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Effect of *Lactobacillus acidophilus* Extract against Selective Enteric Bacterial Pathogens

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Abstract: ***In this study, Lactobacillus species (probiotics) had been examined toward pathogenic bacteria. Potency of Probiotics were examined with physiochemical parameters as temperature, pH, incubation time and concentration. Observed data revealed that, Lactobacillus species showed resistance against pathogenic bacteria's under different temperature, concentration and incubation time. Subsequently, the antimicrobial activities of Lactobacillus bacteria depend on temperature, pH, incubation time and concentration.***

Keywords:- Probiotics, Lactobacillus acidophilus extract (LAE), Bacterial pathogens, Anti-microbial effect, MIC.

I. INTRODUCTION

In the present block of time, foods and microorganisms (especially Bacteria) are emerging simultaneously and have plus and minus impact on living being[1]. Food gives energy to living being while microorganisms can be one of exchanger. On one side, microorganism defence living being from infections & on the other side, it causes disease with food infection that plays a major role in causing diseases amongst human and animal [2, 3] Unhygienic food conditions effect number of bacterial pathogens (*Listeria monocytogene, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Bacillus cereus, Pseudomonas spp., Clostridium spp.*) which eventually leads to cause diseases called food born disease[4]. It causes gastrointestinal disease as dysentery, diarrhea, typhoid etc. Food born disease is a major health issue around the world and affect the economic growth. Food borne diseases affect approximately 2.2 million people and death annually[5]. In the favour of defence, Probiotics act as barrior against food borne diseases. Probiotics are desirable effective microorganism that prevent human bowel of digestive system. As per FDA, Probiotics are used as food as well as medicament[6]. Probiotic is a Greek word which means 'for life' and define for probiotic, a live microorganism. Probiotics are naturally found in alimentary canal and in some foods e.g., yogurt, other milk product as leben raib, jahurt, ajran etc. Probiotics can be bacteria or yeast and *Lactobacillus, Bifidobacterium, Lactococcus, Leuconostoc, Bacillus* are known probiotics. It influenced some properties as gastric juice, bile tolerance, intestinal microbiota balance and survive in the gastrointestinal track of humans and animals [7, 8].

II. TEST PRODUCTS

Some bacteria & yeast expressed significant probiotic properties and *Lactobacillus* is one of a probiotics. The species of *Lactobacillus* (*Lactobacillus acidophilus, Lactobacillus casei.*) used for the extracellular bioactive extract which is used against the most common causative pathogens (*Salmonella typhi, Escherichia coli, Staphylococcus aureus*) of food borne disease and other pathogen which are not causing agent of food borne disease but sometimes it may be responsible for disease causing as *Klebsiella pneumonia*.

III. TEST SCHEDULE

The studies were conducted as protocol and was performing over five months. Following are the study design: -

1) *Activation of Microbes:* Firstly we were inoculated the cultures in nutrient broth and incubate them for 24 hours in 37°C, for their activation (sub culturing).
2) *Preparation of Lactobacillus acidophilus Filtrate:* Freshly prepared culture of *Lactobacillus acidophilus* then used for the collection of cell free supernatants by centrifuge it at 10,000rpm for 15 minutes, these supernatant was filtrated through 0.45 micron filters and it was stored immediately at low temperature, if not used.
3) Detection of Antagonistic activity of *Lactobacillus acidophilus* filtrate against *Klebseilla pneumonia*, *Escherichia coli*, and *Salmonella enteric*.

a) *By Antibacterial/Antimicrobial Activity:* By well diffusion method: - We had prepared agar plates and then spread the nutrient broth of pathogenic bacteria's on the plates, then make a hole/well with the help of sterilized tips and then pour the filtrate of *lactobacillus acidophilus* on that well then kept for the incubation for 24 hours at 37˚C.

