The potential neuroprotective effect of allicin and melatonin in acrylamide-induced brain damage in rats

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Research Article

Abstract

Acrylamide (ACR) is an unsaturated monomer that entered in various fields however, it is a potent neurotoxic. The present study target is to explore the neuroprotective efficacy of allicin and melatonin on ACR-induced neurotoxicity. Thirty-six male adult rats were non-selectively separated into six groups: placebo, allicin (20 mg/kg b.w daily per os), melatonin (10 mg/kg b.w 3 times/week per os), ACR (50 mg/kg b.w daily per os), ACR + allicin and ACR + melatonin with the same doses. The assessment of brain biomarkers, neurotransmitters, antioxidative status, Nrf2 signalling pathway, and histopathological analyses were performed following 21 days. ACR exposure enhanced the brain lipid and DNA oxidative damage as well as a reduction in the GSH levels. The obvious brain oxidative injuries was contributed to distinct brain dysfunction that was assured by alteration of brain neurotransmitters (serotonin, dopamine, acetylcholine, and acetylcholinesterase), and pathological brain lesions. Furthermore, ACR exposure increased hydroxy deoxy guanosine (8-OHdG), tumor necrosis factor (TNF) and amyloid protein (AB-42). Finally, the mRNA transcripts of brain Keap-1, Nrf2, and NF-kB were up regulated after ACR intoxication. Interestingly, allicin and melatonin alleviated the ACR-induced brain damage assessed by normalization of the mentioned analyses. The present study demonstrated the protective role of both allicin and melatonin on ACR-prompted neuropathy by alleviation of redox imbalance and enhancement of neurotransmitters as well as relieving DNA damage and anti-inflammatory effect.

Introduction

Acrylamide (ACR) is an unsaturated monomer which entered in various benefits as waste water control, soil conditioners, tincture production, gel electrophoresis and various commercial applications as: the cosmetic, paper, textile industries (Mehri et al. 2015). However, polyacrylamide is not toxic, ACR is a powerful neurotoxic that causes damages to both CNS and PNS. ACR is not exist in nature and commonly not found in raw or boiled food (Khaneghah et al. 2020) but it is synthesized when sugar-rich diets are processed at high temperature by chemical reaction between asparagine and reducing sugars (Acaroz et al. 2018). ACR exposure had been recorded through ingestion of ACR-containing food, drinking polyacrylamide flocculants-contaminated water, occupational exposure through dermal contact or inhalation of industrial manufacturing dusts (Shinomol et al. 2013). Furthermore, Rifai and Saleh (2020) stated that the tobacco smoking is the main reason of ACR exposure than food. ACR is absorbed just after ingestion and then reached to all body organs and has the ability to pass the placental barrier and also breast milk, thus it transferred to fetus (Ghareeb et al. 2010). ACR is metabolized in two ways; first mechanism for the detoxification of ACR is conjugation with GSH and the other way its epoxidation to the epoxide glycidamide via cytochrome P450 2E1. However, ACR epoxidation to form glycidamide is more important in rodents than in humans (zodl et al. 2007). Excessive brain accumulation of ACR caused excessive inflammatory cell infiltrations and finally cumulative neurotoxicity in both humans and laboratory animals. In rodents and other experimental animals, ACR is a neurotoxic, genotoxic, carcinogenic monomer and induces developmental and reproductive toxicity (Prasad and Muralidhara

2013). Moreover, ACR is responsible for the enhancement of ROS in many cells by increasing the level of lipid peroxidation, and decreasing redox imbalance (Mehri et al. 2012).

Garlic, known as *Allium Sativum*, which used in food and medicine for a long time (Liu et al. 2015). While, Allicin (allyl-2-propenethiosulfinate or diallyl thiosulfinate), is one of the main active biomolecules found in garlic (Ghareeb et al. 2010). Allicin exhibits prophylactic activity against neuronal injury, atherosclerosis, cardiovascular disease, arrhythmia, hyperlipidemia, thrombosis, hypertension, cancer, and diabetes (Asdaq and Inamdar 2011). Moreover, Borlinghaus et al. (2014) discussed that garlic has nutritional, antimicrobial and immune-modulatory activities. The composition of allicin including flavonoids and sulfur-containing compounds diallyl disulphide (DDS), S-allylcysteine (SAC) and diallyl trisulfide (DTS) which are potent antioxidamts (Mikaili et al. 2013). Recently, phytochemicals and other natural products including allicin is able to reverse ACR-induced neuropathy in rodents (Tamimi and AL-Domi, 2019). It is considered as a potent antioxidant by scavenging ROS, inhibiting lipid oxidation and enhancing formation of pro inflammatory messengers (Ghareeb et al. 2010).

Melatonin (MT) is the master neurohormone secreted via the pineal gland at night. It possess a vital function in various physiological processes including circadian rhythms, reproductive, neuroendocrine, cardiovascular, neuroimmunological and oncostatic actions (Tamura et al. 2012). Melatonin also has a protective effects on some of pathological damages as shock, ischemia and inflammation. Melatonin is a potent lipophilic antioxidant against reactive oxygen species and neuroprotective in both *vivo* and *vitro*. It could reduce the redox imbalance and inhibit apoptotic mechanisms (Pan et al. 2015). MT prevents neuronal apoptosis enhanced by ROS and brain injury caused by singlet oxygen, and is effective in reducing hydrogen peroxide-induced lipid peroxidation in brain tissues. Also, MT is reported to prevent the AB and NO toxicities from reaching to CNS (Ahmed et al. 2010). Moreover, MT has a defensive role versus redox imbalance induced by ACR- (Li et al. 2018). Recently, some studies suggested that MT can be applied in individuals suffering from COVID-19 to decrease deaths through reduction of Coronavirus-activated inflammasome (Shneider et al. 2020). Moreover, Reiter et al. (2020) stated that MT level reduced in patients with COVID-19 as COVID-19 may disturb the melatonin synthetic pathway. Melatonin has a powerful effects as an anti-viral which make it a choice for use In COVID-19 infection (Anderson and Reiter 2020).

Our hypothesis is that allicin and melatonin have the ability to regenerate the redox imbalance and brain damage .The current study was done to investigate the mechanistic pathway of ACR-induced neuropathy and the preventive action of allicin and melatonin via the estimation of oxidative stress/antioxidant indices, brain neurotransmitters, Nrf2 signalling pathway, and biochemical and histopathological analyses.

Material And Methods

Reagents and chemicals

Highly purified powdered ACR, allicin, melatonin, and other pharmaceutical grade chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA), ACR was dissolved in distilled water before use. Diagnostic kits for Gene Jet Genomic DNA purification kit obtained from (Thermo Scientific, USA), commercial 8-OH-dG ELISA kit was obtained from (Abcam, Cambridge, UK). Immunoassay kits for the quantitative determination of rat serotonin, Ach and Aβ1−42 were obtained from Biospes Co., China. Immunoassay kit for the quantitative determination of rat dopamine was purchased from (Elabscience, co, USA). The rat TNF-α ELISA kit was purchased from (eBioscience, USA) and Total RNAs Extraction Kit using RNeasy Mini Kit, miScript II RT Kit for Reverse transcription and Rotor-Gene SYBR Green PCR Kit were obtained from (Qiagen, Germany).

