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REPEATED EXPOSURE OF SODIUM TELLURITE ON THE RAT LIVER AND ON THE POTENTIAL MECHANISMS OF THE METALLOID-INDUCED HEPATOTOXICITY

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Abstract: Tellurium (Te) is a semiconductor and is frequently doped with copper, tin, gold or silver. It is also used to color glass and ceramics and is one of the primary ingredients in blasting caps. Little is known about Te biological activity but it is well known for toxicity to human and animals. It has inhibited the lipids profiles and oxidative stress in the brain of mice. Sodium tellurite 4.15, 8.3 and 16.6 mg/kg (1/20, 1/10 and 1/5 of LD_{50}, respectively) was given to male Wistar rats orally in saline for a period of 15 days. On day 16, the blood was collected and the livers were dissected out for biochemical assays. The hepatotoxicity biomarkers [bilirubin, aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP)] were elevated significantly and dose dependently in the serum of Te treated groups as compared to control group. The content of thiobarbituric reactive substances in Te treated groups was increased significantly and dose-dependently as compared to control group. Conversely, the content of glutathione and activities of antioxidant enzymes (glutathione peroxidase, glutathione reductase, glutathione-S-transferase, superoxide dismutase and catalase) were decreased significantly in Te treated groups as compared to control group. No data of inorganic Te compounds on the liver toxicity of rats are available. The aim of the present study was to evaluate the hepatotoxicity of inorganic Te compound. In conclusion, Te accelerated hepatotoxicity and oxidative stress in liver tissue of rats.

Keywords: sodium tellurite, metalloid, hepatotoxicity, oxidative stress

Sulfur, selenium, and tellurium are in the same group - VIA in the Periodic Table. It has been reported that sulfur is less toxic than selenium (1). Sodium tellurite (Te) has applications in various industries; steel, rubber, electronic, ceramic and glass. Te demonstrates properties similar to those of elements known to be toxic to humans and animals (2). The organic tellurium compound, diphenyl ditelluride at the dose of 0.65 μmol/kg in rats has increased 2-fold serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (3). The other organo tellurides, 2-butyltellurium furan and dinaphthalene ditelluride at a dose of 0.75 and 0.125 mmol/kg have also increased the activities of AST and ALT in the liver of rats (4). Conversely, the vinyl telluride derivative, (diethyl-2-phenyl-2-tellurophenylvinyl-phosphonate) has protected the hepatotoxicity in rodents (5). It has also protected the levels of lipid peroxidation (5). Similarly, the other vinyl tellurium compounds, [(Z)-2-(methylthio)-1-(butyltelluro)-1-phenylethene] have shown antioxidant properties *in vitro* and *in vivo* (6). Te accelerates the toxicity of Cd, so it is used as cadmium telluride quantum dots (CdTe-QDs), which accelerates the formation of lipid peroxidation and decreased the activities of catalase (CAT) and superoxide dismutase (SOD) in the liver of mice (7). CdTe-QDs caused cytotoxicity in HepG2 cells and increased the reactive oxygen species (ROS), decreased the level of glutathione (GSH) and activities of CAT and glutathione-S-transferase (GST) (8).

Kaur et al., (9) have reported 2-fold more neurotoxicity of Te than Se on the same parameters.

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Sodium tellurite has been reported to cause neurotoxicity by decreasing the contents of the lipids profiles in cerebrum, cerebellum and brainstem of mice (9). It has also depleted the content GSH and activities of antioxidant enzymes (GPx, GR, SOD, GST and CAT in the cerebrum, cerebellum and brainstem of mice (9). On the other hand, Te has elevated the level of lipid peroxidation in discrete brain areas of the animals (9). Moreover, Te causes toxicity by acting directly as a general oxidizing agent. The above studies of organo-tellurium compounds or CdTe-QDs are available in the literature but the hepatotoxicity caused by inorganic Te compounds is not available which has created our interest to evaluate the devastating toxic effect of Te on the liver of the rats.

