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Efficacy of Copper Metal on Probiotic Bacteria to Combat Dysbiosis

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Abstract—
Objective- *Probiotics are dietary supplements of micro-organisms which when administered in adequate amount confers health benefits to the host. Beneficial microflora such as probiotic bacteria when administered in excess amount interferes with normal body functioning. An imbalance between the microbiota will lead to the condition known as 'dysbiosis' which holds possible links to be a cause of various intestinal tract diseases. However, the emergence of multidrug-resistant pathogens has generated a demand for alternative anti-microbial therapies. Hence in the current research study, an attempt was made to evaluate the efficacy of copper metal against probiotic bacteria Lactobacillus spp. to combat dysbiosis.*
Methods- *In the present study, five isolates of Lactobacillus spp. were isolated from five different curd samples. Dilutions of copper sulphate solutions (100%, 50%, 25% and 12.5%) were prepared and their antimicrobial activities were analyzed.*
Results- *The 100% copper sulphate solution inhibited the growth of all five isolates of Lactobacillus spp. while at 12.5% dilution of copper sulphate, all the isolates were found to be resistant. Antibiotic resistance profile showed that all the five isolates of Lactobacillus spp. were found to be resistant to Ampillicin and sensitive to Clindamycin.*
Conclusion- *According to the current study copper metal helps to balance the gut microbiota which might be useful for maintaining proper body functioning.*

Keywords— *Copper metal; Antibacterial activity; Probiotic bacteria; Dysbiosis.*

I. INTRODUCTION

Intestinal flora is groups of bacteria that live in our intestines. They assist digestion and kill or crowd out harmful or pathogenic organisms. In order for the intestinal tract to work correctly, it must contain only the correct groups of organisms. When the wrong groups of bacteria are present in the intestine, the condition is called as 'intestinal dysbiosis'. Probiotics are dietary supplements that contain correct flora of bacteria or yeasts thought to be healthy for human consumption. Probiotics aimed at improving human health by modulating microbiota. According to currently adopted definition by WHO – Probiotics are "live micro-organisms which when administered in adequate amount confers health benefits to the host" [1].

The correct or friendly flora in the human intestine includes Lactobacilli. They constitute an integral part of healthy gastrointestinal microecology and have received considerable scientific attention. When the good or friendly flora is missing or in low concentration, it allows other bacteria to multiply in the intestine. This will hamper the digestion of food, but can also cause many other symptoms, since the incorrect flora produce toxins that are absorbed into the body. Also, without the proper flora, vitamin deficiencies can develop even if a person eats a wholesome diet. On the other hand, taking antibiotics, especially wide-spectrum antibiotics, often destroys the friendly intestinal flora. This allows other bacteria to grow in the intestinal tract. On the other hand the emergence of multidrug-resistant pathogens has generated a demand for alternative anti-microbial therapies [2]. Thus, intestinal dysbiosis can occur. Dysbiosis is a term for a microbial imbalance or maladaptation on or inside the body, such as an impaired microbiota. There is growing evidence that dysbiosis of the gut microbiota is associated with the pathogenesis of both intestinal and extra-intestinal disorders. Intestinal disorders include inflammatory bowel disease (IBD), irritable bowel syndrome (IBS) and celiac disease, while extra-intestinal disorders include allergy, asthma, metabolic syndrome, cardiovascular disease and obesity. Infection is the common disease caused by dysbiosis of the microbiota. Moreover infectious disease and its treatment have a huge impact on the human microbiota, which further determines the outcome of the infectious disease in the human host [3].

Besides this, drinking water from a copper water utensil has significant health benefits. But it is necessary to drink it in a write way. However, metals have not been explored for their capacity to fight against infectious disease and also for the use of metal ions as anti-microbial agents to fight multidrug-resistant infections [4]. Among metals, copper is an essential micro-nutrient and plays a role in development of body organs, RBCs, absorption of iron and anti-oxidant defense. The human body does not manufacture copper, so it needs to be obtained from food and water. As per Ayurvedic texts, drinking water from copper utensil helps in tuning your digestive system to perform better, copper also helps your body to break down fat and eliminate it more efficiently thus leading to weight loss. It is also said to slow down ageing, and reduces the appearance of fine lines. Copper has a lot of anti-inflammatory properties which helps in arthritis and other inflammatory pains. Drinking water from copper utensil can relieve all the pains caused due to inflamed joints. Copper can also strengthen the immune system. Human body typically has about a 100 mg of Cu and about 1.2 mg/day of dietary intake is recommended in UK, 1.3 mg/day by WHO. Storing water in copper can leech some amount of copper to inside water and it

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reaches around 180ppb in 16 hours. This can go up to 450ppb in 7 days. WHO limit of 2mg/L (2000ppb). So from a dietary point of view drinking 1-2 liters of this water will give you 0.18-0.36mg (~15-30% of total daily requirement). Storing water in copper can also kill some of the harmful bacteria, particularly those related to diarrhea [5].

