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RESEARCH ARTICLE

Protective Effects of N Acetylcysteine and Vitamin E against Acrylamide-induced Neurotoxicity in Rats

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A B S T R A C T

When many widely ingested foods are heated, a toxin called acrylamide (ACR) is created. The aim of our research was to investigate if N acetylcysteine (NAC) and/or vitamin E (Vit E) could provide protection against neurotoxicity induced by ACR. Rats were classified into seven groups of 7 rats; control (saline); NAC (150 mg/kg bw); Vit E (100 mg/kg bw); ACR (20 mg/kg bw orally); ACR+NAC; ACR+Vit E; ACR+NAC+Vit E. Saline, NAC and/or Vit E were administered orally, once daily for 30 days. Acetylcholinesterase (AChE) inhibition, glutathione depletion, and elevated levels of malondialdehyde (MDA), were all observed after ACR intoxication. Furthermore, ACR diminished superoxide dismutase (SOD) and catalase (CAT) activities, as well as inflammatory mediators as interleukins (IL-1, IL-6), and tumor necrosis factor-α (TNF-α). The combined treatment of ACR with NAC or Vit E dramatically reduced both oxidative and biochemical consequences, with a more frequent return to normal values. To conclude, NAC or Vit E supplementation may alleviate ACR-induced neuronal injury, most likely due to NAC or Vit E antioxidant, anti-inflammatory, and anti-apoptotic effects.

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INTRODUCTION

Acrylamide (ACR) is a crystalline monomer with no smell, no color and high chemical reaction which was frequently utilized in wastewater control, the production of cosmetics and the synthesis of dyes (Aboubakr *et al*., 2019). It has also been found in widely consumed foods such cookies, crisps, French fries, crackers, breakfast cereals, and bread (Farouk *et al*., 2021). It can easily pass biological membranes due to its low molecular weight and high water solubility. As a result, it is quickly distributed throughout the body after intake and is readily absorbed from the gastrointestinal system (Yildizbayrak and Erkan, 2018).

Oxidative stress is frequently linked to ACR-induced toxicity. An imbalance between oxidant and anti-oxidant levels results from exposure to ACR, which is required for ACR toxicity (Hamdy *et al*., 2022). Previous studies reported neurotoxicity of ACR (Farouk *et al*., 2021, Fang *et al*., 2022).

N-acetylcysteine (NAC) is a precursor of the antioxidant glutathione (GSH). It can scavenge free radicals, replace depleted GSH and prevent lipid peroxidation (Samuni *et al*., 2013). NAC has neuroprotective effects positively affects effect on neurological disease recovery due to its ability to reduce inflammation (Bhatti *et al*., 2018). Furthermore, NAC penetrated the blood brain barrier and ameliorate changes in brain. Various benefits have been attributed to NAC administration in experimental animals, including increased GSH levels; amelioration of oxidative stress indicators were reduced, suppression of apoptosis caused

by dopamine or necrosis (Hassanen *et al*., 2019). NAC has minimized neuroinflammatory symptoms throughout the past decade (Khan *et al*., 2004). Furthermore, NAC may stop neuronal cells from death by removing free radicals.

One of the essential antioxidants, vitamin E, commonly known as tocopherol, can inhibit the oxidation of polyunsaturated fatty acids by preventing the action of the enzyme lipoxygenase (Conrad *et al*., 2018). Vit E, a naturally occurring, fat-soluble antioxidant, reduces oxidative stress via its anti-inflammatory and antioxidant actions (Esu *et al*., 2022). Several studies have found that natural antioxidants may be beneficial in reducing neurodegeneration (Rehman *et al*., 2019). Vital cellular structures can be secured by vitamin E from damage by both free radicals and oxidation products. In several neurodegenerative diseases, Vit E can defend neuronal tissue (Rahangadale *et al*., 2012, Esu *et al*., 2022).

Therefore, our research objective was to examine how NAC and/or Vit E could alleviate neurotoxicity caused by ACR in the brain tissues of rats.

MATERIALS AND METHODS

Chemicals: Acrylamide was bought from Sigma-Aldrich (USA), NAC (SEDICO, Egypt), and Vit E (Pharco Pharmaceuticals Industries, Alexandria, Egypt). Kits for estimation of MDA (Catalogue No: MD2529), SOD (Catalogue No: SD2521), GSH (Catalogue No; GR2511), and CAT (Catalogue No; MD2529) were obtained from Biodiagnostic CO, Cairo, Egypt. AChE assay kits (Catalogue No; DY757405) were obtained from R&D, Mannheim, Germany. ELISA kits for analyzing cytokines IL-1β (Catalogue No; AB100768) from Abcan Co, Tokyo, Japan) & IL-6 (Catalogue No; 95035) were purchased from Glory Science Co. Ltd, USA and TNF-α (Catalogue No; MBS032310) from (BioSource International, USA) to measure inflammatory processes.

