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A STUDY ON THE ROLE OF *TRIBULUS TERRESTRIS* IN CADMIUM-INDUCED TOXICITY ON SOME ORGANS OF ADULT MALE ALBINO RATS

EMAN AHMED NEGM [1]; ALSHAIMAA A. ALGHRIANY [2]
AND AHMED A. MOHAMMED [3]

[1] Department of Physiology, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt, Fax: 0882366503, Postal code: 71526

[2] Department of Zoology, Faculty of Science, Assiut University, Assiut 71526, Egypt

[3] Department of Animal and Poultry Behavior and Management, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt

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ABSTRACT

Cadmium (Cd) is a dangerous environmental pollutant that causes toxicity to humans and animals even when exposed to a small amount as it is hard to expel from the body affecting all body parts. *Tribulus terrestris* (Tt) is a natural herb used for its medicinal effects around the world. This study directed to detect the possibility of Tt to protect from Cd toxicity in adult male albino rats. Twenty-four rats were separated into four groups, each 6 rats: The first group (C); served as a control without any treatments. Group (2) (Tt + Cd); administrated daily by oral dose of Tt (5 mg/ kg b. wt. liquified in distilled water) for eight weeks then from the start of the ninth week, they daily injected intraperitoneally with Cd (2 mg/kg b. wt.) for eight days. Group (3) (Cd); injected with Cd only at the same dose for eight days. Group (4) (Cd + Tt); firstly, injected with Cd at the same dose for eight days then on the ninth day they had the same Tt dose for eight weeks. In this study, results clarified that Cd application significantly decreased plasma SOD level and the time spent in the opened arms of the elevated plus maze test while increased plasma levels of H_2O_2, CRP, IL-6, immunoreaction of brain cleaved caspase-3 and the time spent in the closed arms of the elevated plus maze test compared to control. Administration of Tt succeeded to increase SOD level and the time spent in the opened arms of the elevated plus maze test; however, it reduced plasma levels of H_2O_2, CRP, IL-6, immunoreaction of brain cleaved caspase-3 and the time spent in the closed arms of the elevated plus maze test in comparison with Cd group. In conclusion, Tt is highly protective for the brain from harmful Cd effects, referring to its antioxidant, anti-inflammatory and antiapoptotic properties.

Keywords: *Tribulus terrestris*, cadmium, brain, behavior, oxidative stress, cytokines, apoptosis, Caspase-3, histopathology.

INTRODUCTON

Cadmium (Cd) is a very toxic heavy metal and ubiquitous environmental pollutants in soil, water, food, smoke and air. It is used in industry for coating steel, plastics and glass. It induces toxicity in various target organs such as the placenta, kidneys, lungs, liver, pancreas, testes, bones and brain (Cuypers *et al.*, 2010). Also, it inhibits the antioxidant potential, thiol status, DNA repair and methylation causing cell impairment (Czeczot and Skrzycki, 2010). Cd accumulates in the hippocampus to a greater

Corresponding author: Eman Ahmed Negm
E-mail address: Emannegm@vet.aun.edu.eg,
Present address: Department of Physiology, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt

extent than in other parts of the brain (Mukherjee *et al.,* 2010). Hippocampal dysfunction is responsible for the behavioral abnormalities. In this direction, exposing rats to Cd increased anxiety and fear; however, it decreased memory (Brenneman *et al.,* 2000). Neuroendocrine alteration (i.e., stimulation of the hypothalamic-pituitary-adrenal axis) has been suggested to be associated with anxiety-like disorders (Morris *et al.,* 2012). Adrenocorticotropic hormone (ACTH), cortisol (CORT), and corticotropin-releasing factor (CRF) are responsible for hypothalamic pituitary adrenal axis dysfunction and the mammalian responses to stress (Kioukia-Fougia *et al.,* 2002).

Also, Cd exposure as a popular environmental contamination has a great adverse effect on human and animal health. Studies discussed the ability of Cd to change the adaptive, mucosal and innate immune in relation to gene expression, microbial infection sensitivity and chemokine production. Its toxicity is often due to the generation of free radicals causing oxidative stress and inflammation that mediated by various pathways leads to inflammatory chemokines and cytokines production (Das and Al-Naemi, 2019).

Interleukin-6 (IL-6) is a crucial pro-inflammatory cytokine and a well-established indicator of inflammation. It is a highly sensitive proinflammatory cytokine to brain damage and can be simply noticed in cerebrospinal fluid and serum (Hergenroeder et al., 2010). It is involved in numerous patho-physiological procedures as it is known to control inflammation, immunity, hematopoiesis, neural growth and bone metabolism. Besides, IL-6 has been involved in autoimmune disease, aging, Alzheimer's disease, osteoporosis, and neuronal injury (Woodcock and Morganti-Kossmann, 2013).

C-reactive protein (CRP) is a key indicator of inflammation, a protein released from hepatocytes due to increased inflammatory cytokines. It has been recorded as a mirror to brain inflammatory indication, as its elevation would relate to the modulation in brain metabolites indicating early brain disorders (Hilal *et al.,* 2018). CRP enhancement is back to the spread of inflammatory reactions and is involved in neurodegenerative defects through stimulation of activator protein 1 (AP-1) (Yang *et al.,* 2007).

Tribulus terrestris (Tt) (family Zygophyllaceae), is usually called a *puncture vine or Gokharu*. It contains ingredients that are medicinally important, such as flavonol, flavonoids, alkaloids, glycosides, terpenoids, tannins, phenol carboxylic acids, and steroidal saponins (Ștefănescu *et al.,* 2020). It is a medicinal and traditional plant that used for thousands of years in various countries. Tt extractions are sold nowadays in Europe and the United States as a food supplement that aims to have an enhancement influence on muscle tone, rejuvenation and athletic activity. Indian and Chinese also used it for the treatment of various diseases, and it is known to enhance men's sexual functions (Gauthaman *et al.,* 2002).

Several studies have shown a promising outcome in using Tt as an anti-anxiety replacement in rats under stressful conditions (Zhang *et al.,* 2008). Also, it has been recorded to have antioxidant influences against many experiments induced oxidative stresses (Kadry *et al.,* 2010). New research has shown that pretreatment with Tt extraction has a cerebrovascular protective property, and significantly reduced mitochondrial changes, apoptosis and cell ischemia. It also has antidiabetic, diuretic, immunomodulatory, hypolipidemic, hepatoprotective, central nervous system protection, anti-inflammatory, anti-bacterial analgesic, anticancer, antispasmodic, larvicidal and anthelmintic functions (Chhatre *et al.,* 2014).

The hippocampus is the area of spatial memory consolidation and crucial information selection. The entorhinal cortex

and the hippocampal formation [dentate gyrus, a series of Cornu Ammonis areas (CA1, CA2, CA3 and CA4), subiculum] convey the environmental sensory impulses that the cortical sensory receives then returned to the cortical region (Andersen *et al.*, 2007). When there are significant temporal gaps between stimulus components, CA1 cells are crucial for linking regularly received input. The CA3 cells of the hippocampus are important for preserving fresh spatial information in short-term memories that last for a few seconds to a few minutes. Damage to the pyramidal cells in the CA1- CA3 areas brain can impair memory and spatial learning (Ma *et al.*, 2010).

The cerebellum is one of the most interesting parts of the body. Even though it looks simple, it has more computing power and can process more information than any other part of the brain. Changes in their normal state seem to cause terrible conditions that spread through the brain's major circuits. The cerebellum is known to maintain motor coordination by recognizing neural patterns and predicting appropriate motions. Cerebellum injury inhibits learning and causes motor abnormalities (Reeber *et al.*, 2013).

Purkinje cells are the fundamental element of cerebellar circuit and can only be found in the cerebellar cortex. They are noteworthy for their enormous, elaborately branched, flat dendritic trees, which give them the ability to process great amounts of information and learn by remodeling their dendrites. These trees are also immediately recognizable by their size. Purkinje cells are required not just for well-coordinated movement but also for other aspects of function, such as cognition and emotion (Beckinghausen and Sillitoe, 2019).

MATERIALS AND METHODS

Drug, kits and Animals

Cadmium chloride ($CdCl_4$) was obtained from C.P. Evans. Co, Egypt. Natural extract of *Tribulus terrestris* was bought from Germany, Elmo. natur, 604289 in Leipzig.

Using ELISA (Dynatech Microplate Reader Model MR 5000, Canada, 478 Bay Street, Suite A213 Midland), plasma C-reactive protein (CRP), pro-inflammatory interleukin-6 (IL-6) and antioxidant marker superoxide dismutase (SOD) levels were detected by ELIZA test kits that bought from SinoGeneClon Biotech Co., Ltd. in YuHang District, Hang Zhou, China, at No. 9 BoYuan Road. Reagent kits purchased from Biodiagnostic, Dokki, Giza, Egypt, to calculate free radical Hydrogen peroxide (H_2O_2) value spectrophoto-metrically.

Twenty-four male adult albino rats (150–200 gm body weight) were acclimatized one week before the experiment, kept in separate cages 12 hours of the light-dark cycle, free feed and drink water, relative humidity (50 ± 10) % and temperature 28 ~ 34 °C were maintained.

Animal grouping and experimental approach:

The protocol was approved (with permission number 17300892) by the Local Experimental Ethical Committee of the Deanship of Scientific Research of the Faculty of Medicine at Assiut University in Assiut, Egypt.

Experimental Design:

Four groups; each consisted of 6 rats: Group (1) (C); acted as a control without any treatments. Group (2) (Tt + Cd); administrated daily by oral dose of Tt (5 mg/ kg b. wt. liquified in distilled water) for eight weeks then from the start of the ninth week, they daily injected intraperitoneally with Cd (2 mg/kg b. wt.) for eight days. Group (3) (Cd); injected with Cd only at the same dose for eight days. Group (4) (Cd + Tt); firstly, injected with Cd at the same dose for eight days then on the ninth day they had the same Tt dose for eight weeks.