Animals, and experimental layout

Thirty-six male rats with 160−180 g were obtained from Animal Breeding Unit, Faculty of Agriculture, Alexandria University. The rats were maintained under controlled supervision with suitable temperature, humidity, and dark/light cycle and free access to rat ration and drinking water. The dealing of rats were done refereeing to international ethical guidelines for the care and use of laboratory animals and the approval of experimental procedures were done by the Experimental Animal Use and Ethics Committee at the Faculty of Veterinary Medicine, Alexandria University, Egypt. The rats were non-selectively divided into six groups (6 rats each); control, allicin group orally gavaged with allicin 20 mg/kg b.w, daily for 21 days (Zhang et al. 2013), melatonin group orally gavaged with melatonin 10 mg/kg b.w, day by day for 21 days (Soliman et al. 2019), ACR-intoxicated group orally gavaged with ACR 50 mg/kg b.w, daily for 15 days (Aydin 2017), allicin + ACR group orally gavaged with ACR and allicin with the same previous doses, melatonin + ACR group orally gavaged with ACR and melatonin with the same previous doses. A day following the last doses, the rats were anesthetized by xylazine/ ketamine (1 mg/kg, 75−100 mg/kg, i.p).The blood was gathered from the inner canthus, and the sera were detached for estimation of Tumor necrosis factor (TNF). The euthanization of rats were done and the brain were rapidly sliced, swilled with chilled normal saline 0.9%, and separated into three fractions; one was used for histopathological analyses, the second part was used for gene expression analyses, and the last part was used for determination of brain neurotransmitters and oxidative stress/antioxidant indices.

Estimation of serum TNF-α and brain amyloid beta1-42

At the end of experiment, blood samples were gathered from the retro-orbital vein of each rat and each sample was collected into clean tubes. The blood samples were left till coagulation and then centrifuged at 3000 rpm for 10 min. The separated sera were kept at -20∘C until used for the estimation of serum activity of TNF by Immunoassay kit (eBioscience, USA). Immunoassay kit (Biospes Co., china) was used for the quantitative determination of rat Aβ1−42 in cortical tissue homogenate.

Oxidative stress indices

The excised cortex tissues were rinsed with saline and then homogenized in ice-cold using phosphate buffer saline (PBS) pH 7.4 in the ratio of 1:10. The homogenate was centrifuged at 10000 rpm, at 4o C for

20 min for supernatant production. Lipid peroxide was estimated after the reaction with thiobarbituric acid and represented as nmole malondialdehyde (MDA)/ g tissue weight (Draper and Hadley 1990). Total glutathione and GSSG contents were determined according to Griffith (1980). 8-OH-dG as index of oxidative DNA damage was determined in DNA samples, using a commercial 8-OH-dG ELISA kit (ab201734, Abcam, Cambridge, UK)

Estimation of brain neurotransmitters

The homogenized excised cortex tissues used for determination Ach, serotonin, and dopamine were estimated using Immunoassay kits. While, the activity of AChE enzyme in cortical tissue homogenates were estimated by the colorimetric method of. (Ellman et al. 1961).

RNA extraction and qRT-PCR

About 100 mg brain tissues were homogenized in liquid nitrogen then, the homogenate was stored at −80°C till RNA isolation. Total RNA was isolated using the RNeasy Mini Kit (Qiagen GmbH, Germany) according to the manufacturer's instructions. cDNA was synthesized from the purified RNA using the Mini Kit, miScript II RT Kit for Reverse transcription(Qiagen, Germany). The reaction mixture included RNA and master mix were placed at 37°C then inactivated at 95°C. The qRT-PCR for the target genes were applied using Rotor-Gene SYBR Green PCR Kit were obtained from (Qiagen, Germany). The primer sequences of all target and reference genes and the PCR conditions were recorded in Table 1. The fold change of mRNA expression was calculated after recording the Ct values for reference and target genes using the 2 − ΔΔCt method.

Table 1
Primer sequences of NRF2, NF-κB, keap-1and GAPDH

Gene	Gene description	Gene bank Accession No.	primer sequence	
NRF2	Nuclear factor erythroid 2 like factor	NM_031789.2	F:	5'-CAAATCCCACCTTGAACACA-3'
			R:	5'-CGACTGACTAATGGCAGCAG – 3'
Keap1	Kelch Like ECH Associated Protein 1	NM_057152	F:	5'-GGACGGCAACACTGATTC-3'
			R:	5'-TCGTCTCGATCTGGCTCATA-3'
NF-κB	Nuclear factor kappa B	NM_199267.2	F:	5'-CAGGACCAGGAACAGTTCGAA-3'
			R:	5'-CCAGGTTCTGGAAGCTATGGAT-3'
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	NM_017008.4	F:	5'-GGGTGTGAACCACGAGAAATA-3'
			R:	5'-AGTTGTCATGGATGACCTTGG-3'

Histopathologic examination

Brain samples from each group were gathered and rapidly fixed in 10% buffered formalin for at least 24 h. Tissue specimens were washed, dehydrated by serial dilutions of alcohol, cleared in xylene, and immersed in paraffin at 60°C in a hot air oven. Paraffin sections of 4–5-μm thickness were prepared and stained with hematoxylin and eosin (HE) according to the method described by Culling, (1983) and examined under the light microscope.

Statistical analyses

SAS software for Windows Version 9.10 (SAS Institute Inc., Cary, NC, USA) was used for the analysis of data. A probability value less than or equal to 0.05 was considered statistically significant. Variables passed the Shapiro-Wilk test of normality, therefore we used univariate analysis of variance (ANOVA) for group effect. Group mean differences were assessed using Tukey's test. Groups are represented as mean and standard error. Pearson's correlation analysis was carried out among selected variables.

Results

Brain oxidative/antioxidant indices

The present data cleared in Fig. 1 showed that ACR lead to a marked elevation in brain MDA (5.55 ± 0.38 nmole/g tissue), and 8-OHdG (21.0 ± 0.71 pg/μg DNA) compared with control values, assuring the neural toxic effect of ACR. In comparison with the ACR group, co-treatment of allicin and melatonin statistically reduced the changed biochemical tests, which was represented by a marked decline in brain MDA and 8-OHdG levels. The data presented in Fig. 2 dedicated that ACR led to a marked in the GSH level (2.15 ± 0.070 nmole/mg protein), and the GSH to GSSG ratio (6.70 ± 0.12) as well as marked increase in the GSSG level (0.32 ± 0.010 nmole/mg protein), in comparison with control values, as well as increased confirmation that ACR is able to generate oxidative imbalance. Co-treatment with allicin or melatonin led to a significant (p < 0.05) decline in the brain GSSG level with subsequent significant increase in the brain GSH level.

Brian neurotransmitters analyses

With regard to brain neurotransmitters, ACR enhanced a significant (p < 0.05) decline in Ach (2.07 ± 0.05 pmol/mg protein), serotonin (1.96 ± 0.10 ng/mg protein), and dopamine (149 ± 2.96 pg/mg protein) levels as well as a marked elevation in AchE (23.7 ± 0.90 U/mg protein) activity upon comparing to the control group proving brain dysfunction and alteration of neurotransmitters levels. Comparing to the ACR group, co-treatment of allicin and melatonin ameliorated the changed biochemical tests, which was proved by a marked elevation in brain neurotransmitters levels as well as the decline of AchE activity (Fig. 3).

Serum tumor necrosis factor and brain amyloid beta 1−42

The data presented in Fig. 4 revealed comparable to control values, ACR induced a marked increase in TNF (188 ± 7.93 ng/ml) and AB1-42 (3.27 ± 0.15 pg/mg protein) levels assuring the neural toxic effect of ACR. Upon comparing to ACR group, co-treatment of allicin and melatonin ameliorated the changed biochemical tests, which was proved by a marked decline in serum TNF, and AB1-42 levels.

