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

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Abstract
Acrylamide (ACR) is an unsaturated monomer that served various fields; however, it is a potent neurotoxin. The target of the present study 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 at the same doses as the preceding groups. The assessment of brain biomarkers, neurotransmitters, antioxidative status, Nrf2 signaling pathway, and histopathological analyses was performed following 21 days. ACR exposure induced brain lipid and DNA oxidative damage as well as reduced the glutathione (GSH) levels. The obvious brain oxidative injuries 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 deoxyguanosine (8-OHdG), tumor necrosis factor-α (TNF-α), and amyloid protein (AB1-42). Finally, the mRNA transcripts of brain Keap-1, Nrf2, and NF-kB were upregulated after ACR intoxication. Interestingly, allicin and melatonin alleviated the ACR-induced brain damage assessed by the normalization of the mentioned analyses. The present study demonstrated the protective role of both allicin and melatonin in ACR-prompted neuropathy by alleviation of redox imbalance and enhancement of neurotransmitters as well as relieving DNA damage and anti-inflammatory effect.

Keywords Acrylamide · Melatonin · Allicin · Neurotransmitters · Oxidative stress

Introduction
Acrylamide (ACR) is a powerful neurotoxin that causes damages to both central and peripheral nervous systems. ACR does not exist in nature and is commonly not found in raw or boiled food (Khaneghah et al. 2020) but it is synthesized when sugar-rich diets are processed at high temperatures by a chemical reaction between asparagine and reducing sugars (Acaroz et al. 2018). ACR exposure had been recorded through ingestion of ACR-containing food, drinking poly-acrylamide flocculants-contaminated water, and occupational exposure through dermal contact or inhalation of industrial manufacturing dust (Shinomol et al. 2013). Furthermore, Rifai and Saleh (2020) stated that tobacco smoking is the main reason for ACR exposure than food. The mechanism of ACR neurotoxicity is mainly recruited to the induction of lipid peroxidation subsequently with the decline in the antioxidant enzymatic activity and reduced glutathione level in the nerve tissue (Zhu et al. 2008). Recently, Farouk et al. (2021) contributed that ACR-induced neurotransmitter perturbation and astrocyte gliosis are other mechanisms of ACR neurotoxicity. Also, ACR caused multiorgan toxicity including the brain in rats through altering the serum biochemical parameters, provoking the pro-inflammatory cytokines, enhancing DNA damage, and inducing oxidative damage (Bin-Jumah et al. 2021).

Garlic, known as *Allium Sativum*, is used in food and medicine for a long time (Liu et al. 2015), and 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 compositions of allicin including flavonoids and sulfur-containing compounds diallyl disulfide (DDS), S-allylcysteine (SAC), and diallyl trisulfide (DTS), are potent antioxidants (Mikaili et al. 2013). Recently, phytochemicals and other natural products including allicin are able to reverse ACR-induced neuropathy in rodents (Tamimi and Al-Domi 2019). It is considered a potent antioxidant by scavenging ROS, inhibiting lipid oxidation, and enhancing the formation of proinflammatory 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 protective effects on some pathological damages as shock, ischemia, and inflammation. Melatonin is a potent lipophilic antioxidant against reactive oxygen species and neuroprotective in both in vivo and in 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 the CNS (Ahmed et al. 2010). Moreover, MT has a defensive role versus redox imbalance induced by ACR (Li et al. 2018).

The neuroprotective effect of allicin and melatonin on ACR neurotoxicity had been recorded previously; however, these studies lack the mechanistic explanation underlying this protection in detail. The current study aimed to assess the protective efficacy of allicin and melatonin on ACR neurotoxicity through the evaluation of their antioxidant, anti-inflammatory, anti-amyloidosis, and neurotransmitter enhancement activities.

