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GC-Mass Analysis of *Ginkgo biloba* and *Cichorium intybus* and their Neuroprotective Effects in a Rat Model of Alzheimer's Disease

*Karrar AR Al-Salim, Areej GH Al-Charak

College of Veterinary Medicine, Al-Qasim Green University, Babylon, Iraq

Abstract

Background: Globally, the records of people who have dementia are rising, which hurts communities and healthcare systems. The current study aimed to determine whether *Ginkgo biloba* (GB) and *Cichorium intybus* (CI) could relieve Alzheimer's disease (AD) by suppressing oxidative stress and neuroinflammation.

Methods and Results: Gas chromatography-mass spectrometry (GC-MS) was used to evaluate the phytoconstituents of GB and CI hydroalcoholic extracts. ELISA assay was used for the assessment of acetylcholinesterase (AChE) and dopamine in the brain tissue and SOD and TNF-α in blood serum.

Forty male albino rats were randomly divided into five groups (eight rats in each group). Group 1 (negative control) rats received only a baseline diet and distilled water. Alzheimer's disease was induced in Group 2 rats by oral administration (100 mg/kg bw) of $AlCl_3$ dissolved in distilled water daily for 28 days (positive control). Rats in Group 3 were orally supplemented concomitantly with both *Ginkgo biloba* extract (GBE) (120 mg/kg bw) once daily for 28 days and $AlCl_3$ (100 mg/kg bw). Rats in Group 4 were orally supplemented concomitantly with both *Cichorium intybus* extract (CIE) (500 mg/kg bw) once daily for 28 days and $AlCl_3$ (100 mg/kg bw). Rats in Group 5 were given 120 mg/kg of GBE and 500 mg/kg of CIE orally for 28 days with oral supplementation of $AlCl_3$ (100 mg/kg bw). In Groups 3-5, GBE and CIE were given one hour before $AlCl_3$ administration.

The results showed that GBE suppressed levels of brain AChE and serum TNF-α in $AlCl_3$-induced AD. CIE improved levels of brain dopamine and serum SOD in $AlCl_3$-induced AD. Moreover, the combined administration of GBE and CIE significantly suppressed the levels of brain AChE and serum TNF-α and improved the level of serum SOD in $AlCl_3$-induced AD, leading to the achievement of negative control values.

Conclusion: The combined use of GBE and CIE can lower the toxic impacts of aluminum chloride on brain neuronal structures, neurotransmission, and oxidative stress it causes, suppressing the development of $AlCl_3$-induced AD.(**International Journal of Biomedicine. 2024;14(3):510-515.)**

Keywords: Alzheimer's disease • rat • *Gingko biloba* • *Cichorium intybus* • *brain* • oxidative stress

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Abbreviations

AD, Alzheimer's disease; **$AlCl_3$**, aluminum chloride; **AChE**, acetylcholinesterase; **BT**, brain tissue; **BW**, body weight; **CI**, *Cichorium intybus*; **CIE**, *Cichorium intybus* extract; **DW**, distilled water; **GC-MS**, gas chromatography-mass spectrometry; **GB**, *Ginkgo biloba*; **GBE**, *Ginkgo biloba* extract; **RT**, retention time; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase; **TNF-α,** tumor necrosis factor-alpha.

Introduction

Alzheimer's disease (AD) is marked by a gradual deterioration in memory, attention, and other cognitive abilities. AD has a lengthy pre-symptomatic period, and it is believed that degenerative changes in the brain start many years before cognitive issues become apparent.[1,2] Approximately 50 million individuals have AD in the world, characterized by cognitive deficits in memory and other cognitive functions, causing death within 3–9 years following diagnosis. Alzheimer's disease is a progressive neurodegenerative brain disorder and is the most common type of dementia in older adults,

significantly affecting individuals' well-being and societal responsibilities.[3,4] In severe stages of AD, individuals lose the ability to carry out simple daily activities and need extensive care, eventually becoming completely bedridden.[5] Treatment options only provide respite from symptoms during the early stage of the disease.[6]

Ginkgo biloba (GB), an acknowledged botanical therapy, has been employed in China since ancient times.[7] GB extract mostly contains flavonoids and terpenoids.[8] GB may help treat AD because it contains different parts that fight free radicals, inflammation, and apoptosis and protect against mitochondrial dysfunction, amyloid formation, and aggregation. Additionally, GB is believed to modulate ion homeostasis and phosphorylation of the tau protein.[9] Many studies indicate that GB notably enhanced the cognitive abilities of AD in rodent models.[10,11] *Cichorium intybus* (CI) was used in ancient Rome, Greece, and Egypt to improve metabolism and digestion.[12] Furthermore, it functions as both a vegetable and a pasture plant and includes glycosides and triterpenoids. These compounds hinder glutamatergic transmission and improve GABAergic transmission.[13] The escalating prevalence of AD in Iraq needs to develop a potential therapeutic program intervention capable of modulating its course.

