

## PROTECTIVE ROLE OF AQUEOUS EXTRACT OF *ATRIPLEX HALIMUS* L. AGAINST BENZENE-INDUCED DAMAGE ON RENAL FUNCTION AND GLOMERULAR CELLS IN RATS

KHAOULA ZEGHIB<sup>1,2\*</sup>, DJAHRA ALI BOUTLELIS<sup>3</sup>

<sup>1</sup>Department of Chemistry, Faculty of exact sciences, University of El-Oued, El-Oued 39000, El-Oued, Algeria. <sup>2</sup>University of El Oued, VTRS Laboratory, Fac. Technology, 39000 El Oued, Algeria. <sup>3</sup>Department of Biology, Faculty of Natural Sciences and Life, University of El-Oued, El-Oued 39000, Algeria. Email: zeghib-khaoula@univ-eloued.dz

Received: 13 November 2018, Revised and Accepted: 01 January 2019

### ABSTRACT

**Objective:** Benzene (BZ) is an important pollutant compound, present in both occupational and general environment that has been linked to adverse health effect. The aim of the current study was to investigate the curative and preventive effect of *Atriplex halimus* L. (Ah) extract against BZ-induced nephrotoxicity in rats.

**Methods:** A total of 30 male albino Wistar rats were divided into five groups (n=6): Control, Ah, BZ, AhP+BZ, and BZ+AhC. BZ (100 mg/kg b.w) was added in their drinking water for 15 weeks. Ah (Aqueous Extract of aerial parts) was given by gavage during the past 30 days of BZ-exposed for curative treatment (AhC) and all the duration of BZ exposure in the animals for preventive treatment (AhP). Some biochemical, oxidative stress parameters, and histopathology of kidney tissue were studied.

**Results:** Obtained results revealed that BZ exposure to rats caused a significant elevation in urea, creatinine, and malondialdehyde levels. Then, it led to reducing the glutathione level, catalase, and glutathione-S-transferase activities when compared to the control animals. Histopathological studies showed a massive damage in kidneys of the BZ-exposed rat. Our results showed that curative treatment of aqueous extract of Ah has a partial correction of the biochemical, oxidative stress parameters, and kidney morphology, but preventive treatment has a dramatic effect on the previous parameters.

**Conclusion:** Results demonstrated the beneficial effects of Ah preventive treatment against stress oxidative and kidney damage induced by BZ.

**Keywords:** *Atriplex halimus*, Benzene, Oxidative stress, Nephrotoxicity.

© 2019 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2019.v12i3.30741>

### INTRODUCTION

Benzene (BZ) with C<sub>6</sub>H<sub>6</sub> formula is the primary aromatic compound [1]. It has been extensively used since 100 years and is still widely used as a solvent and intermediate compound [2] and produced in large quantities in the chemical and fuel industry including gasoline, automobile exhaust, several polymers, resins, synthetic fibers, and cigarette smoke [3]. It is also used in manufacturing products such as rubber, detergents, lubricants, pesticides, and drugs [4]. Workers employed in industries that manufacture or use BZ is exposed to its highest levels [5]. BZ is a ubiquitous environmental pollutant [6] which affects human health includes hematological diseases, reproductive system, respiratory problems, kidney disease, liver injury, and central nervous system [7]. Thus, humans are unavoidably exposed to BZ both occupational and environmental settings and which poses a serious risk to public health [8].

Toxicity of BZ most likely results from oxidative metabolism of BZ to reactive products [9]. These reactive products can lead to the formation of reactive oxygen species (ROS), which can directly damage pivotal molecular targets, such as DNA, proteins, and lipids, leading to the formation of new compounds and modified structures [10], and in this condition; oxidative stress has a critical role in the pathophysiology of several kidney diseases [11,12].

The association between food and health is well established. Recent overwhelming attention to plant products and traditional medicine has focused on natural antioxidant for preventing the tissues from various xenobiotics induced toxicities [13,14]. *Atriplex halimus* L. (Ah) is a famous halophytic shrub for the nutritive and energetic value, not only for livestock but also as food for nomads and the local steppe population.

The young shoots of Guettaf are eaten by the man, preparing its like spinach [15]. Ah also has always been a popular folk remedy for treating hyperglycemia, stomach painkiller, and intestinal worms [16]. In light, these considerations, the aim of the present study is to determine the effects of Ah against BZ-induced nephrotoxicity in rats.

### METHODS

#### Chemicals and reagents

All chemicals used in this study were purchased from Sigma-Aldrich, Mo, USA.

#### Plant material

Aerial parts of Ah were collected in September 2017 from a village in Sidi Khaled of Biskra state, Algeria and were identified by a botanist (Helios Youssef) at the herbarium in the Department of Biology, the University of El-Oued, Algeria. The plant material was washed using water, dried at room temperature, and grounded into powder and then stored at room temperature until use.

#### 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. It was filtered through Whatman 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 [17].

#### Estimation of total phenolic

Total phenolic content was estimated by the Folin-Ciocalteu method. This method initially described by Singleton and Rossi (1965). The

sample of the aqueous extract of Ah (0.2 ml) and 1 ml of 1:10 diluted Folin-Ciocalteu reagent were added after 4 min to 800  $\mu$ l of saturated sodium carbonate (75 g/l). After 2 h of incubation at room temperature, the absorbance was measured at 765 nm. The tests were carried out 3 times to ensure the reproducibility of the results [18]. The total phenolic content was expressed in mg equivalent of gallic acid per gram of sample.

