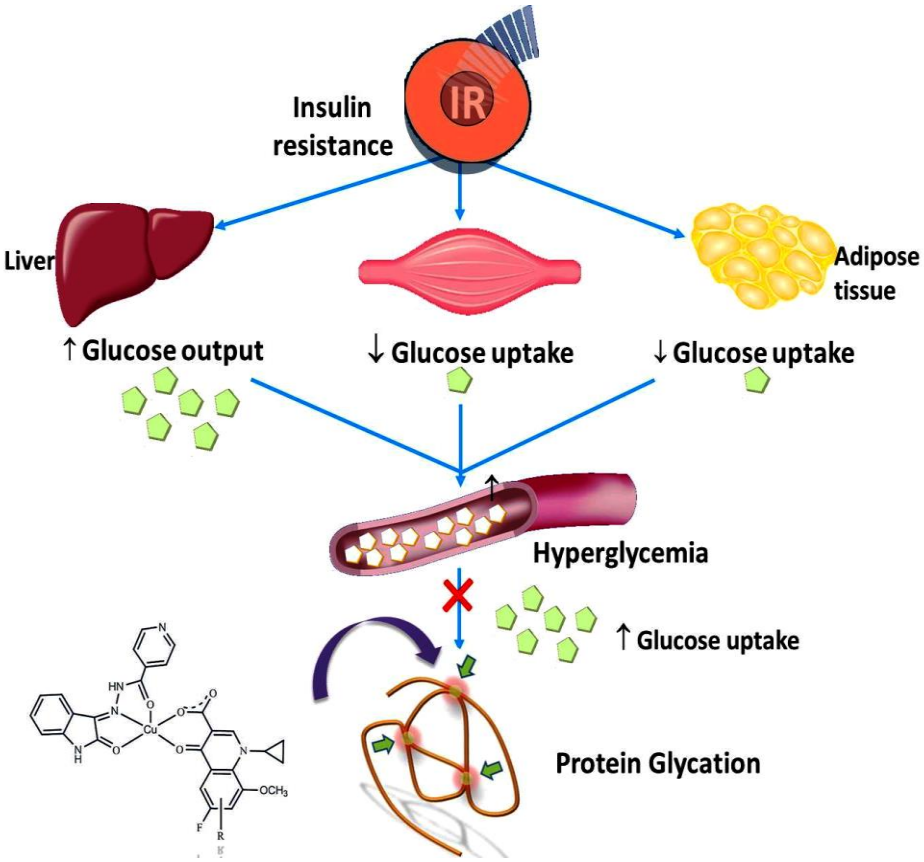
Chapter 7

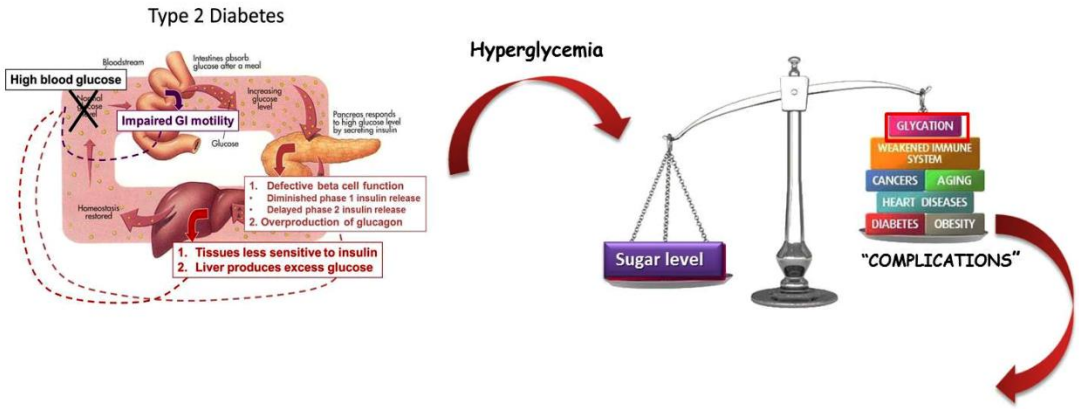
Synthesis and Characterization of Cu (II) Schiff base complexes: Anti-glycation agents.



7.1 Introduction

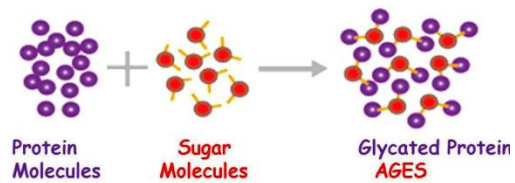
Diabetes mellitus is an endocrine disorder characterized by chronic hyperglycemia, which results from an absolute or relative deficiency of or resistance to insulin. The prevalence of diabetes mellitus is rapidly increasing. The World Health Organisation predicted that the number of adults aged > 20 years with diabetes will increase from 135 million to 300 million in 2025 [1]. Individuals affected by diabetes are prone to long-term complications such as retinopathy, cataract, neuropathy, atherosclerosis, nephropathy, and delayed healing of wounds [2].

Hyperglycemia has a key role in the pathogenesis of diabetic complications. This has been demonstrated by the Diabetes Control and Complications trial [3] and the more recentUK Prospective Diabetes Study [4]. Both of these studies have shown that diabetic patients with poor blood glucose control are more likely to develop chroniccomplications like microvascular stress, cells injury and cell death. [5]



The major biological effects of excessive glycation include:

- Crosslinking of glycated proteins
- Inactivation of enzymes
- Abnormalities of nucleic acid function
- Tissues and organs progressively lose flexibility and function



Advanced glycation end products

- Non enzymatic glycation is a process by which glucose is chemically bound to amino groups of proteins but withou the help of enzymes.

The adverse effects of high plasmaglucose depend upon the type of cells. Cells which express ahigh level of the glucose transporter 1 (GLUT1) are unable to regulate intracellular glucose concentrations and are thus very susceptible to hyperglycaemia-induced damage [6]. Non-enzymatic reaction between reducing sugar and free amino group of proteins, also known as Maillard reaction, leads to the formation of glycated protein termed Amadori product. Further rearrangement, oxidation and reduction of the Amadori product result in the formation of advanced glycation end products (AGEs) such as pentosidine, carboxymethyllysine, crossline and pyralline. Some of these products can react with a free amino group nearby and form crosslinking between proteins [7].

