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Evaluation of effectiveness of ginkgo biloba aqueous extract on some physiological parameters and cerebrum tissue in male albino rats treated with aluminum chloride

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***Abstract*---**The current study aims to know the protective role of the aqueous extract of the *Ginkgo biloba* against cerebrum damage induced by Aluminum chloride (AlCl3) in male rats. 40 adult male rats were used, which were randomly divided into four equal groups (10 animals/group), the first group (G1) administered 1 ml/kg of tap water, the second group (G2) administered 10 mg / kg of AlCl3, the third group (G3) was administered 10 mg/kg of aqueous extract of the *Ginkgo biloba* ,while the fourth group animals (G4) were administered 10 mg/kg of AlCl3 and 10 mg/kg of *Ginkgo biloba* aqueous extract orally and daily for 30 days. Fasting blood samples were collected after the end of the experiment to study the following parameters: concentration of soluble acetylcholinesterase AChE(G1), membrane bound acetylcholinesterase AChE (G4), Neuronal Mitochondrial ATPase (Nmit ATPase), Cytochrome C oxidase (COX) , Tumor Necrosis Factor-α (TNF-α). The results showed that oral administration of AlCl3 caused significant increase ($P<0.05$) in the concentration of AChE (G1), TNF-α, and significant decrease ($P<0.05$) in the concentration of AChE (G4), Nmit ATPase and COX compared with the control group. Group treated with *Ginkgo biloba* showed significant increase ($P<0.05$) in the concentration of Nmit ATPase, COX with a significant decrease ($P<0.05$) in AChE (G1), with no significant difference ($P<0.05$) in of AChE (G4) and TNF-α. The oral administration of AlCl3 with aqueous extract of the *Ginkgo biloba* caused no significant differences ($P<0.05$) in the concentration of AChE (G1), AChE (G4), Nmit ATPase, COX and

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TNF -α. Histological examination showed that oral administration of $AlCl_3$ for 30 days caused cerebrum damage with clear degenerative changes in the nervous tissue with H&E dye, while there was a clear appearance of Aβ plaques spread in the cerebrum after treatment with silver stain. It is concluded from the current study that Aluminum Chloride ($AlCl_3$) causes clear pathological changes in the cerebrum tissue and confirms that treatment with aqueous extract of ginkgo (has a protective role against $AlCl_3$-induced nervous tissue damage in male rats.

***Keywords*---**Cerebrum, Alzheimer, Beta Amyloid, *Ginkgo biloba*, Acetylcholine, Aluminum Chloride.

Introduction

The environmental pollution is one of the main problems that people focus on due to its repercussions on various economic and social activities, as the environment is exposed to various types of pollutants, which negatively affects many environmental characteristics (Yadav et al, 2021), industrial waste is one of the most important sources Pollution has been highlighted by many environmental protection associations and the World Health Organization (WHO), Aluminum is one of the metals that contribute to environmental pollution (Rodríguez & Mandalunis, 2018; Artiola, 2019). It is widely used in the food industry, as it is used in the manufacture of cans that are used to package foodstuffs, soft drinks, cosmetics, cleaning, pharmaceuticals, toothpastes and household pots (Alasfar & Isaifan, 2021). The liquid waste from factories contributes to the pollution of groundwater and soil, so we find that Aluminum is present in the tissues and roots of plants, which negatively affects the growth of these plants (Mold et al, 2019).

Aluminum enters the body through the alimentary tract by food and drinking water, or through the respiratory system by inhaling Aluminum dust, which leads to many lung disorders, or through skin contact with cosmetics and medicines containing Aluminum hydroxide (Soni *et al* ,2001). Brain and the kidneys are the most organs affected by the accumulation of this element, in addition to other organs, including the testes and lungs (Wang et al 2020). It was found that permanent exposure to high concentrations of Aluminum leads to many health problems, the most important of which are central nervous system atrophy, memory loss, impairment of attention also fibrosis and atrophy of lung cell, damage and destruction of liver cell and anemia. The permanent exposure to Aluminum may be associated with the risk of Alzheimer's disease (Missel et al, 2005).