#### Estimation of condensed tannin

The tannins content in the extract was determined spectrophotometrically according to Broadhurst and Jones [19]. Catechin was used to make the calibration curve. 0.5 ml of sample pipetted into a tube in aluminum foil, and mixed with 3.0 ml of vanillin reagent (Freshly prepared 4% w/v of vanillin in methanol), and then added 1.5 ml of concentrated hydrochloric acid and mixed thoroughly. After 15 min of reaction at 20–2°C, the absorbance was measured against water at 500 nm.

#### In vivo study

##### Animals and treatment

A total of 30 adult male albino rats, weighing 227.14 $\pm$ 3.42 g, were taken from the animal house of Pasteur Institute, Algeria. They were placed in five groups of six rats in each and kept in animal's house of Molecular and Cellular Biology Department, University of El-Oued, Algeria. Standard rat food and tap water were available *ad libitum* for the duration of the experiments. Animals were adapted for 2 weeks under the same laboratory conditions of photoperiod (12 h light/12 h dark) with relative humidity 62% and room temperature of 24 $\pm$ 1°C. The experimental procedures were carried out according to the National Institute of Health Guidelines for Animal Care and approved by the Ethics Committee of our Institution. The adult Wistar albino rats were randomly divided into five groups, each containing 6 rats as follow:

- Group 1 was orally received normal water and serving as a control.
- Group 2 was received oral gavage 200 mg/kg b.w (3 days/week) aqueous extracts of Ah for 15 weeks.
- Group 3 treated by 100 mg/kg, bw/day of BZ (Fig. 1) for 15 weeks.
- Group 4 was first treated by BZ only for 11 weeks then treated curatively by 300 mg/kg, bw/day of Ah (BZ+AhC) for 30 days.
- Group 5 was concomitantly administered preventively Ah (200 mg/kg, 3 days/week) with BZ for 15 weeks (AhP+BZ).

During treatment, body weight was recorded periodically during the experiment weeks.

##### Blood collection and preparation of tissue samples

At the end of treatment, rats were fasted for 16 h, anesthetized with chloroform by inhalation, rats were decapitated, and blood samples were transferred into ice-cold centrifuge tubes. The serum was prepared by centrifugation for 10 min at 3000 revolutions/min and utilized for urea, creatinine, albumin, uric acid, and total proteins concentrations, and lactate dehydrogenase (LDH) activity assays. Blood glucose was measured by glucometer. The kidney was rapidly excised, washed, weighed, and stored at –20°C for oxidative stress parameters analysis. Another part of the kidney was excised for histopathological examination.

##### Measurement of biochemical parameters

Serum urea, creatinine, albumin, uric acid, and total proteins concentrations, and LDH activity were measured using commercial kits obtained from Spinreact (Barcelona, Spain).

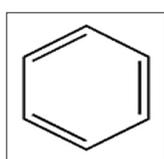


Fig. 1: Chemical structure of benzene [20]

#### Antioxidants measurement

##### Preparation of homogenates

About 1 g of the kidney was homogenized in 9 ml of buffer solution of Tris buffer saline (TBS, pH=7.4). Homogenates were centrifuged at 9000 $\times$  g for 30 min at 4°C, and the obtained supernatant was used for the determination of oxidative stress markers.

##### Determination of malondialdehyde (MDA) level

MDA was measured according to the method described by Quintanilha *et al.* [21]. The MDA reagent (trichloroacetic acid, thiobarbituric acid, and hydrochloric acid) was added to aliquots of the homogenate mixed with 2% (w/v) ethanolic solution of butylated hydroxytoluene. Then, the mixture was heated for 15 min in a boiling water bath. After cooling, the precipitate was removed by centrifugation, and the absorbance was measured at 532 nm. The results were expressed as nmol of MDA/mg protein.

##### Determination of reduced glutathione (GSH) level

GSH concentration was performed with the method described by Ellman (1959) based on the development of yellow color when DTNB is added to compounds containing sulfhydryl groups. In brief, 0.8 ml of tissue homogenate was added to 0.2 ml of 0.25% sulfosalicylic acid and tubes were centrifuged at 2500 g for 15 min. Supernatant (0.5 ml) was mixed with 0.025 ml of 0.01 M DTNB and 1 ml TBS (pH 7.4). Finally, the absorbance at 412 nm was recorded. Total GSH content was expressed as nmol GSH/g tissue [22].

##### Determination of glutathione-S-transferase (GST) activity

GST activity of tissues was measured spectrophotometrically by the method of Habig *et al.* [23]. Using CDNB as an electrophilic substrate that binds to GSH with the participation of the enzyme and forms a colored GSH-substrate complex, detected at 340 nm. The activity of GST was expressed in terms of  $\mu$ mol CDNB-GSH conjugate formed/min/g tissue.

##### Determination of catalase (CAT) activity

CAT activity was assayed by the decomposition of hydrogen peroxide according to Aebi method, and the absorbance of the sample was measured at 240 nm for 1 min using a UV-spectrophotometer. The enzymatic activity was calculated in terms of the international unit/min/g of tissue [24].