NrF2 signaling pathway

With concern to NrF2, ACR intoxication resulted in significant depletion of NrF2 protein expression alongside with upregulation of NrF2 mRNA transcript in brain tissue as a compensatory mechanism to counteract the oxidative insult. However, co-treatment with allicin and melatonin increased the NrF2 protein with restoring the NrF2 mRNA transcript expression in ACR-intoxicated rats (Fig. 5).

Relative mRNA expression of brain NF-kB and Keap-1pathway

ACR significantly upregulated mRNA transcript of brain NrF2, Keap-1, and NF-kB as compared with the control confirming that ACR causes oxidative stress which significantly activated the Nrf2 gene expression. However, ACR significantly increase Keap-1 expression which degrade Nrf2 protein and subsequently the Nrf2 gene expression increased. On the other hand, administration of allicin or

melatonin ameliorated the changed gene expression, which was represented by the marked downregulation of NrF2, Keap-1, and NF-kB mRNA transcripts (Fig. 6).

Histopathological findings

Histopathological evaluations of cortical tissues of the control group, allicin, and melatonin groups revealed normal histological characteristics (Fig. 7, 8). ACR-treated rats showed mild inflammatory cell infiltration in the meninges. Additionally, congestion of submeningeal blood vessels, hemorrhage in the meninges, neuropil spongiosis, and neuronophagia of degenerated neuron. Co-treatment of allicin or melatonin showed congestion of submeningeal and cortical blood vessels in ACR-treated rat.

Discussion

The current study was done to explore the probable neuropathic actions of oral ACR administration, and the preventative action of allicin and melatonin via estimation of brain functions, redox status, gene expression changes and histopathological findings. ACR can induce neurotoxicity, reproductive toxicity and genotoxicity in animal models and human beings. The oxidative stress is the main factor for inducing of acrylamide toxicities (Zamani et al. 2017). The current data revealed that ACR led to marked elevations in the serum TNF level, cortical amyloid protein level, and AchE activity with subsequent decline of brain neurotransmitters (acetylcholine, serotonin, and dopamine) which are potent markers of brain damage. ACR-induced neurotoxicity was alleviated after the co-treatment with allicin and melatonin as confirmed by the marked declines in the serum TNF, cortical amyloid protein level and AchE activity along with elevating brain neurotransmitters. ACR led to the stimulation of inflammatory cells by releasing several cytokines and increasing the expression levels of inflammation-related genes (Acoroz et al. 2018). Serum TNF significantly increased after ACR administration which mediated by stimulating of NF-κB pathway. The nerve terminal is the main site for ACR action where inhibits neurotransmitter release and enhances downstream degeneration (Abd El-monem and Ali 2012). ACR induced marked reduction of dopamine levels in whole brain due to the increase in monoamine oxidase activity that is responsible for the increase of dopamine catabolism (Rawi et al. 2012). Moreover, ACR has the ability to react with intercellular molecules that contain SH, NH_2 or OH and thiol-rich proteins that involved in the release of neurotransmitters (serotonin and DA). This ACR adduction is the cause of defective neurotransmission in ACR-intoxicated laboratory animals (Abozaid et al. 2017a). ACR significantly increase the level of brain Aβ due to acute disruption of transthyretin secretion by ACR. Transthyretin (TTR) acts as a thyroxine transport that circulates in blood and CSF and possess a neuroprotective role by interacting with Aβ (Abozaid et al. 2017b). The TTR-Aβ combination is necessary to illustrate the protective technique of nervous system (Du and Murphy 2010). So, ACR leads to decrease in transthyretin levels with increase in Aβ which involved in neurotoxicity by affecting nerve regeneration (Yao et al. 2014). Aβ level has a marked significance and correlated directly with cholinesterase activity. Aβ elevates AChE activity by promoting calcium entrance, the elevation of AChE expression is a result of the upset of calcium homeostasis (Melo et al. 2003). ROS formed by ACR disturbed the redox status in the brain, which was a cause of the increase in the AChE activity. This elevation of AChE activity in the brain is a potential

indicator of neuronal damage and physiological changes by oxidative stress induced by ACR (Abozaid et al. 2017a).

Our study is in harmony with Zhang et al. (2013) who reported that allicin reversed the elevation of TNF-α level induced by ACR comparing to control group, which indicated attenuation of the brain damage due to oxidative stress by allicin. Allicin inhibited expression of TNF-α which in activated cytokine network and prevented renal injury (Sindhu et al. 2015). Moreover, TNF-α expression was decreased after administration of allicin in monocytes obtained from individuals suffering from vaginal diseases which mediated by prevention of NF-κB pathway (Islam et al. 2008). The co-administration of melatonin significantly modulated serum TNF-α level after ACR administration was due to reduction in ROS production, inflammatory markers, tissue and mitochondrial injury with elevation in endogenous antioxidants, as glutathione after melatonin administration (Hernández-Velázquez et al. 2016). Melatonin inhibited TNF-α formation by down regulation of the PI3K/AKT, ERK, NF- κB signaling pathways, as well as miR-3150a-3p overexpression (Huang et al. 2019). The present data showed that allicin significantly elevated brain dopamine and serotonin levels after ACR intoxication. Allicin significantly reversed dopamine and serotonin levels after treatment of animals with sulpiride (a selective dopamine D2 - receptor antagonist) and p-CPA, a serotonin synthesis inhibitor (Dhingra and Kumar 2008). Also, Murray et al. (2020) reported that ACR cause dopaminergic neurodegeneration with damaging the cholinergic neurons but not the GABAergic neurons. Parvathi (2018) revealed that S-Allylcysteine organosulfur compound that is a constituent of fresh garlic modulated neurotransmitter levels and decreased ROS production. On the other hand, melatonin able to cross through the cell membrane and impaired the apoptotic death in neurons. Also, MT scavenges peroxynitrite and its metabolites which has a vital function in the damage of the dopaminergic neurons. Additionally, it could inhibit neural nitric oxide synthase (nNOS) activity with decline in NO and peroxynitrite production which prevent the activity of tyrosine hydroxylase, a rate limiting enzyme in the synthesis of the dopamine (Ahmed et al. 2010). The gut derived or exogenous melatonin supports serotonin formation by cytochrome P450 (Haduch et al. 2016). This study reveals that allicin significantly decreased amyloid beta peptide after ACR intoxication. In accordance, Kumar et al. (2018) said that allicin had anti-amyloidogenic property which can ameliorate neural diseases. The organosulfur component present in garlic can protect neurons from Aβ induced neurotoxicity and apoptosis by interacting with Aβ and preventing its aggregation. Also, it prevented Aβ fibril formation and also defibrillated Aβ preformed fibrils (Gupta et al. 2009). While, melatonin reduced Aβ level due to its antioxidant and anti-amyloid properties, inhibit Aβ generation and production of amyloid fibrils by a structure-dependent attaching to Aβ (Wang and Wang 2006). Also, Lin et al. (2013) discussed that melatonin binds to Aβ and inhibits its aggregation by the hydrophobic interaction between melatonin with histidine and aspartate residues of Aβ. This study was in harmony with those of Chen et al. (2016) who investigated that allicin act as cholinesterase inhibitor which enhanced cholinergic transmission by preventing the activity of AChE which in turn hydrolyses acetylcholine. More over, administration of garlic essential oil markedly elevated brain-derived neurotrophic factor (BDNF) level and reduced AChE activity which significantly increases the cell proliferation and neuroblast differentiation (Jung et al. 2016). Melatonin has an inhibitory action on AChE activity in dementia induced by

streptozotocin (Agrawal et al. 2009). In addition to prevention of AChE activity, melatonin is used as a drug for many neurological diseases which depend on scavenging of reactive oxygen and nitrogen species, preventing sleeping disturbances and reducing Aβ toxicity (Lin et al. 2013).

