

## Histological criteria of hippocampus as affected by acrylamide and the possible protective potential of saffron

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**ABSTRACT:** Acrylamide (ACR) became an environmental pollutant due to huge and progressive exposure for both experimental animals and humans. Alternative medicine research paid more attention to natural antioxidants. Saffron exhibited obvious antioxidant and medically-beneficial properties. Here, the present study aimed to investigate the possible neuro-protective effect of saffron against acrylamide-induced histopathological alterations in hippocampal tissue of albino rats. Adult male albino rats were categorized equally into 4 groups (n = 8); control, saffron, ACR and ACR + saffron groups. Our results recorded histopathological changes due to exposure of rats to ACR toxicity in hippocampus. The pyramidal cells of Ammon's horn (CA1- CA4) appeared degenerated and necrotic with hemorrhage, decreased thickness and increased vacuolization concomitant to shrinkage and damage of these neurons. The dentate gyrus, in its turn, appeared more affected by ACR exposure of experimental animals at the cellular level where darkly-stained and degenerated granular cells with vacuolization could be detected. Conclusion; the present study confirmed ACR-induced neurotoxicity in rats (50 mg/kg) and suggested the use of saffron extract (80 mg/kg) to prevent or delay neurological damages induced by ACR exposure.

**KEYWORDS:** Acrylamide, Neurotoxicity, hippocampus, Saffron, histopathology.

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### I. INTRODUCTION

ACR is extensively-used as an additive in molecular laboratories, chemical engineering, water purification, mining, textiles, paper manufacturing, cosmetics and protein electrophoresis (Riboldi et al., 2014; Tepe and Çebi, 2019). Natural sources of ACR have been reported recently comprising drinking water, tobacco smoke, Arabic coffee (Qahwa), coffee, tea besides occupational exposure (Khan et al., 2017). ACR has been recorded to be toxic to nervous system (Seale et al., 2012; Nassar, 2017), reproductive system (Favor and Shelby, 2005; Ma et al., 2011) and precancerous to laboratory animals (Hogervorst et al., 2010). Also, ACR has injurious effects in living body tissues such as brain, kidney, liver, testis and intestine (Rawi et al., 2012; Rahangadale et al., 2012; Jangir et al., 2016). ACR is not genotoxic by itself, but it becomes an active Glycidamide (GA) through epoxidation. Cytochrome P450 2E1 (CYP2E1) leads to the formation of GA-DNA and hemoglobin adducts (Alalwani, 2013). It is found to have a high susceptibility for binding to brain tissue (Sumner et al., 1997). Its neurotoxicity damages motor and sensory functions of central and peripheral neurons (LoPachin and Gavin, 2008). Saffron and its metabolites have attained a progressive and increasing importance in modern pharmacological studies due to its health promoting potential (Bagur et al., 2018). Therefore, the current study was planned to evaluate the possible protective effect of saffron against ACR-induced histopathological lesions in hippocampal neurons seeking for a promising role for this medicinal plant in neurotoxicity.

## II. MATERIALS AND METHODS

### Animals

Adult healthy male albino rats (n=32, weighting 200 ±10 g) *Rattus norvegicus* strain were used in this study. Animals were housed in plastic cages under standard conditions of light/dark cycle, temperature (25°C ±2) and humidity (54%). Standard pellets diet and tap water were provided ad libitum for one week before the start of experiment.

### Acrylamide:

Acrylamide (anataze form: C<sub>3</sub>H<sub>5</sub>NO) was purchased as a pure analytical grade from Sigma chemical (St. Louis, MO, USA). It is an odorless and colorless reactive molecule.

### Saffron extract

Saffron, the dried stigmas of *Crocus Sativus* flower were obtained from El-attar market, Taif, Saudi Arabia. Saffron (1 g) was soaked in boiled distilled water (100 ml). After 2 hours it was homogenized in the same distilled water, stirred for 1 hour and filtered. This aqueous extract was lyophilized and stored at 4 °C until further use (Premkumar et al., 2003).

### Experimental protocol

Animals were divided equally into 4 groups as follows: G1: Control animals, G2: Saffron group (administered water extract of saffron, 80 mg/kg), G3: ACR group (exposed to ACR 50 mg/kg) and G4: ACR+ saffron group; where saffron (80 mg/kg B.Wt.) Saffron was given three days prior to acrylamide exposure. Both were given by oro-gastric tube. At the end of exposure period (8 weeks), rats were anesthetized by Na barbitone and scarified by cervical dislocation and brains (hippocampal areas) were rapidly dissected out for histopathological study.

