ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Selenocaffeine And Zinc-Alphatocopherol Complexes as Noval α-amylase and α-glucosidase Inhibitors Potential Source of Anti-Diabetic Drug.

Mohamed I. Kobeasy[1,2]*, A.Y. El-Naggar[1,3], and Sayed A. Fayed[2].

[1]Chemistry Department, Faculty of Science, Taif University, P.O. Box 888, Al-Hawiah, Taif 21974, Saudi Arabia
[2] Department of Biochemistry, Faculty of Agriculture, Cairo University, Giza, Egypt
[3]Egyptian petroleum research institute, Nasr city, Cairo, Egypt

ABSTRACT

This article aimed to synthesis of two anti-diabetic bio-compounds which were included of selenium-caffeine and zinc α–tocopherol. These compounds were chemically characterized microanalytical techniques and infrared spectra. The inhibition activity of these compounds toward α-amylase and α-glucosidase enzymes were assessed. The selenium compound has more efficient than zinc compound toward α-amylase and α-glucosidase enzymes with IC_{50} value of 0.20 and 0.36 , 0.19 and 0.32 µg for selenocaffeine and zinc-alphatocopherol respectively. From the biological analyses, it's clearly obviously that both of these new compounds can be used as anti-diabetic drugs.

Keywords: selenium, caffeine; zinc; α–tocopherol; infrared; α-amylase; α- glucosidase, diabetic agent.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Chronic diabetes gives way to several complications of the illness by damaging the perivascular and cardiovascular systems. These include retinopathy, nephropathy, neuropathy and cardiovascular disorders [1]. The most common complication of diabetes is a diabetic kidney disease (DKD) frequently leading to end-stage renal disease (ESRD) and eventually to renal transplantation [2]. Although the heterogeneous pathogenesis of DKD is still unclear [3,4], the proposed mechanisms associated directly with diabetic complications include increased polyol pathway flux, increased hexosamine pathway and formation of advanced glycation end products (AGEs), and also activation of protein kinase C (PKC) pathway [5,6]. All these pathways are associated with oxidative stress directly or indirectly [7]. As known, increased concentrations of reactive oxygen species (ROS) in living organisms generate oxidative stress which is an important cause of cell injury during diabetes [8]. Since the existing treatment plans are still inadequate, it is important to search for new treatment approaches for DKD [9]. The great majority of the research focused on oxidative stress and antioxidants [10] and several studies reported that selenium administration improve antioxidant levels and protects the body from diabetes [11, 12]. Podocyte loss, thickening of glomerular basement membrane, dysfunction of glomerular endothelium and deposition of extracellular matrix components in the mesangial area are the common structural alterations due to DKD [13,14]. Selenate functions as an integral component of several enzymes such as, glutathione peroxidases, deiodinases and selenoproteins [12]. Glutathione peroxidase was the first determined enzyme protecting cellular membranes from oxidants [15]. In addition to its role in enzyme function, selenate also acts as an antioxidative agent [16]. Selenate prevents the progress of diabetes [16] and it has been suggested as an attractive drug in therapy of diabetes [12].

The cellular zinc levels are tightly regulated, and disturbances of zinc homeostasis have been associated with diabetes mellitus, a disease characterized by high blood glucose concentrations as a consequence of decreased secretion or action of insulin [17]. Dysregulation of Zn homeostatic metabolism within the pancreas impairs a multitude of key processes, including glycemic control [18]. Zn dyshomeostasis, both systemically and in the pancreas, plays an intricate role in the pathology of both type 1 and type 2diabetes mellitus (DM) [19–21]. Suboptimal Zn status has been suggested to decrease insulin secretion from the pancreas [22]. Severe Zn deficiency induces hyperglycemia and hyperinsulinemia [20], directly implicating Zn in systemic glucose regulation.. Consistent with a critical role for Zn in this process, individuals with type 1 DM often have low serum Zn concentrations [23]. The fact that Zn is stored and secreted from the pancreas along with insulin [24] and that it is essential for the synthesis and structural stability of insulin [25] prompted investigators to test the insulin-mimetic and anti-diabetic potential of Zn *in vitro* systems as well as in animal models of diabetes. The first report showing that Zn can mimic insulin was presented in 1980, when it was shown that Zn, in the form of zinc chloride ($ZnCl_2$) can mimic insulin in its ability to stimulate lipogenesis in rat adipocytes [26], even though a connection between Zn and diabetes had been made 14 years earlier, when Quarterman *et al*. demonstrated that Zn deficient animals were less sensitive to insulin and concluded that Zn was in some way involved with insulin action [27].

