Study of the Effects of Complexation of Lead with Metformin, Glimepiride, Vildagliptin and Dapagliflozin in Mice Model

Fahima Aktar[a], Md. Zakir Sultan[b], Mohammad A. Rashid[a]*

Abstract

Lead is abundant in the air, water and soil as a pollutant. It is sometimes consumed by humans in excessive amounts, which can lead to toxicity in the body or may interact with soluble drugs, thus altering their therapeutic efficacy. In this study, four antidiabetic drugs were synthesized to form a complex with metallic lead at different conditions. The formation of four complexes viz. Pb-metformin, Pb-glimepiride, Pb-vildagliptin, and Pb-dapagliflozin was established and confirmed by TLC, DSC, TGA and FT-IR data analysis. The physiological effects of these complexes was then examined using a mice model. After treatment, blood glucose, serum creatinine and uric acid were measured and histopathology of hepatic and nephrotic tissues were studied. Control treatments of metformin, glimepiride, vildagliptin and dapagliflozin reduced blood glucose levels in mice from 31.54 to 19.02 mmol/L, 30.24 to 17.20 mmol/L, 31.50 to 19.70 mmol/L and 30.37 to 17.60 mmol/L, respectively, whereas Pb-met, Pb-glim, Pb-vilda, and Pb-dapa slightly increased blood glucose levels compared to control after 14 days of treatment from 30.60 to 25.82 mmol/L, 30.22 to 29.23 mmol/L, 31.93 to 25.32 mmol/L and 32.25 to 29.32 mmol/L, respectively. The serum creatinine levels increased from 3.38, 3.96, 3.60 and 3.42 mg/dL on metformin, glimepiride, vildagliptin and dagagliflozin respectively to 4.57, 5.36, 5.21 and 5.24 mg/dL on the lead-complex forms of those respective medications. Similarly, the serum acid levels also increased from 42.91, 44.83, 40.21 and 41.49 mg/dL on metformin, glimepiride, vildagliptin and dagagliflozin respectively to 53.13, 57.40, 49.36 and 53.32 mg/dL on the lead-complex forms of those respective medications. Both high creatinine and uric acid level after treatment with lead complexes indicated nephrotic toxicity.

Keywords: Antidiabetic drugs, complexation, Lead, Creatinine, Uric acid

***Significance* | Pb-complexes of antidiabetic drugs increased serum creatinine and serum uric acid levels of mice which suggested cellular damage in kidney of mice.**

*Correspondence: Mohammad A. Rashid
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh
E-mail: r.pchem@yahoo.com

Edited by Aman Shah Abdul Majid, PhD, Quest International University, Malaysia. And accepted by the Editorial Board November 11, 2020 (received for review October 8, 2020)

Introduction

The increased incidence of type 2 diabetes since the 1950s is thought to primarily be due to coincidental alterations in life style, although another potential contributing factor in industrialized countries is exposure to environmental pollutants and chemicals from industrial waste. Population exposure levels of many environmental toxicants have risen proportionally with disease incidence. Of note, there is a particular interest towards metallic lead (Leff et al., 2018). Some metals can be considered xenobiotics e.g., lead, mercury, cadmium and the metalloid arsenic may have negative effects on physiology and in some cases, human exposure levels have been associated with the incidence of diabetes and other related metabolic syndromes (Afridi et al., 2008; Bener et al., 2001; Cave et al., 2010; Chen et al., 2007; Chen et al., 2009; Kolachi et al., 2011; Moon, 2013; pathologies following lead exposures have been well documented (Ris et al., 2004; Searle et al., 2014; Surkanet al., 2007), very little is known

Author Affiliation:
[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh
[b] Centre for Advanced Research in Sciences (CARS), University of Dhaka, Dhaka-1000, Bangladesh

Please cite this article:
Fahima Aktar, Md. Zakir Sultan, Mohammad A. Rashid (2020). Study of the Effects of Complexation of Lead with Metformin, Glimepiride, Vildagliptin and Dapagliflozin in Mice Model. Microbial Bioactives, 3(1), 125-133.

about the effects of lead exposure on the incidence of chronic metabolic diseases such as type 2 diabetes.

