SYNTHESIS AND BIOLOGICAL EVLUAITON OF METAL COMPLEXES OF AN ANTIDIABETIC DRUG, GLICLAZIDE

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ABSTRACT*: The complexes of Co(II), Ni(II), Cu(II), with gliclazide (GCZ) drug ligand have been synthesized and characterized using spectroscopic techniques like IR, UV-Vis., and magnetic susceptibility measurements. Octahedral geometry have been assigned to gliclazide complexes of Co(II), Ni(II), Cu(II). Hypoglycemic activities, antibacterial effects, minimum inhibitory concentrations and LD_{50} of the synthesized complexes have been studied. Among these Complexex Cu(II)-GCZ has exhibited significant hypoglycemic activity, whereas most of these complexes have shown antibacterial effects. Although most of the metal complexes were found to be more toxic than the parent drug (GCZ), but at normal dose of the parent drug (GCZ) the complexes were not found to be much toxic.*

Keywords: Gliclazide; Sulphonylurea; Diabetes; Metal based drugs; Hypoglycemic activity.

INTRODUCTION

With the development of the pharmaceutical sciences, Bio-inorganic chemistry have been found to be the most rapidly emerging field. The applications in this field have high potential in the medicines, as it provides highly useable drugs and concentrate on the modified mechanisms. Moreover, metal bio-chemistry facilitates the scientist to formulate new drugs by using organic and inorganic fields. It is investigated in many researches that inorganic elements play a significant role in the processes of bio-medical sciences. Many previous research reports revealed that the organic compounds used in the formation of the medicine do not have pure organic mode of action. Some of the organic compounds are bio-transformed by metal ions through "metalloenzymes". Furthermore, other have an impact on the metal ion metabolism[1,2] It has been reported that protein enzymes contain metal like manganese, iron, copper, cadmium, chromium and zinc etc. It also provides potential for "therapeutic inhibition or mimicry" [1]. Zinc surrounding structural, catalytic, and regulatory properties, ranging from the impact of Zn shortage to immunological character toward Zn therapy for the poisoning of copper macular degeneration along with the cancer. Furthermore, metal like zinc, vanadium, chromium and copper, etc., reported to have potential to lower blood glucose level (Hypoglycemia). More-likely cobalt has the ability to perform the role in cofactor cobalamin and in some anti-tumor agent. Sulphonylurea derivatives are commonly used as oral hypoglycemic agents for the treatment of type-2 diabetes mellitus. For the last 40 years, Inspite of having side effects like hypoglycemia, problems associated central nervous system and skin reactions, second generation sulfonylureas (SUs) remained effective. These drugs possess enhanced absorption through β-cell membrane of pancreatic islet and extensively bound to plasma protein [3-5]. Their action in diabetes mellitus is by reducing the platelet adhesiveness and aggregation by antagonizing the abnormal fibrin deposition on the vessel wall and by reducing the excessive response of the diabetic micro vessels to adrenaline [1].

Complexes of some existing drugs with certain metal ions proved to have much biological significance[6-8], because complexes some drugs having donor atoms are proved to be more potent compared to the drugs themselves [9-13]. Although, second generation sulfonylureas can act as good ligands for metal complexation having many donor atoms but their complexes have rarely been reported in literature as compared to first generation hypoglycemic sulphonylureas [5,14-16]. However, with the progress in medicinal chemistry, it is necessary to understand the reactions of the metal based compounds in biological system. Keeping in view, we have focused to synthesize different metal complexes of anti-diabetic second generation sulphonylurea drug i.e., gliclazide along with their pharmacological and toxicological studies for the expansion of pharmaceutics.

MATERIAL AND METHOD

All chemicals utilized in the experiments were of analytical grade imported from "Merck, Germany" Fluka, Switzerland and "BDH Chemicals England". Pure drug compound "Gliclazide"was obtained from "E.Merck Co Germany". Chemical "alloxan" was purchased by "Sigma-Aldrich Co.,U.S.A". "IR spectra (in KBr)" was taken out on "Shimadzu FTIR 4200 infrared spectrophotometer". The analysis of these elements was conducted on"CHNS analyzer "Exeter Analytical CE-440".

