Beneficial effect of *Hordeum vulgare* extract against aluminum chloride induced neurotoxicity in Wistar rats

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Abstract
Aluminum is present in medicines and food. Its toxicity induces deleterious effects in various living organisms. At the same time, *Hordeum vulgare* a cereal known as an important nutritional source and also endowed with bioactive molecules. The objective of this study was to evaluate, on the one hand, the modifications induced by aluminum chloride in Wistar rats at the cerebral level and, on the other hand, to test the efficacy of the barley extract, *Hordeum vulgare* (HEV), to restore the harmful effects of this studied metal with a concentration of 13 ml HEV/kg/day for a period of 21 days. The extraction of HEV by maceration resulted in an aqueous extract with a yield of 10.70%. Exposure to AlCl3 at a concentration of 100mg/kg, permitted to observe that the concentration of aluminium at the brain level is significantly high (p<0.05) in the intoxicated rats compared to the control rats. On the other hand, the activity of alkaline phosphatase (PAL), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) indicated a decrease in the intoxicated rats. Indeed, the histological study showed very pronounced lesions in the brains of the poisoned rats resulting in necrosis and cellular spongiosis. In addition, the administration of HEV restored the activity of the various antioxidant enzymes with an improvement in brain tissue architecture in intoxicated rats treated with HEV which justifies the therapeutic virtues of *H. vulgare* in protecting against aluminium chloride-induced neurotoxicity.

Keywords: Aqueous Extract, Antioxidant status, AlO3, *Hordeum vulgare*, Brain

INTRODUCTION
Aluminum is an abundant metallic element on earth and is very used because of its excellent properties (Bai et al. 2012). It represents 8% of the earth's miner-
als. In food its maximum content is not fixed. Aluminum can be used in the preparation of some medicines (Turkez et al., 2010), used in water clarification (flocculation, coagulation, etc.), in materials in contact with food and used as a food additive. It is found in drinking water at a maximum concentration of 0.2 mg/l (Afssaps, 2011). In mammals, aluminum accumulates in the kidneys, liver, heart, blood, bones and brain (Al-Kahtani, 2010). There is experimental evidence that chronic exposure to aluminum not only causes neurological signs resembling progressive degeneration but also leads to changes in neurofilaments in the hippocampus, cerebral cortex, brain stem and spinal cord (Liaquat et al., 2019). The aluminum can produce free radicals in the body. It induces a toxic effect due to its ability to transfer electrons. Aluminum is known to affect the permeability of cell membranes, subcellular organelles, the structure and functions of proteins and the structure of nucleic acids (Taus et al., 2013).

The barley is of the Poaceae family and the Hordeum species, the most common form being Hordeum vulgare. It is considered to be one of the most important food grains since antiquity and today. It ranks fourth in the world in terms of quantity produced and area under cereal cultivation (Zhou, 2010). Barley is a nutritious cereal with many bioactive elements that are beneficial to health. Much research has shown that barley has been associated with protection against diabetes, insulin resistance, ischemic stroke, atherosclerosis, obesity and cancer (Behall et al., 2004; FDA, 2002). Grains contain many nutrients, including dietary fibre, antioxidants (soluble, fat-soluble and insoluble), vitamins, minerals (calcium, magnesium, potassium, phosphorus), sphingolipids and unsaturated fatty acids (Webster, 2002; Murtaugh et al., 2003).

The present study was aimed to observe the impact of aluminum chloride exposure at the brain level in Wistar rats intoxicated by this metal. The number of suffering animals were minimized in accordance with the guidelines of the European Council Directive (86/609/EEC).

The experiments were carried out on albino rats of Wistar strain, from the local animal house of the university, Dr. Moulay Tahar, Saida, Department of Biology. The animals were housed in professional plastic cages (43 x 28 x 15 cm) at the pet shop. The room they occupied was maintained at a temperature of (22 to 23 °C) and was subjected to a constant light cycle (alternating 12 hours of darkness and 12 hours of light). The animals focused Ad libitum at the water bottles and feeders.

