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Study on In-Vitro Antimitotic Module

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ABSTRACT:
Multiple genetic changes occur during the evaluation of normal cells into cancer cells. This evaluation is facilitated in cancer cells by loss of fildety in the processes that replicate, repair and segregate the genome. This insight suggests molecular mechanism for cellular transformation and may help to identify potential targets for improved cancer therapies.Meristematic cells that are situated in the tip of the roots that render the most desirable and suitable raw material to study the different stages of mitosis. To better understand the mitosis and different stages of mitotic processes and the relative length of each stage of mitosis (can be estimated). Root tips of Allium cepa L. have been recommended as a standard for cytogenetic assay in environmental monitoring due to the correlation of these plants with mammalian and non-mammalian test systems. Onion was selected for the antimitotic assay which shows the root growth inhibition that compared with standard antimitotic drug.The Antimitotic assay was selected because this is easy to done and give fastest promising results.

KEYWORDS: Antimitotic, Meristematic, Allium cepa etc.

I. INTRODUCTION:

Cancer is a disease in which some of the body's cells grow uncontrollably and spread to other parts of the body. Sometimes this orderly process breaks down, and abnormal or damaged cells grow and multiply when they shouldn't. These cells may form tumours, which are lumps of tissue. Tumours can be cancerous or not cancerous (benign). The term mitosis is used to describe the duplication anddistribution of chromosomes, the structures that carry the genetic information.

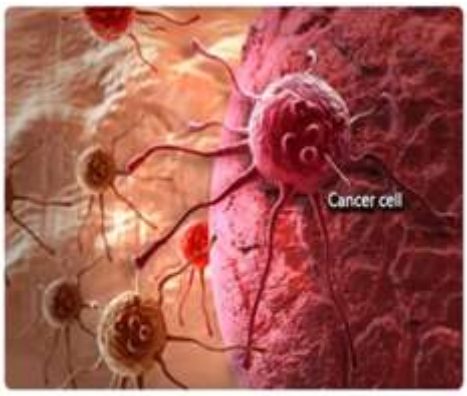

Figure 1

Cancer refers to cells that grow out of central and invade other tissues. Cells may become cancerous due to the accumulations of defects, or mutations in their DNA environmental factors (for example air pollution) and poor lifestyle choices – such as smoking and heavy alcohol use- can also damage DNA and lead to cancer. Cancer occurs when damaged cells grow, divide, and spread normally instead of self-destructing as they should these cells can infiltrate normal body tissues. Many cancerous and the abnormal cells that compose the cancer tissue are further identified by the name of the tissue that the abnormal cells originated from (ex. breast, lungs, colorectal cancer). Cancer can start almost anywhere in the human body,which is made up of trillions of cells. Normally, human cells grow and multiply (through a process called cell division) to form new cells as the body

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needs them. When cells grow old or become damaged, they die, and new cells take their place.

THE FOLLOWING TABLE NO.1 GIVES THE ESTIMATED NUMBERS OF NEW CASES AND DEATHS FOR EACH COMMON CANCER TYPE IN 2022: -

Cancer Type	Estimated Cases	Estimated Deaths
Bladder	81,180	17,100
Breast (female – male)	287,850 – 2,710	43,250 – 530
Colorectal cancer	151,030	52,580
Endometrial	65,950	12,550
Kidney (renal cell and renal pelvis) cancer	79,000	13,920
Leukaemia (all types)	60,650	24,000
Lung (including Bronchus)	236,740	130,180
Melanoma	99,780	7,650
Non-Hodgkin Lymphoma	80,470	20,250
Pancreatic	62,210	49,830
Prostate	268,490	34,500
Thyroid	43,800	2,230

Table No. 1

There are three most common cancers in men, women, and children in the U.S are as follows:-

- **Men:**Prostate, Lung, and Colorectal etc.
- **Women:** Breast, Lung, and Colorectal etc.
- **Children:**Leukaemia IQ, Brain tumours and Lymphoma etc.

The World Health Organization (Who) Provides The Following General Information About Cancer Worldwide: -

- Cancer is a leading cause of death worldwide. It is accounted for 8.2 million deaths (around 22% of all deaths not related to communicable diseases: most recent data from WHO).
- Lung, Stomach, Liver, Colon, and Breast cancer cause the most cancer deaths each year.
- Deaths from cancer worldwide are projected to continue rising with an estimated 13.1 million deaths in 2030 (about a 70% increase).

