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Neuroprotective Effect Of *Cymbopogon nardus* And *Polygonum glabrum* On Alcohol-Induced Alzheimer In Rats

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Abstract:

Alzheimer's disease (AD) is a degenerative neurological condition that gradually worsens over time. It is marked by a decline in cognitive function, including memory loss, and changes in behaviour. AD aetiology is influenced by several variables, including genetics, environment, and lifestyle. Alcohol use is a risk factor that may be changed and has the potential to impact the structure and function of the brain. Prolonged alcohol use may cause neuroinflammation, oxidative stress, and neuronal death, resulting in cognitive impairment and dementia. Nevertheless, the impact of alcohol on the developing brain remains little understood. This research examined the effects of alcohol on the brains of adult rats and explored the possible therapeutic benefits of extracts from Cymbopogon nardus L. and Polygonum glabrum L. for treating Alzheimer's disease. We used a rat model to simulate the drinking behaviour of human adolescents, namely binge ethanol consumption. The rats were subjected to subcutaneous administration of ethanol at a dosage of 2 g/kg every day for a duration of 21 days. Subsequently, rats who had received treatment were exposed to behavioural and biochemical assessments. Our investigation revealed that excessive ethanol consumption has a detrimental effect on the ability of adult rats to acquire and remember spatial information, as shown by the Morris water maze tests. Ethanol furthermore decreased the number of dendritic spines and synaptic proteins in both the hippocampus and cortex, which suggests the occurrence of synaptic injury. In addition, ethanol elevated the concentrations of pro-inflammatory cytokines, oxidative stress indicators, and apoptotic proteins in the brain regions. The administration of extracts effectively corrected the cognitive impairments, loss of synaptic connections, inflammation of brain tissue, oxidative damage, and death of neurones caused by ethanol exposure. The findings of our study indicate that exposing teenage rats to binge alcohol leads to persistent neurodegeneration and cognitive impairment in adulthood. However, these negative effects may be mitigated by the administration of BMCT, AMCT, WCO, and HAEPG. This work offers novel perspectives on the processes behind alcohol-induced brain damage and the potential advantages of using extracts as a therapy to prevent or manage alcohol-related dementia.Utilising any metric to evaluate dementia clinically, whether in individuals with cognitive impairments or in the general population, is inherently constrained. Having a thorough understanding of these

limitations allows us to make informed decisions in selecting appropriate scientific methods to customise our neuropsychological assessment or consider alternate measures.
In the end, there could be a middle ground due to these constraints; yet, scientific knowledge has provided us with a more comprehensive understanding of the progression of dementia than ever before. By using advanced technologies like MRI, fMRI, PET, and SPET scans, together with cognitive testing conducted at certain intervals, including follow-ups, clinicians now have a greater ability to provide a more accurate diagnosis and prognosis compared to previous methods. It is expected that this would help educate service providers in expanding access to individuals with learning difficulties who also have dementia.
Keywords: Alzheimer, oxidative stress, ethanol

INTRODUCTION

Alzheimer's disease (AD) ranks as the sixth leading cause of mortality in the United States. Alzheimer's disease (AD) is the most common neurodegenerative condition.1 Although there is increasing evidence that damage from Alzheimer's disease (AD) begins to occur in the brain throughout middle age, the first clinical symptoms often do not manifest until individuals reach the age of 65 or beyond. This delay is mostly due to the fact that the number of elderly individuals in the community is expanding at a higher rate compared to other age groups.The numbers 2 and 3.

The reason that it is consistently overlooked is that it is the most crucial factor in the development of AD. The condition often remains dormant until middle life, even in those with a hereditary predisposition. Regardless of hereditary predisposition, ageing plays a key role in Alzheimer's disease (AD), clearly showing that the start of the illness is controlled by a process connected to age. Furthermore, apart from Alzheimer's disease (AD), this age-related penetrance is linked to many other grave conditions, including cancer, atherosclerosis, arthritis, and emphysema. This implies that there might be common causes leading to different results. From the first accounts of AD, it has been recognised that the process of identifying AD in older individuals may be difficult. Indeed, a research revealed that neuropathologists, who were unaware of the patients' clinical history, accurately diagnosed Alzheimer's disease in 76% of older individuals who had not previously shown any clinically significant mental disorders. These results reinforce the distinction between senile dementia and AD, with the former being a consequence of old age in general and the latter being caused by an unknown factor.Four Reports indicate that some dietary factors, such as excessive alcohol use, increase the probability of developing AD. The impact of alcohol on cognitive processes and the underlying mechanisms of Alzheimer's disease (AD) remain uncertain. This research investigates the correlation between ethanol exposure and Alzheimer's disease. Different concentrations and durations of ethanol were applied to cell culture samples.5

Alcohol use may contribute to the development of Alzheimer's disease (AD). The research posits a correlation between drinking and severe cognitive impairments, such as alcoholic dementia. Considering the similarities between alcohol use and the effects of AD on cognition, brain disorders, and brain chemistry, it seems plausible that alcohol consumption may increase the probability of acquiring AD. Assessing the potential correlation between alcohol use and AD is particularly difficult because of

the complexities involved in identifying and distinguishing between alcoholic dementia and AD. This study is crucial because there is a physiologically plausible connection between alcohol and AD, and understanding how alcohol affects AD may provide insights into its root causes. Despite being avoidable, regular alcohol intake is associated with AD, making this research even more important.Six Cymbopogon nardus, often known as the "tree of life," is highly valued for its many applications and serves as a vital source of sustenance for millions of people globally, particularly in tropical and subtropical areas. Around 65-75% of copra, which is the desiccated kernel mostly used for extracting oil, consists of oil. Cymbopogon nardus oil is a very promising edible oil for use in the kitchen. The oil is extensively metabolised and consists of a substantial proportion of medium-chain fatty acids (MCFA). By pulverising the dehydrated core of copra, which has an oil content of 60-65%, Cymbopogon nardus oil is obtained. The oil has a naturally pleasant taste like Cymbopogon nardus and consists of 92% saturated fatty acids in the form of triglycerides. The predominant kind of saturated fatty acids present in the oil are MCFAs, which stands for medium-chain saturated fatty acids. Most vegetable oils do not include medium-chain fatty acids (MCFA), however Cymbopogon nardus oil mostly consists of lauric acid (45-56%), which is a kind of MCFA.7

