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Zingerone Protects the Tellurium Toxicity in the Brain Mitochondria of Rats

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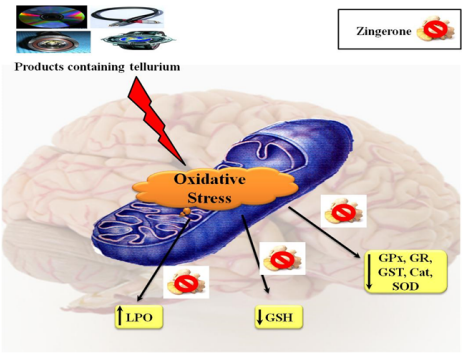
Abstract

Tellurium (Te) has been reported to be toxic to humans but its application in industries is growing at rapid pace. Mitochondria are responsible to generate the chemical energy to regulate the fuelling of cellular processes. The decay of the mitochondria in the brain cells is a primary cause of all neurodegenerative disorders from Parkinson's to dementia. Animals were divided into 5 groups; group 1st was control. Group 2nd received Te as sodium tellurite (4.15 mg, 1/20th of LD_{50}) orally once daily for 15 days. Groups 3rd and 4th were pretreated with zingerone (50 and 100 mg/kg b. wt respectively once daily for 15 days) followed by 15 days treatment with Te (orally once daily). On day 16, after the completion of Te dosing, the animals were sacrificed and brains were taken out to isolate the mitochondria. The content of lipid peroxidation (LPO) was increased significantly and the content of glutathione and activities of antioxidant enzymes; glutathione peroxidase (GPx) glutathione reductase (GR), glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) were decreased significantly in the brain mitochondria of Te treated group as compared to the brain mitochondria of control group and these changes were protected significantly and dose dependently with the treatment of zingerone in Te+Z1 and Te+Z2 groups. The above study indicates that the zingerone which is part of our diet may be used as the best tool for the prevention of Te toxicity.



Keywords: Brain mitochondria; Sodium tellurite; Oxidative stress; Antioxidant enzymes; Zingerone

Introduction

Tellurium belongs to group VIA in periodic table and has physical properties like a metal but chemical properties are more like a nonmetal [1]. Tellurium has been reported to be toxic to humans but its application in industries is growing at rapid pace. A decade back, Kaur et al. [2] have reported the toxicity of Te on brain lipids. Te has accelerated the levels of lipid peroxidation and inhibited the contents of glutathione and activities of antioxidant enzymes like glutathione peroxidase, glutathione reductase and catalase in the cerebrum, cerebellum, and brain stem [3].

Mitochondria are often referred as the powerhouse of the cells and it is found in every cell of the body. They generate the chemical energy in the form of ATP that regulates fuelling of cellular processes. The brain cells need a lot of energy to communicate and control the cell signaling with each other along with parts of the body that may be far away. A part from these, mitochondria has also many other functions, so the mitochondria are essential for normal brain function. The decay of mitochondria in brain cells is a primary cause of all neurodegenerative disorders from Parkinson's to dementia [4].

Oxidative stress is a result of reactive oxygen species (ROS) generated by the dysfunction of mitochondrial energy metabolism [5]. ROS are increasingly recognized as playing an important role in neurodegeneration because of their ability to cause oxidative stress and consequently damage cellular contents. Acute exposure to relatively high levels of oxidants, especially in the presence of calcium, can also induce opening of the mitochondrial permeability transition (MPT), an inner mitochondrial membrane, voltage sensitive, non-selective ion channel which opens to pass large molecular weight solutes between the mitochondrial matrix and cytoplasm, enabling the inner membrane (which is normally impermeable) to become permeable, leading to a "large amplitude swelling" [6].

Zingerone is an active principle of *Zingiber officinale* rhizome having good medicinal value. It has been reported to inhibit lipid peroxidation (LPO) and possess superoxide dismutase (SOD) like activity [7,8]. Previous reports have shown that zingerone quickly metabolized in rats and humans, easily crosses blood-brain barrier, achieves good concentration in systemic circulation, and is eliminated from body within 6 h after oral administration [9,10].

The brain is rich in polyunsaturated fatty acids and is much prone to oxidative stress. It is proposed that brain mitochondria are the main

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organelle responsible for oxidative stress and cause neuronal damage. There are no reports on this issue which has stimulated our interest to select the brain mitochondria for this study and neuroprotective role of zingerone.

