

Research Article

GCMS Analysis, Neuroprotective, Cognitive, and Anti-inflammatory Impact of a Polyherbal Therapy in Alcohol-Induced Dementia in Wistar Rats

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Abstract

Dementia is a neurodegenerative disorder that leads to cognitive decline, and unfortunately, treatment options are quite limited. This study explored the neuroprotective, cognitive, and anti-inflammatory effects of polyherbal therapy (PHT) on alcohol-induced dementia in Wistar rats. The PHT was formulated using *Lantana camara*, *Salvia hispanica*, *Zingiber officinale*, *Curcuma longa*, and *Beta vulgaris* in a 2:1:1:1:1 ratio. Thirty adult female Wistar rats, weighing between 180 and 200 grams, were split into six groups: a control group (receiving just feed and water), an alcohol group 20 % w/v ethanol (2g/kg, i.g.), an alcohol+ donepezil group (2.5mg/kg), and alcohol+ PHT groups (250, 500, and 750mg/kg). Treatment was administered once daily, orally, for 21 days to all groups. Animals were assessed for cognitive performance using the elevated plus maze one hour after the 21st day of treatment and on the 24th day for the classic labyrinth maze experiment. On the following day (day 25), the rats were euthanized with an intraperitoneal (i.p.) injection of ketamine hydrobromide at a dose of 50 mg/kg. GC-MS analysis identified key bioactive compounds with neuroprotective and anti-inflammatory properties, including artemetin (18.26%), nobiletin (2.42%), and baicalin (1.37%). Neurobehavioral tests, such as the Classic Labyrinth Maze and Elevated Plus Maze, demonstrated significant ($p < 0.05$) improvements in spatial learning and memory in the groups treated with PHT compared to those exposed to alcohol. PHT also reduced pro-inflammatory cytokines, such as IL-6 and IL-1 β , in a dose-dependent manner, with the highest dose of 750mg/kg outperforming donepezil. Furthermore, histopathological analysis indicated that PHT helped mitigate alcohol-induced neurodegeneration in the hippocampus, resulting in fewer cases of pyknosis and vacuolation. The LD₅₀ of PHT was determined to be 2500mg/kg, suggesting it is safe for use. These findings imply that PHT provides neuroprotection through mechanisms involving antioxidants and anti-inflammatory effects, presenting a promising multi-target therapeutic strategy for alcohol-induced dementia. More clinical studies are needed to confirm its effectiveness in humans.

Keywords: Dementia; Polyherbal therapy; Donepezil; Neurobehavioral tests; Neurodegeneration; Antioxidants; Histopathological analysis.

1. Introduction

Dementia is a decline in cognitive abilities across various domains that are as severe enough to impact daily social interactions or

work life. In the U.S., Alzheimer's disease (AD) is a major contributor to dementia. AD is a neurodegenerative disease that involves the destruction of neuronal cells, and there is

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no effective cure for the disease [1, 2]. Neurodegenerative diseases usually occur as a result of mild to severe memory dysfunction, which is associated with the acquisition, consolidation, and retrieval of both recent and remote information [1, 3]. AD affects around 5.8 million people. It's important to know that dementia often involves more than one type of brain pathology, typically combining Alzheimer's with issues related to blood vessels in the brain. To diagnose dementia, a thorough history is needed to assess cognitive function [1].

Dementia is becoming an increasingly pressing health issue around the globe, affecting around 50 million individuals as of 2021, according to the WHO [4]. One specific type, known as alcohol-induced dementia, arises from prolonged heavy drinking, which can lead to cognitive decline and difficulties with spatial awareness. While current treatment options have their drawbacks, herbal remedies are gaining interest as potential alternative therapies, as noted by Koch *et al.* [5] and Zahr [6].

Alcohol-induced dementia brings about some serious challenges, particularly with visuospatial skills, which can disrupt everyday life. Conventional treatments often do not work effectively and can be associated with a range of side effects [6, 7]. When tackling cognitive decline, many pharmacological options have limited effectiveness, can

lead to unwanted side effects, or do not offer lasting solutions [8]. This has sparked a growing interest in exploring alternative and complementary methods, such as herbal and poly herbal formulations, which may provide various benefits for managing cognitive issues [9]. These poly herbal formulations, derived from plant sources, are believed to possess neuroprotective, antioxidant, and anti-inflammatory properties, showing promise for enhancing cognitive function and safeguarding against neurodegeneration [10].

Ayurveda, a traditional system of medicine, is an ancient medical tradition that attracts global attention for its health promotion and disease prevention [11]. In the present study, a polyherbal formulation was prepared by combining five traditional plants, namely *Lantana camara*, *Salvia hispanica* L., *Zingiber officinale*, *Curcuma longa*, and *Beta vulgaris*. The aforementioned plants were chosen due to their use in folk medicine for neuroprotective, memory-enhancing, antioxidant, and anti-inflammatory properties.

