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ORIGINAL ARTICLE

Syntheses, characterization, *in vitro* antiglycation and DPPH radical scavenging activities of isatin salicylhydrazidehydrazone and its Mn (II), Co (II), Ni (II), Cu (II), and Zn (II) metal complexes

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Isatin hydrazone;
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Abstract 2-Hydroxy salicylhydrazide isatin hydrazone (L) and its Mn (II), Co (II), Ni (II), Cu (II), and Zn (II), metal complexes were synthesized. ^{1}H NMR, UV–Vis, IR spectroscopy and elemental (CHN/S) analysis techniques were applied for characterization. TG/DTA techniques revealed that all the synthetic compounds are thermally stable up to 300 °C. They were found non-electrolytes in nature. Furthermore, all these complexes were evaluated for antiglycation and DPPH radical scavenging activities. They showed varying degree of activity with IC_{50} values between 168.23 and 269.0 μM in antiglycation and 29.63–57.71 μM in DPPH radical scavenging activity. Mn (II), Co

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(II), Ni (II), Cu (II), and Zn (II), metal complexes showed good antiglycation as well as DPPH radical scavenging activity. The IC_{50} values for antiglycation activity are 168.23 ± 2.37, 234.27 ± 4.33, 257.1 ± 6.43, 267.7 ± 8.43, 269.0 ± 8.56 Ni for Co, Zn, Mn, Cu, and Ni complexes, respectively, while IC_{50} value were found to be 29.63 ± 2.76, 31.13 ± 1.41, 35.16 ± 2.45, 43.53 ± 3.12, 57.71 ± 2.61 μM for Cu, Zn, Mn, Co and Ni complexes, respectively, for DPPH radical scavenging activity. These synthesized metal complexes were found to be better active than standards Rutin (IC_{50} = 294.46 μM) for anti-glycation, and *tert*-butyl-4-hydroxyanisole (IC_{50} = 44.7 μM) for DPPH radical scavenging activity.

1. Introduction

Hydrazones are well known for their interesting bioactivities such as anti-bacterial, anti-fungal (Jaina et. al., 2011; Kumar et al., 2010; Kasabe et al., 2010), anti-convulsant (Verma et al., 2004), anti-inflammatory (Iana et al., 2004), anti-malarial (Harpstrite et al., 2008), analgesic (Pandeya and Rajput, 2012), anti-platelets (Jagadish et al., 2013), anti-tuberculosis (Bhat and Al-Omar, 2013), and anti-cancer activities (Popp and Kirsch, 1961). They also act as herbicides, insecticides, nematicides, rodenticides, and plant growth regulators and are used as plasticizers, stabilizers and antioxidant initiators for polymerization (Akelah et al., 1993; Tarafder et al., 2002; Vicini et al., 2003). Other derivatives are used in analytical chemistry as selective metal extracting agents as well as in the spectroscopy for the determination of certain transition metals (Tantaru et al., 2002).

Metal complexes of hydrazones showed some degree of antibacterial, (Anant and Devjani, 2011), antifungal, (Dhande et al., 2007) and antitumor, (Ainscough et al., 1998) activities. A literature survey reveals that Schiff bases containing N,S and N,O donors efficiently coordinated with transition metal ions. These complexes have been reported as anti-carcinogenic and antiviral agents (Maurya et al., 2010; Girija et al., 2013).

Various isatin derivatives and their metal complexes are reported for their remarkable biological activities (Khan et al., 2012, 2013). Significant antitumor, antifungal, herbicidal, antibacterial and anti-convulsant activities were reported by several researchers (Mithun and De, 2013). Isatin-thiosemicarbazone copper (II) complexes found to have antiviral effect (Padhye and Kauffman, 1985).

Various endogenous (*e.g.* respiratory chain, oxidative enzymes) and exogenous (*e.g.* air pollution, smoking) processes responsible for the production of free radicals in human bodies.

