NEUROPROTECTIVE EFFECTS OF OIL OF COCOS NUCIFERA ON ALUMINIUM CHLORIDE-INDUCED BRAIN DYSFUNCTION IN RATS.

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Abstract

This study examined the neuroprotective effects of fractions- BMCT (Best Medium Chain Triglycerides) AMCT (All Medium Chain Triglycerides) and WCO (Whole Coconut Oil) of *Cocos nucifera* on the learning and memory in aluminium chloride induced brain dysfunction in rats. All Wistar rats of groups II to VI were received respective treatment doses (100/200 mg/kg) 30 minutes before oral administration of Aluminium chloride (100 mg/kg), daily for 42 days. On day 20, 21 and 42 following the start of aluminium chloride administration rats were subjected to evaluation of gross behavioral activity using Open field arena (OFA). Behavioral score recorded using- Line Crossing, Center Square Entries, Rearing and Grooming.

Cognitive performance was evaluated using Morris water maze (MWM) and Elevated Plus Maze (EPM). In MWM- rats were trained on day 19 and 20 to swim to a platform in a circular pool. The latency to reach the visual platform (acquisition latency) was measured. Time latency to find the hidden platform on day 21 and 42 following the start of aluminium chloride administration was recorded and termed the first retention latency (1 RL) (21 day) and the second retention latency (2 RL) (42 day), respectively. In EPM- rats were trained on day 20, each rat was placed at the end of an open arm and the time it took to move from the end of open arm to either of the closed arms was

recorded as initial transfer latency (ITL). On day 21 and 42, the rats were tested for retention latency and termed as first retention transfer latency (1st RTL) and second retention transfer latency (2nd RTL), respectively. The respective treatment groups (100/200 mg/kg, p.o.) significantly protected against aluminium-induced brain dysfunction, as evidenced in OFA, MWM and EPM.

Keywords: MCT's, Coconut Oil, OFA, MWM, EPM

Introduction

Alzheimer disease (AD) is a neurodegenerative disorder showing gradual progressive cognitive and functional deficits as well as behavioral changes and is associated with the accumulation of amyloid-β (AB) and tau proteins in the brain. (**1**)

The diagnostic criteria include a staging system based on early lesions in the entorhinal/perirhinal cortex, then hippocampal Ammon subfields, then association cortex, and finally primary neocortex. This is because the hierarchical pattern of neurofibrillary degeneration among brain regions is so consistent (NIA-RI Consensus 1997). Although it is unclear whether tangles are the cause of either neuronal loss or synaptic loss, there is a strong correlation between the loss of neurons and the loss of synapses. (**2**) The main beginning element may be changes in the creation and processing of amyloid protein, according to this theory. In Western societies, Alzheimer's disease is the most common cause of dementia. In the US, some 5.5 million people are affected, and up to 24 million people may be affected globally. The frequency is anticipated to double every 20 years through 2040 since both developed and developing countries are rapidly ageing. The social aging-related increase is expected to be significant and will have a high financial impact on public health. (**3**) The clinical diagnosis of AD is accurate about 80%–90% of the time when based on clinical indications of slowly progressing dementia and MRI evidence of substantial cerebral cortical atrophy. More complex studies, such as amyloid PET imaging, amyloid and tau concentrations in CSF, and (in the near future) tau PET imaging and plasma amyloid concentration, can be used to get results with higher precision. (**4**) Reactive oxygen species (ROS), which accumulate with age and cause damage to important cell parts such the nucleus, mitochondrial DNA, membranes, and cytoplasmic proteins, are the subject of the long-standing free radical hypothesis of ageing. According to numerous writers over a long period of time, the imbalance

between the formation of free radicals and ROS may have a role in the development of the majority of neurodegenerative illnesses, including AD. (**5**)

NEUROPSYCHOLOGICAL SYMPTOMS OF ALZHEIMER DISEASE The neuropsychological symptoms of Alzheimer disease include

1. Episodic memory impairment (amnesia)

2. Abnormally rapid forgetting (delayed recall failure)

3. Intrusion errors (paraphasias)

4. Deficits in language abilities-Verbal fluency (number of words produced on a given category in a given time eg, animal naming), Semantic categorization (not recognizing objects (words that belong together), Inability to recall over-learned facts

5. Executive function (inability to recognize blue when printed in another color)

6. Working memory (inability to name the country when given a town in the country)

7. Failure of dual tasking (inability to answer a simple question while walking)

8. Attention deficits

9. Visuospatial deficits (clock drawing failure)

10. Functional impairment (decline in instrumental and basic activities of daily living)

In addition, persons with Alzheimer disease may develop apathy early in the disease and agitation and other behavioral disturbances later in the disease. Higher levels of amyloid-b in older persons are linked to anxiety. (**6**)

By 2030, a projected 66 million people worldwide will be living with dementia—a figure set to rise to 115 million by 2050. Alzheimer's disease is the leading cause of dementia in the elderly. Clinically, Alzheimer's disease can be divided into early-onset (ie, patients younger than 65 years) and late-onset (ie, those older than 65 years), whereas pathologically it is characterized by the presence of plaques of amyloid β peptides and intraneuronal tangles of hyperphosphorylated forms of microtubule associated protein tau (MAPT). In our cohort, in 15 years, 27.5% of deaths occurred in individuals with AD. (**7**)

There are presently no effective or disease-modifying medications for AD. The course of AD is accompanied by molecular and clinical processes including as amyloid

accumulation, neuroinflammation, tau accumulation, neuronal degeneration, cognitive decline, and the appearance of behavioural and psychiatric disorders. Clinical trials that aim to prevent these occurrences are being assessed. Research attention has switched to individuals with prodromal or preclinical stages and positive diagnostic biomarkers as a result of recent failures in anti-amyloid trials. In the meanwhile, the amyloid hypothesis has been contested, and in 2019, there were much fewer anti-amyloid phase 3 trials. There are many different objectives for phase 1 and phase 2 trials, and trends indicate that neuroprotection and antineuroinflammation are being targeted more often in both phases, respectively. (**8**)