CAUSES OF CANCER:

1) Chemical or Toxic compound exposures:

- Benzene • Vinyl chloride
- Nickel
  - Benzidine
- Cadmium
  - N- nitrosamines
- Tobacco
  - Cigarette smoke etc.

2) Ionizing radiation:

- Uranium
  - Radon
- Ultraviolet rays from sunlight
- radiation from alpha (α), beta (β), gamma (π), and
- X-ray emitting sources etc.

3) **Pathogens:**

- Human papillomavirus (HPV)
- EBV or Epstein-Barr virus
- Hepatitis viruses B and C Kaposi
- Sarcoma-associated herpes virus (KSHV)
- Helicobacter pylori etc.

4) **Genetics:**

Genes contains information to make proteins and proteins control many important functions like cell growth. Genetic mutations can change how proteins function. Same types of geneticmutations can change proteins in such a way that cause healthy cells to become cancerous.

RISK FACTORS: -

1. Sunlight
2. Cigarettes smoke
3. X-rays
4. Red mate (beef, lamb, pork etc.)
5. Obesity
6. Lack of exercise

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7. Chronic inflammation
8. Harmon's
9. Cells phones due to low energy radiations etc.

SIGNS AND SYMPTOMS: -

It depends on the type of cancer, where it is located, and /where the cancer cells have been spread. The American cancer Society describe 7 warnings that a cancer may be present and which should prompt a person to seek medical attention.

1. Change in bowel / bladder habits.
2. A sore throat that does not heal.
3. Unusual bleeding / discharge.
4. Thickening / lump in the breast, testicles, elsewhere.
5. Indigestion / difficulty in swallowing.
6. Obvious change in the size, shape, colour / thickness of a wart / mole.
7. Nagging cough / hoarseness etc.

OTHERS:

1) Unexplained loss of weight or loss of appetite.
2) A new type of pain in the bones or other parts of the body that may be steadilyworsening, or come and go, but is unlike previous pains one has had before.
3) Persistent fatigue, nausea, or vomiting.
4) Unexplained low-grade fevers with may either persistent or come and go.
5) Recurring infection which will not clear with usual treatment.

DIFFERENT TYPES OF CANCERS:

Cancer can occur anywhere in the body,

- **Solid cancer:**

Ex. Breast, Lung, Prostate cancer etc.

- **Liquid cancer:**

Ex. Blood cancer etc.

ACCORDING TO THE TISSUE DURING WHICH IT PHASES: -

1) Carcinoma cancer:

Cancer that begins within the skin or within the tissue that line or cowl internal organs i.e., skin, lungs, colon, pancreatic, ovarian, epithelial, squamous, basal cell carcinomas, melanomas, papilloma's and adenomas etc. They comprise 80-90% of all cancers.

2) Sarcoma cancer:

Cancer that begins within the bone, cartilage, fat, muscle, blood vessels, alternative connective, supportive tissue i.e., bone, soft tissue cancers etc.

Ex.oestrogenic sarcoma, secretion cancer, sarcoma, malignant tumour, sarcoma, Fibro cancer
etc.

3) Leukaemia cancer:

Cancer that starts in blood- forming tissue like the bone marrow and causes giant no. of abnormal somatic cell to be created and enter into the blood.
Ex. Lymphoblastic leukaemia, Myelogenousleukaemia, T- cell leukaemia, furry – cell
leukaemia etc.

4) Malignant Tumour cancer:

Cancers that happen in plasma cells of the bone marrow.
Ex. Myeloma (Koehler disease) etc.

5) Lymphoma cancer:

Cancers of the system cells. It includes: -
• Hodgkin cancer.
• Don – Hodgkin cancer i.e., WBC cancer.

6) Mixed cancer:

Cancer arises from over one kind of tissue.

7) Central Nervous System cancer (CNS cancer):

Cancer that begins within the tissues of the brain and spinal card.
Ex. Meningioma's, Pituitary adenoids, Primary CNS lymphomas etc.

7 COMMAN CANCERS: -

Cancer is that the ordinal leading reason for death within the US they'll be as follows: -

1) Breast cancer:

Commonest and deadliest within the us. Concerning 1/8 girls can develop invasive at some purpose in her life. Although death rates therefore decreased since 1989, over 40000 US girl's area unit thought to possess died from it in 2015 done.

2) Lung cancer:

It's the ordinal commonest and deadliest for each men and girls. In 2012, over 40000 Americans were diagnosed with it and within the same year over 15000 Americans died from it.

