

Research Article

Alpha-2-Macroglobulin Mitigates Mitochondrial Dysfunction and Neuronal Apoptotic Responses in Pesticide-induced Neurotoxicity

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Abstract

Persistent and repetitive application of pesticides has been linked to adverse effects on human metabolism and the onset of various disorders. Commonly used pesticides, such as carbamates (e.g., Aldicarb, ALD) and organophosphates (e.g., Chlorpyrifos, CPF), are widely applied in potato cultivation and household pest control. Chronic exposure to these substances has been implicated in the early onset of neurodegenerative disorders. Alpha-2-macroglobulin (A2M), a protein known for its regulatory role in oxidative stress, participates in multiple biological processes. Despite its significance, the role of A2M in mitigating mitochondrial-induced neuronal apoptosis triggered by pesticide interference remains poorly understood. This study explores the involvement of A2M in SH-SY5Y neuroblastoma cells exposed to pesticides, focusing on its impact on mitochondrial enzyme expression, inflammatory cytokines, neuronal apoptotic and anti-apoptotic proteins, and the activation of the Nrf2 signaling pathway. Comparative analyses of control and pesticide-exposed SH-SY5Y cells revealed that A2M positively modulates neuronal stress responses. Western blot profiling demonstrated that A2M upregulates anti-apoptotic proteins such as Bcl-2 and Nrf2, while downregulating pro-apoptotic markers, including Bax, Caspase-3, and Caspase-9. Biochemical assays showed that A2M enhances mitochondrial enzyme activity, particularly complexes I and III, while mitigating the reactive oxygen species (ROS) generated by ALD and CPF exposure. Furthermore, A2M was found to reduce DNA damage caused by pro-inflammatory cytokines, which are exacerbated by mitochondrial oxidative stress. These findings highlight the pivotal role of A2M in attenuating pesticide-induced neuronal toxicity through the regulation of mitochondrial function and inhibition of neuronal apoptosis.

Keywords

Alpha-2-Macroglobulin, Nrf2 Pathway, Cytokines, Mitochondrial Dysfunction, DNA Damage, NADH Dehydrogenase

1. Introduction

Neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Multiple sclerosis (MS) are major clinical concern worldwide. Epidemiological evidences reports dietary, genetic, molecular and environmental fac-

tors are leading factors of neurodegeneration [1]. The contaminants accumulate in human body via food chain and environmental factors, thus altering gene expression and modify protein synthesis and significantly affect other bio-

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logical process [2]. Pesticides such as carbamates, pyrethroids, organophosphates are recognized as one of the risk factors involved in epigenetic mechanisms leading to progression of neurodegenerative diseases [3]. Low levels of pesticides accumulate in tissues, impact human brain causing impairment in cognitive functions, memory and some motor functions [4]. Organophosphate (OP) and organochlorine (OC) pesticides are highly lipophilic and can therefore easily enter and accumulate within the mitochondria [5]. These neurobehavioral changes are associated with the development and pathogenesis of various neurological disorders.

One of the prominent neurodegenerative disease worldwide is Alzheimer's disease, which is characterised by loss of neurons and synapses in the cerebral cortex and certain subcortical regions [6]. Preclinical stages of AD can be determined by examining the Cerebrospinal fluid (CSF), neuroimaging of biomarkers to recognise the deposition of β -amyloid plaques, synaptic dysfunction and inflammatory and apoptotic markers of neuronal injury [7, 8]. Some inflammatory responses and immune regulatory factors are intrinsic to AD pathogenesis and act as trigger of AD onset and its clinical symptoms [7]. These factors include pro inflammatory cytokines, apoptotic protein markers, transcription factors etc. [9]. Research suggests that exposure to certain pesticides interferes with normal immune system functioning, leading to increased inflammation [10, 11]. Alteration in immunotoxicity, endocrine disruption and antigenicity have been introduced as the main mechanisms working with pesticides-induced immune dysregulation [12] which contributes to mitochondrial dysfunction in neurons, altered DNA expression and triggered apoptosis [13, 14]. Recent literatures show that pesticide immunotoxicity involves signaling pathways by reciprocal triggering with inflammation through cytokine modulation [15].

Alpha-2 macroglobulin (A2M) is an acute phase protein and major component of the innate immune system, functioning as a pan-protease inhibitor and a chaperone protein [6, 16]. A2M is a tetramer, consisting of bait region located among each identical subunits which functionally inhibits any proteinases present in plasma regardless of their specificity (Figure 1) [16]. It transports and inhibits pro inflammatory cytokines such as interleukins (IL-1 β and IL-6), interferons, insulin, growth hormone, transforming growth factor- β (TGF- β), tumor necrosis factor (TNF- α) and platelet derived growth factor (PDGF) etc. [17]. Affinity of A2M to these cytokines and factors has led to efforts targeting inflammation as a potential strategy for AD pathogenesis. Although, the strategy of targeting anti-inflammatory agents have failed in the patients diagnosed with established AD have failed, which alternatively suggests targeting inflammation and anti-inflammatory substances during early stage before the onset of clinical symptoms of AD [18].

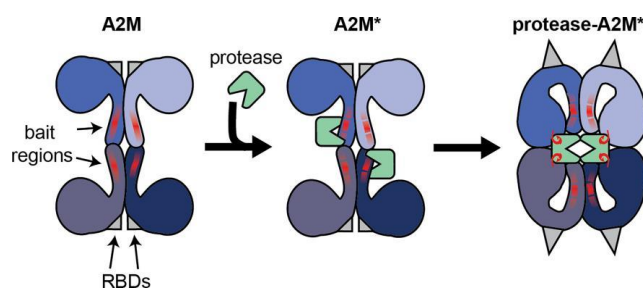


Figure 1. Representation of the interaction between the A2M and proteases. Each subunit contains a protease bait region and a buried receptor binding domain. Proteases recognise the bait region and interacts with it, which changes the protein's conformation resulting in trapping the proteases.

In our previous study, we have investigated the cytoprotective role of A2M against pesticides namely Chlorpyrifos (CPF), Aldicarb (ALD) and Deltamethrin (DLM); which were found to induce neurotoxicity targeting its role in modulating antioxidant enzymes and ROS species markers such as MDA, TBARS in neuronal SH-SY5Y cell lines. We found that A2M shielded the antioxidant system of cells against ROS [19] however some other issues need to be addressed to reach some conclusion about A2M's protective mechanism [20]. This present study evaluates the neuroprotective effects of A2M on pesticides induced mitochondrial dysfunction, inflammatory aberrations, and neuronal apoptosis to identify A2M's ameliorative effective as a strategic approach to early inflammatory and immune-regulatory processes which are intrinsic to AD pathogenesis. Our study focuses whether A2M renders neuroprotection by inhibiting mitochondrial mediated apoptotic pathway and underlying events like apoptosis in neuronal SH-SY5Y cells during pesticides induced neurotoxicity; whether A2M reduces neuronal inflammation by inhibiting pro-inflammatory cytokines in neurons and what mechanism or transcription factors are involved during the process. Hence, to address these issues, we designed the experimental study with SH-SY5Y cells, as continuation to our previous study with pesticides aldicarb (ALD) and chlorpyrifos (CPF) to obtain some novel outcomes.

2. Materials and Methods

2.1. Materials

Pesticides (ALD and CPF) and chemicals (Lithium lactate, NAD, 2, 4-dinitrophenyl-hydrazine reagent; DNPH, 1.0 N hydrochloric acid, Sodium Pyruvate, Ethylenediamine tetraacetic acid; EDTA, 5-5'-dithiobis [2-nitrobenzoic acid]; DTNB, Trichloroacetic Acid; TCA, α -Ketoglutarate, Potassium ferrocyanide, Potassium cyanide, Succinate, Oxaloacetate, Cytochrome c, N-phenyl-p-phenylene diamine, agarose were commercially purchased from Sigma Aldrich, Takara Bio, Thermofisher Scientific etc.

2.1.1. Cell Culture Reagents

SH-SY5Y cells were obtained from NCCS, Pune, India. Dulbecco's modified eagle media (DMEM) and Ham's F12 medium, fetal bovine serum (FBS), 1% penicillin-streptomycin were commercially purchased from Thermofisher scientific.

2.1.2. Buffers

Glycine, sodium phosphate, Tris-HCl (pH 7.2), Tris-HCl 0.1 M (pH 7.5), potassium phosphate buffer (pH 7.4), 0.3 M Phosphate buffer (pH 7.6), dH₂O were prepared in laboratory.

2.1.3. Solutions

1 M NaOH 1 M, Guanidine hydrochloride 8 M, Ethanol/Ethyl Acetate (1:1 v/v), 0.1 M Trisodium isocitrate, 0.015 M Manganese chloride, 0.002 M Thiamine pyrophosphate, 0.25 M Potassium ferricyanide, 3% BSA were purchased from Sigma Aldrich. All other reagents used were of analytical standard.