Studies undertaken over the last several decades have shown that Polygonum glabrum and its constituents possess potent antioxidative, anti-inflammatory, antibacterial, antimicrobial, and antifungal properties8,9,10,11. Studies have shown that Polygonum glabrums have antihypertensive properties in both live organisms and laboratory experiments. Polygonum glabrums and its contents have been extensively researched for their potential in treating prostate cancer, which is a kind of cancer affecting the prostate gland.Twelve Furthermore, a multitude of pre-clinical investigations have shown the beneficial effects of consuming Polygonum glabrum juice or extract on many disorders. For example, these medications enhanced the quality of mouse sperm, reduced neuronal damage, and lowered the accumulation of amyloid in mice models of Alzheimer's disease and brain injury caused by lack of oxygen and blood flow. Administering a single intraperitoneal injection of Polygonum glabrum extracts also had a beneficial impact on fish that were naturally afflicted with the lymphocystis disease virus. This occurred due to the activation of the fish's inherent immune system, resulting in a decrease in mortality caused by infection. Hydro-alcoholic extracts derived from Polygonum glabrum have notable antibacterial properties and may be used for the treatment of dental plaque. Oral use of Polygonum glabrum juice, which is high in ellagic acid, reduces skin issues caused by UV exposure in humans.11

Materials and Methods

Collection, authentication and extraction of plant material

In December 2021, I collected mature and freshly harvested fruits of Cymbopogon nardus (Cymbopogon nardus L.) and Polygonum glabrum (Polygonum glabrum L.) from Alwar, Rajasthan, India. The authenticity of the Cymbopogon nardus (Cymbopogon nardus L.) and Polygonum glabrum (Polygonum glabrum L) fruits was confirmed by botanist Harshad M Pandit, formerly the Head and Associate Professor of Botany. The authentication was conducted in Andheri (West), Mumbai, with reference numbers 85/14909-specimen #: bbg p 08222616 and 14909/85-specimen #:

bbg p 082202611, dated 03/12/2021, respectively.

The shredded Cymbopogon nardus is then inserted into a mesh bag and then put into a pressing machine. In order to extract the maximum amount of Cymbopogon nardus milk, it is necessary to repeatedly apply pressure to the bag. After the pressing process, just the fibre of Cymbopogon nardus is left, while everything else is dried. During the night, the Cymbopogon nardus milk is left outside in a glass jar to undergo separation. The oil is located in the centre, the water settles at the bottom, and the cream ascends to the top.

The water located at the bottom is discarded, while the cream situated at the top may be extracted by skimming. The oil is located in the intermediate stratum; in order to enhance its transparency and cleanliness, it underwent filtration and was thereafter left undisturbed. After the filtration process, the oil may exhibit a small cloudiness. Subsequently, the oil was sealed with a small aperture and stored for about three days. During this period, all the water contained inside evaporates, resulting in the formation of transparent oil.

A hydroalcoholic solution with a concentration of 90% (volume/volume) ethanol was used to extract the peel. The peel, weighing 3 kg, was first physically separated and then soaked in the solution at room temperature for a period of five days in a dark environment. The hydroalcoholic extracts underwent filtration, were subjected to drying at a temperature of 40 °C, and then kept in a freezer at a temperature of -10 °C. The pulps were also extracted, and then the peel extract was mixed with them. [14]

The extracted Medium Chain Triglycerides (MCT) included MCT-1, MCT-2, MCT-3, and MCT-4. Among these, MCT-4 was chosen as the Best Medium Chain Triglycerides (BMCT). Additionally, we also selected AMCT (a mixture of all Medium Chain Triglycerides), WCO (Whole Cymbopogon nardus Oil), and HAEPG (Hydroalcoholic Extract of Polygonum glabrum L) for the purpose of evaluating their neuroprotective activity. Rats were administered this unrefined extract to evaluate its neuroprotective properties in rats exposed to ethanol.

Animals

Bharat Serum and Vaccines Ltd., Ambernath supplied the male Spargue Dawley rats, which weighed between 180 and 300 g. The rats were housed in an environment with a temperature of 24 oC, a humidity level of 60%, a light/dark cycle of 12 hours and a limitless supply of food and water. Experimental methods that follow the 2018 Compendium of CPCSEA, New Delhi, were approved by Sunrise University's animal ethics committee.

Chemicals

Ethanol, Bovine Serum Albumin, Thiobarbituric acid, DTNB (5,5′-dithiobis-(2-nitrobenzoic acid) were purchased from Sigma - Aldrich, Mumbai, India and used in this study. All other chemicals used were of analytical grade.

Dosage Fixation

The therapeutic dosage was determined in a pilot research that used an ethanol-induced animal model of Alzheimer's disease. The study included two different doses of each treatment drug, 200 and 300 mg/kg. Subcutaneous administration of ethanol for 21 days was associated with memory impairment as measured by the Morris water maze and the Elevated plus maze, two behavioural tests. There was no difference in neuroprotection between the 200 and 300 mg/kg treatment dosages, and both reduced memory deterioration. For this reason, we have been utilising the lesser dosages of 100

mg/kg and 200 mg/kg instead of the larger dose of 300 mg/kg.

Experimental Design

This research used 70 fully grown male Sprague Dawley rats, ranging in weight from 180 to 300 g. The research was conducted in a standard laboratory setting, where the animals were provided with food and water on a continual basis and followed a natural 12-hour cycle. From 9:00 am to 17:00 pm, a whole spectrum of investigations were conducted. Oral solutions of all experimental drugs, with the exception of WCO, were prepared in normal saline (pH 7.4). The oral administration solutions of all the medications were prepared fresh at the start of each trial, and 0.5 mL per 100 g of body weight was ingested in total.