##### Protein level assay

The protein content of the supernatant was spectrophotometrically estimated by Bradford method using bovine serum albumin as standard [25].

##### Histopathological study

After rats sacrificed, the part of kidney tissues was removed and immersed in fixative (solution 10% formaldehyde) until the time of slices preparation. It was 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 Leica Rotary Microtome (Wetzlar, Germany), and colored with hematoxylin and eosin. Histopathological evaluation was performed with a light microscope.

##### Statistical analysis

Data were carried out using Student's t-test to compare means among the groups. Differences were considered statically significant at  $p < 0.05$ .

## RESULTS

##### Phenolic and tannin compounds

Total phenolic and condensed tannin compounds were expressed in terms of gallic acid equivalents (mg of GAE/g sample) and of catechin equivalents (mg of Ca/g sample), respectively, using the

following equation based on the calibration curve:  $Y=0.0104x+0.0819$ ,  $R^2=0.9925$  for phenolic compounds and  $Y=0.003x+0.006$   $R^2=0.9882$  for condensed tannin compounds. Total phenolic and condensed tannin contents of aqueous Ah extract were represented in Table 1.

#### Body and relative kidney weight

BZ exposure rats caused a decrease in body weight gain ( $p<0.001$ ) and an increase in relative kidney weight ( $p<0.01$ ) compared to the control rats. Preventive Ah treatment of BZ exposure rats (AhP+BZ) was totally restored the body weight gain ( $p<0.05$ ) and relative kidney weight ( $p<0.05$ ) level as compared to the BZ rats. Curative treatment of the extract aqueous of Ah was significantly ( $p<0.05$ ) increase body weight gain compared to BZ rats, but no effect for on relative kidney weight in

(BZ+AhC) rats compared to BZ rats. No effect on body weight gain and relative kidney weight of Ah alone treatment in rats compared to the control rats (Table 2).

#### Blood biochemical values

As shown in Tables 3 and 4, BZ exposure caused a significant increase ( $p<0.05$ ) in serum creatinine and urea level and a significant decrease in serum protein ( $p<0.01$ ) levels. Furthermore, no effect of Ah alone or BZ on blood glucose, blood ratio urea/creatinine, serum albumin concentration and LDH activity compared to control. Meanwhile, Ah made a recovery in the above-mentioned biochemical parameters for preventive treatment (AhP+BZ) but the low effect for curative treatment (BZ+AhC). In another way, treatment with the aqueous extract of Ah alone has no effect on most of these parameters.

#### Oxidative stress parameters

Results presented in Table 5 show a significant increase ( $p<0.05$ ) in MDA level and a significant decrease in GSH concentration ( $p<0.01$ ), GST ( $p<0.01$ ), and CAT ( $p<0.001$ ) activities in BZ group compared to the corresponding control values. Treatment with Ah partially restored the MDA levels ( $p<0.001$ ), CAT ( $p<0.05$ ), and GST ( $p<0.05$ ) activities

**Table 1: Total phenolic and tannin content**

Compounds	<i>Atriplex halimus</i>
Total phenolic (mg GA eq/g dry wt)	17.183±0.067
Condensed tannin (mg Ca eq/g dry wt)	1.49±0.020

Data are expressed as mean±SE; n=3. GA: Gallic acid, Ca: Catechin, SE: Standard error

**Table 2: Initial body weight, body weight gain, and relative kidney weight in control and experimental groups**

Parameters	Initial body weight (g)	Body weight gain (g/d)	Absolute kidney weight (g)	Relative kidney weight (g/100 g bw)
Control	221.17±7.73	0.74±0.10	1.35±0.031	0.479±0.019
Ah	233±4.71	0.719±0.064	1.49±0.051	0.48±0.011
BZ	234.2±12.1	0.38±0.031***	1.39±0.072	0.51±0.007**
BZ+AhC	229.25±6.34	0.66±0.037 <sup>Φb</sup>	1.43±0.065	0.49±0.01
AhP+BZ	219.25±7.16	0.76±0.072 <sup>Φb</sup>	1.39±0.065	0.475±0.01 <sup>Φb</sup>

Data are expressed as mean±SE; n=6. For Student's t-test significant p value Ah, BZ, BZ+AhC, and AhP+BZ versus control \*\* $p<0.01$ , \*\*\* $p<0.001$  and therapy versus BZ  $\Phi\Phi p<0.01$ . Ah: *Atriplex halimus*, AhC: *Atriplex halimus* curative treatment, AhP: *Atriplex halimus* Preventive treatment, BZ: Benzene, SE: Standard error

**Table 3: Mean blood glucose levels and blood biochemical value in the control and experimental groups**

Parameters	Blood glucose (g/l)	Serum urea (g/l)	Serum creatinine (mg/l)	Blood ratio urea/creatinine
Control	1.026±0.059	0.27±0.017	7.45±0.16	37.58±3.85
Ah	0.93±0.039	0.28±0.009	7.85±0.20	35.66±2.84
BZ	1.038±0.09	0.337±0.015**	8.82±0.38*	37.41±3.94
BZ+AhC	1.05±0.028	0.33±0.02*	8.20±0.34	40.24±5.88
AhP+BZ	0.82±0.19	0.24±0.016 <sup>Φb</sup>	7.65±0.22 <sup>Φb</sup>	31.37±7.27 <sup>Φ</sup>

