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Modulation of Sirtuin-1, Apoptosis and Redox Signaling Pathways by Astringenin: A Potential Parkinsonism Therapeutic Effect

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VARIETIES of cellular stressors are reported to disrupt brain circuits or neuronal pathways that distribute neurotransmitter signals, resulting in subsequent genetic abnormalities, behavioral impairments, and/or mood disorders. For example, unfolded proteins accumulation in the endoplasmic reticulum (ER) induces ER stress-mediated cell death. Astringenin (AST) flavonoid functions as a neuroprotective agent and rescues neurons from various insults. However, the molecular mechanisms underlying the neuroprotective effects of Astringenin are still unclear. Rats received a single intraperitoneal (i.p.) injection of reserpine (RES) (5mg/kg) or exposed to a single dose of 10Gy head ionizing radiation (RAD) followed by AST treatment and sustained for 7 days. The expression of α-synuclein increased following head irradiation or reserpine administration, and endoplasmic reticulum stress factors were increased in brain tissue. AST administration results in upregulation of expression of AMPK and SIRT1, resulting in the inactivation of NF-κB p-65, FOXO1, and caspase-3. In addition, reduction of α-synuclein by AST improved the mobility-deficient behavior in rats. AST has also attenuated the decrease of dopamine, serotonin and BDNF levels. Moreover, AST upregulation of NRF2/HO1 levels was accompanied by enhancements of GSH content and SOD activity, and decreased inflammatory cytokines (TNF-α, IL-1β and IL-6). Such findings suggest that AST could be considered a promising agent that alleviates neuroinflammation by inhibiting ERs-mediated apoptosis signaling, and might possess a parkinsonism therapeutic effect.

Keywords: α-synuclein, Astringenin, ER-stress, Parkinsonism, Radiation.

Introduction

Globally, Parkinson disease (PD) incidence have been doubled during the past 25 years with worldwide estimates in 2019 of over 8.5 million individuals living with PD. Moreover, morbidities and mortalities due to PD are increasing faster than those caused by any other neurological diseases. It is characterized by impaired muscular functions, such as resting tremors and delayed movement, as well as a loss of coordination and balance together with speaking and swallowing difficulties upon progression (Maiti et al., 2017). Parkinson's disease is caused by a loss of nerve cells in the "substantia nigra" nucleus. The primary etiology of the disease remains unknown; although tumors, trauma, hydrocephalus, chemotherapy, drugs, and radiatiotherapy are examples of secondary causes (Reddy et al., 2022). Clinically, radiation is used routinely as both therapeutic and diagnostic tools. Despite its widespread use, there are still gaps in understanding the particular mechanisms of the neurological responses to high-dose ionizing radiation (IR). The central nervous system (CNS) is extremely vulnerable to chemo- and radio-therapy; through multiple physiological mechanisms, for instance, the irradiation-induced radiolytic lesions were reported to boost the overall cellular stress response, and to affect different pathways involved in DNA repair, cell

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cycle progression and survival (Cuccurullo et al., 2019). Moreover, the IR-sensitivity of the neural precursor cells are known to significantly alter the neurogenesis process, suggesting a critical role in cognitive impairment (Smart, 2017; Thabet et al., 2021).

Earlier reports implied that brain oxidative DNA damage and microglial activation contribute to the pathophysiology of PD. However, several studies have recently focused on two critical signalling networks; namely, the Nrf2/HO-1 and SIRT1/AMPK/FOXO pathways, in order to identify novel targets that potentially contribute to the pathophysiology of Parkinsonism (Yu-En Lin et al., 2021; Prasad & Hung, 2021). Many neurological stressors, for example free radical exposure and hypoxia, can disrupt the folding of proteins and cause an accumulation of mis-folded/unfolded proteins in the endoplasmic reticulum (ER) lumen, triggering the un-folded protein response (UP-R). The primary goal of UP-R is to restore normal cellular functions; but if the damage is too serious to heal, the UP-R will eventually cause cell death through apoptotic pathway(s) activation. PRK-like ER kinase (PERK) is activated by phosphorylation during the UP-R, and then it phosphorylates the eIF2, inhibiting most of the protein synthesis and activating ATF-4 translation (Adams et al., 2019). CHOP; a transcription factor which binds to other transcription factors inducing the activation of pro-apoptotic genes, has been reported to be induced by this ER-stress signalling pathway. Aggravation of PD and other neurodegenerative disorders is dependent on the quantity and the type of the mis-folded proteins accumulation, such as beta-amyloid, or α-synuclein (Omura et al., 2013).

Despite decades of rigorous study, no medicine has yet been discovered that can alleviate PD symptoms or hinders disease progression. Astringenin (3, 3', 4',5-tetrahydroxystilbene), a resveratrol (3,4',5-trihydroxystilbene) analogue with significantly stronger antioxidative activity and free radical fighting capability, was also identified in natural sources such as almonds, peanuts, teas, and berries, but mainly in grapes and in the seeds of passion fruits. Aside from performing general physiological actions in a wide range of non-hematopoietic cells, AST has been reported to prevent liver damage after trauma-haemorrhage by reducing the pro-inflammatory cytokines release (Hung et al., 2001; Liu et al., 2011). The present study aims at investigating the possible effectiveness of AST in protecting against the ER stress-mediated neuronal injuries through the activation of each of NF-kB-p65, FOXO1 and Caspase-3 through a pathway involving the inactivation of NRF2/HO-1 and SIRT1/AMPK cascades. The authors studied the proposed effect(s) and mechanism(s) of AST action in male rats exposed to reserpine alkaloid; a Parkinsonism animal model, or to 10 Gy head irradiation as inducers of cellular neuro-inflammations stress.

Materials and Methods

Materials

Reserpine; methyl reserpate 3, 4, 5-trimethoxybenzoic acid, a well-known vesicular monoamine transporter 2 (VMAT2) blocker, used to control blood pressure and known to trigger central catecholamine storage depletion, which has been linked to muscular rigidity, tremors, and akinesia, in this parkinsonism rat model, was provided by Novartis Pharmaceutical Company (Cairo, Egypt). Astringenin; 3, 3′,4′,5-tetra hydroxyl stilbene, was purchased from MedChemExpress, Monmouth Junction, NJ, USA. All other chemicals and solvents used in the current work were purchased from Sigma–Aldrich®, St. Louis, Missouri, USA.