ACR enhances the formation of ROS and the decline of antioxidants that causing neurotoxicity (Zamani et al. 2017). DNA adducts from glycidamide was detected after oral treatment of ACR (Yousef and El-Demerdash, 2006). Excessive ROS can attack DNA macromolecules (Zhang et al. 2013) causing elevation of 8-OHdG levels in ACR treated rats. After ACR administration, GSH levels declined significantly while the MDA levels increased which discussed by the binding of ACR with GSH, thus leads to the decrease of GSH and the induction of LPO (Emekli-Alturfan al. 2012). Moreover, allicin possess a protective role against ROS by exerting antioxidant properties (Bhanot and Shri 2010). Furthermore, diallyl disulfide, one of the highly bioactive compounds of allicin, possess an effective antioxidant properties, which counteract the ACR adverse actions on GSH level and also GST activity. Hong et al. (2019) reported that allicin improvement seems to be due to the alleviation of ACR-induced oxidative stress and DNA damage with significant decrease of 8-OHdG levels in BRL-3A cells. Additionally, allicin protective effects were accompanied by significant reductions in both ROS and 8-OHdG concentrations (Zhang et al. 2013). Melatonin significantly increased total GSH, reduced GSH, GSH/GSSG ratio as well as decrease MDA and 8-OHdG levels. MT protect cells from ROS, LPO, protein degradation and DNA oxidative damage (Tamura et al. 2012). Also, melatonin is able to cross through cell membranes due to its lipophilicity and hydrophilicity (pan et al. 2015). Antioxidant activity of MT leads to prevention the degeneration of sciatic nerve, that evidenced by significant decrease in MDA level (Soliman et al. 2019).

Nrf2, a transcription factor, which activates antioxidant cascade through interaction with the antioxidant response element (ARE) and then production of antioxidants (Satta et al. 2017). While, Keap-1 possess a vital role in the regulation of Nrf2 activity. In our result, ACR caused oxidative stress which significantly upregulated the Nrf2 gene expression. However, ACR significantly increased Keap-1 expression which degrade Nrf2 protein and subsequently the Nrf2 gene expression increased. In harmony with our results Murray et al. (2020) stated that ACR decreased Nrf2 protein. Although, during stress the keap1 is changed and not be capable of promoting proteasomal degradation of Nrf2, leading to the increase of Nrf2 accumulation (Ahmadi and Ashrafizadeh 2019). Also, ACR significantly increase NF-κB expression. ACR-induced inflammatory response as well as oxidative stress. GSH depletion by ACR causes an elevation of ROS, which then stimulates NF-κB signaling pathway to trigger inflammatory response which leads to the stimulation of Nrf2, that has a preventative mechanism to ameliorate the ROS (Pan et al. 2018). After NF-κB activation, inflammatory cytokines, as IL-6, TNF-α, and IL-1β, were released while cell viability reduced. Many neurotoxicants had the ability to promote neurotoxicity through stimulation of the Nrf2/NF-κB pathways (Zhao et al. 2017). In comparison with the ACR group, co-treatment of allicin and melatonin ameliorated the changed gene expression, which was proved by a marked down regulation in Nrf2, Keap-1 and NF-kB. Allicin exerted an inhibitory actions on redox imbalance, inflammatory response, and apoptosis. The allicin treatment decreased the oxidative stress via down regulating Keap-1 expression (Garcia-Trejo et al. 2016) which increased Nrf2 protein and the expression of antioxidant enzymes with subsequently the Nrf2 gene expression decreased as feedback mechanism. Our data along with Li et al.

(2012) who explored that the organosulfur compounds in allicin possess an electrophilic center that act as an attack site for nucleophiles, as specific protein SH-groups found on Keap1. Allicin modulate the Keap1-Nrf2 regulatory pathway, leading to the transcription of cytoprotective genes and the decline of oxidative damage (Marón et al. 2020). Also, allicin alleviated the inflammation caused by diabetic macroangiopathy by attenuating the NF-κB level (Li et al. 2020), as well as TNF- α (Panyod et al. 2020). Moreover, allicin enhanced GPx that contributed negatively to the prevention of NF-κB, TNF- α mRNA expression and ROS in monocytes infected with Mycobacterium tuberculosis (Hasan et al. 2006). On the other hand, MT possess a role of antioxidant defense by the Nrf2 pathway and reducing inflammation by NF-κB inhibition. The melatonin treatment decreased the oxidative stress via down regulating Keap-1 expression (Jung et al. 2009) which increased Nrf2 protein and the expression of antioxidant enzymes with subsequently the Nrf2 gene expression decreased as feedback mechanism. Deng et al. (2015) stated that melatonin increased Nrf2 protein and decreased Keap-1 protein level. Also, melatonin reduced expression of inflammatory cytokines as IL-6, TNF-α, IL-1β and iNOS by NF-kB inhibition (Jung et al. 2009). Further, melatonin inhibits NF-κB by increasing concentrations of IkBα (Perkins 2007).

Conclusion

In conclusion, ACR could promote neurotoxicity, oxidative stress, brain dysfunction, epigenetic changes and histopathological findings contributed to disturbance of neuronal cells, decline of antioxidant status, and upregulation of Nrf2, Keap-1, and NF-kB. The administration of allicin and melatonin potentially revised the adverse effects of ACR by anti-inflammatory ,antioxidant activities and conservation of brain functions.

Abbreviations

ACR Acrylamide

MT Melatonin

nNOS nitric oxide synthase

ANOVA Analysis of variance

ARE Antioxidant response element

GSH Reduced glutathione

GSSG Oxidized glutathione

MDA Malondialdehyde

Nrf2 Nuclear factor erythroid 2 like factor

ROS Reactive oxygen species

SE Standard error

OHdG Hydroxy deoxy guanosine

TNF-α Tumor necrosis factor

Ach Acetylcholine

AchE Acetylcholine esterase

BDNF Brain-derived neurotrophic factor

Aβ Amyloid protein

Keap-1 Kelch-like ECH-associated protein 1

NF-kB Nuclear factor-kappa B

DDS Diallyl disulphide

SAC S-allylcysteine

DTS Diallyl trisulfide

GAPDH Glyceraldehyde-3-phosphate dehydrogenase

TTR Transthyretin

Declarations

Availability of data and materials

All data analyzed during the current study are included in this published article.

Author contributions

Hanan A. Edres: Funding acquisition, Investigation, and. Nabil M. Taha: Conceptualization, Investigation, and. Mohamed A. Lebda: Conceptualization, Investigation, Writing - original draft Writing – original draft, submission and follow up the peer-review process,.and. Mohamed S. Elfeky: Conceptualization, Investigation, and Formal analysis.

MAL carried out all the fieldwork, including samplingwastewaters from the different hospitals, and was a major contributor inwriting the manuscript. DA assisted with the analytical methods. AKoperated the GC-MS. YL designed and managed the entire study.

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Consent to publish

All authors have read and approved the final version of the manuscript for publication.

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

All authors carefully read and approved the study.