Experimental design: Forty-nine male Wister Albino rats (185±20 g) were obtained from the Egyptian Organization for Biological Products and Vaccines. They were acclimatized for 7 days subdivided into seven groups and administered orally once daily for 30 days. Control group (saline); NAC group (150 mg/kg BW; Elsayed *et al*., 2022); Vit E group (100 mg/kg BW; Aboubakr *et al*., 2020); ACR group (20 mg/kg BW; Aboubakr *et al*., 2019); ACR+NAC, ACR+Vit E, and ACR+NAC+Vit E groups.

Sampling and processing: Rats were anaesthetized a day after the last treatment with isoflurane. From the retro-orbital plexus, blood samples were collected. The separated sera were kept at -20°C for subsequent biochemical analysis.

Tissue preparation: The entire brain was dissected, and used for measuring the oxidative stress markers. The brain was homogenized with potassium phosphate buffer and homogenates were centrifuged for 10 minutes at 5000 rpm. Then supernatant was saved at -80°C until their usage to test the oxidative/antioxidant characteristics (Abouakr *et al*., 2021).

Determination of serum AChE activity and proinflammation markers: AChE activity and cytokines (IL-1, IL-6, and TNF-α) were determined by ELISA kits.

Lipid peroxidation and antioxidant enzymes assay: The MDA in brain tissues was measured and activities of CAT, SOD, and the GSH concentration were also determined.

Histopathology examination and immunohisto-chemical localization of GFAP: Hematoxylin and eosin were used to stain the brain sections and the immunohistochemistry method was carried out as outlined by Farouk *et al*. (2021).

Histological/immunohistochemical scoring methods: Ordinal scoring method was used for grading the alterations of each histological parameter in both cerebrum and cerebellum (Meyerholz and Beck, 2018).

Statistical analysis: The data were reported as mean + SE. Using the statistical software package SPSS for Windows (Version 21.0; SPSS Inc., Chicago, IL, USA), data were analyzed using one-way ANOVA followed by Tukey's post hoc test for multiple group comparisons. Differences were judged statistically significant at $P \leq 0.05$.

RESULTS

AChE activity: The administration of ACR resulted in a considerable decrease in AchE activity. Meanwhile, the administration of NAC and/or Vit E to rats showed a modest increase in AchE activity in their brain tissue (Fig. 1).

Inflammatory cytokines markers: The IL-1β, IL-6, and TNF-α level in rat brain tissue were measured to explore the anti-inflammatory effect of NAC and/or Vit E on ACR-induced neurotoxicity. The current findings demonstrated that ACR treatment significantly increased IL-1β, IL-6, and TNF-α levels (Fig. 1). NAC and/or Vit E+ACR showed that NAC and/or Vit E administration suppressed the rise in these parameters, suggesting their anti-inflammatory efficacy against ACR neurotoxicity.

Brain lipid peroxidation and antioxidant status: ACR caused a considerable rise in MDA levels and a significant drop in GSH content, as well as SOD and CAT activities compared to the controls (Fig. 2).

Histopathological findings: Brain sections from control, NAC, and Vit E groups displayed histo-architectures (Fig. 1A-4). In these groups, the cerebral cortex was covered with thin pia mater, then outer molecular layer and various layers of pyramidal cells (Fig. 3A-C). The cerebellar cortex from control, NAC and Vit E groups was also covered by pia mater and its layers were arranged from outer to inner into molecular layer, Purkinje cells layer then granular layer (Fig. 4A-C). On contrary, abnormal histo-architectures were noticed in ACR group in cerebral and cerebellar cortices. The cerebral cortex exhibited significant congestion of

