

## **Synergistic Neuroprotective Effects of Atorvastatin and Rosemary Extract in Experimental Neurotoxicity in Rats: Integrated Toxicological, Anatomical, and Histopathological Evaluation**

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### **ABSTRACT**

#### **Background**

Aluminum chloride (AlCl<sub>3</sub>)-induced neurotoxicity is accompanied by exaggerated oxidative stress, neuroinflammation, mitochondrial damage, neuronal cell death, and incremental loss of memory and cognitive functions. Atorvastatin, in addition to its lipid-lowering action, shows a multitude of pleiotropic anti-inflammatory and antioxidant activities, and rosemary (*Rosmarinus officinalis*) is a rich source of naturally occurring neuroprotective compounds. Yet, very little is known about the impact of simultaneous use of atorvastatin and rosemary extract in AlCl<sub>3</sub>-induced neurotoxicity.

#### **Objective**

This work aims at assessing the efficacy of individual or combinatorial administration of atorvastatin and rosemary extract in ameliorating AlCl<sub>3</sub>-induced neurotoxicity using behavioral, biochemical, molecular, histopathological and anatomical analysis.

#### **Methods**

Seventy adult male Wistar rats were randomly divided into seven experimental groups. One group served as the control group and received distilled water only, whereas the remaining six groups were considered experimental groups. The six experimental groups were orally exposed to AlCl<sub>3</sub> to induce neurotoxicity and were subsequently treated orally with either atorvastatin or rosemary extract alone or with their combination for 6 weeks. The Morris water maze, Y-maze, Open Field, and Rotarod tests were used to assess learning, memory, motor activity, and motor coordination, respectively. Thereafter, oxidative stress markers, inflammatory cytokines, neurotransmitter levels, apoptotic protein expression, antioxidant pathways, and brain histopathological changes were evaluated. The obtained data were analyzed using one-way analysis

of variance (ANOVA), followed by an appropriate post hoc test for multiple comparisons

#### Results:

Aluminum chloride exposure resulted in significant loss of cognitive functions and motor performance along with marked rise in markers of oxidative stress, augmented inflammatory indices, declined endogenous antioxidant capacity, imbalanced levels of neurotransmitters and augmented apoptotic markers in brain tissue accompanied with severe damage of neural tissue ( $p < 0.001$ ).

Both atorvastatin and rosemary extract were found effective in alleviating AlCl<sub>3</sub>-induced oxidative stress, neuroinflammation, neurodegeneration, behavioral and motor deficits. In comparison with their individual effects, the combined treatment significantly protected against AlCl<sub>3</sub>-induced neurotoxicity more effectively and completely. Atorvastatin plus rosemary combination achieved best improvements in all behavioral, biochemical and molecular parameters and reduced the neuronal injury to near normal levels by promoting activation of antioxidant Nrf2/HO-1 signaling, inhibiting inflammatory NF- $\kappa$ B pathway, suppressing apoptotic signals and protecting integrity of neural cells.

#### Conclusion:

The combined treatment of atorvastatin and rosemary extract showed a superior neuroprotective potential against aluminum chloride induced neurotoxicity as compared to the treatment with either drug alone. This enhanced synergistic effect may stem from their combined beneficial actions on oxidative stress, inflammatory response, apoptosis and antioxidant mechanisms that contribute to protection from oxidative stress-induced neural injury.

**Keywords:** Neurotoxicity; Atorvastatin; Rosemary extract; Oxidative stress; Histopathology; Rats.

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## 1. INTRODUCTION

Neurodegenerative diseases are a leading cause of neurological disability and death worldwide, placing a significant burden on individuals and societies. These burdens range from substantial social to economic. Neurodegenerative diseases are characterized by the gradual loss and destruction of nerve cells, leading to a range of negative effects such as cognitive impairment and movement disorders, ultimately resulting in chronic neuroinflammation (**Hasan & Eid, 2020**). In addition, oxidative stress occurs, leading to mitochondrial dysfunction and programmed cell death. One of the most prominent environmental toxins that has emerged as a significant factor in nerve cell damage is aluminum chloride, which has recently garnered considerable attention due to its widespread use and potential toxic effects on the nervous system, Chronic exposure to AlCl<sub>3</sub> stimulates excessive production of various reactive oxygen species, lipid peroxidation, neuroinflammation, cholinergic dysfunction, and neurodegeneration (**Lim et al., 2024**).

Oxidative stress is the central point of Al-induced neurotoxicity where increased production of reactive oxygen species exceeds the endogenous antioxidant systems. It involves decreased levels of GSH and decreased activity of antioxidants like SOD, CAT, and GPx as well as enhanced levels of MDA and subsequently widespread neuronal cell membrane damage. Activation of inflammatory signaling pathways, especially NF- $\kappa$ B nuclear transcription factor leads to induction of inflammatory cytokines including  $\alpha$ -TNF, IL-1 $\beta$  and IL-6 contributing to enhanced neuronal injury (**YURISALDI et al, 2024**).

Numerous experimental studies have identified possible neuroprotective factors that can attenuate Al-induced brain damage. Statins (especially atorvastatin) were extensively researched and although having the property of lowering blood lipid levels, possess potent antioxidant, anti-inflammatory, anti-apoptotic and endothelial protective activities. Experimental evidences suggested atorvastatin is capable of inhibiting oxidative stress, inactivating nuclear transcription factor NF- $\kappa$ B, and suppressing production of inflammatory cytokines, thereby rescuing the neurons from neuronal cell death in various models of neurodegeneration (**Adel et al., 2022**).

Recent interest has focused on natural medicinal plants due to their neuroprotective properties. Rosemary contains a number of beneficial bio-compounds including Carnosic acid, Rosmarinus acid, carnosol and flavonoids that have strong antioxidant and anti-inflammatory activities. Experimental evidences proposed that Rosemary extract boosted up body's

antioxidant systems, reduced free radicals, inhibited lipid peroxidation, increased cholinergic neurotransmission and protect hippocampal neurons against oxidative damage (*Javadi et al., 2023*).

Recent experimental researches have confirmed that rosemary extract acts through multiple neuroprotective molecular pathways including activation of Nrf2/HO-1 antioxidant signaling pathway while inhibition of inflammatory mediators and pro-apoptotic proteins like Bax and caspase-3, therefore rosemary can be a therapeutic approach for oxidative stress-induced cognitive deficits (*Lewandowski et al., 2023*).

Likewise, atorvastatin is known to improve behavioral performance, prevent the AChE activity and restore BDNF and inhibits apoptosis in chemical induced neurotoxicity models. Its multiple actions demonstrate its neuroprotective benefits beyond the ability to lower cholesterol level (*El-Bolkiny et al., 2021*).

While, evidence indicating the neuroprotective abilities of both atorvastatin and rosemary extract were observed from different studies; very limited research comparing the efficacy of both to ameliorate Al chloride induced neurotoxicity with the help of extensive behavior, biochemistry, oxidative stress, inflammation and histopathology has been done. Similarly, only little is known about the effects on antioxidant defense systems, inflammatory mediators, apoptosis and preservation of brain structure after chronic aluminum administration (*Alavi et al., 2021*).

Hence, the aim of this study was to determine the effect of atorvastatin and rosemary extract on Al chloride induced neurotoxicity using an adult model through a Randomized controlled trial.

## 2. PATIENTS AND METHODS

### Study Design

This was a randomized controlled trial of experiments conducted on rats in the Animal Research Laboratory, Department of Pharmacology and Toxicology, Faculty of Medicine, from June 2023 to January 2024. The study included 70 healthy adults male Wistar rats, weighing between 220 and 250 grams and aged between 10 and 12 weeks, divided into 7 groups. The rats were obtained from the Laboratory Animal Research Unit. The conditioning period was designed according to clinical standards used in similar studies. For one week prior to the experiment, the rats were kept under standard laboratory conditions (temperature  $22 \pm 2^\circ\text{C}$ , relative humidity 50-60%, and a 12-hour light/dark cycle), with continuous access to food and water.

### Study Population and Experimental groups

Seventy healthy adults male Wistar rats were randomly assigned to control and six experimental groups, with ten rats in each group ( $n = 10$ ). Group I served as the control group and received distilled water orally throughout the experimental period. Group II received aluminum chloride ( $\text{AlCl}_3$ ) orally to induce experimental neurotoxicity. Group III received atorvastatin alone, while Group IV received rosemary extract alone. Group V received aluminum chloride followed by atorvastatin treatment, whereas Group VI received aluminum chloride followed by rosemary extract treatment. Finally, Group VII received aluminum chloride followed by a combined treatment of atorvastatin and rosemary extract to evaluate their potential synergistic neuroprotective effects against  $\text{AlCl}_3$ -induced neurotoxicity.

### Inclusion Criteria

Clinically healthy male Wistar rats weighing 220–250g, ranging between 10-12 weeks of age were included in the study. Rats were free from any congenital anomalies, infections and neurological disorders. Animals were accustomed to laboratory environments at standard laboratory conditions ( $22 \pm 2^\circ\text{C}$ ;  $55 \pm 5\%$  humidity; 12 h light/12 h dark cycle) for 1 week prior to experimental procedure.

### Exclusion Criteria

Rats showing clinical symptoms (illness), congenital abnormalities, behavioral abnormalities before any experimental treatment, rapid and severe weight loss during acclimatization period, death accidentally during the course of the experiment or incompleteness of experimental data will be excluded from the study (*Mullen et al., 2016*).

### Behavioral Assessment

Behavioral performance was evaluated at the end of six weeks of treatment using standard and validated neurobehavioral tests. Cognitive functions and spatial memory as well as locomotor activity and motor coordination was evaluated through Morris Water Maze, Y- Maze and Open Field and Rotarod tests.

### Biochemical Assessment

After performing behavioral tests blood and brain tissue was collected from each rat and subjected to various biochemical analyses. Pro-inflammatory cytokines, antioxidant enzymes activities, markers of oxidative stress and apoptosis and

neurochemical parameters was analyzed using the commercial ELISA kits and standard spectrophotometric assays at manufacturer's directions.