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4) *Determining the effect of various physiochemical factors on the antagonistic activity of Lactobacillus acidophilus filtrate against Klebsiella pneumonia, Escherichia coli, and Salmonella enteric, Staphylococcus aureus.:* In this present research we were inoculated *Lactobacillus acidophilus* filtrate with *klebsiella pneumonia, Escherichia coli, salmonella enteric,* and *staphylococcus aureus* cultures in a ratio (1:1) then inoculated for 24 hours at 37°C. Generally we use the cells that are in their mid log phase of growth thus we easily estimated the antagonistic activity with the help of optical density assay (600nm).

a) *Effect of Temperature:* Firstly prepare 10ml of nutrient broth (individually for each strain) then it inoculated with 10ml of *Lactobacillus acidophilus* filtrate and 10ml of *klebsiella pneumonia* likewise with another strains of pathogens i.e. *Escherichia coli, salmonella enteric,* and then it was incubated for 24 hours at 25°C, 37°C and 45°C. Study shows that bacteria can easily grow in 37°C.

b) *Effect of Different Concentration:* For this, 4 different concentrations of filtrate were taken. For 5 ml concentration:- 5ml of LAE and 5ml of pathogenic organisms were taken and then it made up by 40ml of nutrient broth. For 10ml concentration:- 10ml of LAE and 10ml of pathogenic organisms were taken and then it made up by 30ml of nutrient broth. For 15ml concentration:- 15ml of LAE and 15ml of pathogenic organisms were taken and then it made up by 20ml of nutrient broth. For 20ml concentration: - 20ml of LAE and 20ml of pathogenic organisms were taken and then it made up by 10ml nutrient broth. The study concluded that the LAE became more effective in respective concentration i, e LAE in 15 ml are most effective against *salmonella enteric,* likewise 10ml for *klebsiella pneumonia,* and 5ml for *Escherichia coli.*

c) *Effect of Different pH:* For this, we worked on least 5 different pH. Initially, pH is same but the concentration may vary i.e. 5ml is good for *Escherichia coli,* 10ml for *salmonella enteric* and 15ml for *klebsiella pneumonia,* all are treated with *Lactobacillus acidophilus* filtrate in pH 3.Secondly, Same activity repeated with different pH i,e LAE treated with pathogens at 5,7,9 pH.

d) *Effect of Different Incubation Period:* In this parameter, *Lactobacillus acidophilus* filtrate treats with different pathogens at different incubation period, i.e. 0, 1, 2, 3, 4, 5 and 24-48 hours.

IV. RESULT

Filtrate of *Lactobacillus acidophilus* treat with pathogenic organisms by well diffusion Method**.**