Data are expressed as mean±SE; n=6. For Student's t-test significant p value Ah, BZ, BZ+AhC, and AhP+BZ versus control \* $p<0.05$ , \*\* $p<0.01$  and therapy versus BZ  $\Phi p<0.05$  and  $\Phi\Phi p<0.01$ . Ah: *Atriplex halimus*, AhC: *Atriplex halimus* Curative treatment, AhP: *Atriplex halimus* Preventive treatment, BZ: Benzene, SE: Standard error

**Table 4: Mean blood glucose levels and blood biochemical value in the control and experimental groups**

Parameters	Serum uric acid (mg/l)	Serum albumin (g/l)	Serum LDH (U/l)	Serum protein (g/l)
Control	19.67±1.33	31.83±1.33	3166±149	72.33±2.47
Ah	14.59±0.55***	39.33±1.41	2227±135**	83.83±3.42*
BZ	17.78±1.83	31.17±1.17	3324±216	68.83±1.14*
BZ+AhC	18.74±1.18	25.40±1.29	3098±118 <sup>Φ</sup>	70±1.96
AhP+BZ	12.00±0.56*** <sup>Φb</sup>	37.6±2.23	2831±187 <sup>Φ</sup>	65.25±2.14*

Data are expressed as mean±SE; n=6. For Student's t-test significant p value Ah, BZ, BZ+AhC, and AhP+BZ versus control \* $p<0.05$ , \*\* $p<0.01$  and \*\*\* $p<0.001$  and therapy versus BZ  $\Phi p<0.05$  and  $\Phi\Phi p<0.01$ . Ah: *Atriplex halimus*, AhC: *Atriplex halimus* Curative treatment, AhP: *Atriplex halimus* Preventive treatment, BZ: Benzene. LDH: lactate dehydrogenase, SE: Standard error

**Table 5: Oxidative stress parameters in kidney of control and experimental groups**

Parameters	MDA (nmol/mg prot)	GSH (nmol/g tissue)	GST (nmol/min/g tissue)	CAT (U/g tissue)
Control	3.78±0.33	2.97±0.21	5.96±0.32	1.16±0.044
Ah	3.98±0.18	2.42±0.31	6.26±0.65	1.21±0.25
BZ	4.52±0.065**	2.28±0.051**	4.40±0.20**	0.70±0.044***
BZ+AhC	5.26±0.085*** <sup>Φb</sup>	2.05±0.3*	5.62±0.42 <sup>Φ</sup>	1.036±0.049 <sup>Φb</sup>
AhP+BZ	3.49±0.19 <sup>Φb</sup>	2.66±0.15 <sup>Φ</sup>	6.31±0.37 <sup>Φb</sup>	0.96±0.07 <sup>Φ</sup>

Data are expressed as mean±SE; n=6. For Student's t-test significant p value Ah, BZ, BZ+AhC, and AhP+BZ versus control \* $p<0.05$ , \*\* $p<0.01$  and \*\*\* $p<0.001$  and therapy versus BZ  $\Phi p<0.05$  and  $\Phi\Phi p<0.01$ . Ah: *Atriplex halimus*, AhC: *Atriplex halimus* Curative treatment, AhP: *Atriplex halimus* Preventive treatment, BZ: Benzene. MDA: Malondialdehyde, GSH: Reduced Glutathione, GST: Glutathione S transferase, CAT: Catalase, SE: Standard error

for curative effect (BZ+AhC) and totally restored the MDA ( $p<0.01$ ), GSH ( $p<0.05$ ) levels and CAT ( $p<0.01$ ), and GST ( $p<0.05$ ) activities for preventive effect (AhP+BZ). In addition, treatment with the plant extract alone does not cause any change in oxidative stress parameters.

#### Correlations between analyzed parameters

The results in Table 6 represent the correlation between serum kidney markers (creatinine and urea) and kidney oxidative stress markers (MDA, GSH, GST, and CAT) for experimental groups. Our results showed that there was a significant positive correlation ( $p<0.05$ ) between serum creatinine concentration and kidney MDA level. However, there was a significant negative correlation between serum creatinine concentration and kidney antioxidative markers GSH ( $p<0.05$ ), GST ( $p<0.001$ ), and CAT ( $p<0.01$ ). In addition, there was a significant positive correlation ( $p<0.05$ ) between serum urea concentration and kidney MDA level and a negative correlation between serum urea concentration with GSH ( $p<0.01$ ) level and GST ( $p<0.01$ ) activity, but there was no significant correlation between serum urea and kidney CAT activity.

#### Histopathological studies

Microscopic observation of histological sections of kidney from the control and Ah treated rat showed normal renal parenchyma with glomeruli, tubules and normal cortical, and medullary areas (Fig. 2a and b). However, histological sections of the BZ exposed rat kidney revealed severe degeneration represented by necrosis and glomerular hypertrophy apparent to their architecture with tubular necrosis, dilatation, and tubular vacuolation. In addition, hemorrhage, foci of inflammation and large space of Bowman, was observed in the kidney sections of this group (Fig. 2c). Kidney section of (AhP+BZ) groups when rats received the aqueous extract of *Atriplex* protective treatment with BZ indicated protection from tubule and glomerulus (Fig. 2e). *Atriplex* curative treatment partially decreased severe damage to kidney tissue (Fig. 2d). The histopathological changes are graded and summarized in Table 7. Histological grading was made according to four severity grades: - (none), + (mild), ++ (moderate), and +++ (severe).