### Neurochemical markers

Levels of acetylcholinesterase (AChE), brain-derived neurotrophic factor (BDNF), dopamine, serotonin, and acetylcholine were measured in homogenized brain tissue using commercially available ELISA kits, according to the manufacturer's instructions. Absorption was measured using a BioTek ELx800™ microplate reader (BioTek Instruments Inc., Winnewse, Vermont, USA), while tissue homogenization was performed using a T25 ultra-fast digital homogenizer (IKA, Germany) under cold conditions to preserve protein integrity. Neuronal activity was then assessed by measuring these neurotransmitters based on the results obtained.

### Inflammatory Markers

Tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), interleukin-10 (ILβ-10), nuclear factor kappa B (NF-κB), and C-reactive protein (CRP) levels were analyzed using enzyme-linked immunosorbent assay (ELISA) to measure the levels of inflammatory cytokines and related markers in brain homogenates) (Khan et al, 2023).

### Histopathological Examination

Histopathological examination was performed by fixing brain tissue in 10% dilute neutral formalin solution, then embedding it in paraffin after standard processing, and cutting it into 4-5 μm sections. These slides were stained with hematoxylin and eosin (H&E), then examined under a light microscope to assess cavitation, glial fibrosis, and neuronal degeneration. Histological changes of damage and cavities were also recorded, as well as the total brain weight, number of neurons, and thickness of the cerebral cortex and hippocampus tissues.

### Ethical approval

All required approvals for the experiment were obtained from the Research Ethics Committee in the faculty of Medicine Al-Azhar university (Assiut) under approval number (MSC/AZ.AST./PHA006/10/215/3/2023).

### Statistical Analysis

The data were statistically analyzed using IBM SPSS Statistics version 29.0 (IBM Corp., Armonk, NY, USA). Values are expressed as mean ± standard deviation (SD). Normality of distribution was checked using the Shapiro–Wilk test, and variances of homogeneity was tested by Levene test. One way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test were applied for comparison among the 7 groups.

Pearson's correlation coefficient was used to estimate association between behavioral, biochemical, oxidative stress, inflammation, neurochemistry, and molecular markers, followed by multiple linear regression analysis, which was applied for the detection of independent predictors of the neuroprotective effect and assessment of individual contributions of various markers on behavioral outcome recovery. A two-sided P-value < 0.05 was considered significant

## 3. RESULTS

**Table 1: Behavioral findings.**

| Variable                               | Group I(control)<br>(N=10) | Group II<br>(N=10) | Group III<br>(N=10) | Group IV<br>(N=10) | Group V<br>(N=10) | Group VI<br>(N=10) | Group VII<br>(N=10) | P-value |
|----------------------------------------|----------------------------|--------------------|---------------------|--------------------|-------------------|--------------------|---------------------|---------|
| <b>Morris Water Maze Latency (sec)</b> |                            |                    |                     |                    |                   |                    |                     |         |
| <b>M±SD</b>                            | 23.22 ± 2.53               | 58.17 ± 3.24       | 22.25 ± 1.81        | 24.43 ± 2.07       | 37.53 ± 2.94      | 37.18 ± 2.83       | 20.94 ± 1.64        | <0.001  |
| <b>Min–Max</b>                         | 20.10–26.80                | 53.90–62.90        | 20.10–26.10         | 21.10–27.60        | 33.90–42.00       | 33.40–41.70        | 18.60–23.80         |         |
| <b>IQR</b>                             | 21.02–25.50                | 56.12–60.73        | 20.88–23.05         | 23.30–26.07        | 34.67–39.43       | 34.60–39.02        | 19.80–22.10         |         |
| <b>Y Maze Alternation (%)</b>          |                            |                    |                     |                    |                   |                    |                     |         |
| <b>M±SD</b>                            | 77.69 ± 1.70               | 47.85 ± 3.32       | 76.00 ± 2.65        | 77.83 ± 2.25       | 62.66 ± 3.48      | 63.39 ± 3.52       | 81.42 ± 2.06        | <0.001  |
| <b>Min–Max</b>                         | 75.10–80.50                | 43.10–52.70        | 73.40–80.80         | 73.30–80.90        | 58.00–67.80       | 58.60–67.50        | 78.20–84.50         |         |
| <b>IQR</b>                             | 76.70–78.78                | 45.28–50.20        | 73.75–76.95         | 76.67–79.38        | 60.15–65.28       | 60.15–66.02        | 79.90–82.85         |         |
| <b>Open Field Crossings (count)</b>    |                            |                    |                     |                    |                   |                    |                     |         |
| <b>M±SD</b>                            | 78.00 ± 3.06               | 41.70 ± 2.79       | 78.40 ± 3.66        | 78.20 ± 3.01       | 60.40 ± 3.06      | 62.30 ± 2.83       | 82.10 ± 2.75        | <0.001  |

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|                           |               |              |               |               |              |              |               |        |
|---------------------------|---------------|--------------|---------------|---------------|--------------|--------------|---------------|--------|
| <b>Min–Max</b>            | 73.00–82.00   | 38.00–45.00  | 73.00–83.00   | 73.00–83.00   | 57.00–65.00  | 56.00–65.00  | 78.00–86.00   |        |
| <b>IQR</b>                | 76.25–79.75   | 39.00–44.00  | 76.50–81.25   | 77.00–78.75   | 58.00–63.00  | 61.25–64.75  | 80.10–84.20   |        |
| <b>Rotarod Time (sec)</b> |               |              |               |               |              |              |               |        |
| <b>M±SD</b>               | 138.50 ± 4.70 | 62.95 ± 4.74 | 140.78 ± 3.99 | 140.93 ± 2.62 | 95.71 ± 5.24 | 97.51 ± 4.18 | 146.42 ± 3.28 | <0.001 |
| <b>Min–Max</b>            | 131.20–144.10 | 57.40–71.50  | 135.30–145.70 | 136.10–145.50 | 88.00–102.80 | 88.00–101.80 | 141.20–151.80 |        |
| <b>IQR</b>                | 134.62–142.30 | 58.98–65.45  | 137.12–144.20 | 139.38–142.30 | 91.25–100.17 | 95.42–100.50 | 144.20–148.60 |        |

## Notes

Data are presented as Mean ± Standard Deviation (SD), Median (Interquartile Range, IQR), and Minimum–Maximum (Min–Max) for each experimental group (n = 10 rats/group). Statistical comparisons among groups were performed using one-way analysis of variance (One-Way ANOVA) followed by Tukey's post hoc multiple comparison test. A p-value < 0.05 was considered statistically significant.

Group I (Control): Rats received distilled water orally throughout the experimental period.

Group II (AlCl<sub>3</sub>): Rats received aluminum chloride (AlCl<sub>3</sub>) orally to induce experimental neurotoxicity.

Group III (Atorvastatin): Rats received atorvastatin alone.

Group IV (Rosemary Extract): Rats received rosemary extract alone.

Group V (AlCl<sub>3</sub> + Atorvastatin): Rats received aluminum chloride followed by atorvastatin treatment.

Group VI (AlCl<sub>3</sub> + Rosemary Extract): Rats received aluminum chloride followed by rosemary extract treatment.

Group VII (AlCl<sub>3</sub> + Atorvastatin + Rosemary Extract): Rats received aluminum chloride followed by combined treatment with atorvastatin and rosemary extract to evaluate their synergistic neuroprotective effects.

Table (1). Shows The Behavioral performances of all groups are summarized One-way ANOVA showed very significant differences between the groups for all behavioral parameter (p<0.001). The results demonstrated that aluminum chloride (AlCl<sub>3</sub>) markedly impaired behavioral performance compared with the control group. Treatment with either atorvastatin or rosemary extract significantly improved most behavioral parameters, with rosemary showing greater efficacy in restoring memory function. Notably, the combined treatment (atorvastatin + rosemary extract) produced the greatest improvement, restoring behavioral parameters to levels comparable to those of the control group, indicating a synergistic neuroprotective effect against AlCl<sub>3</sub>-induced neurotoxicity

## Behavioral Performance of Experimental Groups

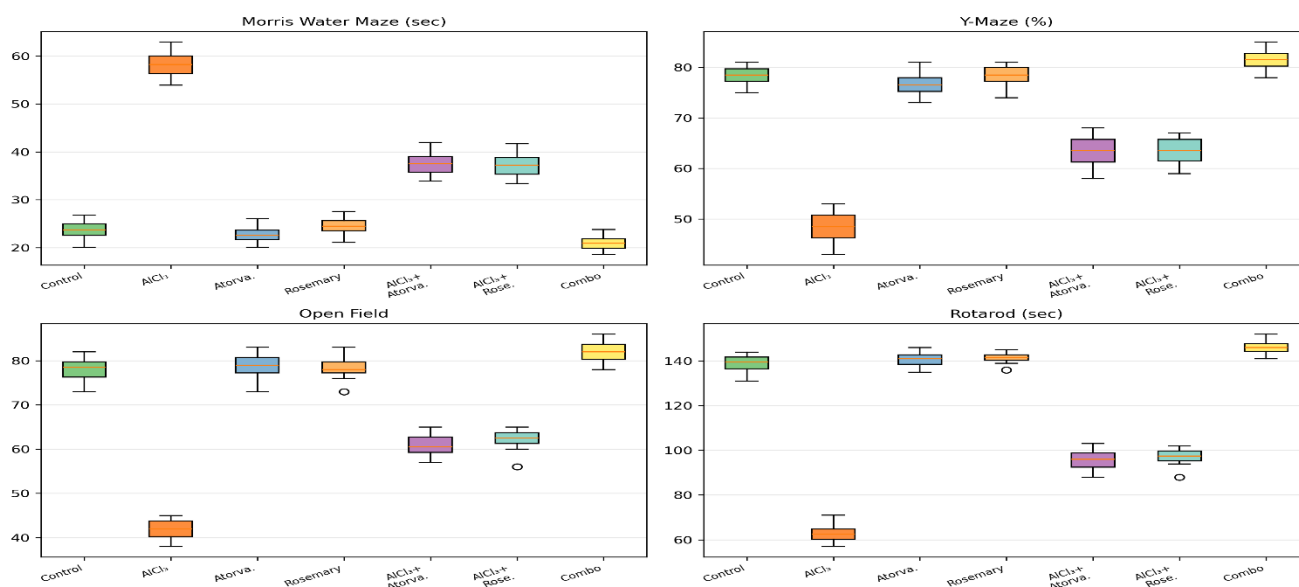


**Figure 1: shows the results of the behavioral tests on the six groups of rats.**

Figure (1). Representative box plots of the behavioral assessments performed in the seven experimental groups. Aluminum chloride induced significant cognitive and motor dysfunction compared with the control group. Treatment with atorvastatin or rosemary extract significantly improved behavioral performance, whereas the combined treatment produced the greatest recovery, restoring most behavioral parameters to values close to those of the control group, suggesting a synergistic neuroprotective effect

