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Estimation the protective role of *Ginkgo biloba* aqueous extract on some hormones and cerebellum 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 cerebellum 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* (GBE), 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 Cortisol, Adrenocorticotropin hormone (ACTH) and growth hormone (GH). The results showed that oral administration of AlCl3 caused significant increase ($P<0.05$) in the concentration of Cortisol, ACTH and significant decrease ($P<0.01$) in the concentration of GH compared with the control group. Rats treated with GBE and that administration of AlCl3 with GBE showed no Significant difference ($P<0.01$) in the concentration of Cortisol, ACTH and GH. Histological examination showed that oral administration of AlCl3 for 30 days caused neuronal damage with clear degenerative changes in the nervous tissue after treatment with H&E dye, while the treatment of nerve tissue with silver stain showed a clear appearance of Aβ plaques spread in cerebellum, with normal composition of nervous tissue with H&E dyes for the group that was

orally administration with AlCl3 with oral administration by GBE with absence of Aβ deposition in the nervous tissue after staining with sulfur stain compared with control group. It is concluded from the current study that Aluminum Chloride ($AlCl_3$) causes clear pathological changes in the cerebellum tissue and confirms that treatment with aqueous extract of *Ginkgo biloba* has a protective role against $AlCl_3$-induced nervous tissue damage in male rats.

Keywords---cerebellum, alzheimer, beta amyloid, ginkgo biloba, growth hormone, cortisol, ACTH, 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 Cortisol, Adrenocorticotropin hormone (ACTH) and growth hormone (GH). Plasma concentration of hormones 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 of the current study showed that oral administration of AlCl3a caused significant increase ($P<0.05$) in the concentration of cortisol and ACTH, these results are agreement with (Kinawy & Al-Eidan, 2018; Murugaiyan & Bhargavan, 2021). The increase in cortisol secretion may be due to the oxidizing nature of aluminum and its role in inducing cellular injury by free radicals via enhancing oxidative stress, weakening antioxidant defense system and altering brain neurochemistry (Liaquat *et al* 2019). Neurologic, which causes a clear imbalance on the nervous system that is reflected in hormonal changes, which can lead to neurodegenerative changes and thus to dementia and cognitive decline (Wong & Herbert, 2004; Jesulola et al, 2017), Studies have shown that stress primarily affects the hypothalamus-pituitary axis (HPA). It has been found a link between hyperactivity of the HPA axis and chronic stress, the persistent stress stimulates an increase in the secretion of adrenocorticotropin releasing hormone (CRH) from the hypothalamus.

Which causes the synthesis and secretion of adrenocorticotropic hormone (ACTH) in the pituitary gland, which in turn stimulates the secretion of cortisol by the adrenal glands. The amount of stress is directly proportional to the level of cortisol in the plasma. It was found that stress causes an increase in the level of cortisol in the blood, which in turn causes a dysfunction in the centers that regulate mood, psychological motivation, memory mechanisms, disorders in the formation of neurons, and impairment in the function of the hippocampus (Justice, 2018; Saveanu & Nemeroff, 2012). It was also found that the toxicity caused by aluminum affects the tissue structure of the pituitary gland, causing a defect in its functions (Olawuyi et al, 2019), since aluminum chloride acts as a stress stimulus, which leads to an increase in corticosterone from the hypothalamus, which increases the release of ACTH from the pituitary gland and thus activates the axis of the pituitary gland as a result of stress caused by aluminum chloride. the activation of this axis causes the release of stimulating factors for this hormone from the hypothalamus, which include corticotropin releasing factor (CRF) and Arginin vasopersin(AVP), which works to stimulate the anterior pituitary gland to secrete adrenocorticotropic hormone (ACTH), which in turn causes the synthesis of cortisol and its release from the adrenal glands (Murugaiyan & Bhargavan, 2021), studies indicate that cytokines, especially (IL-1 and TNF-a), which in turn increases the release of ACTH (John et al, 2015; Arzt et al, 1999).

