

Preventive and curative effects of *Atriplex halimus* L. aqueous extract on benzene provoked hepatic injury in rats

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The present study was designed to estimate the preventive and curative potency of aqueous extract of *Atriplex halimus* L. (Ah) aerial parts against benzene (BZ) intoxication in liver rats.

30 male albino rats were divided into five groups of 6 rats each: Control, Ah, BZ, AhP+BZ, and BZ+AhC. BZ (100 mg/kg b.w) was added in drinking water for 15 weeks. Aqueous extract of *Atriplex halimus* was received intragastrically during the last 30 days of BZ exposure for curative treatment (AhC) and all the duration of BZ exposure for preventive treatment (AhP). At the end the experiment, body weight gain and relative liver weight were estimated and liver enzyme markers (AST, ALT, ALP and LDH) were analysed by spectrophotometry. Histopathological studies on hepatic tissue were also performed by the method of Hematoxylin and Eosin staining.

Benzene administrated to rats caused an alteration in physiological parameters (body and organs weigh) and hepatic enzymes markers (AST, ALT, ALP and LDH). In addition, Histopathological studies showed a massive degeneration in liver tissue in BZ-exposed rats. However, treatment by *Atriplex halimus* especially preventive effect ameliorated most of the adverse effects induced by benzene. Ah restored the altered of physiological, biochemical and histopathological changes.

In conclusion, the present study suggests that *Atriplex halimus* extract could be a substantially promising hepatoprotective agent against benzene toxic effects and may be against other hepatotoxic chemical or drugs.

Keywords: *Atriplex halimus*, Benzene, hepatotoxicity, Rats.

INTRODUCTION

Liver is the most important organ responsible for xenobiotic metabolisms, acting as sensitive target site for substance modulating biotransformation. Many xenobiotics (drugs and

environmental chemicals) are capable of causing some degree of liver injury (Abdou *et al.*, 2015).

Benzene (BZ) is a ubiquitous environmental pollutant; it has been classified as a group 1A carcinogen as well as its toxic effects on the hematopoietic system are well known (Falzone *et al.*, 2016). Benzene can also cause a great number of acute and chronic diseases, which can affect the functions of respiratory, nervous, immune, hepatic, renal, cardiovascular and reproductive systems (D'Andrea and Reddy, 2018). Liver function as the key organ of metabolism and excretion of benzene thus, constantly endowed with the task of detoxification, inducing various disorders to the organ. Majority of benzene metabolism occurs in the liver by cytochrome p4502E1 and has genotoxic properties and play a role in benzene toxicity (Uzma *et al.*, 2008). The general population is exposed to benzene in the atmosphere by inhalation of vapours released by motor vehicles, gasoline service stations, cigarette smoke or by ingestion of polluted foods or water (Wilbur *et al.*, 2007; Falzone *et al.* 2016).

Herbs have been an important source of natural materials used for treatment and/or prevention of several illnesses (Abdou *et al.*, 2015). *A. halimus* is known in folk medicine (Kabbash and Shoeib, 2012). This plant has been used as traditional cures for thousands of years (Walker *et al.*, 2014). In the Arab world, *A. halimus* was used to treat heart disease, diabetes, rheumatism (Parvez *et al.*, 2018), anaemia (Mohammedi *et al.*, 2016), and to cure stomach pains and for intestinal worms (Slama *et al.*, 2018). *A. halimus* is also used to treat urinary tract inflammation (cystitis) and urinary lithiasis; it accompanies any diet that requires tissue drainage and desincrustation of wastes and toxins (Nedjimi *et al.*, 2013). In Algeria, *A. halimus* is mainly used by the local population as a remedy to treat cancer (Alves-Silva *et al.*, 2017), diabetes mellitus and anaemia (Mohammedi *et al.*, 2016).

Given these considerations, this study was carried out to examine the possible hepatoprotective effect of *A. halimus* against benzene induced liver toxicity in rats.