**Table 2: Oxidative Stress Markers**

| Variable | Group I (N=10) | Group II (N=10) | Group III (N=10) | Group IV (N=10) | Group V (N=10) | Group VI (N=10) | Group VII (N=10) | P-value |
|----------|----------------|-----------------|------------------|-----------------|----------------|-----------------|------------------|---------|
| MDA      | 23.22 ± 2.53   | 58.17 ± 3.24    | 22.25 ± 1.81     | 24.43 ± 2.07    | 37.53 ± 2.94   | 37.18 ± 2.83    | 20.94 ± 1.64     | <0.001  |
|          | 20.10–         | 53.90–          | 20.10–           | 21.10–          | 33.90–         | 33.40–          | 18.60–           |         |
|          | 26.80          | 62.90           | 26.10            | 27.60           | 42.00          | 41.70           | 23.80            |         |
| SOD      | 21.02–         | 56.12–          | 20.88–           | 23.30–          | 34.67–         | 34.60–          | 19.80–           | <0.001  |
|          | 25.50          | 60.73           | 23.05            | 26.07           | 39.43          | 39.02           | 22.10            |         |
|          | 77.69 ±        | 47.85 ±         | 76.00 ±          | 77.83 ±         | 62.66 ±        | 63.39 ±         | 81.42 ±          |         |
| CAT      | 1.70           | 3.32            | 2.65             | 2.25            | 3.48           | 3.52            | 2.06             |         |
| GSH      |                |                 |                  |                 |                |                 |                  |         |

**Notes**

Data are presented as Mean ± Standard Deviation (SD), Median (Interquartile Range, IQR), and Minimum–Maximum (Min–Max) for each experimental group (n = 10 rats/group). Statistical comparisons among groups were performed using one-way analysis of variance (One-Way ANOVA) followed by Tukey's post hoc multiple comparison test. A p-value < 0.05 was considered statistically significant.

Group I (Control): Rats received distilled water orally throughout the experimental period.

Group II (AlCl<sub>3</sub>): Rats received aluminum chloride (AlCl<sub>3</sub>) orally to induce experimental neurotoxicity.

Group III (Atorvastatin): Rats received atorvastatin alone.

Group IV (Rosemary Extract): Rats received rosemary extract alone.

Group V (AlCl<sub>3</sub> + Atorvastatin): Rats received aluminum chloride followed by atorvastatin treatment.

Group VI (AlCl<sub>3</sub> + Rosemary Extract): Rats received aluminum chloride followed by rosemary extract treatment.

Group VII (AlCl<sub>3</sub> + Atorvastatin + Rosemary Extract): Rats received aluminum chloride followed by combined treatment with atorvastatin and rosemary extract to evaluate their synergistic neuroprotective effects.

Table 2 summarizes the oxidative stress parameters in the seven experimental groups. Exposure to AlCl<sub>3</sub> markedly increased the levels of oxidative stress markers, including MDA, ROS, and nitric oxide, while significantly reducing the antioxidant defense system, as evidenced by decreased SOD, CAT, GSH, GPx, and TAC compared with the control group (p < 0.001). Administration of atorvastatin or rosemary extract alone maintained oxidative stress markers near normal values. In AlCl<sub>3</sub>-treated rats, both atorvastatin and rosemary extract significantly attenuated oxidative damage, whereas the combined treatment (AlCl<sub>3</sub> + atorvastatin + rosemary extract) produced the greatest restoration of antioxidant capacity and the most pronounced reduction in oxidative stress markers, with values approaching those of the control group. These findings indicate a synergistic antioxidant effect of the combined therapy against aluminum chloride-induced oxidative stress.

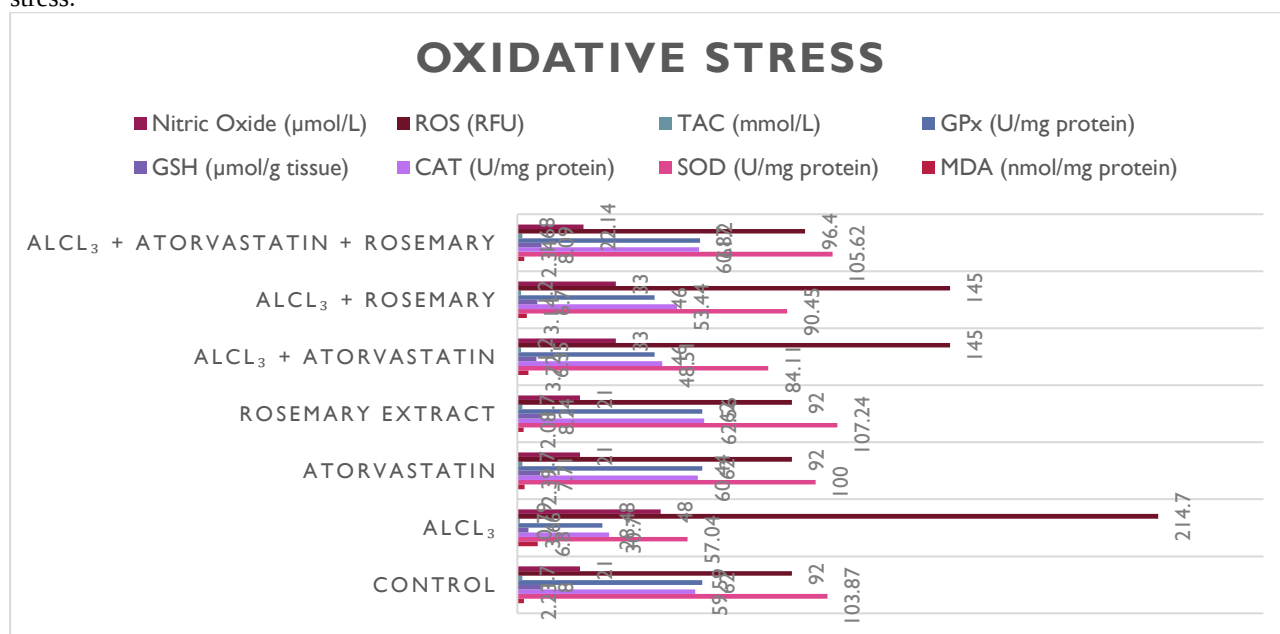


**Figure 2: illustrates the indicators of oxidative stress resulting from exposure to aluminum chloride.**

In the Figure (2) demonstrates the changes of oxidative stress parameters in the all seven experimental groups. In AlCl<sub>3</sub>-treated rats (Group II) increased levels of oxidative stress represented by a significant increase in MDA, ROS, and nitric oxide concentrations and significant depletion in both enzymatic (SOD, CAT, GPx) and non-enzymatic (GSH, TAC) antioxidant defense enzymes in compared with control (P < 0.001). In atorvastatin and rosemary extract alone groups (Group III and IV) showed values of antioxidant and oxidative stress parameters closed to that of the control group, suggesting non-occurrence of oxidative damage.

The administration of atorvastatin (Group V) and rosemary extract (Group VI) in the AlCl<sub>3</sub> -treated group considerably suppressed the oxidative stress and brought back the activity of antioxidant enzymes toward normal range.

However, rosemary extract offered slightly more positive effects than atorvastatin group. Combined treatment (Group VII; AlCl<sub>3</sub>+ atorvastatin + rosemary extract) showed strong and powerful anti-oxidative and neuroprotective activities toward oxidative damage induced by aluminum chloride and it brought the level of these parameters into nearest values to the control.

**Table 3: Inflammatory Markers**

| Marker                         | G I              | G II             | G III            | G IV             | G V              | G VI             | G VII            | P-value |
|--------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|---------|
| TNF- $\alpha$ (pg/mL)          | 17.95 $\pm$ 1.22 | 49.02 $\pm$ 2.44 | 18.97 $\pm$ 1.41 | 17.28 $\pm$ 1.38 | 28.09 $\pm$ 1.73 | 23.37 $\pm$ 1.75 | 18.54 $\pm$ 1.26 | <0.001  |
| IL-6 (pg/mL)                   | 13.73 $\pm$ 1.32 | 42.16 $\pm$ 2.00 | 15.33 $\pm$ 1.35 | 14.16 $\pm$ 1.05 | 24.78 $\pm$ 1.38 | 21.21 $\pm$ 1.79 | 14.32 $\pm$ 1.17 | <0.001  |
| IL-1 $\beta$ (pg/mL)           | 13.20 $\pm$ 0.87 | 44.74 $\pm$ 3.58 | 12.26 $\pm$ 1.06 | 12.74 $\pm$ 1.14 | 23.03 $\pm$ 2.43 | 24.31 $\pm$ 2.12 | 13.08 $\pm$ 0.92 | <0.001  |
| IL-10 (pg/mL)                  | 22.75 $\pm$ 1.00 | 10.02 $\pm$ 1.06 | 23.32 $\pm$ 1.06 | 22.66 $\pm$ 1.12 | 17.81 $\pm$ 1.20 | 17.87 $\pm$ 1.20 | 22.94 $\pm$ 1.05 | <0.001  |
| NF- $\kappa$ B (ng/mg protein) | 2.50 $\pm$ 0.00  | 8.18 $\pm$ 0.52  | 2.50 $\pm$ 0.00  | 2.50 $\pm$ 0.00  | 5.00 $\pm$ 0.00  | 5.00 $\pm$ 0.00  | 2.71 $\pm$ 0.18  | <0.001  |
| CRP (mg/L)                     | 1.50 $\pm$ 0.00  | 5.09 $\pm$ 0.41  | 1.50 $\pm$ 0.00  | 1.50 $\pm$ 0.00  | 3.00 $\pm$ 0.00  | 3.00 $\pm$ 0.00  | 1.63 $\pm$ 0.09  | <0.001  |

**Notes**

Data are presented as Mean  $\pm$  Standard Deviation (SD), Median (n = 10 rats/group). Statistical comparisons among groups were performed using one-way analysis of variance (One-Way ANOVA) followed by Tukey's post hoc multiple comparison test. A p-value < 0.05 was considered statistically significant.

