SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL STUDY OF COMPLEXES OF GROUP-12 ELEMENTS WITH GLICLAZIDE.

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ABSTRACT*: A series of complexes of Gliclazide (GCZ), a second generation hypoglycemic sulphonylurea drug with group 12 elements [Zinc, Cadmium and Mercury] were synthesized. These compounds were characterized by using number analytical and spectroscopic methods. In all these complexes, GCZ ligand coordinates with metal ions in a bidentate way via deprotonated nitrogen (N^b) and carbonyl oxygen atoms of the* the $-SO_2N^bCON^aH-$ *group. A four-coordinated tetrahedral structure is proposed for all these complexes. The Hypoglycemic and antibacterial activity of the synthesized complexes were tested. Further toxicity (LD_{50}) and (MIC) minimum inhibitory concentration measurements were also made for these complexes. A significant antidiabetic (hypoglycemic) activity was shown by Zn (II)-GCZ complex whereas it remained comparable to GCZ drug itself for Cadmium and Mercury complexes. A considerable antibacterial action was shown by cadmium and Mercury complexes against various species of bacteria and found to exhibit a strong inhibitory effect compared to Zn (II)-GCZ complex. Mercury (II)-GCZ complex have given lowest and Zinc (II)-GCZ highest LD50 value. From these observations, it inferred that Cadmium and Mercury complexes possess a considerable antibacterial activity and toxicity with a very small hypoglycemic activity, whereas Zn (II)-GCZ complex exhibited a significant hypoglycemic activity with minimum toxicity*

Keywords: Diabetes; Sulfonylurea; Gliclazide; Hypoglycemic Activity; Antibacterial Activity

Gliclazide (GCZ) M.F: ($C_{15}H_{21}N_3O_3S$) is an important member of hypoglycemic sulfonylurea drugs. Gliclazide (GCZ) is an odorless, tasteless white crystalline powder (mp 165–170°C) It remains very stable under ordinary conditions [1–2]. GCZ is an oral antidiabetic drug used to treat type-2 diabetes mellitus (NIDDM). In many patients Gliclazide alone is not enough to obtain the required level of blood glucose in the body therefore more than one drugs are given to such patients [3]. Usually, a combination of one of the sulphonyl urea drug and metformin is recommended for this purpose [4]. Thus, among the diabetic populations there could be patients who were prescribed second generation sulphonyl urea drugs (gliclazide, glibenclamide, glipizide) or metformin or a combination of two drugs. Many procedures of the simultaneous determination of metformin with one of the sulphonyl urea drugs i.e; gliclazide, glipizide and glibenclamide in multi-component dose forms have been reported [5,6]. No methods for the determination of metaformin and sulfonylurea drugs simultaneously in biological fluids have been given in literature till now[7].

Transition metal elements perform a very essential role in biological and biochemical systems [8,9]. Role of metals in one third of the enzyme system is well established[10] Metals like zinc, chromium, copper, vanadium, selenium, *etc*., have ability to reduce blood sugar level. zinc metal is basically involve in synthesis of insulin, its secretion and signaling, and therefore, stimulate its action on metabolism [11,12].On the basis of different epidemiological and clinical observations it is suggested that decreased level of Zn metal in the body is associated with diabetes[13,14].

The pharmacological and toxicological properties of certain drugs can be modified when administrated in the form of metal complexes. Cu(II) complexes were most frequently studied in this regard and found to be active against various diseases, such as gastric ulcers, rheumatic disease, tuberculosis, and cancers [15,16]. It has been found that some active drug compounds become more potent on coordination with certain transition metal ions [17-21], ulcer

Although, nuclei of the drugs belonging to second generation class of sulphonyl ureas(SUs) contain a number of donor atoms and can act as good ligand But their complexes with metals have rarely been synthesized and reported in literature compared to first generation sulphonyl ureas [22-24] Extensive investigation of these complexes motivated us to synthesize some metal based drug compounds of Gliclazide (GCZ). In continuation of our previous work [25-26], synthesis, structural evaluation by various instrumental techniques and pharmacological studies of metal complexes of Gliclazide (GCZ) with zinc, cadmium and mercury are being reported.

