



Research Article

Virgin Coconut Oil Ameliorates Colchicine Induced Cognitive Dysfunction- A Preclinical Study

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Abstract

Background: Virgin coconut oil (VCO) has been identified as a potential cognitive strengthener associated with Alzheimer's disease (AD). It contains medium chain fatty acids (MCFA) which are absorbed and easily metabolized by the liver to form ketone bodies. Ketone bodies are converted to acetyl Co-A in the brain which then enters the citric acid cycle to provide ATP and also serves as precursors of acetylcholine in neurons. Sunflower oil (SO) contains polyunsaturated fatty acids which has both anti-inflammatory and neuroprotective actions. To compare the neuroprotective effects of VCO and SO on biochemical parameters involved in the cognitive dysfunction induced by colchicine through intracerebroventricular (i.c.v) route. To assess the role of polyphenols and MCFA present in VCO in preventing oxidative stress and its influence on neuroprotection and memory enhancement.

Methods: In the present study, we induced dementia through i.c.v injection of colchicine after giving the diet enriched VCO and SO in rats for 60 days. Rats were sacrificed on the 22nd day after the administration of colchicine. Behavioral parameters were assessed during the study period and biochemical estimations were performed using frontal cortex and hippocampus isolated from rat brain.

Results: From the memory and learning tests by Morris water maze, VCO treated group performed better than SO treated rats. VCO reversed the antagonistic effects induced by colchicine by decreasing the acetylcholinesterase and malondialdehyde levels and increasing the levels of catalase and superoxide dismutase. SO only reduced malondialdehyde levels in cortex and hippocampus.

Conclusion: The results demonstrated potential beneficiary effects of VCO in the cognitive dysfunction induced by colchicine by enhancing acetylcholine levels in the frontal cortex and hippocampus and also by reducing oxidative stress induced by physiological oxidants.

Introduction

Alzheimer's disease (AD), being a progressive brain disorder has a predominant feature that the underlying pathophysiological mechanisms responsible for the disease are active long before the appearance of the clinical manifestation of the disease. In the absence of proper disease curable treatments, prevention of the disease opens a wide range of perspectives. Cognitive impairment can be influenced by a number of factors and the potential of nutrition in dementia has become an interesting topic for both public and scientific fraternity. In recent years, there is increasing evidence that supports the role of diet and nutrition in improving the symptoms of AD.¹ Sporadic form of Alzheimer's is the most common form of AD which is influenced by both genetic and environmental factors, one such factor being diet. The sporadic form of AD can be mimicked by the intracerebroventricular administration of colchicine which is a microtubule dysfunctioning agent

and can cause the destruction of cholinergic neurons as well as contribute to oxidative stress in the brain.²

Coconut oil is an important dietary component of various communities all over the world. But being saturated fatty acid-rich oil; it is unfortunately defamed as hypercholesterolemic compared to polyunsaturated fatty acid (PUFA) rich oils. However, studies reveal that the habitual consumption of coconut oil has no specific role in the causation of cardiovascular disease because the nature of the fatty acid present in coconut oil is less implicated in the accumulation of body fat.³ Coconut oil consists of medium chain fatty acids (MCFA) which are unique unlike other high chain fatty acids because they are easily absorbed and metabolized by the liver to form ketone bodies.⁴ Even though coconut oil and butter belong to the category of saturated fats, the nature and source of fatty acids in both are different. Because coconut oil a plant-based oil and

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consists of MCFA and butter is a dairy product which consists of long chain triglycerides. In a randomized trial conducted in healthy men and women, to ascertain the effects of coconut oil and butter on blood lipids, it was found that low density lipoprotein-cholesterol (LDL-C) concentrations were significantly increased with butter compared to coconut oil. No differences in LDL-C were noted in coconut oil compared with olive oil. Coconut oil significantly increased the high density lipoprotein-cholesterol (HDL-C) compared with butter or olive oil.⁵ In a randomized study of coconut oil versus SO in patients with stable coronary heart disease, coconut oil in comparison with SO did not change the lipid related cardiovascular risk factors, when used as a cooking oil media over a period of 2 years.⁶