Padilla et al., 2010; Zhai et al., 2017). From early childhood, due to exposure to lead-based paints, sporadic exposure to contaminated drinking water, a significant segment of the population has been exposed to low levels of lead. Though the neuronal developmental Few studies have directly examined the correlation between blood lead levels and diabetic incidence. Indeed, Kolachi et al., (2011) found that the level of lead in blood and urine was higher in female diabetic patients compared to non-diabetic patients. Further, a significant positive correlation between blood lead levels and fasting blood glucose was observed in factory workers in the United Arab Emirates, suggesting a possible link between lead exposure and diabetes. They also found a connection between lead exposure and blood pressure (Bener et al., 2001). A strong association between blood lead with markers of oxidative stress in the general population demonstrated that oxidative stress should be considered in the development of lead-mediated diseases, even among people with relatively low exposure to environmental lead i.e., <10 µg/dL (Lee et al., 2006). In fact, it was revealed that Pb^{+2} increases oxidative stress in living organisms (Bokara et al., 2009; Coban et al., 2007; Hunaiti et al., 2000) and oxidative stress is thought to promote the diabetic state by directly impacting cellular signaling by influencing the insulin secretion pathway. A 6-year study concluded that bone lead content was related to progressive elevation of serum creatinine in diabetics (Tsaih et al., 2004).

Four antidiabetic drugs were selected to produce complexes with lead, namely metformin, glimepiride, vildagliptin and dapagliflozin. The chemical structures of metformin, glimepiride, vildagliptin and dapagliflozin are been shown in Figure 1. Metformin, a biguanide group of drugs increases peripheral glucose utilization as well as decreases gluconeogenesis, possibly by its action on membrane phospholipids. It also inhibits glucose uptake from the intestinal lumen (Brunton et al., 2006). Glimepiride is a drug of the sulphonylurea group which mediate hypoglycemia by stimulating insulin release from pancreatic β-cells i.e. it increases insulin release in patients with type 2 diabetes mellitus. This group of drugs may also further increase insulin levels by reducing hepatic clearance of the hormone (Brunton et al., 2006). Vildagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor for clinical use to treat type 2 diabetes mellitus (T2DM). The inhibition mechanism of DPP-4 inhibitors depends on their ability to increase the levels of incretin hormones, glucagonlike peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) in the systemic circulation (Ahren et al., 2011). Dapagliflozin, is a sodium-glucose co-transporter-2 (SGLT2) inhibitor in the kidneys and reduces renal glucose reabsorption, leading to urinary glucose excretion and a reduction in blood glucose levels (Plosker, 2012).

In order to evaluate the effects of complexation of these drugs with lead, the complexes of Pb-metformin (Pb-met), Pb-glimepiride (Pb-glim), Pb-vildagliptin (Pb-vilda) and Pb-dapagliflozin (Pb-dapa) were made and their antidiabetic property was evaluated in mice model. Furthermore, the toxicological studies were performed by measuring the serum creatinine and uric acid level. Herein, we report the results of our preliminary investigations.

Materials and Methods

Materials

Analytical grade solvents, chemicals and lead nitrate were used for all experimental purposes without further purification. The API of antidiabetic drugs metformin (purity 99%), glimepiride (purity 99%), vildagliptin (purity 99%) and dapagliflozin (purity 99%) were received as gift samples from Incepta Pharmaceuticals Ltd., Dhaka, Bangladesh. Alloxan monohydrate (98%) was purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Serum creatinine and uric acid level determination kit (Linear Chemicals, S.L.U., Spain) were purchased locally.

Synthesis of lead (II) complexes with the antidiabetic drugs

The Pb-met, Pb-glim, Pb-vilda and Pb-dapa complexes were synthesized by dissolving each drug e.g. metformin hydrochloride (2 mmol), glimepiride (0.5 mmol), vildagliptin (0.5 mmol), dapagliflozin (0.5 mmol) in 25 mL of methanol and then mixed with 25 mL methanol solution of 1 mmol $Pb(NO_3)_2$. The mixtures were heated at 70-75 ℃ in a water bath (J.P. Selecta, Spain) with occasional stirring for 4.30 hours. Then the mixtures were left overnight for precipitation.

Characterization of synthesized lead complexes with the antidiabetic drugs

Differential scanning calorimetry (DSC)

The phase change properties of the Pb complexes were studied by differential scanning calorimetry (DSC) (DSC-60, Shimadzu, Japan). The range of temperature was up to 300 ℃ and temperature rising rate was 10 °C/ min at a flow rate of 20 mL/ min of nitrogen gas.

Thermogravimetric analysis (TGA)

The thermogravimetric analyses (TGA) of the lead complexes were carried out at temperature up to 600 ℃ by TGA-50 (Shimadzu, Japan). An aliquot of each complex (~3mg) was heated in an aluminum pan with temperature rising rate at 10 °C/ min under nitrogen gas flow rate of 20 mL/min.