The results also showed a significant decrease ($P<0.05$) in the concentration of GH after oral administration with AlCl3. The level of growth hormone in the body is regulated by several hormones including growth hormone releasing hormone and growth hormone release inhibiting hormone secreted from the hypothalamus, (Xu et al, 2014). Aluminum chloride is one of the oxidants that cause oxidative stress (Liaquat et al 2019), growth hormone (GH) is one of the hormones that is an indicator of aging, as levels of growth hormone drop dramatically with age. It's one of the remarkable physiological changes termed somatopoiesis (Bartke, 2019) Decreased growth hormone may adversely affect metabolism and physiological functions, leading to weakness, decreased body mass, increased risk of cardiovascular disease, increased cognitive disorders and sleep disturbances in older adults Age (Maghfirah et al , 2017).

There are several views related to the effect of growth hormone (GH) on antioxidant levels, it has been found that growth hormone affects the function of immune cells, inhibits apoptosis and increases the production of reactive oxygen species (ROS) (Mancini *et al* , 2020) . It was also found that the decline in growth hormone directly affects the mitochondria and its role in regulating cellular balance and also plays an important role in cell differentiation, functions and survival, since this hormone reaches its peak during adolescence and gradually decreases during adulthood and aging and thus is responsible for regulating Mitochondrial mass and function As the organelles responsible for regulating vital processes in eukaryotic cells, and reduced its function is one of the hallmarks of aging (Poudel et al, 2020).

Table 1
Effect of aluminum chloride and aqueous extract of *Ginkgo biloba* on Cortisol, ACTH and GH in white male rats

	Cortisol mIU/ml	ACTH mIU/ml	GH mIU/ml
G1 Control	15.90 ± 0.26 AC	22.07 ± 0.56 A	1.97 ± 0.18 A
G2 10 mg/kg $AlCl_3$	19.78 ± 0.47 B	25.70 ± 1.12 B	0.71 ± 0.21 B
G3 10 mg/kg GBE	15.10 ± 0.29 C	21.23 ± 0.36 A	1.99 ± 0.11 A
G4 10 mg/kg $AlCl_3$+10 mg/kg GBE	16.17 ± 0.34 A	23.04± 0.43 A	1.71± 0.15 A
LSD	1.0199	1.9886	0.4278

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

The results of the current study showed that there were no significant differences (P<0.05) in the concentration of cortisol and ACTH hormones after oral administration of *ginkgo biloba* extract. The reason for this may be due to the protective role of GBE on the hypothalamus and pituitary gland and in enhancing the functions of brain cells. Many Studies have shown the anti-stress effects of GBE. This indicates the presence of inhibitors in GBE for the release of hypothalamic ACTH or CRH. The presence of this inhibitor makes the potential use of the extract important as it will protect the brain from the negative effects of persistently high ACTH levels (Marcilhac et al 1998; Amri et al, 1996).

Treatment with GBE results in decreased synthesis of glucocorticoids by the adrenal glands, suggesting that GBE may exert its stress-relieving effects by maintaining low levels of circulating glucocorticoids and thus opposing the stress-induced increase in glucocorticoids that could have Neurotoxicity effects. Thus, The stress-reducing effects are due to alterations in the function of the central nervous system, as the active substances in the GBE extract transfer cholesterol precursor from intracellular stores to the inner mitochondrial membrane, where the cytochrome P450 side-chain cleavage (P-45Oscc) enzyme is present and affects the structure of peripheral benzodiazepine-type receptors (PBR) that is an essential component of cholesterol transport regulation, thus limiting the amount of mitochondrial cholesterol available for corticosteroid synthesis (Walesiuk et al, 2006).

The results of the current study also showed that there were no significant differences (P<0.05) in the concentration of growth hormone (GH) after oral administration with GBE, due to its role in regulating growth hormone mRNA

expression in the cerebral cortex (Ahlemeyer & Krieglstein). , 2003), GH is an anabolic hormone that stimulates most target cells to grow in size and multiply, and GH receptors are located in the brain and GH may cross the blood-brain barrier through receptor-mediated mechanisms (Watanabe et al, 2001). Studies also indicate the therapeutic effects of GBE on aging-related diseases may be similar to the anti-aging effects of growth hormone secretion caused by activation of the ghrelin receptor, meaning that some of the active ingredients in this herb may mimic ghrelin to stimulate growth hormone release via the same molecular mechanism by activating the Growth Hormone Secretagogue Receptor (GHSR), stimulating growth hormone (GH) secretion, and regulating energy balance (Castaneda et al, 2010; Hsieh et al, 2016)

