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Role of Reduced Glutathione in the Amelioration of Nicotine-Induced Oxidative Stress

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Abstract Nicotine, a major toxic component of cigarette smoke has been identified as a major risk factor for different diseases. This study investigates the role of reduce glutathione (GSH) against nicotine treated liver and kidney toxicity. Results showed that the application of 80 mg GSH per kg body weight per day exert protective effect against nicotine-induced liver and kidney toxicity by modulating the biochemical marker enzyme LDH, lipid peroxidation and augmenting antioxidant defense system. To our knowledge, this is the first finding of this sort.

Keywords Nicotine · GSH · Tissues · Oxidative stress

Nicotine, a pharmacologically active substance in tobacco, is generally regarded to be a primary risk factor in the development of cardiovascular disorders, pulmonary disease and lung cancer (Jung et al. 2001). Nicotine has been reported to induce oxidative stress both in vivo and in vitro (Suleyman et al. 2002). The mechanisms of free radical generation by nicotine are not clear. However, it has been reported that nicotine disrupts the mitochondrial respiratory chain leading to an increased generation of superoxide anions and hydrogen peroxide (Yildiz et al. 1999). Studies showed that increased vitamin C intake is associated with decreased chronic obstructive pulmonary disease in adult smokers (Sargeant et al. 2000). In our laboratory, recent studies reported that vitamin E is able to ameliorate nicotine-induced oxidative stress in tissues (Neogy et al. 2008). Husain et al. (2001) have reported that chronic administration of ethanol and nicotine decreased the level of GSH in the lung and kidney. Also our recent studies explored that the GSH levels were decreased in serum and neutrophil with smoking history of university students (Mahapatra et al. 2008).

However, to our knowledge, no information is available regarding the role of exogenous GSH in nicotine-induced tissue toxicity. Therefore, in this present investigation we have tried to reduce the effect of nicotine-induced oxidative damage in liver and kidney by the GSH in vivo using lipid peroxidation and antioxidant as biomarkers.

Materials and Methods

Adult male albino rats (n = 36) of Wistar strain of body weight 100–120 g were obtained and housed in polypropylene cages and fed standard pellet diet (Hindusthan Lever Ltd, India) for 1 week and water ad libitum. Animals were divided into three groups of almost equal average body weight of twelve animals each. The animals of two groups were induced by subcutaneous injection with nicotine tartrate (dissolved in 0.9% physiological saline) at a dose of 2 mg/kg body weight per day for 15 days. The animals of one of the nicotine treated groups serving as the supplemented group injected GSH i.p. at a dose of 80 mg/kg body weight daily at an interval of 6 h after injection of nicotine tartrate for a period of 15 days. The animals of the remaining group received only the vehicle (0.9% physiological saline), served as control. After completion of treatment the animals were fasted overnight prior to sacrifice by the use of anesthesia. The intact liver and kidney

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were dissected out and weighted. The tissues were then quickly stored at −20°C. Weighed tissues were homogenized in ice-cold 0.25 M sucrose to obtain tissue homogenates used for the analysis in the following methods: (1) Lipid peroxidation was measured according to the method of Ohkawa et al. (1979). Malondialdehyde (MDA) was determined from the absorbance of the pink coloured product (TBARS). (2) Content of conjugated dienes was measured according to the method of Slater (1980). (3) The activity of lactate dehydrogenase (LDH) was measured by the method of Young et al. (1975). (4) GSH and GSSG were measured according to the method of Griffith (1980). (5) Catalase activity was determined at room temperature by using a slightly modified version of Aebi (1983). One unit of CAT activity is equal to the millimoles of H_2O_2 degraded per minute per milligram of protein. (6) SOD activity was estimated by measuring the percentage inhibition of the pyrogallol auto- oxidation by SOD according to the method Marklund & Marklund (1974). (7) The rate of oxidation of reduced glutathione by H_2O_2 as catalyzed by the glutathione peroxides (GPx) present in the homogenate is assayed for the measurement of enzyme activity. Glutathione peroxidase activity was measured according to method of Paglia and Valentine (1967). (8) The activity of glutathione reductase was measured by the method of Miwa (1972). (9) Glutathione-s- transferase activity was measured according to the method of (Habig et al. 1974) and (10) Total protein of tissues was estimated according to the method of Lowry et al. (1951) using bovine albumin as standard. The data were expressed as mean ± SEM. Comparisons of the means of control, nicotine and nicotine with GSH group were made by two-way ANOVA with multiple comparison '*t*'-test, $p < 0.05$ as a limit of significance.