The WHO estimated that 80% of people around the world depend on medicine herbal as an essential part of their health (Dekanski et al 2009). *Ginkgo biloba* was used in traditional Chinese medicine more than 5,000 years ago, and through studies, it was found that the ginkgo plant contains high levels of flavonoids and terpenoids, compounds known for their powerful antioxidant effects. *Ginkgo biloba* is a powerful antioxidant and is known for its ability to

stimulate blood circulation, and the herbal extract can reach the narrowest blood vessels in order to supply oxygen to the heart, brain and all other parts of the body, and this helps to perform mental functions, as the ginkgo plant contains high levels of flavonoids and terpenoids. Compounds known for their powerful antioxidant which reduce the effects of free radicals, they are highly reactive molecules that are produced in the body during normal metabolic functions, such as turning food into energy or removing toxins. (Ansley, 2018; Dziwenka & Coppock, 2021)

Materials and Methods

Forty white male rats were used, divided into four groups (10 animals/group): the first group was dosed with 1 ml of tap water as a control group, the second group was orally dosed with 10 mg/kg of $ALCL_3$, the third group was orally dosed with 10 mg/kg of aqueous extract of *Ginkgo biloba*, while the fourth group orally dosed with 10 mg/kg of $ALCL_3$ + 10 mg/kg of aqueous extract of *Ginkgo biloba*.

Fasting blood samples were drawn after the animals were starved overnight, after a 30 days, 5 ml of blood was withdrawn directly from the heart, the serum was separated by a centrifuge at 3000 rpm for 15 minutes to measure the following parameters: concentration of AChE(G1), AChE (G4), Nmit ATPase, COX and TNF-α . Plasma concentration of AChE(G1), AChE (G4) , Nmit ATPase, COX and TNF-α were measured by ELISA using commercially available kits.

Preparation of the aqueous extract of *Ginkgo biloba*

Dry leaves of the *Ginkgo biloba* were purchased from the local markets in the holy Karbala - Iraq. They were ground by the electric mill to obtain a fine powder used in the extraction. The aqueous extract of the plant was prepared by soaking 50 gm of *Ginkgo biloba* powder in 500 ml of distilled water for 24 hours and stirring the soaked with wooden sticks .Then it was poured into glass containers and then dried in an oven at 40° C. The dry matter was skimmed off to obtain the extract (green color) that was kept in a glass container in the refrigerator. Then administer the extract orally using an oral dose prepared for this purpose. (Bako, 2010)

Result and Discussion

The results showed that oral administration of $AlCl_3$ caused significant increase ($P<0.05$) in the concentration of AChE (G1), TNF-α, and significant decrease ($P<0.05$) in the concentration of AChE (G4), Nmit ATPase and COX compared with the control group .

Acetylcholine (ACh), is a cholinergic neurotransmitter important for learning and memory, AChE is the main enzyme to break down ACh in the synaptic cleft. Several studies have confirmed that AchE molecular forms are affected unequally in pathological conditions, and that changes in the concentration of AchE molecular forms reflect many changes that occur in the brain (García-Ayllón *et al*, 2011; Saez-Valero *et al*, 2000a). AchE(G1) are the most abundant type in the brain and are critically important in the glycosylation process. By adding different

moieties of carbohydrates to AchE from different tissues and from the same tissue, and the defect in this process reflects the disturbances that occur in the brain, and thus causes changes in the molecular forms of AchE (Darreh-Shori, 2006; Saez-Valero *et al*, 2000b). the significant increase (P<0.05) in the concentration of AchE enzyme may be due to oral administration of AlCl3. These changes are due to Al which is a highly toxic substance that affects cholinergic neurons and affects the need for The cerebral hematopoietic system leads to changes in noradrenaline and the cholinergic neurotransmitter, which leads to the destruction of brain cells by generating a number of free radicals due to oxidative stress, causing cell injury and enzyme leakage (Sagae *et al*, 2011) , this increase in AchE activity is due to either the dangerous effect of AlCl3 or the rupture of the cell membrane caused by increased lipid peroxidation, and the decrease in the concentration of AchE (G4) enzyme may be due to the loss of cholinergic neurons (Fishman *et al* , 1986) The brain cholinergic system plays a major role in modulating learning and memory. Decreased acetylcholinesterase (G4) activity and acetylcholine levels in the hippocampus and cerebral cortex have been associated with loss of cognitive function in Alzheimer's patients (John *et al*, 2015), and it was proven that Aluminum reduces the activity of the acetylcholinesterase enzyme in rats and causes damage to the cholinergic centers in the cerebral cortex and memory area (Platt *et al*, 2001).