MCFA present in the virgin coconut oil (VCO) metabolizes into ketone bodies in the liver which is transported through monocarboxylic acid transporters into the brain. In the brain, ketone bodies get converted into acetyl-CoA which then enters the citric acid cycle to produce ATP as well as acetyl-CoA which can also serve as precursors for acetylcholine. So ketone bodies can provide an alternate source of energy during cerebral glucose hypo metabolism which is a characteristic feature of the AD brain and appear early in the disease.⁷ Available clinical trials show that expanding ketone accessibility to the brain by means of moderate ketosis has an unassuming useful impact on cognitive results in gentle to direct AD and in mild cognitive decline.⁸

Invitro biochemical analysis showed that VCO protects cortical neurons from amyloid-beta toxicity by enhancing signaling of cell survival pathways especially on signaling intermediates such as AMPK, Akt, GSK3 β , and ERK.⁹

VCO is extracted directly from the coconut milk, without subjecting to high temperature unlike copra and refined bleached deodorized oils, where high temperature and pressure are applied. There are different methods for the extraction of VCO like cold extraction, hot extraction, fermentation technique and the enzymatic extraction technique. Among all the above methods, the hot extraction method results in the release of bound polyphenols and also provides high yield.¹⁰

Along with coconut oil, SO also forms an important component of cooking oil in most parts of the world. It is rich in PUFA, whose role in AD is already established to be beneficial.¹¹

Coconut oil and SO are two entirely different oils which plays a significant role in day to day dietary regimen. These two oils are taken solely to compare the effects of MCFA in VCO and PUFA in SO in improving AD.

Dietary supplementation of antioxidants, vitamins, polyphenols, polyunsaturated fatty acids, fruits, vegetables are advantageous to AD. In this scenario, because of the relationship between the dietary modifications and AD, it is relevant to analyze the possible neuroprotective effects of dietary oils like coconut oil and SO in modifying the disease condition.

The antioxidant and memory enhancing properties of VCO in normal rats and the possible neuroprotective effects of SO due to the presence of polyunsaturated fatty acids merit the evaluation of these dietary oils in colchicine-induced cognitive dysfunction with the comparison of the existing drug in AD.

Eventhough there are studies are going on for analyzing the effect of coconut oil in AD, it is impossible for us to know how well coconut oil does or does not work in AD because there have not been rigorous, large-scale research studies done. There haven't been any large studies to test the effectiveness of coconut oil; so we just can't say whether it really helps people with AD.

Thus, the present study has been designed to evaluate the neuroprotective and memory enhancing effects of VCO and SO enriched diet against colchicine induced dementia in rats.

Materials and Methods

Animals

Post weaned, male Wistar rats, weighing 60-70g (30 days old) were acquired from Central Animal Research Facility (CARF), Manipal Academy of Higher Education, Manipal. The animals were maintained under controlled conditions of temperature (23 \pm 2°C) and humidity (50 \pm 5%). The animals were kept in groups of 3 per cage prior to the colchicine administration and 1 animal per cage after colchicine administration under standard conditions of 12-h light-dark cycles in sterile polypropylene cages containing sterilized paddy husk as bedding with water and food ad libitum. All the behavioral procedures were carried out between 09:00 and 16:00 hours of the day throughout the study.

Diet composition

A laboratory rodent diet was used as the standard diet. Each rat was given 10-20g standard diet containing 10% of respective test oil daily, started 60 days prior to the colchicine administration and continued till the end of the study. The doses of VCO and SO (10%) was selected based on scientific literature¹² and was found to be effective. To prepare 10% diet of dietary oils, 10 g of respective test oil (VCO and SO) was mixed with 90 g of standard diet. Food intake of rats was noted daily and gain in body weight was recorded weekly.