Behavioral tests

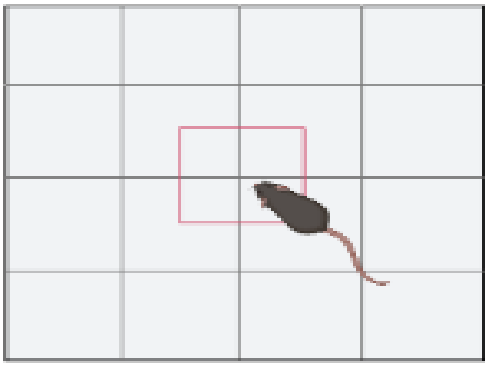
1. Elevated plus maze test

It was used to detect anxiety behaviors (Mohammed *et al.*, 2020**).** We designed the

device from yellowish wood. The device was elevated at a height of 70 cm and the arms length was 60 cm and the width was 10 cm. The closed arms were closed by 50 cm edges while leaving the open arms edges opened to allow the entrance of the rats (Treit *et al.*, 1993)**.** The tested rats were put for 5 min at the device center. Recording the time spent by rats in the various device arms, also, the entered number in the various device parts was calculated. The device was cleaned with alcohol between the tested rats to remove the previous rat odor.

2. Open field test

Anxiety-related behavioral test (Time spent in the central and peripheral squares of the device, and the rearing) (Mohammed *et al.*, 2020)**.** The device was a glass square enclosure (high 70 cm and diameter 100 cm). This test was done at the end of the experiment. The device was separated into peripheral and central squares; the central one was divided into equal-sized 4 divisions. The test started by putting rats in the device's peripheral squares. Alcohol solution was used between the rats to get out of the previous rat odor.



Samples collection

All rat groups were anesthetized, blood collected from the jugular vein in tubes containing EDTA and centrifuged for 10 minutes at 3000 rpm to obtain plasma that was frozen at -20 °C for further biochemical parameters estimation.

For a histological study, the brain was fixed quickly in neutral buffered formalin 10 % (pH 7.2). The paraffin-embedded method was used to make brain sections. Then, they are washed, dried in 70 % to 100 % ethanol solutions to get rid of water, and cleaned in xylene before being set in wax. Using a rotatory microtome, 5 µm thick sections of paraffin blocks were cut, and the paraffin was then removed in xylene. Lastly, the normal staining Hematoxylin and Eosin method was used for general histological examination (Bancroft & Gamble, 2008). The thickness of the pyramidal cell layer in CA1 and CA3 hippocampus regions was measured in micrometers. The Purkinje cells were counted per millimeter from cerebral folia (Maloku *et al.*, 2010). We used a digital camera (Toup Tek ToupView, China, Version: x86, Copyrightc 2019, Compatible: Windows XP/Vista/7/8/10), ImageJ software, and a computer linked to a light microscope (Japan, Olympus CX31) to examine and photograph of the samples.

Immunohistochemical examination of cleaved caspase-3 expression

Tissues from the brain were fixed with formalin and put in a neutral buffered (pH 7.2) 10% solution. Then, they were put in paraffin cleaned, rehydrated in solutions of 100 % to 70 % ethanol, and then rinsed in water. Slides were boiled for 10 minutes in 1 mM ethylenediaminetetraacetic acid (EDTA) to unmask the antigens, and then sections were removed and emerged in 3% hydrogen peroxide for 10 minutes. Each piece was left for an hour in a blocking solution at room temperature. The main cleaved caspase-3 antibody (USA, 1:1000, Novus Biologicals, LLC) was then added to brain sections for 24 hours. The secondary antibodies (USA, 1: 5000, Novus Biologicals, LLC) were then applied for 2 hours. After a 2–3-minute reaction with 3,3′-diaminobenzidine, the slices were stained with hematoxylin for 2–5 minutes (Atia & Alghriany, 2021).

Statistical Analysis:

Statistics were represented in a mean ± standard error (SEM). One-way ANOVA was used to analyze the data followed by Multiple Comparisons of Bonferroni's test with Prism software (version 8.0.1; GraphPad Software,

USA, Inc San Diego, CA. Differences of $p < 0.05$ were significant statistically.

RESULTS

The effect of Tt administration on anxiety-like behaviors of elevated plus maze and open field tests of rats before injection with Cd are presented in Table 1. In the elevated plus maze test, there were no treatment effects on all the measured parameters between the control versus the Tt + Cd group. However, the time elapsed in the opened arms was increased ($P < 0.05$) in the control, Tt + Cd, and Cd + Tt groups in comparison with the Cd groups, but the time passed in the closed arms was decreased in the control and the Tt + Cd groups only in comparison the Cd group ($P < 0.05$). There were no treatment effects on all the parameters of the open field test between all the tested groups ($P > 0.05$).

Effect of Tt supplementation on plasma level of SOD, H_2O_2, CRP and IL-6 in rats injected by Cd is shown in table 2. Rats group injected with Cd only caused a significant decline in SOD concentration ($P < 0.001$) when compared to the control one. However, the addition of Tt either in (Tt + Cd) or (Cd +Tt) groups significantly increased SOD level compared to Cd group ($P < 0.01$ and $P < 0.05$; respectively).

There was a significant increase in H_2O_2 and IL-6 concentrations in rats after Cd injection versus the control ($P < 0.001$). Administration of Tt either in (Tt + Cd) or (Cd +Tt) groups significantly decreased H_2O_2 and IL-6 levels compared to Cd group ($P < 0.01$ and $P < 0.05$; respectively).

CRP plasma level significantly increased in rats after Cd injection compared to the control group ($P < 0.001$). Otherwise, administration of Tt either before and after Cd injection in (Tt + Cd) or (Cd +Tt) groups significantly decreased the CRP levels in rat plasma compared to Cd group ($P < 0.001$ and $P < 0.01$; correspondingly).

Table 1: Shows the effect of Tt administration on anxiety like behaviors of elevated plus maze and open field tests of rats before injection with Cd (no. = 6 in each group).

Treatment	C	Tt + Cd	Cd	Cd + Tt	P value
Elevated plus maze test					
Time spent in opened arms	1.90 ± 0.17^{a}	1.66 ± 0.17^{a}	0.26 ± 0.17^{b}	1.20 ± 0.17^{a}	0.0007
Time spent in closed arms	2.83 ± 0.20^{b}	2.93 ± 0.20^{b}	4.33 ± 0.20^{a}	3.53 ± 0.20^{ab}	0.0030
No. of enters in opened arms	2.00 ± 0.53	2.00 ± 0.53	2.66 ± 0.53	3.00 ± 0.53	0.4823
No. of enters in closed arms	2.00 ± 0.47	1.66 ± 0.47	2.00 ± 0.47	1.66 ± 0.47	0.9159
Open field test					
Time spent in line crossing	4.38 ± 0.05	4.44 ± 0.05	4.43 ± 0.05	4.46 ± 0.05	0.7262
Time spent in line central	16.33 ± 4.51	5.00 ± 4.51	2.33 ± 4.51	2.66 ± 4.51	0.1735
Time spent in rearing	5.33 ± 2.26	10.33 ± 2.26	14.66 ± 2.26	10.66 ± 2.26	0.1043

C: control; Tt*: Tribulus terrestris*, Cd: cadmium. Data are presented as Mean ± SE. Values in the same row followed by different superscript ($^{a, b}$) are significant ($P < 0.05$).

Table 2: Shows the effect of Tt supplementation on plasma level of SOD, H_2O_2, CRP and IL-6 in rats injected by Cd.

Treatment	C	Tt + Cd	Cd	Cd + Tt	P value
SOD (pg/ml)	159.4 ± 1.151^{a}	156.2 ± 0.207^{b}	152.6 ± 0.212^{c}	155.4 ± 0.483^{b}	0.0029
H_2O_2 (mm/L)	0.914 ± 0.004^{a}	0.990 ± 0.003^{b}	1.062 ± 0.020^{c}	1.006 ± 0.007^{b}	0.0015
CRP (ng/ml)	12.92 ± 0.166^{a}	20.78 ± 0.309^{b}	23.30 ± 0.138^{c}	21.82 ± 0.276^{d}	0.3810
IL-6 (ng/L)	122.4 ± 0.977^{a}	146.9 ± 0.431^{b}	152.4 ± 1.053^{c}	148.4 ± 0.568^{b}	0.3089

C: control; Tt*: Tribulus terrestris*, Cd: cadmium; SOD: Superoxide dismutase; H_2O_2: Hydrogen peroxide; CRP: C-reactive protein; IL-6: Interleukin-6**.** Data are presented as Mean ± SE. Values in the same row followed by different superscript ($^{a, b, c, d}$) are significant ($P < 0.05$).

Histopathological examinations

Hematoxylin and eosin-stained hippocampus sections in the control group showed normal structure. The CA1 and CA3 regions were composed of three layers:

- Polymorphic layer: contained glial cells with light- and dark-stained nuclei.
- The pyramidal cell layer contains closely compacted layers of small pyramidal neurons in CA1 (Fig. 1a) and loosely compact layers of huge pyramidal neurons in CA3 (Fig. 2a). The pyramidal cells had prominent nucleoli and rounded vesicular nuclei.
- Molecular layer: contains glial cells with light- and dark-stained nuclei.

In CA1 region sections, the (Tt + Cd) group had normal small pyramidal cells with rounded vesicular nuclei. A few shrunken pyramidal cells contain pyknotic nuclei and spaces around them were observed (Fig. 1b). Cadmium administration in (Cd) group showed a sever decline in the thickening of the pyramidal cell layer. Shrunken pyramidal cells were observed with deeply stained cytoplasm and pyknotic nuclei with spaces around them (Fig. 1c). In the (Cd + Tt) group, there were long degenerated pyramidal cells having pyknotic nuclei and spaces around them, as well as normal small pyramidal cells having round vesicular nuclei (Fig. 1d). Statistically, the thickening of the pyramidal cell layer in CA1 region in (Cd) group significantly declined ($P < 0.01$) versus control group. That thickening increased significantly ($P < 0.05$) in (Tt + Cd) and (Cd + Tt) groups when compared with (Cd) group but was non-significant versus the control one (Fig. 7a).

In CA3 region sections, the (Tt + Cd) group had normal large pyramidal cells and few elongate degenerated pyramidal cells contained pyknotic nuclei and spaces around them (Fig. 2b). Sections from (Cd) group (Fig. 2c) showed a decrease in thickening of the pyramidal cell layer. Shrunken, degenerated pyramidal cells had pyknotic nuclei and spaces around them Empty spaces contain remnants of degenerated cells. **(**Cd + Tt) group displaying elongated, degenerated large pyramidal cells containing pyknotic nuclei and spaces around them, as well as normal large pyramidal cells had rounded vesicular nuclei (Fig. 2d). Statistically, the thickening of the pyramidal cell layer in CA3 region in (Cd) group decreased significantly ($P < 0.01$) versus the control group. That thickening increased significantly ($P < 0.01$) in (Tt + Cd) and (Cd + Tt) groups when compared to (Cd) group but was non-significant versus the control group (Fig. 7b).

