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The Antioxidant Activity of Copper(II) (3,5-Diisopropyl Salicylate)$_4$ and Its Protective Effect Against Streptozotocin-Induced Diabetes Mellitus in Rats

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Abstract Oxidative stress has been suggested as a potential contributor to the development of diabetic complications. In this study, we investigated the protective effect of a strong antioxidant copper complex against streptozotocin (STZ)-induced diabetes in animals. Out of four copper complexes used, copper(II) (3,5-diisopropyl salicylate)$_4$ (Cu(II)DIPS) was found to be the most potent antioxidant–copper complex. Pretreatment with Cu(II)DIPS (5 mg/kg) twice a week prior to the injection of streptozotocin (50 mg/kg) has reduced the level of hyperglycemia by 34 % and the mortality rate by 29 %. Injection of the same dosage of the ligand 3,5-diisopropyl salicylate has no effect on streptozotocin-induced hyperglycemia. The same copper complex has neither hypoglycemic activity when injected in normal rats nor antidiabetic activity when injected in STZ-induced diabetic rats. The protective effect of Cu(II)DIPS could be related to its strong antioxidant activity compared to other copper complexes median effective concentration (MEC)=23.84 μg/ml and to Trolox MEC=29.30 μg/ml. In addition, it reduced serum 8-hydroxy-2′-deoxyguanosine, a biomarker of oxidative DNA damage, by 29 %. This effect may explain why it was not effective against diabetic rats, when β Langerhans cells were already destroyed. Similar protective activities were reported by other antioxidants like Trolox.

Keywords Copper complex · Antidiabetic · Antioxidant · Protective activity · Streptozotocin

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Introduction

Diabetes mellitus is a chronic metabolic disorder which has a strong effect on the quality of almost all aspects of life including health, social, and psychological well-being. It is characterized by abnormally high blood glucose levels due to either insulin deficiency or the inability of cells to respond to insulin. Diabetes leads to serious health complications involving the eyes, kidneys, nerve cells, and blood vessels. In previous studies using a comet assay, increased level of DNA damage in diabetic patients with poor glycemic control was demonstrated [1, 2].

Oxidative stress refers to the imbalance between the production of free radicals and the body's antioxidant defense system leading to tissue damage [3]. Oxidative stress causes serious damage in different biomolecules such as proteins [4], lipids [5], and nucleic acids [6], which leads to the development of many diseases including diabetes mellitus [7]. One of the main free radical scavengers is copper (Cu)–Zn superoxide dismutase [8]. Hyperglycemia increases the generation of free radicals by glucose auto-oxidation [9, 10]. Several studies describe that these oxygen-derived free radicals result in pancreatic β cell dysfunction and apoptosis [11, 12]. It has been proposed that oxidative stress is a major contributor not only to the development of the late complications in diabetic patients but also to insulin resistance and impaired insulin secretion in diabetes [13]. There is convincing experimental and

clinical evidence that hyperglycemia-induced activation of oxidative stress pathways plays a key role in the development of types 1 and 2 diabetes [3, 9, 13–15].

Cu is one of the most frequently occurring elements integrating into essential biochemical pathways [16]. It is an essential element required as a cofactor and/or structural component of numerous metalloenzymes. Copper ion is involved in the pathogenesis of type 2 diabetes [17, 18]. It was found that patients with diabetes had lower zinc and copper concentrations than nondiabetic controls, and homeostasis of trace elements can be disrupted by diabetes mellitus [19]. Treatment with copper-chelating agents could be used as potential therapeutic agents due to their effect in the treatment of glucose and lipid metabolism in type 2 diabetes [20]. Because of its importance for many biochemical pathways, researchers' interest in using copper to synthesize and produce complex compounds has increased. A wide variety of biological activities has been reported for many synthesized copper complexes. They were proven to have anticonvulsant activity [21, 22], antitumor activity [23], anti-inflammatory activity [24], antimicrobial activity [25], and antiulcer activity [26]. Furthermore, copper complexes showed a strong antioxidant activity [24, 25],as well as antidiabetic activity [16, 27–29]. Copper complexes are able to modulate Cu homeostasis in different tissues, resulting in protective effects in several models of degenerative diseases including diabetes. The therapeutic effect could be due to their ability to increase superoxide dismutase activity as reported by Duncan and White [30].

