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Research Article

Metformin-Cefixime Co-administration affects Glucose Regulation and Reno-Pancreatic Histology in Alloxan-induced Hyperglycemic Rats

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Abstract

Type 2 diabetes mellitus (T2DM), is often associated with renal infections and complications, requiring antimicrobials. Metformin (Met) being the first line therapy in T2DM is co-administered with antimicrobial agents when infections coexist. Cefixime (Cef), an oral cephalosporin is effective in treatment of several bacterial infections. The current study investigated the effect of concurrent metformin-cefixime (Met-Cef) administration on glucose regulation, renal function and haematological indices in alloxan induced hyperglycaemic rats. Four groups of five wistar rats were used in the study. Groups I and II were normoglycemic receiving daily oral normal saline (10 ml/kg) and cefixime (400 mg/kg from day 14) respectively. Diabetes was induced with a single i.p. administration of 140 mg/kg alloxan monohydrate in groups III and IV which received 200 mg/kg metformin for 28 days, while group IV received cefixime in addition from day 15. Random blood glucose (RBG) was evaluated on days 8, 14 and 28, while fasting blood glucose (FBG) was evaluated on days 1, 14, 21 and 28. At the end of the study animals were humanely sacrificed and blood obtained was used for the determination of renal and haematological parameters. Relative weights of kidneys and pancreas were determined and histopathological evaluation of the organs also conducted. There was a statistically significant reduction ($p<0.05$) in RBG and a decrease in FBG between metformin treated and Met-Cef treated groups at the end of two weeks co-administration. There was no significant difference in electrolytes, urea, creatinine and haematological parameters. Pancreatic histology showed amelioration of necrosis of pancreatic acini in the Met-Cef treated rats compared to Met only treated rats. Concurrent Met-Cef treatment did not result in any differences in renal histology in comparison with metformin treated group. Data from the study revealed a possible augmentation of the hypoglycaemic effect of metformin by cefixime, without any deleterious effect on renal and haematological parameters.

Keywords: Cefixime, metformin, drug interaction, hyperglycemia, diabetes mellitus

Introduction

Type 2 Diabetes mellitus (T2DM) is a disorder that results from pancreatic insufficiency, insulin resistance or suboptimal responses to insulin mediated actions.[1] The disorder is of global importance resulting in significant morbidity and mortality,[2] with a high prevalence worldwide and a growing incidence.[3] Like cardiovascular and other non communicable diseases, diabetes is emerging as a threat to global development.[4] Contemporary management of diabetes combines lifestyle modification and therapeutic interventions to attain adequate glycaemic control and prevent micro and macrovascular complications.[5] Metformin, a biguanide which is relatively safe, inexpensive and without the hypoglycaemic effects of sulfonylureas and insulin, is recommended worldwide as first line therapy for T2DM.[6] Although severally reported to be contraindicated in patients with renal impairment, others[7] argue that the lactic acidosis so often associated with metformin use could be a consequence of diabetes itself corroborating the postulation of earlier researchers who argued that lactic acidosis has not been evidenced to correlate metformin concentrations.[8] However current opinion still employs caution in administration of this drug particularly in diabetic patients with chronic kidney disease.

Diabetic patients are more susceptible to infections than the general population, suffering a higher frequency and/or severity of such infections.[9] This increased risk of both viral and bacterial infection consequently affects disease prognosis and may result in death.[10] However, some infections are almost exclusively seen in diabetic patients and these include rhino-cerebral mucormycosis which has been reported in both developed and developing countries,[11] including cystitis, complicated urinary tract infections, emphysematous pylonephritis and malignant otitis externa with consequent significant morbidity and mortality.[12]

In diabetic patients, urinary tract infections are usually more severe with associated increase in risk of complications.[13] Studies have also shown that patients with both Type 1 diabetes mellitus

and T2DM are at increased risk of both urinary and respiratory infections and with higher recurrence rates.[14]

