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AMELIORATION OF ALTERED ANTIOXIDANTS STATUS BY GINGER IN STZ INDUCED DIABETIC RAT BRAIN

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Abstract:

The present study was carried out to investigate the protective effect of Ginger on lipid peroxidation (LPO), antioxidants activities of both enzymatic and non-enzymatic in streptozotocin (STZ)-induced diabetic rats brain. Thirty Wistar strain rats were divided into five equal groups: Normal control (NC), Diabetic control (DC), Ginger treated (Gt), and Diabetic plus ginger treated (DC + Gt) and Diabetic plus glibenclamide (Standered reference drug). Experimental diabetes was induced by a single dose of STZ (50mg/kg, i.p.) injection. The oxidative stress was measured by tissue Lipid peroxidation (LPO) level, content of reduced glutathione (GSH) and by antioxidant enzymatic activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) in rat brain. We observed in the present study antioxidant enzymatic activities and glutathione content were decreased in diabetic control rats. Administration of Ginger (200mg/kg day) diabetic rats showed significant increase in the activities antioxidant enzymes when compared to diabetic control rats. From the present results, we conclude that diabetes-induced brain oxidative stress was modulates by ginger treatment, thus ginger can be used as a regular nutrient to care for the brain tissue.

Key words: Diabetes, Ginger, STZ, Antioxidant enzymes and Glibanclamide

Introduction:

Diabetes mellitus is a metabolic syndrome categorized by the defeat of glucose homeostasis as a consequence of defects in insulin emission and functionality. Glucose accumulation in the blood and continued hyperglycemia, often leading to various microvascular and macrovascular complications [1]. In Diabetes mellitus, a leading non-communicable disease with multiple etiologies, affects more than 100 million people worldwide and is considered as one of the five leading causes of death in the world [2]. A state of increased free radical formation has been shown by Diabetes mellitus [3]. The endurance of oxidative stress consequential from improved free radicals has been postulated in diabetes. The harmful influence of diabetes mellitus on metabolism of tissues of various organs is well known. Glucose control plays an important role in the pro-oxidant/antioxidant balance [4]. Antioxidants, which can hunt free radicals in opposition to harm and decompose, have an important role in biological system and may be helpful in the prevention of cancer, heart diseases, ageing and diabetes mellitus. The central nervous system is highly inclined to oxidative stress. Reactive oxygen species (ROS) reliant central nervous disorders have been experiential to be really triggered by the occurrence of free radicals. Anti-oxidant treatment has proved to be extremely useful to combat reactive oxygen species-induced injury in the central nervous system. Synthetic antidiabetic agents create severe side effects, such as hypoglycaemic unconsciousness and renal and hepatic disorders. The plant products usefulness is reported to be recognized to the amount of bioactive substances such as alkaloids, flavonoids, phenolic compounds and essential oils, with antioxidant activity properties [5]. Ginger (Zingiber officinale) is generally addicted as spice for the food flavouring. Various reports have demonstrated that ginger has hypoglycemic, hypocholesterolemic, antirheumatic and antidiabetic properties in humans and experimental animals [6]. Ginger is considered to be an excellent entrant for oral remedy as it is useful, non-toxic and without severe part effects. Many phytochemicals like phenols, flavanoids, terpenoids and other phytochemicals were present in ginger which is responsible for their pharmacological

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activities. Its dried extract contains monoterpenes and sesquiterpenes [7]. However, the effects of ginger have not been studied for its antioxidant actions on diabetic brain parts. Thus, the present study aims to investigate the neuroprotective effect of ginger on oxidative damage in the brain of streptozotocin-induced diabetic rats.

Material and methods:

Animals.

Wistar strain male albino rats aged 3months weighing 180 ±200 g were obtained from Indian Institute of Science Bangalore (IISc). The rats were housed in clean polypropylene cages having 6 rats per cage and maintained under temperature controlled room (27±2°C) with a photoperiod of 12 h light and 12 h dark cycle. The rats were fed with a standard rat pellet diet and water ad libitum. The experiments were carried out in accordance with guidelines and protocol approved by the Institutional Animal Ethics Committee.

Chemicals:

All the chemicals used in this study were of analytical grade obtained from Fisher, sigma, Ranbaxy, Merck.

Induction of diabetes

The animals were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of streptozotocin (STZ) (50 mg/kg body weight) in 0.1 M cold citrate buffer (pH 4.5). The animals were allowed to drink 15% glucose solution overnight to overcome the drug- induced hypoglycemia. The animals were considered as diabetic, if their blood glucose values were above 250 mg/dl on the third day after STZ injection. Ginger treatment was given to the diabetic rats for 30 days.