3) Prostate cancer:

Its commonest found in men in 2013, over 177000 American's were diagnosed with it and over 27000 men died from it.

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4) Colorectal cancer:
It's ordinal greatest killer within the US which will impact each men and girls.

5) Liver cancer:
It develops in concerning 20000 men and 8000 girls annually. Serum hepatitis, C and serious drinking will increase one's risk of developing it.

6) Ovarian cancer:
Concerning 20000 yank girls diagnosed with it annually. For women, it's the eighth commonest cancer, and fifth leading reason for cancer death.

7) Pancreatic cancer:
It's highest morbidity of major cancers. Out of the roughly 53000 Americans diagnosed with it annually, any 8 May 1945 can survive over five years.

STAGES OF CANCER:
The TNM classification of a cancer usually correlates to one of the following 5 stages according to: -
TUMOR (T): - Primary tumour size and or extent.
NODES (N): - Spread of cancer to lymph nodes in the regional area of the primary tumour.
METASTASIS (M): - Spread of cancer to distant sites away from the primary tumour.

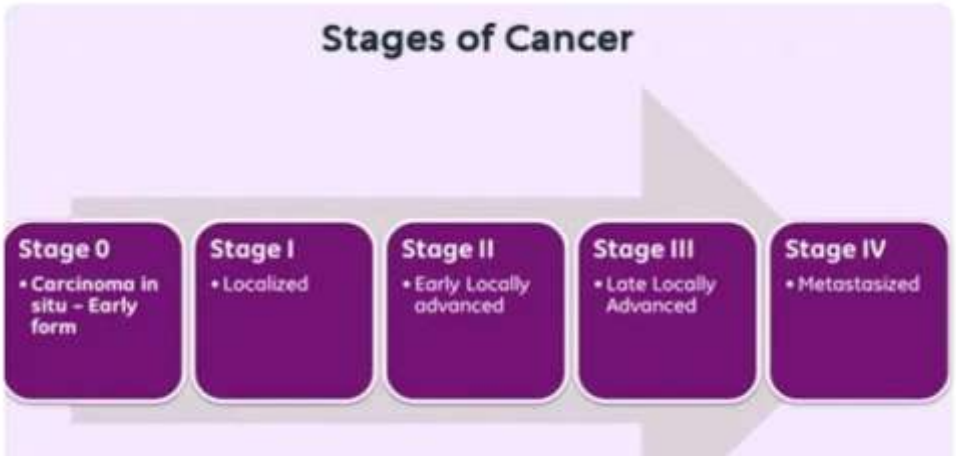

Figure 2

CANCAR DIAGNOSIS:
Various tests may be performed in order to conform a cancer diagnosis it may include: -
1) Mammogram
2) Pap test
3) Tumour matter test
4) Bane scan
5) MRI
6) Tissue Biopsy
7) PET-CT scan

TREATMENT:
The cancer treatment is predicated on the sort of cancer and therefore the stage of the cancer. In some individuals, identification and treatment might occur at a similar time, if the cancer is entirely surgically removed once the doc removes the tissue for diagnostic assay. Though patient treatment or protocol for his or her cancer most treatments have mate one amongst the subsequent components: -

- Surgery • Chemotherapy
- Radiation therapy • Targeted therapy
- Combination therapy • Immunotherapy
- Palliative therapy • Stem cell transplant etc.
- Somatic cell transplant etc.

All although each medical practitioner suggests that diet and sensible nutrition can facilitate a private to combat cancer, though a number of these treatments might facilitate to scale back symptoms, there's no sensible proof they'll cure any cancers.

CELL CYCLE / CELL DIVISION CYCLE: - It is the series of events that takes place in a cell leading to duplication of its DNA and division of cytoplasm and organelles of to produce 2 daughter cells. It is the vital process by which a single celled fertilized egg develops into an inactive organism, as well as the process by which hair, skin, blood cells and some internal organs are renewed.

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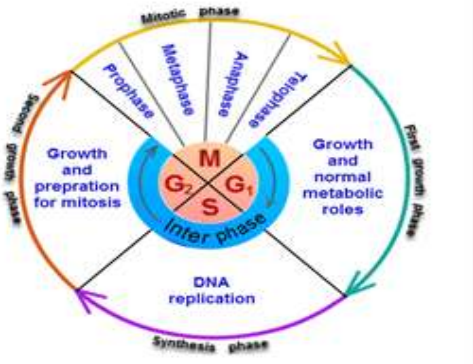

Figure 3

THE EUKARYOTIC CELL CONSIST OF 3 DISTINCT PHASES ARE:

State	Phase	Abbreviations
Resting	Gap 0	Go
Interphase	Gap 1 Synthesis Gap 2	G1 S G2
Cell Division	Mitosis	M

Table No. 2

A) Go- part (Quiescent Phase):
Resting part and cell has stopped dividing. The cell stones with this part.

