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Taurine protects the antioxidant defense system in the erythrocytes of cadmium treated mice

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The present study was undertaken to investigate the protective role of taurine (2-aminoethanesulfonic acid) against cadmium (Cd) induced oxidative stress in murine erythrocytes. Cadmium chloride ($CdCl_2$) was chosen as the source of Cd. Experimental animals were treated with either $CdCl_2$ alone or taurine, followed by Cd exposure. Cd intoxication reduced hemoglobin content and the intracellular Ferric Reducing/Antioxidant Power of erythrocytes, along with the activities of antioxidant enzymes, glutathione content, and total thiols. Conversely, intracellular Cd content, lipid peroxidation, protein carbonylation, and glutathione disulphides were significantly enhanced in these cells. Treatment with taurine before Cd intoxication prevented the toxin-induced oxidative impairments in the erythrocytes of the experimental animals. Overall, the results suggest that Cd could cause oxidative damage in murine erythrocytes and that taurine may play a protective role in reducing the toxic effects of this particular metal. [BMB reports 2008; 41(9): 657-663]

INTRODUCTION

Cadmium (Cd) is a widely distributed and extremely toxic heavy metal capable of causing damage to various organs with an exact cellular mechanism of toxicity far from being completely understood. Cd exposure is known to cause interference with antioxidant enzymes, inhibition of energy metabolism, membrane damage, altered gene expression, and apoptosis (1, 2). Another target is erythrocytes, which contain hemoglobin that transports oxygen and carbon dioxide between the lungs and all tissues of the body. After exposure, Cd enters the blood, binds the erythrocytic membrane, and stimulates formation of the reactive oxygen species (ROS) (3), leading to alterations in the antioxidant system of erythrocytes and imposing oxidative damage upon the membrane (4). Anemia is one of the characteristic, clinical manifestations of chronic Cd intoxication (5) as Cd is known to reduce red blood cell count and the hematocrit value, as well as hemoglobin concentration (6).

Antioxidants belong to various classes of compounds with a wide variety of physical and chemical properties (7, 8) with sulfur-containing amino acids receiving much attention (9). Taurine, (2-aminoethanesulfonic acid), a derivative of cysteine, is abundant in the tissues of many animals and has been reported to protect many of the body's organs against toxicity and oxidative stress due to heavy metals and other toxin exposure (10, 11). The mechanisms of its protective actions are not completely understood but it can be viewed as a direct antioxidant due to its radical scavenging properties or an indirect antioxidant because of its preventative activities against changes in membrane permeability caused by oxidative stress (12, 13).

Although various reports have described the beneficial role of taurine against Cd-induced organ toxicity, no work has been done to elucidate its beneficial role in red blood cells under Cd-induced, pathophysical situations. The aim of the present study is, therefore, to investigate the protective role of taurine against Cd-induced oxidative damage in mice erythrocytes. Hemoglobin contents of the erythrocytes were measured to determine the effect of taurine in Cd-induced anemia while intracellular antioxidant power was determined by the Ferric Reducing/Antioxidant Power (FRAP) assay. Activities of: a) the intracellular antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GP_x), and glucose-6-phosphate dehydrogenase (G6PD); b) the levels of cellular metabolites, such as reduced glutathione (GSH), oxidized glutathione (GSSG), and total thiols; c) the levels of lipid peroxidation end products, lipid hydroperoxide, and protein carbonyl content; and d) the extent of intracellular ROS production, were determined to assess the chemopreventive role of taurine against cadmium-induced oxidative damage in murine erythrocytes.

RESULTS AND DISCUSSION

In this study we observed that $CdCl_2$ caused severe oxidative stress in the erythrocytes of the experimental mice and that oxidative impairment could be prevented by pretreatment with taurine.

From the dose- and time-dependent studies, $CdCl_2$ intoxi-

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cation decreased SOD activities monotonically up to a dose of 2.5 mg/kg body weight, with the time necessary for activity at 3 d (Fig. 1). Results suggest that taurine administration increased SOD activities linearly up to a dose of 100 mg/kg body weight, when applied 5 d prior to $CdCl_2$ intoxication (Fig. 2).