Fourier transform infrared spectrophotometry (FTIR)

The FTIR spectra of lead complexes with the selected antidiabetic drugs were carried out at the wavelength from 400 cm^{-1} to 4000 cm^{-1}. About 100 mg of pure and dried KBr was added to 1 mg of each dried sample, then homogenously mixed with a mortar-pestle

and pressed mechanically to make a pellet under the pressure of 8-10 tons. The prepared disc was placed in the IR beam path for acquiring the spectrum.

Induction of diabetes in mice

Male Swiss Albino mice with average weight of 26 g were purchased from the Department of Pharmacy, Jahangirnagar University, Savar, Bangladesh and maintained on a normal diet and filtered tap water *ad libitum*. The mice were maintained and handled according to the guidelines of Helsinki declaration. Experiment in animal model was carried out after approval of the authority of the Department of Pharmaceutical Chemistry, University of Dhaka. The mice were randomly divided into 9 groups (Group-I to Group-IX) (6 mice in each group). Group-I was treated as control and conceded to feed normal diet only. Neither alloxan nor drug was introduced to the Group-I. Diabetes was induced in the rest of the eight groups (Group-II to Group-IX) by intraperitoneal administration of alloxan at a dose of 150 mg/kg body weight following an overnight fasting. Before induction of diabetes and after a period of three days of alloxan treatment, blood was collected by cutting the tiny portion of tail of the all groups of fasting mice, and serum glucose level was determined immediately by a glucometer (GlucoLeader-yasee, GLM-76, Yasee Co. Ltd., Taiwan). The alloxan-treated rats were considered to be diabetic due to a high serum glucose level (>20 mmol/L). Group-II and Group-III were given 1.50 mg/kg body weight (bw) metformin hydrochloride and Pb-met complex, respectively as solution for 14 days. Similarly, Group-IV and Group-V were given 1.50 mg/kg bw glimepiride and Pb-glim complex, respectively, Group-VI and Group-VII received 1.50 mg/kg bw vildagliptin and Pb-vilda complex, respectively, and Group VIII and Group IX were given dapagliflozin and Pb-dapa complex, respectively orally as solution for 14 days. After 14 days of treatment, blood was drawn by cutting the tail of the all groups of fasting mice and serum glucose levels were determined immediately by a glucometer.

Determination of serum creatinine level

The procedure is based upon a modification of the original picrate reaction. Creatinine under alkaline conditions reacts with picrate ions forming a reddish complex. The formation rate of the complex measured through the increase of absorbance in a prefixed interval of time is proportional to the concentration of creatinine in the sample (Heinegaardet al., 1973; Larsen, 1972).

$$Creatine + Picric\ Acid \xrightarrow{pH>12,37^{\circ}C} Red\ addition\ complex$$

Working reagent, samples and standard were pre-incubated to reaction temperature (37°C). The photometer was set to 0 absorbance with distilled water. One (1.0) mL working reagent and 100 µL sample or standard was pipette into a cuvette and mixed gently. Then cuvette was inserted into the temperature-controlled instrument and stop watch started. The absorbance was measured at 510 nm after 30 seconds (A_1) and after 90 seconds (A_2) of the sample or standard addition. The level of creatinine was determined by using the following equation:

$$\frac{(A_2\text{-}A_1)\ sample}{(A_2\text{-}A_1)\ standard} \times C\ standard = mg/dL\ creatinine = mg/dL \times 88.4 = \mu mol/L$$

Uric acid determination

Uric acid is oxidized by uricase to allantoin with the formation of hydrogen peroxide. In the presence of peroxidase (POD), a mixture of dichlorophenol sulphonate (DCBS) and 4-aminoantipyrine (4-AA) is oxidized by hydrogen peroxide to form a quinoneimine dye proportional to the concentration of uric acid in the sample (Barham et al., 1972; Fossati et al., 1980).

$$Uric\ acid + O_2 + 2H_2O \xrightarrow{Uricase} Allantoin + H_2O_2$$

$$4AA + DCBS \xrightarrow{H_2O_2,\ POD} Quinoneimine + 4H_2O$$

Reagents and samples were kept at room temperature and reagents were pipetted into three different tubes, mixed and rested for 10 minutes at room temperature. The absorbance (A) of the samples and the standard at 520 nm against the reagent blank was recorded and the level of uric acid was determined by using the following equation:

$$\frac{A_{\ Sample}}{A_{\ standard}} \times C\ Standard = mg/dL\ uric\ acid = mg/dL \times 59.5 = \mu mol/L$$

Statistical analysis

All the data are shown as mean ± SEM. Statistical analyses were conducted using Microsoft-Excel 2007.