Te has been reported to bind with sulfhydryl components of squalene monooxygenase, thereby causing blockage in the formation of cholesterol, which could lead to peripheral neuropathy with significant demyelination with the passage of time (10-13). The depleted level of sulfhydryl group makes the cells more susceptible to free radicals.

MATERIALS AND METHODS

Chemicals

Sodium tellurite, oxidized glutathione (GSSG), reduced glutathione (GSH), glutathione reductase (GR), nicotinamide adenine dinucleotide phosphate (NADPH), 1-chloro-2,4-dinitrobenzene (CDNB), ethylene diamine tetraacetic acid (EDTA), 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB), thiobarbituric acid (TBA), (-)-epinephrine, sodium azide, hydrogen peroxide, sulfosalicylic acid and trichloroacetic acid (TCA) were purchased from Sigma-Aldrich, Co., Germany. The kits were purchased from Human Gesellschaft fur Biochemical and Diagnostic mbH, Germany.

Animals and dosing

The male Wistar rats 200-220 g were taken from the Animal House of Jazan University, Jazan, Kingdom of Saudi Arabia. All procedures were performed in accordance with NIH guidelines and Guide for the Care and Use of Animals. These protocols were approved by Jazan University Institutional Animal Care and Use Committee (IACUC). The animals were divided into four groups each having 8 animals. Group 1 was control group and vehicle (saline) was given orally. Groups 2-4 were experimental and sodium telluride 4.15, 8.3 and 16.6 mg/kg body weight (b.w.) (1/20, 1/10 and 1/5 of LD_{50}, respectively; oral LD_{50} in rat is 83 mg/kg) were given orally in saline for 15 days. On day 16, blood was taken out from orbital sinus of overnight fasted animals from each group for biochemical assays. Thereafter, animals were sacrificed and liver of each animal was dissected out. A 10% homogenate of the liver was prepared in 20 mM Tris-HCl (pH 7.4, having protease inhibitors 10 µl/mL). The homogenate was centrifuged at 800 × g for 5 min at 4°C to remove cell debris. The supernatant-1 (S-1) was used for lipid peroxidation and rest of the S-1 was again centrifuged at 10,500 × g for 30 min at 4°C to separate the post mitochondrial supernatant (PMS). The PMS was used for the assays of GSH, GPx, GR, GST, SOD and CAT.

Biochemical assays for liver function tests

The kits of Human Gesellschaft fur Biochemical and Diagnostic were used for the assays of all serum markers such as bilirubin, AST, ALT and ALP.

Non enzymatic assays

Lipid peroxidation (LPO)

The procedure of Utley et al. (14) as modified by Islam et al. (15) was used for the estimation of the rate of lipid peroxidation. In brief, S-1 0.5 mL samples were pipetted in 18 × 150 mm test tubes and incubated at 37 ±1°C in a metabolic shaker (120 cycles / min) for 1 h. Another 0.5 mL of the same homogenate was pipetted in other test tube and placed at 0°C. After 1 h of incubation, 0.5 mL 10% of chilled trichloacetic acid (TCA) followed by 1.0 mL of 0.67% thiobarbituric acid (TBA) were added to each test tube and mixed after each addition. The aliquot of each test tube was transferred to centrifuge tubes and centrifuged at 1000 × g for 10 min at 4°C. Thereafter, supernatants were transferred to other test tubes and placed in the boiling water bath. After 10 min, the test tubes were cooled and the absorbance of the color was read at 535 nm. The rate of thiobarbituric acid reactive substances (TBARS) formed was expressed as nmol of TBARS formed/h/mg protein using molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}.

Glutathione reduced (GSH)

The method of Jollow et al. (16) was used for the assay of GSH. PMS 0.1 mL was precipitated with 0.1 mL of sulfosalicylic acid (4%). The samples were kept at 4°C for 1 h and then subjected to centrifugation at 1000 × g for 10 min at 4°C. The assay mixture contained 0.1 mL supernatant, 0.8 mL phosphate buffer (0.1 M, pH 7.4) and 0.1 mL DTNB (0.4% in phosphate buffer 0.1 M, pH 7.4) in a total

volume of 1.0 mL. The yellow color developed was read immediately at 412 nm. The GSH content was calculated as µmol GSH/mg protein, using molar extinction coefficient of 13.6×10^3 M^{-1} cm^{-1}.