Kostic *et al.* (14) reported that the presence of Cd in an organism decreased the intracellular iron level and caused the decrease in hemoglobin concentration. The results obtained in

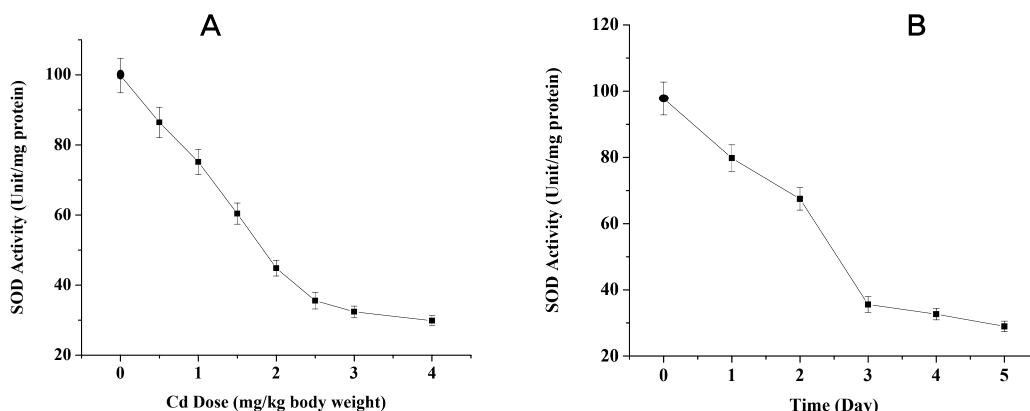

Fig. 1. (A) Dose-dependent effects of $CdCl_2$ on SOD activities in the erythrocytes. Closed circle: SOD activity in normal mice, Closed square: SOD activity in $CdCl_2$-intoxicated mice for 3 d at a dose of 0.5 mg, 1.0 mg, 1.5 mg, 2.0 mg, 2.5 mg, 3.0 mg, and 4.0 mg/kg body weight. Each column represents a mean ± SD, n = 6. "a" indicates the significant difference between the normal control and toxin treated groups ($P^a < 0.01$). (B) Time-dependent effects of $CdCl_2$ on SOD activities in the erythrocytes. Closed circle: SOD activity in normal mice, Closed square: SOD activity in $CdCl_2$-intoxicated mice (2.5 mg/kg body weight, orally) for 1, 2, 3, 4, and 5 d, respectively. Each column represents a mean ± SD, n = 6. "a" indicates the significant difference between the normal control and toxin treated groups ($P^a < 0.01$).

