

Synthesis, Characterization and Antidiabetic Activity of Chromium (III) Metformin Complex

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Abstract

The chromium (III) metformin hydrochloride complex as a diabetic drug model was synthesised by the chemical reaction between chromium (III) chloride hexahydrate and metformin.HCl (Mfn.HCl) in methanol solvent. The $[Cr(Mfn-HCl)_2(Cl)_2].Cl.6H_2O$ complex was characterized using microanalytical measurements, molar conductance, spectroscopic (infrared, and UV-vis.), effective magnetic moment, and thermal analyses. The infrared spectroscopic data in the comparison between free Mfn.HCl ligand and its chromium (III) complex proved that metformin hydrochloride react with chromium(III) ions as a bidentate ligand through its two imino groups. The anti-diabetic activities of the Mfn.HCl drug, chromium salt and Cr (III)-2Mfn.HCl complex were discussed on the male rats. The chromium (III) metformin HCl complex was recorded successful efficiency in the decreasing blood glucose level and HbA1C against diabetic rats. The Cr(III)-2Mfn.HCl complex has succeeded to great extent as antidiabetic drug with enhanced the antioxidant defence system as well as act as pronounced efficient hypoglycaemic agent compared to metformin HCl free drug.

Keywords: Metformin hydrochloride; Chromium(III) complex; Anti-diabetic activity; Spectroscopic; Thermal

Introduction

Metformin hydrochloride (Mfn.HCl) structure was referred in Figure 1. Diabetes is a metabolic syndrome which was characterized by hyperglycemia and glycosuria resulting from the defect in the secretion or the action of insulin, or both of them [1,2]. Some metal complexes or organo-metallic compounds have been used in medicine for centuries. Supplement contains trivalent chromium was needed for a person with type 2 diabetes mellitus, according to its important role in glucose metabolism [3]. The Cr (III) metal ion interacts with the insulin and its receptors on the first step in the metabolism of glucose entry into the cell, and facilitates the interaction of insulin with its receptor on the cell surface [4,5]. Chromium increases insulin binding to cells, insulin receptor number as well as activates insulin receptor kinase leading to increase sensitivity of insulin receptor. Additional studies were urgently needed to elucidate the mechanism of the action of chromium and its role in the prevention and control of diabetes [6]. Metformin, the most common prescribed oral medication in type 2 diabetes, lowers HbA1c around 1.5%, rarely causes hypoglycemia (compared with insulin or sulfonylureas), has relatively few contraindications, its adverse effects are generally tolerable, did not cause weight gain, was cheap, and was highly acceptable among patients [7]. Metformin exerts its main antihyperglycemic effects through activation of AMP-activated protein kinase, resulting in reduced hepatic gluconeogenesis [8]. In addition, moderate improvements in lipid profile and weight reduction have been reported with metformin use [8]. Herein, this paper reports the synthesis, characterization and chromium (III) metformin complex as a prospective antidiabetic candidate

Experimental

Materials

All chemicals, solvents, chromium(III) chloride hexahydrate were commercially available from BDH and were used without further purification. The pure grade metformin hydrochloride drug was received as a gift sample from Egyptian International Pharmaceutical Industrial Company EIPICo.

Synthesis of Cr(III)-2Mfn.HCl

The of metformin hydrochloride drug ligand (2 mmol, 0.332 g) was dissolved in 25 mL methanol then mixed with 25 mL of methanolic solution of (1 mmol, 0.267 g) $CrCl_3.6H_2O$. A mixture of molar ratio of 1:2 was heated at $\sim 80^\circ C$ under reflux for about 3 hours. The mixture was left overnight at room temperature until precipitated. The precipitate obtained was filtered off and washed by diethyl ether then left over anhydrous calcium chloride. The yield of the solid leaf green colour powder product was about $\sim 85\%$. The formula weight of chromium(III) complex is $C_8H_{36}Cl_5CrN_{10}O_6$, molecular weight is 597.74 g mol^{-1} , and the microanalytical data of theoretical and experimental are as follows: theoretical=%C, 16.06; %H, 6.02; %Cr, 8.70; %N, 23.42 and experimental=%C, 15.43; %H, 5.89; %Cr, 8.65; %N, 23.19.

Instruments

IR spectra of the Mfn.HCl and its chromium(III) complex were

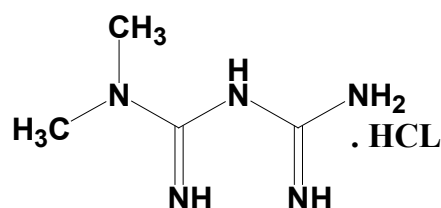


Figure 1: Structure of metformin hydrochloride ligand.

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recorded on Bruker infrared spectrophotometer in the range of 400-4000 cm^{-1} , at Taif University. The electronic spectrum of the Cr(III) complex was measured in DMSO solvent with concentration of 1×10^{-3} M, in rang 200-1100 nm by using Unicam UV/Vis spectrometer. SEM images were obtained using a Jeol Jem-1200 EX II Electron microscope at an acceleration voltage of 25 kV. X-ray diffraction (XRD) patterns of the samples were recorded on X Pert Philips X-ray diffractometer. All the diffraction patterns were obtained by using $\text{CuK}_{\alpha 1}$ radiation, with a graphite monochromator at 0.02°/min scanning rate. Carbon, hydrogen and nitrogen analysis have been carried out in Vario EL Fab. CHNS. The amount of water and the metal content percentage were determined by gravimetric analysis method. The molar conductance of 10^{-3} M solutions of the metformin hydrochloride ligand and its chromium(III) complex in DMF solvent were measured on a HACH conductivity meter model. All the measurements were taken at room temperature for freshly prepared solutions. Differential Thermal Analysis (DTA) and Thermo Gravimetric Analysis (TGA) experiments were conducted using Shimadzu DTA-50 and Shimadzu TGA-50H thermal analyzers, respectively. All experiments were performed using a single loasetop loading platinum sample pan under nitrogen atmosphere at a flow rate of 30 ml/min and a 10°C/min heating rate for the temperature range 25-800°C. The mass susceptibility (X_g) of the solid chromium (III) complex was measured at room temperature using Gouy's method by a magnetic susceptibility balance from Johnson Metthey and Sherwood model. The effective magnetic moment (μ_{eff}) value was obtained using the following equations (1- 3) [9].

$$X_g = \frac{C_{\text{Bal}} L (R - R_0)}{10^9 M} \quad (1)$$

Where:

R_0 = Reading of empty tube

L = Sample length (cm)

M = Sample mass (gm)

R = Reading for tube with sample

C_{Bal} = balance calibration constant = 2.086

$$X_M = X_g \times M \times Wt. \quad (2)$$

The values of X_M as calculated from equation (2) are corrected for the diamagnetism of the ligand using Pascal's constants, and then applied in Curie's equation (3).

$$\mu_{\text{eff}} = 2.84 \sqrt{X_M \times T} \quad (3)$$

Where T = t (°C) + 273

Biological experiments

Forty male Wistar rats (Weighing 200-250 g), were used in all experiments of this study. They were obtained from the Animal House of the National Research Center (Dokki, Giza, Egypt). The animals were maintained in solid-bottom shoe box type polycarbonate cages with stainless steel wire-bar lids, using a wooden dust free litter as a bedding material. We have followed the European community Directive (86/609/EEC) and national rules on animal care. Hyperglycemic rats were weighed and randomly allocated into 4 groups (10 rats each). One group served as hyperglycemic control. Animals were divided into four groups with 10 animals in each group as following:

Group (1):

Saline (control) was normal and injected intraperitoneally (I.P) with 0.1 mL for 30 successive days.

