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Full Length Research Paper

Hepatoprotective effect of cactus extract against carcinogenicity of benzo(a)pyrene on liver of Balb/C mice

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The cactus *Opuntia ficus-indica* is a xerophyte plant it has an increasing interest of nutritional and pharmacological power. In the present study we choose cactus cladode extract (CCE) to investigate its efficacy against benzo(a)pyrene (BAP) a widespread environmental genotoxin classified as probably carcinogenic to humans witch induced liver injury. Our results using balb/c mice clearly showed that BAP induced significant alterations in all tested oxidative stress markers the malondialdehyde level (MDA), the catalase activity and the expression of the heat shock proteins (Hsp 70) and (Hsp 27) which increased. In addition, it induces deoxyriboNucleic acid (DNA) fragmentation in liver and chromosomal aberrations in bone morrow cells. Moreover it increases the expression of anti apoptotic proteins bcl2 and decrease the expression of bax. The treatment of CCE after or before treatment with BAP showed a total reduction of BAP induced oxidative damage for all tested markers, it showed also an anti-genotoxic effect compared to the group treated with BAP alone, CCE was able to restrict the effect of BAP by differential modulation of the expression of p53 which is increased and its associated genes such as bax and bcl2. We concluded that CCE was effective in the protection against BAP hazards.

Key words: Cactus cladode extract, Benzo(a)pyrene, antioxidant activity, anti-carcinogenicity, *in vivo.*

INTRODUCTION

In recent years, interest on plant research has increased all over the world; many plant extracts have shown potent cancer chemopreventive properties as observed in the last decade (Ames, 1998; Beckman and Ames, 1998). In order to find new natural sources of medicinal plants from Tunisia, we studied the efficiency of cactus cladode extract (CCE) from cactus *Opuntia ficus-indica* which is a tree-like cactus belonging to the Cactaceae family. Native to Mexico, this plant is widespread throughout central and South America, Australia, South Africa, and the whole Mediterranean area (Galati et al., 2003; Tesoriere et al. 2004).

The cactus grows in all of the semiarid countries and is mainly cultivated for its fruit (prickly pear) eaten after pealing, is sweet and juicy, and rich in nutritional compounds such as ascorbic acid and polyphenols. These fruits have shown several effect such as antiulcerogenic (Galati et al., 2003), antioxidant (Kuti, 2004; Tesoriere et al., 2004), neuroprotective (Dok-Go et

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Abbreviations: CCE, Cactus cladode extract; **BAP,** benzo(a)pyrene; **MDA**, malondialdehyde level; **Hsp 70**, heat shock proteins 70; **Hsp 27**, heat shock proteins 27; **DNA**, deoxyriboNucleic acid; **PAHs**, polycyclic aromatic hydrocarbons; **DMSO**, dimethyl sulfoxide; **NBT**, nitro blue tetrazolium; **BCIP**, 5-bromo-4-chloro-3-indolyl phosphate disodium salt; **H_2O**, water; **H_2O_2**, hydrogen peroxide; **TBA**, thiobarbituric acid; **TBARS**, thiobarbituric acid-reactive substances; **KCl**, potassium chloride; **UV**, ultra violet; **SD**, standard deviation; **ANOVA**, analysis of variance; **ROS**, reactive oxygen species; **EDTA**, ethylene diamine tetraacetic acid; **SDS**, sodium dodecyl sulfate; **DMSO**, dimethylsulfoxide.

al., 2003). Moreover, prickly pear may be used for the treatment of gastritis, hyperglycemia, arteriosclerosis, diabetes, and prostate hypertrophy (Agozzino et al., 2005). In Chinese medicine cactus pear is used against inflammation and pain (Zou et al., 2005). Different parts of *O. ficus-indica* are used in the traditional medicine of several countries, our recent study showed the potential antigenotoxic activities of cactus cladodes against the mycotoxin zearalenone, a potent estrogenic metabolite (Zourgui et al., 2009). These data has made cactus pear fruits and cladodes perfect candidates for cytoprotective investigations. Most of the investigations have been carried out on CCE whereas very little information is available about the anti-oxidative effect, anticlastogenic effects and apoptotic proprieties of CCE. Chemical carcinogenesis induced in different susceptible strains provides very useful models for understanding the mechanism of carcinogenesis and its modulation. Better knowledge of genetic alterations taking place during carcinogenesis is important for exploring the mechanism of action of different chemopreventive agents, thereby helping in formulating important intervention strategies (Sugata et al., 2009). Benzo[a]pyrene (BAP), one of the most common polycyclic aromatic hydrocarbons (PAHs) constitute a large class of compounds formed during incomplete combustion of organic matter and fossil fuels in industrial processes, automobile exhaust, cigarette smoke and charbroiled food (IARC Monographs, 1983). It is classified as probably carcinogenic to humans (a group 2A carcinogen) by the International Agency for Research on Cancer (IARC Monographs, 1983) like other PAHs; BAP and several of its metabolites produce tumors in newborn mice (Brian et al., 1993) it needs metabolic activation to exert its mutagenic and carcinogenic effects. It is readily oxidized by CYP-1A1, -1A2 and -1B1 enzymes and subsequently hydrolyzed by epoxide hydrolase to yield BaP-4, 5-diol (Irena and Metka, 2009). BAP undergoes a metabolic activation to form reactive intermediates before it is capable of inducing its mutagenic and carcinogenic effects in biological systems (Shabad, 1997; Gelboin, 1980). It has been reported that BAP-quinones are important redox-cycle compounds, which are produced during the metabolic processing of BAP (Kim and Lee, 1997).

The aim of the present study is to find out the protective effect of CCE against BAP *in vivo* using Balb/c mice. To this end, we monitored the malondialdehyde level (MDA) concentrations, catalase activity, of the heat shock proteins (Hsp70 and Hsp 27) expression, chromosome aberrations and deoxyriboNucleic acid (DNA) damage induction revealed by gel electrophoresis and expression of p53, bax and bcl2 major determinant of cell death.

MATERIALS AND METHODS

Chemicals

BAP was obtained from Sigma Chemical Co. (USA). Dimethyl sulfoxide (DMSO) was obtained from Sigma Chemical Co. (St Louis, MO, USA). Nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate disodium salt (BCIP) were from Sigma Aldrich, France. Goat anti-mouse alkaline phosphatase conjugate antibody, mouse anti-heat shock proteins 70 (Hsp 70) and anti-heat shock proteins 27 (Hsp 27) monoclonals antibody (SPA-80) were from Stressgen, USA. Mouse monoclonal anti-p53, anti-bax and anti-bcl2 and the secondary antibody (phosphatase-conjugated) were from Invitrogen. Gen Elute “Mammalian genomic DNA Miniprep Kit sufficient for 70 purifications” was purchased from Sigma Aldrich, USA. All other chemicals used were of the highest grade available from commercial sources.

Extract of cactus cladodes

Young cactus cladodes of *O. 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 in 10 mM Tris-HCl, pH 7.4 at 4°C and centrifuged 30 min at 3500 g at 4°C. The supernatant was collected, dried and stored at -20°C.