Cefixime a third generation cephalosporin has been demonstrated to be very effective in uncomplicated urinary tract infections as single daily doses for durations of five days and above.[15] It has also been shown to produce very large concentrations in renal parenchyma, with reduction in tissue concentrations of cefixime being much slower than that observed in serum.[16] Given the broad spectrum of this agent and its convenient dosing, it is therefore an important agent clinically utilized in the treatment of urinary tract infections in T2DM. Decompensation in T2DM, which further increases the risk and progression of infection, is most times attributed to therapeutic failure due to patient non-compliance or the use of fake and counterfeit drugs. Meanwhile some of these therapeutic failures or negative outcomes in pharmacological actions may be direct consequences of drug/drug interactions which may necessitate modification or alteration in therapeutic drug regimens. The use of metformin and cefixime in the co-morbid state of T2DM and urinary infections thus presents a new clinical challenge with possible beneficial or adverse consequences. Cefixime and metformin are primarily cleared from the system via the kidneys unchanged. In the current study, the effect of metformin and cefixime co-administration on glucose regulation, haematological indices, kidney/pancreatic function and histology were investigated in alloxan induced diabetic wistar rats.

Materials and Methods

Animals

Male and female wistar rats obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria weighing 150-200 gm were used for this study. The animals were placed on standard institutional feed and water *ad libitum*. The animals were treated according to approved institutional animal handling and the CPCSEA 1986 and NIH animal care guidelines. Animals were acclimatized for 72h in the experimental room before the conduct of the main study.

Drugs

Metformin (Merke Sante s.a.s France) and Cefixime (Fredum Pharmaceuticals) were used as the primary investigational drugs in the study. Alloxan monohydrate (Sigma Aldrich, St. Louis, USA) was used for the induction of diabetes.

Induction of diabetes

Diabetes was induced after rats were subjected to overnight fast and they received single intraperitoneal injections of alloxan monohydrate freshly prepared in normal saline, at a dose of 140 mg/kg.[18] Because alloxan is capable of producing fatal hypoglycaemia as a result of massive pancreatic insulin release, the rats were maintained on 5% glucose solution via their drinkers for the 24h following alloxan administration to prevent hypoglycaemia.[19] Seventy-two hours after alloxan administration, blood glucose levels of the rats were determined following an overnight fast using the glucose oxidase method with the aid of an Accu Check Active digital glucometer. Animals with blood glucose levels of 250 mg/dl and above[36] were considered diabetic and included in the main study.

Experimental design/protocol

The study consisted of four groups of rats. Two groups of rats were normoglycemic, with the first group (group I) receiving 10 ml/kg normal saline and serving as negative control. The second normoglycemic group (group II) received cefixime alone at a dose of 400 mg/kg daily for 14 days. Two other groups were made hyperglycaemic with alloxan. The first hyperglycaemic group (group III) received metformin orally at 200 mg/kg daily for 28 days, while the second hyperglycemic group (group IV) received a combination of metformin and cefixime. However, while metformin was administered for the entire 28 days of the study, cefixime was administered orally only in the last 14 days of the study with normal saline being administered along with the metformin in the preceding 14 days. Animals were grouped with five animals per group. Random blood glucose levels were determined on day 8, 14 and day 28, respectively. Fasting blood glucose was determeined on days 1, 14, 21 and 28. At the end of the experimental duration, animals were exsanguinated under light chloroform anaesthesia.[20] Whole blood was used for the determination of haematological indices, while serum obtained from the blood was used for the determination of serum urea, creatinine and electrolytes.

Haematological indices

Whole blood collected from the rats was used to determine selected haematological indices using the methods as described.[21]

Renal function tests

Serum sodium and potassium were determined using the flame photometric method while serum chloride was determined using the mercuric nitrate titrimetric method of Schales and Schales.[22] Determination of serum urea level was conducted based on the diacetyl monoxime method using thiosemicarbazide,[23] and serum creatinine was determined by the Rehberry Method.

Organ weight determination

After the collection of blood, the animals were dissected and the kidneys and pancreas were removed and weighed to determine their weight in relation to the total body weight of the animals.