Ginger ethanolic extract preparation

Fresh ginger rhizomes were purchased nearby and washed by water to eliminate the dissipate. The outer layer of ginger was peeled off and was air dried. Two kilograms of air dried

Herb rhizomes was crushed into well powder automatically and extracted in cold percolation for 24 h with 95% ethanol. The extract was recovered and 95% ethanol was further added to the ginger powder and the extraction was continued. This process was repeated three times. The three extracts were pooled together, combined, filtered and the filtrate was concentrated to dryness under reduced pressure in a rotary evaporator. The resulting ethanolic extract was air dried, finally yielding 80 g of dark brown, gelatinous extract of ginger dried rhizomes. The crude ethanolic extract was used for the experiments without any further purification. Dose equivalent to 100 mg/kg and 200 mg/kg b.w of the ginger, was calculated and suspended in 2%, v/v Tween 80 solution for the experiment.

Grouping of animals

The rats were divided into five groups, six rats in each group and treated as follows:

Group 1. Normal control (NC): this group of rats received vehicle solution (2% of Tween 80).

Group 2. Ginger treatment (Gt): six rats received ethanolic extract of ginger with a dose of 200 mg/kg body weight via oral gavage for 30 days.

Group 3. Diabetic control (STZ 50 mg/kg body weight) (DC): streptozotocin is given intraperitonially for the induction of diabetes to this group.

Group 4. Diabetic + ginger treatment (DC + Gt): diabetic rats received ginger ethanolic extract (200 mg/kg) for a period of 30 days.

Group 5. Diabetic + glibenclamide treatment (DC + Gli): diabetic rats treated with glibenclamide (600 micro gram /kg body weight orally).

After completion of 30 days of treatment, the animals were sacrificed by cervical dislocation and the brain tissues were excised at 4^{0}C. The tissues were washed with ice-cold saline, immersed in liquid nitrogen and immediately stored at 80^{o}C for further biochemical analysis.

Analytical procedure

Brain superoxide dismutase activities were assayed in the tissue homogenates by the method of Misra and Fridovich at 480 nm for 4 min on a Hitachi U-2000 spectrophotometer. Catalase activity was determined at room temperature by using the method of Aebi (1984), Activity of glutathione peroxidase was determined by the method of Flohe and Gunzler (1984), Glutathione reductase (GR) activity was determined according to the method of Carlberg and Mannervik (1985). The

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concentration of reduced glutathione (GSH) in brain homogenates was measured, as described by Akerboom and Sies (1981).

Statistical analysis

Analysis of variance (ANOVA) and Duncan's multiple comparison tests among data were carried out using the SPSS (Version 13.5; SPSS Inc., Chicago, IL, USA) and M.S. Office, excel software for the significance of the main effects (factors), and treatments along with their interactions. Statistical significance was set at $p < 0.05$.

Results

Ameliorative effect of ginger on antioxidant enzyme status in STZ-induced diabetic rats

Significant ($p < 0.01$) decreases in SOD, CAT, GPx, GR activities and GSH level and a high level of MDA were observed in the diabetic control rats compared with normal control rats. Diabetic rats with ginger treatment, showed significant ($p < 0.001$) increases in SOD, CAT, GR, GPx activities and GSH level, and a decrease in MDA level, which reflects restoration of the antioxidant enzyme systems to near-normal values (Figs. 1–6).

Discussion:

Diabetes is accompanied with lipid peroxides increased levels and reactive oxygen species and antioxidant enzymes SOD and CAT activities were decreased which play an important role in scavenging the toxic transitional of partial oxidation [8]. The antioxidative defence system enzymes like SOD and CAT showed lower activities in the brain tissue during diabetes and the results agree well with the earlier published data [9]. The decreased activities of SOD and CAT may be a response to increased production of H2O2 and O2_- by the auto-oxidation of the excess of glucose and non-enzymatic glycation of proteins. Pigeolet et al. [10] have reported the partial inactivation of SOD and CAT activities by hydrogen peroxide and hydroxyl radicals. SOD and CAT was decreased could also be due to their protein expression levels decreased in the diabetic condition, as recently reported in hepatic cells [11].Treatment with ginger extract and *glibenclamide* has reversed the SOD and CAT activities. This could be due to the presence of alkaloids, flavonols, flavones and volatile oils in *ginger*. These compounds may have antioxidant properties, with this property the extract could directly scavenges the superoxide radicals.