MATERIALS AND MEASUREMENTS

The Chemicals utilzed in the experiments were of pure Analytical grade and obtained from "E.Merck Co Germany, Fluka, Switzerland and BDH Chemicals England". Pure drug Gliclazide was donated by "E. Merck Co Germany". Chemical alloxan was imported from "Sigma-Aldrich Co., U.S.A". A "Shimadzu FTIR 4200 infrared spectrophotometer" was used to record the IR spectra (in KBr). Mass Spectra of the prepared compounds were taken on "MAT-312"; ^{1}H- and ^{13}C-NMR (in DMSO-d_6) spectra were recorded on "Bruker 14.1T NMR spectrometer" that operates at 600 MHz frequency. Elemental analysis was performed on CHNS analyzer "Exeter Analytical CE-440".

"Atomic absorption spectrophotometer model AA-680 equipped with GFA-4B Graphite Furnace Atomizer and ASA Arsenic analyzer" was used for the metal analysis. Melting point apparatus, "Mel-Temp MP-D, Mitamura Rikon Kogyo Japan".was used to record Melting points and decomposition points of drug ligand and complexes were recorded by using sealed capillary method.

Antidiabetic activity of drug compounds and complexes was determined by their oral administration to diabetic rabbits (~1.75-2 kg body weight). Blood Glucose level (BGL) was continuously recorded using one touch glucometer and the results were analyzed on "Microsoft Excel program".

"Minimum inhibitory concentration"(MIC) of these compound, various concentrations of complexes and blank solvent (DMSO/water) was monitored for different strains of "gram positive and gram negative bacteria". These strains were grown on "MacConkey agar and blood agar", respectively [27] to check the inhibitory effect of the solvent. Organisms under study were sub-cultured and re-identified by using screening methods. In this a loopful of growth from a colony was taken and inoculated in 10 mL of "Mueller-Hinton broth", having concentration of human body. On the basis of MIC results, antibacterial activity for these compounds was checked against various strains of microorganisms. Only (100mg/cm^3) concentration of complexes was used and their activity was compared with antibiotic "streptomycin sulphate" [28]

Toxicity (LD_{50}) for albino rats 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).

Zinc(II)-Gliclazide Complex

Gliclazide 1.34g (6.0 m.mol) and KOH. 0.336g (6.0 m.mol) were dissolved in 50 ml of ethanol while refluxing under constant stirring. A solution of 1.31g (6 m.mol) zinc (II) acetate in ethanol was added drop wise into the ligand solution under continuous stirring. Initially, the formation of precipitates started after one hour which further stirred for six hours at 50°C. The colorless powdered product obtained was separated by filtration and then washing was given with solvent ethanol and acetone. Finally product was dried at 70°C. Yield: 58%.

The product was colorless and amorphous powder having diamagnetic properties. m.p., 228-230°C (dec.). Melting and decomposition of the complex causes the appearance of a very weak peak at low electron volts area at m/z 710.2=(728.2-H_2O). "IR: (KBr. cm^{-1})" 3285 (s, NH- str), 1646 (C=O str), 1533 (C-N- str.) 1332, 1150 (SO_2, unsym. sym. str), 770 (M-O), 523 (M-N). "UV(λ_{max}DMSO nm)(ε x 10^3)", 0.1 (15649), 0.08 (13157). δ_H (DMSO-d_6); 1.2-1.6 (m, Heterocyclic ring), 2.3 (s, CH_3), 7.2 (d, H^3, H^4), 7.6 (b, H^1, H^2) and 8.7 (b, N^a–H). δ_C (DMSO-d_6); 21.08 (**C_{11}**), 24.19 (**C_2, C_3, C_4**), 30.60 (C_5), 61.0 (**C_1**), 127.28 (C_9), 129.38 (C_8), 137.42 (C_{10}), 143.41 (C_7) and 150.1 (C_6). Anal. Calcd for [$Zn(C_{15}H_{21}N_3O_3S)_2$].$H_2O$: C, 49.48; H, 5.81; N, 11.54; S, 8.81; M, 8.98, C, 49.85; H, 5.70 ; N, 11.10; S, 8.79; M, 9.10

Cadmium(II)-Gliclazide Complex

Cadmium (II) acetate 1.59g (6.0 m.mol) was added with constant stirring into hot solution of 1.34g (6 mmol) gliclazide (GCZ) ligand in ethanol. Into this solution 0.336g (6.0 mmol) of "KOH" taken in 50 ml of absolute alcohol was added. After two hour more refluxing, mixture was allowed to stand overnight. A colorless precipitate was settled down in the bottom of the flask and filtered out. The product was separated by filtration and was given washing with acetone and ethanol solvent. Final product was dried under vacuum at room temperature. Yield: 74%.

