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Effect of some Transition Complexes on Bacteria and Fungi Causes Gastroenteritis in Humans

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ABSTRACT

This study concerned with the synthesis and characterization of some complexes of the ligand. All the complexes are non-electrolytes place , instead crystalline solid compounds insoluble in water , but soluble in organic solvents the negatively charge bidentate ligand coordinated with metal ions through the oxygen atom in the hydroxyl group and the oxygen atom of the aldehyde group. The effect of these complexes on inhibiting the growth of bacteria and fungi isolated from diarrheal patients with intestinal inflammation has been shown to be highly inhibitory and has been the best inhibitory effect of the complex $Zn(OC_{10}H_6CHO)_2$ on bacteria *Shigella dysentariea* inhibition diameter 32 milmeter and on fungus *candida glabrata* in hibition diameter 27 milimeter and there were no toxic effects of these complex on the Laboratory mice are the dose of 2.5 – 15 gm / kg of body weight

Keyword: Transition, Complexes, Bacteria, Fungi, Gastroenteritis.

INTRODUCTION

Gastroenteritis (GIT)

Is a condition called satisfactory inflammation of GLT , which includes both of the stomach and small intestine , which lead to the total of symptoms such as diarrhea , vomiting , abdominal pain and spasm called gastro esophageal[1], is causing the most common cases gastroenteritis in children *Rota* virus and adults *Nor* virus[2,3]. also may occur because many of bacteria *Escherichia coli , Staphylococcus aureus, Salmonella spp, Bacillus spp., Vibrio Cholera* and other types of bacteria it may happen due to the consumption of foods stomach improperly or because of drinking contaminated water[4]. Gastrointestinal tract is more common among infants and children. They cause of death under 5 years age in the developing world[5]. The intestinal bacteria transmitted by eating contaminated food especially beef and their products are healthy cows main treasure and high rate was observed in rural as a result of direct contact with cow or the faces containing the bacteria[6].

MATERIAL AND METHODS

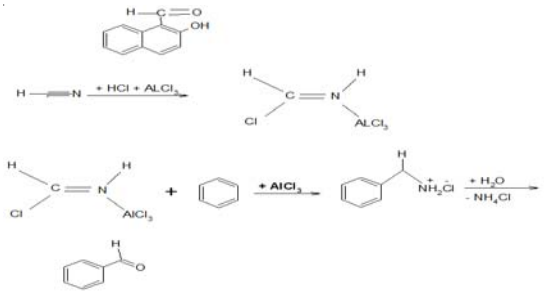
Synthesis of Ligand

Ligand 2-hydroxy-1-naphthaldehyde ($C_{11}H_8O_2$) it is one of the aromatic compound derivatives of naphthalene dark brown crystalline and the degree of melting 76-80°C and boiling point 192°C/27mm Hg (lit) not soluble in water but soluble in organic solvents molecular weigh 172.183 g/mole.

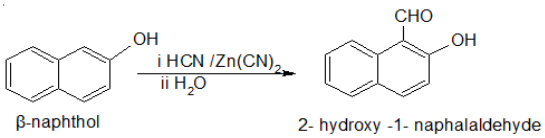
Ligand preparation

Ligand was prepared by the Gattermann-Koch reaction(also known as the Gattermann salicylaldehyde synthesis) is a chemical reaction in which aromatic compounds are formylated by hydrogen cyanide in the presence of a Friedel-Crafts catalyst (e.g $AlCl_3$).It is named for the German chemist ludwing Gattermann and is similar to the Friedel-Craft reaction.

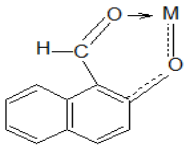
Combination with zinc cyanide. Although it is also highly toxic. Zn $(CN)_2$ is asoild, making it safer to work with than gaseous HCN, additionally, because the reaction uses HCI,$Zn(CN)_2$ also supplies the reaction with $ZnCl_2$ in situ, where it acts as a lewis acid catalyst.