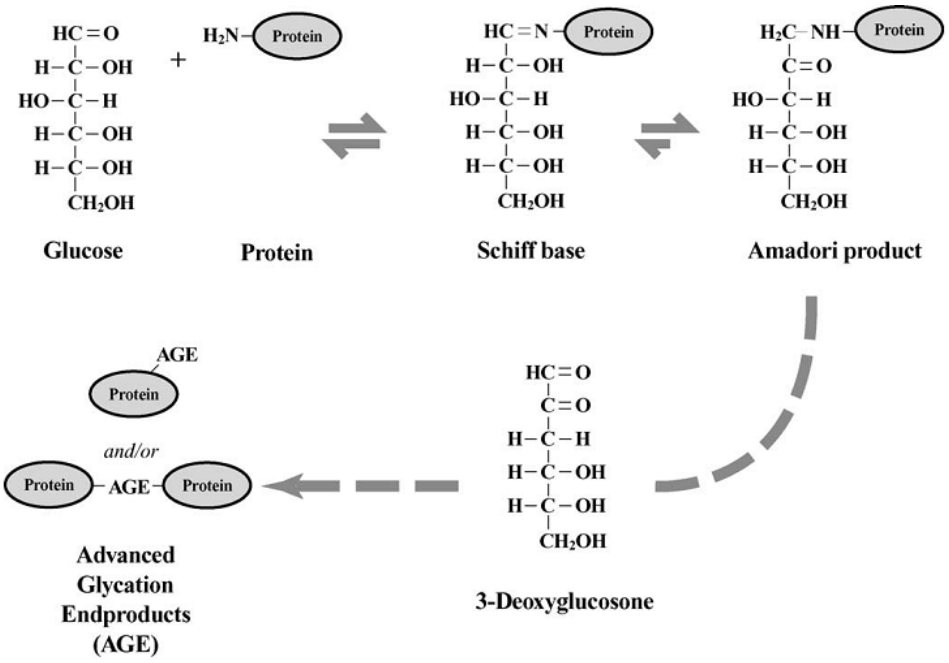


Fig 7.1: Glycation of a protein by glucose and the subsequent formation of AGEs. The initial reaction between glucose and protein amino groups forms a reversible Schiff base that rearranges to a ketoamine or Amadori product. With time, these Amadori products form AGEs via dicarbonyl intermediates such as 3-DG.

AGEs accumulate in vascular wall tissues and on plasma lipoproteins and bind to AGE specific receptors (RAGEs) with ageing. AGEs bind to RAGE at an accelerated rate in diabetic patients and play an important role in the development of diabetes

complications, age-related cardiovascular disease and osteoarthritis [8–10]. Therefore, agents with antiglycation and antioxidant properties may retard the process of AGE formation by preventing further oxidation of Amadori products. In fact, the investigation of compounds with both antioxidative and AGEs inhibition properties may act as preventive agents against diabetic complications

Since at present a number of efficient glycation inhibitors with anti-oxidative properties are very few, the need for novel glycation inhibitors with anti-oxidant properties is still unmet, a lot of efforts have been focused on the discovery of new glycation inhibitors, because of their healing potential [11]. Few molecules have been synthesized that can cleave AGEPs cross-Links and possibly open the opportunity of reversing the steady process of diabetic complications [11]. Aminoguanidine, an inhibitor of AGEs formation was found to prevent retinopathy in diabetic animals and protect them from developments of diabetic vascular complications. It is reported that a schiff base adduct between aminoguanidine and pyridoxal inhibit advanced glycation end product (AGEs) formation [12]. However, aminoguanidine has encountered some toxicity problems in phase III clinical trials. Therefore, discovery of antiglycation agents is an important approach for the treatment of late diabetic complications. Since currently number of effective antiglycating agents is very small, the need of new antiglycating agents is still unmet.

Isatin(2,3-dioxindoles) is a natural product found in a number of plants and has been found as a metabolic derivative of adrenaline in humans. Substituted isatins are also found naturally in plants. A variety of biological activities are associated with Schiff bases of isatin and their metal complexes including analgesic, anticonvulsant, antidepressant, anti-inflammatory ,antimicrobial, and CNS activities [13] .

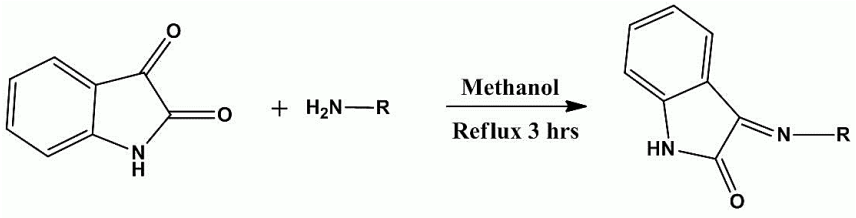
Complications and side effects associated with DM and failure of existing antidiabetic drugs are forcing researchers to come up with long term and sustainable solutions for management of diabetes. Discovery of antiglycation agents is an important approach for the treatment of late diabetic complication. To the best of our knowledge, metal

complexes in this report have never been explored for their antiglycation and antioxidant potential, although some of these complexes have reported for their antitumor activity. Therefore, this study opened the doors to develop such organometallics that could be used to treat diabetic and its complications. In the present study, in vitro antiglycation activity of a series of isatin schiff base derivatives and their copper complexes has been evaluated by BSA fluorescence assay. Complexes have also been investigated for their possible antioxidant (DPPH assay) and SOD mimic properties *in vitro.* These metal complexes show remarkable DPPH radical scavenging and antiglycation activity which may find their importance in the applied medicinal chemistry.

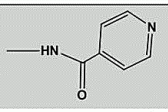
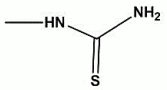
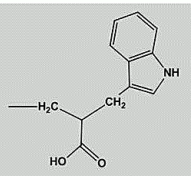
7.2 General synthesis of complexes

(A) Synthesis of Ligands: L1-L3

The preparation of hydrazone's was carried out by refluxing a mixture of isatin (5.0 mmol) and amines (isoniazid, thiosemicarbazide and tryptophan, 5.0mmol) in methanol (20 mL) for 3 hrs (scheme 7.1). The ligands precipitated as yellow solids were filtered, thoroughly washed with water and cold methanol, and finally dried in vacuum. over P_4O_{10}.



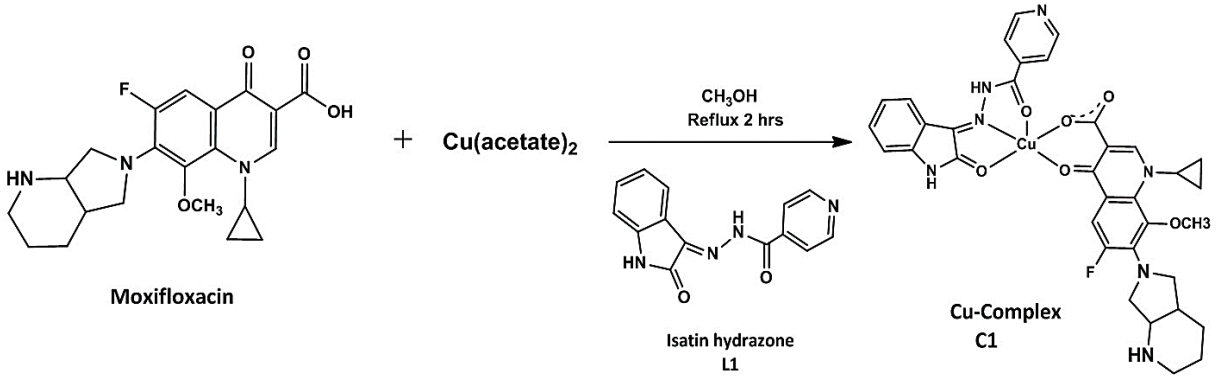
Isatin **Amine** **Isatin hydrazone L1-L3**

Amine		R
Isoniazid	L1	
Thiosemicarbazide	L2	
Tryptophan	L3	

Scheme 7.1

(B) Synthesis of Cu-Complexes: C1-C3

Complex **C1-C3** were prepared by a general synthetic procedure (scheme 7.2). To a methanolic solution of moxifloxacin ((MFL, 1.0 mmol) a solution of copper (II) acetate (1.0 mmol) in MeOH (10 mL) was added at room temperature and stirred for 0.5 h, followed by addition of isatinhydrazones (1.0 mmol, L1-L3) in methanol with constant stirring.The pH of the solution was adjusted to 7.0 using alcoholic ammonia solutions. The reaction mixture was refluxed on a water bath for 2 h and cooled to room temperature. The complexes separated were filtered, washed with MeOH and then dried in vacuum.

