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The Protection Role Nano-Doped Zinc Oxide on F2-Isoprostane As Biomarkers of Oxidative Stress in STZ Induced Diabetic Retinopathy in Male Rats

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ABSTRACT

Oxidative stress (OS) has been suggested as one of basic mechanism behind the development of type 2 diabetes especially diabetic retinopathy (DR). Isoprostanes (IsoPs) are involved in type 2 diabetes and increased in pathological conditions associated with OS. Our present study was designed to prepare Cl: ZnO nanoparticales (Cl: ZnONPs) and evaluate the effect of $ZnCl_2$ and Cl: ZnONPs at (5 mol 10 mg/kg bw) on retinal oxidative stress in STZ-induced diabetic rats. The male albino rats treated with one dose of streptozotocin (60 mg/kg bw). The diabetic rats adminst rated Zn Cl_2 and Cl: ZnONP (5 and 10 mg/kg bw) orally for 3 days. The results investigated that TEM of Cl: ZnONPs has average particle size 14.5-70 nm. Serum insiulin and SOD were decreased and serum glucose and IsoPs were increased significantly ($P \leq 0.05$) 72h after treatment with STZ (60 mg/kg bw) than other groups. Asignificant decreased ($P \leq 0.05$) in glucose and F_2-IsoPs levels and significant increase in insulin and SOD levels have been detected also in retinal in STZ diabetic rats which treated with $ZnCl_2$ and Cl: ZnONPs (5 and 10 mg/kg bw). Cl: ZnONPs (10 mg/kg bw) + (60 mg/kg bw) gave the best results compared to $ZnCl_2$ (5 and 10 mg/kg bw) + STZ and Cl: ZnONPs (5 mg/kg bw) + STZ. In conclusion, Cl: ZnONPs have a highly protective effect against hyperglycemia and diabetic retinopathy oxidative stress, especially at (10 mg/kg bw).

Keywords: Diabetic retinopathy, Streptozotocin, Cl: ZnO nanoparticales, Isoprostanes (Isops), Oxidative stress

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases in which has high blood sugar in person, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produce [1]. All over the world there are a large number of people suffer from diabetes [2], approximately (25.9%) [3].

Oxidative stress (OS) due to the imbalance between pro-oxidant/antioxidant status results in generation of reactive oxygen species (ROS) and free radicals or impaired antioxidant defense system [4], subsequent modification of biomolecules such as protein, lipids and nucleic acids. Excessive generation of ROS and free radicals have been implicated in a variety of pathological events such as ischemia-reperfusion injury, cardiovascular disease [5] hepatorenal syndrome [6], scleroderma [7], smoking [8], coronary reperfusion injury [9], atherosclerosis [10], diabetes mellitus [11,12] and neurodegenerative disease [5]. Oxidative stress appears to be an integral and possibly causative part of the pathogenesis of diabetic retinopathy. Compelling evidence has been provided that both insulin-dependent and non–insulin-dependent diabetic patients are under conditions of oxidative stress and that the complications of diabetes mellitus (thereafter indicated as diabetes) could be partially mediated by oxidative stress [13], and that peroxide formation is increased in elevated glucose solutions [14,15]. Oxidative damage of cellular membranes has been suggested as a common mechanism in a large number of bio pathological conditions. It can be measured by either primary or secondary end products of peroxidation [16]. While secondary end products include thiobarbituric reactive substances (TBARS), F2-like products termed F2-isoprostanes (F2-IsoPs) [17,18]. Increased levels of F2-IsoPs can be found in the plasma of patients affected with type I and type II diabetes [11,19] and in animal models of diabetes [20], and currently are used as

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in vivo indicators of lipid peroxidation [13,11,21,22].