**Distribution of lots:** The animals in our experiment were divided into three groups of 7 rats each.

**Control lot (C):** Consisting of 7 rats receiving only distilled water orally.

**AlCl3 lot:** Consisting of 7 rats receiving 100mg/kg Aluminum chloride in distilled water orally for 12 weeks (Kumar et al., 2009)

**AlCl3-HEV lot:** Consisting of 7 rats receiving aluminum chloride in distilled water orally and H.vulgare aqueous extract at 13 ml/kg by gavage for 21 days (Al-dalain et al., 2008).

**Biochemical tests**

**Determination of the level of aluminum in the brain:** After weaning and treatment with H. vulgare extract, the animals are decapitated and whole blood samples (100 μl) are recovered in a 5 ml haemolysis tube containing 100 μl of 0,1 % newt. After vortex agititation for 30 seconds, deproteinization was achieved by adding 600 μl of HNO3 (1 M). This was followed by a second vortex agitation of the tube contents for 10 minutes at room temperature. The next step was centrifugation of the total tube container at 3000 rpm for 10 minutes. In the end, the blood lead level was determined by a type Atomic absorption spectrophotometer (SHIMA DZU AA6200).

**Measurement of alkaline phosphatase:** The brains of the rats were placed in a 4 ml Potter-Elvehjem homogenizer. Homogenization was carried out in ten volumes of 0,32 M sucrose ice solution. The whole homogenate and the rinsing liquid are centrifuged at 1 000 g for 10 minutes at 4 °C. The supernatant thus represented a crude synaptosomal fraction which was removed and kept in ice for enzyme determination (Adli et al., 2018).

Alkaline phosphatase (PAL) was estimated using the following reaction:

- p-nitrophenylphosphate + H 2 O; - phosphate + p-nitrophenol.

The concentration of p-nitrophenyl released is proportional to the activity of PAL and is measured photometrically at a wavelength of 405 nm.
Measuring the activity of antioxidant enzymes: The brains of the recovered rats were homogenized in a Buffer solution containing 0.5 mM EDTA, 10 mM Tris-HCl (pH: 7.4) 0.32 M sucrose, at 4°C (1 mg tissue per 4 ml buffer solution) using a glass/glass homogenizer. The homogenate was centrifuged at 1 000× g for 15 minutes at 4°C. The resulting supernatant was then centrifuged at 10 000 × g for 15 minutes at 4°C. The pellet constitutes the mitochondrial fraction, and the supernatant was re-centrifuged at 10 000 g/30 minutes. The two pellets thus obtained are solubilized in a buffer solution containing 0.5 mM EDTA, 10 mM Tris-HCl (pH 7.4), 0.32 M sucrose and 0.02 % digitonin (pH 7.4); a second pellet was made at 10 000 × g for 15 minutes at 4°C. The second pellet was centrifuged at 10 000 × g for 15 minutes at 4°C. Finally, the pellet obtained, which constitutes the total fraction of mitochondria, will be solubilized in a sucrose-containing solution (0.32 M at pH = 7.4) (Rotruck et al., 1973). Superoxide dismutase (SOD) [EC 1.15.1.1] was analyzed on the supernatant using the technique of Kakkar et al. (1984), The activities and levels of antioxidant enzymes in the brain, such as glutathione peroxidase (GPx) and catalase (CAT) were analyzed by the method of Rotruck et al (1973). Sinha (1972) and Jollow et al (1973).

Histological study: After sacrifice, brain samples were collected from all groups of rats and fixed in a buffered solution of formalin (10%), dehydrated in ascending grades of ethanol (70-100%), clarified in xylene and cast (the paraffin), blocked, cut to a thickness of 5 µm, routinely stained with haematoxylin and eosin (H&E) dyes (Bancroft, 1975) and examined microscopically at magnification (×40).