Animals and treatments

Adult, healthy balbC (20–25 g) male mice provided from an animal breeding centre (SEXAL, St. Doulchard, France) were used. The animals were kept for acclimatization one week under constant conditions of temperature and a light/dark cycle of 12 h: 12 h. Animals had free access to standard granulated chow and drinking water. Animals were pretreated by intraperitonial administration of CCE (50 mg/Kg.b.w) for 2 weeks. Control animals were treated 3 days a week for 4 weeks by subcutaneous administration of 50 µg/Kg.b.w. BAP. Animals treated by BAP and CCE were divided into two groups: the first group was administered CCE 2 h before each treatment with BAP 3 days a week for 4 weeks. The second group was administered without pre-treatment with CCE but this extract was administrated 24 h after each treatment with BAP 3 days a week for 4 weeks.

All animals were divided in 9 groups of 6 animals per group; treatment as follow:

Group 1, Mice given water (H_2O) (100 µl); Group 2, Mice given DMSO/H_2O (1:1, v: v); Group 3, Mice given CCE 50 mg/Kg b.w; Group 4, Mice given BAP 50 µg/Kg b.w. for 15 days treatment; Group 5, Mice given BAP 50 µg/Kg b.w. + CCE 50 mg/Kg b.w. (before 15 days treatment by BAP); Group 6, Mice given BAP 50 µg/Kg b.w. + CCE 50 mg/Kg b.w. (after 15 days treatment by BAP); Group 7, Mice given BAP 50 µg/Kg b.w. for 30 days treatment; Group 8, Mice given BAP 50 µg/Kg b.w. + CCE 50 mg/Kg b.w. (before 30 days treatment by BAP); Group 9, Mice given BAP 50 µg/Kg b.w. + CCE 50 mg/Kg b.w. (after 30 days treatment by BAP).

Collection of serum and tissue samples

Blood was collected (in heparinized tubes). It was allowed to clot and then centrifuged at 3000 rpm for 15 min. The serum samples were collected and left standing at –20°C until required. Livers of mice were homogenized with a Potter (glass-Teflon) in the presence of 10 mM Tris-HCl, pH 7.4 at 4°C and centrifuged at 4000 rpm for 30 min at 4°C. The supernatant was collected for analysis and the protein concentration was determined in liver extract using Protein BioRad assay (Bradford, 1976).

Determination of catalase activity

Catalase activity was measured in the liver extracts spectro

phometrically at 405 nm, 25°C according to Claiborne (1985). Briefly, 20 µl of the extracts were added in the quartz cuvette contain 780 µl phosphate buffer and 200 µl of hydrogen peroxide (H_2O_2) 0.5 M. The activity of catalase was calculated using the molar extinction coefficient (0.04 Mm^{-1} cm^{-1}). The results were expressed as µmol of H_2O_2/min/mg of proteins.

Evaluation of lipid peroxidation status

The production of lipid peroxides was measured in the sera, the MDA content of the homogenates was determined basically according to the method of Ohkawa et al.(1979) with some modifications. 400 µl serum were homogenized with 1,5 ml 20% acetic acid, 1,5 ml 0,8% thiobarbituric acid (TBA) and 600 µl water and placed 2 h in 85°C. After cooling, an n-butanol and pyridine mixture (15:1, v:v) was added and then shaken vigorously and centrifuged at 2500 g for 10 min. the absorbance of the supernatant was measured spectrophotometrically at 532 nm. The thiobarbituric acid-reactive substances (TBARS) level (expressed as nmol/mg protein) was used as the quantity of MDA.

Protein extraction and Western blot analysis

Equal amounts of proteins (20 µg) were separated by 12% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. Separated proteins were electro-blotted on nitrocellulose membrane in the transfer buffer (10 ml Tris-base, pH 8.3, 96 mM glycine and 10% methanol). The membrane was then blocked in TBS (20 mM Tris-HCl, Ph 7.5, 500 mM sodium chloride) containing 5% of BSA, washed in TTBS (TBS containing 0.3% Tween 20) and probed with an antibody for Hsp 70 or Hsp 27, p53 or bax or bcl2 at a 1:1000 dilution for 6 h at room temperature. The membrane was then washed and incubated with goat anti-mouse alkaline phosphate conjugated at a 1:3000 dilution for 1 h. Next, the membrane was washed and the chromogenic substrate BCIP/ NBT was added to localize antibody binding. Hsp 70, Hsp 27, p53, bax and bcl2 levels were then determined by computer-assisted densitometric analysis (Densitometer, GS-800, BioRad Quantity One).

Chromosome aberration assay

24 h before sacrifice, animals were given a suspension of yeast powder (100 mg/500 µl) to accelerate mitosis of bone-marrow cells. Vinblastine (200 µl; 250 µg/ml) was injected into the animals 45 min before sacrifice in order to block dividing cells in metaphasis. Bone-marrow cells from femurs and tibias were collected, subjected to hypotonic shock (potassium chloride (KCl) 0.075 M) and fixed three times using methanol-acetic acid (Evans et al. 1960). The cells were spread on glass slides that were blazed on a flame for 5 s, then air-dried for conservation at room temperature and finally stained by 4% dilution of Giemsa reagent in water for 15 min. After coding of the slides, the chromosomes of 100 cells in metaphase were examined for abnormalities at a magnification of 1000× using an optical microscope (Carl Zeiss, Germany). This was done for each one of three replicates (300 metaphases per dose level) for negative controls, positive controls and treated groups. Chromosome aberrations were identified according to criteria described by Savage 1975. Metaphases with chromosome breaks, gaps, rings and centric fusions (robertsonian translocation) were recorded and expressed as percentage of total metaphases per group.

Detection of fragmented DNA by agarose gel electrophoresis

Mammalian tissues (livers) are lysed with a chaotropic salt-containing buffer to ensure denaturation of macromolecules. DNA is bound to the spin column membrane and the remaining lysate is removed by centrifugation. A filtration column is used to remove cell debris, after washing to remove contaminants; the DNA is eluted with buffer into a collection tube. The pellet was rinsed with 70% ethanol, dried at room temperature for 2 h and re-suspended in 200 µl of TE (20 mM Tris–HCl pH 8.0,1 mM ethylene diamine tetraacetic acid (EDTA). Loading buffer was added to 10 µg of DNA for each treatment, and the samples were analyzed by electrophoresis on a 1% agarose gel (1 h at 80 V/30 mA) with a TBE running buffer (44 mM Tris–HCl,44 mM boric acid, 50 mM EDTA, pH8.0).

Quantitative analysis of DNA samples was performed by ultra violet (UV) spectrophotometry

(1 OD = 50 µg DNA ml–1, max = 258 to 260 nm).

Each DNA sample was prepared and stored at –80°C prior to use.

Statistical analysis

Experimental values are expressed as mean ± standard deviation (SD). Comparison of mean values between groups was performed by one way-analysis of variance (oneway-ANOVA) followed by post- hoc Tukey test.

Expression of Hsp 70, Hsp 27, p53, bax and bcl2 were determined by Kruskal–Wallis Test. The differences in mean percentages between treated and control groups and among treated groups for numerical aberrations were evaluated using chi-square test (χ 2-test) (Waller et al., 1969). The level of significance was accepted with $P< 0.05$ was used for statistical analysis.

RESULTS

Determination of antioxydant activity of CCE

Evaluation of lipid peroxidation status

Results of the effect of BAP alone and jointly with CCE on the induction of lipid peroxidation in serum of BAP treated animal (50 µg/Kg b.w.) as determined by MDA level are shown in Figure 1, after 15 days of exposure, MDA level increased significantly (6.5± 0.35 nmol/ml serum), on days 30, MDA level is (14.03± 0.15 nmol/ml serum) compared to the control value (4.5± 0.52 nmol/ml serum).