Reactive oxygen species (ROS) (Tarpey et al., 2004; Ravanat et al., 2004) such as singlet oxygen (Bolann and Ulvik, 1987) superoxide (Chamulitrat and Mason, 1989), peroxyl (Yildız and Demiryürek, 1998), hydroxyl (Radi et al., 1993), and peroxynitrite (Henning et al., 2004) radicals are among the most destructive species and produced oxidative stress. Cell matrices, including lipids, proteins and nucleic acids damaged by this oxidative stress and leads to various diseases, *e.g.* diabetes, cancers, cardiovascular and Alzheimer's diseases (Barnham et al., 2004; Rahimi et al., 2005). The endogenous defence systems, such as anti-oxidative enzymes and metal binding proteins (Young and Woodside, 2001) are responsible to balance the super expression of these free radical species.

Oxidative stress and chronic hyperglycaemia generate advanced glycation end-products (AGEs). Glycation process starts with the formation of Amadori products *via* chemical reaction of the amino residue of proteins and sugar molecules. The transformation of these products, even more reactive dicarbonyl compounds by glycoxidation leads to the formation of advanced glycation end products (AGEs), (Rojas and Morales, 2004). Carboxyl-methyllysine (CML) and carboxymethyl-hydroxylysine (CMhL) are AGEs formed by oxidative cleavage of Amadori adducts, whereas pentosidine is formed between crosslinking of lysine and arginine. High level of glycation process observed under ageing and hyperglycaemic patient than healthy persons (Baral et al., 2000). AGEs formation is further enhanced with oxidative stress (Fu et al., 1994; Wu and Yen, 2005; Selvaraj et al., 2002). Diabetic complication such as neuropathy, retinopathy, cataract and atherosclerosis has direct relevance with AGEs (Ahmed, 2005). 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 (Brownlee, 1994), While the horrible incident of type-2 diabetes is increasing, a lot of efforts have been focused on the discovery of new glycation inhibitors, because of their healing potential (Peppa et al., 2003) Few molecules have been synthesized that can cleave AGEPs cross-links and possibly open the opportunity of reversing the steady process of diabetic complications (Monnier, 2003; Vasan et al., 2003).

A significant rising interest in the design of metal compounds as drugs and diagnostic agents is currently observed in the area of scientific inquiry appropriately termed medicinal inorganic chemistry (Walcourt et al., 2004).

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 (Zhong et al., 2007). Therefore, this study opened the doors to develop such organometallics that could be used to treat diabetic and its complications.

In the present work, Schiff base of isatin with 2-hydroxy salicylhydrazide (see Supplementary Information) and its Mn

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(II), Co (II), Ni (II), Cu (II), and Zn (II) complexes were synthesized. The metal complexes were characterized by elemental analysis, IR, UV–Vis, and thermal methods. These metal complexes showed remarkable DPPH radical scavenging and antiglycation activities which may find their importance in the applied medicinal chemistry.

2. Experimental

2.1. Physical parameters

Carbon, hydrogen and nitrogen were estimated by using Perkin Elmer 2400 Series II CHNS/O Elemental Analyzer. The IR spectra and Uv–Vis spectra of isatin hydrazone and its metal complexes were recorded on a HITACHI-270 IR spectrophotometer in the 4000–250 cm^{-1} region in KBr disc and Perkin Elmer Lambda 35 UV–Vis spectrophotometer in the region of 200–800 nm respectively. The ^1H NMR spectrum of the ligand was recorded on a BRUKER 300 MHz spectrometer at room temperature in DMSO-d_6 using TMS as an internal reference. Electron impact mass spectra (EIMS) were recorded on a Finnigan MAT-311A Germany, metal content analyses were made on Perkin Elmer Analyst 800 atomic absorption spectrophotometer. The magnetic moments were carried out at 25 °C on the solid states by Faraday method using Bruker BM6 instrument. Germany. Pre-coated silica gel aluminium plates (Kieselgel 60, 254, E. Merck, Germany) were used for thin layer chromatography (TLC) and 254 and 365 nm UV wavelengths used for visualization of chromatograms. Molar conductance was measured by Thermo scientific Orian 5 Star conductivity meter. Thermograms were evaluated by Perkin Elmer Diamond TG/DTA instrument.