Aluminium was the first metal linked with neurodegenerative disease. Injecting aluminium in to the brain was shown to induce NFTs in animals. Aluminium was shown later to be enriched in AD brains, and specifically in NFTs and SPs. Considerable interest in the aluminium hypothesis of AD was generated by a study that showed that aluminium contamination in drinking water increased the risk of AD by1.5-fold. (**9**) (**Fig. 1**)
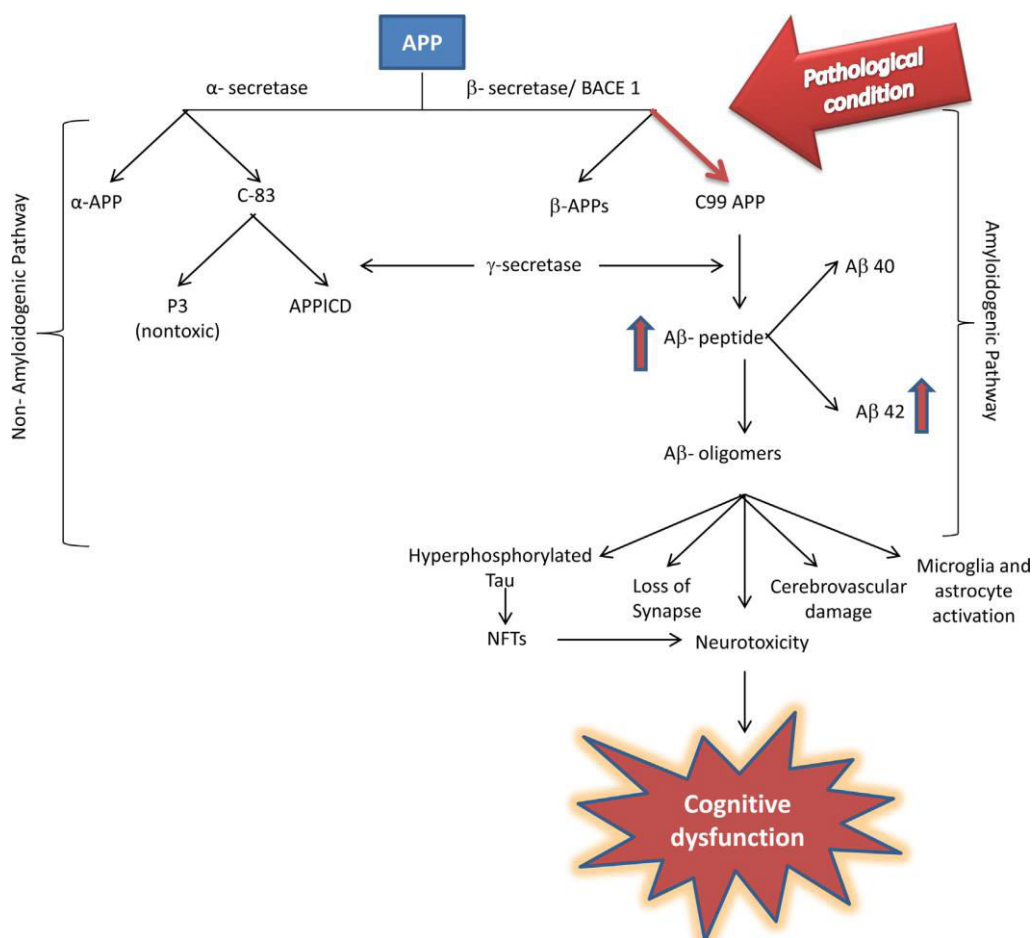
Fig. 1, Amyloid Precursor Proteins (APP) processing pathways

Ultimately, there is much potential on the horizon for AD therapeutics. If administered appropriately, a successfully developed mitochondrial antioxidant provides the vulnerable, aging neuron with a defensive shield against the oxidative cascade of neurodegeneration. Importantly, however, as effective as such therapies may be to those who have yet to enter the neurodegenerative cascade mentioned above, once significant amounts of oxidative damage accumulate within the cell such that the secondary pathologies of AD become apparent, any hope of reversing the course of the disease remains beyond the scope of simple antioxidant therapy. Therefore, while such a preventative treatment strategy is ideal for the young-to-middle aged population, its benefits do not extend to those who present the "oxidative steady state" within affected cells. At that point, secondary or symptomatic therapeutics must also be instituted. (**10**)

Coconut (*Cocos nucifera*), family Arecaceae (palm family) is one of the most important tropical tree crops in the world and is widely known as the "tree of life."

Medium chain triglycerides found in different fractions of coconut oil are regarded as a great solvent for flavourings, essences, and emulsifiers. These fatty acids are used to make emulsifiers, medications, and cosmetics.

The extraction of coconut oil through cold processing is done without the use of heat. This method involves centrifuging the coconut milk (2-8 °C) overnight to separate the oil, which is then collected, filtered, and stored. The simplest and least expensive way is this one.