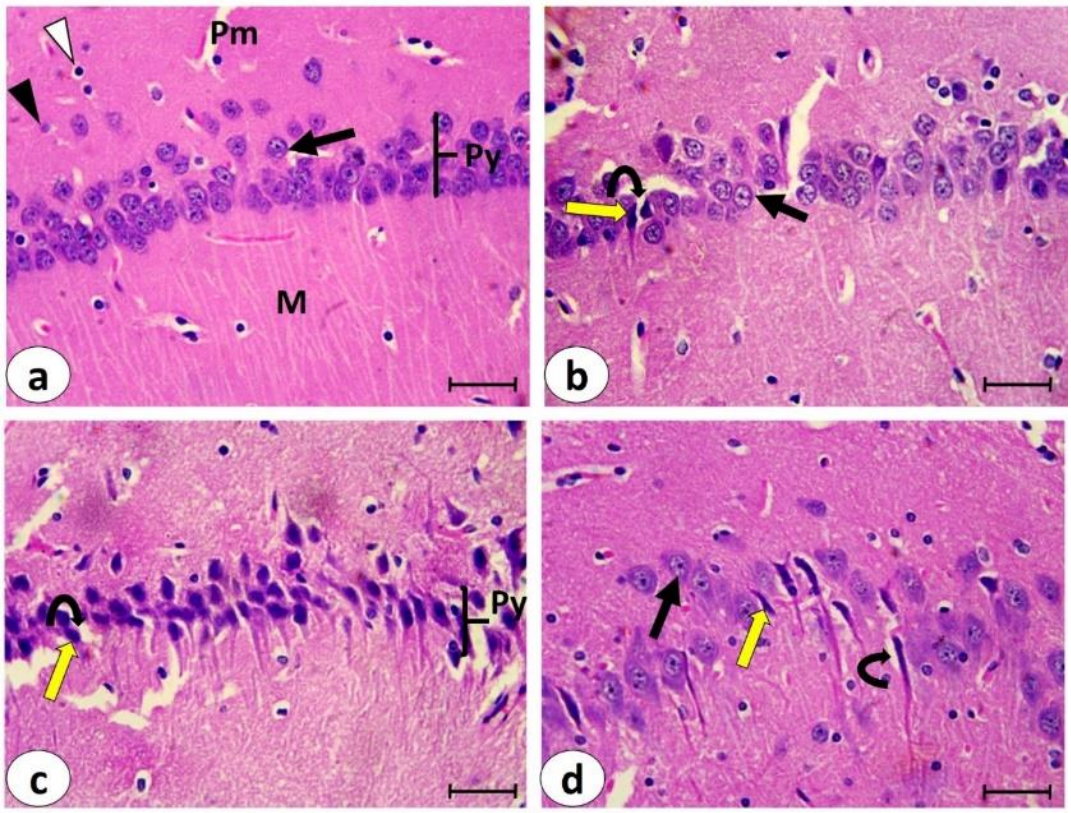


Figure 1: Photomicrographs of CA1 region of hippocampus sections from experimental groups **a**: control group showing polymorphic layer (Pm), pyramidal cell layer (Py), and molecular layer (M). Polymorphic and molecular layers contain light (Δ) and dark (▲)-stained nuclei of glial cells. The pyramidal cell layer contains closely compact layers of small pyramidal neurons (↑) with rounded vesicular nuclei and prominent nucleoli. **b: (Tt + Cd)** group showing normal small pyramidal cells with rounded vesicular nuclei (↑). A few shrunken pyramidal cells with pyknotic nuclei (yellow arrow) and spaces around them (curved arrow) are present. **c**: (Cd) group showing a marked decrease in thickening of the pyramidal cell layer (Py). Shrunken pyramidal cells (yellow arrow) with deeply stained cytoplasm and pyknotic nuclei are shown with spaces around them (curved arrow). **d**: (Cd + Tt) group showing elongate, degenerated pyramidal cells with pyknotic nuclei (yellow arrow) and spaces around them (curved arrow). Normal small pyramidal cells (↑) with rounded vesicular nuclei are shown. (H&E stain, Bar = 50 μm).

The cerebellar cortex sections of the control group clarified normal structure (Fig. 3a). It was composed of lobulations and folia, which differentiated into:

- Outer molecular layer: has stellate cells and basket cells.
- The middle layer of Purkinje cell: has a layer of pear-shaped Purkinje cell containing vesicular rounded nuclei and protuberant nucleoli and are enclosed by glial cells.
- Inner granular layer: has granule cells

In (Tt + Cd) group (Fig. 3b), normal Purkinje cells appeared. Some Purkinje cells were seen with severely stained or fragmented nuclei. Remnants of degenerated Purkinje cells and deeply stained nuclei of basket cells were detected. In (Cd) group (Fig. 3c), the molecular layer was observed with noticeable

deeply stained nuclei of basket cells. The Purkinje cell layer was nearly devoted to Purkinje cells. The still-present Purkinje calls were deeply stained, shrunken, and had pyknotic nuclei. Empty areas from Purkinje cell loss were filled with glial cells. The granular layer is filled with densely stained nuclei of granular cells. In **(**Cd + Tt) group, deeply stained nuclei of basket cells, densely stained shrunken Purkinje cells with pyknotic nuclei, empty areas from Purkinje cell loss, and normal Purkinje cells with rounded vesicular nuclei were detected (Fig. 3d). Statistically, Purkinje cells count per millimeter of Purkinje cell layer in (Cd) group decreased significantly ($P < 0.001$) versus control group. Purkinje cell number increased significantly in (Tt + Cd) and (Cd + Tt) groups when compared with (Cd) group ($P < 0.001$, $P < 0.01$ respectively). Non-significant differences occurred between the numbers of cells in the control and (Tt + Cd) groups. Purkinje cells number significantly declined in (Cd + Tt) group ($P < 0.05$) in comparison with the control one (Fig. 7c).

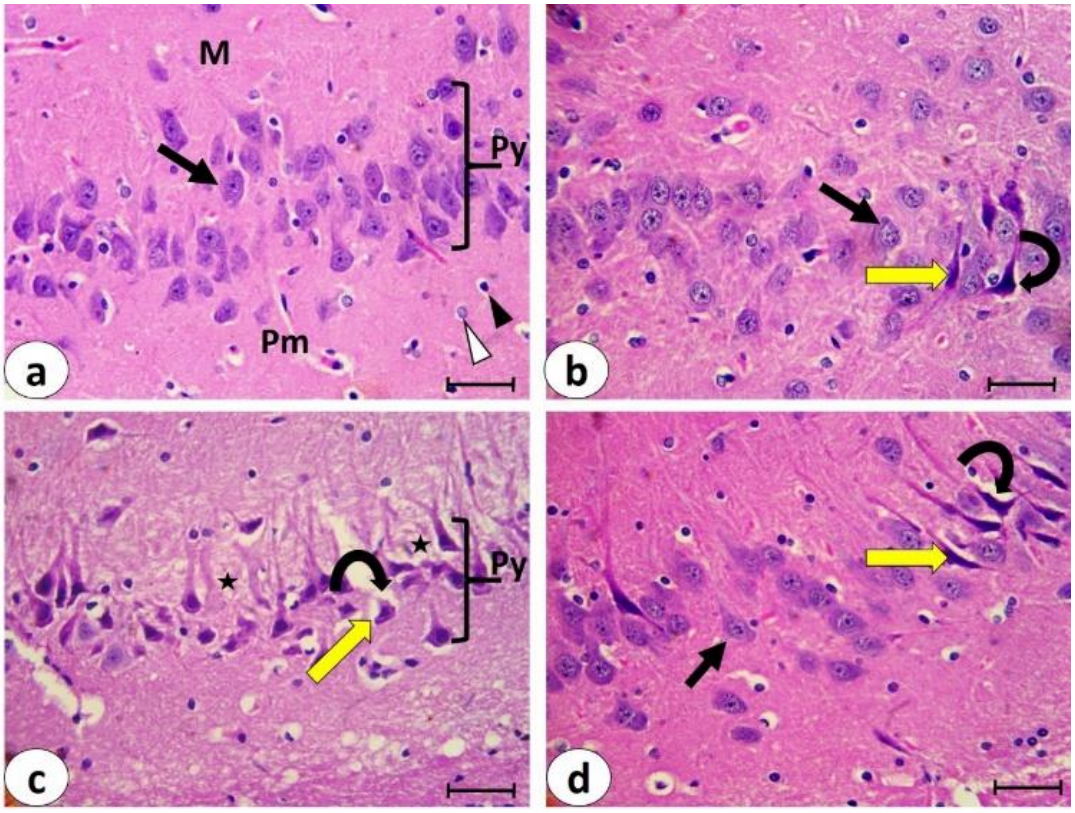


Figure 2: Photomicrographs of CA3 region of hippocampus sections from experimental groups **a**: control group showing molecular layer (M) and polymorphic layer (Pm) contain light (Δ) and dark (▲)-stained nuclei of glial cells. The pyramidal cell layer (Py) contains loosely compact layers of large pyramidal neurons (↑) with rounded vesicular nuclei and prominent nucleoli. **b: (Tt + Cd)** group showing normal large pyramidal cells (↑) and a few elongated degenerated pyramidal cells with pyknotic nuclei (yellow arrow) with spaces around them (curved arrow). **c**: (Cd) group showing: decrease in thickening of the pyramidal cell layer (Py), shrunken degenerated pyramidal cells (yellow arrow) with pyknotic nuclei with spaces around them (curved arrow), and empty spaces contain remnants of degenerated cells (asterisk). **d: (**Cd + Tt) group displaying elongated degenerated large pyramidal cells with pyknotic nuclei (yellow arrow) and spaces around them (curved arrow), as well as normal large pyramidal cells (↑) with rounded vesicular nuclei. (H&E stain, Bar = 50 μm).