Zinc (Zn) is the most abundant trace intracellular element required for a number of cellular processes, including cell proliferation, reproduction, immune function and defense against free radicals [23]. It plays an important role as an antioxidant, protects cellular components from oxidation, [24] an activator for more than three hundred enzymes in the body [25], and in different metabolic pathways including glucose metabolism [26]. Also, Zinc promotes hepatic glycogens through its actions on the insulin pathways, storage, and secretion [27] and thus improves glucose utilization [28]. Due to its fast electron transfer capability, ZnO is a key material for fabrication of bio membranes, and enzymatic detective devices [29]. Many studies have addressed the importance of ZnO as an antioxidant and a therapeutic agent in several free radicals initiating systems [26,30]. Nano-ZnO is a product whose particle diameter is between 1 to 100 nm. More recently, the study of ZnO nanoparticles is a very active area [31,32]. Umrani and Paknikar [33] reported that the antidiabetic effects of ZnONPs through induction of insulin, also, Umrani and Paknikar [23] proved the ability of ZnONPs for controlling of blood glucose in diabetic rats. Soheir et al. [34] investigate the useful effects of high dose of F: ZnO and Cl: ZnO nanoparticles in protection diabetic rats against hyperglycemia and retina against oxidative stress. There is only one study that monitored the effect of Cl: ZnO nanoparticles on diabetic rats, but there is not previous study carried out with Cl: ZnO nanoparticles on the role of isoprostane during diabetic rats. Therefore, this work designed to prepare ZnO nanoparticles doped with chlorine (Cl: ZnOPNs) and reviews the role of $ZnCl_2$andCl: ZnONPs on F2-Isoprostane as biomarkers of oxidative stress in retinal in relation to type II diabetes in rats.

MATERIALS & METHODS

Experimental

The ZnO nanoparticles doped with chlorine was prepared by a chemical solution method from zinc acetate (ZnAc) and oxalic acid. The preparation is as follows: 20 mL of a 0.5 M ethanolic solution of oxalic acid was added drop-by-drop to 20 mL of a 0.1 M ethanolic solution of ZnAc under stirring and maintained at 60°C for 3 h. Then 0.5 M of aqueous solution of ammonium chloride (NH_3Cl) was added to the above solution in order to get at 5% Cl/Zn ratio. A white precipitate was obtained, which was separated by filtration and washed with a mixture of 75:25 (water: Ethanol). This precipitate was dried in an oven at a temperature of 100°C for 24 h. The obtained precursor was finally calcined at 500°C for 2 h, with a heating rate of 5°C/min. All the reagents used in the experiments were of analytical grade and used directly as purchased.

CHARACTERIZATION

The structure was analyzed by FTIR spectra (FTIRNicolet6700**)**. Surface Morphology was analyzed by SEM (Quanta 3D FEG/ FEI) and TEM (JEOL, Tokyo, Japan) operating at 60 kV.

Biological methods

Male albino adult rats (60 animals weighing 130 g ± 5) were obtained from the private market, Helwan, Giza, Egypt, then transported to Animal House of Ophthalmology Research Institute, Giza, Egypt. Rats were housed in individual cages with screen bottoms and fed on basal diet (corn starch 70%, casein 10%, corn seed oil 10%, cellulose 5%, salt mixture 4% and vitamins mixture 1%) for ten days. After equilibration, rats were weighted and divided into six groups (ten animals per each) everyone was assigned to one of the six diet groups (G1: Negative Control (NC), G2: STZ-treated group that injected with a single ip dose of STZ (60 mg/kg b.w), G3: Treated group that received a single dose of STZ (60 mg/kg b.w)+ single daily oral low dose of $ZnCl_2$ (5 mg/kg b.w), G4: Treated group that received a single dose of STZ(60mg/kg b.w) + single daily low dose of Cl: ZnONPs (5 mg/kg b.w), G5: treated group that received a single dose of STZ (60 mg/kg b.w) + single daily oral high dose of $ZnCl_2$ (10 mg/kg b.w), G6: Treated group that received a single dose of STZ (60 mg/kg b.w) + single daily high dose of Cl: ZnONPs (10 mg/kg bw). Rats were sacrificed at 0, 24, 48 and 72 h after STZ treatment. Before the rats were sacrificed, blood was collected from the orbital sinus and serum was prepared and kept frozen at -20°C until the time of assay. Each eye was immediately enucleated, the lens was removed, and the retina was gently peeled away from the pigment epithelium and placed in 500 ı`l ice-chilled 10 mmol/l sodium phosphate buffer, pH 8.0.

Measurements of blood glucose and insulin

Blood glucose (mg/dL) was estimated by glucose oxidase method using the kit supplied by SPINREACT (SantEstevadeBas, Girona, Spain) according to Soheir, Reda and Sheikh [35], we measured blood glucose in all experimental animals before the beginning of the experimental procedures, after streptozotocin injection. After that, blood glucose was monitored in all experimental animals, and results were obtained at 0, 24, 48 and 72 h of the experimental period. Serum insulin was measured using an insulin radio immunoassay kit.