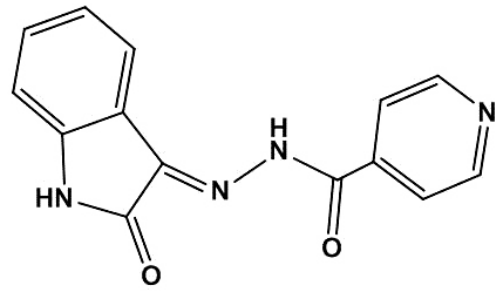


Amine	Isatin Hydrazone	Cu-Complex
Isoniazid	L1	C1
Thiosemicarbazide	L2	C2
Tryptophan	L3	C3

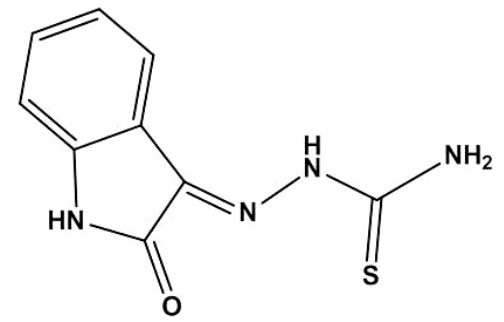
Scheme 7.2

7.3 Physicochemical data of the synthesized Ligands & Complexes

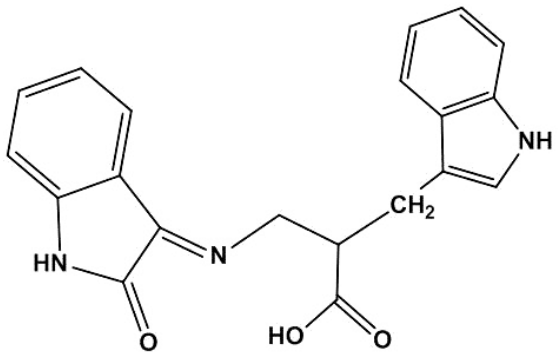
A. N'-(2-oxoindolin-3-ylidene)isonicotinohydrazide **(L1)**

	Yield: 87.8 % Molecular weight: 265.27 g/mol Molecular formula: $C_{14}H_{10}N_4O_2$ Calc. (%): C, 63.15; H, 3.79; N, 21.04 Found (%):C, 63.22; H, 3.70; N, 21.30 λ_{max}: 229nm, 276 nm. ESI-MS [MeOH, *m/z*]: 265.0 [M^+] IR cm^{-1}:1742 (C=O);1678(C=N isatin ring)

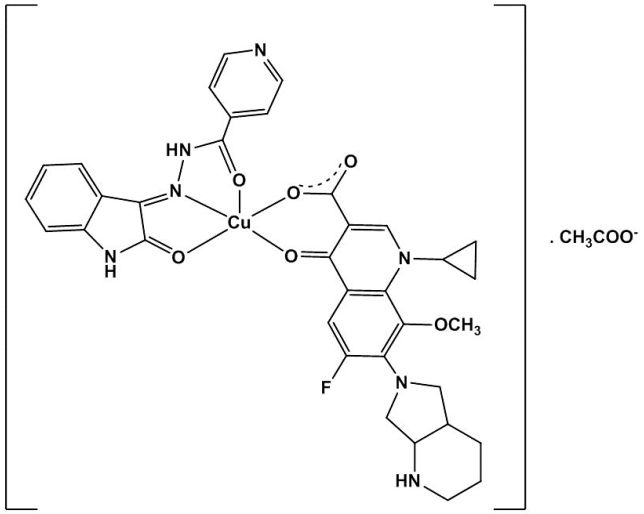
B. 2-(2-oxoindolin-3-ylidene)hydrazinecarbothioamide **(L2)**

	Yield: 82.9% Molecular weight: 220.25 g/mol Molecular formula: $C_9H_8N_4OS$ Calc. (%): C, 49.08; H, 3.66; N, 25.44 Found (%):C, 49.02; H, 3.70; N, 25.12 λ_{max}: 228, 278 nm. ESI-MS [MeOH, *m/z*]: 219.56 [M^+] IR cm^{-1}: 1741 (C=O);1677 (C=N isatin ring)

C. 2-((1H-indol-3-yl)methyl)-3-((2-oxoindolin-3-ylidene)amino)propanoicacid **(L3)**

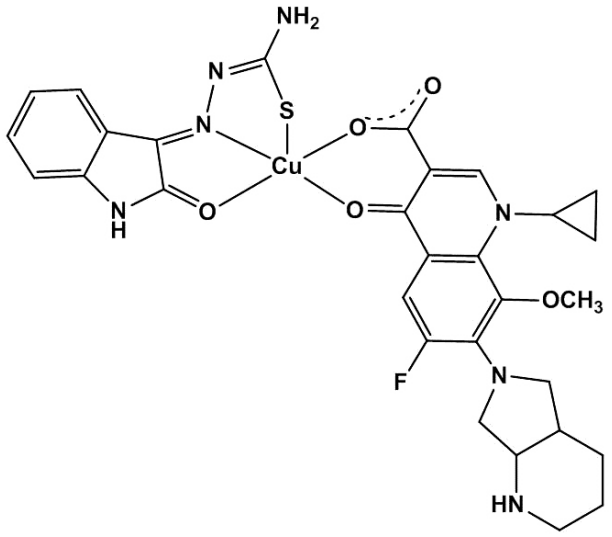
	Yield: 82.9% Molecular weight: 347.37 g/mol Molecular formula: $C_{20}H_{17}N_3O3$ Calc. (%): C, 69.15; H, 4.93 ; N, 12.10 Found (%):C, 68.99; H, 5.00; N, 12.22 λ_{max}: 229,277 nm. ESI-MS [MeOH, *m/z*]: 348.1 [M+1] IR cm^{-1}: 1740 (C=O); 1679(C=N isatin ring)

D. [Cu(L1)(MFL)].CH_3COO^- **(C1)**



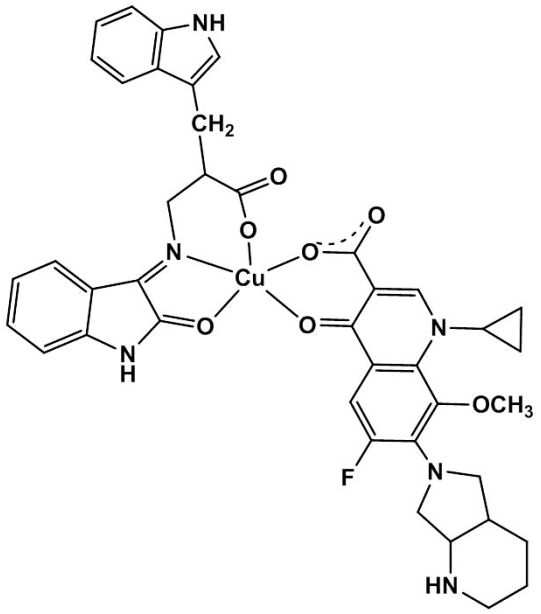
Yield: 65.8 %
Molecular weight: 730.23 g/mol
Molecular formula: $C_{35}H_{34}CuFN_7O_6$
Calc. (%): C, 57.49; H, 4.69; N, 13.41; Cu, 8.69
Found (%):C, 57.60; H, 4.71; N, 13.89 ; Cu, 8.75
λ_{max}: 240, 282, 630 nm.
ESI-MS [MeOH, *m/z*]: 727.2 [M-3]
IR cm^{-1}: 1695(C=O isatin ring); 1580 (C=N), 1461 (COO_{asym}), 1338(COO_{sym}), 1676 $(C=O)_{ketone}$