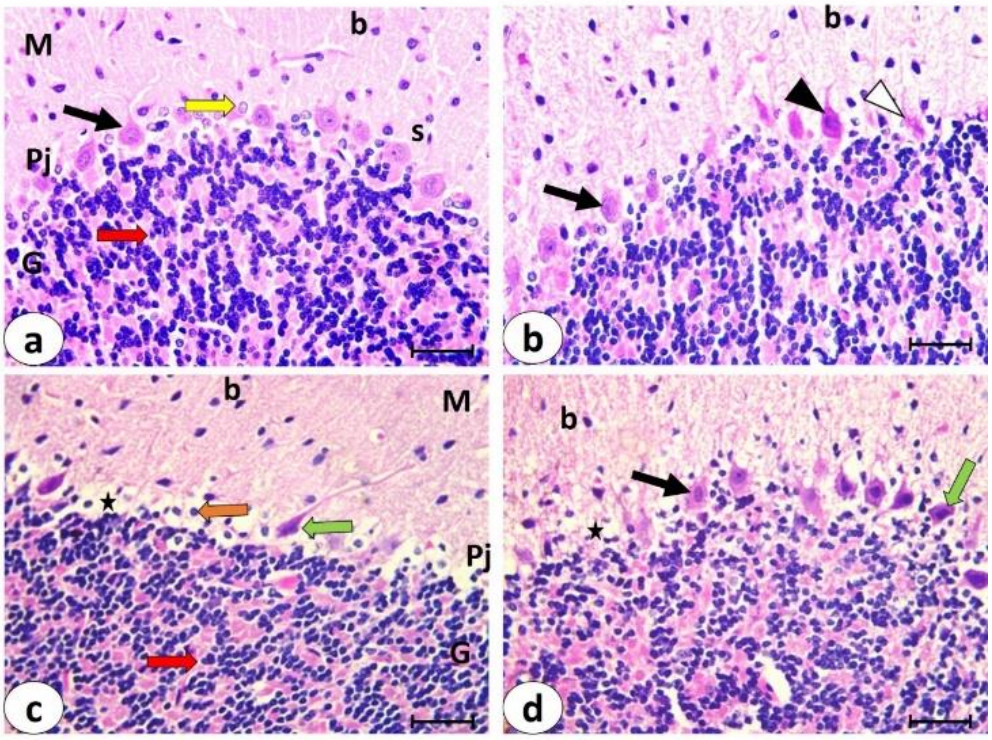


Figure 3: Photomicrographs of cerebellar folia sections from experimental groups **a**: control group showing: molecular layer (M), purkinje cell layer (Pj), granular layer (G), basket cells (b), stellate cells (S), and granular cells (red arrow). Purkinje cells (↑) are pear-shaped with rounded vesicular nuclei and are surrounded by glial cells (yellow arrow). **b: (Tt + Cd)** group showing normal Purkinje cells (↑), deeply stained Purkinje cells with fragmented nuclei (▲), remnants of degenerated Purkinje cells (Δ), and deeply stained nuclei of basket cells (b). **c**: (Cd) group showing noticeable deeply stained nuclei of basket cells (b) in the molecular layer (M). The Purkinje cell layer (Pj) is nearly devoted from purkinje cells. The still-present Purkinje calls are deeply stained, shrunken, and have pyknotic nuclei (green arrow). Empty areas from Purkinje cell loss (asterisk) are filled with glial cells (orange arrow). Granular layer (G) filled with densely stained nuclei of granular cells (red arrow) **d: (**Cd + Tt) group showing deeply stained nuclei of basket cells (b), densely stained shrunken Purkinje cells with pyknotic nuclei (green arrow), empty areas from Purkinje cell loss (asterisk), and normal Purkinje cells with rounded vesicular nuclei (↑). (H&E stain, Bar = 50 µm).

Immunohistochemical examination of cleaved caspase-3 expression

In CA1 region of the hippocampus, the control group showed a negative immunoreaction of cleaved caspase-3 (Fig. 4a)**.** In (Tt + Cd) group, a mild immunoreaction of cleaved caspase-3 was detected (Fig. 4b)**.** A severe positive immunoreaction of cleaved caspase-3 was seen in (Cd) group, as represented by brown color in the nuclei of the small pyramidal cells (Fig. 4c)**.** Group **(**Cd + Tt) showed mild immunoreactivity (Fig. 4d)**.** Statistically, the immunoreaction of cleaved caspase-3 in CA1 from (Cd) group increased significantly ($P < 0.01$) versus the control group. The immunoreaction decreased significantly ($P < 0.01$) in (Tt + Cd) and (Cd + Tt) groups in comparison with (Cd) group, but there was no discernible difference versus the control**.** (Fig. 7d)**.**

In hippocampus CA3 region, the control group showed a negative immunoreaction of cleaved caspase-3 (Fig. 5a)**.** Group (Tt + Cd) showed moderate immunoreactivity (Fig. 5b)**.** Positive immunoreaction of cleaved caspase-3 was detected in (Cd) group, as

represented by brown color in the nuclei of the large pyramidal cells (Fig. 5c)**.** The immunoreaction of cleaved caspase-3 in (Cd + Tt) group was positive (Fig. 5d)**.** Statistically, the immune-reaction to cleaved caspase-3 in CA3 region from (Cd) group increased significantly ($P < 0.05$) versus the control. Non- significant differences occurred between (Cd), (Tt + Cd), and (Cd + Tt) groups. Non-significant difference is present between (Tt + Cd) and control groups (Fig. 7e)**.**

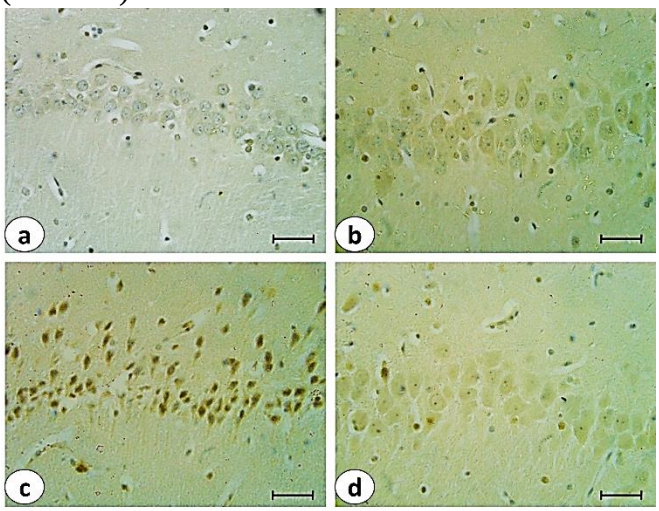


Figure 4: Immunohistochemical detection of cleaved caspase-3 expression in CA1 region of hippocampus from the experimental groups. Bar = 50µm. **a**: control group showing negative immunoreaction of cleaved caspase-3. **b: (Tt + Cd)** group showing mild immunoreaction of cleaved caspase-3. **c**: (Cd) group showing severe positive immunoreaction of cleaved caspase-3 as represented by brown color in the nuclei of the small pyramidal cells. **d: (**Cd + Tt) group showing mild immunoreaction of cleaved caspase-3.

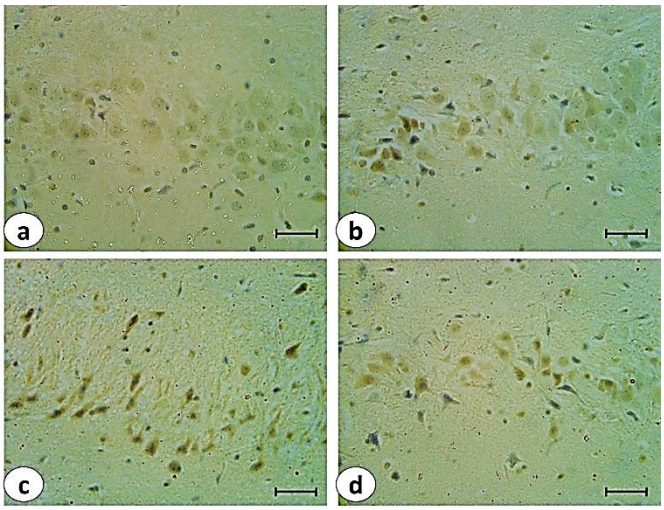


Figure 5: Immunohistochemical detection of cleaved caspase-3 expression in CA3 region of hippocampus from the experimental groups. Bar = 50µm. **a**: control group showing negative immunoreaction of cleaved caspase-3. **b: (Tt + Cd)** group showing moderate immunoreaction of cleaved caspase-3. **c**: (Cd) group showing positive immunoreaction of cleaved caspase-3 as represented by brown color in the nuclei of the large pyramidal cells. **d: (**Cd + Tt) group showing positive immunoreaction of cleaved caspase-3.

Immunohistochemical detection of cleaved caspase-3 expression in cerebellar folia from control (Fig. 6a) and (Tt + Cd) (Fig. 6b) groups showing mild immune-reaction. Group (Cd) showed a severe positive immunoreaction, as represented by brown color in basket cells, Purkinje cells, and granular cells (Fig. 6c)**.** Moderately positive immunoreaction of cleaved caspase-3 detected in **(**Cd + Tt) group (Fig. 6c)**.** Statistically, the immune-reaction of cleaved caspase-3 in cerebellar folia from (Cd) group increased significantly ($P < 0.001$) versus control group. The immunoreaction decreased in (Tt + Cd) and (Cd + Tt) groups significantly ($P < 0.001$, $P < 0.01$ respectively) in comparison with (Cd) group, but there was a non-significant difference present versus control group (Fig. 7f)**.**

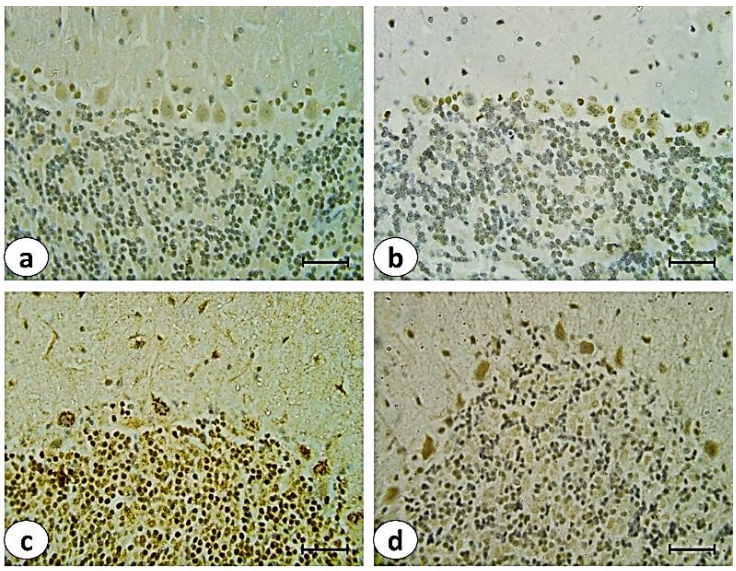


Figure 6: Immunohistochemical detection of cleaved caspase-3 expression in cerebellar folia from the experimental groups. Bar = 50µm. **a**: control and **b: (Tt + Cd)** groups showing mild immunoreaction of cleaved caspase-3. **c**: (Cd) group showing severe positive immunoreaction of cleaved caspase-3 as represented by brown color in basket cells, Purkinje cells, and granular cells. **d: (**Cd+Tt) group showing moderate positive immunoreaction of cleaved caspase-3.