SUPEROXIDE DISMUTASE (SOD) AND F2-ISOPROSTANES (F2-ISOPS) ASSAY

The enzymatic activity of retinal SOD was measured as described by Ohkuma et al. [36] Retinal. F2-IsoPs were measured using a competitive enzyme-linked immunoassay (ELISA) kit according to instructions (Cayman Chemical, Ann Arbor, MI) [37].

Statistical analysis

The results are represented as mean ±SE and statistically analyzed by using one-way ANOVA. Accepted level of significance ($P \leq 0.05$).

RESULTS & DISCUSSION

IR Spectra

Figure 1 shows the FT-IR at sorption spectrum prepared sample (Cl: ZnONPs). The peak at 470 cm^{-1} is the characteristic distinct stretching vibration of zinc oxide. The broad absorption peak at 3400 cm^{-1} can battributed to the characteristic absorption of hydroxyls group. These results agree with Soheir et al. [35]. They reported that the undoped ZnO, an absorotion peak is observed centered at 372nm (3.33 eV), which is in good agreement with previously reported works of ZnO single crystals and Cl: ZnONPs.

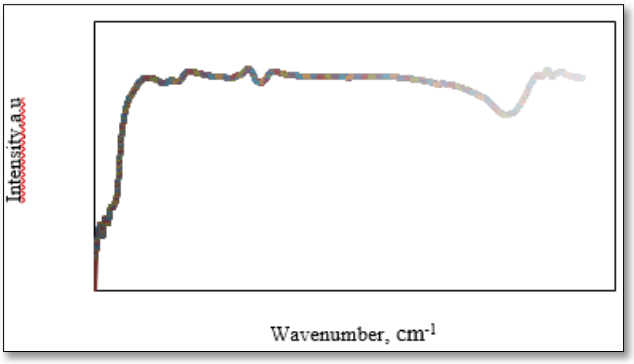

Figure 1. FTIR spectra of Cl: ZnONPs powder.

Scanning electron microscopy (SEM)

Figure 2 shows the SEM images of the Cl: ZnONPs powder prepared with chlorine doping. Cl: ZnONPs shows a rode shape. The results of this study agree with Soheir et al. [35], and they reported that the ZnO nano powders prepared with fluorine and chlorine and doping have average particle size 17.7 and 59.3 nm, respectively.

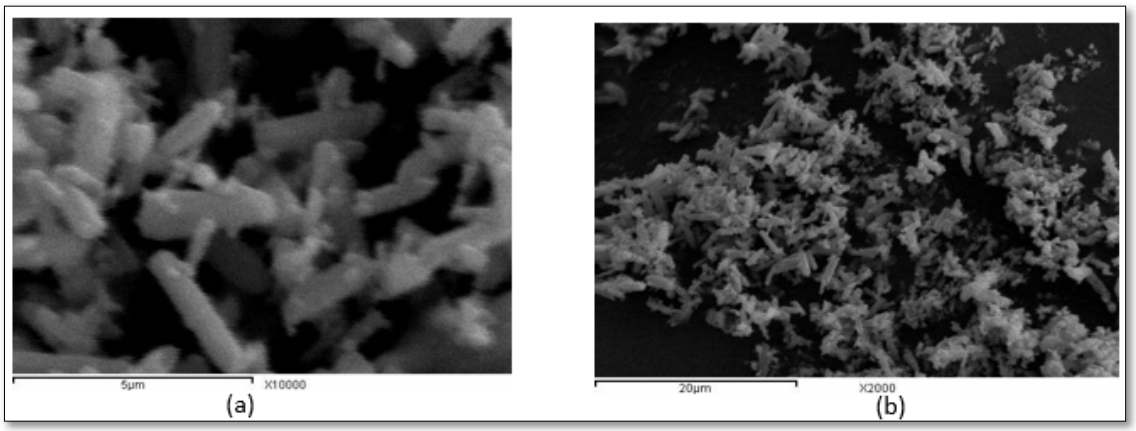

Figure 2. SEM spectra of Cl: ZnONPs powder.

Transmission electron microscoupe (TEM)

Figure 3 shows the Cl: ZnONPs powder prepared with chlorine doping have average particle size 14.5-70.0 nm.