E. [Cu(L2)(MFL)] **(C2)**



Yield: 65.9%
Molecular weight: 684.22 g/mol
Molecular formula:$C_{30}H_{31}CuFN_7O_5S$
Calc. (%): C, 52.66; H, 4.57; N, 14.33; Cu, 9.29
Found (%):C, 52.52; H, 4.61; N, 13.99 Cu, 9.56
λ_{max}: 236,289,642 nm.
ESI-MS [MeOH, *m/z*]: 685.0 [M+1]
IR cm^{-1}: 1692(C=O isatin ring); 1589 (C=N), 1468 (COO_{asym}), 1341 (COO_{sym}), 1682 $(C=O)_{ketone}$

F. [Cu(L3)(MFL)] **(C3)**

	Yield:68.9% Molecular weight: 811.22 g/mol Molecular formula: $C_{41}H_{40}CuFN_6O_7$ Calc. (%): C, 60.69; H, 4.97 ; N, 10.36; Cu, 7.83 Found (%):C, 60.99; H, 5.00; N, 10.22; Cu, 7.85 λ_{max}: 239,285, 638 ESI-MS [MeOH, *m/z*]: 810.0 [M-1] IR cm^{-1}: 1688(C=O isatin ring); 1591 (C=N), 1465 (COO_{asym}), 1344(COO_{sym}), 1678 $(C=O)_{ketone}$

7.4 Results and discussions

7.4.1 Synthesis and spectral properties

Mixed ligand isatin-Schiff base copper(II) complexes [Cu(L1)(MFL)].CH_3COO^- **(C1)**, [Cu(L2)(MFL)] **(C2)** and [Cu(L3)(MFL)] **(C3)** were obtained from methanol solution, by reaction of copper actetate with MFL and isatin hydrazones (condensation of different amines and isatin) in 1:1:1 molar ratio (Scheme 7.2). The isolated compounds were characterized by physicochemical and analytical methods.

The ESI-MS spectra of the ligands **L1–L3** and their complexes **C1–C3** showed molecular ion peaks at m/z values equivalent to their molecular weights. The m/z values of all the complexes are in well agreement with the proposed composition Furthermore the composition and purity of the complexes have been confirmed by their C, H, N elemental analysis.

The NMR spectra of the ligands **L1-L3** (Fig. 7.2, Table 7.1) are in well agreement with their proposed structures. The indolinic N-H shows a one proton singlet at a δ value of ~14 ppm whereas the N-H proton of the diazenyl group (N=NH) shows a one proton singlet at a δ value of ~11 ppm confirming the condensation of isatin with amines. Rest of the signals can be attributed to the aromatic protons of the indolinic ring and the substitution on the diazenyl group.

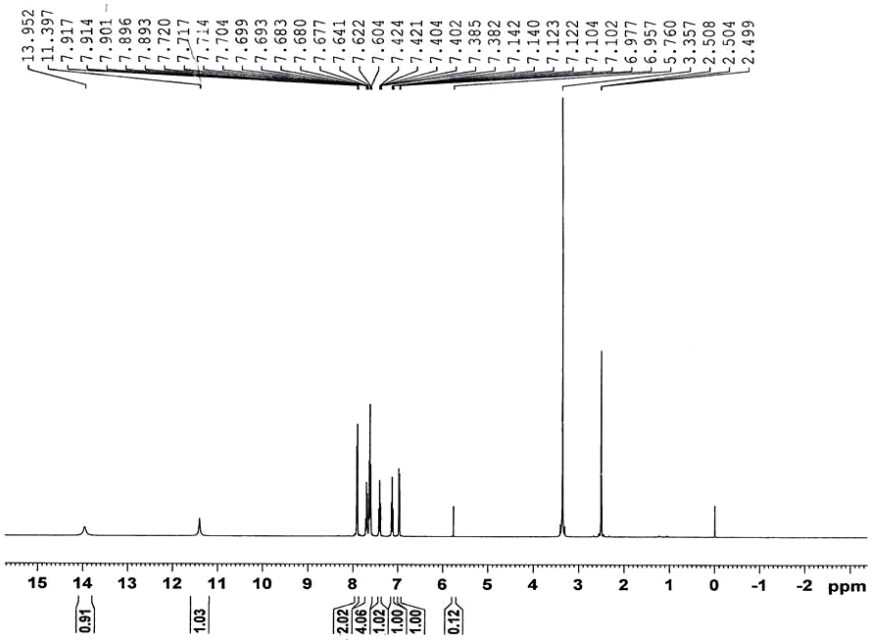


Fig 7.1: [1]HNMR spectra of **L1**

Table 7.1: [1]HNMR data of Ligands **L1-L3**.

Compounds	NMR Signals
L1	14.00 (s, 1H, N=NH), 11.44 (s, 1H, indolinic NH), 8.87-8.85 (d, 2H, pyridinyl α-H), 7.78-7.77 (dd, 2H, pyridinyl β-H), 7.61 (broad s, 1H, indolinic Ar-H), 7.42-7.39 (dd, 1H, indolinic Ar-H), 7.13-7.09 (dd, 1H, indolinic Ar-H), 6.96-6.95 (d, 1H, indolinic Ar-H)
L2	12.46 (s, 1H, N=NH), 11.21 (s, 1H, indolinic NH), 9.05 (s, 2H, NH_2), 7.65-7.64 (d, 1H, indolinic Ar-H), 7.35-7.32 (dt, 1H, indolinic Ar-H), 7.10-7.06 (dt, 1H, indolinic Ar-H), 6.93–6.91 (d, 1H, indolinic Ar-H)
L3	11.0(s, 1H, O–H), 9.87 (s, 1H, indolinic NH), 8.05 (s, 1H, NH amide), 7.86-7.90 (d, 1H, indolinic Ar-H), 7.42 -7.45 (dt, 1H, indolinic Ar-H), 7.25-7.56 (dt, 1H, indolinic Ar-H),7.11–7.21 (d, 1H, indolinic Ar-H)

In IR spectra of ligands, peaks were observed at ~1742 $cm^{-1}$for -C=O and ~1678 cm^{-1} for C=N streching respectively (Fig. 7.2 (a)). The absorption patterns of complexes appear quite similar to that of the free ligand with the shifts in peak values attributed to coordination (Fig. 7.2(b)). The -C=O peaks were shifted to lower wavelength and appeared at 1695, 1692 and 1688 cm^{-1} for C1, C2 and C3 respectively, indicating that –C=O group is involved in coordination. Peak for –C=N at ~1678 cm^{-1} in free ligands is also shifted to lower frequency between 1580-1591 cm^{-1}on coordination with Cu (II) suggesting coordination of the schiff bases through the azomethine nitrogen. Peaks in the range of 520–529 and 450–462 cm^{-1} are due to Cu-O and Cu-N bonds respectively. Similarly the shifts in the pyridone carbonyl $\nu(CO)_{MFL}$ and carboxylate $\nu(COO)_{MFL}$ stretching frequencies of moxifloxacin in complexes indicate the binding of these groups with the metal ion. All the important stretching values have been tabulated in Table 7.2.