Group (2):

Control diabetic group was injected (i.P) by a single dose of STZ (50 mg/Kg body weight) [10] for induction of diabetes.

Group (3):

Diabetic (STZ) + Metformin: diabetic group treated with Metformin (I.P) by a dose (150 mg/kg) [11] for successive 30 days.

Group (4):

Diabetic (STZ) + Metformin/ Cr^{+3} : diabetic group treated with Metformin/ Cr^{+3} (I.P) by a dose (150 mg/kg) daily for successive 30 days.

Group (5):

Diabetic (STZ) + Chromium salt ($\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$): diabetic rats treated with chromium salt (I.P) by a dose (150 mg/Kg) daily for successive 30 days.

Induction of hyperglycaemia: Hyperglycemia was induced by a single i.p. injection of STZ (50 mg/kg) [12]. Briefly, rats were weighed and injected with STZ dissolved in a citrate buffer (0.1 M, pH 4.5). After 72 h blood samples were withdrawn from the retro-orbital venous plexus under light ether anesthesia and the plasma was separated by centrifugation for the determination of glucose level. The treatment was carried out for 30 days after 72 h from STZ injection. Only rats with plasma glucose levels more than 230 mg/dl were selected and considered as hyperglycemic animals that have been subjected to further experimentation. At 7 days post-induction of hyperglycemia, blood glucose was assayed by the glucose oxidase method, using a glucometer. The animals were carefully monitored every day and weighed every week during the experiment.

Collection of blood and organs: Blood samples of the fasted rats were collected from the medial retro-orbital venous plexus immediately with capillary tubes (Micro Haematocrit Capillaries, Mucaps) [13]. About 9 mL of blood collected in two tubes from each animal, one with EDTA for obtaining plasma, the second was allowed to clot for 30 min. Then, the blood in two tubes was centrifuged at 3,000 rpm for 15 min to separate serum and plasma for different biochemical analyses.

Lipid profile: Triglycerides, cholesterol and high density lipoprotein-cholesterol (HDL-c) were determined using the commercial kits. Low density lipoprotein-cholesterol (LDL-c) levels were calculated by using the following formula of Muruganandan et al., [14] $\text{LDL-c} = \text{total cholesterol} - (\text{HDL-c} + \text{triglycerides})/5$. Volatile low density lipoprotein-cholesterol (VLDL-c) levels were calculated by using the following formula of Prakasam et al., [15]: $\text{VLDL-c} = \text{triglyceride}/5$. The risk ratio was calculation by dividing the total cholesterol by HDL-c.

Antioxidant abilities

Assessment of lipid peroxidation as oxidative indicator:

Bioindicator in tissues the thiobarbituric acid reactive substances (TBARS) levels as an index of malondialdehyde (MDA) production were measured by the method described by Ohkawa et al., [16]. MDA, an end product of lipid peroxidation reacts with TBA-TCA complex to form a colour complex at high temperature exhibiting an absorption maximum at 535 nm.

Determination of enzymatic and non-enzymatic antioxidants:

The principle of SOD activity ability was based on the inhibition of nitro blue tetrazolium (NBT) reduction. Illumination of riboflavin in the presence of O_2 and electron donor like methionine generates

superoxide anions and this has been used as the basis of ability of SOD. The reduction of NBT by superoxide radicals to blue colour formazan was followed at 480 nm [17]. Reduced glutathione level (GSH) as non-enzymatic antioxidant was estimated based on the method of Beutler et al., [18].

Determination of blood glucose level, Hb, HbA1c: Glucose was estimated by O-toluidine method of Sasaki et al., [19]. Hb was estimated by cyanmethaemoglobin method of Drabkin and Austin [20]. HbA1c was estimated by the method of Sudhakar and Pattabiraman [21] with modification by Bannon [22].

Insulin level and C-peptide: Insulin in pancreatic homogenates was determined by Immulite Insulin (Diagnostic Products Corporation, Los Angeles) which depends on a two-site chemiluminescent enzyme-labelled immunometric ability [23]. Serum C-peptide was measured by radioimmunoassay (Medgenix Diagnostics) as described by Kumar et al., [24]. All chemicals and reagents were of pure analytical grade.

Pancreatic homogenates preparation: At time of death, pancreas tissues were dissected, cleared of lymph nodes and fat, blotted, washed from blood and weighed. The pancreas was immediately homogenized in 5 ml cold 2 M acetic acid for 5 s. The extract was centrifuged at 15 000 r.p.m. for 10 min, and the resulting supernatant was frozen at -80°C until further analysis of insulin.

Electron microscopy: The third portion of the pancreas was immediately cut into small cubes and transferred to ice-cold fixation buffer (1.25% v/v glutaraldehyde in 0.1 mM cacodylate-HCl buffer, 0.1 M sucrose, and 2 mM calcium chloride pH 7.2) and prepared for transmission electron microscopy [25].

Statistical analysis: Data were collected, arranged and reported as mean \pm standard error of mean (S.E.M) of four groups (Each group was considered as one experimental unit), summarized and then analyzed using the computer program SPSS/version 15.0) The statistical method was one way analyzes of variance ANOVA test (F-test), and if significant differences between means were found, Duncan's multiple range test (Whose significant level was defined as $P < 0.05$) was used according to Snedecor and Cochran, [26] to estimate the effect of different treated groups.

Results and Discussion

Chemical composition

The elemental analysis shows that Cr (III) formed complex with Mfn.HCl in 1:2 (Cr(III): Mfn.HCl) molar ratio. The synthesized Cr(III) complex is leaf green and soluble in dimethylsulfoxide and dimethylformamide, partially soluble in hot methanol and insoluble in water and some other organic solvents. The conductivity of chromium(III) metformin HCl complex is measured in DMF solvent at room temperature, that show the molar conductance value of 10^{-3} mol/cm³ concentration is 66 ohm⁻¹cm²mol⁻¹. This data reflect that Cr(III)/Mfn-HCl complex is slightly electrolytic nature [27]. The slightly electrolytic value may be due to the contribution of the one chloride anion in the outer sphere of chelating skeleton of the Cr(III) metformin complex.

The infrared absorption bands are one of the important tools of analyses used for determining the mode of chelations. The most significant bands of metformin HCl ligand can be classified into two groups: i) NH vibrational spectra of primary (-NH₂), secondary (-NH) and imino (-C=NH) groups and ii) C-N and C=N vibration bands of different amino groups (-NH₂, -NH and -C=NH). According to the

two fundamental vibrational groups mentioned above, the metformin HCl free ligand can be interpreted as follows: N-H Vibrations: i- N-H vibrations; the N-H stretching of C=N-H group occurs in the region 3400-3100 cm⁻¹. Usually the frequency of this vibration decreases in the presence of the hydrogen bond [28]. The broad bands at 3370 and (3294 and 3174) cm⁻¹ have been assigned to N-H asymmetric and symmetric stretching vibrations, respectively [28]. The band at 1570 cm⁻¹ has been assigned for NH₂ in the plane deformation vibrations [28]. The bands of the medium-to-weak intensities at 935, 798 and 735 cm⁻¹ are due to N-H wagging. ii) The strong absorption band at 1627 cm⁻¹ is due to C=N stretching vibration [29]. The medium-to-weak intensity bands in the IR spectra at 1271, 1168, and 1061 cm⁻¹ have been assigned to C-N stretching vibrations of aliphatic amine compounds. Medium-to-weak intensity bands at 639, 582, 541, and 419 cm⁻¹ are due to CNC deformation vibrations [29-32].