B) Interphase: -

a) G1- Phase (Gap/Growth Phase):

It's a series of changes that takes place in an exceedingly freshly fashioned cell and it's nuclear before it becomes capable of division once more, additionally known as preparative part / Intermitotic. The cell produces several essential materials i.e. proteins and ribosomes. The dimensions might increase its energy production and overall metabolism and making ready it for S-part. It takes concerning 10-12hrs.

b) S-Phase (Synthetic Phase):

DNA is synthesized/ duplicated. Its lakes concerning 6-8 hrs. All of the chromosomes are replicated i.e., everybody consists of two sister chromatids. Deoxyribonucleic acid quantity is doubled and no. of chromosomes square measure unchanged. Rate of polymer transcription and macromolecule synthesis square measure terribly low.

c) G2-Phase:

It happens alternative deoxyribonucleic acid replication and it's an amount of macromolecule synthesis and fast cell growth to organize the cell for cell division. Throughout this part microtubules begin to acknowledge to create a spindle (preprophase) and additionally checked at the G2- stop for any deoxyribonucleic acid harm among the chromosomes.

C) M-Phase (Cell division/Mitosis/Chromosome separation):

It consists of nuclear division (karyokinesis). Its short amount of your time and it's advanced and extremely regulated.

Antimitotic Activity Study On Allium Cepa Root Tips (Onion Roots)

Mitosis:

It is a type of cell division in which one cell (the mother divides to produce two new cells) daughters that are genetically identical to itself i.e., DNA of the cell's nucleus is split into 2 equal sets of chromosomes.

PHASES OF MITOSIS:

There are 4 phases i.e.

1) Prophase: - a) Early phase (prophase)
b) Late phase (prometaphase)
2) Metaphase
3) Anaphase
4) Telophase

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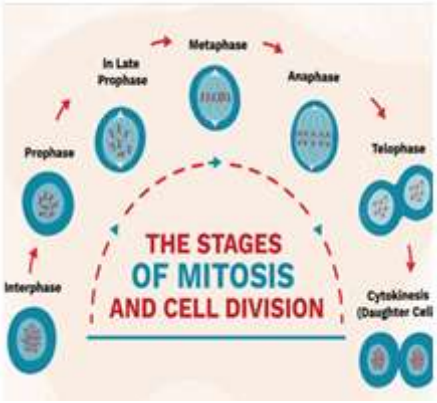

Figure 4

1) PROPHASE:

In prophase, the chromatin condenses into discrete chromosomes. The nuclear envelope breaks down and spindles form at opposite poles of the cell. Prophase (versus interphase) is the first true step of the mitotic process. During prophase, a number of important changes occur:

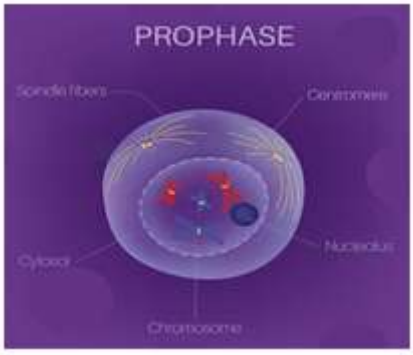

Figure 5

- Chromatin fibres become whorled into bodies with every chromosome having 2 chromatids joined at a structure.
- The mitotic spindle composed of microtubules and proteins forms within the living substance.
- The 2 tries of centrioles (formed from the replication of 1 pair in interphase) move removed from each other towards opposite ends of the pole thanks to the perpetuation of the microtubules that type between them.
- Polar fibres, that square measure microtubules that structure the spindle fibres reach from every cell pole to the cell's equator.
- Kinetochores, that square measure specialised regions within the centromeres of chromosomes, attach to a kind of tubule referred to as complex body part fibres.
- The complex body part fibres "Interact" with the spindle polar fibres connecting the kinetochores to the polar fibres.
- The chromosomes begin to migrate toward the cell centre.