The current study aimed to determine whether *Ginkgo biloba* (GB) and *Cichorium intybus* (CI) could relieve Alzheimer's disease (AD) by suppressing oxidative stress and neuroinflammation.

Materials and Methods

All chemicals utilized in this research were of analytical quality and sourced from several medical and commercial services firms.

Plant Material

In September 2023, dried leaves of GB and CI were bought at a local market in the Al-Hilla province, Iraq. The leaves were rinsed using water and subsequently dried at 33°C for one week to dehydrate the plants. Then, the raw material was crushed into a powder and submitted to the hydroalcoholic extraction process using a hot plate apparatus with stirring.[14,15]

GC-MS Analysis

Gas chromatography-mass spectrometry (GC-MS) was used to evaluate the phytoconstituents of CI and GB. The GC-MS analysis was conducted via an Agilent 7820A GC system connected to a mass spectrometer (Agilent, USA). The analytical column employed was an Agilent HP-5ms Ultra Inert (30 mm × 250 µm × 0.25 µm) as shown in Table 1.

Animals

The experiments were conducted on 40 three-month-old male Wistar albino rats weighing 160–200 g. Rats were housed in specialized cages in ideal, normal settings for a period of two weeks to allow for adaptation. The rats lived in a quiet, air-conditioned environment with a temperature range of 20–25 °C and a 12:12-h light-dark cycle. A standard rodent diet was used, and free access to water was provided during the experiment.

Table 1.

Experimental conditions for the GC-MS analysis.

Parameter	Value
Analytical Column	Agilent HP-5ms Ultra Inert (30 mm × 250 µm × 0.25 µm)
Injection Volume	1 µL
Pressure	11.933 psi
Carrier Gas	Helium 99.99%
Injection Type	Splitless
Temperature (°C)	
GC Inlet Line	250 °C
Aux heaters	300 °C
Injector	250 °C
Oven Program Temperature	
First Ramp	60 °C hold for 3 min
Second Ramp	60 °C increased to 180 °C, rate 7 °C/min
Third Ramp	180 °C increased to 280 °C, rate 8 °C/min
Fourth Ramp	280 °C hold for 3 min

Study Design

Forty male albino rats were randomly divided into five groups (eight rats in each group). Group 1 (negative control) rats received only a baseline diet and distilled water. Alzheimer's disease (AD) was induced in Group 2 rats by oral administration (100 mg/kg BW) of $AlCl_3$ dissolved in distilled water daily for 28 days (positive control).[3] Rats in Group 3 were orally supplemented concomitantly with both *Ginkgo biloba* extract (GBE) (120 mg/kg bw) once daily for 28 days and $AlCl_3$ (100 mg/kg bw).[16] Rats in Group 4 were orally supplemented concomitantly with both *Cichorium intybus* extract (CIE) (500 mg/kg bw) once daily for 28 days and AlCl3 (100 mg/kg bw).[17] Rats in Group 5 were given 120 mg/kg of GB and 500 mg/kg of CI orally for 28 days with oral supplementation of $AlCl_3$ (100 mg/kg bw). In Groups 3-5, GB and CI were given one hour before $AlCl_3$ administration.

Biomarker Assessment

The serum was separated from the blood samples, placed in sterile Eppendorf tubes, and frozen at −20 °C. The SOD and TNF-α levels in the serum were assessed using ELISA kits from (BT-Lab, China). Following the collection of blood samples, all animals were euthanized. 0.4 g of the brain was homogenized with an electric homogenizer in 1 ml of ice-cold phosphate-buffered saline (PBS). The blended tissue was spun at 5000 /min for 5 min. The supernatant was used to assess acetylcholinesterase (AChE) and dopamine levels using ELISA kits from (BT-Lab, China).

