Antioxidant and anti-hypoglycemic activity of Zinger officinale root powder in STZ induced diabetic rats

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Abstract

Diabetes mellitus is a potentially morbid condition affecting a large number of people around the world. Animal model of diabetes is vital for the understanding of progression of various aspect of the disease and its pathogenesis and its cure by use of new therapeutics. In the present study the antioxidant and anti-diabetic potential of Zinger root powder (ZRP) was evaluated in streptozotocin induced diabetic rats (STZ). Diabetes in rats were induced by administering streptozotocin of 45 mg/kg of body weight 15 to overnight fasted rats. ZRP 100 mg/kg of body weight was orally administered to group C and Pioglitazone to group D at a dose of 20 mg/kg of body weight for 6 weeks respectively for six weeks. Lipid peroxide levels were also measured in normal, diabetic and treated animals. ZRP treated rats showed significant decrease in biochemical parameters like blood sugar level (BS), triglycerides (TG), and Malondialdehyde (MDA) levels as compared to diabetic rats. However, antioxidant activity was observed low in diabetic groups as compared to the healthy control groups, which was ameliorated by treatment of ZRP. On the basis of our results we obtained from the study we conclude that ZRP not only useful in controlling the blood sugar level but are also helpful against streptozotocin-induced oxidative toxicity by strengthening the antioxidant potential.

Key word- Diabetes, Oxidative stress, Toxicity, Anti-oxidant

Introduction

Diabetes is most common chronic metabolic diseases spread worldwide. It is assessed that there are approximately 366 million people globally suffers with diabetes and it is expected that figure will rise to 552 million people by 2030globally (Skyler,2012).Diabetes is a chronic metabolic disorder and rigorouscontrol of blood glucose and pressure have reduced the chance of developing diabetes-associated microvascular and macrovascular complications.

Apart from other metabolic defect, oxidative stress, reported to increase in diabetes and play major role in underlies aetiology of diabetes complications (Tagang et al.,2016). Long time before, a combining hypothesis was put forward, which state that hyperglycaemia along with excessive free fatty acids cause increase in production of reactive oxygen species (ROS) (Wellen,2005). Increase ROS can activate various intracellular signalling pathways such as the polyol pathway, the advanced glycation endproducts and receptors (AGE/RAGE) pathway etc., ultimately leading to β-cell dysfunction, and insulin resistance, development of diabetic complications. Indeed, developing evidence suggests that hyperglycaemia also alter antioxidant defences, which in turn cause various kind of cellular damage. Alteration of levels of antioxidants such glutathione (GSH), superoxide dismutase (SOD), catalase and glutathione reductase (GR)etc, were also reported in the diabetic patients. So, decreasing oxidative stress not only improve the complications associated with diabetes but also help in treatments of dyslipidemia and diabetes.Thoughtful and educating medication is vital for the controlling of diabetes and its associated complications. Though large number of allopathic drugs are available in the market for the treatment of DM, however, have various undesired side effects (Kasetti et al.,2012). Hence, in the scientific examination to discover plant based alternative therapy for the treatment ofdiabetes. The importance of plants based products in

the treatment of diabetes is widely acceptedand a large number of plants have been identified for their active role in the treatment of diabetes(Pushparaj et al.,2000).

Medicinal plants are complex mixtures of various compounds that can act synergistically and modulate the activity of disease targets.

Zinger is a traditionally flowering spicy plant grows in parts of China, India and has been grown in many countries in the Southeast Asia and South Asia.Zinger has staring potential for treating a number of ailments including degenerative disorders (arthritis and rheumatism), digestive health, cardiovascular disorders and cancer. Zinger have been reported to have potent antimicrobial, antioxidant, immunomodulatory activities. In the present study we used powder of Zingerto assess the anti-diabetic activity in STZ induced diabetic rats.

Methodology

Experimental animal model

Male Wistar rats were used weighing 150-180 grams to induce diabetes for the present study. Animals were secured from the animal house of the University. Rats were allowed free access to feed on a standard chow diet and water *ad libitum*. The rats were acclimatized under laboratory condition of temperature (25 ± 2°C) with light dark cycle (14/10hr). Ethical clearance was acquired from University Animals Ethical Committee for present study.