Histopathological evaluation

After animals were dissected, the kidneys and pancreas were removed and preserved in 10% formalin solution before being processed for histopathological evaluation. Thin Hematoxylin and Eosin stained sections of about 6 microns were prepared following the method as previously described.[24]

Results and Discussion

The random and fasting blood glucose of diabetic rats were significantly ($p<0.001$) higher than the non diabetic controls at the beginning of the study, establishing hyperglycemia. However, treatment with 200 mg/kg metformin reduced random blood glucose significantly ($p<0.05$) by the 8th day in the diabetic rats . Random blood glucose in the Met-Cef group was significantly lower ($p<0.01$) than that of the Met group by day 28. There was a statistically significant and pronounced reduction in random blood glucose levels ($p<0.01$) in Met-Cef treated rats between Day 1 and Day 28. However, cefixime alone did not result to any change in glucose levels (Fig. 1). Fasting blood glucose levels were also significantly ($p<0.01$) reduced in the group that received metformin alone by day 14 and 28, while there was significant reduction ($p<0.001$) by day 14, 21 and 28 in the metformin-cefixime group. The fasting blood glucose in the met-cef group on day 28 was markedly reduced compared to that of the met alone group although not statistically significant (Fig. 2).

In addition, Met-Cef co-administration did not significantly affect urea and creatinine levels in hyperglycaemic rats as compared to control and other drug treated groups, though rats in the cefixime alone group had slightly higher serum creatinine levels (Fig 3).

No significant differences were observed in serum sodium, potassium chloride and bicarbonate ion concentration of the rats in all treated groups in comparison with the control (Table 1). The relative weights of the kidney of rats treated with metformin were slightly higher than those of Met-Cef and cefixime treated groups, while there was no significant difference in the relative weights of the pancreas across the treatment groups (Table 2). There was also no statistically significant difference ($p>0.05$) in the haematological parameters investigated between rats treated with metformin alone and those that received both Met-Cef compared to the control (Table 3).

The kidney of non diabetic control and cefixime treated rats showed normal tubules and epithelial cells (Plates I-II). However, the drug treated diabetic groups showed some renal tubular collapse and necrosis of tubular epithelial cells with mononuclear cellular infiltration (Plate III-IV). Co-administration of cefixime and metformin ameliorated the alloxan induced changes in pancreatic histology as typified by absence of focal areas of necrosis of pancreatic acini which were observed in diabetic rats that received metformin alone (Plate VII-VIII). However, the pancreas of non diabetic rats showed no pathological alterations (Plate V-VI).

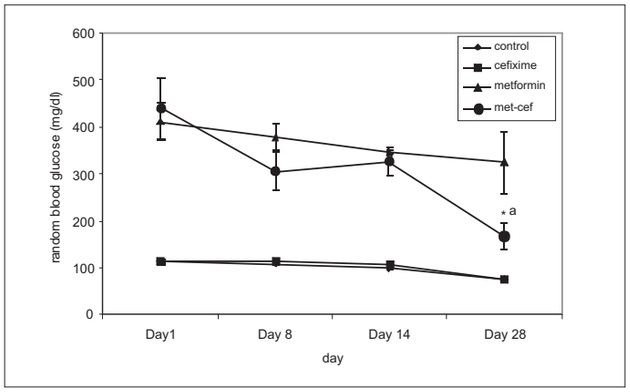

Fig. 1: Effect of metformin-cefixime co administration on random blood glucose (RBG) levels in wistar rats.*statistically significant decrease ($p<0.01$) in RBG of met-cef group as against met alone; a = statistically significant decrease ($p<0.01$) in RBG in met-cef group on day 28 in comparison to day 1 (ANOVA and Tukey post hoc). Values are mean ± SEM, $n=5$

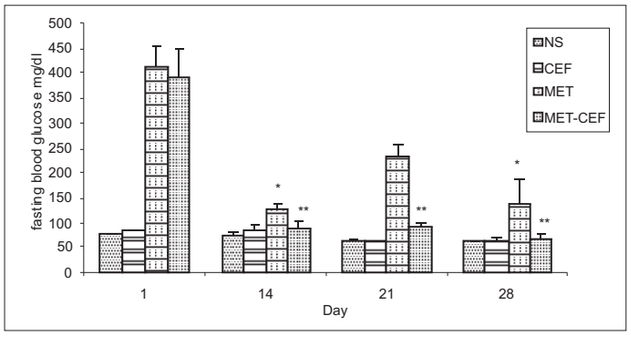

Fig. 2: Effect of metformin-cefixime co administration on fasting blood glucose (FBG) in Wistar rats. *statistically significant decrease ($p<0.01$) in FBG in metformin group on day 14 and 28 in comparison with day 1; **statistically significant decrease ($p<0.001$) in met-cef group in comparison with its value on day 1(ANOVA and Tukey post hoc). Values are Mean ± SEM, $n=5$