A significant amount of lower chain fatty acid glycerides can be found in coconut oil. The oil is very resistant to oxidation from the atmosphere. The oil exhibits low iodine value, high saponification value, high amount of saturated fatty acids, and liquidity at 27 °C. The family of lipids known as medium chain triglycerides (MCT) consists of three saturated fats attached to a glycerol backbone. Each fat molecule in MCT has between 6 and 12 carbons, which sets it apart from other triglycerides. (**11**)

Materials and Methods

Plant Material

Medium Chain Triglycerides were isolated from oil of *Cocos nucifera.* Ketone bodies generated by fatty acid oxidation can serve as alternative metabolites for aerobic energy

production. The ketogenic diet, which is high in fat and low in carbohydrates, mimics the metabolic state of starvation, forcing the body to utilize fat as its primary source of energy. As ketones are efficiently used by mitochondria for ATP generation may also help to protect vulnerable neurons from free radical damage. (**12**)

Animals

Male Albino Wistar rats (180–300 g) were procured from National Institute of Biosciences, Bhor, Pune and maintained at 12/12 h light/dark cycle, 24 ^{0}C temperature and 60 % humidity with food and water *ad libitum*. The experimental protocols comply with the Compendium of CPCSEA, New Delhi, 2018 and were approved by the Animal Ethics Committee of the LSHGCT's Gahlot Institute of Pharmacy (Reg. No: 1485/PO/Re/S/11/CPCSEA, Proposal No. GIP/IAEC/2021/13/1).

Chemicals

Aluminium chloride, Bovine Serum Albumin, Thiobarbituricacid,DTNB were purchased from Sigma - Aldrich, Mumbai, India and used in this study. All other chemicals used were of analytical grade.

Dosage Fixation

To determine the treatment dose, we have conducted pilot study where the AlCl$_3$-induced experimental model of AD, two different doses of fractions of MCT and WCO (200 and 300 mg/kg) were used. It was observed that oral administration of AlCl$_3$ for 42 days caused memory dysfunction which was confirmed by behavioral (Morris water maze test and Elevated plus maze test) and histopathological studies.

It was observed that 200 and 300 mg/kg treatment doses showed similar reduction in Al levels and memory loss. As a consequence, we have confirmed the 200 mg/kg as higher dose and selected 100 mg/kg as lower dose for further study.

Experimental Design

For oral administration the solutions of aluminium chloride and all drugs under evaluation were made freshly at the beginning of each experiment and ingested total volume is 0.5 ml/100 g body weight. Aluminium chloride dissolved in sterile water, administered orally at a dose of 100 mg/kg daily for 42 days to all groups excluding Group-I. Animals to be divided into following groups (each of 7 rats); as follows:

Group I (Control): Included normal healthy rats that received vehicle P.O. (Saline) 0.5

mL/100 g body weight to be given daily for 42 days.

All the groups from II to VIII will receive respective doses 30 minutes before oral administration of Aluminium chloride (100 mg/kg), daily for 42 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.

On day 43 rats were sacrificed, brains were excised and used for the biochemical estimation.

Biochemical assessment

Measurement of brain Lipid peroxidation (**13-15**)

The level of Lipid peroxides was estimated by Thiobarbituric acid (TBA) reaction method described by Ohkawaet al. The TBAtestis often saidtomeasurem alondialdehyde (MDA)formedin peroxidizinglipid systems. So, the results are expressed as nmoles/ mg protein.

EstimationofbrainNitrite (**16**)

The use of the Griess diazotization reaction to spectrophoto metrically detect nitrite formed by the spontaneous oxidation of NO under physiological conditions. The detection limit for this method is 1.0 μM nitrite. The Griess reaction can also be used to analyze nitrate via its catalytic reduction to nitrite. Sulfanilic acid is quantitatively converted to a diazonium salt by reaction with nitrite in acid solution. The diazonium salt is then coupled to N-(1-naphthyl) ethylenediamine, forming an azo dye that can be spectrophotometrically quantitated based on its absorbance at 548 nm.

Read nitrite concentrations corresponding to the absorbance of experimental samples from the standard plot**,** results are expressed as nmoles/ mg protein.

Estimationofbrain Reduced glutathione (**17**)

Reduced glutathione measured by the method of (Ellaman 1959). Glutathione was

determined by its reaction with DTNB to yield a yellow chromophore which wasmeasured spectrophotometrically at412nm. The GSH concentrations of the samples were derived from the standard curve prepare dusing known amounts of GSH and expresse das ug/mgprotein.

Estimation of brain Superoxided ismutase (SOD) (**18**)

Super oxidedis mutase scavengesthe super oxide ($O*_2$)and thus providesa firstline defenseagainst freeradi caldamage. SOD is afamily of met alloenzy methatcatalyzes the dismutation of super oxideanion ($O*_2$) to hydrogenperoxide andmolecular oxygen. SOD measurement was carried out on the ability of SOD to in hibitspontaneous oxidation of epinephrine to adrenochrome.
The results are expressed as units (U) of SOD activity per mg protein.

Estimation of catalase (CAT) (**19**)

InU.V.rangeH_2O_2showsacontinualincreaseinabsorptionwithdecreasingwavelength. Catalase catalyses the rapid decomposition of hydrogen peroxide towater.The decomposition of H_2O_2can be followeddirectly by the decreasein absorbanceat 240nm. The difference in absorban ceperunitisa measure of catalase activity.

Estimation of brain Glutathione S-transferase (**20**)

Glutathione S-transferases initiating the detoxication alkylating agents by catalyzing its reaction with the -SH group of glutathione, thereby neutralizing their electrophilic sites and rendering the products more water-soluble. Glutathione conjugates are thought to be metabolized further by cleavage of the glutamate and glycine residues, followed by acetylation of the resultant free amino group of the cysteinyl residue, to produce the final product, a mercapturic acid.
A unit of activity is defined as the amount of enzyme catalyzing the formation of 1 μmole of product per min under the conditions of the specific assay.