Results and Discussion

The drugs (e.g. metformin, glimepiride, vildagliptin and dapagliflozin) were left to react with $Pb(NO_3)_2$ at 70-75°C for 4.5 hours, with the resultant formation of crystalline and amorphous Pb-drug complexes. The formation of drugs complexes was confirmed by TLC, DSC, TGA and FTIR studies.

Firstly, TLC of the Pb-drug complexes was carried out using methanol-dichloromethane in different ratios for different complexes. The spot of each of the complex appeared at different places from their precursor drugs (**Table 1**). Each individual spot indicated the formation of a new complex.

Table 1. R_f values of standard drugs and their lead complexes on silica gel F_{254} TLC plate.

Item	Mobile phase	Rf value
Metformin HCl	Methanol/dichloromethane (2:8)	0.5
Pb-metcomplex		0.45
Glimepiride	Methanol/dichloromethane (7:3)	0.6
Pb-glim complex		0.75
Vildagliptin	Methanol/dichloromethane (8:2)	0.7
Pb-vilda complex		0.55
Dapagliflozin	Methanol/dichloromethane (7:3)	0.5
Pb-dapa complex		0.3

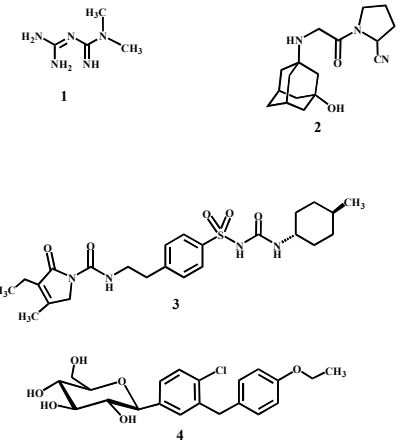
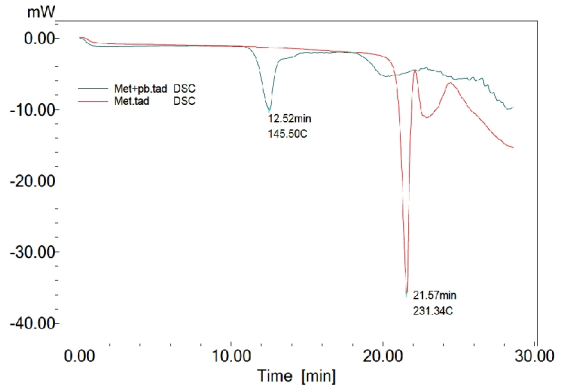
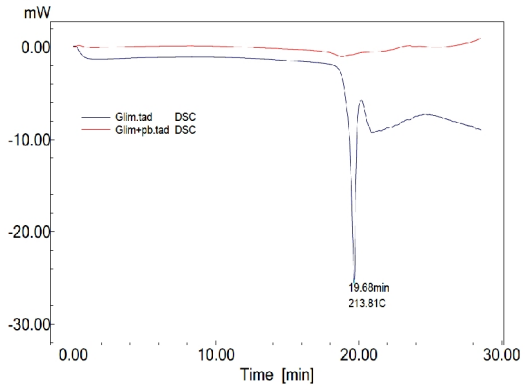


Figure 1. Structures of metformin (1), vildagliptin (2), glimepiride (3), and dapagliflozin (4).



(A)



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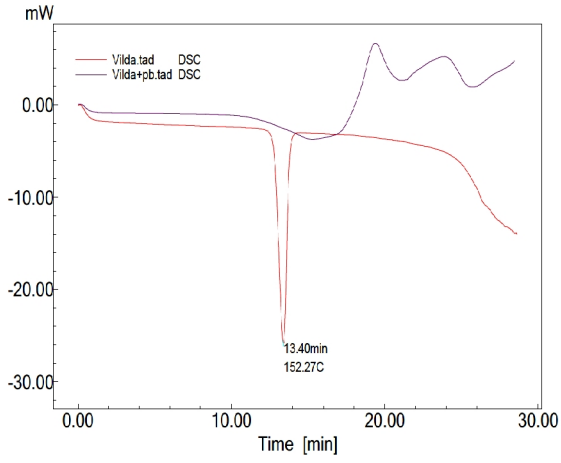
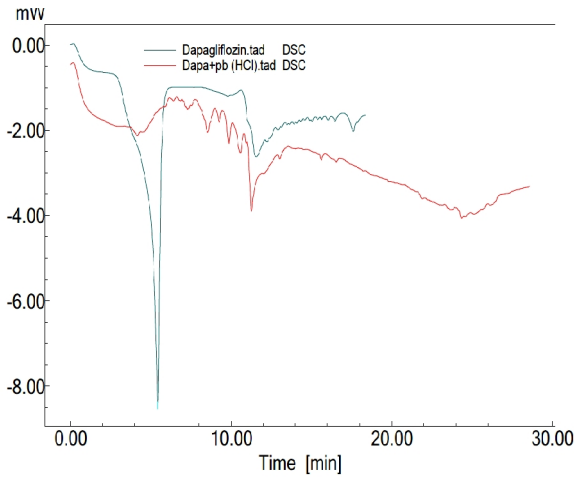