2.2. Methods

2.2.1. Purification and Characterization of Human A2M

The A2M protein was isolated and purified from human blood plasma using ammonium sulfate precipitation followed by gel exclusion chromatography as per the method described in previous study [19]. A 5% (w/v) native PAGE was performed and the gel was stained with Coomassie brilliant blue R-250 (0.15% in 10% acetic acid). The gel was destained for 12 h in the destaining solution (10% acetic acid), and the purified A2M formed a single band on the gel.

2.2.2. Cell Culture

The SH-SY5Y cell line was cultured in a medium containing 1:1 DMEM and Ham's F12 medium, 10% FBS, and 1% penicillin-streptomycin [21]. The cells were treated with a standard solution of pesticides and A2M, accordingly, to perform the experiments. Cells were used at 3-7 passages. The cells were divided into five groups based on the treatment with pesticides and proteins to obtain results for various stress markers under standard conditions (37 °C and 5% CO₂). Cells were marked under various groups:

- 1) *Group I*: It was the control group comprising only SHSY5Y cells.
- 2) *Group II*: It consisted of SH-SY5Y cells incubated with ALD and CPF (5 µM each) separately under standard conditions for 3 h.
- 3) *Group III*: It comprised 2 sets of cells treated with pesticides ALD and CPF separately and each set subsequently treated with A2M (2 µM) under standard conditions for 3 h.
- 4) *Group IV*: This group consisted of A2M alone treated

SH-SY5Y cells for 3 h.

2.2.3. Analysis of Pathophysiological Marker of Cell Membrane Damage; Assay of Lactate Dehydrogenase (LDH)

LDH release is generally considered as marker of cellular damage and cell death upon acute or chronic injury in muscle and brain cells [22]. In the assay, the lactate is acted upon by lactate dehydrogenase to form pyruvate in the presence of NAD. The pyruvate forms pyruvate phenyl hydrazone with 2,4 dinitrophenyl hydrazine. The colour substrate developed is read at 420nm. The enzyme activity was expressed as IU/L (µmole pyruvate liberated/min/litre) and tissue enzyme activity was expressed as µmoles pyruvate liberated/min/mg protein [23].

2.2.4. Analysis of Neurotoxicity; Acetylcholinesterase (AChE) Assay

AChE activity is measured to assess exposure to neurotoxic compounds such as organophosphate and carbamate pesticides in humans. It is a reliable biomarker of neurotoxicity attributed to its dose-dependent response to exposure to pollutants [24]. AChE enzymatic activity was assayed using standard method [25]. AChE hydrolyses the acetylthiocholine to produce thiocholine and acetate. The liberated thiocholine reduces the dithiobis-nitrobenzoic acid (DTNB) liberating nitrobenzoate, which absorbs at 405 nm. The change in absorbance was recorded at 412 nm for 2 min at 30 sec interval using UV spectrophotometer and the activity was expressed as µmoles of acetylcholine iodide hydrolyzed /min/mg protein.

2.2.5. Analysis of Protein Carbonyls; Major Hallmark of Oxidative Damage

Oxidative stress is considered as sign of several neurodegenerative diseases, including Alzheimer's, Parkinson's, and Huntington's disease [19]. Protein carbonyl levels are estimated to measure the extent of protein oxidation to predict the oxidative stress in early stages leading to neurodegeneration. The measurement of protein carbonyls involves reaction of carbonyl group with 2,4-dinitrophenylhydrazine (DNPH), which leads to the formation of a stable 2,4-dinitrophenyl (DNP) hydrazone product. The optical density of each sample was read at 365 nm against the control. The level of protein carbonyls was expressed as nmoles /mg protein [26].

2.2.6. Analysis and Estimation of Mitochondrial Enzymes

Mitochondrial TCA cycle is a primary source of cellular energy during which acetyl-CoA is oxidised to produce carbon dioxide, ATP and NADH. Various enzymes participate during this cycle to form intermediate substrates ultimately leading to the release of acetyl-CoA. Excessive free radicals

generated by some toxicants damage the inner mitochondrial membrane, leading to compromised mitochondrial energy production and metabolism in the brain [27]. To assess the mitochondrial functioning, we performed the estimations of following TCA cycle enzymes-

Assay of Isocitrate dehydrogenase: The enzyme activity was assayed according to the standard method. Isocitrate dehydrogenase catalyzes the decarboxylative oxidation of threo-DS-isocitrate (2R-3S-isocitrate) by NADP⁺ or NAD⁺, yielding α -ketoglutarate, carbon dioxide, and NADPH or NADH. The reaction is magnesium/manganese dependent. Reduction of NADP⁺ /NAD⁺ to NADH is read spectrophotometrically at 420 nm. The isocitrate dehydrogenase activity was expressed as nmoles of α -ketoglutarate liberated/hr/mg mitochondrial protein [28].

Assay of α -Ketoglutarate dehydrogenase: α -KGDH activity was determined from the rate of reduction of NAD⁺ in the presence of α -ketoglutarate (potassium salt). The colour substrate was measured at 460 nm. The activity of α -ketoglutarate dehydrogenase was expressed as nmoles of ferrocyanide liberated/hr/mg of mitochondrial protein [29].

Assay of Succinate dehydrogenase: Succinate dehydrogenase catalyzes the oxidation of succinate to fumarate with the sequential reduction of enzyme-bound FAD and non-heme-iron. Reaction mixture consisted of 1 ml of phosphate buffer, 0.1 ml of EDTA, 0.1 ml of BSA, 0.3 ml of sodium succinate, 40 mM sodium azide and 50 μ M 2,6-dichloroindophenolate. This mixture was added to the cell lysate. The change in OD was recorded at 15 seconds interval for 5 min at 600 nm. The succinate dehydrogenase activity was expressed as nmoles of succinate oxidized/min/mg of mitochondrial protein [30].

Assay of Malate dehydrogenase: Malate dehydrogenase is an oxidoreductase involving nicotinamide adenine dinucleotide the decrease in absorbance due to the oxidation of NADH is followed. Oxaloacetic acid reacts with NADH and H⁺ and releases Malic acid and NAD. Reaction was recorded at 340 nm. The enzyme activity was expressed as nmoles of NADH oxidized/min/mg of mitochondrial protein [31].

2.2.7. Analysis of Mitochondrial Dysfunction

Mitochondrial damage signifies the disruption of mitochondrial respiratory chain enzymes. Mitochondria are both producers as well as targets of ROS, which increases oxidative damage. Upon continuous damage, mitochondria lose their functional integrity and release more ROS molecules compromising neuronal functioning and accelerating neurodegenerative process. To analyse mitochondrial dysfunction, biochemical assay of ATPase activity and NADH dehydrogenase was performed.

ATPase activity assay: Na⁺ / K⁺ ATPase activity is the process by which the sodium-potassium adenosine triphosphatase (Na/K-ATPase) enzyme transports ions across the cell membrane. NKA dysfunction has been linked to mitochondrial dysfunction in neurodegenerative diseases [32].

Na⁺ / K⁺ ATPase transports Na⁺ / K⁺ against concentration gradient at the cost of ATP molecule liberating an inorganic phosphate (Pi). Na⁺ / K⁺ ATPase activity was estimated from the amount of Pi liberated in the reaction. The blue colored substrate was read at 620 nm against blank in UV spectrophotometer. Enzyme activity was expressed as μ moles of phosphorus liberated/min/mg protein at 37 °C [33].

Assay of NADH dehydrogenase: NADH dehydrogenase complex or NADH-ubiquinone oxidoreductase is a large, multisubunit protein complex that transfers electrons from NADH to ubiquinone (coenzyme Q10) in the respiratory chain of mitochondrial complex I. To the reaction mixture containing phosphate buffer, potassium ferricyanide and NADH, cell mitochondrial lysate suspension was added. The change in OD was measured at 400 nm as function in time dependent manner [34]. The activity of NADH dehydrogenase was expressed as nmoles of NADH oxidized/min/mg mitochondrial protein.

Assay of Cytochrome-c-oxidase: Cytochrome-c-oxidase is a terminal enzyme in the cellular respiration which reduces oxygen to water. This process creates an electrochemical gradient that powers the synthesis of adenosine triphosphate (ATP), which is used to fuel cellular processes. The activity of cytochrome-c-oxidase was assayed by the standard method in which the enzyme oxidizes the reagent (N-phenyl-p-phenylene diamine) to color end product. The enzyme activity was expressed as nmoles of cytochrome oxidized /min/mg of mitochondrial protein [35].