Results and Discussion

The levels of MDA and conjugated dienes were significantly increased in liver by 75% and 179.89% and in kidney by 32.2% and 221.55%, respectively (Figs. 1, 2). After supplementation with GSH there was a significant diminution of MDA and conjugated dienes content in liver by 29.6% and 42.39% and, in kidney by 11.5% and 27.37%, respectively. The activity of biochemical marker enzyme LDH was significantly lower in nicotine treated rats compared with control in liver and kidney by 39.09% and 30.68%, respectively. GSH supplementation elevated the LDH activity in liver by 36.93% and in kidney by 22.12%, respectively (Fig. 3). Enhanced lipid peroxidation and conjugated dienes in liver and kidney is a characteristic observation in nicotine treated rats. Nicotine, a potent carcinogen, plays a key role in the pathogenesis of liver, kidney and lungs (Husain et al. 2001). The mechanism of free

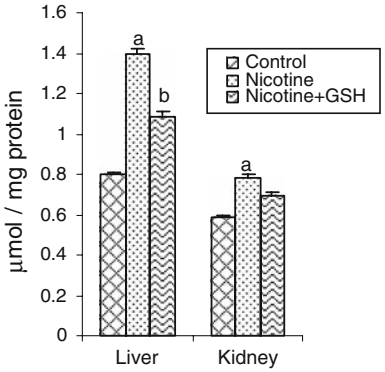

Fig. 1 Changes the MDA content in liver and kidney after co-administration of GSH in nicotine treated rats. Data represents mean ± SE, N = 12. *a* $p < 0.05$ compared to control, *b* $p < 0.05$ compared to nicotine

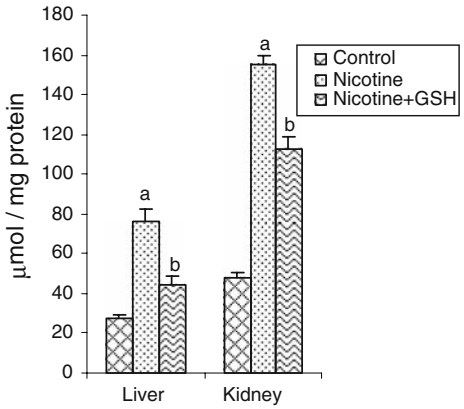

Fig. 2 Changes the CD content in liver and kidney after co-administration of GSH in nicotine treated rats. Data represents mean ± SE, N = 12. *a* $p < 0.05$ compared to control, *b* $p < 0.05$ compared to nicotine

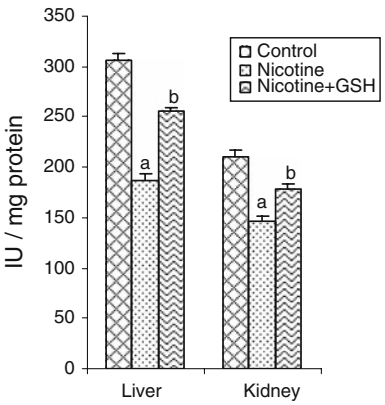

Fig. 3 Changes the LDH activity in liver and kidney after co-administration of GSH in nicotine treated rats. Data represents mean ± SE, N = 12. *a* $p < 0.05$ compared to control, *b* $p < 0.05$ compared to nicotine

radical generation by nicotine is not clear. However, it has been reported that nicotine is chemotactic for polymorphonuclear (PMN) leucocytes and enhances the responsiveness of PMN leucocytes to activated complement C5a, thus generating oxygen free radical (Wetscher et al. 1995). Further, nicotine disrupts the mitochondrial respiratory chain leading to the increased generation of superoxide anions and hydrogen peroxide (Yildiz et al. 1999). Thus, the decreased activity of marker enzyme LDH and increased the level of MDA and conjugate dienes in liver and kidney of nicotine treated rats in the present study may be due to excessive generation of free radicals by nicotine.