2.2. Materials and methods

Reagent grade chemicals were used throughout the studies. Bovine serum albumin (BSA) was purchased from the Research Organics (Cleveland, USA), while other chemicals isatin, salicylic acid, manganese, cobalt, nickel, copper, and zinc acetates, glucose, trichloroacetic acid (TCA), sodium azide (NaN_3), dimethyl sulfoxide (DMSO), sodium chloride (NaCl), sodium dihydrogen phosphate (NaH_2PO_4), potassium dihydrogen phosphate (KH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), potassium chloride (KCl), and sodium hydroxide (NaOH) were purchased from Sigma–Aldrich, USA. Sodium phosphate buffer (pH 7.4), was prepared by mixing Na_2HPO_4 and NaH_2PO_4 (67 mM) containing sodium azide (3 mM). Phosphate buffer saline (PBS) was prepared by mixing NaCl (137 mM), Na_2HPO_4 (8.1 mM), KCl (2.68 mM), and KH_2PO_4 (1.47 mM) and pH 10 was adjusted with NaOH (0.25 mM). BSA (10 mg/mL) and glucose anhydrous (50 mg/mL) solutions were prepared in sodium phosphate buffer. Standard test solutions were prepared in DMSO (1 mM/mL).

2.3. Synthesis of 2-hydroxy methyl salicylhydrazide and isatin hydrazone

2-Hydroxy methyl salicylate and isatin hydrazone were synthesized according to previously reported methods and their spectroscopic results are in close agreement with reported compounds (László, 2001; Buu et al., 1953; Ali et al. 1989).

2.4. Synthesis of Mn (II), Co (II), Ni (II), Cu (II), and Zn (II) metal complexes

Methanolic solution of isatin hydrazone (1 mmol) and ammonium acetate was mixed with different metal acetates (0.5 mmol), in 2:1 ligand:metal ratio and was refluxed for 6 h. The solvent was evaporated and coloured precipitate was obtained (Table 1), washed with water to eliminate any unreacted metal salt, dried and characterized by UV–Vis, IR, CHN and TG/DTA analysis.

2.5. Antiglycation activity assay

This test was used to evaluate the ability of the candidate compounds to inhibit the methyl glyoxal mediated development of fluorescence of BSA (see Supplementary Information).

2.6. DPPH radical scavenging assay

The free radical scavenging effects of all the metal complexes as well as legend with the DPPH radical were evaluated by

Table 1 Physical parameters of Isatin Hydrazone Metal Complexes.

S.no.	Ligand/metal complexes	Empirical formula	M.W.	Colour	M.P (°C)	% yield	M:L ratio	Molar conductance (μs/cm)	Elemental analysis % Found (cal.)			
									C	H	N	M
1	Ligand	$C_{15}H_{11}N_3O_3$	281	Yellow	335.	85	–	2.76	64.05 (64.03)	3.94 (3.83)	14.94 (14.9)	–
2	$Mn(L)_2$	$C_{30}H_{20}N_6O_6Mn$	615	Yellow	342	81	1:2	4.16	58.55 (58.45)	3.28 (3.18)	13.65 (13.3)	8.93 (8.81)
3	$Co(L)_2$	$C_{30}H_{20}N_6O_6Co$	618	Brown	352 decomp	93	1:2	8.89	58.17 (58.16)	3.25 (3.15)	13.57 (13.5)	9.5 (9.4)
4	$Ni(L)_2$	$C_{30}H_{20}N_6O_6Ni$	619	Mustard	393	88	1:2	1.99	58.19 (58.18)	3.26 (3.16)	13.57 (13.3)	9.48 (9.38)
5	$Cu(L)_2$	$C_{30}H_{20}N_6O_6Cu$	624	Chocolate brown	338	85	1:2	2.42	57.54 (57.22)	3.23 (3.11)	10.18 (9.9)	13.47 (13.24)
6	Zn $(L)_2$	$C_{30}H_{20}N_6O_6Zn$	625	Selective yellow	367decomp	83	1:2	2.98	57.57 (57.55)	3.22 (3.11)	13.43 (13.2)	10.45 (10.35)

M.W = Molecular weight, M.P. = Melting Point, M:L ratio = Metal:Ligand ratio.