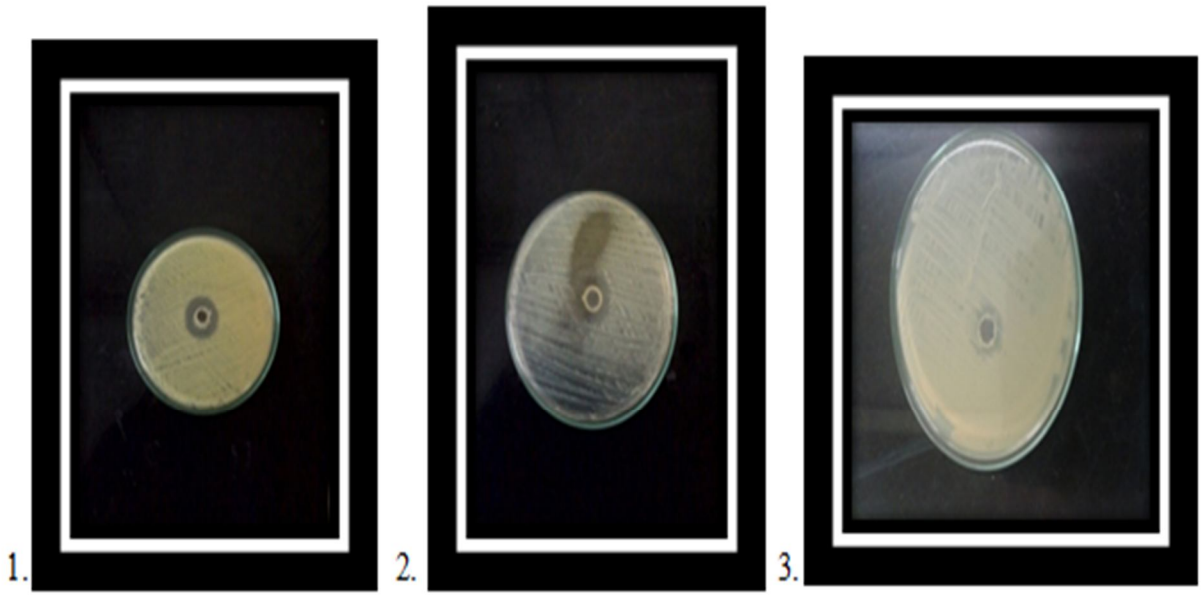
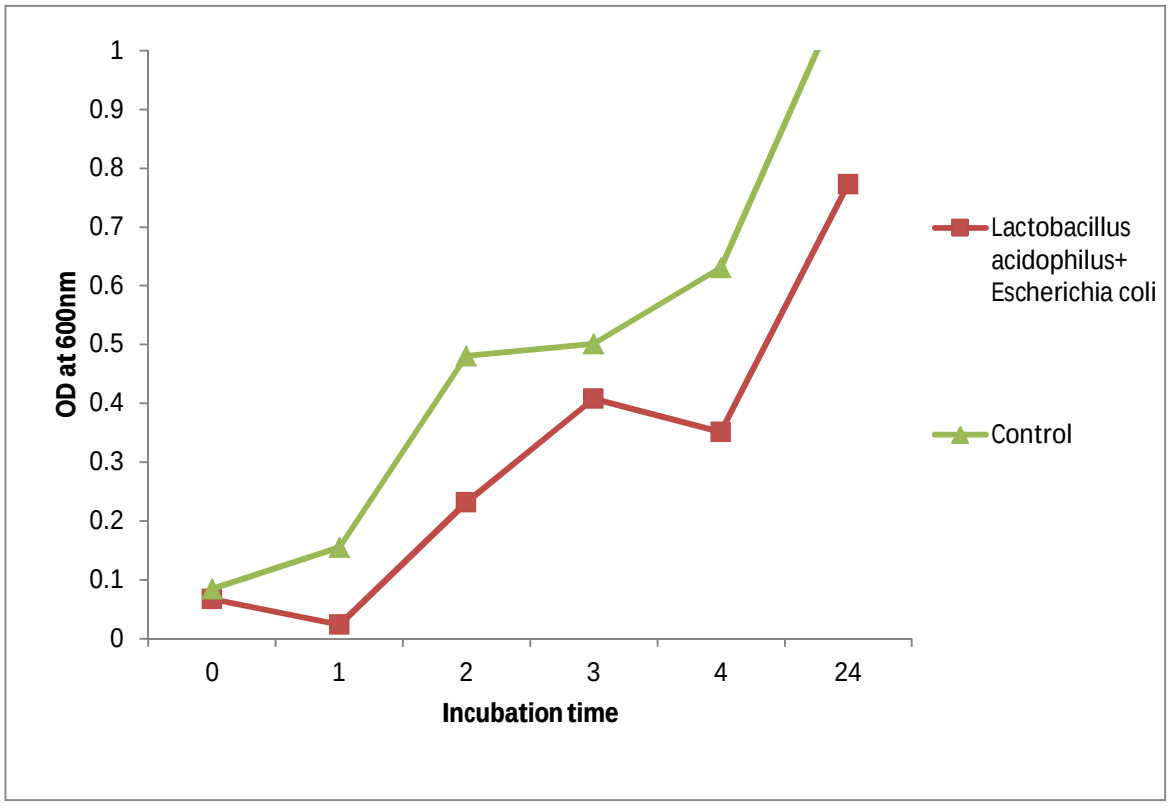


Fig .1. Zone showed inhibition by *Lactobacillus acidophilus* against *klebsiella pneumonia.* Fig.2. Zone showed inhibition by *Lactobacillus acidophilus* against *Escherichia coli.*Fig.3. Zone showed inhibition by *Lactobacillus acidpphilus* against *Salmonella Enterica.*(*Staphylococcus aureus* is not sensitive to the *lactobacillus acidophilus* filtrate. So it was eliminated from the study)

lactobacillus acidophilus filtrate showed different inhibition for bacterial pathogengs under following concentration, pH, temperature and incubation time (Minimum inhibitory concentration)

