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Study the effect of Taraxacum officinale on the animal model of AlCl3 induce Alzheimer disease in rats.

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ABSTRACT

Background

Alzheimer's disease is characterized by progressive deficits in memory, cognition, and behavioral impairments that ultimately lead to dementia. Two main pathogenic processes contribute to its development: oxidative stress and neuroinflammation.

Objectives:

This study aimed to study the effects of *taraxacume officinallis* (TOE) on hippocampus of the Rats and attenuate the symptom of Alzheimer disease induced by AlCl3.

Materials and Methods:

In this case control research, 48 male albino rats in good health were split equally into six groups. The purpose of the three-month study was to assess how different therapies affected these rats. The experimental setup's specifics are as follows:

1. Healthy Control Group: This group was the initial control and was not given any medication.
2. AlCl3 Group: Each day, 10 mg/kg of AlCl3 was administered intraperitoneal
3. AlCl3 + Donepezil Group: This group was administered 10 mg/kg of AlCl3 IP daily in addition to 5 mg/kg of oral donepezil.
4. AlCl3 plus 200 mg/kg of TOE Group: The fourth group was administered an oral dose of 200 mg/kg of TOE extract in addition to 10 mg/kg of AlCl3 IP daily.
5. AlCl3 + TOE 400 mg/kg Group: The fifth group was administered 400 mg/kg of TOE extract orally daily in addition to 10 mg/kg of AlCl3 IP. 6. AlCl3 + TOE 600 mg/kg Group: The sixth

group was administered an oral dose of 600 mg/kg of TOE extract daily in addition to 10 mg/kg of AlCl3 IP each day.

Neurobehavioral analysis using an eight-arm maze was performed on day 31. Following the behavioral tests, rats were killed and remove their heads, and brain tissue samples were collected and homogenized to obtain tissue supernatant. Biochemical tests were then conducted on these samples to measure the levels of interleukin 6 (IL-6) and total antioxidant capacity (TAOC).

Results:

Comparing the effects of *TOE* with the aluminum chloride group, the main results indicate that rats in groups treated at different dose with *TOE,* spent less time finding food in the maze. This suggests that *TOE* effectively decrease Alzheimer's-like symptoms in rats.

Biochemical analysis further revealed that groups 5 and 6, treated with *TOE*showed a significant decrease in IL-6 levels and a significant increase in TAOC compared to the AlCl3 group. These findings support the hypothesis that (TOE) has shown potential anti-Alzheimer-like activity, particularly in reversing Alzheimer's disease symptoms induced by aluminum chloride in male rats through its anti-inflammatory and antioxidant properties.

In **conclusion**, this study experimentally demonstrates that *Taraxacum officinale* exhibits potential anti-Alzheimer activity by mitigating neuro inflammation and oxidative stress.

Key words: Alzheimer disease, Aluminum chloride ,Oxidative stress Taraxacume officinalle

Introduction: -

Alzheimer's disease (AD) is one of the most common neurodegenerative disease and accounts for more than 80% of dementia cases worldwide in elderly people. It leads to the progressive loss of mental, behavioral, functional decline and ability to learn. In AD, cerebral extracellular senile plaques and intra neuronal neurofibrillary tangles(NFT) are two of the major histopathological lesions leading to the progression of the pathogenesis in this disease(1). In 1906, during the autopsy of a, demented patient Alois Alzheimer made the first discovery of plaques and NFTs. In AD, plaques and NFTs are localized to areas in the brain that correspond to clinical symptoms β-amyloid plaques are thought to play the central role in AD pathogenesis (2).The development of AD has been associated with both neuro inflammation and oxidative stress. Neuro inflammation and oxidative stress are the two main

mechanisms in microglial cell activation that lead to progressive neuronal degeneration and provide a prospective therapeutic target in AD. The generation of free radicals such as reactive oxygen species (ROS), reactive nitrogen species (RNS), and inducible nitric oxide synthase (iNOS) as well as the activation and release of pro-inflammatory cytokines such as interleukin 1-β (IL-1β), interleukin 6 (IL-6), and tumour necrosis factor (TNF-α).