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the approach of Blois (Navnath et al., 2010) under the same condition (see Supplementary Information).

3. Result and discussion

3.1. Chemistry

The literature reveals that these metal complexes were not so much studied so it is needed to synthesize and discussed its various parameters as well as their biological potential. The metal complexes of isatin hydrazone with Mn-II, Co-II, Ni-II, Cu-II, and Zn-II were synthesized by mixing metal acetates with ligand in 1:2 M ratio in methanol in the presence of ammonium acetate. During refluxing colour of the solution was changed and it was taken as indication for complexation. After 6 h solvent was evaporated and coloured precipitates were obtained which were washed with water as well as methanol in order to remove any unreacted material. These precipitates were dried and subjected towards UV–Vis, IR spectroscopy, CHN/S and TG/DTA analysis for structural confirmation.

3.2. Colour, solubility and melting point

All the synthesized metal complexes were found to be coloured, nonhygroscopic in nature, stable in air. The metal complexes were found to be soluble in DMSO, while they were insoluble in other common organic solvents such as DCM, Hexane, and partially soluble in ethanol and methanol. The melting points of the complexes were found to be in the range of 335–367 °C Table 1.

3.3. Molecular formula of metal complexes

Elemental analyses (CHN/S) give satisfactory results for all the compounds. CHN/S values are in close agreement with expected molecular formulae assigned to these complexes Table 1, suggesting 1:2 metal–ligand stoichiometric ratios. This 1:2 metal–ligand relationship, indicating the tridentate nature of the ligand. The IR and TG/DTA data confirm that there is no role of water molecule in coordination. The possible reaction between metal salt and ligand is given below (see Fig. 1).

3.4. Molar conductance

The molar conductance values of 1×10^{-3} M solution of metal complexes in DMSO are in the range of 5.90–11.65 Ω^{-1} cm^{-2} - mol^{-1} for all the metal complexes. The low conductance values indicated the non-electrolytic nature of the complexes Table 1.

All the data have been summarized in Table 1.

3.5. Electronic spectra

The electronic spectral data of the complexes are presented in Table 2. The electronic spectrum of free Schiff base showed a band around 341 nm characteristic of π–π^* transitions. In the metal complexes, this band is shifted to a longer wave length with increasing intensity. This shift may be attributed to the donation of the lone pair of electrons of nitrogen of Schiff base to metal ion, i.e. ligand to metal charge transfer (LMCT) bands. In the view of previous literature that bathochromic shift is a sign of complexation, so, the bathochromic shifting of peak from 341 nm to 413, 418, 410, 420, 405, for Mn, Co, Ni, Cu and Zn metal complexes respectively was taken as evidence for the complex formation Table 2.

$$2LH + M(OAc)_2.nH_2O \xrightarrow[\text{Reflux 6h}]{NH_4CH_3COO} [M(L)_2] + 2CH_3COOH + nH_2O$$

Fig. 1 Reaction scheme for the synthesis of metal complexes.

3.6. Magnetic moment

Magnetic studies have been used for the confirmation of the geometry of complexes. The magnetic moment values 5.85 B.M for the MnL_2 were found to be in close agreement with the octahedral structure with five unpaired electrons. The magnetic moment of CoL_2 complex was 4.13 B.M indicating an octahedral environment around the metal ion. NiL_2 complexes showed magnetic moment values 3.09 B.M higher than that of normal value 2.87 B.M. These higher values were due to orbital contribution. Cu (II) complex showed *μeff* 1.77 B.M suggesting an octahedral structure with one unpaired electron. ZnL_2 complex was found to be diamagnetic as expected (Fuxin et al., 2004) Table 2.