#### DISCUSSION

The finding of this study indicates that BZ-induced nephrotoxicity in the rat through the increase relative kidney weight, serum urea and creatinine levels, and alters renal antioxidant and oxidative stress parameters and also caused histopathological alterations in kidney tissue. However, our study indicated that Ah has a dramatically preventive effect on BZ-induced nephrotoxicity through the potential restoration of biochemical and histopathological parameters in kidney tissue rat. As well, in a curative mode, Ah partially restored the renal

damage, suggesting that *Atriplex* exhibits inhibitory action thus causing nephroprotection.

BZ exposure at a dose (100 mg/kg) resulted in a significant decrease in body weight and increase in relative kidney weight. Ibrahim *et al.* reported a significant decline in the body weight gain of the animals intoxicated with BZ. Moreover, according to him; this 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 [26]. Then, cotreatment of *Atriplex* with BZ restored the weight of the body and kidney as normal, which can indicate the prevention against the injury effect of BZ on kidney as evidenced macroscopically.

The increased levels of urea and creatinine are considered as the marker of impaired kidney functions [27,28]. Thus, in our study, an increase of plasma urea and creatinine reflected the diagnosis of renal damage. These results were inconsistent with the study of Khan and Yusufi [29]. On other, occupational exposure to BZ in petrol stations was showed that serum creatinine was significantly higher in the exposed group than in the unexposed employees [30]. Aqueous extract of Ah preventive treatment decreased the raised serum urea and creatinine and maintained healthy kidney function, but low effect, we observed for curative treatment in these parameters. Moreover, BZ orally administrated to rats caused a significant decrease in serum protein concentration and less increase in serum LDH activity. However, BZ

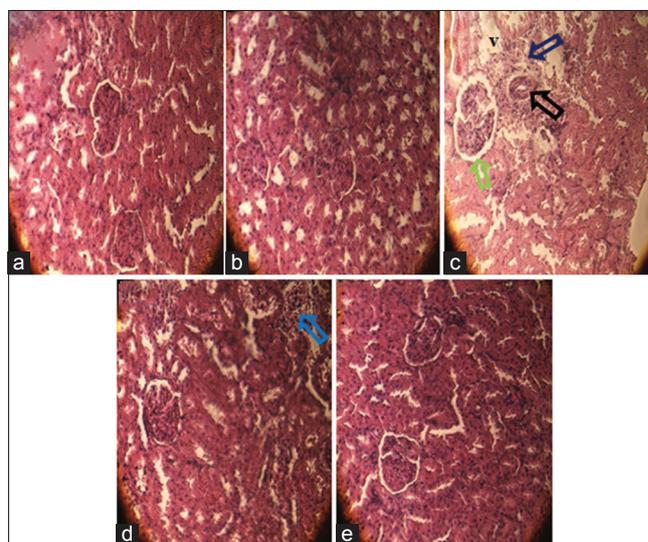


Fig. 2: (a) The histological examination of kidney from a control rat showing normal architecture  $\times 400$ . (b) Kidney section of *Atriplex halimus* treated rats showing a healthy renal architecture  $\times 400$ . (c) Benzene exposed rats kidney showing focus of inflammation (blue arrow), glomerular necrosis (black arrow), large space of bowman (green arrow), and vacuolation (V)  $\times 400$ . (d) *A. halimus* curative treated rat kidney showing a moderate degree of kidney damage and few inflammatory cell, protection from tubules, and glomerulus degradation  $\times 400$ . (e) Kidney sections of benzene rats prevented by *A. halimus* showed the normal appearance of tubules and glomerulus  $\times 400$

Table 6: Correlation coefficients and the significant levels of different kidney marker components in rats

Component vs.	Creatinine		Urea	
	R	P	R	P
Kidney MDA	0.65	0.04	0.88	0.001
Kidney GSH	-0.75	0.012	-0.76	0.01
Kidney GST	-0.9	0	-0.83	0.003
Kidney CAT	-0.81	0.005	-0.52	0.111

Table 7: Semi-quantitative recording of architectural damage on histopathological analysis of the kidney of control and treated rats

Parameters	Dilatation and tabular vacuolation	Inflammatory infiltration	Tubular and glomerular necrosis	Large space of Bowman	Hypertrophy glomerular
Control	-	-	-	-	-
Ah	-	-	-	-	-
BZ	+++	++++	++++	++++	+++
BZ+AhC	+	++	++	+	+
AhP+BZ	-	-	-	+	-

Ah: *Atriplex halimus*, AhC: *Atriplex halimus* Curative treatment, AhP: *Atriplex halimus* Preventive treatment, BZ: Benzene

exposure did not affect markedly serum glucose, uric acid, and albumin concentration.

Recent studies reported that BZ-initiated toxicity has been linked to the production of ROS [31]. More specifically BZ can be enzymatically bioactivated to reactive metabolites (catechol, benzoquinones, hydroquinone, and phenol), which can produce ROS, decreases antioxidant activity, and hence increases oxidative stress [32,33]. Kidney is one of the major tissue sites of BZ's metabolites and BZ's conjugated metabolites for phenol, catechol, and hydroquinone, phenyl sulfate and hydroquinone glucuronide, and muconic acid [34,35]. These compounds form covalent adducts with diverse macromolecules such as nucleic acids and proteins in the kidney [36].