MATERIALS AND METHODS

1. Plant material

1.1. Collection and identification of *Atriplex halimus* L

Atriplex halimus L. aerial parts (stems, leaves, seeds) were collected in September 2017 from Sidi Khaled village in Biskra state, Algeria; and were identified by the botanist (Pr. Youssef Halis) at the herbarium in the Center for Scientific and Technical Research on Arid Regions (CRSTRA) of Biskra, Algeria. The plant material was washed using water, dried at room temperature, and grounded into powder and then stored at room temperature until use.

1.2. Preparation of aqueous extract

About 10 g of the aerial parts powder of Ah was soaked in 100 ml of distilled water and kept at room temperature in the dark for 24 h. Then, it was filtered through filter paper. After extraction, the solvent was removed using a rotary evaporator and incubated at 40°C to dry completely. The extract was weighed and stored in a refrigerator at 4°C for future analysis (Murugan and Parimelazhagan, 2014).

2. Animals

2.1. Animals and Maintenance

A total of 48 adult male albino rats, weighing 224.98 ± 3.81 g, were obtained from the animal house of Pasteur Institute, Algeria. They were placed and kept in animal house of Molecular and Cellular Biology Department, University of El-Oued, Algeria. Animals were adapted for 2 weeks under the same laboratory conditions of photoperiod (12 h light/12 h dark) with relative humidity $64 \pm 2\%$ and room temperature of $19 \pm 1^\circ\text{C}$. Standard rat food (Table 1) and tap water were available ad libitum for the duration of the experiments.

Table 1: Standard diet composition of rats (Southon *et al.*, 1984).

Primary materials	Quantity (g/kg)	Percentage (%)
Starch (Maize)	326	32.6
Sucrose	326	32.6
Protein (soy)	168	16.8
Cellulose (bran)	40	4
Minerals	40	4
Vitamin	20	2
Oil	80	8

2.2. Experimental design

The adult Wistar albino rats were randomly divided into eight groups, each containing 6 rats as follow: Groups 1: was serving as a control and received normal water; Groups 2: was treated orally by gavage 200 mg/kg, b.w (3 days/week) of aqueous extract of *Atriplex halimus* L (Ah) for 15 weeks; Groups 3: received in drinking water 100 mg/kg, b.w/day of benzene (BZ) daily for 15 weeks; Group 4: was concomitantly administered preventively Ah 200 mg/kg, 3 days/week) with BZ for 15 weeks (AhP+BZ); Group 5: was firstly treated by BZ only for 11 weeks then treated curatively by 300 mg/kg, b.w/day of Ah (BZ+AhC) for 30 days. Body weight was recorded periodically during the experiment weeks.

2.3. Samples preparation

At the end of treatment, rats were fasted for 16 h, anaesthetized with chloroform by inhalation, and then decapitated. The blood samples were transferred into serum separating tubes and prepared by centrifugation for 10 min at 3000 revolutions/min and it was utilized for biochemical parameters analysis.

We made a longitudinal abdominal opening in animals and excise the liver. After removing all connective tissue from the organs, the liver were weighed, and fixed in formaldehyde (10%) for histopathological examination.

2.4. Biochemical parameters analysis

ALT, AST, ALP, and LDH activities were measured by kinetic colorimetric method using technical cards and commercial kits obtained from Spinreact and BioSystems (Barcelona, Spain).

2.5. Histopathological study

After rats sacrificed, the parts of studied tissues were removed and immersed in fixative (10% formaldehyde) until the time of slices preparation. The samples were dehydrated in ascending graded series of ethanol, cleaned with toluene, and immersed in paraffin. Sections of 4~6 mm were prepared from paraffin blocks using a Histoline Rotary Microtome, and coloured with hematoxylin and eosin. Histopathological evaluation was performed with a light microscope.