Statistical analysis was performed using the statistical software package SPSS version 26.0 (SPSS Inc, Armonk, NY: IBM Corp). Baseline characteristics were summarized

as frequencies and percentages for categorical variables and as mean ± standard error of the mean (SEM) for continuous variables. Multiple comparisons were performed using a one-way ANOVA and Tukey HSD post-hoc test. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

Assessment of Phytochemicals of Plants by GC-MS

Using the National Institute of Standards and Technology (NIST) database, the compound's name, molecular weight, chemical formula, peak area, and biological activity of GB and CI were determined (Figures 1 and 2, Tables 2 and 3). The relative proportion of each component was determined by comparing its average peak area to the total area. Our GC-MS analysis of the two extracts exhibited that oleic acid was the most abundant fatty acid.

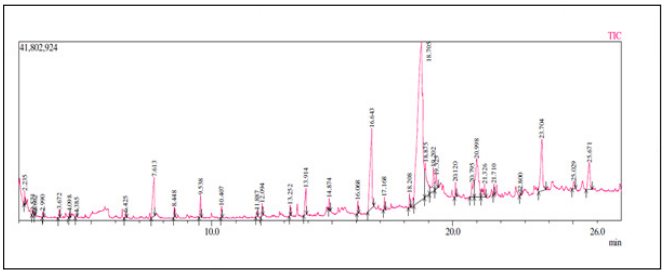

Fig. 1. *GC-MS analysis of Cichorium intybus hydroalcoholic extract.*

Table 2.

GC-MS analysis of Cichorium intybus hydroalcoholic extract.

No.	Compound	RT (min)	Chemical formula	MW	Area %	BA
1	Oleic Acid	18.70	$C_{18}H_{34}O_2$	282	47.35	Antimicrobial
2	n-Hexadecanoic acid	16.64	$C_{16}H_{32}O_2$	256	9.67	Anti-inflammatory
3	Hexatriacontane	20.99	$C_{36}H_{74}$	506	7.08	Antioxidant
4	Tetracontane	23.71	$C_{40}H_{82}$	562	6.48	Antioxidant Antibacterial
5	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	7.61	$C_6H_8O_4$	144	3.70	Antioxidant
6	Dotriacontane	25.67	$C_{32}H_{66}$	450	3.35	Antioxidant
7	Tetracosamethyl-cyclododecasiloxane	19.20	$C_{24}H_{72}O_{12}Si_{12}$	888	2.58	Antioxidant Antibacterial
8	Cyclooctasiloxane, hexadecamethyl	14.87	$C_{16}H_{48}O_8Si_8$	592	2.01	Antimicrobial Anti-inflammatory
9	5-Hydroxymethyl-furfural	9.53	$C_6H_6O_3$	126	1.16	Antioxidant
10	Cyclohexane, octadecyl	25.02	$C_{24}H_{48}$	336	0.65	Antimicrobial
11	Vanillin	11.88	$C_8H_8O_3$	152	0.31	Antioxidant Anti-inflammatory Antimicrobial

MW, molecular weight; BA, biological activity; RT, retention time

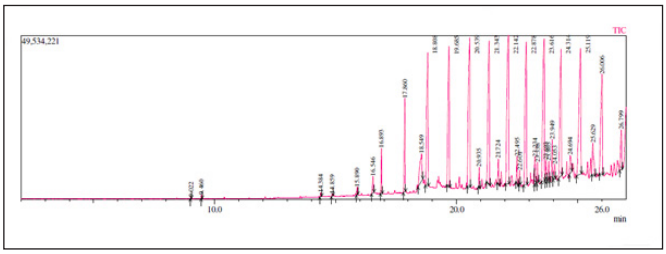

Fig. 2. *GC-MS analysis of Gingko biloba hydroalcoholic extract.*

Table 3.

GC-MS analysis of Ginkgo biloba hydroalcoholic extract.

No.	Compounds	RT (min)	Chemical formula	MW	Area %	BA
1	n-Hexadecanoic acid	16.54	$C_{16}H_{32}O_2$	256	0.57	Anti-inflammatory Antioxidant
2	Docosane	17.86	$C_{22}H_{46}$	310	2.94	Antioxidant
3	Oleic Acid	18.549	$C_{18}H_{34}O_2$	282	2.37	Antimicrobial
4	Tetracosane	18.80	$C_{24}H_{50}$	338	6.84	Antimicrobial Anticancer
5	Tetratriacontane	19.68	$C_{34}H_{70}$	478	7.07	Antibacterial Antioxidant
6	2-Methylpenta-cosane	20.93	$C_{26}H_{54}$	366	9.09	Antibacterial Antioxidant
7	Hexatriacontane	21.34	$C_{36}H_{74}$	506	18.59	Antioxidant
8	Dotriacontane	22.88	$C_{32}H_{66}$	450	8.30	Antimicrobial Antioxidant
9	Tetracontane	23.61	$C_{40}H_{82}$	562	16.41	Antimicrobial antioxidant
10	Pentatriacontane	25.12	$C_{35}H_{72}$	492	9.50	Antimicrobial antioxidant