2) METAPHASE:

In metaphase, the spindle reaches maturity and the chromosomes align at the metaphase plate (a phase that is equally distant from the two spindle poles) during this phase, a number of changes occur:

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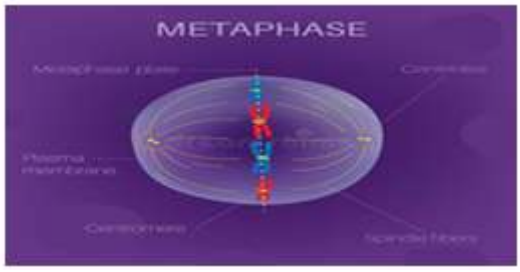

Figure 6

- The nuclear membrane disappears fully.
- Polar fibres (microtubules that structure the spindle fibres) still extend from the poles to the centre of the cell.
- Chromosomes move arbitrarily till they attach (at their kinetochores) to polar fibres from each side of their centromeres.
- Chromosomes align at the metaphase plate at right angles to the spindle poles.
- Chromosomes square measure command at the metaphase plate by the equal forces of the polar fibres pushing on the centromeres of the chromosomes.

3) ANAPHASE:

In phase of cell division, the paired chromosomes (sister chromatids) separate and start moving to opposite ends (poles) of the cell. Spindle fibres not connected to chromatids lengthen and elongate the cell. At the tip of phase of cell division, every pole contains an entire compilation of chromosomes. Throughout phase of cell division, the subsequent key changes occur:

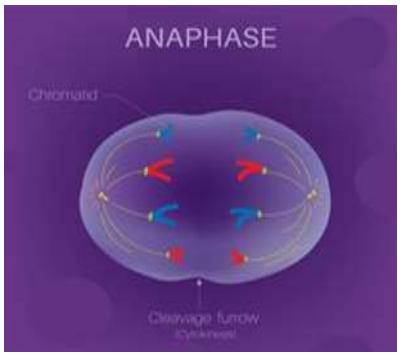

Figure 7

- The paired centromeres in every distinct body begin to move apart.
- Once the paired sister chromatids break free each other, every is taken into account a “full “chromosome. They’re named as "Daughter chromosomes".
- Through the spindle equipment, the girl chromosomes move to the poles at opposite ends of the cell.
- The girl chromosomes migrate to the bodily structure initial and therefore the centromere fibres become shorter because the chromosomes close to a pole.
- In preparation for Telophase, the 2 cell poles conjointly move more apart throughout the course of phase of cell division, every pole contains a whole compilation of chromosomes.

4) TELOPHASE:

In Telophase, the chromosomes area unit cordoned off into distinct new nuclei within the rising female offspring cells. The subsequent changes occur:

- The polar fibres still lengthen.
- Nuclei begin to create at opposite poles.

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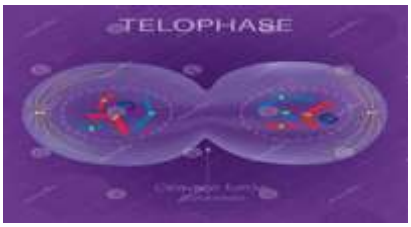

Figure 8

- The nuclear envelopes of those nuclei kind from remnant items of the parent cell's nuclear envelope and kind items of the endomembrane system.
- Nucleoli conjointly re-emerge.
- Chromatin fibres of chromosomes disentangle.
- After these changes, Telophase/mitosis is essentially complete. The genetic contents of 1 cell are divided equally into 2.

EXPRIMENTAL DESIGNS:

1) Roots developments: -

a. The experiments were planned as per the standard protocol for allium test onions were descaled and placed on glass cups which was filled with tap water.
b. Kept it in BOD incubator at 24^0C for 48 hours then 2-3cm roots are allow to germinate.
c. These roots are used for further process.

2) Sample preparation:

a. Different extracts of plant (successive extraction) are triturate.
b. Try to dissolve in distil water and then undissolved portion was removed.
c. The system mainly consists of highly polar solvent like Pet ether, Benzene, Chloroform,
d. Water, Ethanol and Acetone are soluble in water.

3) Standard preparation:

a. Methotrexate used as a standard of concentration 5mg/ml.

Control Root tip treatment with distil water.

4) Treatment:

a. Developed roots are kept in different extract for 3 hours in at 18^0 C.

5) Fixation:

a. Then these roots tips were cut and place in fixing solution caronys fixative for 24 hours at cool temperature.