(C)



(D)

Figure 2. Overlaid DSC thermograms: metformin and Pb-met (**A**), glimepiride and Pb-glim (**B**), vildagliptin and Pb-vilda (**C**), dapagliflozin and Pb-dapa (**D**).

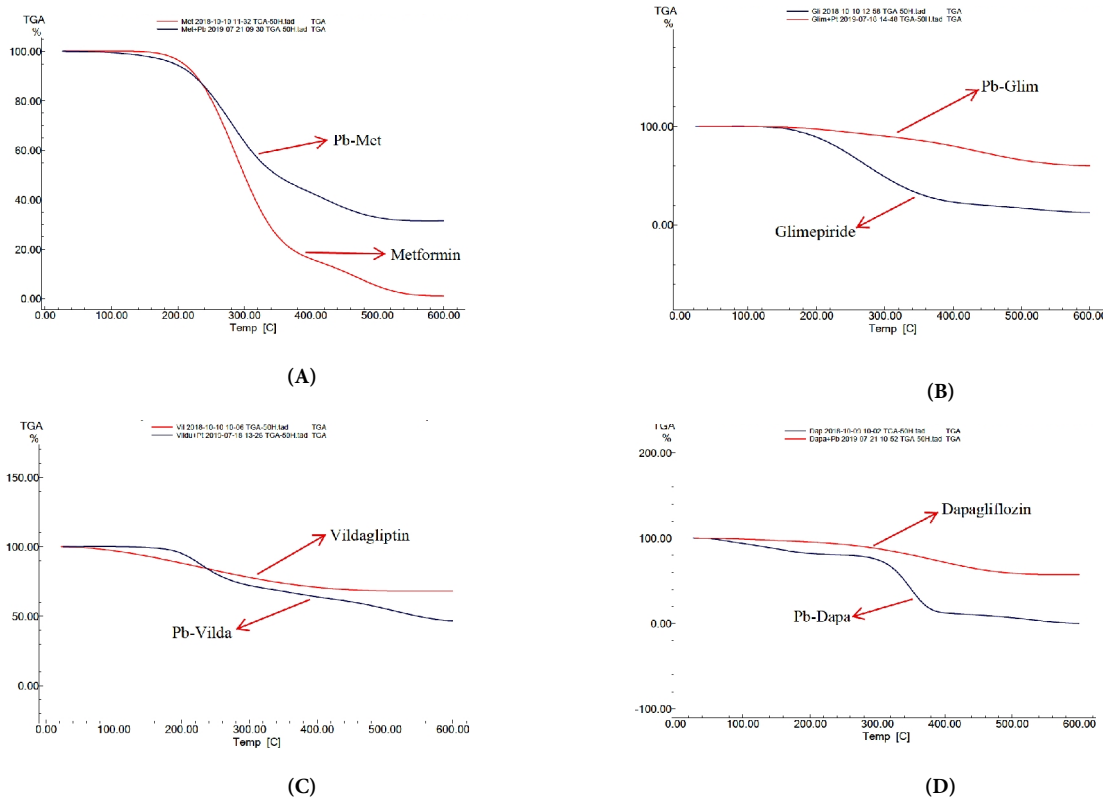


Figure 3. Overlaid TGA thermograms: metformin and Pb-metformin complex (**A**), glimepiride and Pb-glimepiride complex (**B**), vildagliptin and Pb-vildagliptin complex (**C**), dapagliflozin and Pb-dapagliflozin complex (**D**).

