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Studies On Toxic Effect of Food Preservatives On Human Gut Microbiomes

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ABSTRACT

In the present study toxic effect of different concentrations of citric acid and acetic acid on human gut microbiome were analysed by performing antimicrobial activity adopting disc diffusion method. The results revealed that both the food preservatives viz; citric acid and acetic acid found toxic for *Escherichia coli* and *Saccharomyces cerevisiae*. However, No inhibitory effect against *Lactobacillus spp* was recorded. It was also observed that the inhibitory effect was found directly proportional to the concentrations of food preservatives. The study will helpful to design the Biological exposure indices and suitable limit of food preservative that could be safe for human and human gut microflora.

Key Words: Food Preservative, Microbiome, Antimicrobial, *Escherichia coli*, *Saccharomyces cerevisiae* and *Lactobacillus spp.*

INTRODUCTION

All living organism needs food to live. Foods is source of carbohydrates, fats, proteins, vitamins, or minerals. These nutrients require to produce energy, stimulate growth and maintain life. However, the food has limited shelf life. Hence it is very necessary to increase the shelf life of food for maintain the quality. Preservation of food can be accomplished by certain food preservatives (Sanjay Sharma 2015). Preservatives are food additives that play an important role in making foods last longer or taste better. Specifically, preservatives help to control and prevent the deterioration of food, providing protection against spoilage from micro-organisms (e.g., bacteria, yeast, moulds), life-threatening botulism and other organisms that can cause food poisoning (García-García, R., & Searle, S. S. 2016). High-risk foods such as meat, seafood, dairy, and cheese serve as a breeding ground for potentially dangerous micro-organisms, therefore, the addition of a preservative is usually required to ensure food safety (Canadian Institute of Food Safety. (2021). Food preservative indirectly introduced to foods (during the manufacturing process, by packaging, or during transport or storage) (FDA, 2017; FDA, 2018). Food Preservatives currently used are natural or synthetically produced. Salt and sugar are the natural food preservatives used for the preservation pickles, sauerkraut and jams. Sodium benzoate, Benzoic acid, Sodium sorbate, Potassium sorbate, Sodium nitrite are chemical

preservatives that exhibit Antimicrobial effect on bacteria, molds, insects and other microorganisms. Some substances used as Antioxidants (that act as free radical scavengers) are Vitamin E, Vitamin C, Pine Bark Extract, Grape Seed Extract, Sodium Erythorbate Sodium Diacetate, Sodium Succinate, Sodium Dehydro Acetate, Succinic Acid and Ascorbic Acid, Parabens, Erythorbic Acid, Proplyphenols. Also some Chelating agents work as preservatives for example Disodium ethylenediaminetetraacetic acid (EDTA), Polyphosphates, Citric acid and Ascorbic acid Monosodium Glutamate (MSG) Disodium Guanylate and Disodium Inosinate are used as food flavouring agents (M.I. Anon. (1991). Beside of this Kinderlerer and P. Hatton. (1990) reported the harmful effects of food preservatives on human health Sulfites are common preservatives used in various fruits, may have side effects in form of headaches, palpitations, allergies, and even cancer. Nitrates and Nitrites are used as curing agents in meat products. It gets converted into nitrous acid when consumed and is suspected of causing stomach cancer. Benzoates are used in foods as antimicrobial preservatives, and have been suspected to cause allergies, asthma and skin rashes. Sorbates/sorbic acid are added to foods as antimicrobial preservatives. However, the antimicrobial effect of chemical food preservative on human gut microbiome is poorly investigated. Hence, present study was carried out to analyse the antimicrobial effect of some chemical preservative on human gut microflora.

The community of commensal, symbiotic, and pathogenic microorganisms that inhabit all kinds of multicellular organisms are called ‘Microbiome”. The human microbiome has co-evolved with the human being as a unity called holobiont or hologenome (Salvucci, 2016). The microbiota includes bacteria, fungi, protozoa and viruses. The human gut microbiota is estimated to encompass 10^{13} to 10^{14} resident microorganisms. (Sender et al., 2016). The human microbiota is composed primarily of bacteria from either phylum Bacteroidetes (mostly Bacteroides or Prevotella species), that are gram negative, or Firmicutes (mostly *Clostridium and Lactobacillus spp*), that are gram positive (Consortium, 2012). The majority are strict anaerobes (97 %), mostly belonging to the phyla Firmicutes (64 %), Bacteroidetes (23 %), Proteobacteria (8 %), and Actinobacteria (3 %); low numbers of the phyla *Fusobacteria*, Verrucomicrobia, and TM7 (2 %) are also present. Fungi and archaea comprise less than 1 % of the total gut microbiota (Cardinelli et al., 2015.)The gut microbiota plays an important role in the normal functioning of the host organism. The benefits are mutual: the microorganisms are supported by the food humans eat and play a key role in health throughout human life. Next to digestion they are involved in establishing the immune system, the defence against pathogens, the endocrine system and mental health. Disruption of the normal equilibrium may induce metabolic and brain related disease. Most microorganisms reside in the distal part of the human gut (colon). As they play a role in the digestion of residual substrates, they contribute to their host in the synthesis of vitamins (vitamins K and B12, thiamine, and riboflavin and folate) and essential amino acids. Fermentation products of dietary fibres and carbohydrates such as butyrate, propionate, and acetate (short-chain fatty acids, SCFAs) act as a major energy source for intestinal epithelial cells and may therefore strengthen the mucosal barrier (Simpson and Campbell, 2015; Singh et al., 2017). In the present study the antimicrobial effect of common food preservative citric acid and Acetic acid (Vinegar) on human gut microbiomes such as *E.coli, Lactobacillus spp* and *S.cerevisiae.* (yeast) was analysed.

