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ANTIOXIDANT PROTECTION ON ESCHERICHIA COLI AGAINST UV RADIATION

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Abstract: The main motive of the experiment was to investigate the efficiency of antioxidants in DNA damage repair when subjected to UV radiation. Main approach of the experiment was to investigate the concentration of the antioxidants that efficiently help in DNA damage repair. Method: Wild type *E. coli* as the experimenting organism and Vitamin C as an antioxidant were taken. Both the *E. coli* was serially diluted by a factor of (10^{-1} - 10^{-5}) dilution and Vit-C was taken in the concentration of (0 µg/ml, 45 µg/ml, 50 µg/ml). Then the *E. coli* cultures grown with different concentrations of Vit-C were spotted on to LB media by Drop Plate Technique. The culture plates were subjected to the UV sensitivity assay by exposing to UV radiation at 254 nm. Result: After the UV sensitivity assay, the culture plates were kept for overnight incubation. Incubated plates with 50 µg/ml Vit-C and UV exposure, showed the maximum growth till 10^{-5} dilution factor, when compared with the control plate with UV exposure. The plates with 45µg/ml Vit-C with UV exposure, showed the growth till 10^{-4} dilution when compared with the control plate with UV exposure. The further analysis of the change in protein expression in analyzed by SDS-PAGE. Conclusion: from the experiment we can conclude that Vit-C had efficiently reduced the cytotoxic effect of UV exposure by repairing the damaged DNA. It has left some scope of investigating the anti-cancer and anti-aging effects of Vit-C in the human system.

Index Terms - DNA damage, UV radiation, Oxidative stress, Antioxidant, Vitamin C, Drop plate technique, UV sensitivity assay.

I. INTRODUCTION

Bacteria in their natural environment are often exposed to various external and internal mutagens, which cause DNA damage. Such external and internal mutagens are, UV radiation, γ-radiation, plant toxins, chemicals, etc. and metabolic byproducts, reactive oxygen species, etc. respectively. For the survival, bacteria have developed a number of protective mechanisms, such as the SOS response, base excision repair (BER), nucleotide excision repair (NER) to prevent DNA damage (Nora Goosen et al., 2007). Bacteria environmentally, are prone to UV damage to different degrees depending on the fidelity and the accuracy of the DNA repair mechanism they are using. UV exposure cause formation of reactive oxidative species (ROS) inside the bacterial cell. Sudden increase of ROS generates oxidative stress by the alteration or photodamage of various biomolecules like-proteins, fats and nucleic acids (DNA, RNA). UV radiation cause pyrimidine dimer, which leads to interruption of transcription. Defective transcription ultimately generates mutation and subsequently cell death (Daniel L. Jones, 2017). Here employing *Escherichia coli* as a model organism for the study of the role of its survival rate with effect of the Vitamin C as the antioxidant. Antioxidants are a class of molecules that are capable of inhibiting the oxidation of another molecule. Antioxidants are said to prevent the damage of cells caused by ultraviolet radiation (Adella A. Fejeran et al., 2008). Antioxidants are natural way of providing the cells with adequate defense against attack by reactive oxygen species (ROS).

Other important benefits of antioxidants include:

- Repairing damaged molecules - Some unique types of antioxidants can repair oxidized molecules by donating a hydrogen atom. This is very important when the molecule is a critical one, like our DNA (Dunnet et al., 2003).
- Blocking metal radical production - Some antioxidants have a chelating effect that binds with the toxic metals like mercury, arsenic, and prevent any chemical reaction from taking place. Water-soluble chelating agents can also escort toxic metals out of our body through our urine.
- Stimulating gene expression and endogenous antioxidant production - Antioxidants such as flavonoids can stimulate genes expression by attaching to our DNA to protect it from free radicals' attacks.
- Promoting cancer cells to "commit suicide" - some antioxidants can exhibit anti-cancer properties that halt cancer growth and force some cancer cells to self-destruct (apoptosis).

On the basis of solubility antioxidants can be categorized either as hydrophobic (fat soluble, like- vitamin A, D, E, K) or hydrophilic (water soluble, like-vitamin B, C, P). Vitamin-C has a wide range of astonishing health benefits. As an antioxidant, vitamin C can help battle oxidation by acting as a major electron donor, maintain optimal electron flow in our cells, Protect proteins, lipids, and another vital molecular element. To observe the antioxidant effect on the survival rate of wild strain of *E. coli* under UV sensitivity assay, we have taken Vitamin C (ascorbic acid) as antioxidant. Vitamin C is the most important antioxidant that can protect bio-membranes against lipid peroxidation damage by eliminating peroxyl radicals in the aqueous phase before the latter can initiate peroxidation. Vitamin C is located in the aqueous phase of cells, where it contributes to radical scavenging. Vitamin C is a potent antioxidant; it easily gives up electrons to provide stability to reactive species such as ROS. In some stress condition, it was seen that vitamin C act as pro-oxidant and associated with overproduction of free radicals, such as cancer cell. There the disturbance of the pro-oxidant-antioxidant balance leading to potential tissue damage (Aratirika Chakraborthy et al., 2014).