Animals

Forty- eight male albino rats of Wistar strain, 5-month-old, average weight 160 -180 g, obtained from the breeding unit animals house of the National Center for Radiation Research and Technology (NCRRT), were used in the current work. The animals were accommodated in standard cages; under controlled conditions of temperature with 12h light/dark cycles. Animal experimentation procedures were designed in consistence with the guidelines of the Guide for the Care and Use of Laboratory Animals (1996, National Academy Press publication) and in accordance with the recommendations for proper laboratory animal care provided by the Research Ethics Committee of the NCRRT, Cairo, Egypt (Approval serial No. of the study is 70A / 21).

Methods

Irradiation process

An indoor shielded Canadian Gamma Cell-40 (^{60}Co) biological irradiator (Atomic Energy

of Canada Ltd., Ottawa, Ontario, Canada) constructed at the NCRRT (Egyptian Atomic Energy Authority, Cairo, Egypt) was used for rats' irradiation. Head irradiation was carried out at a dose rate of 1.144 K Gy/h (as calculated by the Dosimetry Department of the NCRRT). The skulls of rats were exposed to a single shot dose of 10 Gy according to Abdel-Aziz et al. (2021) and a lead shield to attenuate the radiation dose by more than 98% was used for the protection of the other animal body parts.

Experimental design

After a week of acclimatization, rats were randomly assorted into six experimental groups; 8 rats each, as follows: Group-1 control: normal rats. Group-2 RES: single intraperitoneal (i.p.) injection of reserpine (5mg/kg body weight) according to Duty & Jenner (2011). Group 3: normal rats treated with AST i.p. in a dose of 20 mg/ kg body weight /day suspended in 0.3mL 0.5% freshly prepared carboxymethyl cellulose solution for 7 consecutive days. Group 4: reserpinized rats as in group 2 and treated with AST as in group 3 for 1 week; starting after one hour of reserpine administration. Group 5: head irradiated; the rats were anesthetized via i.p. injection of pentobarbital (6mg/kg body weight) and after 10min they were exposed to a single irradiation dose of 10 Gy. Group 6: head irradiated AST-treated; rats were exposed to a single irradiation dose of 10Gy as in group 5 and treated with AST as in group 3 one hour following irradiation. At the end of one-week of AST administration following irradiation or reserpine injection, rats were euthanized under deep urethane anaesthesia, and sacrificed by decapitation. The brains were dissected out of the skull, split on ice cold saline then homogenized as a whole in ice cold phosphate buffered saline then stored at -80°C for further biochemical study.

Behavioral assessments

The establishment of Parkinsonism was assessed after 24h from reserpine administration, by the observation of muscular tremors, bradykinesia and rigidity in rats which were quantified by the "Catalepsy test". This test consists of two parts; the first of which was the "grid test", where each animal was hung by its paws on a vertical mesh (25.5cm wide X 44cm high; each two wires are 1cm apart), and the time until the animal moves its paws or make its first move was recorded. The second test part was the "Bar test"; where the animal was placed with both forepaws on a bar (9cm high from the base and parallel to it), and the time elapsed until the first movement of the paw was recorded (Alam & Schmidt, 2002).

Biochemical assays

Cellular stress, neuroinflammation and apoptosis biomarkers: Rat whole brain tissue contents of malondialdehyde (MDA) and reduced glutathione (GSH) as well as superoxide dismutase (SOD) activity was determined according to the methods of Yoshioka (1979), Beutler et al. (1963), and Minami & Yoshikawa (1979), respectively. Brain IL-1β, TNF-α and IL-6 inflammatory cytokines have also been assessed using Enzyme-Linked Immunosorbant Assay (ELISA) kits, according to the kits' instructions. The brain NF-kB-p65 level was quantified using the ELISA rat kit provided by MyBioSource (USA). Furthermore, brain-derived neurotic factor (BDNF) tissue content was determined using rat-specific ELISA kits (BDNF, CSB-E04504r). Moreover, brain tissue Nrf-2 content (Nrf-2, OKAG00918) was assessed using the rat ELISA kit Aviva® Systems Biology, USA. In addition, rat brain HO-1 content was quantified using rat ELISA kits (HO-1; CSB-E08267r) by CUSABIO®, Houston, TX, USA. Brain contents of Caspase-3 and tyrosine hydroxylase (TH) were measured using rat-specific ELISA kit (Cusabio Biotech, LTD., Wuhan, China). A microplate reader (model 680Bio-Rad, USA) was used to measure absorbance at the wavelength(s) specified for each of the parameters.

Detection of Neurotransmitters: Brain content of dopamine was determined according to Ciarlone (1978a), by oxidation in an alkaline media to produce a luminous product. Similarly, brain serotonin was quantified as described by Ciarlone (1978b) using its fluorescence performance when heated with o-phthalaldehyde in a strong acidic medium. The fluorescence intensity was related to the concentrations according to the constructed standard curves.

Western blotting analysis

Homogenized rat brain samples were used for Western blotting analysis based on the procedure described by Mingone et al. (2003), using lysis buffer (Sigma-Aldrich®, St. Louis, MD, USA). Total tissue protein was assessed using BCA kit (Thermo Fisher Scientific), then each sample was loaded onto sodium dodecyl sulphate–

polyacrylamide gel electrophoresis (SDS–PAGE); 8% and transferred to a nitrocellulose membrane; Amersham Bioscience®, Piscataway, New Jersey, USA. The membranes were incubated overnight at 4°C in 5% fat-free milk blocking buffer to which 10mmol Tris–HCl (pH 7.4), 150mmol NaCl and Tris-buffered saline together with Tween-20 (0.05%) were added. After washing, membranes were incubated with each of the antibodies of α-synuclein rabbit mAb (1:1,000, cat. no. 4179), anti-SIRT1 rabbit mAb (1:1,000, cat. no. 9475), anti-FOXO1 rabbit mAb (1:1000, cat. no. 2880), AMPK, ATF-4, phospho-eIF2α, phospho-PERK and CHOP on a roller shaker overnight at 4°C. Chemiluminescence detection was carried on by Amersham® detection kit, based on the manufacturer's recommendations. Then, the protein levels have been quantified utilizing the densitometric analysis technique using a scanning laser densitometer (Biomed® Instruments). Results were calculated after normalization using the housekeeping protein; β-actin, expression.