Materials and Methods

Collection of Food Preservatives

Two food preservative samples such as Citric acid and Acetic acid were purchased from "Arjav" shop of Washim market. The citric acid was manufactured by the "Shanti spices" Pvt. Ltd. Jalgaon, (M.S) India and the Acetic acid is manufactured by the "Universal Agrofood" Nagpur (M.S) India. Both the Food preservatives were transported in microbiology research laboratory, R.A. College, Washim (M.S), India.

Preparation of Standard stock solutions

The standard stock solutions of citric acid and Acetic acid were prepared by dissolving 1gm of citric acid in 10ml of distilled water and 1ml of Vinegar in 9ml distilled water separately and obtained standard stock solutions of food preservatives 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. (Daniel *et al.*, 1973).

Toxicity of food preservatives

The toxic effect of citric acid and acetic acid on three members of human gut microflora viz *Escherichia coli*, *Saccharomyces cerevisiae* and *Lactobacillus spp* were evaluated by performing antimicrobial activity adopting disc diffusion method (Sadar et al 2017).The effect of food preservative on *Saccharomyces cerevisiae* could also demonstrate possible toxic effect on human cell. The turbidity of enriched *Escherichia coli*, *Lactobacillus spp* and *Saccharomyces cerevisiae* were accurately matched with McFarland standard solution. (Deshmukh 1997).100µl enriched cultures of *Escherichia coli* and *Lactobacillus spp* 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 preservative 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 was 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 were incubated at 37^{0} C for 24 hours and potato dextrose agar plates were incubated at 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 preservatives 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 DISCUSSIONS

Toxic effect of citric acid and acetic acid was analysed against three members of human gut microflora viz *Escherichia coli*, *Saccharomyces cerevisiae* and *Lactobacillus spp*. The toxic effect of food preservatives 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 both the food preservatives were presented in table 1.Present study indicates that higher dilutions reflected by decrease in diameter of

zone inhibition. From the results it was observed that citric acid was found 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*. Whereas, 10, 1 , 0.1 and 0.01mg/ml concentrations of citric acid was found inhibitory to *Saccharomyces cerevisiae* . Xiong et al., (1999) reported that citric acid has been found at the 4th position in its antibacterial activity showing inhibition of all the three isolates of *S. aureus ,Bacillus subtilis* and showing no inhibition of Gram-negative bacteria i.e. *E. coli* isolates. had earlier reported the inhibitory activity of citric acid against *S. aureus besides Salmonella spp* and *Clostridium botulinum* was not used in present study. In a study carried out by Sorrel (1989), citric acid was investigated for its effect on inhibition of bacteria, yeast and molds. However, *S. aureus Bacillus subtilis, Salmonella* and *Clostridium botulinum.* Similarly, in case of acetic acid the inhibitory effect was observed at 10, 1 , 0.1 and 0.01mg/ml concentrations against *Escherichia coli*. However, all the dilutions of acetic acid were showed toxic effect on *Saccharomyces cerevisiae.* Leesmith et al, (2005) reported the effectiveness of acetic acid against *S. anatum* and found that acetic acid (1%) with pH was at 3.18 could inhibit the growth of *S. anatum*. Doores (1993) reported that bacteria inhibited by acetic acid include *Bacillus spp., Clostridium spp., L. monocytogenes, P. aeruginosa, E. coli and S. aureus* The present study noted tremendously different results about *Lactobacillus spp*. It was observed that all the tested concentrations of citric acid and acetic acid showed no inhibitory effect against *Lactobacillus spp.* It indicates that *Lactobacillus spp* have potential to tolerate both the preservative up to 10mg/ml. The results of present investigation found in accordance with scientific data published by (Silva and Lindon ;2016) in review article. Their synoptical review describes side effects of twenty five food preservatives. The enlisted side effects are skin rashes, itching, difficulty in breathing, sneezing or gastrointestinal upsets on human health. However, in present study antimicrobial effect of food preservative analysed against few members of human gut microbiome. The antimicrobial activity of food colors against common pathogens *Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa* has also studied. (Singh et al., 2005) They also reported that the decrease in viability of bacteria depends on the concentration of food colors .These results are in agreement with the findings of present study. However, food preservatives were used in our investigation instead of food colors.

CONCLUSIONS

It was concluded from the study both the tested food preservative viz citric acid and acetic acid exhibit inhibitory action against *Escherichia coli* and *Saccharomyces cerevisiae.* However, *Lactobacillus spp* showed no sensitivity against any tested concentrations of both the food preservative. The effect on *Saccharomyces cerevisiae* 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 Biological exposure indices and suitable limit of food preservative that could be safe for human and human gut microflora . However, further research is needed to evaluate toxicity of food preservatives against other human gut flora.

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Table 1:Toxic effect of citric acid and acetic acid on test Microorganisms

E.C.-Escherichia coli, S.C.-Saccharomyces cerevisiae, L.B.-Lactobacillus, NZ- No Zone, C- Control, Mg-Milligram, Ml-Milliliter

Sr. no.	Concentrations (mg/ml)	Zone of Inhibitions (mm)					
		Citric Acid			Acetic acid		
		E.C.	S.C	L.B.	E.C.	S.C.	L.B.
1	10	14.3	16	NZ	15	14.3	NZ
2	1	14	15	NZ	14.8	14.1	NZ
3	0.1	13.7	14.8	NZ	14.5	13.8	NZ
4	0.01	NZ	14.5	NZ	13	13.6	NZ
5	0.001	NZ	NZ	NZ	NZ	13.2	NZ
6	C	NZ	NZ	NZ	NZ	NZ	NZ

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