Stress conditions can be generally divided into three main categories: Nutritional, Environmental and Internal. To understand how antioxidant works, it's first necessary to establish an understanding of free radicals. Free radicals are atoms that have an unpaired electron in an atomic orbital and are capable of independent existence. (Pharmacogn Rev, 2010). An atomic orbital typically holds two electrons, but during certain reactions a molecule can be left with only one, unpaired, electron in its atomic orbital. An odd number of electrons make the molecule unstable and highly reactive, resulting in a species that is known as a free radical. The energy of UV radiation penetrating the cell and encourages such reactions within cells. Since, free radicals are highly reactive, they want to either donate that single electron to another molecule or accept an electron from another molecule so that they can become stabilized. The "donor" molecule may become unstable. While a certain level of free radical production is normal but overtime free radicals can accumulate and damage cells through vicious chain reactions. The problem arises when the free radicals react with important cellular components, such as DNA, proteins, carbohydrates, or the cell membrane. When this happens, the cells become dysfunctional or undergo apoptosis (cell death). *E. coli* has developed, defense mechanism against free radicals by antioxidants. Antioxidants work through many different mechanisms, with the main objective being to interfere with oxidative processes that produce harmful free radicals. They act by scavenging free radicals and "breaking the chain" of reactions, by binding metal ions, or by removing oxidative damaged biomolecules. (Karger et al., 2001).

Unfortunately, the *E. coli* naturally, does not produce or ingest enough antioxidants to neutralize all of the free radicals, which lead to oxidative stress. Essentially, oxidative stress is an imbalance between the production of free radicals and the ability of the cell to counteract or detoxify their harmful effects through neutralization by antioxidants. There are many types of radiation damage to bacterial cell. The types of damage depend on the cells being irradiated, the dose and the rate of exposure, and the time after exposure that is being assayed for a radiation effect. Oxidative stress is strongly implicated on the genetical and molecular level of the cell responded to the oxidative stress. In *E. coli.*, the respond to oxidative stress invoke by two distinct stress responses, the peroxide stimulation and the superoxide stimulation, depending on whether the stress is mediated by peroxides or the superoxide anion. The products of OxyR and SoxRS-regulated genes, such as catalases and superoxide dismutase, are involved in the prevention of oxidative damage, whereas others, such as endonuclease IV, play a role in the repair of oxidative damage (Aaron Daugherty et al., 2011).

Antioxidants act as a defense mechanism that protects against oxidative damage, and include compounds to remove or repair damaged molecules. It can prevent/retard the oxidation caused by free radicals. Sufficient intake of antioxidants is supposed to protect against diseases (Celiktar et al., 2007).

Oxidation process is a chemical reaction that produces free radicals, leading to chain reactions that may damage cells. The antioxidant effect of vitamin C has been well documented (Bindhumol et al., 2003). Oxidation of molecules leads to the formation of free radicals. These free radicals when present can cause harmful chain reactions which may be responsible for cell death. Antioxidant neutralizes these radicals by donating their electrons, ending in taking the electrons. The majority of free radicals that damage biological mechanism are oxygenated radicals and these are known as 'reactive oxygen species' (ROS). ROS are formed during UV light irradiation, or by X-rays, gamma rays (Cadenas E, 1989).

Vitamin C also enhances iron absorption by reducing Fe^{+3} to Fe^{2}"from non-heme iron sources (Hacișevki 2009, Halliwell et al., 1999). In the presence of redox - active ions (iron, copper), vitamin C acts as a prooxidant, contributing to the formation of hydroxyl radicals, that may lead to lipid, DNA, or protein oxidation (Rouhier N Lemaire et al., 2008).