Induction of diabetes

To induce diabetes, a freshly prepared solution of streptozotocin (45 mg/kg of body weight in 0.1 M citrate buffer, pH 4.5) was injected intraperitoneally to overnight fasted rats. Nicotinamide at a dose of 230 mg/kg body weight was given 15 minutes prior to STZ injection for the development of stable type 2 diabetes mellitus (Szkudelski et al.,2012). After 48 hours of STZ administration, fasting blood sugar (BS) levels were measured.

Plant material

Zinger root powder (ZRP) were obtained in the month of March from the local market of Delhi was air dried at room temperature. Dried ZRP were crushed in an electric grinder machine to a fine powder and stored in an air-tight container, until further use.

Grouping of animals

After induction rats were divide randomly into 4 groups with 6 rats each.

Group A: Healthy control (normal saline)

Group B: Diabetic control (normal saline)

Group C: Diabetic treated with ZRP (100 mg/kg of body weight) orally daily (Zammel et al.,2021)

Group D: Diabetic treated with Pioglitazone (20 mg/kg of body weight) orally daily

All the rats were treated with 6 weeks.

Glycemic parameters and hormonal assays:

At the end of the study rats were fasted overnight and blood was drawn from retro orbital plexus on week 6 of the study for the estimation of Glycosylated hemoglobin and Glucose. Serum was separated for the estimation of serum triglycerides (TG) (Fortress diagnostics, United Kingdom) and Insulin (Mercodia rat ELISA kit, Sweden) respectively using commercially available kits. Plasma glucose (Fortress diagnostics, United Kingdom), HbA1c (For tress diagnostics, United Kingdom).

Oxidative Stress parameters

Assessment of Lipid Peroxidation and Reduced glutathione content

Lipid Peroxidation (LPO) was determined in serum as previously described method by Satoh (Satho et al.,1978). Briefly, 0.5 ml serum was first precipitated with 20% TCA. After obtaining precipitate, it was suspended in 0.05 N sulphuric acid and TBA (0.07% in 1 M sodium sulfate) and incubated for over half an hour in boiling water bath. The MDA–TBA

adduct thus formed was extracted with butanol and absorbance were measured at 532 nm. The results are expressed as nM/ml.

Reduced glutathione content (GSH) was estimation in whole blood as method published by Tietze et al.,1969. Briefly, the reaction mixture (1 ml) contained glutathione reductase (1 unit), NADPH [0.2 µM/ml in 0.01 M/0.005 M phosphate EDTA buffer (pH 7.5)] and 25 µl of hemolysate. On addition of DTNB, the absorbance of the chromophoric product formed was measured at 412 nm. The result is expressed as µM/ml blood.

Estimation of Superoxide dismutase and Catalase activity

Superoxide dismutase (SOD) and Catalase (CAT) activity in rat serum was measured using commercially available standard kit as described in user manual.

Statistical analysis: All values are expressed as Mean± SD.One way ANOVA followed by Tukey's test is applied separately for all the above mentioned parameters to analyse the data. Difference was assumed to be significant at the level of $p < 0.05$.

Results

Effect of ZRP on body weight:

In the present study we found a decrease in the body weight in diabetic rats which on oral treatment of ZRP prevented the loss of body weight. (Table 1)

Table 1: Effect of ZRP on body weight in diabetic (n = 6 in each group) rats.

Body Weight	**Control**	**Diabetic**	**ZRP treated**	**PG Treated**
Week 0	150±2.5	151±3.75	152±2.2	150.2±3.1
Week 4	151±1.72	145±1.12	155.1±1.25	158.2±2.5

Effect of ZRP on level of insulin

As shown in figure 1 serum insulin levels were found to be decrease in diabetic rats which after week 4 of the study we observed a significant increase in serum insulin levels in treated

rats in comparison to diabetic rats and the results were comparable to Pioglitazone (PG) treated group rats.