The copper(II) complex with 3,5-diisopropyl salicylate (Cu(II)DIPS) was found to have an anticonvulsant activity by preventing Metrazol and maximal electroshock-induced seizures [31]. Other copper(II) salicylate derivatives, including aspirinate complexes, were effective in preventing maximal electroshock-induced seizures without having any effect on Metrazol-induced seizures [21, 32]. Copper(II) acetate imidazole was reported to have a hypoglycemic activity [28] and a protective action against strychnine- and thiosemicarbazide-induced seizures [33]. In all cases of antidiabetic, anti-inflammatory, or anticonvulsant activities, the copper complexes were found to be more active and effective than their parent ligands or copper(II) inorganic forms [28, 32, 34].

Herein, our goal is to test in vitro the antioxidant activity of four copper complexes and to investigate the most potent copper complex in vivo for its possible antidiabetic activity using streptozotocin (STZ)-induced diabetic animals.

Materials and Methods

Reagents and Materials

Fluorescein sodium salt was purchased from Sigma-Aldrich, cat. no. F6377 (0.189 g). A stock solution of fluorescein sodium salt was prepared by dissolving it in 50 ml of phosphate buffer solution (PBS) (75 mM, pH 7.0). The working solution (4 μM) was obtained by subsequent dilution in PBS. α,α′-Azodiisobutyramidine dihydrochloride (AAPH) was purchased from Sigma-Aldrich, cat. no. 44,091-4, and was freshly prepared at a concentration of 40 mM prior to injection. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Sigma-Aldrich, cat. no. 23,881-3. It was prepared fresh by serial dilution in PBS from a 10 mg/ml stock solution. The analysis was performed using a flat-bottom 96-well plate and microplate, TECAN, GENios reader (GENios Serial number 12900400892; Firmware V 6.01, 13 May 2004, GENios; XFLUOR4 version V4.50).

Copper(II) chloride, copper(II) acetate, and its methyl derivatives were purchased from Sigma-Aldrich (Milwaukee, WI). Copper(II) acetate complexes were synthesized and characterized in the Chemistry Department at Birzeit University. Dextrostix strips were purchased from Ames (Miles, Paris), and STZ was purchased from Sigma-Aldrich (St. Louis, MO).

The DNA Damage enzyme-linked immunosorbent assay (ELISA) kit, catalog no. EKS-350, was purchased from Assay Designs/Stressgen, Inc., 5777 Hines Drive, Ann Arbor, MI 48108, USA. All other chemicals used were of analytical grade and were purchased from Sigma-Aldrich Chemicals Co. (St. Louis. MO).

In Vitro Studies

Screening for the antioxidant activity of the copper complexes was performed as previously described in detail [34, 35]. Reaction mixtures consisted of 25 μl sample or Trolox (as a standard) mixed with 125 μl of fluorescein (4 μM) and incubated for 10 min at 37 °C in the microplate. AAPH solution (45 μl) was injected using a multichannel pipette, and the microplate was shaken. The fluorescence (excitation=485 nm, emission=535 nm) was recorded every 2 min for 120 cycles. The quantification of the antioxidant activity was based on the calculation of the area under the curve, as proposed by Prior and Cao [36]. Samples from the different copper complexes were screened for their antioxidant activity, by the dilution of 10 mg of each sample in 1 ml dimethyl sulfoxide (DMSO) and measured in duplicate.

Induction of Diabetes in Rats and Administration of Drugs

Male Sprague–Dawley rats weighing 100–150 g were fasted for 12 h but were allowed water ad libitum before use in these experiments. Experimental diabetes was induced by subcutaneous (SC) injection of 50 mg STZ/kg body mass dissolved in 100 mM sodium acetate buffer, pH 5.2. Diabetes was assessed by monitoring blood glucose levels in fasted rats after 1, 2, and 3 weeks following injection of STZ. Rats were considered diabetic when blood glucose levels were above 200 mg/dl.