3. Statistical analysis

Data was expressed as mean $\pm$ standard deviation ($M \pm SD$) of six animals. Statistical analysis was carried out by using Student T-test to compare means between two groups. Results were evaluated using the Minitab and EXCEL software. Differences were considered significant at $p \leq 0.05$.

RESULTS

Body weight gain and relative liver weight

BZ exposure to rats caused a decrease in body weight gain ($p < 0.001$) and in relative liver weight ($p < 0.001$) compared to the control rats. Preventive Ah treatment of BZ exposure rats (AhP+BZ) was restored the body weight gain ($p < 0.01$), relative liver weight ($p < 0.01$) as compared to BZ rats. Curative treatment of the aqueous extract of Ah was significantly ($p < 0.05$) increase body weight gain and relative liver weight compared to BZ rats (Table 2).

Table 2: Initial body weight, body weight gain, and relative liver weight in control and experimental groups.

Parameters	Initial body weight (g)	Body weight gain (g/d)	Relative liver weight (g/100 g bw)
Control	221.17±7.73	0.74±0.10	2.340±0.088
Ah	233±4.71	0.719±0.064	2.319± 0.052
BZ	234.2±12.1	0.38±0.031 ***	2.097±0.033 ***
AhP+BZ	219.25±7.16	0.76±0.072 ^b	2.216±0.033 ^{**b}
BZ+AhC	229.25±6.34	0.66±0.037 ^b	2.291±0.085 ^a

Biochemical parameters

Results presented in Table 3 showed a significant decrease in liver ALT ($P<0.001$) and serum ALP ($P<0.05$) activities, and non-significant change in liver AST and serum LDH activities in benzene-intoxicated group when compared with the control group. However, treatment with aqueous extract of *A. halimus* extract along with benzene provided protection to these enzymes activities. *Atriplex* curative treatment attenuated the decrease of ALT ($P<0.001$) and the increase of LDH activities but did not affect the serum ALP level when compared with benzene group. Treatment with Ah alone decreased serum LDH activity only.

Table 3: Liver and serum enzymes activities in the control and experimental groups.

Parameters	Liver ALT (U/l)	Liver AST (U/l)	Serum ALP (U/l)	Serum LDH (U/l)
Control	219.9±11.5	434.1±46.1	343.5±16.5	3166±149
Ah	206.5 ±8.54	422.8 ±45.9	319.6 ±19.7	2227±135 **
BZ	144.68±9.06 ***	472.8±32.4	234.0±23.6 *	3324±216
AhP+BZ	188.8±12.2 ^{*b}	404.3 ±33.1	371.5±29.1 ^c	2831±187 ^a
BZ+AhC	201.1± 16.1 ^b	550.20 ±8.83 ^{***c}	222.6±29.7 *	3098±118 ^a

Histopathological study

The light micrographs of liver tissues demonstrated normal architecture of hepatic cells with normal central and portal area in the control and Ah groups (Figure 1, 2), while BZ-intoxicated rats revealed severe pathological changes manifested by degeneration and dilatation of the central vein, hepatic cell necrosis, congestion and dilatation of hepatic sinusoids, and accumulation of inflammatory cells around central vein. Moreover, portal areas showed fibrous expansion of most portal areas, severe infiltration of portal tract areas with inflammatory cells, congestion and dilatation of the portal vein and oedema (Figure 3). However, livers of BZ rats treated with *A. halimus* extract markedly reduced and attenuated the histological alterations

(Figure 4). Moderate congestion and dilatation of the central and portal vein and mild inflammation was showed in these groups (Figure 5).