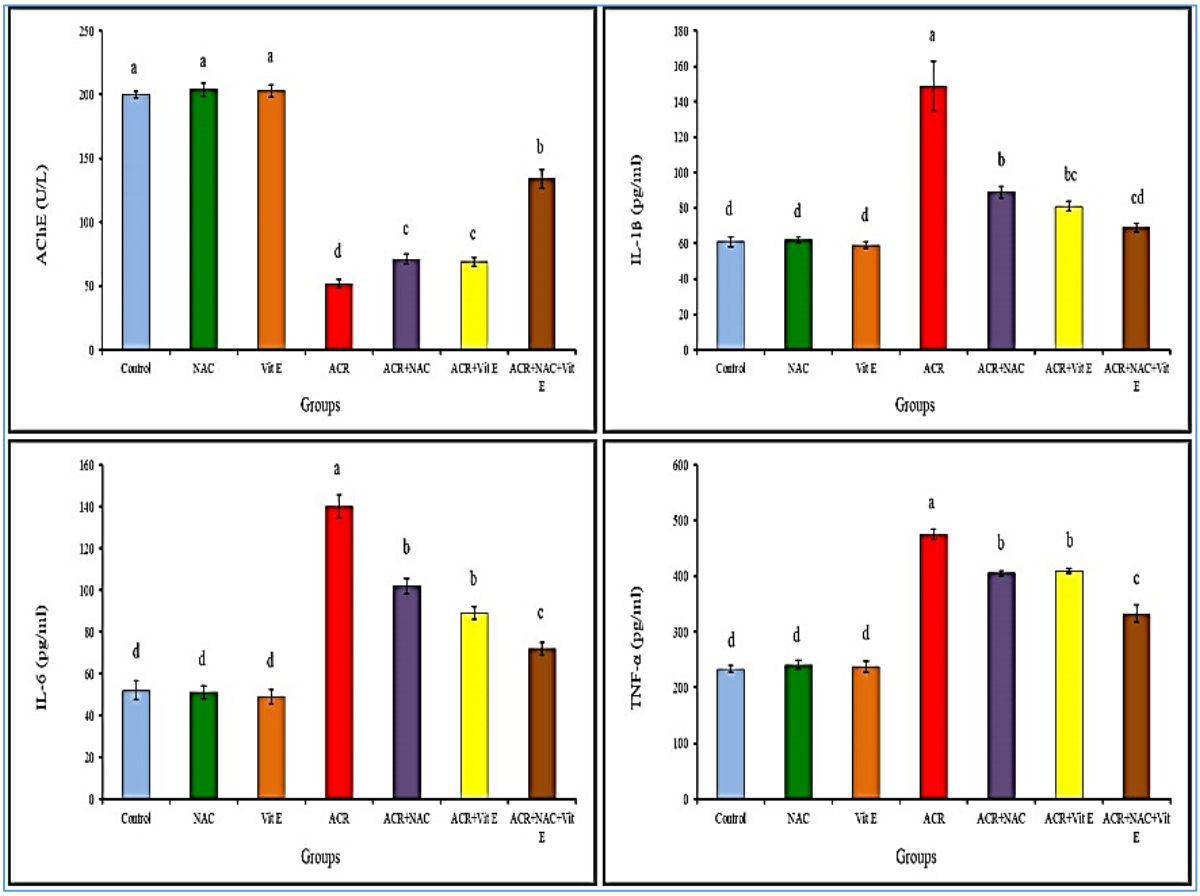

Fig. 1: Effect of NAC and/or Vit E on serum AChE activity and IL-1β, IL-6,TNF-α levels against ACR induced neurotoxicity in rats (n=7).