The product is a colorless amorphous powder which is diamagnetic in nature. m.p., 280-285°C (dec). Melting and decomposition of the complex causes the appearance of a very weak peak at low electron volts area at m/z; 757.2. IR.(K Br, cm^{-1}) 3210 (s, -NH), 1637 (C= O), 1558 (C- N), 1330, 1165 (SO_2), 766 (M- O), 528 (M-N). "UV (λ_{max};DMSOnm)", "(ε x 10^3)": 1.19 (38260), 2.06 (45847), 0.07 (32210). δ_H (DMSO-d_6);1.1-1.6 (m, Heterocyclic ring), 2.5 (b, CH_3), 7.3 (b, H^3, H^4), 7.8 (b, H^1, H^2), 8.5 (b, N^a–H).δ_C (DMSO-d_6); 21.08 (C11), 24.20 (C2, C3, C4), 61.63 (C1), 127.51 (C9), 129.05 (C8), 143.12 (C7), 145.41 (C6) while C_{10} has disappeared. Anal. Calcd. for [$Cd(C_{15}H_{20}N_3O_3S)_2$]: C, 47.58; H, 5.32; N, 11.10; S, 8.47; M, 14.85; found; C, 47.47; H, 5.31; N, 10.96; S, 8.44; M, 14.,14

Mercury(II)-Gliclazide Complex

Murcury(II) acetate 1.89g "(5.0 mmol)" of was mixed with 100 mL solution of alcohol containg 1.34g (6 mmol) ligand and 0.336g (10.0 mmol) of KOH, with continuous stirring at normal temperature. The mixture was stirred for 1 hour. A colourless product was settled down in the bottom of the reaction flask. The product was separated by filtration and given washing with ether and ethanol solvent. Final product was dried under vacuum at room temperature. Yield: 91%.

The product is a colorless amorphous powder which is diamagnetic in nature. m.p.,187-190°C (dec). Melting and decomposition of the complex causes the appearance of a weak molecular ion peak at low electron volts area at m/z 666.2=(756.2-5H_2O). IR: (K Br, cm^{-1}) 3270 (N H), 1719 (C= O), 1596 (C-N) 1355, 1142 (SO_2), 708 (M-O), 509 (M-N). UV (λ_{max} DMSO, nm), (ε x 10^3): 3.29 (30864), 0.41 (36493), 0.32 (48076). δ_H (DMSO-d_6); 1.0-1.5 (m, Heterocyclic ring), 8.8 (s, N^a–H), 7.8 (d, H^1, H^2), 7.4 (d, H^3, H^4), 2.4 (s, CH_3). δ_C (DMSO-d_6); 21.08 (C11), 23.95 (C2, C3, C4), 30.08 (C5), 79.23 (C1), 127.38 (C9), 128.91 (C8), 142.37 (C7), 151.41 (C6), while C_{10} has disappeared..Anal. Calcd. for K[$Hg(C_{15}H_{20}N_3O_3S)(OAc)_2$].5$H_2O$: C, 30.18; H, 4.8; N,3.7; S, 4,24; M, 26.52 found: C, 29.85; H, 4.50; N,3.46; S,4,22; M, 26.57

RESULTS AND DISCUSSION

The complexes of "Zn(II),Cd(II)" and "Hg(II)" with gliclazide (GCZ) ligand (**I,II**) were prepared by reacting together appropriate molar quantities of the drug ligand and metal salts in ethanol/methanol/acetone solvents and are characterized by determination of metals atom and different elements in them (C, H, N, S and metal) and different spectroscopic techniques like absorption, IR, ^{1}H- NMR and

^{13}C-NMR. Magnetic susceptibility measurements for paramagnetic complexes were also carried out.

The elemental and metal analyses of all the complexes clearly verified the formulae $[Zn(C_{15}H_{21}N_3O_3S)_2].H_2O$, $[Cd(C_{15}H_{21}N_3O_3S)_2]$ and $K[Hg(C_{15}H_{21}N_3O_3S)(OAc)_2].5H_2O$.These formulae suggest four coordinated environment around Zn(II), Cd(II) and Hg(II) ions, provided ligand is coordinated in a bidentate mode.