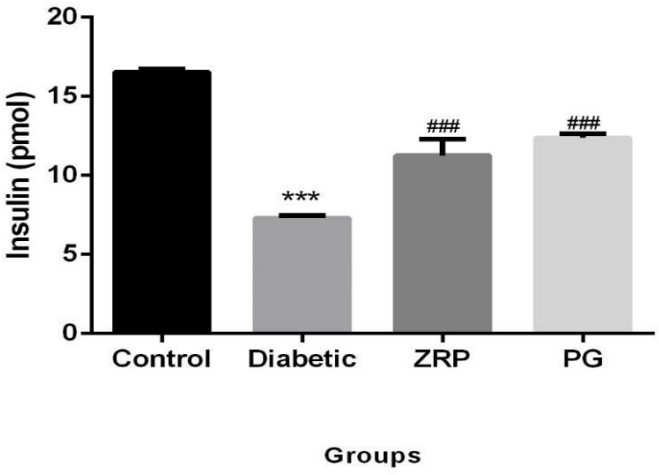

Figure 1: Effect of ZRP and PG on insulin level in diabetic rats (n = 6 per group) rats. ***Significantly different from control; ### significantly different from induced group ($p < 0.05$).

Effect of ZRP on level of TG

As shown in figure 2 serum TG levels were increased in diabetic rats which on the end of the study was significantlydecreased in insulin levels in rats treated with ZRP in comparison to diabetic rats and the results were comparable to Pioglitazone (PG) treated group rats.

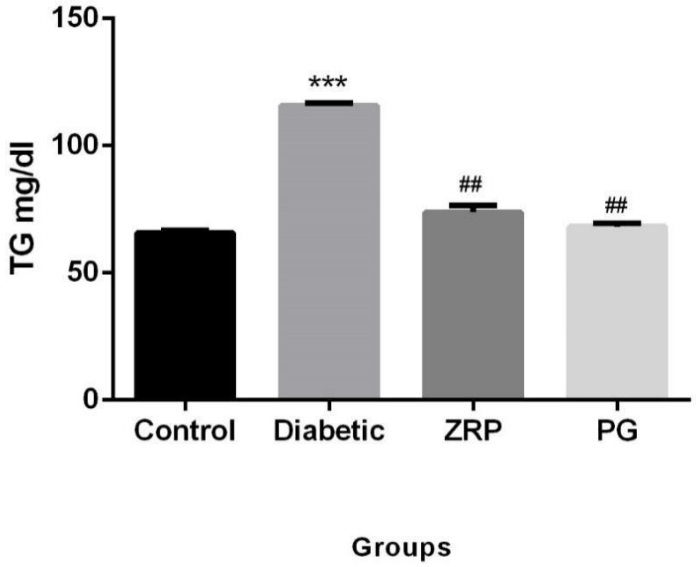

Figure 2: Effect of ZRP and PG on TG level in diabetic rats (n = 6 per group) rats. ***Significantly different from control; ### significantly different from induced group ($p < 0.05$).

Effect of ZRP on level of HbA1c

As expectedplasmaHbA1c levels were increased significantly in diabetic rats which on the treatment with ZRPlevel of HbA1cwas decreased in comparison to diabetic rats and the results were comparable to Pioglitazone (PG) treated group rats.

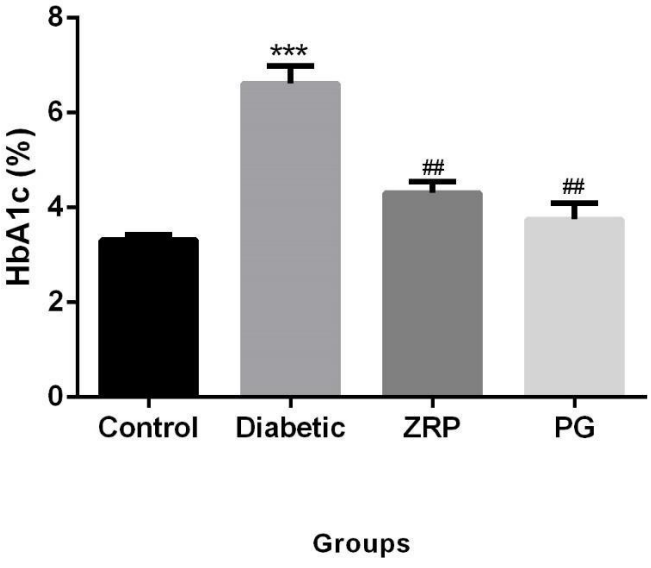

Figure 3: Effect of ZRP and PG on HbA1c level in diabetic rats (n = 6 per group) rats. ***Significantly different from control; ### significantly different from induced group ($p < 0.05$).

