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Full Length Research Paper

Improvement effect of green tea on hepatic dysfunction, lipid peroxidation and antioxidant defence depletion induced by cadmium

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We have evaluated the antioxidant effect of green tea on cadmium-induced hepatic dysfunction and stress oxidant in rats. Adult male Wistar rats were administered cadmium by injection with 20 µmoles/Kg bw/3 days for six months. Results revealed a significant ($p < 0.05$) liver dysfunction, lipid peroxidation and a decline in antioxidant enzymes activities in the liver of cadmium-treated rat compared to control. Compared to vehicle control, the activity of lactate dehydrogenase (LDH), gammaglytamyl transferase (GGT), acid phosphatase (PAC), phosphatase alkaline (PAL), as well as bilirubin and thiobarbituric acid-reactive substances (TBARs) rates were significantly ($p < 0.05$) increased. Moreover, antioxidants enzymes activities such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase, were significantly ($p < 0.05$) decreased in the liver of cadmium-treated rats. The oral administration of five percent aqueous green tea extract along with cadmium treatment for six months caused a significant ($p < 0.05$) improvement in cadmium-induced toxicity by significantly decreasing ($p < 0.05$) the activities of enzymatic markers of liver dysfunction (LDH, GGT, PAC, PAL activities as well as bilirubin rate). Indeed, green tea extract significantly increased ($p < 0.05$) the enzymatic antioxidants activities (SOD, catalase, GPX) in of rats liver compared with those given cadmium alone. Thus, the oral administration of green tea along with cadmium significantly ($p < 0.05$) improves cadmium-induced liver dysfunction and stress oxidant in rats' liver.

Key words: Cadmium, liver dysfunction, green tea, antioxidants enzymes and lipid peroxidation.

INTRODUCTION

The major cause of hepatic diseases is metal pollution. Among the potent toxic substances is cadmium. Cadmium (Cd) is used industrially to manufacture electroplates, batteries, alloys and fuels (Waisberg et al., 2003). The increasing industrial use of Cd causes soil, air and water contamination. Exposure to cadmium is associated with liver tumors and toxicity (Waalkes et al., 2000). Various studies have shown that cadmium toxicity in liver seems to be crucially mediated by the production of reactive oxygen species causing necrosis in various rats organs (Xu et al., 1999), lipid peroxidation ((El-Maraghy et al., 2001) and decrease in antioxidant enzymes (Sarkar et al., 1998).

Epidemiological studies have strongly suggested that diet plays an important role in the prevention of chronic diseases (Niggeweg et al., 2004). Polyphenolics, commonly found in fruits, vegetables and grains, provide chemoprotective effects to combat oxidative stress in the body and maintain balance between oxidants and antioxidants to improve human health (Adom and Liu, 2002). An imbalance caused by oxidants excess leads to oxidative stress, resulting in damage to DNA and protein and inc-

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Abreviations: **LDH**, lactate dehydrogenase; **GGT**, gammaglytamyl transferase; **PAC**, acid phosphatase; **PAL**, phosphatase alkaline; **TBARS**, thiobarbituric acid-reactive substances; **SOD**, superoxide dimutase; **GPX**, glutathione peroxidase.