A 20% solution of ethanol in sterile normal saline was administered subcutaneously to all groups except the control group at a dosage of 2 g/kg. Each of the following sets of animals included seven rats:

Group I (Control): Vehicle P.O. 0.5 mL/100 g body weight was given daily for 21 days.

All the groups from II to X received respective doses, 30 minutes before subcutaneous injection of ethanol at a dose of 2 g/kg daily for 21 days.

Group II (Untreated AD): Vehicle P.O. (Saline) 0.5 mL/100 g body weight.

Group III (BMCT 100 mg/kg): Oral dose of BMCT 100 mg/kg.

Group IV (BMCT 200 mg/kg): Oral dose of BMCT 200 mg/kg.

Group V (AMCT 100 mg/kg): Oral dose of AMCT 100 mg/kg.

Group VI (AMCT 200 mg/kg): Oral dose of AMCT 200 mg/kg.

Group VII (WCO 100 mg/kg): Oral dose of WCO 100 mg/kg.

Group VIII (WCO 200 mg/kg): Oral dose of WCO 200 mg/kg.

Group IX (HAEPG 100 mg/kg): Oral dose of HAEPG 100 mg/kg.

Group X (HAEPG 200 mg/kg): Oral dose of HAEPG 200 mg/kg.

On days 12, 13, 14, and 21, all animals underwent behavioural evaluation utilising cognitive performance and gross behavioural activity. The animals were terminated by administering Xylazine (5-13 mg/kg) and Ketamine (40-100 mg/kg) by intraperitoneal injection. This was done after 21 days of daily administration of the medicines being examined, in addition to ethanol. Following the incision of each rat's skull, the brains were expeditiously extracted and rapidly chilled using isotonic saline. A sterile NaCl solution (0.9%) was used to thoroughly cleanse the whole brain. Subsequently, a 10 percent brain homogenate was produced by using a homogenizer and sodium phosphate buffer (30 mM, pH 7.4). In order to remove any residual cell debris, the homogenate was subjected to centrifugation at a speed of 20,000 revolutions per minute for a duration of 2 hours, after which the resulting supernatants were collected for further analysis.

Behavioral studies Open Field Test[15]

The apparatus was constructed from white plywood, with dimensions of 72 by 72 cm, and included walls of 36 cm in height. Rats may be seen within the device due to the fact that one of its walls is made of clear Plexiglas. Through the transparent Plexiglas floor, he could see the green lines that had been delineated on the ground using a marker. The dimensions of each square on the floor were 18 by 18 centimetres, as a

result of the presence of lines. A central square of 18 cm x 18 cm was drawn in the middle of the open area. The centre square is used due to the high locomotor activity and frequent crossing of test chamber boundaries seen in some rat strains during test sessions. The centre plaza is surrounded by ample space, effectively distinguishing it from the other neighbourhoods. The rats were placed either in the centre of the open field or in a corner nearby for a duration of five minutes in order to allow them to explore the apparatus.

The frequency of each rat's exploration of the centre square of the wall and its 12 neighbouring periphery squares was individually recorded, along with a variety of grooming behaviours including licking the fur, cleaning the face, and scratching. For a duration of five minutes, we tallied the quantity of animals that were assuming an upright position, namely by standing on their hind limbs while periodically resting their forelegs against the wall. During this time, they were engaged in the activities of smelling and attentively surveying their environment. After the five-minute test, the rats were returned to their cages, and the open field was sanitised using 70% ethyl alcohol and allowed to dry before the next testing. Rats were administered ethanol subcutaneously and then put in the open field arena (OFA) on days 13, 14, and 21 to evaluate their overall behavioural activity.

Assessment of cognitive performance by the Morris water maze task[16]

The Morris water maze was used to assess the capacity to learn and remember information. As the foundation of the Morris water labyrinth, a big circular pool of 150 cm in diameter and 45 cm in height, filled to a depth of 30 cm with water at 28 ±1oC, was divided into four equal quadrants by two threads that were fastened at right angles to each other. There was enough illumination and a chamber with four coloured flag clues—one in each quadrant—around the pool. Throughout the study, these irrelevant hints serve as a memory enhancer. A 4.5-centimeter-diameter circular platform was positioned 1 centimeter above the water on one pool quadrant during the acquisition phase. The retention phase used the same platform, but this time it was placed 1 cm below water level. The platform's location remained unaltered during both periods of examination. There were a total of four trials for each animal, with a five-minute interval between each. The animal was carefully dropped into the water in a different quadrant of the pool for each experiment. Then it was 120 seconds till the animal had to locate the platform. After that, the animal had 20 seconds to stay still on the platform. If the animal did not reach the platform within the 120-second time limit, its escorts were permitted an additional 20 seconds to remain there.

The animals were trained for two consecutive days during the maze acquisition phase. During the acquisition phase, the rats were randomly put into one of four starting locations before being thrown into the water. The time it took to reach the visual platform was measured, also known as acquisition latency.

After the acquisition phase, the animals had a retention phase in a maze. To measure how well they remembered the task, they were tested again. At random, each animal was released from the side of the pool that was facing the wall. On days 14 and 21, the time it takes to find the underwater platform is called first retention latency (1 st RL), and on days 21 and 22, it's called second retention latency (2nd RL). Measurements of acquisition and first and second retention latency began on days 12, 13, 14, and 21 after subcutaneous ethanol therapy in rats with Ethanol-induced Alzheimer's disease.

Elevated plus maze[17]

The elevated plus maze included two open arms that were 50 × 10 cm in size, flanked by two closed arms that were 40 cm high and of the same size. The arms are joined by the middle square, which is 10 × 10 cm in size. Memory acquisition was evaluated on the thirteenth day. Separate rats were placed at the tips of open arms that were angled away from the central square. The time it takes to transition from an open arm to a closed arm is known as the initial transfer latency (ITL). Once ITL was recorded, the animals were given five minutes to explore the labyrinth before being returned to their original cage. A rat's memory recall was assessed by placing it in a similar manner on an open arm. The retention latency was recorded twice, once after the first transfer delay (the first RTL) and once after the second RTL (second RTL). After rats started receiving ethanol subcutaneously on day 13, they underwent initial transfer latency on day 14, and first and second retention transfer latency on days 14 and 21, respectively.