3.7. IR spectroscopy

In IR spectra of ligand, peaks were observed at 1731 cm^{-1} —C═O and 1667 cm^{-1} for —C═N starching respectively. A sharp band at 1516 cm^{-1} might be due to imin-ol tautomerism found in the target compound. The absorption patterns of complexes look quite similar to that of the free ligand which is in agreement that coordination occurs through tautomeric forms with some alteration in peak values. In metal complexes spectra —C═O peak was not observed due to complete enolization comes chelating effect or shifted towards lower wavelength. The IR peaks for Mn, Co, Ni, Cu and Zn complexes appeared at 1710, 1683, 1675, 1685, 1683 and 1705 cm^{-1} respectively, this is a sign of an indication that —C═O group involved in coordination while peak for C═N at 1667 cm^{-1} underwent 68–44 cm^{-1} suggesting azomethine group also playing part in coordination. N—N stretching peaks at 1009 cm^{-1} incremented 10–15 cm^{-1} due to C═N—N═C— formation in case of metal complexes. Peaks at 1606 cm^{-1} Mn, 1588 (Co), 1593 (Ni), 1588 (Cu) and 1606 (Zn) for C—O also supporting the coordination effect of C═O after enolization. Peaks in the range of 512–526 and 420–480 cm^{-1} are due to M—O and M—N respectively Table 3.

3.8. Thermogravimetric analysis

Thermal behaviour and decomposition pattern of the metal complexes was established by recording thermo gravimetric analysis (TGA), differential of thermogram (DTG) and differential thermal analysis (DTA) curves. The thermo gravimetric (TG) curves of representatives Schiff base and its respective metal complexes (see Supplementary Information). No weight loss was observed upon heating till 300 °C, and thus, ruling out the presence of water molecules. Isatin hydrazone undergoes decomposition in single stage between the temperature range

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Table 2 UV–Vis and magnetic moment data of isatin hydrazone metal complexes.

Compound	Ligand	$Mn(L)_2$	$Co(L)_2$	$Ni(L)_2$	$Cu(L)_2$	$Zn(L)_2$
Wavelength λ (nm)	341	345, 413	325,418	391, 410	361, 420	343, 405
μ_{eff} B.M	–	5.85	4.13	3.09	1.77	Diamagnetic

Table 3 IR spectroscopic data of isatin hydrazone metal complexes.

S. no.	Compound	NH/OH (cm^{-1})	ν(C=O) (cm^{-1})	ν(C=N) (cm^{-1})	ν(C–O) (cm^{-1})	ν(N–M) (cm^{-1})	ν(M–O) (cm^{-1})
1	Ligand	3162	1731	1667	1516	–	–
2	$Mn(L)_2$	3254	1710	1660	1606	420	520
3	$Co(L)_2$	3257	1683	1615	1588	452	512
4	$Ni(L)_2$	3395	1675	1632	1593	461	518
5	$Cu(L)_2$	3320	1685	1608	1588	480	526
6	$Zn(L)_2$	3245	1705	1539	1606	442	513

320–350 °C and 55.38% weight loss was observed with DTG peak at 341 °C, while an endothermic peak at 333.01 °C, and an exothermic peak at 344.71 °C were observed in DTA thermogram.

MnL_2 complex decomposed into two stages (Stage I: 250–280 °C with 6% and Stage II: 300–350 °C with 19% weight loss). DTG peaks were observed at 271 °C and 339 °C. An endothermic at 257 °C and exothermic at 343 °C were observed in DTA. TGA Curve of Co-complex indicates that weight loss occurred in 3-stages, (Stage I: 325–335 °C that is 9.7%, Stage II: 390–400 °C 29.3%, and Stage III, 410–510 °C and loss is 40.4%), the residue about 10% remained which may be the metal oxide. The overall 90% weight loss may be due to the decomposition of both the ligand molecules. DTG peaks were observed at 335, 390, and 410 °C for decomposition in 3 steps. An endothermic peak was observed at 333 °C which may be due to melting of one molecule of hydrazone, an exothermic peak was also observed at 435 °C due to some chemical or physical phenomenon. TGA study of Ni-complex indicates that weight loss in two stages (Stage I: 360–390 °C, weight loss 30.00% and Stage II: 392–430 °C, weight loss 13.00%), and maximum loss is observed *i-e* DTG at 395 °C .While its DTA thermogram showed one endothermic peak at 390 °C. TG Curve of Copper Complex indicated that weight loss occurred in 2 stages, (Stage I: 318–350 °C, 35.91%, Stage II: 352–500 °C that is 14.26% weight loss) with DTG peaks at 318 °C and 327 °C. In DTA thermogram two exothermic peaks 319 and 331 °C were observed. In ZnL_2 complex 41% weight loss was observed in two stages, (Stage I: 300–345 °C that is 10.5%, Stage II: 360–400 °C 29.37%) with DTG peaks at 333 and 391 °C. DTA thermogram showed two endothermic peaks at 331 and 390.58 °C. The above study revealed that all the complexes are thermally stable up to 300 °C. Mostly complexes were decomposed into 2 stages although Co II complex showed decomposition in 3 steps, while ligand degradation occurred in a single step. Only Mn II complex showed minor loss of 6% in the range of 250–280 °C. The thermal stability profile of metal complex as Co > Ni > Cu,Zn > Mn, While according to total weight loss thermal stability found to be as Mn > Zn > Ni > -Cu > Co (see Table 4).