Groups of 6–12 animals were injected intraperitoneally (IP) twice a week with 5 mg/kg body mass of Cu(II)DIPS, or with the ligand (DIPS) or with the same volume of vehicle solution (DMSO), to test their effect in the following groups: A, in normal rats for hypoglycemic activity; B, in diabetic rats for antidiabetic activity; and C, injection to normal rats twice a week before induction of diabetes to test their protective activity.

At the end of the experiments male rats from groups A, B, and C were anesthetized with ether. At the stage of light anesthesia, characterized by loss of spontaneous movement and pain sensation but with positive corneal reflex, blood samples (1 ml) were drawn from the femoral artery into test tubes. The blood samples were centrifuged at 2,000×*g* for 10 min; serum was isolated and stored at −80 °C for DNA damage test. Internal organs were removed for measuring the relative weight.

Measurement of DNA Damage

Cu(II)DIPS was tested for its possible effect in reducing DNA damage and oxidative stress. The DNA Damage ELISA kit from Assay Designs (EKS-350) is a fast and sensitive competitive immunoassay for the detection and quantitation of 8-hydroxy-2′-deoxyguanosine (8-OH-dG) in serum samples. 8-OH-dG has become a biomarker of oxidative DNA damage and oxidative stress, and the method uses an 8-OH-dG monoclonal antibody to bind in a competitive manner. The procedure is described in detail as published previously [37–40]. The DNA Damage ELISA kit was used for the detection and quantitation of 8-hydroxy-2′-deoxyguanosine in serum samples of control and treated animals.

Statistical Treatment of Data

Values were expressed as mean ± standard error of the mean (SEM). Number of experiments is indicated in parentheses. Results were compared, where appropriate, using unpaired two-tailed Student's *t* test. Differences were considered statistically significant if $P<0.05$. Statistical analysis was performed using the Microsoft Excel and GraphPad Prism version 6.

Results

Antioxidant Activity of Copper Complexes

The oxygen radical absorbance capacity (ORAC) assay used in our in vitro experiments is a common and popular tool used to determine the antioxidant capacity of any substance, and it is directly estimated by comparison to the standard curve of Trolox (the well-known antioxidant), as previously described [34, 35]. From our results in Fig. 1 and Table 1, Cu(II)DIPS was found to have the best antioxidant activity, compared to the other tested copper complexes, with minimal effective plasma concentration=23.84 μg/ml.

Effect of Cu(II)DIPS on Blood Glucose Levels in Normal and Diabetic Rats

Glucose concentration was monitored once a week in overnight-fasted rats before and after injection of STZ (50 mg/kg). Intraperitoneal injection of Cu(II)DIPS 5 mg/kg to normal rats has no significant effect on blood glucose levels after 7, 14, or 21 days. Similar results were obtained when the same copper complex was injected to STZ-induced diabetic rats, for the same periods as shown in Table 2.

Protective Effect Against Induction of Diabetes

SC injection of STZ 50 mg/kg to normal rats significantly increased blood glucose levels in fasted rats from 73.4±1.7 (24) to 315.9±26.2 (18)mg% after 7 days and to 360.7± 28.5 (9)mg% after 21 days, an increase of 4.3- and 4.9-fold, respectively (Fig. 2 and Table 3). Pretreatment with the most potent copper complex Cu(II)DIPS (5 mg/kg) twice a week had no effect on blood glucose levels before injection of STZ. However, after injection of STZ (50 mg/kg), blood glucose levels were increased to 218.3±23.4 (26)mg% after 7 days and to 244.9±31.6 (15)mg% after 21 days, an increase of 3- and 3.3-fold, respectively. The hyperglycemic effect of STZ was reduced by 34 % after pretreatment with Cu(II)DIPS. Pretreatment with the ligand 3,5-diisopropyl salicylate has no effect in reducing a blood glucose level in STZ-induced diabetic rats, and no effect on the development of the diabetic process (Fig. 3).

Mortality rate due to STZ injection was also reduced significantly following pretreatment with Cu(II)DIPS from 33 to 21 % after 7 days, from 42 to 29 % after 14 days, and from 54 to 43 % after 21 days (Table 3).

