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*Original Research Article*

# Evaluation of Toxicity of Synthetic Food Colors on Human Normal Flora and Yeast

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## ABSTRACT

The present work was undertaken to study the toxic effect of synthetic food colors against human normal flora and Yeast. Four colors viz Orange, Yellow, Red and Green; derivatives of synthetic dyes were used. The toxic effect of all food colors were analyzed by performing antimicrobial activity adopting disc diffusion method. The results indicate that almost all food colors found inhibitory for test organisms i.e., *Escherichia coli*, *Staphylococcus albus* and *Saccharomyces cerevisiae*. It was also observed that the inhibitory effect was found directly proportional to the concentrations of food colors. Hence, it is recommended that dilute synthetic food colorants up to safe level before add into food, Food products; drinks etc. or natural colorants seemed to be the best choice as food colorants.

***Key words***: Synthetic food colors, Human Normal flora (*Escherichia coli*, *Staphylococcus albus*) and Yeast (*Saccharomyces cerevisiae)*, Disk diffusion Method, Anti-microbial Activity.

## INRODUCTION

Food colors are substances which added to food, food products and drink to changes its color to improve the visual quality of food for attraction of the consumer. [1] Food colors are classified as natural and synthetic food colors. Natural Food Color is derived from variety of sources such as seeds, fruits, vegetables, insect and microorganisms without any chemical treatment. In prehistoric times, natural colorants were usually obtained from the sources like flowers, leaves, berries, blossoms, barks and roots. [2] Synthetic food colors are usually water-soluble chemical substances which have been made in factory and can be used in foods without any further processing. Ponceau 4R, Carmoisine, Erythrosine, Tartrazine, Sunset Yellow FCF are the examples of synthetic food colors. [3,4,5] In the middle of the nineteenth century, introduction of synthetic color marked the decline in the use of natural colors to color foods, drugs and cosmetics. [6] Synthetic colors are reliable and economical for restoring the original shade of the foods compared to the natural colorants which are expensive and less stable. [7] However, the synthetic food colorants showed adverse effect on human health. It has been reported that consumption of synthetic foods color additives could sometimes lead toxic effects on liver, kidney and testes. [8,9] The administration of synthetic food colorants decreased the percentage of high density lipoprotein cholesterol (HDL-C), glutathione secretion (GSH), superoxide dismutase (SoD), and plasma immune-system and significantly increased plasma lipid lipoprotein, total cholesterol (LDL-C), lipid peroxidase, blood glucose, plasma urea and creatinine and increased activities of

alkaline phosphatase, acid phosphatase, and lactate dehydrogenase. [10] Previous studies investigated the metabolic and toxicological disorders induced by the administration of specific food colorant additives to rats and other mammals. [11,12,13] The nutritional hazards of synthetic food colors have been detected in the liver and kidney. [14] However, to our knowledge, synthetic food colors still least touched to investigate it's toxic effect on human normal flora. "Normal flora" is resident flora consists of relatively fixed types of microorganisms that inhabit the skin or mucous membrane of certain areas of the body and plays a definite role in maintaining health and normal function. [15,16] Therefore, the present investigation was planned to elucidate toxicity of synthetic food colors on human normal flora and yeast(*Saccharomyces cerevisiae)*.

## MATERIALS AND METHODS

### Collection of Food Colors

Four food colors samples viz. Orange, Red, Yellow and Green were purchased from "Arjav" shop of Washim market. All the food colors were synthetic food color preparations manufactured by the "Asian food products," Ahmadabad, Gujarat, India. Orange, Yellow and Green colors were the derivative of Tartrazin and Red color is the derivative of Carmosine. All food colors were transported in microbiology research laboratory, R.A. College, Washim (M.S), India.