Therefore, the conditions in the intestine must change to support the friendly intestinal flora, but not the pathogenic or harmful bacteria. Hence in the current research study, an attempt was made to evaluate the efficacy of copper metal on probiotic bacteria *Lactobacillus* spp. to combat dysbiosis.

II. Materials and Methods

- *Collection of Curd Sample:*

Five different curd samples were collected from the local market of the Nagpur region, Maharashtra (India) and transported to the microbiology laboratory aseptically for further processing.

- *Isolation of Probiotic bacteria (Lactobacillus spp.) from Curd Samples:*

A loopful of each curd sample was streaked on de Man Rogosa Sharpe medium (MRS medium) and the plates were incubated at 37˚C for 24 hours [6]. After incubation, the isolated colonies were identified on the basis of morphological, cultural and biochemical characteristics [7].

- *Preparation of Copper Sulphate Dilutions:*

Four different dilutions of copper sulphate solution viz. 100%, 50%, 25% and 12.5% were prepared in sterile distilled water.

- *Agar well Diffusion Method:*

Lactobacillus spp. was inoculated on MRS agar plate and the plate was incubated at 37^0C for 24 hours. The culture was then inoculated in MRS broth and incubated 37^0C for 18 hours. A suspension (100µL) was spread over the MRS agar plate. The wells of 6 mm diameter were cut into the agar medium with a sterilized cork borer. Then 50µl of each of the $CuSO_4$ dilutions were added into different wells. Three types of antibiotic discs viz. Ampicillin (10mcg), Clindamycin (2mcg) and Erythromycin (15mcg) were obtained from Hi-media Laboratories Pvt. Ltd. Mumbai and were placed simultaneously on the plate lawned with *Lactobacillus* spp. The plates were incubated at 37^0C for 24 hours. The diameter of the zone of inhibition around each well was measured and recorded [8] [9]. The diameter of the zone of inhibition produced by each antibiotic disc was measured and recorded using a zone measurement scale and the isolates were classified as "resistant" or "sensitive" based on the standard interpretative chart according to Clinical and Laboratory Standards Institute (CLSI) guidelines [10].

III. Results and Discussion

In the present study, five *Lactobacillus* spp. were isolated from five different curd samples for evaluating the efficacy of copper on it. Different dilutions of copper sulphate solutions (100%, 50%, 25% and 12.5%) were prepared in sterile distilled water. The copper antimicrobial activity increases proportionally to its concentration. It was observed that as dilution factor of copper sulphate solution increases the zone of inhibition was to be decreasing proportionally. It was reported that copper sulphate solution (100%) was found to inhibit all five isolates of *Lactobacillus* spp. showing 15mm, 16mm, 13mm, 22mm and 16mm inhibition zone for *Lactobacillus* spp. I, II, III, IV and V respectively. At 12.5% dilution of copper sulphate, all the isolates were found to be resistant (Table I). Although metals and metal oxides are known to be toxic at relatively high concentrations, they are not expected to be toxic at low concentrations [11].

Antibiotic resistance profile showed that all the five isolates of *Lactobacillus* spp. were found to be resistant to Ampillicin (AMP) and sensitive to Clindamycin (CD) while three isolates of *Lactobacillus* species I, II and IV were found to be resistant and two isolates of *Lactobacillus* species III and V were found to be sensitive to Erythromycin (E) (Table II). With the emergence of antibiotic resistance, the interest for evaluating antimicrobial properties of metals has tremendously increased in accordance with public health. Copper is a trace element required for several essential biological processes that exhibit remarkable structural and functional conservation from bacteria to human. Owing to the high antibacterial properties, copper is used in the antimicrobial studies [12]. This led to many investigations of the various properties of copper metal as potential antimicrobial agent against antibiotic resistant bacteria.

The assessment of activity index of 100% CuSO4 solution was obtained by comparing the resultant zone of inhibition (mm) of 100% CuSO4 solution with the standard reference antibiotic Clindamycin [13].

$$\text{Activity Index} = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the Standard}}$$

TABLE I. Antimicrobial activity of 100%, 50%, 25% and 12.5% CuSO4 solution five against different isolates of *Lactobacillus* spp.

Sr. No.	Probiotic bacteria	CuSO4 (100%)	CuSO4 (50%)	CuSO4 (25%)	CuSO4 (12.5%)
		Zone of Inhibition(mm)			
1	LSI	15mm	14mm	10mm	R
2	LSII	16mm	12mm	12mm	R
3	LSIII	13mm	14mm	11mm	R
4	LSIV	22mm	17mm	15mm	R
5	LSV	16mm	11mm	R	R

Where, LS- *Lactobacillus* spp.; R-Resistance

TABLE II. Antimicrobial activity of Copper ring and antibiotics solution against five different isolates of *Lactobacillus* spp.