However, animals were treated with CCE (50 mg/kg b.w.) before or after treatment with BAP a sharp decrease in MDA level was noticed at both times 15 days and 30 days, MDA level decreased significantly to reach the control level.

Determination of catalase activity

Figure 2 illustrated the effect of BAP in the presence or absence of CCE on catalase activity. BAP induced a marked increase in catalase activity after 15 days and 30 days exposure on liver extracts. The treatment with BAP in presence of CCE added before or after BAP treatment showed a noticeable decrease of this activity. At day 30, the highest level of catalase activity was decreased by 40% in liver when CCE is added.

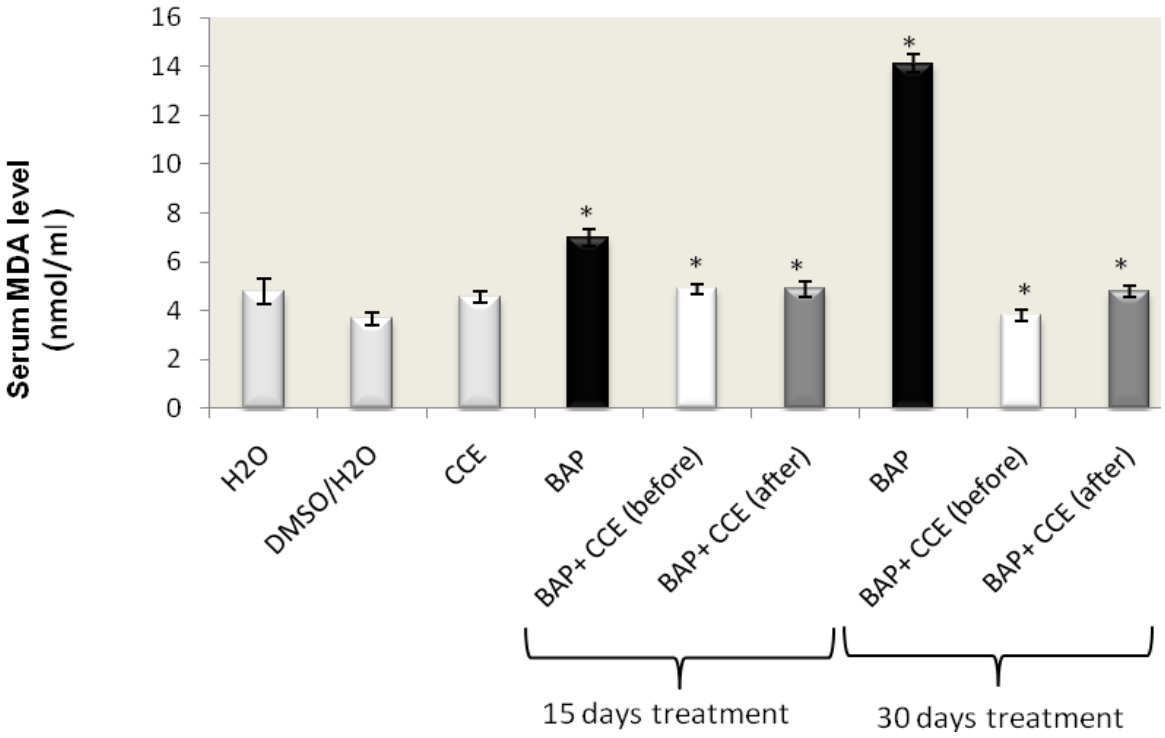

Figure 1. Lipid peroxydation as determined by MDA level in serum of Balb/c mice exposed to BAP (50µg/Kg b.w.) for 15 days then 30 days and prevention by cactus cladode extract (50mg/Kg b.w) before or after BAP administration. Results were expressed as means ± S.D. from independent experiments. (*) indicated significant difference ($p < 0.05$) from control.

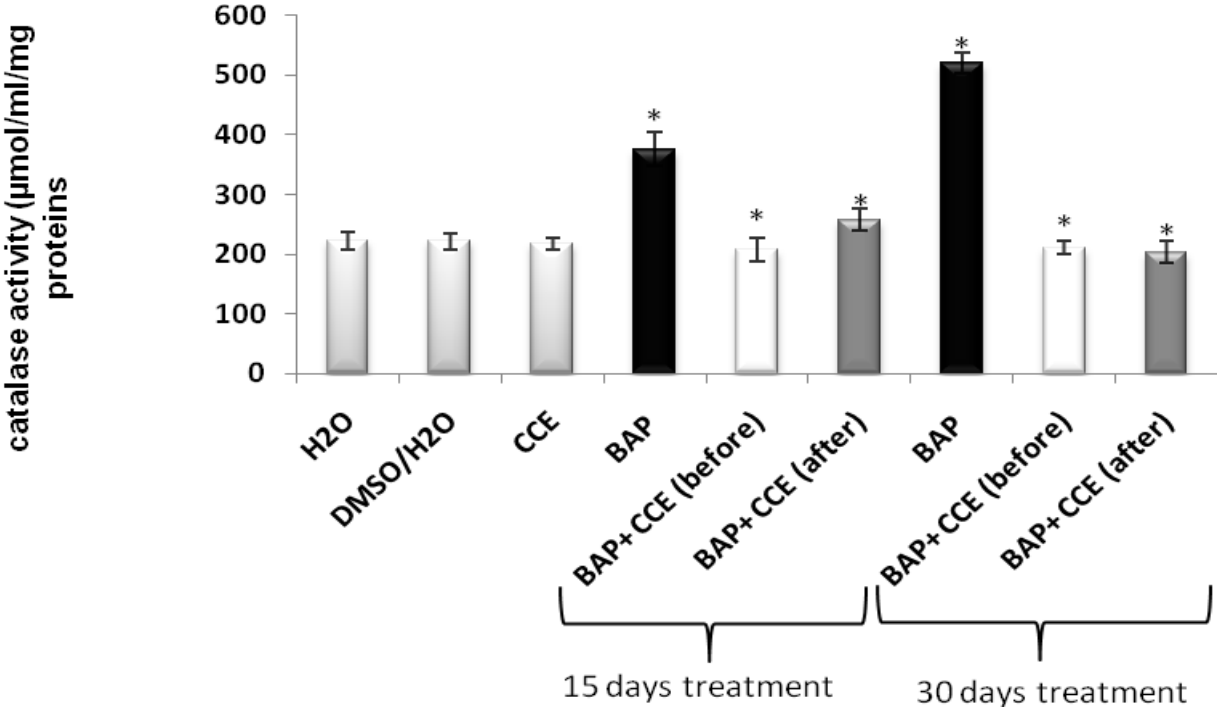

Figure 2. Effect of CCE (50 mg/Kg b.w.), before and after treatment by BAP induced catalase enzyme activity in mice. Results were expressed as means ± S.D. (*) indicated significant difference ($p < 0.05$) from control.