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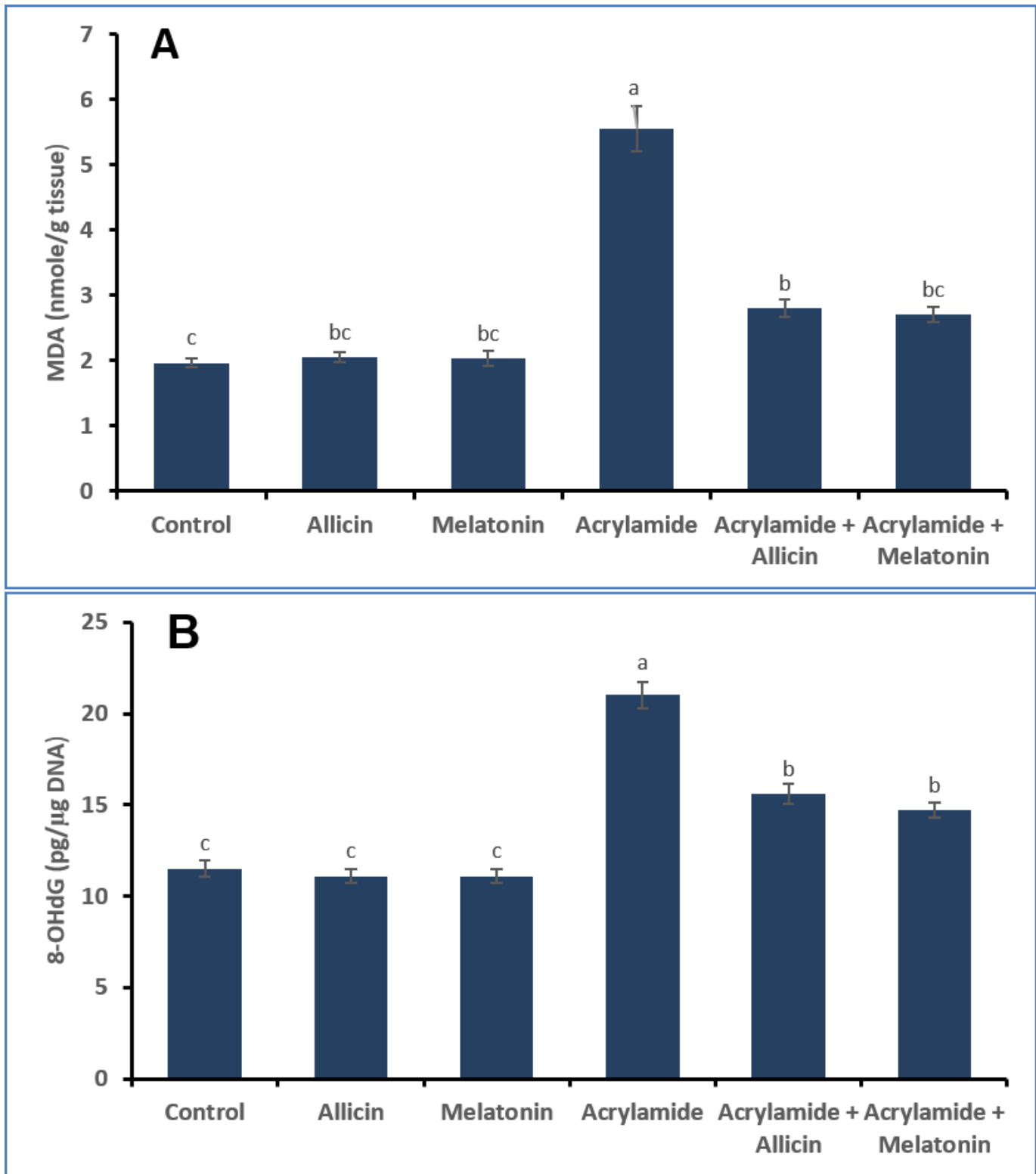
Figures

