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Hematological evaluation of red blood cell count as a peripheral indicator in aluminum-induced Alzheimer's disease in rats treated with gum Arabic and Fenchol

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Abstract
Background: Alzheimer's disease (AD) is a progressive neurodegenerative disorder influenced by environmental and genetic factors. It is primarily characterized by beta-amyloid plaque accumulation, synaptic dysfunction, and cognitive decline. Despite existing pharmacological therapies, there is no definitive cure for AD, prompting growing interest in natural compounds with antioxidant and neuroprotective potential that may also influence systemic physiological responses.
Objective: This study aimed to evaluate the therapeutic potential of gum Arabic and Fenchol in a rat model of Alzheimer's disease induced by aluminum chloride ($AlCl_3$), with a particular focus on assessing red blood cell (RBC) count as a peripheral hematological indicator of neurodegeneration and treatment efficacy.
Methods: Thirty-six adult male rats were randomly assigned to six groups: (1) a negative control group receiving standard feed and water, (2) a positive control group treated orally with $AlCl_3$ (17 mg/kg/day), (3) a group treated post-induction with Fenchol (2 mL, 5 mg/80 mL), (4) a group treated with gum Arabic (2 mL, 10 g/100 mL), (5) a group receiving a combination of Fenchol and gum Arabic, and (6) a memantine-treated group (2 mL, 1.57 g/25 mL). After one month of treatment, red blood cell counts were analyzed to evaluate systemic hematological responses.
Results: The positive control group (G2) exhibited a marked reduction in RBC count (7.00±0.40 million/µL), significantly lower than all other groups ($p = 0.000$). Treatment with Fenchol (G3) and gum Arabic (G4) led to significant increases in RBC levels (8.50±0.30 and 8.80±0.30 million/µL, respectively). The combination group (G5) and memantine group (G6) showed the highest RBC counts (9.20±0.20 and 9.00±0.30 million/µL, respectively), comparable to the negative control (G1, 9.00±0.30 million/µL). ANOVA revealed highly significant differences among groups ($p<0.00001$), with LSD post hoc tests confirming the therapeutic superiority of all treatments over the positive control. The progressive restoration of RBC counts across treated groups may reflect systemic recovery and mitigation of oxidative or inflammatory damage associated with $AlCl_3$-induced neurotoxicity.
Conclusion: Gum Arabic and Fenchol demonstrated promising systemic protective effects in $AlCl_3$-induced Alzheimer's disease, as evidenced by significant restoration of red blood cell counts. These findings suggest that RBC count may serve as a valuable peripheral biomarker for assessing disease severity and therapeutic efficacy in neurodegenerative models. Further research is warranted to explore the mechanistic link between neurodegeneration and hematological alterations.

Keywords: Alzheimer's disease, red blood cells, gum Arabic, Fenchol, neurotoxicity, aluminum chloride, natural compounds, systemic biomarkers

Introduction
The human brain is a highly integrated and functionally dynamic organ responsible for governing a wide range of physiological and behavioral processes, including cognition, memory, autonomic regulation, and sensory integration (Pray *et al*., 2015) [18]. Among the most devastating neurological disorders impacting brain function is Alzheimer's disease (AD), a progressive and age-associated neurodegenerative condition characterized by impaired synaptic communication, neuronal loss, and the gradual decline of cognitive performance. Globally, AD ranks among the leading causes of death, with mortality rates exceeding those of several major cancers, including breast and prostate cancer (Hunsberger *et al*., 2019) [8].
According to the World Health Organization (WHO), approximately 55 million people are currently affected by dementia, a number projected to increase to 78 million by 2030 and 139 million by 2050. This alarming trend not only signifies a substantial public health challenge but also imposes a dramatic socioeconomic burden, with global costs estimated at US$1.3 trillion in 2019 and anticipated to reach US$2.8 trillion by 2030 (Shin, 2022) [24].

Pathologically, AD is characterized by extracellular accumulation of amyloid-beta (Aβ) plaques and intracellular neurofibrillary tangles composed of hyperphosphorylated tau proteins, leading to widespread neuroinflammation, oxidative stress, and progressive neural degeneration (Shalan, 2024; Ayuob *et al*., 2018) [21, 2].