Assays of antioxidant enzymes

Glutathione peroxidase (GPx)

The method of Mohandas et al. (17) was used for the assay of GPx. In brief, the reaction mixture consisted of phosphate buffer (0.1 M, pH 7.0), 0.1 mL of 20 mM EDTA, 0.1 mL of 20 mM sodium azide, glutathione reductase (1U/mL), 0.1 mL of 20 mM of glutathione, 0.1 mL of 4 mM NADPH, 0.1 mL of 5 mM of hydrogen peroxide and 0.1 mL of PMS in a total volume of 2 mL. The disappearance of NADPH at 340 nm was recorded per min for 3 min at room temperature. The enzyme activity was calculated as nmol NADPH oxidized/min/mg protein using molar extinction coefficient of 6.22×10^{-3} M^{-1} cm^{-1}.

Glutathione reductase (GR)

GR was assayed by the method of Carlberg and Mannervik (18) as modified by Mohandas et al. (17). In brief, the assay mixture consisted of phosphate buffer (0.1 M, pH 7.6), 0.1 mL of 2 mM NADPH, 0.1 mL of 10 mM EDTA, 0.1 mL of 20 mM oxidized glutathione and 0.1 mL of PMS in a total volume of 2 mL. The enzyme activity was quantitated per min for 3 min at room temperature by measuring the disappearance of NADPH at 340 nm and was 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)

The activity of GST was measured by the method of Habig et al. (19). The reaction mixture consisted of phosphate buffer (0.1 M, pH 6.5), 0.1 mL of 20 mM of reduced glutathione, 0.1 mL of 20 mM of 1-chloro-2,4-dinitrobenzene (CDNB) and 0.1 mL of PMS in a total volume of 2.0 mL. The change in absorbance was recorded at 340 nm per min for 3 min 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)

The activity of CAT was measured by the method of Claiborne (20). In brief, the assay mixture consisted of phosphate buffer (0.1 M, pH 7.4), 0.1 mL of 180 mM of hydrogen peroxide and 0.05 mL PMS to give a total volume of 3.0 mL. Change in absorbance was recorded at 240 nm per min for 3 min. The catalase activity was calculated in terms of nmoles of H_2O_2 consumed/min/mg protein by using molar extinction coefficient of 43.6 M^{-1} cm^{-1}.

Superoxide dismutase (SOD)

SOD activity was measured spectrophotometrically as described previously by Stevens et al. (21) by monitoring the autooxidation of (-)-epinephrine at pH 10.4 for 3 min at 480 nm. The reaction mixture contained glycine buffer (50 mM, pH, 10.4) and 0.2 mL of PMS. The reaction was initiated by adding 0.05 mL of 20 mM (-)-epinephrine. The enzyme activity per min for 3 min was calculated in terms of nmol (-)-epinephrine protected from oxidation/min/mg protein using molar extinction coefficient of 4.02 ×103 M^{-1} cm^{-1}.

Estimation of protein

Protein was estimated according to the method of Lowry et al. (22) using bovine serum albumin as standard.

Table 1. Effects of sodium tellurite on bilirubin, AST, ALT and ALP in the serum of rats.