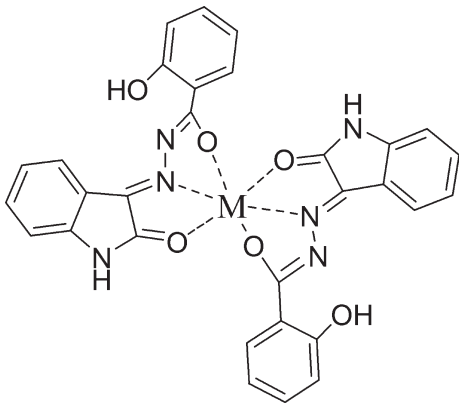
3.9. Structural interpretation

On the basis of the above spectroscopic, elemental analysis, molar conductance, magnetic moment and thermal analysis results the proposed structures for the metal (II) complexes are shown (Fig. 2). It is concluded that the ligand and metal

Table 4 Thermogravimetric data of isatin hydrazone metal complexes.

S. no.	Compound	*n*	Temperature (°C)	Total weight loss (%)	DTG/T_{max} (°C)	DTA (Endo) (°C)	DTA (Exo) (°C)
1	Ligand	1	320–350	55.38	341	333	344
2	$Mn(L)_2$	2	Stage-I: 250–280	25	271	257	343
			Stage-II: 300–350		339	334	
3	$Co(L)_2$	3	Stage-I: 325 335	90	335	333	435
			Stage-II: 390–400		390		
			Stage-III: 410–510		410		
4	$Ni(L)_2$	2	Stage-I: 360–390	43	395	390	–
			Stage-II: 392–430				
5	$Cu(L)_2$	2	Stage-I: 318–350	50	318	–	319
			Stage-II:352–500		327		331
6	$Zn(L)_2$	2	Stage I: 300–345	41	333	331	–
			Stage II: 360–400		391	390	

n = no of stages of weight loss.



M= Mn, Co, Ni, Cu, Zn

Fig. 2 Possible structure of metal complexes.

are in 2:1 ratio and metal coordinated *via* N and O atoms of the ligand in such a manner that they form octahedral complexes and these complexes are non-electrolyte in nature.