Expression and statistical analysis of results: The results were expressed as the mean (M) of the individual values, assigned the standard error to the mean (SEM). The comparison of several means was carried out by an analysis of variance (ANOVA) with the intoxication factor (Al, C) and/or the treatment factor (HEV, Al) followed possibly by the Post-Hoc Student-Newman-Keuls test: A probability *p ≤0.05 indicates a significant difference. **p<0.01; indicates a highly significant difference; ***p<0.001 indicates a highly significant difference compared to controls. Statistical analyses were performed using SigmaStat software (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION
Extract yield and principal compounds: The extraction of plant matter from Hordeum vulgare resulted in a yield of 10.70%. This result is at variance with the work of Abu-Serie et al., (2019), which reported a return of 12.27%. This difference in yield can be attributed to several factors, mainly the geographical origin of the plants and pedoclimatic conditions (Sefidkon, 2002).

Determination of AlCl3 and PAL at brain level: The results showed that AlCl3 concentration in the brain was significantly elevated (p < 0.05) in the intoxicated rats compared to the control rats (Table 1). This accumulation in the brain confirms the capacity of AlCl3 to penetrate the blood-brain barrier (Chalansonnet et al., 2018). In addition, there are reports that aluminium can accumulate in various human organs such as the brain and bones (Abdel-Wahab, 2012; Kalaiselvi et al., 2014). Other studies have indicated that a concentration of aluminum chloride (34 mg/kg bw) induced neurotoxicity and accumulation in the brain in adult male albino Wistar rats that were injected with AlCl3 for 7 days (Yuan et al., 2012). Sanchez-Iglesias et al. (2007) identified aluminium accumulation in the ventral midbrain, cerebellum, hippocampus, cortex and striatum of rats after treatment with aluminum chloride by oral administration or intraperitoneal injection. In addition, Domingo et al. (1996) showed that administration of aluminum nitrate with doses of 0.50 and 100 mg/kg body weight/d in drinking water over a period of 6.5 months accumulated in the olfactory bulb, cortex, and thalamus in rats. PAL is one of the key enzymes that indicate central nervous system (CNS) function, and its activity will allow further study of the impact of neurotoxicity in rats. This enzyme is synthesized at the choroid plexus, and its membrane localization at synapses and Ranvier’s nodes, as well as its activity regulated by the level of afferent activity and neuronal maturation suggest an important role in nerve impulse transmission and regulation (Fonta et al., 2005). Results indicate that PAL activity, which reflects neurotoxicity in rats, is significantly lower in the brains of AlCl3-exposed rats compared with non-exposed rats (Table 1).

Brain activity of oxidative status enzymes: The high concentration of transition metals has the potential to cause an overproduction of nitrogen and oxygen free radicals in the body, and they are likely to react near their formed location. If these free radicals do not neutralize themselves, oxidative damage forms in the subcellular systems and this are due to the incapacity of the cellular defense system. As a result, aluminum chloride is a transition metal and has the potential to modify the activity of enzymatic antioxidants. (Abdel-Wahab, 2012; Kalaiselvi et al., 2014). In another series of experiments, the exploration of antioxidant enzymes at the brain level in rats exposed to AlCl3 revealed considerable perturbations, with a significant (*p < 0.05, **p < 0.001) decrease in the activity of the different enzymes (CAT, GPx and SOD) compared to the control group (Table 2). These results show that exposure to AlCl3 induces a reduction.
in the activities of antiradical enzymes in the brain (Sanet and Wages., 2018). The administration of aluminum chloride (AlCl3; 34 mg / kg bw) showed a reduction in antioxidant enzymes (SOD, CAT and GPx) in albino Wistar rats (Al-Olayan et al., 2015).

Some studies have described that high Al levels can induce oxidative stress and cause lipid peroxidation in the cerebellum, brain stem, diencephalon and hippocampus. In these tissues, the production of antioxidant enzymes was expected to counteract oxidative stress. Other studies have shown that SOD activities decreased in the hippocampus, cerebellum and brainstem tissues of animals with high AlCl3 content. Several other studies that have also identified a significant increase in antioxidant enzymes (SOD, CAT and GPx) in control, intoxicated and intoxicated rats treated with HEV.

Table 1: Al levels and cerebral PAL activity in control, intoxicated and intoxicated rats treated with HEV.