Biomarker enzymes (Unit)	Control	Te 4.15 mg/kg	Te 8.3 mg/kg	Te 16.6 mg/kg	**'F' value (MS treatment/ MS residual)**
Bilirubin (mg/dL)	0.34 ± 0.01	0.66* ± 0.03 (92.42 %)	1.12** ± 0.06 (226.53 %)	1.5 ** ± 0.06 (291.54 %)	120.83
AST (U/L)	18.00 ± 0.23	61.60** ± 0.64 (242.22 %)	69.76** ± 0.96 (287.55 %)	70.54** ± 1.86 (291.89 %)	510.30
ALT (U/L)	6.6 ± 0.31	18.2** ± 0.28 (175.75 %)	21.34** ± 0.45 (223.33 %)	25.18** ± 0.53 (281.51 %)	390.86
ALP (U/L)	515.47 ± 0.68	1290.9** ± 46.31 (150.44 %)	1309.67** ± 45.16 (154.08 %)	1380.87** ± 50.28 (167.89 %)	99.02

Note: Te toxicity has significantly elevated the hepatotoxicity biomarkers in the serum of rats. Values are expressed as the mean ± S.E.M. of 8 rat/group. Values in parentheses show the percentage increase with respect to control.*$p < 0.05$, **$p < 0.001$ *vs.* control.

Statistics

The one-way ANOVA and *post hoc* Dunnett's test were used for the significance and $p < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Table 1 shows the effects of sodium tellurite on the biomarkers of liver function tests (bilirubin, AST, ALT and ALP). The content of bilirubin was increased significantly (*$p < 0.05$, **$p < 0.001$) and dose dependently in the serum of the rats treated with various doses of Te as compared to control group. The rise in bilirubin level is one of the most important clinical indications of the severity of necrosis, and its accumulation further indicates the binding, conjugation and excretory capacity of hepatic cells (23). The significantly elevated level of bilirubin in the serum indicates that Te is hepatotoxic. No other data of inorganic or organic Te compounds on the content of bilirubin is available.

AST and ALT are the most sensitive biomarkers of liver toxicity that can directly indicate the extent of hepatic damage and toxicity. Administration of Te to rats caused significant elevation (*$p < 0.001$) on the activities of AST and ALT in the serum, indicating hepatotoxicity of Te. Elevation of ALP may be found in a large number of disorders such as gallstone disease, alcohol abuse and drug-induced hepatitis, or in less common disorders such as primary biliary cirrhosis or biliary tumors (24, 25). There is no report of inorganic tellurium compounds on the toxicity of liver biomarkers but organo tellurides: 2-butyltellurium furan, dinaphtha-

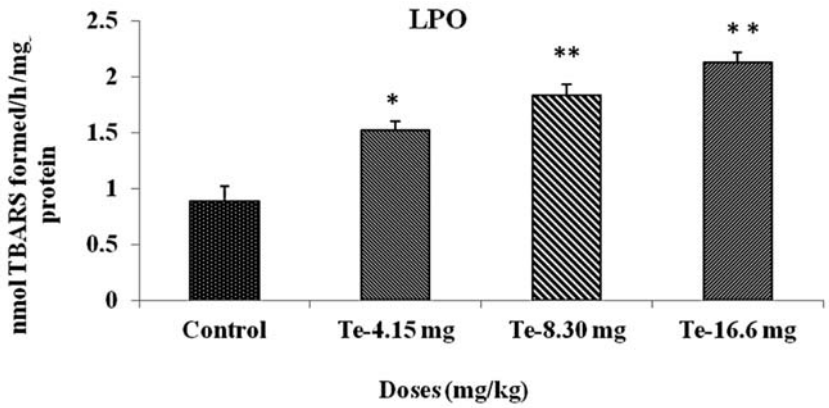

Figure 1. Effect of various doses of sodium tellurite on the content of thiobarbituric acid reactive substances (TBARS) in the liver of rats. The level of TBARS was increased significantly and dose dependently in sodium tellurite (4.15, 8.3 and 16.6 mg/kg) treated groups as compared to control group. The values are expressed as the mean ± S.E.M (n = 8 rat/group). *$p < 0.05$ and **$p < 0.01$ *vs*. control