Composition of caronys fixative:
Glacial acetic acid – 25ml
Ethanol- 75ml

6) Squash preparation:

a) Hydroxylation:

These roots were hydrolysed with 1N HCl by keeping it with HCI in oven at 60^0 for 10 min.

b) Staining:

Transfer roots into 2% acetocarmine stain for 20 min.

c) Slide preparation: -

Take a stain root, cut it from the tip (which get dark stain) where meristematic cells are present. Then place cover slip on it and observe under microscope at 40 X objective and then count the cells.
Find out mitotic index using following formula,

$$\text{Mitotic I} = \frac{(\text{P}+\text{M}+\text{A}+\text{T})}{\text{N}} \cdot 100\%$$

II. RESULTS: -

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Figure 9

Figure 10

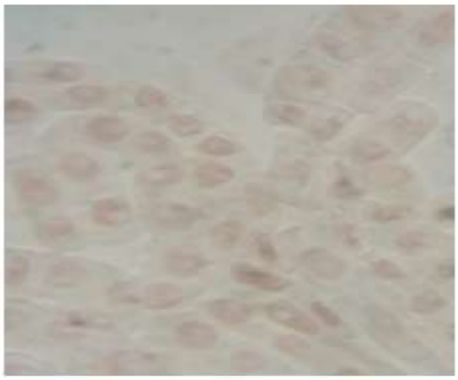

Figure 11
1. Prophase

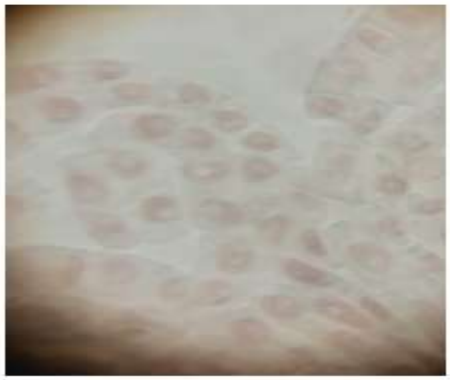

Figure 12
2. Metaphase

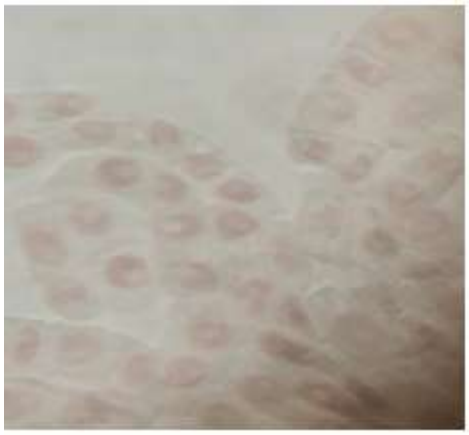

Figure 13
3) Anaphase

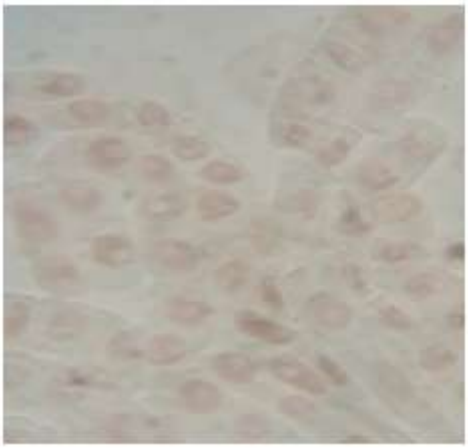

Figure 14
4) Telophase

III. CONCLUSION: -

The Allium monocot genus check has typically been wonted to judge DNA harm like body aberrations and disturbance within the mitotic cycle because of its cycle length and its reaction within the presence of the many well-known agent agents. The foremost frequent abnormalities (stickiness and phase of cell division bridges) were

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because of chromatin granule pathology or spindle failure. Body bridges result from body and/or strand breaks, indicating the clastogenic impact. In onion L. root tip model scheme of plant cells is usually used as a check for investigation environmental pollution factors, toxicity of chemical compounds and evaluating potential malignant neoplasm properties. The antimitotic activity was screened mistreatment onion root meristematic cells that are used extensively in screening of medication with antimitotic activity this division is analogous to the preceding cancer division in humans. Hence, these meristematic cells are often used for preliminary screening of medication with malignant neoplasm activity. Therefore, these meristematic cells are often evaluated for screening of medication with potential antimitotic activity.Allium monocot genus assay is taken into account a fast, sensitive and reproducible bioassay for police investigation toxicity and genotoxicity.

ACKNOWLEDGEMENT: -

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