Statistical analysis

Statistical data analysis were carried on using the statistical software "Statistical Program for Social Science" SPSS; version 15.0 by applying one-way analysis of variance (ANOVA) test followed by a posttest for multiple comparisons. Data are presented as a mean of 8 values ± standard error (SE); where the difference among means was considered to be significant at $P< 0.05$.

Results

Effects of AST on reserpine or irradiation-induced α-synuclein expression and catalepsy score in rats

In the current study, each of reserpine and head irradiation induced progressive increases in the rats' cataleptic behavior associated with significant decrements in both muscle activity and motor coordination when compared to normal rats. However, AST administration attenuated the development of rigidity and enhanced the motor co-ordination as compared with the untreated reserpinized or irradiated animals (Fig. 1 B & C). Furthermore, the levels of α-synuclein protein expression in each group were quantified; results shown in Fig. 1A revealed an acute stress-induced rise in α-synuclein expression as compared to its expression levels in the normal groups. As shown in Fig. 1, AST significantly attenuated acute stress-induced upregulation of α-synuclein as compared to the untreated groups.

Effects of AST on reserpine or irradiation-induced alterations of neurotransmitters, TH and BDNF expression in rats

The ELISA assessed the effects of acute cellular stress induced by reserpine or cranial irradiation on the levels of TH and BDNF proteins. As shown in Fig. 2 A & B, acute stress resulted in decrement of brain TH and BDNF expressions as compared to those recorded in the normal group. However, AST markedly up-regulated TH and BDNF expressions as compared to the untreated groups. The present findings indicated a significant reduction in dopamine and serotonin (5-HT) tissue contents in RES and irradiated rats as compared to the normal ones, while an elevation in the AST-treated group in both RES+AST and RAD+AST rats was observed as compared to the untreated groups (Fig. 2 C & D).

Effects of AST on reserpine or irradiation-induced alterations of brain redox status in rats

In the present study, each of reserpine and head irradiation induced a status of oxidative stress indicated by the observed increment in brain MDA contents, the decrease in SOD activity and the decrement in GSH contents are shown in Table 1. On the other hand, AST-treated groups; RES + AST and RAD +AST, showed a significant correction in each of the above detailed parameters as compared to the untreated groups; RES and RAD.

Effects of AST on reserpine or irradiation-induced alterations of brain inflammatory status in rats

Brain tissue contents of the pro-inflammatory cytokines IL-1β and IL-6 showed a significant increase in RES and RAD groups when compared to the normal group (Fig. 3 A & B). However, the treatment with AST induced a significant decline in IL-1β and IL-6 brain contents as compared to the untreated groups (Fig. 3). These data confirmed that RES or gamma-radiation caused an oxidative stress status together with an inflammatory cascade in brain tissue; however, AST offered corrective effects against these injuries. In the same line; as shown in Fig. 3 C & D, irradiation or injection of reserpine significantly increased the brain contents of TNF-α and NF-κB-p65 as compared to the control group. On the other hand, i.p. administration of AST significantly reduced the elevation of TNF-α and p65 (NF-κB subunit) content as compared to the untreated RES and RAD groups.