Analyses of metals in various complexes was carried out on "atomic absorption spectrophotometer model AA-680" equipped with "GFA-4B Graphite Furnace Atomizer" with "ASA Arsenic analyzer" by applying an accepted procedure [17]. "Melting point apparatus", "Mel-Temp MP-D", "Mitamura Rikon Kogyo Japan" was used to determine the melting point and decomposition points of complexes and ligands drug itself. "Sealed capillary tube" process was adapted

to do this."Absorption spectra" of metal complexes and pure ligand was obtained through "Perkin-Elmer Lambda 20 spectrometer". The values of magnetic moment "μ_{eff}" of different complexes were obtained by "Chyo Balance MSB-10" [17,18].

Antidiabetic actions of the complexes and parent drug were checked by their oral administration to diabetic rabbits "(~1.75-2 kg body weight)" as studied earlier [17]. The data of the results was analyzed with the help of "Microsoft Excel program".

Minimum inhibitory concentration(MIC)" of the complexes was determined by using various concentration of blank and solvent "(DMSO/water)" against various strains of "gram

positive and gram negative organisms", which were grown-up on "MacConkey agar" and "blood agar", correspondingly [19] to check the inhibition effect of the solvent. Organisms under test were sub-cultured and re-identified by screening methods using a loopful of growth from a single colony. These were inoculated in 10 mL of "Mueller-Hinton broth", keeping up the concentrations as per human body. On the premise of "MIC" comes about, antibacterial action of complexes was checked against various strains of microorganisms utilizing just a single dilution of 100mg/ cm^3 of them and compared with standard antibiotic "streptomycin sulphate" [20].

Toxicity (LD_{50}) for albino rates of the synthesized complexes at different concentrations (50, 100, 200, 400, and 800 mg/5 mL) was determined by the "Reed-Muench Method" by using water as a solvent.

SYNTHESIS AND CHARACTERIZATION OF THE COMPOUNDS

Gliclazide **"**IR: (KBr, cm^{-1}) 3375 (s, NH Amide.), 3210 (NH thionyl), 1704 (C=O), 1591 (C-N)" 1345, 1163 (SO_2). UV (λ_{max} DMSO, nm), (ε x 10^3): 3.86 (30674), 2.76 (36101), 3.21 (40000), 2.32 (45045). δ_H (DMSO-d_6);1.2-1.7 (m, Heterocyclic ring), 2.3 (s, CH_3), 7.4 (d, H^3, H^4), 7.6 (d, H^1, H^2), 8.1 (b, N^a–H) and 10.0 (b, N^b–H). δ_C (DMSO-d_6); 61.73 (C1), 21.08 (C11), 24.20 (C2, C3, C4), 127.54 (C9), 129.34 (C8), 137.42 (C10), 143.59 (C7), 152.07 (C6).

Cobalt(II)-Gliclazide Complex; To the refluxing solution of 1.340g (6 mmol) of the ligand in 100 mL of ethanol containing 0.336g (6 mmol) potassium hydroxide, 0.705g (3 mmol) of cobalt(II) acetate dissolved in ethanol was slowly added. The solution was further refluxed for two hours. Pinkish product was separated out. The mixture was kept as such at room temperature overnight. The product was separated by filtration, given washing with ethanol, acetone correspondingly and dried out at 80°C. Yield: 88 %.

The product obtained was a pink colored amorphous powder, m.p 230–232.0 °C (dec.). Melting and decomposition of complex causes a weak molecular ion peak at low electron volt area at *m/z:*703.7=(739.7–$2H_2O$). "IR (KBr, cm^{-1}) 3290 (s, NH), 1669 (C=O), 1528 (C–N), 1330, 1160 (SO_2), 773 (M-O) and 492 (M-N)". UV (λ_{max} DMSO, nm), (ε x 10^3): 0.06 (19960), 0.02 (15432), 3.38 (30674). Complex is paramagnetic in nature, having "μ_{eff} **(B.M)"** = 4.49 *Anal.* Calcd for $[Co(C_{15}H_{21}N_3O_3S)_2(OH_2)_2]$: C, 48.71; H, 6.0; N, 11.36; S, 8.67; M, 7.97; Found: C, 48.70; H, 5.85; N, 11.36; S, 8.65; M, 7.96.

