches were transferred into other container which contained natural pond water to enable them recover from the effect of toxic extract of *Calotropis procera* and to act in normal behaviour. Each and every leech movement were observed from time to time and record that how many leeches were dead after 1, 2, 3, 4 hr. of exposure. The effect of *Calotropis procera* on the behaviour of adult leech were reflected by toxic property of extract. This plant extract shows effect on leeches were very fast causing death followed by the very aggressive behaviour as early as few minutes of exposure.

**Keywords:** Calotropis Procera, Aquatic Toxicity, Water Contamination, Latex, Behavioural changes, Water Pollution

## 1. Introduction

A 2001 study on the leech fauna of the Marathwada region of Maharashtra found 14 species of leeches, with both aquatic and terrestrial varieties. In Marathwada region of Maharashtra there are 14 Species of leeches these includes both aquatic (living in water) and terrestrial (land-dwelling), (S. D. Kondhekar And G. K. Kulkarni, 2001). This study also mentions two commonly found fresh water leeches i.e. *Hirudo Birmanica* (scavengers/ predators) and *Poecilobdella viridis* (fish parasite). They are found in all types of aquatic habitats, particularly in and around the freshwater bodies (A. Levenstein et al., 2022). Leech population from all over the world has been decreasing due to several factors like habitat degradation, aquatic pollution, over harvesting, climate changes, invasive species. From these causes one is aquatic pollution. This aquatic pollution is of various types as: Chemical pollution, Nutrient pollution, Microbial pollution, Thermal pollution, Oil spills, Plastic Pollution. Among these types of pollution, one is chemical pollution. Aquatic fauna may, in some cases, take up the contaminant distributed from a food ingestion route supplementing other routes but also taking intake materials accidentally with inspiration (M. Ahmed et al., 2016). And when we study chemicals pollution, then toxic plant extracts can be considered a form of chemical pollution in aquatic environments in very small amount but they show their effect on the aquatic ecosystems. Aquatic animals can be exposed to pollutants directly through the ingestion, absorption or uptake routes as well as indirectly through the food web pathways involving the uptake of background food or exposure via predators or prey (Salgado Costa et al., 2021)

The plant extract of *C. procera* has been known to possess biological properties like antioxidant, hypolipidemic, hepatoprotective, anticancer, aphrodisiac, anti-allergic,

anti-ulcer, insecticidal and molluscidal (Pandey et al., 2016). Whenever toxic chemicals were released in water bodies, they show direct impact on total aquatic fauna (Samadder et al., 2023). Since there are very few information on the toxic effect of plant extract on behavioural activity of fresh water leech. Therefore, this study was carried with the main objective of determining toxic effect of *Calotropis procera*'s extract on behavioural activity of fresh water leech.

## 2. Materials and Methods

The extract of *Calotropis procera* (Common Name- Rui) were tested in vitro for behavioural impact on fresh water leech (*Poecilobdella viridis*). The behavioural activity of leeches was determined by exposing 8 adult leeches of near about same weight and size (2 in each container) to tap water with 1 ml of plant extract for 1 hr. at room temperature, 1 ml of plant extract for 2 hr. at room temperature, 1 ml of plant extract for 3 hr. at room temperature, 1 ml of plant extract for 4 hr at room temperature.



*Calotropis procera*

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Volume 13 Issue 8, August 2024

Fully Refereed | Open Access | Double Blind Peer Reviewed Journal

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According to ayurvedic systems of medicine, the plants which grows under adverse environmental condition may have a number of medicinal characteristics (Y. Morad et al., 2023) and vice versa. Leaves of *Calotropis procera* were used for extraction process. In this process of extraction, I do follow some basics condition i.e. young healthy and disease-free plant which contain high amount of desired compound, fresh leaves of plants because leaves are generally good for general extraction process. Collected leaves were washed in normal cool water and pat them dry thoroughly and ensure that leaves not get infected by any mold. Place the leaves between absorbent paper and apply pressure using heavy objects or a plant press. Change the paper every few days until the leaves are completely dry and brittle. This process can take several weeks. After completion of this process the leaves were crushed into powder form to increase the surface area. *Calotropis procera* has well known in traditional medicinal systems for its pharmaceutical importance. phytochemicals identification from *C. procera* explains its common traditional applications (Darra Wadhawani et al., 2021).

**Chemical Composition of *Calotropis procera*:**

Chemical pollution refers to the introduction of harmful chemicals into the environment. While these chemicals often come from industrial sources, any harmful substance not naturally present in an amount that disrupts the ecosystem can be considered a pollutant. *Calotropis procera* Contains Kaempferol, Quercetin and Myricetin and some other chemicals also for anti-fungal and other pharmacological actions through the involvement in enzymatic system. (Salgado Costa et al., 2021). *C. procera* is widely known for its medicinal properties used to treat various ailments (Kaur et al., 2021). *C. procera* has been reported to contains cardioactive, alkaloids, flavonoids, resins, tannins and glycosides. Animals that accidentally consumes *Calotropis* sp. Shows a wide variety of clinical signs which includes diarrhea, frothy salivation, anorexia and respiration issue. Plant species which are beneficial to human being due to their chemical composition, anti-cancer, antimicrobial, anti-diarrheal, antiseptic, analgesic and anti-inflammatory properties often produce poisonous secondary metabolites. (Rashel Ahmed et al., 2021). Toxic plant extracts contain specific chemical compounds that are harmful to aquatic life. These can be alkaloids, glycosides, or other toxins that disrupt biological functions. Just like industrial chemicals, toxic plant extracts can cause harm to aquatic organisms, disrupt food chains, and alter the overall health of the ecosystem. There is details information on how *C. procera* is responsible for intoxication in livestock and humans, however there are rare chances of its ingestion by aquatic animals (M. Ahmed et al., 2016). Even if the original purpose of the plant extract wasn't to harm aquatic life (e.g., natural pesticide use), its presence in water bodies can have negative consequences.