2.2.8. Isolation and Analysis of DNA Fragmentation by Agarose Gel Electrophoresis

DNA from SH-SY5Y cells after pesticides treatment and A2M treatment was isolated and extracted by using kit from Qiagen and following the manufacturer's instruction. Sample DNA in microfuge tube was dissolved in TAE buffer (pH: 8.0) containing 40% sucrose and a pinch of bromophenol blue marker dye. Agarose gel was prepared and the apparatus was set up equipped with the constant power supply of 150 V. When the gel was run completely, the supply was removed and gel was then visualized by ethidium bromide (EtBr) in UV trans-illuminator [36].

2.2.9. Western Blotting Analysis

The nucleic components were isolated using a nucleic/cytosolic fractionation kit (Takara Bio) according to the manufacturer's instructions. The protein content of each cellular extract was quantified by the Bradford assay. An equal amount of protein from each sample was separated by SDS-poly-acrylamide gel electrophoresis (10% gel) and transferred to the nitrocellulose membrane. The membranes were blocked with 5% skimmed milk and incubated with primary antibodies for TNF- α , NF- κ B, p53, iNOS, IL-1 β , Caspase-3, Caspase 9, anti Bax and anti-Bcl-2 antibody (Santa-Cruz Scientific, USA) and HRP-conjugated secondary antibody

(Thermo Fisher, USA) followed by enhanced chemiluminescence detection (Thermo scientific, USA). The nuclear fraction was incubated with anti-Nrf2 and anti- β actin antibody (Santa-Cruz Scientific, USA) for normalization of positive loading control. Relative band intensities were quantified using Image analysis software.

2.2.10. Statistical Analysis

All the grouped data was evaluated using SPSS software. Hypothesis testing method included one-way analysis of variance (ANOVA), followed by least significant difference (LSD) test. $P < 0.05$ indicates statistical significance. All the results were expressed as mean $\pm$ S.D for triplicates in each group.

3. Results

3.1. Purification of A2M

Figure 2 shows the results from the purification of human A2M from human plasma. Native gel electrophoresis of human A2M consisted of several fractions to rule out the purest fraction containing A2M. In this figure, lane 1 consist of human blood plasma, lane 2 consisting of 20-40% ammonium sulfate fraction and lane 3 has purified A2M (180 KDa), obtained after gel filtration chromatography.

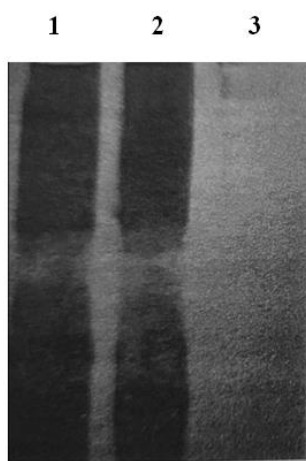


Figure 2. Native gel electrophoresis of human A2M consisting of the purified A2M (lane 3).

3.2. Effect of A2M and ALD/CPF on Pathophysiological Marker Enzyme Ldh in Control and Experimental Group of SH-SY5Y Cells

Table 1 & Figure 3 show the effect of ALD, CPF and A2M on Lactate dehydrogenase (LDH); a pathophysiological marker enzymes in control and experimental group of SH-

SY5Y cells. The results indicated that the levels of LDH were significantly ($P < 0.05$) increased in group II when compared with control cells (group I). However, supplementation of A2M to pesticides treated cells significantly lowered the LDH levels (group III) as compared to group II. A2M alone treated cells did not show any significant change in the enzyme's activity (group IV). A2M alone did not cause significant change in LDH activity suggesting its neutral role in cellular injury (group IV), but interaction with LDH was found to restore its protective mechanism against pesticides induced injury, attributed to A2M's trapping mechanism (group III).

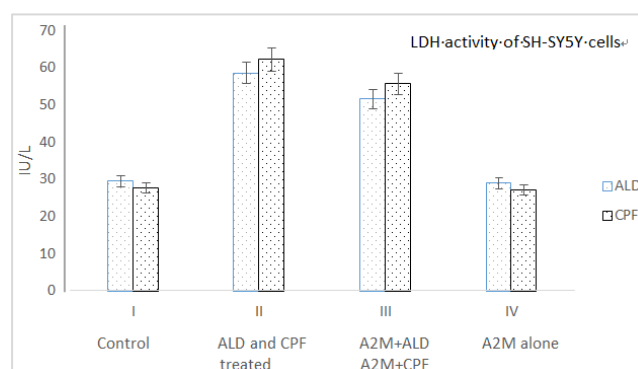


Figure 3. LDH: Lactate dehydrogenase. Activity is expressed as IU/L. Results are expressed as mean + SD for different sets of experiments (3 set/enzyme/group for ALD treated and A2M induced cells, 3 set/enzyme/group for CPF treated and A2M induced cells).

Table 1. Effect of pesticides ALD and CPF and treatment with A2M on pathophysiological marker LDH on control and experimental group of SH-SY5Y cells. Group I represents control, Group II represents ALD treated cells and CPF treated cells, Group III represents treatment of pesticides induced cells with A2M and group IV represents A2M treated cells alone.

Groups	ALD	CPF
I	29.44±3.51	27.57±2.22
II	58.53 ± 4.21	62.13±5.73
III	51.49±2.33	55.55±3.88
IV	27.13±3.61	23.62 ± 2.14

3.3. Effect of A2M and ALD/CPF on the Activities of AChE in SH-SY5Y Cell Homogenate of Control and Experimental Groups

The effects of ALD, CPF and A2M on the activity of acetyl cholinesterase (AChE) in control and experimental group of SH-SY5Y are shown in Table 2 and Figure 4. The obtained results showed that a significant ($p < 0.05$) inhibition in

the activity of AChE in ALD and CPF treated cells (group II) compared with control (group I). However, upon simultaneous treatment of A2M with ALD and CPF was able to preserve the AChE activity levels (group III). The present re-

sults suggest the healthy role of A2M in significantly countering the harmful effects of pesticides ALD and CPF. A2M alone treated cells did not cause any significant alteration on the AChE activity (group IV) compared to control.

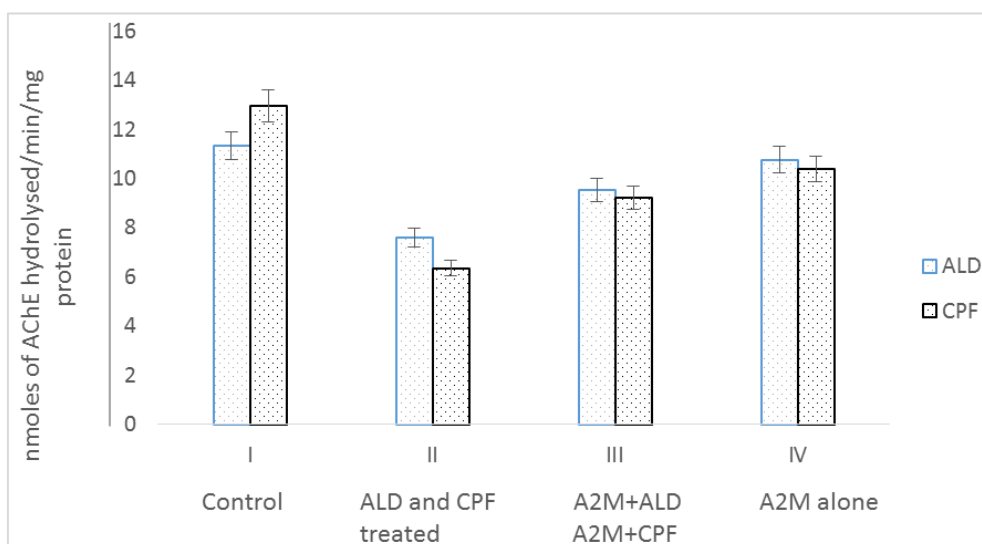


Figure 4. Results are expressed as mean + SD for different sets of experiments (3 set/ enzyme/group for ALD treated and A2M induced cells, 3 set/enzyme/group for CPF treated and A2M induced cells).

Table 2. Effect of pesticides ALD and CPF and treatment with A2M on AChE levels in control and experimental group of SH-SY5Y cells. Group I represents control, Group II represents ALD treated cells and CPF treated cells, Group III represents treatment of pesticides induced cells with A2M and group IV represents A2M treated cells alone.