### Preparation of Standard stock solutions

The stock solutions of all food colors were prepared by dissolving 1gm of color in 10ml of distilled water separately and all the food colors were serially diluted to form the different concentrations such as (10mg/ml, 1mg/ml,0.1mg/ml,0.01mg/ml, and 0.001mg/ml) were kept in to screw cap tube and preserved at $4^{o}$ C in refrigerator. [17]

### Toxicity of food colors against test organisms

The toxic effect of all food colors on *Escherichia coli*, *Staphylococcus albus* and *Saccharomyces cerevisiae* were evaluated by performing antimicrobial activity adopting disc diffusion method. [18] The effect of synthetic food colors on *Saccharomycescerevisae* was carried out to demonstrate possible toxic effect on human cell. The turbidity of enriched *Escherichia coli*, *Staphylococcus albus* and *Saccharomycescerevisiae* were accurately matched with McFarland standard solution. [19] 100µl enriched cultures of *Escherichia coli* and *Staphylococcus albus* aseptically spread over sterile Muller Hinton's agar plates. Whereas, seed layer of enriched culture of *Saccharomyces cerevisiae* prepared on Potato dextrose agar by spread plate technique. There after sterile paper discs of size 6 mm(diameter) separately soaked in to each dilution of each food color and aseptically placed on respective Petri plates of seed layer containing Muller Hinton's agar and potato dextrose agar plates. Whereas, the paper discs soaked into sterile distilled water and place separately on seed culture containing Muller Hinton's agar plates and potato dextrose agar plates aseptically and considered as control. All the Muller Hinton's agar plates and potato dextrose agar plates incubated at $37^{0}$ C for 24 hours and room temperature for 3 days respectively. After incubation period all the plates were observed for the zone of inhibition to evaluate the toxic effect of food colors on test organisms over control. The experiment carried out in triplicates hence, the results were recorded in terms of mean zone of inhibition in results.

## RESULTS AND DISCUSSION

Four synthetic food colors Orange, Red, Yellow and Green which are derivatives of derivative of Tartrazin and Carmosin were used to analyze the toxicity on *Escherichia coli*, *Staphylococcus albus* and *Saccharomyces cerevisiae*. The toxic effect of all food colors were assessed by antimicrobial activity adopting disc diffusion method and the results were measure in terms of zone of inhibitions. The results on inhibitory effect of different concentrations of all food color were

summarized in table 1.The diameter of zone was recorded in each concentration of each food color. Present study indicates that higher concentration reflected by decrease in diameter of zone inhibition. From the results it was observed that the orange food color was inhibitory to *Escherichia coli*, at its 10, 1&0.1mg/ml concentrations respectively .Thereafter, further decrease in concentration doesn't showed any inhibitory effect on the growth of *Escherichia coli*. Similarly in case of *Staphylococcus albus* the inhibitory effect was observed upto 0.1mg/ml. However, the yeast cells are inhibited by the orange food colour only up to 1mg/ml concentration of the dye. This indicates the use of orange food color may be safe to use only upto0.1mg/ml concentration.

In case of yellow food color 10, 1&0.1mg/ml and 10, 1, 0.1& 0.01 mg/ml concentrations displayed inhibitory effect against *Escherichia coli* and *Staphylococcus albus* respectively. Whereas, inhibition of yeast cell does not recorded in all the concentrations of yellow food color. It indicates that the use of yellow food color upto 0.001 mg/ml concentration may be safe for *Escherichia coli* and *Staphylococcus albus*. However, further dilutions of yellow food color require to achieve safe concentration for the Yeast cell.

At concentrations 10&1mg/ml Green food color found inhibitory to *Escherichia coli* and all decreased concentrations (0.1, 0.01 &0.001 mg/ml) were found safe for *Escherichia coli*. However, terrifically different results were recorded in case of *Staphylococcus albus*. The Green food color does not showed inhibitory effect at any concentration against *Staphylococcus albus*. The zone of inhibitions of Green food color against Yeast obtained in 10, 1 &0.1mg/ml concentrations. This indicates that 0.01 mg/ml was found safe for Yeast cell.