Figure 1

Brain tissues oxidative stress. (A) MDA (nmole/g tissues). (B) 8-OHdG (pg/μg DNA). Data were analyzed with one-way ANOVA followed by Tukey's multiple comparison test. P < 0. Error bars represent mean± SD. n= 5.
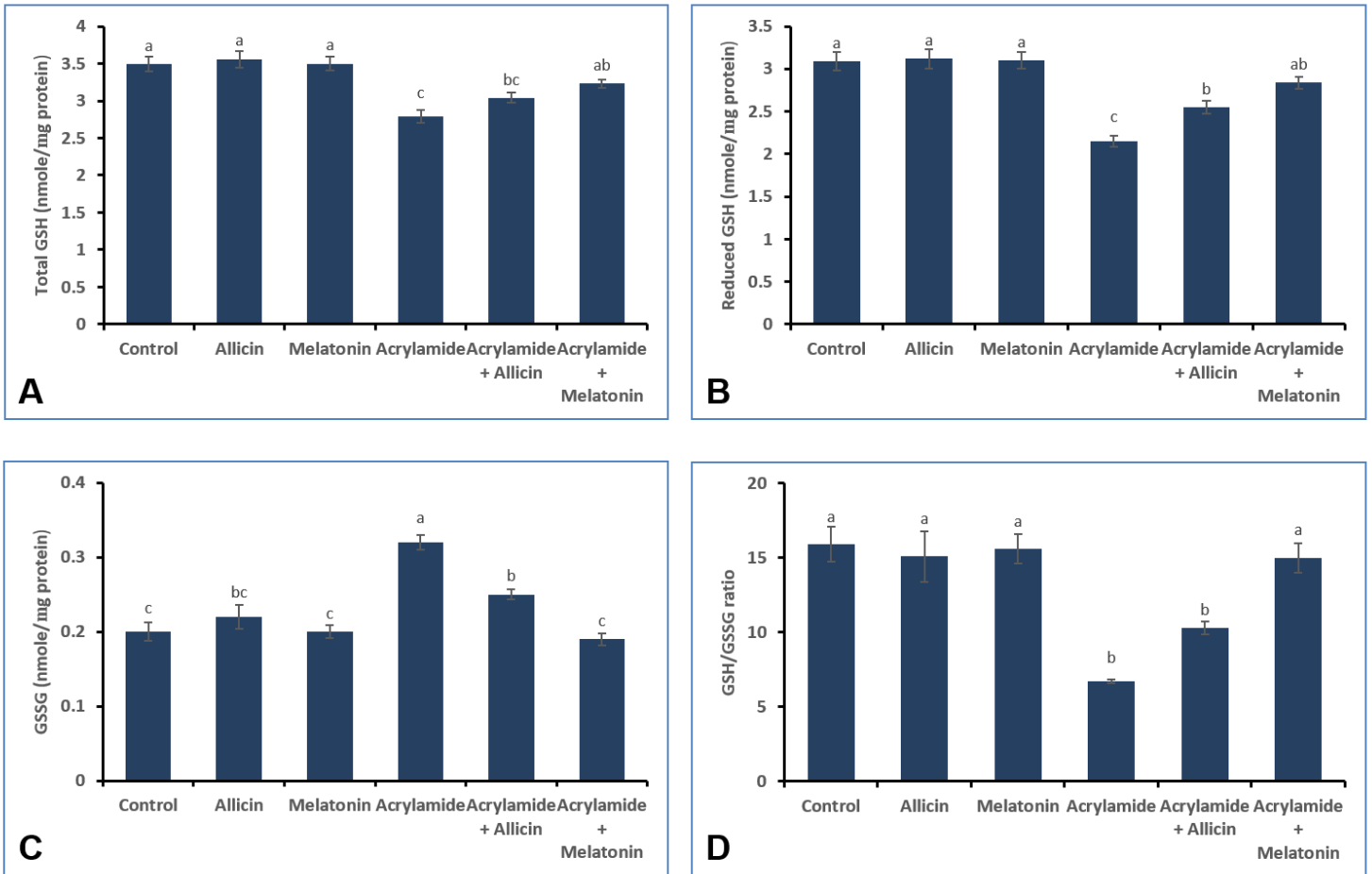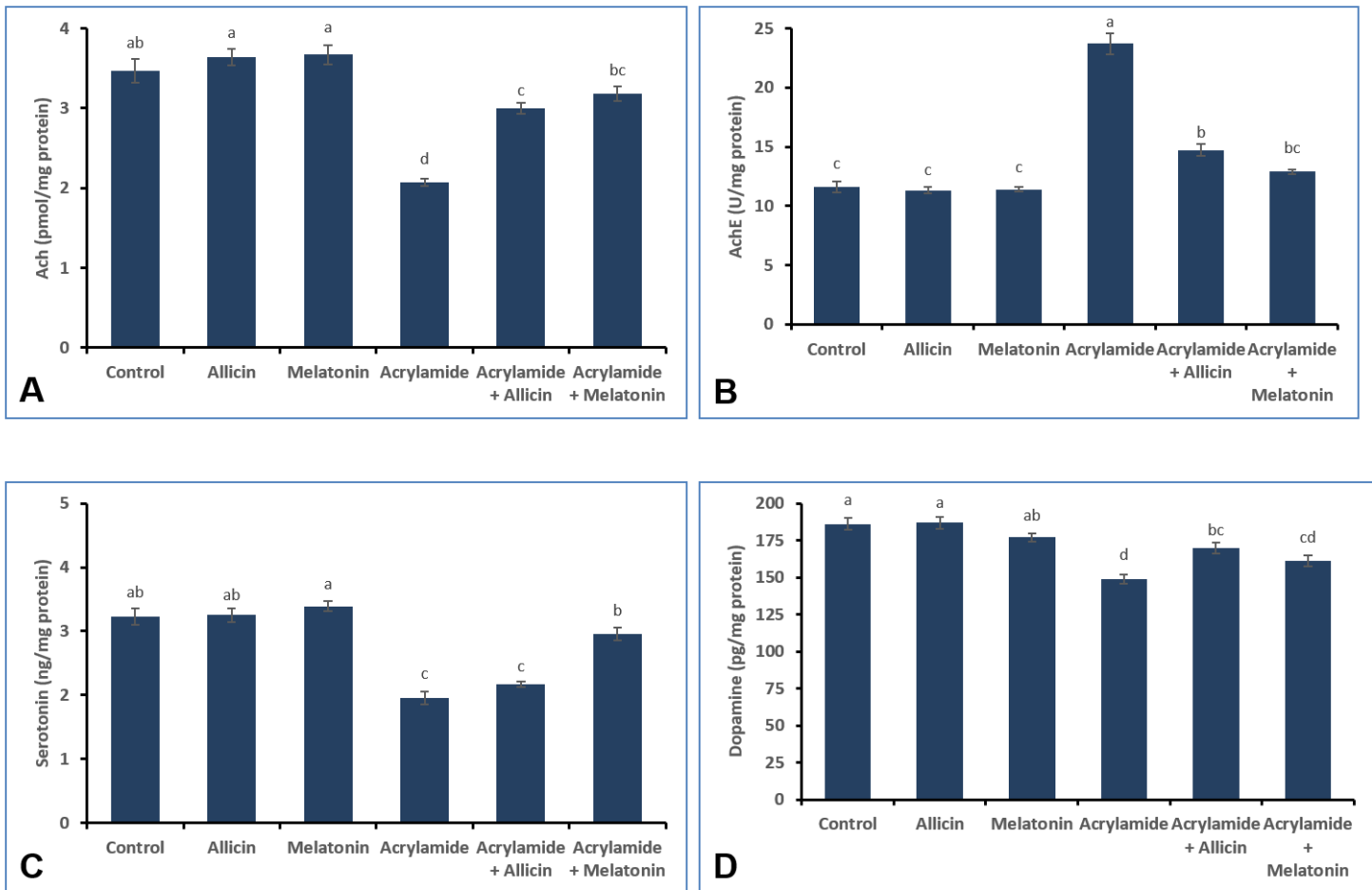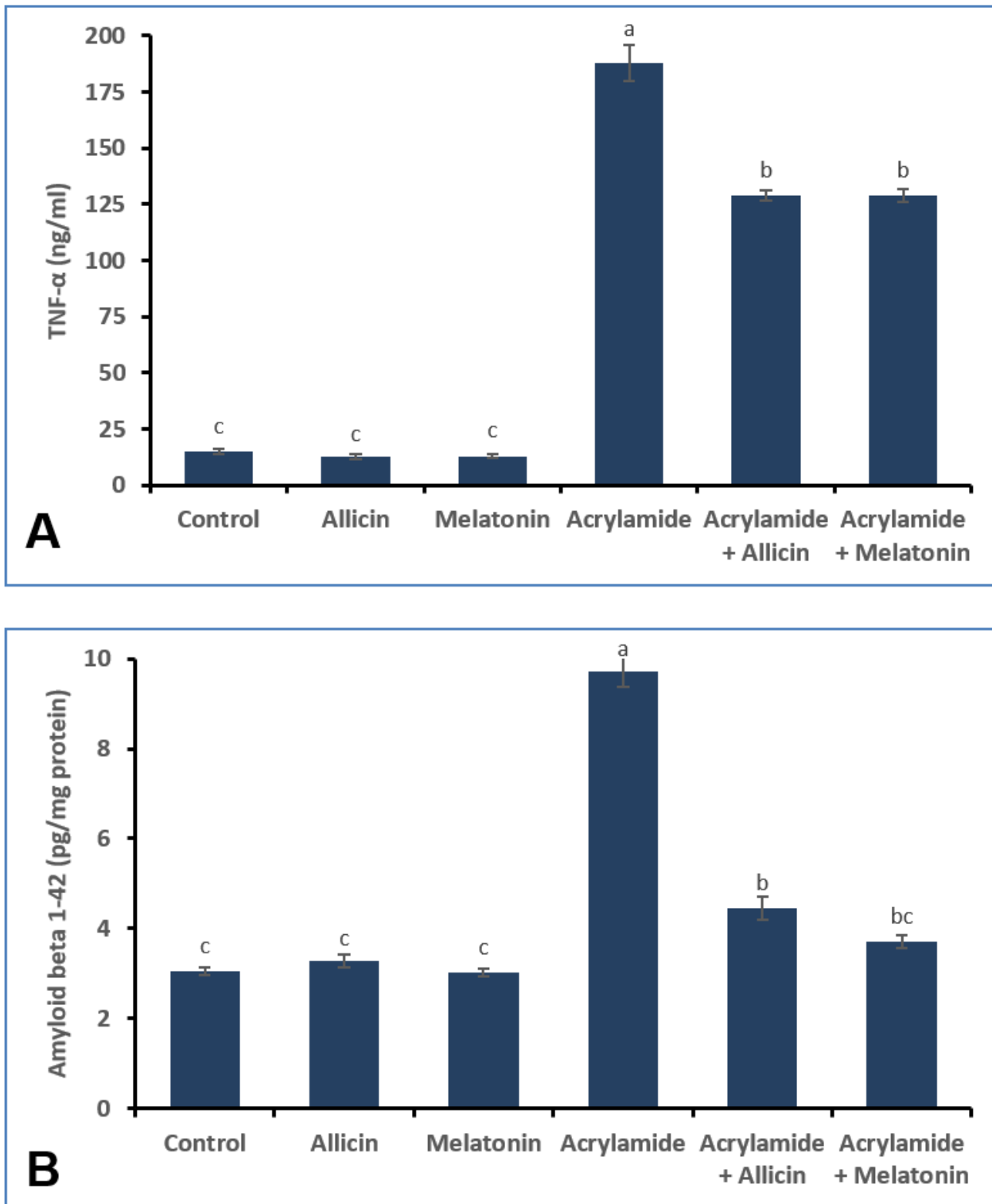
Figure 2

Brain tissue glutathione redox. (A) Total GSH (nmole/mg protein). (B) Reduced GSH (nmole/mg protein). (C) GSSG (nmole/mg protein). (D) GSH/GSSG ratio. Data were analyzed with one-way ANOVA followed by Tukey's multiple comparison test. P < 0.05. Error bars represent mean± SD. n= 5.