Estimation of brain acetylcholinesterase (**21**)

Themethod of AchE activity estimationis popularly known as Ellman's method,developed by George Ellmanin 1961. Thiocholine released because of the cleavage of acetylcholineiodide by A ChE is allowed to react with the SH reagent 5, 5 -

dithio- bis-(2, nitrobenzoic acid) (DTNB),which is reduced to thionitrobenzoic acid, a yellow coloured anion with absorptionmaxima at412nm.The extinction coefficientof the thionitrobenzoic acidis1.36 ×104/ molar/ centimeter. The concentration of thionitrobenzoic acid is detected using a UV spectro photo meter which is taken as adirectestimate of the AChE activity, expressed in nmoles/min/gram.

Estimation of brain total proteins (Modified Biuret, End Point Assay) (**22**)

The peptide bonds of proteins react with cupric ions in alkaline solution to form a coloured chelate, the absorbance of which is measured at 578nm. The Biuret Reagent contains Sodium-Potassium Tartrate, which helps in maintaining solubility of this complex at alkaline pH. The absorbance of final colour is proportional to the concentration of Total Protein in the Sample in g/dL.

Estimation of brain Aluminium (**23**)

The aluminium was analyzed by the wet acid digestion method of Zumkley in the hippocampus and cortex of the brain. A mixture of 2.5 ml of perchloric acid/nitric acid (1:4 by volume) was added to the tissue and then placed in a sand bath at 40°C to 50°C for 44 h until the point where a white ash or residue was obtained. Residues were then dissolved in 2.5 ml of 10 mM nitric acid. This sample (in liquid form) was placed in the sample holder of an atomic absorption spectrophotometer. The total concentration of aluminium was calculated in μg/g of tissue.

Behavioral studies

Open Field Test-(**24**)

This has walls that were 36 cm thick, was made of white plywood, and measured 72 by 72 cm. Since one of the walls was made of transparent Plexiglas, rats could be seen inside the contraption. The clear Plexiglas floor allowed for visibility of the green lines that had been marked with a marker on the ground. The floor was split into sixteen 18 × 18 cm squares by the lines. The open field had a central square (18 cm x 18 cm) drawn in the centre of it. Because some rat strains exhibit great locomotor activity and frequently cross the lines of the test chamber during a test session, the middle square is used. Additionally, the central square has enough room around it to indicate that it is different from the neighbouring areas. Rats were placed in the open field's middle or

one of its corners, and they were free to explore the equipment for five minutes. The number of times each rat explored the wall's 12 nearby outer squares and the centre square was counted individually. Numerous grooming behaviours, such as licking the fur, washing the face, or scratching. For five minutes, the number of rearing, or standing on the hind limbs and occasionally leaning on the wall with the forelegs, smelling, and looking around, was noted. Rats were put back in their cages after the five-minute test, and the open field was wiped with 70% ethyl alcohol and left to dry in between experiments.

Rats were repetitively subjected to OFA for evaluation of gross behavioral activity on day 20, 21 and 42 starting from oral administration of Aluminium chloride.

Assessment of cognitive performance by the Morris water maze task-(**25**)

The Morris water maze was used to test memory acquisition and retention. A big circular pool (150 cm in diameter, 45 cm in height, filled to a depth of 30 cm with water at $28 \pm 1°C$), which served as the Morris water maze's foundation, was split into four equal quadrants by two threads fixed at right angles to one another. The pool was positioned in a room that was well-lit and had numerous coloured light cues. These outside cues serve as the reference memory and remain constant during the course of the investigation. During the acquisition phase, a circular platform with a diameter of 4.5 cm was positioned in one pool quadrant at a height of 1 cm above the water. For the retention phase, the identical platform was positioned 1 cm below the water's surface. The position of the platform was not changed in any quadrant during assessment of both phases. Each animal was subjected to four consecutive trials with a gap of 5 min. The animal was gently placed in the water of the pool between quadrants facing the wall of the pool, with the drop location changed for each trial. The animal was then allowed 120 seconds to locate the platform. Next, the animal was allowed to stay on the platform for 20 seconds. If the animal failed to reach the platform within 120 seconds, it was guided to the platform and allowed to remain there for 20 seconds.

Maze acquisition phase (training)-

Animals received two consecutive daily training sessions. During the acquisition phase, each rat was put into the water in any one of four starting positions, the sequence of which was selected randomly. The latency to reach the visual platform (acquisition

latency) was measured.

Maze retention phase (testing for retention of the learned task)-

Animals were tested twice after the acquisition phase, each animal was released randomly from one of the edges facing the wall of the pool to assess memory retention. Time latency to find the hidden platform first time was recorded and termed the first retention latency (1st RL) and the second time as second retention latency (2nd RL), respectively.

Rats were subjected to acquisition latency on day 19 & 20, and first & second retention latency on day 21 and 42 starting from oral administration of Aluminium chloride.

Elevated plus maze-(**26**)

The elevated plus maze consisted of two opposite open arms (50 × 10 cm), crossed with two closed arms of same dimensions with 40 cm high walls. The arms are connected with central square (10 × 10 cm). Acquisition of memory was assessed on day 13 after colchicine injection. Rats were placed individually at one end of an open arm facing away from the central square. The time taken to move from open arm and enter into one of the closed arms was recorded as initial transfer latency (ITL). Animals were allowed to explore the maze for 5 minutes after recording ITL and returned to its home cage. Retention of memory was assessed by placing a rat similarly on an open arm and retention latency was noted twice after initial transfer latency and termed as first retention transfer latency (1st RTL) and second retention transfer latency (2nd RTL), respectively.

Rats were subjected to initial transfer latency on day 20, and first & second retention transfer latency on day 21 and 42 starting from oral administration of Aluminium chloride.

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. $p < 0.05$ was considered significant.