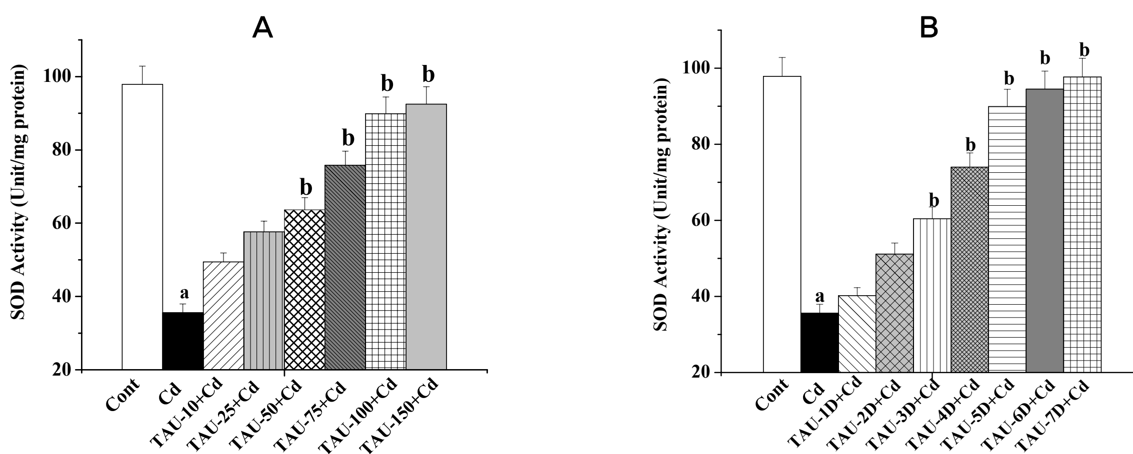

Fig. 2. (A) Dose-dependent effect of taurine (TAU) on SOD activities against $CdCl_2$-induced oxidative stress in the erythrocytes. Cont: SOD activity in the erythrocyte of the normal mice, Cd: SOD activity in the erythrocyte of the $CdCl_2$-treated mice, TAU-10 + Cd, TAU-25 + Cd, TAU-50 + Cd, TAU-75 + Cd, TAU-100 + Cd, TAU-150 + Cd: SOD activities in the erythrocytes of the TAU-treated mice for 5 d at a dose of 10, 25, 50, 75, 100, and 150 mg/kg body weight prior to $CdCl_2$ intoxication (at a dose of 4 mg/kg body weight, for 3 d). Each column represents a mean ± SD, n = 6. "a" indicates the significant difference between the normal control and toxin treated groups and "b" indicates the significant difference between the toxin-treated and taurine-treated groups ($P^a < 0.01$, $P^b < 0.01$). (B) Time-dependent effects of taurine (TAU) on SOD activities against $CdCl_2$-induced oxidative stress in the erythrocytes. Cont: SOD activity in the erythrocyte of the normal mice, Cd: SOD activity in the erythrocyte of the $CdCl_2$ treated mice, TAU-1D + Cd, TAU-2D + Cd, TAU-3D + Cd, TAU-4D + Cd, TAU-5D + Cd, TAU-6D + Cd and TAU-7D + Cd: SOD activities in the erythrocytes of the TAU-treated mice for 1, 2, 3, 4, 5, 6, and 7 d, respectively, at a dose of 100 mg/kg body weight prior to $CdCl_2$ intoxication. Each column represents a mean ± SD, n = 6. "a" indicates the significant difference between the normal control and toxin treated groups and "b" indicates the significant difference between the toxin-treated and taurine-treated groups ($P^a < 0.01$, $P^b < 0.01$).

the present study clearly showed that Cd intoxication increased intracellular Cd accumulation and simultaneously decreased hemoglobin content in the erythrocytes of the toxin-treated mice (Table 1), which is in agreement with prior findings (15, 16). Taurine pretreatment, however, ameliorated the toxic effects of Cd and played a protective role against anemia.

Bauman *et al*. (3) reported that post intake and absorption, Cd enters into the blood stream where it binds to the erythrocytic membrane and plasma albumin. Sarkar *et al*. (17) reported that Cd-induced elevation of lipid peroxidation played an important role in the deleterious effects upon the erythrocytes. In the present investigation, increased production of intracellular ROS was observed in the erythrocytes of the toxin-treated group. In addition, lipid peroxidation and protein carbonylation have also been elevated in the erythrocytes of the $CdCl_2$-intoxicated experimental mice (Table 1). Pretreatment with taurine reduced intracellular ROS formation, lipid peroxidation, and protein carbonylation. Results indicate that taurine treatment was very effective in the prevention of Cd-induced oxidative damage.

To prevent biological macromolecules from oxidative damage, antioxidant enzymes are considered to be the second line of cellular defense. In the present study, a significant decrease in the activities of antioxidant enzymes was observed in the red blood cells of the toxin-treated animals (Table 2). The reduction in SOD activity in the erythrocytes of the Cd-exposed animals may be due to the accumulation of superoxide radical anions as suggested by earlier reports (18). Cadmium intoxication also decreased the activities of CAT and GPx, an effect potentially explained by their influence on hydrogen peroxide as a substrate (formed in excess in the process of the dismutation reaction of the super oxide radical anion). The intracellular redox status is generally maintained by the coordinated activities of GR and GPx (19). Exposure of cells to large fluxes of ROS due to Cd intoxication reduced the activities of those antioxidant enzymes (GR and GPx). Cd intoxication decreased the activities of other thiol-based antioxidant enzymes (GST and G6PD) through modification of the −SH (thiol) groups as well. Treatment with taurine prior to Cd administration increased activities of the antioxidant enzymes, possibly by stimulating the elimination of excess ROS or binding

Table 1. Cadmium concentration, hemoglobin content, MDA, protein carbonylation and the rate of intracellular ROS formation in the red blood cells of the normal and experimental mice