DISCUSSION

Cd is one of the most harmful and toxic compounds to the environment, it is thought to cause air contamination, especially in industrial settings**.** Cd chronic exposure affects the nervous system resulting in Parkinsonian symptoms as vasomotor and olfactory dysfunction, headache, peripheral neuropathy, learning disabilities, declined equilibrium and concentration loss (Okuda *et al.*, 1997)**.** Also, it caused raise in aggressiveness, impaired memory development, motor hyperactivity and changed social behavior (Desi *et al.*, 1998).

In the present study, results show a significant decrease in plasma SOD and an increase in H_2O_2 levels of rats after Cd injection that agrees with (Lakshmi *et al.*, 2012) who found a reduction in the antioxidant markers such as SOD with an increase in peroxidation indicators in rats under Cd effect. Cd induces membrane disturbances and oxidative stress in the nervous system suggesting that it causes DNA damage in the presence of H_2O_2 probably due to the generation of hydroxyl ions. Cd has been shown to reduce protein sulfhydryl bound groups and glutathione which enhance free radical release, oxidative stress and detrimental effects like peroxidation of proteins, lipids, carbohydrates and DNA molecules (Badisa *et al.*, 2007; Pari and Murugavel, 2007). Also, Cd blocks factors that are responsible for gene activation which produce antioxidant enzymes (Liu *et al.*, 2020).

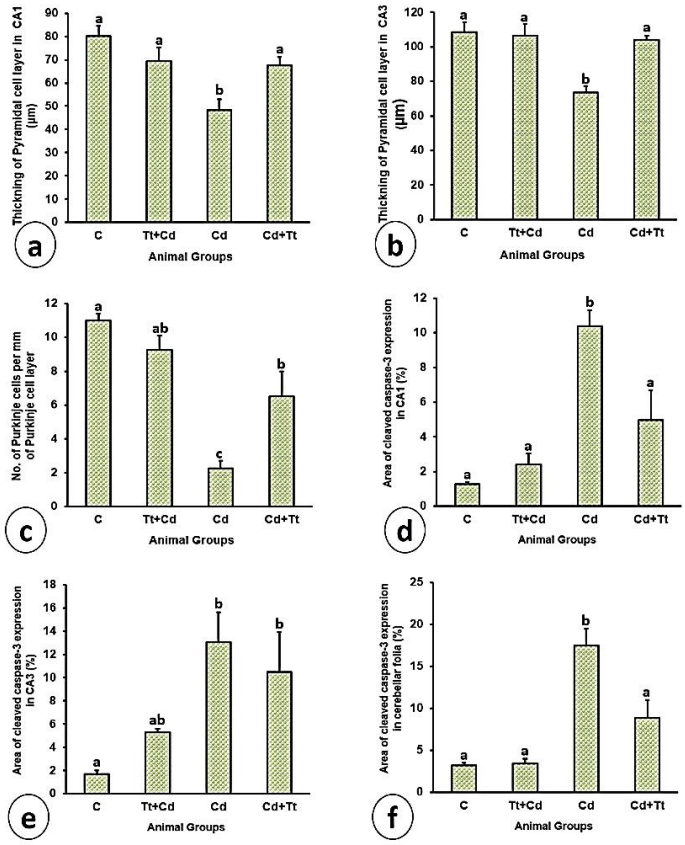


Figure 7: a: thickening of pyramidal cell layer in CA1 region of hippocampus (µm). **b:** thickening of pyramidal cell layer in CA3 region of hippocampus (µm). **c:** number of Purkinje cells per millimeter of Purkinje cell layer from cerebellar folia. **d:** percentage of area of cleaved caspase-3 expression in CA1 region of hippocampus. **e:** percentage of area of cleaved caspase-3 expression in CA3 region of hippocampus. **f:** percentage of area of cleaved caspase-3 expression in cerebellar folia. Values in the column with unlike superscript letters are significantly different at ($P < 0.05$). Data represents mean ± S.E.M.

Also, our outcomes approved that Cd significantly increased rat plasma levels of CRP and IL-6 in comparison to control rats that agree with (Razzuoli *et al.,* 2018) who used porcine epithelial cells to evaluate the influence of Cd on protein release, pro-inflammatory gene, and infection susceptibility in a model of Salmonella typhimurium. They recorded that epithelial cells could absorb Cd rapidly and concentrate it, increasing pro-inflammatory chemokines and cytokines (IL-8, IL-6 and IL-18). Also, (Kundu *et al.,* 2011) showed that a small Cd dose resulted in the enhancement of epidermal growth factor receptor which is a vital reason for the enhancement of proinflammatory cytokines such as IL-6, IL-1 and tumor necrosis factor-alpha (TNF-α). Other, recorded significantly high gene expression IL-6 mRNA in cells treated with Cd (Afolabi *et al.,* 2012). Besides, Cd has proinflammatory properties causing upregulation and stimulation of many intracellular signaling pathways mainly as activator protein 1 (AP-1), cyclooxygenase-2 (COX-2), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), TNF-α, and inducible isoform nitric oxide (iNOS) which stimulate IL-6, IL-8, IL-1β and CRP production (Olszowski *et al.,* 2012).

In addition, we found that Cd enhanced CRP level than that of the control similar to a prior study that showed that Cd injection was combined with sever increased levels of rat plasma CRP (Yildirim *et al.,* 2018). Also, (Xiao *et al.,* 2019) showed that there is a positive relation between type 2 diabetes risk

and urinary Cd, supported by high plasma CRP level. This can be explained as Cd exposure may interrupt tissue homeostasis and harm endothelial cells, which leads to stimulation of certain cell types as neutrophils, and Kupffer cells, with the production of inflammatory cytokines like CRP from macrophages (Odewumi et al., 2016). CRP acts as a brain inflammatory marker, as elevated CRP was noticed in cerebrovascular disease and neuro-degeneration because CRP enhances the blood-brain barrier permeability (Hsuchou *et al.,* 2012). This elevated CRP leads to increased entry and decreased outflow of Amyloid β (Aβ) through the blood-brain barrier and directly motivates neuronal formation of Aβ in the brain leading to many brain defects (Hilal *et al.,* 2018). Therefore, inflammation may induce neuronal apoptosis, influencing synaptic plasticity and suppressing hippocampal neurogenesis (Semmler *et al.*, 2005). A past study showed that a cytokine-chemokine combination, containing CRP and IL-6, was related to deep cortical atrophy (Baune *et al.,* 2009).

Thus, Cd causes changes in gene expression including different mechanisms such as inflammatory and oxidative stress reactions leading to neurodegenerative defects (Razzuoli *et al.,* 2018). So, suppression of chemokines and cytokines (IL-8 and IL-6) generations may be the way to prevent inflammation produced by Cd in the brain and angiogenesis in gliomas (Phuagkhaopong *et al.,* 2017). Also, Cd caused endoplasmic reticulum (ER) malfunction with an increase in reactive oxygen species (ROS), which leads to cell apoptosis and mitochondrial dysfunction (Morales *et al.,* 2006).

Histologically, in hippocampus CA1 and CA3 regions, Cd administration to rats caused a marked decline in the pyramidal cell layer thickening. Shrunken of small and large pyramidal cells, degenerated with pyknotic nuclei and spaces around them. These results were confirmed by (Al Olayan *et al.,* 2020) who said that the nervous system is the primary known system of Cd exposure targets. Ebokaiwe *et al.,* (2022) explained that the adult brain's hippocampus function is compromised by cd due to disruptions in calcium transmission and neurogenesis. The vacuolation in the neuropil around the cells could be caused by the cells shrinking and pulling back their processes because of damage to the cytoskeleton, which leaves pericellular gaps (Afifi & Embaby, 2016). The uneven shape and loss of the form of pyramidal cells might be linked to the inefficiency of the cytoskeleton. Alterations in mitochondrial permeability and an increase in cytosolic calcium can both contribute to mitochondrial damage alongside oxidative stress. Rapid cell death results from damage to mitochondria and its function (Afifi & Embaby, 2016). Cd changes the shape of the axons and dendrites and encourages the release of ROS in neurons of hippocampus, striatum and the parietal cortex (Rai *et al.,* 2022). Antioxidant enzymes such SOD, glutathione peroxidase and catalase, have their levels and activities reduced by Cd exposure. Oxidative stress is triggered by all of this, causing enhancement in lipid breakdown and neuronal injury across the brain (Pulido *et al.,* 2019).

In this study, Cd induced many degenerative alterations in the cerebellum as noticeable deeply stained nuclei of basket cells in the molecular layer, the Purkinje cell layer was nearly devoted from Purkinje cells and the still-present Purkinje calls were deeply stained, shrunken, and had pyknotic nuclei. Empty areas from Purkinje cell loss were filled with glial cells. The granular layer was filled with densely stained nuclei of granular cells. These discoveries are consistent with those of (Bi *et al.,* 2023) who found that Cd exposure declined Purkinje cell number, increased Purkinje cell degeneration with pyknosis, and caused dendrites disappearance and cerebellum degeneration. Prior research determined that Cd caused the degeneration of the Purkinje cell layer and the granular cell layer in the brains of rodents and chickens (Bekheet, 2011). In addition, sublethal Cd exposure led to degenerative alterations in cerebellum Purkinje cells (Stoev *et al.,* 2003). Cd enhanced cerebellum neurotoxicity which

was linked to changes in genes related to response to heat shock (Bi *et al.,* 2023). Previous studies also showed that Cd exposure greatly caused severe mitochondrial splitting by increasing mitochondrial absorption to calcium and translocation of dynamin1-like (DNM1L) to the mitochondria, which caused damage to the mitochondria and death of neural cells (Deng *et al.*, 2023). Moreover, (Wahdan *et al.,* 2014) said that the initiated influence of Cd was in Purkinje cells of the cerebellum because of the high ability of heavy metals to precipitate in their cytoplasm. Also, (Jackson, 2008) mentioned that the influence of Cd on the blood-brain barrier may inhibit nutrition and enhance hypoxia, which influenced the Purkinje cell's structure causing cell degeneration or necrosis.