Examples of $Zn(CN)_2$ being used in this way include the synthesis of 2- hydroxy -1-naphthaldehyde and Mesitaldehyde preparation of ligand . The Ligand (L) was prepared as follows[7].



Ligand be charged negatively (L^-) after the loss of a proton (H^+) from a hydroxyl group (OH) bidentate ligand coordinated with metel ion through the oxygen atom in the hydroxyl group (-OH) and the oxygen atom of the carbonyl group shown in Figure(1).



Collection of samples

Sixty four clinical specimens collected for children and adult , distributed between 20 stool sample adults of both sexes and 44 sample of stool of children less on 10 years of age suffer from diarrhea for the duration of January 2016 until august 2016 from different hospitals in Baghdad , collect stool by wooden sticks and placed in a sterile tube containing 2 ml of saline solution then cultured on selective isolation specific media such as macconkey media , blood agar media and initially diagnosed by examination of morphometric traits developing colonies then microscopic examination by using gram stain[8,9]. Then differential Biochemical test like Idols test, methyl red, vogas proskauer, citrate utilization, urea hydrolysis, triple sugar iron test and diagnosis by growth on cultural media like Eosin methyl in blue media, *Salmonella – Shigella agar*, mannitol salt agar then to confirm used EPI 20 E and API 20 staph[10] . and diagnosed the fungi by culturing on the sabouraud dextrose agar, CHROM agar[11] .

Preparation of complexes

The chemical was used ligand 2-hydroxy-1-naphthaladehyde and equipped company (E. Merck) and metal salts equipped from different companies Fluka, Merck & Co., Ridel-de-Haen age and Al-don Chemical Inc.

Complexes were prepared by taking 0.25 gm (1 mmol) from ligand according molar ratios mentioned earlier and has melting this quantity at the lowest possible amount of ethanol (10-15) ML then add salt solution of the dissolved in ethanol between (0.33-1) mmol,(0.1-0.24) gm move mix continuous manner where it observed the emergence of a deposit has been nominated and washed with distilled water and ethanol and then returned crystallized with ethanol and dry temperature 50°C that some of the complexes

needed preparation to a temperature higher than 60°C and on the water bath they both complexes $[Mn(OC_{10}H_6CHO)_2]$,$[Ni(OC_{10}H_6CHO)_2]$ and attended all the complexes in the Neutral surroundings complexes of 2-hydroxy-1-naphaladehyde with some metal salt[12].

Study the effect of trans elements compounds on growth of bacteria

Diffusion method used in the agar the note the effect of chemical compounds on the growth of bacteria isolated. Cultured Muller Hinton agar in with sterile swab. loaded bacteria containing 1.5×10^8 cell / mL , worked wells on the media ager by cork borer and put the prepared concentrations of each compound by 50 micrometer by each well with a DMSO (Dimethyl sulfoxide). Control to confirm that the inhibitory has no effect on the growth of bacteria and leave the microletter in the laboratory temperature for 15 min. then incubated a temperature 37°C in 24 h. and an average of three replicates for each isolated and Identified the complexes effectiveness by measuring the diameter area around each hole and the conference has been three replicate[13] .

Study the effect of trans element on the growth of fungi

Fungi prepared from suspension fungal and cultured on the sabouraud dextrose agar and worked in with holes by cork borer and put trans elements compounds in the well and petridishes were let 15 min. at laboratory temperature and then incubated 24 h at 37 with three replicates of each concentration of the transelement and read results by measuring inhibition zone of bacteria around the wells[14].

Minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC) of trans element complex.

The method of turbidity was used[15]. In addition to the control tube, add 0.8 mL of sterile brain heart infusion broth (media of bacteria) and other sterile sabouraud dextrose broth (Media of fungi) , to the small test tubes . then add 0.1 mL of the concentration of the selective elements except the control tube and then add 0.1 mL of the bacterial suspension compare with Mac far land tube number 0.5. The tubes were incubated at 37°C for 18-24 hours. The result were coloured on the basis of the turbidity observation and 100 mew L were taken from the bacterial mixture or fungal mixture and incubated for a period of 24 h and temperature 37°C and recorded the result on the basis of the presence of growth number of colonies or lack of growth.