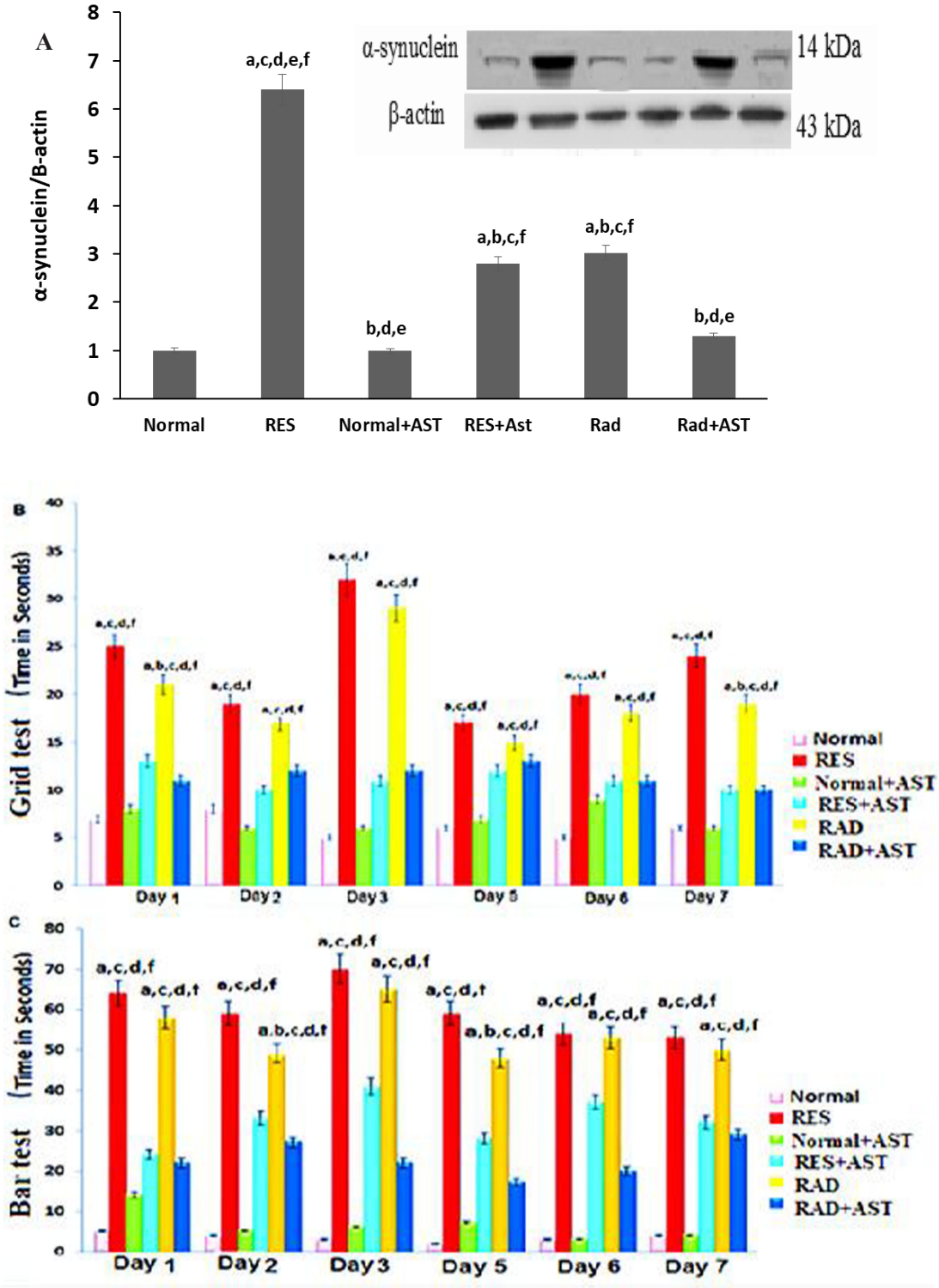

Fig. 1. Expression of α-synuclein and behavioral/motor alterations in brain of rats exposed to head irradiation or reserpine injection with or without AST at different time points. (A) Quantitative analysis and western blotting membranes of α-synuclein protein expression in the brain tissues of different studied groups; normalized to β-actin. (B) Time records of rats' performance in the grid test and (C) Time records of rats' performance in the bar test [Data are represented as means ± SEM (n = 8) and values are considered statistically significant at P< 0.05; "a", "b", "c", "d", "e" and "f" denote significant difference from normal, AST, RES, RES+AST, RAD, and RAD+AST groups, respectively]

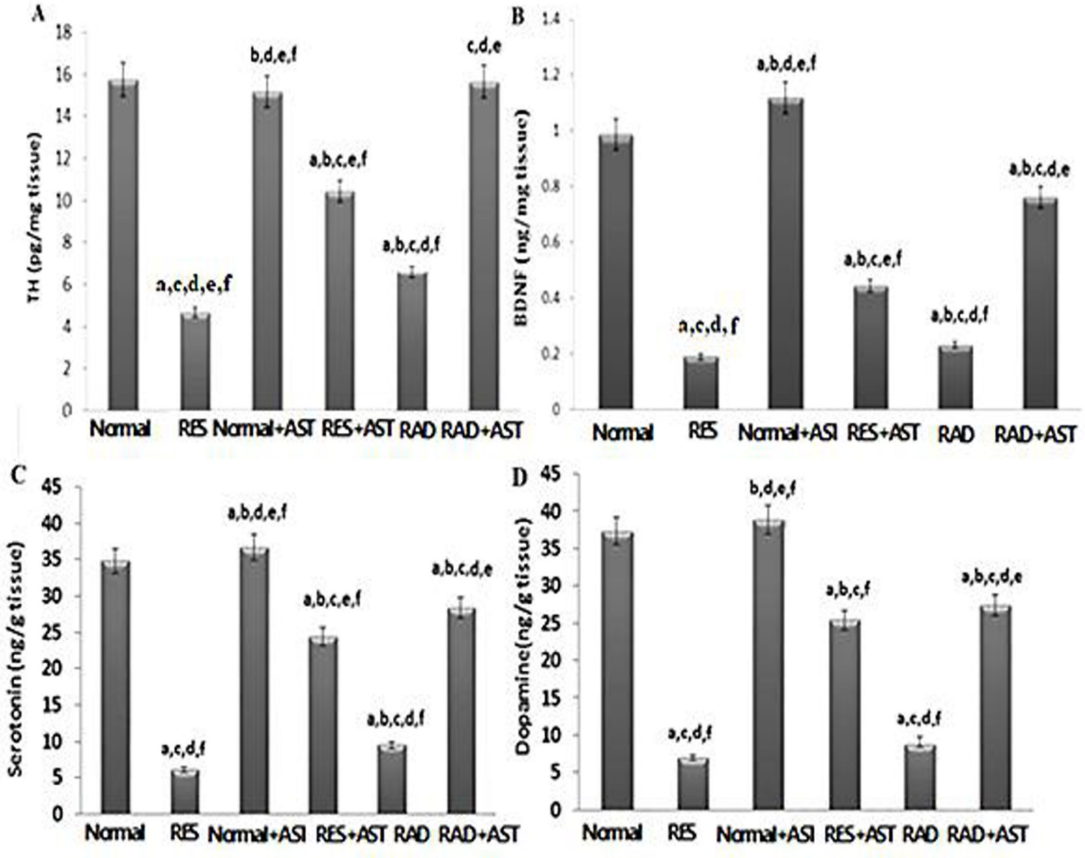

Fig. 2. Effects of AST on reserpine or irradiation-induced alterations of brain tissue expressions of A) TH, B) BDNF, C) Serotonin (5-HT) and D) Dopamine [Data are represented as means ± SEM (n = 8), and values are considered statistically significant at P< 0.05; "a", "b", "c", "d", "e" and "f" denote significant difference from normal, AST, RES, RES + AST, RAD, and RAD+AST groups, respectively]

TABLE 1. Effects of AST on reserpine or irradiation-induced alterations of rat brain tissue contents of MDA, GSH and SOD activity

Parameters / Groups	MDA (nmol/g tissue)	SOD (U/ g tissue)	GSH (µmol /g tissue)
Normal	112.80±1.72	5.30±0.99	62.60 ± 3.24
RES	169.72±6.76 ^{acde}	0.91±1.31 ^{acde}	27.29 ± 3.54 ^{acde}
Normal+AST	103.86±2.26 ^{bef}	5.11± 0.67 ^{be}	69.11 ± 1.27 ^{be}
RES + AST	119.84±4.92 ^{bef}	4.45± 2.57 ^{be}	51.63 ± 1.62 ^{be}
RAD	178.52±7.04 ^{acdf}	0.76±1.87 ^{acdf}	29.40±0.30 $^{a\,c\,d\,f}$
RAD+AST	136.52±4.02 ^{abcde}	4.52±2.53 $^{b\,c\,e}$	50.61±3.16 $^{a\,b\,c\,e}$

Data are presented as means ± SE. (n=8), and values are considered statistically significant at p < 0.05; "a", "b", "c", "d", "e" and "f" denote significant difference from normal, AST, RES, RES + AST, RAD, and RAD+AST groups, respectively.