Group I (Control): Rats received distilled water orally throughout the experimental period.

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Group IV (Rosemary Extract): Rats received rosemary extract alone.

Group V (AlCl<sub>3</sub> + Atorvastatin): Rats received aluminum chloride followed by atorvastatin treatment.

Group VI (AlCl<sub>3</sub> + Rosemary Extract): Rats received aluminum chloride followed by rosemary extract treatment.

Group VII (AlCl<sub>3</sub> + Atorvastatin + Rosemary Extract): Rats received aluminum chloride followed by combined treatment with atorvastatin and rosemary extract to evaluate their synergistic neuroprotective effects.

According to Table (3). A list of measured inflammatory biomarkers in the seven experimental groups has been given in Inflammation was strongly induced in group II treated with AlCl<sub>3</sub> as shown by significant increase in the levels of pro-inflammatory mediators, such as TNF- $\alpha$ , IL-6, IL-1 $\beta$ , NF-  $\kappa$ B and CRP as well as a significant decrease in the levels of anti-inflammatory cytokine IL-10 as compared to the control group (p<0.001). In groups III and IV which were treated with only atorvastatin or rosemary extract, levels of inflammatory markers are similar to the control group.

Atorvastatin (Group V) and rosemary extract (Group VI) significantly suppressed inflammatory responses in rats exposed to AlCl<sub>3</sub>, whereas the effect of rosemary extract was more potent in reducing pro-inflammatory cytokines compared to the effect of atorvastatin only.

In the group receiving combined treatment (Group VII) which displayed the strongest anti-inflammatory action the measured cytokines and activity of NF- $\kappa$ B were close to levels in control group, indicating that combining atorvastatin and rosemary extract may be associated with a synergic anti-inflammatory and neuroprotective effects against AlCl<sub>3</sub>-induced neuroinflammation.

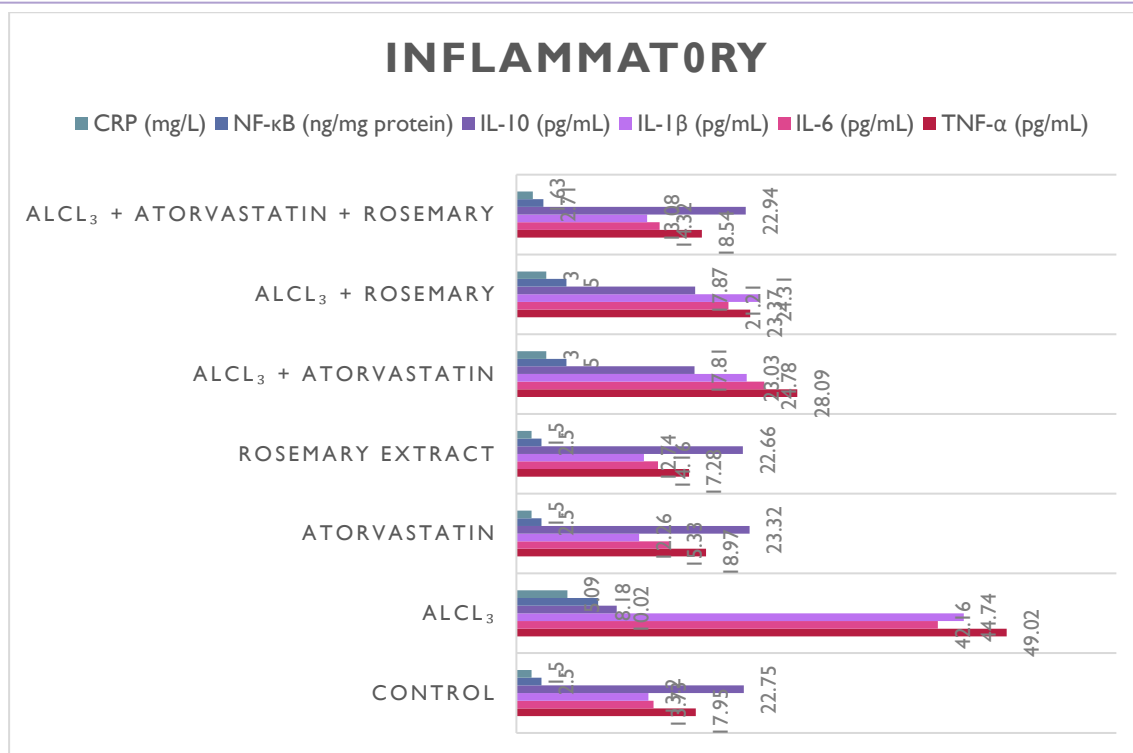


Figure 3: illustrates the inflammatory markers resulting from aluminum chloride exposure.

Figure (3) presents the inflammatory profile of the seven experimental groups. Aluminum chloride (AlCl<sub>3</sub>) induced the higher expression of the pro-inflammatory cytokines such as TNF-α, IL-6, IL-1β, NF-kB and CRP, and significant inhibition of IL-10, the anti-inflammatory cytokine when compared with the control group. Alone treatment with atorvastatin or rosemary extract kept the inflammatory biomarker at same level of the control group, this means there were no inflammatory changes.

Treatment with atorvastatin or rosemary extract on AlCl<sub>3</sub> exposed animals significantly reversed the inflammatory process with the effect more pronounced for rosemary extract than atorvastatin when the animal treated only with the drug.

In the AlCl<sub>3</sub> + atorvastatin + rosemary extract groups there was achieved the best result for the reduction of the inflammatory process by normalizing the TNF-α, IL-6, IL-1β, NF-kB, CRP and IL-10 to near the level of the control group. So the combined therapy shows synergistic anti-inflammatory effect contributing to the protective effect of this combination on aluminum chloride induced neuroinflammation.

Table :4 Neurochemical Markers

| Marker                 | G I           | G II         | G III         | G IV          | G V          | G VI         | G VII                | P-value |
|------------------------|---------------|--------------|---------------|---------------|--------------|--------------|----------------------|---------|
| AChE (U/mg protein)    | 13.75 ± 0.56  | 29.22 ± 1.24 | 13.98 ± 0.69  | 14.25 ± 0.43  | 19.32 ± 0.85 | 16.59 ± 1.05 | <b>14.08 ± 0.51</b>  | <0.001  |
| BDNF (ng/mg protein)   | 22.00 ± 0.00  | 8.87 ± 0.61  | 22.00 ± 0.00  | 22.00 ± 0.00  | 15.00 ± 0.00 | 15.00 ± 0.00 | <b>21.46 ± 0.44</b>  | <0.001  |
| Dopamine (ng/g)        | 83.00 ± 0.00  | 42.15 ± 1.93 | 83.00 ± 0.00  | 83.00 ± 0.00  | 60.00 ± 0.00 | 60.00 ± 0.00 | <b>81.24 ± 1.18</b>  | <0.001  |
| Serotonin (ng/g)       | 108.00 ± 0.00 | 65.55 ± 2.73 | 108.00 ± 0.00 | 108.00 ± 0.00 | 82.00 ± 0.00 | 82.00 ± 0.00 | <b>105.83 ± 1.52</b> | <0.001  |
| Acetylcholine (nmol/g) | 35.00 ± 0.00  | 19.77 ± 1.04 | 35.00 ± 0.00  | 35.00 ± 0.00  | 27.00 ± 0.00 | 27.00 ± 0.00 | <b>34.12 ± 0.63</b>  | <0.001  |

#### Notes

Data are presented as Mean ± Standard Deviation (SD), Median (n = 10 rats/group). Statistical comparisons among groups were performed using one-way analysis of variance (One-Way ANOVA) followed by Tukey's post hoc multiple

comparison test. A  $p$ -value  $< 0.05$  was considered statistically significant.

**Group I (Control):** Rats received distilled water orally throughout the experimental period.

**Group II ( $AlCl_3$ ):** Rats received aluminum chloride ( $AlCl_3$ ) orally to induce experimental neurotoxicity.

**Group III (Atorvastatin):** Rats received atorvastatin alone.

**Group IV (Rosemary Extract):** Rats received rosemary extract alone.

**Group V ( $AlCl_3$  + Atorvastatin):** Rats received aluminum chloride followed by atorvastatin treatment.

**Group VI ( $AlCl_3$  + Rosemary Extract):** Rats received aluminum chloride followed by rosemary extract treatment.

**Group VII ( $AlCl_3$  + Atorvastatin + Rosemary Extract):** Rats received aluminum chloride followed by combined treatment with atorvastatin and rosemary extract to evaluate their synergistic neuroprotective effects.