MDA is one of the major lipid peroxidation products and is used as a marker of cell membrane destruction and tissue damage [37]. Our data demonstrated that BZ administration caused a significant increase in MDA levels as compared to control group in the kidney tissue, which reflects extensive lipid peroxidation process in the kidney of rats after BZ exposure. Our results are in good agreement with Çavuşoğlu *et al.* who found that BZ administration at a dose 50 mg/kg of body weight for 50 days caused a significant increase in kidney MDA [38].

Results from the present study demonstrate that the levels of endogenous antioxidants both enzymatic and non-enzymatic (GSH and CAT, GST) are significantly lowered in the BZ-treated group compared to the normal control rats, which are supported by the findings of Verma and Rana [39] who reported that BZ administration decreased the level of GSH in rats kidney. Furthermore, Hetal [40] demonstrated that BZ exposure leads to a decrease in antioxidant enzymes activity. The sulfhydryl reduced GSH is a small intracellular thiol (-SH) molecule, play an essential role in the detoxification processes [41]. Furthermore, previous studies indicated that GSH depletion is one of the important consequences of toxic injury; which might be consumed during the metabolic reaction of BZ [42]. CAT and GST antioxidant enzymes play a critical role in the protection of tissues from the deleterious effects of BZ. GST represent a major group of detoxifying enzymes [43], which catalyze the detoxification of BZ oxepin, metabolite potential reactive, and carcinogenic of BZ through conjugation with GSH and excretion in the urine as S-phenylmercapturic acid [44]. Finally, as an H<sub>2</sub>O<sub>2</sub> scavenger, CAT has been shown to play an important role in the protection of cells against toxicity from the hydroquinone metabolite [45]. Therefore, the decreased activities of these antioxidant enzymes indicated a failure of the antioxidant defense system in BZ group, may have been due to oxidation reactions initiated by BZ metabolites and the influx of ROS induced by BZ exposure.

Extract Ah treatment, (especially preventive treatment) dramatically decrease MDA level and increase GSH, CAT, and GST level in the BZ group. This effect can be attributed to the activity of its antioxidants and the ROS scavenging capacity. In this study, aqueous extract of Ah contains higher levels of total phenolic and condensed tannin. Many researchers reported a significantly high correlation between the high antioxidant activity of plant extracts and its high total phenolic compounds. Further, according to the previous research, Ah contain a wide range of bioactive metabolites. It is a source of Vitamins A, C, and D, polyphenols, flavonoids, tannins, alkaloids, saponins, and resins [46]. These constituents are good indicators for the antioxidant activity of this plant that may be involved in the prevention of cellular damage and many human diseases, caused by free radicals [47,48]. These antioxidants molecules interact with free radicals and terminate the chain reaction before vital molecules are damaged. They donate an electron to stabilize a free radical [49]. Therefore, it may be suggested that Ah treatment can reduce the BZ toxicity by decreasing the oxidative stress through the activation of antioxidant defense systems, the ROS scavenging and the inhibition of LPO in BZ administered rats. These observations might indicate that *Atriplex* has protective effects on BZ-induced oxidative stress and it may potentially reduce the unfavorable effects of BZ in the kidney.

Histopathological observation of renal tissue sections from the BZ group confirmed the biochemical results and showed clear signs of nephrotoxicity. Stained sections reveal they marked severe glomerular and tubular degeneration, glomeruli hypertrophy, loss of brush border membrane, vascular congestion, inflammatory cell infiltration, vacuolization of tubular cells, tubular dilatation, and increased number of pyknotic nuclei. Overproduction of ROS in chronic BZ exposure can cause damage of important biomolecules (proteins, lipids, and DNA) and increase intra-renal pro-inflammatory and fibrogenic factors, which can lead to cell death (necrotic) and chronic inflammation associated with tissue renal damage [50]. However, *Atriplex* improved the renal histological changes with normal glomeruli and tubules in preventive effect. This protective effect of the extract may be attributed to the presence of antioxidant and anti-inflammatory components in this plant.

## CONCLUSION

The current study demonstrates that the administration of Ah can reduce (or completely prevent) several toxicities induced by BZ in kidneys. This protective potential may involve the powerful antioxidant properties of this plant by eliminating free radicals and oxidative damages caused by BZ. Hence, dietary supplementation with Ah could be a useful method to protect populations at high risk of environmental and/or occupational BZ chronic intoxication, and for scavenging the free radicals generated in the kidney.

## ACKNOWLEDGMENTS

The first author would like to thank the Faculty of Sciences of Nature and Life, University of El Oued, Algeria, for the permission to utilize the institute facilities to make this work.

## AUTHOR'S CONTRIBUTIONS

The conception of the study, drafting the article and interpretation of data was done by Khaoula Zeghib author; Djahra Ali Boutlelis author was responsible for the acquisition of data and analysis.

## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

## REFERENCES

1. Sonawane B, Bayliss D. Toxicological Review of Benzene (Non cancer effects) (CAS No. 71-43-2). In: Support of Summary Information on the Integrated Risk Information System. Washington, DC, USA: U.S. Environmental Protection Agency; 1998.
2. Mathews JM, Etheridge AS, Matthews HB. Highlight dose-dependent metabolism of benzene in hamsters rats and mice. *J Environ Sci* 1998;44:14-21.
3. Çavuşoğlu K, Yalçın E, Yapar K, Gür B, Çiçek F. The protective role of grape seed extract against chronic toxicity of benzene in swiss albino mice. *Cumhuriyet Sci J* 2014;35:1-11.
4. Maltoni C, Ciliberti A, Cotti G, Conti B, Belpoggi F. Benzene, an experimental multipotential carcinogen: Results of the long-term bioassays performed at the bologna institute of oncology. *Environ Health Perspect* 1989;82:109-24.
5. Verma Y, Rana, SVS, Singh N. Simultaneous exposure to benzene and alcohol in petrol pump operators. *Int J Pharm Sci Res* 2016;7:2606-10.
6. Medinsky MA, Schlosser PM, Bond JA. Critical issues in benzene toxicity and metabolism: The effect of interactions with other organic chemicals on risk assessment. *Environ Health Perspect* 1994;102 Suppl 9:119-24.
7. Emara AM, El-Bahrawy H. Green tea attenuates benzene-induced oxidative stress in pump workers. *J Immunotoxicol* 2008;5:69-80.
8. Falzone L, Marconi A, Loreto C, Franco S, Spandidos DA, Libra M, *et al.* Occupational exposure to carcinogens: Benzene, pesticides and fibers (Review). *Mol Med Rep* 2016;14:4467-74.
9. El Batsh MM, Zakaria SS, Gaballah HH. Protective effects of alpha-lipoic acid against benzene induced toxicity in experimental rats. *Eur Rev Med Pharmacol Sci* 2015;19:2717-24.
10. Derouiche S, Zeghib K, Gharbi S, Khelef Y, Feriani A. Protective effects of aristolochia longa and aquilaria malaccensis against lead

