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CYTOTOXIC ANALYSIS OF SEED EXTRACT OF *CYCAS CIRCINALIS* L. USING THE *ALLIUM CEPA* CHROMOSOME ABERRATION ASSAY

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ABSTRACT

In this study, cytotoxic abnormalities in *Allium cepa* by the seed extract of *Cycas circinalis,* was tested. Different concentrations like 20%, 40%, 60%, 80%, and 100% of seed extract of *Cycas circinalis* were prepared after shade drying. In order to compare with standard, a control was also maintained. The mitotic indices and chromosomal aberration of control and treatment were calculated. From this study it is proved that, seed extract of *Cycas circinalis* has capacity to change mitotic indices and chromosomal make up. From this it is clear that *Cycas circinalis* seed extract has mito-depressive activity on cell division. As the concentration of extract increases, mito-depressive activity also increases. Abnormalities like Nuclear lesions, Strap shaped nucleus, Chromosome Bridges, Micronuclei and Multinuclei were also observed in different concentrations.

Key words: Cytotoxic abnormalities, *Cycas circinalis, Allium cepa*, mito-depressive activity, chromosomal aberration.

INTRODUCTION

Medicinal plants are the backbone of traditional medicine, which means more than 3.3 billion people in the less developed countries use medicinal plants on a regular basis. Herbs are staging a comeback and herbal 'renaissance' is happening all over the world. However, there is an end for the blind dependence on synthetics and people are returning to herbals with the hope of safety and security (Davidson-Hunt.2000). Plants are the source of medication for preventive, curative, protective or promotive purposes (Sidhu, et.al 2007). Plant-derived foods help in the prevention of lifestyle associated diseases. Several groups of constituents in plants have been identified as potentially health promoting in animal studies, including cholesterol lowering factors, antioxidants, enzyme inducers and others (Dragsted, et al 2006,). Drugs are derived either from the whole plant or from their parts, like leaves, stem, bark, root, flower, seed, etc. Some drugs are prepared from excretory plant products such as gum, resins and latex. Traditional medicines derive the scientific heritage from rich experiences of early civilization (Shailajan, et al 2005).

Although ancient sages through trial and error methods have developed herbal medicines, the reported uses of plant species do not certify their efficacy (Rout et.al 2009). About 40% of all medicines on the market today have been derived directly or indirectly from natural sources; However, the safety of these plants has been questioned recently because of fatalities (Ernst and Pittler, 2002). Reports of the patients experiencing negative health consequences caused by the use of herbal medicines are also on the rise (Ewu, 2010). There is a need to analyse the physiological effects of such concoctions. Reports on ethno-medicinal uses of plant species require pharmacological screenings, chemical analyses, and tests for their bioactive activities.

Pharmacological screening of plant extracts provides insight to both their therapeutic and toxic properties as well as helps in eliminating the medicinal plants or practices that may be harmful (Prance et.al, 1994).

Toxicology is the important aspect of pharmacology that deals with the adverse effect of bio active substance on living organisms. The data of the acute toxicity studies of medicinal plants is obtained in order to increase the confidence in its safety to human, particularly for use in the development of pharmaceuticals (Gullo et. al, 2006). A key stage in ensuring the safety of drugs is to conduct toxicity tests in appropriate animal models, and acute toxicity studies are just one of a battery of toxicity tests that are used (Kathryn Chapman et.al, 2007). Acute toxicity refers to the adverse effects that occur on first exposure to a single dose of a substance.

In order to initiate the search for drugs from plants, the anti-mitotic activity of the extracts were tested by *Allium cepa* assay. The *Allium cepa* root meristem assay is considered widely as a practical and reliable system for the screening of environmental mutagens and carcinogens (Fiskc - jo, 1985; Stich et al., 2013). As the patterns of divisions in onion cells and animal somatic cells are similar, an extract which is able to inhibit the cell division in *Allium cepa* root cells, will be effective in human/animal cells. Thus it is possible that chemicals that affect plant chromosomes will also affect the chromosomes of animals. Hence these meristematic cells of plants can be used for preliminary screening of anti-mitotic / anti-cancer activity of extracts / drugs .The onion root tip assay is used by many researchers to screen several plant extracts to evaluate their anti-mitotic activity. Cytotoxic assays were conducted on a wide spectrum of plants by several earlier workers using different test materials. The extracts caused different kinds of chromosome aberrations in dividing and non dividing cells of *AIIium cepa* such as micronucleus, multinucleated cells in the interphase stage, bridges, stickiness, non-congression metaphase, laggards, polyploidy and disturbed anaphase.