The activities of SOD and CAT were significantly reduced in liver by 64.42% and 55.04% and in kidney by 67.19% and 50.02%, respectively (Fig. 4, 5). GSH supplementation increased the activity of SOD in liver by 98.11% and in kidney by 102.9%. But with the supplementation of GSH, the activity of CAT elevated in liver by 22.23% and in kidney by 75%. Previous reports have shown the decreased activity of SOD and CAT in the tissues of nicotine treated rats (Ashakumary and Vijayammal 1996). Thus decrease in the activity of SOD observed in the present study could be due to a feed back inhibition or oxidative inactivation of enzyme protein due to excess ROS generation. Catalase, which acts as preventive antioxidant, plays an important role in protection against the deleterious effects of lipid peroxidation**.** The inhibition of CAT activity is suggestive of enhanced synthesis of superoxide anion during the ingestion of nicotine since superoxide anion is a powerful inhibitor of catalase.

The levels of GSH and GSSG were significantly diminished in liver by 55.56% and 52.17% and in kidney by 34.39% and 57.89%, respectively (Fig. 6, 7). Supplementation with GSH showed significant elevation of GSH and GSSG contents in liver by 97.45% and 80% and in kidney by 38.3% and 43.75%, respectively. Previous

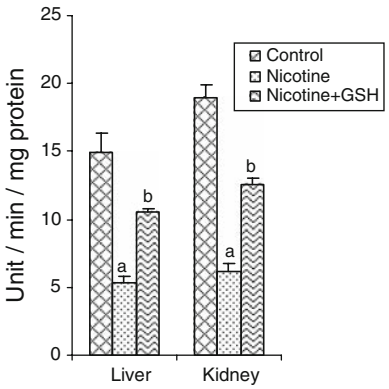

Fig. 4 Changes the SOD activity in liver and kidney after co-administration of GSH in nicotine treated rats. Data represents mean ± SE, N = 12. *a* $p < 0.05$ compared to control, *b* $p < 0.05$ compared to nicotine

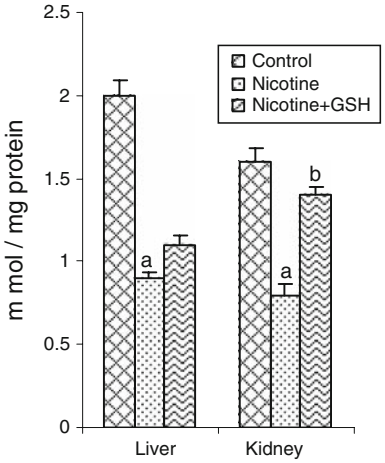

Fig. 5 Changes the CAT activity in liver and kidney after co-administration of GSH in nicotine treated rats. Data represents mean ± SE, N = 12. *a* $p < 0.05$ compared to control, *b* $p < 0.05$ compared to nicotine

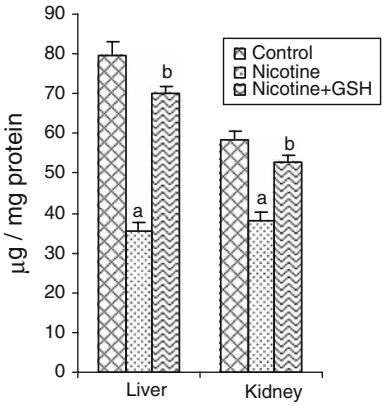

Fig. 6 Changes the GSH content in liver and kidney after co-administration of GSH in nicotine treated rats. Data represents mean ± SE, N = 12. *a* $p < 0.05$ compared to control, *b* $p < 0.05$ compared to nicotine

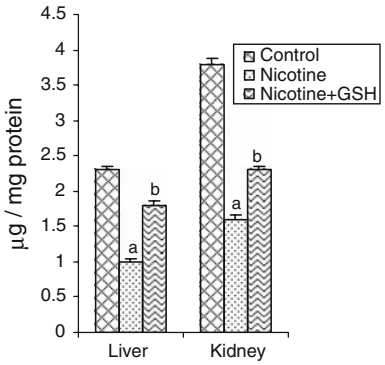

Fig. 7 Changes the GSSG content in liver and kidney after co-administration of GSH in nicotine treated rats. Data represents mean ± SE, N = 12. *a* $p < 0.05$ compared to control, *b* $p < 0.05$ compared to nicotine

studies have suggested that superoxide anion and hydrogen peroxide are the main source of nicotine-induced free radicals depleting the cellular antioxidant (Helen et al. 2000). GSH plays a crucial role in protecting the liver and kidney from oxidative stress by detoxifying exogenous toxicants and quenching reactive oxygen species (ROS).