Biochemical assessment

Measurement of brain Lipid peroxidation[18-20]

The amount of lipid peroxides was determined using the thiobarbituric acid (TBA) reaction technique established by Ohkawa et al. According to popular belief, the TBA test may detect levels of malondialdehyde (MDA) produced by peroxidizing lipid systems. Findings are presented in nanomoles per milligramme of protein.

Estimation of brain Nitrite[21]

Using the Griess diazotization method, nitrite may be spectrophotometrically detected as a product of NO's spontaneous oxidation under physiological circumstances. This approach can detect nitrite at a concentration of 1.0 μM. Another way to analyse nitrate is by using the Griess reaction, which involves catalytic reduction of nitrate to nitrite. A diazonium salt may be quantitatively produced by reacting sulfanilic acid with nitrite in an acidic solution. Finally, the diazonium salt is combined with N-(1-naphthyl) ethylenediamine to produce an azo dye whose absorbance at 548 nm may be measured spectrophotometrically. Reading nitrite concentrations from the absorbance of experimental samples from the standard plot yields results expressed as nmoles/ mg protein.

Estimation of brain Reduced glutathione(GSH)[22]

The Ellaman method is used to find reduced glutathione. A spectrophotometric detection at 412 nm is made possible when glutathione combines with DTNB to form a yellow chromophore. The amounts of GSH in the samples were reported as ug/mg protein, and a standard curve was created using known values of GSH.

Estimation of brain Superoxide dismutase (SOD)[23]

Scavenging superoxide (O*2), superoxide dismutase provides a first barrier against free radical damage. Enzymes belonging to the superoxide dismutase (SOD) family break down superoxide anion (O*2) into oxygen molecules and peroxide. We tested SOD's capacity to inhibit the spontaneous oxidation of epinephrine to adrenochrome. Data are expressed as units of superoxide dismutase (SOD) activity per milligramme of protein.

Estimation of catalase (CAT)[24]

The absorption of H2O2 increases monotonically with decreasing wavelength in the ultraviolet (UV) spectrum. Catalase is a catalyst that speeds up the process of reducing hydrogen peroxide to water. The

reduction in absorbance at 240 nm is directly associated with H2O2 oxidation. The change in absorbance per unit is a measure of the catalase activity.

Estimation of brain Glutathione S-transferase (GST)[25]

When detoxication alkylating chemicals engage with glutathione's -SH group, glutathione S-transferases catalyse the reaction. This makes the byproducts of the alkylating agents more water-soluble by neutralising their electrophilic sites. Metabolising glutathione conjugates to mercapturic acid likely involves cleaving the glutamate and glycine residues and acetylating the cysteinyl residue's free amino group. One mole of product per minute under the circumstances of the specific test is catalysed by the amount of enzyme that is known as a unit of activity.

Estimation of brain acetylcholinesterase[26]

In 1961, George Ellman developed the Ellman's approach, a way to estimate AChE activity. When cholinergic iodide is hydrolyzed by AChE, the resulting thiocholine reacts with the SH reagent 5, 5-dithio-bis-(2, nitrobenzoic acid) (DTNB), which is reduced to thionitrobenzoic acid, a yellow anion with an absorbance maximum at 412 nm. The extinction coefficient of thionitrobenzoic acid is 1.36 104 molar/cm. Thionitrobenzoic acid concentrations, measured in nmoles/min/gram using a UV spectrophotometer, serve as a direct measure of AChE activity.

Estimation of brain total proteins[27]

A coloured chelate is produced when cupric ions in an alkaline solution react with protein peptide links; its absorbance is then measured at 578 nm. The solubility of this complex at an alkaline pH is maintained with the help of Sodium-Potassium Tartrate, which is an ingredient of the Biuret Reagent. There is a direct correlation between the absorbance of the final colour and the total protein content (g/dL) of the sample.

Estimation of BDNF[28]

A 96-well strip plate pre-coated with an antibody specific for BDNF is used in the Boster PicokineTM Rat BDNF Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit for the measurement of Rat BDNF. A biotinylated antibody that is specific to BDNF is used as the detecting antibody. Mouse monoclonal antibodies were used for both the detection and capture processes. An immunogen-enhanced recombinant Rat BDNF is part of the package. The expression system is NS0, and the immunogen sequence is H129-R247. The analytical validation of the kit has been completed and the reagents are ready to be used.

Estimation of amyloid beta[29]

You may track the expression of several target proteins in different cell lines and the effects of different stimulation conditions with the help of the Colorimetric Cell-Based ELISA Kit. To qualitatively ascertain the concentration of the target protein, an indirect ELISA format is used. In general, main (1°) antibodies attach to the target protein, whereas secondary (2°) antibodies that are HRP-conjugated bind to the Fc region of the 1° antibody. The 2° antibody-conjugated HRP enzyme is able to catalyse a colorimetric reaction upon substrate addition because of this contact.

Statistical Analysis

Values are expressed as the mean ± SD. The behavioral assessment data and biochemical estimations were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett test. $P < .05$ was considered significant.

Results

Animal behavioural parameters Open Field Arena (OFA)

The effects of ethanol induction on mobility and activity were alleviated to the greatest extent by BMCT 200 mg/kg and HAEPG extract. Medications that treat Alzheimer's disease have shown that animals may continue to walk and respond normally even after being exposed to these substances.

