Open Science Journal of Pharmacy and Pharmacology
2018; 6(3): 21-25
http://www.openscienceonline.com/journal/osjpp

Ameliorative Effects of Combined Administration of Lycopene and/or Zinc on Biomarkers of Oxidative Stress in Alloxan- Induced Diabetic Wistar Rat

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To cite this article

Goji Anthony Donatus Teru, Muhammed Kabir Ahmed, Isah Anthony Denis, Medina Sani. Ameliorative Effects of Combined Administration of Lycopene and/or Zinc on Biomarkers of Oxidative Stress in Alloxan- Induced Diabetic Wistar Rat. *Open Science Journal of Pharmacy and Pharmacology.* Vol. 6, No. 3, 2018, pp. 21-25.

Received: April 30, 2018; **Accepted**: May 31, 2018; **Published**: July 2, 2018

Abstract

Diabetes mellitus remains a burdensome and public health problem with increasing global. The current global prevalence of diabetes mellitus, 285 million people in 2010, is estimated to be 439. This work was designed to study the effects of combine administration of lycopene and zinc on some biomarkers of oxidative stress in alloxan- induced diabetic rats. Apparently healthy albino rats weighing between 150g and 200g were used. The rats were randomly allotted into six groups, each containing five albino rats respectively. Five of the groups (II, III IV V and VI) were induced with diabetes by single intraperitoneal (*i.p*) injection of freshly prepared in 0.1 mol/L citrate buffered solution (pH 4.5) of alloxan (Sigma Aldrich, St. Louis, MO, USA) at a dose of 150 mg/kg body weight. Control (vehicle) rats were injected with equal volume of 0.1 mol/L citrate buffer. Four days after alloxan injection, diabetes induction was confirmed by measuring fasting blood glucose level in a tail vein blood samples using ACCU-CHEK compact plus glucometer (Roche, France). Rats with glucose level of 200 mg/dl or higher were considered as diabetic. After the induction of diabetes the rats were treated using the Lycopene and zinc separately and in combination respectively according to group daily, whereas, the other group (I) was not given any treatment and this served as the normal control, providing a baseline data. The results indicated that oral supplementation of lycopene (10 mg/kg b.w /day) and zinc (20mg/kg b.w /day) separately or in combination for 4 weeks of treatment exhibited significant alterations in the alloxan-induced-type-1 diabetes. The result showed that there was an increase in the serum CAT activity, SOD and GPx levels of the group treated with lycopene supplement as compared to the diabetic control group. The result also showed a significant $p<0.05$ increase in CAT, SOD and GPx levels in the group treated with zinc supplement when compared with the diabetic control group. There was also a significant $p<0.05$ increase in SOD and GPx levels in the group treated with zinc and lycopene as compared with the diabetic control. There was also a decrease in MDA concentrations for the groups treated with lycopene, zinc and the combined regimen as compared to the diabetic control. In conclusion, our finding indicates better ameliorative effects of combined treatment with lycopene and zinc for the oxidative stress in alloxan-induced diabetic rats.

Keywords

Diabetes, Alloxan, Lycopene, Zinc, Wistar Rats

1. Introduction

There is increasing evidence that complication related to diabetes are associated with oxidative stress induced by the generation of free radicals [1]. In diabetes, oxidative stress has been found to be mainly due to an increased production of oxygen free radicals and a sharp reduction of antioxidant defences [2]. Hence, compounds with antioxidative properties would be useful antidiabetic agents [2]. Streptozotocin induced diabetes mellitus is associated with the generation of reactive oxygen species causing oxidative damage [3]. Diabetic and experimental animal models exhibits oxidative stress due to persistent and chronic hyperglycaemia, which there by depletes the activity of antioxidative defense system and thus promotes de novo free radicals generation [4]. In spite of the availability of several oral hypoglycemic drugs, diabetes is still one of the main causes of many micro- and macro-vascular complications such as retinopathy leading to blindness, end stage renal disease and cardiovascular complications [5]. It is characterized by defects in insulin secretion and/or insulin action resulting in impaired metabolism of glucose, lipid and protein [6]. In diabetes, inability of excess glucose to enter insulin dependent tissues, such as skeletal muscles and adipocytes due to deficiency of or insensitivity to insulin, results in accumulation of glucose in the blood and other non-insulindependent tissues such as pancreas and brain [7]. The aim of this study therefore is to investigate the effect of the combine administartion of lycopene and zinc on some biomarkers os oxidative stress in alloxan- induced type 1 diabetic rats.