Vitamin C is thought to be an important water-soluble antioxidant which is reported to neutralize ROS and reduce the oxidative stress. (Rouhier N Lemaire et al., 2008). Vitamin C has been shown to be an effective scavenger against oxygen and nitrogen oxide species, such as superoxide radical ion, hydrogen peroxide, the hydroxyl radical, and singlet oxygen. This property of vitamin C has vital processes in protection of cellular components from free radical-induced damage. In addition, vitamin C is effective in regenerating the antioxidant form of vitamin E by reducing tocopheroxyl radicals. This process protects membranes and other compartments of the cell from free radical-induced damage. The fur mutation leading to an intracellular overload of iron also increased sensitivity of growing *E. coli* cells to osmotic upshift. Using lacZ fusions, it was shown that expression of antioxidant genes sox and katE was stimulated by an increase in osmolarity. (Smirnova, *et al.*, 2000). ROS are oxygen derived small molecules which are produced as intermediates in the redox reactions, such as ozone, superoxide, singlet oxygen and hydrogen peroxide. So, ROS play important roles in cellular signaling processes and trigger diseases (Chen S et al., 2013).

Due to the generation of reactive oxygen species, Vitamin C (L-ascorbic acid) functions as biological co-factor and antioxidant due to its reducing properties. The topical application showed the application of vitamin C, correlates with protection of the cell from UVB damage. This is due to the reducing properties of the molecule. (Dermatol et al., 1992)

II. MATERIALS AND METHODS

1. **Bacterial strain:**
   The bacterial strain that was used in this experiment is a wild strain of *Escherichia coli* was taken from Department of Microbiology, Maharani Science College, Bengaluru.
2. **Media:**
   E. coli was routinely grown in the Luria-Bertani broth (LB) and in the Luria-Bertani Agar (LB agar). Different amount of the components for preparing LB broth and LB agar is listed in the TABLE 1.
3. **Vitamin-C:**
   We have used a drug store vitamin-C tablet for our experiment, Abbott LIMCEE- Chewable vitamin C 500 mg tabs (orange flavor). As this tablet is easily available.
4. **SDS-PAGE:** For protein assay we have done SDS-PAGE. Different component for the preparation of the gel is listed below in the TABLE 2 and different other chemicals like dyes are listed in the TABLE 3.

TABLE 1:
SHOWING COMPOSITION OF LB BROTH AND LB AGAR.

LB BROTH (l00ml)	Peptone- lg, Yeast extract- 0.5g, NaCl- 1g
LB AGAR (l00ml)	Peptone- 1g, Yeast extra- 0.5g, NaCl- lg Agar- 1.6g

TABLE 2:
SHOWING COMPOSITION OF Stacking and Separating gel in SDS-PAGE.

STACKING GEL	**SEPARATING GEL**
Distilled water	Distilled water
30% Acrylamide	30% Acrylamide
1 M Tris HC1 (pH-6.8)	1 M Tris HCl (pH-8.8)
10% APS (Ammonium Persulfate)	10% APS (Ammonium Persulfate)
TEMED (Tetramethyl ethylenediamine)	TEMED (Tetramethyl ethylenediamine)

TABLE 3:
Other components of SDS-PAGE, Lysis buffer and Dyes.

LYSIS BUFFER	10 mM tris-HCL pH 8.0, 10% glycerol, 150 mM NaC1, l mM EDTA, l mM β-mercaptoethanol (β-ME).
STAINING DYE	0.04% Coomassie Brilliant blue (R — 250) 10% glacial acetic acid and 40% methanol, make up to 500 ml.
TREATMENT DYE	250 mM tris HCL pH 6.8, 10% SDS, 30% glycerol, 10 mM, β -mercaptoethanol and 0.01% bromophenol blue.

5. ***E. coli* growth assay:**

E. coli slant was taken from Microbiology department from Maharani College, Bengaluru. Then for reviving it was cultured twice in 3 ml of LB broth. Then a single colony from the overnight incubated plate was again inoculated in a 3 ml LB broth and kept for overnight incubation at 37 °C in a rotary shaker at 150 rpm. After that, 1% of the overnight grown culture was inoculated in a 3 ml LB broth and incubated 37 °C in the rotary shaker 150 rpm.

6. Vitamin C assay:

Vitamin C (Abbott LIMCEE- chewable vitamin C 500 mg tabs- pack of 15 tabs- orange flavor) tablet was dissolved completely in 20 ml of distilled water to make a stock solution of a concentration of 25 mg/ml. From that stock solution, 1 m1 of vitamin C solution was dissolved in 10 ml of distilled water to make a working solution of a concentration of 2.5 mg/ml. For the Vitamin C assay, we had mixed vitamin C into two 10 ml LB broth with a concentration of 45 µg/ml and 50 µg/ml respectively. And one, 10 ml LB broth without Vitamin C was taken as control. After that 1ml of overnight grown *E. coli* culture was mixed into each of the 10 ml LB broth and kept for 2 h of incubation at 37°C and 150 rpm. After 2 h, Optical density (OD) was taken in 600 nm wave length. OD for the control was 0.47 OD, flask with 45 µl vit-C concentration, had an OD of 0.55 OD and the flask with 50 µl concentration of vit-C had an OD of 0.66 OD at A600 nm.