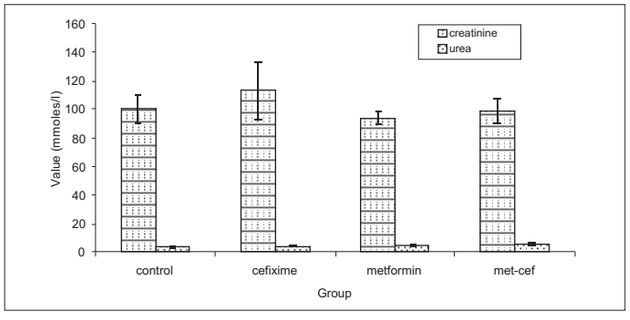

Fig. 3: Effect of metformin-cefixime co-administration on urea and creatinine in Wistar rats. No statistically significant difference ($p>0.05$, ANOVA and Tukey post hoc). Values are means ± SEM of 5 observations

Table 1. Effect of metformin-cefixime co-administration on some serum electrolytes

Group	Sodium (mmoles/L)	Potassium (mmoles/L)	Chloride (mg%)	Bicarbonate (mg%)
Control	133.5±4.249	4.800±0.1155	96.17±2.810	24.00±2.556
Cefixime (400mg/kg)	127.2±8.470	4.900±0.4848	95.60±3.124	23.40±1.860
Metformin (200mg/kg)	137.8±11.05	4.525±0.3683	96.67±4.055	28.00±0.817
Met(200mg/kg)-Cef(400mg/kg)	140.2±8.558	4.860±0.5870	96.80±3.929	25.20±2.223

No statistically significant differences in the electrolyte levels between the groups ($p>0.05$) ANOVA and Tukey post hoc). Values are mean ± SEM, n=5.

Table 2. Effect of metformin-cefixime co-administration on relative kidney and pancreas weights

	Relative Organ Weight (Percentage)	
Group/Organ	**Kidney**	**Pancreas**
Control	0.6383±0.04045	0.6683±0.0817
Cefixime	0.6060±0.03265	0.6740±0.1065
Metformin	0.7533±0.09597	0.7733±0.1650
Metformin-Cefixime	0.6360±0.04578	0.7900±0.1990

No statistically significant differences in the sizes of tissues ($p>0.05$, ANOVA and Tukey post hoc), Values are mean ± SEM, n = 5.

Table 3. Effect of metformin-cefixime co-administration on some hematological parameters

Group	PCV (%)	RBC ($\times10^{12}$/L)	WBC ($\times10^{9}$/L)
Control	44.83±0.749	4.130±0.2709	1.608±0.04715
Cefixime (400mg/kg)	44.60±1.208	4.498±0.5040	1.536±0.05810
Metformin (200mg/kg)	46.25±1.031	4.448±0.5156	1.490±0.09460
Met(200mg/kg)-Cef(400mg/kg)	43.00±1.183	4.784±0.3789	1.836±0.19540

No statistically significant difference ($p>0.05$, ANOVA and Tukey post hoc). Table shows mean ± SEM of PCV, RBC and WBC, n = 5.

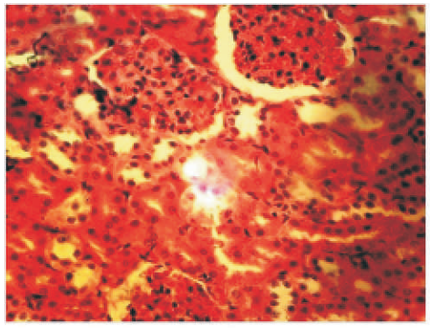

Plate I: Photomicrograph of a section of the kidney of a control rat (H & E ×400). There were no observable pathological findings.

