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EFFECT OF CAPECITABINE ON BIOLOGICAL MATERIALS (ONION ROOT TIPS, BACTERIAL AND HUMAN DNA)

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ABSTRACT

Environment means the natural world, as a whole or in geographical area, especially effected by human activity. Recent studies have shown that environment is not only polluted by contaminants but also by improper disposal of drugs. Since 1990's water contamination by pharmaceuticals has been one of the environmental issues of concern. Most of the pharmaceuticals are deposited in environment through human consumption and excretion and are often filtered ineffectively by waste water treatment plants which are not designed to manage them. Once in the water they can have diverse subtle to moderate effects on organisms.Chemotherapy drugs are designed to kill rapidly growing cells such as those in a cancer tumor. Due to their disruptive action on DNA replication, practically all biological entities could be harmed by these drugs. The aquatic organisms are particularly vulnerable to these drugs. We took an anticancer chemotherapeutic drug Capecitabine (Xeloda) as an example and studied it's effect on cell division and DNA kinetics. Xeloda is diluted to different concentrations and tested on Onion root tips, Bacterial DNA and Human DNA extracted from bacterium E.coli and human blood respectively. In mitosis we observed that roots exposed to the drug solution for about 2, 3, and 4 hours at a concentrations 1mg, 3mg, 5mg and 7mg showed cell and chromosomal damage . The bacterial and human DNA were exposed to the drug solutions of various concentrations and were incubated at different time periods, later the optical density values were taken at 610nm . The result obtained showed that the DNA is denatured and damage indicated by increasing trend in values while increase in concentration as well as incubation time.

KEYWORDS: Capecitabine, Cell division (Onion root tips), DNA denaturation.

INTRODUCTION

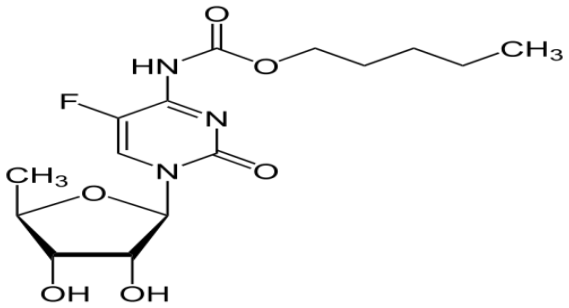
The word chemotherapy is mostly referred to the use of medicines or drugs to treat the cancer. Chemotherapy (chemo) can kill the cancer cells that had spread through the part of the body away from the original tumor. Hence chemo works throughout the body.

- Chemo may be used to shrink a tumor before surgery or radiation therapy. Chemo used in this way is called *neoadjuvant* therapy.
- It may be used after surgery or radiation therapy to help kill any remaining cancer cells. Chemo used in this way is called *adjuvant* therapy.

Chemotherapy is commonly given at regular intervals called cycles. A cycle may be a dose of one or more drugs followed by several days or weeks without treatment. [1]

Capecitabine being one of the chemotherapy drug which is supplied as a biconvex, oblong film - coated white of white crystalline powder which was developed more than 50 years ago by "Heilderlberg".[2] Capecitabine is an anticancer ("antineoplastic" or "cytotoxic") chemotherapy drug.[3] It is orally – administered drug which is a deoxycytidine derivative and fluorouracil PRODRUG which is rationally designed as an example for capecitabine that is used as an ANTINEOPLASTIC ANTI – METABOLITE.[9] A prodrug is a pharmacological substance that is administrated in a inactive form which is metabolized in vivo inside the body into an active metabolite. Capecitabine is a fluoropyrimidine carbamate that is converted into active 5-FU by the action of three enzymes: an esterase, a deaminase, and a phosphorylase.[9] It is mostly used in treatment of Metastatic colon or Rectal cancer and Metastatic Breast cancer.