7. Drop plate technique:

As reported the DOT plate technique is more efficient as compare to spread plate technique. (Hossein Naghili *et al*., 2013) At equivalent 0.4 OD, all the three *E. coli* cultures (control, culture with 45 µl vit-C and 50 µl vit-C) were collected in same amount and centrifuged at 10,000 rpm for 15 min. Then the cell pellets were resuspended in 1ml distilled water. After that, each of the three cell suspension cultures were diluted to 10^{-1} to 10^{-5} dilutions, forming a total of 15 diluted cultures (3 cultures X 5 dilutions = 15 diluted cultures). Then, the 2µl of diluted cultures were spotted onto a total 9 LB plates supplemented with 0 µg/ml, 45 µg/ml and 50 µg/ml of vitamin-C respectively. All the details of the plates are given in the TABLE 4.

8. UV Sensitivity assay:

All the test LB plates were then irradiated with UV radiation (254 nm) with different time span. Three LB plates (Control-1, Vit-C 45 µg/ml-1, Vit-C 30 µg/ml-1) were radiated with UV for 45 seconds. Then another three LB plates (Control-2, Vit-C 45 µg/ml-2, Vit-C 30 µg/ml-2) were exposed to UV for 30 seconds. And last three LB plates (Control-3, Vit-C 45µg/ml-3, Vit-C 30µg/ml-3) were kept without any exposure of UV. After UV sensitivity assay all the LB plates were incubated at 37 °C for overnight in dark.

TABLE 4:
All details of the 9 LB plates.

PLATE 1	CONTROL 1 (dilution from 10^{-1} to 10^{-5})	UV exposure for 45 second.
PLATE 2	CONTROL 2 (dilution from 10^{-1} to 10^{-5})	UV exposure for 30 second.
PLATE 3	CONTROL 3 (dilution from 10^{-1} to 10^{-5})	Without UV exposure.
PLATE 4	Vit-C 45µg/ml-1 ((dilution from 10^{-1} to 10^{-5})	UV exposure for 45 second.
PLATE 5	Vit-C 45µg/ml-2 ((dilution from 10^{-1} to 10^{-5})	UV exposure for 30 second.
PLATE 6	Vit-C 45µg/ml-3 ((dilution from 10^{-1} to 10^{-5})	Without UV exposure.
PLATE 7	Vit-C 50µg/ml-1 ((dilution from 10^{-1} to 10^{-5})	UV exposure for 45 second.
PLATE 8	Vit-C 50µg/ml-2 ((dilution from 10^{-1} to 10^{-5})	UV exposure for 30 second.
PLATE 9	Vit-C 50µg/ml-3 ((dilution from 10^{-1} to 10^{-5})	Without UV exposure.

9. Protein extraction assay:

Separately, overnight grown cultures of *E. coli* was then inoculated to 10 ml of LB broth with 0.0 µg/ml and 50 µg/ml of vit C and incubated at 37°C at 150 rpm until the OD of the culture reached to 0.4 to 0.5 OD at A600 nm. Then the cultures were exposed to UV radiation at 254nm for 1 minute and the cultures were subsequently recovered by incubating in 37 °C at 150 rpm for 1hr. After that the cultures were chilled on ice for 30 minutes and then centrifuged at 10,000 rpm for 15 minutes at 4°C. Then the pellets were resuspended in lysis buffer and lysed by sonication (Model No. GEX 750, Ultrasonic Processor) on ice at 30% duty cycles in a pulse mode. The sonicated suspension was centrifuged at 13000 rpm for 1 h at 4°C. The protein concentration of cell free lysates of both control and with vitamin C were estimated by Nanodrop.

10. SDS-PAGE for protein pattern:

The protein samples (10 µl -100 µg)) of both control and Vitamin C treated, were loaded on to 10 % SDS – PAGE, run at 30 mA for 1.30 h at RT. Then the gel was stained with staining solution for 1h and destined with destining solution for 1h.

III. RESULT & DISCUSSION

1. Effect of the Vitamin-C on UV protection:

The effect of vitamins C on the protection of *E. coli* against UV radiation was tested by comparing the size of colonies between the control plate and the plate with vitamins that were exposed to UV radiation and without UV exposure. The result indicated that, the presence of vitamins had positive effect on the *E. coli* growth and survivability. Plates with vitamin C showed greater growth and resistant against UV when compared to the control. Also, the study concluded that higher the concentrations of vitamin C, higher the survivability and growth of *E. coli* colonies in the presence of UV rays, when compared between the different concentrations of vitamin C plates.