**Material and methods**

**Reagents and chemicals**

Highly purified powdered ACR, allicin, melatonin, and other chemicals of pharmaceutical-grade 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 were obtained from (CAT# ab156900, Thermo Scientific, USA), and commercial 8-OH-dG ELISA kit was obtained from (Abcam, CAT# ab201734, Cambridge, UK). Immunoassay kits for the quantitative determination of rat serotonin (CAT# K6880), Ach (CAT# MBS169077), and Aβ1-42 (CAT# BYEK2615) were obtained from Biopes Co., China. An immunoassay kit for the quantitative determination of rat dopamine was purchased from Elabscience, Co, USA (CAT# E-EL-0046). The rat TNF-α ELISA kit was purchased from eBioscience, USA (CAT# 560479), and the total RNA 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 protocol**

Thirty-six male Wistar rats, 4-weeks old weighing 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 with free access to rat ration and drinking water with 2-week acclimation period. The dealing of rats was done refereeing to the 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 (Approval No: AU-IACUC 09-12-19). 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. 2020); ACR-intoxicated group orally gavaged with ACR 50 mg/kg b.w, daily for 15 days (Aydın 2017); allicin + ACR group orally gavaged with ACR and allicin with the same previous doses; and 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 separated sera were kept at −20°C until used for the estimation of serum TNF-α and brain amyloid beta1-42.

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

At the end of the 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 until 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-α by an immunoassay kit (eBioscience, USA). The 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-buffered saline (PBS) pH 7.4 in the ratio of 1:10. The homogenate was centrifuged at 10,000 rpm, at 40 °C for 20 min for supernatant production. Lipid peroxide was estimated after the reaction with thiobarbituric acid and represented as nanomole malondialdehyde (MDA)/ g tissue weight (Draper and Hadley 1990). The total glutathione and GSSG contents were determined according to Griffith (1980). 8-OH-dG as an 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 cerebral homogenate was used for the determination of Ach, serotonin, and dopamine using immunoassay kits according to the manufacture instructions, while the activity of the AChE enzyme in cerebral homogenates was estimated by the colorimetric method as described by 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 until 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 mixtures including RNA and master mix were placed at 37 °C and then inactivated at 95 °C. The qRT-PCR for the target genes was applied using the Rotor-Gene SYBR Green PCR Kit 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.

**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 a light microscope.

**Statistical analyses**

SAS software version 22.0 for Windows (IBM, Armonk, NY, 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 clarified in Fig. 1 showed that ACR leads 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 indicated that ACR led to a marked decline in the GSH level (2.15 ± 0.070 nmole/mg protein), and the GSH to GSSG ratio (6.70 ± 0.12) as well as a 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 a subsequent significant increase in the brain GSH level.

**Brain 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 that compared to the 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 the 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

Concerning 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 by restoring the Nrf2 mRNA transcript expression in ACR-intoxicated rats (Fig. 5).

**Relative mRNA expression of brain NF-κB and Keap-1 pathway**

ACR significantly upregulated mRNA transcript of brain Nrf2, Keap-1, and NF-κB as compared with the control confirming that ACR causes oxidative stress which significantly activated the Nrf2 gene expression. However, ACR significantly increased the Keap-1 expression which degrades 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-κB mRNA transcripts (Fig. 6).

**Histopathological findings**

Histopathological evaluations of meninges and cerebrum cortex of the rat’s brain (Fig. 7) showed that the control (a), allicin (b), and melatonin (c) groups revealed normal histological structure of the meninges and cerebrum cortex that noticed normal pia matter, outer molecular layer, and pyramidal cells. On the other side, H&E sections of ACR-treated groups revealed abnormal histopathological findings of cerebral cortex and meninges (d, e, f). The meninges showed prominent mild inflammatory cell infiltration (black arrows, d) and, additionally, announced congestion of sub-meningeal blood vessels (black arrows, e), evident hemorrhage (star, e) in the meninges, neuropil spongiosis, and neuronophagia of degenerated neuron in the cerebral region (black arrows, f). Co-treatment of allicin with ACR showed mild congestion of blood vessels in the meningeal region (black arrows, g) and cortical region (blue arrows) with normal histopathological findings of meninges and cerebrum (h) while co-treatment of melatonin with ACR showed mild congestion of cortical blood vessels (black arrows, i) with normal histopathological findings of meninges and cerebrum (j).
Discussion

The current study was done to explore the probable neurotoxicologically mechanism of oral ACR administration, and the preventative efficacy of allicin and melatonin via estimation of brain functions, redox status, gene and protein expression Nrf2 signaling pathway, and histopathological findings. ACR toxicity is mediated via induction of DNA and lipid oxidative damage, pro-inflammatory cytokines, and depletion of antioxidant activities in the brain, liver, and kidneys (Elhelaly et al. 2019). The current data revealed that ACR led to marked elevations in the serum TNF-α level, cortical amyloid protein level, and AchE activity with a 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-α, 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 (Acraoz et al. 2018).