Groups	ALD	CPF
I	11.33 ± 0.94	12.96 ± 0.55
II	7.59 ± 0.81	6.34 ± 0.46
III	9.51 ± 0.35	9.20 ± 0.68
IV	10.75 ± 0.72	10.36 ± 0.59

3.4. Effect of A2M and ALD/CPF on the Levels of Protein Carbonyl in Mitochondrial Lysate of Control and Experimental Group of SH-SY5Y Cells

Pesticides act as pro-oxidants and triggers oxidative stress in multiple organs. Table 3 & Figure 5 illustrates the effects of ALD, CPF and A2M on the protein oxidation in control and experimental group of SH-SY5Y cells. Protein carbonyl

(PC) is the marker for protein oxidation. There was a significantly increased PC ($p < 0.05$) contents was shown in ALD and CPF treated SH-SY5Y cells (group II) as compared with control group (group I), confirming the susceptibility of neuronal cells to oxidative stress. However, administration of A2M to ALD and CPF treated cells, A2M significantly decreased ($p < 0.05$) the PC level (group III) compared with ALD and CPF treated cells. No significant difference was observed in cells treated with A2M alone (group IV) when compared to control cells (group I).

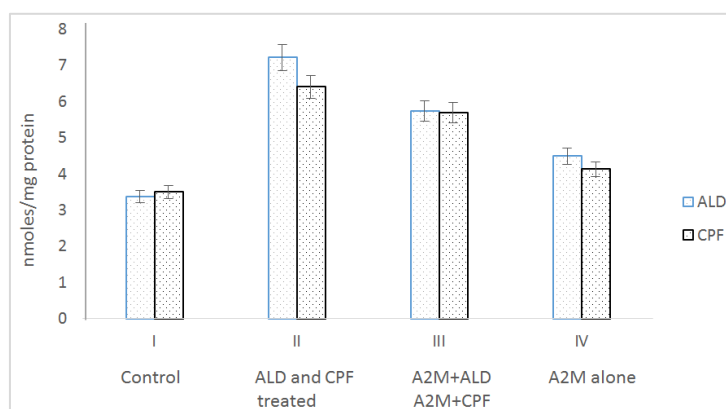


Figure 5. Results are expressed as mean + SD for different sets of experiments (3 set/ enzyme/group for ALD treated and A2M induced cells, 3 set/enzyme/group for CPF treated and A2M induced cells).

Table 3. Effect of pesticides ALD and CPF and treatment with A2M on protein carbonyls in control and experimental group of SH-SY5Y cells. Group I represents control, Group II represents ALD treated cells and CPF treated cells, Group III represents treatment of pesticides induced cells with A2M and group IV represents A2M treated cells alone.

Groups	ALD	CPF
I	3.36±0.15	3.49±0.16
II	7.21 ±0.55	6.40±0.66
III	5.73±0.27	5.69±0.35
IV	4.49±0.22	4.12±0.08

3.5. Analysis and Estimation of ALD, CPF and A2M on the Activities of Mitochondrial Tca Cycle Enzymes in Control and Experimental Group of SH-SY5Y Cells

The effects of pesticides ALD and CPF treated control and experimental groups of SH-SY5Y cells and their further treatment with A2M on mitochondrial TCA cycle enzymes and complex I and III are shown in Table 4. The results indicated that the activities of mitochondrial enzymes were significantly ($P<0.05$) decreased in pesticides induced cells

(group II), compared with the control cells (group I). However, treatment with A2M in combination with the pesticides alleviated their negative effects and significantly increased ($P<0.05$) the activities of the mitochondrial enzymes, especially Isocitrate dehydrogenase and α -Ketoglutarate, as compared to other enzymes (group III). A2M alone treated cells did not show any significant changes in the mitochondrial enzyme activities (group IV). However, the values obtained in group IV are comparatively higher than group III, suggests the protein's maintenance role for mitochondrial enzymes and their activities.

Table 4. Effect of pesticides ALD and CPF and treatment with A2M on mitochondrial TCA cycle enzymes in control and experimental group of SH-SY5Y cells. Group I represent control, Group II represents ALD treated cells and CPF treated cells, Group III represents treatment of pesticides induced cells with A2M and group IV represents A2M treated cells alone.

Enzymes	Pesticides	I	II	III	IV
Isocitrate dehydrogenase (ICDH)	ALD	33.63±3.92	24.93±1.86	30.43±3.54	33.39±3.34
	CPF	31.30±3.80	28.91±1.74	30.55±3.21	31.20±3.09
α -Ketoglutarate dehydrogenase (α -KGDH)	ALD	2.45±0.24	1.34±0.21	1.51±0.04	2.34±0.41
	CPF	2.10±0.19	1.05±0.15	1.19±0.05	1.91±0.36

Enzymes	Pesticides	I	II	III	IV
Succinate dehydrogenase (SDH)	ALD	5.63±0.53	4.16±0.14	3.73±0.22	5.55±0.27
	CPF	4.91±0.47	3.19±0.08	3.59±0.16	4.72±0.21
Malate dehydrogenase (MDH)	ALD	4.31±0.12	3.23±0.13	3.78±0.92	4.37±0.41
	CPF	3.36±0.09	2.55±0.17	2.87±0.81	3.20±0.15

Units: ICDH- nmoles of ketoglutarate formed/hr/mg protein; α - KGDH - nmoles of ferrocyanide formed/hr/mg protein; SDH - nmoles of succinate oxidized/min/mg protein; MDH -nmoles of NADH oxidized/min/mg protein.

Values are expressed as mean + SD for different sets of experiments (3 set/ enzyme/group for ALD treated and A2M induced cells, 3 set/enzyme/group for CPF treated and A2M induced cells) with ($P<0.05$) as statistical significance.

3.6. Analysis of Mitochondrial Dysfunction in Control and Experimental Group of SH-SY5Y Cells

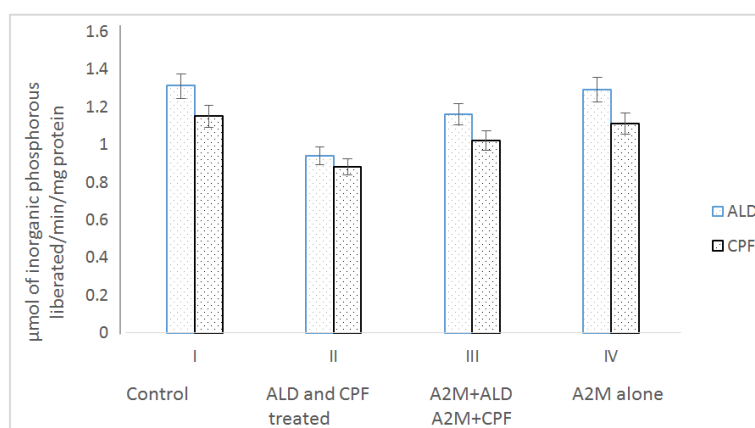


Figure 6. Results are expressed as mean + SD for different sets of experiments (3 set/ enzyme/group for ALD treated and A2M induced cells, 3 set/enzyme/group for CPF treated and A2M induced cells).

Figure 6 represent the activity of Na^+ / K^+ ATPase pump in response to the ALD, CPF and A2M treated group in control and experimental group of SH-SY5Y cells. Na^+ / K^+ ATPase activity was significantly ($P<0.05$) decreased in ALD and CPF induced rats (group II) suggesting the impairment of ATPase activity could be one of the underlying biochemical mechanism that leading to early cognitive CNS dysfunctions. However, administration of A2M with ALD and CPF treated cells, was able to prevent the inhibition of this en-

zyme pump (group III), thereby indicating that A2M protect ATPase from pesticides induced depletion of its activity. A2M alone treated cells did not cause any significant alteration on the ATPase activity (group IV) compared to control group of SH-SY5Y cells. The effect of pesticides ALD and CPF treated SH-SY cells for complex I (NADH dehydrogenase) and complex III (Cytochrome-c-oxidase) and the effect of A2M, are given in Table 5.

Table 5. Effect of pesticides ALD and CPF and treatment with A2M on mitochondrial dysfunction of complex I NADH Dehydrogenase and complex III cytochrome-c-oxidase in control and experimental group of SH-SY5Y cells. Group I represents control, Group II represents ALD treated cells and CPF treated cells, Group III represents treatment of pesticides induced cells with A2M and group IV represents A2M treated cells alone.

Enzymes	Pesticides	I	II	III	IV
NADH Dehydrogenase (NADH-DH)	ALD	16.51±2.53	14.33±1.61	15.77±2.39	16.49±2.92
	CPF	14.48±2.12	12.39±1.55	14.16±1.93	14.25±2.51
Cytochrome c-oxidase	ALD	8.66±0.36	5.43±0.44	6.36±0.25	8.42±0.95

Enzymes	Pesticides	I	II	III	IV
	CPF	7.91±0.21	4.55±0.22	5.34±0.33	7.51±0.51

Units in which the values are expressed are as follows:

NADH-DH - nmoles of NADH oxidized/min/mg protein.

Cytochrome-c-oxidase - nmoles of cytochrome/min/mg protein.

Values are expressed as mean + SD for different sets of experiments (3 set/ enzyme/group for ALD treated and A2M induced cells, 3 set/enzyme/group for CPF treated and A2M induced cells) with ($P < 0.05$) as statistical significance.