### Histopathological study

Hippocampal specimens after 10% formalin fixation were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in melted paraffin wax at 58°C. Paraffin blocks were sectioned at 3-4 micron thickness. The obtained tissue sections were collected on glass slides, dewaxed and processed for hematoxylin and eosin staining (Levison et al., 1996).

### Ethics Approval and Consent to Participate:

This protocol was approved by The Institutional Animal Care and Use Committee of Zagazig University, Sharkia, Egypt (ZU-IACUC/1/F/108/2020).

## III. RESULTS AND DISCUSSION

### Results

Histologically, hematoxylin and eosin-stained sections of control animals revealed the characteristic two main compartments of hippocampal tissue. These are the hippocampus proper and dentate gyrus. The hippocampus proper appeared formed of Cornu Ammonis (differentiated into CA1, CA2, CA3 and CA4). The first two composed of small pyramidal cells and CA3 and CA4 comprise large pyramidal cells. CA4 projects into the concavity of dentate gyrus (the second compartment of hippocampal proper) (Fig.1). CA1 and CA2 regions showing 5 – 6 compact layers of small pyramidal cells most of them with two vesicular nuclei (Fig.2). CA3 and CA4 regions showing few layers of large pyramidal cells with vesicular nuclei. Molecular layer (M) exhibits glial cells and scattered nerve cells within neuronal processes (axons and dendrites) (Fig.3). The dentate gyrus comprised granule cells (gc) and basket cells (bc). The basket cells lying at the granule – hilar border and some of them are found inside the granular layer. Most of granule cells appeared also having double nucleoli (Fig.4). Investigating the Hand E stained sections of saffron group showing the same histological criteria in all areas of hippocampus proper as those of controls.

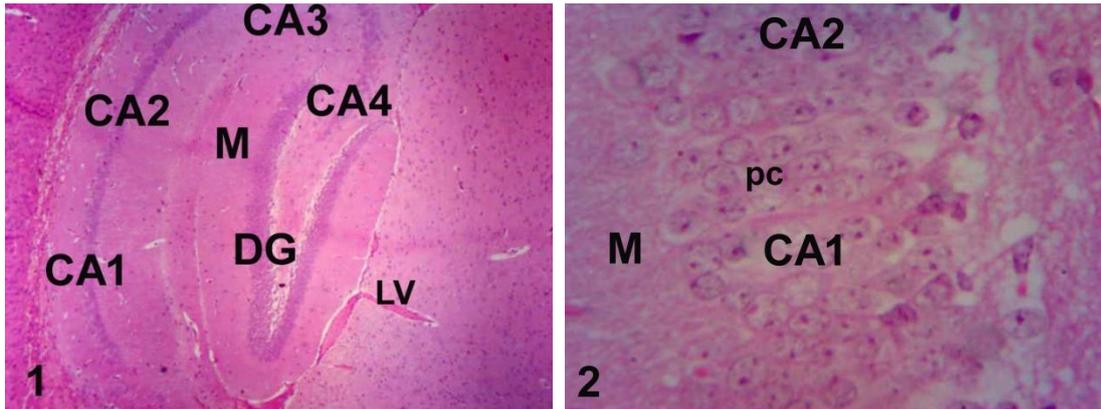


Fig. 1. Section from normal control mouse (H & E, x100) showing hippocampus proper formed of Cornu Ammonis (CA) as CA1, CA2, CA3 & CA4. Dentate gyrus (DG) embracing CA4. Note lateral ventricle (LV). M denotes molecular layer. Fig. 2. Section from normal control mouse (H & E, x1000) showing 5–6 compact layers of small pyramidal cells (pc) of CA1 and CA2. Molecular layer (M).