4. Biological screening

4.1. Antiglycation activity

Isatin hydrazone and its metal complexes were screened for their anti-glycation potential Table 5. Ligand (IC_{50} = 413.18 ± 6.21 μM) itself found to be weaker active, although; all the metal complexes CoL_2 (IC_{50} = 168.23 ± 2.37 μM), ZnL_2 (IC_{50} = 234.27 ± 4.33 μM), MnL_2 (IC_{50} = 257.1 ± 6.43 μM), CuL_2 (IC_{50} = 267.7 ± 8.43 μM), NiL_2 (IC_{50} = 269.0 ± 8.56 μM) were showed significant antiglycation activity and found to be better active than the standards Rutin (IC_{50} = 294.46 μM), used in antiglycation activity. Among these complexes CoL_2 (IC_{50} = 168.23 ± 2.37 μM), complex showed higher activity and it is far better active than the standard routine. However, ZnL_2, MnL_2, CuL_2, and NiL_2 comparatively less active than CoL_2 complexes, although they were found to have better activities than the standard rutin. By portraying the activity pattern of theses complexes, *i.e.* $CoL_2 > ZnL_2 > MnL_2 > CuL_2 > NiL_2$, it is concluded that these complexes may have competent capability to bind protein or glucose and inhibit the further progression of glycation. Among these CoL_2 has protein or glucose binding ability in greater extent. It is recently reported that Co Salen complex can efficiently bind with the BSA by axial coordination (Li et al., 2013). The HOSalenCo was inserted into the hydrophobic cavities of the BSA and formed the BSA/HOSalenCo conjugation. So, in the same manner our synthesized ligands may bind with the BSA and slot into hydrophobic cavities which might be responsible for suppressing further glycation phenomenon, while other complexes also capable, but could not bind as much efficiently as CoL_2, So they also bind with the BSA protein but in lesser extent than CoL_2.

Table 5 Antiglycation activity of ligand and respective complexes.

S. no.	Compound	IC_{50} ± SEM[a] (μM)
1	Ligand	413.18 ± 6.21
2	MnL_2	257.1 ± 6.43
3	CoL_2	168.23 ± 2.37
4	NiL_2	269.0 ± 8.56
5	CuL_2	267.7 ± 8.43
6	ZnL_2	234.27 ± 4.33
7	Rutin	294.46 ± 1.50

[b]NA, Not active.
[c]Rutin, standard inhibitor for anti-glycation activity.
[a] SEM is the standard error of the mean.

Table 6 Antioxidant activity of ligand and respective complexes.

S. no.	Compound	IC_{50} ± SEM (μM)
1	Ligand	54.14 ± 2.43
2	MnL_2	35.16 ± 2.45
3	CoL_2	43.53 ± 3.12
4	NiL_2	57.71 ± 2.61
5	CuL_2	29.63 ± 2.76
6	ZnL_2	31.13 ± 1.41
7	*tert*-butyl-4-hydroxyanisole	44.7 ± 1.21

[a]SEM is the standard error of the mean.
[b]NA, Not active.
[c]Rutin, standard inhibitor for anti-glycation activity.

4.2. Antioxidant assay

Isatin hydrazone and its metal complexes were evaluated for DPPH radical scavenging activity Table 6. All the complexes showed remarkable antioxidant potential with IC_{50} values 29.63 ± 2.76, 31.13 ± 1.41, 35.16 ± 2.45, 43.53 ± 3.12, 57.71 ± 2.61 μM Cu, Zn, Mn, Co and Ni respectively, and almost all the complexes found to have better activities than standard *tert*-butyl-4-hydroxyanisole (IC_{50} = 44.7 μM). The ligand isatin hydrazone (IC_{50} = 54.14 ± 2.43 μM) was found to be lesser active than standard as well as its respective complexes except Ni complex. Copper (IC_{50} = 29.63 ± 2.76), and zinc (IC_{50} = 31.13 ± 1.41) complexes showed remarkable antioxidant activity while manganese complex (IC_{50} = 35.16 ± 2.45 μM) was found to be a good antioxidant too. The Cobalt complex also showed significant activity (IC_{50} = 43.53 ± 3.12 - μM). Nickel complex was found to be active in the DPPH radical scavenging activity, but surprisingly it was found to be lesser active than the standard and it looks like complexes as well as ligand. So, the order of activity of these complexes is as Cu > Zn > Mn > Co > Ni. This significant activity of the complexes might be due to the stabilization of free radical by the metal ion with phenolic moiety.

5. Conclusion

Isatin hydrazone and its Mn (II), Co (II), Ni (II), Cu (II), and Zn (II) complexes were synthesized and characterized by various spectroscopic techniques and investigated for DPPH radical scavenging and antiglycation activity. The results showed that these complexes have DPPH radical scavenging and glycation inhibition potential. These complexes may be effective to cure diabetic complications. However, further studies on the mechanisms of antioxidation and antiglycation are required.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.arabjc.2015.02.015.

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