3.7. Effect of A2M and ALD/CPF on DNA Fragmentation of SH-SY5Y Cells

DNA pattern analysis of SH-SY5Y cells treated with pesticides ALD and CPF (lane 2) and exposed to A2M (lane 3) later is shown in Figure 7, along with the control group of cells (group I, lane 1) and A2M induced cells alone (lane 4). This agarose gel electrophoresis was performed to assess the DNA damage in neuronal cells after being introduced by the pesticides and to investigate the role of A2M. Pesticides induced cells (group II, lane 2) gave smear like pattern suggesting DNA contamination or DNA degradation (highlighted in lane 2). However, when the group II was treated with A2M, the band in lane 3 (group III) showed prevented DNA smear and fragmentation as compared to lane 2. Lane 1 and Lane 4 (group IV) showed no fragmentation.

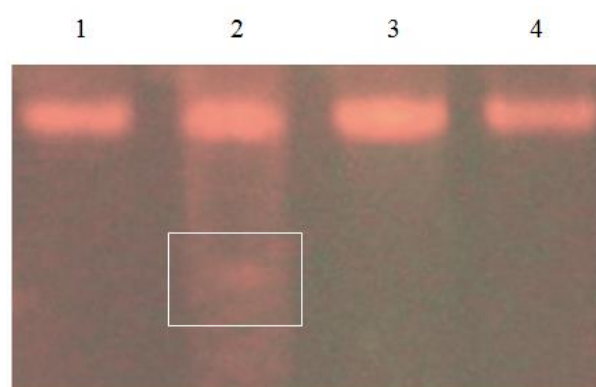


Figure 7. DNA agarose gel electrophoresis results of control and experimental groups of SH-SY5Y cells. Lane 1- SH-SY5Y cells, Lane 2- SH-SY5Y cells + ALD/CPF, Lane 3- SH-SY5Y cells+ ALD/CPF + A2M, Lane 4- SH-SY5Y cells+ A2M.

3.8. Effect of Pesticides and A2M on Expression of Inflammatory Marker Proteins in Control and Experimental Group of SH-SY5Y Cells

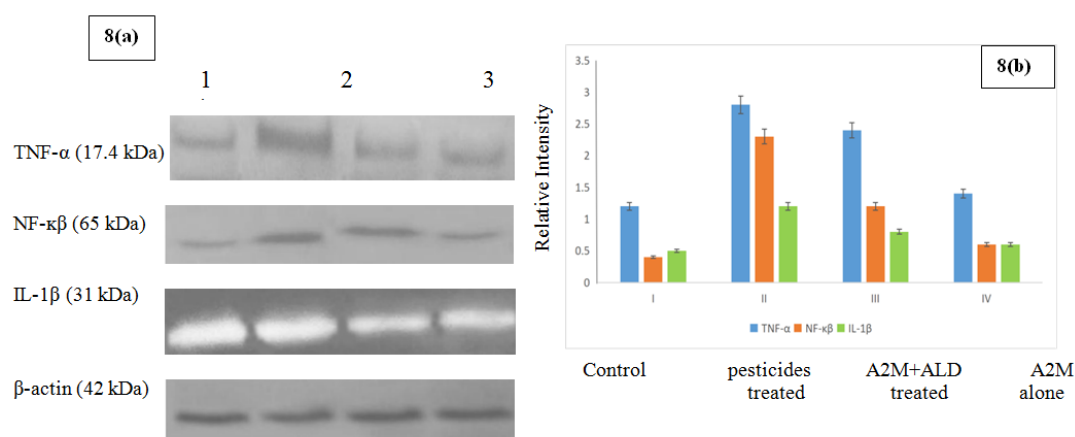


Figure 8. 8(a): Western blot expression of inflammatory marker proteins in control and experimental group of SH-SY5Y cells. Lane 1- SH-SY5Y cells, Lane 2- SH-SY5Y cells + ALD/CPF, Lane 3- SH-SY5Y cells+ ALD/CPF + A2M, Lane 4- SH-SY5Y cells+ A2M. 8(b): Relative intensity of the proteins expressed in control and experimental groups of SH-SY5Y cells.

A2M binds with cytokines such as TNF- α , NF- κ B and IL-1 β . These cytokines promote neurotoxicity and neuroin-

flammation by silencing cell survival mechanisms, promoting Fas signals, enhancing glutamate levels from microglia,

increasing Ca^{2+} permeability in NMDA receptors etc. [37]. A2M traps and neutralize these cytokines and decrease the inflammation in various cells. In Figure 8a, western blot expressions of these inflammatory markers are shown in control and experimental groups of SH-SY5Y cells.

Pesticides induced neuronal SH-SY5Y cells (group I) showed increased expression of these markers (lane 2) as compared to control cells (group II) (lane 1). Lane 3 shows SH-SY5Y cells induced with pesticides and treated with A2M (group III) showed decreased expression of these inflammatory markers as compared their Lane 2 expressions. A2M treated cells (lane 4) didn't show any significant expression of these inflammatory markers. Figure 8b represents the data expressing the respective protein levels which were quantified using the image analysis software and results are expressed in relative intensity. The values represent relative

levels of protein expression.

3.9. Effect of Pesticides and A2M on Bax, Bcl-2, Caspase-3, Caspase-9; Apoptotic Protein Expressions in Control and Experimental Group of SH-SY5Y Cells

The mitochondria-dependent pathway is one of the most important pathways of apoptosis, which is regulated by the B cell lymphoma-2 (e.g., Bcl-2) family proteins, including pro-apoptotic proteins (Bax) and anti-apoptotic proteins (e.g., Bcl-2). Caspase-3 is a proteolytic enzyme that regulate other caspases (e.g., Caspase-3), cause DNA damage and alter cell's morphology to induce apoptosis during cancer progression and neuronal degeneration [38].

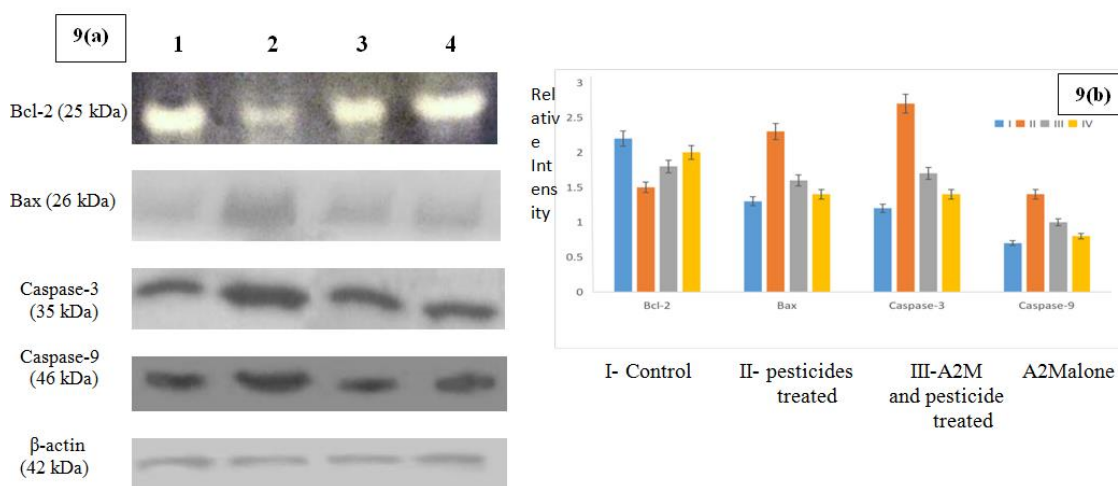


Figure 9. 9(a): Western blot expression of apoptotic marker proteins in control and experimental group of SH-SY5Y cells. Lane 1- SH-SY5Y cells, Lane 2- SH-SY5Y cells + ALD/CPF, Lane 3- SH-SY5Y cells+ ALD/CPF + A2M, Lane 4- SH-SY5Y cells+ A2M. 9(b): Relative intensity of the proteins expressed in control and experimental groups of SH-SY5Y cells.

There is a small amount of knowledge regarding the expression patterns and role of A2M in apoptosis. So, in our study, results in Figure 9 suggests the positive role of A2M in regulating the apoptotic marker proteins. In Figure 9a, western blot expressions of these apoptotic markers proteins (Bax, Bcl-2, Caspase-3, Caspase-9) are shown in control and experimental groups of SH-SY5Y cells. Pesticides induced neuronal SH-SY5Y cells (group II) showed increased expression of these markers (lane 2) as compared to control cells (lane 1), but in Bcl-2 expression, pesticides were shown to reduce its expression (*positive loading control β -actin was used but not shown*).