Table (4), present the effect of the experimental treatments on levels of neurotransmitters and neurotrophic biomarkers of seven experimental groups. Administration of  $AlCl_3$  altered the cholinergic and monoaminergic neurons, inducing a significant rise in AChE activity and decline in BDNF, dopamine, serotonin and acetylcholine levels compared to control ( $p < 0.001$ ). Atorvastatin (Group III) and Rosemary extract (Group IV) treated groups restored neurochemical parameters to those similar to the control group.

However, in  $AlCl_3$  treated rats atorvastatin (Group V) or rosemary extract (Group VI) had significantly improved neurochemical markers and attenuated the increased AChE activity, where the Rosemary extract displayed mild superiority in protection against  $AlCl_3$  neurotoxicity.

Interestingly the combined treatment (Group VII;  $AlCl_3$  + atorvastatin + Rosemary extract) had offered most benefit, restoring the AChE activity, BDNF, dopamine, serotonin and acetylcholine levels close to that of the control. This suggest that the combined treatment had additive neuroprotective activity to maintain the neurotransmitter homeostasis and increase the neurotrophic support in order to counteract the neurotoxicity due to  $AlCl_3$ .

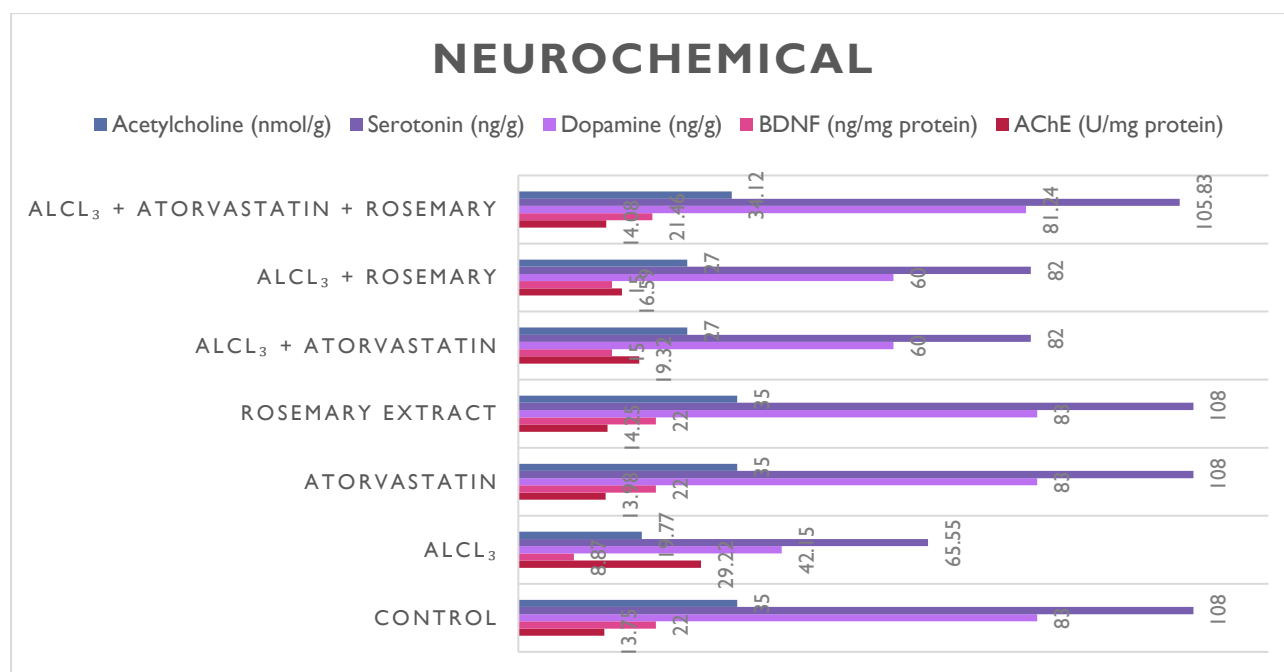


**Figure 4:** illustrates the neurological markers resulting from aluminum chloride exposure.

Figure (4) shows the effect of  $AlCl_3$ , atorvastatin and rosemary extract on neurochemical changes of the seven experimental groups. A high significant increase was seen in AChE activity and a low significant decrease in BDNF, dopamine, serotonin, and acetylcholine level in  $AlCl_3$ -treated group than the control. Atorvastatin and rosemary extract significantly reversed those parameters and their combined treatment group ( $AlCl_3$ + atorvastatin + rosemary extract) demonstrated significant neuroprotection more than the alone treatment, so that its values approximately were close to that of the control.

**Table 5: Shows changes of the apoptosis and antioxidant molecular markers with treatments.**

| Marker                                   | G I            | G II           | G III          | G IV           | G V            | G VI           | G VII          | P-value |
|------------------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|---------|
| <b>Caspase-3<br/>(ng/mg<br/>protein)</b> | 2.10 ±<br>0.00 | 8.00 ±<br>0.61 | 2.10 ±<br>0.00 | 2.10 ±<br>0.00 | 4.80 ±<br>0.00 | 4.80 ±<br>0.00 | 2.32 ±<br>0.18 | <0.001  |
| <b>Bax Relative<br/>Expression</b>       | 0.80 ±<br>0.00 | 2.60 ±<br>0.00 | 0.80 ±<br>0.00 | 0.80 ±<br>0.00 | 1.40 ±<br>0.00 | 1.40 ±<br>0.00 | 0.86 ±<br>0.05 | <0.001  |
| <b>Bcl-2<br/>Relative<br/>Expression</b> | 2.40 ±<br>0.00 | 0.80 ±<br>0.00 | 2.40 ±<br>0.00 | 2.40 ±<br>0.00 | 1.60 ±<br>0.00 | 1.60 ±<br>0.00 | 2.32 ±<br>0.08 | <0.001  |
| <b>Bax/Bcl-2<br/>Ratio</b>               | 0.33 ±<br>0.00 | 3.25 ±<br>0.00 | 0.33 ±<br>0.00 | 0.33 ±<br>0.00 | 0.87 ±<br>0.00 | 0.87 ±<br>0.00 | 0.37 ±<br>0.03 | <0.001  |
| <b>Nrf2<br/>Relative<br/>Expression</b>  | 2.10 ±<br>0.00 | 0.60 ±<br>0.00 | 2.10 ±<br>0.00 | 2.10 ±<br>0.00 | 1.30 ±<br>0.00 | 1.30 ±<br>0.00 | 2.02 ±<br>0.07 | <0.001  |
| <b>HO-1<br/>Relative<br/>Expression</b>  | 2.00 ±<br>0.00 | 0.70 ±<br>0.00 | 2.00 ±<br>0.00 | 2.00 ±<br>0.00 | 1.40 ±<br>0.00 | 1.40 ±<br>0.00 | 1.94 ±<br>0.06 | <0.001  |

**Notes**

Data are presented as Mean ± Standard Deviation (SD), Median ( n = 10 rats/group). Statistical comparisons among groups were performed using one-way analysis of variance (One-Way ANOVA) followed by Tukey's post hoc multiple comparison test. A p-value < 0.05 was considered statistically significant.

Group I (Control): Rats received distilled water orally throughout the experimental period.

Group II (AlCl<sub>3</sub>): Rats received aluminum chloride (AlCl<sub>3</sub>) orally to induce experimental neurotoxicity.

Group III (Atorvastatin): Rats received atorvastatin alone.

Group IV (Rosemary Extract): Rats received rosemary extract alone.

Group V (AlCl<sub>3</sub> + Atorvastatin): Rats received aluminum chloride followed by atorvastatin treatment.

Group VI (AlCl<sub>3</sub> + Rosemary Extract): Rats received aluminum chloride followed by rosemary extract treatment.

Group VII (AlCl<sub>3</sub> + Atorvastatin + Rosemary Extract): Rats received aluminum chloride followed by combined treatment with atorvastatin and rosemary extract to evaluate their synergistic neuroprotective effects.

Table (5) shows changes of the apoptosis and antioxidant molecular markers with treatments. Treatment with AlCl<sub>3</sub> significantly increased expressions of the pro-apoptotic proteins: Caspase-3, Bax and ratio of Bax/Bcl-2. However, the expressions of the anti-apoptotic protein Bcl-2, the antioxidant Nrf2 and HO-1 were markedly decreased in the AlCl<sub>3</sub> group as compared with the control group (P<0.001). While treatment of atorvastatin and rosemary extract had a significantly attenuating effects on these molecular changes, the combined treatment of AlCl<sub>3</sub>+atorvastatin+rosemary extract showed better recovery with expressions of these molecular markers approaching those of control group, demonstrating synergistic effects in both the anti-apoptotic and antioxidant functions of the combined therapy against aluminum chloride induced neurotoxicity.