CONCENTRATION	INCUBATION PERIOD (in hour)	ORGANISM					
		Salmonella enteric		*Klebsiella pneumonia*		*Escherichia coli*	
		Control	Experimental	Control	Experimental	Control	Experimental
5 ML	0	0.139 ±0.011	0.004 ±0.011	0.206 ±0.011	0.183 ±0.012	0.084 ±0.010	0.067 ±0.011
	1	0.144 ±0.010	0.012 ±0.012	0.571 ±0.013	0.135 ±0.010	0.155 ±0.011	0.023 ±0.012
	2	0.260 ±0.012	0.021 ±0.010	0.331 ±0.011	0.223 ±0.012	0.481 ±0.010	0.231 ±0.010
	3	0.338 ±0.011	0.286 ±0.011	0.468 ±0.010	0.298 ±0.011	0.501 ±0.012	0.408 ±0.013
	4	0.448 ±0.013	0.103 ±0.012	0.520 ±0.011	0.284 ±0.013	0.631 ±0.010	0.351 ±0.011
	24	0.503 ±0.010	0.373 ±0.013	0.558 ±0.012	0.215 ±0.012	1.120 ±0.013	0.773 ±0.010

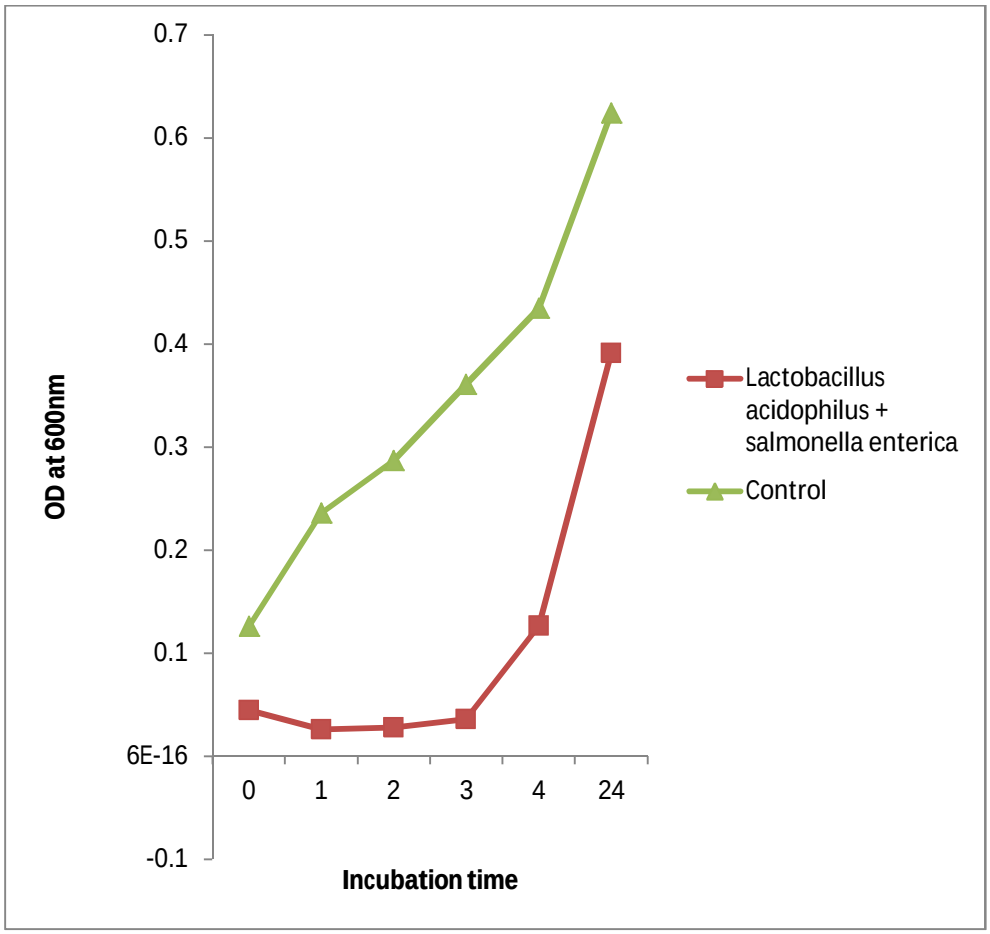
Table: - 1. Effect of LAE on pathogenic bacteria in 5ml concentration