Medicinal plants are extensively used by indigenous peoples in several tribal people and very effectively applied for the treatment of various diseases with limited or no side effects (Saha, 2023).

**Preparation of solution and Experiments:**

In this study we are going to do three step experiment in which the concentration of plant extract goes increase. Preparation of extract solution as, take 1 gm of plant extract and 1000 ml of water. Then allow to settle for 24 hrs. after that two leeches were dropped in the container for 1 hr., 2 hr., 3 hr., and 4 hr. respectively. Again, 2gm of plant extract added in 1000 ml of water. Like this 5gm of plant extract added in 1000 ml of water. Lastly the observation carried out to examine change in behaviour if leeches.

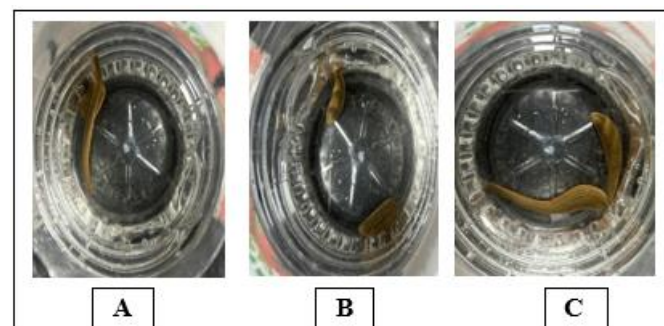
After complete experiment all leeches were separated in a normal water to observe mortality.

**Table 1: Container A**

Solution	Avg. Body Length (cm)	Avg. Weight (gm)	Exposure period	Observation
1 gm of fine extract + 1000 ml of water	16 +/- 2	20 +/- 5	1 hr.	Extended and swimming
			2 hr.	Crawling, moving, walk-like at the bottom
			3 hr.	Elongating, Stretching body at the bottom
			4 hr.	Anchored at top-bottom

**Table 2: Container B**

Solution	Avg. Body Length (cm)	Avg. Weight (gm)	Exposure period	Observation
2 gm of fine extract + 1000 ml of water	16 +/- 2	20 +/- 5	1 hr.	Normal swimming
			2 hr.	Extended with head swaying & crawling
			3 hr.	Anchored & reaching
			4 hr.	Elongating & moving in any direction



**3. Result and Discussion**

Solution	Avg. Body Length (cm)	Avg. Weight (gm)	Exposure period	Observation
5 gm of fine extract + 1000 ml of water	16 +/- 2	20 +/- 5	1 hr.	Extended & swimming
			2 hr.	Crawling
			3 hr.	Swimming in any direction
			4 hr.	Elongating & moving in any direction

According to observation of table 1: Container A contains 1 gm of fine extract of plant extract and 1000 ml of tap water, in this solution 2 leeches of near about same body weight and length and given different exposure as: 1 hr., 2 hr., 3 hr., 4 hr. among these 1 and 2 hr. exposures shows normal behavioural activity of leeches. 3 hr. exposure shows fast movement of leeches. And 4 hr. Exposure shows Anchored at top of the container. Which shows the plant extract have some impact of behavioural patterns of leeches.

According to observation of table 2: Container A contains 2 gm of fine extract of plant extract and 1000 ml of tap water, in this solution 2 leeches of near about same body weight and length and given different exposure as: 1 hr., 2 hr., 3 hr., 4 hr. among these 1 and 2 hr. exposures shows normal behavioural activity of leeches. 3 hr. exposure shows fast movement of leeches. And 4 hr. Exposure shows Elongated & Contraction & Moving in any direction. Which also shows some more impact of plant extracts on behavioural patterns of leeches.

According to observation of table 3: Container A contains 5 gm of fine extract of plant extract and 1000 ml of tap water, in this solution 2 leeches of near about same body weight and length and given different exposure as: 1 hr., 2 hr., 3 hr., 4 hr. among these 1 and 2 hr. exposures shows normal behavioural activity of leeches. 3 hr. exposure shows fast movement in any direction and 4 hr. Exposure shows Elongated & Contraction & Moving in any direction. Which also shows some more impact of plant extracts on behavioural patterns of leeches. Further observation of table 2: container B and table 3: container C shows movement of leeches were as fast as compare to container A. this shows as if we increase in amount of plant extract in the solution then responses of leeches also increased by movement.

#### 4. Conclusion

From this study we have understand that, in nature some plants are toxic and other are not. Among these toxic plants some are directly or indirectly harm human being as well as all flora & fauna. Aquatic ecosystem already gets polluted by so many reasons. Out of this one in a negligible form that is plant extract. Which goes by water run-off to the aquatic ecosystems. Exactly this study focuses on natural hazards of aquatic ecosystems. Some toxic plant shows chronic or acute toxicity on aquatic life. There is also the issue of bioaccumulation which also threat to all living world. after the bioaccumulation of chemicals, enters to human food web. And threat to humans.

In this study if we increase the concentration of plant extract then the behaviour of study animals shows some unaccepted pattern of movement. On the basis of this movement we say that the aggression goes rises as concentration increases. Finally, half of the test animal's dead. Which means plant extract have negative impact on it.

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