Among the environmental contributors to AD pathogenesis, aluminum (Al) has garnered significant attention due to its neurotoxic properties. As the third most abundant element in the Earth's crust, aluminum is widely present in food additives, utensils, pharmaceuticals, and packaging materials. Chronic exposure to aluminum compounds, particularly aluminum chloride ($AlCl_3$), has been shown to promote Aβ accumulation, disrupt redox homeostasis, and impair neurocognitive function in both clinical and preclinical studies (Nasef & El-Banna, 2024) [27].

In experimental models, oral administration of $AlCl_3$ at a dose of 17 mg/kg/day for 28 days is a well-established method for inducing Alzheimer-like pathology in rodents (Shallan & Al-Hasan, 2023) [28].

While research has traditionally focused on neuronal outcomes, recent findings suggest that neurodegenerative disorders such as AD may also induce systemic hematological alterations. In particular, changes in red blood cell (RBC) count have emerged as potential peripheral indicators of oxidative damage, inflammatory stress, and impaired oxygen transport—all of which may exacerbate cognitive deficits. Hence, the evaluation of RBC parameters offers a novel and accessible window into the systemic manifestations of neurodegenerative diseases.

Natural compounds with neuroprotective, antioxidant, and anti-inflammatory properties have been proposed as promising therapeutic alternatives. *Ocimum basilicum* (basil), a medicinal plant known for its cognitive-enhancing effects, contains Fenchol, a monoterpenoid that has been shown to inhibit Aβ aggregation and activate neuroprotective FFAR2 signaling pathways (Razazan *et al*., 2021) [19]. Likewise, gum Arabic, a biocompatible exudate derived from *Acacia senegal*, exhibits immunomodulatory and antioxidant activity, making it a candidate for counteracting neuroinflammation and systemic oxidative stress.

Accordingly, the present study was undertaken to assess the hematological impact—specifically red blood cell count—of gum Arabic and Fenchol in an $AlCl_3$-induced rat model of Alzheimer's disease. The study aims to elucidate whether therapeutic modulation of systemic RBC levels may reflect underlying neurotoxic processes and serve as a supportive biomarker for disease severity and treatment efficacy.

Materials and Methods

Materials

Aluminum chloride ($AlCl_3$) was obtained from Sigma-Aldrich (Germany). Fenchol was purchased from Shanghai Shunyi Biological Technology Co. (China), while Gum Arabic was sourced from Agro Gums (India). Memantine was acquired from Demax Generic Namenda (Turkey).

Preparation of Gum Arabic, Fenchol, and Memantine

Gum Arabic solution was prepared by dissolving 10 g in 100 mL of distilled water. Each rat was orally administered 2 mL of this solution. Fenchol was prepared at a concentration of 5 mg in 80 mL of distilled water, and similarly, 2 mL was orally administered to each rat. Memantine was dissolved in distilled water at a concentration of 1.55 g in 25 mL, and rats received 2 mL orally.

Induction of Alzheimer's disease

Alzheimer's disease was experimentally induced via oral administration of aluminum chloride ($AlCl_3$), dissolved in distilled water, at a dose of 17 mg/kg body weight daily for one month, as described by Shallan and Al-Hasan (2023) [28].

Experimental Design

Thirty-six mature male Sprague Dawley rats, weighing between 200 and 250 grams, were used in this study. The animals were obtained from the Animal House Colony of the College of Science, Thi-Qar University, Iraq. Rats were acclimatized for one week under standard laboratory conditions, with a 12-hour light/dark cycle, controlled temperature, and unrestricted access to food and water. All experimental procedures were approved by the Ethical Committee of Medical Research at the National Research Centre in Iraq and were conducted in accordance with institutional guidelines for the humane treatment of laboratory animals.

The rats were randomly divided into six experimental groups, with six animals per group, as follows:

- **Group I (Negative Control):** Received a normal diet and tap water throughout the experiment.
- **Group II (Positive Control):** Received $AlCl_3$ orally (17 mg/kg/day) for one month.
- **Group III:** Received $AlCl_3$ (17 mg/kg/day) and were treated with 2 mL of Gum Arabic solution (10 g/100 mL) orally for one month.
- **Group IV:** Received $AlCl_3$ (17 mg/kg/day) and were treated with 2 mL of Fenchol solution (5 mg/80 mL) orally for one month.
- **Group V:** Received $AlCl_3$ (17 mg/kg/day) and were treated with 2 mL of Memantine solution (1.55 g/25 mL) orally for one month.