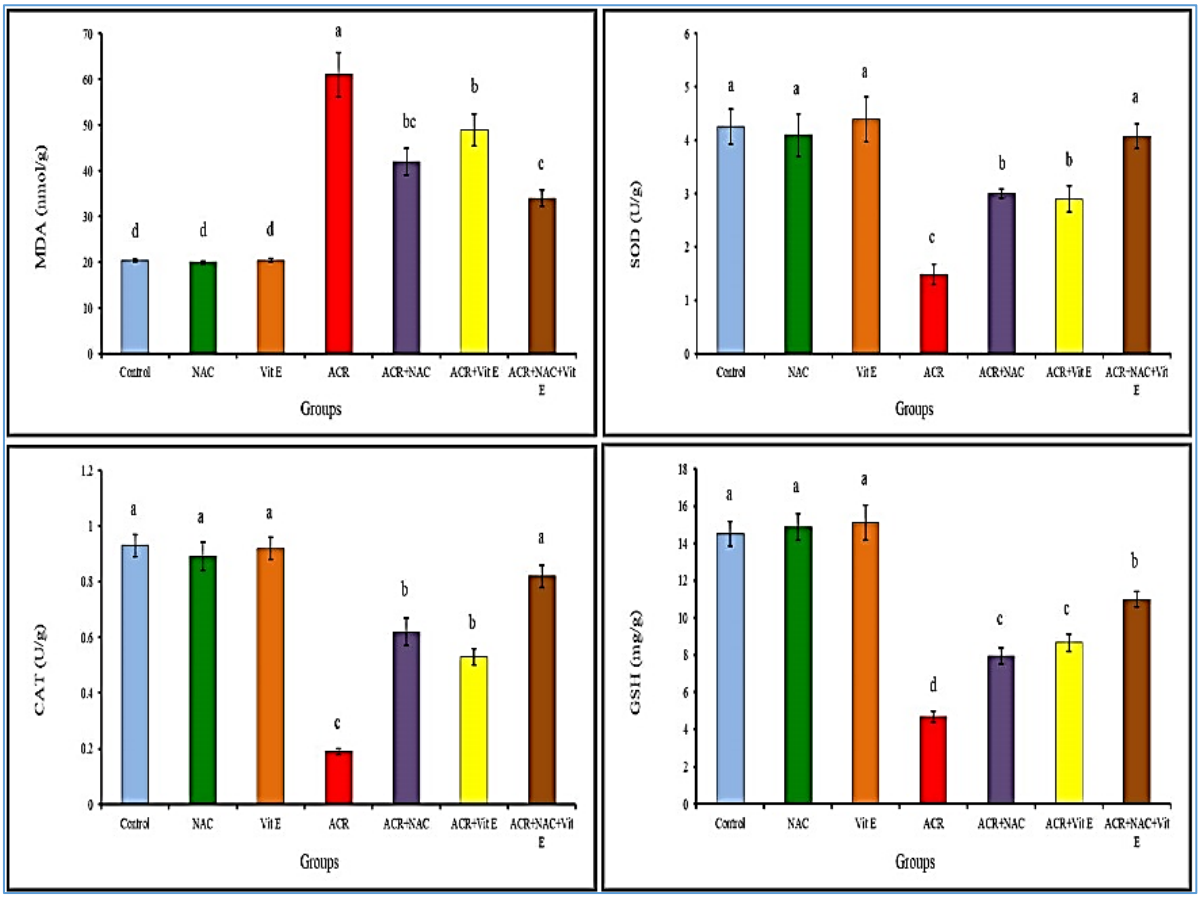

Fig. 2: Effect of NAC and/or Vit E on oxidative stress parameters against ACR induced neurotoxicity in rats (n=7).

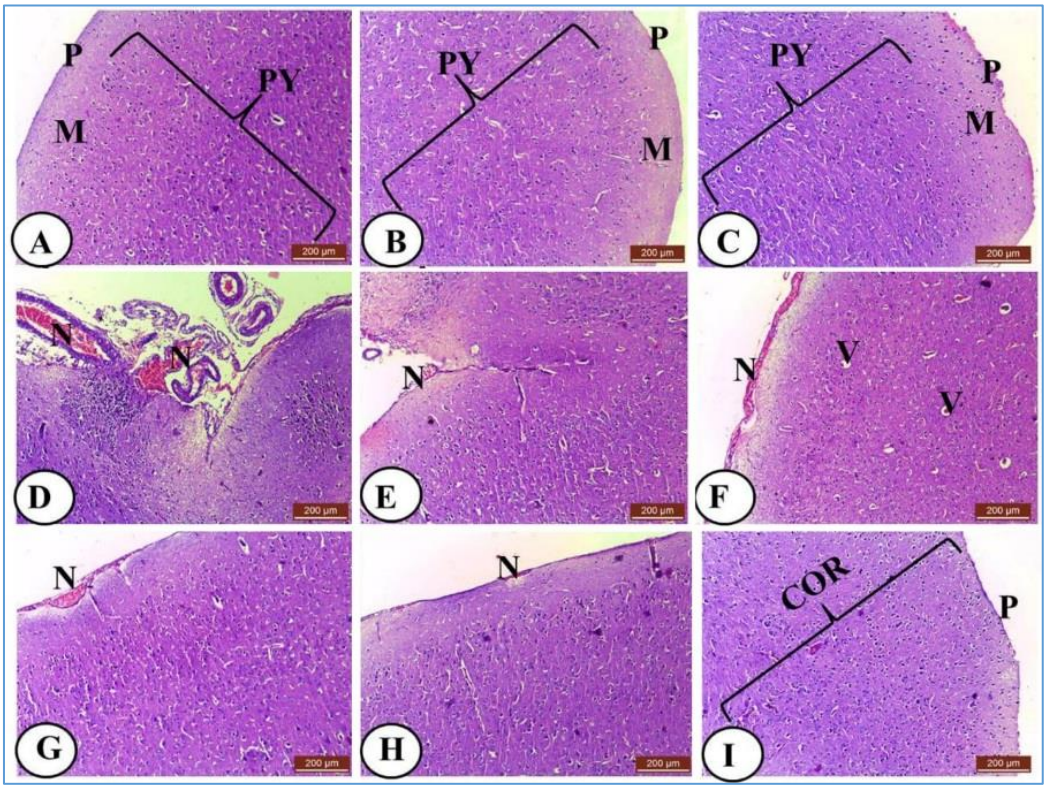

Fig. 3: Histological sections of cerebrum from control (A), NAC (B), Vit E (C) and ACR (D-F), ACR+NAC (G), ACR+Vit E (H) and ACR+NAC+Vit E (I) groups. Control, NAC, and Vit E groups showed thin cover of pia mater (P), then outer molecular layer (M) and various layers of pyramidal cells (PY). D-F; ACR treated rats showed several histological changes; congestion (C) of the cortical blood vessels and pia mater, degenerated neurons with pyknotic nuclei (arrow), and vacuolar spaces (V) around the pyramidal cells. ACR+NAC and ACR+Vit E showed mild congestion (G) of pia mater and mild degenerated cells compared to ACR-exposed rats. ACR+NAC+Vit E showed restoration of the normal cerebral architecture of pia mater (P) and cortical layers (COR). H&E stain, scale bars=200µm.