Changes in Relative Weight of Internal Organs

Table 4 shows that pretreatment with Cu(II)DIPS; while it has significantly reduced the level of STZ-induced hyperglycemia and the mortality rate, it has no significant effect in relative weight of any of the following organs: the testis, epididymis, seminal vesicles, heart, kidneys, spleen, liver, or brain.

STZ-Induced DNA Damage and the Protection by Cu(II)DIPS

DNA damage was estimated by measuring the concentration of 8-OH-dG in the blood serum following injection of STZ

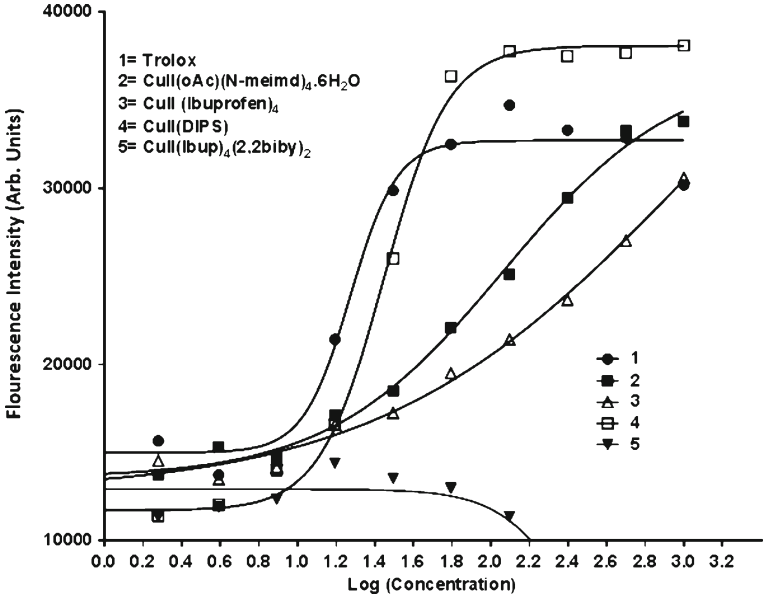

Fig. 1 Fluorescein (4 μM) was incubated in the presence of AAPH (40 mM) and *1* Trolox, *2* Cu(II)(OAc)(*N*-meimd)$_4$·6H$_2$O, *3* Cu(II)(ibuprofen)$_4$, *4* Cu(II)DIPS, or *5* Cu(II)(ibup)$_4$(2,2bipy)$_2$. The reaction was followed by the change in the fluorescence intensity of fluorescein (excitation=485 nm, emission=535 nm) in phosphate buffer solution (75 mM, pH 7.0). Cu(II)DIPS showed the highest fluorescence intensity compared to all other tested copper complexes

50 mg/kg for 7 days. Figure 4 shows a significant increase of 29 % in 8-OH-dG concentration in the serum blood of STZ-induced diabetic rats, which indicates an oxidative stress produced by a significant increase in DNA damage. Pretreatment with Cu(II)DIPS has prevented the increase in DNA damage by reducing the serum concentration of 8-OH-dG to normal values and reducing the severity of hyperglycemia as shown in Fig. 2.

Discussion

In Vitro Studies

From our in vitro studies using the ORAC assay, it was clear that all copper complexes used have an antioxidant activity compared to the standard curve of Trolox®. The antioxidant activity of the copper complexes was rated as follows, starting with the copper complex having the highest antioxidant activity: Cu(II)DIPS→Cu(II)(OAc)(*N*-methylimidazole (meimd))$_4$·6H$_2$O→Cu(II)(ibuprofen (ibup))$_4$(2,2-bipyridine (bipy))$_2$→Cu(II)(ibuprofen)$_4$. The only limitation of the method is that samples contain free radical generators like H$_2$O$_2$ which interfere with the procedure and cannot be analyzed using this technique [35].

Cu(II)DIPS with a median effective concentration of 23.84 μg/ml was the most potent and was selected for the in vivo studies. Compounds with salicylate skeleton are well-known as good scavengers of hydroxyl radicals [41] and were reported to have a cytoprotective activity like Trolox, an effective scavenger of both superoxide and hydroxyl radicals [42].