Pesticides were shown to increase expression of Bax, Caspase-3 and Caspase-9, but decreased expression of Bcl-2 (Lane 2). Lane 3 shows SH-SY5Y cells induced with pesticides and treated with A2M (group III) showed slightly decreased expression of Bax, Caspase-3 and Caspase-9, but

slightly increased expression of Bcl-2 as compared their Lane 2 expressions, while in Bcl-2 expression profile, A2M was found to restore Bcl-2 expression (Lane 4) and not much significant effect was observed upon the activity of Bax, Caspase-3 and Caspase-9. Figure 9b represents the data expressing the respective protein levels which were quantified using the image analysis software and results are expressed in relative intensity. The values represent relative levels of protein expression.

3.10. Effect of Pesticides and A2M on Nrf2 Expression in Control and Experimental Group of SH-SY5Y Cells

Nrf2 is a transcription factor that regulates cellular redox balance and inflammation. It coordinates cellular defense processes against the pathological hallmarks of neurodegen-

eration, such as oxidative stress, neuroinflammation, mitochondrial dysfunction, and protein aggregation. Oxidative stress is associated with neuronal cell death during the pathogenesis of neurodegenerative diseases. There is a limited yet ongoing research shows that A2M helps in regulating Nrf2/KEAP1 pathway in necrosis [39]. So we decided to investigate A2M's function in Nrf2 pathway during pesticides induced neurotoxicity in neuronal cells.

Western blot analyses of Nrf2 in cytosolic and nucleus fraction of the control and experimental group of SH-SY5Y cells is shown in Figure 10. Pesticides induced group II showed slight reduction in expression of nuclear and cytosolic Nrf2 (lane 2) when compared to the control (lane 1). However, when group II was treated with A2M (group III), it resulted in significant increased expression of Nrf2 in its cytosolic and nuclear fractions as compared to the observations in group II, suggesting its role in balancing and regulating Nrf2 activity during pesticides induced neurotoxicity in neuronal SH-SY5Y cells. In group IV, A2M treatment alone didn't have any noticeable observation in Nrf2 expression pattern (lane 4). β -actin was used as positive loading control.

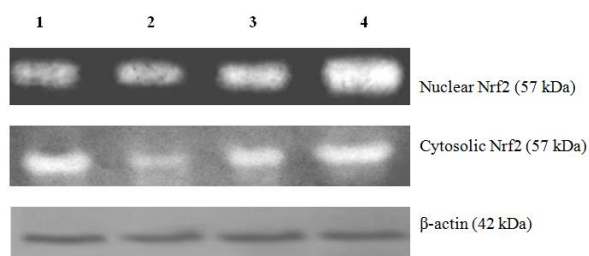


Figure 10. Western blot expression of Nrf2 nuclear and Nrf2 cytosolic fraction in control and experimental group of SH-SY5Y cells.

4. Discussion

Pesticides such as organophosphates and carbamates have found to provoke multiple deleterious and harmful effects in human body but most of them operate via the production of oxidative stress damage [19]. They accumulate in cells and over time, they start producing ROS and damage biomolecules such as lipids, proteins and DNA, thereby interfering with the biochemical processes ultimately leading to inflammation induced apoptotic cell death in living beings [20]. Long term exposure to pesticides can cause inflammation in neuronal cells and the brain, which can lead to neurodegeneration. They activate glial cells, astrocytes and microglia, which produce pro-inflammatory factors such as the tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), interleukin-1 β (IL-10), nitric oxide (NO), and cyclooxygenase-2 (COX-2) that further damage neurons. This leads to neuroinflammation which leads to development of neurodegenerative diseases like Alzheimer's and Parkinson's [40].

Various studies have shown the role of neuroinflammatory

markers in the occurrence, diagnosis, and treatment of neurodegenerative diseases [41]. A2M is a serum protein in the human circulatory system that act as guardian during inflammatory cellular injury by trapping and functionally inhibiting proteases. It also binds several cytokines, including interleukin (IL)-6, platelet-derived growth factor (PDGF), nerve-growth factor, tumor-necrosis factor (TNF)- α , and IL-1 β , making it a crucial component during the interactions between several cytokines and the process of inflammation, by decreasing these inflammatory mediators.

The present study highlights the impact of pesticide exposure on systemic oxidative stress, inflammation, and protease-antiprotease balance, with particular focus on the role of α 2-Macroglobulin (A2M). Pesticide-treated cell lines demonstrated elevated lipid peroxidation, diminished antioxidant enzyme activities, and increased pro-inflammatory cytokine expression, all of which were accompanied by histological evidence of cellular injury. These findings indicate that pesticides disrupt homeostatic defense mechanisms, overwhelming endogenous antioxidants and leading to persistent tissue damage. The decline in A2M activity observed under pesticide stress further suggests impairment of the protease-trapping function, which may facilitate unchecked proteolytic activity and exacerbate inflammatory responses. Such alterations are consistent with earlier reports linking pesticide toxicity to redox imbalance and protease dysregulation, thereby establishing A2M as a sensitive biomarker for pesticide-induced pathophysiology.

The comparative analysis across all four groups reveals distinct patterns in antioxidant defense, mitochondrial function, cholinergic activity, and cellular integrity. In the control group (I), physiological homeostasis was evident with robust Nrf2 activity, optimal mitochondrial enzyme function, normal acetylcholinesterase (AChE) activity, and intact cellular morphology. In contrast, pesticide exposure (Group II) markedly suppressed Nrf2 signaling, leading to impaired antioxidant responses and heightened oxidative stress. This suppression coincided with significant mitochondrial dysfunction, characterized by reduced activity of key oxidative phosphorylation enzymes, alongside pronounced inhibition of AChE, a recognized marker of pesticide-induced neurotoxicity. Histological analysis further confirmed severe cellular injury in this group, including necrosis, infiltration, and structural disruption, demonstrating the systemic toxicity induced by pesticide challenge.

The introduction of A2M in pesticide-exposed animals (Group III) produced a clear protective effect. Nrf2 activity was significantly restored compared to Group II, suggesting that A2M can re-activate endogenous cytoprotective pathways, though not to the levels observed in control or A2M-alone animals. Likewise, mitochondrial enzyme function improved relative to pesticide-only exposure, reflecting partial restoration of bioenergetic capacity, while AChE activity was less inhibited, indicating mitigation of neurotoxicity. Histologically, Group III tissues exhibited reduced necrosis

and cellular infiltration, though residual signs of damage persisted. Animals treated with A2M alone (Group IV) showed optimal outcomes, with enhanced Nrf2 activity, preserved mitochondrial enzyme function, normal AChE activity, and intact tissue morphology, closely resembling control conditions but with evidence of A2M's baseline protective potential. Collectively, these findings establish that pesticide exposure severely impairs antioxidant, mitochondrial, and cholinergic systems, while A2M supplementation confers substantial but incomplete protection under toxic stress. The comparative outcomes underscore A2M's dual role as a therapeutic mediator under pesticide challenge and a physiological stabilizer under normal conditions.

Notably, the administration of exogenous A2M demonstrated a protective role against pesticide toxicity, as reflected in the comparative outcomes of Group III (pesticides + A2M) versus Group IV (A2M alone). A2M supplementation significantly attenuated oxidative stress, restored antioxidant defense to near-normal levels, reduced inflammatory mediator expression, and ameliorated histopathological alterations, although complete normalization was not achieved compared to the A2M-only group. These results confirm the multifunctional protective role of A2M, acting as a protease inhibitor, cytokine carrier, and regulator of immune responses, which collectively contribute to its ability to mitigate toxic insults. However, the persistence of mild oxidative and inflammatory stress in the pesticide + A2M group underscores that while A2M confers substantial protection, it may require either higher doses, combinatorial therapy with antioxidants, or adjunctive interventions to fully restore systemic homeostasis. Taken together, the study demonstrates that A2M is not only a target of pesticide toxicity but also a promising therapeutic candidate to counteract its adverse effects.

5. Conclusion

This study elucidates the mechanisms underlying pesticide (ALD and CPF)-induced neuronal toxicity, emphasizing their role in triggering mitochondrial-mediated apoptosis and subsequent inflammatory apoptotic cell death in SH-SY5Y cells. Based on our previous study, we continued the investigation of A2M in mitochondrial enzymes and its relationship with inflammatory and apoptotic marker proteins, in response to pesticide-mediated neuronal injury. The investigation highlights the pivotal role of Alpha-2-Macroglobulin (A2M) in counteracting these adverse effects. A2M effectively mitigates oxidative stress in mitochondria, enhances cellular antioxidant defense systems, facilitates DNA repair, and suppresses neuronal inflammation and apoptosis. A schematic conclusion of the study is given below in the Figure 11.