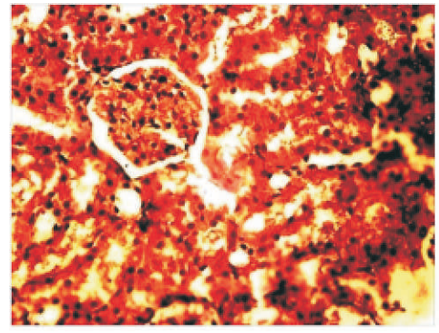

Plate II: Photomicrograph of a section of the kidney of a cefixime treated rat (H & E ×400). There were no observable pathological findings.

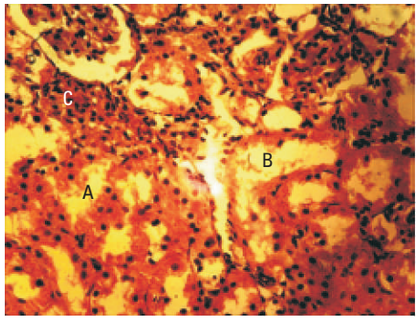

Plate III: Photomicrograph of a section of the kidney of a metformin treated alloxan induced hyperglycaemic rat (H & E ×400). Kidney of metformin only treated group showing some renal tubular collapse (A), focal area of necrosis of renal tubular epithelial cells (B) and mononuclear cellular infiltration (C).

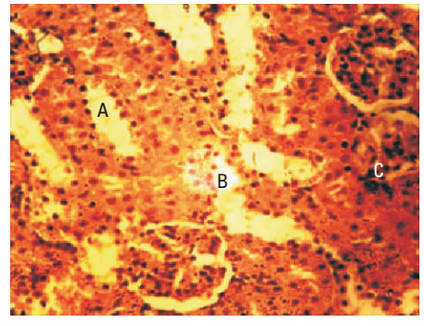

Plate IV: Photomicrograph of a section of the kidney of a metformin-cefixime treated alloxan induced hyperglycaemic rat (H&E ×400). Kidney of cefixime-metformin treated group also showing some renal tubular collapse (A), focal area of necrosis of renal tubular epithelial cells (B) and mononuclear cellular infiltration (C).

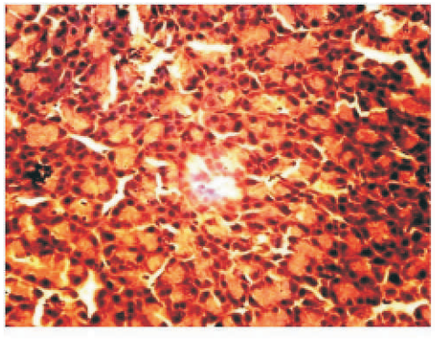

Plate V: Photomicrograph of a section of the pancreas of a control rat (H&E ×400). There were no observable pathological findings.

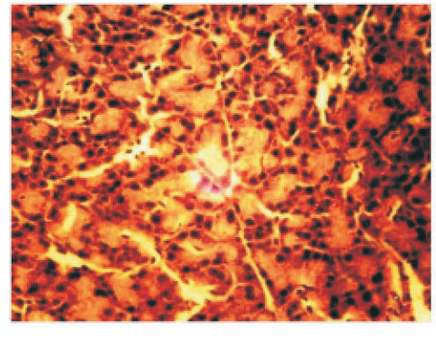

Plate VI: Photomicrograph of the section of the pancreas of a cefixime treated rat (H&E ×400). No significant histopathological findings were seen.