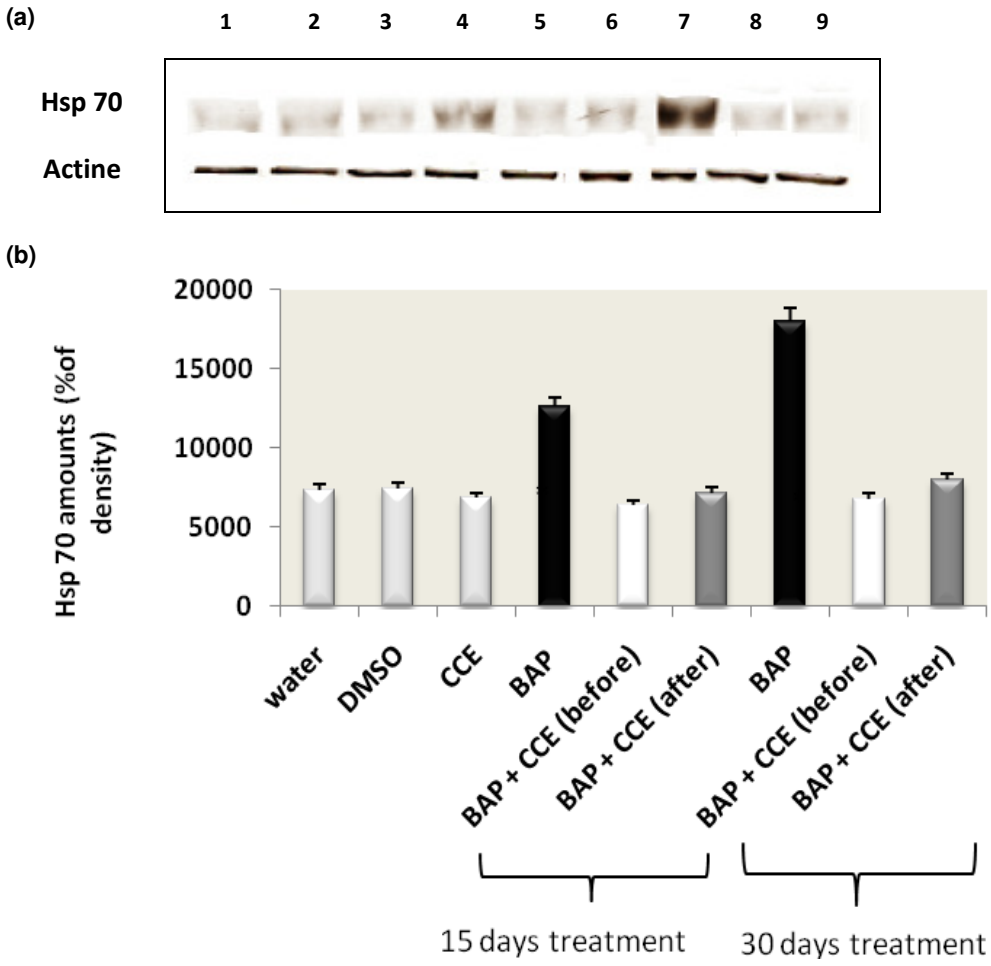

Figure 3. Immunoblot (a) and densitometric (b) analysis of Hsp 70 in liver of control and treated animals. The protein was separated on 12% SDS-PAGE and blotted with anti-Hsp70 antibody. The intensity of the protein band was scanned by densitometry. Significantly different as compared to controls ($p < 0.005$). The results are representative of nine independent experiments as follow: 1: Mice given H2O (100 µl), 2: Mice given DMSO/H2O (1:1, v: v), 3: Mice given CCE 50 mg/Kg .b.w, 4: Mice given BAP 50 µg/Kg b.w. for 15 days treatment, 5: Mice given BAP 50 µg/Kg b.w. + CCE 50 mg/Kg b.w. (before 15 days treatment by BAP), Group 6: Mice given BAP 50 µg/Kg b.w. + CCE 50 mg/Kg b.w. (after 15 days treatment by BAP), 7: Mice given BAP 50 µg/Kg b.w. for 30 days treatment, 8: Mice given BAP 50 µg/Kg b.w. + CCE 50 mg/Kg b.w. (before 30 days treatment by BAP), 9: Mice given BAP 50 µg/Kg b.w. + CCE 50 mg/Kg b.w. (after 30 days treatment by BAP).

Determination of Hsp70 and Hsp27expressions

Figures 3a and b showed the western blotting and densitometry analysis of hsp 70 expressions in the liver of control and treated animals. BAP exposed mice showed significantly increased expression of hsp 70 after 15 days and remarkably after 30 days exposure on liver extract compared to control groups. Administration of CCE before or after BAP exposure decreased significantly the hsp 70 expressions. This decrease of hsp 70 reached the basal expression observed in control groups. For Hsp 27 expression, similar results were found (Figures 4a and b).

Determination of anti-genotoxicity activity of CCE of cactus

Eventual induction of chromosome aberrations and its prevention by cactus

Animals treated with BAP alone (50 µg/kg b.w.) showed a significant increase in chromosome aberrations in bone marrow cells, mainly centric fusions and chromosome breaks. Control groups which were treated with H_2O, H_2O/ dimethylsulfoxide (DMSO) or CCE showed a similar basal and low percentage of total chromosome aberrations (respectively 3.78 ± 0.68; 4.55 ± 1.03 and

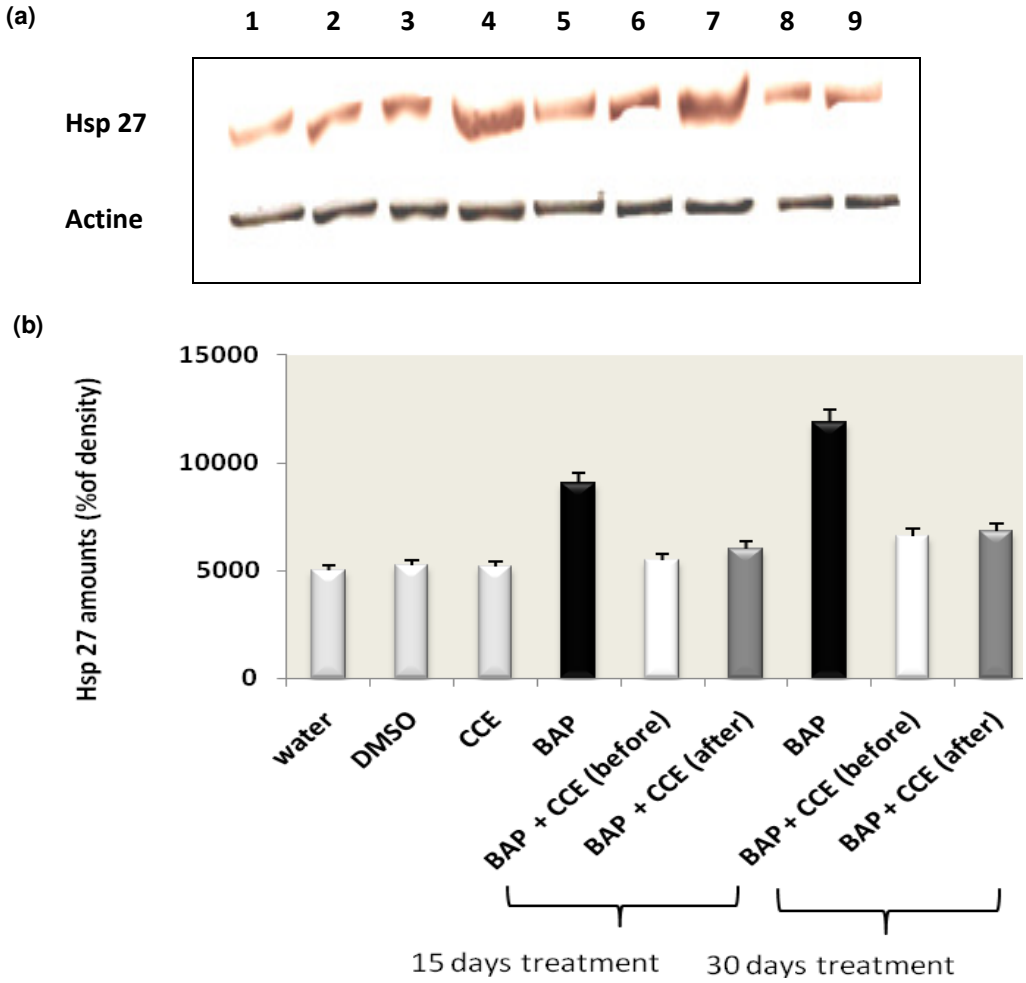

Figure 4. Immunoblot (a) and densitometric (b) analysis of Hsp 27 in liver of control and treated animals. The protein was separated on 12% SDS-PAGE and blotted with anti-Hsp 27 antibody. The intensity of the protein band was scanned by densitometry. Significantly different as compared to controls ($p < 0.005$).