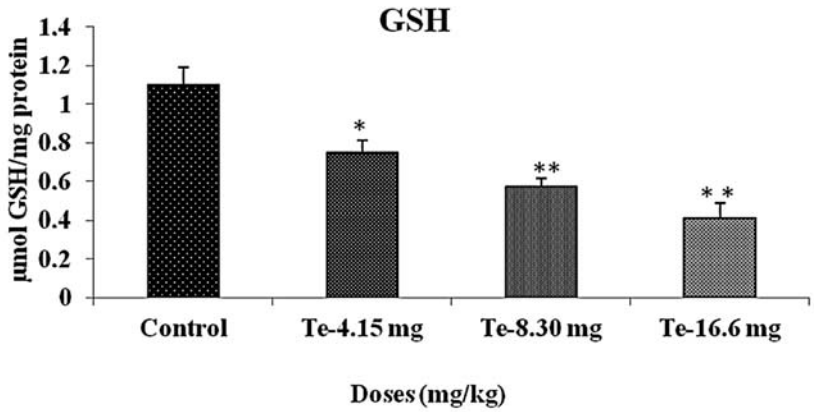

Figure 2. Effect of various doses of sodium tellurite on the content of glutathione (GSH) in the liver of rats. The level of GSH was decreased significantly and dose dependently in sodium tellurite treated groups (4.15, 8.3 and 16.6 mg/kg) as compared to control group. The values are expressed as the mean ± S.E.M (n = 8 rat/group). *$p < 0.05$ and **$p < 0.001$ *vs*. control

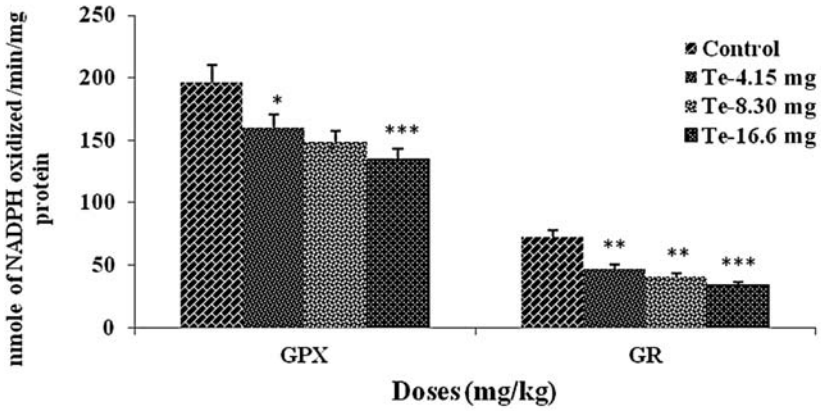

Figure 3. Effect of various doses of sodium tellurite on activities of glutathione peroxidase (GPx) and glutathione reductase (GR) in the liver of rats. The activities of GPx and GR were decreased significantly and dose dependently in sodium tellurite (4.15, 8.3 and 16.6 mg/kg) treated groups as compared to control group. The values are expressed as the mean ± S.E.M (n = 8 rats/group). *p < 0.05, **p < 0.01 and ***p < 0.001 *vs*. control

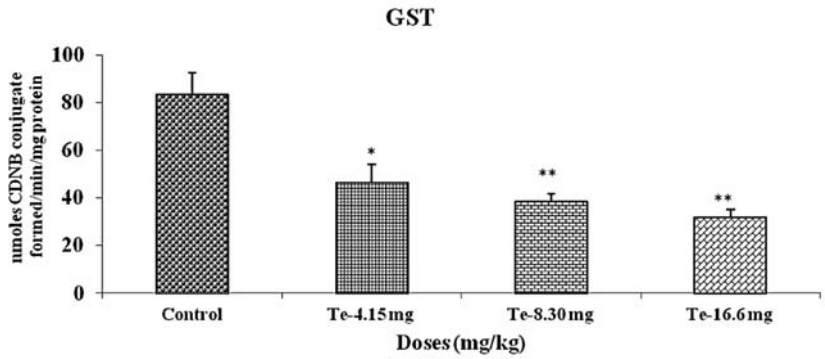

Figure 4. Effect of various doses of sodium tellurite on the activity of glutathione-S-transferase (GST) in the liver of rats. The activity of GST was decreased significantly and dose dependently in sodium tellurite (4.15, 8.3 and 16.6 mg/kg) treated groups as compared to control group. The values are expressed as the mean ± S.E.M (n = 8 rat/group). *p < 0.01 and **p < 0.001 *vs*. control