Sr. No.	Probiotic bacteria	Ampicillin	Clindamycin	Erythromycin
1	LSI	R	10mm	R
2	LSII	R	16mm	R
3	LSIII	R	12mm	13mm
4	LSIV	R	10mm	R
5	LSV	R	10mm	11mm

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It was observed that 100% CuSO4 solution exhibited 1.50, 1.00, 1.08, 2.20 and 1.60 antibacterial activity (LS I, LS II, LS III, LS IV and LS V respectively) when compared with the standard antibiotic Clindamycin (CD) (Table III).

TABLE III. Activity Index (AI) for 100% CuSO4 Solution on Probiotic bacteria

Sr. No.	Probiotic Bacteria	CuSO4 Solution (100%)
1	LSI	1.50
2	LSII	1.00
3	LSIII	1.08
4	LSIV	2.20
5	LSV	1.10

IV. CONCLUSION

With the emergence of antibiotic resistance, antimicrobial agents have recently gained immense interest in accordance to public health. Copper acts as the potential antimicrobial agent. Hence in the present investigation, the efficacy of Copper metal was evaluated against probiotic bacteria to fight against the concept of dysbiosis of the microbiota. According to the current study copper metal helps to balance the gut microbiota thereby maintaining proper body functioning. Use of copper vessels for storing drinking water is one of the best ways of maintaining healthy digestive system.

REFERENCES

[1] Food and Agriculture Organization of the United Nations (FAO), World Health Organization (WHO), "Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria," 2001.

[2] L. D. Wilson, "Your Intestinal Flora," https://drlwilson.com/ARTICLES/FLORA.htm, 2017.

[3] C. Casen, H.C. Vebo, M. Sekelja, F.T. Hegge, M.K. Karlsson, Ciemniejewska, S. Dzankovic, C. Froyland, R. Nestestog, L. Engstrand, P. Munkholm, O.H. Nielsen, G. Rogler, M. Simren, L. Ohman, M.H. Vatn, and K. Rudi, "Deviations in human gut microbiota: A novel diagnostic test for determining dysbiosis in patients with IBS or IBD," *Alimentary Pharmacology and Therapeutics*, vol. 42 issue 1, pp. 71-78, 2015.

[4] G. Grass, C. Rensing, and M. Solioz, "Metallic copper as an antimicrobial surface," *Appl. Environ. Microbiol.*, vol. 77, pp. 1541–1547, 2011.

[5] A. Malhotra, "Does drinking water kept overnight (or for a long interval) in a copper utensil has any medical benefit?" https://www.quora.com/, 2016.

[6] J. C. De Man, M. Rogosa, and M.E. Sharpe, **"**A medium for the cultivation of Lactobacilli," *J. Appl. Bacteriol.*, vol. 23, pp. 130–135, 1960.

[7] A. Bhardwaj, M. Monica Puniya, K.P.S. Sangu, S. Kumar, and T. Dhewa, "Isolation and Biochemical Characterization of *Lactobacillus* species Isolated from Dahi," *J. Dairy Sci. Technol.*, 2012.

[8] A. W. Bauer, W. M. M. Kirby, J. C. Sherris, and M. Turck, "Antibiotic susceptibility testing by a standardized single disc method," *Am J ClinPathol.*, vol. 45, pp. 493-496, 1966.

[9] A. Chakraborty, and J. Bhowal, "Isolation, Identification and Analysis of Probiotic Properties of *Lactobacillus* spp. from Selected Regional Dairy Product," *Int. J. Curr. Microbiol. Appl. Sci.*, vol. 4, issue 6, pp. 621-628, 2015.

[10] CLSI, "Performance standards for antimicrobial susceptibility testing: 17th Informational supplement, Approved standard M100-S17, Wayne, USA," *Clinical and Laboratory Standards Institute*, 2007.

[11] I. Chopra, "The Increasing Use of Silver Based Products as Antimicrobial Agents: A Useful Development or a Cause for Concern?" *J Antimicrob Chemother*, vol. 59, issue 4, pp. 587-590, 2007.

[12] W. H. D. Jong and P. J. A. Borm, "Drug delivery and nanoparticles: applications and hazards," *Int. J. Nanomedicine*, vol. 3 issue 2, pp. 133-149, 2008.

[13] P. Singariya, P. Kumar and K. K. Mourya, "Antimicrobial activity of fruit coat (calyx) of *Withania somnifera* against some multi drug resistant microbes," *Int. J. Biol. Pharm. Res.*, vol. 3 issue 2, pp. 252-258, 2012.