2.67 ± 0.45). Co-administration of cactus before or after BAP treatment decreases significantly the total chromosomal aberrations (Table 1). However, the protection by cactus extract was not total it reaches 70% (Figure 5).

Eventual DNA fragmentation by agarose gel electrophoresis induced by BAP and its prevention by cactus

Results obtained after agarose gel electrophoresis are shown in Figure 6; No specific DNA fragments were observed for control groups (lines 1, 2, 3). However, in animals treated by BAP (50 µg/kg b.w.) for 15 days and 30 days (lines 4 and 7, respectively) induced a significant DNA fragmentation in animals liver cells. Simultaneous treatment of mice with CCE before or after BAP exposure for 15 days and 30 days showed a significant restoration of DNA (lines 5, 6, 8 and 9, respectively).

Apoptosis Status in the presence of BAP alone or added by CCE

Determination of p53 expression

Figures 7a and b show the western blotting and densitometry analysis of p53 expression in liver of controls and treated animals. After 15 days and 30 days exposure to BAP alone, p53 expression was found to be significantly decreased by 50% compared to controls but it increased by 34–64% before or after treatment by CCE. The CCE treated group did not have any significant effect on the expression of p53.

Table 1. Percentage of different type of chromosomal damage induced by BAP and reverted with cactus cladodes extract before or after treatment with BAP.

	Centric fusion	Ring	Break	Gap	Total
Control water	1.5 ± 0.67	1.00 ± 0.45	1.28 ± 0.33	0 ± 0.00	3 .78 ± 068
Control DMSO/H2O	2.33 ± 1.07	1± 1.24	1.22 ± 0.55	0 ± 0.00	4.55 ± 1.03
Control CCE	0.67 ± 0.33	1 ± 0.51	0 ± 0.00	1.00 ± 0.78	2.67 ± 0.45
BAP 15 days treatment	10.16 ± 0. 67*	6 .5 ± 1 .07*	10 ± 1.15*	2 ± 0.54*	28 .66 ± 0.43*
BAP+ CCE (before)	4 ± 0.56*	3.67 ± 1.12*	2 ± 1.34*	1 ± 0.45	10.67 ± 0.28*
BAP + CCE (after)	6.33 ± 0.78*	5 ± 1.00*	2 ± 1.67*	1 ± 0.17	14.33 ± 1.18*
BAP 30 days treatment	18.5 ± 1.87*	7 ± 0.87*	13.67 ± 1 .56*	5.5 ± 0.33*	44.67 ± 1.45*
BAP+ CCE (before)	5 ± 0.15*	4 ± 1.67*	3 .33 ± 1.34*	2 ± 1.96	14.33 ± 0.47*
BAP+ CCE (after)	6 ± 1.08*	5.5 ± 1.78*	3 ± 1.33*	2.67 ± 0.45*	17 .02 ± 1.07*

(*) Significant compared with group 1(water) (P < 0.05).

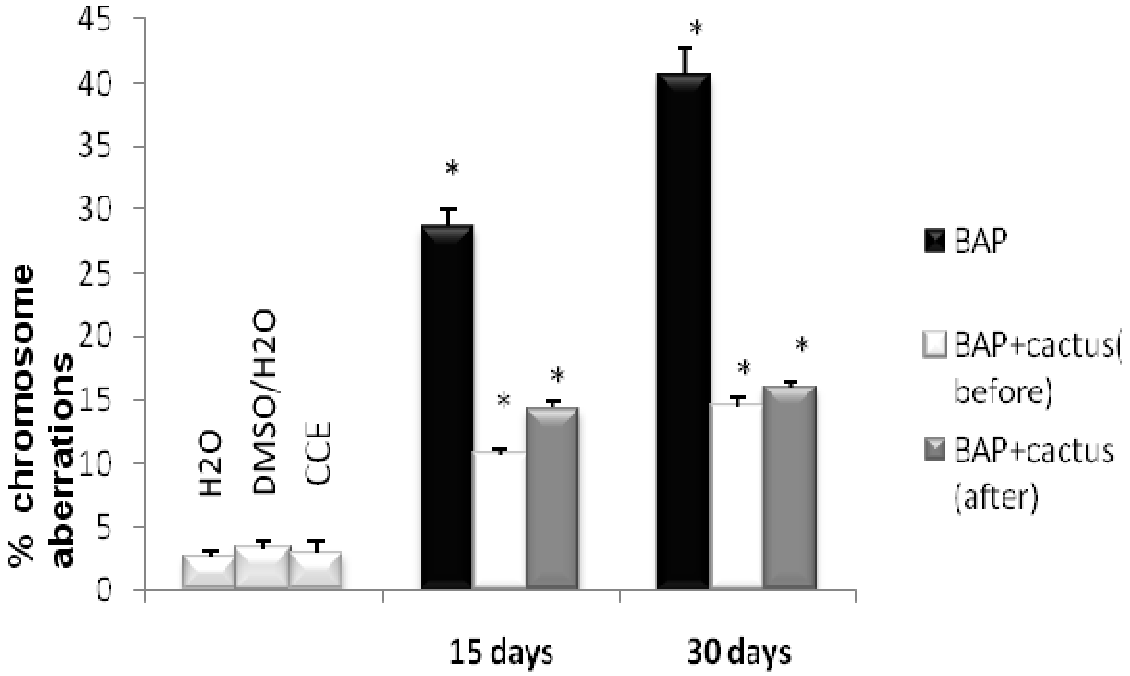

Figure 5. Effect of cactus cladodes on chromosomal aberrations in bone marrow cells of BAP treated Balb/c mice. Results are expressed as mean ± standard deviation (n = 3). (*) indicated significant difference (p < 0.05) from control.

Determination of bax expression

BAP induces the expression of bax genes in liver as evidenced for example of immunoblotting illustrated in Figure 8a, which was further, confirmed by results of scanning densitometry (Figure 8b). This induction is decreased significantly in liver by BAP treatment, but the administration of CCE before and after BAP exposure for 15 and 30 days treatment increases the amounts of bax (Figures 8a and b). The CCE treated group did not have any significant effect on the expression of bax.