Nickel(II)-Gliclazide Complex;

Gliclazide 3.23g (10 mmol) dissolved in 100 mL of ethanol by heating while stirring under reflux for fifteen min. Hexahydrated nickel(II) chloride 2.37g (10 mmol) dissolved in 30 mL of water was added drop wise in to the ligand solution kept at room temperature .The reaction mixture was acidic (pH 4.93) at this stage. The pH of the solution was raised to 8.5 with ethanolic KOH solution. The resulting solution was refluxed for four hours and it was then evaporated on the water bath to reduce the volume to one half. The reaction mixture was cooled to normal temperature and kept in freezer for 12 hours. Parrot green product was separated and washed with ethanol, acetone respectively and finally dried at 80°C.Yield;54%

Green, tinny crystalline product having , mp 231-233 °C, (dec.). Melting and decomposition of complex causes the appearance of a weak molecular ion peak at low electron volt area at *m/z* 857.7=(893.7-$2H_2O$). IR (KBr, cm^{-1}) 3280 (s, NH) , 1645 (C=O), 1587 (C–N), 1331, 1165 (SO_2), 771 (M-O) and 529 (M-N). UV "(λ_{max}DMSO/H_2O, nm)", "(ε x 10^3)" : 0.12 (11834), 0.05 (15384), 5.9 (32894). Complex is paramagnetic in nature having μ_{eff} **(B.M)** = 2.99. *Anal.* Calcd. for $K_2[Ni(C_{15}H_{21}N_3O_3S)_2(OH_2)(OH)].2H_2O$: C, 43.0; H, 5.41; N, 9.40; S, 7.18; M, 6.57; Found: C, 42.89; H, 5.32; N, 9.53; S, 7.17; M, 6.95.

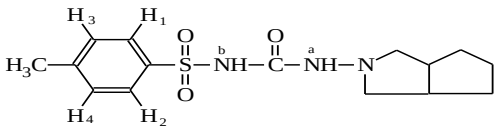
Copper(II)-Gliclazide Complex;

Gliclazide 1.94g "(6 mmol)" and KOH 0.336g "(6 mmol)" dissolved in 100 mL of alcohol with continuous stirring while heating under reflux. A suspension of 0.60g (3 mmol) copper (II) acetate in ethanol was drop wise added to the refluxing mixture. A greyish blue microcrystalline product began to form immediately. The resulting mixture was refluxed with constant stirring for one hour to complete the reaction. The resulting mixture was kept at room temperature for few minutes. Product was filttered out and given washing with acetone, chloroform and ether orrespondingly. Final product was dried at 70°C. Yield : 90%.

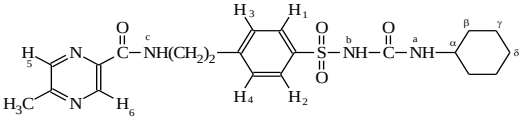
The product is blue colour amorphous powder , mp 205 °C (dec.) . Melting and decomposition of complex causes the appearance of a weak molecular ion peak at low electron volt area at *m/z* 708.3=(744.3 - $2H_2O$). "IR(KBr, cm^{-1})" 3270 (s, N-H), 1693 (C=O), 1614 (C– N), 1341, 1155 (SO_2) , 765 (M- O) and 511 (M – N). UV (λ_{max} DMSO, nm), (ε x 10^3): 5.99 (15015), 2.763.88 (33674). Complex is paramagnetic in nature having "μ_{eff}**(B.M)"** = 1.97. "*Anal.*Calcd". for $[Cu(C_{15}H_{21}N_3O_3S)_2(OH_2)_2]$: C: 48.41; H, 5.96; N, 11.29; S, 8.62; M, 8.54; Found: C , 44.40; H, 5.83; N, 11.27; S, 4.40; M, 8.57.

RESULTS AND DISCUSSION

The complexes of "Co(II), Ni(II), Cu(II) with Gliclazide(GCZ)" (**I,II**) were synthesized by reacting together particular molar quantities of the ligand and metal salts in ethanol /methanol/acetone solution and are characterized by their elemental (C, H, N, S) and metal analysis and different spectroscopic techniques like absorption, IR, UV-Visible etc. Magnetic susceptibility measurements for paramagnetic complexes were also carried out



(I)

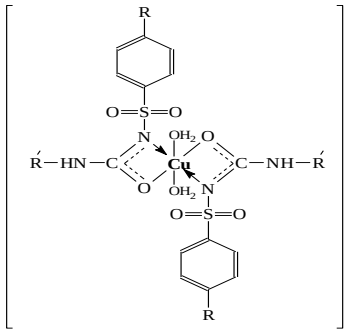


(II)

Cobalt(II), Nickle(II) and Copper(II)-"Gliclazide Complexes"

The data of elemental analysis for these complexes fit nicely into the formulae $[Co(C_{15}H_{21}N_3O_3S)_2(OH_2)_2]$,$K_2[Ni(C_{15}H_{21}N_3O_3S)_2(OH_2)(OH)]$ $.2H_2O$ and $[Cu(C_{15}H_{21}N_3O_3S)_2\ (OH_2)]$. These formulae indicate the octahedral arrangement of the ligands around these metal ions depending upon their mode of coordination.