Table (1) Effect of Aluminum chloride and aqueous extract of *Ginkgo biloba* on enzymatic parameters in white male rats

	AchE (G1) Unit/ml	AChE(G4) Unit/ml	MiT ATPase ng/ml	CYT c xidase units/ml	TNF α mg/dl
G1 Control	9.21± 0.16 AC	8.96 ± 0.15 A	20.35 ± 0.41 A	220.63 ± 0.57 A	2.67 ± 0.15 A
G2 10 mg/kg $AlCl_3$	10.04±0.16 B	5.83 ±0.26 B	8.12± 0.49 B	192.83 ± 0.68 B	4.68 ± 0.39 B
G3 10 mg/kg GBE	8.84 ± 0.14 C	9.06 ±0.17 A	21.69 ± 0.36 C	223.02± 0.44 C	2.67 ± 0.12 A
G4 10 mg/kg $AlCl_3$+10 mg/kg GBE	8.64 ±0.14 C	8.66± 0.15 A	19.22 ± 0.37 A	218.91 ± 0.43 A	2.87± 0.14 A
LSD	0.4479	0.5449	1.1978	1.5579	0.6728

mean ± standard error, n = 10/group, the different letters indicate the presence of significant differences vertically under the probability level P<0.05

Mitochondria are responsible for many metabolic pathways, and neurons have a high density of mitochondria, which explains why they are particularly prone to energy-dependent defects caused by mitochondrial abnormalities (Chen et al, 2013). Alteration in mitochondria as a result of exposure to toxins and an imbalance between the production of free radical species and the activity of antioxidant systems are factors that are involved in the development of neurodegenerative disorders and aging. In fact, the occurrence of oxidative stress and an increase in redox processes affect the function of mitochondria themselves. Aluminum has a high ability to change the respiratory chain processes in mitochondria of brain. However, the extent and nature of this change appears to be variable depending on the conditions of exposure to this element.

(Iglesias-González *et al*, 2017). Studies have shown that exposure to AlCl3 causes the generation of free radicals and the occurrence of oxidative stress, which in turn leads to functional changes such as depletion ATP in the neuron, (Takahashi et al 2009).

The increase in TNF-α concentration may be due to AlCl3-induced neurotoxicity, which causes the accumulation of many abnormal proteins, such as beta amyloid, especially Aβ-42 and free radicals including (NO, ROS and RNS), which cause cellular stress and inflammation. It was found that the accumulation of Aluminum is accompanied by the release of cytochrome c from mitochondria, which leads to increased production of free radicals that leads to oxidative stress by increasing Oxidative damage to biomolecules and increased production of proinflammatory cytokines. Moreover, it leads to increased gene expression of TNF-α which plays an important role in the neurodegeneration of neuroinflammation associated with Aluminum -induced neurotoxicity (Kawahara, 2005),