Results

Effect BMCT, AMCT and WCOon lipid peroxidation, nitrite, reduced glutathione, glutathione S-transferase, superoxide dismutase, catalase activity and total protein in whole brains of rats treated with aluminium chloride

Chronic administration of aluminium chloride significantly raised MDA and nitrite concentration, depleted reduced GSH, and decreased glutathione S-transferase, superoxide dismutase, catalase activities and total protein in the whole brain compared to naive rats ($p < 0.05$). However, chronic BMCT, AMCT and WCO (100 and 200 mg/kg) administration to the rats significantly attenuated oxidative damage (as indicated by reductions in MDA, nitrite concentration and reduced GSH, and increased glutathione S-transferase, superoxide dismutase, and catalase activities) as compared to Untreated rats. (**Table 1**).

Groups	MDA (nmol MDA/ min/mg protein)	Nitrite (µmol/mg)	GSH (µg/g)	SOD (unit/ mg)	Catalase (units/mg protein)	GST (µmol/ ml/min)	Total protein (g/dl)
Control	4.89**** ± 0.15	244.3**** ± 7.8	130.8**** ± 2.02	45.02**** ± 2.07	2.66**** ± 0.04	97.0**** ± 5.21	5.71**** ± 0.07
Untreated	7.99 ± 0.09	625.7 ± 17.7	42.7 ± 0.88	15.13 ± 1.27	1.39 ± 0.01	34.5 ± 0.80	1.70 ± 0.09
BMCT 100	7.66*** ± 0.08	446.5**** ± 14.2	92.2**** ± 1.51	23.00**** ± 0.44	1.86**** ± 0.02	58.3**** ± 1.04	2.51**** ± 0.09
BMCT 200	6.22**** ± 0.27	289.8**** ± 3.4	113.9**** ± 1.86	37.27**** ± 0.87	2.04**** ± 0.04	79.5**** ± 1.31	3.42**** ± 0.08
AMCT 100	7.56**** ± 0.05	521.6**** ± 7.5	76.2**** ± 1.54	20.02**** ± 0.65	1.69**** ± 0.03	50.5**** ± 1.04	2.40*** ± 0.05
AMCT 200	6.15**** ± 0.18	452.6**** ± 9.9	97.5**** ± 1.25	32.60**** ± 1.39	1.82**** ± 0.03	71.7**** ± 1.31	2.74**** ± 0.25
WCO 100	7.77* ± 0.05	555.3**** ± 7.8	72.3**** ± 3.74	18.45**** ± 1.30	1.57**** ± 0.03	49.0**** ± 1.04	2.38*** ± 0.57
WCO 200	5.65**** ± 0.23	484.3**** ± 3.6	86.4**** ± 1.86	29.77**** ± 0.41	1.68**** ± 0.03	70.1**** ± 1.31	2.68**** ± 0.29

Effect BMCT, AMCT and WCO on lipid peroxidation, nitrite, reduced glutathione,

glutathione S-transferase, superoxide dismutase, catalase activity and total protein in whole brains of rats treated with aluminium chloride (Table 1)

Effect of BMCT, AMCT and WCO on acetylcholinesterase (AChE) activity in aluminium chloride treated rats

Chronic aluminium chloride treatment significantly increased the whole brain AChE activity compared to control rats. However, chronic BMCT, AMCT and WCO (100 and 200 mg/kg) administration to the rats significantly attenuated AChE activity, as compared to the Untreated rats ($p < 0.05$) (**Fig. 2**).
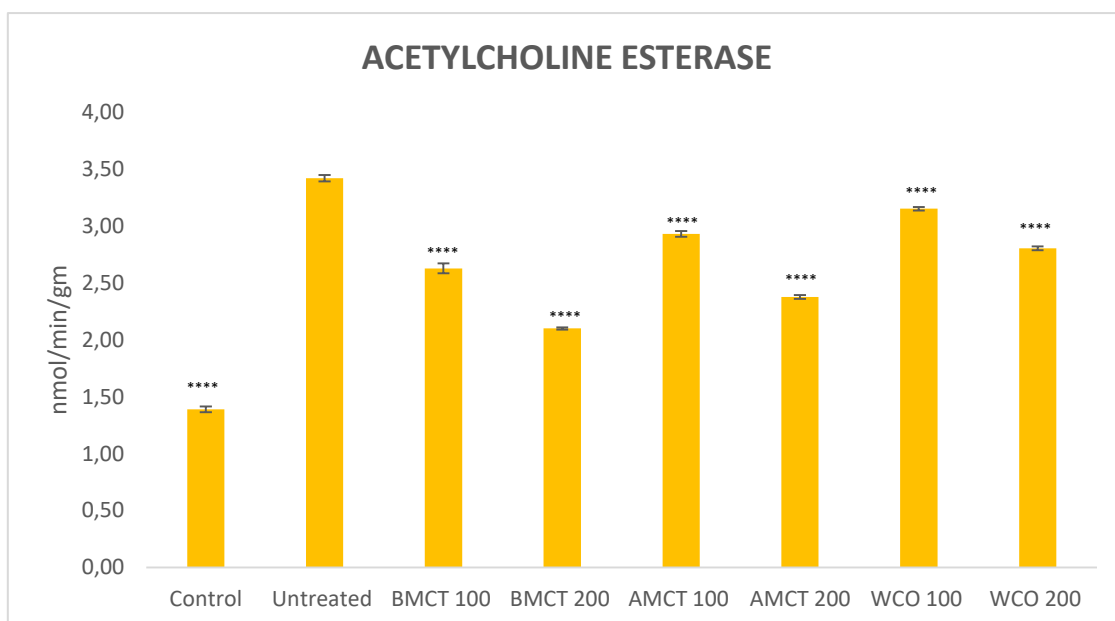
Fig. 2, Effect of BMCT, AMCT and WCO on acetylcholinesterase (AChE) activity in aluminium chloride treated rats.