Extraction of VCO

Hot extraction process of VCO¹³ involves following steps; Solid endosperm of the mature coconut was crushed, made into slurry and squeezed through a clean cloth to obtain coconut milk. It was then refrigerated for a period of 2 hours which resulted in the separation of coconut milk into cream and skim milk (creamy phase). Then the upper creamy layer was collected and heated in a wok. For the first hour of heating, stove setting is set between medium and high and reduced the stove setting to low, when the oil starts to separate from the coagulated protein. Then the

oil was separated from the coagulated protein by pouring the mixture through a muslin cloth. The extracted oil was subjected to oil drying in a double boiler at a very low temperature for about 15–20 minutes to ensure that moisture is at its minimum level and to decrease the chances of rancidity. SO (Freedom brand) was purchased from a local market.

Selection of doses

Dose selection of colchicine as 15µg/5µL ACSF was based on the previous study.¹⁴ The dose for the standard drug donepezil was taken as 2mg/kg based on the literature reports.¹⁵

Preparation and administration of drugs

Colchicine (TCI chemicals, India) and donepezil solutions were made freshly on each day of administration. Cognitive dysfunction in rats was induced by the administration of colchicine through i.c.v route at a dose of 15µg of drug dissolved in 5µL of ACSF. Donepezil was dissolved in normal saline and administered by oral gavage at a dose of 2mg/kg. Donepezil administration was started 4 days prior to the administration of colchicine and continued till the 21st day of colchicine administration. Animals were divided into 8 groups with 6 animals in each group. The experimental protocol is depicted in Figure 1.

1. Normal control received saline (5 ml/kg p.o.)
2. Sham control received Artificial cerebrospinal fluid (ACSF) (5µL i.c.v.)
3. Disease control received colchicine (Col) (15 µg/5 µL ACSF i.c.v.)
4. Standard treatment received Donepezil (DPL) (2 mg/kg p.o.)
5. Standard rodent diet+ VCO group received 10-20 g rodent diet + 10% Virgin coconut oil (VCO)
6. Standard rodent diet + Sunflower oil (SO) group received 10-20 g rodent diet + 10% SO
7. Standard rodent diet + VCO + colchicine group received 10-20 g rodent diet + 10% VCO + colchicine (15 µg/5µL ACSF i.c.v)

8. Standard rodent diet + SO + colchicine received 10-20 g rodent diet + 10% SO + colchicine (15µg/5µL ACSF i.c.v)

Intra-cerebroventricular administration of colchicine

Animals weighing 200-350 g were anaesthetized with thiopentone sodium (45 mg/kg, i.p.). The head of the rat was positioned in the stereotaxic apparatus (Neurostar, Germany) in which the frame was previously calibrated and cleaned with 70% alcohol. After properly positioning the animal in the apparatus, a midline sagittal incision was made in the scalp and bregma was exposed. Following the fixing of neuro-syringe (Hamilton syringe 703N series, 26 gauge) within the apparatus exactly above and as close as to exposed bregma, concerned coordinates of the injection site were entered using the software (Stereodrive). The coordinates of the injection site above which Hamilton syringe was placed were 0.8 mm posterior to bregma, 1.8 mm lateral to the sagittal suture and 3.6 mm below the cortical surface. Once, the injection site was identified, that point of skull was drilled for placing the Hamilton syringe into the lateral ventricle. The sham control group was injected with ACSF (ACSF; in millimoles/litre: 147 NaCl, 2.9 KCl, 1.6 MgCl₂, 1.7 CaCl₂, and 2.2 dextrose) and disease control group was injected with 15µg of colchicine dissolved in 5µL ACSF. After administration, Hamilton syringe was kept in the same position for 2 minutes without moving to prevent the back diffusion. The drilled hole was filled with dental cement and fixer. The skin over the scalp was sutured and povidone-iodine solution was applied to prevent infection. Post operatively, rats were kept in separate cages and monitored.¹⁶

Measurement of body weight

Animals were weighed one day before the administration of colchicine (day -1) and on the last day of the study period (day 21).