Effects of AST on reserpine or irradiation-induced alterations of brain Nrf2 and HO-1 expression in rats

The HO-1 is an Nrf2 target gene that is crucial to Nrf2-mediated NF-κB suppression. The obtained results revealed that each of reserpine and radiation significantly lowered brain Nrf2 protein level in rats. Moreover, the impaired HO-1 content reflected the reduced Nrf2 signalling in reserpine or radiation -intoxicated rats. Nevertheless, AST treatment of RES or RAD rats almost normalized the brain Nrf2/ HO-1 contents (Fig. 3 E & F) as compared to the untreated rats.

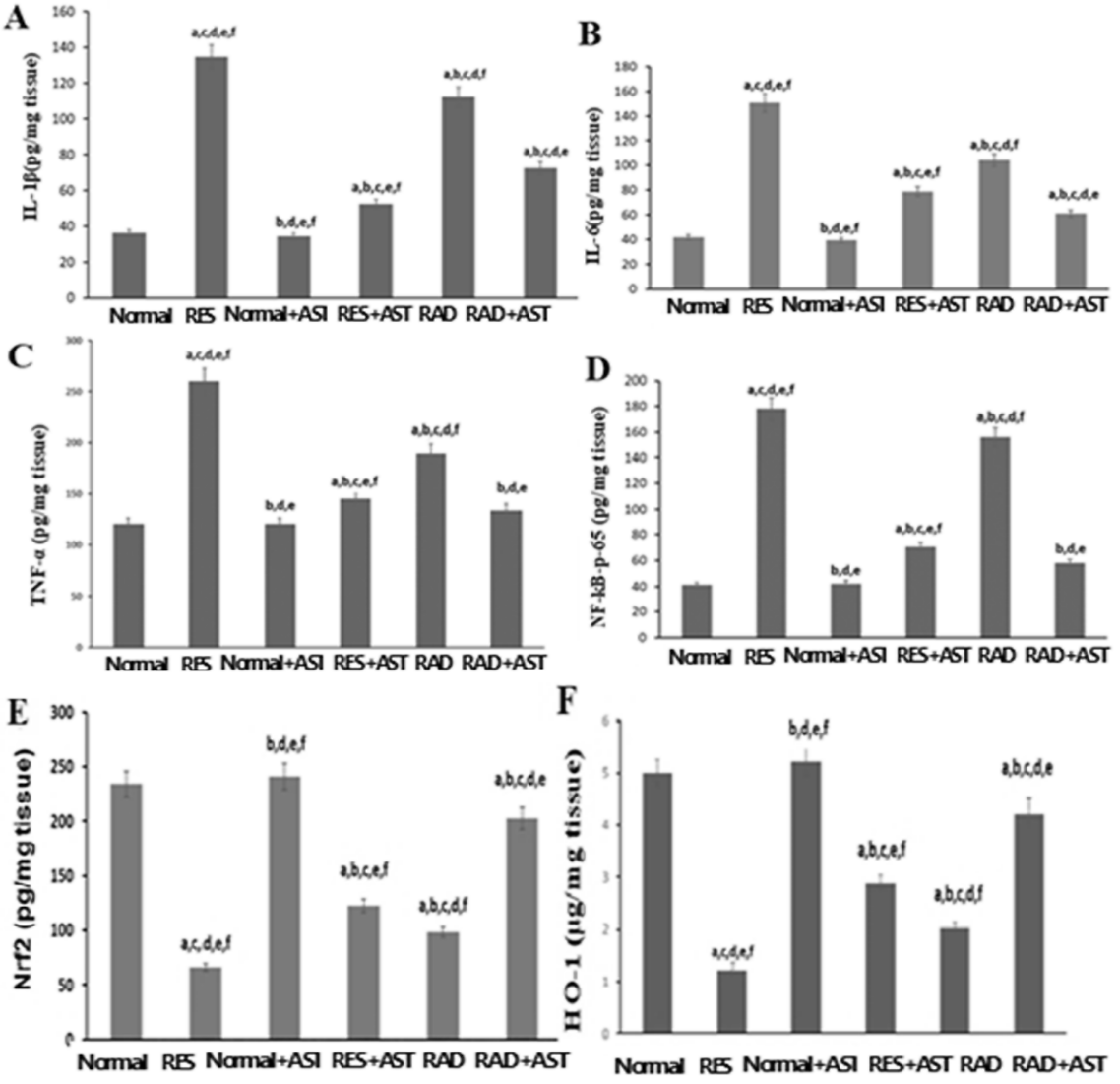

Fig. 3. Effects of AST on reserpine or irradiation-induced alterations of rat brain tissue expressions of (A) IL-1β, (B) IL-6, (C) TNF-α (D) NFkB-p65 (E) NRF2 and (F) HO-1 [Data are presented as means ± SE. (n=8), and values are considered statistically significant at $P < 0.05$; "a", "b", "c", "d", "e" and "f" denote significant difference from normal, AST, RES, RES + AST, RAD, and RAD+AST groups, respectively]

Effects of AST on reserpine or irradiation-induced alterations of the expression of brain cell survival mediators in rats

The circulatory regulation mechanism between AMPK and SIRT1 reinforces the important role of balance in the cellular processes. SIRT1 participates in the regulation of the apoptosis process by the upregulation of the AMPK pathway and the inhibition of pro-apoptotic molecules, attenuating the over-expression of the Caspase-3. The expression of AMPK and SRIT1 proteins showed a significant decrement in reserpine injected group, while Caspase-3 increased in both RES and RAD groups, while the AST-treated groups showed a significant correction of such status (Fig. 4) as compared to the untreated groups.

