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Toxicological Evaluation of *Eclipta alba* using Brine Shrimp (Artemia salina L.) Model

Akshay R.Yadav*, Dr. Shrinivas K. Mohite

[1-2]Department of Pharmaceutical Chemistry, Rajarambapu College of Pharmacy, Kasegaon, Dist- Sangli, Maharashtra, India

*Corresponding author E-mail:- akshayyadav24197@gmail.com

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ABSTRACT

Brine shrimp lethality assay is the most useful tool for tracking the biological behavior of different plant species. This method is useful in advance for the toxicity evaluation of plant extracts. Eclipta alba leaves have long been in use in the Indian Local Medicine System for their antioxidant and anti-inflammatory properties. The toxicity of Eclipta alba herb extracts using this assay was determined within a concentration range of 1mg/ml, 100µg/ml, 10µg/ml and 1µg/ml of the herbal extract being tested. Most studies of the toxicity with a Brine Shrimp lethality test measure toxicity after 24 hours of exposure to the examined sample. The Brine shrimp lethality assay is a perfect method for assessing the toxic potential of plant extracts.

Keywords: Eclipta alba, Brine shrimp lethality assay, Toxicity testing, Artemia salina

I. INTRODUCTION

Now-a-days brine shrimp lethality assay is commonly used to check the bioactive chemicals cytotoxic effect. This is a preliminary screening of plant extracts for toxicity. Subsequently animal model for establishment is recommended[1]. Other top assays at the bench are inhibition of crown gall tumors on potato tuber disks, frond proliferation inhibition in duckweed and yellow fever larvae lethality test. Between them, the lethality test for brine shrimps is the shortest, low cost and effective one[2-3]. The nauplii about 22 mm long, are large enough to observe in a laboratory without high magnification and small enough to hatch in vast amounts without extensive workspace[4]. This is a rapid and thorough test for bioactive compounds of either natural or synthetic origin. It is also a cheap and simple test, since no aseptic techniques are required. It easily uses a large number of species for statistical testing and needs no special equipment, and needs fairly low sample amounts (2-20 mg or less)[5].This in vivo test has been used successively since its introduction for the bioassay-guide fractionation of active cytotoxic and antitumor agents. The expression of poisonous toxicity indicates the state of adverse effects arising from the contact between the toxicant and cell. This interaction is subject to the toxic chemistry and the cell membrane because it may occur in the surface of the cells, the cell body or in the underlying tissues,

and also in the extracellular matrix. Before the toxicants are bound to critical organ including liver and kidneys, toxic effects may occur. In the present analysis of Eclipta alba herb extracts for the brine shrimp lethality test, to determine its toxic properties[6-7]. Microwave extraction has proved to be more effective and efficient than its conventional counterpart, the soxhlet extraction method. The Soxhlet extraction, which is a standard technique, is a continuous solvent extraction method[8-23]. Extraction systems are used to conduct routine solvent extractions of soils, sediments, sludge, polymers and plastics, pulp and paper, biological tissues, textiles and food samples[24-36]. Experiments have proved that microwaves, in comparison with the soxhlet extraction, use a lesser volume of solvent and sample and perform extraction at a much faster rate[26-37]. In the discovery of effective medicines for prevention and treatment, an outbreak of coronavirus disease (COVID-19) caused by the novel extreme acute respiratory syndrome coronavirus-2 (SARS-CoV-2) poses an unprecedented obstacle[37-51]. The proximity to the patient during dental care, high generation of aerosols, and the identification of SARS-CoV-2 in saliva have suggested the oral cavity as a potential reservoir for COVID-19 transmission. Soon, someday, you might be making your own drugs at home. That is because researchers have adapted a 3D printer from basic, readily available medicinal active agents fed into a drug delivery system[52-54].

II. METHODS AND MATERIAL

Plant Material

Eclipta alba has been collected from Karad, Maharashtra, India. Department of Botony, YashwantraoChavan College of Science, Karad has identified the plant and authenticated it.