The presence of the IR spectral bands of the imino group (-C=NH) in the Cr(II) complex are shifted in comparison with the metformin HCl free ligand with significant intensity. This indicates that metformin is coordinated to the metal ions through the nitrogen atom of the imino group. The second evidence which denies the displacement of imino group, is the presence of the stretching vibration band of $\nu(\text{C=NH})$ almost shifted and not similar to that of the metformin free ligand. The infrared spectra of distinguish bands of water molecules concerning hydrated Mfn-HCl complex exist with overlapping the characteristic bands of the amino group. There is no definite borderline between lattice and coordinated water molecules, especially in the stretching of OH and bending $\delta(\text{H}_2\text{O})$ vibration. In addition to these interpretations the new bands at 454 and 421 cm⁻¹ are assigned to the stretching vibration motions of $\nu(\text{M-N})$ [33].

Metformin hydrochloride free ligand has absorption in the ultraviolet regions at 228, 262, 284 and 375 nm and in some cases these bands extends over to higher wavelength region due to conjugation. New bands due to charge transfer spectra from metal to ligand (M-L) or ligand to metal (L-M) can be observed and this data can be processed to obtain information regarding the structure and geometry of the complexes [34]. Electronic spectra of Cr(III) complex was recorded in DMSO with 10^{-3} mol/cm³. UV-visible peaks corresponding to the $\pi \rightarrow \pi^*$ transitions in the Mfn-HClCr(III) complex was observed at 278 and 309 nm. This transitions ($\pi \rightarrow \pi^*$) could be assigned to the aromaticity of the double bond. The peaks belonging to $n \rightarrow \pi^*$ transitions are recorded at wavelengths 432 nm. This is most probably due to the $n \rightarrow \pi^*$ transitions of imine (=NH), primary (-NH₂), secondary (-NH), and tertiary (-N(CH₃)₂) amino groups. The transition in visible region located at 635 nm for Cr(III) complex can be attributed to the ligand-to-metal charge transfer bands LMCT from the electronic lone pairs of adjacent nitrogen coordinated to the Cr(III) ions. The electronic spectrum of the Mfn.HCl ligand exhibited maximum band at 375 nm, which could be assigned to the $n \rightarrow \pi^*$ transition of the imine (=NH), primary (-NH₂), and secondary (-NH) amino groups. This band show a red shift of the absorbance intensity in Cr(III) complex. This clearly indicates the coordination of the imine nitrogen atom with the metal atom. The solid reflectance spectrum of Cr(III) complex display three bands in the range 18,691 (ν_1), 22,472 (ν_2) and 25,126 cm⁻¹, characteristic to an octahedral geometry. These bands may be assigned to the transitions ${}^4\text{A}_{2g} \rightarrow {}^4\text{T}_{2g}$ (F) ν_2 and ${}^4\text{A}_{2g} \rightarrow {}^4\text{T}_{2g}$ (F) ν_1 , respectively, and third one is due to the charge transfer. Various ligand field parameters are calculated. The Nephelauxetic parameter β is obtained by using the relation: $\beta = B(\text{Complex})/B(\text{Free ion})$. The β values indicate that the complex has appreciable covalent character. Chromium(III) complex shows magnetic moment in the range 3.67 BM recorded at room

temperature, corresponding to three unpaired electrons. This value is close to the spin only value [35]. On the basis of the above discussion, the suggested structure of the chromium(III) Mfn-HCl complex can be represented as in (Figure 2).

The homogeneity, surface morphology and chemical composition of Mfn-HCl free ligand and Cr^{III} complex were studied using SEM (Figure 3). The surface morphology of SEM micrograph reveals the well uniform nature of the [Cr(Mfn-HCl)₂(Cl)₂].Cl.6H₂O complex with variant grain sizes and shapes. Clear large grains are obtained with agglomerates. The distribution of the grain size is homogeneous. X-ray

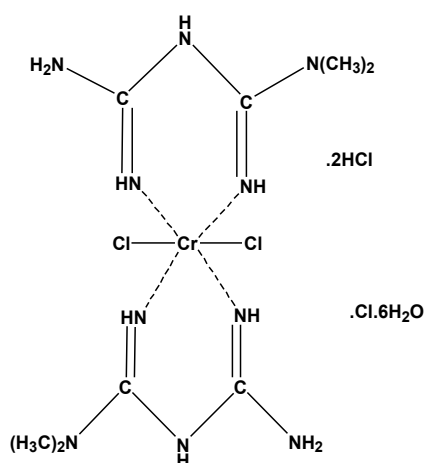


Figure 2: Suggested chelating of [Cr(Mfn-HCl)₂(Cl)₂].Cl.6H₂O complex.

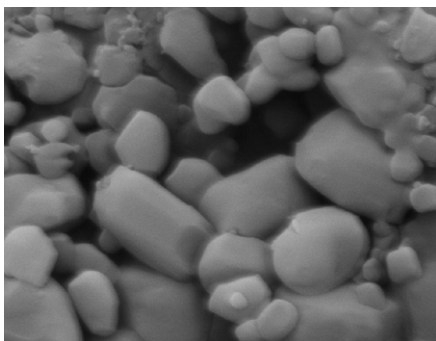


Figure 3a: SEM photos of Mfn-HCl free drug.

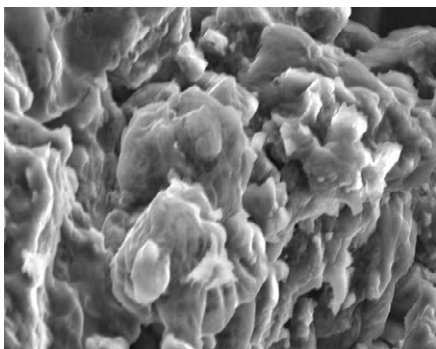


Figure 3b: SEM photos of [Cr(Mfn-HCl)₂(Cl)₂].Cl.6H₂O complex.

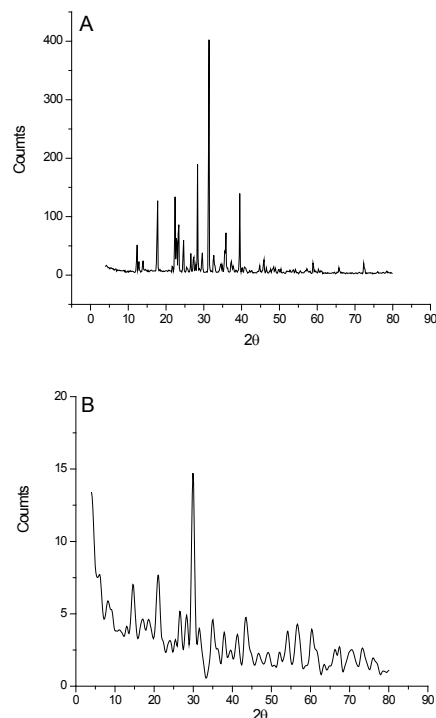


Figure 4: XRD patterns of A- Mfn-HCl free drug and B- [Cr(Mfn-HCl)₂(Cl)₂].Cl.6H₂O complex.

powder diffraction patterns in the range of $5^\circ < 2\theta < 80^\circ$ of the Mfn-HCl free ligand and Cr^{III} complex were done. The diffractograms collected for these compounds are given in (Figure 4). The definite diffraction data like angle ($2\theta^\circ$), interplanar spacing (d value, Angstrom), and relative intensity (%) have been discussed. The X-ray patterns refer to the amorphous nature for Cr(III)/Mfn-HCl complex. The variable diffractograms of Cr^{III} complex can be attributed to the formation of new structure. The maximum diffraction patterns of Mfn-HCl and Cr^{III} complex exhibited at 2θ [36] (relative intensity)=31 (100%) and 29 (100%), respectively. The crystallite size could be estimated from XRD patterns by applying FWHM of the characteristic peaks using De by-Scherrer equation 4 [37].

$$D = K\lambda / \beta \cos\theta \quad (4)$$

Where D is the particle size of the crystal gain, K is a constant (0.94 for Cu grid), λ is the X-ray wavelength (1.5406 Å), θ is the Bragg diffraction angle and β is the integral peak width. The particle size was estimated according to the highest value of intensity compared with the other peaks.