The *Allium cepa* is commonly used as a test organism because it is cheap, easily available and has advantages over other short-term tests. Among the endpoints of *Allium cepa* root chromosomal aberrations, detection of chromosomal aberration have been the most used one to detect genotoxicity/ antigenotoxicity along the years. The mitotic index and chromosomal abnormalities are used to evaluate genotoxicity and micronucleus analysis is used to verify mutagenicity of different chemicals. The *Allium cepa* root chromosomal aberration assay is widely used to determine genotoxic and antigenotoxic effects of different plant extracts.(Namita Khanna*and Sonia Sharma, 2000).

Cycas circinalis is a dioecious plant, 6-20 ft high, occurring wild in south India and Orissa, and occasionally grown in Indian gardens. The leaves are feathery, spirally arranged, even-pinnately compound, lanceolate, evergreen. The flowers are dioecious. The fruit is elongated dry or hard. 1-3 inches in size. The plant is not self fertile. It can fix nitrogen. Sago is similar to that obtainable from palms is extracted from the trunk. According to IUCN category it is ‘Endangered species.’

Cycas as food **:** Cycas have been a source of food and medicine for many people in South India. These plants are well-known to be highly poisonous and must be carefully processed to remove toxins, before they are consumed. If they are not properly processed they can cause vomiting, liver damage and even death. There is also evidence they have neurotoxic effects. It is used as narcotic, poultice and in treatment for stomach diseases.

In this study, cytotoxic abnormalities in *Allium cepa* is tested by using the seed extract of *Cycas circinalis*. Different concentrations of seed extract of *Cycas circinalis*, like 20%, 40%, 60%, 80% and 100%, are used and mitotic indices and chromosomal aberrations are tested.

MATERIALS AND METHODS

A) Preparation of Crude Extract

Extract was prepared at room temperature by Soxhlet extraction method, taking ethanol as solvent

B) Mitosis in Onion Root Tip

An onion *(Allium cepa L.)* cell possess eight chromosomes (2n=16). It belongs to Liliaceae family. Cell division occurs rapidly in growing root tips of sprouting seeds or bulbs. An onion root tip is a rapidly growing part of the onion and thus many cells will be in different stages of mitosis. The onion root tips can be prepared and squashed in a way that allows them to be flattened on a microscopic slide, so that the chromosomes of individual cells can be observed easily. The super coiled chromosomes during different stages of mitosis present in the onion root tip cells can be visualized by treating with DNA specific stains, Acetocarmine stain.

Mitotic Index: The percentage of cells undergoing mitosis or it is defined as the ratio of no. of cells in the dividing phase to the total number of cells observed. This will help to identify the region of most mitotic activities. Mitotic index helps us to quantify the cell division. The meristematic region in the root tip is the actively growing region and thus the mitotic index is high.

Mitotic index = n/N × 100

n: Total number of dividing cells observed
N: Total number of cells in the field of microscope

Mitotic index is used to quantify the differences in cell division when environmental parameters are changed.

Different types of chromosomal aberrations: Nuclear lesions, Chromosome bridge, Ring chromosome, Micronuclear interphase, Chromosome clumping, Strap shaped nuclei, Polyploid cells, Disoriented metaphase, Leggad, Disturbed metaphase, Disturbed anaphase, Disturbed telophase etc.

Plant Materials used:

The seeds of *Cycas circinalis* L. is the selected material for the study. They were collected from various localities of Kannur District. They were shade dried at room temperature for 10-15 days. Later they were made into fine powder. This powder was stored in dry and airtight container and used for further studies.