In this paper, we discuss the efficient of zinc with α-tocopherol and selenium with caffeine as biological compounds, respectively, an overview of its insulin-mimetic and anti-diabetic effects.

MATERIALS AND METHODS

Chemicals

Caffeine (Aldrich), DL- α–tocopherol (Applichem GmbH Company, Germany), $Zn(NO_3)_2.6H_2O$, SeO_2, α–amylase and α- glucosidase were purchased from (Aldrich).

Preparation of seleno caffeine complex

A selenium metal (1 mmol) and caffeine (4 mmol) was refluxed toluene solvent (50 mL) for 24 hrs at 60 ^oC. The resultant light brown solution were reduced to *ca*. ⅓ of its volume and cooled to room temperature. The solid beige complex obtained was then collected by filtration, washed with little amount of toluene and dried in *vacuo* over anhydrous $CaCl_2$. Elemental analysis, Calcd: %C= 44.91, %H= 4.71, %N= 26.19, %Se= 9.23; Found: %C= 44.43, %H= 4.55, %N= 26.08, %Se= 9.12.

2-3-Preparation of zinc-α–tocopherol complex

A solution of α–tocopherol (2 mmol in 50 mL methanol)was added to a solution of zinc(II) nitrate hexahydrate (1mmol in 20 mL methanol). The resulting mixtures were refluxed for 2hrs under continuous stirring. The obtained viscous liquid with yellowish brown colored was obtained without precipitation. The despite numerous attempts, we have not be able to collect any product in solid powder form.Elemental analysis, Calcd: %C= 66.29, %H= 9.59, %N= 2.67, %Zn= 6.22; Found: %C= 66.02, %H= 9.43, %N= 2.57, %Zn= 6.01.

Instrumentals

The elemental analyses of carbon, hydrogen and nitrogen contents were performed using a Perkin Elmer CHN 2400 (USA). The infrared spectra were recorded on Bruker FTIR Spectrophotometer (4000–400cm^{-1}).

α- Amylase inhibition assay

The α-amylase inhibitory activity was determined by the method described [28] with slight modification.A total of 500 µL of two complexes and 500µL of 0.02 M sodium phosphate buffer (pH6.9 with 0.006 M NaCl) containing α-amylase solution (1.0 U/mL) was incubated at 25 °C for 10 min. After pre incubation, 500µL of 1% starch solution was added to 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl). The reaction mixture was then incubated at 25 °C for 10 min. The reaction was stopped by adding 1.0 mL of 3,5 dinitrosalicylic acid (DNS) color reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted by adding 10 mL of distilled water, and the absorbance was measured at 540 nm.The α-amylase inhibitory activity was calculated according to the following equation:

$$\text{Inhibition (\%)} = (A_{control} - A_{sample})/A_{control} \times 100$$

where $A_{control}$was the absorbance of the control (without complex), A_{sample} was the absorbance in the presence of complex.

α-Glucosidase inhibition assay

A modified version of the assay described by [29]. A volume of 500 µl of two complexes were diluted with 100 µL of o.1 M potassium phosphate buffer (pH 6.9) containing α-glucosidase solution (1.0 U/mL) and was incubated in 96-well plates at 25 °C for 10 min. After pre incubation, 50 µL of 5 mM p-nitrophenyl-α-glucopyranoside solution in 0.1 M potassium phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25 °C for 5 min. The α-glucosidase inhibitory activity was expressed as percentage of inhibition and was calculated as follows:

$$\text{Inhibition (\%)} = (A_{control} - A_{sample}) / A_{control} \times 100$$

Where *A control* was the absorbance of the control (without complexes), *A sample* was the absorbance in the presence of complexes.