The activation of cysteine proteases (caspases) is the most well-known biochemical marker of early and late phases of apoptosis. Active caspase-3 detection in cells is an important issue for detecting apoptosis generated by a wide range of apoptotic signals. Caspase-3 has a key role in necrotic and apoptotic cell death. Caspases begin as inactive zymogens or precursors that are activated by proteolytic cleavage to an active enzyme which further proteolytically cleave cellular proteins or other caspases (Kumar, 1999).

In the current study, immune-histochemical examination of cleaved caspase-3 expression explained that Cd increased the immunoreactivity significantly in CA1, CA3 regions and in the cerebellum. These results come in line with those of (Pulido *et al.*, 2019) who found that in the three hippocampal regions, there was an enhancement in immunoreactive cell numbers of caspase-3 and caspase-9 after administration of Cd. In the hippocampus of Cd-poisoned rats, the exposure to Cd enhanced mRNA levels of Caspase3, Caspase-9 proteins, and Bax while reduced the Bcl-2 amount, which resulted in apoptosis. (Zhu *et al.,* 2021).

Attention is being paid by several studies to natural product-based therapies to alleviate the deleterious effects of metal toxicity. Administration of Tt has shown promising results in reducing oxidative stress, inflammation, attenuating histopathological changes and increasing non-enzymatic and enzymatic antioxidant amounts (Zhang *et al.,* 2008). In the current research, applying Tt enhanced the open arms time spent; while declined the time consumed in the elevated plus maze test closed arms. Spending more time in the closed arm measures wall-hugging or thigmotaxis behavior and is a marker of anxiety-related behavior. In agreement with our findings, previous studies indicated that Tt influence at the elevated plus maze test could be attributed to the role of the Tt in normalizing hypothalamic pituitary adrenal axis hyperactivity, it is believed to contribute to several anti-anxiety roles, and regulating elevated cortisol levels (Bratt *et al.,* 2001; Wang *et al.,* 2013).

Also, we found that administration of Tt before or after Cd, induced SOD while reducing H_2O_2 levels. This matches with (Lakshmi *et al.,* 2012) who approved that Tt with Cd significantly declined the Cd load in the kidney and liver and caused restoration of lipid peroxidation and antioxidant parameters when compared to Cd group. Exposure to a single dose of Cd causes oxidative toxicity in the male reproductive system by enhancing ROS, lipid peroxidation and altering antioxidant enzymes while the pretreatment with Tt protects against this damage (Pavin *et al.,* 2018). Additionally, (Dutt-Roy *et al.,* 2017) detected that the treatment with Tt extracts increased the superoxide dismutase (SOD) and catalase while inhibiting the malondialdehyde (MDA) concentrations. Moreover, pretreatment with Tt inhibited apoptosis in H_2O_2-damaged cells (Wang *et al.,* 2009) as Tt contains flavonoids and polyphenol carboxylic acids which have a powerful antioxidant activity built on their facility to give hydrogen. Added, that polyphenols are able of scavenging hydroxyl

(HO•), superoxide (O2•-) and peroxyl (RO2•) radicals (Zhang *et al.*, 2015).

Besides, our findings presented that Tt succeeded in decreasing CRP and IL-6 plasma levels when applied before or after Cd compared with Cd group. That matched with (Yang *et al.*, 2022) who found that TST (total saponins of *Tribulus*) significantly decreased IL-6 and TNF-α expression in RAW 264.7 cells induced by lipopolysaccharides. Also, the results of Zhang *et al.*, (2023) showed that gross saponin of *Tribulus terrestris* fruit (GSTTF) significantly declined TNF-*α* and IL-6 amount generated in brain injury which exerts inflammation procedures and neuro-toxicity after middle cerebral artery occlusion. Moreover, GSTTF has an anti-inflammatory effect as it could inhibit phosphorylation of nuclear factor kappa B (NF-*κ*B) which is a main inflammatory issue that interferes with immunological receptors in a huge manner.

In addition, (Oh *et al.*, 2012; Lee *et al.*, 2017) identified that Tt ethanolic extract decreased the lipopolysaccharide-induced inflammatory cytokines expression, as IL-6, IL-4, IL-10 and TNF-α through inhibition of mitogen-activated protein kinase p38 (Akt/MAPKs) and inactivation of NF-*κ*B. Additionally, it succeeded in reducing the PGE2 levels (another significant inflammatory factor) and COX-2 immunoreactivity signals (a central enzyme in the inflammatory steps). Also, (Zhao *et al.*, 2021) recommended that Tt showed potent anti-inflammatory properties via inhibition of NF-*κ*B/iNOS-NO and Akt/MAPKs pathways (NO generation via macrophages is a significant marker of inflammation, which leads to cell oxidative stress and is controlled by iNOS).

From the histological aspect, the pretreatment with Tt in the (Tt + Cd) group ameliorated the cd has raised alteration in cells of the hippocampus CA3 and CA1 areas and in the cerebellum to a greater extent than the posttreatment in the (Cd + Tt) group. Tt administration in both groups increased the thickening of small and large pyramidal cells of the hippocampus CA3 and CA1 areas and increased the number of Purkinje cells significantly compared to Cd group. These findings matched with Chauhdary *et al.*, (2019) who found that Tt reversed the neurofibrillary tangles, pigmentation, neuronal loss and neuroinflammation caused by aluminum chloride. Recent research has found that Tt extraction, particularly the saponins, showed cerebrovascular defensive properties. Tt pretreatment was observed to minimize apoptosis in H9c2 cells, mitochondrial alterations, and ischemia-related damages (Zhang *et al.*, 2023). Mohamed *et al.*, (2023) demonstrated that Tt extract reduces histological damage and oxidative stress, hence improving kidney function. Tt was discovered to have a high phenolic content as well as superior radical scavenging properties. Pretreatment with Tt extract reduces oxidative stress through many mechanisms, including inhibition of superoxide free radicals and the restoration of total antioxidant capacity by keeping endogenous enzymatic/non-enzymatic antioxidants constant within the normal levels (Kilany *et al.*, 2020).

In the current study, immunohisto-chemical examination of cleaved caspase-3 expression explained that Tt administration in CA1 of the hippocampus and the cerebellum decreased the immunoreactivity significantly when compared to Cd. However in the hippocampus CA3 region, no significant difference occurred in cleaved caspase-3 expression between the (Cd), (Tt + Cd), and (Cd + Tt) groups. These investigations were confirmed by Liu *et al.*, (2008) who cleared that Tt could reduce apoptosis caused by hypoxia and reoxygenation. Tt pretreatment dramatically reduced apoptotic percentage in H_2O_2-damaged cardiocytes (Wang *et al.*, 2009). Methods of Tt versus myocardial apoptosis could be related to the inhibition of the mitochondrial apoptosis pathway after PKCepsilon activation (Wang *et al.*, 2009). Tt extract inhibits cell oxidative stress and apoptosis by activating protein kinase C and decreasing proapoptotic proteins like Bax and caspase-3 while raising the amount of Bcl-2

anti-apoptotic protein that may be related to its antioxidant character as it consists of flavonoids, that can regulate a group of enzymes responsible for cell detoxification, division, inflammation, proliferation and immune response (Kilany *et al.,* 2020).

CONCLUSIONS

In conclusion, Tt possesses improvement in biochemical, histochemistry and behavioral parameters that are connected with its antioxidant, anti-inflammatory and antiapoptotic properties which were able to protect from Cd insult.

CONFLICT OF INTEREST

The authors affirm that they do not have any competing interests.

REFERENCES

Afifi, O. and Embaby, A. (2016): Histological Study on the Protective Role of Ascorbic Acid on Cadmium Induced Cerebral Cortical Neurotoxicity in Adult Male Albino Rats. *Journal of Microscopy and Ultrastructure, 4*(1).

Afolabi, O.K.; Oyewo, E.B.; Adekunle, A.S.; Adedosu, O.T. and Adedeji, A.L. (2012): Impaired lipid levels and inflammatory response in rats exposed to cadmium. *EXCLI journal* 11, 677.

Al Olayan, E.M., Aloufi, A.S., AlAmri, O.D., El-Habit, O.H. and Abdel Moneim, A.E. (2020): Protocatechuic acid mitigates cadmium-induced neurotoxicity in rats: Role of oxidative stress, inflammation and apoptosis. *Science of the Total Environment, 723*.

Andersen, P.; Morris, R.; Amaral, D.; O'Keefe, J. and Bliss, T. (2007): The hippocampus book. Oxford University Press.

Atia, M.M. and Alghriany, A.A.I. (2021): Adipose-derived mesenchymal stem cells rescue rat hippocampal cells from aluminum oxide nanoparticle-induced apoptosis via regulation of P53, Aβ, SOX2, OCT4, and CYP2E1. *Toxicology Reports, 8*.

Badisa, V.L.D.; Latinwo, L.M.; Odewumi, C.O.; Ikediobi, C.O.; Badisa, R.B.; Ayuk-Takem, L.T.; Nwoga, J. and West, J. (2007): Mechanism of DNA damage by cadmium and interplay of antioxidant enzymes and agents. *Environmental Toxicology: An International Journal* 22, 144-151.

Bancroft, J.D. and Gamble, M. (2008): Theory and practice of histological techniques. Elsevier health sciences.

Baune, B.T.; Ponath, G.; Rothermundt, M.; Roesler, A. and Berger, K. (2009): Association between cytokines and cerebral MRI changes in the aging brain. *Journal of geriatric psychiatry and neurology* 22, 23-34.

Beckinghausen, J. and Sillitoe, R.V. (2019): Insights into cerebellar development and connectivity. *Neuroscience Letters, 688*, 2–13.

Bekheet, S.H.M. (2011): Comparative effects of repeated administration of cadmium chloride during pregnancy and lactation and selenium protection against cadmium toxicity on some organs in immature rats' offsprings. *Biological Trace Element Research, 144*, 1008–1023.

Bi, S.S.; Talukder, M.; Sun, X.T.; Lv, M.W.; Ge, J.; Zhang, C. and Li, J.L. (2023): Cerebellar injury induced by cadmium via disrupting the heat-shock response. *Environmental Science and Pollution Research, 30*(9).

Bratt, A.M.; Kelley, S.P.; Knowles, J.P.; Barrett, J.; Davis, K., Davis, M. and Mittleman, G. (2001): Long term modulation of the HPA axis by the hippocampus: behavioral, biochemical and immunological endpoints in rats exposed to chronic mild stress. *Psychoneuroendocrinology* 26, 121-145.