Effect of BMCT, AMCT and WCO on aluminium concentration in aluminium chloride treated rats

Aluminium chloride treatment significantly increased the aluminium concentration in the hippocampus and cortex of rats compared to control. However, chronic BMCT, AMCT and WCO (each 100 and 200 mg/kg) treatment significantly attenuated the aluminium concentration in the hippocampus and cortex compared to Untreated rats ($p < 0.05$) (**Fig. 3**).
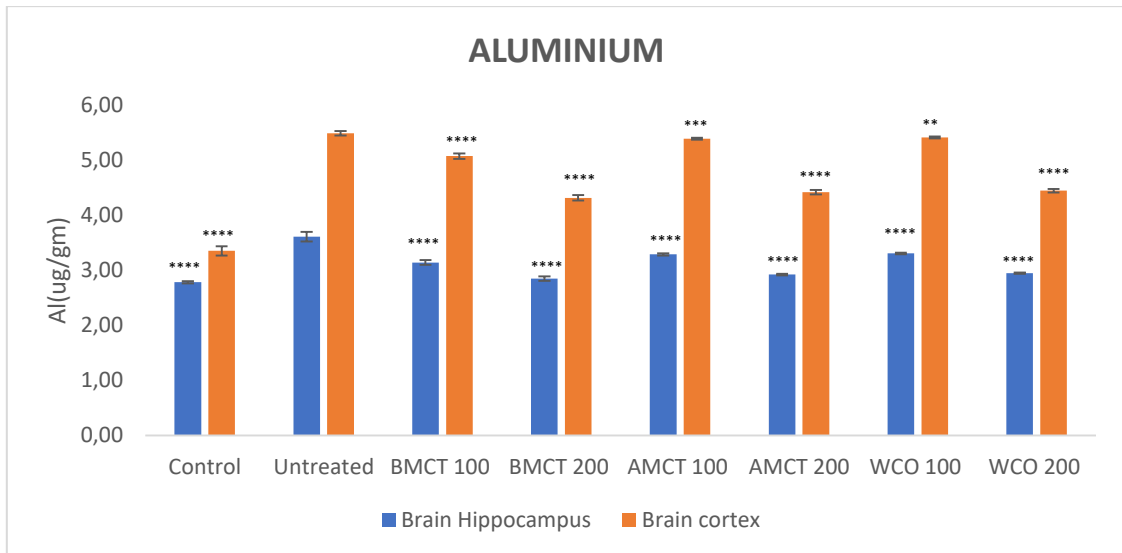
Fig.3, Effect of BMCT, AMCT and WCO on aluminium concentration in aluminium chloride treated rats.

Effect of BMCT, AMCT and WCO on Movement and activities of animals using open field testin aluminium chloride induced rats

The AlCl3 group exhibited a significant decrease (P<0.05) in peripheral and central movements and rearing and grooming activities. However, the oral administration of BMCT, AMCT and WCO (each 100 and 200 mg/kg) to AlCl3 treated rats showed a significant increase in movement and activities (P<0.05) compared to the $AlCl_3$ group. (**Fig. 4 and 5**).
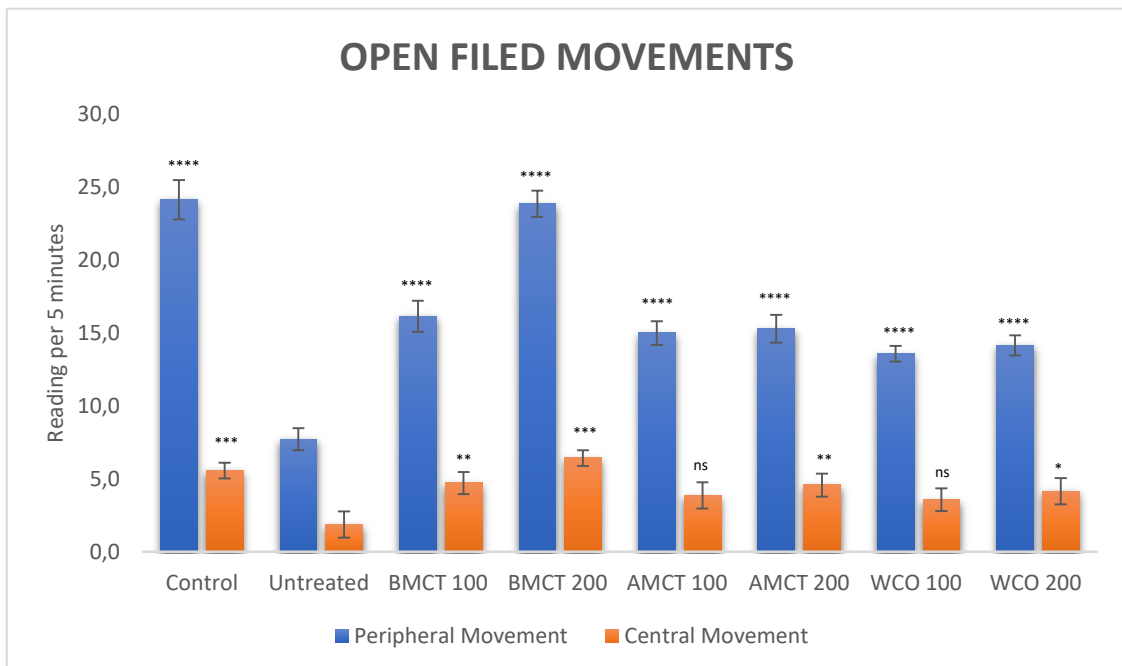