Biochemical markers

Table 1 shows the changes of serum glucose levels induced by $ZnCl_2$ and Cl: ZnONPs in diabetic rats. Experimental groups treated by $ZnCl_2$ and Cl: ZnONPs (5 and 10 mg/kg bw) for 3 days, showed a significant ($P \leq 0.05$) decreased in serum glucose levels, it found to be (138.022, 116.094 mg/dl), (117.962, 102.964 mg/dl) at 5 and 10 mg/kg bw), respectively after 72 h. Additionally, Cl: ZnONPs (10 mg/kg bw)+STZ (60 mg/kg bw) treated group results were close to normal values that showed in NC group. This showed a great anti-diabetic activity of zinc oxide nanoparticles, as zinc has been elucidated to be a potent metal that improves glucose utilization and metabolism through its potent influence on enhancement of hepatic glycogenesis through actions on the insulin signaling pathway [35]. Alkaladi et al. [38] showed a great reduction in blood glucose level in diabetic groups treated with ZnONPs, SNPs and insulin. However, ZnONPs induce more reduction than SNP. Rehal et al. [31] reported that at 30, 60, 90 and 120 min, the plasma glucose levels in the STZ-induced rats. That was treated with Vildagliptin, ZnONPs, and various combinations of the two, groups, for 7 weeks showed significant improvements compared to the diabetic group (group II). The maximum improvement in the reduced plasma glucose levels was observed in the rats treated with the combination therapy (Vildagliptin plus 10 mg/kg/d ZnONPs, group IIIh) compared to diabetic group and the other treated groups [39]. Also, a great reduction in blood glucose level in diabetic groups treated with low and high

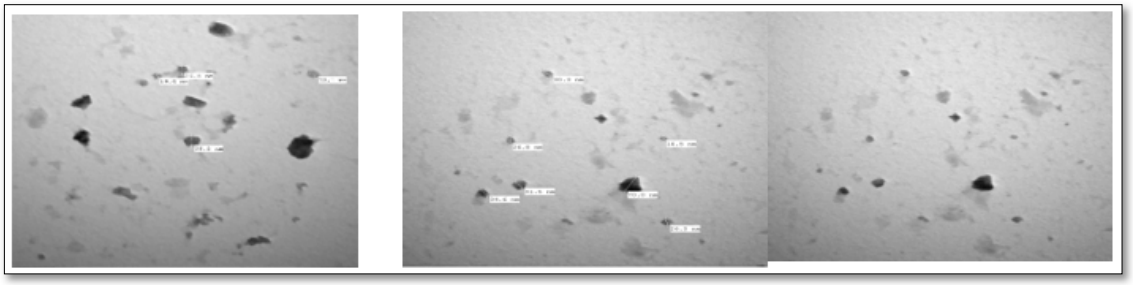

Figure 3. TEM spectra of Cl: ZnONPs powder.

dose of F: ZnONPs and Cl: ZnONPs, it found to be 119.03, 117.10, 108.97 and 103.97 mg/dl after 72 h, respectively [35].

Table 1. Effect of zncl2 and Cl: znonps (5 and 10mg/Kg bw) on Serum glucose concentration in diabetic rats.

Time / Treatments	Glucose (mg/dl)			
	0 h	**24 h**	**48 h**	**72 h**
G1 (NC)	$94.080^{b} \pm 0.078$	$94.808^{d} \pm 0.121$	$93.572^{f} \pm 0.169$	$94.932^{f} \pm 0.099$
G2 (PC)	$93.986^{b} \pm 0.062$	$154.392^{a} \pm 0.615$	$203.278^{a} \pm 0.524$	$379.524^{a} \pm 0.474$
G3	$94.556^{a} \pm 0.124$	$101.602^{b} \pm 0.175$	$141.104^{b} \pm 0.272$	$138.022^{b} \pm 0.154$
G6	$93.160^{c} \pm 0.257$	$101.602^{b} \pm 0.175$	$109.184^{c} \pm 0.287$	$116.094^{c} \pm 0.272$
G5	$94.178^{ab} \pm 0.171$	$97.040^{c} \pm 0.048$	$121.104^{d} \pm 0.272$	$117.962^{d} \pm 0.045$
G6	$94.002^{b} \pm 0.015$	$95.380^{d} \pm 0.192$	$97.654^{e} \pm 0.208$	$102.964^{e} \pm 0.039$
LSD	**0.417**	**0.840**	**0.908**	**0.693**

G1(NC) = Negative control, G2 (PC) = Positive control, G3 = STZ + $ZnCl_2$ (5 mg/kg bw), G4 = STZ + Cl: ZnONPs (5 mg/kg bw), G5 = STZ + $ZnCl_2$ (10 mg/kg bw), G6 = STZ + Cl: ZnONPs (10 mg/kg bw)