Brenneman, K.A.; Wong, B.A.; Buccellato, M.A.; Costa, E.R.; Gross, E.A. and Dorman, D.C. (2000): Direct olfactory transport of inhaled manganese (54MnCl2) to the rat brain: toxicokinetic investigations in a unilateral nasal occlusion model. *Toxicology and applied pharmacology* 169, 238-248.

Chauhdary, Z.; Saleem, U.; Ahmad, B.; Shah, S. and Shah, M.A. (2019): Neuroprotective evaluation of Tribulus terrestris L. In aluminum chloride induced Alzheimer's disease. *Pakistan*

Journal of Pharmaceutical Sciences, *32*(2).

Chhatre, S.; Nesari, T.; Somani, G.; Kanchan, D. and Sathaye, S. (2014): Phytopharmacological overview of Tribulus terrestris. *Pharmacognosy reviews* 8, 45.

Cuypers, A.; Plusquin, M.; Remans, T.; Jozefczak, M.; Keunen, E.; Gielen, H.; Opdenakker, K.; Nair, A.R.; Munters, E. and Artois, T.J. (2010): Cadmium stress: an oxidative challenge. *Biometals* 23, 927-940.

Czeczot, H. and Skrzycki, M. (2010): Cadmium–element completely unnecessary for the organism. *Advances in Hygiene and Experimental Medicine* 64.

Das, S.C. and Al-Naemi, H.A. (2019): Cadmium toxicity: Oxidative stress, inflammation and tissue injury.

Deng, P.; Zhang, H.; Wang, L.; Jie, S.; Zhao, Q.; Chen, F.; Yue, Y.; Wang, H.; Tian, L. and Xie, J. (2023): Long-term cadmium exposure impairs cognitive function by activating lnc-Gm10532/m6A/FIS1 axis-mediated mitochondrial fission and dysfunction. *Science of The Total Environment*, *858*, 159950.

Desi, I.; Nagymajtenyi, L. and Schulz, H. (1998): Behavioural and Neurotoxicological Changes Caused by Cadmium Treatment of Rats during Development. Journal of Applied Toxicology, 18, 63-70.

Dutt-Roy, R.; Kayalvizhi, E. and Chandrasekhar, M. (2017): Evaluation of the antidepressant activity of Tribulus terrestris in diabetic depression in rat model. *Int. J. Pharm. Sci. Res* 8, 5392-99.

Ebokaiwe, A.P.; Ushang, O.R.; Chimezie, I.P. and Chiamaka, N. (2022): The mechanism of the neuroprotective effect of zinc against cadmium-induced behavioral impairments in male Wister rats: Focus on tryptophan degradation pathway, oxidative-inflammatory stress, and histologic evidence. *Toxicology*, *472*.

Gauthaman, K.; Adaikan, P.G. and Prasad, R.N.V. (2002): Aphrodisiac properties of Tribulus Terrestris extract (Protodioscin) in normal and castrated rats. *Life sciences* 71, 1385-1396.

Hergenroeder, G.W.; Moore, A.N.; McCoy, J.P.; Samsel, L.; Ward, N.H.; Clifton, G.L. and Dash, P.K. (2010): Serum IL-6: a candidate biomarker for intracranial pressure elevation following isolated traumatic brain injury. *Journal of neuroinflammation* 7, 1-13.

Hilal, S.; Ikram, M.A.; Verbeek, M.M.; Franco, O.H.; Stoops, E.; Vanderstichele, H.; Niessen, W.J. and Vernooij, M.W. (2018): C-reactive protein, plasma amyloid-β levels, and their interaction with magnetic resonance imaging markers. *Stroke* 49, 2692-2698.

Hsuchou, H.; Kastin, A.J.; Mishra, P.K. and Pan, W. (2012): C-reactive protein increases BBB permeability: implications for obesity and neuroinflammation. *Cellular physiology and biochemistry* 30, 1109-1119.

Jackson, J.R. (2008): Heavy metal neurotoxicity and associated disorder: Biochemical Mechanisms. *Science*, *291*, 860–867.

Kadry, H.; Abou, B.L. and Gindi, E.L. (2010): Antioxidant activity of aerial parts of Tribulus alatus in rats. *Pak J Pharm Sci* 23, 59-62.

Kilany, O.; Abdou, R.; El-Beltagy, M. and Mohammad, H. (2020): Protective Effects of Tribulus terrestris Against Gentamicin Mediated Nephrotoxicity, Oxidative Damage and Apoptosis in Male Rats. *Egyptian Academic Journal of Biological Sciences, B. Zoology*, *12*(1).

Kioukia-Fougia, N.; Antoniou, K.; Bekris, S.; Liapi, C.; Christofidis, I. and Papadopoulou-Daifoti, Z. (2002): The effects of stress exposure on the hypothalamic–pituitary–adrenal axis, thymus, thyroid hormones and glucose levels. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 26, 823-830.

Kumar, S. (1999): Mechanisms mediating caspase activation in cell death. In *Cell Death and Differentiation* (Vol. 6, Issue 11).

Kundu, S.; Sengupta, S. and Bhattacharyya, A. (2011): EGFR upregulates inflammatory and proliferative responses in human lung adenocarcinoma cell line (A549), induced by lower dose of cadmium

chloride. *Inhalation toxicology* 23, 339-348.

Lakshmi, G.D.; Kumar, P.R.; Bharavi, K.; Annapurna, P.; Rajendar, B. and Patel, P.T. (2012): Protective effect of Tribulus terrestris linn on liver and kidney in cadmium intoxicated rats.

Lee, H.H.; Ahn, E.K.; Hong, S.S. and Oh, J.S. (2017): Anti-inflammatory effect of tribulusamide D isolated from Tribulus terrestris in lipopolysaccharide-stimulated RAW264. 7 macrophages. *Molecular medicine reports* 16, 4421-4428.

Liu, C.; Zhu, Y.; Lu, Z.; Guo, W.; Tumen, B.; He, Y.; Chen, C.; Hu, S.; Xu, K. and Wang, Y. (2020): Cadmium induces acute liver injury by inhibiting Nrf2 and the role of NF-κB, NLRP3, and MAPKs signaling pathway. *International journal of environmental research and public health* 17, 138.

Liu, X.M.; Huang, Q.F.; Zhang, Y.L.; Lou, J.L.; Liu, H.S. and Zheng, H. (2008): Effects of Tribulus terrestris L. saponion on apoptosis of cortical neurons induced by hypoxia-reoxygenation in rats. *Journal of Chinese Integrative Medicine*, *6*(1).

Ma, L.; Liu, J.; Li, N.; Wang, J.; Duan, Y.; Yan, J.; Liu, H.; Wang, H. and Hong, F. (2010): Oxidative stress in the brain of mice caused by translocated nanoparticulate TiO2 delivered to the abdominal cavity. *Biomaterials, 31*(1), 99–105.

Maloku, E.; Covelo, I.R.; Hanbauer, I.; Guidotti, A.; Kadriu, B.; Hu, Q.; Davis, J.M. and Costa, E. (2010): Lower number of cerebellar Purkinje neurons in psychosis is associated with reduced reelin expression. *Proceedings of the National Academy of Sciences, 107*(9), 4407–4411.

Mohamed, S.S.; Zeima, N.M. and El-Melmoslemany, A.M. (2023): The Effect of Tribulus Terrestris Fruits and Urtica dioica Leaves Extracts on Renal Calculus Induced by Sodium Oxalate in Experimental Rats. *Bulletin of the National Nutrition Institute of the Arab Republic of Egypt, 61*(1), 100–137.

Mohammed, A.; Hamdy, H.; Abdelrady, G., Seliman, M. and Mahmoud, U. (2020): Effect of zinc oxide nanoparticles on anxiety-related behaviors in male rats. *SVU-International Journal of Veterinary Sciences* 3, 123-132.

Morales, A.I.; Vicente-Sánchez, C.; Jerkic, M.; Santiago, J.M.; Sánchez-González, P.D.; Pérez-Barriocanal, F. and López-Novoa, J.M. (2006): Effect of quercetin on metallothionein, nitric oxide synthases and cyclooxygenase-2 expression on experimental chronic cadmium nephrotoxicity in rats. *Toxicology and applied pharmacology* 210, 128-135.

Morris, M.C.; Compas, B.E. and Garber, J. (2012): Relations among posttraumatic stress disorder, comorbid major depression, and HPA function: a systematic review and meta-analysis. *Clinical psychology review* 32, 301-315.

Mukherjee, R.; Desai, F.; Singh, S.; Gajaria, T.; Singh, P.K.; Baxi, D.B.; Sharma, D.; Bhatnagar, M. and Ramachandran, A.V. (2010): Melatonin protects against alterations in hippocampal cholinergic system, trace metals and oxidative stress induced by gestational and lactational exposure to cadmium. *Excli Journal* 9, 119.

Odewumi, C.O.; Latinwo, L.M.; Ruden, M.L.; Badisa, V.L.D.; Fils-Aime, S. and Badisa, R.B. (2016): Modulation of cytokines and chemokines expression by NAC in cadmium chloride treated human lung cells. *Environmental toxicology* 31, 1612-1619.

Oh, J.S.; Baik, S.H.; Ahn, E.-K.; Jeong, W. and Hong, S.S. (2012): Anti-inflammatory activity of Tribulus terrestris in RAW264. 7 Cells (54.2). *The Journal of Immunology* 188, 54-2.

Okuda, B.; Iwamoto, Y.; Tachibana, H. and Sugita, M. (1997): Parkinsonism after acute cadmium poisoning. *Clinical neurology and neurosurgery* 99, 263-265.

Olszowski, T.; Baranowska-Bosiacka, I.; Gutowska, I. and Chlubek, D. (2012): Pro-inflammatory properties of cadmium. *Acta Biochimica Polonica* 59.

Pari, L. and Murugavel, P. (2007): Diallyl tetrasulfide improves cadmium-induced alterations of acetylcholinesterase, ATPases and oxidative stress in brain of rats. *Toxicology* 234, 44-50.

Pavin, N.F.; Izaguirry, A.P.; Soares, M.B.; Spiazzi, C.C.; Mendez, A.S.L.; Leivas, F.G.; Dos Santos Brum, D. and Cibin, F.W.S. (2018): Tribulus terrestris protects against male reproductive damage induced by cyclophosphamide in mice. *Oxidative Medicine and Cellular Longevity* 2018.