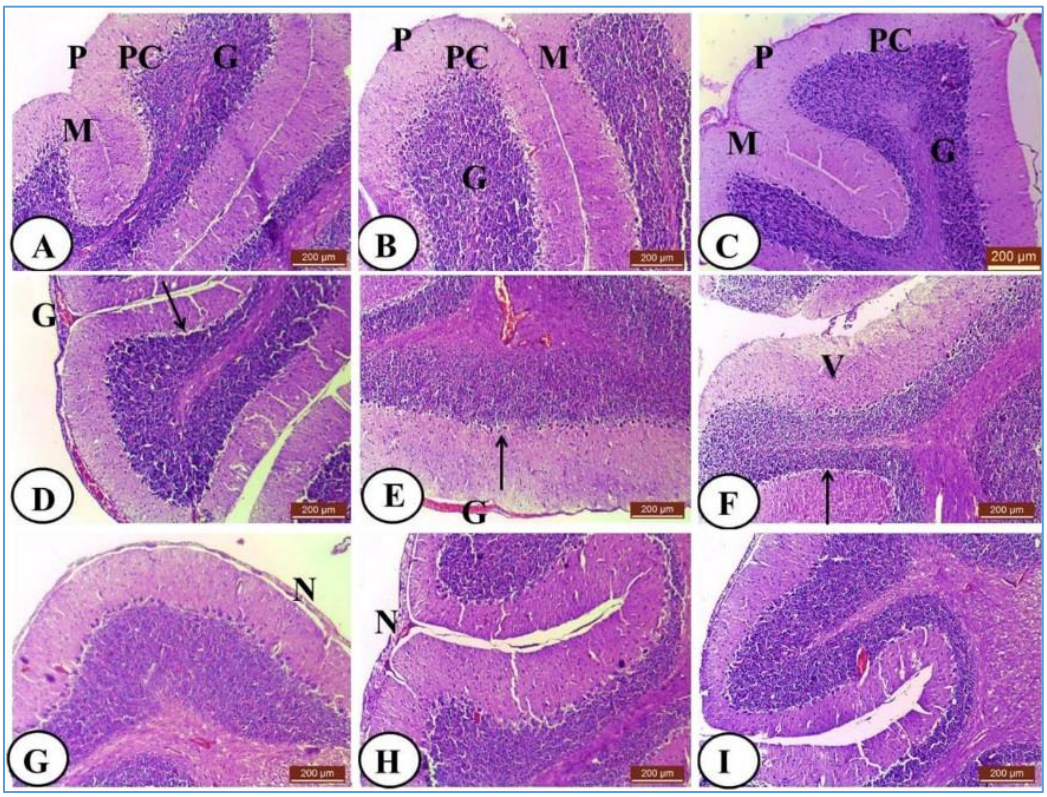

Fig. 4: Histological sections of cerebellum from control (A), NAC (B), Vit E (C) and ACR (D-F) groups. Control, NAC, and Vit E groups showed external pia mater (P) then molecular (M), Purkinje cell (PC) and granular (G) layers. ACR treated rats showed several histological changes; The pia mater of cerebellum showed a marked congestion (N) as well as vacuolation of molecular layer (V) and degeneration of some Purkinje cells (arrow). ACR+NAC and ACR+Vit E showed mild congestion (N) of pia mater and mild degenerative changes compared to ACR-treated rats. ACR+NAC+Vit E showed restoration of the normal cerebellar cell layers.H&E stain, scale bars=200µm.