Materials and Method

Chemicals

Glutathione (oxidized and reduced), nicotinamide adenine dinucleotide phosphate reduced form (NADPH), thiobarbituric acid (TBA), 1-chloro-2,4-dinitrobenzene (CDNB), sulfosalisylic acid, 5-5′-dithio-bis-2-nitrobenzoicacid (DTNB), sucrose; HEPES, ethylene diamine tetraacetic acid (EDTA), sucrose, percoll and zingerone (≥ 96%) were purchased from M/S Sigma-Aldrich, Germany. Other chemicals were of reagent grade.

Animals

Male Wistar rats of 250-300 g obtained from Central Animal House of College of Pharmacy, Jazan University were used in this study. They were housed in polypropylene cages in an air-conditioned room for a 12-h light/dark cycle. The animals were divided into four groups and each group comprising eight animals. Group-1 was control and vehicle was given orally and group-2 was experimental and sodium tellurite was given at a dose of 4.15 mg/kg b. wt (1/20 of LD_{50}) [oral LD_{50} in rat is 83 mg/kg [11]] orally once daily for 15 days. Groups-3 and -4 were zingerone pretreated (50 and 100 mg/kg b. wt. once daily for 15 consecutive days orally, respectively). The dose of Te (in terms of LD_{50}) was same as described [2]. The doses of zingerone were same as we described earlier [12]. Zingerone was dissolve in warm (45°C) distilled water to make it miscible and allow to cool slowly at room temperature [12]. All procedures were performed in accordance with NIH guidelines and the Guide for the Care and Use of Animals. These protocols were approved by the Jazan University, Institutional Animals Care and Use Committee (IACUC).

Isolation of mitochondria

Overnight fasted rats were sacrificed and their brains were taken out. Brain from each rat was used for the preparation of mitochondria as described by Nagy and Antonio [13]. In brief, 10% homogenate (w/v) was prepared in isolation buffer I (0.32 M sucrose; 5.0 mM HEPES, pH 7.5; 0.1 mM EDTA) with a Teflon–glass homogenizer at 1000 rpm with five strokes up and five strokes down at 4°C. The homogenate was centrifuged at 1000 *g* for 10 min at 4°C. Pellet-1 (P-1) containing nuclear and cell debris was discarded. The supernatant-1 (S-1) was further centrifuged at 12,500 *g* for 20 min at 4°C. The pellet-2 (P-2) was re-suspended in isolation buffer (3 mL/g of original wt. of the tissue) and homogenized gently by hand in a Teflon–glass homogenizer. Pure mitochondria were isolated on Percoll gradients of 8.5%, 10%, and 20% in buffer II (0.25 M sucrose, 5.0 mM HEPES, pH 7.2, 0.1 mM EDTA-K^+) and centrifuged at 15,000*g* for 20 min at 4°C. The bottom layer of 20% Percoll was resuspended in 10 mL of isolation buffer and further centrifuged at 15,000*g* for 10 min at 4°C. The pellet was resuspended in an appropriate amount of phosphate buffer (0.1 M, pH 7.6) to give 2.5 mg protein/mL.

TBARS content

TBARS content was estimated by the method of Utley et al. [14] as modified by Islam et al. [15] Mitochondria 0.25 ml was pipetted into a 15 × 100 mm test tube and incubated at 37°C in a metabolic shaker for 1 h. An equal volume of same mitochondria was pipetted into a centrifuge tube and placed at 0°C. After 1 h of incubation, 0.25 ml of 5% (w/v) chilled TCA and 0.5 ml 0.67% TBA were added and centrifuged at 4000 × g for 10 min. Thereafter, supernatant was transferred to other test tubes and placed in a boiling water bath for 10 min. The absorbance of pink colour produced was measured at 535 nm. The TBARS content was calculated by using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} and expressed as nanomoles of TBARS formed/hr/mg of protein.

Reduced glutathione (GSH) content

GSH content was measured by the method of Jollow et al. [16] with slight modification. Mitochondria were mixed with 4% sulfosalisylic acid (w/v) in 1:1 ratio (v/v). The test tubes were incubated at 4°C for 1 h, and centrifuged at 4000 × g for 10 min at 4°C. The assay mixture contained 0.1 ml of supernatant, 1.0 mM DTNB and 0.1 M phosphate buffer pH 7.4 in a total volume of 3.0 ml. The yellow colour developed was read immediately at 412 nm in a spectrophotometer (Shimadzu-1601, Japan). The GSH content was calculated as micromoles GSH/ mg protein, using a molar extinction coefficient of 13.6×10^3 M^{-1} cm^{-1}.