MW, molecular weight; BA, biological activity; RT, retention time

Brain Dopamine and AChE

After 28 days of the experiment, brain dopamine levels were suppressed by $AlCl_3$ compared to the negative control and increased after oral administration of CIE and GBE+CIE but reached the level of negative control only in the CIE group (Table 4). Brain levels of AChE increased in the $AlCl_3$ group significantly compared to negative control and decreased significantly after oral administration of GBE, CIE, and GBE+CIE combination, reaching the level of negative control in the GBE group and GBE+CIE group.

Antioxidant and Inflammatory Markers

The antioxidant status of serum by assessment SOD showed that $AlCl_3$ administration suppressed SOD level significantly compared to the negative control. CIE and GBE+CIE administration allowed to increase SOD level compared to positive control. SOD levels reached the level of negative control only in the GBE+CIE group. The serum

TNF-α level increased in the $AlCl_3$ group compared to the negative control and significantly decreased after GBE and GBE+CIE administration compared to the positive control. TNF-α levels reached the level of negative control in the GBE and GBE+CIE groups.

Discussion

This research highlighted the therapeutic potential of two famous herbs, GB and CI, in treating $AlCl_3$-induced AD in rats. The abundance of bioactive chemicals, the variety of secondary metabolites in GB and CI, and their byproducts have contributed to its rising popularity. Several beneficial biological effects, such as antibacterial, anti-inflammatory, antioxidant, anti-cancer, and antimicrobial, have been linked to the many components in both GB and CI.[7,12] Our GC-MS analysis of the GB and CI hydroalcoholic extracts found several phytoconstituents like phenols, alkaloids, and unsaturated fatty acids possessing antioxidant abilities, protecting the brain from oxidative stress. Moreover, the two extracts exhibited that oleic acid was the most abundant fatty acid, which can directly regulate both the synthesis and the activity of antioxidant enzymes.

Aluminum (Al) intoxication poses a significant risk to the brain since it has a strong affinity for receptors and may readily pass across the blood-brain barrier, ultimately leading to its accumulation in the brain.[14,18] Additionally, it has the potential to stimulate the generation of free radicals, which can lead to brain damage, particularly in the areas of the brain responsible for memory and learning.[19,20] Oxidative stress has a crucial role in the development of neurotoxicity, which is a key pathogenic event in the basic process of neurodegeneration in AD.[21,22] Antioxidants are a promising component in preventing the onset and progression of AD. Al intoxication induces severe oxidative stress by increasing the pro-oxidant effects of iron in the brain and reducing the activity of antioxidant enzymes.[23]

These studies indicated that GBE and CIE contain antioxidant compounds: phenols, flavonoids, and unsaturated fatty acids. It is safe to assume that the studied extracts may modulate AD by acting as antioxidants and anti-inflammatory agents. Our results align with previous research indicating that GBE contains approximately 22.0–27.0% flavonoids, 5.0–7.0% terpene lactones, and ginkgolic acids at levels below 5 mg/kg.[8,24]

The phytoconstituents of CIE identified in this study align with previous findings.[25,26] These studies also identified polyphenols, flavonoids, phenols, and fatty acids as the main components. Furthermore, the flavonoids examined in GBE and CIE of the current investigation are well-documented for their antioxidant properties. The administration of flavonoids could be a good strategy for modulating symptoms related to neurotoxicity.[7,8]

Among the herbal extracts, GBE is one of the most investigated herbal remedies for cognitive disorders and AD. Flavonoids and terpenoids are the primary components found in standardized GBE.[27] Anti-oxidation, anti-inflammation, anti-apoptosis, defense against mitochondrial dysfunction, amyloidogenesis, and the β-amyloid peptide (Aβ) aggregation, modulation of phosphorylation of tau protein, ion homeostasis, and even induction of growth factors are possible mechanisms of action of GBE.[28]

Dopamine and acetylcholine are neurotransmitters vital in conveying information between neurons and are strongly linked to the preservation of learning and memory in the brain.[29] Acetylcholinesterase (AChE), an acetylcholine-hydrolyzing enzyme, consistently colocalizes with the amyloid deposits and may contribute to the generation of amyloid proteins. An increase in AChE levels around amyloid plaques and neurofibrillary tangles is a common feature of AD neuropathology. A decrease in brain acetylcholine (ACh) level is implicated in the pathophysiology of cognitive dysfunction occurring in AD. Enhancing ACh levels by inhibiting the AChE activity has a beneficial impact on cognitive performance.[30]

Table 4.