The thermal degradation behaviour of Cr(III)/Mfn-HCl drug complex is one of the interesting tools to confirm the composition and assessment of the role of metal ions. The thermal analysis of metformin hydrochloride free ligand shows two main consecutive steps of mass loss at the temperature ranges (181-410 °C) and (410-600 °C). At the first step (181-410 °C), the mass loss of 80.70% with maximum rate (T_{DTG}) at 271 °C, corresponds to an endothermic volatilization of hydrogen chloride molecule together with $C_4N_3H_7$ fragment (calcd.=80.67%) at T_{dta} of 234 °C. The mass loss (19.30%) at the second step (410-600 °C) of DTG at 504 °C as assigned to the exothermic release of H_4N_2 fragment (calcd.=19.33%) at T_{dta} of 516 °C.

The TG/DTG curves recorded for the $[\text{Co}(\text{Mfn-HCl})_2(\text{Cl})_2] \cdot \text{Cl} \cdot 6\text{H}_2\text{O}$ complex are given in Figure 5. This curve, which characterizes and compares the thermal decomposition behaviour of the Mfn-HCl ligand and its Cr(III) complex, shows seven (weak-to-very strong intensities) successive degradation steps at 30-97, 97-168, 168-315, 315-47, 447-490, 490-546 and 666-740°C. The first-to-third step at T_{DTG} of 60, 131 and 2272°C, the mass loss of 17.00% is consistent with the evolution of six uncoordinated water molecules (Cal. 18.07%). Consequently, the fourth-to-seventh steps existed at 315-447, 447-490, 490-546 and 666-740°C with endothermally (T_{dig}) at 360, 465, 514 and 700°C, respectively, are assigned to the decomposition of two Mfn-HCl and three chlorine atoms. The final residual is chromium oxide ($\text{CrO}_{1.5}$) (Cal. 12.71%, found 12.00%).

The kinetic and thermodynamic parameters were determined using non-isothermal methods. The non-isothermal kinetic analysis for the thermal decomposition of Mfn-HCl ligand and Cr(III) complex in this work was carried out by the application of the Coats-Redfern [38] and Horowitz-Metzger method [39] methods. From the TGA curves (TG/DTG) recorded for the successive steps in the decomposition process of Mfn-HCl ligand and its Cr(III) complex, it was possible to determine the following characteristic thermal parameters for each reaction step as follows: Initial point temperature of decomposition (T_i): the point at which DTG curve starts deviating from its base line. Final point temperature of decomposition (T_f): the point at which DTG curve returns to its base line. Peak temperature, i.e. temperature of maximum rate of mass loss (T_{DTG}): the point obtained from the intersection of tangents to the peak of DTG curve. Mass loss at the decomposition step (Δm): it is the amount of mass that extends from the point T_i up to the point T_f on the TG curve. The material released at each step of the decomposition is identified by attributing the mass loss (Δm) at a given step to the component of similar weight calculated from the molecular formula of the investigated compounds, comparing that with literatures of relevant compounds considering their temperature. This may assist identifying the mechanism of reaction in the decomposition steps taking place in the complex under study. Activation energy (E^*) of the decomposition step: the integral method used is the Coats-Redfern equation [38] for reaction order $n \neq 1$, which when linearized for a correctly chosen n yields the activation energy from the slope;

$$\log \left[\frac{1 - (1 - \alpha)^{1-n}}{T^2(1-n)} \right] = \log \frac{ZR}{qE_a} \left[1 - \frac{2RT}{E_a} \right] - \frac{E_a}{2.303RT} \quad \text{for } n \neq 1$$

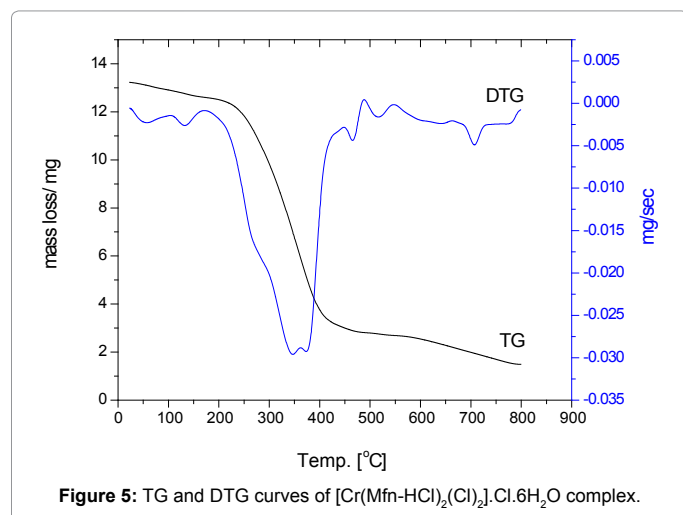


Figure 5: TG and DTG curves of $[\text{Cr}(\text{Mfn-HCl})_2(\text{Cl})_2] \cdot \text{Cl} \cdot 6\text{H}_2\text{O}$ complex.

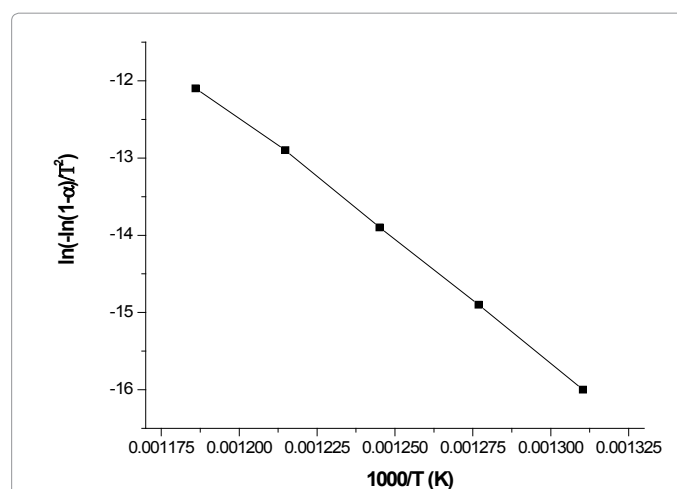


Figure 6: Coats-Redfern (CR) plot of $[\text{Cr}(\text{Mfn-HCl})_2(\text{Cl})_2] \cdot \text{Cl} \cdot 6\text{H}_2\text{O}$ complex.