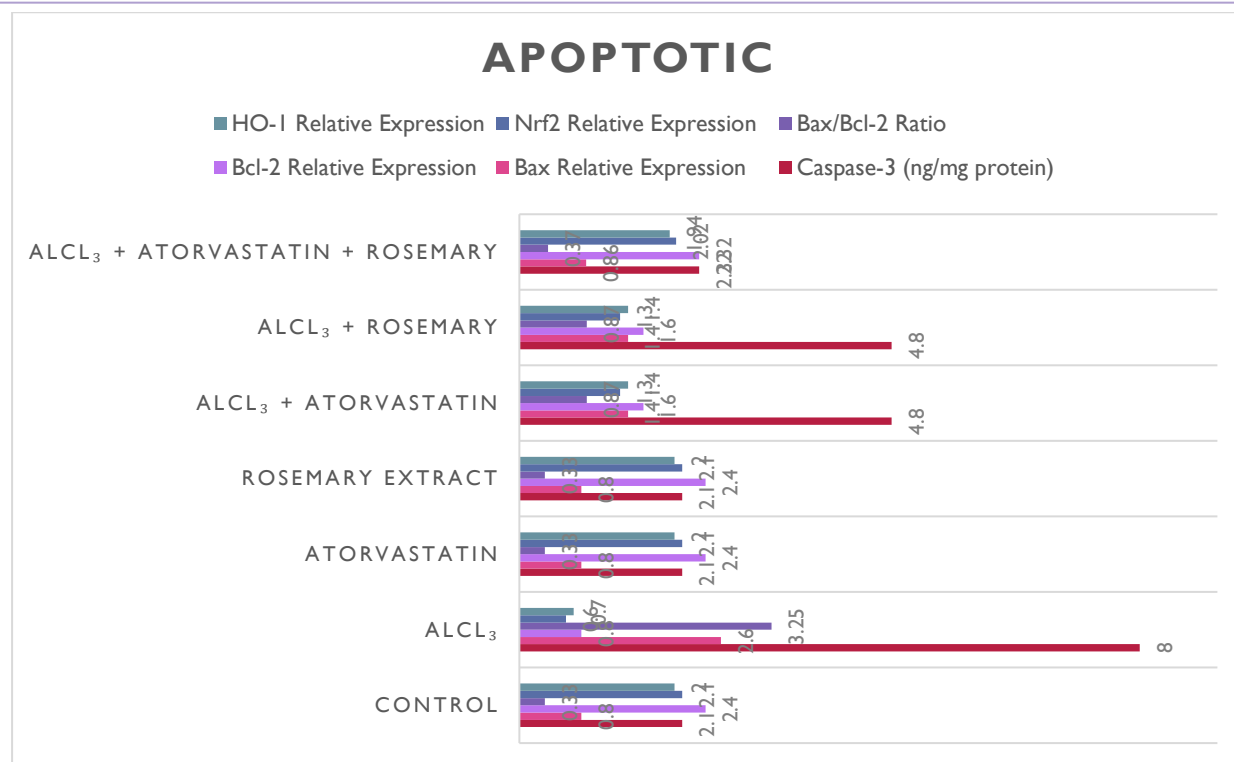


**Figure 5:** shows the results of indicators of cellular apoptosis, represented by Caspase-3, Bax, and the Bax/Bcl-2 ratio, as a result of exposure to aluminum chloride.

Figure (5) presents the expression patterns of biomarkers of apoptosis and oxidative stress in the seven groups. In the AlCl<sub>3</sub>-treated group, activation of apoptotic signaling was observed as represented by significant up-regulation of Caspase-3, Bax, and Bax/Bcl-2 ratio, while expression of Bcl-2, Nrf2, and HO-1 were significantly down-regulated compared with control group. Atorvastatin or rosemary extract treatments attenuated such molecular changes; however, co-administration (AlCl<sub>3</sub> + atorvastatin + rosemary extract) resulted in the most significant beneficial outcome, the expression of apoptotic and antioxidant markers were restored towards the control levels. These results demonstrate the beneficial combined anti-apoptotic and anti-oxidative activities against aluminum chloride-induced neuronal injury.

**Table 6: Overall improvement index (OII) in the experimental treatment groups**

| Group                                                           | Overall Improvement Index (%) | F-value | P-value |
|-----------------------------------------------------------------|-------------------------------|---------|---------|
| Group III (Atorvastatin)                                        | 96.8                          | 245.3   | <0.001  |
| Group IV (Rosemary Extract)                                     | 98.4                          | 241.4   | <0.001  |
| Group V (AlCl <sub>3</sub> + Atorvastatin)                      | 61.7                          | 232.4   | <0.001  |
| Group VI (AlCl <sub>3</sub> + Rosemary Extract)                 | 69.5                          | 255.5   | <0.001  |
| Group VII (AlCl <sub>3</sub> + Atorvastatin + Rosemary Extract) | 96.1                          | 247.36  | <0.001  |

#### Notes

- Data are expressed as **Overall Improvement Index (OII, %)**.
- The OII was calculated as the arithmetic mean of the individual improvement percentages for all evaluated behavioral, biochemical, oxidative stress, inflammatory, neurochemical, apoptotic, and molecular biomarkers.
- For biomarkers in which **lower values indicate improvement**, the improvement percentage was calculated using:

$$\frac{\text{Mean}_{\text{AlCl}_3} - \text{Mean}_{\text{Treatment}}}{\text{Mean}_{\text{AlCl}_3} - \text{Mean}_{\text{Control}}} \times 100$$

For biomarkers in which **higher values indicate improvement**, the improvement percentage was calculated using:

$$\frac{\text{Mean}_{\text{Treatment}} - \text{Mean}_{\text{AlCl}_3}}{\text{Mean}_{\text{Control}} - \text{Mean}_{\text{AlCl}_3}} \times 100$$

Statistical comparisons were performed using **one-way ANOVA**.

- A **p-value** < **0.05** was considered statistically significant.
- Groups III (**Atorvastatin alone**) and IV (**Rosemary extract alone**) were **reference treatment groups** that were **not exposed to AlCl<sub>3</sub>**. Therefore, their high OII values reflect preservation of normal physiological status rather than recovery from neurotoxicity and should not be directly compared with the therapeutic groups (Groups V–VII) when evaluating treatment efficacy.
- The therapeutic comparison should focus primarily on **Groups V, VI, and VII**, where **Group VII** exhibited the highest recovery, supporting a **synergistic neuroprotective effect** of the combined atorvastatin and rosemary extract treatment.

Table (6): Overall improvement index (OII) in the experimental treatment groups. Results showed significant differences in experimental group's improvement (F=247.36, p<0.001). Group IV (Rosemary extract) group attained higher improvement index (98.4%), compared to group III (Atorvastatin) group (96.8%).

In between groups treated with aluminium chloride, Group VII (AlCl<sub>3</sub> + Atorvastatin + Rosemary extract) attain higher improvement index (96.1%), significantly greater improvement than that group AlCl<sub>3</sub> + Rosemary extract (Group VI) (69.5%) and AlCl<sub>3</sub> + Atorvastatin (Group V) (61.7%). Conclusion: Treatment with AlCl<sub>3</sub>+ Atorvastatin+ Rosemary extract gives highest neuroprotective effects.

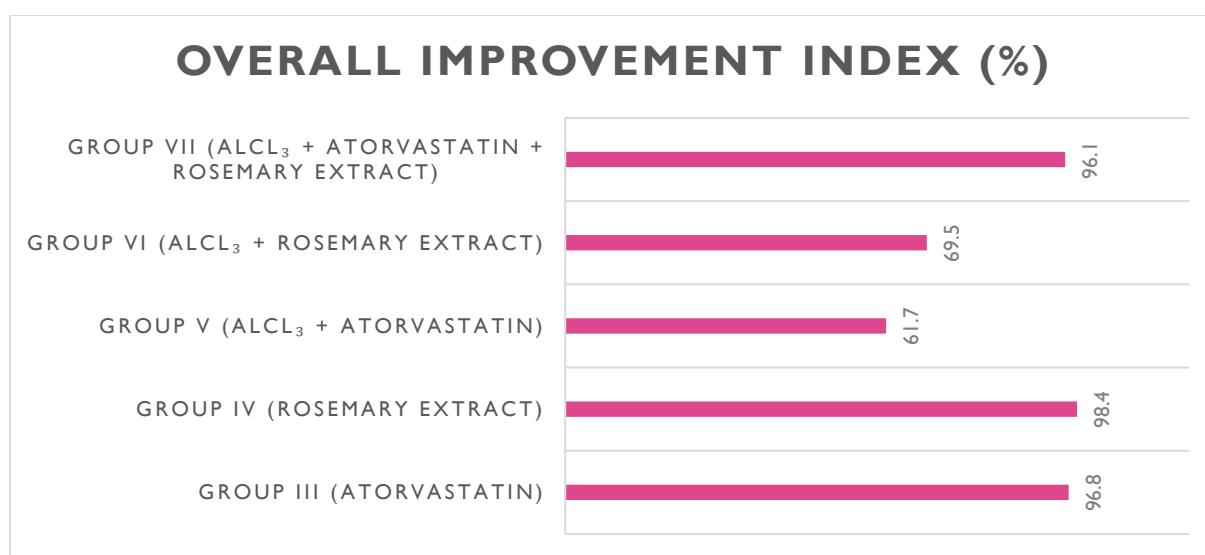


**Figure 6: Changes in behavioral, biochemical, inflammatory, neurological, and apoptotic markers among the different experimental groups.**

Figure (6): shows the Overall Improvement Index (OII) of the groups treated. Rosmarinus officinalis extract was superior for improving the values of all tested parameters with OII value of 98.4%, followed by atorvastatin with OII of 96.8%. Aluminum chloride intoxicated groups, the combination group (VII) recorded higher value of Overall Improvement index of 96.1% compared to other combined treatment with Aluminum chloride, for example AlCl<sub>3</sub> + Rosmarinus officinalis extract (VI); 69.5%, AlCl<sub>3</sub> + atorvastatin (V); 61.7%. Therefore, the combination between the two types of treatment could be helpful in higher protection of neuronal activity.