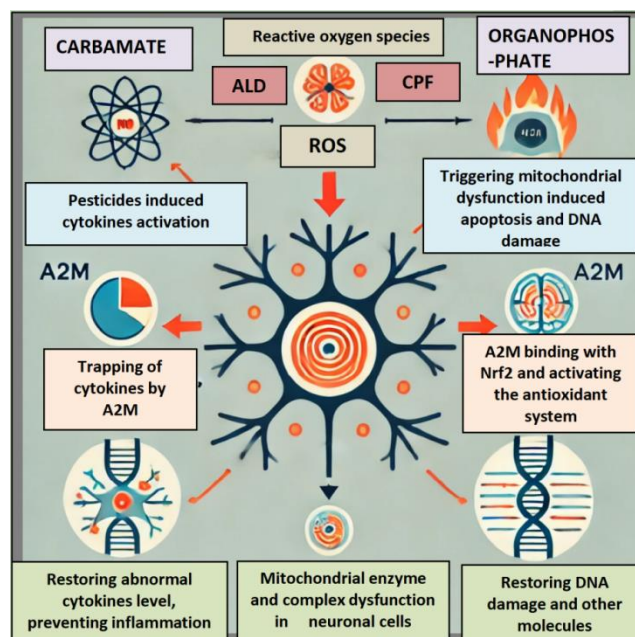


Figure 11. Graphical representation of highlighting A2M's role in mitigating oxidative stress in mitochondria by enhances cellular antioxidant defense systems, facilitating DNA repair and suppressing of neuronal inflammation and apoptosis.

Building on these findings, future studies should extend the protective role of alpha-2-macroglobulin (A2M) beyond *in vitro* models to *in vivo* systems of pesticide-induced neurotoxicity, enabling validation of its neuroprotective potential in the context of cognitive and behavioral outcomes. Critical next steps include determining whether A2M can cross the blood-brain barrier or exert its effects through systemic immune modulation, and assessing its efficacy against a broader spectrum of pesticides relevant to environmental exposure. Mechanistically, further investigation is needed to define how A2M interfaces with additional stress pathways, such as ER stress, autophagy, and protein aggregation, which are central to Alzheimer's and Parkinson's disease pathology. Translational approaches could also focus on developing A2M-derived mimetics or small molecules with improved stability and brain penetration.

Finally, evaluating plasma A2M levels as potential biomarkers in pesticide-exposed human populations could provide early diagnostic insight and establish its relevance as a therapeutic target for neurodegenerative disease prevention. This protective action preserves neuronal integrity and functionality, reducing the likelihood of pesticide-induced neurodegeneration. Importantly, this research provides the first evidence of A2M's interaction with apoptotic markers and mitochondrial enzyme complexes, offering novel insights into its therapeutic potential for combating pesticide-mediated neuronal injury during preclinical stage.

Abbreviations

DNPH	2,4-dinitrophenylhydrazine
DTNB	5,5-dithiobis (2-nitrobenzoic acid)
AChE	Acetylcholinesterase
ATP	Adenosine Triphosphate
ALD	Aldicarb
A2M	Alpha-2-macroglobulin
AD	Alzheimer's Disease
BSA	Bovine Serum Albumin
CSF	Cerebrospinal Fluid
CPF	Chlorpyrifos
DLM	Deltamethrin
DNA	Deoxyribonucleic Acid
DMEM	Dulbecco's Modified Eagle Medium
FBS	Fetal Bovine Serum
iNOS	Inducible Nitric Oxide Synthase
ICDH	Isocitrate Dehydrogenase
LDH	Lactate Dehydrogenase
MDH	Malate Dehydrogenase
MDA	Malondialdehyde
MS	Multiple Sclerosis
NAD	Nicotinamide Adenine Dinucleotide
NADH	Nicotinamide Adenine Dinucleotide (NAD) + Hydrogen (H)
NADPH	Nicotinamide Adenine Dinucleotide Phosphate (Hydrogen)
Nrf2	Nuclear Factor Erythroid 2-Related Factor 2
PD	Parkinson's Disease
PAGE	Polyacrylamide gel Electrophoresis
PC	Protein Carbonyls
ROS	Reactive Oxygen Species
SDS	Sodium Dodecyl Sulphate
TBARS	Thiobarbituric Acid Reactive Substances
TCA	Tricarboxylic Acid
TAE	Tris, Agarose, e
TGF- β	Tumor Growth Factor- β
TNF- α	Tumor Necrosis Factor- α

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Author Contributions

Swati Dixit is the sole author. The author read and approved the final manuscript.

Data Availability Statement

The data supporting the findings of this study are available within the article and its supporting materials.

Conflicts of Interest

The author declares no conflict of interest.

References

- [1] Chin-Chan M, Navarro-Yepes J, Quintanilla-Vega B. Environmental pollutants as risk factors for neurodegenerative disorders: Alzheimer and Parkinson diseases. *Front Cell Neurosci.* 2015; 9: 124. <https://doi.org/10.3389/fncel.2015.00124>
- [2] Yu G, Su Q, Chen Y, Wu L, Wu S, Li H. Epigenetics in neurodegenerative disorders induced by pesticides. *Genes Environ.* 2021b; 43(1): 55. <https://doi.org/10.1186/s41021-021-00224-z>
- [3] Vellingiri B, Chandrasekhar M, Sabari SS, Gopalakrishnan AV, Narayanasamy A, Venkatesan D, Iyer M, Kesari K, Dey A. Neurotoxicity of pesticides - A link to neurodegeneration. *Ecotoxicol Environ Saf.* 2022 (243), 113972. <https://doi.org/10.1016/j.ecoenv.2022.113972>
- [4] Yu G, Guo Z, Li H. Analyses of epigenetic modification in environmental pollutants-induced neurotoxicity. *Methods Mol Biol.* 2021a; 2326: 123-141. https://doi.org/10.1007/978-1-0716-1514-0_9
- [5] Reddam A, McLarnan S, Kupsco A. Environmental Chemical Exposures and Mitochondrial Dysfunction: A Review of Recent Literature. *Curr Environ Health Rep.* 2022; 9(4): 631-649. <https://doi.org/10.1007/s40572-022-00371-7>
- [6] Varma VR, Varma S, An Y, Hohman TJ, Seddighi S, Casanova R, Beri A, Dammer EB, Seyfried NT, Pletnikova O, Moghekar A, Wilson MR, Lah JJ, O'Brien RJ, Levey AI, Troncoso JC, Albert MS, Thambisetty M. Alpha-2 macroglobulin in Alzheimer's disease: a marker of neuronal injury through the RCAN1 pathway. *Mol Psychiatry.* 2017; 22(1): 13-23. <https://doi.org/10.1038/mp.2016.206>
- [7] Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, et al. Neuroinflammation in Alzheimer's disease. *Lancet Neurol.* 2015; 14: 388-405. <https://doi.org/10.1038/s41577-024-01104-7>
- [8] Lagrange J, Lecompte T, Knopp T, Lacolley P, Regnault V. Alpha-2-macroglobulin in hemostasis and thrombosis: An underestimated old double-edged sword. *J Thromb Haemost.* 2022; 20(4): 806-815. <https://doi.org/10.1111/jth.15647>
- [9] Chen Z, Balachandran YL, Chong WP, Chan KKY. Roles of Cytokines in Alzheimer's Disease. *Int J Mol Sci.* 2024; 25(11): 5803. <https://doi.org/10.3390/ijms25115803>
- [10] Li G, Li D, Rao H, Liu X. Potential Neurotoxicity, Immunotoxicity, and Carcinogenicity Induced by Metribuzin and Tebuconazole Exposure in Earthworms (*Eisenia fetida*) Revealed by Transcriptome Analysis. *Sci. Total Environ.* 2022; 807: 150760. <https://doi.org/10.1016/j.scitotenv.2021.150760>
- [11] Cestonaro LV, Macedo SMD, Piton YV, Garcia SC, Arbo MD. Toxic effects of pesticides on cellular and humoral immunity: an overview. *Immunopharmacol Immunotoxicol.* 2022; 44(6): 816-831. <https://doi.org/10.1080/08923973.2022.2096466>