Infrared spectral data of all these complexes have shown a same kind of pattern. The (N-H str) of $-SO_2NHCO-$ moiety, in the spectra of the free ligand has disappeared on coordination of its deprotonated nitrogen atom. The C–N str. frequency of the $-SO_2NCO-$ moiety appeared at 1532 cm^{-1} for Zn(II)-GCZ, at 1558 cm^{-1} for "Cd(II)-GCZ" and at 1596 cm^{-1} for Hg(II)-GCZ complex compared to the ligand at 1591 cm^{-1}.

The C=O str. band of $-SO_2NHCO-$ in drug ligand (1704 cm^{-1}) has shifted at 1646 cm^{-1} in Zn(II), at 1637cm^{-1} in Cd(II) and at 1719 cm^{-1} in Hg(II)-GCZ complexes. The energies of observed bands suggested a bidentate mode of coordination of the ligand for all these complexes. This coordination take place via carbonyl oxygen atom and deprotonated imido nitrogen atom.

The stretching frequencies bands of $\nu_{asy}(SO_2)$ and $\nu_{sym}(SO_2)$ remained at the same positions in the spectra of complexes as well as drug ligand. This showed no interaction between metal ions and oxygen atoms of the sulphonyl group. New bands in the spectra of Zn(II)-GCZ complex at 770 cm^{-1} and at 523 cm^{-1} are due to M–O and M–N linkage respectively. Moreover, for Cd(II)and Hg(II)-GCZ complexes the bands at 766 cm^{-1},708 cm^{-1} and 628 cm^{-1},509 cm^{-1} are probably assigned to M-O and M–N linkages respectively.

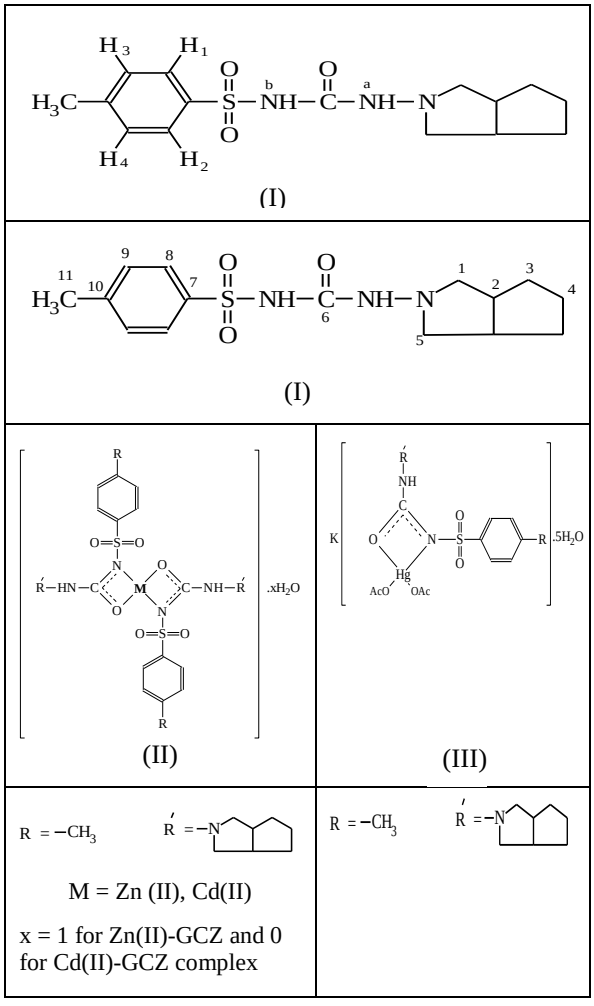
In "Zn(II)-GCZ", "Cd(II)-GCZ" and "Hg(II)-GCZ" complexes metals belong to d^{10} system hence, no d-d transition is expected in absorption spectra. Due to this absorption spectra of complexes remained transparent in vis. region. Their spectra consist of a number of intense bands (30,000-45,000 cm^{-1}) in the ultraviolet region. These bands may correspond to the ligand bands and indicate the formation of complexes.