Our results revealed that $ZnCl_2$ and Cl: ZnONPs (5 and 10 mg/kg bw) +STZ (60 mg/kg bw) were significantly ($P \leq 0.05$) increased serum insulin level (290.40, 328.40 uIV/ml),(310.40, 369.80 uIU/ml) at (5 and 10 mg/kg bw), respectively, compared to (PC)(177.40 uIU/ml) **(Table 2)**. The serum insulin levels were significantly decreased in the diabetic group compared to the control group [39]. This result may be due to zinc could enhance the glucose stimulated insulin secretion from rat isolated pancreatic islets [40]. Umrani and Paknikar [34] demonstrated that ZnONPs did not possess the risk of hypoglycemia in living organisms so it can act as an insulin secretor/due to accumulation of zinc in the secretory vesicle of B cells using transporter 8 [41]. Zinc transporters are also identified in adipose tissues and liver [35,42]. Treatment with ZnONPs. Induced a significant increase in the serum insulin levels compared to the diabetic group (NC) [39]. The groups administration high dose of F: ZnONPs and Cl: ZnONPs gave best results (326.40 and 367.80 pg/ml), respectively, compared to PC (175.40 pg/ml). ZnONPs and SNP could increase serum insulin level in diabetic groups, it was found (79.4% and 3%), respectively if compared with diabetic groups treated with insulin (97.3%). It appeared that ZnONPs also induced more insulin secretion if compared to the effect of SNPs [31]. ZON treatment showed a trend towards a dose-dependent increase in nonfasted serum insulin levels (35% increase at 10 mg/kg dose). However, no significant effects were seen on fasted serum insulin levels after ZON treatment, indicating no risk of hypoglycemia [34]. The best results were observed in diabetic rats treated with ZnONPs in a dose of 10 mg/kg b.w [43].

Isoprostanes in contrast to lipid hydroperoxides are chemically stable end products of lipid peroxidation, and the measurement of their levels in plasma or urine may permit a sensitive and specific method for detection of lipid oxidative damage in vivo [22]. In the present study the retinal F2-IsoPs levels were significantly ($P \leq 0.05$) increased in the diabetic group (0.7162 pg/ml bw) compared to the NC (0.2762pg/ml bw) after 72h **(Table 3)**. Treatment with$ZnCl_2$ and Cl: ZnONPs (5 and 10 mg/kg bw) +STZ (60 mg/kg bw) induced a significanty decreased of F_2-IsoPs the retina levels

Table 2. Effect of ZnCl2 and Cl: ZnONPs (5 and 10mg/Kg bw) on Serum Insulin concentration in diabetic rats.

Time / Treatments	Insulin (uIU/ml)			
	0 h	**24 h**	**48 h**	**72 h**
G1 (NC)	471.40^{bc}±0.91	476.00^{a} ±1.08	475.40^{a} ±1.45	480.60^{a} ±2.71
G2 (PC)	478.60^{a}±1.01	336.80^{e}±0.56	205.60^{f}±0.58	177.40^{f}±0.91
G3	469.20^{c}±0.71	354.00^{d}±0.30	303.80^{e}±0.35	290.40^{e} ±0.22
G4	474.00^{b} ±0.98	394.00^{c} ±0.30	367.00^{c} ±1.28	328.40^{c} ±0.22
G5	477.00^{a} ±0.93	394.40^{c} ±0.22	343.40^{d} ±0.38	310.40^{d} ±0.22
G6	471.60^{bc}±1.27	415.20^{b} ±0.78	389.00^{b} ±4.51	369.80^{b} ±1.05
LSD	**2.924**	**1.861**	**5.960**	**3.700**

G1 (NC) = Negative control, G2 (PC) = positive control, G3 = STZ + $ZnCl_2$ (5mg/kg bw), G4 = STZ + Cl: ZnONPs (5mg/kg bw), G5 = STZ + $ZnCl_2$ (10mg/kg bw), G6 = STZ + Cl: ZnONPs (10mg/kg bw)

compared to (PC). The results of Cl: ZnONPs (10 mg/kg bw) +STZ (60 mg/kg bw) was close to normal value that showed in NC group **(Table 3)**. Increase in plasma level of total F_2-IsoPs in type 2 diabetic nephropatic patients ($P \leq 0.01$) with respect to controls [44]. Increased F2-IsoPs synthesis during diabetes appears to be responsible in part for the increase in renal TGF-β, α well-known mediator of diabetic nephropathy [6]. Plasma F_2-IsoPs levels in STZ-induced diabetic rats were significantly higher than in nondiabetic animals [45].