(3) all of which engage in the neuro inflammatory response and oxidative stress observed in AD. Aluminum is one of the major neurotoxins that used to induction of AD. The prolonged and extensive exposure of the aluminum toxicity is the root cause for the development of the AD that damages the neurons by increasing oxidative stress (4) To keep cells in a state of equilibrium, pro-oxidant and antioxidant mechanisms must be in balance. This equilibrium is upset in Alzheimer's disease, which results in inflammation and oxidative stress, both of which promote neurodegeneration.(5) The World Health Organization (WHO) oddly supports the use of traditional medicinal plants for both prevention and therapy. Because TOE has antioxidant qualities as well as the ability to scavenge radicals, inhibit enzymes, chelate metals, donate hydrogen, and quench singlet oxygen, it was selected for this investigation.(6) Because TOE has a higher phenolic and flavonoid content, it has excellent radical scavenging action and antioxidant capacity. The antioxidant characteristics of TOE are passed to the antioxidant status of tissues. (7) Therefore, the purpose of this work is to create an AlCl3-induced AD model in rats in order to assess the effects of TOE.

MATERIALS AND METHODS: -

Animals

In this experiment, 48 adult male Albino rats, weighing 200–300 grams, were included. The rats were kept in the University of Babylon's College of Medicine's Animal House. They were housed at 25 degrees Celsius, with a fourteen-hour day and ten-hour night cycle. They also had unlimited access to food and water. After two weeks of acclimatization, the animals were split into six groups at random. Based on the results of the experiment. The research was conducted from November 2023 to February 2024 at the University of Babylon's College of Medicine

Plant Preparation:

According to document No. 2063 dated 3/11/2024, the dried plant was authorized to be TOE with assistance from the College of Agriculture, Plant Department, Al-Qasim Green

University. In Babylon, Iraq, gather around 30 grams of air-dried TOE plant material from nearby marketplaces.

Then Grind the dried plant material into a fine powder and dissolve the powdered plant material in 300 ml of ethanol (75%). Perform the extraction using a Soxhlet extractor for 4 hours. Maintain the temperature between 80°C and 85°C during the extraction process. After extraction, remove the solvent from the extract using an oven. Store the resultant extract at -20°C until further use. We dilute 1000 mg in 5 ml DW then we take 0.25 ml for group 4: 0,5 ml for group 5 and 0.75 ml for group 6.

AlCl3 dilution:

A stock solution of 300 mg of AlCl3 power was mixed with 15 ml of D.W., and then 1 ml of this stock solution was added to 3 ml of D.W. Finally, 0.5 ml of this solution was given intraperitoneal to each rat.

Donepezil dilution:

broke up 5 mg of the tablet, added 4 ml of D.W, and then gave the rats 1 ml of the resulting solution orally once a day for 30 days.

Study Design:

Group I:(normal control group), rats were administered normal saline intraperitoneal (i.p.) once daily for 30days.

Group II: (Alzheimer's induced group), rats were received AlCl3 (10 mg/kg, i.p.) once daily for 30 days.

Group III: (donepezil group), rats were undergoing same procedure as group II plus they were received donepezil (5 mg/kg) orally by gastric gavage for 30days at the same time.

Group 1V: *(taraxacume officinalle* group 200 mg): rats were undergoing same procedure as group II plus they were received 200 mg /kg orally by gastric gavage for 30days at the same time.

Group V: (*taraxacume officinalle* group 400 mg); rats were undergoing same procedure as group II plus they were received 400 mg /kg orally by gastric gavage for 30days at the same time.

Group V1: (*taraxacume officinalle* group 600 mg): rats were undergoing same procedure as group II plus they were received 600 mg /kg orally by gastric gavage for 30 days at the same time.

On day 31, twenty-four hours following the final dosage, behavioral assessments were conducted.

to compare Alzheimer development and treatment effectiveness. Each animal was placed within the center of eight arm maze consistently contains food. Animals undergo training to learn that only one arm of the maze consistently contains food. Then each animal euthanized. Their skulls were dissected, her brain was gently extracted from the rat skull, and each brain was divided into two halves. One half of the rat's brain from each group was dissected, rinsed with normal saline, weighed, and placed in sterilized Eppendorf tubes. These samples were subsequently frozen at -20°C for preservation and later homogenized in phosphate buffer with a pH of 7.4 for further biochemical analysis**.**

Behavioral Test:

The eight-arm radial maze serves as a widely employed behavioral task in neuroscience, particularly for studying spatial learning and memory in rodents. Constructed by the researcher using wood, the maze dimensions are 50 cm in length, 35 cm in height, and 11 cm in width, featuring a central area measuring 40 cm. on the day 31 rats are placed within the center of the maze and are allowed to explore in search of food located at the end of one of the maze's arms. The time taken for an animal to locate the arm leading to food serves as a measure of its spatial learning and memory abilities(8).

Assessments of IL6 and TOAC: - The enzyme-linked immunosorbent assay (ELISA) was utilized to evaluate the levels of IL6 and TOAC.

Statistical Analysis: -

The results of the study are statistically tabulated using the SPSS. The statistical equations used to find the significant differences are One-way ANOVA and the post hoc test.