The ligand ^{1}H-NMR signals appearing between 1-1.7 became broad multiplet upon coordination with the metal ions. As a result of involvement of -NHCONH moiety in coordination, the signals of N^a–H proton shifted little more to downfield at 8.7 ppm in Zn(II), 8.5 ppm in Cd-(II), and 8.8 ppm in Hg(II)-GCZ complexes. On complexation with these metals the signal in the most downfield region at 10.0 ppm for N^b–H proton has disappeared. This showed the involvement of $-SO_2N^bCO-$ group in complex formation with metal. Deshielding of N-H proton causes an coordination of ligand with metal center may force the two aromatic rings to be parallel to each other resulting in an up field shift. The ^{1}H-NMR confirms the involvement of N^b–H in bond formation as suggested by IR spectral studies.

On the basis of comparison of ^{13}C NMR spectral data of the complexes, new assignment for various carbons have been made. In Zn-GCZ complex the peaks at 143.41 ppm, 129.38 ppm, 127.28 ppm and 137.42 ppm were assigned to the signals of phenyl ring. The resonance peak due to (C=O) carbon C^6 was shifted a up field at 150.01ppm, 145.41 ppm and 151.41 ppm in the spectra of Zn(II)GCZ, Cd(II)GCZ and Hg(II)-GCZ complexes respectively compared with ligand 152.07ppm.. The signal due to C^2, C^3, C^4 were observed at 20-40 ppm and for C^5 at 30.60 ppm. The methyl carbon (C^{11}) resonates most up field at 21.08 ppm.These observations reveal that in all these complexes, GCZ coordinates with metals Zn(II), Cd(II) and Hg(II) in a bidentate mode through nitrogen N^b and carbonyl O atom of the $-SO_2N^bCO-$ moiety. A four coordinated tetrahedral structure is proposed for all these complexes **(III), (IV)**

HYPOGLYCEMIC ACTIVITY

The hypoglycemic activity of these complexes was compared with control as well as parent drug (GCZ). The decrease in mean blood glucose level of control and treated groups of alloxan diabetic rabbits are reported in Table 1. Hypoglycemic action of the complexes and parent drug Gliclazide-GCZ was compared by giving particular doses of these compounds orally to



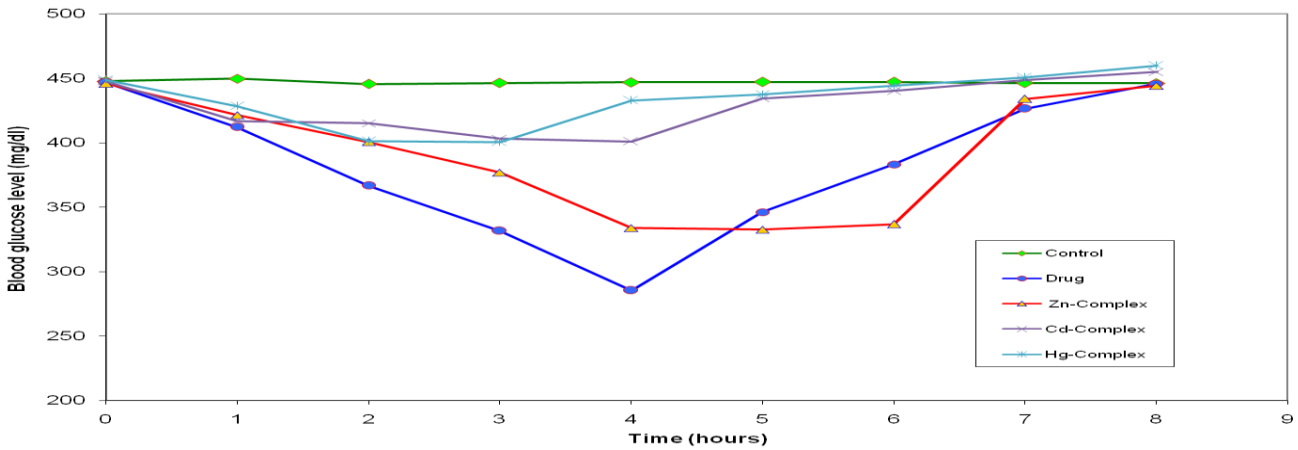


Figure 1Change in mean blood level (mg/dl) of alloxan diabetic rabbits treated with gliclazide(GCZ) drug and Zn(II), Cd(II), Hg(II)-GCZ-BCD complex

different groups of diabetic rabbits. The change in (BGL) was recorded at the start of experiment (0hrs) and after each hour upto 8 h. Among these complexes Zn(II)–GCZ complexes presented significant hypoglycemic action as compared to the diabetic animals treated original drug and control groups.The results were then calculated statistically. The change in (BGL) was plotted as function of time. The profiles of various curves obtained are shown in Fig. 1.