Statistical analysis

The obtained data were statistically analyzed according to the method described in literature [30].

RESULTS AND DISCUSSION

Infrared studies

The FTIR spectral data of the free caffeine chelate and its seleno complex are comparable. The changes in the spectrum of caffeine is presence at 1700-400 cm^{-1} range due to vibration of carbonyl, imidazole, pyrimidine and methyl groups [31]. The IR spectrum of free caffeine at has medium intensity band at 1548 cm^{-1} due to the stretching vibrations of imidazol and pyrimidine, this band existed in seleno complex at 1538 cm^{-1} [32]. The bands observed within the region 1500-1100 cm^{-1} for the seleno complex are attributed to the vibration motions of the $\delta(CH_3)$, ν (imidazole) and $\rho_r(CH_3)$. The newly vibrations appeared in the spectrum of

ISSN: 0975-8585

the seleno complex compared to the free caffeine spectrum are those due to the ν(Se-N) at 443 cm^{-1} [31]. The infrared spectrum of free caffeine chelate exhibited a strong band at 1660 cm^{-1} which is attributed to ν(C=N). This band is shifted to lower wave numbers in the seleno complex by 10 cm^{-1}, indicating that the (C=N) group is involved in complex formation. FTIR spectrum of the Zn(II) complex has characteristic bands at 3289, 3036 and 2801 cm^{-1} due to the stretching (O–H) of *α*–tocopherol molecules, ν(C–H) aliphatic and aromatic moieties of *α*–tocopherol ligand [33]. The shifted in the wavenumber of –OH phenolic group indicates the formation of bond between the oxygen of phenolic group and Zn(II) ion. The assignments of the other different ν(Zn–O) vibrations in zinc(II) complex is observed at 487 and 449cm^{-1} [33].