Figure 3

Brain tissue neurotransmitters. (A) ACh (pmol/mg protein). (B) ACHE (U/mg protein). (C) Serotonin (ng/mg protein). (D) Dopamine (pg/mg protein). Data were analyzed with one-way ANOVA followed by Tukey's multiple comparison test. p < 0.05Error bars represent mean± SD. n= 5.

Figure 4
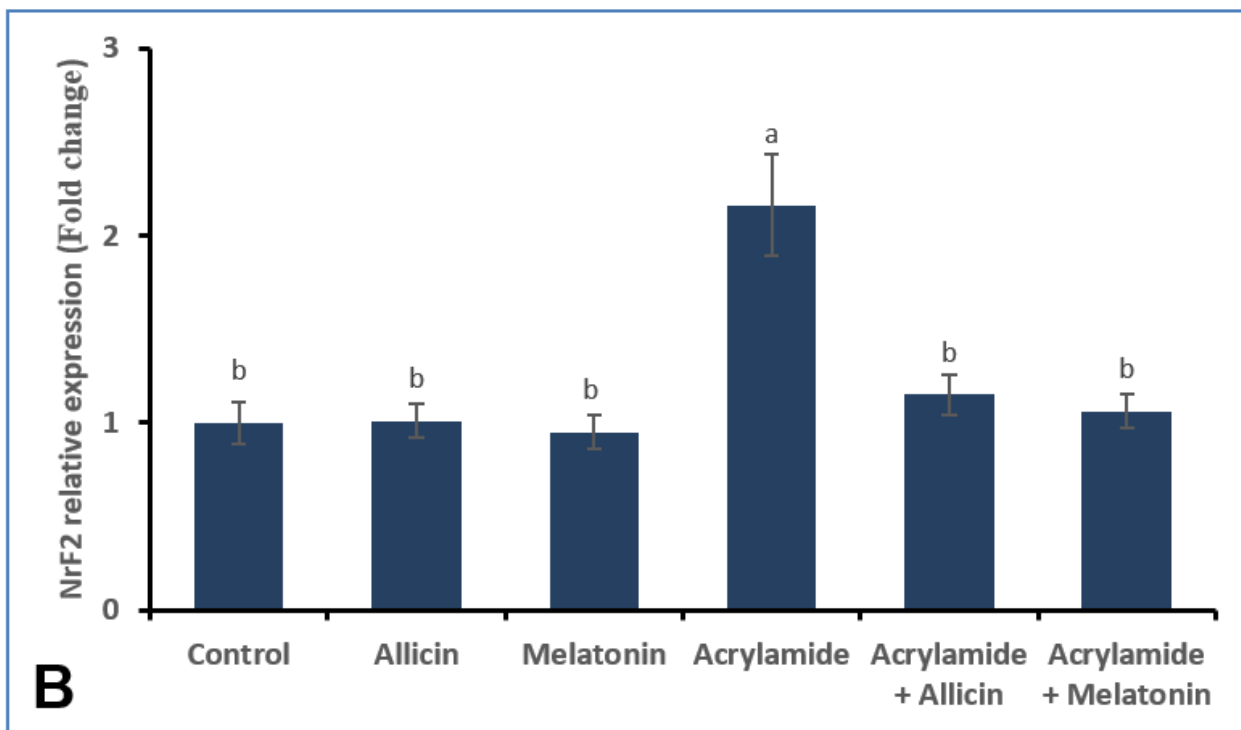
Serum tumor necrosis factor and Brain tissue amyloid beta1-42. (A) TNF-α (ng/ml). (B) Aβ1-42 (pg/mg protein). Data were analyzed with one-way ANOVA followed by Tukey's multiple comparison test. $p < 0.05$ Error bars represent mean± SD. n= 5.

Figure 5

NrF2 protein and mRNA relative fold change expression of brain tissue. (A) NrF2 protein (μg/mg protein. (B) NrF2 mRNA expression. Data were analyzed with one-way ANOVA followed by Tukey's multiple comparison test. $p < 0.05$ Error bars represent mean± SD. n= 5.
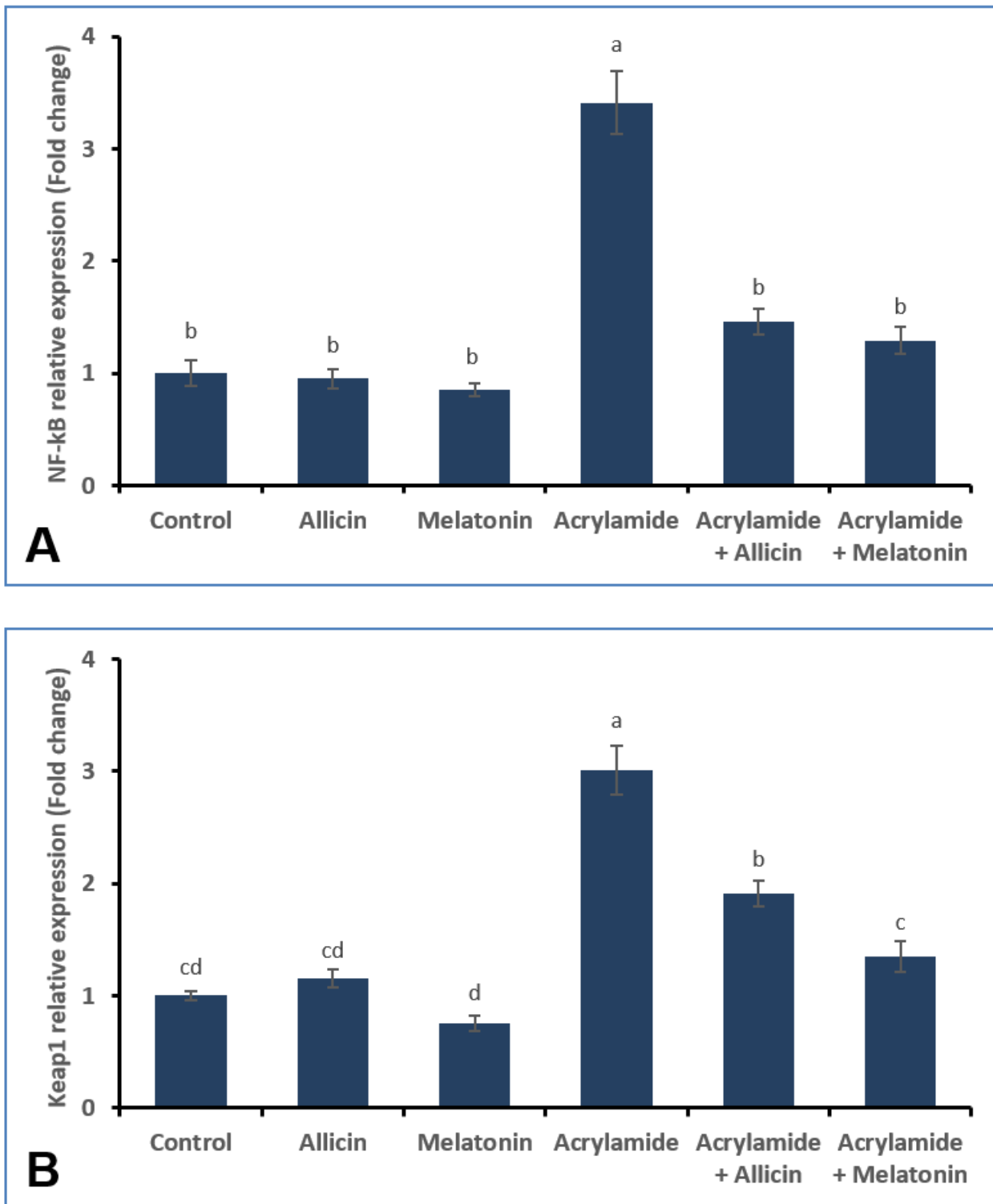
Figure 6