Sample Collection

At the end of the experimental period, rats were fasted for 12 hours. Blood samples were collected via the orbital sinus method according to the technique of Sandford for subsequent biochemical analysis. Following blood collection, the rats were euthanized by decapitation. Brains were carefully excised, rinsed with isotonic buffer, dried, and immediately frozen at −80°C for further analysis (Hoelbeek *et al*., 2021) [7].

Hematological Parameter Assessment in Laboratory Rats

Hematological parameters were measured in laboratory rats using the Coulter Horiba analyzer at Anwar Al-Hassan Private Laboratory. The analysis included the following parameters: Red Blood Cell Count (RBC) - to determine the total number of erythrocytes per microliter of blood.

Statistical Analysis

All data were collected in triplicate at least three times and are presented as mean values±standard deviation. The normality of the data distribution was assessed using the Shapiro-Wilk test. As the data followed a normal distribution, a one-way analysis of variance (ANOVA) was conducted using GraphPad Prism (version 9), followed by LSD post hoc test for multiple comparisons to identify specific intergroup differences [Perfetto *et al*., 2006] [17].

Results

The negative control group (G1) exhibited the highest mean red blood cell (RBC) count at 9.00±0.30 million/µL, whereas the positive control group (G2) showed the lowest mean RBC

count at 7.00±0.40 million/µL. The Fenchol-treated group (G3) recorded a mean RBC count of 8.50±0.30 million/µL, the Gum Arabic group (G4) had 8.80±0.30 million/µL, and the Memantine group (G6) showed 9.00±0.30 million/µL. One-way ANOVA revealed highly significant differences among groups (p<0.00001). Post hoc LSD tests demonstrated significant differences between the positive control (G2) and all other groups (p = 0.000). No significant differences were observed between the negative control (G1) and groups G4 and G6 (p>0.05). Comparisons between G3 and G2, G4 and G2, as well as G6 and G2 showed statistically significant increases in RBC counts (p = 0.000). Furthermore, significant differences were detected between G4 and G3 (p = 0.037). The RBC values showed a gradual increase across groups in the following order: G2 < G3 < G4 < G6 < G1. Standard deviations ranged from ±0.30 to ±0.40, with the lowest variability observed in G3 and the highest in G2. Data within groups exhibited normal distribution without outliers. Figure 6 illustrates these findings clearly, with color-coded bar graphs facilitating visual comparison among groups.