Lethal dose of moderation of transelement complexes on the laboratory mice by mouth dosage.

We have used 35 Swiss Webster albino mice for each trans element compound and dosage gradual dose (2.5,5,7.5,10,12.5,15) gram /kg of body weight with total control and repeated the experience the same number of other mice were observed during 96 hours[16].

RESULT AND DISCUSSIONE

Out of 87 patients, 64 were found suffering with inflammation of stomach and intestine in the hospitals of Iraq. as well as, seven types of bacteria and four species of fungi depending on microscopic, phenotypic, biochemical test and API staph, API 20 E as it is shown in the following table1.

Table.1: Bacterial and fungi isolation from in patients

Bacteria sp.and fungi	Total number	%
Escherichia coli	23	26.40
Acinetobacter sp	5	5.70
Shigella dysentariea	8	9 .1
Staph aureus	10	11. 4
Bacillus subtilus	9	10 . 3
Enterobacter clocae	12	13. 6
Morganella morganii	9	10 . 3
Candida albicans	6	6 .8
Candida tropicalis	2	2.20
Candida glabrata	2	2.20
Candida para psilosis	1	1

All the complexes were prepared of interaction the ligand 2-hydroxy-1-naphaladehyde with metal ions Cd^{+2} , Zn^{+2}, Vo^{+2}, Cr^{+3}, Ni^{+2}, Mn^{+2} the reactions required the following molar ratios were used Ligand – metal 1 : 1) with M^{+1} ions ,(1:2) with M^{+2} ions, 1:3 with M^{+3} ions All the complexes are non electrolytes, the general formula has been given for the complexes [$M^{+n}(OC_{10}H_6CHO)_m$)]

where M= metal ions $n^+ = +1, +2, +3, +4$. m = 1- 4 the negatively charge bidentate Ligand coordinated with metal ions through the oxygen atom in the hydroxyl group(-OH) and the oxygen atom of the carbonyl group.

The effect of the transelements complex on the growth of bacteria isolated from cases of diarrhea

The transelements of the positive results in inhibiting the growth of bacteria and these results are different according to the type of transelements and bacteria type as $[Cd(OC_{10}H_6CHO)_2]$,[$Zn(OC_{10}H_6CHO)_2$,$Mn(OC_{10}H_6CHO)_2$] are effectiveness inhibitory clear growth of bacteria.

While the effectiveness of element [Vo $(OC_{10}H_6CHO)_2$] inhibition on the growth of bacteria *Acinetobacter sp*, *Enterobacter cloacae*, *Shigella dysentery, Bacillus subtitles* and *Morganella Morganaii* while *Staphylococcus aureus* and *Escherichia* coli_ are resist for $[Vo(OC_{10}H_6CHO)_2]$ $[Ni(OC_{10}H_6CHO)_2]$ showed effectiveness on different species of bacteria but the bacteria *Morganella morganii* were resistant to them while [Cr $(OC_{10}H_6CHO)_3$] no effectiveness against bacteria isolated and doesn't have a (DMSO) intended for the solvent of trans elements no inhibitory effectiveness when used a control in bacterial species as shown in the table 2.

The impact of these trans elements complex may return the effect on bacteria according to their ability to solubility in membranes Lipid so the work inhibitory either be outside the cell or the cell surface or intra cellular cell. The effect of the trans element of bacterial cells in term of their effect on the cell membrane or impermeability so this transformation from an optional permeability to random permeability and thus allow the passage of toxic Material[17] or out material and essential elements of the cell and some of trans elements affect the enzymes produced by the cell, which prevents bacterial work or interference to the cell through the bacterial cell membrane and prevent metabolic reactions in the cell, such as respiratory reactions and thus play elements to the inhibition of bacterial cell growth[18] Figures. 2,3,4,5.