Inhibition of α–amylase and α- glucosidase activity

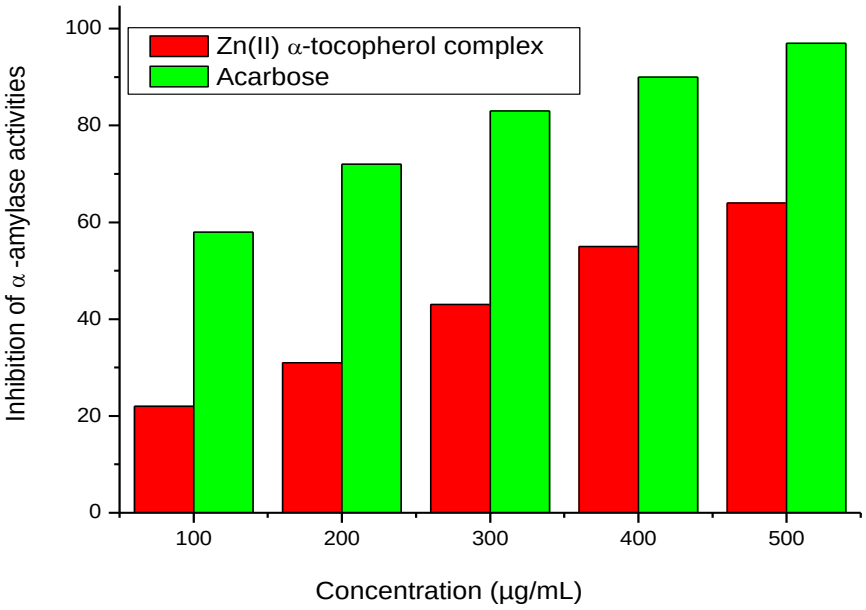

Figure 1: Inhibition of α–amylase activities by zinc(II) α–tocopherol complex

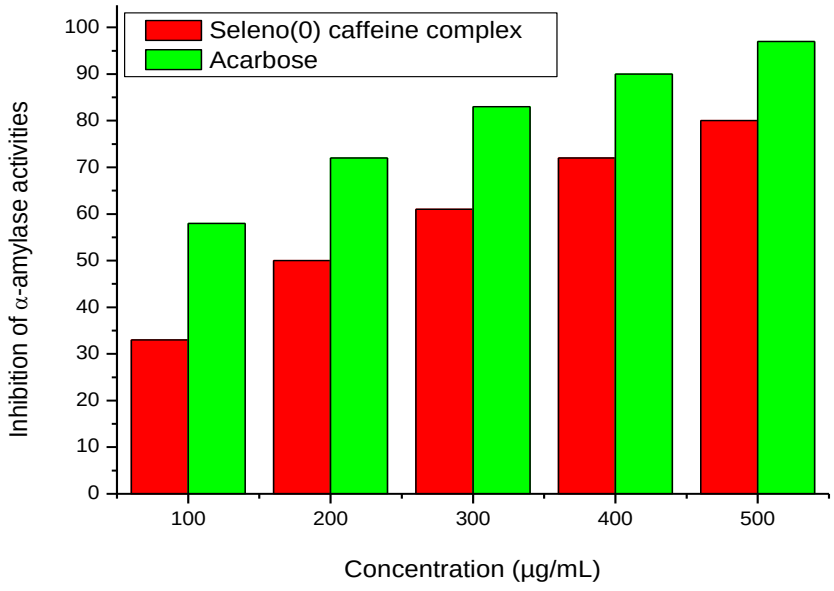

Figure 2: Inhibition of α–amylase activities by seleno caffeine complex

ISSN: 0975-8585

The α–amylase and α- glucoside inhibitory properties of the two complexes are presented in Figs.1-4. The two complexes inhibited α–amylase and α-glucosidase in a dose dependent manner the IC_{50} value for α–amylase and α- glucosidase for two complexes were found to be 0.20 and 0.36, 0.19 and 0.32µg for selenocaffeine and zinc(II) α–tocopherol complexes, respectively. Inhibition of enzymes involved in the hydrolysis of carbohydrates such as α–amylase and α–glucosidase has been exploited as a therapeutic approach for controlling postprandial hyperglycemia [34]. The inhibition activity of α-amylase was extended and might be responsible for decreasing the rate of glucose absorption and concentration of postprandial serum glucose [35]. This effect would delay the degradation of starch and oligosaccharides, which would in turn cause a decrease in the absorption of glucose and consequently inhibit the increase in postprandial blood glucose. In the human species α–amylase is present in both salivary and pancreatic secretions, This enzyme is responsible for cleaving large malto–oligosaccharides to maltose which is then a substrate for intestinal α–glucosidase [36].The inhibitory effects of two complexes may be due to α–tocopherol exerts a protective role against diabetes induced peripheral muscle dysfunction. This effect is probably mediated via a free radical scavenging mechanism or modification of Ca^{2+} homeostasis also highly inhibition effect of seleno caffeine due to its components from caffeine and selenium which have high antioxidant than zinc-α-tocopherol [37].