The chemical name for capecitabine is 5'-deoxy-5'-fluoro-N-[(pentyloxy) carbonyl] – cytidine and has a molecular weight 359.35 .Its aqueous solubility is of 26 mg/ml at 20^{0}_{C}. Capecitabine is a light peach – colored 150 mg and a peach – colored 500 mg tablet. The inactive ingredients in xeloda include: anhydrous lactose croscarmellose sodium, hydroxypropyl methylcellulose , microcrystalline cellulose, magnesium stearate and purified water. The peach or light peach film coating contains hydroxypropyl methycellulose, talc, titanium dioxide, and synthetic red and yellow iron oxides.[3] Structural formula of capecitabine.



Trade name – XELODA-(3,4-dihydroxy-5-methyltetrahydrofuran-2-yl)-5-fluoro-2-oxo-1*H*-pyrimidin-4-yl]carbamate,Formula-$C_{15}H_{22}FN_3O_6$

COMPONENT: Compound is of a fluropyrimidine carbamate with antineopalstic activity .
In human body "Normal cells" stop dividing when they come in contact with the like cells but cancerous cells lose this ability. Therefore cancer cells have no limit in cell division. The ability of chemotherapy is to kill cancer cells depends on its ability to halt cell division. Usually the drug work by damaging RNA and DNA that tells the cell how to copy itself. Capecitabine which is a prodrug of an antitumor agent, 5 fulorouracil (5-FU) it is mimic of uracil, a building block of RNA and DNA synthesis and is poorly tumor selective. Its therapy causes high incidences of toxicity in bone marrow and gastrointestinal tract, central nervous system and skin.[8] .If cells are unable to divide they die. The faster the cells are dividing, the more likely is that chemotherapy will kill the cells, causing the tumor to shrink. Chemotherapy does not know the difference between the normal cell and cancerous cells. The Normal cells grow back and live healthy mean while leading to side effects.[4] Capecitabine, being one of the chemotherapy antimetabolite is similar to the normal substance within the cell like pyrimidine analog . Capecitabine is an oral 5-FU prodrug that was found to have an activity similar to that of intravenous 5-FU in a patients suffering from colorectal cancer. Hence when the cells incorporate these substance into the cellular metabolism, they are unable to divide.[11 - 12] Capecitabine is activated initially through hepatic metabolism and finally to 5 FU at the level of cancer cells through the action of thymidine phosphorylase, also known as platelet – derived growth factor, which is expressed higher levels in cancer cells than in surrounding normal cells.[13 – 14]

Capecitabine is a nucleosidic metabolic inhibitor. The mechanism and action of capetcitabine is Nucleic acid synthesis inhibitor. Capacitabine is a prodrug, that is enzymatically converted to flurouracil (antimetabolite) in the tumor by thymidine phosphorylase . The greater the thymidine phosphorylase enzyme in tumor tissues allow the

targeted intra - tumoral to realse the 5-FU susbsequently less systemic toxicity compared with the infusions of 5-FU.[10] The 5-FU later inhibits the DNA synthesis and slows growth of tumor tissues . After the absorption, it is metabolized in liver to the intermediate 5'- deoxy – 5 fluorouridine , which is subsequently converted into 5 – fluorouracil (5- Fu) by intracellular thymidine phosphorylase . 5 – FU exerts cytotoxic effects on the cell by direct incorporation into DNA and RNA as well as by inhibiting thymidylate synthase .Since thymidine phosphorylase is present at 3-10 fold higher concentration in cancer cells compared to normal cells , capecitabine's cytotoxic effect is selective for cancer cells.

High dose may lead to severe side effects .Side effects of Capecitabine are usually seen at the onset and duration of the chemotherapy. It's side effects and severity depends on how much of the drug is given.

Few side effects are commonly seen in patient's taking capecitabine are.[4 , 7]

- Poor appetite
- Abdominal pain
- Low White blood cell count
- Low platelet count
- Mouth sores
- Numbness or tingling of hands or feet
- Swelling of the feet and ankles
- Fever
- Constipation
- Eye irritation
- Shortness of breath
- Headache
- Chest , joint , muscle , back bone pain
- Dizziness
- Insomnia
- Dehydration
- Blood Clots
- Cough
- Excessive sleepiness , confusion , very rare seizures
- Loss of balance

- Nail changes , darkening of skin
- Taste changes

The side effects of capecitabine go away after the treatment is completed. There are many options to help prevent the xeloda side effects.