2. Materials and Methods

Alloxan (Sigma Aldrich Inc.st Louis, MO, USA), Lycopene (Sigma), Zinc, (Sigma Aldrich Inc.st Louis, MO, USA), Glucose. All other chemicals and drugs were obtained commercially and were of analytical grade.

2.1. Experimental Animals

Thirty Wistar male rats weighing between 200 to 250g (aged six to eight weeks) were obtained and housed in the animal house unit of the Department of Human Physiology, Ahmadu Bello University, Zaria. The normal standard rat chow and tap water were provided *ad libitum* during the experiment. Animals were stabilized to acclimatize to animal house environment for one week before commencement of the experiment. The study protocol was approved by the Institutional Animal Ethnic Committee of the University, Ahmadu Bello University, Zaria.

2.2. Methodology

2.2.1. Induction of Diabetes Mellitus

Diabetes was chemically induced by intraperitoneal (*i.p*) injection of freshly prepared in 0.1 mol/L citrate buffered solution (pH 4.5) of alloxan (Sigma Aldrich, St. Louis, MO, USA) at a dose of 150 mg/kg body weight. Control (vehicle) rats were injected with equal volume of 0.1 mol/L citrate buffer. Four days after alloxan injection, diabetes induction was confirmed by measuring fasting blood glucose level in a tail vein blood samples using ACCU-CHEK compact plus glucometer (Roche, France). Rats with glucose level of 200 mg/dl or higher were considered as diabetic [8]. Glucose levels of diabetic rats were checked before starting of treatment, so that animals could be homogenously and randomly distributed between the groups.

2.2.2. Experimental Design

Apparently normal healthy rats were used as normal control and diabetic-induced rats and were randomly alloted into six groups (n=5):

GROUP 1- Normal untrated were given olive oil 1 ml/kg daily orally for 4 weeks

GROUP 2- Diabetic untreated were given olive oil 1ml/kg daily orally for 4 weeks.

GROUP 3- Diabetic were treated with Lycopene 10 mg/kg daily orally for 4 weeks.

GROUP 4- Diabetic were treated with Zinc 20 mg/kg daily orally 4 weeks.

GROUP 5- Diabetic were treated with Lycpoene 10 mg/kg + Zinc 20 mg/kg daily orally for 4 weeks.

GROUP 6- Diabetic were treated with Glibenclamide 2mg/kg daily orally for 4 weeks.

2.3. Blood Collection

At the end of the experimental period of four weeks, overnight fasted animals were anaesthesized by halothane About 5 mL of blood was aseptically collected by cardiac puncture from each rat using a 5 mL syringe. The blood sample was placed in a bottle without anticoagulant for serum sample for biochemical analyses.

2.4. Antioxidant Enzymes Activities (Catalase, Superoxide Dismutase and Glutathione Peroxidase GPx)

Superoxide dismutase assay (SOD): Activity of superoxide dismutase in the rat serum was determined using NWLSS SOD assay kit (Product NWK-SOD02, Specificity: Cu/Zn, Mn and Fe Superoxide Dismutase, Sensitivity: 5 U/mL). The assay kit was based on the principle of superoxide inhibition of autooxidation of hematoxylin as described by [9].

2.4.1. Catalase Assay (CAT)

Catalase activity was assessed using NWLSS CAT activity assay kit (Product NWK-CAT01, Specificity: Catalase, Sensitivity: 6.0 U Catalase/mL). Catalase enzyme activity was measured, based on the principle of catalase consumption of H_2O_2 substrate at 240 nm [10].

2.4.2. Gluthathione Assay (GPx)

Glutathione Peroxidase activity was assessed using NWLSS^{TM} cGPx (GPx1) Enzyme-Linked Immunosorbent

Assay (ELISA) assay kit (Product NWK-GPX02, Specificity: Glutathione peroxidase, Sensitivity: 12.5 Pg/ml). The NWLSS™ cGPx assay will be based on a sandwich ELISA, where sample GPx concentration will be determined by comparing the 450 nm absorbance of sample wells to that of known standards [11].

