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Comparative Effects of Organic and Inorganic Substances in the Repair and Renaturation of DNA

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Abstract: ***In this particular experiment, three creams are tested comparatively in DNA renaturation. They are Turmeric (organic), Patanjali (semi-organic) and Fair and lovely (synthetic). DNA is extracted from the buccal mucosal cells of nearly 30 students selected randomly of age group 18-20 years. DNA is left for denaturation. DNA renaturation kinetics is done using the above mentioned solvents (5 mL of 1% solution in distilled water). Optical Density values were then calculated for each of the sample. It was seen that turmeric was the most effective one in renaturing DNA, followed by the creams of Patanjali and fair & lovely.***

Keywords: renaturation, optical density, colorimeter

1. Introduction

Cosmetics such as face washes, shampoos and packs have been introduced with the criteria of enhancing a certain feature of the human body. Each type of product displayed on shelves, all claim to be dermatologically approved for usage (ref 2, ref 4). In this experiment, we would be testing the function of face creams from three different sectors of departmental cosmetics.

The first one being Fair and Lovely, which has been advertised to be the leading brand of cream, women buy for instant whitening of the face and removal of tan lines (ref 1). The second sector being tested is Patanjali, whose market value over the last year has increased tremendously due to its advertisement of using "organic products". Finally, this experiment will be using home remedies and all-natural products (turmeric powder) to set as a control and compare the effects between the three.

Fair and Lovely contains harmful chemicals such as Phenoxyethanol, which is stated to cause skin or lung cancer (ref 2, ref 3). Along with which, it includes glycerin, aloe juice, and Shea butter all of which are bonded together with the use of preservatives.

Patanjali on the other hand, uses higher amounts of organic matter and hence changes color over a period of time providing evidence that organic solvents have been added. This experiment would highlight the renaturation of denatured DNA by application of the three types of enhancers. A comparative data would be analyzed to produce a report citing which products work upon the process of renaturation of DNA.

Objective

Analysis of the Optical Density values to understand the absorption of the sample by the DNA.

2. Materials and Methods

Materials required

Saline water, ethanol, food colors, liquid soap, turmeric powder, fairness creams of Fair & Lovely and Patanjali, Sodium hydroxide, distilled water.

Methodology of experiment

For gaining easiest access to cells of the human body, buccal cells of the mouth/ buccal cavity were used. The buccal mucosal cells of around 30 students were collected who belonged to the age group of 18-20 years. This provided a clean, non-exposed suspension of cells, from which DNA can be extracted.

The procedure has been divided into three parts:

Extraction of DNA

1) Clean mouth and rinse buccal cavity.
2) To a beaker containing about 15 mL of water, add salt (NaCl) to it and stir till the salt is completely dissolved. Gargle this water and spit back into the beaker. The role of the salt is to neutralize the charge of the DNA's sugar phosphate backbone.
3) To this beaker, add about 4-5 drops of detergent/ liquid soaps. Shake gently and keep still. Detergent contains sodium lauryl sulfate, which helps in disintegrating the membranes of the cells, pulling apart the lipids and proteins that make up the membranes surrounding the cell and nucleus. Once these membranes are broken apart, the DNA is released from the cell.
4) In another beaker take 10-15 mL of ethanol and add 3-4 drops of food colouring to it. Mix it well.
5) Now gently add the ethanol mixture to beaker containing sample without much shaking. Two layers of liquids are formed. Slowly mix the contents. Food colouring now stains the exposed DNA, so that the strands are visible to the naked eye.
6) Once the DNA is visible (as strands), it is carefully transferred into an eppendorf to which Sodium Hydroxide and water has been added. In DNA isolation or extraction, NaOH (Sodium hydroxide) is used as alkaline lysis buffer. It helps in the stabilization of DNA and prevents it from further denaturation.
7) This eppendorf is then stored at refrigerator conditions for further usage.

Preparation of cream solutions

Weigh 1 gm of sample and mix it in with 100 mL of water (in case of turmeric, ethanol is used as a dissolving medium). This was repeated for each of the samples; Patanjali, Fair and Lovely and Turmeric. These samples were then stored in a sterile environment.

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Optical density values

1) The colorimeter was adjusted to an absorption value of wavelength 446.
2) The soluble DNA of each sample, which had been extracted, was poured into three separate sterile small beakers.
3) 1 ml of each of the sample solutions was introduced into these beakers. They are immediately transferred into a cuvette and kept in the colorimeter to know the Optical density value. This is known as the 0^{th} minute reading. Values are recorded with a time interval of 10 minutes, until four readings are obtained.

Statistical analysis

1) The observed data of the optical density values of the three creams has been shown in the form of a line graph to make a comparative study of the effects of the creams.(fig 1)
2) ANOVA has been performed for the observed data.

3. Results and Discussion

From the figure (fig.1), it can be observed that there is a significant decrease in the colorimetric values of the samples in which turmeric was added. Following closely behind, Patanjali's Optical Density values also show a consistent decrease. The Optical Density values for Fair & Lovely, however, showed the least amount of decrease. The values remained either consistent or showed a low decline.

Graph

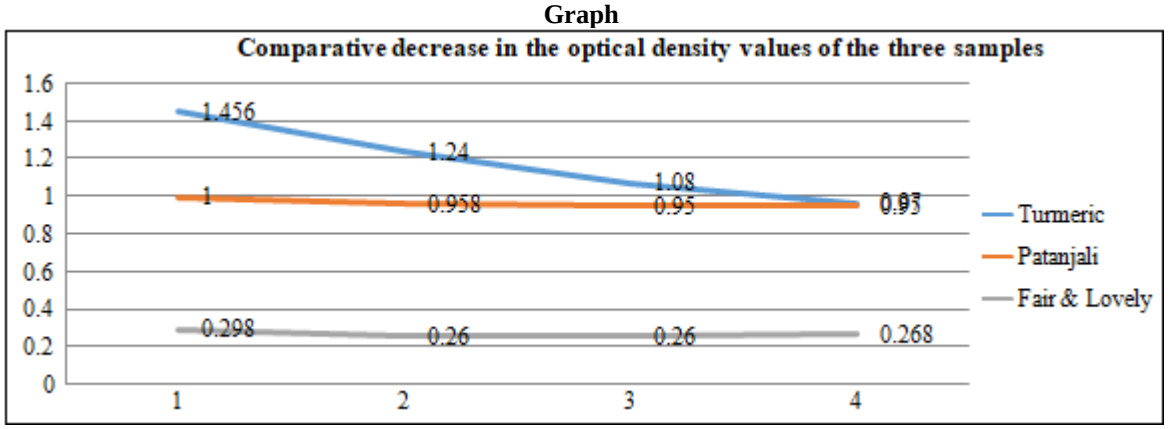

Figure 1

ANOVA (Analysis of Variance)

ANOVA single factor analysis has been carried out at 5% (0.05) level of significance (los). The P-value obtained is 6.20705E.06 (0.00000602) which is less than 0.05(los). This indicates that there is a significant difference in the optical density values due to different cream samples that were used to renature the DNA at 5% level of significance.

4. Conclusion

It can be inferred from the line graph that the decrease in the optical density values are comparatively higher in the case of turmeric than in Patanjali or fair & lovely. Also, the P-value from ANOVA provides support of the significant decrease in the values. Therefore, it can be concluded that turmeric is most effective in renaturing the degraded DNA.

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