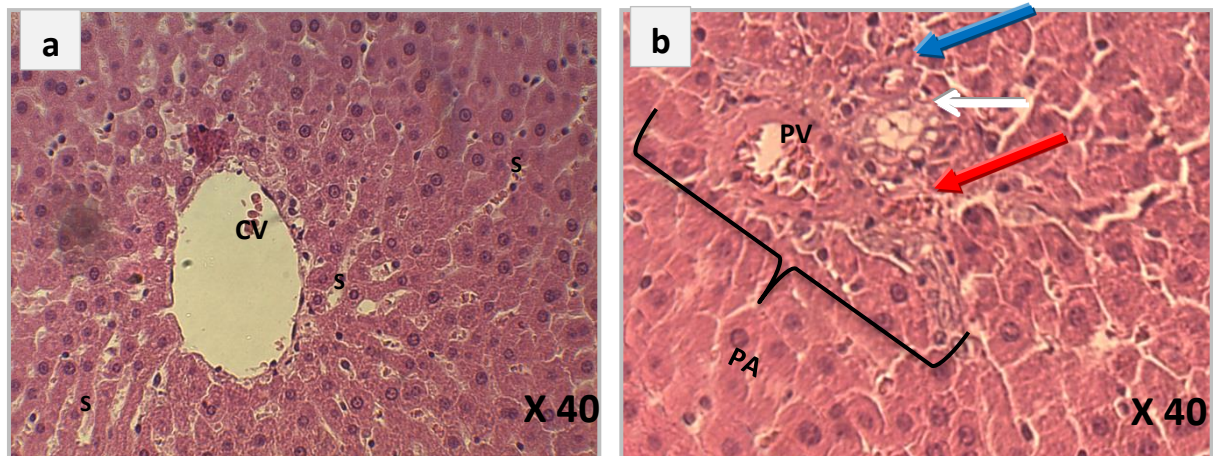


Figure 1: Liver section from control group showing normal structural morphology in (a) the central area, sinusoids (S) and central vein (CV) and (b) normal portal area including portal vein (PV), bile duct (blue arrow), artery (white arrow) and portal tract (red arrow).

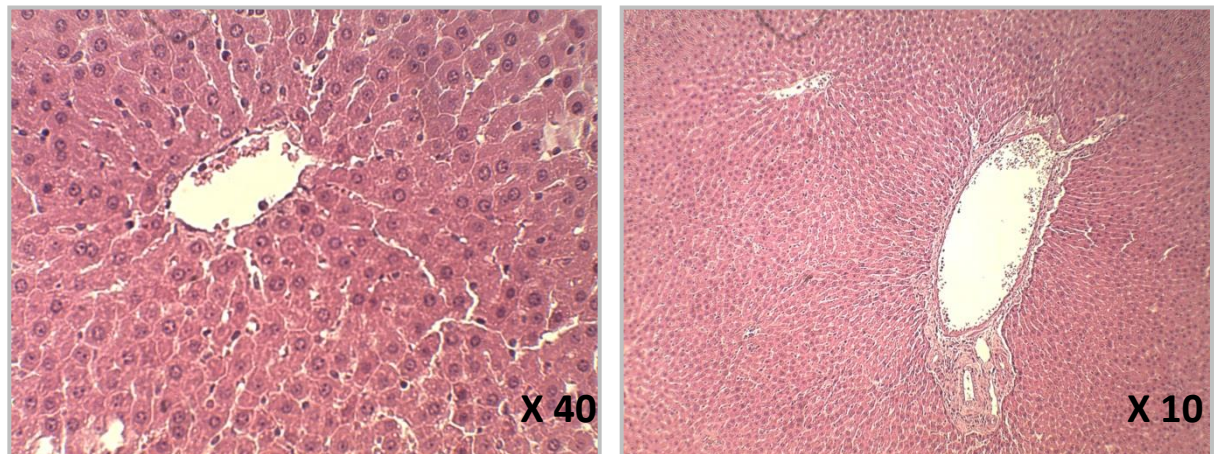
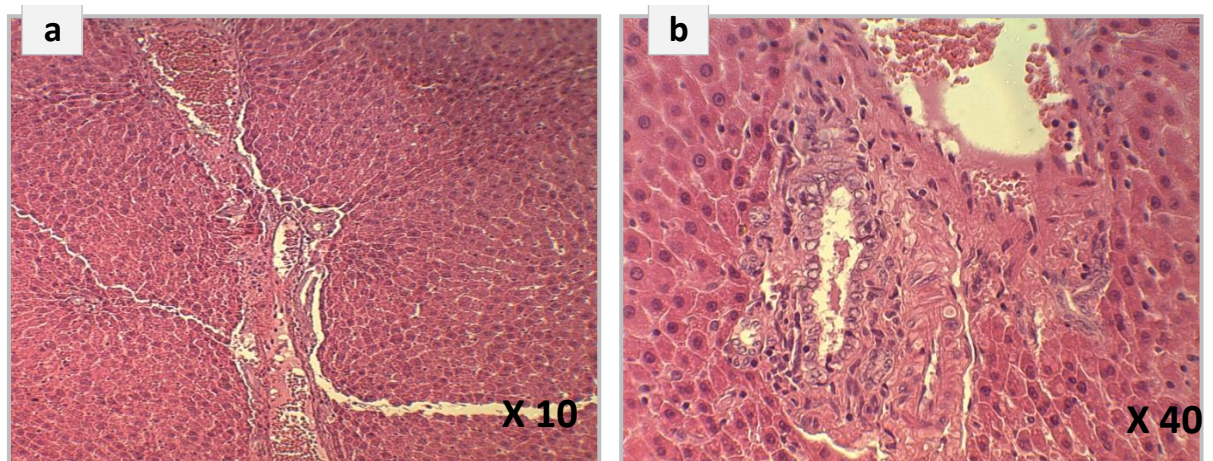