Lantana camara, a member of the family Verbenaceae, is a widely cultivated ornamental shrub with common names including Lantana, Wild Sage, Surinam Tea Plant, Spanish Flag, and West Indian Lantana. Recent studies have shown the possible beneficial use of *L. camara* in contemporary medicine [11]. *Salvia hispanica* L., commonly known as chia, is an herbaceous annual from the Lamiaceae family. It is a native plant of southern Mexico and northern Guatemala, now cultivated across tropical and subtropical regions for its nutritional, medicinal, and industrial uses [12].

Zingiber officinale Roscoe belongs to the Zingiberaceae family. It is a perennial herb with thickened rhizomatous roots and is widely recognized for its additional roles as an anti-inflammatory, analgesic, warming agent, and cholesterol-lowering herb [13]. *Curcuma longa* L., a member of the Zingiberaceae family, is commonly known as turmeric. It is indigenous to Southwest India. The rhizomes are used as a spice, in cosmetics, and in traditional medicine [14].

Beta vulgaris, commonly referred to as beetroot, belongs to the Chenopodiaceae family. It is an annual or biennial plant native to the Middle East but now grown worldwide across Europe, Asia, and the Americas. Current research supports its beneficial role in the management of cardiovascular and other clinical conditions [15].

Thus, it's crucial to investigate how polyherbal therapy affects visuospatial abilities, including spatial learning, memory, and navigation skills, in animal models [16]. This study aims to explore the GC-MS analysis and the neuroprotective, cognitive, and anti-inflammatory effects of polyherbal therapy on alcohol-induced dementia in Wistar rats.

2. Materials and Methods

2.1. Animals

The current study occurred in the animal house at Alex-Ekwueme Federal University, located in Ndufu Alike, Ebonyi State, Nigeria (AE-FUNAI). We brought in 30 healthy female Wistar rats, each weighing between 180 and 200 grams and around 6 months old. After giving them two weeks to acclimate in well-ventilated wired cages, we randomly divided the rats into five groups. They were maintained at a comfortable room temperature of $23 \pm 2^\circ\text{C}$, with a 12-hour light/dark cycle and a relative humidity of

50 ± 5%. The rats had unrestricted access to water and food (raw chow from Vital Feeds Nigeria Ltd., Jos).The animals were handled with care, following the guidelines set by the National Institutes of Health for the use and care of laboratory animals. Ethical approval was obtained from the Faculty of Basic Medical Sciences' Ethics Committee at Alex Ekwueme Federal University, Ndufu-Alike, Ebonyi State. The approval number is: AEFUNAI 2025/00979.

2.2. Plant Collection and Identification

Lantana camara (leaves), *Salvia hispanica* L (seeds), *Zingiber officinale* (rhizome), *Curcuma longa*(rhizome), and *Beta vulgaris* (seeds) were all gathered from a local market in Abakaliki, Ebonyi State, Nigeria. Fresh, mature leaves of *Lantana camara* and *Zingiber officinale* were verified by a botanist in the Department of Pharmacognosy at the University of Uyo. A voucher number (UUPH17AI) was assigned, and samples were deposited at the Herbarium. *Zingiber officinale* was identified in the Department of Botany and Ecological Studies and deposited at the herbarium with voucher number UUH4099 (Uyo).

2.3. Preparation and Extraction of the Plant Extract

After air drying, the plant materials were individually ground into a fine powder using a pulverizer and then sifted through a sieve. The finely powdered samples from five different plants were blended in a 2:1:1:1:1 ratio to create a uniform mixture. The specific order of mixing for these five plants is detailed in [Table 1](#) [17].

Table1.CompositionofPolyherbalFormulation

Botanicalname	Family	Partused	Weight
<i>Zingiber officinale</i>	Zingiberaceae	Rhizome	50g
<i>Salvia hispanica</i>	Lamiaceae	Seed	50g
<i>Betavulgaris</i>	Chenopodiaceae	Root	50g
<i>Curcuma longa</i>	Zingiberaceae	Rhizome	50g
<i>Lantana camara</i>	Verbenaceae	Leaves	100g

2.4. AcuteToxicityStudy(LD50)

The median lethal dose(LD₅₀)was determined using the method described by Lorke [18]. In this study, the

polyherbal formulation was administered orally. The doses ranged from 1000 to 5000 mg/kg. The rats were monitoreddailyfor14days.Atanydoselevelgiven, the study found no toxicological reaction or mortality.

2.5. Gas Chromatography-MassSpectroscopy(GC- MS) Analysis of Polyherbal Formulation

The active phytochemicals were evaluated using a GC– MS (Model: QP2010 plus Shimadzu, Japan) as previously done in Liu's work [19], which consisted of an AOC-20i auto-sampler and gas chromatograph interfaced to a mass spectrometer (GC–MS). The mass spectrum was analyzed using the National Institute of Standards and Technology (NIST) database, which contains more than 62,000 patterns. The fragmentation pattern spectra of the unknown components were compared to those of known components stored in the NIST library(NIST11).The relative percentage of each phytochemical compound was calculated by comparing its average peak area to the total area. The names, molecular weights, and structures of the components of the test materials were also determined [20].