Estimation of glutathione peroxidase (GPx)

GPx activity was determined by the method of Mohandas et al. [17]. The reaction assay consisted of phosphate buffer (0.05 M, pH 7.0), EDTA (1mM), sodium azide (1 mM), glutathione reductase (1 EU/ml), glutathione (1 mM), NADPH (0.2 mM), hydrogen peroxide (0.25 mM) and 0.1 ml of mitochondria in the final volume of 2 ml. The disappearance of NADPH at 340 nm was recorded at room temperature. The enzyme activity was calculated as nmol NADPH oxidized/min/mg protein by using molar extinction coefficient of 6.22×10^3 $M^{-1}cm^{-1}$.

Glutathione reductase (GR)

Glutathione reductase activity was measured by the method of Carlberg and Mannervik [18] as modified by Mohandas et al. [17]. The reaction mixture consisted of phosphate buffer (0.1 M, pH 7.6), NADPH (0.1 mM), EDTA (0.5 mM), oxidized glutathione (1 mM) and 0.05 ml of mitochondria in a total volume of 2 ml. The enzyme activity was quantified at room temperature by measuring the disappearance of NADPH at 340 nm and calculated as nmol NADPH oxidized/min/mg protein using molar extinction coefficient of 6.22×10^3 M^{-1} cm^{-1}.

Glutathione-S-transferase (GST)

Glutathione-S-transferase (GST) activity was measured by the method of Habig et al. [19]. The reaction mixture consist of phosphate buffer (0.1 M, pH 6.5), reduced glutathione (1 mM), 1-chloro-2,4-dinitrobenzene (CDNB, 1.0 mM) and 0.1 ml of mitochondria in a total volume of 2.0 ml. The change in absorbance was recorded at 340 nm and enzyme activity was calculated as nmoles CDNB conjugate formed/min/mg protein using molar extinction coefficient 9.6×10^3 M^{-1} cm^{-1}.

Catalase (CAT)

CAT activity was measured by the procedure of Claiborne et al. [20]. Briefly, the assay mixture consisted of 0.05 M PB (pH 7.0), 0.019 M H_2O_2 and 0.05 ml of mitochondria in a total volume of 3.0 ml. The change in absorbance was recorded at 240 nm. Catalase activity was calculated in terms of nmol H_2O_2 consumed/min/mg protein using molar extinction coefficient of 43.6×10^3 M^{-1} cm^{-1}.

Superoxide dismutase (SOD)

Superoxide dismutase activity was measured spectrophotometrically, as described previously by Stevens et al. [21]

by monitoring the auto-oxidation of (−)-epinephrine at pH 10.4 for 3 min at 480 nm. The reaction mixture contained glycine buffer (50 mM, pH, 10.4) and 0.1 ml of mitochondria. The reaction was initiated by the addition of (−)-epinephrine. The enzyme activity was calculated in terms of nmol (−)-epinephrine protected from oxidation/min/mg protein using the molar extinction coefficient of $4.02 \times 10^3\ M^{-1}\ cm^{-1}$.

Protein estimation

Protein was estimated by the method of Lowry et al. [22] using bovine serum albumin as standard.

Statistical analysis

Results are expressed as mean ± SEM. The result was analyzed by one-way ANOVA followed by Tukey's test. The p value ≤ 0.05 was considered significant.

Results

Effect of zingerone on TBARS content in the brain mitochondria

The effect of zingerone on TBARS content was measured to demonstrate the oxidative damage on the membrane. TBARS content in the brain mitochondria was elevated significantly ($p<0.01$) in Te treated brain mitochondria as compared to the brain mitochondria of the control group animals which was protected significantly ($p<0.05$) and dose dependently in Te+Z1 and Te+Z2 groups as compared to Te group (Figure 1).

Effect of zingerone on GSH content in the brain mitochondria

The GSH level was decreased significantly ($p<0.05$) in the brain mitochondria of Te group as compared to the brain mitochondria of control group. Pretreatment with zingerone in the groups Te+Z1 and Te+Z2 has protected the GSH level significantly and dose dependently (Figure 2).

Effect of zingerone on the activities of antioxidant enzymes in the brain mitochondria

The activities of antioxidant enzymes (GPx, GR, GST, SOD and catalase) were decreased significantly in the brain mitochondria of Te treated group as compared to the brain mitochondria of control group. On the other hand, these activities were protected significantly and dose dependently in Te+Z1 and Te+Z2 groups of the brain mitochondria as compared to the brain mitochondria of Te group (Table 1).