1.1. COMPARISION BETWEEN ALL THE PLATES OF CONTROL, VITAMIN-C:

(45 µg/ml, 50 µg/ml) (without UV, Plates exposed to UV for 30 sec, Plates exposed to UV for 45 sec

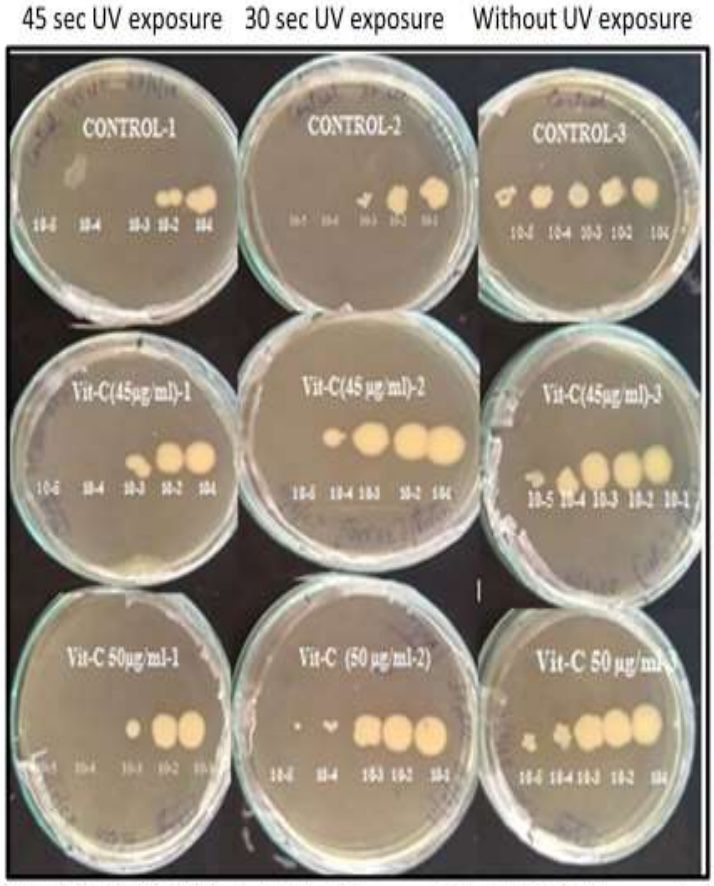

Fig 1: FIRST ROW FROM LEFT TO RIGHT, Control-1 (45s UV exposure),Control-2 (30s UV exposure), Control-3 (without UV exposure). SECOND ROW FROM LEFT TO RIGHT, Vit-C 45 µg/ml (45s UV exposure), Vit-C 45 µg/ml (30s UV exposure), Vit-C 45 µg/ml (without UV exposure). THIRD ROW FROM LEFT TO RIGHT, Vit-C 50 µg/ml (45s UV exposure), Vit-C 50 µg/ml (30s UV exposure), Vit-C 50 µg/ml (without UV exposure).

The Fig-1 depicting the comparison. Through these plates we concluded that the Vit-C showed antioxidant property. The growth of the *E. coli* in presence of Vit-C was more even after UV exposure as compare to the control. From the figure all the three plates which were not exposed to UV (control-3, Vit-C, 45 µg/ml and Vit-C 50 µg/ml) showed growth but the amount of growth was more in the plates with vitamins. The plates with highest concentration (Vit -C, 50 µg/ml) showed better growth than the other plates. The plates which were exposed to UV for 30 sec, in case of control showed growth till 10^{-3} dilution and also the growth was less as compared to the control plate without UV exposure. Whereas the other plates with Vitamin C showed growth till 10^{-4} for the concentration 45 µg/ml and till 10^{-5} for Vitamin C concentration of 50 µg/ml. For UV exposure of 45 sec, the plates showed less growth as compare to the other plates. In case of the control plate, it showed growth till 10^{-2}. Whereas the other plates with vitamin C showed growth till 10^{-3}. But in this case the plate with Vitamin C of concentration 45 µg /ml showed more growth than the Vitamin C of concentration 50 µg/ml. So, we can conclude that Vitamin C has antioxidant property that can neutralize the effect of UV exposure in the cell. (Adella A. Fejeran, 2008)

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1.2. COMPARISION BETWEEN ALL THE PLATES OF CONTROL, VITAMIN-C

(45 µg/ml, 50 µg/ml) (Plates exposed to UV for 30sec, Plates exposed to UV for 45sec)