Parameters	Normal control	Toxin control	TAU + Cd	VitC + Cd
Cadmium concentration (ppm)	0.03 ± 0.002	14.94 ± 0.76[a]	8.32 ± 0.39[b]	7.96 ± 0.41[c]
Hemoglobin content (g/dl)	13.14 ± 0.66	8.66 ± 0.54[a]	11.75 ± 0.51[b]	12.26 ± 0.61[c]
MDA (nmol/mg protein)	22.17 ± 1.12	44.43 ± 2.14[a]	27.53 ± 1.39[b]	25.79 ± 1.35[c]
Lipid hydroperoxide (nmol/mg protein)	2.73 ± 0.15	4.81 ± 0.25[a]	3.25 ± 0.14[b]	3.17 ± 0.16[c]
Protein carbonylation (nmol/mg protein)	11.62 ± 0.54	25.78 ± 1.31[a]	15.54 ± 0.76[b]	12.84 ± 0.0.66[c]
Rate of intracellular ROS production (nmols of DCF/min/mg protein)	42.45 ± 2.14	89.73 ± 4.51[a]	58.34 ± 2.89[b]	54.86 ± 2.75[c]

Values are expressed as mean ± SD, for 6 animals in each groups. "a" values differs significantly from normal control ($P^a < 0.01$); "b" values differs significantly from toxin control ($P^b < 0.01$); "c" values differs significantly from toxin control ($P^c < 0.01$).

Table 2. Effect of cadmium and taurine on the antioxidant enzymes, the thiol based antioxidants, their metabolite and intracellular Ferric Reducing/Antioxidant Power (FRAP) in the red blood cells of the normal and experimental mice

Name of the antioxidant enzymes	Activities of the antioxidant enzymes			
	Normal control	Toxin control	TAU + Cd	VitC + Cd
CAT (μmol/min/mg protein)	146.83 ± 7.05	69.74 ± 3.38[a]	125.87 ± 6.15[b]	139.32 ± 6.78[c]
GST (μmol/min/mg protein)	1.96 ± 0.09	0.85 ± 0.05[a]	1.85 ± 0.08[b]	1.94 ± 0.09[c]
GR (nmol/min/mg protein)	45.07 ± 2.21	15.49 ± 1.82[a]	40.51 ± 2.04[b]	43.32 ± 2.12[c]
GPx. (nmol/min/mg protein)	168.39 ± 8.38	98.82 ± 4.96[a]	156.94 ± 7.81[b]	161.47 ± 8.07[c]
G6PD (nmol/min/mg protein)	13.09 ± 0.65	3.87 ± 0.25[a]	10.48 ± 0.55[b]	12.32 ± 0.63[c]
GSH (nmol/mg protein)	90.42 ± 4.26	41.18 ± 3.58[a]	78.58 ± 3.91[b]	86.43 ± 4.44[c]
GSSG (nmol/mg protein)	18.63 ± 1.01	27.18 ± 1.33[a]	20.19 ± 1.14[b]	18.96 ± 1.09[c]
Total thiols (nmol/mg protein)	178.68 ± 8.24	86.45 ± 4.44[a]	159.54 ± 7.88[b]	171.66 ± 8.54[c]
FRAP levels (% over control)	100 ± 4.58	39.67 ± 3.41[a]	92.48 ± 4.54[b]	95.87 ± 4.69[c]

Values are expressed as mean ± SD, for 6 animals in each groups. "a" values differs significantly from normal control ($P^a < 0.01$); "b" values differs significantly from toxin control ($P^b < 0.01$); "c" values differs significantly from toxin control ($P^c < 0.01$).

with the Cd species to prevent the toxin from perpetrating any further oxidative damage.

Maintenance of normal cellular functions in the presence of oxygen largely depends on the efficiency of the defense mechanisms against free-radical mediated oxidative stress. Thiols are considered to be the first line of cellular defense against Cd-mediated oxidative damage. GSH functions by detoxifying various xenobiotics as well as scavenging free radicals and is consequently converted to its oxidized form, glutathione disulfide (GSSG). Cd-induced cellular toxicity includes alterations in the biological activity of thiol-containing proteins *via* structural modification (20). Chiu *et al*. (21) reported that treatment of erythrocytes with thiol-reactive substances shortened *in vivo* survival of those cells. Remarkable decreases in the levels of total thiols and GSH, along with the increased level of its metabolite, GSSG, has been observed (Table 2) in the erythrocytes of the Cd-intoxicated animals, which may be due in part to the binding ability of this metal ion with various intracellular sulfhydryl groups. Reduction in intracellular antioxidant power was also observed in the erythrocytes of the Cd-intoxicated animals (Table 2). Treatment with taurine before Cd exposure could, however, prevent the toxin-induced alterations.