A prominent imbalance between reactive oxygen species production and endogenous anti-oxidant defence mechanism has been confirmed by reduced activity of GPx and GR in diabetic rats in the present study. Decreased GPx and GR activities indicate production of lipid peroxides and elevated H_2O_2 production. Treatment with ginger significantly potentiates above enzyme activities and the results are in agreement with the previous reports [12]. Thus, these results suggest that ginger has effective antioxidant activity by free radicals scavenging and oxidants/anti-oxidant homeostasis restoring developed during diabetic condition. In the present study we have observed significant reduced in Glutathione levels in brain during diabetic condition. The detoxification pathway of reactive oxygen species involves glutathione oxidation to glutathione disulfide (GSSG), ensuing in GSH level decreased diminution of tissue GSH content improves cellular damage caused by oxidative stress in accordance with previous Publications [13]. Ozbek et al [14] found that untreated diabetes caused generally lower levels of GSH in different brain region. With administration of ginger extract enhanced the GSH content in the diabetic rats. The increases in the content of GSH may protect cellular proteins against oxidation through glutathione redox cycle and also directly detoxifies reactive oxygen species generated from exposure to STZ. GSH content and GPx activity significant increase in diabetic rats treated with ginger indicate an adaptive mechanism in response to oxidative stress.

Conclusion:

From the above findings we concluded that ginger extract could be effective glibenclamide (like the anti-diabetic drug) in preventing the diabetic-induced turbulence in brain antioxidant enzyme status and lipid peroxidation. This was exposed by enhanced activities of antioxidant enzymes and recovered brain cells from injuries by ginger treatment in the diabetic rats. These results could further suggest that possible use of ginger as a nutraceutical supplement to cope

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with diabetic-induced damaging effects and to protect brain cells from reparation.

References

1. D. Control, C Trial. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. The New England journal of medicine. 2005; 353(25):2643.
2. P. Zimmet,. Z. Diabetes epidemiology as a tool to trigger diabetes research and care. Diabetologia 2005; 42, 499–518.
3. C Feillet-Coudray, Rock, E., Coudray, C., Grzelkowska, K., Azais-Braesco, V., Dardevet, D. Lipid peroxidation and antioxidant status in experimental diabetes. Clin. Chem. Acta 1999; 284, 31–43.
4. Sochor M, Baquer NZ, and McLean P.Glucose over-and underutilization in diabetes: comparative studies on the change in activities of enzymes of glucose metabolism in rat kidney and liver," Molecular Physiology 1985 vol. 7, pp. 51–67,
5. Tiwari, A. K., & Rao, M. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. Current Science 2002; 83, 30–38.
6. Young, H.Y., Luo, Y.L., Cheng, H.Y., Hsieh, W.C., Liao, J.C., Peng, W.H. Analgesic and anti-inflammatory activities of [6]-gingerol. J. Ethnopharmacol 2005; 96 (1–2), 207–210.
7.He,X.G.,Bernart,M.W.,Lian,L.Z.,&Lin,L.Z. High-performance liquid chromatography–electrospray mass spectrometric analysis of pungent constituents of ginger. Journal of Chromatography 1998; 796(2), 327–334.
8. D.Veera Nagendra Kumar, K.R. shanmugam, Ch. Ramakrishna. G. Narasimhulu., T. Lavanya, S.Rajeswara reddy4, K. Sathyavelu reddy. Neuroprotective effect of *pimpinella tirupatiensis* tuberous root aqueous extract on brain antioxidant status in STZ- induced diabetic rats. World journal of pharmaceutical research 2015; Volume 4, Issue 10, 2424-2435.
9. Sindhu, R. K., Koo, J. R., Roberts, C. K., & Vaziri, N. D. Dysregulation of hepatic Superoxide dismutase, catalase and glutathione peroxidase in diabetes response to insulin and antioxidant therapies. Clinical and Experimental Hypertension 2004; 26, 43–53.
10. Pigeolet, E., Corbisier, P., Houbion, A., Lambert, D., Michiels, C., Raes, M., et al. Glutathione peroxidase, superoxide dismutase and Catalase inactivation by peroxides and oxygen derived free radicals. Mechanisms of Ageing and Development 1990; 51, 283–297.
11. Sindhu, R. K., Koo, J. R., Roberts, C. K., & Vaziri, N. D. Dysregulation of hepatic Superoxide dismutase, catalase and glutathione peroxidase in diabetes response to insulin and antioxidant therapies. Clinical and Experimental Hypertension 2004; 26, 43–53.
12. Levy, Y., Zaltzberg, H., Ben-Amotz, A., Kanter, Y., Aviram, M. B-Carotene affects antioxidant status in non-insulin dependent diabetes mellitus. Pathophysiology 1999; 6, 157–161.
13. Matcovis B, Varga SI, Szaluo L, Witsas H. The effect of diabetes on the activities of the peroxide metabolic enzymes. Hormone and Metabolic Research 1982 14: 77-79.
14. Ozbek E, Turkoz Y, Gokdeniz R, Davarci, M, Ozugurlu F. Increased nitric oxide production in the spermatic vein of patients with varicocele. European Urology 2000; 37(2).