Preparation of Psidium guajava Leaf Powder

Fresh Eclipta alba herb were collected and air dried for 10 days. The dried leaves were then crushed into a blender to form a coarse powder. The powder was collected in an air-tight jar, and stored away from sunlight in a cool and dry place.

Preparation of Plant Extract

Extraction of Eclipta alba was done by microwave assisted extraction further filtered and excess solvent present was evaporated and dried extract were collected and subjected for activity studies.

Brine Shrimp Toxicity (BST) Assay:

Preparation of seawater

38gm sea salt (without iodine) was weighed, dissolved in one litre of distilled water and filtered off to get clear solution.

Hatching of brine shrimp

Brine shrimp eggs were collected from pet shops was used as the test organism. Seawater was taken in the small tank, and shrimp eggs were moved to one side of the tank, and sealed on this side. The shrimp was allowed to hatch for two days and be matured like nauplii. Constant supply of oxygen was rendered during the process of hatching. The hatched shrimps are drawn to the light (phototaxis), and so egg shell-free nauplii from the illuminated portion of the tank was collected. The nauplii was taken by a pipette from the fish tank and filtered to improve visibility in fresh clear sea water, and 10 nauplii was taken carefully by micropipette.

Preparation of Reagents

Serial dilution of extract

Clean test tubes have been taken and labelled. An analytical balance was measured against plant extract of 10mg. Dissolving 10mg of plant extract (soluble in water) in 1ml of water then prepared stock solution. Concentrations of 1 mg/ml, 100µg/ml, 10µg/ml, and 1µg/ml were prepared from stock solution using serial dilution. Then 1 ml of prepared solution was taken into the test tubes which contain 10 nauplii and 1 ml

of seawater. After 24 hours the number of dead nauplii was counted.

Calculation

The mortality endpoint of this bioassay is defined as the absence of controlled forward motion during 30 seconds of observation. The percentage of nauplii lethality for each concentration was calculated. For each tube, count the number of dead and the number of live nauplii, and determine the % death[55-58].

$$\% \text{ death} = \frac{\text{Number of dead nauplii}}{\text{Number of dead nauplii} + \text{Number of live nauplii}} \times 100$$

III. RESULTS AND DISCUSSION

Different extracts of Eclipta alba used for the cytotoxicity test with help of brine shrimp. It is very useful for BSLA to screen a wide range of extract for their different bioactivities. From results it was found that the methanolic extract shows less % death of nauplii in different concentrations compared to the other extracts.

Table 2. Results of Brine shrimp lethality assay of Eclipta alba extracts

Sr.no	Test subs.	% death nauplii			
		1 mg/ml	100 µg/ml	10 µg/ml	1 µg/ml
1	Methanolic extract	70	30	20	10
2	Ethyl acetate extract	100	70	40	20
3	Ethanolic extract	80	50	30	10
4	Aqueous extract	100	60	50	30

IV. CONCLUSION

While it may be a somewhat inadequate brine shrimp lethality test when it comes to clarifying the mechanism of action, the bioactivity of plant extracts is very beneficial. Indeed, the brine shrimp lethality test proved a convenient way to monitor the biological activities of a number of herbal types that are used in traditional medicine during our studies. There was a great need to use animal model in vivo experiments to gather specific data that could be extrapolated to humans. For years, researchers have used rats and other animal models. The ethics and economic concerns have recently restricted this kind of research. There is also extensive use of alternative toxicity tests to assess the toxicity potentials of plant products. BSLA appeared to be a good approach particularly as in vivo testing could still be classified. Artemia Salina nauplii has shown a strong link to many other animal models, and is one of the alternative for biological toxicity testing on herbal extracts. In the Brine shrimp lethality test, the preliminary knowledge regarding toxicity offers a supportive forum for more toxicity research.

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