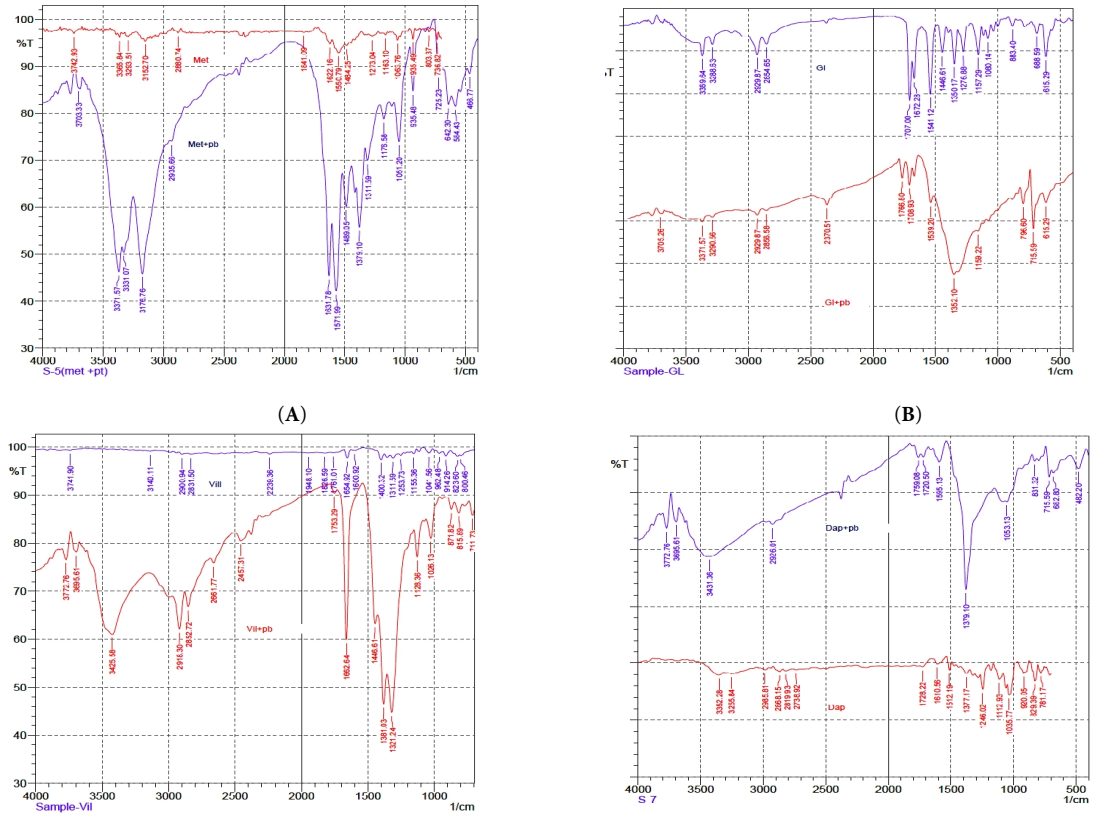


Figure 4. Overlaid IR spectra of metformin and Pb-met complex (**A**), glimepiride and Pb-glim complex (**B**), vildagliptin and Pb-vilda complex (**C**) and dapagliflozin and Pb-dapa complex (**D**).

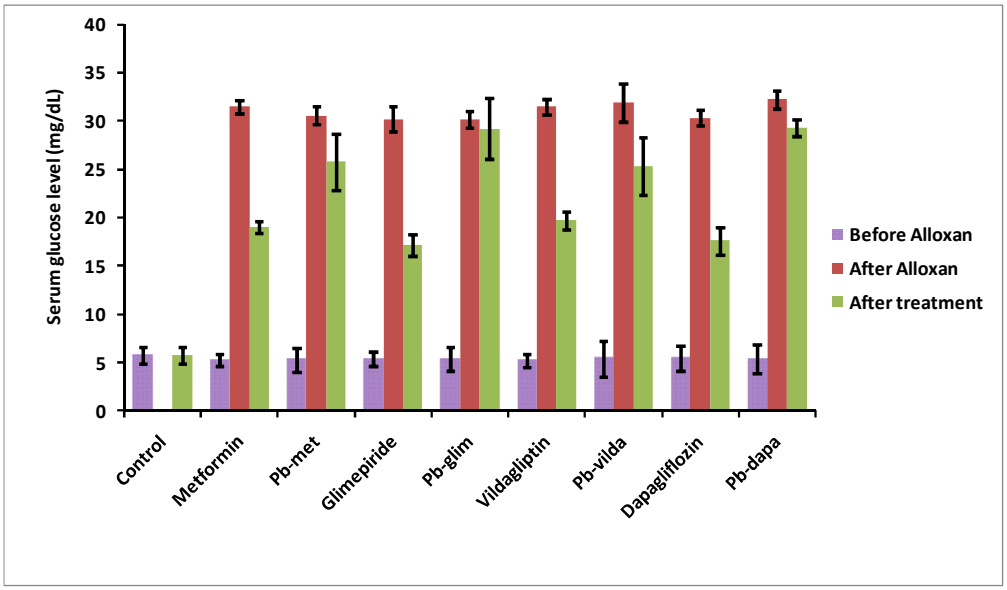


Figure 5. *In vivo* antidiabetic activity of pure drugs (metformin, glimepiride, vildagliptin and dapagliflozin) and their Pb-complexes (Pb-met, Pb-glim, Pb-vilda and Pb-dapa) in a mouse model.