The results of these experiments have shown that zinc(II)-GCZ complex shown very good antdiabetic activity compared to the parent drug (GCZ) itself and Cd(II)–GCZ and Hg(II)–GCZ complexes. After two hours the blood glucose level of the groups treated with Zn(II)-GCZ complexes (400.60 ±36) 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 Zn(II) complexes (334.00 ±2.73) and of Cd(II)–GCZ and Hg(II)–GCZ compleses was (401.12±7.5 and 433.00±3.46) respectively. Thus BGL level of complexes remained higher than the drug loaded group (285.60 ±3.57) at the end of four hour time duration. After which blood glucose level in all these groups of animals starts increasing except Zn(II)-GCZ complex (332.80±3.30) which remained low even after 5 hour of time duration. This increase in (BGL) was rapid in Cd(II)–GCZ and Hg(II)–GCZ complexes between 4^{th}-5^{th} hour of experiment and showed less hypoglycemic activity than parent drug itself. After eight hours the BGL of both the complexes and drug loaded groups reach to maximum. Thus Zn(II)-GCZ complex showed slower onset of action with prolonged time duration as compared to parent drug(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 [30]. 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 this purpose, metal based drug compounds were also trialed for their toxicity (LD_{50}). LD_{50} value for gliclazide is reported to be greater than 3g/kg b.w., orally [31].

LD_{50} value for cadmium (II)-gliclazide complex was found to be 2.89g/kg b.w. orally in rats. This is the highest LD_{50} value in the series of gliclazide complexes synthesized in this work. This compound is therefore, found to be least toxic than all the complexes tested for their toxicity. It should be noted that toxicity of cadmium(II)-gliclazide complex is comparable to the parent compound gliclazide (3g/kg b.w., orally in mice). As the experimental animal model used in the present work is different, hence, the results are expected to be different. On this basis we can say that Cd (II)-GCZ complex might be less toxic than the parent drug. The toxicity of cadmium ion is greater than many metal ions like arsenic, cobalt, copper etc [32]. The higher LD_{50} value of cadmium complex shows that complex is less toxic than both of its constituents, ligand and metal. LD_{50} data of the complexes is given in Table-2

The LD_{50} value of zinc(II) and mercury(II) complexes were found to be 2.20 and 1.71g/kg b.w. respectively hence observed to be more toxic than the parent compound gliclazide. The toxicity value of zinc(II)-sulphate is 0.63g/kg b.w. This may be a cause of lower LD_{50} value of the zinc(II)-GCZ complex. Also LD_{50} of mercury is smaller than zinc [33]. This may further decrease the LD_{50} value of mercury(II)-gliclazide complex 1.71g/kg b.w. On the basis of these observations it is inferred that Cd(II)-GCZ is least toxic (LD_{50} 2.89g/kg) and Hg(II)-GCZ is most toxic (LD_{50} 1.71g/kg) of all these complexes of gliclazide drug.

ANTIBACTERIAL ACTIVITY

On the basis of results obtained from the above discussion, it was considered to be of interest from clinical point of view to do comparative study of these complexes against a standard streptomycin sulphate. Antibacterial action of these compounds was checked against various organisms such as: *Escheria coli(C), Staphylococus eureus, Staphylococus coagulase, Escheria coli and Streptococus and "gram negative bacteria"such as: "Pseudomonas(C)", Escheria "col"i, Salamonella thphi(R).Pseudomonas, Escheria coli, Enterobacteria coloacae, Enterobacteria faecalis, Proteus mirabilis, Klepsiella pneumonia and Salimonella sensitive* using only one concentration (100 μg/cm^3), of these complexes their activity was compared with standard

antibiotic streptomycin sulphate. Results of this study are reported in Table 3.

Most of the complexes of GCZ ligands have shown no activity against the organisms used in this study compared to streptomycin sulphate.Complexes of Zn(II), Cd(II), and Hg(II) I) have shown considerable activity for these organism.