Extraction:

50 gm dried powder of *Cycas circinalis* seed was mixed in 300 ml of ethanol in Soxhlet extractor. Kept the extract in Soxhlet extractor 5 days. After these days, the extract was poured into petridishes equally and dried. Prepared different concentrations like 20%, 40%, 60%, 80% and 100% of the dried extract. Took a set as control.

Preparation

Actively growing onion root tips are required for this activity. Allow at least 2–4 days for new roots to grow. To grow root tips, obtain healthy 5–6 onion bulbs. Remove any dried, old root growth from the bottom of the bulbs. Place each onion bulb into a wet sand which was sterilised. After two days took out the onions, wash with distilled water and then put into solution of different concentrations like 20%, 40%, 60%, 80%, 100%, control respectively. After two hour the onions taken out and did cytotoxicity experiment.

Procedure

1. Cut roots from onion plant using a scalpel.
2. Trim the tip of each root to 1 cm; use only the tapered end of the root tip.
3. Use forceps to place 2–3 root tips (use only the 1-cm tips) on a glass microscope slide.
4. Place them in a solution containing Hydrochloric acid (1N) and ethanol in 1:1 concentration
5. Allow the root tips to soak in the mixture for 1 minutes.
6. After one minute, Put the material in water and wash off the solution.
7. Place the root tip on a slide. Use a paper towel to blot away excess water.
10. Using a clean, graduated pipette, add 2–3 drops of Aceto carmine stain to the root tip.
11. Allow the root tips to soak in the stain for 3 minutes.
12. Use a paper towel to blot away excess stain.
15. Place a cover slip on the root tissue. Gently apply pressure on the cover slip to squash the root tissue.
16. Using low magnification on the microscope, focus on the root cells. Switch to medium power or high power as necessary to easily visualize the inside of the onion root cells.
17. Study all of the squashed tissue to locate cells in each stage of the cell cycle.

Repeat same procedure for all concentrations (Flinn Scientific, Inc. 2016)

RESULTS AND DISCUSSION

From the result it is clear that, *Cycas circinalis* seed extract of different concentration shows variation in mitotic indices and large number of chromosomal aberrations are also noted, Mitotic index of control set is 35.29. 20% concentration shows a decrease in mitotic index i.e., 30.90 .As the concentration of solution increases the mitotic index also decreases. 40% concentration shows a decrease in mitotic index i.e., 27.43 .60% concentration shows a decrease in mitotic index i.e., 23.04 .80% concentration shows a decrease in mitotic index i.e., 17.31 and 100% concentration shows further decrease in mitotic index 7.55. From this it is clear that *Cycas circinalis* seed extract has mito-depressive activity on cell division. As the concentration of extract increases mito-depressive activity also increases.

In control only a very little chromosomal aberration is noticed. Only nuclear lesions are visible. The percentage of abnormality is very low.

But in 20% concentration, there is high inter phase abnormalities like Lesions, Strap shaped nucleus, etc. Prophase abnormality and metaphase abnormality are also prominent like Clumping, Disorientation etc. In anaphase chromosomal bridges are also observed. . The most prominent abnormality in anaphase stage was the formation of chromosome or Chromatid Bridge. Such bridges sometimes persisted during telephase. The separation of the daughter chromosomes to the polar became incomplete and remained connected together by these chromatic bridges.

In 40% concentrated solution, Inter phase and prophase abnormalities are at higher rate. Lesions, Strap shaped nucleus, micronuclei can be observed. Chromosomes fragmentation

was induced and was quite clear at interphase as micronuclei. The formation of unbalanced daughter nuclei leading to somatic instability. Extract involves in disruption of the spindle apparatus or interference with normal chromatid separation. In interphase stage the occurrence of micronuclei and multinuclei were frequently noticed. The production of micronuclei may be as a result of laggard chromosome fragments which may be surrounded by nuclear membrane, such micronuclei may be persisted in the following prophase. Metaphase clumbing and polypoid cells can be observed. But anaphase abnormalities could not be observed.