2.6. Animal Groupings

[Figure 1](#)shows the experimental protocol and study timeline of this current work. The experimental animals were divided into several groups as follows:

- Group1**received just feed and water.
 - Group2**was administered 20% w/v ethanol (2g/kg,*i.g.*) for 21 days.
 - Group 3** received 20% w/v ethanol (2 g/kg, *i.g.*) along with 2.5 mg/kg of donepezil for 21 days.
 - Group4** was administered 20 % w/v ethanol (2g/kg, *i.g.*) and 250mg/kg of PHT for 21days.
 - Group5** received 20 % w/v ethanol (2g/kg,*i.g.*) and 500 mg/kg of PHT for 21 days.
 - Group6**wasadministered 20% w/v ethanol (2g/kg,*i.g.*) and 750 mg/kg of PHT for 21 days.
- The administration of alcohol (ethanol) was performed intragastrically at a concentration of 20 % w/v, at a dose of 2 g/kg, to deliver the target ethanol mass while minimizing gavage volume and animal distress [21, 22].

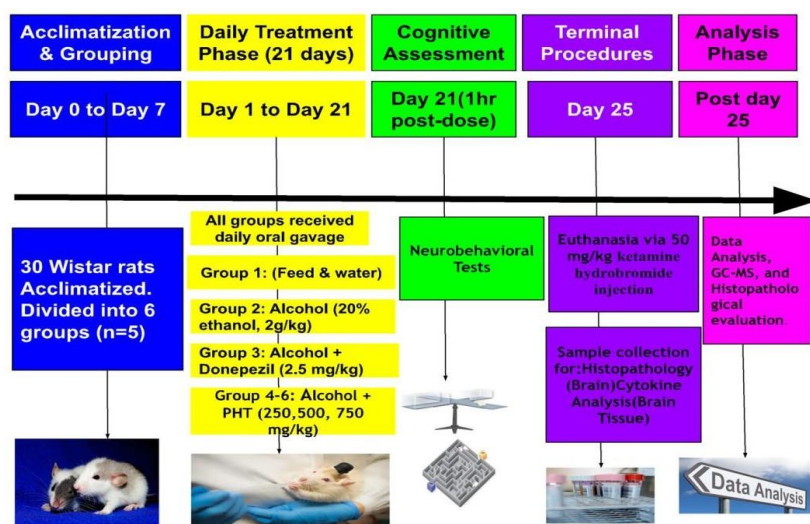


Figure 1. A Diagram showing the experimental design and study timeline (Alcohol-Induced Dementia & PHT Treatment)

2.7. Elevated Plus Maze

This test was done on day 22 following 21 days of treatment. It was carried from 8:00 a.m. to 10:00 a.m. The maze was elevated 45 cm off the ground and featured two open arms that crossed each other (30 cm x 5 cm x 25 cm) with two closed arms of the same dimensions. This setup is utilized for studies on anxiety and memory. The experiment spanned two days. On the first day, each animal was positioned at the end of an open arm, facing away from the central platform. Transfer latency was the time it took the animals to move from the open arm to the enclosed arm, serving as a measure of learning. This process was repeated on Day 2 to assess memory recall. Each animal had 90 seconds to enter one of the closed arms. If it did not do so, it was gently guided into one of the closed arms and allowed to stay there for 20 seconds to help it consolidate its memory. If the animal managed to enter the closed arm, the time taken was recorded using a stopwatch; if it did not, it was noted as 90 seconds. After each trial, the maze was cleaned with a cloth soaked in 70% ethyl alcohol and left to dry, ensuring that any olfactory cues were eliminated [23].

2.8. Classic Labyrinth Test

Day 24 marked the testing phase, during which animals were placed in a square labyrinth situated in a behaviour assessment room, lit with low-intensity white light. Initially, they were allowed 10 minutes to explore freely. Subsequently, five trials were administered: the first four

training trials rewarded the animals with a biscuit for reaching the goal, while the test trial was without a reward. The time taken (latency) to move from the starting point to the exit was recorded [24].

2.9. Animal Sacrifice and Sample Collection

After euthanizing the rats on day 25 with an intraperitoneal (i.p.) injection of ketamine hydrobromide at a dose of 50 mg/kg, the scalp was skinned and the brain excised, which were then fixed in normal saline. The samples were ground and homogenized in a phosphate buffer (0.1 mM, pH 7.0). We centrifuged the homogenate at 3000 rpm for 15 minutes at 4°C and then collected the supernatant.