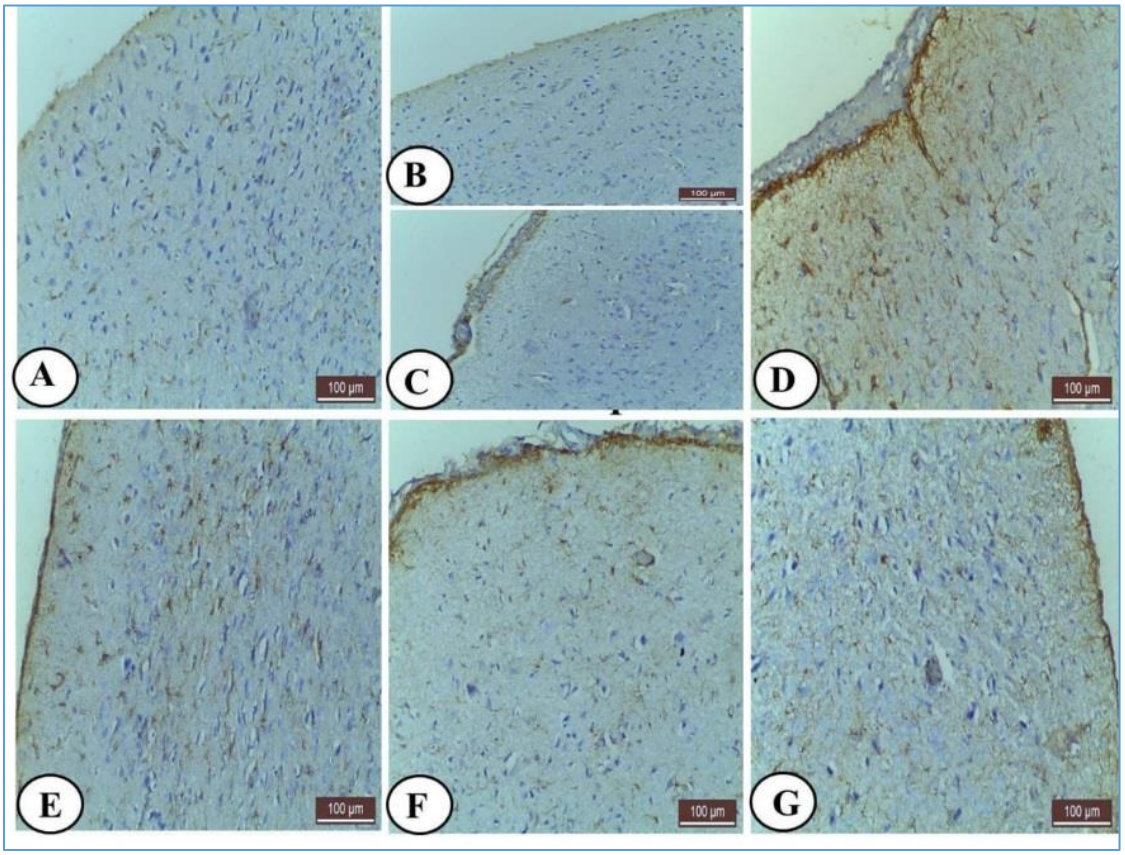

Fig. 5: Immunohistochemical staining of GFAP in cerebral sections from all experimental groups. Control (A), NAC (B) and Vit E (C) groups showed weak GFAP staining in the neuronal cells. D; ACR-treated group (D) revealed strong GFAP staining. ACR+NAC (E), ACR+Vit E (F) and ACR+NAC+Vit E (G) groups, showed moderate immunoreaction in comparison to ACR group. Scale bars = 100µm.