In 60% concentrated solution, almost every types of abnormalities are observed.

In 80% concentration, anaphase and telophase abnormalities are very low.

In 100% concentration, only inter phase and prophase abnormalities can be seen. They are at higher rate. This is because the cells are get arrested at interphase or prophase itself. so the metaphase, anaphase and telophase abnormalities are very low.

As the concentration of the solution increases, the inter phase abnormalities increases.

Prophase abnormality decreases from 20% to 60% but in 80% and 100% it is higher.

Metaphase abnormality decreases as concentration of solution increases. Anaphase and telophase abnormalities are very low.

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Table 1
Cytotoxic effects of different concentrations of *Cycas circinalis* seeds on *Allium Cepa* root

Concentration	Time	Mitotic index	Interphase	Lesions	Strap shaped nucleus	Micronuclei	Total interphase abnormalities	Prophase	Total prophase abnormalities	Metaphase	Clumping	Polyploid cells	Disorientation	Total metaphase abnormalities	Anaphase	Bridge	Total anaphase abnormalities	Telophase	Total Telophase abnormalities
Control	2 hrs	35.29	218	3	0	0	3	80	0	5	0	0	0	0	3	0	0	5	0
20%	2 hrs	30.90	105	71	22	0	84	27	21	32	20	0	12	32	6	1	1	3	0
40%	2 hrs	27.43	132	56	26	36	118	15	13	28	12	11	0	23	0	0	0	2	0
60%	2 hrs	23.04	156	112	8	0	128	24	10	17	8	4	0	12	3	0	3	0	0
80%	2 hrs	17.31	180	122	4	11	139	51	44	13	0	10	0	10	0	0	0	0	0
100%	2 hrs	7.55	227	160	0	20	180	21	21	0	0	0	0	0	0	0	0	0	0

Table 2
Effects of different concentrations of *Cycas circinalis* seed on *Allium Cepa* root cell division

Concentration	Duration of treatment in hours	Total number of cells analysed	Total number of dividing cells	Total number of normal cells	Total number of abnormal cells	Mitotic index
Control	2 hrs	221	78	218	3	35.29
20%	2 hrs	220	68	21	138	30.90
40%	2 hrs	228	45	20	152	27.43
60%	2 hrs	243	56	97	146	23.04
80%	2 hrs	283	49	90	193	17.31
100%	2 hrs	278	21	31	247	7.55

Figure1
Chart showing Cytotoxic analysis of *Cycas circinalis* seed on *Allium Cepa* root.