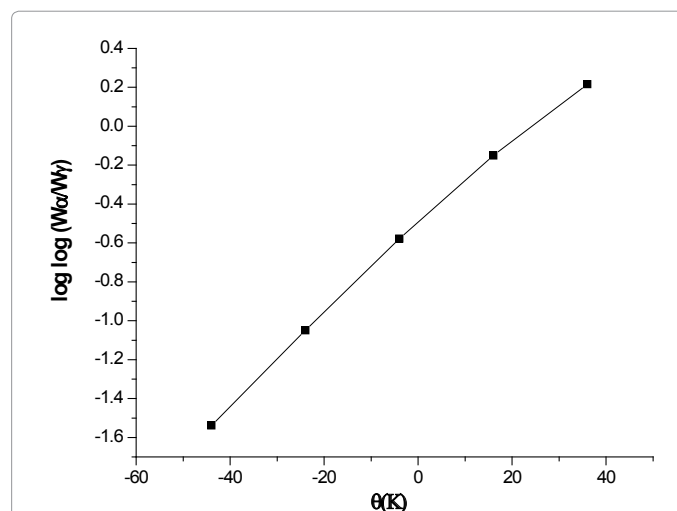


Figure 7: Horowitz-Metzger (HM) plot of $[\text{Cr}(\text{Mfn-HCl})_2(\text{Cl})_2] \cdot \text{Cl} \cdot 6\text{H}_2\text{O}$ complex.

$$\log \left[\frac{-\log(1 - \alpha)}{T^2} \right] = \log \frac{ZR}{qE_a} \left[1 - \frac{2RT}{E_a} \right] - \frac{E_a}{2.303RT} \quad \text{for } n = 1$$

Where: α =fraction of weight loss, T =temperature (K), n =order of reaction, Z =pre-exponential factor, R =molar gas constant, E_a =activation energy and q =heating rate. The activation energies (E_a) are calculated from the slopes of the best fit straight lines ($r \approx 1$) obtained when the plots of the Coats-Redfern equation [38] are used for the best values of reaction order (n). Order of reaction (n): it is the one for which a plot of the Coats-Redfern expression gives the best straight line (Figure 6) among various trial values of n that are examined, i.e., by trial and error for various trial values of n , estimated by the Horowitz-Metzger method [39] (Figure 7). The thermodynamic parameters: entropy change (ΔS^*), enthalpy change (ΔH^*) and free energy of activation change (ΔG^*) were calculated using the following equations:

$$\Delta S^* = R [\ln (Zh/KT)] \quad (5)$$

$$\Delta H^* = E_a - RT_s \quad (6)$$

$$\Delta G^* = \Delta H^* - T_s \Delta S^* \quad (7)$$

Where, Z, K and *h* are the pre-exponential factor, Boltzman and Plank constants, respectively [40].

The negative ΔS^* values indicate that the activated complex has more ordered structure than the reactants and the reactions are slower than normal [41]. The positive values of ΔG^* indicate the non-spontaneous character for the reactions at the transition-state. The positive ΔH^* values show endothermic transition-state reactions [42]. From the abnormal values of Z, the reactions of the complexes at the transition-state can be classified as a slow reaction [43]. The higher stability of the Cr(III) complex than that of the Mfn-HCl ligand may be due to the formation of two stable 6-membered rings structures in the metal complexes [44] and the higher is the molecular symmetry the more stable is the molecule [45].

Biological evaluation

Effect on Superoxide Dismutase (SOD), Total Antioxidant Capacity (TAC), Malondialdehyde (MDA) and Glutathione Reduced (GSH):

Effect on SOD: The results revealed that the administration of Metformin/Cr³⁺ to diabetic rats afforded slight significant decrease when compared to control group while afforded highly significant increase in SOD activity as compared to diabetic control group (STZ). It was recorded from table 1 that diabetic untreated group elicited highly significant decrease in SOD when compared to normal control group. However, diabetic group treated with Metformin elicited significant decrease in SOD activity when compared with normal control group by 31.65%. However, Chromium salt treated group elicited non-significant decrease in SOD activity when compared with normal control group.

Effect on GSH%: Regarding the effect of Metformin and Metformin/Cr³⁺ complex on GSH content, It was shown that diabetic untreated group elicited highly significant decrease in GSH content as compared to normal control group while combination of Metformin/Cr³⁺ induced slight significant decrease in GSH content when compared to normal control group by 5.21% followed by diabetic group treated with Metformin as compared to normal control group. The chromium salt treated group elicited slight decrease in GSH level as shown in table 1.

Effect on MDA%: Table 1 illustrates that administration of STZ only to rats afforded highly significant increase in MDA content by 71.73% as compared to normal control group while diabetic group treated with Metformin/Cr³⁺ afforded non-significant increase in MDA content as compared to normal control group. While other diabetic group treated with Metformin showed significant increase in MDA

content but the effect was much less intense in diabetic group treated with Metformin as compared to diabetic untreated group, a slight decrease was recorded in chromium salt treated group as compared to normal control group.

Effect on TAC%: It was apparent from table 1 that administration of STZ only to rats afforded highly significant decrease in TAC activities as compared to normal control group by 65.22%. Meanwhile, administration of Metformin/Cr³⁺ afforded significant increase in TAC activities when compared to diabetic untreated group while elicited slight decrease when compared to normal control group. While another diabetic treated group with Metformin exhibited significant decrease in TAC % activity as compared to normal control group. The chromium salts treated group afforded slight decrease in TAC activity as compared to normal control group and showed non-significant changes as compared to Metformin/Cr³⁺ complex treated group. But the diabetic group that recorded the highly TAC% value is the diabetic group treated with Metformin/Cr³⁺ as compared to normal control group and other diabetic groups .

ROS include superoxide free radicals, hydrogen peroxide, singlet oxygen, nitric oxide (NO), and peroxynitrite [52] that if expressed at increased concentrations can lead to cellular injury and demise through oxidative stress [53]. Most ROS occur at low levels and are scavenged by endogenous antioxidant systems that include superoxide dismutase (SOD), glutathione peroxidase, catalase, and small molecule substances such as vitamins C, D, E, and K [54,55]. Yet, one vitamin in particular, namely nicotinamide may be considered to stand-alone among antioxidants since nicotinamide influences multiple pathways tied to both cellular survival and cellular death. In several scenarios, nicotinamide is a robust cytoprotectant that addresses both early membrane PS externalization and later genomic DNA degradation [56,57] during oxidative stress in a way that is different from other vitamin entities and these findings go hand in hand with our results. In addition, nicotinamide prevents membrane PS exposure in vascular cells [58] that can reduce risk for cardiovascular disorders.

Oxidative stress is one of the most dangerous effects on the cellular activities and thus according to our results complexation between Cr³⁺ and Metformin greatly scavenged free radical molecules and thus decreased MDA level as it is the final end product of lipid peroxidation and also increased the enzymatic capacities of SOD and GSH and thus improving liver function activities and thus enhancing the conversion of blood glucose into glycogen and thus decreasing blood glucose level which reflect the solution for diabetes mellitus complications.

Effect on blood glucose level, Hb, HbA1C

Effect on blood glucose level: It was clear from Table 2 that the administration of STZ in its recommended dose afforded highly significant increase in blood glucose level when compared with normal control group. While administration of Metformin to diabetic rats afforded significant increase in blood glucose level when compared with normal control group but still showed significant decrease in blood glucose level as compared to diabetic untreated group, Meanwhile administration of Metformin/Cr³⁺ complex to diabetic rats afforded non-significant increase in blood glucose level when compared with normal control group but showed more better results than other diabetic treated and untreated groups. The chromium salts treated group elicited slight significant increase in blood glucose level as compared to normal control group.

