Open Access Journal

Indian Journal of Medical Research and Pharmaceutical Sciences

August 2018;5(8) ISSN: ISSN: 2349-5340

DOI: 10.5281/zenodo.1341611 Impact Factor: 4.054

EVALUATION FOR CYTOTOXIC POTENTIAL OF LEAF EXTRACT OF CALOTROPIS GIGANTIA

Hadpad Shobha*, Gharge Varsha, Jadhav Rahul, Lokhande Tulashiram & Sharma Ajaykumar

Pharmaceutics, Gourishankar Institute of Pharmaceutical Education & Research, Limb, Satara, Maharashtra, India

Keywords:

C.gigantia, leaf extract, Cytotoxic Potential, % inhibition.

Abstract

In the present study we have utilized the BSLB model (Brine shrimp lethality bioassay) and *Allium cepa* root meristem (ACRM) models to evaluate the cytotoxic activity of ethanolic leaf extract of *c. gigantia*. In BSLB model LC_{50} value of leaf extract was found to be 67.25 µg/ml while LC_{50} value of 5-flurouracil was found to be 66.01 µg/ml extract produced dose dependent growth inhibition.

In ACRM model incubation of onion bulbs in different concentration of extract produced a growth retarding effect that was associated with a decreased in root number. Standard cytotoxic drug 5-flurouracil and non- cytotoxic drugs aspirin served as controls like 5-flurouracil. Ethanolic leaf extract significantly inhibited the growth of roots. In ACRM model % inhibition of root growth in leaf extract (ethanolic) after 48hrs was found to be 60.64% (1mg/ml), 72.35% (10mg/ml) and after 96hrs was found to be 85.85% (1mg/ml), 98.31% (10mg/ml) % inhibition of root growth in 5-flurouracil after 48hrs was found to be 63.83% (1mg/ml), 85.106% (10mg/ml) and after 96hrs was found to be 73.46% (1mg/ml), 96.46% (10mg/ml).

Introduction

Extract produced dose dependent growth inhibition. Ethanolic leaf extract of *C.gigantia* exhibits potent cytotoxic property comparable to standard drug. Plant has been useful sources of clinically relevant antitumor compound. *Calotropis gigantia* Linn (Asclepiadaceae) is a shrub found throughout India [1]. Leaves and aerial parts of the plant are reported for antidiarrheal activity, anticandida activity, anticancer activity, antibacterial activity and antioxidant activity [2]. The traditionally C.gigantia is used as analgesic [3] cures tooth ache and earache [4, 5] sprain [6] an anxiety [7, 8] epilepsy [9] and mental disorder [10]. Ayurvedic preparation containing Calotropis gigantia and leaves were extensively used by ayurvedic physicians for treatment of disorder diabetes mellitus, bronchial asthma [11, 12] chloroform extract of C.gigantia leaves prevent insulin induced resistance in high fructose diet [13] . Various scientific studies reported this plant as contraceptives for human [14], sedative, anxiolytic and anticonvulsant [15] .our study demonstrated the cytotoxic activity of ethanolic extract of leaf of Calotropis gigantia plant by reported model of BSLB and Allium cepa root model (ACRM).

Indian Journal of Medical Research and Pharmaceutical Sciences
August 2018;5(8) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.1341611 Impact Factor: 4.054

Figure:

Figure No.1: Calotropis gigantia

Materials and Method

Material

The *Calotropis gigantia* leaves were collected from Satara District. The plant was identified and authenticated by head of department, Botany, Yashvantrao Chavan College, satara. All the chemicals used for experimental purpose were of laboratory grade. Brine shrimp eggs were purchase from Aquatic remedies Chennai and standard drug i.e. 5-flurouracil was gifted by Ozone international, Mumbai.

Dried leaves of *Calotropis gigantia* was subjected for extraction with ethanol in soxhlet apparatus. Extract was filtered dried at 50^0c.

Methods

Determination of physical constant

1. Ash Value
    a. Total ash
    b. Water soluble ash
    c. Acid insoluble ash
2. Loss on Drying

Preliminary Pharmacognostic Characteristics:

In present study, the all leaves plants were investigated for its macroscopic Characteristics.

Construction of soxhlet apparatus:

A Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. It was originally designed for the extraction of a lipid from a solid material. However, a Soxhlet extractor is not limited to the extraction of lipids. Typically, a Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a significant solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance.