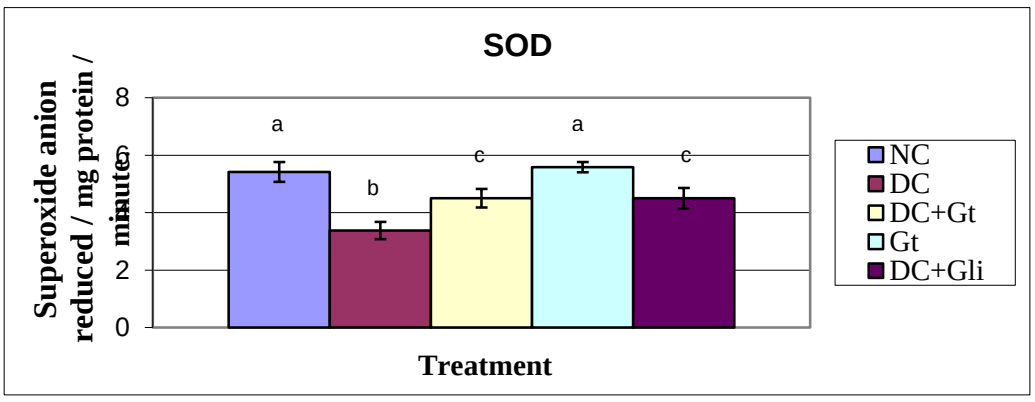

Fig.1: Changes in SOD activity in the brain of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with *Ginger* (DC+Gt), *Ginger treated* (Gt), Diabetic rats treated with *Glibenclamide*. Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at p<0.01.

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Fig.2: Changes in CAT activity in the brain of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with *Ginger* (DC+Gt), *Ginger treated* (Gt), Diabetic rats treated with *Glibenclamide*. Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at p<0.01.

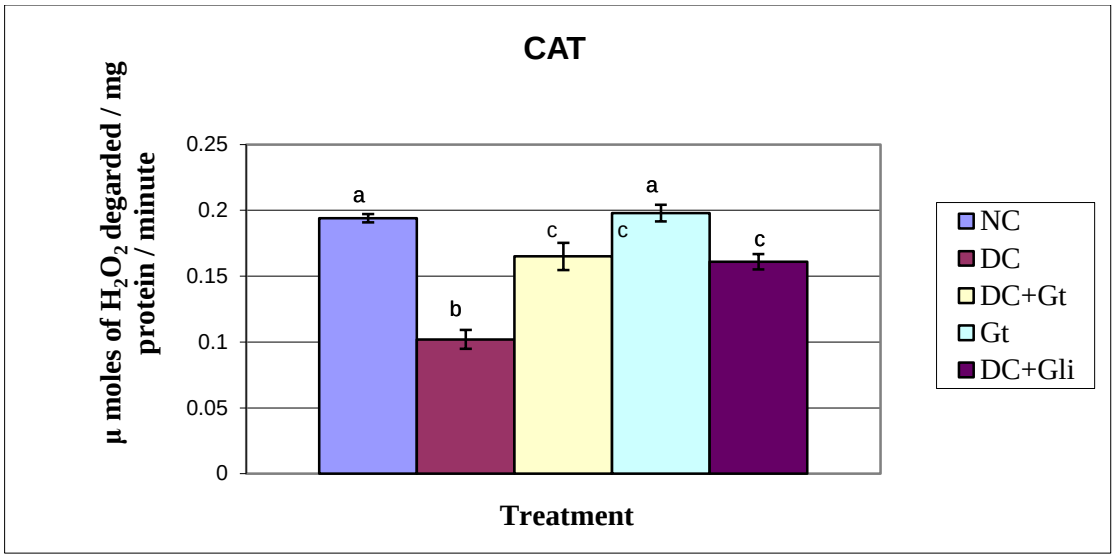

Fig.3: Changes in GPx activity in the brain of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with *Ginger* (DC+Gt), *Ginger treated* (Gt), Diabetic rats treated with *Glibenclamide*. Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at p<0.01.