<table>
<thead>
<tr>
<th>Brain concentration</th>
<th>Control</th>
<th>ALCL3</th>
<th>ALCL3-HEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al (µg/g)</td>
<td>0,98 ± 0,18*</td>
<td>6,65 ± 0,36*</td>
<td>4,29 ± 0,25*</td>
</tr>
<tr>
<td>PAL (µg p-nitrophenol/ mg protein/ minute)</td>
<td>3,66 ± 0,34*</td>
<td>1,86 ± 0,23*</td>
<td>2,19 ± 0,56*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; *p < 0.05

Table 2: Cerebral antioxidant enzyme activity (SOD, GPx, CAT) in control, intoxicated and intoxicated rats treated with HEV.

<table>
<thead>
<tr>
<th>Brain concentration</th>
<th>Control</th>
<th>ALCL3</th>
<th>AlCl3-HEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/mg de protein)</td>
<td>5,98 ± 0,28***</td>
<td>3,75 ± 0,11***</td>
<td>4,51 ± 0,44***</td>
</tr>
<tr>
<td>GPx (U/mg de protein)</td>
<td>19,44 ± 0,17*</td>
<td>11,51 ± 0,61*</td>
<td>13,92 ± 0,21*</td>
</tr>
<tr>
<td>CAT (U/mg de protein)</td>
<td>0,76 ± 0,34*</td>
<td>0,51 ± 0,22*</td>
<td>0,59 ± 0,85*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; ***p < 0.001, *p < 0.05

Histological study: Histological study of the cerebellum of AlCl3 intoxicated rats revealed marked damage, with cellular necrosis and cellular spongiosis (Fig. 1). Histological investigations revealed that exposure to AlCl3 caused progressive alterations of the brain. The results corroborated the previous findings of Abdel Moneim (2012) and Bhadauria (2012) as AlCl3 has been implicated in the pathogenesis of several clinical disorders and histopathological changes in the brain of rats.

Histopathological changes in the brain have been reported in several studies in rats for periods ranging from 21 days to 1 year following administration of drinking water or food to which aluminum salts {AlCl3, AlF3 or C3H 4AlO2 (Al citrate)} were added at varying doses (15, 20 et 40 mg Al/kg).

In some cases, cytoplasmic vacuolization, astrocyte swelling, and nuclear vacuolization of neurons and inclusions in the brain parenchyma (Liaquat et al., 2019) and in other studies, multifocal neuronal degeneration, damaged and abnormal neurons, and decreased neuronal density in certain brain regions (cerebral cortex, subcortical region, hippocampus and base of the brain) in rats (Al-Olayan et al., 2015). Deformation, vacuolation of the nuclei and neurocellular degeneration in the hippocampus in rats, although this degeneration is distinct from that of Alzheimer's disease (Somova, 1996).

It has been postulated that the neuropathological effects observed in some of these studies may result from the increased bioavailability of aluminum administered as citrate and fluoride (ATSDR, 1999).

A study realized by Platt (Platt et al., 2001), whose goal was to immunocytochemically characterize the neurotoxosomovactivity of aluminum in rats, revealed a concentration of aluminum in the white matter of the middle striatum and the corpus callosum.

Intracerebroventricular administration of aluminum (5,4 µg) was performed daily for 5 weeks in adult male rats. The results of this study showed that the neurotoxicity of aluminum at the brain level in rats affects both glial response and cholinergic projections, which is consistent with the observations of aluminum-induced cognitive defects in this species (Platt et al., 2001).

_Hordeum vulgare_ is a good natural source of vitamins and minerals, and its extract is widely consumed as a functional food for health reasons (Yamaura, et al., 2015). They are a rich source of the powerful antioxidants saponarin and lutonarin (Benedet et al., 2007) and also have physiological activities, including lipiodlowering (Yu et al., 2002a), anti-diabetic (Yu et al., 2002b), and anti-ulcer (Ohtake et al., 1985) effects, as well as an antidepressant effect in mice (Yamaura et al., 2012), via its antioxidant action.