Powder extraction in progress. The sample is placed in the thimble. Normally a solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser. The solvent is heated to reflux. The solvent vapour travels up a

Indian Journal of Medical Research and Pharmaceutical Sciences
August 2018;5(8) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.1341611 Impact Factor: 4.054

distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. The thimble ensures that the rapid motion of the solvent does not transport any solid material to the still pot. This cycle may be allowed to repeat many times, over hours or days**.**

Figure No.2: A Soxhlet extractor

During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded.

Working of soxhlet apparatus:

The soxhlet assembly is a continuous extractor which is generally suitable for the extraction of alkaloids from powdered plant materials with the help of organic solvents. In this instance, the powdered drug is usually moistened with dilute ammonia solution and then packed loosely in the thimble of the soxhlet apparatus; and the organic solvent affords a deep penetration of the moist drug therapy allowing the greatest possible extraction of the alkaloids from exposed surfaces of the cell and tissues of the crude drug. Once, the extraction is ascertained to have competed, the solvent is filtered and evaporated in a Rotary Thin –Film Evaporator and residue is treated further for the isolation of the individual alkaloids.

Extraction:

In the present study, the dried, leaves and powder of *calotropis gigantia Linn*belonging to family Asclepiadaceae were reduced to coarse powder (# 40 size mesh) and around 200gm. was subjected to hot continuous extraction

Indian Journal of Medical Research and Pharmaceutical Sciences
August 2018;5(8) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.1341611 Impact Factor: 4.054

(soxhlet) with, ethanol and then water. After the effective extraction, solvent were evaporated to dryness and the extract obtained with each solvent was weighed. Its percentage yield obtained was shown in Table no. 2

Determination of Ash Values

Ash values are helpful in determining the quality and purity of a crude drug, especially in the powdered form. The objective of washing vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in an analytical determination. On incineration, crude drugs normally leave an ash usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The total ash of a crude drug reflects the care taken in its preparation. A higher limit of acid-insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high.

Total ash value

Weighed accurately about 2 to 3 g of the powdered drug in a tarred silica crucible. Incinerated at a temperature not exceeding 450^0C for 4 hrs.Until free from carbon, cooled and weighed. Calculated the percentage of ash with reference to air-dried drug using following formula,

$$\% \text{ Total ash value} = \frac{\text{Wt. of total ash}}{\text{Wt. of crude drug taken}} \text{ X } 100$$

Water soluble ash value: The ash was boiled with 25 ml of water. Filtered and collected the insoluble matter on an ashless filter paper, washed with hot water and ignited in a tarred

crucible at a temperature not exceeding 450^0C for 4 hrs. Cooled in desiccators, weighed and subtracted the weight of insoluble matter from the total weight of ash. The difference in weight represented weight of water soluble ash. Calculated the percentage of water soluble ash with reference to the air-dried drug using the following formula-

$$\% \text{ Water soluble ash value} = \frac{\text{Wt. of total ash - Wt. of water insoluble ash}}{\text{Wt. of crude drug taken}} \text{ X } 100$$

Acid insoluble ash value

Boiled the ash for 5 min with 25 ml of 2 M HCl. Filtered and collected the insoluble matter on an ashless filter paper, washed with hot water and ignited in a tarred crucible at a temperature not exceeding 450^0C for 4 h. Cooled in a desiccators and weighed. Calculated the percentage of acid insoluble ash with reference to the air-dried drug using following formula,

$$\% \text{ Acid insoluble ash value} = \frac{\text{Wt. of acid insoluble ash}}{\text{Wt. of crude drug taken}} \text{ X } 100$$

Loss On Drying

Loss on drying is the loss of mass expressed as per cent w/w. The test for loss on drying determines both water and volatile matter in the crude drug. Moisture is an inevitable component of crude drug, which must be eliminated as far as possible. An accurately weighed quantity of about 5 g of powdered drug was taken in a tarred porcelain dish. The powder was distributed evenly. The porcelain dish kept open in vacuum oven and the sample was dried at a temperature 110°C for 2 hrs.Until a constant weight was recorded. Then it was cooled in a desiccator to room temperature, weighed and recorded. % Loss on drying was calculated using the following formula.

$$\% \text{ Loss on drying} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample}} \text{ X } 100$$

Indian Journal of Medical Research and Pharmaceutical Sciences
August 2018;5(8) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.1341611 Impact Factor: 4.054

Extractive Values

The extractive values for various solvents of air-dried sample were evaluated.