Effect of ZRP on level of MDA

MDA levels is a marker of oxidative stress, in our study we found asignificantincrease in MDA level in diabetic rats which on the treatment with ZRPlevel of MDA was decreased in comparison to diabetic rats and the results were comparable to Pioglitazone (PG) treated group rats.

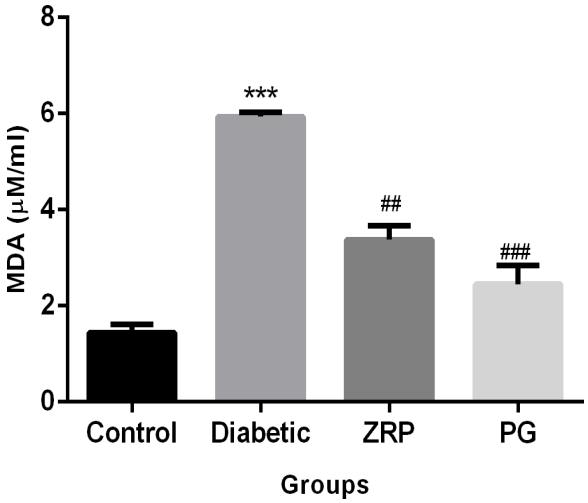

Figure 3: Effect of ZRP and PG on MDA level in diabetic rats (n = 6 per group) rats. ***Significantly different from control; ### significantly different from induced group ($p < 0.05$).

Effect of ZRP on level of GSH

In our study we found asignificant decrease in GSH content in diabetic rats which on the treatment with ZRPlevel of GSH was increased in comparison to diabetic rats. This shows antioxidant activity of ZRP.

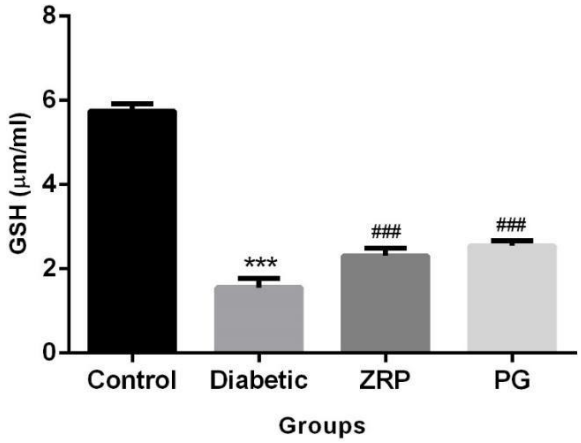

Figure 4: Effect of ZRP and PG on GSH level in diabetic rats (n = 6 per group) rats. ***Significantly different from control; ### significantly different from induced group ($p < 0.05$).

Effect of ZRP on level of SOD

In our study we found asignificant decrease in SOD content in diabetic rats which on the treatment with ZRPlevel of GSH was increased in comparison to diabetic rats and the results were comparable to PG treated rats.

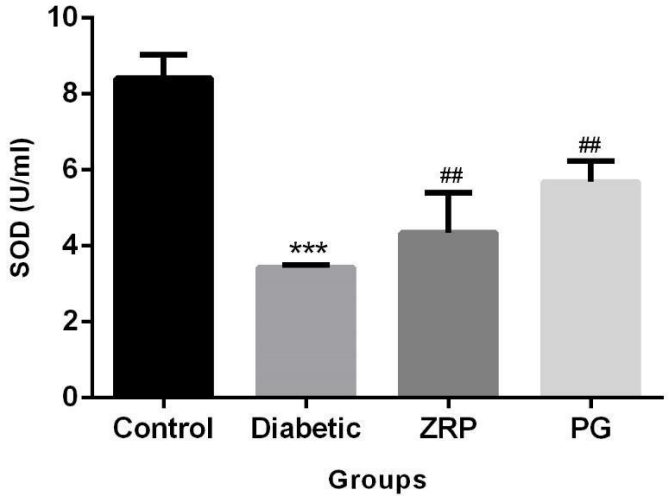

Figure 4: Effect of ZRP and PG on SOD level in diabetic rats (n = 6 per group) rats. ***Significantly different from control; ### significantly different from induced group ($p < 0.05$).