Graph: - 1 Effect of LAE on *Escherichia coli*.

CONCENTRATION	INCUBATION PERIOD	ORGANISM			
		Salmonella enterica		*Klebsiella pneumonia*	
		Control	Experimental	control	Experimental
10ML	0	0.126± 0.010	0.045± 0.011	0.314± 0.010	0.167± 0.013
	1	0.236± 0.012	0.026± 0.010	0.498± 0.010	0.142± 0.012
	2	0.287± 0.011	0.028± 0.013	0.549± 0.013	0.230± 0.011
	3	0.361± 0.013	0.036± 0.012	0.446± 0.010	0.401± 0.012
	4	0.435± 0.011	0.127± 0.011	0.626± 0.011	0.618± 0.010
	24	0.624± 0.010	0.391± 0.010	1.589± 0.012	0.964± 0.011

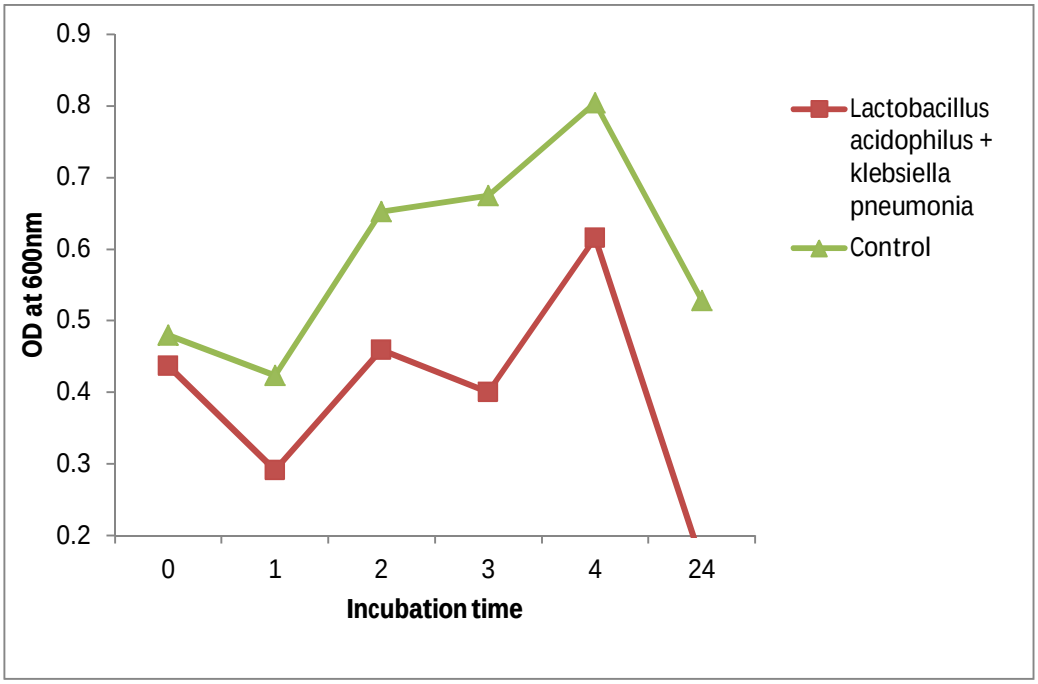
Table:-2 Effect of LAE on pathogenic bacteria in 10ml concentration.