Group treated with *Ginkgo biloba* showed significant increase ($P<0.05$) in the concentration of Nmit ATPase, COX with a significant decrease ($P<0.05$) in AChE (G1), With no Significant difference ($P<0.05$) in of AChE(G4) andTNF-α, .The oral administration of AlCl3 with aqueous extract of the *Ginkgo biloba* caused no significant differences ($P<0.05$) in the concentration of AChE(G1), AChE(G4), Nmit ATPase, COX and TNF -α. *Ginkgo biloba* extract has a direct effect on the cholinergic system responsible for perpetuating the cognitive functions of the brain, through its role in activating the muscarinic acetylcholine receptor, which affects the transmission of information between nerve cells through its role in increasing the activity of the neurotransmitter acetylcholine ACh and reducing the activity of AChE (Marucci et al 2021) It is known that extracellular choline levels depend on the metabolism of choline-containing phospholipids. Choline levels are increased in several pathological conditions including ischemia and excitotoxicity, due to activation of phospholipase A2 and hydrolysis. For phosphatidylcholine, several studies have confirmed that *Ginkgo biloba* extract is characterized by its ability to maintain the normal structure of the phosphatidylcholine membrane and maintain the normal functions of mitochondria (Mdzinarishvili et al, 2012), *Ginkgo biloba* (GBE) works to scavenge free radicals as a result of containing many active compounds, including ginkgolide, the flavonoid, which acts as electron donors and thus reduces oxidative damage to neurons (Zuo et al, 2017), and it has been found to improve learning and treat memory impairments caused by AICI3 in mice due to its inhibition of AChE enzyme expression in the Hippocampus (Qi-Hai et al, 2006) In addition, GBE has the ability to reduce Aβ plaque production by modulating the Amyloid Precursor Protein (APP) pathway and thus has a role in protecting neurons from the toxicity of these plaques that malfunction in Synapse functions (Bastianetto & Quirion, 2002), Several studies have shown that *Ginkgo biloba* plays an effective role in supporting mitochondrial functions through its role in protecting against changes in oxidative phosphorylation, which leads to an increase in the level of ATP production (astre et al, 1998), in addition to its role in activating the electron transport chain complexes. cytochrome c oxidase, cytochrome c reductase and NADH, as well as maintaining intracellular calcium balance (Kwon et al, 2004), in addition to GBE's role as an antioxidant and has

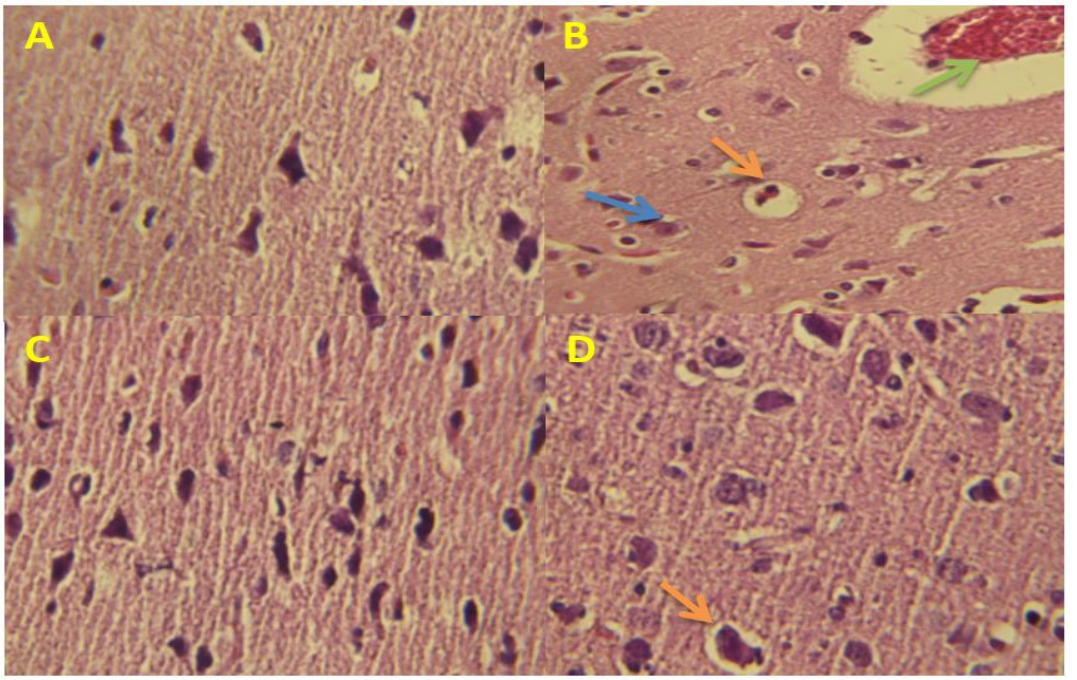
protective effects for various cell types, including neurons and astrocytes, as it works to protect mitochondria from While it captures many free radicals, including hydroxyl radical (OH-), suer oxide (O2-), nitric oxide (NO), hydrogen peroxide (H_2O^2) and peroxynitrite (ONOO) (Cheung, & Yew, 2020).