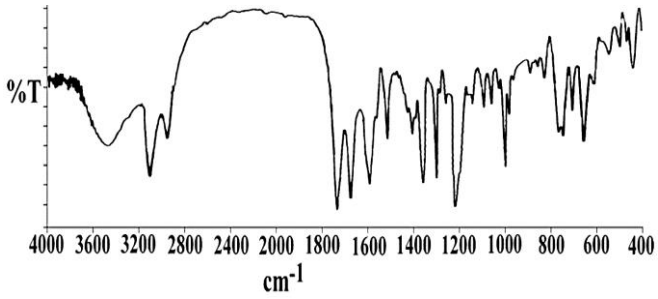


Fig 7.2(a): IR spectra of **L1**

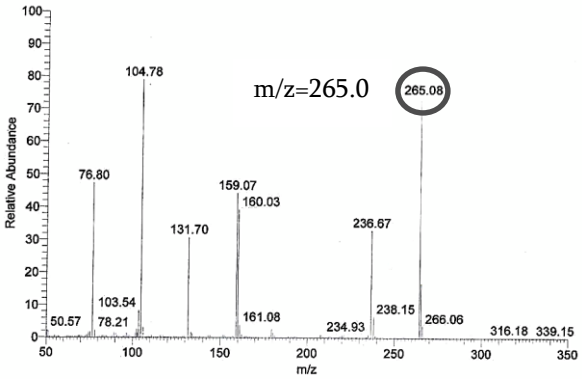


Fig 7.3(a): ESI-MS spectra of **L1**

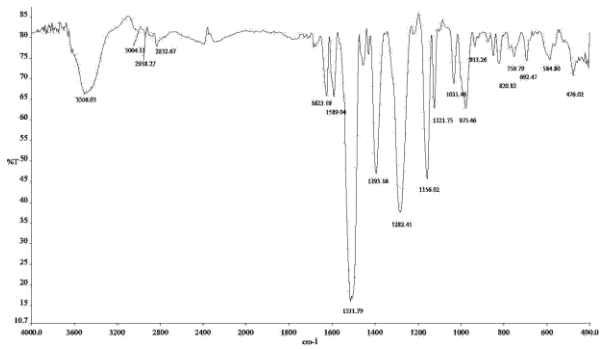


Fig 7.2(b): IR spectra of **C1**

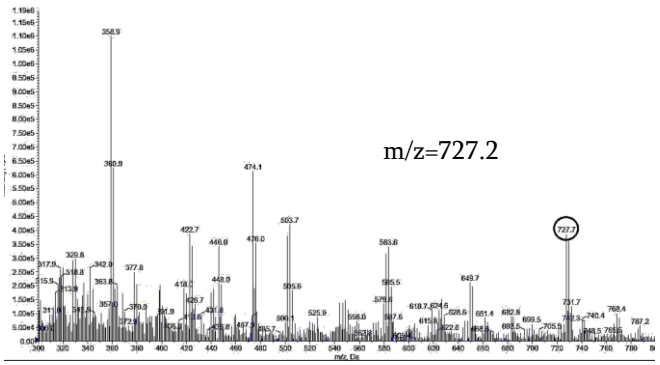


Fig 7.3(b): ESI-MS spectra of **C1**

Table 7.2: Characteristic IR bands (4000–400 cm^{-1}) of **L1-L3** and **C1-C3.**

Compounds	IR stretching values (cm^{-1})						
	Isatin hydrazine		M-N	M-O	MFL	υ_{COO} assym	υ_{COO} sym
	(C=O)	(C=N)			$\upsilon_{(C=O)p}$		
MFL	-	-	-	-	1708	1624 (Free COOH)	
L1	1742	1678	-	-	-	-	-
L2	1741	1677	-	-	-	-	-
L3	1740	1679	-	-	-	-	-
C1	1695	1580	520	450	1676	1461	1338
C2	1692	1589	526	452	1682	1468	1341
C3	1688	1591	529	462	1678	1465	1344

The electronic absorption spectra of isatinhydrazones (**L1-L3**) and Cu (II) complexes (**C1-C3)** were recorded in methanol in the range 200-900nm. The absorption maxima bands are listed in Table 7.3 and there spectra of **L1** and **C1** are given representatively in Fig. 7.4. The UV-vis spectrum of isatinhydrazones shows characteristic strong bands ~229 nm and ~276 nmdue to n-π* and π-π* transitions respectively. These bands are red shifted on complexation with copper. All the complexes shows characteristic bands at ~420 nm and a broad band at 630-642 nm due to Ligand to metal charge transfer (MFL→ Cu^{2+}) and d-d transitions respectively.

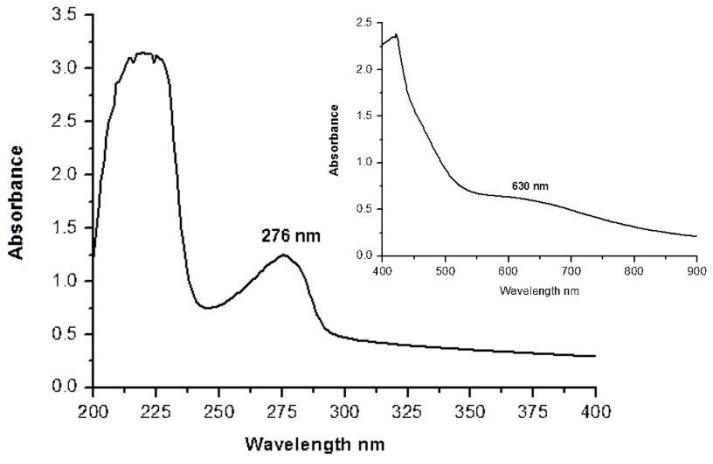


Fig 7.4: Electronic spectra of **L1.**
Inset: Visible spectra of **C1.**

Table 7.3: Electronic spectral data of **C1-C3.**

	π-π* nm	n-π* nm	d-d nm
L1	229	276	-
L2	228	277	-
L3	229	278	-
C1	240	282	630
C2	236	289	642
C3	239	285	638

The ESR spectrum of **C2** was recorded in DMSO at 77 K (Fig. 7.4). The frozen solution spectrum shows a well resolved four line spectrum and no features characteristic for a dinuclear complex, which confirms the mononuclear nature of the complex. The spin Hamiltonian parameters for the copper complex were calculated from the spectrum. The observed order (A_{II}= 149 x10^{-4} cm^{-1}> $A_{\perp}$= 50x10^{-4} cm^{-1}; g_{II} = 2.38cm^{-1}> $g_{\perp}$ = 2.18cm^{-1}) indicates that the complex has an axially elongated square pyramidal geometry [14]. The values $g_{||}$>$g_{\perp}$>2.0023 is typical of axially symmetric Cu(II) having one unpaired electron in $d_{x^2-y^2}$orbital of copper(II) ions giving $^2B_{1g}$ as the ground state.