The IR spectra of Co(II)-GCZ, Ni(II)-GCZ and Cu(II)-GCZ complexes showed that the band due to N–H stretching of $-SO_2NHCO-$ group of ligand at 3210 cm^{-1} has vanished on complex formation with metal ions. The (C–N stretch) of $-SO_2NCO-$ group of ligand at 1591 cm^{-1} has transferred to 1528 cm^{-1} in Co(II), 1587 cm^{-1} in Ni(II) and 1614 cm^{-1} in Cu(II)-GCZ complexes respectively. Carbonyl stretching in ligand (1704 cm^{-1}) has shifted to 1669 cm^{-1} in Co(II), 1645 cm^{-1} in Ni(II) and 1693 cm^{-1} in Cu(II) complexes of GCZ. So in all these complexes, ligand is coordinated with metal ions in a bidentate mode through nitrogen atom and carbonyl oxygen atoms of $-SO_2NCO-$ moiety forming a four membered chelate. The "$\nu_{asym}(SO_2)$" stretching in ligand (1345 cm^{-1}) has shifted to 1330 cm^{-1}, 1331 cm^{-1} and 1341 cm^{-1}. "$\nu_{sym}(SO_2)$" stretching in ligand (1157 cm^{-1}) has shifted to 1160 cm^{-1}, 1165 cm^{-1} and 1155 cm^{-1} correspondingly. Thus ν_{asym} (SO_2) and ν_{sym} (SO_2) frequencies of the ligand remained almost unshifted. This observation shows that SO_2 group is not participating in complexation. The bands brcause of M - N stretching was observed at 492 cm^{-1}, 529 cm^{-1} and 485 cm^{-1} and band a due to M – O stretch was seen at 773 cm^{-1}, 771 cm^{-1} and 765 cm^{-1} for "Co(II),Ni(II)andCu(II)"-gliclazide complexes respectively. Depending upon these observations it is assumed that the drug ligand is coordinated to metal in a bidentate mode through nitrogen atom and carbonyl oxygen atoms of$-SO_2NCO$ group.An octahederal environment is therefore proposed

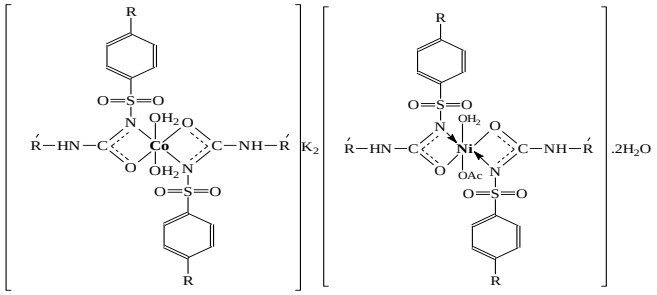


(V)

$R = -CH_3$ $\quad R' = -N$

around the central metal atoms in these complexes. (**III, IV, and V)**.Absorption spectrum of Co(II)-GCZ complex consists of a broad band at 19,960 cm^{-1} "(ε = 60 L $mol^{-1}cm^{-1}$)" and a very weak band at 15,432 cm^{-1} "(ε = 20 L $mol^{-1}cm^{-1}$)" and a high intensity charge transfer band at 30674 cm^{-1}"(ε = 3383.L.mol^{-1}.cm^{-1})" in UV region. Co(II) being d^7 system tends to form regular octahedral complexes exhibiting three crystal field bands [21-23]. The observed band in the spectrum of Co(II)-GCZ complex at 15,432 cm^{-1} "(ε= 20 L mol^{-1} cm^{-1})" is therefore assigned to the $^4T_{1g} \rightarrow {}^4T_{2g}$ transition and at 19960 cm^{-1} with molar absorptivity "(60 $Lmol^{-1}cm^{-1}$)"is assigned to transition $^4T_{1g} \rightarrow {}^4T_{1g}(P)$. Low intensity values of these bands suggest an octahedral environment around the metal. The high intensity bands at 30,674 cm^{-1} with molar absorptivity (ε = 3380 L mol^{-1} cm^{-1}) is a charge transfer band.