Table. 2: Diameter rate of Inhibition of the growth of Bacteria Isolated from cases of Diarrhea impact Transelement Complex

Bacteria	$[Cd(OC_{10}H_6CHO)_2]$							$[Zn(OC_{10}H_6CHO)_2]$							$[Vo(OC_{10}H_6CHO)_2]$							$[Cr(OC_{10}H_6CHO)_3]$							Control DMSO
	10%	30%	50%	70%	90%	10%	30%	50%	70%	90%	10%	30%	50%	70%	90%	10%	30%	50%	70%	90%	10%	30%	50%	70%	90%				
E.coli	18	19	21	23	24	15	16	17	18	19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				-
Acinetobacter sp	19	19	20	21	22	21	22	23	24	24	15	15	16	17	18	-	-	-	-	-	-	-	-	-	-				-
Shigella dysentariae	24	25	26	27	28	27	28	30	31	32	19	20	21	22	23	-	-	-	-	-	-	-	-	-	-				-
Enterobacter clocae	24	25	26	27	28	21	22	23	24	25	31	14	15	16	17	-	-	-	-	-	-	-	-	-	-				-
Morganella morganii	23	24	25	26	27	21	22	23	24	25	12	13	14	15	16	-	-	-	-	-	-	-	-	-	-				-
Staph aureus	20	21	22	24	25	17	18	19	20	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				-
Bacillus subtilus	19	19	20	21	23	21	21	22	23	24	12	13	14	15	15	-	-	-	-	-	-	-	-	-	-				-

	[Mn $(OC_{10}H_6CHO)_2$]					[Ni$(OC_{10}H_6CHO)_2$]					Control DMSO
Bacteria	**10%**	**30%**	**50%**	**70%**	**90%**	**10%**	**30%**	**50%**	**70%**	**90%**	**-**
E.coli	12	13	16	17	18	-	-	12	13	13	-
Acinetobacter sp	17	17	18	19	20	-	-	13	14	15	-
Shigella dysentariae	24	25	26	27	28	25	26	27	28	29	-
Enterobacter clocae	20	21	22	22	23	17	18	19	20	21	-
Morganella Morganii	14	15	16	17	18	-	-	-	-	-	-
Staph aureus	16	17	18	19	20	16	17	18	19	20	-
Bacillus subtilus	21	22	22	23	23	-	12	15	15	16	-

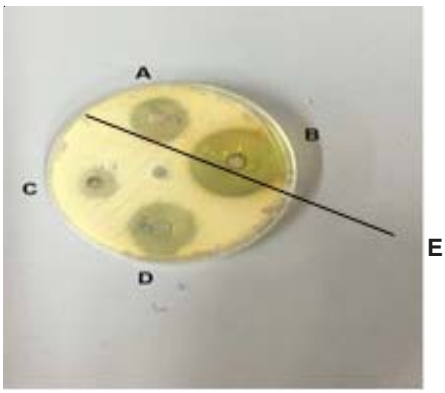


Fig. 2. Effect of A - Mn $(OC_{10}H_6CHO)_2$. B - Zn$(OC_{10}H_6CHO)_2$. C - Ni $(OC_{10}H_6CHO)_2$. D -Cd $(OC_{10}H_6CHO)_2$ E. DMSO control on bacteria *Acientobacter sp* by concentration 90%

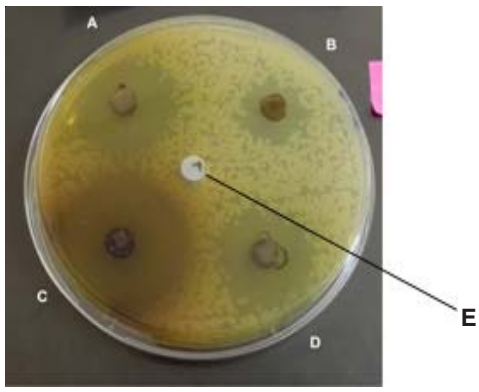


Fig. 3. Effect of A- Zn$(OC_{10}H_6CHO)_2$. B- Vo$(OC_{10}H_6CHO)_2$. C- Cd$(OC_{10}H_6CHO)_2$ .D- Mn$(OC_{10}H_6CHO)_2$. E. DMSO control on bacteria *Enterobacter clocae* by concentration 90%