1) Ethanol soluble extractive.
2) Aqueous soluble extractive

Ethanol soluble extractive value:-

20 grams of air-dried leaf powder of *Calotropis gigantia* was macerated with 200 ml of Ethanol in a closed flask, shaking frequently during the first 6 hours and allowed to stand for 18 hours separately. Thereafter, it was filtered rapidly taking precaution against loss of Ethanol. Evaporated 25 ml of filtrate to dryness in a tarred flat bottom shallow dish dried at 105^0 C and weighed. Percentage alcohol soluble extractive was calculated with reference to the air-dried drug.

Aqueous soluble extractive value:-

20 grams of air-dried leaf powder of *Calotropis gigantia* added to 200 ml of water at 80^0C in a stoppered flask separately. It was then shaken well and allowed to stand for 10 minutes so as to cool it and filtered. 5ml of filtrate was transferred to an evaporating dish, which was 7.5 cm in diameter, the solvent was evaporated on water bath, allowed to dry for 30 minutes, finally dried in an oven for 2 hours at 100^0C and residue was weighed. Percentage of water-soluble extractives was calculated with reference to the air-dried drug. The results are shown in table no.3-

Qualitative Chemical Investigation

The ethanol and aqueous extracts were subjected to qualitative chemical investigation.

Chromatographic Studies:

Thin Layer Chromatography- studies were carried out for various extracts to confirm the presence of different phytoconstituents in these extract. TLC is a mode of liquid chromatography, in which, the extract is applied as a small spot or band at the origin of thin sorbent layer supported on a glass/plastic/metal plate. The mobile phase migrates through the stationary phase by capillary action. The separation of solutes takes place due to their differential absorption/ partition coefficient with respect to both mobile and stationary phases. Each separated component has same migration time but different migration distance.

The mobile phase consists of a single solvent or a mixture of solvents. Although, a number of sorbent like silica gel, cellulose, polyamide, alumina, chemically modified silica gel etc. are used, silica gel (type 60) is most commonly used sorbent. Handmade plates are prepared by using techniques like pouring, dipping or spraying. Now days, readymade precoated plates are also available. The plates need to be activated at 110^0c for 1h. This removes water/ moisture loosely bound to silica gel surface.

The retardation factor (R_F) is calculated using following formula,

R_F = Distance traveled by sample from base line/ Distance traveled by solvent from base line

Thin Layer Chromatography

The extracts were subjected to thin layer chromatography for the presence of phytoconstituents. In this technique, the Silica gel-G (for TLC) was used as an adsorbent and plates were prepared by spreading technique, then air dried for an over-night and activated for one hour at 110°C and used.

Cytotoxic activity-

Brine shrimp lethality bioassay (BSLB)

Eggs of Brine shrimp were kept for hatching in sterile artificial sea water under constant aeration for 72 hrs. PH was adjusted to 8.5 using 1 N NaOH to avoid death of larvae.

After 48hrs yeast solution was added in order to feed larvae. After 72hrs hatching takes place and active nauplii were collected and used for assay.

Indian Journal of Medical Research and Pharmaceutical Sciences
August 2018;5(8) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.1341611 Impact Factor: 4.054

8 concentrations of plant extract, 8 concentrations of standard drug (5-flurouracil) (10, 20, 50, 100, 500, 1000, 2000, 5000) µg/ml were tested to determine dose response relationship and control group was set with vehicle used for dilution.

30 nauplii were drawn through a glass capillary in petriplate containing 10 ml artificial sea water. After 24hrs live nauplii were counted and LC_{50} value was estimated.

Percent lethality was determined by comparing surviving nauplii of test and control. LC_{50} value were obtained from concentration verses % lethality using statistical model.