DM affects both young and older individuals [59] and complexation of Cr³⁺ with Metformin succeed to great extent in reducing blood

Groups	SOD (U/mg)	TAC%	MDA(U/mg)	GSH (U/mg)
1 st	2.78 ± 0.36 ^{ab}	100.00 ± 1.01 ^a	104.52 ± 4.63 ^d	95.25 ± 4.15 ^a
2 nd	0.81 ± 0.17 ^d	34.78 ± 5.96 ^d	368.36 ± 6.57 ^a	22.35 ± 1.95 ^d
3 rd	1.90 ± 0.15 ^c	68.25 ± 5.24 ^c	156.53 ± 3.85 ^b	62.96 ± 8.52 ^c
4 th	2.25 ± 0.14 ^b	80.69 ± 7.95 ^b	106.38 ± 4.10 ^{cd}	90.25 ± 6.45 ^b
5 th	2.20 ± 0.11 ^b	79.25 ± 3.25 ^{bc}	110.41 ± 3.24 ^c	90.58 ± 4.07 ^b

Data presented as mean ± S.E. (n=10).

Low density lipoprotein cholesterol (LDL-c)=Total cholesterol—(HDL-c+ triglyceride)/5, volatile low density lipoprotein cholesterol (VLDL-c) = Triglycerides/5, Risk ratio=Total cholesterol/high density lipoprotein cholesterol (HDL- c)

Means within the same column in each category carrying different litters are significant at (P ≤ 0.05) using Duncan's multiple range tests, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

Table 1: Effect of Metformin and Metformin/Cr³⁺ complex on Superoxide dismutase (SOD), Total antioxidant capacity (TAC), Malondialdehyde (MDA) and Glutathione reduced (GSH) in normal and diabetic rats.

glucose level and this explain the important role of Cr⁺³ in decreasing blood glucose level after complexation with Metformin. Supplement contains trivalent Chromium is needed for a person with type 2 diabetes mellitus, according to its important role in glucose metabolism [61] and this is greatly confirmed and reinforced our findings as the combination of Metformin with Cr(III) afforded a significant reduction in blood glucose level as compared to diabetic untreated group.

It was well known that hyperglycemia is the hallmark of diabetes. Our findings are greatly supported by Hai-yan et al. [46] as they showed that an increase in blood glucose was observed after treating with alloxan intraperitoneally and after 15 day's administration of Chromium methionine (CrMet), the blood glucose levels significantly decreased in comparison with the diabetic control group. These findings indicated that CrMet had hypoglycemic effect on AID mice.

Our results are in accordance with Sahin et al. [62] as they indicated that the anti-hyperglycemic activity of CrMet was superior to that of CrCl₃·6H₂O and equivalent to that of CrNic. Ghiasi et al. [63] found out that supplemental CrMet in the diet for 6 weeks could significantly decrease the blood glucose levels of fructose-fed diabetic rat.

Effect on Hb%: The results revealed that diabetic untreated group (STZ) elicited highly significant decrease in Hb content when compared to normal control group as shown in table 2. While treatment of diabetic rats with Metformin/Cr⁺³ elicited non-significant decrease in Hb content as compared to normal control group, while, other diabetic group treated with Metformin showed significant decrease in Hb content when compared to normal control group. While, chromium salts treated group elicited slight decrease in Hb level as compared to normal control group.

Effect on HbA1C: After 4 weeks post administration of STZ and other treatments, the diabetic untreated group afforded significant increase in HbA1c compared to normal control group followed by diabetic group treated with Metformin by 23.69% as compared to normal control group. Whereas treatment of diabetic rats with Metformin/Cr⁺³ afforded non-significant increase in HbA1c as compared to normal control group and thus showing the best results in treating diabetes mellitus table 2, meanwhile, chromium salt treated

Groups	Blood glucose level (mg/dl)	Hb (g/dl)	HbA1C (g/dl)
1 st	74.92 ± 7.50 ^e	12.43 ± 2.95 ^{ab}	7.30 ± 1.20 ^d
2 nd	348.19 ± 30.52 ^a	8.04 ± 1.54 ^e	9.58 ± 1.03 ^a
3 rd	140.80 ± 9.82 ^b	10.20 ± 1.99 ^d	9.40 ± 1.01 ^b
4 th	82.40 ± 8.76 ^{de}	12.30 ± 1.92 ^b	7.31 ± 1.24 ^d
5 th	86.24 ± 5.20 ^c	11.20 ± 1.42 ^c	7.53 ± 1.04 ^{cd}

Data presented as mean ± S.E. (n=10).

Means within the same column in each category carrying different litters are significant at (P ≤ 0.05) using Duncan's multiple range tests, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

Table 2: Effect of Metformin and Metformin/Cr⁺³ complex on blood glucose level, Hb, HbA1C in normal and diabetic rats.

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-c (g/dl)	LDLc (g/dl)	VLDLc (g/dl)	Risk ratio
1 st	73.22 ± 5.78 ^d	90.23 ± 4.23 ^d	38.52 ± 2.88 ^a	28.63 ± 9.95 ^d	18.04 ± 2.96 ^c	1.90 ± 0.45 ^d
2 nd	269.32 ± 11.03 ^a	220.75 ± 10.66 ^a	5.45 ± 2.45 ^e	58.63 ± 7.09 ^a	44.15 ± 3.41 ^a	49.41 ± 0.55 ^a
3 rd	97.36 ± 6.54 ^b	119.48 ± 4.23 ^b	24.44 ± 1.32 ^d	39.52 ± 2.49 ^b	23.89 ± 2.85 ^b	3.98 ± 0.32 ^b
4 th	74.25 ± 1.72 ^d	94.15 ± 5.44 ^{cd}	32.36 ± 3.98 ^b	29.75 ± 4.60 ^{cd}	18.83 ± 1.95 ^c	2.29 ± 0.57 ^c
5 th	76.25 ± 3.20 ^{cd}	93.20 ± 2.10 ^{cd}	31.25 ± 1.20 ^c	28.50 ± 3.54 ^d	18.64 ± 1.20 ^c	2.10 ± 0.24 ^c

Data presented as mean ± S.E. (n=10).

Means within the same column in each category carrying different litters are significant at (P ≤ 0.05) using Duncan's multiple range tests, where the highest mean value has the symbol (a) and decreasing in value were assigned alphabetically.

Table 3: Effect of Metformin and Metformin/Cr⁺³ complex on the lipid profile in normal and diabetic rat.

group afforded non-significant increase in glycated haemoglobin as compared to normal control group.

In uncontrolled or poorly controlled diabetes, there is an increased glycosylation of a number of protein including haemoglobin [60]. Glycosylated hemoglobin (HbA1C) was significantly increased in diabetic animals and this increase was found to be directly proportional to the fasting blood glucose level. During diabetes, the excess glucose present in blood reacts with hemoglobin. Therefore, the total hemoglobin level is decreased in diabetic rats [64]. In this study a decrease in total hemoglobin during diabetes has been observed in diabetic untreated group and this may be due to the formation of glycosylation hemoglobin. Administration of Metformin/Cr⁺³ to diabetic rats prevent a significant elevation in glycosylated hemoglobin. Thereby increasing the level of total hemoglobin in diabetic rats. This could be due to the improved glycaemic control produced by this new complex.

Effect on lipid profile

Effect on cholesterol: Table 3 and Figure 8 demonstrates that diabetic untreated rats (STZ) afforded a significant increase in cholesterol level as compared to normal control group while treatment of diabetic rats with Metformin/Cr⁺³ elicited non-significant changes in cholesterol level when compared with normal control group while other diabetic group treated with Metformin afforded significant elevation in cholesterol when compared with normal control group while both groups treated with either Metformin or Metformin/Cr⁺³ showed significant decrease in cholesterol level when compared with diabetic untreated group but the effect was more intense in diabetic group treated with Metformin/Cr⁺³. The non-significant increase in cholesterol level was reported in group treated with CrCl₃·H₂O treated group as compared to normal control group.