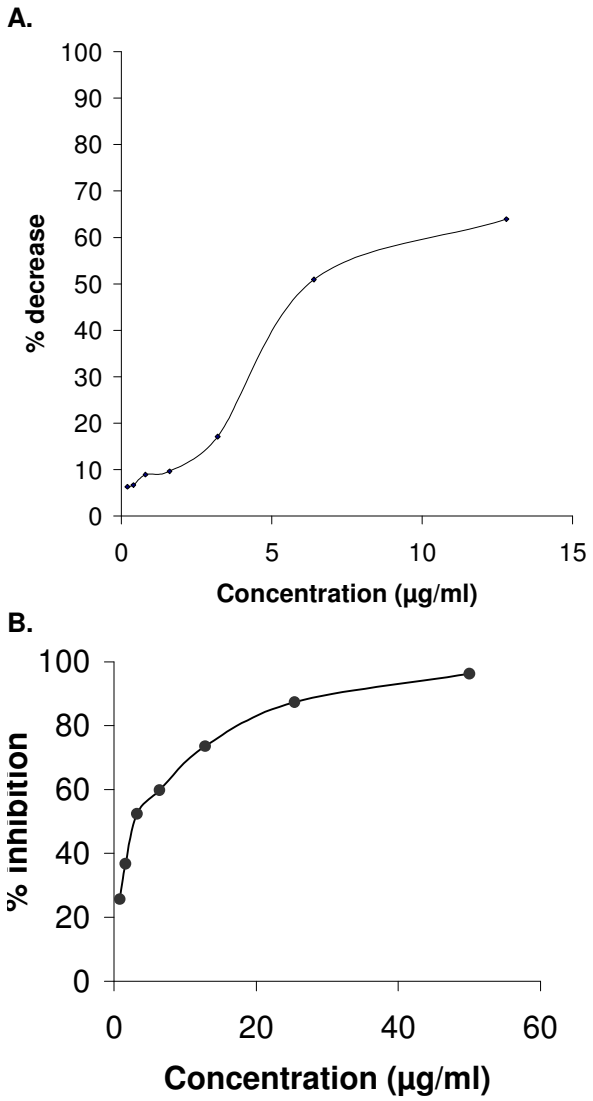

Figure 1. Scavenging effects of aqueous green tea extract against the radicals DPPH (A) and NBT / Riboflavin (B).

reases the risk of degenerative diseases such as cancer (Moller and Loft, 2004; Sander et al., 2004). Green tea is prepared from tea plant *Camellia sinensis*. These are rich in flavonoids, and in green tea most important polyphenolic compound. Studies have shown that tea possesses diverse pharmacological properties which include anti-inflammatory (Mutoh et al., 2000), anti-mutagenic (Steele et al., 2000), antiangiogenic (Jung and Ellis, 2001), anti-aging effects (Esposito et al., 2002), and preventive effects against cancers.

The present study was designed to assess the protective effect of green tea infusion by evaluating the free radicals scavenging capacity using the DPPH and NBT / riboflavin *in vitro. In vivo*, the liver dysfunction parameters rate, lipid peroxidation, and antioxidant enzymes activity in treated were measured over a six-month period.

MATERIALS AND METHODS

Animals and treatment

Adult male wistar rats weighing approximately 140 – 152 g were obtained from the Central Pharmacy, Tunisia. They were provided with animal feed and water *ad libitum* and maintained in 12 h light / dark cycles at 24 ± 4°C. Animals were divided into four groups (six rats per group) and caged separately. Group 1 (untreated control) animals were injected under cutaneous 0.9%. NaCl. Group 2 (group Cd) was injected under cutaneous 20 µmoles of cadmium/kg bw/3 days (Wallkes et al., 2000). Group 3 (group Cd + GT) was treated with cadmium and given 5% green tea extract instead of drinking water for six months. Group 4 (Group GT) was given only 5% tea. On completion of the treatment, the animals were decapitated and the arterio-venous blood collected. After centrifugation of the blood at 1000 g, 4°C, the liver dysfunctions parameters (LDH, acid phosphatase, gamma-glytamyl transferase, phosphatase alkaline activeties and bilirubin rate) were determined in the serum. The antioxidant enzymes activities (SOD, catalase and GPX) and the TBARs rates were determined in the liver.

Preparation of green tea extract

Green tea was extracted using the method described elsewhere (Itharat et al., 2004) and modified in our laboratory. Briefly, the plant samples weighing about 50 g were individually extracted in 1 L water at 40°C for 60 min. After filtration, the extract was kept at 4°C. The extract was prepared and given each day. For DPPH and NBT/Riboflavin tests, green tea was extracted by ethanol.

Biochemical determination

The free radicals scavenging activity of Green tea was determined using the 1,1-diphenyl-2-picryldrazil (DPPH) method (Ohinishi et al., 1994) and nitroblue tetrazolum (NBT) reduction method (McCord and Fridovich, 1969). The activities of lactate dehydrogenase (LDH), gamma-glytamyl transferase (GGT), acid phosphatase (PAC), phosphatase alkaline (PAL), and bilirubin were assayed using commercial kits from Sigma Munich (Munich, Germany) and Boehringer–Mannheim (Mannheim, Germany). The lipid peroxidation in the liver of control and all treated groups was measured by the quantification of thiobarbituric acid reactive substances (TBARS) determined by the method of Buege and Aust (1984). The super oxide dismutase activity in the liver of control and treated groups was assayed by the modified spectrophotometric method of Marklund and Marklund (1975). Of glutathione peroxidase and catalase activities in the liver of control and all treated groups were assayed by the modified method of Pagila and Valentine (1967) and Aebi (1984), respectively. The protein rate was determined by Lowry et al. (1951).

Statistical analysis

Means ± SEM were calculated for the different groups of rats. The statistical evaluation of the data was achieved using Student's t-test as described by Garret (1956). A difference was considered significant at $p \leq 0.05$.