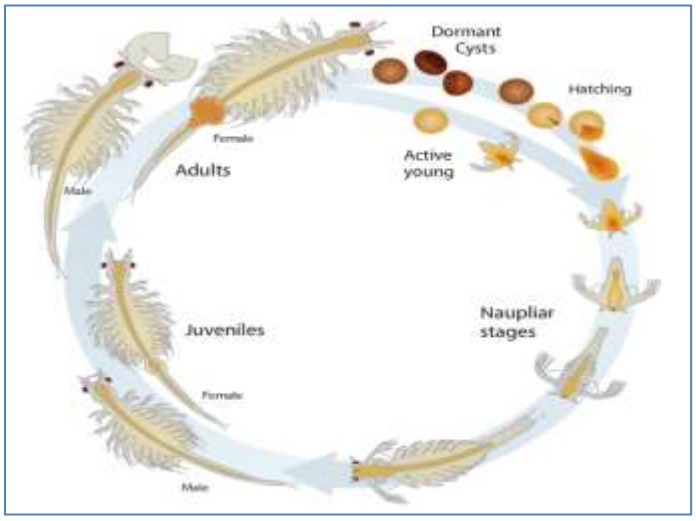

Figure No.3: Brine shrimp lethality bioassay (BSLB)

***Allium cepa* root tip meristem (ACRM) model-**

Locally available onion bulb (Allium cepa 50g ± 10g) were obtained and grown in the dark over 100 ml tap water at ambient temperature until the roots have grown to approximately 4-5 cm length. The base of each of the bulbs were suspended on the extract inside 100 ml beakers, root length (newly appearing roots not include) and root number at 0, 48, 96, hrs.For each concentration of extract and control was measured. The percentage root growth inhibition after treating with ethanolic extract at 48, and 96 hrs.Was determined. Working dilutions of all the drug were made in water. 5-flurouracil (standard) as well as extracts of leaf was used at 1mg/ml and 10 mg/ml concentration.

Microscopic examination and determination of mitotic index-

The root tips (2-3mms) were collected, placed in 1 N HCl for 5 minutes, squashed and finally stained with 2% aceto–orceine. For each root tip the number of mitotic and total meristematic cells were counted in 5-8 fields using high power (100x) light microscope. In all 400-500 cells were counted and cells manifesting different stages of mitosis i.e., interphase and prophase (p), metaphase (m), anaphase (a) and telophase (t) were recorded. The mitotic index was calculated using the following formula;

$$\text{Mitotic index} = \frac{P+M+A+T}{\text{Total cell}} \times 100$$

Open Access Journal

Indian Journal of Medical Research and Pharmaceutical Sciences

August 2018;5(8) ISSN: ISSN: 2349-5340

DOI: 10.5281/zenodo.1341611 Impact Factor: 4.054

Preliminary Pharmacognostic Characteristics

Table no 1 In present study, the all leaves plants were investigated for its macroscopic characteristics.

Sr no	Parameter	Observation of *calotropis gigantia*
1.	Colour	Green
2.	Odour	Aromatic
3.	Taste	Characteristics

Table No. 2 Observation table of Nature, Colour, Yield of Calotropis gigantia extract

No.	Extracts	ture of Extract	Colour	Weight (g)	%Yield(w/w)
1	Ethanolic	Solid	Brown	9.1	7.6
2	Aqueous	Solid	Brown	10.2	12.8

Fig no.4 (a)

Fig.no.4 (b)

Determination of Ash Values

Table No. 3 Physical Constants of plants

Sr. No.	Physical Constants	*Calotropis gigantia*
1.	Ash Value (% w/w) • Total Ash • Acid Insoluble Ash • Water Soluble Ash	 80.5 % 4 2.8
2.	Loss on Drying (% w/w)	4.6
3.	Extractive Values (%) ➢ Ethanol soluble extractive. ➢ Aqueous soluble extractive	 0.80 1.60

Indian Journal of Medical Research and Pharmaceutical Sciences
August 2018;5(8) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.1341611 Impact Factor: 4.054

Table No. 4 Qualitative Chemical Investigation of Calotropis gigantia Extract

Sr No.	Name Of The Test	*Calotropis gigantia*	
		Ethanol	**Aqueous**
1.	Test for sterols	-	-
2.	Test for Triterpenoids	_	_
3.	Test for glycosides	-	-
4.	Test for carbohydrates	_	_
5.	Test for alkaloids	+	+
6.	Test for flavonoids	-	-
7.	Test for tannins	+	+
8.	Tests for proteins	-	_
9.	Test for amino acids	-	_
10.	Test for fats	_	_
11	Test for Volatile oils	_	_