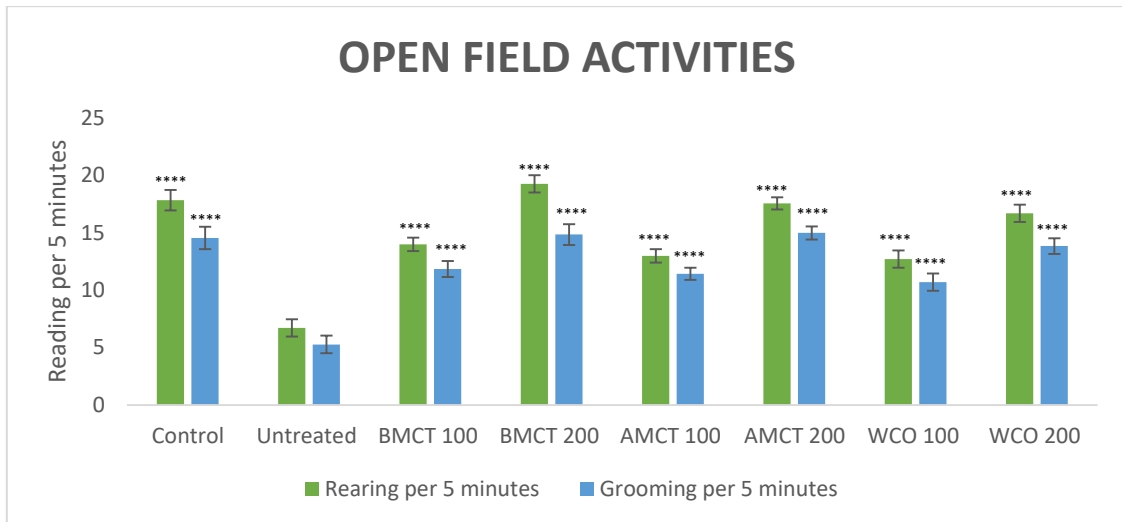Fig.4, Effect of BMCT, AMCT and WCO on Movement of animals using open field test in aluminium chloride induced rats.

Fig.5, Effect of BMCT, AMCT and WCO on activities of animals using open field test in aluminium chloride induced rats.

Effect of BMCT, AMCT and WCO on memory performance in the Morris water maze task in aluminium chloride induced rats

Aluminium chloride treated rats significantly delayed acquisition latency to reach the visual platform compared to the control group, indicating memory deficits. BMCT, AMCT and WCO treatment significantly improved this memory performance (i.e., shortened average acquisition latency) on day 19 and 20 ($p < 0.05$) in the aluminium chloride treated group. Following training, the visual platform was hidden. Aluminium chloride induced group (Untreated) was then found to significantly delay average acquisition latency (on day 20) and retention latencies (1st and 2nd RL on day 21 and 42, respectively) to escape onto the hidden platform compared to the control group. These results suggested that aluminium chloride caused significant cognitive impairment. Further, chronic BMCT, AMCT and WCO treatment (each 100 and 200 mg/kg) significantly improved memory performance (increased memory retention) for the 1st and 2nd RL on days 21 and 42, respectively, compared to the Untreated rats.
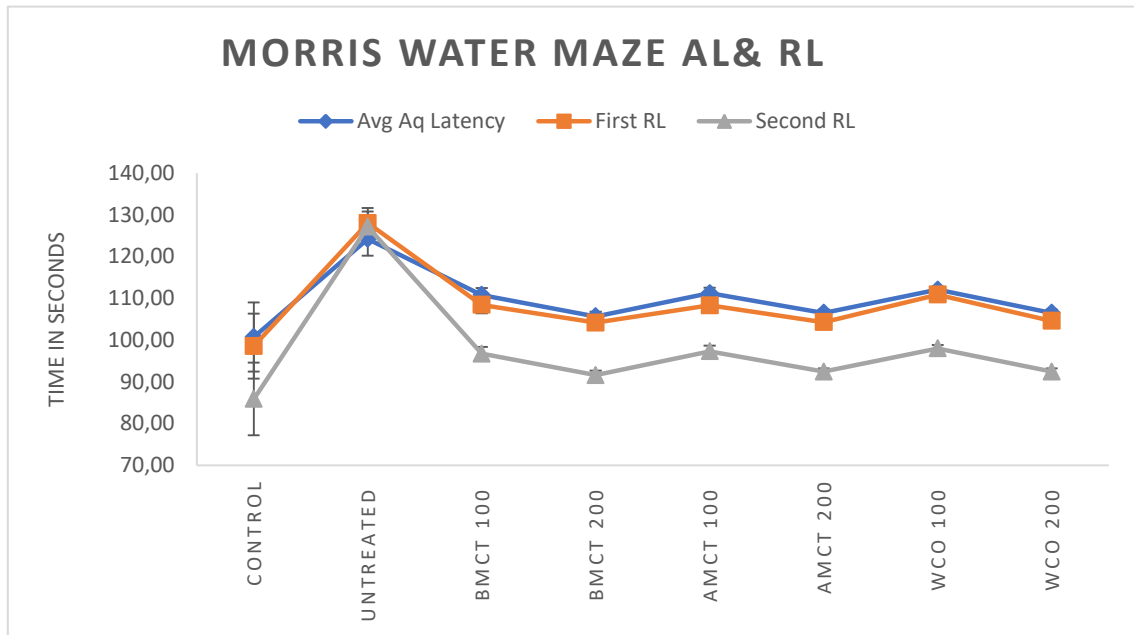
(**Fig. 6**).

Fig.6, Effect of BMCT, AMCT and WCO on memory performance in the Morris water maze task in aluminium chloride induced rats.