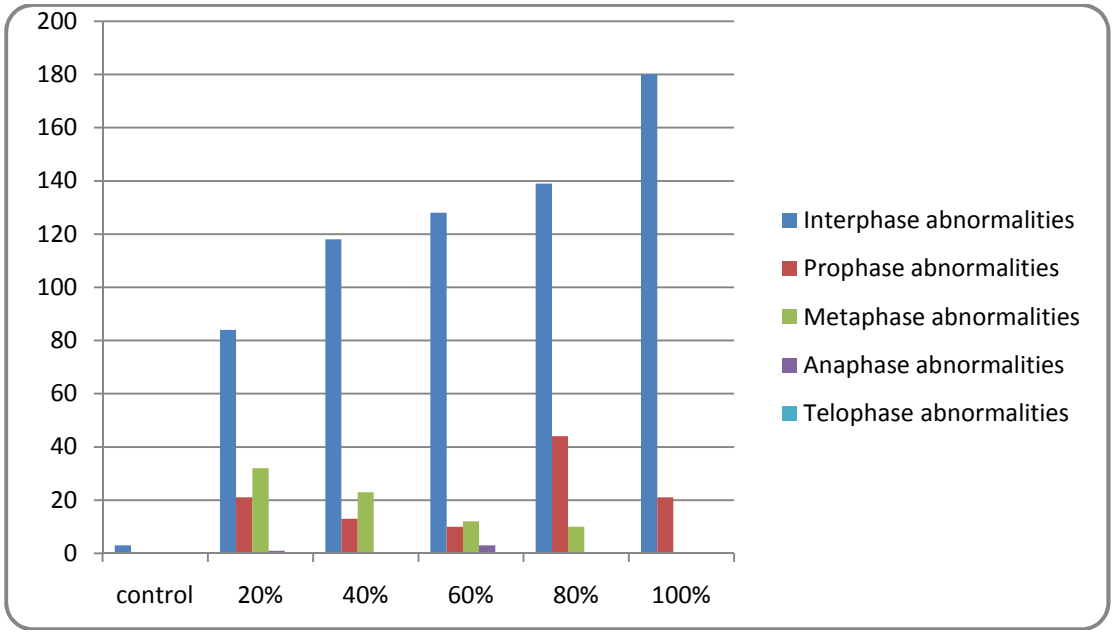


Table3
Cytotoxic analysis of *Cycas circinalis* seed *Allium Cepa* root

Concentration of solution	Interphase abnormalities	Prophase abnormalities	Metaphase abnormalities	Anaphase abnormalities	Telophase abnormalities
control	3	0	0	0	0
20%	84	21	32	1	0
40%	118	13	23	0	0
60%	128	10	12	3	0
80%	139	44	10	0	0
100%	180	21	0	0	0

Figure2
Chart showing Mitotic index of different concentration of *Cycas circinalis* seed on *Allium Cepa* root