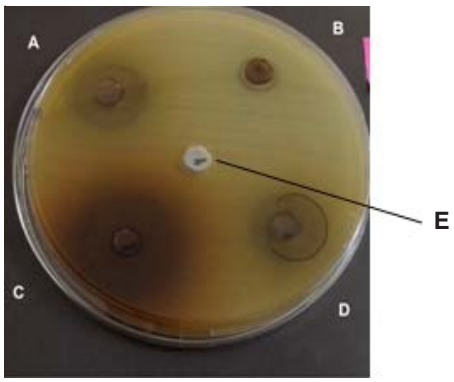


Fig. 4. Effect of A- Zn$(OC_{10}H_6CHO)_2$. B- Vo$(OC_{10}H_6CHO)_2$. C- Cd$(OC_{10}H_6CHO)_2$ .D- Mn$(OC_{10}H_6CHO)_2$ .E.DMSO control on bacteria *E.coli* by concentration 90%

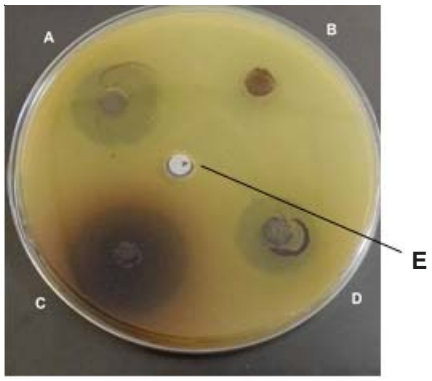


Fig. 5. Effect of A- Zn$(OC_{10}H_6CHO)_2$. B- Ni $(OC_{10}H_6CHO)_2$. C- Cd $(OC_{10}H_6CHO)_2$. D- Mn $(OC_{10}H_6CHO)_2$ .E . DMSO control on bacteria *M.morganii* by concentration 90%

Table. 3 : Diameter rate of inhibition of the growth of fungi isolated from cases of diarrhea impact trans element complex measured by millimeter

Fungi	[Cd ($OC_{10}H_6CH O)_2$] 10%]	[Cd ($OC_{10}H_6CH O)_2$] 30%	[Cd($OC_{10}H_6C_{10}H_6CHO)_2$]	[Cd ($OC_{10}H_6CH O)_2$] 70%	[Cd($OC_{10}H_6CH O)_2$] 50%	[Zn($OC_{10}H_6CHO)_2O)_2]_2$ 10%	[Zn($OC_{10}H_6CH O)_2O)_2]$ 50%	[Zn($OC_{10}H_6CH O)_{22}30\%]$ 70%	Zn($OC_{10}H_6CH O)_{2j}$ 90%	Vo($OC_{10}H_6CH O)_2$] 10%	Vo($OC_{10}H_6CH O)_2$] 30%	Vo($OC_{10}H_6CH O)_{22}o(_2$ 50%	Vo($OC_{10}H_6CHO)_2$] 70%	Vo[($OC_{10}H_6CH O)_{21\Delta}$ control 90%
c.albicans	21	22	23	23	25	20	21	22	23	24	-	9	10	11	12	-
c.tropicalis	13	16	17	18	18	10	13	14	15	16	-	-	-	-	-	-
C.glabrata	23	24	25	26	27	20	21	21	22	22	-	12	13	15	16	-
c.parapsilosi	15	16	17	18	19	12	14	16	17	18	-	-	-	-	-	
	Mn	Mn_2	Mn	Mn_2	Mn_2	Ni ($_2$	Ni (	Ni (	Ni	Ni ($_2$	Cr($_2$	Cr (	Cr (	Cr (2	Cr_2	-
	(10%	30%	(50%	70%	90%	10%	30%	50%	70%	90%	10%	30%	50%	70%	90%	
c.albicans	-	12	14	15	16	14	15	16	17	18	-	-	-	-	-	
c.tropicalis	-	-	-	-	-	17	18	20	21	21	-	-	-	-	-	
c.glabrata	10	12	13	14	15	14	14	15	16	17	12	13	14	15	15	
c.parapsilosis	-	-	-	-	-	-	12	15	16	16	-	-	-	-	-	