Our obtained results are greatly in accordance with Hai-yan et al. [46] as they tested the anti-diabetic activity of Chromium methionine (CrMet) in detail. Their obtained results showed that CrMet had beneficial effects on glucose and lipid metabolism, and might possess hepatoprotective efficacy for diabetes.

Effect on triglycerides: It was clear from table 3 and Figure 8 that diabetic untreated group showed significant elevation in TG levels as compared to normal control group by 59.12%. However, diabetic group treated with Metformin/Cr⁺³ afforded non-significant increase in TG levels as compared to normal control group while showed significant decrease in TG levels as compared to diabetic untreated group. On the other hand, other diabetic group treated with Metformin elicited significant increase in TG levels when compared to normal control group but the best results and the less TG increment was noticed in group treated with Metformin/Cr⁺³. Meanwhile, chromium treated group elicited non-significant increase in Triglycerides level as compared to normal control group.

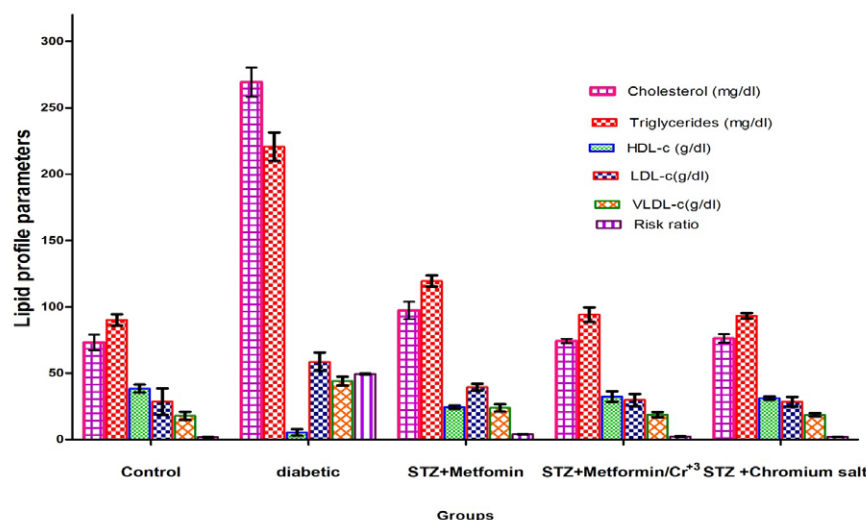


Figure 8: Effect of $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ (Chromium salt), Mfn-HCl and Mfn-HCl / Cr^{+3} complex on the lipid profile in normal and diabetic rat.

Effect on HDL-c: The administration of diabetic rats with Metformin/ Cr^{+3} afforded slight decrease in HDL-c levels when compared to normal control group while diabetic untreated group elicited highly significant decrease in HDL-c levels as compared to normal control group. At the mean time the diabetic group treated with Metformin elicited significant decrease in HDL-c level with respect to normal control group Figure 8. Chromium treated group afforded slight significant decrease in HDL-c level as compared to normal control group.

Effect on LDL-c: At the same time, Table 3 and Figure 8 demonstrates that administration of Metformin/ Cr^{+3} to diabetic rats exhibited non-significant changes in LDL-c levels as compared to a normal control group. Meanwhile diabetic untreated group induced significant increase in LDL-c levels as compared to a normal control group and also as compared to diabetic group treated with Metformin. Regarding the chromium treated group, it is afforded non-significant decrease in LDL-c level in comparison with normal control group.

Effect on vLDL-c: Table 3 and Figure 8 illustrates the effect of Metformin and Metformin/ Cr^{+3} complex on vLDL-c, there was significant increase in diabetic untreated group in response to administration of STZ only to rats. At the same time, the treatment of diabetic rats with Metformin/ Cr^{+3} afforded significant decrease in vLDL-c as compared to diabetic rats by 57.34%. While showed non-significant increase when compared to normal control group. At the meantime, diabetic group treated with Metformin showed significant decrease in vLDL-c when compared to diabetic untreated group and showed significant increase when compared with normal control group.

Effect on risk ratio: It was clear from table 3 and Figure 8 that the administration of STZ to rats elicited the highest risk value as compared to normal control group and other diabetic treated groups and the best group that succeed in reducing the risk factor is the diabetic group treated with Metformin/ Cr^{+3} and this group showed non-significant increase in risk ratio as compared to a normal control group. The results revealed that the diabetic group treated with Metformin/ Cr^{+3} showed the lowest risk ratio as compared to control group and the diabetic untreated group followed by diabetic group treated with Metformin.

The chromium salt treated group elicited slight significant increase in risk ratio as compared to normal control group but still much better than other diabetic groups.

Free fatty acids can lead to ROS release and contribute to mitochondrial DNA damage and impaired pancreatic β -cell function [47]. In patients with type 2 DM, skeletal muscle mitochondria have been described to be smaller than those in control subjects [48]. In addition, a decrease in the levels of mitochondrial proteins and mitochondrial DNA in adipocytes has been correlated with the development of type 2 DM [49]. Insulin resistance in the elderly also has been associated with elevation in fat accumulation and reduction in mitochondrial oxidative and phosphorylation activity [50]. An association also exists with insulin resistance and the impairment of intracellular fatty acid metabolism in young insulin-resistance offspring of parents with type 2 DM [51] and this greatly agreed with our results that confirming the increased level of fatty acids and increasing the level of LDL, Triglycerides and cholesterol and decreasing their levels significantly in diabetic group treated with Metformin/ Cr^{+3} as compared to normal control group and this is correlates to Cr^{+3} role in decreasing fatty acids level and thus enhancing the properties of Metformin.

Effect on insulin level: Concerning the effect of Metformin and their Cr^{+3} complexes on insulin level, the results revealed that administration of Metformin/ Cr^{+3} complexes afforded non-significant increase in insulin level as compared to normal control group while administration of STZ only to rats elicited significant decrease in insulin level when compared to normal control group. The other diabetic treated group with Metformin elicited significant decrease in insulin level by 60 % as compared to normal control group as shown in table 4. There is no significant change reported in group treated with chromium salt treated group. The Cr(III) interact with the insulin and its receptors on the first step in the metabolism of glucose entry into the cell, and facilitates the interaction of insulin with its receptor on the cell surface [65] and thus our results come in harmony with these findings as the combination of Metformin with Cr^{+3} increased the level of insulin in diabetic rats and the best results was shown in diabetic group treated with Metformin/ Cr^{+3} as they showed the high value of insulin level by 1.76 % as compared to normal control group and by 65.11% increment as compared to diabetic untreated group and this go side by side in

Groups	Insulin ($\mu\text{U/ml}$) in pancreatic homogenates	Serum C-peptide (pmol/mL)
1 st	$17.52 \pm 1.05^{\text{ab}}$	$3.87 \pm 1.01^{\text{ab}}$
2 nd	$6.22 \pm 0.53^{\text{e}}$	$1.12 \pm 1.03^{\text{d}}$
3 rd	$12.43 \pm 1.51^{\text{d}}$	$2.44 \pm 1.25^{\text{c}}$
4 th	$15.55 \pm 1.87^{\text{c}}$	$3.85 \pm 1.34^{\text{b}}$
5 th	$16.99 \pm 0.96^{\text{bc}}$	$3.45 \pm 1.10^{\text{b}}$

Table 4: Effect of Metformin and Metformin/ Cr^{+3} complex on insulin level and serum C-peptide in normal and diabetic rat.