Determination of bcl2 expression

Figures 9a and b showed the Western blotting and densitometry analysis of bcl2 expression in liver of controls and treated animals. After 15 days and 30 days exposure to BAP alone, pro-apoptotic protein bcl2 expression was found to be significantly increased

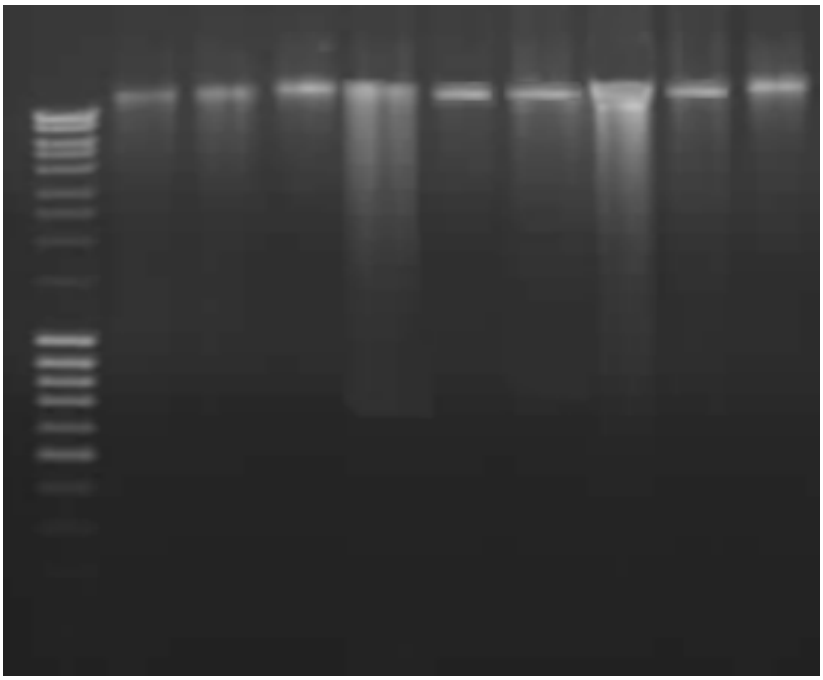

Figure 6. DNA fragmentation of mice liver extracts induced by BAP and prevention of CCE revealed by agarose gel electrophoresis. The results are representative of nine independent experiments: (1) H2O; (2) DMSO/H2O; (3) Cladode 50 mg/Kg; (4) BAP 50 µg/Kg (15 days treatment); (5) BAP 50 µg/Kg + cladodes 50 mg/Kg (before);(6) BAP 50 µg/Kg + cladodes 50 mg/Kg (after); (7) BAP 50 µg/Kg (30 days treatment); (8) BAP 50 µg/Kg + cladodes 50 mg/Kg (before); (9) BAP 50 µg/Kg + cladodes 50 mg/Kg (after).

compared to controls, but it decreased before or after treatment by CCE. The CCE treated group did not have any significant effect on the expression of bcl-2.

DISCUSSION

Chemoprevention has emerged as a very effective preventive measure against carcinogenesis. Many bioactive compounds present in edible as well in herbal plants have revealed their cancer chemopreventive potential. Our goal was to investigate the effect of CCE on BAP induced toxicity in mice balb/c. To this end, we evaluated the effect of CCE 50 mg/kg b.w. tested in Balb/c by monitoring its effects on oxidative stress, genotoxicity by BAP; we chose this dose because our studies have shown that it can do a good prevention against toxicity induced by mycotoxine zearalenone (Zourgui et al., 2009).

BAP is classified as probably carcinogenic to humans (a group 2A carcinogen) by the International Agency for Research on Cancer (IARC Monographs, 1983). Like other PAHs, it needs metabolic activation to exert its mutagenic and carcinogenic effects (Irena and Metka, 2009). To evaluate the oxidative stress, we monitored lipid peroxidation, in fact, the most common group of indices used to assess oxidative stress is that of peroxidation products of lipids considered as a late biomarker of oxidative stress and cellular damage, the MDA (Draper et al., 1993; Kim and Lee, 1997; Dotan et al., 2004). In the present study, exposure to BAP (50 µg/kg b.w) which can cause tumors in more 25% of the animals (Stephen et al., 1978) it induces a marked increase in MDA formation in liver but administration of CCE significantly reduced this induction which dropped to the control level (Figure 1). Cheu et al. (1997) and Kim et al. (2000) supported the earlier findings that BAP induced oxidative stress and increased lipid peroxidation, it has already been proposed that the higher toxicity could be due to an increase of the amount of carcinogenic BAP metabolites and reactive oxygen species (ROS), both generated by the metabolic activation of BAP by cytochrome P450 1A1 (CYP1A1) (Kim and Lee, 1997, Zourgui et al., 2008). Also, to assess BAP oxidative induced damages in balb/c mice, we dosed catalase activity. Our results clearly showed that BAP enhanced catalase activity and this activation was significantly reduced when animals were treated with CCE before or after BAP treatment (Figure 2). The preventive effect of CCE was total and catalase activity was reduced to its basal level and similarly Zourgui et al. (2008) reported that cactus was effective in the protection against