Graph: - 2. Effect of LAE on *Salmonella Enterica*

CONCENTRATION	INCUBATION PERIOD	ORGANISM					
		Salmonella enteric		*Klebsiella pnemonia*		*Escherichia coli*	
		Control	Experimental	control	Experimental	Control	
15ML	0	0.410± 0.011	0.256± 0.010	0.480± 0.012	0.437± 0.011	0.356± 0.010	0.292± 0.012
	1	0.598± 0.010	0.396± 0.011	0.424± 0.011	0.291± 0.010	0.524± 0.011	0.297± 0.010
	2	0.465± 0.012	0.386± 0.012	0.652± 0.011	0.459± 0.011	0.645± 0.012	0.557± 0.011
	3	0.480± 0.011	0.382± 0.011	0.675± 0.010	0.400± 0.010	0.665± 0.010	0.635± 0.012
	4	0.560± 0.012	0.495± 0.010	0.804± 0.012	0.616± 0.012	0.793± 0.011	0.780± 0.011
	24	0.489± 0.010	0.461± 0.011	0.528± 0.010	0.163± 0.011	0.845± 0.012	0.786± 0.010

Table:- 3. Effect of LAE on pathogens in 15ml concentration.



Graph:-3. Effect of LAE on *Klebsiella Pneumonia*

1) *Result:* Following Data depicts, the action of Lactobacillus acidophilus filtrate or extract against pathogen's under different concentration, pH and incubation period.

Serial No.	ORGANISM	TEMPERATURE	CONCENTRATION	pH	INCUBATION PERIOD
1.	*Salmonella enteric*	37^0C	10 ML	5 pH	1-4 hours
2.	*klebsiella pneumonia*	37^0C	15ML	8 pH	24 hours
3.	*Escherichia coli*	37^0C	5ML	7 pH	0-3 hours

V. DISCUSSION

Present study showed that, milk products are rich in probiotics whereas, French national institute for agricultural research state that, fermented foods are the rich source of probiotics and it may change the composition of gut bacteria to prevent digestive conditions[6,7] Emory University explained that probiotic bacteria helps to maintain intestinal health by promoting the growth of the cells while the recent work on probiotics explain about the bacteriocin that kills harmful pathogens and help to keep digestive system healthy[8]. According to the study, *lactobacillus acidophilus* has extracellular metabolite that inhibits the growth of pathogenic bacteria while study conducted in Michigan State University found that *lactobacillus reutrii* has been more effective to reduce gut inflammation and for the treatment of inflammatory bowel disease. Allen SJ in 2011 suggest that probiotics may serve as a functional food in the treatment of diarrhea[9], in addition to this, present study focus on the effect of Lactobacillus acidophilus extract on food borne disease. Lactobacillus acidophilus extract has ability to destroy the intracellular growth of pathogens that causes food borne disease [10] hence for the betterment of digestive system probiotics are often prescribed. At last, remaining challenges includes identifying mechanisms of action to provide the basis of more refined hypothesis-driven clinical trials [11]. The correct combination and concentration of probiotics applied to the appropriate gastrointestinal disorders may improve the efficacy of this approach for diarrhoea and other Food borne diseases [13]

VI. CONCLUSION

The present study reveals the antagonist activity of lactobacillus acidophilus extract/filtrate against pathogenic enteric bacteria. All activity took place in optimum temperature at 37^0C. In 10 ml concentration and acidic media, *Salmonella enteric* showed sensitivity against LAE in 0-4 hours. *klebsiella Pneumonia* was different from other organisms. It showed inhibition in 24 hours and in 15ml concentration and alkaline media. *Escherichia coli* were very easy to inhibit by LAE in 5ml concentration and it took less than 3 hours for processing. Therefore LAE showed capability to inhibit pathogenic organisms under above variation because LAE has extracellular metabolites and bacteriocin that eventually inhibit salmonella enteric and *Escherichia coli* in short span of time. Whereas *klebsiella Pneumonia* is made up of lipoploysaccharides, and has antigen O & k, and LAE took more than 24 hours for inhibition. Hence Lactobacillus acidophilus destroyed *klebsiella Pneumonia* by inhibiting intracellular growth and trancellular passage in alkaline media. And by this present study we concluded that in physiochemical factors (pH, temperature, incubation period, concentration) the ability of *Lactobacillus acidophilus* extract (extracellular metabolites) becomes more effective and it shows better inhibition