The results of the current study showed that there were no significant differences ($P<0.05$) in the average concentration of TNF-α after oral administration of *Ginkgo biloba* , the reason may be due to Anti-inflammatory effects of ginkgo Inflammation is closely related to oxidative stress and in the inflammatory response. Studies have shown this effect of GBE and its role in protecting the memory area against neuronal death. In conjunction with a significant decrease in the number of microglia and small astrocytes, flavonoids can regulate enzyme-mediated signaling pathways. Tyrosin kinase, phosphoinositide 3-kinase, protein kinase C, and mitogen-activated protein kinase thus inhibit microglia cytokine activation and iNOS induction. Inflammatory conditions such as TNF-α (Cheung & Yew, 2020)

Histological examination showed that oral administration of AlCl3 for 30 days caused cerebrum damage with clear degenerative changes in the nervous tissue with H&E dye, while there was a clear appearance of Aβ plaques spread in the cerebrum after treatment with silver stain. The occurrence of these tissue changes may be due to the ability of Aluminum to cross the blood-brain barrier (Abdul-Rassoul et al, 2009). The reason for the appearance of Aβ plaques is that AlCl3 causes an imbalance in the intracellular calcium (Ca+) balance, so it causes the depolarization of mitochondria, leading to an increase in the generation of ROS (O2-, H2O2 -,OH), which in turn reduces the activity of Cytochrome oxidase and reduces the production of ATP energy, as well as causes an increase Activation of the amyloidogenic and nonamyloidogenic APP pathway by activation of α-secretases, γ-secretases and β-secretases that affect the APP protein and cause the generation of increased amounts of Aβ plaques (Kumar & Singh, 2015; Itkin *et al*, 2011; Zatta *et al*, 2000), It was also found that an increase in Aluminum in the synaptic cleft causes an imbalance in the ionic and affects the cholinergic system, causing a decrease in the concentration of Ach and an increase in the concentration of AchE, leading to increased formation of Aβ plaques and an increase in protein phosphorylation. P-tau in nervous tissue (Silveyra *et al* 2011; Dey & Singh, 2022) In addition, it was found that Aβ causes a defect in the synthesis of neurons due to its ability to increase the production of ROS in the nerve cell, and the occurrence of cellular calcium imbalance, in addition to that it affects the activity of a large number of enzymes, including the flavoprotein-linked enzyme And NADPH oxidase leads to an increase in the generation of free radicals, a decrease in GSH, and a defect in the mitochondrial respiratory chain, and thus the occurrence of cell death (Sadigh-Eteghad *et al*, 2015; Abramova *et al*, 2004).