Table 3. Effect of $ZnCl_2$ and Cl: ZnONPs (5 and 10mg/Kg bw) on retinal F2-isoprostans concentration in diabetic rats.

Time / Treatments	F2-isoprostant (pg/ml)			
	0 h	**24 h**	**48 h**	**72 h**
G1 (NC)	0.2776^{a} ±0.001	0.2750^{e} ±0.000	0.2762^{e} ±0.001	0.2762^{e} ±0.001
G2 (PC)	0.2766^{a} ±0.002	0.5622^{a} ±0.003	0.6622^{a} ±0.003	0.7162^{a} ±0.003
G3	0.2758^{a} ±0.002	0.3620^{b} ±0.003	0.3652^{b} ±0.001	0.3282^{b} ±0.003
G4	0.2762^{a} ±0.002	0.3044^{c} ±0.002	0.3038^{c} ±0.001	0.3048^{c} ±0.002
G5	0.2774^{a} ±0.001	0.2942^{d} ±0.002	0.2956^{d} ±0.001	0.2952^{d} ±0.001
G6	0.2748^{a} ±0.001	0.2724^{e} ±0.002	0.2760^{e} ±0.001	0.2764^{e} ±0.001
LSD	**0.004**	**0.006**	**0.005**	**0.006**

G1(NC) = Negative control, G2 (PC) = positive control, G3 = STZ + $ZnCl_2$ (5mg/kg bw), G4 = STZ + Cl: ZnONPs (5 mg/kg bw), G5 = STZ + $ZnCl_2$ (10 mg/kg bw), G6 = STZ + Cl: ZnONPs (10 mg/kg bw)

$ZnCl_2$ and Cl: ZnONPs (5 and 10 mg/kg bw) +STZ (60 mg/kg bw) treatment to STZ-induced diabetic rats are shown in **Table 4**. The results show significantly ($P \leq 0.05$) increased in retina SOD activity (16.29, 21.95 U/ml), (17.15, 24.12 U/ml) after treatment with (5 and 10 mg/kg bw), respectively for 72 h than PC group (11.31 U/ml). ZON treatment increased serum SOD and catalase activity, suggesting antioxidant effects [34].

CONCLUSIONS

Based on our results, we can conclude that $ZnCl_2$ and Cl: ZnONPs lead to activation of insulin mechanism of action and induction its synthesis. Cl: ZnONPs + STZ increased SOD and decreased F_2-IsoPs activates significantly ($P \leq 0.05$). Furthermore, the results elucidated the beneficial effects of

Table 4. Effect of ZnCl2 and Cl: ZnONPs (5 and 10mg/Kg bw) on retinal SOD concentration in diabetic rats..

Time / Treatments	SOD (U/ml)			
	0 h	**24 h**	**48 h**	**72 h**
G1 (NC)	$25.56^{a} \pm 0.2$	$25.36^{a} \pm 0.2$	$25.24^{a} \pm 0.17$	$25.09^{a} \pm 0.03$
G2 (PC)	$25.36^{ab} \pm 0.17$	$20.19^{f} \pm 0.03$	$14.22^{f} \pm 0.02$	$11.31^{f} \pm 0.17$
G3	$25.08^{bc} \pm 0.07$	$21.04^{e} \pm 0.02$	$17.63^{e} \pm 0.08$	$16.29^{e} \pm 0.11$
G4	$25.17^{abc} \pm 0.05$	$22.47^{c} \pm 0.04$	$19.32^{c} \pm 0.04$	$21.95^{c} \pm 0.15$
G5	$24.93^{c} \pm 0.13$	$21.81^{d} \pm 0.12$	$18.94^{d} \pm 0.05$	$17.15^{d} \pm 0.09$
G6	$24.82^{c} \pm 0.14$	$24.38^{b} \pm 0.23$	$23.57^{b} \pm 0.23$	$24.12^{b} \pm 0.02$
LSD	**0.399**	**0.392**	**0.360**	**0.320**

G1 (NC) = Negative control, G2 (PC) = positive control, G3 = STZ + $ZnCl_2$ (5mg/kg bw), G4 = STZ + Cl: ZnONPs (5mg/kg bw), G5 = STZ + $ZnCl_2$ (10 mg/kg bw), G6 = STZ + Cl: ZnONPs (10 mg/kg bw)

high dose of Cl: ZNONPs gave the best results protection retinal against oxidative stress and controlling hyperglycemia.

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