MATERIALS AND METHODS

1 –DIFFERENT CONCENTRATIONS OF XELODA DRUG

- 1mg/ml

A tablet of 500mg was dissolved in 500ml of water. The solution was left overnight.

- 3mg/ml

A tablet of 500mg was dissolved in 167ml of water. The solution was left overnight.

- 5mg/ml

A tablet of 500mg was dissolved in 100ml of water. The solution was left overnight.

- 7mg/ml

A tablet of 500mg was dissolved in 72ml of water. The solution was left overnight.

2 – MITOSIS IN ONION ROOT TIPS

Four samples of onion root tips were taken which were exposed to different concentrations of drug at different time intervals (2hrs, 3hrs and 4 hrs) and the remaining one sample unexposed was referred as standard.

Standard procedure to obtain stages of mitosis was followed. The cells were viewed under the microscope.

3 – EXTRACTION OF DNA FROM E.COLI

The kit used is HIMEDIA Hiper Bacterial Genomic DNA Extraction Teaching Kit (Solution Based).[5]

Allow the bacterial cell pellet collection tube to thaw at room temperature. Add 180µl of lysis solution and resuspend the pellet by gentle pipetting. Add 20µl of proteinase K solution to the above collection tube, vortex for 10-15 seconds, and incubate for 5minutes at room temperature (15-25^{0}C). Add 20µl of RNase. A solution to the above collection tube, vortex for 10-15 seconds, and incubate for 5minutes at room temperature (15-25^{0}C). Centrifuge at 10,000rpm for 10minutes. Transfer the supernatant into a new collection tube without

disturbing the small white pellet. Add 1ml of isopropanol to the lysate and mix by gentle inversion till white precipitate is seen, centrifugate at 10,000rpm for 10minutes carefully discard the supernatant without disturbing the white pellet. Add 500µl of prewash solution and re suspend the pellet by gentle pipetting. Centrifuge at 10,000rpm for 10minutes. Discard the supernatant carefully without disturbing the white pellet. Add 500µl of wash solution and resuspend the pellet by gentle pipetting. Centrifuge at 10,000rpm for 10minutes.Carefuuly discard the supernatant without disturbing the white pellet. Air dry the pellet for 10 minutes at room temperature . Re suspend the pellet in100µl of the elution buffer add incubate at 55^{o}_{c} for 10 minutes. Centrifuge at 10,000rpm for 10minutes and transfer the supernatant containing pure genomic DNA into a collection tube.

4 –HUMAN DNA

The human DNA sample was provided by "PARAM SCIENCE AND TECHNOLOGY"[6]

Bacterial DNA and human DNA samples were exposed to the Xeloda drug solution with variable concentrations at different time intervals.

RESULTS AND DISCUSSIONS

OD values of Xeloda treated DNA samples were taken at 610nm using colorimeter. The values varied for different concentrations of drug used.

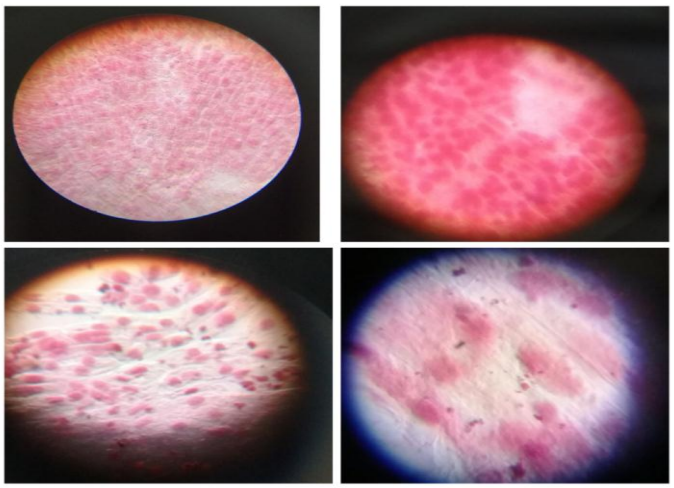
- The effect of Xeloda drug on mitotic cells showed a delayed effect at lowest concentration whereas gradual increase in density over time intervals in other concentrations.
- When DNA samples were exposed to Xeloda drug of different concentrations, a slight increase in the OD values was observed in the beginning, followed by stability over time.