Taurine is a conditional amino acid that possesses one amino group and one sulfonate group. Using these functional groups, it can bind heavy metal ions and stimulate excretion of such compounds. Thus far, there is little evidence describing direct inhibition of the production of free radicals by taurine, but it has been suggested that it may bind Cd, forming a complex that is readily excreted (22).

In addition to transport properties, recent studies revealed that erythrocytes play an important role in the body's immune system (23). Being a toxin, Cd damages the erythrocyte's membrane, ultimately disrupting normal body functions. Many food-stuffs are known to be the rich source of taurine. The outcome of our study is clear evidence that taurine can prevent Cd-induced oxidative impairments in erythrocytes. Thus Cd-induced alterations in normal physiological functions can be ameliorated by taurine uptake.

Although the preventive mechanism of action of this amino acid is not fully clear, it may be speculated that amino and sulfonate groups may bind with Cd and reduce its overall intracellular bioavailability. Further studies are necessary to ascertain the exact binding site of taurine with Cd and are in progress.

MATERIALS AND METHODS

Chemicals

Bovine serum albumin (BSA), Bradford reagent, and taurine (2-aminoethane sulfonic acid) were purchased from Sigma-Aldrich Chemical Company, (St. Louis, MO) USA. All other necessary reagents of analytical grade were bought from Sisco research laboratory, India. All reagents were used without further purification.

Animal protocol

Male Swiss-albino mice weighing between 20-25 g were acclimatized under laboratory conditions 2 wk prior to experimentation. All studies were performed in conformity with the guidance for care and standard experimental animals study ethical protocols.

Determination of dose and time dependent effects of $CdCl_2$ by the SOD assay

To establish the dose of $CdCl_2$ necessary for maximum damage in murine erythrocytes, mice were randomly allocated into 8 groups, each consisting of 6 mice, and were treated as follows. The first group served as a normal control (receiving only water as a vehicle). The remaining 7 groups were treated with 7 different doses of $CdCl_2$, orally (0.5 mg, 1.0 mg, 1.5 mg, 2.0 mg, 2.5 mg, 3.0 mg, and 4.0 mg/kg body weight for 3 d).

To determine the time needed for $CdCl_2$-induced maximum damage in murine erythrocytes, experiments were carried out with 6 groups of animals consisting of 6 animals in each group. The first group received water as a vehicle and served as a normal control. $CdCl_2$ was administered orally to the other 5 groups at a dose of 2.5 mg/kg body weight for 1, 2, 3, 4, and 5 d respectively.

Twenty-four hours after the final dose of $CdCl_2$, all the animals were sacrificed, blood samples were collected in heparinized tubes, hemolysates prepared, and the SOD assay performed with the hemolysates.

Determination of dose and time dependent activity of taurine by SOD assay

For this study, mice were randomly distributed into 8 groups, each consisting of 6 animals. The first 2 groups served as a normal control (receiving only water as a vehicle) and a toxin control (receiving $CdCl_2$ at a dose of 2.5 mg/kg body weight for 3 d, orally), respectively. The remaining 6 groups of animals were treated with 6 different doses of taurine (10 mg, 25 mg, 50 mg, 75 mg, 100 mg and 150 mg/kg body weight for 5 d, orally) followed by $CdCl_2$ intoxication (2.5 mg/kg body weight for 3 d, orally, once daily).

To determine the time-dependent effects of taurine, mice were divided into 9 groups, each consisting of 6 animals. The first 2 groups served as a normal control (receiving only water as a vehicle) and a toxin control (receiving $CdCl_2$ at a dose of 2.5 mg/kg body weight for 3 d, orally) respectively. The other 7 groups were treated orally with taurine at a dose of 100 mg/kg body weight, once daily, for 1, 2, 3, 4, 5, 6, and 7 d prior to $CdCl_2$ intoxication (2.5 mg/kg body weight for 3 d, orally, once daily).

Twenty-four hours after the final dose of $CdCl_2$, all the animals were sacrificed, blood samples were collected in heparinized tubes, hemolysates prepared, and the SOD assay performed with the hemolysates.