2.10. Determination of Pro-Inflammatory Cytokines

Brain tissues were homogenized on ice in PBS (1:9:1:20, w/v) that contained protease inhibitors, clarified at $10,000 \times g$ (10–15 min, 4 °C), and supernatants were stored at -80 °C. Total protein was measured (BCA) for normalization, and IL-6 and TNF- α were measured in the homogenates by sandwich ELISA according to the manufacturer's instructions, with absorbance read at 450 nm and concentrations expressed as pg/mg protein [25].

2.11. Histopathological Studies

After conducting behavioral and biochemical evaluations, the brains of several groups were perfusion-fixed using 4% paraformaldehyde in a 0.1 M phosphate buffer solution.

Following excision, the brain was post- fixed in the same fixative overnight at 48 °C. After the brains had been properly soaked in paraffin, they were stained with hematoxylin and eosin. The lesions were examined with a microscope at 400 magnification.

2.12. Statistical Analysis

The data were presented as mean ± SEM and analyzed using one-way ANOVA, followed by Tukey’s multiple comparison tests. AP value of less than 0.05($p<0.05$) was deemed statistically significant. The statistical analysis was performed using GraphPad Prism 8.0 software.

3. Result and Discussion

3.1. LD50 of Polyherbal Therapy

The LD50 of the PHT,as determined by Lork's method,

was 2500 mg/kg.

3.2. GC-MS Analysis of Polyherbal Therapy(PHT)

The chromatogram for the Polyherbal Therapy Extractis illustrated in **Figure 2**. **Tables 2a-c**list the phytochemicals in the plants used for this therapy and their pharmacological properties. The most abundant component is Artemetin, making up 18.266% of the extract, with a retention time of 9.905 minutes. It is well- known for its antimalarial and anticancer effects. Robinetin and Myricetin both account for 3.988 % each and exhibit strong antioxidant and anticancer characteristics, with retention times of 14.101 minutes and 14.310 minutes, respectively. Additionally, Nobiletin (2.421%) and Baicalin (1.370%) are present in significant amounts from a pharmacological perspective, with retention times of 13.903 minutes and 13.696 minutes, respectively.

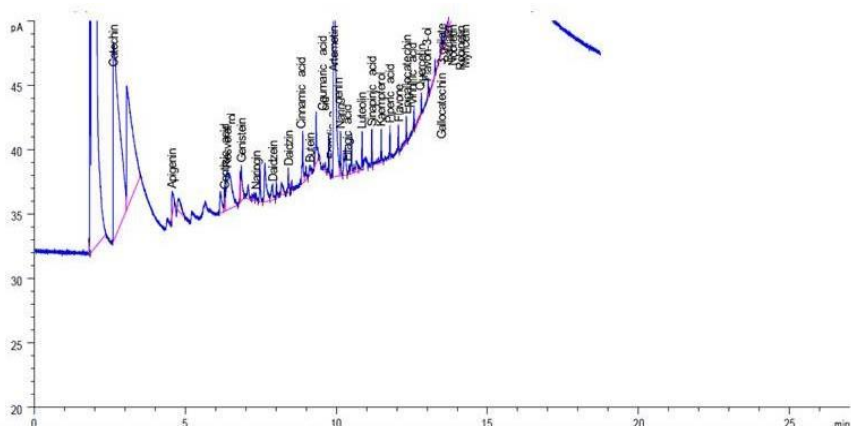


Figure2:TheGC-MSchromatogramofthepolyherbaltherapyextract

Table2a.Phytochemicalsandtheirpharmacologicalproperties

RetTime (min)	CompoundName	Amount(ppm)	MolecularWeight (g/mol)	BiologicalActivities
7.334	Naringin	0.178	580.53	Anti-oxidant,anti-inflammatory,hepatoprotective
7.884	Daidzein	0.770	254.24	Phytoestrogenwhichareanticancer,alsoimprove bonehealth.
8.402	Daidzin	0.575	416.38	Anti-oxidant,neuroprotective,improves menopausal symptoms.
8.880	Cinnamicacid	1.303	148.16	Antimicrobial,anti-oxidant,anti-inflammatory
9.110	Butein	0.389	272.25	Anticancer,anti-inflammatory,anti-oxidant
9.326	Coumaricacid	1.519	164.16	Anti-oxidant,antimicrobial,UV-protective
9.618	Ferulicacid	0.131	194.18	Anti-oxidant,anti-inflammatory,photoprotective
9.905	Artemetin	18.266	374.35	Antimalarial,anti-inflammatory,cytotoxicto cancer cells
10.130	Naringenin	0.723	272.25	Anti-oxidant,cardioprotective,antiviral
10.425	Ellagicacid	0.222	302.19	Anticancer,anti-oxidant,anti-mutagenic