It is reported that g_{II} is 2.4 for copper-oxygen bonds, 2.3 for copper-nitrogen bonds [14]. For mixed copper-nitrogen and copper-oxygen systems, there is a small variation in the point of symmetry from octahedral geometry [15]. For the present copper complex, the g_{II} value (2.38) is in between 2.3-2.4, this shows that the complex is having mixed copper-nitrogen and copper oxygen bonds. In axial symmetry, reflects the spin interaction between Cu(II) centres in solid polycrystalline complexes. If G > 4.0, the local tetragonal axes are aligned parallel or only slightly misaligned. If G <4.0 significant exchange coupling is present and the misalignment is appreciable. The observed value for the exchange interaction parameter for the copper complex (G = 4.4) suggests that the local tetragonal axes are misaligned. The value of exchange interaction term G>4 indicates absence of Cu-Cu interaction, thus supporting proposed monomeric structure [16].

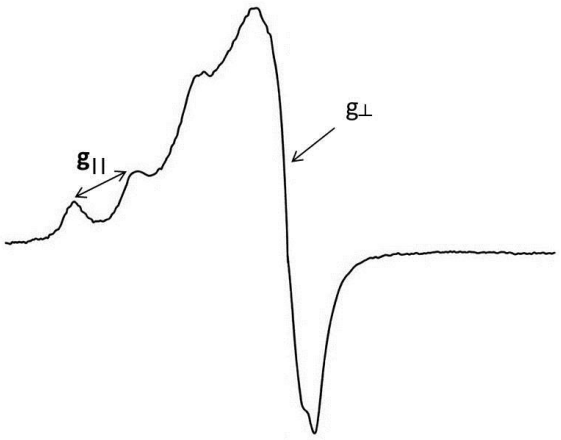


Fig 7.5 : ESR spectra of **C2** in DMSO. EPR conditions: Temperature, 10K; microwave power, 5.0 mW; Modulation amplitude, 1G; microwave frequency, 9.1 Ghz

7.4.2 Biological Studies

7.4.2.1 Antiglycation activity

Pilot experiments were undertaken to work out the time of incubation needed to glycated BSA(10mg/ml) in the presence of glucose (50 mg/ml). BSA was incubated with and without glucose in phosphate buffer saline for 5, 10, 15, 20 and 25 days at 37 °C. BSA sample when subjected to UV-Vis spectroscopic analysis, showed a characteristic peak at 280 nm. In the prescence of glucose a decrease in absorbance was obtained upto 13, 29, 61, 69 and 71% after 5, 10, 15, 20 and 25 days of incubation respectively.

Since maximum hypochromicity was obtained after 20 days of incubation and further incubation did not result in any significant change (Fig. 7.6), BSA was incubated for 20 days with 50 mg/ml of glucose in phosphate buffer saline.

The observed hypochromicity could be due to modification or changes in the micro environment of the aromatic amino acids residues in BSA. It has been reported that glycation induces unfolding of the protein leading to cross linking and aggregation responsible for a change in conformation of protein which results in hypochromicity [17].

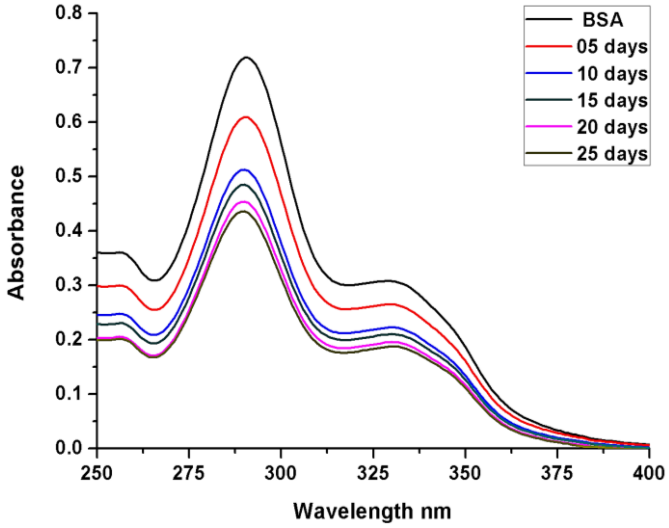


Fig 7.6:UV-Visible spectral profile of native BSA and BSA glycated with glucose incubated for , 10 , 15, 20 and 25 days .

Isatin hydrazones and their metal complexes were screened for their antiglycation potential. Isatin hydrazones (**L1-L3)** and their metal complexes **C1-C3** exhibited a varying degree of antiglycation activity with IC_{50} values ranging between 92.92 and

100.05 µg/ml for ligands and between 28.92 and 100.05 µg/ml for complexes (Table 7.4). The ligands were found to be less active whereas the metal complexes have significant antiglycation activities with **C1** showing the highest activity (IC_{50} = 28.42 ± 2.37 µg/ml) (Fig.7.7) comparable to that of standartd rutin (IC_{50}= 25.46±1.50 µg/ml). However, **C2** and **C3** are comparatively less active than **C1**, although they were found to have better activities than the ligands. The order of antiglycation activity of compounds is **C1>C2>C3>L1>L2>L3,** which reveals that activity of ligands was enhanced on complexation. By portraying the activity pattern of theses complexes, it is concluded that these complexes may have competent capability to bind protein or glucose and inhibit the further progression of glycation.

Table 7.4: Antiglycation activity of ligands (**L1-L3**) and complexes (**C1-C3**).

	Rutin	**L1**	**L2**	**L3**	**C1**	**C2**	**C3**
IC_{50} µg/ml	25.46	92.08	96.56	100.0	28.42	48.27	58.02

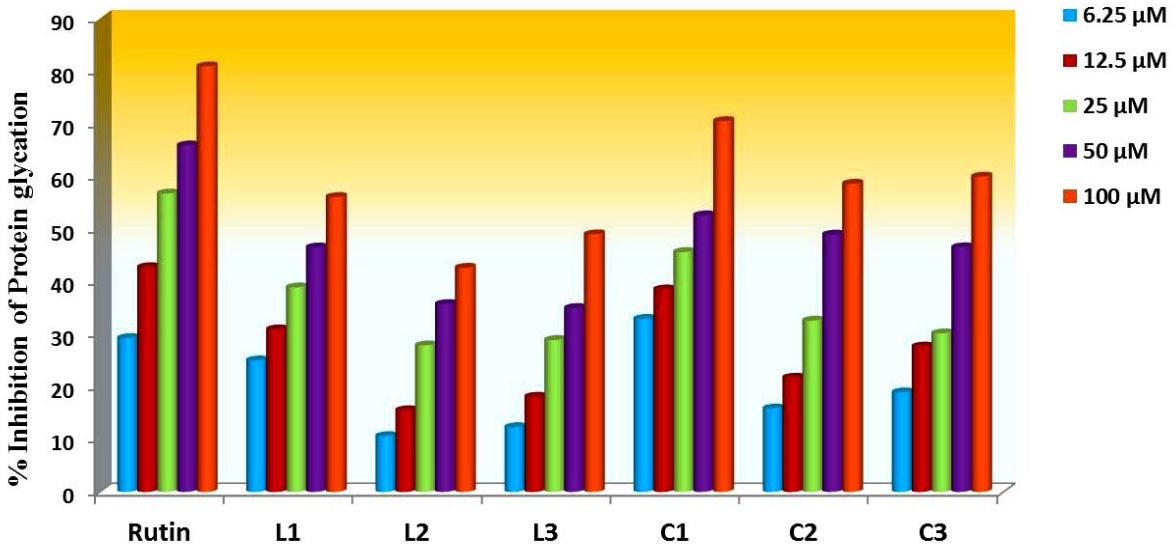


Fig 7.7: Dose-dependent inhibition of BSA glycation in the presence of **L1-L3** and **C1-C3**.