Ethical approval: -

In order to acquire this authorization, on August 8, 2023, a local ethics committee reviewed and approved the consent form, subject information, and study protocol under reference number 4-17.

RESULTS: -

Eight arm mazes:

There was a highly significant increase in the time taken to find food in the AlCl3 group compared to the control group ($p < 0.05$). Treatment with donepezil, a well-established

Alzheimer's medication, significantly reduced the time to find food compared to the Alzheimer's model group ($p < 0.05$). Additionally, administration of TOE (presumably a test substance) at doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg significantly reduced the time to find food compared to the Alzheimer's group ($p < 0.05$).

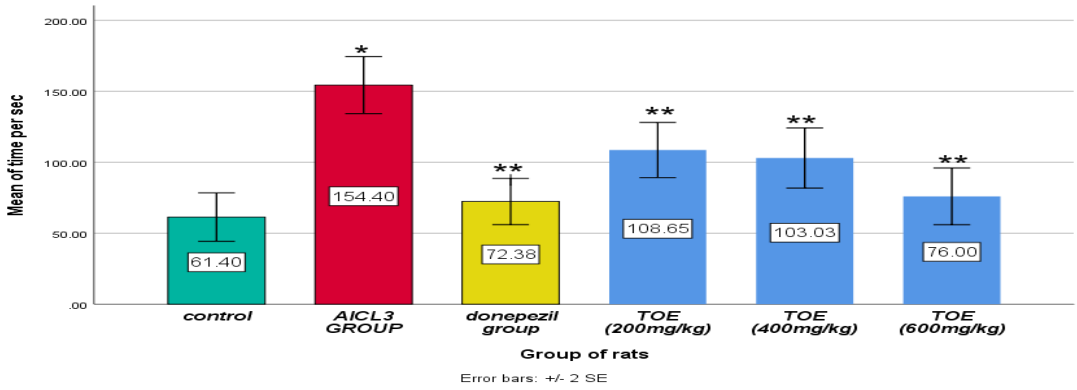

Figure3.8 Mean of time in eight arm maze that show the effect of *T. officinale* extract (TOE) on spatial learning and memory in the eight-arm maze test.

* $p<0.05$ compared to the control group; ** $p<0.05$ compared to the Alzheimer's model group.

Effect of *T. officinalis* Extracts on the Biochemical investigations:

Interleukin -6 (IL-6):

The AlCl3 group had a significantly elevated IL-6 levels as compare with the control group ($p<0.05$). indicating increased systemic inflammation in the Alzheimer's model. Treatment with donepezil significantly reduced plasma IL-6 levels ($p<0.05$) compared to the Alzheimer's model group, demonstrating its anti-inflammatory effect. Similarly, administration of TOE at a dose of(400mg/kg) and (600 mg/kg) also led to a significant reduction in plasma IL-6 levels ($p<0.05$) compared to the Alzheimer's model group. However, and although the TOE (200 mg/kg) group showed trends towards decreased IL-6 levels, the differences were not statistically significant compared to the Alzheimer's model group.