mRNA relative fold change expression of brain tissue. (A) NF-KB. (B) Keap1mRNA expression. Data were analyzed with one-way ANOVA followed by Tukey's multiple comparison test. $p < 0.05$ Error bars represent mean± SD. n= 5.
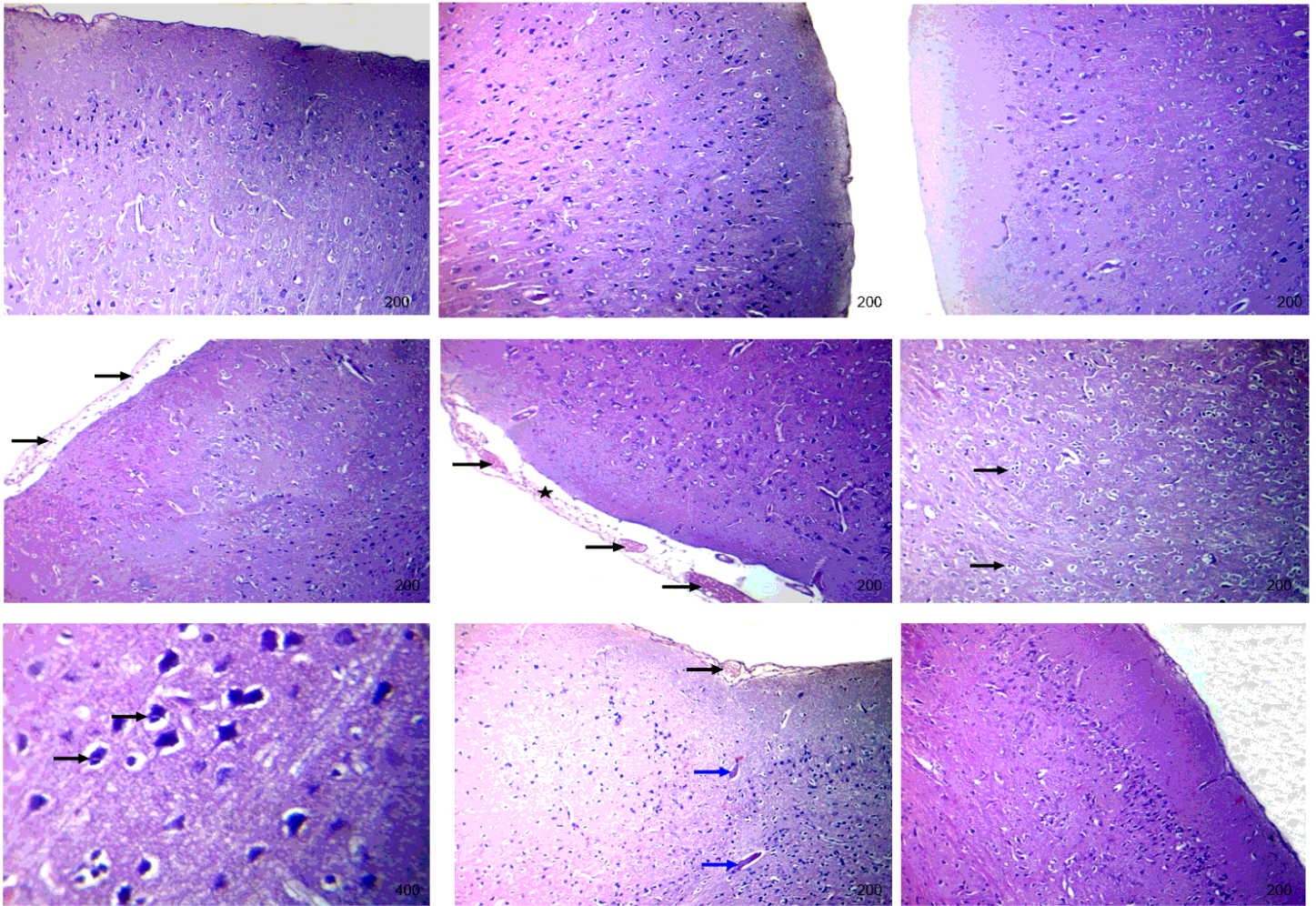
Figure 7

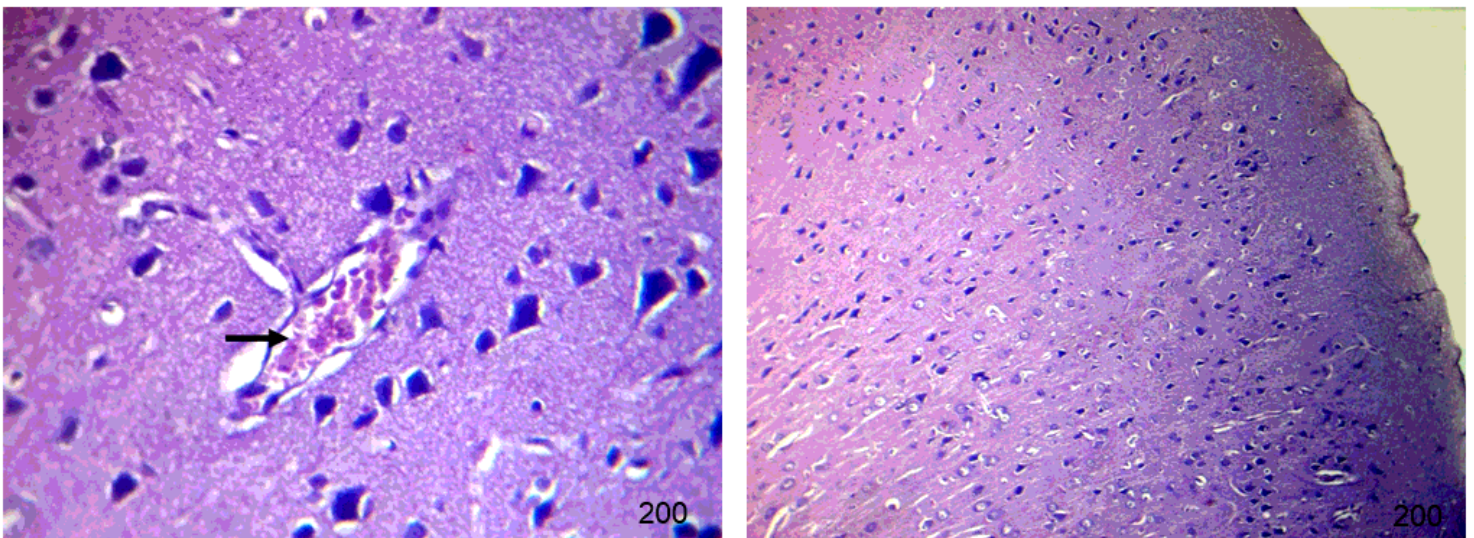Cerebellum histopathology. H&E (X400). Scale bar= 20 µm.

Figure 8

Histopathological evaluations of cortical tissues of the control group, allicin, and melatonin groups revealed normal histological characteristics (Fig 7, 8).