7.4.2.2 Antioxidant Activity

Free radicals that are generated in many bioorganic redox processes may encompass oxidative damage in various components of the body (lipids, proteins and DNA) and have been implicated in aging and a number of life-limiting chronic diseases such as cancer, hypertension, cardiac infraction, atherosclerosis, rheumatism, cataracts and others[18,19]. Efforts to counteract the damage caused by these species are gaining acceptance as basis for novel therapeutic approaches and the field of preventive medicine is experiencing an upsurge of interest in medically useful antioxidants. Antioxidants protect against glycation-derived free radicals and may have therapeutic potential. Vitamin E (800 mg per day) has been shown to reduce levels of glycated haemoglobin [20] and accumulation of AGEs in the arterial walls of diabetic patients [21].

Aminoguanidine, an inhibitor of AGEPs formation was found to prevent retinopathy in diabetic animals and protect them from developments of diabetic vascular complications. However, aminoguanidine has encountered some toxicity problems in phase III clinical trials [22].Efforts are now being made to develop new and safe synthetic antiglycation agents [23]. A recent study has shown that compounds with combined anti-glycation and antioxidant properties such as aminosalicylic acid are more effective than aminoguanidine in protecting endothelial cells against the harmful effects of high glucose and AGEs *in vitro* [24].There is growing interest in synthetic compounds with combined anti-glycation and antioxidant properties as they may have reduced toxicity. Indeed, a number isatin Schiff bases with antioxidant activity have been reported to inhibit glycation, at least in vitro [25].

7.4.2.2.1 Super oxide dismutase activity

The antiglycation activity of compounds has been shown to be related to their antioxidant properties and synthesized compounds were evaluated for their superoxide dismutase activity using the NBT assay [26].

The SOD activity has been expressed in terms of IC_{50} values, implying inhibition of superoxide radical at 50% concentration. The lower the IC_{50} value, the higher is the potency of the compound in dismuting the free radical. In the present case, **C1** was found to be most potent compound with IC_{50} value of 9.01µg/ml (Fig. 7.8). The activities of ligands and complexes are in the order **C1 > C2 > C3 > L1> L2 > L3,** which is in agreement with their antiglycation activity. All the metal complexes were found to have higher antioxidant potentials than their respective isatinhydrazones. From the data, we conclude that complexes possess higher electron scavenging ability compared to igands. The mechanism of action for non-enzymetic system is shown in Fig. 7.9. The higher antioxidant activity of complexes is due to a vacant coordination site that permits the bonding of superoxide anion.

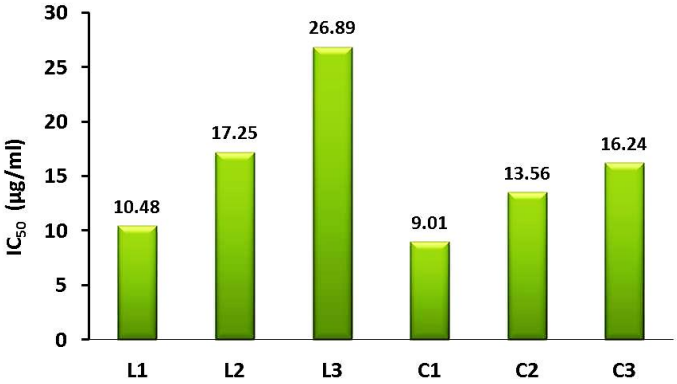


Fig 7.8: SOD Scavenging activity of compounds. Values are mean ± SEM and significantly P< 0.05

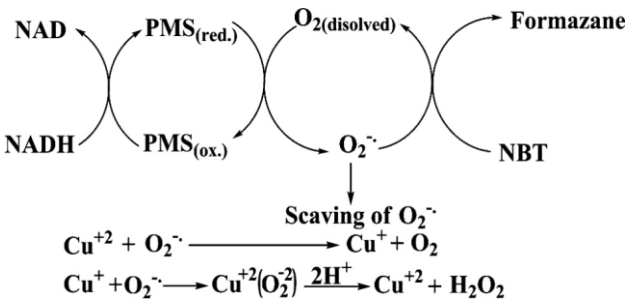


Fig 7.9: Mechanism for SOD-like activity performed using the NBT/NADH/PMS system.

7.4.2.3 DPPH activity:

The DPPH(1,1-diphenyl-2-picryl hydrazyl) radical scavenging activity of the compounds was measured according to the method of Blios [27]. The DPPH radical is a stable free radical and due to the presence of an odd electron, it shows a strong absorption band at 517 nm in visible spectrum. If this electron becomes paired off in the presence of a free radical scavenger, this absorption vanishes resulting in decolorization stoichiometrically with respect to the number of electrons taken up. Various concentrations of the experimental complexes were taken and the volumes were adjusted to 100 µL with methanol. About 5 mL of 0.1 mM methanolic solution of DPPH was added to the aliquots of samples and shaken vigorously. Negative control was prepared by adding 100µL of methanol in 5 mL of 0.1 mM methanolic solution of DPPH. The tubes were allowed to stand for 20 min at 27°C. The absorbance of the sample was measured at 517 nm against the blank (methanol).The IC_{50} values were calculated using equation 2.8.

Lower the IC_{50}, greater the antioxidant activity of the free radical scavengers. The activities of ligands and complexes are in the order **C1 > C2 > C3 > L1> L2 > L3,** which is in agreement with their antiglycation activity. Consistent with the fact that the antiglycation property of the complexes is related to their antioxidant activity, **C1** (IC_{50}=10.06 µg/ml) showed the highest radical scavenging activity (Fig 7.10). All the metal complexes were found to have higher antioxidant activity than their respective isatinhydrazones. From the data, we conclude that complexes possess higher electron scavenging ability compare to ligands.