Effect of BMCT, AMCT and WCO on memory performance in the elevated plus maze in aluminium chloride induced rats

The ITL on day 20 for each rat was relatively stable and showed no significant variations. All rats entered the closed arm within 60 seconds. Following training, Control, Untreated (Aluminium chloride-induced) and chronic BMCT, AMCT and WCO treatment (each 100 and 200 mg/kg) rats entered the closed arm quickly and the retention transfer latencies (1st RTL and 2nd RTL) to enter the closed arm on days 21 and 42 were shorter compared with the ITL on day 20 of each group respectively.

In contrast, Aluminium chloride induced rats performed poorly throughout the experiment and did not show any change in the retention transfer latencies on days 21 and 42 compared with the ITL on day 20, demonstrating that Aluminium chloride induces a marked memory impairment. Chronic administration of BMCT, AMCT and WCO treatment (each 100 and 200 mg/kg) 30 minutes before the Aluminium chloride administration significantly decreased the retention latencies on days 21 and 42 following Aluminium chloride administration ($p < 0.05$ as compared to Untreated group)
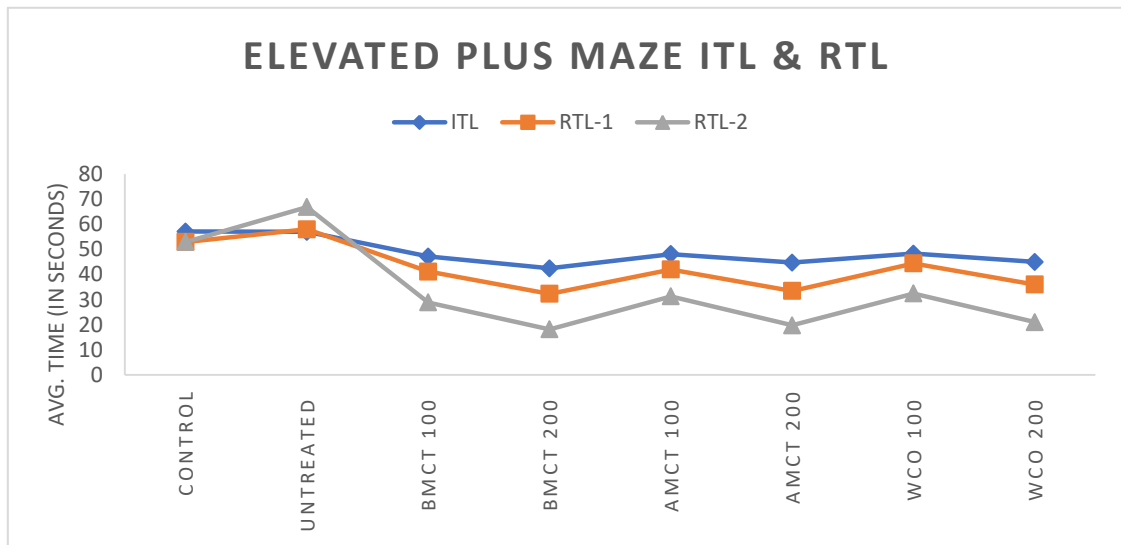
(**Fig. 7**).

Fig.7, Effect of BMCT, AMCT and WCO on memory performance in the elevated plus maze in aluminium chloride induced rats.

Discussion

Aluminium is a ubiquitous metal and has been implicated in the etiology of neurodegenerative disorders and cognitive dysfunction, where it exacerbates brain oxidative damage (**27**), causes neuronal inflammation and induces impairment in working memory, visuoperception, attention and semantic memory (**28**). Aluminium also functionally alters the blood brain barrier and produces changes in the cholinergic and noradrenergic neurotransmission (**29**). It causes impaired glucose utilization, increased free-radical generation and lipid peroxidation as well as changes in phosphoinositide metabolism and protein phosphorylation, thereby causing severe neurotoxicity.

Our results showed that oral administration of $AlCl_3$ for 42 days caused a significant and greater aluminium accumulation (**Fig. 3**) in hippocampus and cortex (**30,31**). Specific high affinity transferrin receptors (TfR) present in the blood brain barrier allow aluminium to enter the brain (**32**). It accumulated in all of the rat brain's areas, with the greatest amount in the hippocampus, the area responsible for memory and learning (**33**). Additionally, Crapper et al. (**34**) noted that AD patients' brains had an increased Al concentration.

The present study examined the therapeutic potential of Coconut oil-WCO and its fractions BMCT and AMCT in the prevention of accumulation of $AlCl_3$.

The increased antioxidant enzyme levels associated with significant protection against oxidative stress may be the mechanism responsible for memory improvement and motor activity in this model.

In addition, a decrease of brain AChE activity was also inhibited by Coconut oil-WCO and its fractions BMCT and AMCT treatment. Observation supports that modulation of cholinergic neurotransmission is involved in the improvement in memory function.

Administration of aluminium chloride resulted in progressive deterioration of spatial memory as determined by Morris water maze, Elevated plus maze which was regained due to treatment of Coconut oil-WCO and its fractions BMCT and AMCT.

Therefore, the present study highlights that Coconut oil-WCO and its fractions BMCT and AMCT improves behavioral activity which was evaluated by using OFA test and biochemical function in the aluminium-treated brain, an effect that could be partially correlated with its anti-oxidant properties. However, further cellular studies are required to understand the effect of Coconut oil-WCO and its fractions BMCT and AMCT on oxidative stress in different experimental systems.

Conclusions

In conclusion, the present study highlights that the Coconut oil-WCO and its fractions BMCT and AMCT treatment attenuated aluminium chloride induced aluminium loading, cholinergic deficit and memory loss. However further research is needed to study the precise mechanism of action of Coconut oil-WCO and its fractions BMCT and AMCT against Al induced neurotoxicity.

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