EFFECT ON MITOTIC CELLS

At 1mg concentration, the drug showed its effect at 4hrs of exposure, while at 2 and 3hrs of exposure the cells did not show any damage. At 3mg concentration, the drug showed its effect at all intervals of time, likewise in 5mg and 7mg similar effects were seen and cells ceased their division.

- **1 mg**

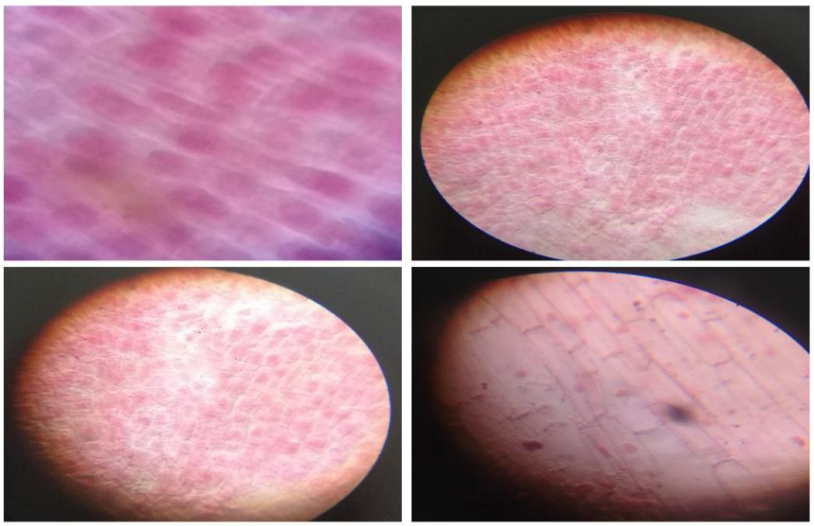
NORMAL **2 HOURS**



3 HOURS **4 HOURS**

- **3mg**

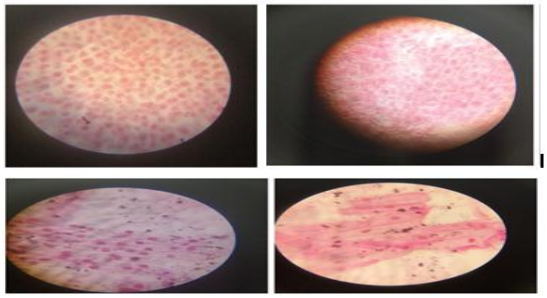
NORMAL **2 HOURS**



3 HOURS **4 HOURS**

- **5 mg**

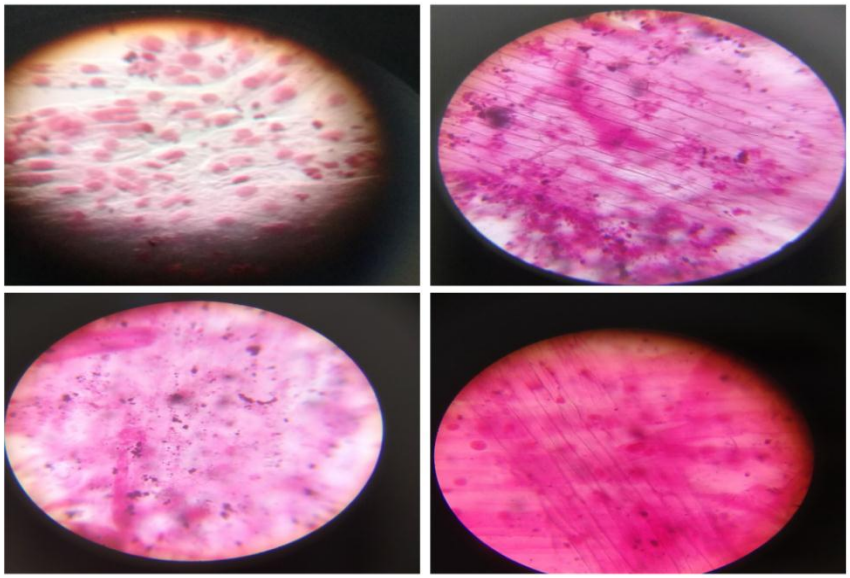
NORMAL **2 HOURS**



3 HOURS **4 HOURS**

➢ **7mg**

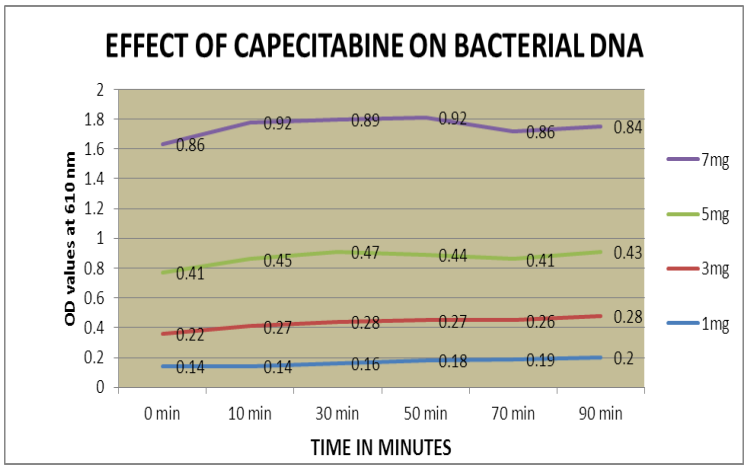
NORMAL **2 HOURS**



3 HOURS **4 HOURS**

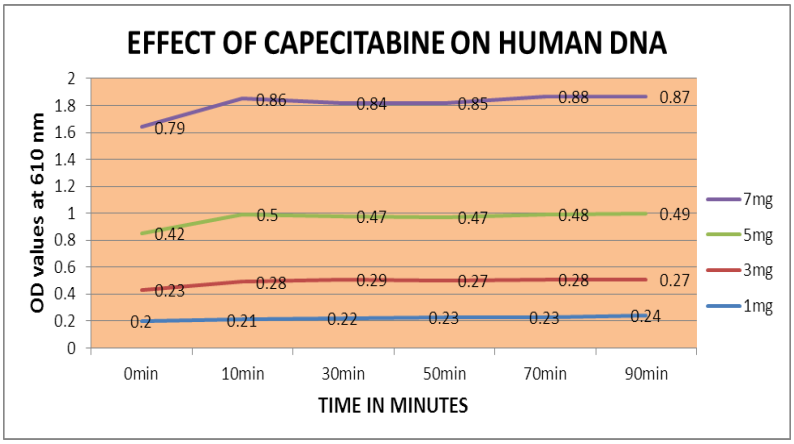
EFFECT ON BACTERIAL DNA

At different concentrations, with increase in time a gradual increase in OD values were also observed signifying denaturation in DNA.



EFFECT ON HUMAN DNA

At different time intervals, the OD values obtained showed an increase suggesting denaturation of DNA at different concentrations.



CONCLUSION

Xeloda has shown to be highly effective on cancer cells, discontinuing their division. Our study centered on the effect of this particular drug on normal DNA and the results obtained presented values that clearly stated the denaturation of DNA. It is evident from this study that normal DNA and dividing plant cells when exposed to this drug, showed precarious effect, the same consequence may also be observed when such chemotherapeutic drugs are dumped in environment damaging biodiversity. This study further helps the other research studies on anti cancer drugs (chemotherapeutic) showing similar effects and a caution is necessary when applied to a larger extent in diseased population.

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