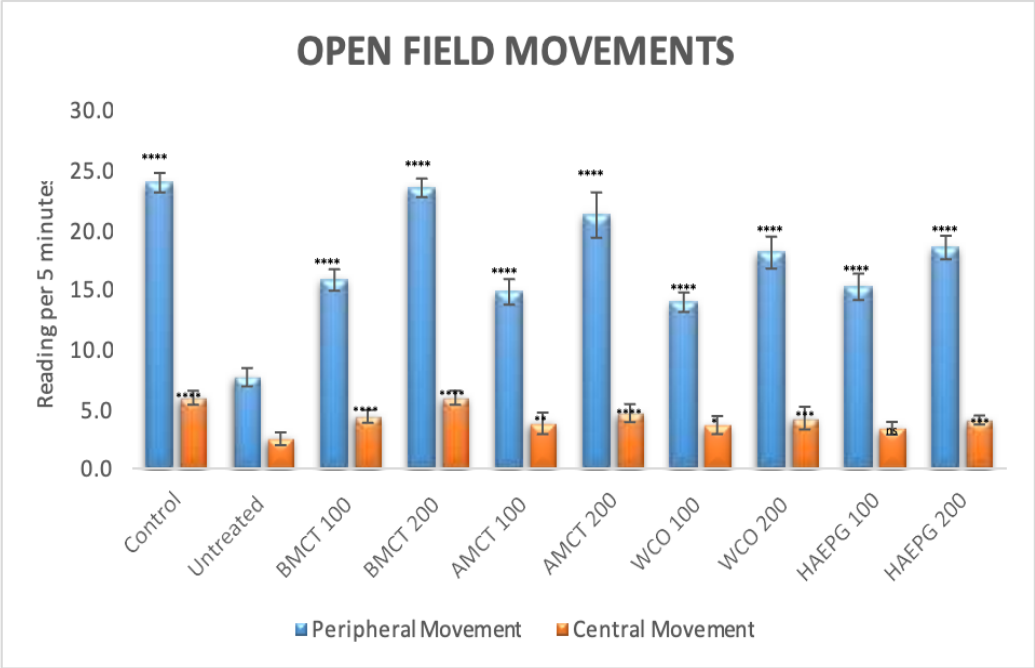

Figure 1: Effect of treatment drugs on movements in OFA of animals

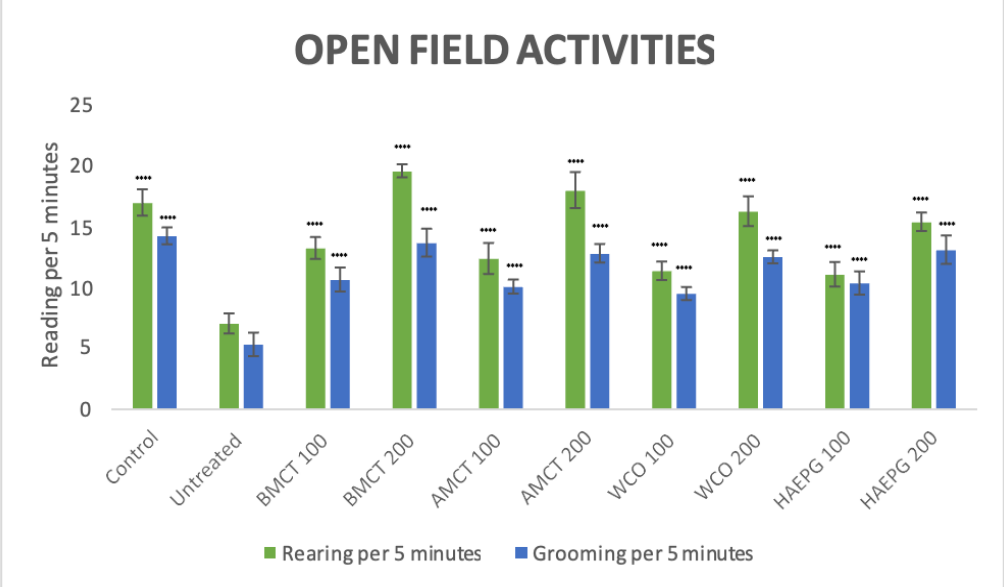

Figure 2: Effect of treatment drugs on open field activities in OFA of animals

Assessment of cognitive performance by the Morris water maze (MWM) task

When given the Morris water maze, the BMCT, AMCT, WCO, and HAEPG all led to considerable improvements in spatial navigation task recall. A significant decrease in IAL, 1st RL, and 2nd RL in the treated group proved this. This finding provides further evidence that the prophylactic use of medium chain triglycerides and HAEPG may improve cognitive performance in the areas of learning and memory. It is clear from these findings that ethanol induction leads to oxidative stress, central neuronal damage, and impaired learning and memory. Untreated AD rats exhibited noticeably longer first and second retention latencies (1st and 2nd RL), as well as an initial acquisition latency (IAL) that was significantly longer than the control group in the Morris water maze task. Nevertheless, the current study was able to overcome this memory impairment by comparing the treatment groups.

Treatment groups outperformed the untreated group, in which animals failed to reach the platform within the allotted time, according to data acquired by comparing the time spent on platform (TSOP). This proves beyond a reasonable doubt that the memory of the treatment group is unaffected by the individual AD-inducing drugs.

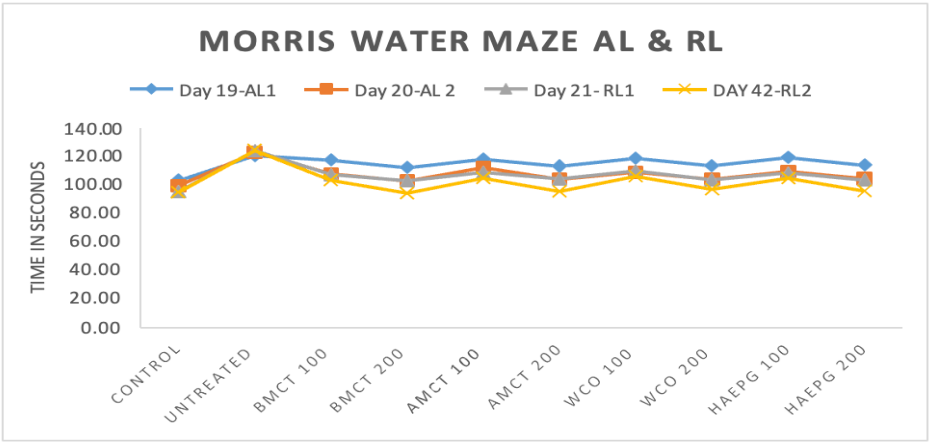

Figure 3: Effect of treatment drugs on acquisition and retention latencies in Morris water maze task of animals.