Ethanolic extract **Aqueous extract**

Fig no.5 TLC

Table No-5 TLC Profile

Extract	**Observation**		**R_f values**
	No. of spots	**Colour of spots**	
Ethanolic	1	Yellowish Orange	0.5
Aqueous	1	Yellowish	0.5

Indian Journal of Medical Research and Pharmaceutical Sciences
August 2018;5(8) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.1341611 Impact Factor: 4.054

Mobile Phase- Ammonia: Ethyl acetateBrine shrimp lethality activity of ethanolic leaf extract of *Calotropis gigantia* shows that mortality increases as concentration of extract increases. The ethanolic extract of Calotropis gigantia have shown the LC_{50} value of 67.25ug/ml while LC_{50} of 5-flurouracil was found to be 66.01µg/ml. *Calotropis gigantia* has potent cytotoxic effect similar to standard.

Fig no 5 average mortality for control

Table no 6 Observation of average mortality for control

control	concentration	No. of petriplate	No. of shrimp tested	Average mortality after 24hrs	% mortality after 24hrs
Without drug	-	1	30	00	00

Table no 7: Observation of average mortality and LC50 value: 5-flurouracil

Sr.no.	Concentration ug/ml	No. of petriplate	No. of shrimp test	Average mortality after 24 hrs	% average mortality	LC_{50} ug/ml
1	10	1	30	5	17	66.01
2	20	2	30	6	20	
3	50	3	30	10	34	
4	100	4	30	22	74	
5	500	5	30	26	87	
6	1000	6	30	28	94	
7	2000	7	30	30	100	
8	5000	8	30	30	100	

Table no 8: Observation of average mortality and LC_{50} value of ethanolic extract of leaf

Sr.no.	Concentration ug/ml	No. of petriplate	No. of shrimp test	Average mortality after 24 hrs	% average mortality	LC_{50} ug/ml
1	10	1	30	5	17	
2	20	2	30	6	20	
3	50	3	30	15	50	

Indian Journal of Medical Research and Pharmaceutical Sciences
August 2018;5(8) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.1341611 Impact Factor: 4.054

4	100	4	30	20	67	67.25
5	500	5	30	26	87	
6	1000	6	30	29	97	
7	2000	7	30	30	100	
8	5000	8	30	30	100	

Table no 9: Observation of average mortality and LC_{50} value of Aqueous extract of leaf

Sr.no.	Concentration ug/ml	No. of petriplate	No. of shrimp test	Average mortality after 24 hrs	% average mortality	LC_{50} ug/ml
1	10	1	30	5	17	60.25
2	20	2	30	6	20	
3	50	3	30	8	27	
4	100	4	30	18	60	
5	500	5	30	22	74	
6	1000	6	30	25	84	
7	2000	7	30	30	100	
8	5000	8	30	30	100	

***Allium cepa* root model-**

(a) Control *(b) 5-flurouracil treated* *(c) Extract treated*

Figure 7: Allium cepa bulb showing the effect of different of roots of c.gigantea on root length following 96hrs of incubation

The cytotoxic effect of ethanolic extract of *Calotropis gigantia* leaf was also evaluated on the *Allium cepa* root meristems. A progressive increase in root number and root length was observed in control group. The root length in control group at 0, 48, and 96hrs was 5.36 cm (n=21), 6.30cm (n=23), 6.49cm (n=28) respectively. The leaf extract produced dose and time dependent growth inhibition. Incubation of bulbs in different concentration of cytotoxic agents produced a growth retarding effect that was associated with a decrease in the root number. *Allium cepa* root tip meristem growth inhibition was highest with significance of ($p<0.01$) at the 10mg/ml concentration after 48hrs for ethanolic leaf. The root length after 0, 48 and 96hrs with significance of ($p<0.01$) at 10mg/ml was found to be 4.88 (n=67), 5.14 (n=55), 4.98 (n=53) respectively. Extract produced dose and time dependent percentage growth inhibition, shows maximum 72.35%root growth inhibition after 48 hrs. The ethanolic leaf extract of *C .gigantia* exhibits potent cytotoxic property comparable to standard anticancer drug.

Indian Journal of Medical Research and Pharmaceutical Sciences
August 2018;5(8) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.1341611 Impact Factor: 4.054

Table 10: Observations for Allium cepa root length and root number attained following incubation with extract of C.gigantia leaf and standard drug.

