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Cactus (*Opuntia ficus-indica*) cladodes prevent oxidative damage induced by the mycotoxin zearalenone in Balb/C mice

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Abstract

Zearalenone (ZEN) is one of the most widely distributed fusarial mycotoxins which is encountered at high incidence in many foodstuffs. ZEN was associated with different reproductive disorders in animals. Several *in vivo* studies have shown that ZEN is hepatotoxic, haematotoxic and causes several alterations of immunological parameters. Furthermore, evidence of its cytotoxicity and genotoxicity has recently emerged from several reports.

The aim of the current study was (i) to find out whether oxidative stress could be relevant for ZEN induced toxicity *in vivo* using Balb/c mice and (ii) to evaluate the safety and efficacy of cactus cladodes *Opuntia ficus* to prevent the deleterious effects of ZEN. To this end, the effect of a single dose of ZEN (40 mg/kg b.w.) alone and with extract of cactus cladodes (25, 50 and 100 mg/kg b.w.) on the induction of oxidative stress was monitored in kidney and liver by measuring the MDA level, the protein carbonyls generation, the catalase activity and the expression of the heat shock proteins (Hsp).

Our results clearly showed that ZEN induced significant alterations in all tested oxidative stress markers. Oxidative damage seems to be a key determinant of ZEN induced toxicity in both liver and kidney of Balb/c mice. The combined treatment of ZEN with the lowest tested dose of cactus extracts (25 mg/kg b.w.) showed a total reduction of ZEN induced oxidative damage for all tested markers. It could be concluded that cactus cladodes extract was effective in the protection against ZEN hazards. This could be relevant, particularly with the emergent demand for natural products which may counteract the detrimental effects of oxidative stress and therefore prevent multiple human diseases.

Keywords: Zearalenone; Cactus cladodes; *Opuntia ficus*; Oxidative stress; MDA induction; Proteins cabonyls; Catalase activity; Hsp expression

1. Introduction

Mycotoxins are food contaminants produced by fungi. The pathologies in humans and animals as well as economical loss are important consequently mycotoxins have become a worldwide preoccupation (For review, see Hussein and Brasel, 2001; Bennett and Klich, 2003). While much attention has been given to the study of the mycotoxins produced by *Aspergillus* and *Penicillium* species, much less concern has been paid to fusarial toxins, although compelling evidence have recently implicated the *Fusarium* mycotoxins in livestock disorders in different parts of the world (D'Mello et al., 1999; Eriksen and Alexander, 1998; Cavret and Lecoeur, 2006). Zearalenone (Fig. 1), abbreviated as ZEN, is one of the most widely distributed fusarial mycotoxins which is encountered at a high incidence in many important corps intended for human and animal consumptions (Bottalico, 1998; Muller et al., 1998; Scudamore and Patel, 2000).

It is acknowledged that ZEN is of relatively low toxicity, with an LD50 value of 500 mg/kg body weight as determined with mice (Flannigan, 1991). However, its role as a mammalian endocrine disrupter is being recognized, with

Abbreviations: ZEN, zearalenone; Hsp, heat shock protein; MDA, malondialdehyde.

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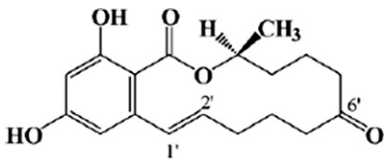

Fig. 1. Chemical structure of zearalenone.

effects in both males and females of different species. ZEN and some of its metabolites have been shown to bind competitively to estrogen receptors (ERα and ERβ) in a number of *in vitro* or *in vivo* systems and to activate transcription of estrogen-responsive genes (Mehmood et al., 2000; Kuiper et al., 1998; Mayr, 1988 Turcotte et al., 2005). Therefore, ZEN was associated with several reproductive disorders in animals (Eriksen et al., 2000; LeGuevel and Pakdel, 2001; D'Mello et al., 1999). In addition, ZEN was found to be hepatotoxic, it induces liver lesions (Maaroufi et al., 1996; Obremski et al., 1999; Conkova et al., 2001). It is equally haematotoxic and causes several alterations of immunological parameters (Maaroufi et al., 1996; Murata et al., 2003; Abbes et al., 2006a,b). Recently, in our laboratory, several studies have been conducted and have shown that ZEN is cytotoxic and exhibits a geneotoxic potential *in vitro* and *in vivo* through induction of micronuclei, chromosome aberrations, DNA fragmentation, cell cycle arrest, etc. (Abid-Essefi et al., 2004; Ouanes et al., 2003, 2005; Abbes et al., 2006a,b; Abbes et al., 2007; Boussema et al., 2007).