Serum TNF-α significantly increased after ACR administration which was mediated by stimulating the NF-κB pathway. The nerve terminal is the main site for ACR action where neurotransmitter release is inhibited and downstream degeneration is enhanced (Abd Elmonem 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. 2010). Moreover, ACR has the ability to react with intercellular molecules that contain SH, NH₂, or OH and thiol-rich proteins that are involved in the release of neurotransmitters (serotonin and DA). This ACR adduction is the cause of defective neurotransmission in ACR-intoxicated laboratory animals (Abouzaid et al. 2017a). ACR significantly increased 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 possesses a neuroprotective role by interacting with Aβ (Abouzaid et al. 2017b). The TTR-Aβ combination is necessary to illustrate the protective technique of the nervous system (Du and Murphy 2010). So, ACR leads to a decrease in transthyretin levels with an increase in Aβ which is involved in
neurotoxicity by affecting nerve regeneration (Yao et al. 2014). Aβ level has a marked significance and is 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 (Abouzaid et al. 2017a).

Our study is in harmony with that of Zhang et al. (2013) who reported that allicin reversed the elevation of TNF-α level induced by ACR comparing to the control group, which indicated attenuation of the brain damage due to oxidative stress by allicin. Allicin inhibited the expression of TNF-α which inactivated 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 were mediated by prevention of the NF-κB pathway (Islam et al. 2008). The co-administration of melatonin significantly modulated serum TNF-α level after ACR administration which was due to the reduction in ROS production, inflammatory markers, and 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 downregulation 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 causes dopaminergic neurodegeneration, 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 was able to cross through the cell membrane and impaired the apoptotic death in neurons. Also, MT scavenges peroxynitrite and its

Fig. 4 Serum tumor necrosis factor-alpha and brain amyloid beta1-42 concentrations. 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. Columns with different lowercase letters are significantly different at p < 0.05 Error bars represent mean± SD. n= 5
metabolites which has a vital function in the damage of the dopaminergic neurons. Additionally, it could inhibit neural nitric oxide synthase (nNOS) activity with a decline in NO and peroxynitrite production which prevents the activity of tyrosine hydroxylase, a rate-limiting enzyme in the synthesis of 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. Accordingly, 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 and inhibited 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 a cholinesterase inhibitor which enhanced cholinergic transmission by preventing the activity of AChE which in turn hydrolyses acetylcholine. Moreover, administration of garlic essential oil markedly elevated brain-derived neurotrophic factor (BDNF) level and reduced the 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 the prevention of AChE activity, melatonin is used as a drug for many neurological diseases which depend on scavenging 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 cause neurotoxicity (Zamani et al. 2017). DNA adducts from glycidamide were 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 was discussed by the binding of ACR with GSH, thus leading to the decrease of GSH and the induction of LPO (Emekli-Alturfan et al. 2012). Moreover, allicin possesses a protective role against ROS by exerting antioxidant properties (Bhanot and Shri 2010). Furthermore, diallyl disulfide, one of the highly bioactive compounds of allicin, possesses an effective antioxidant property, 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 a 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 and GSH/GSSG ratio, and decreased MDA and 8-OHdG levels. MT protects 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 the prevention of the degeneration of the sciatic nerve evidenced by a significant decrease in MDA level (Soliman et al. 2020).