Groups	Concentration	Root length (cm)		
		0 hrs	48 hrs	96 hrs
Control	-	5.36 n= 21	6.30 n= 23	6.49 n= 28
Ethanol	1 mg/ml	5.62 n= 65	5.99 n= 68	5.78 n= 70
	10 mg/ml	4.88 n= 67	5.14 n= 55	4.98 n= 53
5-flurouracil	1 mg/ml	3.85 n= 60	4.19 n= 69	4.15 n= 70
	10 mg/ml	3.94 n= 50	4.08 n= 50	3.98 n= 49
Aqueous	1 mg/ml	3.75 n= 45	4.15 n= 48	4.10 n= 50
	10 mg/ml	3.90 n= 35	4 n= 30	3.92 n= 29
Aspirin	1 mg/ml	3.70 n= 64	4.10 n= 66	4.5 n= 70
	10 mg/ml	3.85 n= 65	3.5 n=60	4.20 n= 58

Table11: Root growth after treating with drugs

Groups	Concentration	Root growth after (cm)	
		48 hrs	96 hrs
Control	-	0.94	1.30
Ethanol extract	1 mg/ml	0.37	0.16
	10 mg/ml	0.26	1.90
5-flurouracil	1 mg/ml	0.34	0.3
	10 mg/ml	0.14	0.04
Aqueous extract	1 mg/ml	0.40	0.35
	10 mg/ml	0.10	0.02
Aspirin	1 mg/ml	0.4	0.8
	10 mg/ml	0.35	0.35

Indian Journal of Medical Research and Pharmaceutical Sciences
August 2018;5(8) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.1341611 Impact Factor: 4.054

Table 12: % Inhibition of root for different extract at 48 & 96 hrs

Extract	Concentration	% inhibition	
		48 hrs	96 hrs
Control	-	-	-
Ethanol extract	1 mg/ml	60.64	85.85
	10 mg/ml	72.35	98.31
5-flurouracil	1 mg/ml	63.83	73.46
	10 mg/ml	85.106	96.46
Aqueous extract	1 mg/ml	57.44	69.07
	10 mg/ml	89.36	98.23
Aspirin	1 mg/ml	57.44	29.20
	10 mml	62.76	69.02

Table 13: Mitotic index in Allium cepa meristems following incubation with various drugs.

Groups	Concentration	Mitotic Index		
		0 hrs	48 hrs	96 hrs
Control	-	64	62	64
Ethanol extract	1 mg/ml	62	48	47
	10 mg/ml	64	50	39
5-flurouracil	1 mg/ml	65	58	50
	10 mg/ml	67	60	58
Aqueous extract	1 mg/ml	61	47	45
	10 mg/ml	60	49	47
Aspirin	1 mg/ml	50	45	44
	10 mg/ml	52	48	45

Mitotic Index-

The mitotic cells were counted in the root meristem in above groups at 0, 48, and 96 hrs, of incubation with each drug. The mitotic index ranged between 62 & 64 in the. As compared to 62 at 0hr while at 96hrs the cellular morphology was lost. Treatment with 5-flurouracil at 1mg/ml and 10mg/ml concentration. Brought down the

Indian Journal of Medical Research and Pharmaceutical Sciences
August 2018;5(8) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.1341611 Impact Factor: 4.054

mitotic index to 64 and control group over a period of 96hrs. The mitotic index at 10mg/ml concentration of ethanolic extract was 50 at 48hrs39 at 96 hrs. Aspirin produced only a marginal decrease in mitotic index at 1mg/ml & 10mg/ml concentration