Table2b.Phytochemicalsandtheirpharmacologicalproperties

RetTime (min)	CompoundName	Amount(ppm)	MolecularWeight (g/mol)	BiologicalActivities
10.844	Luteolin	0.773	286.24	Anti-inflammatory,neuroprotective,anticancer
11.168	Sinapinicacid	0.625	224.21	Anti-oxidant,anti-inflammatory,antimicrobial
11.477	Kaempferol	0.719	286.24	Anti-oxidant,anticancer,cardioprotective
11.769	Piperic acid	0.394	216.19	Bioenhancer(improvesdrugabsorption),anti- oxidant
12.046	Flavone	0.432	222.24	Anti-oxidant,anti-inflammatory
12.229	Epicatechin	-	290.27	Anti-oxidant,improvescardiovascularhealth.
12.310	Epigallocatechin	0.464	306.27	Anti-oxidant,anticancer,antiviral
12.563	Vanillicacid	0.335	168.14	Anti-oxidant,antimicrobial,anti-inflammatory
12.804	Quercetin	0.444	302.23	Anti-oxidant,anti-inflammatory,antiviral

Table2c.Phytochemicalsandtheirpharmacologicalproperties

RetTime (min)	CompoundName	Amount(ppm)	MolecularWeight (g/mol)	BiologicalActivities
13.038	Flavon-3-ol	0.253	238.24	Generalanti-oxidantandanti-inflammatory activity (subclass of flavonoids)
13.483	Gallocatechin3-gallate	0.445	458.37	Anti-oxidant,anticancer,antimicrobial
13.696	Baicalin	1.370	446.36	Anti-inflammatory,antiviral,neuroprotective
13.903	Nobiletin	2.421	402.39	Anti-inflammatory,neuroprotective,anticancer
14.101	Robinetin	3.911	302.23	Anti-oxidant,antifungal,anticancer
14.310	Myricetin	3.988	318.24	Anti-oxidant,antidiabetic,anticancer
16.296	Tangeretin	-	372.37	Anticancer,anti-inflammatory,neuroprotective
18.155	Cinnamicacid	-	148.16	Antimicrobial,anti-oxidant(duplicate entry)
18.155	Cinnamicacid	-	148.16	Antimicrobial,anti-oxidant(duplicate entry)
28.232	Syringicacid	-	198.17	Anti-oxidant,antimicrobial,anti-inflammatory
31.656	Rosmarinic acid	-	360.31	Anti-oxidant,anti-inflammatory,neuroprotective

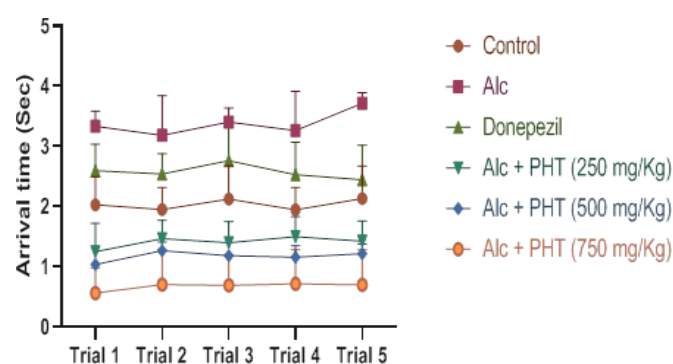
3.3. ClassicLabyrinthMazeTest

The results indicate that the group treated with alcohol and donepezil experienced an increase in arrival time latency compared to the control group. In contrast, the PHT-treated groups showed a decrease in arrival time latency compared to the control. Specifically, there was a reduction in arrival time activity in the PHT groups compared to those treated with 2.5 mg/kg donepezil. Additionally, the 500 mg/kg and 750 mg/kg PHT doses exhibited a decrease in activity compared to the 250 mg/kg PHT dose. Notably, the 750 mg/kg PHT showed low arrival time latency compared to the 250 mg/kg and 500 mg/kg PHT (**Figure 3**).

3.4. Effect of PHT on Transfer Latency across Groups by Day 1

There is a notable decrease in transfer latency ($P < 0.0001$) for the 2g/kg alcohol, 250mg/kg PHT, and 500mg/kg PHT compared to the control. Contrastingly,

the 2.5 mg/kg donepezil and 750 mg/kg PHT show a significant increase in transfer latency ($P < 0.0001$) compared to the 2 g/kg alcohol. Additionally, the 750 mg/kg PHT exhibits an increase in transfer latency ($P < 0.0001$) compared to both the 250mg/kg and 500 mg/kg PHT (**Figure 4**).