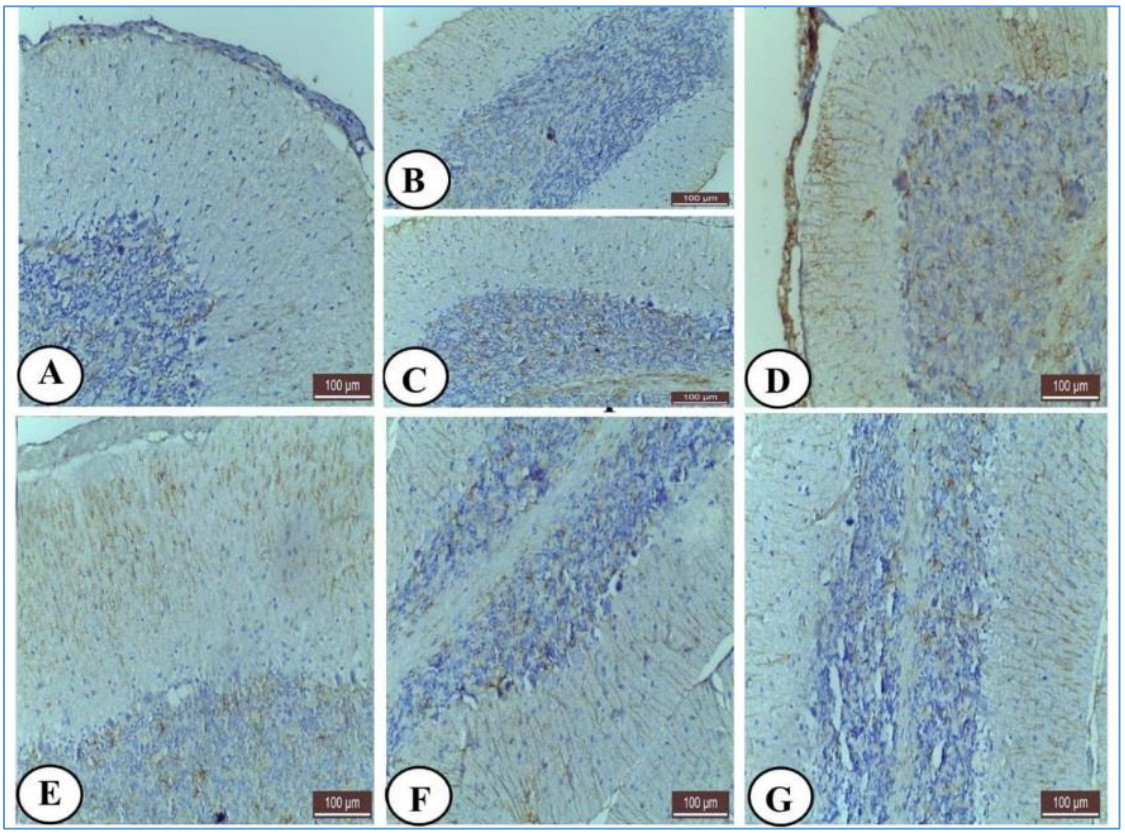

Fig. 6: Immunohistochemical staining of GFAP in cerebellar sections from all experimental groups. Control (A), NAC (B) and Vit E (C) groups showed weak GFAP staining in the neuronal cells. ACR group (D) revealed strong GFAP staining. ACR+NAC (E), ACR+Vit E (F) and ACR+NAC+Vit E (G) groups, showed moderate immunoreaction in comparison to ACR group. Scale bars = 100µm.

Table 1: Ordinal scores of histological changes in cerebrum from different groups.

	Control group	NAC group	Vit E group	ACR group	ACR+NAC group	ACR+Vit E group	ACR+NAC+Vit E group
Congestion of Pia mater	0±0^{c}	0±0^{c}	0±0^{c}	3.91±0.2^{a}	1.77±0.3^{b}	1.82±0.2^{b}	0±0^{c}
Congestion of vessels	0±0^{c}	0±0^{c}	0±0^{c}	3.89±0.5^{a}	0±0^{c}	0±0^{c}	0±0^{c}
Pyknotic nuclei	0±0^{c}	0±0^{c}	0±0^{c}	3.80±0.4^{a}	1.84±0.3^{b}	1.81±0.3^{b}	0±0^{c}
Vacuolar spaces	0±0^{c}	0±0^{c}	0±0^{c}	3.77±0.3^{a}	1.80±0.5^{b}	1.79±0.7^{b}	0±0^{c}

All values expressed as the mean ± SE. Superscript letters within the same rows were significant (P ≤ 0.05).

Table 2: Ordinal scores of histological changes in cerebellum from different groups.

	Control group	NAC group	Vit E group	ACR group	ACR+NAC group	ACR+Vit E group	ACR+NAC+Vit E group
Congestion of Pia mater	0±0^{c}	0±0^{c}	0±0^{c}	3.80±0.2^{a}	1.86±0.4^{b}	1.76±0.5^{b}	0±0^{c}
Vacuolar molecular layer	0±0^{c}	0±0^{c}	0±0^{c}	3.30±0.4^{a}	0±0^{c}	0±0^{c}	0±0^{c}
Purkinje degeneration	0±0^{c}	0±0^{c}	0±0^{c}	3.78±0.6^{a}	1.89±0.6^{b}	1.98±0.8^{b}	0±0^{c}

All values expressed as the mean ± SE. Superscript letters within the same rows were significant (P ≤ 0.05).

Table 3: PS, IS, and TS ± SE for GFAP immunohistochemical expressions in cerebrum from different groups.

	Control group	NAC group	Vit E group	ACR group	ACR+NAC group	ACR+Vit E group	ACR+NAC+Vit E group
PS	1.16±0.03	1.24±0.02	1.23±0.05	4.26± 0.0	3.57±0.2	3.80±0.3	3.11±0.1
IS	0.50±0.05	0.68±0.02	0.76±0.03	3.46±0.03	2.43±0.3	2.40±0.0	2.20±0.4
TS	1.66±0.06^{c}	1.92±0.06^{c}	1.99±0.06^{c}	7.62±0.03^{a}	6.00±0.0^{b}	6.20±0.2^{b}	5.31±0.0^{b}

PS proportional scores; IS intensity scores; TS total scores. All values expressed as the mean ± SE. Superscript letters within the columns of TS for each organ were significant (P ≤ 0.05).

Immunohistochemical findings: The immunohistochemical expression of GFAP from ACR group revealed significantly strongest immunoreaction of glia cells in cerebrum (Fig. 5D) and cerebellum (Fig. 6D) compared to significantly weak GFAP immunoreaction in cerebral (Fig. 5A-C) and cerebellar (Fig. 6A-C) sections from control, Vit E, and NAC groups. However, NAC, Vit E with ACR and ACR+Vit E+NAC revealed moderate GFAP cerebral (Fig. 5E-G) and cerebellar (Fig. 6E-G) immunoreactions compared to ACR group. A summary of GFAP immunohistochemistry scoring was shown in Table 3.

DISCUSSION

Neurotoxicity of ACR was indicated by oxidative stress. ACR neurodegeneration has previously been documented (Fang *et al*., 2022). ACR induced oxidative stress caused by the production of ROS, which results in an antioxidant imbalance (Farouk *et al*., 2021).