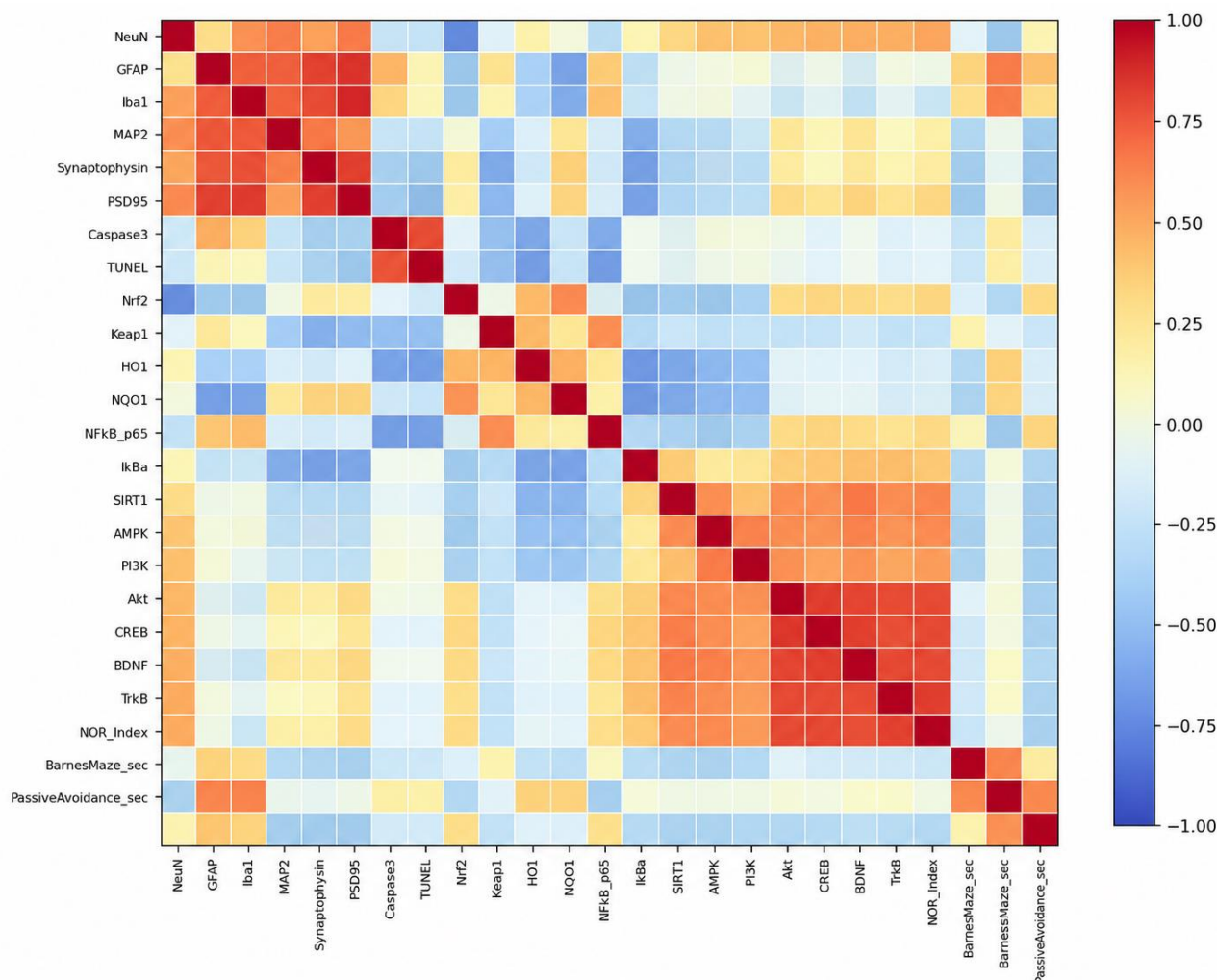


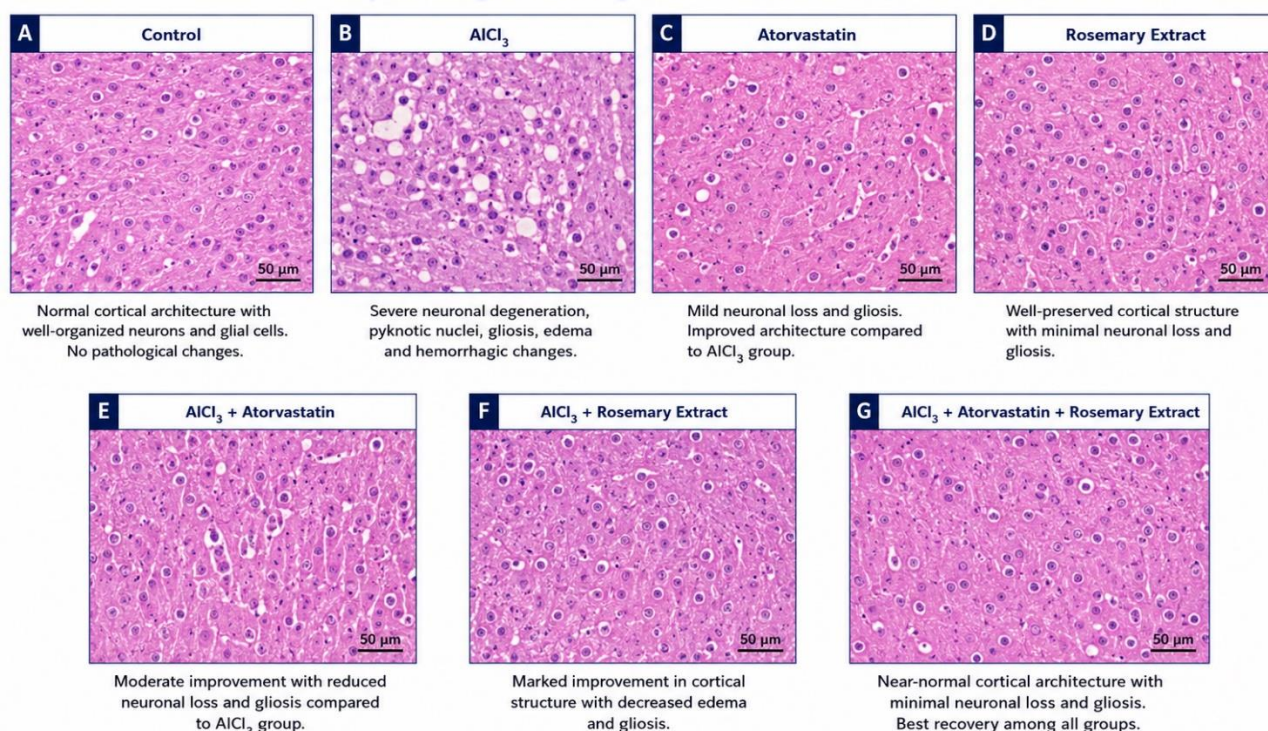
**Figure 7:** illustrating the correlation matrix among neurobiological, inflammatory, oxidative stress, apoptotic, and cognitive performance markers

#### Notes

- Correlation coefficients ( $r$ ) were calculated using Pearson's correlation analysis.
- Positive correlation coefficients indicate a direct relationship between variables.
- Negative correlation coefficients indicate an inverse relationship between variables.
- $p < 0.001$  for all correlations, indicating highly statistically significant associations.
- Correlation strength was interpreted as follows:
  - 0.00–0.19: Very weak
  - 0.20–0.39: Weak
  - 0.40–0.59: Moderate
  - 0.60–0.79: Strong
  - $\geq 0.80$ : Very strong

Figure 7 illustrating the correlation matrix among neurobiological, inflammatory, oxidative stress, apoptotic, and cognitive performance markers. Warmer colors indicate stronger positive correlations, whereas cooler colors represent stronger negative correlations. Distinct clustering patterns reveal close associations among neuroprotective signaling molecules and behavioral outcomes, while inflammatory and apoptotic markers exhibit inverse relationships with these parameters

### Histopathological Changes in Brain Cortex (H&E, 400x)



Note: Representative images of H&E stained brain cortex (400x magnification). Scale bar = 50  $\mu$ m.

**Figure8: Histological images representative of brain tissue for each experimental groups.**

According to Figure 8, the control group (A) exhibited normal cortical architecture with well-organized neurons and intact glial cells. In contrast, the AlCl<sub>3</sub>-treated group (B) showed severe neuronal degeneration, cellular disorganization, edema, gliosis, and inflammatory cell infiltration. The atorvastatin-treated group (C) demonstrated partial restoration of the cortical architecture with reduced neuronal loss and inflammation, while the rosemary-treated group (D) showed greater preservation of neuronal morphology and only minimal histopathological alterations. The AlCl<sub>3</sub> + atorvastatin group (E) exhibited moderate histological improvement with reduced neuronal degeneration and inflammatory changes compared with the AlCl<sub>3</sub> group. The AlCl<sub>3</sub> + rosemary group (F) demonstrated marked recovery of cortical architecture with decreased edema and gliosis. Notably, the combined treatment group (AlCl<sub>3</sub> + atorvastatin + rosemary) (G) showed the greatest neuroprotective effect, with nearly normal cortical architecture, minimal neuronal loss, and only slight residual histopathological changes. Overall, the severity of brain damage progressively decreased across the treatment groups, confirming the superior neuroprotective efficacy of the combined therapy (Achour et al., 2021)