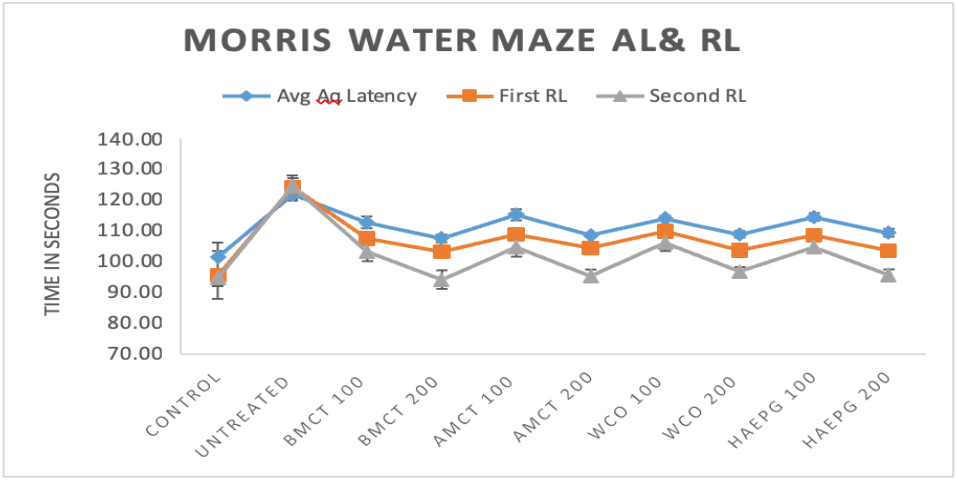

Figure 4: Effect of treatment drugs on average of acquisition and retention latencies in Morris water maze task of animals

Regardless of dose or time point, rats administered MCTs and HAEPG exhibited less working memory impairments than rats given ethanol to induce AD. Treatment groups demonstrated experimentally impacted memory retention after 21 days after AD injection with ethanol.

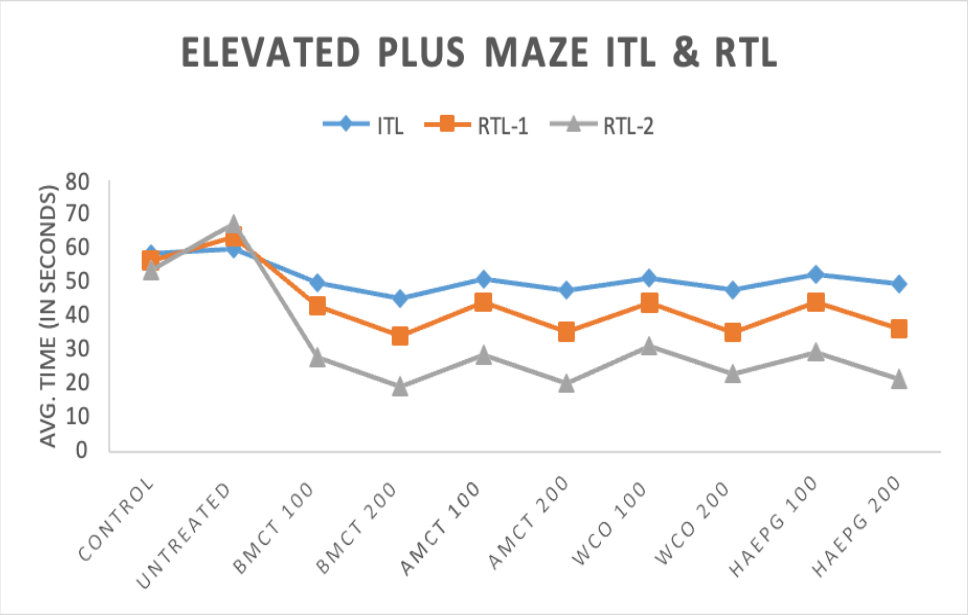

Figure 5: Effect of treatment drugs on Initial and retention transfer latency in Elevated plus maze of animals

Biochemical assessment

Estimation of effect of treatment drugs on lipid peroxidation, nitrite, reduced glutathione, superoxide dismutase, catalase, glutathione S-transferase, Acetylcholine esterase, total protein, brain derived neurotropic factors and amyloid beta in supernatants of whole brains of rats induced with ethanol.

The brain supernatants of the rats showed elevated levels of protein carbonyls and lipid peroxidation, with reduced amounts of SOD, GSH, and CAT. The amounts of glutathione peroxidase, superoxide dismutase (SOD), catalase, and frontal cortical areas of the rat brain were shown to increase after therapy. In order to protect neurones from ethanol-induced neurodegeneration, antioxidants like BMCT, WCO, and HAEPG help restore enzyme levels that have dropped.

Across all research models, the untreated group had elevated total protein levels. Protein levels are decreased with the continual injection of several therapeutic medicines. In ascending order, the HAEPG, WCO, AMCT, and BMCT are all determined to be successful. Brain oxidative stress was reduced and superoxide dismutase (SOD) and catalase (CAT) levels were dramatically increased with dose-dependent treatment with BMCT, AMCT, WCO, and HAEPG. It seems from the results that HAEPG, BMCT, AMCT, and WCO all protect neurones via an antioxidant mechanism. Treatment groups, particularly BMCT and AMCT, decreased AChE levels relative to the control group, suggesting that these candidates have a role in neuronal transmission and neurodegeneration prevention.

Table 1: Effect of treatment drugs on lipid peroxidation, nitrite, reduced glutathione, superoxide dismutase, catalase, glutathione S- transferase, Acetylcholine esterase and total protein in whole brains of rats induced with ethanol.