The mechanisms whereby all these effects are induced are still not totally understood and it is unlikely that the overall ZEN toxicity is due to its estrogenicity solely. It is of note that Takemura et al. (2007) have recently confirmed the low affinity of ZEN towards ERα and ERβ with a relative binding affinities about 4.3% and 6.0% respectively, as compared to 17 β-estradiol. Therefore, other mechanisms that target general cellular compartments, such oxidative stress, could be involved. However, up to now, there are no available data regarding the involvement of oxidative stress induced *in vivo* after ZEN exposure.

In recent years, a special interest was given to the study of natural products and healthy foods for improving overall well being and for prevention of diseases including cancer (Ness and Powles, 1997; Steinmetz and Potter, 1996; Youdim and Joseph, 2001). In this line, in our laboratory, we have been interested in the study of the protective potential afforded by natural compounds towards ZEN toxicity. As such, we have already demonstrated that almost all ZEN toxic effects mentioned above are significantly prevented *in vivo* and *in vitro* using Vitamin E (Abid-Essefi et al., 2003; Ouanes et al., 2003, 2005; El Golli et al., 2006; Hassen et al., 2007). Another set of experiments has shown a significant prevention by the phyllisilicate clay mainly due to its tight binding to ZEN, resulting in a reduction of toxin bioavailability (Abbes et al., 2006a,b,2007). Recently, a special interest is given to the cactus prickly pear belonging to *Opuntia* species. This plant is pointed out as relevant health promoting food with a great number of potentially active nutrients. Indeed, according to several studies, both cactus fruit and cladode yield high values of important nutrients such as minerals, vitamins as well as further antioxidants (Kader, 2002; Ramadan and Morsel, 2003; Stintzing et al., 2002, 2005; Stintzing and Carle, 2005; Tesoriere et al., 2005). Besides, several studies have reported its efficiency in the treatment of several diseases. As such, cactus extract exhibit anti-tumoral (Zou et al., 2005), anti-viral (Ahmad et al., 1996), anti-inflammatory (Loro et al., 1999; Park et al., 2001) and antioxidant effects (Tesoriere et al., 2003, 2004; Gentile et al., 2004). These data have made cactus pear fruits and cladodes perfect candidates for cytoprotective investigations.

In this context, the aim of the present study is (i) to find out whether oxidative damage could be relevant for ZEN induced toxicity *in vivo* using Balb/c mice and (ii) to investigate the preventive potential of cactus cladodes. To this end, the effect of ZEN whether alone or jointly with cactus cladodes extract on the induction of oxidative stress was investigated at several levels in kidney and liver, both prominent sites for intense oxidative processes. Thus, we have monitored the MDA concentrations, the protein carbonyls generation, catalase activity and the expression of the heat shock proteins (Hsp).

2. Materials and methods

2.1. Chemicals

Zearalenone, Triobarbituric acid (TBA); Trichloroacetic acid (TCA) and Butul hydoxyl toluene (BHT) were obtained from Sigma Chemical Co. (St. Louis, MO), 2,4-dinitro-phenylhydrazine (2,4-DNPH) and guanidine were from Prolabo (France), Mouse anti-Hsp 70 (SPA-810), 27 (SPA-800) and 90 (SPA-830) monoclonal antibodies were from Stressgen, USA. All other chemicals used were of analytical grade.

2.2. Extract of cactus cladodes

Young cactus cladodes of *Opuntia ficus-indica* (2–3 weeks of age) collected from the local area were washed with water, chopped into small pieces and then pressed using a hand-press, homogenized with 10 mM Tris-HCl, pH 7.4 at 4 °C and centrifuged 30 min at 5000*g* at 4 °C. The supernatant was collected, dried and stored at −20 °C. Prior to use, the extract was dissolved in ethanol:water mixture (1:1, v:v).

2.3. Animals treatments

Thirty Blab/c mice (Sexual, St. doulchard, France) were used (average body weight 20 ± 0.3 g; age: 6 weeks old). These mice were given a standard granulated food and drinking water and were divided into six groups as follows:

Group 1: Mice given Ethanol/water (1:1, v:v)

Group 2: Mice given cactus cladodes extract at 100 mg/kg b.w.

Group 3: Mice given ZEN in Ethanol/water (1:1, v:v) at 40 mg/kg b.w., corresponding to 8% of the LD50, this concentration was chosen according to previous investigations (Ouanes et al., 2003, 2005; Abbes et al., 2006a,b,2007).

Group 4: Mice given 24 h prior to ZEN administration a dose of cactus cladodes extract of 25 mg/kg b.w., then the dose of ZEN 40 mg/kg b.w.

Group 5: Mice given 24 h prior to ZEN administration a dose of cactus cladodes extract of 50 mg/kg b.w., then the dose of ZEN 40 mg/kg b.w.