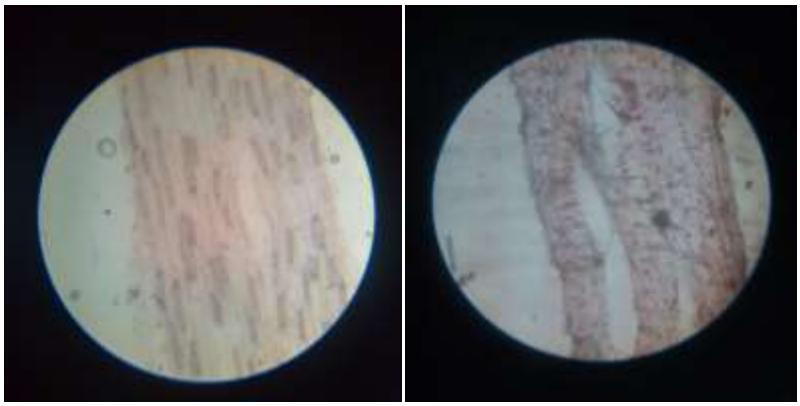

Fig no 8 Mitotic Index

Discussion

In the present study BSLB activity of ethanolic extract of *C.gigantia* showed that mortality is increased as concentration of extract increases. There is linear dose effect relationship between extract concentration & LC_{50} value. Ethanolic extract show LC_{50} value 67.25 while LC_{50} of 5-flurouracil was found to be 66.01 µg/ml. LC_{50} value for extract of leaf was found to be lower than 100ug/ml. Thus leaf extract of *C.gigantia* is bioactive containing physiologically active.

ACRM – *Allium cepa* root tip meristems have been widely used for the evaluation of cytotoxic and antimitotic activity of various compounds. In the present study we have tested the cytotoxic and anti-mitotic effect of ethanolic leaf extract against standard anticancer drugs, 5-flurouracil in *Allium cepa* root tip meristem model compared it with non-cytotoxic drugs aspirin.

The cytotoxic effect of ethanolic leaf extract was comparable to that of 5-flurouracil and both agents inhibited root growth and mitosis to a significant are growing have been used, probably drug might have mitosis inhibitory activity further study is required to clear this aspect, thus, this study demonstrates that ethanolic &aqueous leaf extract of *C.gigantia* exhibits potent cytotoxic property comparable to standard anticancer drugs.

Conclusion

The result of study revealed that ethanolic & aqueous extract of *C.gigantia* exhibits potent cytotoxic activity. Thus further isolated lead compound might be utilized for development of novel anticancer drug.

Acknowledgement

It is not easy to express our emotions in words especially when author have to say thanks to our research guide Miss Belwalkar Shraddha for his valuable guidance throughout the course of this project. The authors are sincerely thanks to the Gourishankar Institute of Pharmaceutical Education & Research, Limb, Satara, India for providing the facilities to complete this research work.

References

1. Pirzoda, Q.,R., 2003.International workshop cum training course on natural products, pharmaceutical and Kamuhabwa A, Ishim c, Witte p. Cytotoxicity of some medicinal plant extract used in Tanranium traditional medicine. J Ethnopharmacol 2000; 70:143-149.
2. Atari c, A Review on pharmacological And Biological properties of Calotropis gigantia, International Journal of Recent scitific Research, 2014, 5(4). Page no- 716-719.
3. Kiritikar KR, Basu BD, Indian medicinal plant, sudhindra Nath Basu, Allahabad, 1995, 3, page no-609.

August 2018;5(8) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.1341611 Impact Factor: 4.054