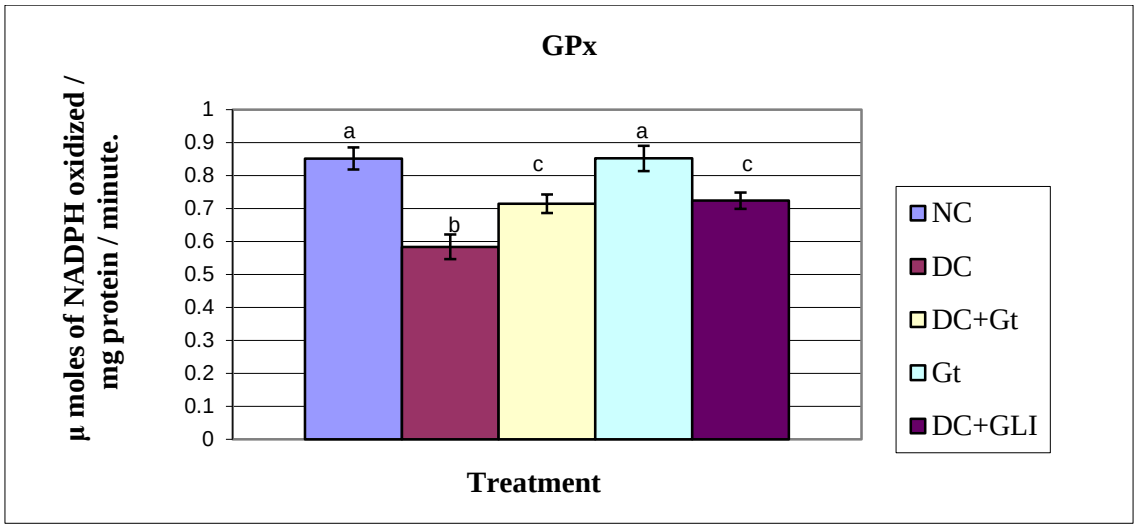

Fig.4: Changes in GR activity in the brain of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with *Ginger* (DC+Gt), *Ginger treated* (Gt), Diabetic rats treated with *Glibenclamide*. Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at p<0.01.

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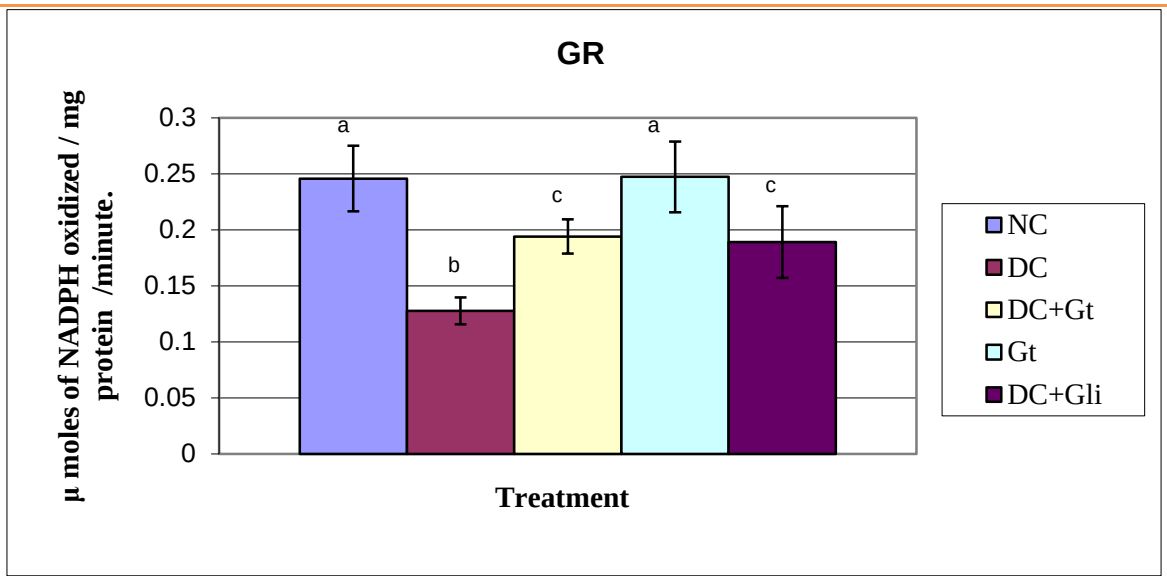

Fig.5: Changes in GR activity in the brain of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with *Ginger* (DC+Gt), *Ginger treated* (Gt), Diabetic rats treated with *Glibenclamide*. Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at p<0.01.