NISEB JOURNAL Vol. 11, No. 4, October 31, 2011
Printed in Nigeria

1595-6938/2011 $5.00 + 0.00

http://www.nisebjournal.org

NISEB 2011109/11407

Biochemical effects of *Pleurotus tuberregium* on lipid peroxidation and the activities of superoxide dismutase and catalase

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(Received May 20, 2011; Accepted June 29, 2011)

ABSTRACT: **Objective:** This work investigated the probable protective effects of an aqueous extract of *Pleurotus tuberregium* on cadmium-induced oxidative stress and activities of some antioxidant enzymes in the heart and testes of male albino rats.
Method: Male albino rats were assigned into four groups of ten animals each in this study that lasted for 4 weeks. The control group was administered normal saline whereas the other groups were all orally treated with 3 mg/kg body weight cadmium chloride and two of the test groups were also gavage-fed different doses of aqueous extract of *Pleurotus tuberregium* (either 500 or 1 000 mg/kg body weight). Lipid peroxidation and the activities of superoxide dismutase (SOD) and catalase (CAT) as well as the concentration of cadmium were determined in the heart and testes of the animals.
Results: The concentration of cadmium in the heart and testes of the rats that were not treated with the aqueous extract of *Pleurotus tuberregium* were significantly ($p<0.05$) higher when compared with the controls. However, administration of *Pleurotus tuberregium* appeared to reduce the level of cadmium in these organs towards values comparable with the controls. The concentration of malondialdehyde also exhibited the same trend. The activities of SOD and CAT were significantly ($p<0.05$) decreased in the rats treated with cadmium compared with the controls. However, administration of the aqueous extract of *Pleurotus tuberregium* seemed to ameliorate the effect of cadmium on the activities of SOD and CAT as there was no significant difference ($p>0.05$) when compared with the controls.
Conclusion: The results of this study suggest that the aqueous extract of *Pleurotus tuberregium* may be able to considerably reduce the oxidative stress engendered by cadmium in rats.

Keywords: *Pleurotus tuberregium*, malondialdehyde, antioxidant enzymes, cadmium.

Introduction

It has been reported that exposure of humans and rodents to concentrations of cadmium as low as 1 – 2 mg Cd/kg body weight results in an impairment of the male reproductive system as well as cardiovascular dysfunction (Adaikpoh and Obi, 2009; Benoff et al., 2000). One of the mechanisms that have been suggested for the toxicity of cadmium is that it promotes an early oxidative stress due to its long retention in tissues and organs and ability to cause the production of free radicals (Kowalczyk et al., 2003).

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It is believed that antioxidants should be one of the important components of an effective treatment of cadmium poisoning (Kara et al., 2007). So far, numerous metal-chelating agents and synthetic antidotes such as butylhydroxyanisole (BHA), butylhydroxytoluene, N-acetyl cysteine, monoisoamyl 2,3-dimercaptosuccinate and mannitol have been employed to reduce cadmium-induced oxidative burden (Henneburg et al., 2006; Tandon et al., 2003). However, toxicological studies have shown that these chelators accentuate the oxidative stress engendered by cadmium especially in testes (Jones et al., 1988). This may partly account for the increasing interest in the use of naturally-occurring phytochemicals in the management of oxidative stress.

Edible mushrooms may play a role in ameliorating the deleterious effects of oxidative stress considering their high content of phenolic compounds with antioxidant properties as well as mineral elements like K, P, Zn, CU and Se (Chirinang and Intarapichet, 2009; Mattila et al., 2001). One of such edible mushrooms that grow naturally in Nigeria is *Pleurotus tuberregium* which has been attributed with antitumour, antifungal, anti-inflammatory and some other beneficial health effects (Ijeh et al., 2009).

It is worthy of note that notwithstanding the apparent widespread use of mushrooms as dietary supplements based on the folkloric belief that they confer numerous health benefits, there is surprisingly very scarce information in the literature about the effect of extracts of *Pleurotus tuberregium* on the activities of antioxidant enzymes in animal models that have been subjected to oxidative stress. Thus, the aim of this study is to investigate the probable protective role of an aqueous extract of the mushroom on cadmium-induced oxidative stress in the testes and heart of rats.

Materials and Methods

Experimental design

Forty (40) male albino Wistar rats (150—180 g) that were used for this study were purchased from the Department of Pharmacology of the University of Benin. They were made to acclimatize to our laboratory conditions for two weeks before there were assigned to four groups of ten animals each; the distribution was such that the variation in weight was not more than 5 g. The animals were housed in clear wire cages with mesh floor in a warm and well-aerated room; they were allowed unlimited access to feed and deionized water.

The first group served as the control and was administered normal saline but not exposed to cadmium or the aqueous extract of *P. tuberregium.* The second group was orally given cadmium chloride (3 mg/kg body weight) alone once weekly while the remaining two groups received cadmium, once weekly, and also graded daily dosage (500 mg/kg body weight and 1000 mg/kg body weight) of the aqueous extract of *P. tuberregium*. The animals were handled according to the guiding principles enunciated in the NIH guide for laboratory animal welfare—Vol 3, 1985.