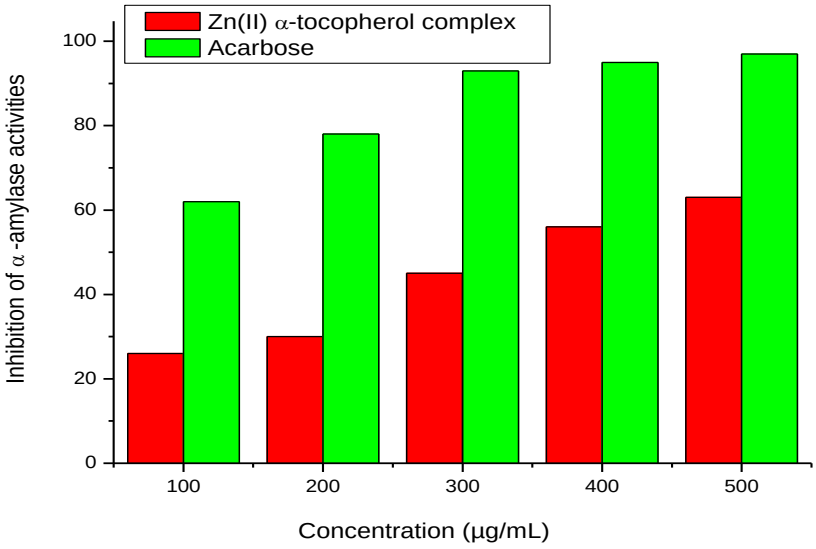

Figure 3: Inhibition of α–glucosidase activities by zinc(II) α–tocopherol complex

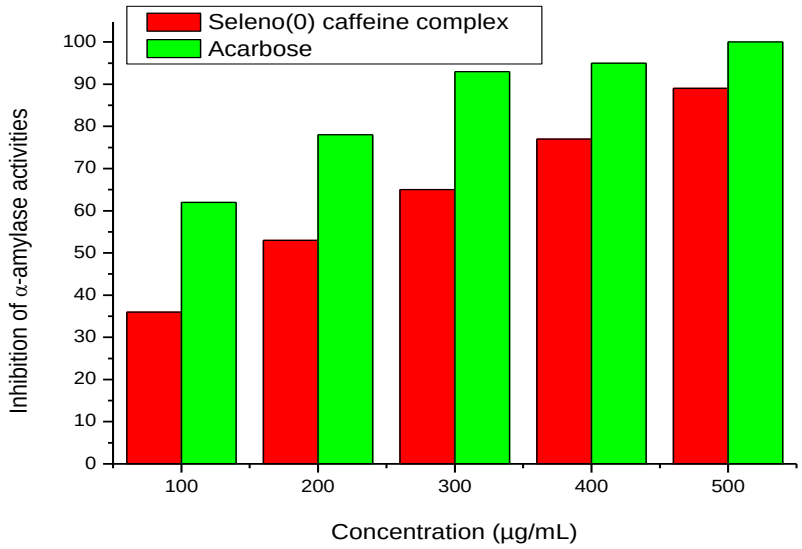

Figure 4: Inhibition of α–glucosidase activities by seleno caffeine complex

ACKNOWLEDGEMENT

This work was supported by grants from Vice President for Graduate Study and Research, Taif University, Saudi Arabia under project Grants No. 5240-437-1.