- [12] Lee GH, Choi KC. Adverse effects of pesticides on the functions of immune system. *Comp Biochem Physiol C Toxicol Pharmacol*. 2020; 235: 108789. <https://doi.org/10.1016/j.cbpc.2020.108789>
- [13] Alimova AA, Sitnikov VV, Pogorelov DI, Boyko ON, Vitkalova IY, Gureev AP, Popov VN. High Doses of Pesticides Induce mtDNA Damage in Intact Mitochondria of Potato In Vitro and Do Not Impact on mtDNA Integrity of Mitochondria of Shoots and Tubers under In Vivo Exposure. *Int J Mol Sci*. 2022; 23(6): 2970. <https://doi.org/10.3390/ijms23062970>
- [14] Mokarizadeh A, Faryabi MR, Rezvanfar MA, Abdollahi M. A comprehensive review of pesticides and the immune dysregulation: mechanisms, evidence and consequences. *Toxicol Mech Methods*. 2015; 25(4), 258-278. <https://doi.org/10.3109/15376516.2015.1020182>
- [15] Costa C, Briguglio G, Catanoso R, Giambò F, Polito I, Teodoro M, Fenga C. New perspectives on cytokine pathways modulation by pesticide exposure. *Curr Opin Toxicol*. 2020; 19: 99-104. <https://doi.org/10.1016/j.cotox.2020.01.002>
- [16] Dixit S, Ahsan H, Khan FH. Interaction of Human Alpha-2-Macroglobulin with Pesticide Aldicarb Using Spectroscopy and Molecular Docking. *Protein Pept Lett*. 2021a; 28(3): 315-322. <https://doi.org/10.2174/0929866527666200921165834>
- [17] Dixit S, Ahsan H, Khan FH. Interaction of organophosphate pesticide chlorpyrifos with alpha-2-macroglobulin: Biophysical and molecular docking approach. *J Immunoassay Immunochem*. 2021b; 42(2): 138-153. <https://doi.org/10.1080/15321819.2020.1837161>
- [18] McCaulley ME, Grush KA. Alzheimer's disease: exploring the role of inflammation and implications for treatment. *Int J Alzheimers Dis*. 2015; 2015: 515248. <https://doi.org/10.1155/2015/515248>
- [19] Dixit S, Ahsan H, Khan FH. Cytoprotective effect of alpha-2-macroglobulin against chlorpyrifos aldicarb and deltamethrin induced generation of ROS in neuronal SH-SY5Y cells. *Bio-med Res Ther*. 2023; 10(2): 5537-5544. <https://doi.org/10.15419/bmrar.v10i2.792>
- [20] Dixit S, Ahsan H, Khan FH. Pesticides and plasma proteins: unexplored dimensions in neurotoxicity. *Int J Pest Manag*. 2022b; 69(3): 278-287. <https://doi.org/10.1080/09670874.2021.1917725>
- [21] Dixit S, Ahsan H, Khan FH. Interaction of synthetic pyrethroid deltamethrin with human alpha 2 macroglobulin: spectroscopic and molecular docking studies. *Protein Pept Lett*. 2022a; 29(4): 284-292. <https://doi.org/10.2174/0929866529666220203095706>
- [22] Kaja S, Payne AJ, Singh T, Ghuman JK, Sieck EG, Koulen P. An optimized lactate dehydrogenase release assay for screening of drug candidates in neuroscience. *J Pharmacol Toxicol Methods*. 2015; 73: 1-6. <https://doi.org/10.1016/j.vascn.2015.02.001>
- [23] Kumar P, Nagarajan A, Uchil PD. Analysis of Cell Viability by the Lactate Dehydrogenase Assay. *Cold Spring Harb Protoc*. 2018; 2018(6). <https://doi.org/10.1101/pdb.prot095497>
- [24] Bernal-Rey DL, Cantera CG, Dos Santos Afonso M, Menéndez-Helman RJ. Seasonal variations in the dose-response relationship of acetylcholinesterase activity in freshwater fish exposed to chlorpyrifos and glyphosate. *Ecotoxicol Environ Saf*. 2020; 187: 109673. <https://doi.org/10.1016/j.ecoenv.2019.109673>
- [25] Sun W, Chen L, Zheng W, Wei X, Wu W, Duysen EG, Jiang W. Study of acetylcholinesterase activity and apoptosis in SH-SY5Y cells and mice exposed to ethanol. *Toxicol*. 2017; 384: 33-39. <https://doi.org/10.1016/j.tox.2017.04.007>
- [26] Colombo G, Clerici M, Garavaglia ME, Giustarini D, Rossi R, Milzani A, Dalle-Donne I. A step-by-step protocol for assaying protein carbonylation in biological samples. *J Chromatogr B*. 2016; 1019: 178-190. <https://doi.org/10.1016/j.jchromb.2015.11.052>
- [27] Vujic A, Koo ANM, Prag HA, Krieg T. Mitochondrial redox and TCA cycle metabolite signaling in the heart. *Free Radic Biol Med*. 2021; 166: 287-296. <https://doi.org/10.1016/j.freeradbiomed.2021.02.041>
- [28] Gavel R, Mishra SP, Khanna S, Khanna R, Shah AG. Analysis of Isocitrate Dehydrogenase -2 (IDH-2) Activity in Human Serum as a Biomarker in Chemotherapy Patients of Breast Carcinoma: A Case-Control Study. *J Clin Diagn Res*. 2017; 11(5): BC05-BC08. <https://doi.org/10.7860/JCDR/2017/21886.9842>
- [29] Gibson GE, Xu H, Chen HL, Chen W, Denton TT, Zhang S. Alpha-ketoglutarate dehydrogenase complex-dependent succinylation of proteins in neurons and neuronal cell lines. *J Neurochem*. 2015; 134(1): 86-96. <https://doi.org/10.1111/jnc.13096>
- [30] Jardim-Messeder D, Caverzan A, Rauber R, de Souza Ferreira E, Margis-Pinheiro M, Galina A. Succinate dehydrogenase (mitochondrial complex II) is a source of reactive oxygen species in plants and regulates development and stress responses. *New Phytol*. 2015 Nov; 208(3): 776-89. <https://doi.org/10.1111/nph.13515>
- [31] Mansouri S, Shahriari A, Kalantar H, Moini Zanjani T, Haghi Karamallah M. Role of malate dehydrogenase in facilitating lactate dehydrogenase to support the glycolysis pathway in tumors. *Biomed Rep*. 2017; 6(4): 463-467. <https://doi.org/10.3892/br.2017.873>
- [32] Zhang X, Lee W, Bian JS. Recent Advances in the Study of Na⁺/K⁺-ATPase in Neurodegenerative Diseases. *Cells*. 2022; 11: 40-75. <https://doi.org/10.3390/cells11244075>
- [33] Rule CS, Patrick M, Sandkvist M. Measuring In Vitro ATPase Activity for Enzymatic Characterization. *J Vis Exp*. 2016; 23(114): 54305. <https://doi.org/10.3791/54305>
- [34] Wang J, Yang C, Chen X, Bao B, Zhang X, Li D, Du X, Shi R, Yang J, Zhu R. A high effective NADH-ferricyanide dehydrogenase coupled with laccase for NAD⁺ regeneration. *Bio-technol Lett*. 2016; 38(8): 1315-1320. <https://doi.org/10.1007/s10529-016-2106-3>

- [35] Simard ML, Mourier A, Greaves LC, Taylor RW, Stewart JB. A novel histochemistry assay to assess and quantify focal cytochrome c oxidase deficiency. *J Pathol.* 2018 Jul; 245(3): 311-323. <https://doi.org/10.1002/path.5084>
- [36] Kašuba V, Milić M, Rozgaj R, Kopjar N, Mladinić M, Žunec S, Vrdoljak AL, Pavičić I, Čermak AMM, Pizent A, Lovaković BT, Želježić D. Effects of low doses of glyphosate on DNA damage, cell proliferation and oxidative stress in the HepG2 cell line. *Environ Sci Pollut Res Int.* 2017; 24(23): 19267-19281. <https://doi.org/10.1007/s11356-017-9438-y>
- [37] Sun C, Cao C, Zhao T, Guo H, Fleming BC, Owens B, Beveridge J, McAllister S, Wei L. A2M inhibits inflammatory mediators of chondrocytes by blocking IL-1 β /NF- κ B pathway. *J Orthop Res.* 2023; 41(1): 241-248. <https://doi.org/10.1002/jor.25348>
- [38] Chota A, George BP, Abrahamse H. Interactions of multidomain pro-apoptotic and anti-apoptotic proteins in cancer cell death. *Oncotarget.* 2021; 12(16): 1615-1626. <https://doi.org/10.18632/oncotarget.28031>
- [39] Fang S, Wu S, Chen P. Alpha-2-macroglobulin mitigates glucocorticoid-induced osteonecrosis via Keap1/Nrf2 pathway activation. *Free Radic Biol Med.* 2024; 225: 501-516. <https://doi.org/10.1016/j.freeradbiomed.2024.09.048>
- [40] Hossain MM, Liu J, Richardson JR. Pyrethroid Insecticides Directly Activate Microglia Through Interaction With Voltage-Gated Sodium Channels. *Toxicol Sci.* 2017; 155(1): 112-123. <https://doi.org/10.1093/toxsci/kfw187>
- [41] Rauf A, Badoni H, Abu-Izneid T, Olatunde A, Rahman MM, Painuli S, Semwal P, Wilairatana P, Mubarak MS. Neuroinflammatory Markers: Key Indicators in the Pathology of Neurodegenerative Diseases. *Molecules.* 2022; 27(10): 3194. <https://doi.org/10.3390/molecules27103194>