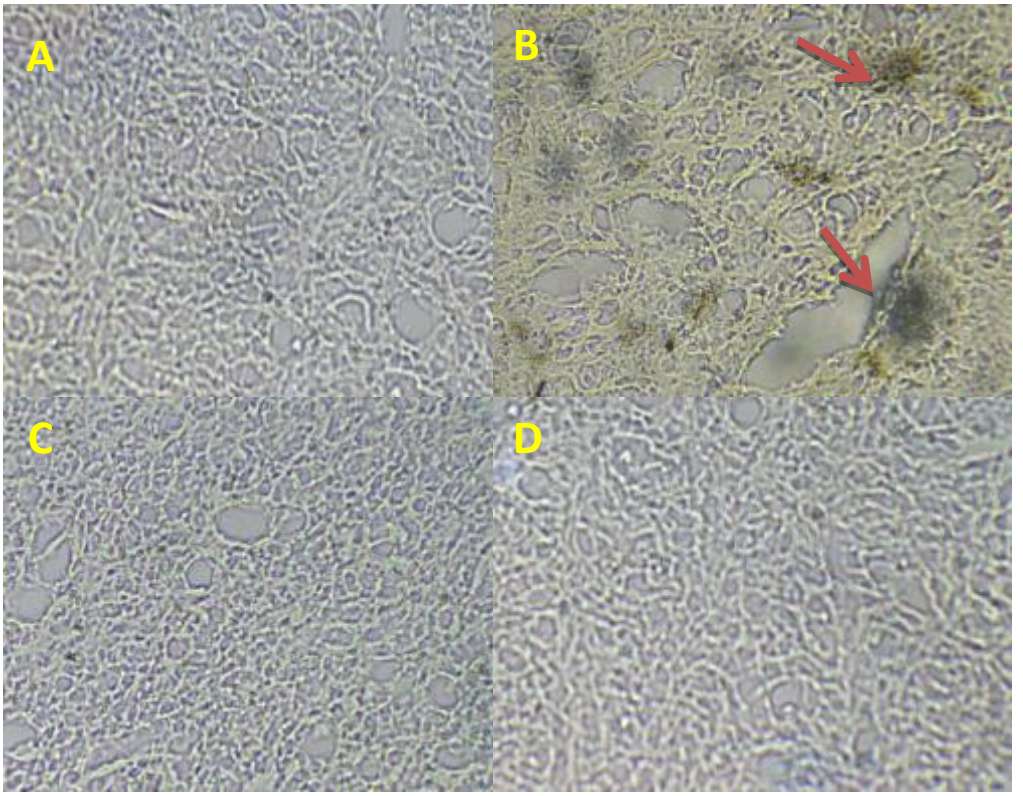
Histological Examination of tissues for rats treated with 10 mg/kg of AlCl3 with 10 mg/kg of *Ginkgo biloba* showed no deposition of Aβ plaques in cerebrum tissue and normal appearance of neurons compared to the control group. This is due to the role of *Ginkgo biloba* in preventing the formation of Aβ plaques, many studies have confirmed that GBE inhibits the production of Aβ plaques by altering the APP protein pathway through the secretory pathways (Cheung, & Yew, 2020).

Several studies have confirmed the effectiveness of GBE in reducing Aβ toxicity, and reducing oxidative stress, maintain the concentration of cellular calcium and protect the cellular DNA from damage thus maintain the normal structure of the neuron (Bastianetto & Quirion, 2002), it was also found that it inhibits the inflammatory pathways caused by Aβ plaques and reduce its effect on the nervous tissue through the inhibition of IL -6 and TNF-α and thus reduce the production of inflammatory cytokinins (Cheung, & Yew, 2020), in addition, it was found that GBE has the ability to remove Aβ plaques formed by chloride Aluminum in the nervous tissue also shows strong activity against Aβ plaques, increasing its disposal across the blood-brain barrier, in addition to stimulating the immune response, degrading Aβ and activating glial cells (DeFeudis &Drie, 2000). GBE increases the activity of antioxidant enzymes and increases the gene expression of glutamatecysteine ligase, which is the main enzyme for building GSH, leading to a decrease in ROS production (Zhou & Chen et al. 2017), and it was found that GBE stimulates neurons to remove proteins affected by oxidative stress and get rid of Aβ plaques (Singh et al, 2019), in addition to the antioxidant property that GBE possesses, which enables it to maintain the normal appearance of tissues (Zuo, et al 2017).



Figure(1) shows a histological section of white male rats cerebrum stained with hematoxylin and eosin (H&E 400X) (A) control group in which the normal structure of cerebrum tissue (B) group of rats that were dosed of 10 mg/kg of AlCl3 for 30 days, it show clear degenerative changes in the cerebrum tissue with blood congestion (green raw) with appearance of gaps in the nervous tissue and (orange raw) the dissolution of the nuclei of neurons (blue raws), (C) cerebrum of rats that were dosed with 10 mg/kg of GBA the normal structure of cerebrum tissue is observed, (D) rats that were dosed with 10

mg/kg of AlCl3 + 10 mg/kg of GBA , it is noted that there are few gaps in the nervous tissue (orange raw).



Figure(2) shows a histological section of white male rats cerebrum stained with silver stain (400X) (A) control group in which there was not observed of Aβ plaques (B) group of rats that were dosed of 10 mg/kg of AlCl3 for 30 days, clear appearance of Aβ plaques (red raw), (C) cerebrum of rats that were dosed with 10 mg/kg of GBA with no Aβ plaques observed, (D) rats that were dosed with 10 mg/kg of AlCl3 + 10 mg/kg of GBA , there was not Aβ plaques observed.

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