Figure 2: Liver section from Ah group showing normal architecture.



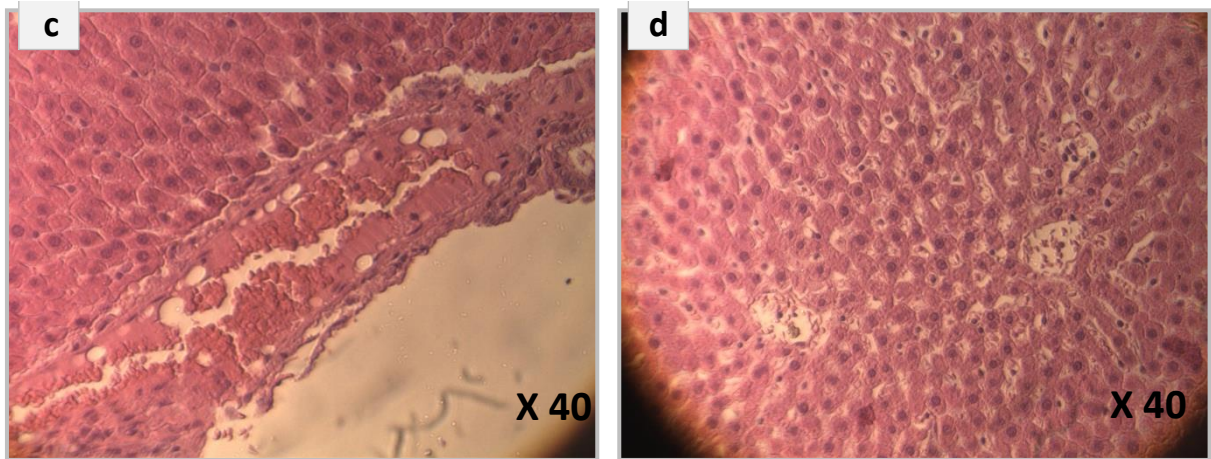


Figure 3: Liver section from BZ group showing: **(a)** fibrosis, congestion, **(b)** fibrosis and lymphocytic inflammation in portal area, **(c)** lymphocytic inflammation, congestion and edema in central vein **(d)** sinusoidal congestion and dilatation, cytoplasmic vacuolation.