Data presented as mean \pm S.E. (n=10).

Means within the same column in each category carrying different litters are significant at ($P \leq 0.05$) using Duncan's multiple range tests, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

1st group: control, 2nd group: STZ, 3rd group: STZ+Metformin, 4th group: STZ +Metformin/ Cr^{+3} , 5th group: STZ + $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$.

confirming our results that reported the success of Cr^{+3} complexes with Metformin in reducing blood glucose level and increasing insulin level and thus alleviating the side effects of diabetes mellitus and improving characterization of Metformin/ Cr^{+3} . So we consider the first author to clarify this improving effect of Metformin/ Cr^{+3} on diabetes mellitus reducing complications. Chromium increases insulin binding to cells, insulin receptor number as well as activates insulin receptor kinase leading to increase sensitivity of insulin receptor [66].

Effect on serum C-peptide: It was apparent from Table 4 that administration of Metformin/ Cr^{+3} afforded non-significant increase in C-Peptide as compared to normal control group while the diabetic untreated group showed the less serum C-Peptide value among all the diabetic treated groups, while administration of Metformin only induced significant decrease in c-Peptide as compared to control group but showed significant increase in c-peptide as compared to normal control group. $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ treated group elicited non-significant decrease in serum C-peptide level as compared to normal control group. The connecting peptide, or C-peptide, is a short 31-amino-acid protein that connects insulin's A-chain to its B-chain in the proinsulin molecule. In the insulin synthesis pathway, first preproinsulin is translocated into the endoplasmic reticulum of beta cells of the pancreas with an A-chain, a C-peptide, a B-chain, and a signal sequence.

Measuring C-peptide can help to determine how much of natural insulin is producing as C-peptide is secreted in equimolar amounts to insulin. C-peptide levels are measured instead of insulin levels because C-peptide can assess a person's own insulin secretion even if they receive insulin injections, and because the liver metabolizes a large and variable amount of insulin secreted into the portal vein but does not metabolize C-peptide, meaning blood C-peptide may be a better measure of portal insulin secretion than insulin itself [67]. A very low C-peptide confirms Type 1 diabetes and insulin dependence and is associated with high glucose variability, hypoglycaemia and increased complications and this is an important concept that explain our findings as the more c-peptide value was found in diabetic group treated with Metformin/ Cr^{+3} and thus we can say the higher C-peptide, the higher insulin level and to best our knowledge, this is the first report on the effect of Metformin/ Cr^{+3} on ameliorating the C-peptide value and consequently increased insulin level.

Ultrastructural results

Acinar cells: Ultrastructure of control pancreas showed acinar cells with euchromatic nuclei, well-developed cisternae of rough endoplasmic reticulum, mitochondria and numerous electron dense secretory granules of variable sizes in the apical part (Figure 9a).

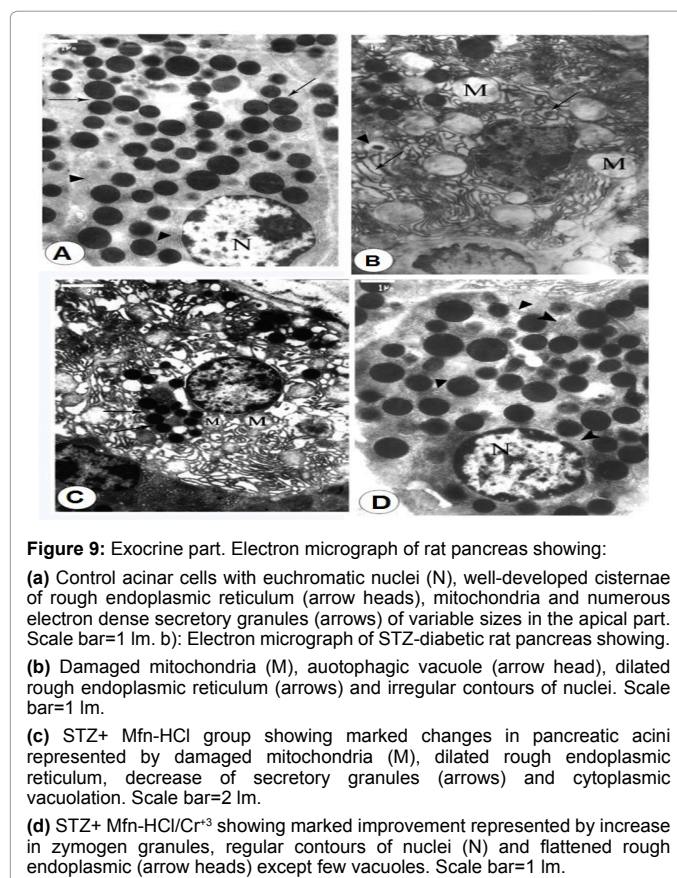


Figure 9: Exocrine part. Electron micrograph of rat pancreas showing:

(a) Control acinar cells with euchromatic nuclei (N), well-developed cisternae of rough endoplasmic reticulum (arrow heads), mitochondria and numerous electron dense secretory granules (arrows) of variable sizes in the apical part. Scale bar=1 μm . b): Electron micrograph of STZ-diabetic rat pancreas showing:

(b) Damaged mitochondria (M), autophagic vacuole (arrow head), dilated rough endoplasmic reticulum (arrows) and irregular contours of nuclei. Scale bar=1 μm .

(c) STZ+ Mfn-HCl group showing marked changes in pancreatic acini represented by damaged mitochondria (M), dilated rough endoplasmic reticulum, decrease of secretory granules (arrows) and cytoplasmic vacuolation. Scale bar=2 μm .

(d) STZ+ Mfn-HCl/ Cr^{+3} showing marked improvement represented by increase in zymogen granules, regular contours of nuclei (N) and flattened rough endoplasmic (arrow heads) except few vacuoles. Scale bar=1 μm .

Electron microscopic examination of the diabetic untreated group showed marked changes in pancreatic acini represented by dilated rough endoplasmic reticulum, a decrease of secretory granules, cytoplasmic vacuolation, damaged mitochondria, autophagic vacuole and irregular contours of nuclei (Figure 9b). STZ + Metformin group showed marked changes in pancreatic acini represented by damaged mitochondria (M), dilated rough endoplasmic reticulum, decrease of secretory granules and cytoplasmic vacuolation (Figure 9c). Pancreatic acini after supplementation with Metformin/ Cr^{+3} in diabetic rats showed marked improvement represented by increase in zymogen granules, regular contours of nuclei and flattened rough endoplasmic reticulum except few vacuoles (Figure 9d). Electron microscopic examination confirmed the presence of apoptotic changes manifested as nuclear pyknosis, indentation of nuclear membrane, chromatin release into the cytoplasm, swollen mitochondria, dilation of Golgi apparatus vesicles and disappearance of secretory granules in STZ diabetic group and the amelioration in these changes was clearly appeared in metformin/ Cr^{+3} diabetic treated group.

Conclusion

The chemical interaction between chromium (III) chloride hexahydrate and metformin HCl (Mfn.HCl) produce diabetic mimetic model of chromium (III) metformin hydrochloride. The infrared spectroscopic results were proven that metformin hydrochloride reacted with chromium (III) ions as a bidentate ligand through its two imino groups. The chromium (III) Metformin complex has succeeded in decreasing blood glucose parameters in diabetic rats and proving its antidiabetic performance and thus proving the efficiency of metformin and Chromium (III) complex in elevating antioxidant capacities.

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