Effect of ZRP on level of CAT

In our study we found asignificant decrease in CAT content in diabetic rats which on the treatment with ZRPlevel of GSH was increased in comparison to diabetic rats and the results were comparable to PG treated rats.

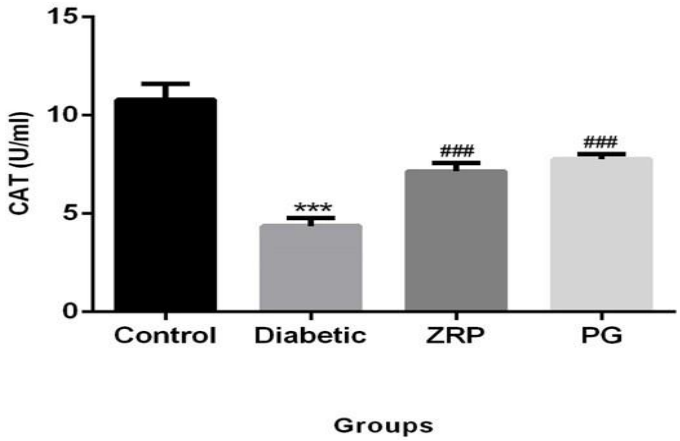

Figure 5: Effect of ZRP and PG on CAT level in diabetic rats (n = 6 per group) rats. ***Significantly different from control; ### significantly different from induced group ($p < 0.05$).

Discussion

Oxidative stress plays anessential role in cellular injury as a result of hyperglycemia, lipid peroxidation, and little activities of antioxidant enzymes (Giugliano et al.,1996). High glucose level generate free radicalthat are very toxic to the cell, which interact with the lipid membrane and produce lipid peroxides causing cellular injury (Kesavulu et al.,2001; Aydin et al.,2001; Pandey et al.,2010).A definitevolume of ROS/oxidative stress is necessary to perform normal metabolic progressions since ROS play severalregulatory roles inside the

cells (Gomes et al.,2012). Though uncontrolled synthesis of ROS is noxious. Various studies shows that oxidative stress acts as intermediary of insulin resistance and its development to glucose intolerance and stabilization of diabetes mellitus (Negre-Salvayre et al.,2009).

In the present study we found increased level of plasma lipid peroxide in STZ-induced diabetic rats along with a decreased level of enzymatic and non-enzymatic anti-oxidant that are in accordance with previously reported documents that also showshigh serum lipid peroxide levels and diminished antioxidant status in diabetic subjects (Khosla et al.,2000; Sato,1979). After treatment with herbal hypoglycemic agents ZRP we found not only a significant decrease in sugar level but they also significantly reduces the lipid peroxidation of lipid in diabetic rats. Furthermore, after induction of diabetes we also found that level of antioxidant GSH,SOD and CAT was decreased which is supposed to form primary defence mechanism against ROS.GSH is the important endogenous antioxidant and accountable for removal of ROS (Khan et al.,2015). Treatment with ZRP increased the GSH level and consequently due to this level of ROS decreases. Further, this reduction in the level of ROS may also help in the amending the antioxidant defense system such as SOD, CAT in diabetic rats which also believe to associated with pathogenic mechanism of diabetes. In our study as expected the level of SOD, CAT was found to be decreased in diabetic rats. After treatment with ZRP level of SOD and CAT get ameliorated and the reversal in their content after treatment may be due to decreased oxidative toxicity, indicating the free radical hunting activity and their defensive act against cellular damage. The above in vivo antioxidant status supports the hypothesis that antioxidant may have role in treatment of diabetes.