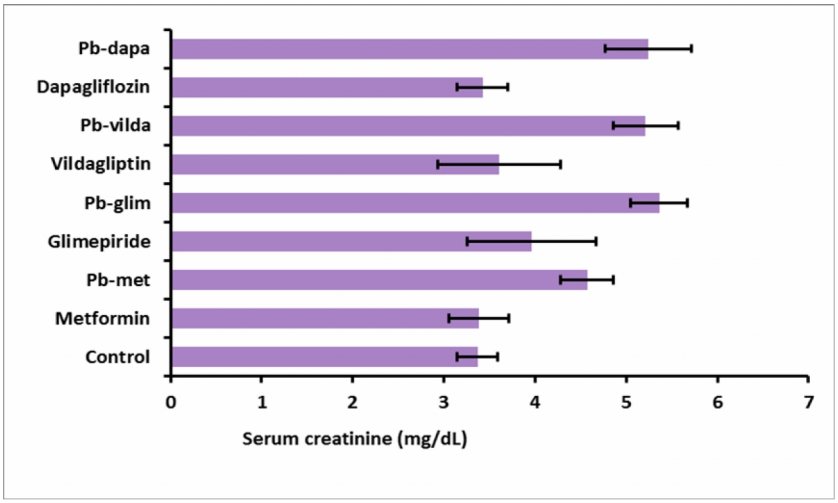


Figure 6. Serum creatinine levels before and after treatment with pure drugs (metformin, glimepiride, vildagliptin and dapagliflozin) and their Pb-complexes (Pb-met, Pb-glim, Pb-vilda and Pb-dapa) in a mouse model.

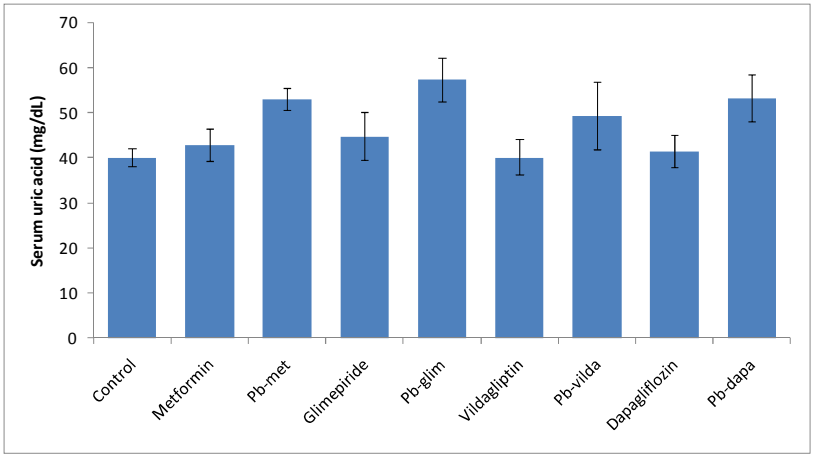


Figure 7. Serum uric acid levels before and after treatment with pure drugs (metformin, glimepiride, vildagliptin and dapagliflozin) and their Pb-complexes (Pb-met, Pb-glim, Pb-vilda and Pb-dapa) in mice model.

The percentages of weight loss for pure antidiabetic drugs and their complexes were analyzed by thermogravimetric analyses. For standard metformin, 4.29% was degraded at 205°C, whereas, Pb-metformin complex displayed completely different degradation pattern (**Fig. 3A**).

As for glimepiride, 2.36% degradation occurred at 160°C and 87.70% degradation at 600°C, whereas the Pb-glimepiride complex showed 1.45% degradation at 175°C and 40% degradation at 600°C (**Fig. 3B**). Vildagliptin showed 2.47% degradation at 92°C and 31.89% degradation at 600°C but the Pb-vildagliptin showed a strikingly different degradation pattern i.e. 2.55% degradation at 185°C and 45% degradation at 600°C (**Fig. 3C**). For pure dapagliflozin, the thermal breakdown pattern showed 17% degradation at 189°C and 94% at 516°C and Pb-dapagliflozin showed different degradation (**Fig. 3D**).

The FTIR spectra exhibited characteristics of specific molecular vibrations and stretching that serve to identify functional groups of the compounds. The FTIR spectra of pure metformin, glimepiride, vildagliptin, dapagliflozin and their Pb-complexes are displayed in (**Fig. 4**). As the pure drugs and their complexes displayed different IR patterns, this strongly suggests that they are different compounds.