Table 1 Antioxidant activity of copper complexes

	Median effective concentration (μg/ml)
Trolox	29.73
Cu(II)DIPS	23.84
Cu(II)(OAc)(*N*-meimd)$_4$·6H$_2$O	115.6
Cu(II)(ibup)$_4$(2,2bipy)$_2$	232.0
Cu(II)(ibuprofen)$_4$	1,054

Median effective concentration of the copper complexes was calculated as the concentration which produces 50 % of the maximum effect. Values are expressed in nanogram per milliliter

In Vivo Studies

When repeated doses of Cu(II)DIPS 5 mg/kg were injected IP twice a week to normal rats, they had no hypoglycemic activity since no significant changes on blood glucose levels were obtained as shown in Table 2. Similar results were obtained when the same treatment with Cu(II)DIPS was applied to STZ-induced diabetic rats. This indicates no antidiabetic activity of Cu(II)DIPS. Previous results have shown an antidiabetic activity with the same copper complex [27]. Injection of the copper complex to diabetic rats is not effective in reducing blood glucose or preventing tissue damage, since most of the β cells are already destroyed. Previous reports have shown that treatment of diabetic rats with antioxidant therapy may not only be too late but may also miss a large fraction of the target, non-oxidatively derived carbonyl compounds, which contribute to tissue damage [14]. Therefore, antioxidants are more effective when applied before the induction of diabetes or in the early stages of the process. Two to four weeks following injection of STZ, no significant changes were observed in the total body weight or relative weight of internal organs. Similar results were reported

Table 2 Effect of Cu(II)DIPS on blood glucose levels in normal and diabetic rats

	0 time	7 days	14 days	21 days
Normal rats				
Control (DMSO)	77.3±3.2 (12)	89.8±4.2 (12)	93.6±2.6 (12)	74.2±2.5 (6)
Cu(II)DIPS (5 mg/Kg)	79.7±2.2 (13)	74.1±3.7 (13)	95.5±2.2 (13)	73.0±1.7 (7)
Diabetic rats				
Control (DMSO)	380.4±30.5 (6)	354.8±42.4 (6)	390.1±28.7 (6)	409.5±18.5 (6)
Cu(II)DIPS (5 mg/Kg)	391.4±25.3 (14)	314.5±52.3 (6)	337.3±45.2 (10)	413.0±23.3 (12)

Normal animals were injected IP with Cu(II)DIPS 5 mg/k, and control animals were injected with the same volume of the vehicle solution (DMSO). The same treatments were applied to diabetic animals. Blood glucose was measured in the tail tip of fasted animals after 0, 7, 14, and 21 days of the copper complex injection. Values are mean ± SEM for the number of experiments indicated in parentheses. Glucose levels were expressed in milligram percent

previously [43]; they observed significant reductions in body weight only after 6 weeks of STZ injection.

Pretreatment with Cu(II)DIPS prior to the injection of STZ has reduced the severity of hyperglycemia by 34 % (Fig. 2), in addition to reduction in mortality rate. The protective effect of Cu(II)DIPS was not achieved when we used the same dosage and treatment of the ligand 3,5-diisopropyl salicylate. Prior administration of Cu(II)DIPS at doses of 48 or 120 mg/kg 15 min before injection of streptozotocin has attenuated the severity of STZ-induced diabetes. In the same experiments, diisopropyl salicylate has no superoxide dismutase activity and no effect on the severity of STZ-induced diabetes [27]. Similar cytoprotective effects of copper complexes were reported against alloxan-induced diabetes [41].

Deficiency of some essential trace elements, such as copper and zinc, may play a role in the development of diabetes mellitus [19, 44, 45]. Diabetes is associated with increased oxidative stress [46–48], and the total antioxidant status in diabetes is lower than that of age-matched controls [47, 49]. Therefore, antioxidants, by reducing the damage caused by hyperglycemia, could be useful in the prevention and management of chronic diabetic complications [47, 50]. In addition to the importance of trace elements such as copper as cofactors of antioxidant enzymes, their deficiency may be associated with increased oxidative stress. On the other hand, copper ions are potentially harmful to cells and are involved in the development of type 2 diabetes. Copper-chelating agents exert beneficial effects on the pathogenesis of diabetes [20], and production of the reactive oxygen species (ROS) is facilitated in the presence of copper ions through the Fenton reaction [51, 52].