At the end of the study period, the rats were fasted overnight and sacrificed by partial decapitation and the heart and testes were excised stored at 4 ^{o}C until analyses which was within 48 h.

Preparation of aqueous extract of *Pleurotus tuberregium*

Pleurotus tuberregium was obtained from the Mycology unit of the Department of Plant Biology and Biotechnology of the University of Benin. The shoot of the plant was sundried for 3 days to reduce the moisture content and then pulverized using a Binatone® electric grinder. 50 g of the resulting powder was soaked overnight in 500 ml of deionized water. This mixture was then filtered through a Whatman® filter paper. The resulting homogenous filtrate was then concentrated using an in-house rotavaporation procedure to give a concentrate that was stored at 2 ^{o}C until use. A stock solution of 500 mg/ml was then prepared by dissolving the concentrate in deionized water. The appropriate volume of this stock solution was administered to the rats daily with particular attention to the dosage per body weight.

Preparation of homogenates of the tissues

This was achieved by the method described by Adaikpoh et al. (2007). In this method, weighed portions of the tissues were homogenized in ice cold 0.05 M phosphate buffer pH 7.8. The homogenates were centrifuged at 10,000 g for 15 min and the clear supernatant obtained was immediately used for the analysis of antioxidant enzymes. For

the assay of malondialdehyde, tissues were homogenized in normal saline. After centrifugation, the supernatant was used for the assay.

Cadmium analysis

The concentration of cadmium in the tissues studied was determined by atomic absorption spectrophotometry (Varian AA 1475 Spectrophotometer). The test metal was dissolved in deionized water and used as standard. In all the determinations, blanks were prepared to determine the effect of reagent purity on the levels of the metal.

Biochemical assays

The level of malondialdehyde (an index of lipid peroxidation) was assayed by the method of Hunter *et al*. (1963) as modified by Guttrigde and Wilkins (1980). The assay involved the determination of Thiobarbituric Acid Reactive Substances (TBARS). Values for TBARS are reported as Malondialdehyde (MDA) and quantitated using a molar extinction coefficient of 1.56×10^5 M/cm and expressed as µmole MDA g^{-1}. The activity of catalase was estimated by the method described by Cohen *et al*. (1970). Superoxide dismutase (SOD) activity was achieved by the method of Misra and Fridovich (1972) and total protein estimation by Lowry *et al*. (1951).

Statistical analysis

The results of this study were expressed as Mean ± SEM. Differences between the groups were determined by one-way ANOVA. Statistically significant differences between means were determined by the Duncan's multiple range test (Sokal and Rohlf, 1969).

Results

The concentration of cadmium in the heart and testes of the rats that were not treated with the aqueous extract of *Pleurotus tuberregium* was significantly ($p<0.05$) higher when compared with the controls (Table 1). However, administration of the aqueous extract of *Pleurotus tuberregium* appeared to result in a reduction in the level of cadmium in these organs towards values that were not significantly ($P>0.05$) different from the controls (Table 1). Similar trends were recorded for the degree of lipid peroxidation, represented by the concentration of malondialdehyde, in the presence of cadmium alone and both cadmium and the aqueous extract of *Pleurotus tuberregium* (Table 1). The activities of superoxide dismutase (SOD) and catalase (CAT) were significantly ($p<0.05$) decreased in the rats treated with cadmium compared with the controls (Table 2). However, administration of the aqueous extract of *Pleurotus tuberregium* seemed to ameliorate the effect of cadmium on the activities of SOD and CAT as there was no significant difference ($p>0.05$) when compared with the controls (Table 2).

Table 1: Effect of aqueous extract of *Pleutorus tuberregium* on lipid peroxidation levels in the heart and testes of rats exposed to cadmium orally

Biochemical parameter	Control	Control + cadmium	Control + cadmium + 500mg/kg *Pt*	Control + cadmium + 1 000mg/kg *Pt*
Cadmium				
Heart	ND	1.67 ± 0.21^a	0.55 ± 0.08^b	0.25 ± 0.04^c
Testes	ND	3.21 ± 0.32^a	0.88 ± 0.11^b	0.35 ± 0.02^c
Malondialdehyde				
Heart	1.34 ± 0.04^a	4.55 ± 0.11^b	1.51 ± 0.09^a	1.37 ± 0.14^a
Testes	5.95 ± 2.08^a	10.83 ± 1.61^b	6.07 ± 1.55^a	5.80 ± 0.68^a

Values are expressed as Mean ± SEM, n=10. Mean of the same row followed by different letter differs significantly ($p<0.05$). Values of cadmium are expressed in µg Cd g^{-1} tissue. Malondialdehyde (MDA) levels are expressed as mmole MDA g^{-1} tissue. ND means Not Detectable

Table 2: Effect of aqueous extract of *Pleutorus tuberregium* on heart and testes antioxidant enzyme activities in rats exposed to cadmium

Biochemical parameter	Control	Control + cadmium	Control + cadmium + 500mg/kg *Pt*	Control + cadmium + 1 000mg/kg *Pt*
Heart				
SOD	4.41 ± 0.10[a]	2.06 ± 1.15[b]	3.41 ± 0.14[a]	4.29 ± 0.51[a]
Catalase	1.86 ± 0.26[a]	0.73 ± 0.10[b]	1.53 ± 0.23[a]	1.94 ± 0.13[a]
Testes				
SOD	11.42 ± 2.96[a]	2.98 ± 0.75[b]	11.20 ± 2.54[a]	14.75 ± 0.73[a]
Catalase	5.43 ± 1.33[a]	0.88 ± 0.28[b]	3.18 ± 0.57[c]	4.73 ±0.68[a,c]

Values are expressed as Mean ± SEM, n=10. Mean of the same row followed by different letter differs significantly ($p<0.05$). SOD and catalase activities are expressed as Units/mg protein.