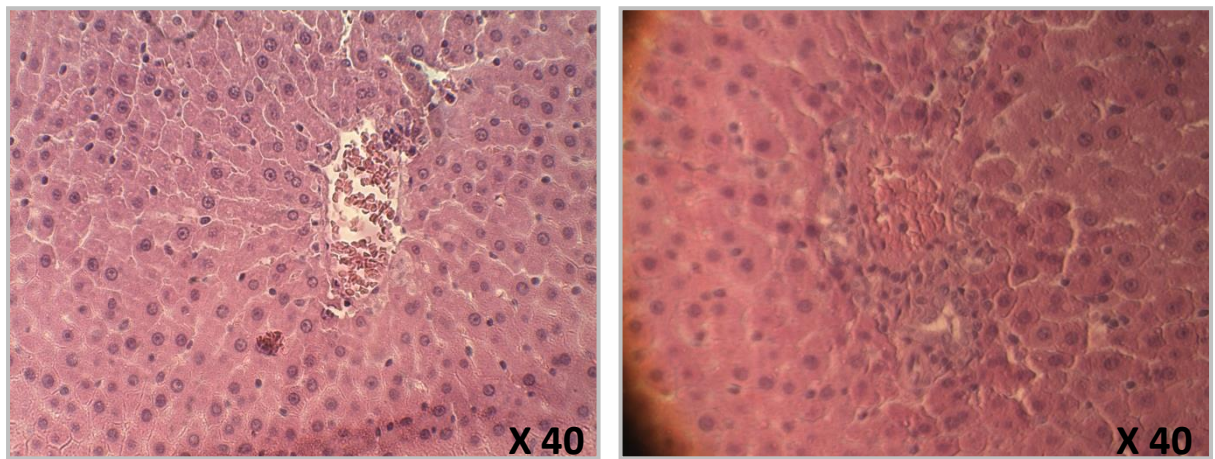


Figure 4: Liver section from AhP+BZ group showing mild degeneration in central and portal areas.

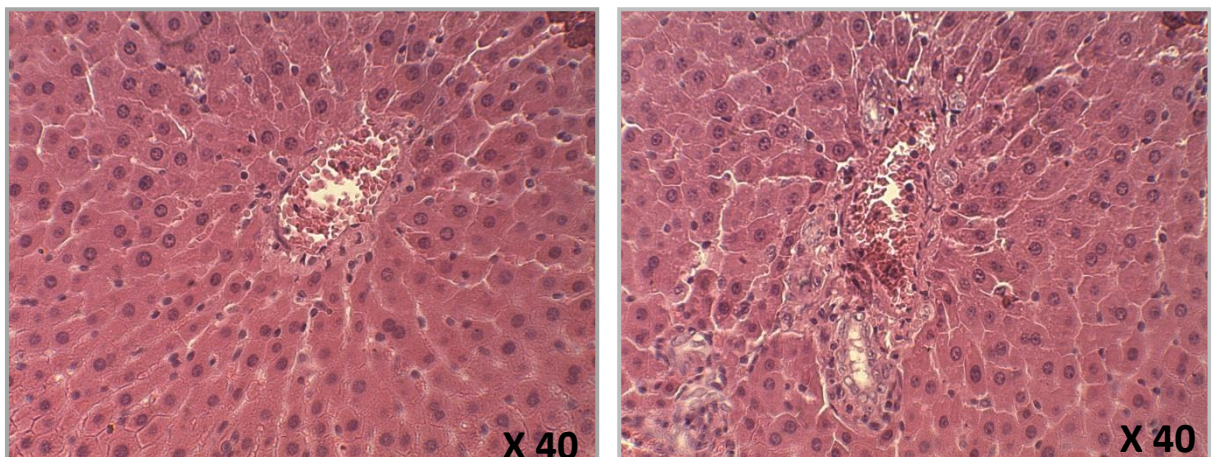


Figure 5: Liver section from BZ+AhC group showing: a moderate degree of liver damage.