4. Girach RD, Ahmad A, Ahmad M. Observation on ethanobotany of the Bhunjia-a tribe of Sonabera plateau, kalahandi, Orissa .Ethno botany 1993; 5:84.
5. Allen TF. Handbook of material medica & Homeopathic Therapeutics, Jain publisher (p) Ltd, New DELHI, 1994, PP-251.
6. Manandhar MP, Folk-lore Medicine of Chitwan district Nepal, Ethnobotany, 1990-2:33.
7. Nanu R Rathod, Havagiray R Chitme, Hypoglycemic Effect of Calotropis gigantia linn. Leaves and Flowers streptozotocin-Induced Diabetic Rats OMAN Medical Journal, 2011.26 (2) pp-104-108.
8. Sharma v.Dravyaguna Vigyan, Evaluation of Anti-inflamentory activity of latex of C.procera in different modes of inflammation, Chaukhambala Bharti Academy, Varanasi, 2001, 2, PP-435.
9. Gaurav Kumar, Loganathan Karthik, Kokati venkata Bhoskra Rao; Antibacterial Activity of Aqueous extract of calotropis gigantia leaves-An In Vitro study, Molecular and microbiology Research laboratory,2010 4(2). Pg-141-144.
10. Tenzin Choedon, Ganeshan Manthan, Sonera Arya, Anticancer and Cytotoxic properties of the latex of calotropis procera transgenic mouse model of hepatocellular carcinoma,word Journal of Gastroenterology,2006,12(16) pp-2517-2522.
11. Mitra A, Chakraborty s, Auddy B, Tripathi p, sens s, Evaluation of chemical cofree-radical scavenging activity of swarna bhasma(gold ash),an Ayurvedic drug. J Ethanopharmaco (2-3)147-153, 10.1016 (50378-8741 (02)00008-[pubmed] [Cross Ref].
12. Chetty M, Sivaji k, Rao Kt. Maristematic region of Allium cepa root tip, chitter district Andhra Pradesh, lethed. Tirupa Published By Student Offset Printer; 2008, PP-202.
13. Nanu R, Raghuveer I, Chitme HR, Ramesh Chandra. Prevention of hifh fructose diet induced Ir resistance by NY catanthes arbortristis and calotropis gigantia in rats. Pharmacognocy magazine 2006 (suppl 19):58-63.
14. Srivastava SR, Keshri G, Bhargavan B, Singnc,Singh MM. Pregnancy interaptive activity of the roots of calotropis gigantia Linn. In rats contraception 2007; 75:318-322.
15. Argal A, Pathak AK, CNS activity of calotropis gigantia roots. J Ethnopharmacol 2006; 106:142-145.
16. KD Tripathi, Essential of medical pharmacology, seventh Edition.pg-857-858.
17. Jose luis carball;Zaira L Hernanda,Maria D, A Comparision between two brine cytotocity in marine natural property, BMC Biotechnology Journal 2002 2(17).472-675.
18. Migliore L, Civitareale c, Brambila G, Didelapis GD: Toxicity of several important agricultured antibiotics to Artemia a.wat Res 1997, 31:1801-1806.
19. Mathews RS, Aretemia salina as a test organism Br measuring superoxide-mediated toxicity. Free Radia Biol.med.1995; 18(5):919-21.
20. Shinho k. Anti-tumor glycol proteins from chlorella and other species. JPN Kokai Tokkyo Koha.1984:105.
21. Raut G.R.Dubey H,Tenpe R.C,yeole G.R,Patole m.A,cytotoxic activity of ethanolic root extract of calotropis gigantia, International Journal of Drug development &Research ,2011,3(4)101-108.
22. Pisutthanan s, Plianbangchang p,Pisutthanon N,Ruanruay s,Muanrit o,Brine shrimp Lethality Activity of Thai medicinal plants in the Family meliaceae,Naresuan uni J2004;12(2)13-18.
23. R.Sehgal, S.Roy & V.L.Kumar, Evaluation of cytotoxic potential of latex of calotropis procera and podophyllotoxin in Allium cepa root model.2006, 30(1), 9-13.
24. Akinboro A,Bakare A.Cytotoxic and genotoxic effect of aqueous extract of five medicinal plants on Allium cepa Linn.J.Ethnopharmacol 2007;112:470-475.
25. Oyedare B,Bakare A, Akinboro A.Genotoxicity assessment of water extracts of acimum gratissimum, morinda Lucida and citrus media using the Allium cepa assay.Bol Latinoam cariba plant med Aromat 2009; 8(2):97-103.
26. Seth c, misra v, Chauhan c, singh R.Genotoxicity of cadmium on root meristems cells of Allium cepa: cytogenetic and comet assay approach, Ecotoxicology and Enviro safety 2008; 71:711-716.
27. Sharma CBSR (1983).Plant meristems as monitors of genetic toxicity of environmental chemicals.curr sci.52:1000-1002.
28. Rudolph; et al (1998),'correlation between mitotic and ki-67 labelling indices in paraffin embedded carcinoma specimens Human pathology .29; 1216-1222.doi: 10.1016/s0046-8177(98)90248-9.
29. Baak,J.P.A; Gudlaugsson E;Skalandl Gua L.H.R; KIOS,J;Lende, T.HSoiland,H.V;JANSSEN E.A.M;Zur Hausen A.(2008)"Proliferation is the strongest prognosticator in node-negative breast cancer; significance

August 2018;5(8) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.1341611 Impact Factor: 4.054

error sources, alternative and comparison with molecular prognostic marker "Breast cancer Research and Treatment.115(2)241-254.doi:10.100710549-008-0216-7,PMID 18665447.

30. Urry; et al. (2014).Mitotic effect of the aqueous leaf extract of Cymbopogan citratus in Allium cepa root tips.
31. Ddexcel practical materials created by salters Nuffield Advanced Biology, Copyright university of York sciences Education group.