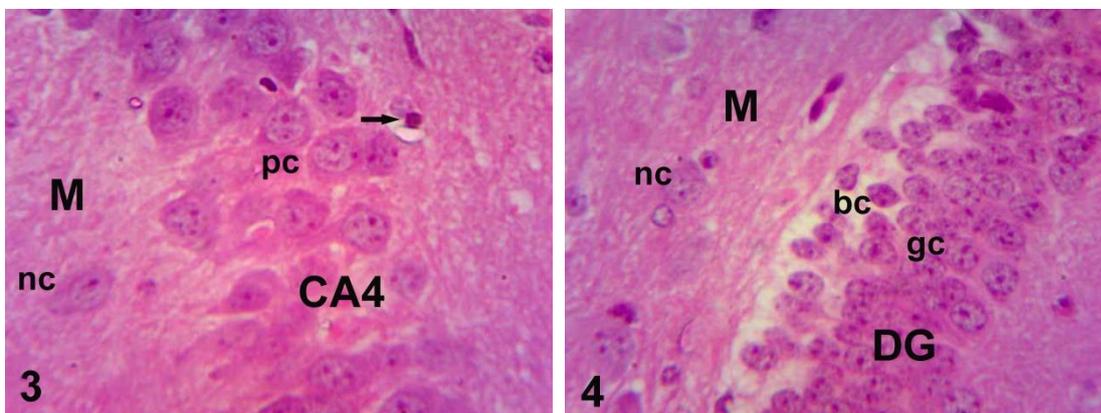


Fig. 3. Section of control mouse (H & E, x1000) showing few layers of large pyramidal cells (pc) in CA4, with vesicular nuclei. Molecular layer (M) showing glial cell (→) and nerve cells (nc). Fig. 4. Section of control mouse (H & E, x1000) showing layers of compact granular cells having dark nuclei in dentate gyrus (DG), granule cells (gc) and basket cells (bc). The basket cells located at the granule – hilar border and some of them shifted to the granular layer. Most of granule cells exhibited two nuclei. M: molecular layer with nerve cells (nc).

The exposure of rats to ACR induced marked histopathological changes in hippocampus. The pyramidal cells of Ammon's horn appeared degenerated and necrotic with hemorrhage, decreased thickness and increased vacuolization due to shrinkage and damage of these neurons. (Fig.5). Hippocampal neurons of CA4 appeared with decreased thickness of layer of large pyramidal cells in some areas (\*) with vacuolization (v) and hemorrhage (h) (Fig. 6). The dentate gyrus of hippocampus was more affected by ACR exposure of experimental animals at the cellular level where darkly-stained degenerated granular cells (gc) with vacuolization (v) could be detected. At higher magnification, neurons of the hilar portion indicating highly-destructed and darkly-stained granular cells with vacuolization (v) in the dentate gyrus (Figs. 7, 8).

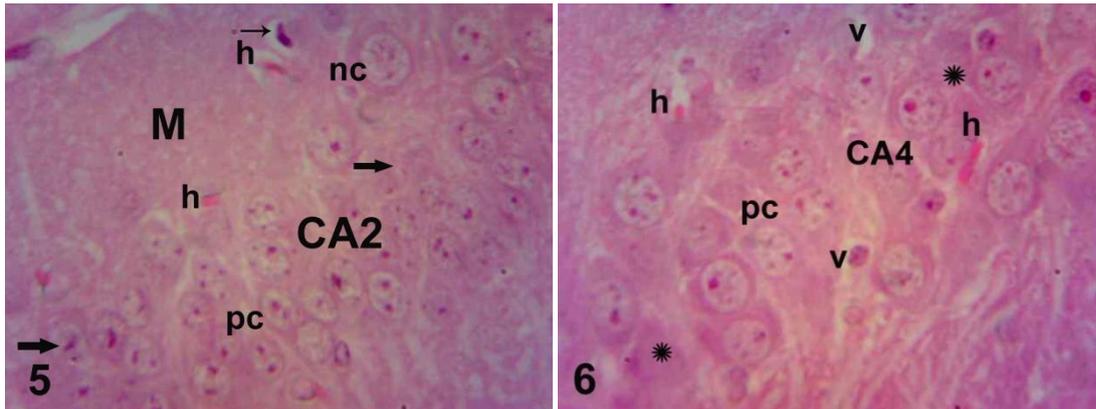


Fig. 5: Section from ACR group (H & E, x1000) showing decreased thickness of layer of small pyramidal cells of CA1 and CA2 in some areas (→), hemorrhage (h). Fig.6: Section from ACR group (H & E, x1000) showing decreased thickness of layer of large pyramidal cells of CA4 in some areas (\*), vacuolization (v), hemorrhage (h).