Neurotransmitters and markers of oxidative stress and neuroinflammation.

Group	Dopamine in BT (ng/g)	AChE in BT (ng/g)	Serum TNF-α (pg/mL)	Serum SOD (ng/mL)
Positive control ($AlCl_3$) [1]	10.71±0.80	28.44±1.15	7.75±0.57	4.42±0.64
Negative control (DW) [2]	24.77±1.59	14.12±1.26	3.54±0.37	17.86±1.12
GBE [3]	13.80±1.22	17.94±0.97	5.44±0.35	6.82±0.81
CIE [4]	19.64±1.39	20.74±1.29	6.16±0.48	11.83±0.79
GBE+CIE [5]	16.85±1.10	13.05±1.29	4.54±0.52	15.92±0.56
One-way ANOVA	F=18.7615 P=0.0000 P_{1}=0.0000 P_{1-3}=0.4283 P_{1-2}=0.0005 P_{1-5}=0.0180 P_{1-4}=0.0000 P_{2-4}=0.0598 P_{2-3}=0.0019 P_{3-4}=0.0259 P_{2-5}=0.4409 P_{4-5}=0.5263	F=26.5317 P=0.0000 P_{1}=0.0000 P_{1-3}=0.0000 P_{1-2}=0.0017 P_{1-5}=0.0000 P_{1-4}=0.2010 P_{2-4}=0.0069 P_{2-3}=0.9681 P_{3-4}=0.4835 P_{2-5}=0.0621 P_{4-5}=0.0017	F=11.8237 P=0.0000 P_{1}=0.0000 P_{1-3}=0.0168 P_{1-2}=0.1525 P_{1-5}=0.0008 P_{1-4}=0.0623 P_{2-4}=0.0059 P_{2-3}=0.5634 P_{3-4}=0.8081 P_{2-5}=0.6550 P_{4-5}=0.1405	F=50.6627 P=0.0000 P_{1}=0.0000 P_{1-3}=0.2576 P_{1-2}=0.0000 P_{1-5}=0.0000 P_{1-4}=0.0000 P_{2-4}=0.0003 P_{2-3}=0.4564 P_{3-4}=0.0023 P_{2-5}=0.0000 P_{4-5}=0.0142

Cholinergic transmission primarily affects cognition, learning, and memory. Transmission impairment levels are associated with dementia severity.[31]

Aluminium is a strong cholinotoxin that provokes alterations in cholinergic transmission.[3] This neurotoxic ability of Al remarkably enhances the AChE activity[32] and thereby elevates the breakdown of ACh in the brain. It was exhibited in our study, where $AlCl_3$ triggered increasing the AChE content in the brain tissues. In our study, the administration of 120 mg/kg of GBE and 500 mg/kg of CIE, both alone and in combination, significantly suppressed brain AChE levels in $AlCl_3$-induced AD.

Alzheimer's disease is closely associated with Aβ-induced neuroinflammation and neuronal injury through the release of proinflammatory and cytotoxic factors.[33] Numerous studies have described elevated TNF-α levels in biological fluids in AD patients.[34-36] Stimulation of primary rodent and human microglial cell cultures with Aβ induces the release of high levels of TNF-α.[37-39] In our study, $AlCl_3$ triggered increasing the TNF-α in the serum. In our study, the increase in serum TNF-α induced by $AlCl_3$ significantly decreased with the administration of GBE (120 mg/kg) alone and in combination with 500 mg/kg of CIE.

In conclusion, the combined use of GBE and CIE can lower the toxic impacts of $AlCl_3$ on brain neuronal structures, neurotransmission, and oxidative stress it causes, suppressing the development of $AlCl_3$-induced AD.

Ethical Approval

The animals were housed in keeping with the rules for good laboratory practice (GLP). The experiments were performed in accordance with the norms for the humane treatment of animals, which are regulated by the International Guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care, followed the protocol approved by the Institutional Animal Care and Use Committee of the Veterinary Medicine Collage at the Al-Qasim Green University.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

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***Corresponding author**: Karrar AR Al-Salim. College of Veterinary Medicine, Al-Qasim Green University, Babylon, Iraq. E-mail: hamza14shukri72@gmail.com*