lene ditelluride and diphenyl ditelluride have significantly increased the activities of AST and ALT in the serum of rats (26). In the present study, the activity of alkaline phosphatase was increased significantly (**p < 0.001) and dose dependently in the serum of rats. The increased activity of ALP was supported by Srivastava et al. (27) in liver and kidney with the treatment of 2-butyltellurium furan and dinaphthalene ditelluride.

Figure 1 shows the effect of various doses of sodium tellurite on the contents of lipid peroxidation in the liver of rats. The level of TBARS was increased significantly (*p < 0.05; **p < 0.01 and ***p < 0.001) and dose dependently in Te treated groups as compared to control group. Vinyl telluride derivative - (Z)-1-(4-methylphenylsulfonyl)-2-(phenyltelluro)-2-phenylethene has increased hepatic lipid peroxidation in rats. Conversely, other vinyl telluride derivative; (Z)-2-(methylthio)-1-(butyltelluro)-1-phenylethene has reducing effect on lipid peroxidation *in vitro*. At very low concentration, the CdTe-QDs (500 nmole/mL) has increased the TBARS concentration in the liver tissue (7). A supporting data of sodium tellurite toxicity on TBARS in the brain of mice have been reported (9).

The content of GSH was decreased significantly (*p < 0.05 and **p < 0.001) and dose dependently in Te treated groups as compared to control group (Fig. 2). GSH also plays a crucial role in regulation of the expression of several redox-sensitive antioxidant and anti-inflammatory genes (28, 29). Thus, GSH inhibition in the liver has increased the suscep-

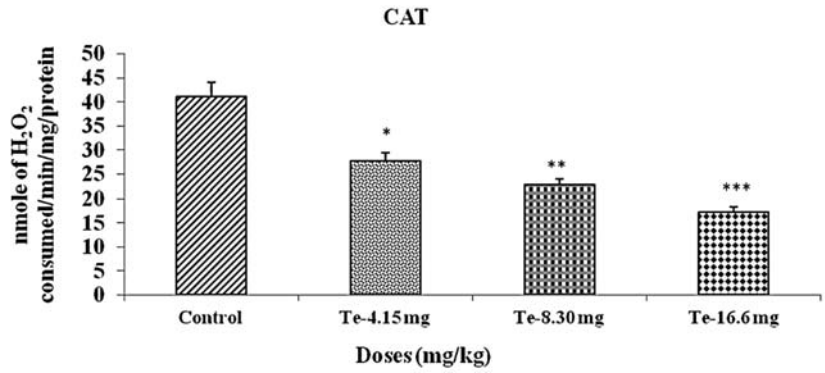

Figure 5. Effect of various doses of sodium tellurite on the activity of catalase (CAT) in the liver tissue of rats. The Te intoxication has decreased the activity of CAT significantly and dose dependently (4.15, 8.3 and 16.6 mg/kg) in the liver in treated groups as compared to control group. The values are expressed as the mean ± MSE. (n = 8 rat/group). *p < 0.05, **p < 0.01 and ***p < 0.001 *vs.* control