DISCUSSION

In order to search new bioactive molecules contained in plants, characterized by effective treatment of different diseases and fewer side effects, it is necessary to carry out pharmacological studies on plants used by traditional healers. In this study, we evaluate the possible pharmacological effects of *A. halimus* against hepatotoxicity induced by benzene.

Decreased body weight is an important indicator for assessing the deterioration of health status (Ullman-Culleré and Foltz, 1999). In our study, BZ exposure at a dose (100 mg/kg) resulted in a significant decrease in body weight and relative liver weight. In agreement with Ibrahim *et al.* (2011) who reported a significant decline in the body weight gain of the animals intoxicated with BZ, suggesting that reduced of the body weights may be due to the overall increased degradation of lipids and proteins as a result of the direct effects of BZ.

In contrast, treatment by *Atriplex* especially preventively with BZ restored the weight of the body, liver near to normal, which can indicate the beneficial effect of Ah against BZ as evidenced macroscopically.

Liver function was assessed by examining the activities of AST, ALT, ALP, and LDH in rats. The results demonstrated that the activities of liver ALT and serum ALP were reduced after benzene intoxication. Our data were consistent with Adeyemi *et al.* (2009) who reported a reduction in liver ALT, AST activities and elevation on serum LDH activity of rats exposed to benzene. Also, the study of Hegazy and Kamel, (2014) showed that occupational exposure to benzene affected four important hepatic marker enzymes activities (LDH, ALP, ALT and AST) in serum.

As a reason for our observation, liver enzymes ALT, AST, and ALP are known markers enzymes for the assessment of the functional integrity of the liver cells. The decrease of liver ALT activities after BZ exposure may be due to the leakage of this enzyme from the liver cytosol into the blood stream (Çavuşoğlu *et al.*, 2015). Liver cell membranes were damaged by benzene via the generation of ROS, leading to lipid peroxidation of biomembrane causing leakage of cellular components (El-Shakour *et al.*, 2015). Alkaline phosphatase is a marker enzyme for plasma membrane; two abnormal levels of alkaline phosphatase have been proposed: increase or decrease in normal levels. Damage to biological membrane may lead to any of the two abnormalities (Adeyemi *et al.*, 2009). Lactate dehydrogenase is an important enzyme in biological processes; it is considered as specific indicators for hepatic dysfunction and damage (Dere and Ari, 2009).

On the other hand, Our results show that the treatment of rats by *A. halimus* significantly restored the liver enzymatic activity, thus indicating that Atriplex inhibits liver damage caused by BZ.

Histopathological observation of liver tissue sections from the BZ group confirmed the biochemical results and showed clear signs of hepatotoxicity. Stained sections reveal severe degeneration in liver tissue, large damage of central and portal liver areas, fibrosis, inflammatory cell infiltration, vascular congestion, vacuolization, and dilatation. Overproduction of ROS in chronic BZ and SB exposure can cause damage of important biomolecules (proteins, lipids, and DNA) and increase intra-tissular pro-inflammatory and fibrogenic factors, which can lead to cell death (necrotic) and chronic inflammation associated with renal and hepatic tissues damage (Cottone *et al.*, 2008). However, Atriplex improved the hepatic and renal histological changes induced by BZ. The histoprotective effect of Ah is due to the presence of antioxidant and anti-inflammatory components in this plant.

CONCLUSION

In light of the results obtained, the present study affirmed a beneficial ability of *A. halimus* aqueous extract to prevent and reduce BZ -induced liver damage. It also reveals that *A. halimus* is a robust medicinal herb for developing as a phytomedicine against hepatic disorders. Hence, dietary supplementation with Ah could be a useful method to protect populations at high risk of BZ and SB chronic intoxication.

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