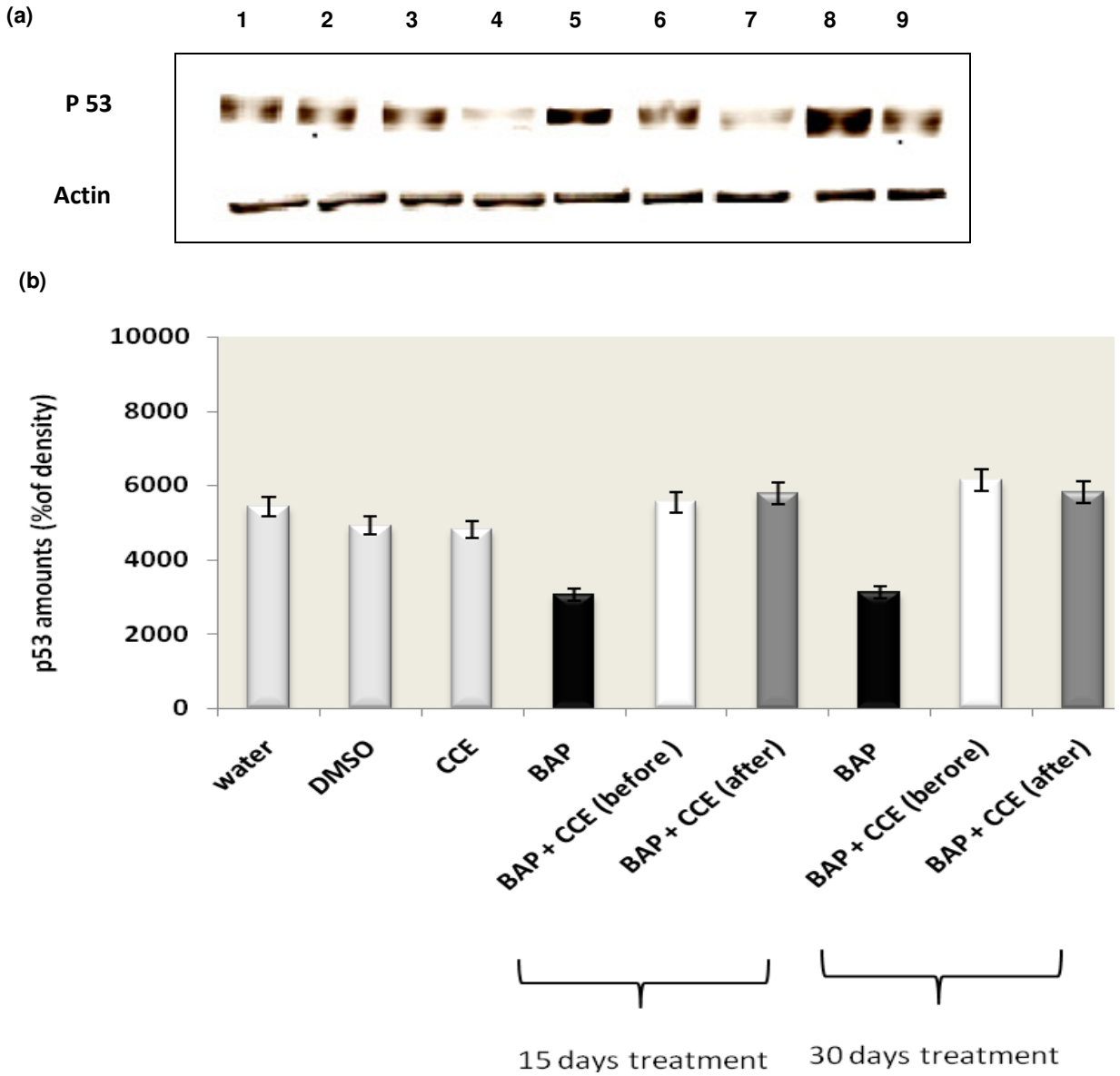

Figure 7. Immunoblot (a) and densitometric (b) analysis of p53 in liver of control and treated animals. The protein was separated on 12% SDS-PAGE and blotted with anti-p53 antibody. The intensity of the protein band was scanned by densitometry. Significantly different as compared to controls ($p < 0.005$).

oxidative stress induced by the mycotoxin Zearalenone and reduced significantlty catalase activity.

For further study of oxidative stress in BAP induced toxicity, we monitored early markers of oxidative stress including antioxidant enzymes as Hsp 70 and Hsp 27, may be altered in the presence of lower levels of oxidative stress and before the biomarkers of severe oxidative stress attributed to cytotoxicity appear. Hsp response is a conserved and physiological adaptive response to various stress conditions, including exposure to heat shock, inflammatory stimuli, infection, and oxidative stress. In fact, after oxidative insult, Hsp are induced and play a key role in cell protection and repair (Ritossa, 1962; Welch, 1993). Several published data has reported that many sources of oxidative stress can lead to the up-regulation of the Hsp 70 as well as small Hspsuch as Hsp 27 at levels where overt oxidative damage is not observed (Beyersmann and Hechtenberg, 1997; Fehrenbach and Northoff, 2001).

Our results clearly demonstrated that BAP alone induced a sharp elevation in the expression level of both Hsp70 and Hsp 27 in liver after 15 days and especially after 30 days treatment Interestingly, when animals were injected by CCE before or after administration of BAP, a

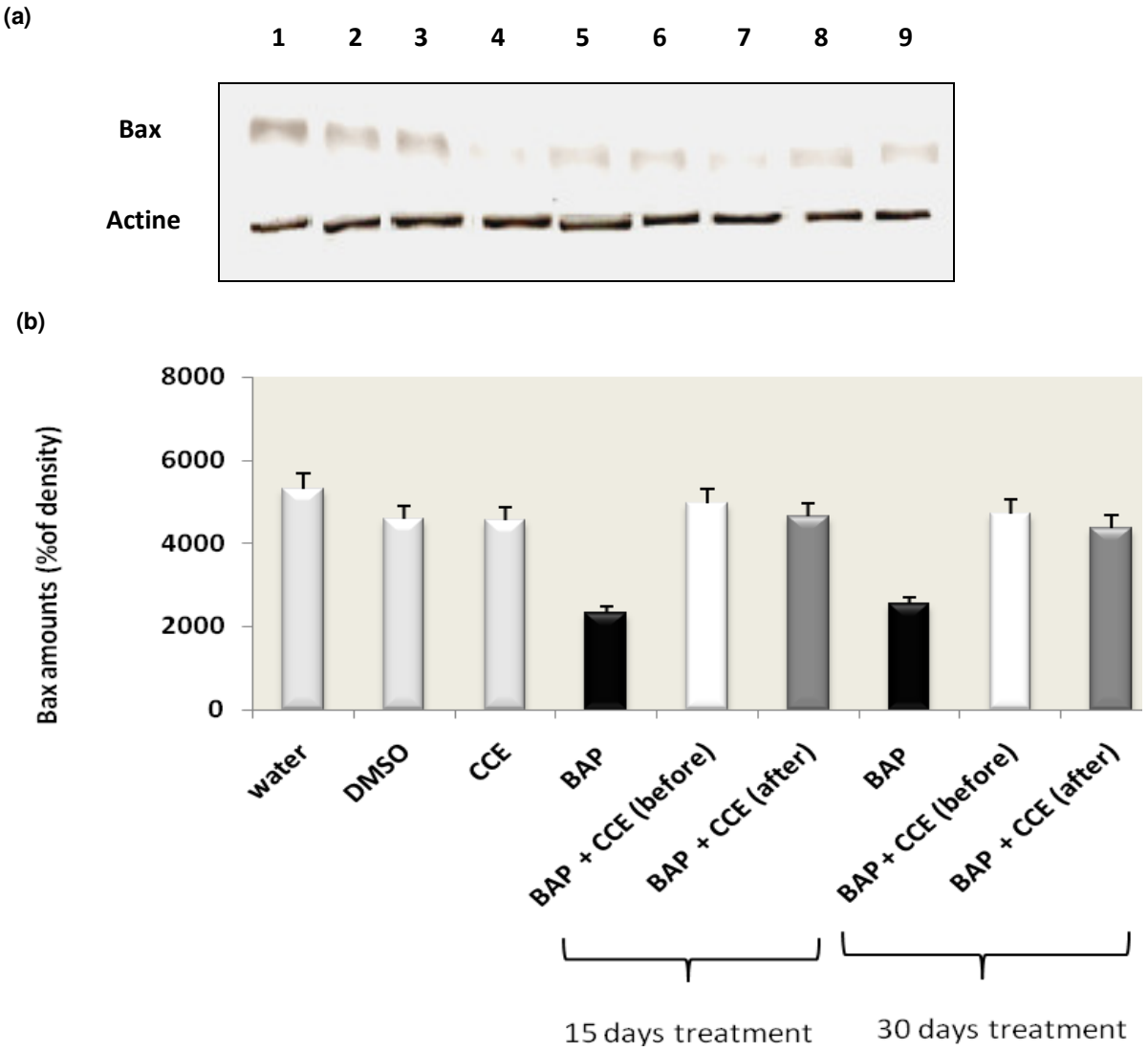

Figure 8. Immunoblot (a) and densitometric (b) analysis of bax in liver of control and treated animals. The protein was separated on 12% SDS-PAGE and blotted with anti-bax antibody. The intensity of the protein band was scanned by densitometry. Significantly different as compared to controls ($p < 0.005$).

sharp decrease of Hsp 70 and Hsp 27 levels were observed (Figures 3a, b and 4a, b). Cactus is able to prevent and protect against oxidative damage is certainly associated to the presence of several antioxidants as ascorbic acid, Vitamin E, carotenoids, reduced glutathione, flavonoids and phenolic acids actually detected in fruits and vegetables of different varieties of cactus (Kuti, 2004; Tesoriere et al., 2005; Panico et al., 2005; Shim et al., 2006). Oxidative stress is important as direct and indirect initiators as well as promoters of genotoxicity and apoptotic process, In order to assess the mechanism of genotoxicity we have performed the chromosome aberrations assay in bone marrow cells and DNA fragmentation in liver. Our result showed after 15 and 30 days treatment with a strong induction of BAP chromosome aberration strongly on 30 days with 40% and this induction was reduced to reach 10% and 15% in the presence of CCE respectively before or after BAP intoxication (Figure 5). To confirm the preventive effect of cactus against BAP genotoxicity, we tested it against DNA fragmentation induced by BAP. Indeed, we showed firstly that treatment with BAP (50 µg/kg b.w.) induced a significant DNA fragmentation in liver cells of treated animals. Simultaneous treatment of mice with BAP and CCE showed a significant restoration of DNA integrity (Figure 6). These results are in accordance with our recently published report involving preventive effect of cactus cladode extract against genotoxicity induced by single intraperitonial treatment by the mycotoxin zearalenone (Zourgui et al., 2008). It is possible that