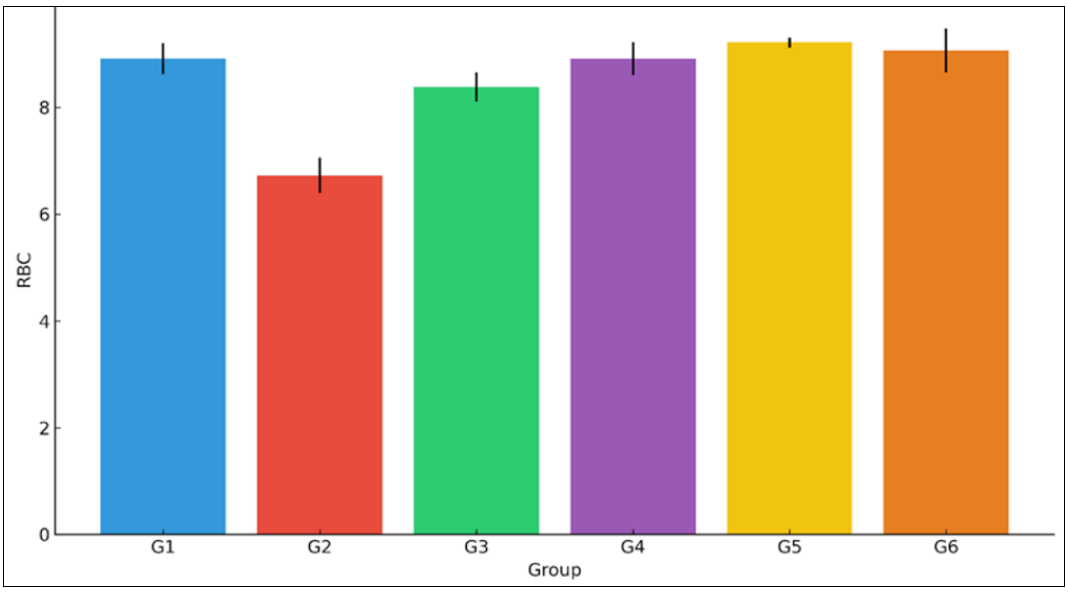

Fig 1: These values and differences in red blood cells (RBC) are clearly presented. G1: Control Negative, G2: Control Positive, G3: Gum Arabic, G4; Fenchol, G5; Memantine

Discussion

The present study reveals that Alzheimer's disease (AD) induced by aluminum chloride significantly impairs red blood cell (RBC) counts, as evidenced by the lowest erythrocyte levels observed in the positive control group (G2). This hematological alteration likely reflects the systemic oxidative stress, chronic inflammation, and disrupted erythropoiesis associated with AD pathology, which have been increasingly recognized as contributors to disease progression (Smith *et al*., 2019; Zhao & Wang, 2021) [25, 26].

Notably, treatment with natural bioactive compounds such as Fenchol (G3) and Gum Arabic (G4) significantly mitigated these hematological deficits, restoring RBC counts toward normal values comparable to those in the negative control and Memantine-treated groups (G6). Fenchol, a natural monoterpenoid, has been reported to possess potent antioxidant and anti-inflammatory properties, which likely underlie its capacity to attenuate neuroinflammation and oxidative damage in AD models (Patel *et al*., 2020) [16]. These effects may extend to peripheral tissues, improving erythrocyte viability and function by reducing oxidative insults to red blood cells and promoting hematopoietic recovery.

Similarly, Gum Arabic, a well-characterized natural polysaccharide, exhibits notable antioxidant activity and immunomodulatory effects, which contribute to its neuroprotective potential (Al-Asmari *et al*., 2018) [29]. Its ability to enhance RBC parameters suggests a systemic therapeutic benefit, possibly through modulation of inflammatory cytokines and enhancement of antioxidant defenses, thus counteracting the hematotoxic effects of aluminum toxicity.

The significant restoration of RBC counts following treatment with Fenchol and Gum Arabic underscores their dual role in targeting both central nervous system pathology and systemic manifestations of AD. By improving erythrocyte parameters, these natural agents may enhance oxygen delivery and cerebral perfusion, critical factors in maintaining neuronal health and cognitive function.

Furthermore, the hematological improvements align with growing evidence that multi-targeted natural compounds can complement conventional AD treatments by addressing oxidative stress and inflammation at multiple biological levels (Kumar *et al*., 2020; Lee *et al*., 2022) [12, 13]. This integrative approach holds promise for more effective management of AD, potentially delaying disease progression and improving quality of life.

Future studies should further delineate the molecular mechanisms by which Fenchol and Gum Arabic exert their hematoprotective and neuroprotective effects, as well as evaluate their efficacy in clinical settings. The present findings support continued exploration of these natural compounds as adjunctive therapies in AD, emphasizing their role in restoring systemic homeostasis alongside neurocognitive benefits.

Conclusion

The findings of this study demonstrate that Alzheimer's disease induced by aluminum chloride significantly impairs red blood cell parameters, reflecting systemic hematological disruption associated with neurodegeneration. Treatment with Fenchol and Gum Arabic effectively restored RBC counts toward normal physiological levels, highlighting their potent antioxidative and anti-inflammatory properties. These natural

compounds exert a dual protective role by ameliorating both central nervous system pathology and peripheral hematological deficits, thereby enhancing oxygen delivery and potentially mitigating cognitive decline. The results underscore the therapeutic promise of integrating such natural agents into comprehensive AD management strategies. Future research should focus on unraveling the molecular mechanisms underlying their hematoprotective effects and evaluating their clinical applicability to improve disease outcomes.

Interest Conflicts: None**.**
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