**Figure3:**BarChartshowingthearrivaltimeactivityofrats

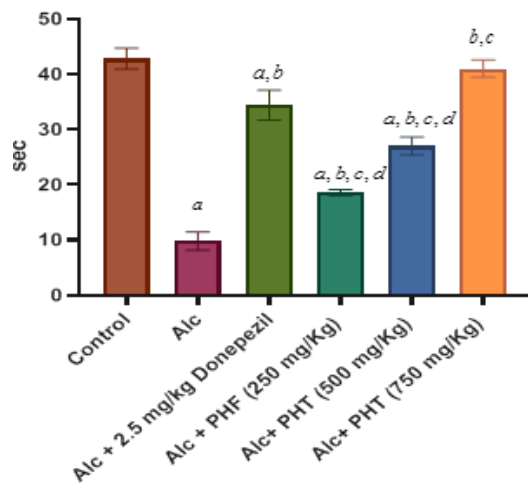


Figure4:EffectofPHTonTransferlatencyacrossGroupsby Day1:a=($p<0.0001$,Alc),b=($p<0.0001$,2.5mg/kgdonepezil), c=($p<0.0001$,Alc+250mg/kgPHTgroup),d=($p<0.0001$,Alc 500 mg/kg PHT).mean $\pm$ SEM and analyzed using one-way ANOVA: (F = 266.6 (5,24), $p<0.0001$).

3.5. Effect of PHT on Transfer Latency across Groups by Day 2

There is a significant increase in transfer latency ($P<0.0001$) for the 2g/kg alcohol, 2.5mg/kg donepezil, and 250mg/kg PHT groups compared to the control.

Conversely, the 2.5 mg/kg donepezil and all PHT doses (250 mg/kg, 500 mg/kg, and 750 mg/kg) show a significant decrease ($P<0.0001$) in transfer latency compared to the 2 g/kg alcohol. Additionally, the 750 mg/kg PHT demonstrates a decrease ($P<0.0001$) in transferlatencycomparedtoboththe250mg/kgand500 mg/kg PHT (Figure 5).

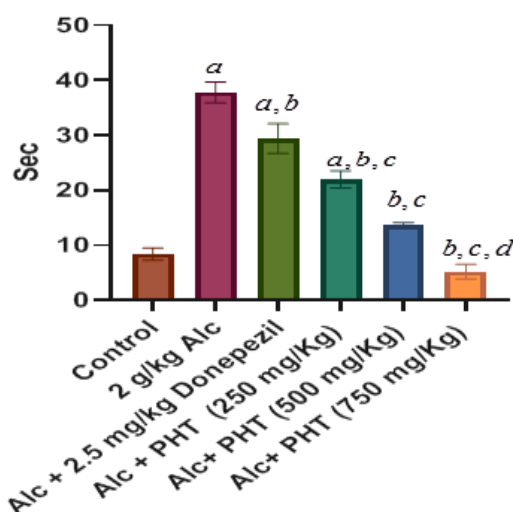


Figure5:EffectofPHTonTransferlatencyacrossGroupsby Day 1: a=($p<0.0001$,Alc), b=($p<0.0001$, 2.5 mg/kg donepezil), c=($p<0.0001$, Alc + 250 mg/kg PHT), d=($p<0.0001$, Alc 500 mg/kg PHT). mean $\pm$ SEM and analyzed using one-way ANOVA: (F = 286 (5,24), $p<0.0001$).

3.6. EffectofPolyherbalFormulationonInterleukin-6.

There is a notable rise ($P<0.0001$) in interleukin-6 activity levels when examining 2g/kg alcohol, 2.5mg/kg donepezil, and both 250 mg/kg and 500 mg/kg doses of PHT compared to the control. Interestingly, 2.5 mg/kg donepezil shows a decrease ($P<0.0001$) in interleukin-6 activity compared to the 2g/kg alcohol. Additionally, the PHT doses exhibit a significant reduction ($P<0.0001$) in interleukin-6 activity in a dose-dependent manner when compared to the alcohol (Figure 6).

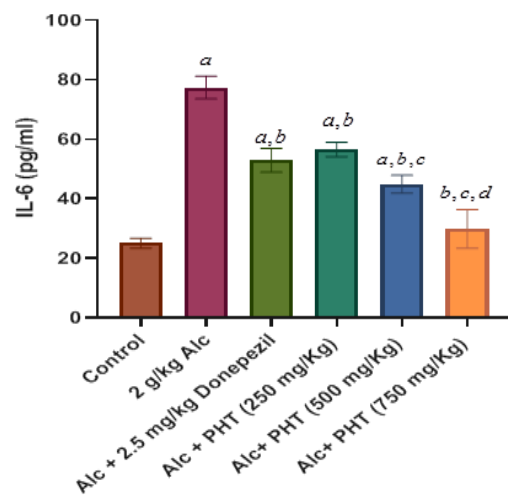


Figure6: Effect of PHT on Transfer latency across Groups by Day1: The analysis showed a highly significant difference in interleukin-6 activity levels among treatment groups (F=120.3; $p<0.0001$) a=($p<0.0001$, Alc) b=($p<0.0001$, 2.5mg/kg donepezil)c=($p<0.0001$, Alc+250mg/kg PHT)d=($p<0.0001$, Alc+500mg/kg PHT). mean $\pm$ SEM and analyzed using one- way ANOVA: (F = 120.3 (5,24), $p<0.0001$).