The test organism *Escherichia coli*, *Staphylococcus albus* and Yeast cell were inhibited by Red color at (10&1mg/ml), (10, 1&0.1mg/ml) and (10&1mg/ml) concentrations respectively. The result indicates that the concentrations of Red color bellow from 0.1, 0.01and 0.1 mg/ml may be safe for *Escherichia coli*, *Staphylococcus albus* and Yeast cell respectively.

**Table 1:- Effect of food colors on test organisms.**

| Sr. no. | Concentrations (mg/ml) | Zone of Inhibition(mm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Orange | | | Yellow | | | Green | | | Red | | |
| | | E.C. | S.A. | Yeast | E.C. | S.A. | Yeast | E.C. | S.A. | Yeast | E.C. | S.A. | Yeast |
| 1 | 10 | 10 | 11 | 10 | 9.33 | 9.66 | 9.66 | 8.33 | N.Z. | 9.33 | 9.6 | 10 | 9.11 |
| 2 | 1 | 9.5 | 10.5 | 7.3 | 8 | 8.5 | 8 | 7 | N.Z. | 8.88 | 7.3 | 9.77 | 7.55 |
| 3 | 0.1 | 7 | 8.55 | N.Z. | 6.55 | 7 | 7 | 7 | N.Z. | 6.55 | N.Z. | 7.66 | N.Z. |
| 4 | 0.01 | N.Z. | 6.33 | N.Z. | N.Z. | 6.5 | 6.99 | N.Z. | N.Z. | N.Z. | N.Z. | N.Z. | N.Z. |
| 5 | 0.001 | N.Z. | N.Z. | N.Z. | N.Z. | N.Z. | 5.33 | N.Z. | N.Z. | N.Z. | N.Z. | N.Z. | N.Z. |
| 6 | C. | N.Z. | N.Z. | N.Z. | N.Z. | N.Z. | N.Z. | N.Z. | N.Z. | N.Z. | N.Z. | N.Z. | N.Z. |

**E.C.-*Escherichia coli*, S.A.-*Staphylococcus albus*, Yeast – *Saccharomyces cerevisiae*, N.Z. –No Zone, C-Control**

## DISCUSSION

The obtained observation in present investigation indicates that the synthetic food colors were able to inhibit the normal flora *Escherichia coli* and *Staphylococcus albus* of human being. The results of present study found accordance with the experimental findings of Daniel. [17] They had tested the effect of 42 dyes on bacterial growth. Their results indicate that many dyes have the potential to inhibit the growth of bacteria. They also reported that the basic dyes are more inhibitory than acidic and negative dyes. The antimicrobial activity of natural dyes against common pathogens *Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa* has also studied. [20] They also reported that the decrease in viability of bacteria depends on the concentration of dyes. These results are in agreement with the findings of present study. However, synthetic colors were used in our investigation instead of natural dyes.

The effect of synthetic food colors on *Saccharomyces cerevisiae* carried out to demonstrate possible effect on human cell. The result of present investigation indicates that all four colors have the potential to inhibit the growth of *Saccharomyces cerevisiae* on solid medium at different concentrations. Naveen studied the toxic effect of the synthetic dyes Brilliant blue on liver, kidney and testes functions in rat. His study revealed that Brilliant blue treatment decreases serum ACP and testosterone concentration. [9] The histopathological examinations showed the alteration in kidney include congestion and hemorrhage with infiltration, Thick walled blood vessels and deformation of the structure of glomeruli. Whereas, the alterations of liver includes focal necrosis of hepatocytes, infiltration and vacuolation. The high concentration and longtime administration of food color has adverse effect on children's immunity. [21]

## CONCLUSIONS

From the observations of present study it may be concluded that almost all tested food colors exhibit inhibitory action against *Escherichia coli*, *Staphylococcus albus* and Yeast cell (*Saccharomyces cerevisiae)*. The effect on yeast cell could demonstrate the effect on human cell. It is evident from the present study that toxicity is directly proportional to the concentration of food colors. The study will helpful to design the threshold limit value of synthetic food colors for human consumption. However, further research is needed to evaluate toxicity of synthetic food colors against other normal flora of human being.

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