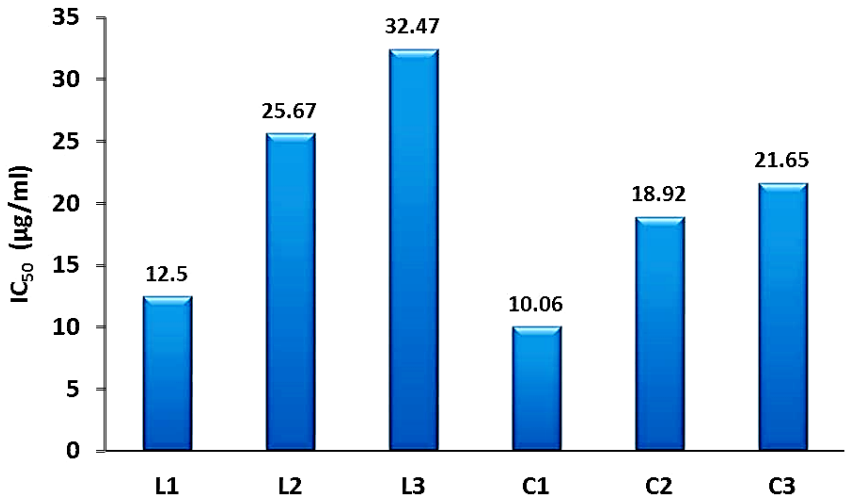


Fig 7.10: DPPH Scavenging activity of compounds. Values are mean ± SEM and significantly P< 0.05

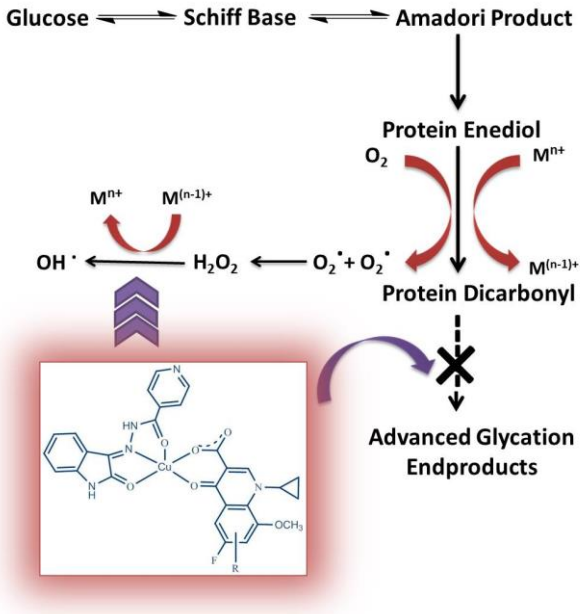


Fig 7.11: Antioxidant activity of complexes.

Autoxidative glycation where glucose is converted to a dicarbonyl ketoaldehye via its enediol radical. This ketoaldehyde can react with a protein amino group to form a ketoimine capable of forming AGEs. These steps are catalysed by transition metals (M) and the superoxide radical generated can be converted to the hydroxyl radical via Fenton reaction.

7.4.2.4 DCFDA Study

Monosaccharides, like glucose, exist in equilibrium with their enediol, which can undergo autoxidation to form an enediol radical [28]. This radical reduces molecular oxygen to generate the superoxide radical ($O_2^{\cdot-}$) and becomes oxidised itself to a dicarbonyl ketoaldehyde that reacts with protein amino groups forming a ketoimine. This is referred to as autoxidative glycation and is outlined in Fig. 10. Ketoimines are similar to, although more reactive, than Amadori products and participate in AGE formation. Oxidation reactions are also involved in AGE formation which is accelerated in the presence of oxygen and reduced in anaerobic conditions [29]. The term glycoxidation is used to describe autoxidation of Amadori products to AGEs as shown in Fig. 7.12. In the presence of molecular oxygen, the Amadori products are converted to protein dicarbonyl compounds via a protein enediol generating the superoxide radical [30]. The protein dicarbonyl compounds can participate in AGE formation and referred to as glycoxidation products. Once formed, the superoxide radicals can be converted to the highly reactive hydroxyl radical ($OH^{\cdot}$) via the Fenton reaction. ROS and the oxidative damage are thought to play an important role in diabetes. Thus, establishing their precise role requires the ability to measure ROS accurately and the oxidative damage that they cause. There are many methods for measuring free radical production in cells.

The most straightforward techniques use cell permeable fluorescent and chemiluminescent probes. 2'-7'-Dichlorodihydrofluorescein diacetate (DCFDA) is one of the most widely used techniques for directly measuring the redox state of a cell. After diffusion into the cell, DCFDA is deacetylated by cellular esterases to a nonfluorescent compound, which is later oxidized by ROS into 2',7'-dichlorofluorescein (DCF), a highly fluorescent dye. In the present study, the images taken using fluorescence microscopy of 3T3LI cells (glycated protein), revealed prominent green fluorescence (Fig. 7.12(a)). Observation of prominent green fluorescence of DCF indicates excessive ROS generation. On treatement with

complexes at IC_{50} values (SOD assay) for 24 hrs, decrease in the intensity of green fluorescence was observed (Fig. 7.12(b)).

This decrease in the intensity can be correlated to absence of ROS. The lower is the fluorescence intensity, higher is the potency of the complexes in dismuting the free radical. From the data, we conclude that all the 3 complexes possess electron scavenging ability *in vitro*.

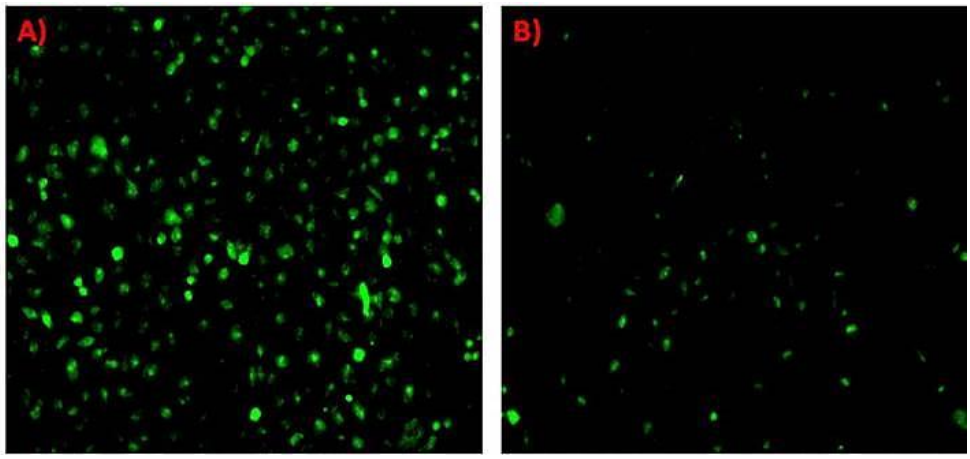


Fig 7.12: Microphotographs of 3T3L1 cells were stained with DCFDA and observed under confocal microscope: (A) 3T3L1 cells without treatment; (B) in the presence of C1; incubated at 37˚ C and 5% CO_2 for 24h.

7.5 Conclusion

Glycation is not only a major cause of AGE-mediated protein modification, but it also induces oxidation-dependent tissue damage, leading to development of complications of diseases including diabetes. Reactive oxygen species generated during glycation and glyoxidation, such as superoxide, are able to oxidize side chains of amino acid residues in protein to form carbonyl derivatives and also diminish an oxidative defence of protein by decreasing thiol groups, leading to damage of cellular proteins. Apart from a significant role in directly resisting against these free radicals, most antioxidants also possess the ability to inhibit oxidative damage-mediated glycation and glycoxidation. Therefore development of antioxidant and antiglycation agents is a key approach for the genuine treatment of late diabetic complications.

In the Present study Isatin hydrazones and their mixed ligand Cu (II) complexes with MFL were synthesized and characterized by various spectroscopic techniques and investigated for DPPH radical scavenging and antiglycation activity. The results showed that, Complexes (**C1-C3**) significantly inhibits glycation of BSA. Complexes potently reduced oxidative stress and free radicals in vitro, including scavenging of DPPH, and superoxide anion. Thus, the ability of Complexes to modulate glycation-mediated BSA oxidation might be from its anti-oxidant activity. These complexes may have potential to cure diabetic complications. However, further studies on the mechanisms of antioxidation and antiglycation are required.

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