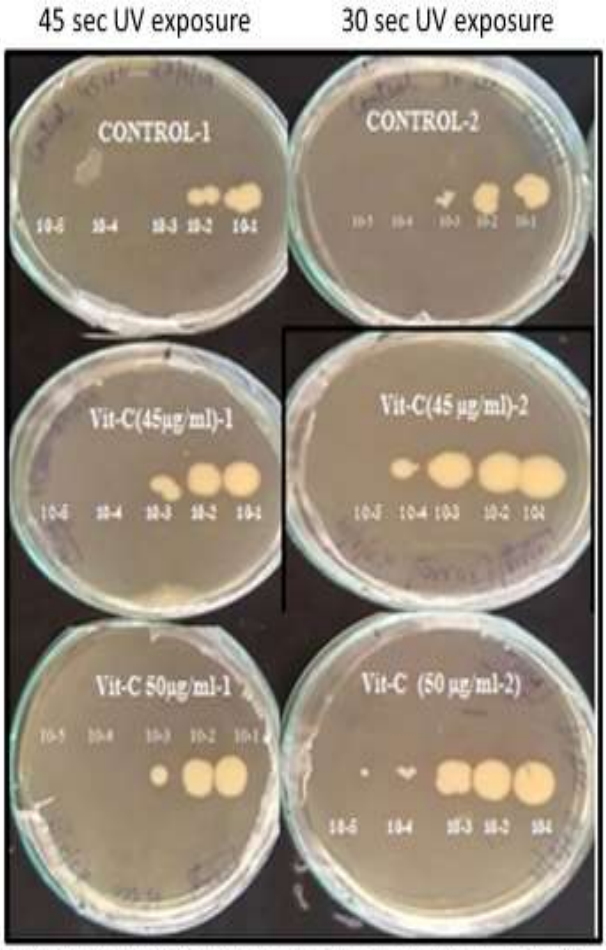

Fig 2: FIRST ROW, FROM LEFT TO RIGHT, Control-1 (45s UV exposure), Control-2 (30s UV exposure). SECOND ROW, FROM LEFT TO RIGHT, Vit-C 45 µg/ml-1 (45s UV exposure), Vit-C 45 µg/ml-2 (30s UV exposed). THIRD ROW, FROM LEFT TO RIGHT, Vit-C 50 µg/ml-1 (45s UV exposure), Vit-C 50 µg/ml-2 (30s UV exposure).

From this Fig-2, we can conclude that the control plates without Vitamin when exposed to UV for 30 sec showed growth till the dilution of 10-3; and when it was exposed to the UV for 45 sec it showed growth till 10-2 dilution only. The plates with Vitamin C (45 µg/ml) exposed to UV for 30sec showed growth till dilution 10-4 when compared to the Control plates. The plates with (45µg/ml) showed growth till 10-3 when exposed to UV for 45 sec, this may be because of more UV rays absorbed by the cells having same Vitamin concentration; therefore, if the concentration of UV increases it overpowers the vitamin present in the media. The plates with 50 µg/ml showed more growth as compare to 45 µg/ml (Vitamin C) till dilution 10-5 when exposed to UV for 30 sec and the plates with Vitamin C 50 µg/ml when exposed to UV for 45sec showed growth till 10-3 dilutions only. Therefore, we can conclude that when UV concentration is more it overpower the concentration of the Vitamins. (Adella A. Fejeran, 2008).

1.3. COMPARISION BETWEEN ALL THE PLATES OF CONTROL, VITAMIN-C:

(45 µg/ml, 50 µg/ml) (without UV, Plates exposed to UV for 45sec)