2.5. Determination of Lipid Peroxidation

Determination of Serum Malondialdehyde (MDA)

The level of thiobarbituric-acid reactive substance, malondialdehyde (MDA), as an index of lipid peroxidation was evaluated. Quantitative measurement of lipid peroxidation of MDA was determined using NWLSS™ MDA assay kit (Northwest Life Sciences Specialities, Product NWK-MDA01, Vancouver WA, and Specificity: Malondialdehyde, Sensitivity: 0.08 μM). The principle is based on the reaction of MDA with thiobarbituric acid (TBA), forming an MDA-TBA adducts that absorbed strongly at 532 nm [12].

3. Statistical Analysis

All data were expressed as Mean±. SEM and data were entered and analyzed using statistical package SPSS (version 23) followed by one way analysis of variance (ANOVA) with multiple comparisons. The Tukey's post-hoc test was used to determine difference between groups. Values of $p<0.05$ was considered as statistically significant [13].

4. Results

Effect of Treatment on Serum Malondialdehyde and Enzymatic Anti-oxidants in Alloxan- Induced Type 1 Diabetes

The result in (Table 1) revealed that, the injection of Alloxan significantly $p< 0.05$ decreased serum enzymatic antioxidant activity GPx, SOD and CAT respectively and significantly $p< 0.05$ increased MDA level in the serum of diabetic rats. The levels of lipid peroxidation, measured as malondialdehyde (MDA), were significantly ($p<0.05$) elevated in diabetic control compared to non-diabetic pancreas (Table 1). MDA levels in glibenclamide-treated diabetic rats differ from the diabetic control rats. On the other hand, the diabetic rats that received a combination of lycopene and zinc exhibited a significant ($p< 0.05$) decrease in MDA concentrations compared to diabetic control rats.

Significant ($p< 0.05$) decrease in the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and Catalase were observed in diabetic control and alloxan-treated rats (Table 1). There was a significant ($p< 0.05$) decrease in catalase (CAT) activity in diabetic control compared to the corresponding non-diabetic control group. The diabetic rats administered lycopene in combination with zinc showed significantly ($p< 0.05$) decrease in CAT activity (Table 1). The glutathione peroxidase (GPx) activity was significantly ($p< 0.05$) increased in the lycopene+zinc treated group compared to nondiabetic control group (Table 1). Also, the diabetic rats administered lycopene in combination with zinc showed significantly ($p< 0.05$) increase in SOD activity when compared to the diabetic control (Table 1).

Table 1. *Effects of Olive oil, Lycopene and/or Zinc on Biomarkers of Oxidative Stress in Alloxan- Induced diabetic Wistar rats.*

	Catalase IU/L	Superoxide Dismutase IU/L	Glutathione Peroxidase IU/L	Malondialdehyde IU/L
Group 1: Normal + Olive oil (1ml/kg)	4.44 ± 0.4	2.7.62 ± 02.35	38.12 ± 1.86	5.60 ±0.33
Group 2: Diabetic + Olive oil (1ml/kg)	1.47 ± 1.51	5.01± 0.30	19.01 ±0.84	189.8 ±3.28
Group 3: Diabetic + Lycopene (10mg/kg)	9.96 ±0.92	41.5 ±3.64*	77.5 ±2.21*	16.42±1.47*
Group 4: Diabetic + Zinc (20mg/kg)	4.90 ±0.50	42.78 ± 1.87*	48.68 ±2.57*	19.64± 1.27*
Group 5: Diabetic + Lycopene+Zinc	1.15 ±0.18	30.50 ±2.58*	66.6 ±1.42*	14.24 ±1.29*
Group 6: Diabetic + Glibenclamide (2mg/kg)	5.10 ±0.04	7.81± 0.41	18.31±0.38	19.0± 3.85