The magnetic moments (μ_{eff}) for "octahedral Co(II)-GCZ complexes at ordinary temperature are between 4.7 and 5.2 B.M.[23].The observed magnetic moment for this complex is 4.49 B.M. On the basis of these observation, an octahedral structure is suggested for Co(II)-GCZ complex (**III**).



(III) **(IV)**

$R = -CH_3$ $\quad R' = -N$

Absorption spectrum of Ni(II)-GCZ complex consists of two broad bands centered at 11,834 cm^{-1} "(ε =120 $Lmol^{-1}cm^{-1}$)" and 15,384 cm^{-1} "(ε=59 L $mol^{-1}cm^{-1}$)". A high intensity band at 32,894 cm^{-1} "(ε = 5990 L mol^{-1} cm^{-1})" has been observed in UV region which may be a charge transfer band. As Ni(II) is a d^8 system, hence, in octahedral environment three spin allowed transitions are expected for Ni(II). The bands due to these transitions may thus be assigned to $^3A_{2g} \rightarrow {}^3T_{2g}$ (9,000-11,000 cm^3), $^3A_{2g} \rightarrow {}^3T_{1g(F)}$ (14,000-18,500 cm^{-1}) and $^3A_{2g} \rightarrow {}^3T_{1g(P)}$ (25,000-30,000 cm^3) whereas, molar absorbance of the bands is found within range (1-100 L mol^{-1} cm^{-1}). The observed bands in the spectrum of Ni(II)-GCZ are therefore assigned to the transition $^3A_{2g} \rightarrow {}^3T_{1g}(F)$ (15384 cm^{-1}) and 11834 cm^{-1} to $^3A_{2g} \rightarrow {}^3T_{2g}$. Third band at 32,894 cm^{-1} is a charge transfer band. From d orbital splitting pattern and energy level diagrams both revealed that these complexes have two unpaired electrons. The magnetic moment (μ_{eff}) ranges from 2.9 to 3.4 B.M. [23]. The measured magnetic moment of Ni(II)-GCZ complex is found to be 2.99 B.M., which corresponds with the expected value confirming an octahedral structure for Ni(II)-GCZ complex (**IV**).

The absorption spectrum of Cu(II)-GCZ complex consists of only one broadband at 15,015 cm^{-1} (ε = 190 L mol^{-1} cm^{-1}) in visible region. Another high intensity charge transfer band is observed at 33,674 cm^{-1} (ε = 3800 L mol^{-1} cm^{-1}). Cu(II) is a d^9 system. The absorption spectrum of octahedral Cu(II) complexes consists of a single absorption band due to electronic transition $^2E_g \rightarrow {}^2T_{2g}$. However, due to John-Teller distortion and low symmetry of the complexes, usually 2 or 3 absorption bands have been reported and assigned to these

transition [21-23]. The observed band at 15015 cm^{-1} in the spectrum of Cu(II)-GCZ is therefore assigned to transition $^2A_1 \rightarrow {}^2B_2$. It is a d-d transition band with molar absorptivity value "(36 $Lmol^{-1}cm^{-1}$)" which is quite low as compared to the ε values of other transition bands.

The magnetic moment (μ_{eff}) of Cu(II)-GCZ complex is generally ranges 1.75-2.20 B.M. [23]. The experimentally determined magnetic moment (μ_{eff}) for Cu(II)-GCZ complex is 1.96 B.M. On the basis of

these results an octahedral structure was proposed for this complex in which remaining sites of the octahedron are satisfied by water molecule(**V**).

HYPOGLYCEMIC ACTIVITY OF COMPLEXES OF GLICLAZIDE

The complex of Cu(II) with gliclazide has shown significant antidiabetic activity, whereas, the remaining complexes found ineffective for hypoglycemic activity and are not discussed here.The consolidated results are shown in table1.