Administration of 200 mg/kg of metformin caused significant reduction in the blood glucose levels of the hyperglycaemic rats [25]. Results of this study demonstrate that Met-Cef co-administration may probably increase hypoglycaemic effect of metformin typified by the reduction in both fasting and random blood glucose in the combination group. Cefixime and metformin are both excreted renally via glomerular filtration and tubular secretion. Previous data showed increase in plasma concentration of metformin in the presence of cephalexin due to competitive renal excretion.[17] Being a cephalosporin, a similar mechanism may underlie the increased hypoglycaemic action of metformin due to its accumulation in plasma. The concurrent administration of metformin and cefixime produced no changes on the haematological parameters in all the treated groups that received either or both drugs. Although long time use of metformin has been reported to be associated with vitamin B_{12} deficiency related haematological abnormalities,[26-27] this was not the case in this 28-day study. Cefixime administration is associated with a number of haematological anomalies e.g. Leucopaenia, neutropaenia and eusinophilia, and is seen in less than 2% of the population.[28] Because cefixime is most often prescribed for a period lasting between 7 and 14 days, the possibility of an additive or cumulative deleterious effect on haematological parameters may be limited.

Varying degrees of electrolyte abnormalities have been reported in diabetes and have been seen in diabetic coma, progression of diabetic nephropathy and other diabetic complications such as cardiovascular diseases.[29] The study reveals only the mean potassium level of diabetic animals in Metformin alone group to be slightly lower, and this effect has also been demonstrated by earlier studies.[30] This may be a beneficial effect of metformin as elevated potassium ions are known to inhibit glucagon secretion in addition to stimulating the secretion of insulin,[31] while elevated sodium ion is associated with increased risk of cardiovascular diseases. It is thus of importance that electrolytes be monitored not only as possible consequence of other concurrently administered drugs but may also provide important information regarding renal function as an important parameter in disease prognosis and outcome of therapeutic interventions.

Serum urea and creatinine levels remain very useful indices in monitoring renal function. Metformin is one of the drugs substantially excreted by the kidneys and the risk of accumulation and life threatening Metformin Associated Lactic Acidosis (MALA) increases with the degree of impairment of renal function and abnormal creatinine clearance from any cause. As such, metformin is contraindicated in any condition predisposing to increase lactic acid production or decrease lactic acid metabolisim. Conversely, the use of cefixime has been associated with increase in serum creatinine levels,[32] which is also evident from this study. Metformin-

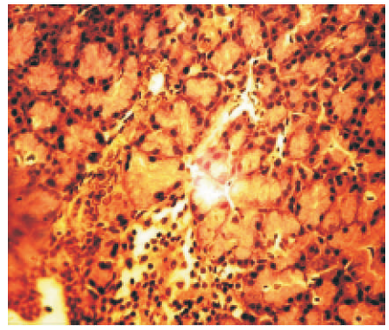

Plate VII: Photomicrograph of the section of the pancreas of a metformin treated alloxan induced hyperglycaemic rat (H & E ×400). The plate showed focal areas of necrosis of pancreatic acini with less cellular endocrine portion of pancreas (A).

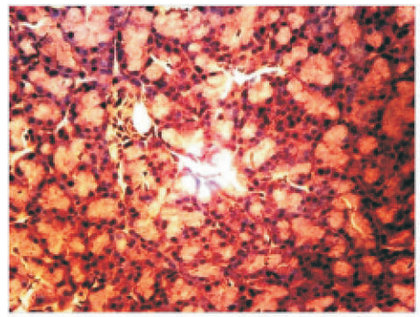

Plate VIII: Photomicrograph of a section of the pancreas of a metformin-cefixime treated alloxan induced hyperglycaemic rat (H&E ×400). No significant histopathological findings were seen.

Cefixime co-administration did not however alter urea and creatinine levels in hyperglycaemic rats for the duration of 14 days. There is however the need to monitor kidney function when this combination is used for longer periods or in other situations that may predispose patients to possible accumulation of metformin, or increase in creatinine levels.

The relative kidney and pancreas weights of the drug treated groups and control showed no statistical difference in sizes, although the diabetic animals showed an increase in kidney weight compared to non diabetic control. Previous data shows increase kidney and pancreas weights in alloxan induced hyperglycemia.[30] An absence of significant difference in relative organ weight, may not necessarily predict an absence of pathological conditions or functional alterations. This is evidenced from the pathological lesions seen in the pancreas of animals in the metformin treated group. However, the possibility of an amelioration of this effect exists with co-administration of metformin and cefixime as no significant histopathological findings were seen in the pancreas of animals in this group. This could probably result from protection of pancreatic cells from

progressive damage enhanced by alloxan and/or the enhancement of the regeneration of these cells. This is further corroborated by the significant reduction in blood glucose of the Met-Cef treated animals at the end of the study in comparison to Meformin alone treated animals.