Histological examination with H&E dye showed that oral administration of AlCl3 for 30 days caused cerebellum damage with clear degenerative changes in the nervous tissue with loss of nuclei , in addition to the loss of association between the granular and Molecular layers, while there was a clear appearance of Aβ plaques spread in the cerebellum 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),

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 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 cerebellum tissue and normal appearance of neurons compared to the control group. This is due to the role of GBE 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 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).

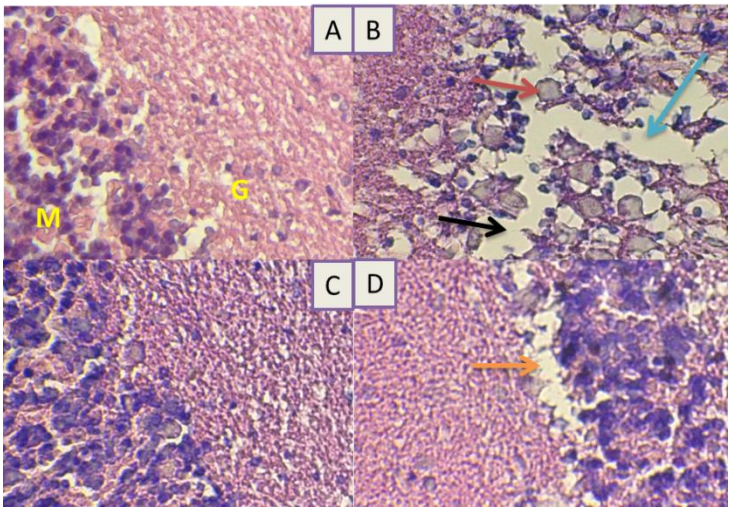

Figure (1) shows a histological section of the cerebellum of white male rats stained with H&E 400X (A) represents control group in which the normal structure of cerebellum tissue is observed. (B) group of rats that were dosed of 10 mg/kg of AlCl3 for 30 days, showed clear destructive changes in cerebellum tissue (blue arrow), nuclei loss and degeneration (red arrow), in addition to the loss of association between the granular and Molecular layers (black arrow),(C) cerebellum of rats that were dosed with 10 mg/kg of GBA , where the normal structure of cerebellum tissue is observed. (D) rats that were dosed with 10 mg/kg of AlCl3 + 10 mg/kg of GBA , it is noted the normal composition of the granular and molecular layers with presence of space between the two layers (orange arrow).

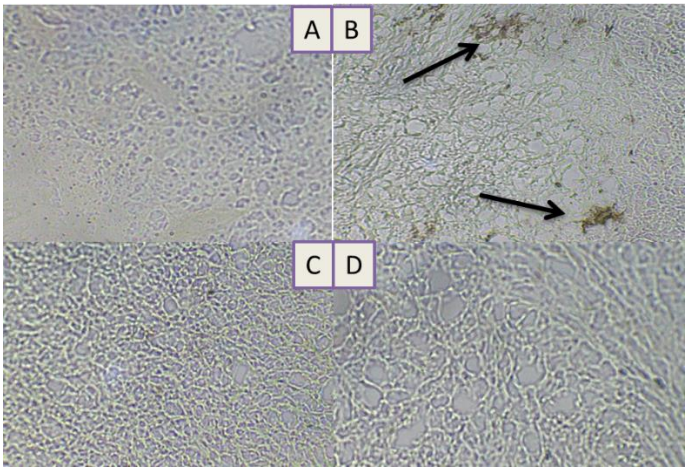

Figure 2 shows a histological section of white male rats cerebellum stained with silver stain (400X) (A) control group in which there was not observed of Aβ plaques (B) group of rats that were dosed of 10 mg/kg of AlCl3 for 30 days, clear appearance of Aβ plaques (black raws), (C) cerebellum of rats 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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