Nrf2, a transcription factor, activates the antioxidant cascade through interaction with the antioxidant response element (ARE) and then the production of antioxidants (Satta et al. 2017), while Keap-1 possesses 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 degrades 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, this leads to the increase of Nrf2 accumulation (Ahmadi and Ashrafizadeh 2019). Also, ACR significantly increases NF-κB expression. ACR induced inflammatory response as well as oxidative stress. GSH depletion by ACR causes an elevation of ROS, which then stimulates the NF-κB signaling pathway to trigger an 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, such as IL-6, TNF-α, and IL-1β, were released while cell viability was reduced. Many neurotoxicants had the ability to promote neurotoxicity through the 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 downregulation in Nrf2, Keap-1, and NF-kB. Allicin exerted inhibitory actions on redox imbalance, inflammatory response, and apoptosis. The allicin treatment decreased the oxidative stress via downregulating Keap-1 expression (García-Trejo et al. 2016) which increased Nrf2 protein and the expression of antioxidant enzymes with subsequently the Nrf2 gene expression decreased as a feedback mechanism. Our data are in accordance with that of Li et al. (2012) who explored that the organosulfur compounds in allicin possess an electrophilic center that acts as an attack site for nucleophiles, as specific protein SH-groups found on Keap1. Allicin modulated 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 possesses a role of antioxidant defense by the Nrf2 pathway and reducing inflammation by NF-κB inhibition. The melatonin treatment decreased the oxidative stress via downregulating Keap-1 expression (Jung et al. 2009) which increased Nrf2 protein and the expression of antioxidant enzymes with the subsequent decrease of Nrf2 gene expression as a feedback mechanism. Deng et al. (2015) stated that melatonin increased Nrf2 protein and decreased Keap-1 protein level. Also, melatonin reduced the expression of inflammatory cytokines as IL-6, TNF-α, IL-1β, and iNOS by NF-kB inhibition (Jung et al. 2009). Furthermore, melatonin inhibits NF-κB by increasing concentrations of IkBα (Perkins 2007).

Collectively, ACR initiates the oxidative damage of lipid and DNA, inhibition of master antioxidant gene and Nrf2, provoking the pro-inflammatory cytokines that increase the amyloid protein, and inducing perturbation of neurotransmitters implicated in ACR neurotoxicity. The present explanation coincided with that of Abdel-Daim et al. (2020) who mentioned that ACR induced lipid and DNA oxidative stress and pro-inflammatory cytokines and decreased the enzymatic and non-enzymatic antioxidants. ACR upregulated the gene expression of Nrf2 while initiating the induction of oxidative stress; the conflict between these results was solved that the ACR decreasing Nrf2 protein expression was explained by...
increasing the gene Keap-1 expression. Allicin and melatonin mediated their neuroprotection through modulating the Nrf2 signaling pathway, pro-inflammatory cytokines, and amyloid proteins. Kahkeshani et al. (2015) reported that allicin shows different mechanisms in reducing ACR toxicity including the antioxidative, anti-apoptotic, anti-inflammatory, and reduction of chemical absorption due to enhanced fecal passage.

**Conclusion**

In conclusion, ACR could promote neurotoxicity, oxidative stress, brain dysfunction, epigenetic changes, and histopathological findings contributed to disturbance of neuronal cells, a 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 and antioxidant activities and conservation of brain functions.

**Abbreviations**

Ach, Acetylcholine; AchE, Acetylcholine esterase; ACR, Acrylamide; ANOVA, Analysis of variance; ARE, Antioxidant response element; Aβ31–42, Amyloid protein1-42; BDNF, Brain-derived neurotrophic factor; DDS, Diallyl disulfide; DTS, Diallyl trisulfide; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; GSH, Reduced glutathione; GSSG, Oxidized glutathione; Keap-1, Kelch-like ECH-associated protein-1; MDA, Malondialdehyde; MT, Melatonin; NF-kB, Nuclear factor-kappa B; nNOS, Nitric oxide synthase; Nrf2, Nuclear factor erythroid 2 like factor; 8-OHdG, 8-Hydroxy deoxyguanosine; ROS, Reactive oxygen species; SAC, S-Allylcysteine; SE, Standard error; TNF-α, Tumor necrosis factor; TTR, Transthyretin

**Author contribution**

H.A.E: funding, investigation, and methodology; N.M.T: idea, design, and supervision; M.A.L: design, validation, writing the manuscript draft, manuscript revision, and editing; M.S.E: data collection and analysis, software, and visualization.

**Data Availability**

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

**Declarations**

**Ethics approval and consent to participate**

All authors carefully read and approved the study.

**Consent for publication**

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

**Competing interests**

The authors declare no competing interests.

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