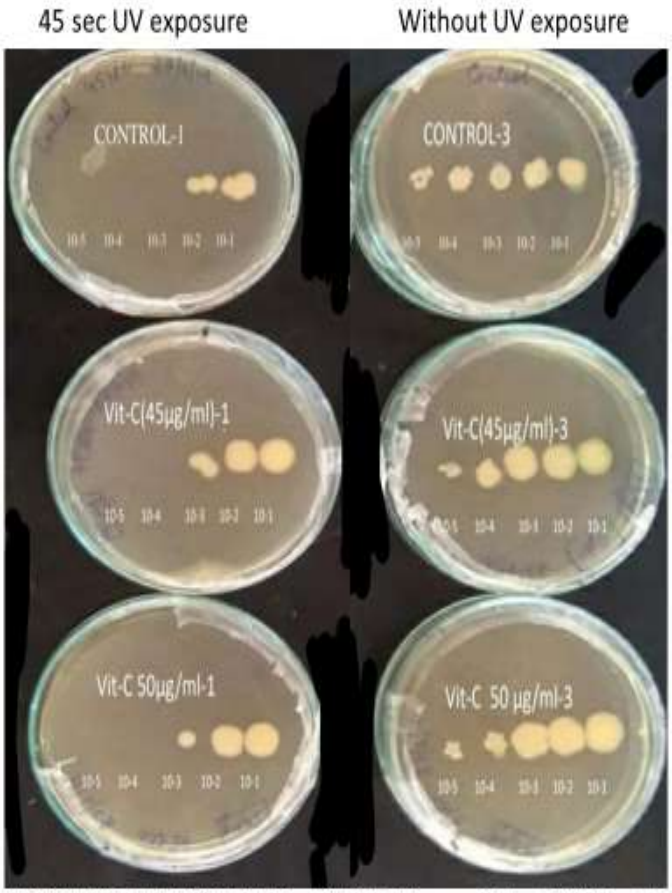

Fig 3: FIRST ROW, FROM LEFT TO RIGHT, Control-1 (45s UV exposure), Control-2 (without UV exposure). SECOND ROW, FROM LEFT TO RIGHT, Vit-C 45 µg/ml-1 (45s UV exposure), Vit-C 45 µg/ml-3 (without UV exposure). THIRD ROW, FROM LEFT TO RIGHT, Vit-C 50 µg/ml-1 (45s UV exposure), Vit-C 45 µg/ml-3 (without UV exposure).

From the Fig-3, we can conclude that even when the plates were exposed to UV for 45sec, the higher concentration of Vitamin C in plate with 50 µg/ml showed comparative good growth. The plates of control and 45µg/ml Vitamin C plates showed less growth when they were exposed to UV for 45 sec. The control plates without UV showed growth in all dilutions, the growth was in the uniform manner. The plates with Vitamin C (50 µg/ml, 45µg/ml) showed more colony size when compared to the control plates which were not exposed to UV.

1.4. COMPARISION BETWEEN ALL THE PLATES OF CONTROL, VITAMIN-C:

(45 µg/ml, 50 µg/ml) (without UV, Plates exposed to UV for 30sec)

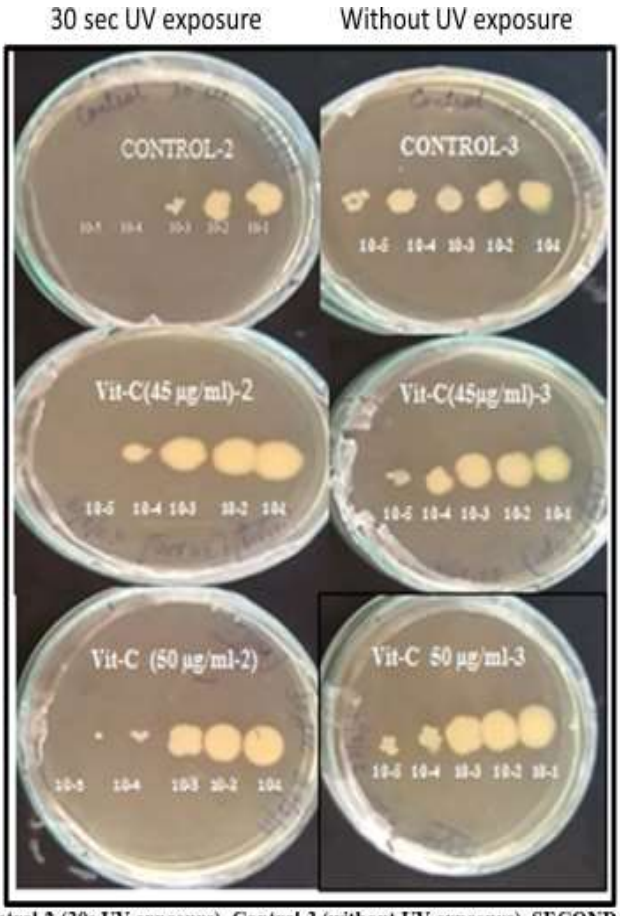

Fig 4: FIRST ROW, LEFT TO RIGHT, Control-2 (30s UV exposure), Control-3 (without UV exposure). SECOND ROW, LEFT TO RIGHT, Vit-C 45 µg/ml-2 (30s UV exposure), Vit-C 45 µg/ml-3 (without UV exposure). THIRD ROW, LEFT TO RIGHT, Vit-C 50 µg/ml-2 (30s UV exposure), Vit-C 50 µg/ml-3 (without UV exposure).