AChE activity is a well-known biomarker used to investigate the neurotoxic effects of drugs or chemicals. It is an enzyme which converted acetylcholine to choline and acetate. Results from the present study showed that compared to normal controls, rats intoxicated with ACR had considerably reduced levels of AChE. Furthermore, we discovered that treatment with NAC or Vit E significantly attenuated ACR-induced decreases in serum AChE levels. Similar findings were reported for NAC against neurotoxicity of chlorpyrifos in rats (Mahmoud *et al*., 2019), and Vit E against neurotoxicity induced by methidathion in rats (Sutcu *et al*., 2006).

Higher levels of IL-1β, IL-6, TNF-α were reported after ACR toxicity. Excessive ROS generation and oxidative stress increased proinflammatory genes expression and cytokines release (Aboubakr *et al*., 2021). The current findings are consistent with those obtained by Bin-Jumah *et al*. (2021). Also, Treatments with NAC and Vit E significantly decreased the rises in serum IL-1, IL-6, and TNF-induced by the ACR; this also reported for NAC (Abdel-Wahab and Moussa, 2019) and Vit E (Al-Rasheed *et al*., 2014).

NAC has anti-inflammatory and antioxidant characteristics that are attributed to its ROS scavenging and antioxidant upregulation actions (Elsayed *et al*., 2022). Furthermore, a previous research has shown Vit E's anti-inflammatory activity (Rossato *et al*., 2015).

ACR dramatically lowered CAT and SOD activities, as well as GSH levels, and induced a sharp increase in MDA levels in brain when compared to control rats (Abdel-Daim *et al*., 2020). Activities of SOD, GSH and CAT were considerably higher in the ACR+NAC and ACR+Vit E groups than in the ACR group. MDA levels were markedly lower in the ACR+NAC and ACR+Vit E groups than in the ACR treated group. The above findings clearly illustrated the efficacy of NAC and/or Vit E in saving rat brains from oxidative stress caused by ACR intoxication.

ACR-induced neurotoxicity is severe and has clinical significance; hence there is increased interest in using neuroprotective substances. Antioxidant substances are mentioned as potential elements in the suppression of ACR neurotoxicity due to the significant role that oxidative stress plays in ACR-induced neurotoxicity. The neuroprotective effects of NAC and Vit E via oxidative stress inhibition have been well documented (Mahmoud *et al*., 2019).

Previous investigations have confirmed up the idea that GSH plays a part in the benefits that NAC has. The key to NAC action was presumed to be the elevation of GSH levels in the oxidized-stressed brain following NAC treatment (das Neves Duarte *et al*., 2012). It acts as an L-cysteine residue to maintain GSH synthesis and is transformed in the body into metabolites that promote glutathione synthesis. Thus, it keeps intracellular GSH levels stable, improves detoxification, and directly scavenges free radicals (Mahmoud *et al*., 2019). Chlorpyrifos poisoning demonstrated NAC's direct interaction with ROS, increasing SOD activity and reducing the brain's sensitivity to these oxidants (Mahmoud *et al*., 2019).

Neurotoxicity caused by ACR was counteracted by Vit E. Numerous investigations have established the role of this vitamin as an antioxidant and antiapoptotic agent (Ungurianu *et al*., 2021). Vit E induced an improvement in the oxidative/antioxidative balance. Also, Foroutanfar *et al*. (2020) proved that ACR drastically increased MDA levels and lessened GSH levels in the cerebral cortex; in contrast, vitamin E treatment greatly reduced MDA levels and increased GSH levels when compared to the ACR

group. However, Rahangadale *et al*. (2012) mentioned that Vit E is not able to protect rats from ACR toxicity.

The current study revealed several cerebral and cerebellar degenerative changes after ACR exposure that was proven by histopathological sections in addition to overexpression of GFAP. The congested pia mater, degenerated neuronal cells with pyknotic nuclei and vacuolation were detected which were in similarity with Mansour *et al*. (2017). On contrary, co-administration of NAC and/or Vit E markedly counteracted the pathological effects of ACR on brain tissues.

ACR treated group revealed strong expression of GFAP that was similar to the findings of Farouk *et al*. (2021). GFAP is biomarker for gliosis (O'Callaghan and Sriram 2005) indicating the adverse effect of ACR on brain tissues. However, NAC and/or Vit E administration decreased the expression of GFAP in both cerebral and cerebellar tissues.

Conclusions: In conclusion, ACR induced oxidative damage and inflammation in the brain. However, to a large extent, NAC and Vit E offered chemoprotective roles on ACR-induced-toxicity in brain tissues. Treatment with NAC or Vit E reduced the ACR-induced neurotoxicity, as evidenced by a considerable increase in serum AChE and a decrease in proinflammatory cytokines, as well as an improvement in the oxidative/ antioxidative balance.

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Ethics approval: The present study was approved by the Ethical Committee of the Agricultural Research Center, Egypt (ARC-AH-2202).

Authors contribution: MA, ST, AF, and AE, contributed in study experimental work, design, and writing. ME and MA; performed the histopathological and immunohisto-chemical. AS and AS analyzed tissue and sera samples. All authors approved the final version and interpreted the data.

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