REFERENCES

[1] K. Alberti, P.F. Zimmet, Diabet. Med. 539-553 (1998).
[2] A. Ravida, L. Musante, M. Kreivi, I. Miinalainen, B. Byrne, M. Saraswat, M. Henry, P. Meleady, M. Clynes, H. Holthofer, Kidney Int. (2015).
[3] A.S. Krolewski, Kidney Int. 55 (1999) 1582.
[4] S. Schön, C. Prante, S. Müller, M. Schöttler, L. Tarnow, J. Kuhn, K. Kleesiek, C. Götting, Kidney Int. 68 (2005) 1483–1490.
[5] M. Brownlee, Nature 414 (2001) 813–820.
[6] J. Wada, H. Zhang, Y. Tsuchiyama, K. Hiragushi, K. Hida, K. Shikata, Y.S. Kanwar, H. Makino, Kidney Int. 59 (2001) 1363–1373.
[7] F. Giacco, M. Brownlee, Circ. Res. 107 (2010) 1058–1070.
[8] S.A. Jewell, P.G. Petrov, C.P. Winlove, Biochim. Biophys. Acta Biomembr. 1828 (2013) 1250–1258.
[9] K. Shahzad, F. Bock, W. Dong, H. Wang, S. Kopf, S. Kohli, S. Ranjan, J. Wolter, C. Wacker, R. Biemann, Kidney Int. 87 (2015) 74–84.
[10] Y. Xu, L. Nie, Y.-G. Yin, J.-L. Tang, J.-Y. Zhou, D.-D. Li, S.-W. Zhou, Toxicol. Appl. Pharmacol. 259 (2012) 395–401.
[11] S. Stackhouse, P.L. Miller, S.K. Park, T.W. Meyer, Diabetes 39 (1990) 989–995.
[12] S. Stapleton, Cell. Mol. Life Sci. 57 (2000) 1874–1879.
[13] J. Jefferson, S. Shankland, R. Pichler, Kidney Int. 74 (2008) 22–36.
[14] G. Wolf, S. Chen, F.N. Ziyadeh, Diabetes 54 (2005) 1626–1634.
[15] M. Messarah, F. Klibet, A. Boumendjel, C. Abdennour, N. Bouzerna, M.S. Boulakoud, A. El Feki, Exp. Toxicol. Pathol. 64 (2012) 167–174.
[16] G.N. Schrauzer, Biol. Trace Elem. Res. 33 (1992) 51–62.
[17] J. Jansen, W. Karges, L. Rink, J. Nutr. Biochem. 20 (2009) 399–417.
[18] S.L. Kelleher, N.H. McCormick, V. Velasquez, V. Lopez, Adv. Nutr. 2 (2011) 101–111.
[19] M.Y. Jou, A.F. Philipps, B. Lonnerdal, J. Nutr. 140 (2010) 1621–1627.
[20] A.G. Hall, S.L. Kelleher, B. Lonnerdal, A.F. Philipps, J. Pediatr. Gastroenterol. Nutr. 41 (2005) 72–80.
[21] P. Ohly, C. Dohle, J. Abel, J. Seissler, H. Gleichmann, Diabetologia 43 (2000) 1020–1030.
[22] A.M. Huber, S.N. Gershoff, J. Nutr. 103 (1973) 1739–1744.
[23] W.B. Kinlaw, A.S. Levine, J.E. Morley, S.E. Silvis, C.J. McClain, Am. J. Med. 75 (1983) 273–277.
[24] S.L. Howell, D.A. Young, P.E. Lacy, J. Cell Biol. 41 (1969) 167–176.
[25] A.B. Chausmer, J. Am. Coll. Nutr. 17 (1998) 109–115.
[26] L. Coulston, P. Dandona, Diabetes 29 (1980) 665–667.
[27] J. Quarterman, C.F. Mills, W.R. Humphries, Biochem. Biophys. Res. Commun. 25 (1966) 354–358.
[28] Worthington Biochemical Crop., Alpha amylase. In: V Worthington (Eds.). Worthington Enzyme Manual. Freehold, (1993a), p 36.
[29] Worthington Biochemical Corp., Maltase-α-glucosidase. In: V. Worthington (Eds.). Worthington Enzyme Manual. Freehold, (1993b)p.261.
[30] G.W.Sndecor, W.G.Cochran, Statistical methods 7th ed. Lowa state Univ., press,Amer,Iowa USA(1980).
[31] F. Ucun, A. Saglam, V. Guclu, Spectrochimi.Acta Part A, 67 (2007) 342-349.
[32] L. David, O. Cozar, E. Forizs, C. Craciun, D. Ristoiu, C. Balan, Spectrochim. Acta Part A, 55(1999)2559–2564.
[33] E. Colacio, J.D. Lopez, J.M. Salas, Can. J. Chem., 61 (1983) 2506-2508.
[34] Y.J. Shim, H.K. Doo, S.Y. Ahn, Y.S. Kim, J.K. Seong, I.S. Park, J.Ethnopharmacol., 85(2003)283-287.
[35] Y.I. kwon, E. Apostolidis, Y.C. Kim, K. Shetty, J.Med.Food, 10(2007) 266-275.
[36] N. Ramasubbu, C. Ragunath, P.J. Mishra, L.M. Thomas, G. Gyemant, L. Kandra, Eur.J.Med.Chem., 271(2004)2517-2529.
[37] M. Hasan, B. Alshuaib, S. Singh, A.M. Fahim, Endocr Res., 29(4) (2003) 419-428.