Conclusion

From the above study it was reported that the ZRP exhibits antihyperglycemic in addition to antioxidant effects in STZ-induced diabetic rats, thereby vindicating its

ethnopharmocological use. Further chemical and pharmacological investigations required to know about the mechanism of action of ZRP in diabetes treatment.

References

1. Skyler, J. Atlas of diabetes. Springer Science & Business Media. 2012.
2. Aluwong T, Ayo JO, Kpukple A, Oladipo OO. Amelioration of Hyperglycaemia, Oxidative Stress and Dyslipidaemia in Alloxan-Induced Diabetic Wistar Rats Treated with Probiotic and Vitamin C. Nutrients. 2016 May 5;8(5):151.
3. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. J Clin Invest. 2005 May;115(5):1111-9.
4. Kasetti RB, Nabi SA, Swapna S, Apparao C. Cinnamic acid as one of the antidiabetic active principle(s) from the seeds of Syzygiumalternifolium. Food ChemToxicol. 2012 May;50(5):1425-31.
5. Pushparaj P, Tan CH, Tan BK. Effects of Averrhoabilimbi leaf extract on blood glucose and lipids in streptozotocin-diabetic rats. J Ethnopharmacol. 2000 Sep;72(1-2):69-76.
6. Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. Diabetes Care. 1996 Mar;19(3):257-67.
7. Kesavulu MM, Rao BK, Giri R, Vijaya J, Subramanyam G, Apparao C. Lipid peroxidation and antioxidant enzyme status in Type 2 diabetics with coronary heart disease. Diabetes Res ClinPract. 2001 Jul;53(1):33-9.
8. Aydin A, Orhan H, Sayal A, Ozata M, Sahin G, Işimer A. Oxidative stress and nitric oxide related parameters in type II diabetes mellitus: effects of glycemic control. ClinBiochem. 2001 Feb;34(1):65-70.
9. Pandey KB, Mishra N, Rizvi SI. Protein oxidation biomarkers in plasma of type 2 diabetic patients. ClinBiochem. 2010 Mar;43(4-5):508-11.

10. Gomes EC, Silva AN, de Oliveira MR. Oxidants, antioxidants, and the beneficial roles of exercise-induced production of reactive species. Oxid Med Cell Longev. 2012;2012:756132.
11. Negre-Salvayre A, Salvayre R, Augé N, Pamplona R, Portero-Otín M. Hyperglycemia and glycation in diabetic complications. Antioxid Redox Signal. 2009 Dec;11(12):3071-109.
12. Khosla P, Bhanwra S, Singh J, Seth S, Srivastava RK. A study of hypoglycaemic effects of Azadirachtaindica (Neem) in normalandalloxan diabetic rabbits. Indian J PhysiolPharmacol. 2000 Jan;44(1):69-74.
13. Sato Y, Hotta N, Sakamoto N, Matsuoka S, Ohishi N. and Yagi K. Lipid peroxide level in plasma of diabetic patients. Biochem. Med. 1979;21,102-107.
14. Khan MA, Subramaneyaan M, Arora VK, Banerjee BD, Ahmed RS.Effect of Withaniasomnifera (Ashwagandha) root extract on amelioration of oxidative stress and autoantibodies production in collagen-induced arthritic rats.J Complement Integr Med. 2015;12(2):117-25.
15. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. ClinChimActa 1978;90:37–43
16. Tietze F. Enzymatic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal Biochem 1969;27:502–22.
17. Szkudelski T. Streptozotocin-nicotinamide-induced diabetes in the rat. Characteristics of the experimental model. ExpBiol Med (Maywood). 2012 May;237(5):481-90.
18. Zammel N, Saeed M, Bouali N, Elkahoui S, Alam JM, Rebai T, Kausar MA, Adnan M, Siddiqui AJ, Badraoui R. Antioxidant and Anti-Inflammatory Effects of *Zingiberofficinale roscoe* and *Allium subhirsutum*: In Silico, Biochemical and Histological Study. Foods. 2021 Jun 15;10(6):1383.