IJP (2018), Vol. 5, Issue 7 **(Research Article)**

Received on 27 March, 2018; received in revised form, 12 June, 2018; accepted, 17 June, 2018; published 01 July, 2018

IN-VITRO CYTOTOXICITY OF ASSAY OF LEAVES OF *PORTULACA QUADRIFIDA* USING BRINE SHRIMP ASSAY

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Keywords:

Brine shrimp assay ,Cytotoxicity, SPSS Software, *Portulaca quadrifida*

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ABSTRACT: *Portulaca quadrifida* Linn. (Portulacaceae) is commonly known as chicken weed in English. In traditional system it is used to treat rheumatism and gynaecological diseases, urinary tract infections, worm diseases, dysentry and dermatitis. To assess cytotoxic potential of hydroalcoholic extract o f *P. quadrifida* leaves using brine shrimp assay. Potassium dichromate was considered as standard reference drug. The concentrations of 10, 100, 1000 µg/ml were used in the experiment. The mature shrimps were taken and it was treated with the extract and the standard in the respective concentrations. LC_{50} value was calculated for both standard and extract and the probit analysis were made by using SPSS software. From the probit analysis the cytotoxicity of the extract was determined by comparing it with the standard. Graph was plotted for both the standard and the extract, the extract was found to be non- toxic in nature.

INTRODUCTION: About plant: *P. quadrifida* is mostly found in bare patches of ground and among rocks, on sandy or stony soils, from sea-level up to 2000 m altitude. Mostly it is grows on sand or sandy loams. *P. quadrifida* may occur on alkaline soils but is not so common on saline soils [1].

Cytotoxicity Studies: The term cytotoxicity is the quality of being toxic to the cells. When a particular cell is being treated with a cytotoxic compound it can result in a variety of cell fates such as necrosis in which the cell membrane will lose its integrity and cell lysis will take place. The membrane integrity can be assessed by monitoring the passage of substances that are normally sequestered inside cells to the outside.

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DOI:

10.13040/IJPSR.0975-8232.IJP.5(7).437-439

Article can be accessed online on:
www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.5(7).437-39

Brine Shrimp assay is to measure the cytotoxicity of the extract of *Portulaca quadrifida* Linn, as it's a new method and it hasn't been carried out in many plants. In this method brine shrimp (*Artemia salina*) was used to determine the cytotoxicity when the extract was used as a feed substance for it and kept for few h. This procedure determines the LD_{50} values in microgram/ml of extract in the brine medium. The cytotoxicity studies are done to prove whether the plant is harmful in nature or not, since plants are used world-wide mainly as medicines and cosmetics. The cytotoxicity analysis was carried out on *Portulaca quadrifida* as it was found to be an anticancer drug and thus by doing the studies we wanted to prove whether it was toxic or not.

MATERIALS AND METHODS:

Preparation of Plant Extract: The leaves were initially separated and washed thoroughly with water and distilled water. The leaves were allowed to air dry for about 10 - 20 days. It is then finely powdered and placed in a container [3].The powdered crude drug was subjected to extraction with

hydroalcohol (1:1 ratio of ethanol and distilled water). The crude drug was subjected to cold maceration for 72 h and the extract was filtered out. The filtered extract was subjected to distillation to remove the solvent and further evaporated to concentrate the extract [4].

Brine Shrimp Lethality Assay:

Hatching of *Artemia salina* Cysts: 33.3g of red sea salt was dissolved in 500ml of sterilized distilled water. About 100g of cysts were allowed to soak in sterilized distilled water to bring back to room temperature. Then it is transferred to a separating funnel consisting of sterilized sea water. Suitable hatching environment should be maintained [5].

Dilution of Extracts: A concentration of 1000µg/ml, 100µg/ml, 10µg/ml were prepared. A stock of 10mg in 10ml of sterilized distilled water was prepared. From stock 1ml was pipetted out to give 1000µg/ml of concentration. 0.1ml of stock was pipetted out and the volume made up to 1ml with sterilized distilled water to produce a concentration of 100µg/ml. Similarly, 0.01ml of stock was pipetted out and the volume made up to 1ml with sterilized distilled water to produce a concentration of 10µg/ml [6].

Plating of Nauplii to the Extracts: Potassium dichromate was used as an agent which induces cytotoxicity to cells. A solution of potassium dichromate was prepared with the same sterilized distilled water with a concentration of 1% potassium dichromate. In two different 24 well plates, 1% potassium dichromate and the dilutions prepared with extract were added in triplicates. Potassium dichromate was also prepared to same concentration as that of the extract. With 1ml of potassium dichromate and extract in the plates, the volume was made up with sterilized sea water to 3/4th of the capacity of the wells. Sterilized distilled water and sea water was used as control [6].