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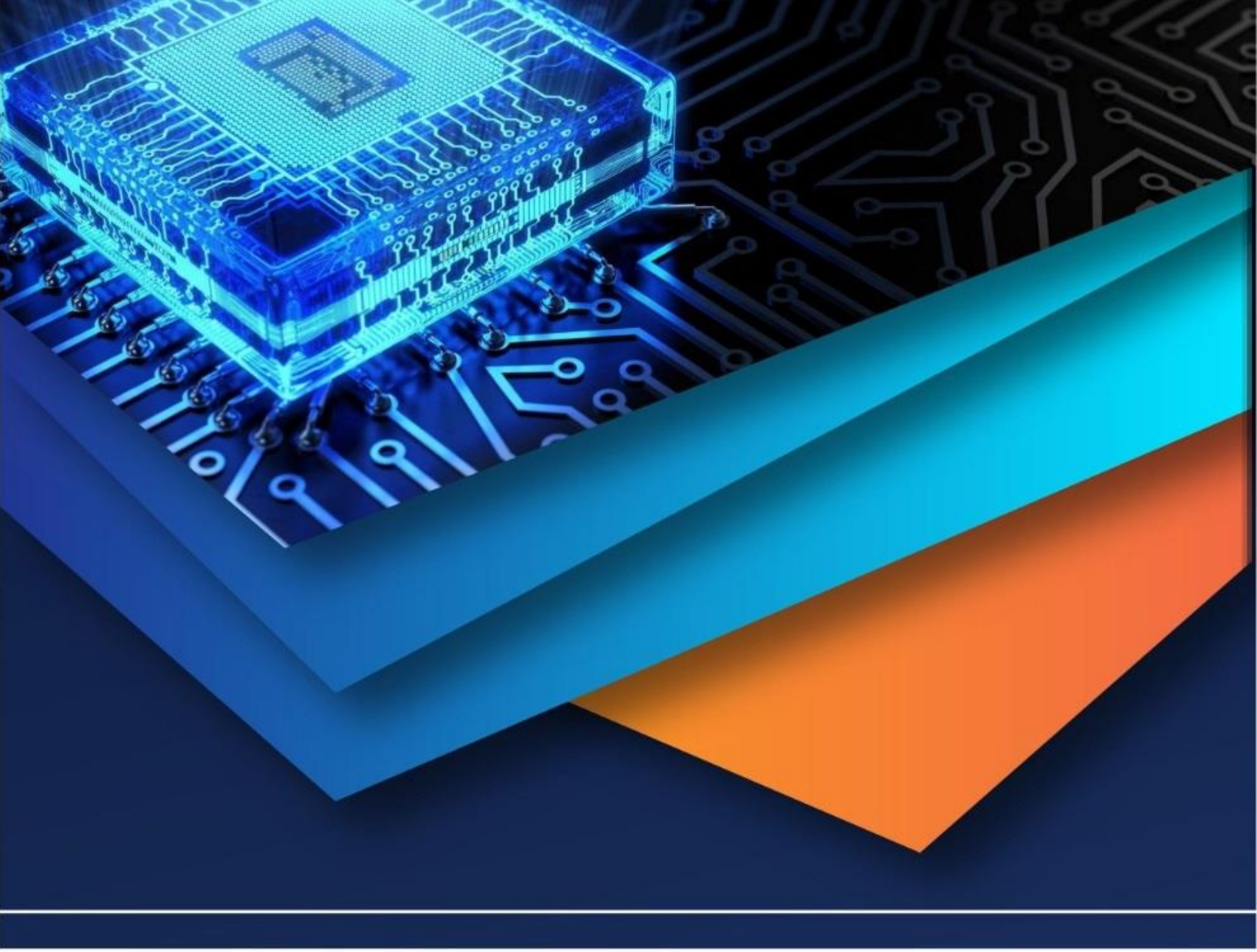

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