The unique stretching peak of -NH of metformin at 3742.93 cm^{-1} was obtained in the downfield at 3703 cm^{-1} for Pb-metformin complex (**Fig. 4A**). Similarly, the -NH stretching vibration peak of glimepiride recorded at 3369.64 cm^{-1} shifted to 3371.57 cm^{-1} for Pb-glimepiride. In vildagliptin the principle peak for -OH vibration at 3140.11 cm^{-1} moved up field to 3425.58 cm^{-1} for Pb-vildagliptin. The -OH vibration peak for dapagliflozin recoded at 3352.28 cm^{-1} shifted in the IR spectra of the corresponding Pb-drug complexes to 3431.36 cm^{-1}. These modifications in the IR spectra suggested the formation of complexes between the lead and drugs under investigation.

The Pb-drug complexes and pure antidiabetic drugs were administered to alloxan-induced diabetic mice. The pure drugs significantly reduced blood glucose level as compared to control mice which received distilled water and normal food. In contrast, Pb-drug complexes did not show significant effect in reducing blood glucose levels after 14 days of treatment (**Fig. 5**). In fact, after 14 days of treatment with metformin, glimepiride, vildagliptin, dapagliflozin, the average glucose levels of mice reduced from 31.54 to 19.02, 30.24 to 17.20, 31.50 to 19.70 and 30.37 to 17.60 mmol/L, respectively, whereas Pb-met, Pb-glim, Pb-vilda and Pb-dapa did not reduce blood glucose levels considerably and that were found as 25.82, 29.23, 25.32 and 29.32 mmol/L, respectively.

There were no significant changes in the renal function of mice as measured by serum creatinine and serum uric acid levels after 14 days treatment with pure antidiabetic drugs viz. metformin, glimepiride, vildagliptin and dapagliflozin. However, the Pb complexes with those respective drugs increased both serum creatinine and uric acid levels, strongly indicating renal impairment (**Fig. 6** and **Fig.**7). It was found that after 14 days of treatment with metformin, glimepiride, vildagliptin, dapagliflozin, the serum creatinine level of mice increased from 3.38 to 4.57, 3.96 to 5.36, 3.60 to 5.21 and 3.42 to 5.24 mg/dL, respectively for Pb-met, Pb-glim, Pb-vilda and Pb-dapa. The serum uric acid levels also increased after treatment with Pb-met, Pb-glim, Pb-vilda and Pb-dapa compared to pure drugs. The uric acid level of Pb-met was measured at 53.13 mg/dL whereas for metformin the level was found to be 42.91 mg/dL. Likewise, for Pb-glim it was measured at 57.40 mg/dL whereas it was 44.83 mg/dL for glimepiride; for Pb-vilda it was measured at 49.36 mg/dL whereas it was 40.21 mg/dL for vildagliptin; and for Pb-dapa it was measured at 53.32 mg/dL whereas it was 41.49 mg/dL for dapagliflozin, respectively.

Conclusion

Pb was left to react with four antidiabetic drugs viz.metformin, glimepiride, vildagliptin and dapagliflozin at 70-75°C to form complexes which were characterized by studying their physicochemical properties and functional groups using DSC, TGA, TLC and FTIR spectrophotometry.

The Pb-drug complexes and pure antidiabetic drugs were administered to alloxan-induced diabetic mice. It was found that pure drugs significantly reduced blood glucose levels as compared to control mice which received distilled water and normal food after 14 days of treatment. However, Pb-drug complexes did not show significant effect in reducing blood glucose levels after 14 days of treatment. On the other hand, the Pb-complexes did increase serum creatinine and serum uric acid levels in mice, which suggests kidney tissue damage in mice.

These drugs are used in tandem with insulin, therefore it is important to verify the potential impact of lead-insulin on patients. However, there is a possibility that these administered drugs might be exposed to lead (Pb) via the patient being exposed to lead from industrial waste, drinking water and the environments. Therefore, this requires more comprehensive studies, including *in vivo* models to verify the beneficial and/or untoward effects of these Pb-drug complexes in diabetic patients.

Acknowledgement

The authors acknowledge the Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh; Department of Pharmaceutical Chemistry, University of Dhaka, Dhaka-1000, Bangladesh and Centre for Advanced Research in Sciences (CARS), University of Dhaka, Dhaka-1000, Bangladesh for providing support and facilities to carry out this research work.

Author Contribution

FA has designed and implemented the work, MZS and MAR has given the idea and supervised the research work.

Competing financial interests

There is no competing financial interest.

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