The hypoglycemic activity of this complex was compared with control as well as parent drug (GCZ). The decrease in mean blood glucose level(BGL) of control and treated groups of alloxan diabetic rabbits are reported in Table 1.The Cu(II)-GCZ complex has shown a significant hypoglycemic activity and decrease sugar level(BGL) compared to control. After two hours the blood glucose level of the groups treated with Cu(II)-GCZ complex (374.80 ±10.35) was greater than the group of animals treated with GCZ drug (366.20 ±6.76) but less than control (445.60 ±12.68). After four hours time period, the BGL of Cu(II)-GCZ complex (301.00 ± 2.73±2.73) was higher than the drug loaded group (285.60 ±3.57), after which blood glucose level in all these groups of animals starts increasing. This increase was rapid in Cu(II)-GCZ complex between the 4^{th}-5^{th} hour of the experiment. After eight hours the BGL of both the complexes and drug loaded groups reach to minimum. The profiles of various curves obtained are shown in Fig.1. On the basis of these observations, it is inferred that Cu(II)-GCZ complex show hypoglycemic activity, but to a lesser extent compared to the parent drug (GCZ). Cu(II)-GCZ complex remains effective for four hours parallel to GCZ drug, although its glucose lowering capacity is less than that of GCZ.

Table-1 Change in Mean Blood Glucose Level (mg/dl) of Alloxan Diabetic Rabbits Treated with 1.0 mg/kg B.W of Gliclazide Drug and Its Complexes.

Time After Administration	Aloxan Diabetic Control	Alloxan Diabetic Rabbits Treated with Standard GCZ	Alloxan Diabetic Rabbits Treated with Cu-GCZ
0 Hr.	447.80 ± 12.72	446.80 ± 6.37	457.74 ± 5.51***
1 Hr.	449.40 ± 14.08	412.20 ± 2.00	417.00 ± 4.30***
2 Hrs.	445.60 ± 12.68	366.20 ± 6.76	374.80 ± 10.3
3 Hrs.	446.40 ± 11.83	331.80 ± 4.20	362.20 ± 10.4*
4 Hrs.	447.00 ± 13.34	285.60 ± 3.57	301.00 ± 2.73*
5 Hrs.	447.20 ± 11.68	346.20 ± 2.23	417.60 ± 3.64*
6 Hrs.	447.20 ± 12.33	383.40 ± 1.07	428.00 ± 4.69*
7 Hrs.	446.40 ± 12.30	626.60 ± 7.53	440.40 ± 6.80***
8 Hrs.	446.20 ± 13.21	445.80 ± 9.60	452.00 ± 7.32

* p < 0.001 Significant relative to GCZ., ** p < 0.01 Significant relative to GCZ, *** p < 0.05 Significant lative to GCZ, GCZ = Gliclazide

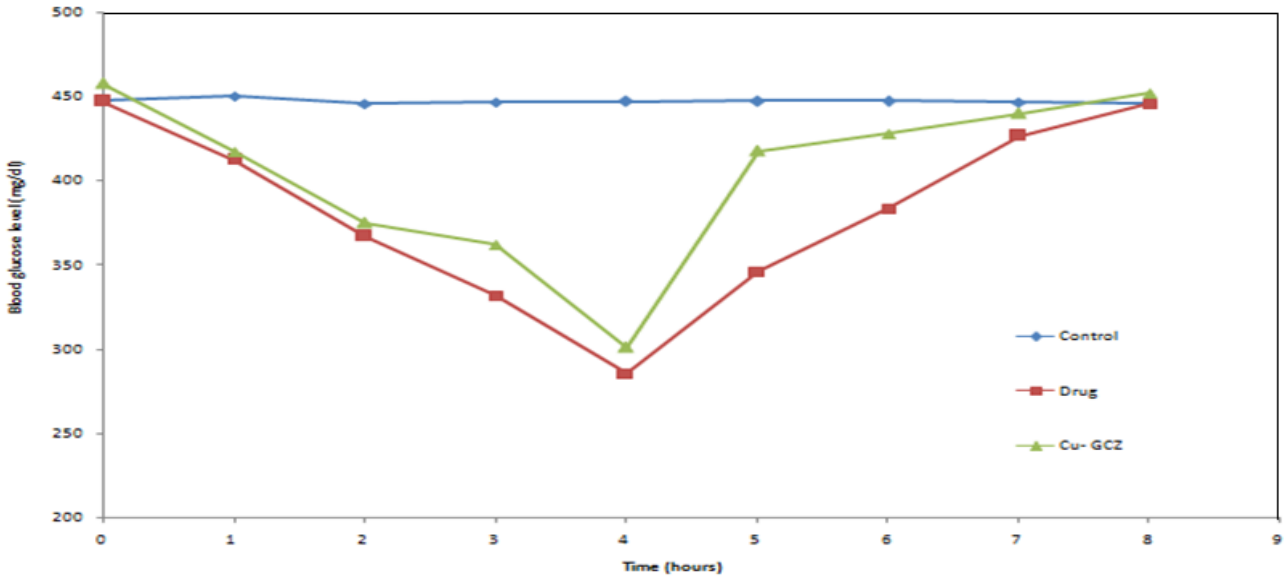


Figure 1 Change in mean blood level (mg/dl) of alloxan diabetic rabbits treated with gliclazide(GCZ) drug and Cu(II)-GCZ complex