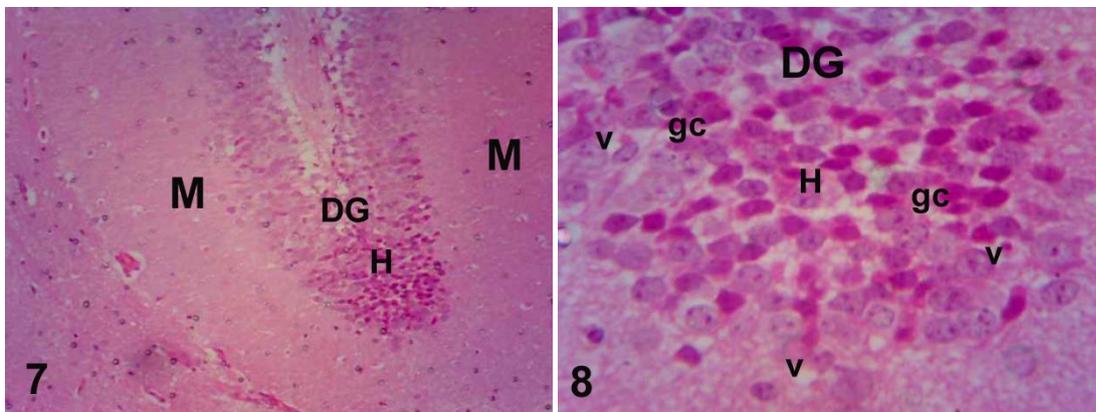
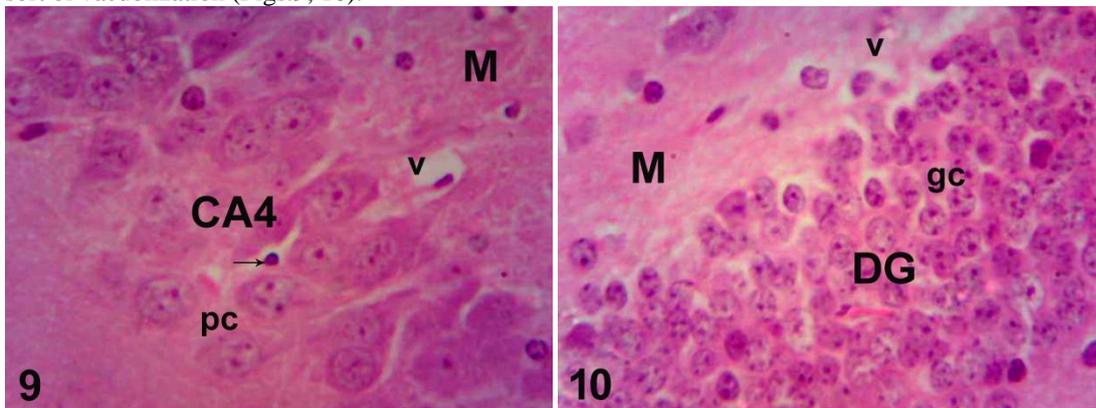


Fig.7: Section from ACR group (H & E, x1000) showing hilum (H) of dentate gyrus (DG) exhibiting darkly-stained degenerated granular cells (gc) with vacuolization (v). Molecular layer (M). Fig. 8: Section from ACR group (H & E, x1000) showing a large magnification of the last photo at the hilar portion indicating highly-destructed gc (darkly-stained) and vacuolization (v) in the DG.

ACR-exposed animals treated with saffron showing partial restoration of the normal histological pattern by both areas (cornu Amonis and dentate gyrus) with good improvement in the preservation of cells of the molecular layer in response to treatment with saffron except with persisted forms of apoptotic glial cells and some sort of vacuolization (Figs.9, 10).



Figs. 9, 10: Sections from ACR-exposed animals treated with saffron (H & E, x1000) showing partial restoration of the normal histological features by the cornu Amonis area (CA4) and dentate gyrus (DG), with their pyramidal

cells and granular cells (pc, gc respectively) with good appearance of molecular layer (M) with persisted forms of apoptotic glial cells (arrow) and vacuolization (v).