RESULTS

Radicals scavenging activity on DPPH and NBT

In vitro, the antioxidant activity of Green tea extract was

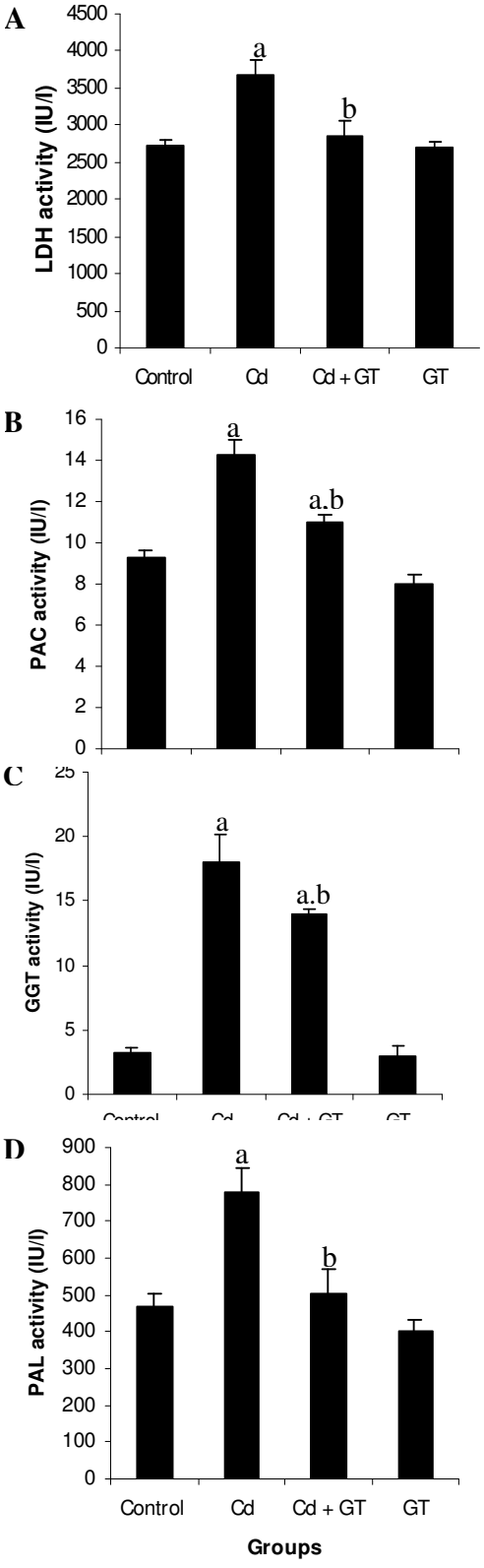

Figure 2. Effect of cadmium and green tea infusion in the LDH (2A), PAC (2B), GGT (2C), PAL (2D) activities. Values are mean ± SEM; n = 6. [a]As compared to control group: $p<0.05$. [b]As compared to Cd group: $p<0.05$.

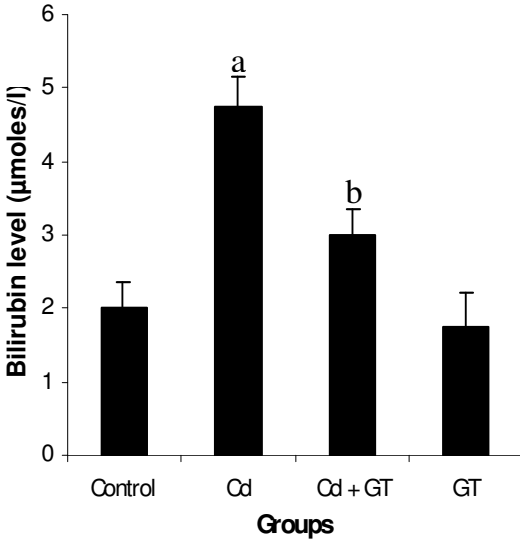

Figure 3. Effect of cadmium and green tea infusion in the bilirubin level. Values are mean ± SEM; n = 6. [a]As compared to control group: $p<0.05$. [b]As compared to Cd group: $p<0.05$.

evaluated by its ability to scavenge DPPH and NBT free radicals. GT extract showed a scavenging activity with a percentage decrease, versus the absorbance of DPPH and NBT standard solution of 63.9 and 87.3% respectively at a concentration of 12.8 and 25.4 µg/ml, respectively (Figure 1).