The Cu(II)-GCZ complex have shown a significant hypoglycemic activity and decrease sugar level(BGL) compared to control. After two hours the blood glucose level of the groups treated with Cu(II)-GCZ complex (374.80 ±10.35) was greater than the group of animals treated with GCZ drug (366.20 ±6.76)

but less than control (445.60 ±12.68). After four hours time period, the BGL of Cu(II)-GCZ complex (324.00 ±2.73) was higher than the drug loaded group (285.60 ±3.57), after which blood glucose level in all these groups of animals starts increasing. This increase was rapid in Cu(II)-GCZ complex between 4^{th}-5^{th} hour of experiment. After eight hours the BGL of both the complexes and drug loaded groups reach to minimum. The profiles of various curves obtained are shown in Fig.1

On the basis of these observations it is inferred that Cu(II)-GCZ complex show hypoglycemic activity but to a lesser extent compared to the parent drug (GCZ). Cu(II)-GCZ complex remains effective for four hours parallel to GCZ drug, although its glucose lowering capacity is less than that of GCZ.

.TOXICITY (LD_{50}) OF METAL COMPLEXES OF GLICLAZIDE

Drugs used in therapeutic, usually show certain adverse affects, but selective toxicity of certain chemicals and biological substances may make them useful therapeutic agents [24]. Hence, before introduction of a new drug, it is necessary to observe their toxicity with a view to find its therapeutic index, usually in terms of LD_{50}. For the purpose, metal based drug compounds were also trialed for their toxicity (LD_{50}) and is reported in table 2. LD_{50} value for gliclazide is reported to be greater than 3g/kg b.w., orally [25]

LD_{50} value of cobalt(II)-gliclazide complex was found to be 1.47g/kg b.w. orally in rats. This value shows that toxicity of this complex is more than the parent drug (3g/kg b.w.) and is found to be the most toxic compound in the series of complexes synthesized. Cobalt(II)-GCZ complex therefore shows relatively higher toxicity.

LD_{50} value for Cu(II)-gliclazide complex was found to be 2.05g/kg b.w., orally. The toxicity of this complex is less than that of Co(II) complex, but still more than the parent drug (3g/kg b.w. orally). LD_{50} value for Ni(II)-gliclazide complex was found to be 2.28g/kg orally in rats, which is still less than the parent drug (3g/kg b.w. orally).This is the highest LD_{50} value in the series of complexes synthesized in this work. The Ni(II)-GCZ complex is therefore, found to be less toxic than Co(II)-GCZ and Cu(II)-GCZ complexes but more toxic than the GCZ drug itself.

Table-2: Toxicity (LD_{50}) Data of Metal Complexes

S.No	Compounds	Toxicity (LD_{50}) g/kg B.W.
5.	Co(II)-GCZ	1.479
7.	Ni(II)-GCZ	2.28
8.	Cu(II)-GCZ	2.051

ANTIBACTERIAL ACTIVITY; On the basis of results obtained from the above discussion, it was considered of interest from clinical point of view to do comparative study of these complexes against a standard streptomycin sulphate. Ativity of complexes on different strain of organism was checked:such as *Escheria coli(C), Staphylococus eureus, Staphylococus coagulase, Escheria coli and Streptococus and "gram negative bacteria" as "Pseudomonas" (C), Escheria coli, Salamonella thphi(R).Pseudomonas, Escheria coli, Enterobacteria coloacae, Enterobacteria faecalis, Proteus mirabilis, Klepsiella pneumonia and Salimonella sensitive* utilizing just one concentration (100mg/ cm^3) of the complexes. their action was compared with typical antibiotic streptomycin sulphate. Results of this study are reported in Table 3.