Experimental setup

The animals were divided into 4 groups each, consisting of 6 mice, and were treated as follows:

Group 1: Normal control (animals receiving only water as vehicle).

Group 2: Toxin control (animals receiving $CdCl_2$ orally at a dose of 2.5 mg/kg body weight for 3 d).

Group 3: Animals treated with a single dose of taurine (orally at a dose of 100 mg/kg body weight) for 5 d, followed by $CdCl_2$ (2.5 mg/kg body weight, orally) intoxication for the next 3 d.

Group 4: Vitamin C was administered orally at a dose of 100 mg/kg body weight for 5 d prior to $CdCl_2$ (2.5 mg/kg body weight for 3 d, orally) intoxication and served as positive control.

The animals were sacrificed under light, ether anesthesia and blood samples were collected in heparinized tubes.

Estimation of cadmium content in erythrocytes

Cadmium contents in the erythrocytes of all experimental animals were determined according to the method of Pari *et al*. (24). The estimation of metal content was performed by an Atomic Absorption Spectrophotometer (Perkin Elmer Model No. 3100) using suitable cathode lamps.

Preparation of hemolysate

After collecting blood samples in heparinized tubes, centrifugation was performed at 1000 g for 15 min to remove the buffy coat. The packed cells obtained at the bottom were washed thrice with phosphate buffer saline (0.9% NaCl in 0.01 M phosphate buffer, pH 7.4). A known amount of erythrocytes was lysed with hypotonic phosphate buffer. The hemolysate was obtained after removing the cell debris by centrifugation at 3000 g for 15 min and used for the following experiments.

Determination of protein concentration

Protein concentrations of experimental samples were determined by the method of Bradford (25) using crystalline BSA as standard.

Estimation of hemoglobin content

Hemoglobin content in the normal and experimental red cell lysates were estimated by following the method of Dacie and Lewis (26).

Estimation of MDA, protein carbonyl content, and lipid hydroperoxide

The lipid peroxidation, in terms of malondialdehyde (MDA), protein carbonylation, and lipid hydroperoxide formation, were estimated according to the method of Esterbauer and Cheeseman (27), Uchida and Stadtman (28), and Jiang *et al*. (29), respectively.

Measurement of intracellular ROS production

The extent of intracellular ROS production was measured using 2, 7-dichlorofluorescein diacetate (DCFDA) as a probe, following the method of LeBel and Bondy (30) as modified by Kim *et al*. (31). The formation of DCF was measured at the excitation wavelength of 488 nm and an emission wavelength of 510 nm for 10 min using a fluorescence spectrometer (HITACHI, Model No F4500).

Assay of antioxidant enzymes

The activities of antioxidant enzymes were measured in the hemolysates following the methods as described in (32, 33) for SOD and (34-38) for CAT, GST, GR, GPx, and G6PD, respectively.

Assay of cellular metabolites

Cellular GSH level was measured according to the method of Ellman (39) by using 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent) as the key reagent. A standard curve was drawn using different known concentrations of GSH solution. With the help of this standard curve, GSH contents were calculated.

GSSG contents were determined by following the method of Hissin and Hilf (40), using 0.04 M N-ethylmaleimide (NEM), 0.3 M Na_2HPO_4, and DTNB. The results were expressed as nmol/mg of protein.

Total thiol levels were estimated according to the method of Sedlak and Lindsay (41), calculated using a molar extinction coefficient of 13,600 $M^{-1}cm^{-1}$.

Ferric reducing antioxidant power (FRAP) assay

The intracellular antioxidant power of the erythrocytes was determined by the FRAP assay following the method of Benzie and Strain (42). Briefly, 50 μl of sample was added to 1.5 ml freshly prepared and prewarmed (37°C) FRAP reagent (300 mM acetate buffer, pH 3.6, 10 mM 2, 3, 5-triphenyltetrazolium chloride (TPTZ) in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ in a ratio of 10:1:1) and incubated at 37°C for 10 min. Absorbance of the sample was recorded against a reagent blank (1.5 ml FRAP reagent + 50 μl distilled water) at 593 nm.

Statistical analysis

All values are represented as a mean ± S.D. (n = 6). Statistical differences among different groups were analyzed by Student's *t*-test. P values of 0.05 or less were considered significant.

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