The kidneys of the alloxan induced hyperglycemic rats that received drug treatment showed marked tubular damage and necrosis of tubular epithelial cells. The degenerative changes in the histology of kidney brought about by alloxan administration are similar to earlier observations.[33] However, earlier reports suggest a renoprotecctive effect of metformin in diabetic animals through its antioxidant activities.[34-35] The above report employed other models of type 2 diabetes mellitus and experiments were for longer periods. This may partly explain why no ameliorative effects of metformin on the kidneys were seen in this present study.

Conclusion

The outcome of this study was observed to be a possible potentiation of the glucose lowering effect of metformin with co administration with cefixime, with a possible amelioration of pancreatic damage associated with alloxan administration. Renal function was apparently unaffected and this would be of benefit while treating infections with cefixime in the presence of metformin. The effect of this drug combination on other aspects of the metabolic syndrome in diabetes will require investigation to enable a greater degree of prediction on the safety and efficacy issues related to the combination.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Mizokami-Stout K, Cree-Green M, Nadeau KJ. Insulin resistance in type 2 diabetic youth. Curr Opin Endocrinol Diabetes Obes 2012; 19:255-262.
2. Cekic V, Vasovic V, Jakovljevic V, Mikov M, Sabo S. Hypoglycaemic action of stevioside and a barley and brewer's yeast based preparation in the experimental model on mice. Bosn J Basic Med Sci 2011; 11:11-16.
3. Khan MU. Lifestyle modification in the prevention of Type II Diabetes Mellitus. OMJ 2012; 27:170-171.
4. Alwan A. Raising the priority accorded to diabetes in global health and development: A promising response. Int J Diabetes Mellit 2010; 2:139-140
5. Fowler MJ. Microvascular and macrovascular complications of diabetes. Clin Diabetes 2008; 26:77-82.
6. Senior PA. Type 2 diabetes, metformin and lactic acidosis-defining the risk and promoting safe practice. Diab Med 2012; 21:161-163
7. Heaf JG, van Biesen W. Metformin and chronic renal impairment: a story of choices and ugly ducklings. Clin Diabetes 2012; 29:97-101
8. Lipska KJ, Bailey CJ, Inzucchi SE. Use of metformin in the setting of mild-to-moderate renal insufficiency. Diabetes Care 2011; 34:1431-1437.
9. Schaberg DS. Case Study: Infections in Diabetes Mellitus. Diabetes Spectrum 2002; 15:37-43.
10. Mohan V, Unnikrishnan R, Thomas N, Bhansali A, Wangnoo SK, Thomas K. Pneumococcal infections and immunization in diabetic patients. J Postgrad Med 2011; 57:78-81
11. Anitha PK, Choudhury MK, Nair S, Devi SC, Dhodapkar R, Nagaraja M, Gayad S. Successful management of rhino-cerebral mucormycosis in a patient with diabetic ketoacidosis. JIAM 2011; 12:314-316.
12. Chin-Hong PV. Infections in patients with diabetes mellitus: importance of early recognition, treatment, and prevention. Adv Stud Med 2006; 6:71-81.
13. Hakeem LM, Bhattacharyya DN, Lafong C, Janjua KS, Serhan JT, Campbell IW. Diversity and complexity of urinary tract infection in diabetes mellitus. Br J Diabetes Vasc Dis 2009; 9:119-125.
14. Muller, LMAJ, Gortner KJ, Hak E, Goudzwaard WL, Schellevis FG, Hoepelman AIM, Rutten GEHM. Increased risk of common infections in patients with type 1 and type 2 diabetes mellitus. Clin Infect Dis 2005; 41:281-288.
15. Hsu G, Chou M. Efficacy of Cefixime in Urinary Tract Infection. J Med Sci 1993; 14:159-164.
16. Leroy A, Oser B, Grise P, Humbert G. Cefixime penetration in human renal parenchyma. Antimicrob Agents Chemother 1995; 39:1240-1242.
17. Jayasagar G, Krishna Kumar M, Chandrasekhar K, Madhusudan RC, Madhusudan RY. Effect of cephalexin on the pharmacokinetics of metformin in healthy human volunteers. Drug Metabol Drug Interact 2002; 19: 41-48.
18. Herrera C, García-Barrantes PM, Binns F, Vargas M, Poveda L, Badilla S. Hypoglycemic and antihyperglycemic effect of Witheringia solanacea in normal and alloxan-induced hyperglycemic rats. J Ethnopharmacol 2011; 133:907-910.
19. Hamden K, Alloucheb N, Damakb M, Elfekia A. Hypoglycemic and antioxidant effects of phenolic extracts and purified hydroxytyrosol from olive mill waste in vitro and in rats. Chem Bio Interact 2009; 180: 421-432.
20. Edem GO. Hypoglycemic effects of ethanolic extracts of alligator pear seed (Persea Americana Mill) in Rats. Eur J Sci Res 2009; 33: 669-678.
21. Ibu JO, Adeniyi KO. A Manual of Practical Physiology. Jos University Press Ltd, Jos: Nigeria, pp 17-21, 26-28, 1989.
22. Schales O, Schales SS. A simple and accurate method for the determination of chloride in biological fluids. J Biol Chem 1941; 140:879-884.