LDH, PAC, GGT, and PAL activities in serum

The serum activities of LDH, PAC, GGT, and PAL increased as a result of Cd administration (by 35, 55, 421, and 66% respectively) compared to control (Figure 2). In the rats that received at the same time cadmium and tea extract, the four enzymes activities increased but remained lower in rats that received only cadmium (by 22, 29, 22, and 35% respectively).

Bilirubin level

The bilirubin concentration increased by 137% in the serum rats that received Cd. In the rats that received Cd + green tea extract, protective effects were observed by maintaining bilirubin rate lower by 36% compared to the rats that received only Cd (Figure 3).

SOD, Catalase, and GPX activities in liver

The liver activities of SOD, catalase, and GPX decreased as a result of Cd administration (by 63, 47, and 29% respectively) compared to control (Figure 4). In the rats that received at the same time Cd and green tea extract a

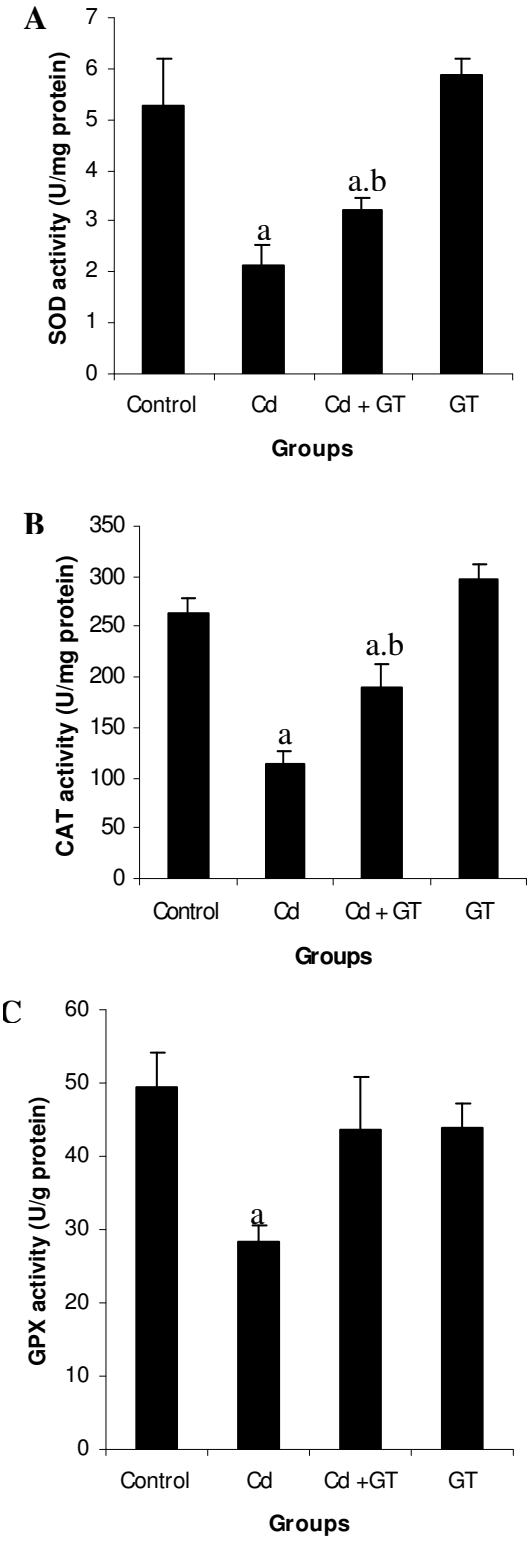

Figure 4. Effect of cadmium and green tea infusion in the hepatic SOD, CAT, and GPX activities. Values are mean ± SEM; n = 6. SOD activity-Units/min/mg protein. Catalase activity- µmoles H_2O_2 consumed / mg protein/min. GPX activity-µmoles of GSH oxided /min/g protein. [a]As compared to control group: $p<0.05$. [b]As compared to Cd group: $p<0.05$.

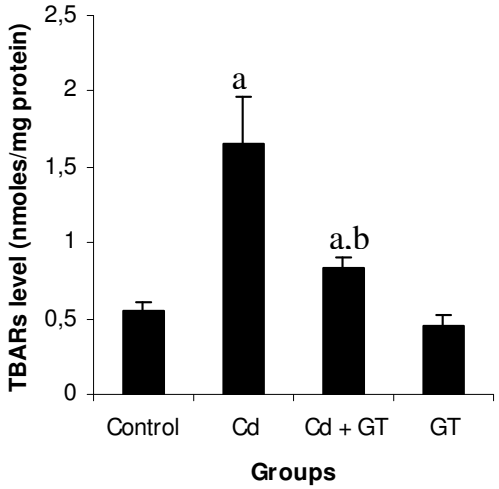

Figure 5. Effect of cadmium and green tea infusion in the hepatic TBARs rate.
Values are mean ± SEM; n = 6.

protective effect was observed. The antioxidant enzymes activities was higher (by 129, 55, and 15% respectively) compared to the rats that received only Cd.