From the Fig-4, we can conclude that when control plates were not exposed to UV (Control-3); It shows all the colonies; Control 2 shows the colony till dilution 10^{-3} when exposed to UV for 30 sec. The plates having 45 µg/ml of vitamins without UV showed more amounts of growth when compared to the control plates and growth were seen till 10^{-5} dilutions. The plates with 45 µg /ml vitamins when exposed to UV showed growth even till 10^{-4}, as compare to control which was exposed to UV for 30 sec. The plates having 50 µg/ml of Vitamin showed maximum growth when compared to the other plates of control, and plates with 45 µg/ml when it was not exposed to UV. The Vitamin C with 50 µg/ml when exposed to UV for 30 sec was able to show the growth till 10-5. Hence, we can conclude that Vitamin C act as an antioxidant which is Concentration dependent.

1.5. Protein extraction and analysis from E. coli:

E. coli is a very versatile host for the production of heterologous proteins, and various protein expression systems have been developed which allow the production of recombinant proteins in *E. coli.* And the protein expressed when introduced with the vitamin was checked. For protein extraction, 2 bacterial cultures of with and without (control) vitamin were lysed by sonication and SDS-PAGE was run to analyses the protein pattern.

TABLE 5:
Protein estimation by Nanodrop

SAMPLES	PROTEIN ESTIMATION Conc. (mg/m1)
Control	38.1 mg/ml
50 µg/ml	17.2 mg/ml

1.6. SDS-PAGE for analysis of protein pattern:

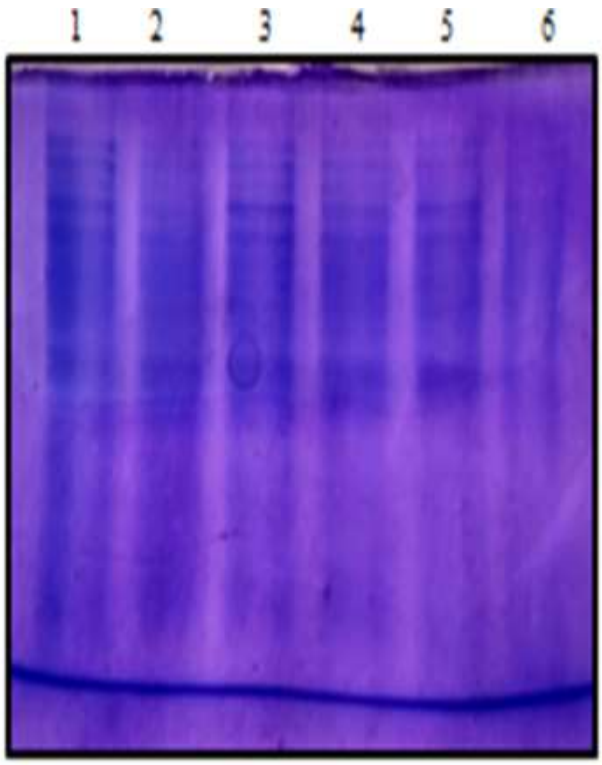

Fig 5: SDS-PAGE OF PROTEIN EXTRACTED FROM E. COLI INCUBATED WITH 50µg/ml(CONC.) VITAMIN-C AND WITHOUT ANY VITAMIN-C(CONTROL). LANE1: Sample with vit-C(5ul); LANE2: Control(5ul); LANE3: Sample with vit-C(7ul); LANE4: Control(7ul); LANE5: Sample with vit-C(10ul); LANE6: Control(10ul)

To check the change in the protein expression SDS-PAGE was done. Protein expressed in the control showed difference as compare to the sample that incubated with vitamin. The expression of protein in the control was less as compare to the proteins expressed in the sample with Vitamin C. The difference in the protein expression can be seen and further we can find the different gene which get activates because of the vitamin C.

IV. CONCLUSION

The present study was an attempt to understand the antioxidant property of Vitamin C, how it regulates the growth of *E. coli* when oxidative stress is given to the cells. In the presence of vitamin C even when the plates were exposed to UV was able to survive when compared with the control.

The plates with more Vitamin C showed more growth as compared to the control plates which were exposed to UV was not able to grow after 10^{-3} dilutions. The dilutions also affected the growth of the organisms as more the dilutions less the organism and therefore when exposed to the UV it got killed. The plates having Vitamin C as the supplements were able to show growth even at lower dilutions.

From the plates we can conclude that protein expression may have changed because of the Vitamin C which was introduced in the media. Therefore, to check the protein expression changes the *E. coli* cells were sonicated and proteins were extracted it was further analyzed in the SDS - PAGE.

The more prominent bands were observed in the supernatant which was derived from the culture having Vitamin C rather than the control. Hence, we can say that the changes are there in the growth and the protein expression pattern of the *E. coli* cells. Thus, the Vitamin C has the antioxidant property.

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