Groups	MDA(nmol MDA/min/ mg protein)	Nitrite (umol/mg)	GSH (ug/g)	SOD (unit/mg)	CAT (units/mg protein)	GST (umol/ml/ min)	AchE (nmol/ min/gm)	Total protein (g/dl)
Control	5.21****	218.3****	125.2****	45.40****	2.54****	132.9****	1.41****	5.61****
	±	±	±	±	±	±	±	±
	0.14	6.5	2.02	2.07	0.04	5.09	0.03	0.07
Untreated	8.32****	598.2****	37.1****	15.51****	1.27****	71.0****	3.43****	1.60****
	±	±	±	±	±	±	±	±
	0.09	17.7	0.88	1.27	0.01	0.80	0.03	0.09
BMCT	7.99****	418.9****	86.6****	23.38****	1.74****	97.6****	2.64****	2.41****
100	±	±	±	±	±	±	±	±
	0.09	14.2	1.51	0.44	0.02	2.17	0.04	0.09
BMCT	5.97****	262.2****	108.2****	37.65****	1.91****	118.8****	2.12****	3.32****
200	±	±	±	±	±	±	±	±
	0.23	3.4	1.86	0.87	0.04	3.25	0.01	0.08
AMCT	7.89****	494.0****	70.5****	20.40****	1.57****	91.3****	2.95****	2.30****
100	±	±	±	±	±	±	±	±
	0.05	7.5	1.54	0.65	0.03	4.84	0.02	0.05
AMCT	6.45****	425.1****	91.8****	32.98****	1.70****	109.9****	2.39****	2.64****
200	±	±	±	±	±	±	±	±
	0.15	9.9	1.25	1.39	0.03	2.90	0.02	0.25
WCO 100	8.10****	527.7****	66.7****	18.83****	1.45****	87.2****	3.17****	2.36****
	±	±	±	±	±	±	±	±
	0.05	7.8	3.74	1.30	0.03	3.18	0.02	0.51
WCO 200	6.17****	456.8****	80.7****	30.15****	1.56****	106.6****	2.82****	2.52****
	±	±	±	±	±	±	±	±
	0.25	3.6	1.86	0.41	0.03	1.31	0.02	0.40
HAEPG	8.04****	498.6****	68.6****	21.29****	1.57****	91.0****	3.27****	2.30****
100	±	±	±	±	±	±	±	±
	0.12	7.5	1.78	0.30	0.02	4.34	0.04	0.24
HAEPG	6.40****	434.0****	84.3****	31.01****	1.71****	111.1****	2.88****	2.41****
200	±	±	±	±	±	±	±	±
	0.11	22.7	1.60	0.50	0.01	1.31	0.04	0.42

The present study successfully overcame a significant memory impairment caused by ethanol which is reflected in all treatment groups especially BMCT found most effective out of all.

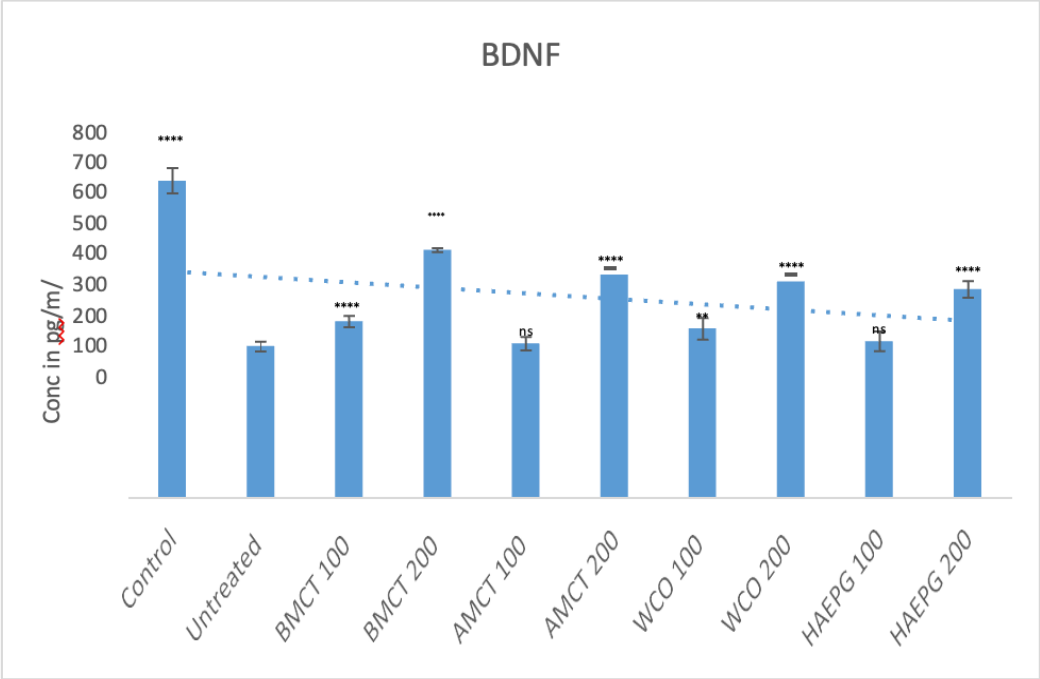

Figure 6: Estimation of BDNF using Boster Picokine ELISA kit Catalog number: EK0308

After being exposed to several drugs that induce Alzheimer's disease, rats' memory and learning capacities showed a significant decline. Group II rats treated with AD had considerably lower levels of brain derived neurotrophic factor (BDNF) than group I rats treated with no AD; nevertheless, BDNF levels increased dramatically after AD therapy across the board, particularly in the BMCT group.