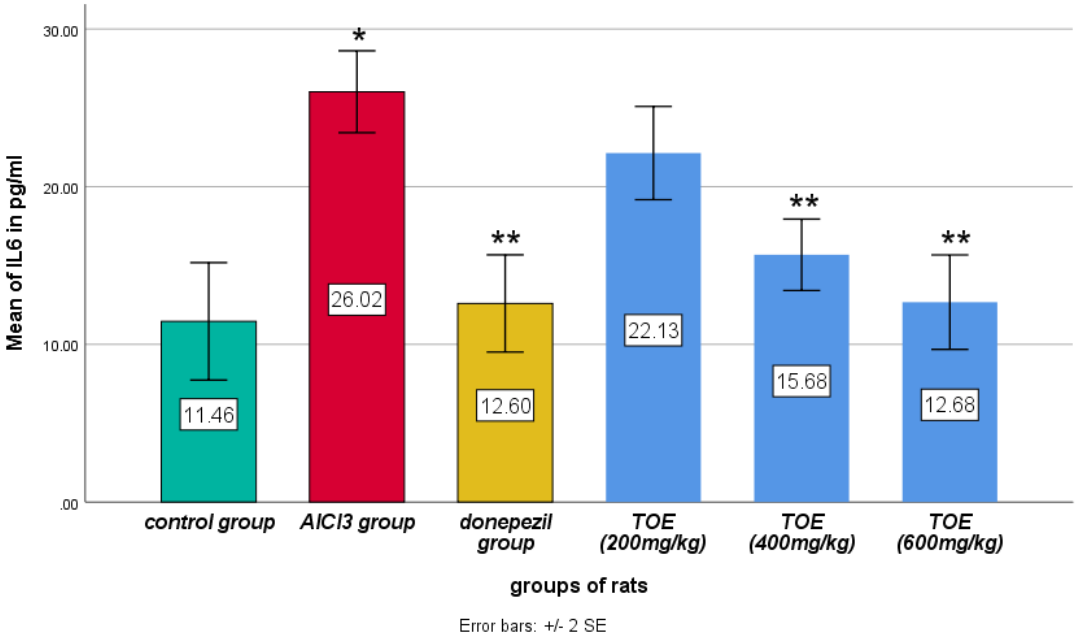

Figure 3.10. Effect of *T. officinale* extract (TOE) on the levels of IL-6 in rats.

p<0.05 compared to the control group; ** p<0.05 compared to the Alzheimer's model group.

Total antioxidant capacity (TAOC): -

Total antioxidant capacity (TAOC) levels were significantly reduced in the Alzheimer's model group compared to the control group ($p < 0.05$), indicating a decrease in overall antioxidant defense in the Alzheimer's model. Administration of TOE at doses of 400 mg/kg and 600 mg/kg significantly increased TAOC levels ($p < 0.05$) compared to the Alzheimer's model group, suggesting their potential to enhance antioxidant capacity.

Furthermore, there was a significant increase in TAOC levels in the brains of rats treated with donepezil compared to the Alzheimer group ($p < 0.05$).

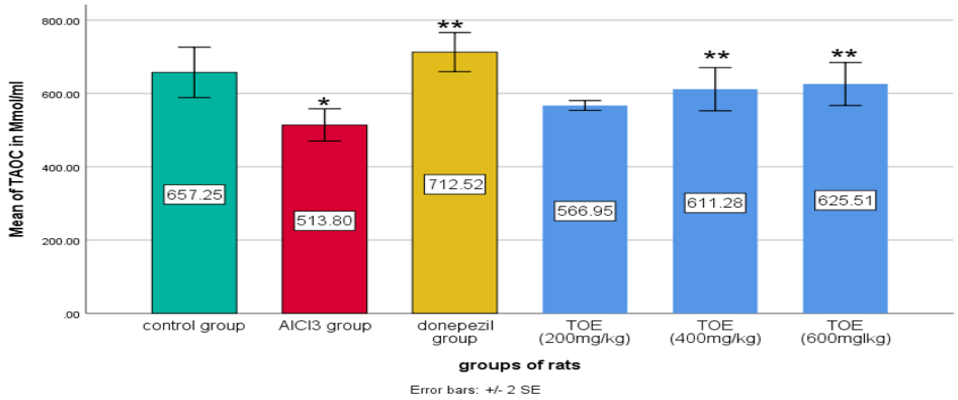

Figure 3. Effect of *T. officinale* extract (TOE) on rat's brain level of TAOC

* p<0.05 compared to the control group; ** p<0.05 compared to the Alzheimer's model group

DISCUSSION: -

The exact mechanism behind AD pathophysiology is not fully understood, oxidative stress and neuroinflammation are considered key factors in the development and progression of the disease. These processes can lead to neuronal damage and contribute to the neurodegenerative cascade observed in AD (9) Despite the fact that AD has no known cure, research is being done to create treatment strategies that can stop or delay the disease's progression. Unfortunately, at the moment, only small-scale clinical trials or animal research are used to develop such medicines.(10). Due to its neuroprotective properties, TOE may aid in preventing oxidative brain damage. This study was therefore created to look into TOE potential anti-Alzheimer impact in rats.

The result show that AlCl3 groups significantly longer time to find food in the maze compared to control groups, indicating impaired spatial learning and memory. This finding aligns with the overall conclusions of the study regarding the impact of Alzheimer's induction on cognitive function in rats(11). AlCl3 is absorbed into the blood-brain barrier (BBB) and accumulated in the brain, primarily in the hippocampus, responsible for memory and learning. Prolonged accumulation of aluminum (Al) causes neurotoxicity by the development of neurofibrillary tangles and amyloid aggregates(12)The elevation of oxidative stress and neuroinflammation induced by AlCl3 can further exacerbate memory deficits by impacting neuronal function and synaptic plasticity, crucial for memory formation and retrieval (13) after *Taraxacume officinalle* treatment the time of the rat to find food was significantly decreased compare with the AlCl3 group this result suggest that *T.officinallis* has neuroprotective and antioxidant properties. The total antioxidant activity has been increased, and the extract decreased the reactive oxygen species (ROS) production (6)