1 (non diabetic 1 ml/kg), 2 (diabetic 1ml/kg), 3 (diabetic treated with lycopene 10 mg/kg) 4 (diabetic treated with zinc 20 mg/kg) 5 (diabetic treated with lycopene 10 mg/kg+ zinc 20 mg/kg) 6 (diabetic treated with glibenclamide 2mg/kg); Values are mean ± SD of 5 rats; **Mean values are significantly ($p < 0.05$) different compare to the normal control (group 1). *Mean values are significantly ($p < 0.05$) different compare to diabetic control rats (group 2).
Note: Values are mean ± SEM, n=5, *$p< 0.05$ versus diabetic group, **$p< 0.05$ versus Normal group

5. Discussion

Results obtained revealed a significant $p<0.05$ increase in the serum catalase activity, superoxide dismutase levels and glutathione peroxidase levels of the group treated with lycopene as compared to the diabetic group that did not ingest lycopene. This is in agreement with research conducted and described by [14], who reported a significant increase in SOD and GPx levels following supplementation with lycopene in diabetic animals. Increase levels of these important cytosolic enzymes like SOD, CAT and Gpx in the present study indicates that lycopene lowers oxidative stress.

The result also recorded a significant ($p< 0.05$) in the serum catalase activity, superoxide dismutase level and glutathione peroxidase levels of the group treated with Zinc supplement as compared to the diabetic control group. This is in agreement with the findings of [15], which showed that serum levels of CAT, SOD, and GPx were increased after supplement with zinc. The present study indicates that zinc help to improve antioxidant status in diabetes. This could probably result from the supportive role played by zinc on the body's antioxidant defence systems. Such roles include; acting as a co-factor for superoxide dismutase (Isoforms 1 and 3) regulating glutathione metabolism and metallothionein expression, competing with iron and copper in the cell membrane and also inhibiting the nicotinamide adenine

dinucleotide phosphatase-oxidase (NADPH-oxidase) enzyme

There were also significant $p<0.05$ increase superoxide dismutase and glutathione peroxide levels of the group treated with zinc and lycopene combined together as compared with diabetic control group. This indicates that the use of the combined regimen of the zinc and lycopene has a better effect on oxidative stress. The increase in catalase after the combined treatment with lycopene and zinc was insignificant.

The result showed significant $p<0.05$ decrease in the MDA concentration for the group treated with lycopene as compared with the diabetic control group. This is in line with the study carried by [16], who reported that plasma MDA level was significantly decrease following lycopene supplememtation in STZ induced diabetic Wistar rats. This indicates that lycopene has an antioxidant property in preventing lipid peroxidation seen in diabetes. This is a likely outcome of the fact that lycopene is an efficient singlet oxygen quencher in the group of carotenoid which has a potentially powerful antioxidant property ([17]. Several clinical trials suggest that it lowers oxidative stress particularly by preventing LDL oxidation [18].

Also the result showed significant $p<0.05$ decrease in the MDA concentration for the group treated with zinc supplements as compared with the diabetic control group. This is in concordance with the research undertaken by [19] and showed that zinc supplementation in diabetes prevent lipid peroxidation in liver of diabetic rat. This indicates that zinc plays a role in lowering oxidative damage in diabetes. The significant $p<0.05$ decrease in MDA concentration for the group treated with both lycopene and zinc supplements as compared to the diabetic control group indicates that combined treatment has greater effect in protecting the tissue from oxidative damage

6. Conclusion

In conclusion, the present study revealed that oral administration of combined lycopene and zinc supplements ameliorate oxidative stress and lowers the MDA (an index of lipid peroxidation) in alloxan induced diabetic Wistar rats to a greater extent than the use of individual regimen.

Acknowledgements

The authors wish to thank Mallam Ya'u Bello casual staff of the Department of Human Physiology, ABU, Zaria for the care of the experimental animals throughout the period of this research work.

References

[1] Paul, c., Okey, O and Angomuo, O. (2015). Oxiadtive Stress in Diabetes Mellitus. *International Journal of Biological Chemistry*, 9 (3): 92-109.

[2] Uhuo, E. M., Ogugua, V., Joshua, N and Paker, E. (2015). Effects of Solvent Extracts of *Colatropis gigantean* leaf on Oxiadtive Parameters in Diabetic Rabbits. *International Journal of Medicine and Medical Sciences*, 7 (6): 108-116.