The activities of GPx, GR and GST were significantly decreased in liver by 63.30%, 57.37% and 37.69% and in kidney by 53.40%, 47.90% and 36.05%, respectively (Figs. 8, 9, 10). GSH supplementation increased the activity of GPx, GR and GST in liver by 94.73%, 59.74% and 25.26% and in kidney by 52.62%, 48.29% and 3.13%, respectively. GPx has a well-established role in protecting cells against oxidative injury. GPx utilizes GSH as a substrate to catalyse the reduction of organic hydroperoxides and hydrogen peroxide (Ray and Husain 2002). Therefore, the excess H_2O_2 and lipid peroxides generated during nicotine ingestion are efficiently scavenged by GPx

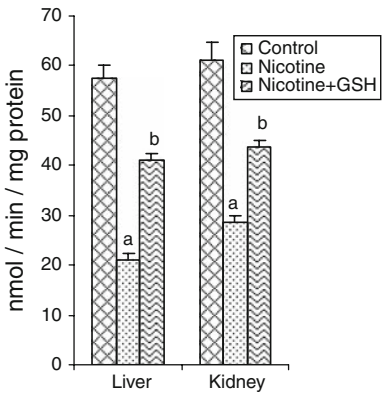

Fig. 8 Changes the GPx activity in liver and kidney after co-administration of GSH in nicotine treated rats. Data represents mean ± SE, N = 12. *a* $p < 0.05$ compared to control, *b* $p < 0.05$ compared to nicotine

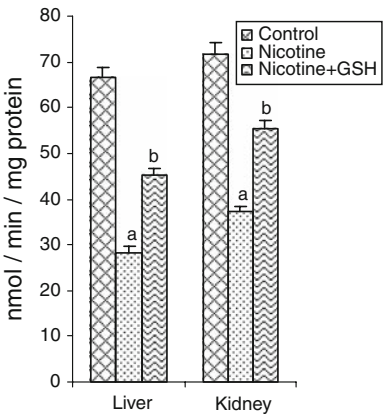

Fig. 9 Changes the GR activity in liver and kidney after co-administration of GSH in nicotine treated rats. Data represents mean ± SE, N = 12. *a* $p < 0.05$ compared to control, *b* $p < 0.05$ compared to nicotine

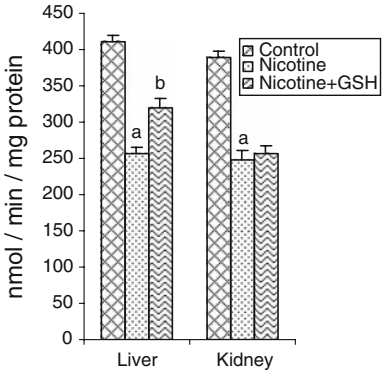

Fig. 10 Changes the GST activity in liver and kidney after co-administration of GSH in nicotine treated rats. Data represents mean ± SE, N = 12. *a* $p < 0.05$ compared to control, *b* $p < 0.05$ compared to nicotine

activity. The depression of this enzyme activity reflects perturbations in normal oxidative mechanisms during nicotine ingestion. There are alternative functions for GSH in cellular metabolism independent of its antioxidant properties. GSH also participates in the detoxification of xenobiotics as a substrate for the enzyme glutathione-S-transferase. Husain et al. (2001) have reported that chronic administration of ethanol and nicotine decreased the level of GSH and activities of GPx, SOD and CAT in the lung and kidney. In the present study, the depletion of GSH, GPx, GR, GST, SOD and CAT in liver and kidney of nicotine treated rats may be due to enhanced utilization during detoxification of nicotine.

Administration of GSH significantly enhanced the antioxidant status in liver and kidney of nicotine treated rats and protected cells against the damaging effects. It is reported that vitamin C may protect the smokers from cigarette smoke–induced oxidative damage and associated degenerative diseases (Panda et al. 2000).

These findings indicate that nicotine treatment at the present dose and duration induces oxidative damage of both liver and kidney. These changes can be attenuated by the GSH supplementation. However, more detailed studies are needed to know the exact mechanism of nicotine-induced oxidative damage. The study with varied dose and duration and other free radical scavengers and antioxidative supporters are also valuable to get the means by which toxic impacts of nicotine can be ameliorated.

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