## Discussion

Hippocampus is a region responsible for memory and cognitive abilities (Sun et al., 2009; El-Marasy et al., 2018). It is highly susceptible to oxidative stress which is induced by chemicals and toxicant such as ACR resulting in neuronal degeneration and necrosis, synaptic transmission dysfunction and subsequently memory and cognitive impairment (Lynch, 2004; Jafarian et al., 2015). Exposure of rats to ACR toxicity, in the current study, induced several histopathological lesions in hippocampus. The pyramidal cells of Ammon's horn (CA1- CA4) appeared degenerated and necrotic with hemorrhage, decreased thickness and increased vacuolization due to shrinkage and damage of these neurons. The dentate gyrus, in its turn, was more affected by ACR exposure of experimental animals at the cellular level where darkly-stained and degenerated granular cells with vacuolization could be detected. The authors attributed these changes to the activity of ACR that liberate the free radicals which lead a phase of degeneration in neuronal tissue. Our results extend and confirm other previous ones; Kunnel et al. (2019) reported that lesions due to ACR- induced oxidative stress could be attributed to generated reactive oxygen species (ROS), lipid peroxidation (LPO) and mitochondrial dysfunction which lead to cellular necrosis and apoptosis. Other investigators recorded atrophy, nuclear pyknosis and neuronal injury, as ACR toxicity in rats, in hippocampal neuronal cells (Mehri et al., 2015; Mansour et al., 2017; Aboubakr et al., 2018). Zhao et al. (2020) recorded neuronal necrosis in the hippocampus, and degeneration of Purkinje cells and reduction of granular layer cells in cerebellum of rats exposed to ACR. They explained that ROS may attack cell membranes during exposure, and lower antioxidant enzyme activity leading to malondialdehyde (MDA) increase (Jiang et al., 2018). The latter results are necrosis and degeneration of nerve cells (Zhu et al., 2008; Ghorbel et al., 2016). The damage of various cells in hippocampus that we observed, in the current study, further supported LoPachin et al. (2007) found that exposure to ACR may also lead to central and/or peripheral distal axonopathy. Radad et al. (2020) showed that gavage of ACR to rats induced neuronal degeneration in hippocampus and cerebellum. Also, Lai et al. (2017) found that toxic effects on the growth and development of hippocampal neurons were recorded due to ACR. El-Bakry et al. (2013) and Al-Gholam et al. (2016) attributed ACR-induced neuronal lesions to its oxidative damage and reduced activity of some antioxidant enzymes. Hippocampus is susceptible to oxidative stress upon exposure to chemicals and toxicants (Wang et al., 2005), which induced neuronal death via apoptosis and resulted in neuronal damage and memory impairment (Kudryashov et al., 2002). Moreover, Elblehi et al. (2020) reported that dentate gyrus of hippocampus showed disorganization and vacuolization of granule cells (may be due to mild edema in brain tissue), as well as disorganized degenerated and necrotic pyramidal cells in CA4. Excessive neuronal apoptosis in hippocampus contributes to loss of memory (Sun et al., 2009). Correspondingly, Park et al. (2010) stated that during the postnatal period, ACR can suppress neurogenesis in the dentate gyrus. Also, brain of ACR-treated rats showed neurons with coarse and clumped hyperchromatic chromatin with granular or amorphous gray cytoplasm. These changes confirmed the degenerative effect of ACR on brain tissue (Mannaa et al., 2006; Ahmed et al., 2010). Wei et al., (2015) demonstrated that the ACR has neurotoxic effect on both CNS and PNS by aggregation of neurofilaments in their large axons. The alterations and high levels of neurofilaments in axons were the main source of degeneration (Lee et al., 1993). Erdemli et al. (2016, 2018) reported that ACR administration significantly increased MDA and total oxidant status levels in rat brain tissues, and decreased total antioxidant status (glutathione and neurotrophic factor levels) as well as the number of neurons in cerebral cortex. It is conclude that exposure to ACR during pregnancy should be avoided and adequate amounts of antioxidants, such as vitamin E, should be consumed. Zhang et al. (2020) demonstrated that exposure to acrylamide decreases the density of noradrenergic axons and the level of nor-epinephrine (NE) in hippocampus of male rats. NE level and density of noradrenergic axons might be more susceptible to ACR exposure than the neurogenesis in rats. Also, histopathological alterations of Qusti and Al-Qahtani (2015) showed hemorrhage, shrinkage in neurons, disappearances of nuclei and ischemic damage (ischemic injury) to the nervous system resulting in red neuron. The soma (cell body) is shrunken, the cytoplasm is intensely eosinophilic, and the nucleus is pyknotic with no discernible nucleolus. The neuronal damages in ACR-intoxicated rats, with observed histopathological changes in hippocampus are cerebral cortex, cerebellum, and sciatic nerve, as well as increased plasma LDH activity. LDH is an important brain injury biochemical marker and considered a cytosolic metabolic enzyme and its release into the blood indicates various cellular damages (Ingebrigtsen and Romner, 2002; Farhana and Lappin, 2020). Several inflammatory cytokines were reported in activated microglia and astrocytes (Zhao et al., 2017a; Zhao et al., 2017b; Pan et al., 2018; Yan et al., 2019) suggesting that the pathogenesis of ACR-induced neuropathy was attributed to neuro-inflammation (Liu et al., 2020).