The Western blotting analysis indicated that the levels of FOXO1 in the RES or RAD groups were considerably greater than in the normal control group (Fig. 4). In comparison to the RES and RAD groups, AST administration lowered FOXO-1 expression in AST treated groups.

Effects of AST on reserpine or irradiation-induced alterations of brain CHOP expression

In the current study, reserpine and radiation induced an overexpression of a variety of ER

stress markers in rat brain neurons; namely, the PERK–ATF4–CHOP pathway. As shown in Fig. 5 A, B and C, reserpine and head irradiation induced the expression of these markers in the brain. However, AST suppressed reserpine and radiation-induced upregulation of CHOP (Fig. 5 D). ER stress promotes PERK that phosphorylates eIF2, resulting in a reduction of the ER's biosynthetic burden by suppressing protein synthesis and promoting ATF4. Then, ATF4 induces CHOP transcription. The treatment with AST significantly reduced the phosphorylation of PERK and eIF2, as demonstrated in Figure 6. Each of reserpine and irradiation-induced an increment in ATF4 expression which was considerably reduced by AST therapy (Fig. 5 C).

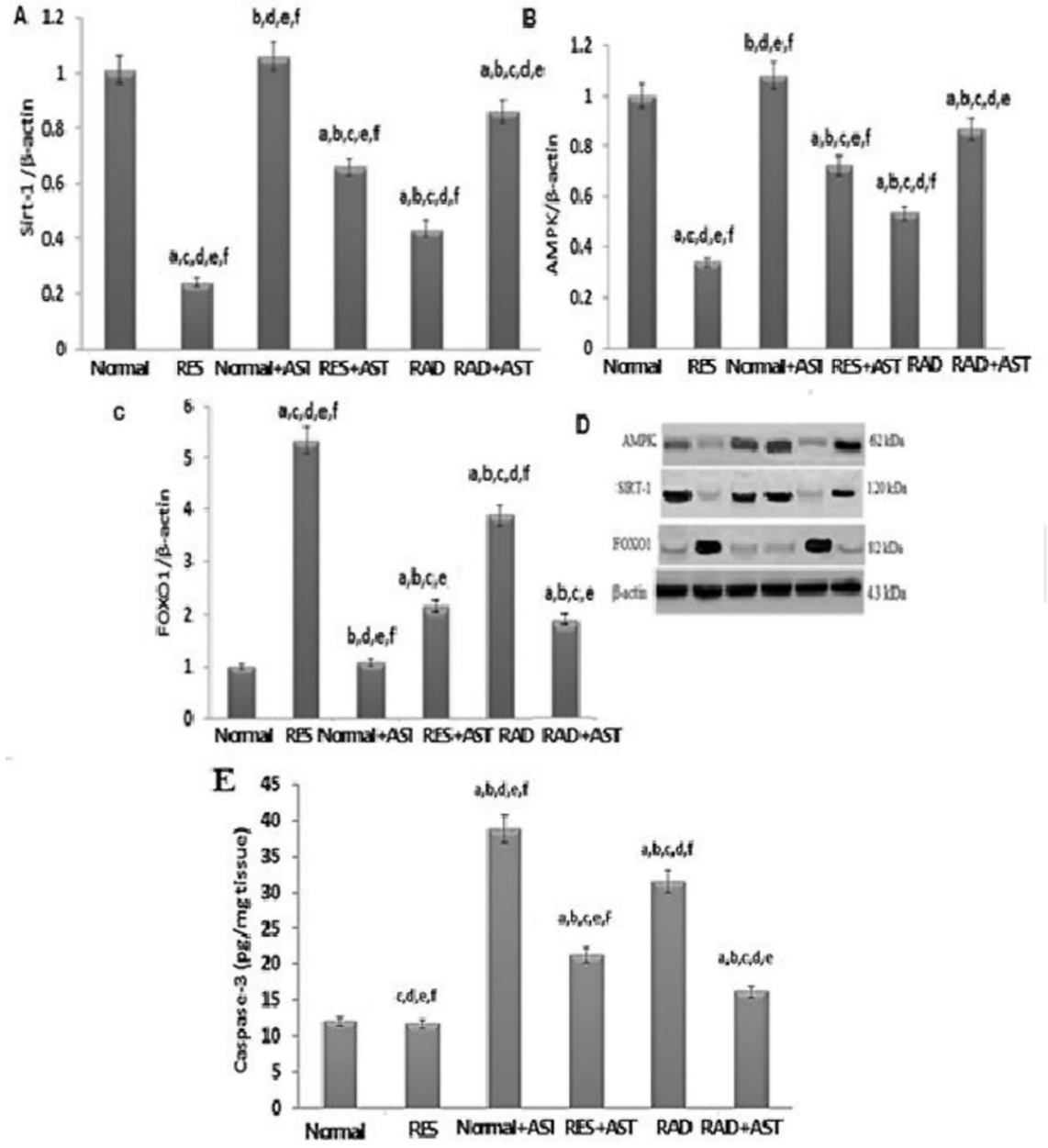

Fig. 4. Effects of AST on reserpine or irradiation-induced rat brain expressions of A) SIRT-1, B) AMPK and C) FOXO-1 of cell survival pathway. The protein expressions of SIRT-1, AMPK, FOXO-1 proteins are represented in comparison to β-actin (43kDa) (D) as housekeeping protein for Western Blotting analysis [Data are presented as means ± SE. (n=8), and values are considered statistically significant at $P < 0.05$; "a", "b", "c", "d", "e" and "f" denote significant difference from normal, AST, RES, RES + AST, RAD, and RAD+AST groups, respectively]