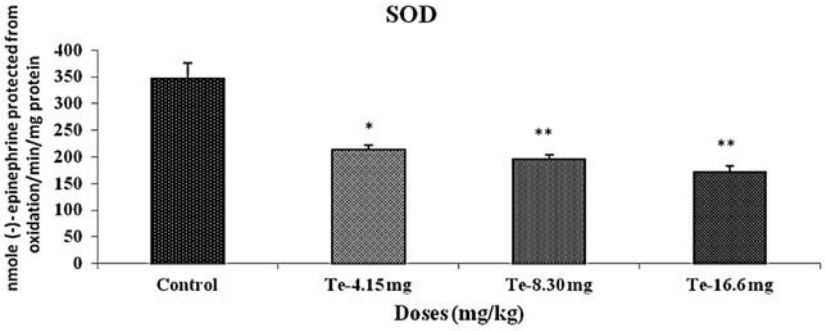

Figure 6. Effect of various doses of sodium tellurite on the activity of superoxide dismutase (SOD) in the liver tissue of rats. The Te intoxication has decreased the activity of SOD significantly and dose dependently (4.15, 8.3 and 16.6 mg/kg) in the liver as compared to control group. The values are expressed as the mean ± S.E.M (n = 8 rat/group). *p < 0.01 and **p < 0.001 *vs.* control

tibility of plasma membranes towards peroxide attacks. The loss of GSH and formation of protein–glutathione mixed disulfide (PrSSG) may result in various membrane dysfunctions, such as inhibition of Na^+K^+-ATPase activity (30). The significantly depleted content of GSH has also been reported in the liver and kidney of rats treated with organo-tellurium compound, bis-(tetraphenylphosphonium) tetracyanato-bis-p-methoxy-p-phenyl tellurate and bis-(tetraheptylammonium) tetraiodocyclopentane tellurate (27). Sodium telluride has also depleted the contents of GSH in the brain of mice (9).

Reactive free radicals such as superoxide and hydroxyl can damage lipid, protein, or DNA and cause cell death (8). Se and Te have been known for some time as thiol-reactive reagents, although the nature of the chemistry is still not completely understood (7). The generation of overproduction of free radicals, which might have caused oxidative damage to the membrane lipid and protein, ultimately led to a decrease in GSH content and activity of antioxidant enzymes. The activity of NADPH dependent antioxidant enzymes; GPx and GR was decreased significantly (*p < 0.05; **p < 0.01 and ***p < 0.001) and dose-dependently in the PMS of the liver of Te treated groups as compared to control group (Fig. 3). GPx plays a predominant role in removing the excess free radicals and hydroperoxides and is a major defense system against oxidative stress (31). GR also plays a significant role in catalyzing the oxidized glutathione (GSSG) to GSH. During the normal catalytic reaction of GPx and GR, the consumed glutathione is recycled. The decreased activities of GPx and GR were also reported in the brain of the mice with the treatment of sodium telluride (9).

During the generation of glutathione-S-conjugates by GST, the GSH is consumed by the cells due to which the total intracellular GSH contents are

lowered which caused more harm to the cells. The activity of GST was decreased significantly (*p < 0.01 and **p < 0.001) and dose dependently in the PMS of the liver of Te treated groups as compared to control group (Fig. 4). The decreased activity of GST was supported by the treatment of sodium tellurite in the brain of mice as well as organo-tellurium compounds; bis-(tetraphenylphosphonium) tetracyanato-bis-p-methoxy-p-phenyl tellurate and bis-(tetraheptylammonium) tetraiodocyclopentane tellurate in liver and kidney of mice (9, 27).

The activity of other antioxidant enzymes such as SOD (*p < 0.01 and **p < 0.01) and CAT (*p < 0.05 and**p < 0.01 and ***p <0.001) were also decreased significantly and dose dependently in Te treated groups as compared to control group (Figs. 5 and 6). SOD converts highly toxic superoxide into less toxic H_2O_2 (32) and CAT detoxifies H_2O_2 into H_2O and oxygen. It has been reported that defense against H_2O_2, which is the most toxic molecule in the body, is mediated primarily by the glutathione system. The Te has depleted the activities of these enzymes due to which the level of H_2O_2 was not detoxified and caused liver toxicity. Kaur et al. (9) have reported the decreased activities of these enzymes in the discrete brain areas of mice. No other data of the Te (inorganic or organic) on the liver of SOD and CAT are available. So this is our first report on the toxicity of sodium tellurite on the activities of SOD and CAT in the liver of rats.

CONCLUSION

The toxicity of Te on hepatic biomarkers indicates that Te caused hepatotoxicity and generates free radicals which cause oxidative stress and more severity to the liver.

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