**Neuroprotective effect of saffron**

In the present work, supplementation of saffron extract to ACR-exposed rats improved the histological pattern of hippocampus against ACR neurotoxicity due to its antioxidant activity; These results are in consistent with those of other investigators; Qusti and Al-Qahtani (2015) reported that oral administration of saffron with ACR to experimental rats showed semi normal tissue in molecular layer but some hemorrhage and shrunken neurons are still present in hippocampus. Soeda et al. (2001) showed that crocin not only has the anti-apoptotic effect, but also prevents their death. The onset which suggests that crocin inhibits neuronal death induced by both an internal and an external apoptotic stimulus in ischemia (highly differentiated neurons cells). The effectiveness of crocin on ischemia/reperfusion injury in mice micro vessels was investigated by Zheng et al. (2007) and the beneficial effect of its pretreatment (20 and 10 mg/Kg) against cerebral ischemia through the inhibition of oxidizing reactions and modulation of the ultra-structure of cortical microvascular endothelial (CMEC) was shown in mice. Moreover, saffron is beneficial for curing nervous pains and brain damages (Bisset and Wichtl, 2001). Delkhosh-Kasmaiea et al. (2018) showed that safranal produced improving effects on metabolic and behavioral alterations as well as histopathological and biochemical changes in hippocampus. Researchers have reported beneficial effects of saffron in some aspects of diabetes mellitus (DM). For example, saffron improved blood glucose concentration, serum SOD and catalase (CAT) activities, and MDA level in STZ (60 mg/kg)-induced T1DM in rats (Samarghandian et al., 2013). Dopaminergic neuron death and reactive oxygen species (ROS) generation was inhibited with safranal treatment in a rat model of rotenone-induced Parkinson's diseases (Pan et al., 2016). Moreover, safranal recovered neuronal loss in the CA1 and CA3 subfields of the hippocampus in a rat model of transient cerebral ischemia (Sadeghnia et al., (2017). Beside to exert antidepressant effect, safranal ameliorated brain MDA level elevation as well as GSH activity reduction in the brain tissue of chronic immobilization stressed rats (Samarghandian et al., 2017). Long-term safranal treatment improved functional, histopathological and biochemical alterations induced by sciatic nerve crush injury in rats (Tamaddonfard et al., 2014). The study of Shafahi et al., (2018) showed that crocin has antioxidant activities and decreases MDA level; also, an increase in SOD and GSH levels in the hippocampus was reported after methamphetamine-induced neurotoxicity. This result according to a previous study shows that the inhibition of SOD promotes methamphetamine neurotoxicity, and also in the previous study demonstrated crocin via enhancing the activities of GSH reductase and  $\gamma$ -glutamyl cysteinyl synthase ( $\gamma$ -GCS), and increasing the GSH levels inhibits apoptosis in PC-12 cells Hirata et al., 1996; Ochiai et al., 2004). Guo et al. (2020) demonstrated the anti-apoptotic, antioxidant and anti-inflammatory effects of curcumin on ACR-induced neurotoxicity in rats, suggesting the use of curcumin to prevent or delay neurological damages induced by ACR exposure. In line with the evidences from humans and animals (Pennisi et al., 2013; Wang et al., 2018; Sun et al., 2018; Goudarzi et al., 2019; Adewale et al., 2015), their study showed that the 4-week exposure of rats to ACR at the dose of 40 mg/kg caused a significant body weight loss, progressive deficits in motor function and adverse pathological outcome in the cortex and hippocampus of rats. Importantly, the present data revealed that curcumin administration could efficiently rescue ACR-induced weight loss and neurobehavioral deficits, relieve the neuropathological damages in brain. Other studies have shown that the abnormal gait of rats is closely related to the cerebellar nerve injury induced by AA (Dortaj et al., 2018; Pan et al., 2015). Erdemli et al. (2021) reported that ACR gavage caused disruption of biochemical, histopathological and cognitive functions in adult rats particularly brain. However, vitamin E provided protection against neurotoxicity in adult rats. Conclusion; the present study confirmed ACR-induced neurotoxicity in rats and suggested the use of saffron extract (80 mg/kg) to prevent or delay neurological damages induced by ACR exposure

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