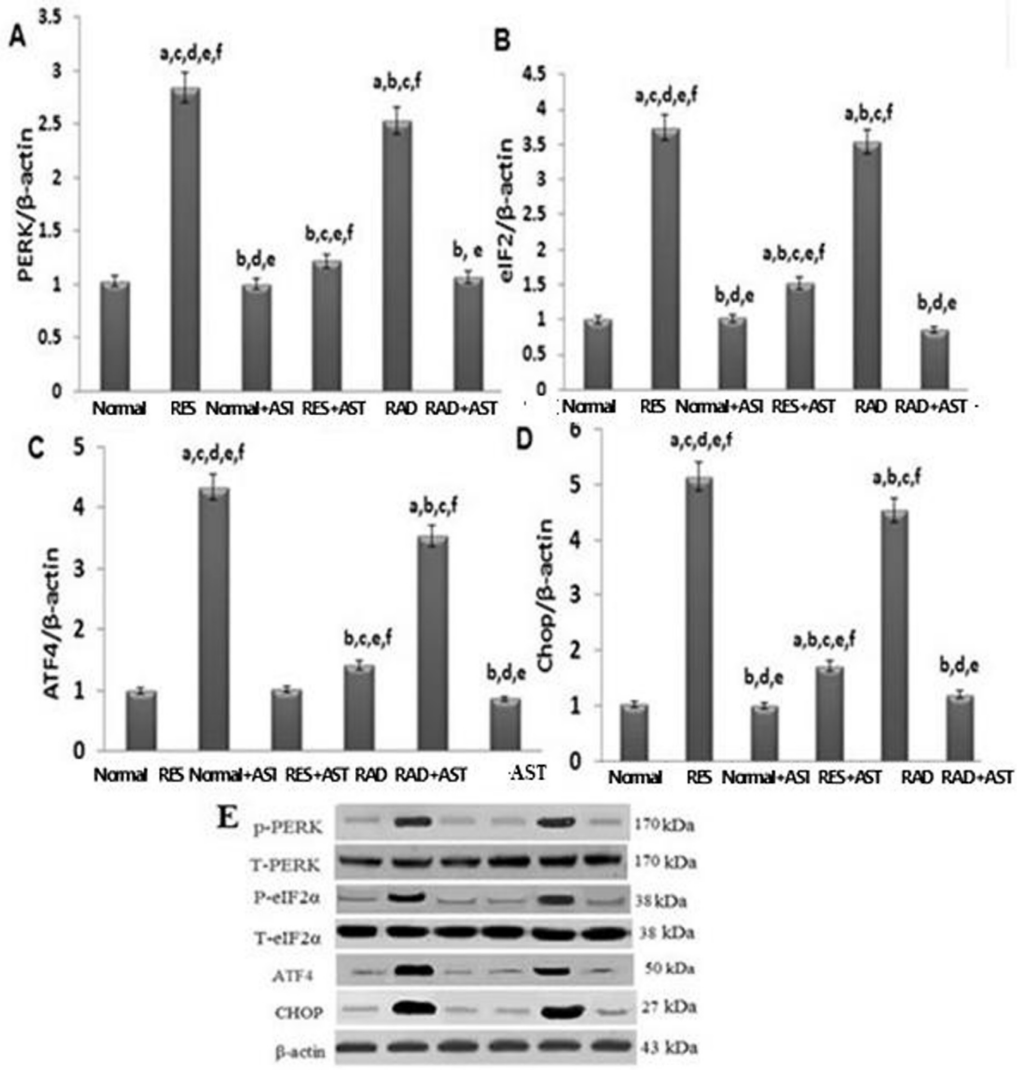

Fig. 5. Effects of AST on reserpine or irradiation-induced rat brain expressions of PERK, eIF2, ATF4 and, chop proteins represented in comparison to β-actin (43kDa) as housekeeping protein for Western Blotting analysis [Data are presented as means ± SE. (n=8), and values are considered statistically significant at P < 0.05; "a", "b", "c", "d", "e" and "f" denote significant difference from normal, AST, RES, RES + AST, RAD, and RAD+AST groups, respectively]

Discussion

Endoplasmic reticulum stress, redox signalling, inflammation, and neurodegeneration have been reported to be involved the pathophysiology of Parkinson's disease (PD). In the current work, the authors investigated the neuroprotective mechanisms of astringenin (AST); a natural derivative of resveratrol flavonoid, in an animal model of Parkinsonism induced by reserpine; a neurotoxin that causes brain dopamine depletion within hours. Moreover, AST effects were also investigated against head irradiation as another neuro-stressor (Zhang et al., 2020; Constanzo et al., 2020). Herein, the reported catalepsy scores; in both grid and bar tests, indicated that AST effectively improved the motor function of neuro-intoxicated rats, implying the possible effectiveness of AST against radiation and toxin-induced Parkinsonism. Similarly, when compared to the normal control group, stressors exposure (RES or RAD) showed higher α-synuclein expression that was suppressed by AST treatment.

The main pathological event of PD is the intracellular DA stores' depletion. However, TH; the rate-limiting enzyme in DA synthesis from tyrosine, is one of the therapeutic targets

for PD management, through the stimulation of TH expression and the consequent release of DA into the striatum of the substantia nigra. In the present study, the degree of TH expression, DA, serotonin and BDNF levels in RES and RAD groups were considerably lower than those reported in the normal groups. On the other hand, the present findings revealed that treatment with AST restored the levels of DA, serotonin and BDNF; it also improved the catalepsy scores implying the partial restoration of functions and viability of DAergic neurons, which could be attributed to the inhibition of α-synuclein toxicity through the formation of soluble non-toxic α-synuclein/polyphenol oligomers (Temsamani et al., 2016). Resveratrol and AST can mediate the transportation of proteins such as transthyretin, facilitating their passage into the circulation and brain fluid. In similar studies, resveratrol has been previously reported to guard against brain injury after crossing the blood–brain barrier (Wang et al., 2002; Rossi et al., 2008).