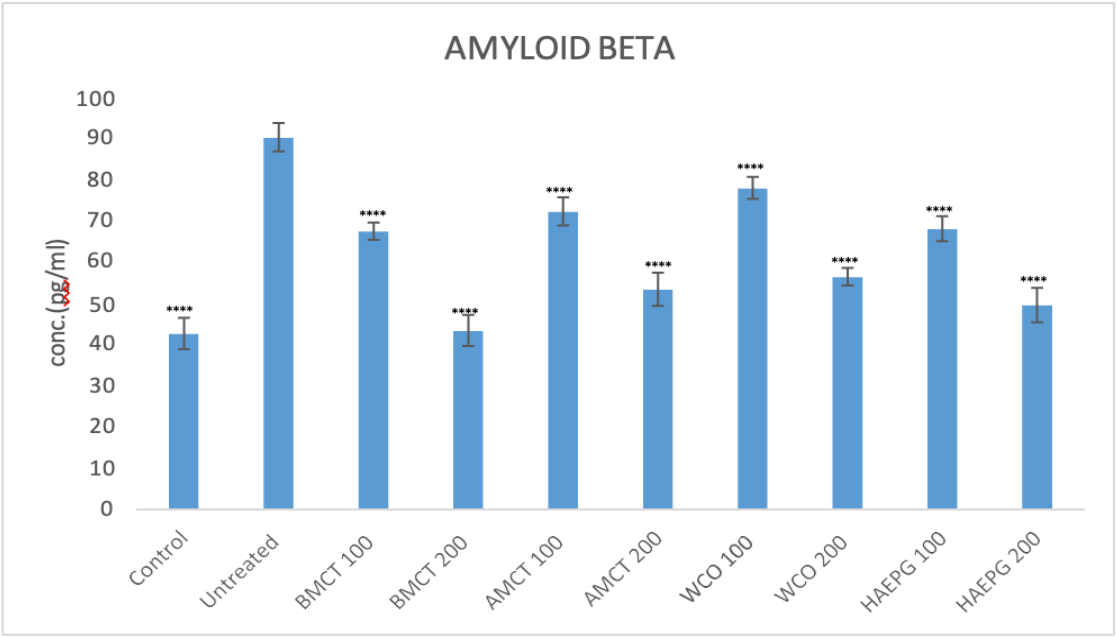

Figure 7: Estimation of Aβ using Boster Picokine ELISA kit, Catalog number: EKC1937

The average levels of Aβ in brain tissue increased significantly. The levels of Aβ peptide in the tissues of all treatment groups were considerably lower than those of the untreated AD rats.

Treatment meds have a role in neuroprotection against AD-induced different agents, as shown by the considerable reductions in Ab levels seen in all four treatment groups (BMCT, AMCT, WCO, and HAEPG).

Discussion

Millions of individuals throughout the globe suffer with Alzheimer's disease, a neurological illness that wreaks havoc on their memories, thoughts, and actions. The imbalance between the generation and removal of reactive oxygen species (ROS) in cells is known as oxidative stress, and it is one of the potential causes of Alzheimer's disease (AD). Reactive Oxygen Species (ROS) are chemicals that have the potential to harm cellular components, including lipids, proteins, and nucleic acids. This harm may result in cellular malfunction and eventual death.

The effects of BMCT, AMCT, WCO, and HAEPG on the biochemical and behavioural responses of rats given ethanol were shown in this research. Cognitive impairment, elevated cholinesterase activity, lipid peroxidation, and glutathione (GSH) levels were seen in rats who developed an alcohol dependence by an ethanol infusion. Rats given ethanol showed considerable and dose-dependent improvement in cognitive impairments after receiving chronic therapy with BMCT, AMCT, WCO, and HAEPG.

A byproduct of lipid peroxidation and a diagnostic for polyunsaturated fatty acid (PUFA) lipid peroxidation is malonaldehyde. Further, it adds to the buildup of oxidative stress. How much oxidative stress the body is experiencing is shown by its MDA level. There has been a reduction in the MDA level. According to a large body of research, the connection between oxidative stress and the development of mental and neurological diseases has long been recognised.[31]

Glutathione is a naturally occurring antioxidant that is mostly present inside cells in its reduced form. It inhibits the generation of hydroxyl radicals by interacting with free radicals. This protective mechanism involves the conversion of reduced glutathione to its oxidised form by the activity of the enzyme glutathione peroxidase.32 In this study, BMCT boosted levels of antioxidants glutathione and catalase, which protect cells from oxidative stress. It also reduced levels of malondialdehyde (MDA), an indicator of lipid peroxidation, and controlled the activity of the neurotransmitter acetylcholinesterase. Cymbopogon nardus oil may alter levels of acetylcholinesterase, a protein that is increased in Alzheimer's disease, according to some research. It may also have antioxidant and anti-inflammatory effects.

Results from open field tests and the Morris water maze test showed that the treated groups in the ethanol-induced rats exhibited intact learning and memory function; this highlights the promise of all of the treatment drugs and their function in Alzheimer's disease.

In this work, the fractions of Cymbopogon nardus L. and the hydroalcoholic extract of Polygonum glabrum L. may have reduced oxidative stress in ethanol-induced rats, which might have protected them from memory loss associated with ethanol.

Conclusion

The results of this investigation demonstrate that hydroalcoholic extract of Polygonum glabrum L. fruit, Cymbopogon nardus oil, or

fractions thereof obtained from fresh Cymbopogon nardus L. plant flesh may provide neuroprotection when taken over an extended period of time. The extracts successfully protect the rats against memory loss and brain dysfunction, even while the rodents are exposed to neurotoxicity from subcutaneous ethanol. Along with the data already presented, a review of the extracts' effectiveness showed that BMCT, with its superior neuroprotective properties, was the clear front-runner over AMCT, HAEPG, and WCO. Furthermore, some biochemical tests show that restoring antioxidant enzymes might mitigate Alzheimer's disease risk factors related to oxidative stress. The decrease in acetylcholinesterase levels in the treatment groups suggests that the extracts may work by influencing cholinergic neurotransmission, which is involved in memory function enhancement. Nevertheless, in order to comprehend the precise neuroprotective mechanism of the components in the hydroalcoholic extract of fruits of Polygonum glabrum L., more thorough research is necessary.

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