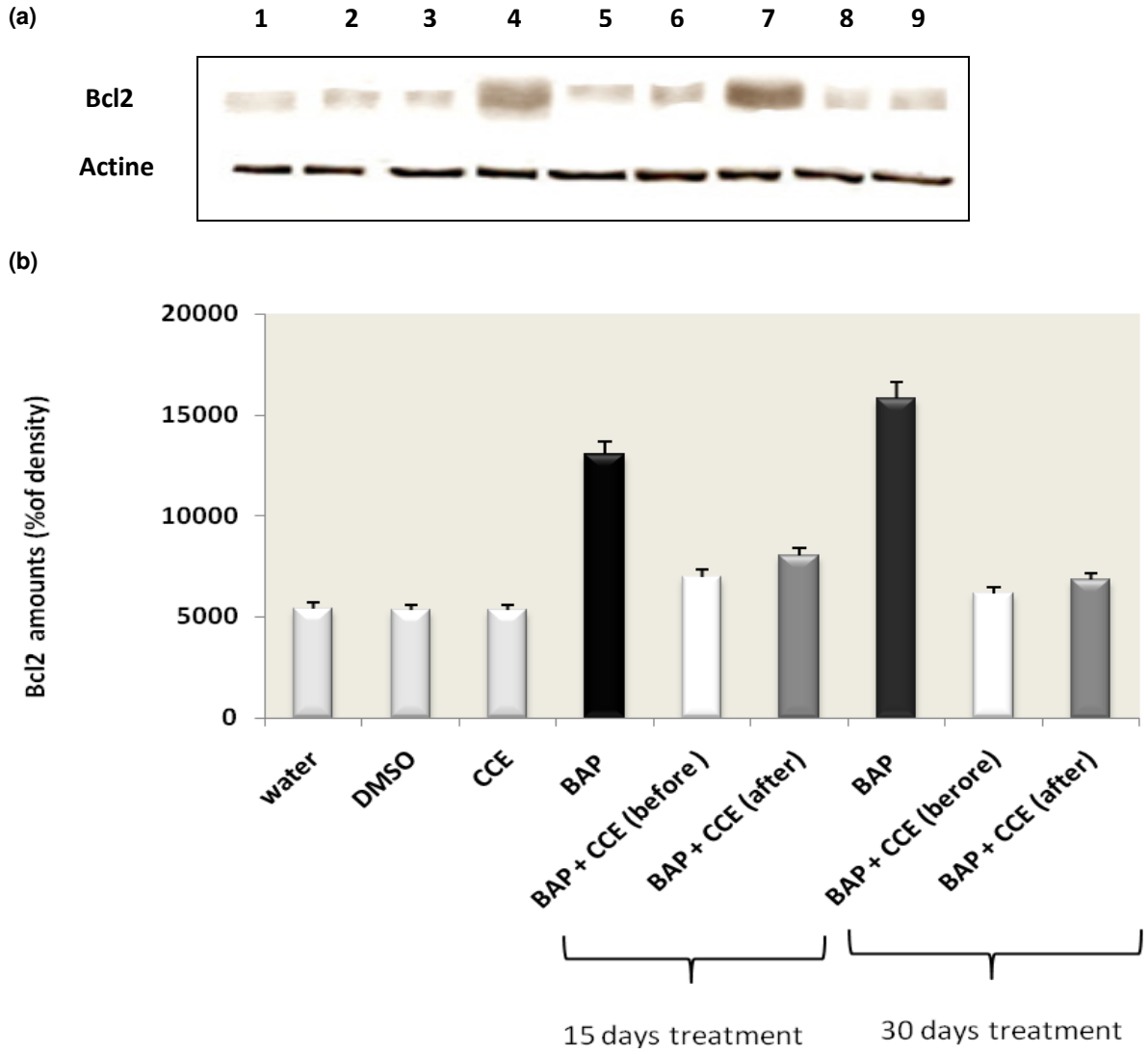

Figure 9. Immunoblot (a) and densitometric (b) analysis of bcl2 in liver of control and treated animals. The protein was separated on 12% SDS-PAGE and blotted with anti-bcl2 antibody. The intensity of the protein band was scanned by densitometry. Significantly different as compared to controls ($p < 0.005$).

the bioactivation of BAP goes through reactive intermediates, like epoxides, that may produce DNA and protein adducts. The formation of covalent DNA adducts is an important first step in the initiation of PAH induced carcinogenesis (Hogan et al., 1981; Stowers and Anderson, 1985). Altogether, our results clearly demonstrate the antigenotoxic potential of cactus cladodes extracts which efficiently protect mice from clastogenic effects and DNA damages of BAP. The protection afforded by CCE against BAP genotoxicity is likely due to its ability to inhibit oxidative process induced by the BAP which is suggested as a key determinant of BAP induced toxicity. In this study we provide evidence that CCE possess anti- carcinogenic properties against BAP classified as probably carcinogenic to humans a group 2A carcinogen by the International Agency for Research on IARC (1983). The modulatory effect of CCE on BAP toxicity was suggested to carry out through alterations in cell death pathway, p53 and the ratio of Bax/Bcl-2 plays an important role in determining whether cells will undergo apoptosis. Our study shows that treatment by BAP for 15 and 30 days induces a high expression of antiapoptotic proteins bcl-2 and down-regulation of bax and p53 (Figures 9a, b; 8a, b and 7a, b). Similar to our results Sugata and al. (2009) demonstrated that BAP interplay of p53 and bcl-2 genes has an important role in the initiation of lung carcinogenesis. Either an over expression of anti apoptotic proteins bcl-2 and the negative regulator of p53 is often found in human lung cancer (Jiang et al., 1995;

Higashiyama et al., 1997) and probably it is the same for liver cancer because BAP is reported to induce the expression of bax in a p53-dependent manner in lung cancer cell lines (Nakanishi et al., 2000). It is evident from the present study that apoptosis was found to occur in opposite directions during BAP induced toxicity on Balb/C mice. Whereas, higher Bax/Bcl2 ratio suggested an increase of apoptosis in animals treated with CCE along with BAP treatment, this extract has been shown to induce apoptosis via inhibition of bcl-2 expression and induction of p53 and bax expression (Figures 9a, b, 8a, b and 7a, b). This indicates that CCE modulate the p53 dependent apoptotic pathway to restrict the BAP toxicity.

Conclusion

Our study clearly demonstrates interesting properties of CCE such as its potential and ability to induce apoptosis. Because many bioactive compounds present in edible as well in herbal plants have revealed their cancer chemopreventive potential. The cactus extract can be an excellent candidate for the inclusion in humans and animal food diet and it can be an anti-cancer drugs and thus may reduced the development of secondary tumors.

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Conflict of interest

None of the authors has any potential conflicts of interest.

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