The antibacterial activity of coordination compounds is well recognized. Both, the type of ligand and of central metal atom effect the "bacteriostatic" and "bactericidal" action of the complexes. Complexes of Cd(II) and Hg(II) with GCZ have good antibacterial activity against all these bacterial strains (Table 3). Cadmium and mercury ions have been reported to exert antifungal activity [34]. Cd(II)-GCZ and Hg(II)-GCZ complexes have shown considerable activity against "*E.coli", Staph. eureus, B. "bronchiseptica", M. luteus, M. flavas* and *"Sacchromyces" cereviciae.* Antibacterial study rervealed that these complexes can find their use as antibacterial agents.

MINIMUM INHIBITION CONCENTRATION

Antibacterial studies of these complexes were performed in DMSO/water solution, as they were insoluble in water. The concentration of solution was kept in range 10-1280 μg/cm^3.

The minimum inhibitory concentration (MIC) was determined against bacteria referred above. In order to study the solvent inhibitory effect upon these bacteria, MIC was also determine using blank solvent.

Minimum inhibitory concentration data have been reported in Table 4. These observations show that metal complexes of mercury(II) and Cd(II) of GCZ drugs are active against almost all the strains of gram positive and gram negative bacteria used, with MIC value from 320 μg/cm^3 to 1280 μg/cm^3. Zn-GCZ complex shows activity against all the gram negative bacterial strains. Mercury complexes of Gliclazide has shown good activity against these bacterial strains with "MIC" range 80 μg/cm^3 to 640 μg/cm^3. Hg(II)-GCZ is only active against E. coli (C) (MIC 80-320 μg/cm^3). Cd(II)-GCZ Complex is also active against most of "gram positive and gram negative bacteria". Cd(II)-GCZ complex is more active against *E. coli* (MIC 320 μg/cm^3) and against *Pseudomonas (C), E. coli* and *Pseudomonas* (MIC 640 μg/cm^3)

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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	AlloxanDiabetic Rabbits Treated	Alloxan Diabetic Rabbits Treated	Alloxan Diabetic Rabbits Treated	Alloxan Diabetic Rabbits Treated with Hg-GCZ
0 Hr.	447.80 ± 12.72	446.80 ± 6.37	445.8 ± 6.09	447.74 ± 5.51***	448.8 ± 5.08
1 Hr.	449.40 ± 14.08	412.20 ± 2.00	421.60 ± 3.13*	417.0 ± 4.30***	428.60 ± 2.14*
2 Hrs.	445.60 ± 12.68	366.20 ± 6.76	400.60 ± 3.36	415.20 ± 4.32	401.50 ± 3.37
3 Hrs.	446.40 ± 11.83	331.80 ± 4.20	377.00 ± 3.67*	403.20 ± 09.34*	400.6 ± 3.84*
4 Hrs.	447.00 ± 13.34	285.60 ± 3.57	334.00 ± 2.73*	401.12±7.5	433.00±3.46
5 Hrs.	447.20 ± 11.68	346.20 ± 2.23	332.80 ± 3.30	434.4 ± 3.20	437.4 ± 4.30
6 Hrs.	447.20 ± 12.33	383.40 ± 1.07	377.00 ± 3.67*	440.20 ± 6.08*	444.20 ± 6.08*
7 Hrs.	446.40 ± 12.30	426.60 ± 7.53	434.04 ± 8.20	448.80 ± 7.92*	450.80 ± 7.92*
8 Hrs.	446.20 ± 13.21	445.80 ± 9.60	444.20 ± 6.08*	455.00 ± 8.3***	460.00 ± 7.3***

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

S.No	Compounds	Toxicity (LD_{50}) g/kg B.W.
5.	Zinc-Gliclazide	2.203
7.	Cadmium-Gliclazide	2.891
8.	Mercury-Gliclazide	1.714

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***
Zn-GCZ	–ve	–ve	–ve	–ve	–ve	–ve	–ve
Hg-GCZ	15 mm	Slightly +ve	Slightly +ve	Slightly +ve	Slightly +ve	Slightly +ve	Slightly +ve
Cd-GCZ	16 mm	16 mm	15 mm	15 mm	17 mm	15 mm	15 mm
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).

Compound	Gram-Negative Bacteria										Gram-Positive Bacteria		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Zn-GCZ	1280	–	1280	1280	–	1280	1280	1280	–	1280	–	1280	1280
Cd-GCZ	640	640	1280	640	320	1280	1280	–	640	640	320	640	320
Hg-GCZ	640	320	1280	640	320	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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