The antibacterial activity of coordination compounds is well recognized. Both the natures of the ligand and central atom and caused effect upon "bacteriostatic and bactericidal activities" of these compounds [17]. The copper (II)-GCZ complex showed antibacterial activity against all the bacterial strains, particularly it shows strong activity against *Sacchromyces cereviceae*, comparable to the streptomycin sulphate. The presence of Cu(II)-ion exert inhibitory activity against *E. coli* [26]. It also shows antibacterial activity against *staphylocucus epidermitis* and *staphylococus aureus* [27]. (Table 4).The complex Co(II)-GCZ have not shown any activity against these organism. Ni (II)-GCZ complex rendered active for "*E.coli", "Staph eureus", B. "bronchiseptica", M. luteus, M. flavas* and *"Sacchromyces" cereviciae.* Complexes exhibiting antibacterial activity may find their utility as antibacterial agents. Detailed study may find out whether these complexes will prove to be good "antibacterial" mediator from "therapeutic" concerns.

MINIMUM INHIBITION CONCENTRATION

Antibacterial study of the complexes was done in "DMSO/water" solution using concentration range of 10-1280 μg/cm^3. It is because most of the complexes rendered insoluble in water. These complexes were first dissolve in DMSO and then added water to get required dilutions. Finally the minimum inhibitory concentration (MIC) was determined against bacteria referred above. MIC was additionally led utilizing blank solvent for checking inhibitory action of the solvent on these microbes. MIC information are given in Table 4.

Ni(II)-GCZ complex shows weak inhibitory effect against gram negative bacteria in the concentration range 1280 μg/cm^3 while against gram positive bacteria no inhibition is shown by this complex even above this concentration. Co(II)-GCZ complex shows very weak inhibition only against *Staph. coagulase, Streptococus, E. coli, Pseudomonas (C),* and *Proteus mirabilis* in the concentration range of 1280 μg/cm^3 while against all other strains no inhibition was shown by these complexes even above this concentration.

Copper(II)-GCZ complex exhibit strong inhibitory effect

against gram negative *Ent. faecalis* (MIC 160-640 μg/cm^3) while in case of remaining gram negative bacteria, it shows inhibition at a very high concentration (MIC 1280 μg/cm^3), but *Klepsiella pneumonia, "E.coli", "Pseudomonas"* and *"Proteus mirabilis"* have shown no activity even at a concentration higher than 1280 μg/cm^3.

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Table-3: Antibacterial Activity of Metal Complexes against Gram Positive and Gram Negative Bacteria.

Standard organism = Streptomycin in Sulphate, Concentration =100 μg/cm^3 Solvent = DMSO

Compound	Zone of Inhibition of Organisms						
	Gram Negative bacteria			*Gram Positive bacteria*			
	E. coli	***B. bron-chiseptica***	***S. cerevisiae***	***Staph. aureus***	***M. luteus***	***M. flavus***	***B. subtilis***
Co-GCZ	–ve	–ve	–ve	–ve	–ve	–ve	–ve
Ni-GCZ	Slightly +ve	Slightly +ve	Slightly +ve	Slightly +ve	Slightly +ve	Slightly +ve	–ve
Cu-GCZ	Slightly +ve	Slightly +ve	19mm	15mm	Slightly +ve	Slightly +ve	Slightly +ve
Streptomycin Sulphate Standard	22 mm	20 mm	18 mm	25 mm	20 mm	18 mm	27 mm

Slightly +ve = Less than 15 mm –ive = No activity at 100 μg/cm^3 concentration

Table-4: Minimum Inhibitory Concentration Data of Metal Complexes against Different Bacteria (MIC μg/cm^3).

Compounds	Gram Negative Bacteria										Gram positive Bacteria		
	1	2	3	4	5	6	7	8	9	10	11	12	13
Co-GCZ	1280	-	-	–	1280	–	–	1280	–	–	–	1280	1280
Ni-GCZ	1280	1280	1280	-	1280	1280	1280	1280	–	1280	1280	1280	1280
Cu-GCZ	1280	1280	1280	-	-	1280	640	-	–	1280	1280	1280	1280

*1. Pseudomonas (C)** *2. E. coli (C)** *3. Salmonella typhi (R)* *4. Pseudomonas*
5. E. coli *6. Ent. Cloacae* *7. Ent. Faccalis* *8. Proteus mirabilis*
9. Klebsiella pneumoniae *10. Salmonella sensitive* *11. Staph. aureus* *12. Staph. coagulase*
13. Streptococcus ***(* = mutant gene of the bacteria ¶ = resistant gene of the bacteria)***

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