TBARs rate

Lipid peroxidation is a potential mechanism of cell injury. The TBARs concentration increased by 126% in liver after six months of treatment with Cd compared to control (Figure 5). A protective effect of green tea against the Cd-induced oxidative effects was observed. In fact, the TBARs concentration in the liver of rats that received Cd and green tea at the same time was lower respectively by 44%.

DISCUSSION

Cadmium, a heavy metal, is among the elements which are present in soils, sediments, air and water. Unlike most metals, cadmium use began fairly recently with its large-scale application (Stoeppler, 1991). The classification of cadmium as a human and animals toxicity in liver is supported by strong evidence from animal experiments. In rodents, cadmium-induced toxicity is seen in various organs such as liver (Waisberg et al., 2003). Various studies have shown that cadmium toxicity seems to be crucially mediated by the induction of cells damage in various rats organs (Xu et al., 1996) by the production of reactive oxygen species. Cadmium is known to induce the production of hydroxyl radicals (O'Brien and Salacinski, 1998), superoxide anions, nitric oxide and hydrogen peroxide (Galan et al., 2001; Stohs et al., 2001). It also increases the rates of lipid peroxidation in liver (El-Maraghy et al., 2001) and decreases the anti-oxidant enzymes activities (Tatrai et al., 2001).

The results of this paper have confirmed that chronic cadmium intoxication causes liver dysfunction and toxicity by significant changes in cells damage and antioxidant parameters in rat liver. The increase in LDH, GGT, PAL and PAC activities result from damage cells and liver dysfunction.

Moreover, Cd induced the decrease of SOD, catalase and GPX activity and the increase of TBARs rate. The imbalance between antioxidant/oxidant was also observed by other authors (Casalino et al., 2002; Casalino et al., 1997; Tatrai et al., 2001) caused by the induction of the hydroxyl radicals production (O'Brien and Salacinski, 1998), superoxide anions, nitric oxide and hydrogen peroxide (Galan et al., 2001; Stohs et al., 2001).

The defence against long-term cadmium exposure consists of both antioxidants synthesized in the tissues and exogenous antioxidants supplied e.g. with diet. The present paper revealed that in animals intoxicated with cadmium, green tea partially prevents liver dysfunction and alterations of antioxidative parameters induced by cadmium. The protective effect of green tea is connected with its components that possess scavenging free radicals properties demonstrated in this study by DPPH and NBT/riboflavin in vitro tests.

Indeed, tea extract enhances the expression of intracellular endogenous antioxidants such as SOD, catalase and GPX by maintaining their activities higher compared to the control rats (Cd) and other antioxidants enzymes such as glutathione, glutathione reductase, glutathione-S-reductase, and quinone reductase (Khan et al., 1992; Valerio et al., 2001). In fact, a direct genomic effect of tea flavonoids with estrogens responsive elements caused an induction of the enzymes antioxidants expression (Vina et al., 2005).

In addition, tea flavonoids might protect against toxicity in liver through the inhibition of oxidative damage. The oxidation of DNA is likely to be important causes of mutations that can potentially be reduced by dietary flavonoids which are one-electron donors. They serve as derivatives of conjugated ring structures and hydroxyl groups that have the potential to function as *in vitro* antioxidants by scavenging superoxide anion (Robak and Gryglewski, 1988), singlet oxygen (Husain et al., 1987), lipid peroxyradicals (Torel et al., 1986), and/or stabilizing free radicals involved in oxidative processes through hydrogenation or complexing with oxidizing species (Shahidi et al., 1992). Another mechanism proposed for protection against cancer by dietary flavonoids may include the induction of Phase II detoxification enzymes in cells. Modification of cellular detoxification enzymes could be a major mechanism for protection against the toxic effects (Talalay et al., 1995).

Conclusion

It can be concluded that multiple pre-treatment with the green tea attenuates liver dysfunction by the attenuation of the increase of LDH, GGT, PAL, and PAC activities as well as bilirubin rate. In addition, green tea inhibits lipid peroxidation and induces the activity of anti-oxidant enzymes such as SOD, catalase, and GPX. Therefore, flavonoid-rich food may play a protective role against cadmium-mediated liver dysfunction and toxicity and other diseases such as cancers.

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