A significant increment of CHOP, p-PERK, p-eIF2, and ATF4 expression were recorded in the RES and RAD groups as compared to the normal control group. In an earlier study; in the same line with the present findings, exposure of rats to ER stress inducers results in a vicious cycle together with the oxidative stress leading to the activation of ER chaperones, resulting in a well-characterized UPR (Corazzari et al., 2017; Mansour et al., 2022). The current findings indicated that AST-induced neuro-protection against ER stress has been achieved, at least in part, by hindering the phosphorylation of both PERK and eIF2 with reduced ATF4 expression; with a consequent suppression of CHOP expression. The findings of the present study are in line with an earlier study; which reported that AST decreased the production of ER stress biomarker CHOP and IRE-1α in liver injury in mouse (Wen et al., 2018). Lately, the transcription factor CHOP has drawn attention for its positive impact in neurodegenerative diseases; where the overexpression of CHOP promotes apoptosis, whereas its inhibition protects against neuronal death (Almanza et al., 2019). In the current work, the authors observed a decrement in caspase-3 expression in AST treated groups; that might be attributed to its antioxidative and free radical scavenging activities. In addition, our results imply that AST anti-ER stress is dependent on the activation of the SIRT-1/AMPK pathway. Furthermore, ER stress causes an imbalance in the production and clearance of α-synuclein followed by disruption of SIRT-1/AMPK signalling pathway, stimulation of Foxo1; finally leading to neuronal apoptosis. In the same line; herein, the investigators observed an inhibition in SIRT1 and AMPK expression with an increment in FOXO-1 in the PD and RAD groups as compared to the normal group. According to recent research (Jiao & Gong, 2020), several cellular brain types such as astrocytes, microglia, and hypothalamus and cerebellum neurons have been reported to express Sirt-1 proteins; whose activation was reported to reduce the pro-inflammtory cytokines expression; IL-6, IL-1β and TNF-α (Fernando & Wijayasinghe, 2021). Sirt1 has also been demonstrated to suppress osteoblast apoptosis triggered by oxidative stress through the FOXO-1/catenin pathway (Huang et al., 2020). In the current study, AST administration significantly increased AMPK and SIRT-1 expression, which is associated with reduced FOXO-1 expression. Previous studies (Sin et al., 2015; Yao et al., 2018) reported that resveratrol act as neuroprotector through modulating the SIRT1/FOXO-1 pathway; probably through SIRT1-induced deacetylation of FOXO-1 inhibiting its activity, which is in line with the current findings. AST also, was previously reported to prevent oxidative stress and apoptosis via boosting AMPK phosphorylation (Moustafa et al., 2021a).

Increased ROS abundance alters protein and nucleic acid structure and function, break peptide bonds, modify protein cross-linking or base modification, hydrolysis and DNA fragmentation. It can also strike the biomembrane's polyunsaturated fatty acids inducing lipid peroxidation, decrement of membrane fluidity, loss of membrane integrity, membrane brittleness, and flow disruption across the cell or organelle membrane (Juan et al., 2021). In the current study, excessive ROS production following RES or irradiation was followed by increased brain lipid peroxidation along with GSH and SOD depletion. In such conditions, neuronal and immunological brain cells interact, causing alterations in neurotransmitters and proinflammatory cytokine secretions (Abdel-Aziz et al., 2021). Herein, restoration of a balanced redox status has been achieved by AST implicating its neuroprotective effects against apoptosis; similarly, it has been previously reported to attenuate ROS-induced apoptosis, α-synuclein-induced cytotoxicity and neurodegeneration in Alzheimer's disease (Fu et

al., 2016). AST, also, offered a neuroprotective effect against experimental toxin-induced neurotoxicity in rat hippocampal and frontal cortex neurons (Kim et al., 2007).

In the present study, the stressors-induced oxidative stress status was accompanied by a significant rise in the pro-inflammatory cytokines; IL-1β, TNF-α and IL-6, production. On the cellular level, TNF-mediated cytotoxicity cascade starts with the binding of TNF to its cell surface receptors activating various intracellular pathways including the G protein-mediated phospholipases activation, the ROS formation, and the endonucleases-induced nuclear DNA damage (Jayaraj et al., 2019). Furthermore, recent studies have reported that pro-inflammatory cytokines such as interleukins, TNF-α and interferon-γ are released in response to radiation doses of 10 and 25 Gy resulting in disruption of neuronal survival pathways (Thabet et al., 2021). In this study, rats exposed to RES or radiation had significantly higher NF-κB (p-65) brain levels, associated with an inhibition of transcription factors Nrf-2 and HO-1 expression; probably induced by ROS overproduction. Since Nrf2 is a critical component in the cellular response to oxidative stress, and its activation is involved in regulating intracellular pathways associated to ROS and antioxidant enzyme activities (Moustafa et al., 2021b). Under normal conditions, Nrf2 generally binds to Keap1 in the cytoplasm as a cofactor, but in the oxidative stress status, Nrf2 is activated by phosphorylation, uncoupled from Keap1, then trans-located into the nucleus, and joined with the anti-response element regulating HO-1 expression (Zhan et al., 2021). As for the HO-1 expression, it can be triggered by a variety of stressors, including ROS, stimulating the production of biliverin and carbon monoxide, as well as bilirubin, and therefore contribute to the in vivo clearance of ROS, which is crucial in limiting the inflammatory processes and peroxidation damage to the brain (Ahuja et al., 2021). On the other hand, AST induced an activation of Nrf2/HO-1 and subsequently blocked the NF-κB pathway. These results are in line with those of the study of Eid & Abdel-Naim (2020), who observed a significant AST-induced modulation of NF-κB /Nrf2/HO-1 axis in prostatic hyperplasia in rats. Similarly, resveratrol-induced enhancement of Nrf2 expression has previously been related to HO-1 in a rat systemic inflammation model (Lee et al., 2010). In the same context, Liu et al. (2011) reported that AST beneficial effects against hepatic damage following hemorrhage are likely mediated by a Akt-dependent HO-1 up-regulation. Furthermore, AST therapy reduced behavioral disorders and brain damage in a mouse model of aging via the induction of Nrf2, HO-1, and NOQ1 expression, implying that AST might be used as an anti-inflammatory therapeutic strategy of age-related disorders (Kim et al., 2018).

To summarize, in the present work, AST has been found to protect brain neurons against ER stress-induced apoptosis via activating the Sirtuin-1 and redox Nrf2/HO-1 signalling pathways. Furthermore, AST neuroprotective action might be linked to its remarkable antioxidant effects that inhibited the expression of PERK-eIF2-ATR4 chaperon's factors and the accumulation of FOXO-1. Such effects propose AST as a promising candidate as an adjuvant therapy in neurodegenerative disorders to act against disease progression.

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