Compared to animals treated with Alcl3, the donepezil group a well-established Alzheimer's medication, significantly reduced the time to find the food this might be because of the increased the levels of acetylcholine (ACh) in the brain of the rat because of Donepezil is an acetylcholinesterase inhibitor (AChEI**)** (14)

IL-6 is a classical proinflammatory cytokine that plays essential roles in the development, differentiation, and degeneration of neurons. It can trigger neuronal degeneration and cell death in neurodegenerative disorders like Alzheimer's disease (15)**.**The IL-6 level increased significantly in the aluminum chloride induced group compared to the control group, which

agrees with the previous study by (16)**.** AlCl3 exposure leads to neuro inflammation, which is characterized by the activation of immune cells, such as microglia, and the release of pro-inflammatory cytokines like IL-6. This neuro inflammatory response contributes to the progression of Alzheimer's disease (17)**.**Administration of TOE after, at a doses of (400mg/kg)and (600 mg/kg) also led to a significant reduction in IL-6 levels compared to the Alzheimer's model group. this result suggest that (TOE) have been shown to inhibit the production of pro-inflammatory cytokines, including IL-6, in a dose-dependent manner (18) TOE leaves had the strongest anti-inflammatory effects because they downregulated pro-inflammatory cytokines, NO, PGE2, and iNOS and COX-2 expressions. Lutein and cichoric acid, which prevent NF-κB pathway phosphorylation, are the primary ingredients in TOE that have an anti-inflammatory impact.

(19) The third ingredient, taraxasterol, reduces inflammation by inhibiting the NF-κB pathway, It is believed that NF-κB is an important target for anti-inflammatory treatments.(20) IL_6 level was also significantly decrease in the donepezil rats group as compare with Alzheimer group, which agrees with the previous study by (21). This suggests that donepezil may have anti-inflammatory properties that could be beneficial in the context of AD.

The Total Antioxidant Capacity (TAOC) plays a crucial role in neurodegeneration by serving as a marker of the competence of the anti-oxidative system in various neurodegenerative diseases. In conditions such as Alzheimer's disease, the antioxidative system's effectiveness, as reflected by TAOC, is a significant factor in disease progression (22).

Total antioxidant capacity (TAOC) levels were significantly reduced in the Alzheimer's model group compared to the control group, suggesting a decrease in overall antioxidant defense in the Alzheimer's model. this agree with the study (23) Aluminum leads to the generation of free radicals in the brain, which could lead to a kind of neurodegeneration comparable with those observed in AD .This is especially so because of the high oxygen turnover and enhanced ROS level in the brain thereby leading to reduced antioxidant activities, resulting in toxicity of Aβ and later development of neurodegeneration (24) Repeated administration of AlCl3 raised the level of NO in the brain, an observation consistent with previous studies(25) This NO is implicated in memory and learning processes and its neuroprotective activities in the CNS There are three isoforms of nitricoxide synthesizing enzyme—neuronal nitric

oxide synthase (NOS)—nNOS, endothelial nitric oxide synthase—eNOS and inducible nitric oxide synthase— iNOS, and expression of all three is deranged during aluminum intoxication. This leads to a rise in the level of NO which is implicated in neurodegeneration through diverse mechanisms including oxidative and nitrative stress (26) Administration of TOE at doses of 400 mg/kg and 600 mg/kg significantly increased TAOC levels compared to the Alzheimer's model group, indicating their potential to enhance antioxidant capacity. This agree with the study(7) *Taraxacum officinale* contains compounds like polyphenols, vitamins, terpenes, chlorogenic acid, and taraxasterols that contribute to its antioxidant potential. Polyphenols act as antioxidants by scavenging free radicals and reactive oxygen species (ROS), reducing oxidative stress, and protecting cells from damage (27) Also there is significant increase in the TAOC in donepezil rat group compare with Alzheimer induced group and this result is agree with the study of(24) Donepezil treatment reduced reactive oxygen species (ROS) levels in the cortex and hippocampus of rats with vascular dementia(14)

CONCLUSIONS

The principal finding of this research is that T. Officinale is a safe and useful plant that reduces the symptoms of AD. The results of the study support this conclusion by demonstrating that this plant effectively raises TAOC levels and decreases IL6 levels in brain tissues. Therefore, it is strongly advised that individuals with Alzheimer's disease use T. Officinale as a dietary supplement. Moreover, its extract can be used in conjunction with anti-alzheimer medications as a co-treatment. Lastly, it is advisable to look into even larger concentrations of T. officinale extract and/or use more rats during longer experimental periods in order to further identify the plant's primary active ingredients.

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