Phuagkhaopong, S.; Ospondpant, D.; Kasemsuk, T.; Sibmooh, N.; Soodvilai, S.; Power, C. and Vivithanaporn, P. (2017): Cadmium-induced IL-6 and IL-8 expression and release from astrocytes are mediated by MAPK and NF-κB pathways. *Neurotoxicology* 60, 82-91.

Pulido, G.; Treviño, S.; Brambila, E.; Vazquez-Roque, R.; Moreno-Rodriguez, A.; Peña Rosas, U.; Moran-Perales, J.L.; Handal Silva, A., Guevara, J.; Flores, G. and Diaz, A. (2019): The Administration of Cadmium for 2, 3 and 4 Months Causes a Loss of Recognition Memory, Promotes Neuronal Hypotrophy and Apoptosis in the Hippocampus of Rats. *Neurochemical Research*, *44*(2).

Rai, N.K.; Ashok, A.; Rai, A.; Tripathi, S.; Nagar, G.K.; Mitra, K. and Bandyopadhyay, S. (2022): Corrigendum to "Exposure to As, Cd and Pb-mixture impairs myelin and axon development in rat brain, optic nerve and retina" [Toxicology and Applied Pharmacology 273 (2013) 242–258, (Toxicology and Applied Pharmacology (2013) 273(242-258) (S0041008X13001920), (10.1016/j.taap.2013.05.003). In *Toxicology and Applied Pharmacology* (Vol. 450).

Razzuoli, E.; Mignone, G.; Lazzara, F.; Vencia, W.; Ferraris, M.; Masiello, L.; Vivaldi, B.; Ferrari, A.; Bozzetta, E. and Amadori, M. (2018): Impact of cadmium exposure on swine enterocytes. *Toxicology Letters* 287, 92-99.

Reeber, S.L.; Otis, T.S. and Sillitoe, R.V. (2013): New roles for the cerebellum in health and disease. *Frontiers in Systems Neuroscience*, *7*, 83.

Semmler, A.; Okulla, T.; Sastre, M.; Dumitrescu-Ozimek, L. and Heneka, M.T. (2005): Systemic inflammation induces apoptosis with variable vulnerability of different brain regions. *Journal of chemical neuroanatomy* 30, 144-157.

Ştefănescu, R.; Tero-Vescan, A.; Negroiu, A.; Aurică, E. and Vari, C.-E. (2020): A comprehensive review of the phytochemical, pharmacological, and toxicological properties of Tribulus terrestris L. *Biomolecules* 10, 752.

Stoev, S.D.; Grozeva, N.; Simeonov, R.; Borisov, I.; Hubenov, H.; Nikolov, Y.; Tsaneva, M. and Lazarova, S. (2003): Experimental cadmium poisoning in sheep. *Experimental and Toxicologic Pathology*, *55*(4).

Treit, D.; Menard, J. and Royan, C. (1993): Anxiogenic stimuli in the elevated plus-maze. *Pharmacology biochemistry and behavior* 44, 463-469.

Wahdan, M.H.; Ismail, A.K.; Saad, M.F.; Meguid, E.A.; Rashidy, A.H. and Rana, M.F. (2014): Effect of Cadmium Exposure on the Structure of the Cerebellar Vermis of Growing Male Albino Rat. *International Research Journal of Applied and Basic Sciences*.

Wang, S.-S.; Ji, Y.-S.; Li, H. and Yang, S.-J. (2009): Mechanisms of gross saponins of Tribulus terrestris via activating PKCepsilon against myocardial apoptosis induced by oxidative stress. *Yao Xue Xue Bao= Acta Pharmaceutica Sinica*, *44*(2), 134–139.

Wang, Z.; Zhang, D.; Hui, S.; Zhang, Y. and Hu, S. (2013): Effect of tribulus terrestris saponins on behavior and neuroendocrine in chronic mild stress depression rats. *Journal of Traditional Chinese Medicine* 33, 228-232.

Woodcock, T. and Morganti-Kossmann, M.C. (2013): The role of markers of inflammation in traumatic brain injury. *Frontiers in neurology* 4, 18.

Xiao, L.; Zhou, Y.; Ma, J.; Cao, L.; Zhu, C.; Li, W.; Wang, D.; Fan, L.; Ye, Z. and Chen, W. (2019): Roles of C-reactive protein on the association between urinary cadmium and type 2 diabetes. *Environmental Pollution* 255, 113341.

Yang, M.; Oppong, M.B.; Di, J.; Yuan, Q.; Chang, Y.; Jiang, M.; Cao, S.; Dong, P.; Li, L. and Xie, Y. (2022): Steroidal saponins with anti-inflammatory activity

from Tribulus terrestris L. *Acupuncture and Herbal Medicine* 2, 41-48.

Yang, Z.; Yang, S.; Qian, S.Y.; Hong, J.-S.; Kadiiska, M.B.; Tennant, R.W.; Waalkes, M.P. and Liu, J. (2007): Cadmium-induced toxicity in rat primary mid-brain neuroglia cultures: role of oxidative stress from microglia. *Toxicological sciences* 98, 488-494.

Yildirim, S.; Celikezen, F.C.; Oto, G.; Sengul, E.; Bulduk, M.; Tasdemir, M. and Ali Cinar, D. (2018): An investigation of protective effects of litium borate on blood and histopathological parameters in acute cadmium-induced rats. *Biological trace element research* 182, 287-294.

Zhang, H.N.; Hu, S.Y.; Li, Y.H. and Zhang, C.H. (2008): Clinical observation of Baisong tablets for depression. *Hunan Zhong Yi Yao Da Xue Xue Bao* 28, 48-49.

Zhang, H.; Guo, W.; Li, X.; Xie, S.; Liu, Y.; Chen, G.; Wang, Y. and Xu, Y. (2023): Supplementation with Tribulus Terrestris Extract Exhibits Protective Effects on MCAO Rats via Modulating Inflamm-ation-Related Metabolic and Signaling Pathways. *ACS omega* 8, 2306-2314.

Zhang, X.; Li, J.; Ito, Y. and Sun, W. (2015): Simultaneous quantification of five steroid saponins from Dioscorea zingiberensis CH Wright in rat plasma by HPLC–MS/MS and its application to the pharmacokinetic studies. *Steroids* 93, 16-24.

Zhao, W.-R.; Shi, W.-T.; Zhang, J.; Zhang, K.-Y.; Qing, Y.; Tang, J.-Y.; Chen, X.-L. and Zhou, Z.-Y. (2021): Tribulus terrestris L. extract protects against lipopolysaccharide-induced inflammation in RAW 264.7 macrophage and zebrafish via inhibition of Akt/MAPKs and NF-κB/iNOS-NO signaling pathways. *Evidence-based Complementary and Alternative Medicine* 2021.

Zhu, Y.; Luan, P.; Liu, X.; Bao, J.; Liu, Q.; Cai, J.; Yang, J. and Zhang, Z. (2021): Crosstalk between autophagy and apoptosis regulates cerebral cortex and cerebellum neurodegeneration induced by cadmium in swine via the PI3K/AKT/AMPK pathway. *Ecotoxicology and Environmental Safety*, *228*.

دراسة عن دور تريبولوس تيريستريس في السمية الناتجة عن الكادميوم علي بعض أعضاء ذكور الجرذان البيضاء البالغة

إيمان أحمد عبد الغني نجم ، أحمد عبدالعليم عبد الحفيظ محمد ، الشيماء أحمد إبراهيم الغرياني

Email: Emannegm@vet.aun.edu.eg, Assiut University web-site: www.aun.edu.eg

إن الكادميوم (Cd) ملوث بيئي خطير يسبب سمية للإنسان والحيوان حتى إذا تعرض لكمية قليلة حيث يصعب طرده من الجسم مما يؤثر على جميع أجزاء الجسم. تريبولوس تيريستريس (Tt) هو عشب طبيعي يستخدم لتأثيراته الطبية في جميع أنحاء العالم. تهدف هذه الدراسة إلى الكشف عن إمكانية Tt للحماية من سمية الكادميوم في ذكور الجرذان البيضاء. تم تقسيم أربعة وعشرون جرذا إلى أربعة مجموعات، كل مجموعة 6 جرذان: المجموعة (1) :مجموعة ظابطة دون أي علاجات. المجموعة (2) (Tt + Cd) ؛ تعطى يومياً جرعة بالفم من Tt (5 مجم / كجم من الوزن مذابة في الماء المقطر) لمدة ثمانية أسابيع, ثم من بداية الأسبوع التاسع تم حقنها يومياً داخل الصفاق بالكادميوم (2 مجم / كجم بالوزن) لمدة ثمانية أيام. المجموعة (3) (Cd) ؛ يتم حقنها بـ Cd فقط بنفس الجرعة لمدة ثمانية أيام. المجموعة (4) (Cd + Tt) ؛ أولاً ، تم حقنها بالكادميوم بنفس الجرعة لمدة ثمانية أيام ثم في اليوم التاسع تم إعطائها نفس جرعة Tt لمدة ثمانية أسابيع. في هذه الدراسة، أوضحت النتائج أن الكادميوم يقلل بشكل كبير من مستوى ديسموتاز الفوق أكسيد في البلازما، والوقت الذي يقضيه في أختبار الأذرع المفتوحة للمتاهة المرتفعه، كما أدي إلي زيادة مستويات البلازما من بيروكسيد الهيدروجين وبروتين سي التفاعلي و الانترلوكين- 6 وكذلك التفاعل الهستوكيميائي المناعي للكاسباس 3 المشقوق في الدماغ، والوقت الذي يقضيه في أختبار الأذرع المغلقة للمتاهة المرتفعه مقارنة بمجموعة التحكم. بينما نجحت التريبولوس تيريستريس في زيادة مستوى الديسموتاز فوق أكسيد، والوقت الذي يقضيه في الذراعين المفتوحين؛ ومع ذلك ، فقد خفضت مستويات البلازما من بيروكسيد الهيدروجين وبروتين سي التفاعلي والانترلوكين- 6 وكذلك التفاعل الهستوكيميائي المناعي للدماغ المشقوق كاسباس 3 ، والوقت الذي يقضيه في في أختبار الأذرع المغلقة للمتاهة المرتفعه مقارنة بمجموعة الكادميوم. في الختام ، وجد أن التريبولوس تيريستريس يحمي الدماغ بشكل كبير من تأثيرات الكادميوم الضارة ، مشيرًا إلى خصائصه المضادة للأكسدة والمضادة للالتهابات ومضادة لإلتهام الخلايا.