Discussion

Mitochondria are essential to maintain the battle against entropy that is necessary to sustain life. They provide energy required for almost all cellular processes. In cellular respiration, highly reactive molecules called free radicals are formed within mitochondria which react with macromolecules leading to degeneration of the cells. Perhaps the best known free radical produced in this way is the superoxide radical, $O_2^{\cdot-}$. Free radicals are potentially very damaging to the cell components such as proteins and genetic material like DNA and RNA. If too many free radicals are released in the mitochondria, the damage can be severe, resulting ultimately the death of the cells. Living in an oxygenated environment has made us more susceptible to superoxide and peroxide reactions. Brain is the organ with high concentration of lipids and very much susceptible to ROS [23]. Moreover some glutathione xenobiotics conjugates formed in liver are able to cross blood brain barrier making central nervous system more vulnerable to ROS [24]. Mitochondria are

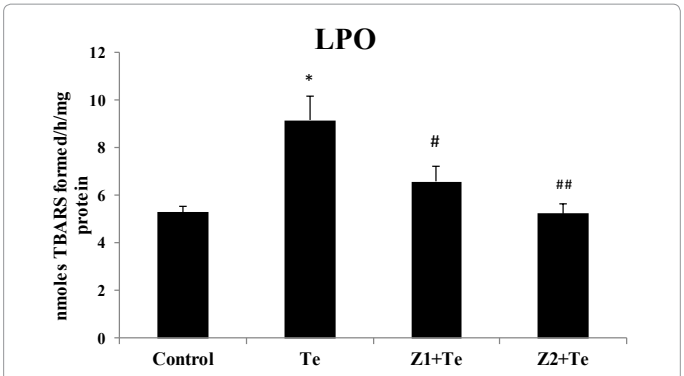

Figure 1: Effect of Te on the content of LPO in the rat brain mitochondria. Te treatment has increased the content of TBARS significantly as compared to control group. Its content was protected significantly and dose dependently in groups (Z1+Te and Z2+Te) as compared to Te group. The data are expressed as Mean ± S.E.M of 8 animals. *p<0.05 Te vs. control group and #p<0.01, ##p<0.001 Z1+Te and Z2+T2 groups respectively vs. Te group.

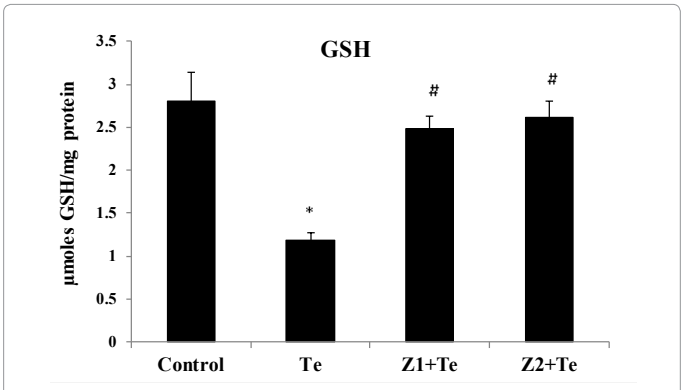

Figure 2: Effect of Te (4.15 mg/kg, 1/20 of LD_{50}) on the content of GSH in the brain mitochondria of rats. The level of GSH was decreased significantly and dose dependently as compared to control. The various doses of zingerone (Z1 50 and Z2 100 mg/kg b. wt) have protected the content of GSH significantly and dose dependently. The data are expressed as Mean ± S. E M of 8 animals. *p<0.01 Te vs. control and #p<0.001 Te vs. Z1+T and Z2+T group.

one of the sites for production of ROS in the brain. Hence we have isolated the brain mitochondria for the estimation of oxidative stress induced by Te toxicity. Selenite and tellurite have been known as thiol-reactive reagents [25] although the nature of the chemistry is still not completely understood [26]. In this study we have tried to explore one of the mechanisms of Te toxicity and its prevention by zingerone.

LPO is the marker of oxidative stress and its content was increased significantly by Te treatment suggesting the role of oxidative stress induced by Te. Zingerone, an alkaloid principle of *Zingiber officinale* rhizome, has been reported to possess the activity like SOD [7,8]. This activity of zingerone was helpful in the protection of the LPO level in the Te group treated with and zingerone. Glutathione is the body's master antioxidant. This tripeptide γ-L-glutamyl-L-cysteinyl-glycine is the thiol compound present in higher concentration in the cells of all organs. It is the major endogenous antioxidant produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen species, as well as maintaining the exogenous antioxidants such as vitamins C and E in their reduced forms, which is the active form of GSH [27]. In this study, the level of GSH was depleted significantly in Te treated group which was restored significantly with the treatment of zingerone.