Fig. 6. Effect of A- Cd (OC_{10} H_6 CHO) $_2$. B - Ni (OC_{10} H_6 CHO) $_2$. C - Zn (OC_{10} H_6 CHO) $_2$.D- Mn (OC_{10} H_6 CHO) $_2$. E. DMSO control on fungi *candida parapsilosis* by concentration 90%

Fig. 7. Effect of A- Ni(OC_{10} H_6 CHO) $_2$. B-Mn ($OC_{10}H_6$ CHO) $_2$. C - Cd(OC_{10} H_6 CHO) $_2$.D- Zn(OC_{10} H_6 CHO) $_2$. E. DMSO control on fungi *candida glabrata* by concentration 90%

Fig . 8. The anatomical grade of the Webster albino swiss mice after drainge complex $_{[\text{Cd (OC10H6CHO)2 }]}$

Table. 4: MIC and MBC of transelement affecting the growth of bacterial and fungal isolates

Bacterial SP	MIC [Cd (OC10H6 CHO)2]	MIC [Cd (OC10H6 CHO)2]	MBC [Cd (OC10H6 CHO)2]	MIC [Zn (OC10H6 CHO)2]	MBC [Cd (OC10H6 CHO)2]	MIC Vo (OC10H6 CHO)2]	MBC [Vo (OC10H6 CHO)2]	MIC Cr ([C10H6 CHO)2	MBC [Cr (OC10H6 CHO)3]	MIC [Mn (OC10H6 CHO)2]	MBC [Mn (OC10H6 CHO)2]	MIC [Ni (OC10H6 CHO)2]	[Ni (OC10H6 CHO)2]
E.coli	50%	50%	70%	70%	90%	-	-	-	-	90%	-	90%	- MBC
Acinetobacter	50%	50%	70%	10%	30%	70%	100%	-	-	90%	-	90%	-
Sh.dysentarie	5%	5%	10%	5%	10%	30%	50%	-	-	5%	10%	5%	10%
E.clocae	5%	5%	10%	10%	30%	90%	-	-	-	10%	20%	70%	90%
M.Morganii	5%	5%	10%	10%	30%	90%	-	-	-	90%	-	-	-
S.aureus	10%	10%	30%	50%	70%	-	-	-	-	70%	90%	70%	90%
B.subtilus	50%	50%	70%	30%	40%	90%	-	-	-	10%	50%	90%	-
C.albicans	10%	10%	30%	10%	30%	90%	-	-	-	90%	-	90%	-
C.tropicalis	90%	90%	-	90%	-	-	-	-	-	-	-	30%	50%
C.glabrata	5%	5%	10%	30%	50%	90%	-	90%	-	90%	-	90%	-
C.parapsilosis	90%	90%	-	90%	-	-	-	-	-	-	-	90%	-

The trans elements complexes are highly effective on fungi belonging to the types of candida as in the table (3) and Fig 6,7 this could go back to the inhibitory potency of these fungi to effect mitochondria or endoplasmic and plasma membrane and these to inhibit fungus[19].

The trans elements complex were effective in inhibiting and killing different bacterial and fungal isolates . The best inhibitory agent was [Cd ($OC_{10}H_6CHO$)$_2$] on bacteria *Enterobacter cloacae , Sh .dysenteries* and *candida sp.* with concentration 5% it is best to kill the same bacteria and fungus with concentration 10% as shown the table(4) and there is no effective toxicity or loss on laboratory mice exposed to different concentrations of trans element and there are no histological or hemorrhagic effects on laboratory mice at dissection after 96 h at different concentrations as shown figure 8.

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