23. Natelson S, Scott ML, Beffa CA. Rapid method for the estimation of urea in biologic fluids. Am J Clin Pathol 1951; 21: 275-281.

24. Tulpule SS, Ghaji A. Light microscope and histology staining procedures. Hand book for laboratory technologists and medical students. Ahmadu Bello University Press: Zaria-Nigeria. pp. 24-34, 1987.

25. Sheehan MT. Current therapeutic options in type 2 diabetes mellitus: a practical approach .Clin Med Res 2003; 1:189-200.

26. Liu KW, Dai LK, Jean W. Metformin-related Vitamin B12 deficiency. Age Ageing 2006; 35:200-201.

27. de Jager J, Kooy A, Lehert P, Wulfelle MG, Kolk J, Bets D, Verburg J, Donker AJM, Stehouwer CDA. Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomised placebo controlled trial. BMJ 2010; 340:c2181- c2187.

28. Stockley IH. General consideration and an outline survey of some basic interaction mechanism. In: Stockley I.H. (Ed) Stockley's Drug Interactions. The Pharmaceutical Press: London (UK), pp. 1- 692, 2002.

29. Shadid SM, Mahboob T. Electrolyte and Na^{+}- K^{+} - ATPase - Potential risk factors for development of Diabetic nephropathy. Pak J Pharm Sci 2008; 21:72-179.

30. Vallon V, Richter K, Blantz RC, Thomson S, Osswald H. Glomerular hyperfiltration in experimental diabetes mellitus: potential role of tubular reabsorption. J Am Soc Nephro 1999; 10:2569-2576.

31. Smith SS, Bhatena SJ, Wilkins SD, Recant L. Potassium ion concentration alters glucagon secretion independently of insulin in the isolated rat pancreas. Diabetologia 1982; 22:188-193.

32. Tally FP, Desjardins RE, McCarthy EF, Catwright K. Safety profile of cefixime. Paediatr Infect Dis J 1987; 6:976-980.

33. Ragavan, B, and Krishnakumari S. Effect of T. Arjuna stem bark extract on histopathology of liver, kidney and pancreas of Alloxan induced diabetic rats, Afr J Biomed Res 2006; 9:189 - 197.

34. Alhaider AA, Korashy HM, Sayed-Ahmed MM, Mobark M, Kfoury H, Mansour MA. Metformin attenuates streptozotocin-induced diabetic nephropathy in rats through modulation of oxidative stress genes expression. Chem Biol Interact 2011; 192:233-342.

35. Kim J, Shon E, Kim C, Kim JS. Renal podocyte injury in a rat model of type 2 diabetes- is prevented by metformin. Exp Diabetes Res 2012; 2012: 210821-210829.

36. Morani AS, Bodhankar SL. Neuroprotective effects of early treatment with pioglitazone and pyridoxine in alloxan induced diabetic rats. Pharmacologyonline 2007; 418-428.