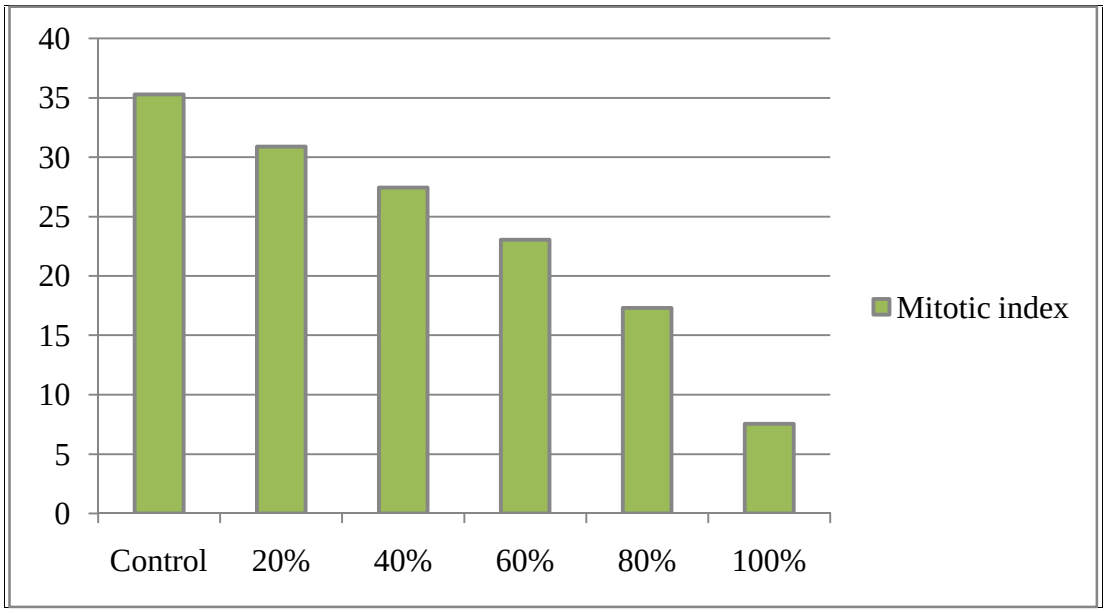
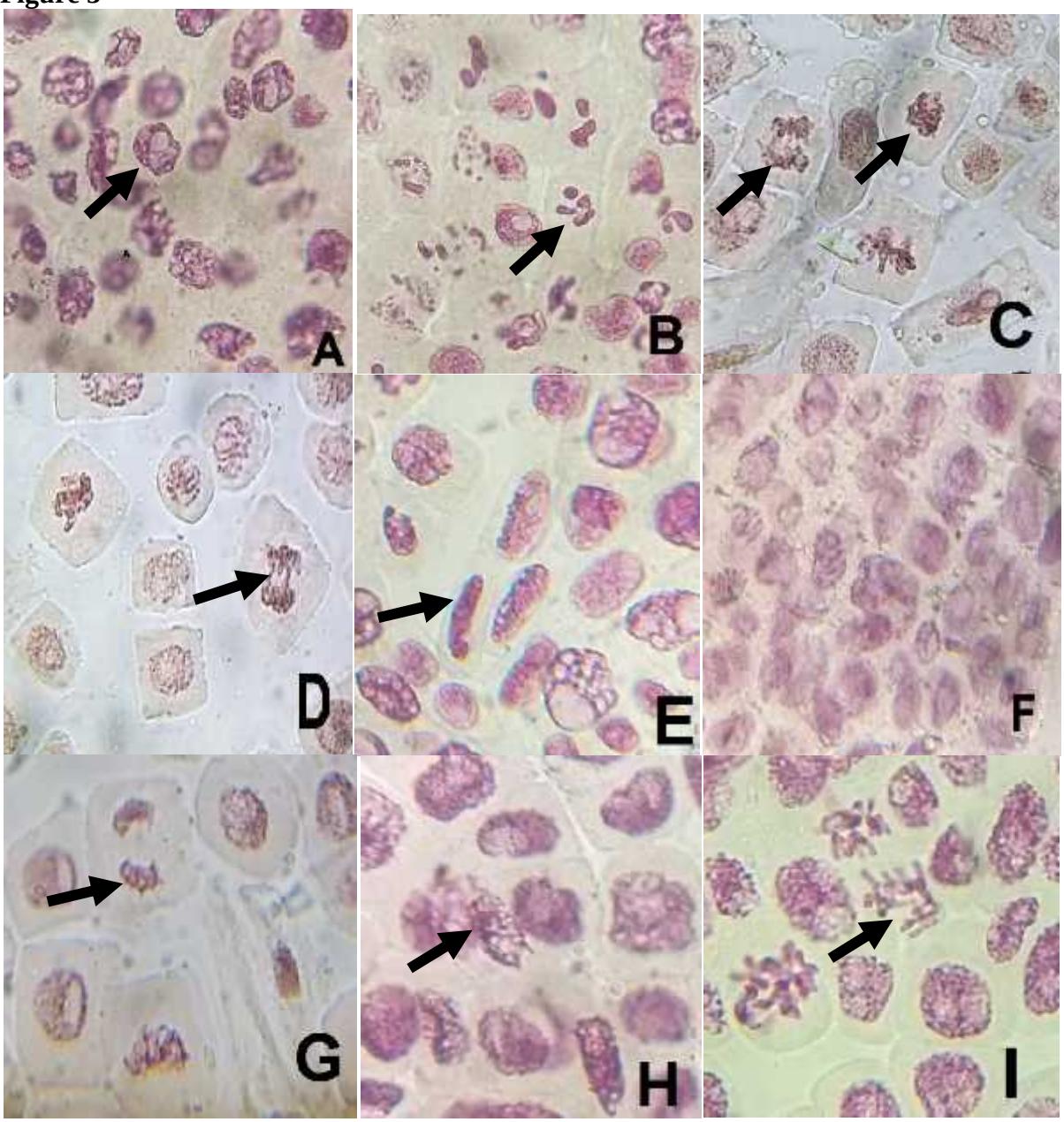


Table 4
Mitotic index of Different concentration of *Cycas circinalis* seed on *Allium Cepa* root

Concentration of the solution	Mitotic index
Control	35.29
20%	30.9
40%	27.43
60%	23.04
80%	17.31
100%	7.55

Figure 3



A. Nuclear lesions B. Micronuclei formation C. Clumbing and bridge formation D. Anaphase with bridges E. Strap shaped nuclei F. Denatured cells G. Disorientation of chromosomes H. Polyploid condition I. Leggard formation.

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