3.7. Effect of Polyherbal Formulation on Interleukins-1 β

There is a notable increase ($P<0.0001$) ininterleukin-1 β activity among the 2g/kg alcohol and 2.5mg/kg donepezil groups compared to the control. Additionally, there is a significant ($P<0.0001$) dropininterleukin-1 β levels in the 750mg/kg PHT compared to the 2g/kg alcohol, 2.5mg/kg donepezil, and control groups. Furthermore, there is a significant ($P<0.0001$) reduction in interleukins-1 β activities in 750 mg/kg PHT when compared to the 250 mg/kg and 500 mg/kg PHT (Figure 7).

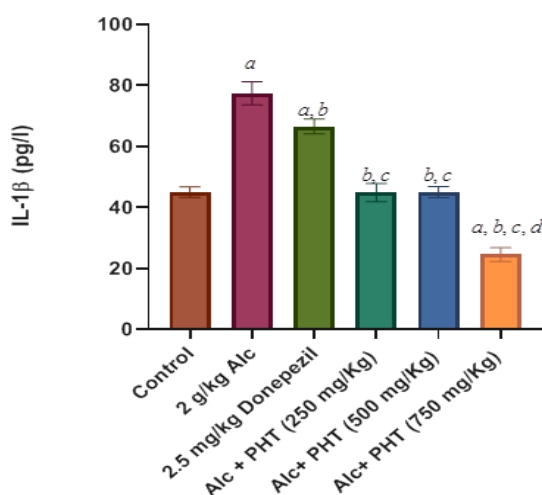


Figure7: Effect of PHT on Transfer latency across Groups by Day 1: a=($p < 0.0001$, Alc), b=($p < 0.0001$, 2.5 mg/kg donepezil), c=($p < 0.0001$, Alc + 250 mg/kg PHT), d=($p < 0.0001$, Alc 500 mg/kg PHT). mean $\pm$ SEM and analyzed using one-way ANOVA: ($F = 257.4$ (5,24), $p < 0.0001$).

3.8. Histology

Figure 8 shows the photo micrographs of the Cornu

Ammonis Area (CA1) of the hippocampus from various animal groups. In the control, a clear polymorphic cell layer (PL) and well-defined pyramidal neurons are visible, each with centrally located nuclei in the pyramidal cell layer (PCL), along with an intact molecular layer (ML). Moving on to the 2 g/kg alcohol group, the photomicrograph reveals significant pyknosis in most pyramidal neurons (marked with **arrows**) and some intracytoplasmic vacuolations (indicated by **circles**). In the 2.5 mg/kg donepezil group, the image shows several rejuvenated pyramidal neurons with in the pyramidal cell layer (again, marked with **arrows**). For the 250 mg/kg PHT group, the photomicrograph highlights a few rejuvenated pyramidal neurons in the pyramidal cell layer (**arrows**), but there are also some pyknotic pyramidal neurons (**circles**). In the 500 mg/kg PHT group, we observe rejuvenated pyramidal neurons (arrows) along side a few instances of pyknosis (**circles**). Lastly, the photomicrograph of the 750mg/kg PHT group shows several rejuvenated pyramidal neurons in the pyramidal cell layer (arrows), with only a handful of vacuolations (**circles**).