The advantage of copper complexes is their ability to modulate copper homeostasis, and their potential therapeutic

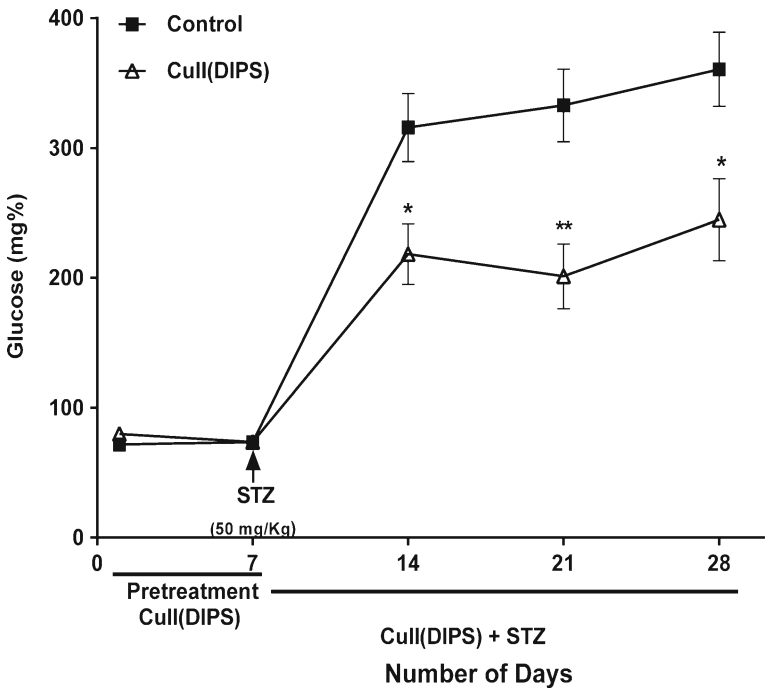

Fig. 2 The development of the diabetes process following pretreatment for 1 week with Cu(II)DIPS, before the injection of STZ (50 mg/kg). Control animals were pretreated for the same period with the vehicle solution (DMSO). Values are mean ± SEM. *$P \leq 0.05$; **$P \leq 0.001$

Table 3 Effect of pretreatment with Cu(II)DIPS on blood glucose and mortality rate after the induction of streptozotocin in diabetic rats

	No STZ		Treatment with STZ (50 mg/kg)		
Pretreatment	1st day	7th day	1st week	2nd week	3rd week
Blood glucose (mg%)					
Control (DMSO)	71.7±2.6 (19)	73.4±1.7 (24)	315.9±26.2 (18)	332.9±27.9 (13)	360.7±28.5 (9)
Cu(II)DIPS (5 mg/kg)	79.8±1.9 (23)	73.6±1.2 (28)	218.3±23.4 (26)*	201.3±25.0 (20)**	244.9±31.6 (15)*
Mortality rate					
Control (DMSO)	0 %	0 %	33 % (8/24)	42 % (10/24)	54 % (13/24)
Cu(II)DIPS (5 mg/kg)	0 %	0 %	21 % (6/28)	29 % (8/28)	43 % (12/28)

Mortality rate is the number of diabetic rats killed by STZ after 7, 14, or 21 days compared to the total number of rats treated. Values are mean ± SEM for the number of experiments indicated in parentheses

usage will most likely be due to their ability to increase superoxide dismutase (SOD) activity leading to relief of oxidative stress. The use of copper complexes is an attempt to increase their efficacy and reduce nonspecific cytotoxicity.