[3] Shatadal, G., Sudip, B., Kahkashan, R and Parames, C. (2015). Curcumin Protects Rats Liver from STZ-induced diabetic Pathophysiology by Counteracting Reactive Oxygen Species and inhibiting the activation of P53 and MAPKs mediated Stress response Pathways, Vol 2 Pages 365-376.

[4] Nagy, M. A. (2015). Biochemical Effects of *Cleome droserifolia* on Alloxan-Induced Diabetes in Rats: Role of Insulin, Oxidative Stress and Inflammation, *Biochemistry an Indian Jourmal*, 9 (2): 61-67.

[5] Aaetha, C., Rajeev, C and Shalini, J. (2016). Microvascular and Macrovascular complications in Diabetes Mellitus: Distinct or Continnum. *Indian Journal of Endocrinology and metabolism*, 20 (4): 546-551.

[6] Chan, D. C. and Watts, GF. (2011). Dyslipidaemia in the metabolic syndrome and type 2 diabetes: pathogenesis, priorities, pharmacotherapies. *Expert Opin Pharmacother* 12 (1): 13-30.

[7] Piero, M. N., Nzaro, G. M and Njagi, J. M. (2015). Diabetes Mellitus- a Devastating Metabolic Disoder. *Asian Journal of Biomedical and Pharmaceutical Sciences*, 4 (40): 1-7.

[8] Hfaiedh, N., Murat, J. C. and Elfeki, A. (2013). Diabetes-induced damages in rat kidney and brain protective effects of natural antioxidants. *Journal of Nutrition and Food Science*, 3: 4-6.

[9] Martin, J. P., Dailey, M. and Sugarman, E. (1987). Negative and positive assays of superoxide dismutase based on hematoxylin autoxidation. *Archives of Biochemistry and Biophysics*, 255: 329-336.

[10] Beer, R. F. and Sizer, I. W. (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *Journal of Biological Chemistry,* 195: 133-140.

[11] Takebe, G. (2002). A comparative study on the hydroperoxide and thiol specificity of the glutathione peroxidase family and selenoprotein. *Journal of Biological Chemistry*, 277 (43): 41254-41258.

[12] Janero, D. R. (1990). Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free-Radical Biology and Medicine*, 9: 515-540.

[13] Duncan, R. C., Knapp. R. G. and Miller, M. C. (1977). "Test of Hypotheses in Population Means" In *Introductory Biostatistics for Health Science*, N. Y.: *John Wiley and SonInc*, 71-6.

[14] Singh, K., Bal, B. S., Chopra, S., Singh, S., and Malhotra, N. (2012). Ameliorative Effect Lycopene on Lipid Peroxidation and certain antioxidant enzymes in diabetic patients. *Journal of Diabetus*, 6 (5): 3-4.

[15] Ranasinghe, P., Pigera, S., Galappatthy, P., Katulanda, P. and Constantine, G. R. (2015). Zinc and diabetes mellitus: understanding molecular mechanisms and clinical implications. *Daru Journal of Pharmaceutical Sciences*, 23 (1): 44.

[16] Eze, D. E., Yusuf, T., Abubakar, A., Suleiman, O. S., Rabiu., M. K. and Mohammed, A. (2017). Lycopene Ameliorates Diabetic-Induced Changes in Erytrocytes Osmotic Fragility and lipid peroxidation in Wistar Rats. *Journal of Diabetes Mellitus* 7 (3): 71-85.

[17] Stahl, W., Heinrich, U., Aust, O., Tronnier, H. and Sies, H. (2006). Lycopene-rich products and dietary photoprotection. *Photochemical and Phototherapy Research,* 15 (2): 238-242.

[18] Chen, J., Yang,-Song, Y., and Zhang, L. (2013). Effect of Lycopene supplementation on oxidative Stress: An Exploratory Systematic Review and Meta- Analysis of Randomised Controlled Trials. *Journal of Medicinal Food,* 16 (5): 361-374.

[19] Cruz, K. J. C., Oliveira, A. R. S., and Marreiro, D. N (2015). Antioxidant role of zinc in diabetes mellitus. *World Journal of Diabetes* 6 (2): 333-337.