Table 1: Effect of various doses of zingerone (Z) on the activity of antioxidant enzymes in brain mitochondria of Te toxicated rats.

Groups	GPx (nmol of NADPH oxidized/min/mg protein)	GR (nmol of NADPH oxidized/min/mg protein)	GST (nmol of NADPH oxidized/min/mg protein)	CAT (nmol of H_2O_2 consumed/min/mg protein)	SOD (nmol (−)-epinephrine protected from oxidation/ min/mg protein)
Control	667.49 ± 51.12	787.30 ± 60.15	1816.12 ± 199.60	241.98 ± 12.52	1270.94 ± 199.60
Te	453.87* ± 38.62 (-32.01 %)	419.29* ± 43.73 (-50.16 %)	1064.44* ± 128.71 (-41.39 %)	177.82* ± 37.94 (-26.51 %)	644.93* ± 52.02 (-49.26 %)
Z1+Te	608.35# ± 65.60 (34.03 %)	629.64* ± 94.81 (50.16 %)	1300.22# ± 118.08 (22.13 %)	225.07* ± 19.14 (26.57 %)	977.11# ± 93.13 (51.50 %)
Z2+Te	634.59# ± 49.09 (39.82 %)	715.90## ± 54.78 (70.74 %)	1587.9#9 ± 83.97 (49.19 %)	236.39* ± 18.21 (32.93 %)	1072.5## ± 160.04 (66.29 %)

Te toxicity leads to significant depletion on the activity of antioxidant enzymes (GPx, GR, GST, CAT and SOD) in Te groups as compared to control group (*p<0.05 vs. control group). Administration of zingerone (50 and 100 mg/kg b. wt.) significantly improved the activity of these enzymes in Z1+L and Z2+L groups as compared to Te group (#p<0.05, ##p<0.05 vs. Te group). Values in parentheses show the percentage increase or decrease with respect to their control. Values are expressed as Mean ± SEM of 8 animals.

All antioxidant defenses are interconnected [28]; hence disruption of one would disrupt the whole microenvironment and eventually could lead to a catastrophe. Glutathione peroxidase (GPx) is a well-known seleno-enzyme that functions as an antioxidant. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and detoxify the free hydrogen peroxide to water. The decrease activity of GPx was unable to catalyze GSSG to GSH and hamper the balance of the pool of GSH and GSSG. The zingerone treatment has protected the activity of GPx which might have maintained the GSSG and GSH pool in the brain mitochondria. Within the cells, GSH is regenerated from GSSG by the reaction catalyzed by GR, which is a ubiquitous cytosolic enzyme required for the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH), concomitantly oxidizing the reduced nicotinamide adenine dinucleotide phosphate (NADPH) in a reaction essential for the stability and integrity of cells [29]. During the normal catalytic reactions of GPx and GR, the glutathione is not consumed but recycled. In contrast, during the generation of glutathione-S-conjugates by glutathione-S-transferase (GST) [30,31] or by release of glutathione from cells [32,33] the level of total intracellular glutathione is lowered which makes more harm to the cells.

Previous reports have shown that zingerone quickly metabolized in rats and humans, easily crosses blood-brain barrier, scavenge free radicles and achieves good concentration in systemic circulation [9, 10,34]. Catalase is usually located in a cellular, bipolar environment organelle called peroxisome [35]. It catalyzes the decomposition of hydrogen peroxide to water and oxygen. H_2O_2 is one of the very toxic indigenous toxicant, its increased contents is extremely harmful to the cells. The decreased activity of catalase with Te was not able to detoxify the H_2O_2 but the treatment with zingerone has protected the activity of catalase which helped in the detoxification of H_2O_2 to H_2O. SOD converts highly toxic superoxide to low toxic H_2O_2 [36], which is further detoxified to H_2O by catalase and GPx. Since the activities of these antioxidant enzymes were depleted significantly by Te toxicity and zingerone has protected these activities significantly and dose dependently in the groups of Te+Z1 and Te+Z2 confirming the role of oxidative stress of Te toxicity and antioxidant properties of zingerone. The results showed the potential of zingerone in protecting the functions of the brain mitochondria. The above study indicates that the zingerone which is part of our diet may be used as the best tool for the prevention of Te toxicity. Therefore, it can be recommended that further understanding the mechanism of neuroprotection of zingerone on Te toxicity will provide an avenue to explore its bio-molecules for the potential use in pharmaceutical formulations.

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