- induced acute liver injury in rats. *J Acute Dis* 2017;6:193-19.
11. Tucker PS, Scanlan AT, Dalbo VJ. Chronic kidney disease influences multiple systems: Describing the relationship between oxidative stress, inflammation, kidney damage, and concomitant disease. *Oxid Med Cell Longev* 2015;2015:806358.
  12. Oh GS, Kim HJ, Shen A, Lee SB, Khadka D, Pandit A, *et al*. Cisplatin-induced kidney dysfunction and perspectives on improving treatment strategies. *Electrolyte Blood Press* 2014;12:55-65.
  13. Afravy M, Angali K, Khodadadi A, Ahmadizadeh M. The protective effect of buffalo's milk against toluene induced-nephrotoxicity in rats. *J Nephrothol* 2017;6:174-9.
  14. Basu S, Das M, Sen A, Datta G. Protective role of crude extract of *Amorphophallus Campanulatus* against ethanol-induced oxidative renal damage. *Asian J Pharm Clin Res* 2018;11:240-50.
  15. Nedjimi B, Guit B, Toumi M, Beladel B, Akam A, Daoud Y. *Atriplex halimus* Subsp. *schweinfurthii* (*Chenopodiaceae*): Description, écologie et utilisations pastorales et thérapeutiques. *Fourrages* 2013;216:333-8.
  16. Kabbash AM. Macroscopic and microscopic characterization of *Atriplex halimus* L. growing in egypt and *in vitro* evaluation of its cytotoxic activity. *W J Pharm Pharm Sci* 2016;5:84-100.
  17. Murugan R, Parimelazhagan T. Comparative evaluation of different extraction methods for antioxidant and anti-inflammatory properties from *Osbeckia parvifolia* Arn. An *in vitro* approach. *J King Saud Univ Sci* 2014;26:267-75.
  18. Li HB, Cheng KW, Wong CC, Fan KW, Chen F, Jiang Y. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chem* 2007;102:771-6.
  19. Broadhurst RB, Jones WT. Analysis of condensed tannins using acidified vanillin. *J Sci Food Agric* 1978;29:788-94.
  20. Snyder R, Hedli CC. An overview of benzene metabolism. *Environ Health Perspect* 1996;104 Suppl 6:1165-71.
  21. Quintanilha AT, Packer L, Davies JM, Racanelli TL, Davies KJ. Membrane effects of vitamin E deficiency: Bioenergetic and surface charge density studies of skeletal muscle and liver mitochondria. *Ann N Y Acad Sci* 1982;393:32-47.
  22. Weckbecker G, Cory JG. Ribonucleotide reductase activity and growth of glutathione-depleted mouse leukemia L1210 cells *in vitro*. *Cancer Lett* 1988;40:257-64.
  23. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem* 1974;249:7130-9.
  24. Aebi H. "Catalase *in vitro*". *Methods Enzymol* 1984;105:121-6.
  25. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-54.
  26. Ibrahim KS, Saleh ZA, Farrag AR, Shaban EE. Protective effects of zinc and selenium against benzene toxicity in rats. *Toxicol Ind Health* 2011;27:537-45.
  27. Kadeche L, Bourogaa E, Saoudi M, Boumendjel A, Djeflal A, El Feki A, *et al*. Ameliorative effects of vanillin against metribuzin-induced oxidative stress and toxicity in rats. *Int J Pharm Pharm Sci* 2017;9:56-62.
  28. Moirangthem RS, Gunindro N, Takhellambam DS, Khurajiam SD, Meena N, Rita S. Protective effect of *Phyllanthus fraternus* against cyclophosphamide induced nephrotoxicity in rats. *Int J Basic Clin Pharmacol* 2017;6:984-9.
  29. Khan S, Yusufi AN. Effect of benzene on the enzymes of carbohydrate metabolism, brush border membrane (BBM) and oxidative stress in kidney and other rat tissues. *Biol Med* 2009;1:28-41.
  30. Neghab M, Hosseinzadeh K, Hassanzadeh J. Early liver and kidney dysfunction associated with occupational exposure to sub-threshold limit value levels of benzene, toluene, and xylenes in unleaded petrol. *Saf Health Work* 2015;6:312-6.
  31. Badham HJ, Renaud SJ, Wan J, Winn LM. Benzene-initiated oxidative stress: Effects on embryonic signaling pathways. *Chem Biol Interact* 2010;184:218-21.
  32. Fenga C, Gangemi S, Teodoro M, Rapisarda V, Golokhvast K, Docea AO, *et al* 8-hydroxydeoxyguanosine as a biomarker of oxidative DNA damage in workers exposed to low-dose benzene. *Toxicol Rep* 2017;4:291-5.
  33. Uzma N, Kumar BS, Hazari MA. Exposure to benzene induces oxidative stress, alters the immune response and expression of p53 in gasoline filling workers. *Am J Ind Med* 2010;53:1264-70.
  34. Wilbur S, Wohlers D, Paikoff S, Keith LS, Faroon O. ATSDR evaluation of health effects of benzene and relevance to public health. *Toxicol Ind Health* 2008;24:263-398.
  35. Kalf GF. Recent advances in the metabolism and toxicity of benzene. *Crit Rev Toxicol* 1987;18:141-59.
  36. Bayliss D, Jinot J, Sonawane B. Toxicological Review of Benzene (CAS No. 71-43-2). In Support of Summary Information on the Integrated Risk Information System; EPA/635/R-02/001F. Washington, DC, USA: U.S. Environmental Protection Agency; 2002.
  37. Derouiche S, Rezzag MO, Serouti A. Triazinone herbicide metribuzin induced acute liver injury: A study of animal model. *J Acute Dis* 2018;7:152-7.
  38. Çavuşoğlu K, Yalçın E, Oruç E, Gür B, Çiçek F. The effects of grape seed extract against toxicity of benzene on liver and kidney tissues of albino mice: Biochemical evaluation. *Turk J Biochem* 2015;40:66-73.
  39. Verma Y, Rana SV. Modulation of CYP4502E1 and oxidative stress by testosterone in liver and kidney of benzene treated rats. *Indian J Exp Biol* 2008;46:568-72.
  40. Hetal R. Evaluation of benzene induced histopathological alteration in rat. *Int J Adv Res* 2016;4:563-7.
  41. Sairazi SM, Sirajudeen KN, Muzaimi M, Swamy M, Asari MA, Sulaiman SA. Tualang honey attenuates kainic acid-induced oxidative stress in rat cerebellum and brainstem. *Int J Pharm Pharm Sci* 2017;9:155-62.
  42. Abd-Elshakour A, El-Ebriar AS, Ibrahim YH, Abd-Elmoneim AE, El-Mekawy AM. Effect of benzene on oxidative stress and the functions of liver and kidney in rats. *J Environ Occup Sci* 2015;4:34-9.
  43. Elsayed AS. Hematotoxicity and oxidative stress caused by benzene. *Pyrex J Biomed Res* 2015;1:74-80.
  44. Zarth AT, Murphy SE, Hecht SS. Benzene oxide is a substrate for glutathione S-transferases. *Chem Biol Interact* 2015;242:390-5.
  45. Ahmad S, Agrawal R, Agrawal DK, Rao GS. Bioreactivity of glutathionyl hydroquinone with implications to benzene toxicity. *Toxicology* 2000;150:31-9.
  46. Chikhi I, Allali H, Dib MA, Medjdoub H, Tabti B. Antidiabetic activity of aqueous leaf extract of *Atriplex halimus* L. (*Chenopodiaceae*) in streptozotocin-induced diabetic rats. *Asian Pac J Trop Dis* 2014;4:181-4.
  47. Kaouachi A, Derouiche S. Phytochemical analysis, DPPH antioxidant activity and Acute toxicity of bark aqueous extracts of *Pinus halepensis*. *Res J Chem Env Sci* 2018;6:86-91.
  48. Mohammadi Z. Resistance, pharmacology properties and nutritional value of a shrub from arid environments *Atriplex halimus*. *Res J Med Plant* 2016;10:10-8.
  49. Mohammadi A, Ahmadizadeh M. Effects of antioxidants on xenobiotics-induced nephrotoxicity. *J Renal Inj Prev* 2018;7:56-7.
  50. Cottone S, Lorito MC, Riccobene R, Nardi E, Mulè G, Buscemi S, *et al*. Oxidative stress, inflammation and cardiovascular disease in chronic renal failure. *J Nephrol* 2008;21:175-9.