From our results, injection of Cu(II)DIPS to diabetic rats with blood glucose levels above 300 mg% had no effect on blood glucose, since the β Langerhans cells were already destroyed. Pretreatment with the same concentration of the copper complex has prevented the destruction of β Langerhans cells due to their antioxidant activity and removal of the free radicals. Previous reports have shown that treatment of STZ-induced diabetic mice with copper sulfate resulted in decreased blood glucose levels, improved pancreas morphology, and preserved β cell function [53]. There was also reduced lipid peroxidation, suggesting that perhaps Cu sulfate exerts its effect through relief of oxidative stress [30]. Oxidative stress is increased in diabetes due to overproduction of ROS and decrease efficiency of antioxidant defenses as a result of hyperglycemia [54]. Oxidation of DNA occurs during the development of diabetes [55]; therefore, pretreatment with copper complexes having a strong antioxidant activity before injection of STZ could protect against the induction of diabetes.

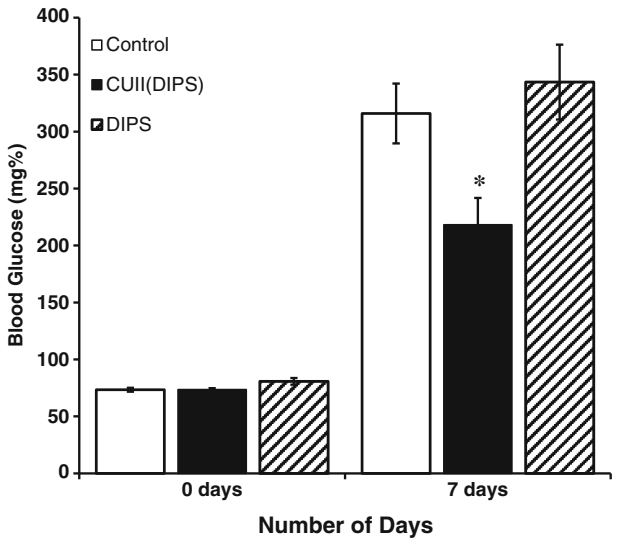

Fig. 3 Blood glucose levels were measured in normal and STZ-induced diabetic rats following pretreatment with Cu(II)DIPS or with the ligand DIPS or with the vehicle solution DMSO. Values are mean ± SEM. *$P \leq 0.05$, compared to diabetic rats pretreated with DMSO

Increased 8-OH-dG has been proposed as a biomarker of oxidative DNA damage [56], and the measurement of its concentration is a sensitive method for measuring the extent of DNA damage in vivo and in vitro [57]. In our results, pretreatment with Cu(II)DIPS before injection of STZ protected the rats against STZ-induced DNA damage as shown in Fig. 4. Other antioxidants like ascorbic acid and Trolox were reported to prevent STZ-induced elevation of DNA damage in the liver and kidneys of mice [58, 59].

Table 4 Effect of Cu(II)DIPS on relative weight of different body organs

	Control + STZ	Cu(II)DIPS + STZ
Total body weight (g)	200.00±7.00 (4)	187.00±6.00 (6)
Glucose (mg%)	382.20±63.50 (4)	210.00±61.00 (6)*
Testis	1.37±0.05 (4)	1.37±0.05 (6)
Epididymis	0.34±0.02 (4)	0.38±0.01 (6)
Seminal vesicles	0.22±0.03 (4)	0.25±0.03 (6)
Heart	0.33±0.01 (4)	0.34±0.01 (6)
Kidneys	0.91±0.07 (4)	0.87±0.05 (6)
Spleen	0.27±0.05 (4)	0.27±0.03 (6)
Liver	3.57±0.18 (4)	3.28±0.15 (6)
Brain	0.82±0.01 (4)	0.84±0.03 (6)

The relative weight is the weight of the different organs compared to the total body weight in each animal. Cu(II)DIPS 5 mg/kg was injected IP twice a week before and during treatment with STZ (50 mg/kg). Control animals were injected at the same time with the same volume of the vehicle (DMSO). At the end of the experiments, rats were anesthetized with ether, and the selected organs: the heart, kidneys, spleen, liver, brain, testis, epididymis, and seminal vesicles were removed and weighed for the measurement of relative weight. Values shown are mean ± SEM for the number of experiments indicated in parentheses