RESULT AND DISCUSSION:

TABLE 1: NAUPLII OBSERVED IN HYDROALCOHOLIC PLANT EXTRACT OF *PORTULACA QUADRIFIDA*

Concentration	No. of Nauplii Alive (24 h)				No. of Nauplii Alive (48)			
	T_1	T_2	T_3	Average	T_1	T_2	T_3	Average
10 µg/ml	10	10	10	10	10	10	10	10
100 µg/ml	10	10	10	10	10	10	10	10
1000 µg/ml	8	7	8	8	7	8	7	7
Control	10	10	10	10	10	10	10	10

TABLE 2: NAUPLII OBSERVED IN POTASSIUM DICHROMATE

Concentration	No. of Nauplii Alive (24 h)				No. of Nauplii Alive (48 h)			
	T_1	T_2	T_3	Average	T_1	T_2	T_3	Average
10 µg/ml	8	8	7	8	7	6	7	7
100 µg/ml	5	4	5	5	3	4	4	4
1000 µg/ml	3	3	2	3	2	2	3	2
Control	10	10	10	10	10	10	10	10

Where, T_1- Trial 1, T_2 -Trial 2, T_3 -Trial 3

TABLE 3: LETHALITY DOSE FOR POTASSIUM DICHROMATE AT 24 h

Log Concentration	Exposure Concentration (µg/ml)	Confidence Limits for Concentration	
		Lower	Upper
10	1.812	0	17.648
20	8.060	0	47.334
30	23.639	0	118.369
40	59.283	0.099	461.894
50	140.004	9.487	12160.922
60	330.639	61.07	4742346.880

TABLE 4: LETHALITY DOSE FOR POTASSIUM DICHROMATE AT 48 h

Log Concentration	Exposure Concentration (µg/ml)	Confidence Limits For Concentration	
		Lower	Upper
10	1.812	0	9.092
20	8.060	0	22.698
30	23.639	0	48.0346
40	59.283	0.098	112.218
50	140.004	0.182	448.755
60	330.639	10.408	13871.776

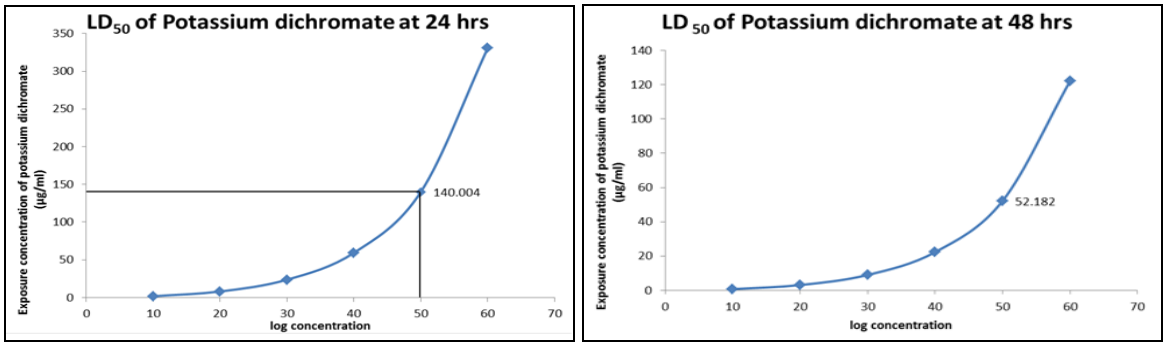

FIG. 1: LETHALITY DOSE FOR POTASSIUM DICHROMATE AT 24 h and 48 h

From the number of shrimps that was alive after the treatment of extract and the standard the LC_{50} value was calculated for both standard and the extract and the Probit analysis were made by using SPSS software. Using Probit analysis the cytotoxicity of the extract was determined by comparing it with the standard. Graph was plotted for both the standard and the extract, the extract was found to be non- toxic in nature.

CONCLUSION: The Brine shrimp results in this study are interpreted as follows:

LC_{50}< 1.0µg/ml - Highly toxic
LC_{50} 1.0 – 10.0µg/ml - Toxic
LC_{50} 10.0 – 30.0µg/ml - Moderately Toxic
LC_{50} <100µg/ml- Mildly Toxic
LC_{50}>100µg/ml - Non-Toxic

The brine shrimp test for the plant *Portulaca quadrifida* results indicate that the leaf extract had LC50 values > 100µg/ml which suggests that they are practically non-toxic when compared to that of Potassium dichromate. So, In conclusion the leaf extracts of *Portulaca quadrifida* exhibited against the brine shrimp. This may be due to non-toxic active principles present in the extract. This confirms that the leaf can be used as a nutraceutical.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: Nil

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How to cite this article:
Kumaran RT and Naidu N: *In-vitro* cytotoxicity of assay of leaves of *Portulaca quadrifida* using brine shrimp assay. Int J Pharmacognosy 2018; 5(7): 437-39. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.5(7).437-39.