In our study, the administration of HEV in intoxicated rats showed a considerable reduction (62, 62 %) of AlCl3 levels in the brain. Some studies indicate that _H. vulgare_ is a good phytostabilization plant because of its capacity to accumulate heavy metals (Cd and Hg) (Dago et al., 2014). Entire grains are also an important dietary source of fat-soluble, water-soluble and water-insoluble antioxidants. These cereal antioxidants include: phytic acid,
tocotrienols, phenolic acids, selenium and vitamin E. These multifunctional antioxidants are available throughout the gastrointestinal tract for a long period of time after consumption in immediate to slow-release forms. (Tsai et al., 2004; Webster, 2002). The prophylactic efficacy of phytic acid can be attributed to its capacity to complex with multivalent cations. The rats treated preventively and curatively with PA exhibited significantly reduced levels of aluminum in the brain. Another reason that IP6 can chelate heavy metal ions, that it has twelve negatively charged hydroxyl groups makes PA a highly negatively charged molecule (Ferry et al., 2002; Daley et al., 2013). This suggests that PA can cross the blood-brain barrier and acts at the brain level. Sanchis and Adrover (2017) showed that PA binds to the surface of organs and is recovered from the blood. Cúneo et al. (2006). showed that the accumulation of heavy metals such as lead in bones, kidneys and liver was inversely proportional to the amount of phytate ingested. These data support the hypothesis that phytic acid may prevent excessive intestinal absorption and accumulation of aluminum in the tissues of laboratory animals. PA has been shown to reduce cadmium-induced toxicity in male rats (Tsao et al. 1997; Rimbach et al. 1998; Mohamed et al., 2015). In all cases, the known chelating ability of PA has been translated into its application as a detoxifying agent against heavy metals such as Cd and Pb (Sanchis and Adrover 2017; Reddy et al., 2000).

In addition, antioxidants can protect the body’s cells from free radical damage. In this respect, antioxidants have the ability to neutralize the harmful effects of reactive oxygen species. There are different types of antioxidants among them enzymatic antioxidants, food-borne antioxidants and plant antioxidants. (Jan et al., 2015). As a result, plants contain a wide range of chemicals and antioxidants or bioactive molecules through which free radicals can be neutralized, thus preventing the progression of many chronic diseases induced by oxidative stress (Dehghan et al., 2011).

The European health grain project has started a collaborative study to evaluate cereal grains for the composition of bioactive compounds. This research group examined 10 barley varieties for antioxidants and other health-promoting compounds (Andersson et al., 2008). Antioxidants identified in barley include fat-soluble vitamins, including tocopherols and tocotrienols, phenolic compounds, flavonoids including proanthocyanidins and anthocyanidins, and alkylresorcinols (Shewry, 2014).

Treatment resulted in a significant increase in SOD, CAT and GPx activity in AlCl3-exposed rats compared...
to that of intoxicated and untreated rats due to the antioxidant activity of barley. Our results are consistent with those of Akalin Çiftci et al. (2015) who found that barley extract also inhibited lipid peroxidation and increased antioxidant enzymes (GSH, SOD, and catalase) in rats fed a hyperlipidic diet, and that barley extract (H. vulgare L.) had potent antioxidant activity (Thatiparthi et al., 2019). Administration of H. vulgare extract in intoxicated rats showed that the treatment significantly improved brain architecture, to some extent, altered brain histopathology. Since Hordeum vulgare is known by its composition of polyphenols (Thatiparthi et al., 2019); polyphenols, including flavonoids, are well-known antioxidants. Recently, in a critical review of antioxidants, neuroprotective properties in the central nervous system of barley polyphenols have been reported (Giacalone et al., 2011). Barley also contains the flavonoids saponarin and lutonarin which have powerful antioxidant activities. These flavonoids are thought to be responsible for the biological activities of barley (Kamiyama and Shimamoto, 2012).

Conclusion

The present study concluded that exposure to aluminium causes oxidative stress and brain dysfunction of Wistar rats by reducing the activity of various antioxidant enzymes (CAT, GPx and SOD). The histological study confirmed the results of this toxicity, which resulted in cell necrosis in the cerebellar cortex. The administration of HVE to rats previously intoxicated with aluminium chloride induced an improvement of all the studied parameters such as the enzymes of the oxidative status and rehabilitation of this system by improving the architecture of the brain. Thus, the neuro-protection against the harmful effects of aluminium was due to the bioactive molecules such as antioxidants found in the H. vulgare plant.

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