Discussion

It has been reported that about 80% of the world population depend on plant-based drugs (WHO, 1996). In Nigeria and most developing countries of the world, there is a great reliance on plant preparations for the treatment and management of numerous diseases despite the availability of orthodox medicine (Nwabuisi, 2002). Apart from plants, mushrooms and their extracts have also been exploited for their apparently diverse medicinal value such as antimicrobial, antihypertensive, antitumour and immunomodulatory activities (Fillipie and Umek, 2002; Hu *et al*., 2006; Jong *et al*., 1991; Ngai *et al*., 2006). The focus of this study was to investigate the effects of administering an aqueous extract of *Pleurotus tuberregium* on lipid peroxidation and the antioxidant status in rats that had been orally exposed to cadmium.

In this study, we observed a significantly ($P<0.05$) higher accumulation of cadmium in the testes and heart of rats exposed to cadmium compared with the controls (Table 1). This finding is in consonance with those of Amara et al. (2008) and Salama and El-Bahr (2007) who also reported an accumulation of the toxicant in rat testes. However, administration of the aqueous extract of *Pleurotus tuberregium* seemed to prevent this accumulation in both organs studied. This might be attributed to the presence of numerous divalent cations such as Zn which may competitively inhibit the binding of cadmium to tissue proteins (Akindahunsi and Oyetayo, 2006; Amara et al., 2008). Another reason for this reduction might be due to the presence of dietary fibres in the mushroom that may act as metal chelators (Wong and Cheung, 2005).

The results of this work also indicate that there was a significantly ($P<0.05$) increased concentration of malondialdehyde (MDA) in the testes and heart of rats exposed to cadmium compared with the controls. This agrees with the findings of Asagba et al. (2007), Kara et al. (2007) and Salama and El-Bahr (2007) who suggested that oxidative cellular damage might be the reason for the increase in malondialdehyde levels. The apparently higher accumulation of cadmium in the testes compared with the heart might be related to the long-established report of a high amount of cadmium-binding proteins in the testes (Cahill et al., 1983; Suzuki et al, 1998).

One of the prominent defence strategies that the body deploys to stem the deleterious effects of cadmium-induced lipid peroxidation are superoxide dismutase (SOD) and catalase. This study revealed a significant reduction ($P<0.05$) in these enzymes in the presence of cadmium alone (Table 2). However, administration of the aqueous extract of *Pleurotus tuberregium* in the presence of cadmium seemed to increase the activities of these antioxidant enzymes back to levels that were not significantly ($P>0.05$) different from those of the controls (Table 2). The probable beneficial effects of the mushroom extract might be attributed to its high content of such mineral elements as Zn, Mg and Se which are cofactors of SOD and glutathione peroxidase (Adejumo and Awosanya, 2005; Akindahunsi and Oyetayo, 2006).

Bauer et al. (1980) have long established that one mechanism by which cadmium diminishes the activity of SOD is by displacing Zn from the Cu-Zn-SOD molecule which effectively inactivates the enzyme. Administration of the mushroom extract then provides an abundant supply of the requisite mineral elements which may stoichiometrically restore the activity of the enzyme back to values that are comparable with the controls. Amara et al. (2008) have

postulated that Zn reduces the cytotoxicity of cadmium by promoting its efflux from the testicular cells as well as a probable induction of the synthesis of metallothionine—a cadmium-binding protein.

It was also observed that there was a significant decrease ($P<0.05$) in the activity of catalase in both organs studied in the presence of cadmium alone (Table 2). This finding agrees with the work of Amara et al. (2008) but is at variance with that of Salama and El-Bahr (2007) who reported an increase in the activity of the enzyme. This discrepancy may be due to a difference in relation to the method of administering the cadmium. Treatment with the extract of *Pleurotus tuberregium* appeared to restore the activity of this enzyme back to values that were not significantly ($P>0.05$) different from the control (Table 2). It is possible that the mushroom extract relieves the inhibition of cadmium by providing a rich supply of beneficial trace elements that might prevent the direct inhibitory binding of cadmium to the sulphydryl (—SH) groups of catalase and thus restore its activity.

In conclusion, the findings that we have reported in this study suggest that administration of an aqueous extract of *Pleurotus tuberregium* might mitigate cadmium-induced lipid peroxidation in rats by a probable reduction in the accumulation of cadmium as well as by a possible induction of the antioxidant defence mechanism.

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