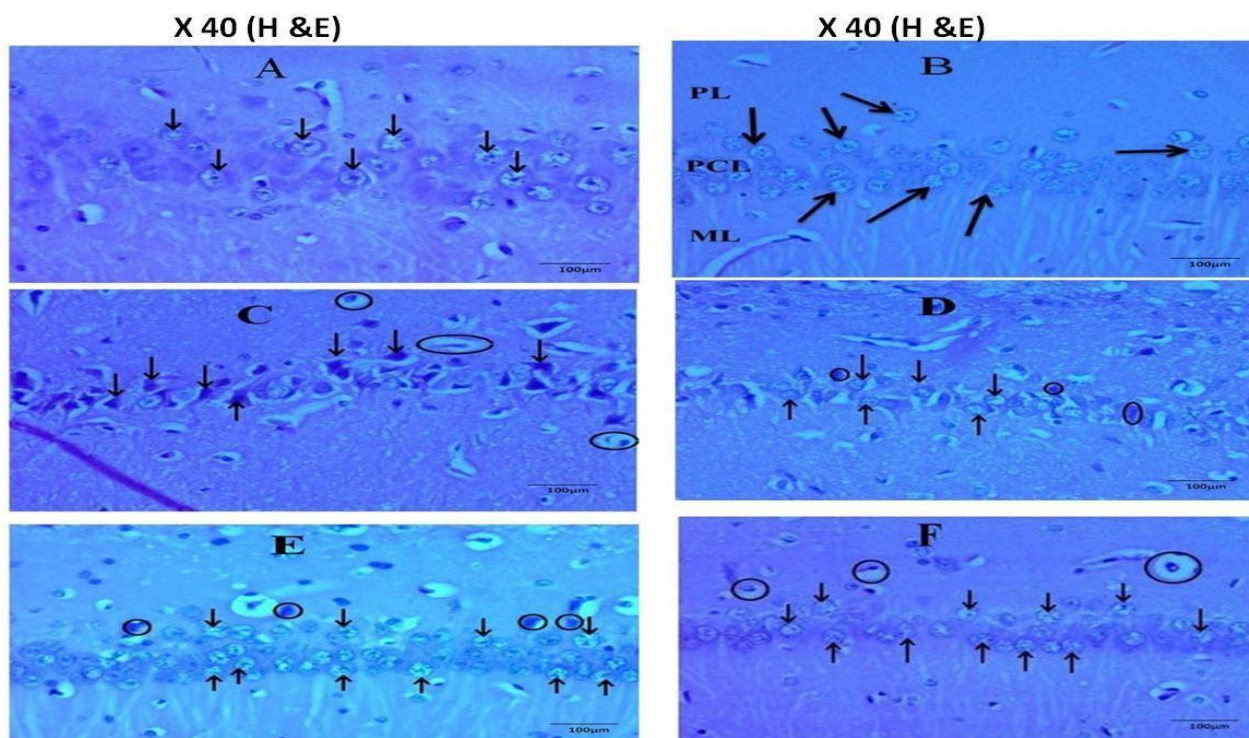


Figure8: Section of the Cornu Ammonis Area(CA1) of the hippocampus. Group 1(A),Group2(B),Group3(C),Group 4 (D),Group 5 (E), Group 6 (F). (Haematoxylin and eosin (H&E), 4x magnification; Scale Bar: 1 mm = 100 μ m).

3.9. Discussion

This study investigated the GC-MS analysis and cognitive and neuroprotective potential of a polyherbal therapy (PHT) on visuospatial functions in alcohol-induced dementia in Wistar rats. The research revealed that PHT contains various neuroprotective compounds. For instance, Baicalin (1.370%) is known for its ability to reduce neuroinflammation and oxidative stress associated with neurodegenerative disorders [26]. Numerous studies have shown that this compound can effectively lessen the severity of conditions like depression, stroke, and central nervous system degenerative disorders by providing antioxidant and anti-inflammatory benefits, regulating programmed cell death, and mitigating mitochondrial dysfunction [27, 28, 29]. Additionally, Nobiletin (2.421%) has been found to boost cognitive function through cAMP response element-binding protein-brain-derived neurotrophic factor (CREB-BDNF) signaling in Alzheimer's models [30, 31]. Luteolin (0.773%) has been reported to protect neurons from excitotoxicity and microglial activation [32].

Rosmarinic acid plays a role in reducing β -amyloid aggregation and enhancing synaptic plasticity [33]. Furthermore, both Artemetin (18.266%) and quercetin (0.444%) have been shown to inhibit the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and cyclooxygenase-2 (COX-2) pathways [34, 35]. Baicalin also suppresses TNF- α and IL-6 in microglial cells [36]. The dose-dependent reduction in pro-inflammatory cytokines (Figures 5 and 6) aligns with quercetin's ability to suppress IL-1 β in neuroinflammation [37]. Notably, high-dose PHT outperformed donepezil, likely due to its multi-target anti-inflammatory effects, contrasting with donepezil's focus on cholinergic mechanisms.

In the classic labyrinth experiment, the shorter arrival times in the PHT groups compared to those treated with alcohol or donepezil suggest that spatial learning has improved (see Figure 2). This boost in spatial learning may be attributed to compounds such as Nobiletin and Baicalin, which are known to enhance the release of acetylcholine in the hippocampus and support long-term potentiation (LTP) [38].

In the elevated plus maze experiment, the memory retention observed on Day2 was notably better in rats treated with PHT (Figure 4), indicating that flavonoids may

play a role in strengthening synaptic connections [39]. The reduced transfer latency points to an improvement in hippocampal-dependent memory, likely driven by neurogenesis and synaptic plasticity facilitated by flavonoids [40]. The dose-dependent improvements align with research showing that Nobiletin offers neuroprotection in a dose-responsive manner [41]. PHT was effective in reversing alcohol-induced neurodegeneration, such as pyknosis and vacuolations, especially at higher doses (Figure 6). The flavonoids in PHT, such as Baicalin and Luteolin, inhibit apoptosis by modulating the B-cell lymphoma 2/Bcl-2-associated X protein (Bcl-2/Bax) pathway and promote neuronal survival [42].

4. Conclusion

This study demonstrates that PHT exhibits impressive neuroprotective, cognitive, and anti-inflammatory benefits, as supported by various behavioral, biochemical, and histological analyses.

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Conflict of interest

The authors declared that there is no conflict of interest.

Data availability

All data associated with this study are present in the paper.

Authors Contributions

UA, Inwang: Designed the experiments, performed experiments and collected data, discussed the results and strategy, supervised, directed and managed the study.

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Using artificial intelligence chatbots

There was no use of artificial intelligence in the making of this article.

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