*$P \leq 0.05$

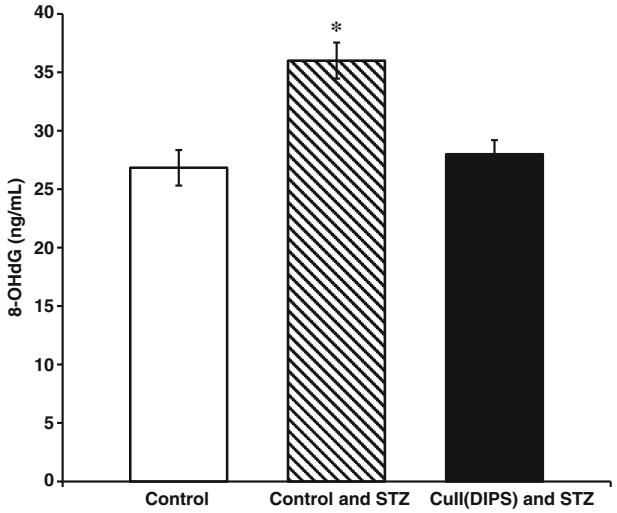

Fig. 4 The concentration of 8-OH-dG in the serum blood was measured in control rats treated with DMSO, in STZ-induced diabetic rats, and in diabetic rats pretreated with Cu(II)DIPS. Values are mean ± SEM measured (in nanogram per milliliter). *$P \leq 0.05$

Several clinical studies have shown high levels in 8-OH-dG in diabetic patients compared to normal healthy participants [60]. The measurement of oxidative DNA damage in leukocytes by means of the comet assay is a suitable marker for the evaluation of systemic oxidative stress in diabetic patients [15], and higher levels of 8-OH-dG in mononuclear leukocyte DNA have been found in types 1 and 2 diabetic patients [60–62]. Free radical formation in oxidative stress cases results from an increased oxidation of DNA, proteins, carbohydrates, or unsaturated fatty acids [12]. In our experiments, we measured DNA damage as reflected by serum 8-OH-dG levels.

Oxidative stress has been found to be due to an increased production of oxygen free radicals, and a sharp reduction of antioxidant defenses has been observed in diabetes [63]. STZ is a glucosamine–nitrosourea compound that can cause pancreatic β cells destruction capable of inducing insulin-dependent diabetes mellitus. STZ and alloxan which induce diabetes mellitus are well-known for their effect in inhibiting superoxide dismutase enzymatic activity [64, 65]. Therefore, intravenous injection of copper–zinc superoxide dismutase before STZ prevented diabetes in rats [66, 67], and copper complexes which have a superoxide dismutase-like activity could have an antidiabetic effect [27, 68].

One of the roles of copper complexes relies on the delivery of copper or modulation of copper homeostasis. The therapeutic success of copper complexes as therapeutics is perhaps due to their ability to increase SOD activity leading to relief of oxidative stress, as reported by Duncan and White [30]. One of the protective mechanisms could be the ability of antioxidant copper complexes like Cu(II)DIPS to scavenge free radicals in the human body, thereby decreasing the amount of free radical damage to molecules like DNA.

Oxidative stress plays an important pathophysiological role in the onset of diabetes and in the development of the diabetic complications [3]. From our recent results, it is very clear that Cu(II)DIPS, which has an antioxidant activity, could affect the onset of diabetes, and further studies are needed to evaluate its effect on the development of the diabetic complications.

Conclusions

Copper(II) (3,5-diisopropyl salicylate)$_4$ (Cu(II)DIPS) has an antioxidant activity similar to that of Trolox. It has no hypoglycemic activity when injected into normal rats and no antidiabetic activity when injected into diabetic rats. On the other hand, pretreatment with Cu(II)DIPS has a protective effect against diabetes induced by streptozotocin, by reducing mortality rate and the severity of diabetes (40 % reduction in blood glucose levels).

It is suggested that Cu(II)DIPS protective effect is related to its antioxidant activity and its potency to reduce the oxidative stress which was measured by total serum DNA damage. Further studies are necessary to elucidate the mechanism of action of copper complexes.

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