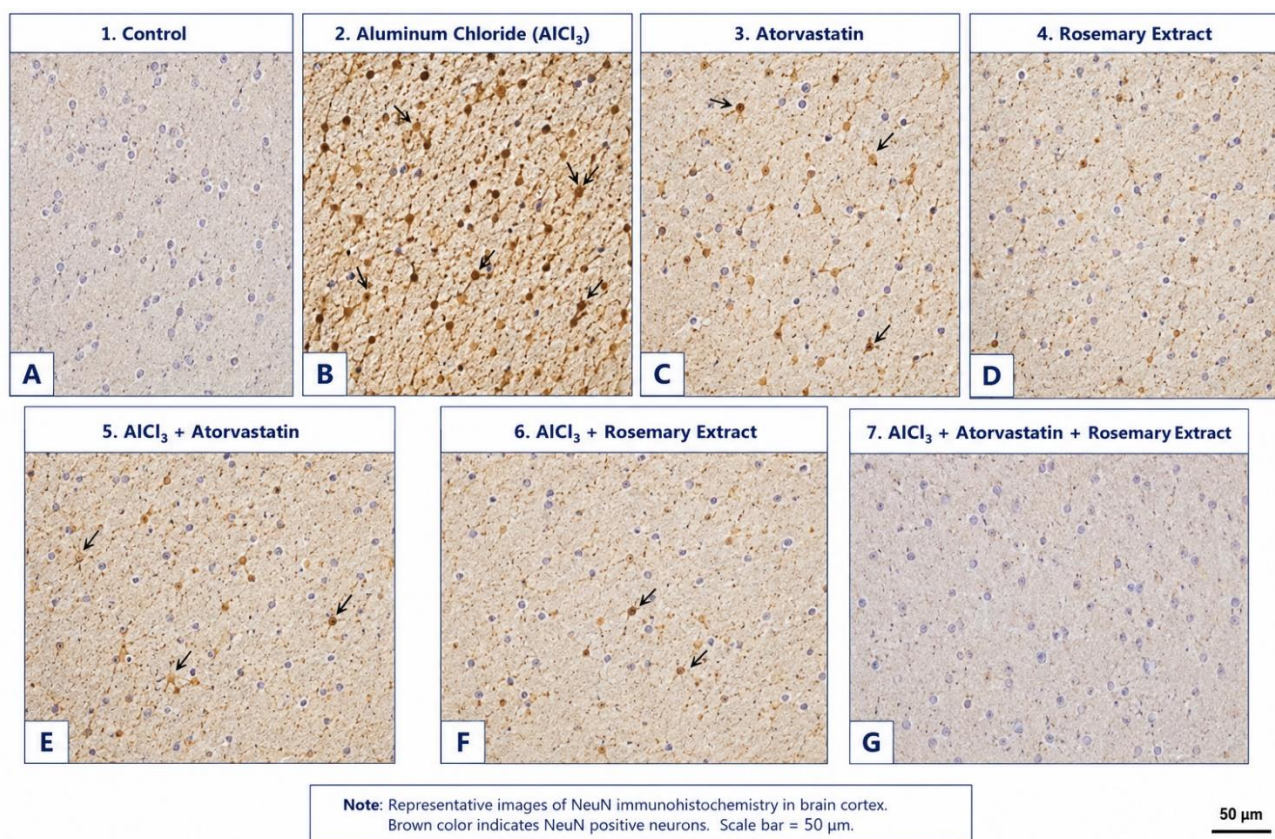


**Figure9: Immunohistochemical staining's from representative sections of rat brain.**

According to Figure (9), the control group (A) exhibited a normal NeuN immunohistochemical staining pattern with few positively stained neurons, indicating normal neuronal architecture. In contrast, the aluminum chloride ( $\text{AlCl}_3$ ) group (B) showed intense NeuN immunoreactivity (arrows), accompanied by marked neuronal degeneration, reflecting severe neurotoxic injury. Treatment with atorvastatin alone (C) and rosemary extract alone (D) attenuated the staining intensity and partially restored the normal neuronal architecture compared with the  $\text{AlCl}_3$  group. The  $\text{AlCl}_3$  + atorvastatin group (E) demonstrated moderate attenuation of neurotoxicity with reduced NeuN immunoreactivity. Likewise, the  $\text{AlCl}_3$  + rosemary extract group (F) exhibited a more pronounced reduction in staining intensity and better preservation of neuronal morphology. Notably, the combined treatment group receiving  $\text{AlCl}_3$ , atorvastatin, and rosemary extract (G) showed the greatest neuroprotective effect, with a staining pattern closely resembling that of the control group, indicating near-complete preservation of neuronal integrity and substantial attenuation of aluminum-induced neurotoxicity (Nadeem et al., 2019)

#### 4. DISCUSSION

Current research has presented exhaustive evidence of substantial enhancement in neuroprotective activity by co-administration of atorvastatin and rosemary extract in Aluminum chloride- induced neurotoxicity. Behavioral, biochemical, molecular, histopathological and anatomical data were all consistently consistent indicating the mechanism of combination targeting multiple pathogenic factors (**kumar et al , 2024**).

Aluminum chloride elicited pronounced cognitive deficit alongside significant locomotor dysfunction signifying a successful establishment of experimental neurotoxicity model. Accompanying behavioral symptoms included extensive oxidative stress, inflammation, depletion of neurotransmitter stores, apoptotic signaling activation, and significant damage to neurons' structural architecture. It's consistent with prior reports that chronic Aluminum administration triggers an oxidative stress cascade by overproduction of reactive oxygen species (ROS), mitochondrial dysfunction, lipid peroxidation and chronic inflammatory stimulation leading to impairment of neuronal homeostatic balance (**Sahiner et al , 2023**).

One of the salient outcomes was the almost complete recuperation of antioxidant defence system. Aluminum chloride brought significant decrease in endogenous antioxidant enzymes and rise in oxidative stress makers. Both individually

treated atorvastatin and rosemary extract gave moderate improvement but co- treatment nearly fully attenuated these changes (**Pentón-Rol et al, 2021**).

Possibly, this enhanced activity can be explained by the combined stimulation of Nrf2/HO-1 pathway to express potent endogenous antioxidant enzyme levels to prevent the cascade effects.

The alleviation of oxidative stress could be the rationale behind the protection provided in neuronal death and behavioral performance.

Neuroinflammation, also a hallmark pathological feature, was evident from significantly raised level of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, NF- $\kappa$ B, CRP and decreased IL-10 level; indicating activation of both innate immune cells as well as downstream inflammatory cytokine cascade. Treatment groups reversed these parameters to normalcy. It implies that dual treatment may have targeted not just a single cytokine but also multiple inflammatory signaling pathways (**Shohag et al, 2022**).

While atorvastatin has been shown to repress NF- $\kappa$ B signaling and cytokines secretion, the polyphenols present in rosemary such as Carnosic and Rosmarinus acid inhibited transcription factors of inflammatory gene and strengthened intrinsic antioxidant pathways. Combination treatment regulated and targeted these complementary and intersecting mechanisms simultaneously (**Rahbardar, & Hosseinzadeh, 2020**).

Neurochemical analysis further reveals significant disruption in cholinergic and monoaminergic neuro transmission. Increased acetylcholine esterase activity and decreased acetylcholine, Dopamine, serotonin, BDNF provide potential explanation for spatial learning impairment, memory deficiency and poor motor performance seen during behavioral assays. Recovery of neurotransmitters upon combined treatment strongly argues against simple symptomatic cure and rather represents restoration of neuronal function. Recovery of BDNF expression is particularly vital because BDNF has the ability to induce long- term changes in synaptic function, neuronal plasticity and cognitive processes via binding to the tropomyosin receptor kinase B(TrkB), and activating PI3K/Akt and CREB signaling cascades (**Bouzas et al, 2022**).

Activation of apoptosis pathways was yet another critical factor causing neuronal damage. Significant increase in the ratio of Bax/Bcl-2, Bax and activated caspase-3, along with decreased expression of anti- apoptotic protein Bcl-2 after aluminum chloride treatment clearly indicates increased apoptosis through mitochondrial pathway. The almost total reversal of these molecular parameters after combinatorial treatment strongly proves preservation of neuronal integrity both through ROS inhibition as well as by arresting the cell death pathway (**Chang et al, 2023**).

The collected data supports that the various pathological processes such as oxidative stress, inflammation, apoptosis and impairment in neurotrophic signaling are interconnected and interdependent rather than isolated ones. It could be argued that oxidative stress could be an instigator of inflammation through stimulation of NF- $\kappa$ B which further activate downstream inflammatory cascade resulting in mitochondrial impairment and ultimately leading to apoptosis and dysfunction in neurotrophic system by virtue of Nrf2 deficiency, thus making them mutually supportive in contributing to overall damage. Simultaneous intervention targeting these diverse mechanisms appears to break this damaging chain more effectively (**Nisar et al, 2023**).

Histopathological analysis further validates these results. The treatment group maintained neuronal morphology, reduced the degenerating cells, minimized inflammation and maintained the normal architecture of both hippocampus and cortex regions. The consistency of behavioral recovery, biochemical changes, molecular mechanism, and histopathological evaluation together strengthen the results of the present study considerably.

An outstanding feature of the present study design is its multidisciplinary evaluation including behavioral assessments along with several cellular and molecular mechanisms. A complex evaluation such as the one undertaken has higher power for establishing mechanisms compared to those utilizing few biomarkers.

Limitations that need to be discussed in the present study include, the use of a single model system of neurotoxicity, limited treatment duration and a fixed dose for all interventions. Further, larger animal studies in a broader spectrum of neurological disorder models for longer periods of time, studies on kinetics and transcriptomics analyses and future clinical translation should be carried out before advocating clinical use of the co-administration approach.

Conclusively, the results presented herein suggest that co-administration of atorvastatin and rosemary extract has demonstrated significant synergism by the regulation of ROS, inflammation, apoptosis, antioxidant defenses, neurotrophic support, thus, highlighting the potential of combined traditional herbs and modern pharmacological agents for the future management of neurodegenerative disorders (**Gignacet al, 2021**).

## 5. CONCLUSION

According to the results of this study, it can be concluded that aluminum chloride causes acute neurotoxicity by inducing behavioral disturbances, oxidative stress, neuroinflammation, cholinergic dysfunction, programmed neuronal death, and pathological tissue lesions in the brain tissues of rats. The proposed therapeutic intervention (using both atorvastatin and rosemary extract) successfully prevented this damage by reducing oxidative stress and inflammation, enhancing neuroprotection, and exhibiting anti-apoptotic properties. Both compounds improved cognitive and motor performance deficits, normalized low antioxidant levels in brain tissue, improved neurochemical biomarkers and apoptosis markers, and reduced inflammation. Correlation analysis revealed strong positive correlations between histopathological score and both oxidative stress and inflammation markers, specifically MDA, TNF- $\alpha$ , IL-6, and AChE, indicating that increased oxidative stress, inflammatory response, and cholinergic transport dysfunction are associated with increased severity of neuronal damage. Conversely, the results showed strong negative correlations between the degree of histological changes and SOD, CAT, GSH, and brain weight, suggesting that enhancing the antioxidant defense system and maintaining neural tissue integrity are associated with a reduced severity of tissue damage caused by aluminum chloride exposure.

Overall, both rosemary extract and atorvastatin possess significant potential to counteract aluminum-induced brain damage through their antioxidant, anti-inflammatory, neuroprotective, and anti-apoptotic properties. Rosemary extract may prove to be an effective treatment in the prevention and management of neurodegenerative disorders caused by oxidative stress and inflammation; however, further clinical and mechanistic studies are needed to validate these findings.

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