Behavioral assessment

Assessment of locomotor activity

Locomotor activity¹⁷ was evaluated on days 3, 7 and 14

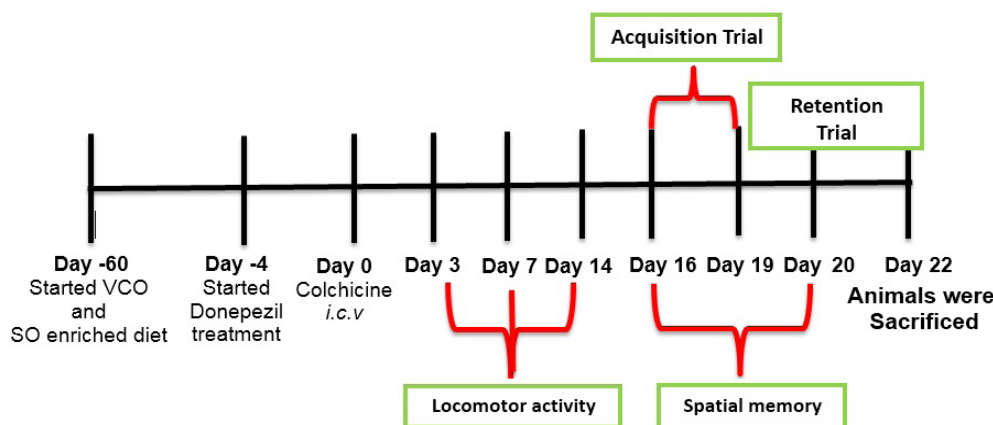


Figure 1. Schematic representation of treatment protocol.

of colchicine administration in a closed square shaped area fitted with infrared light sensing photocells using actophotometer. Each rat was individually placed for 3 minutes to acclimatize and then the counts were recorded for a period of 10 minutes. The apparatus was placed in light and sound attenuated and ventilated room.

Assessment of spatial learning and memory

The acquisition and retention trials of spatial memory task were evaluated by using Morris water maze apparatus.¹⁸ The maze consists of circular black colored tank filled with water and maintained at 27°C. Water pool was divided into four equally divided parts and arbitrarily assigned as A, B, C and D quadrants. An escape platform of 10 cm diameter was submerged 2 cm below the water surface and placed constant in the D quadrant throughout the acquisition trial of 4 days.

Data was recorded by a video camera connected to computer tracking software (Any maze, Ugo Basile, Italy) fixed above the center of the pool.

Acquisition trial

Rats were trained for 4 consecutive days to find the escape platform in the D quadrant. Acquisition trials were started on the 16th day of colchicine administration and continued till the 19th day. All the rats were subjected to one session of 3 trials per day with an inter-interval of 5 minutes. Then the rat was allowed to swim for a period of 60 seconds to locate the hidden platform. After reaching the platform, rat was allowed to stay on the platform for 30 seconds and then removed. If the rat failed to reach the escape platform within 60 seconds, it was gently guided to the platform and allowed to stay on it for 30 seconds. To eliminate the quadrant effects, the rat was placed in 3 quadrants.

Retention trial

After 4 days of training, on day 5, retention trial was performed in which the escape platform was removed and placed in the quadrant just opposite to the quadrant where the platform was placed and the animal was allowed to swim freely for 60 seconds.

Biochemical assessment

Serum cholesterol estimation

At the end of the experimental period, animals were mildly anaesthetized with diethyl ether and blood samples were collected from all the animals by retro orbital sinus puncture into microcentrifuge tubes after overnight fasting. The tubes were centrifuged at 6000rpm for 10 minutes at 4°C. After centrifugation, serum was separated and analyzed by CHOD-POD method with the help of diagnostic kit provided by Aspen laboratories using ELISA micro plate reader.

Brain biochemical estimations

After the completion of study period, animals were anaesthetized by diethyl ether followed by cervical

dislocation. Brain was removed and hippocampus and frontal cortex were isolated according to procedure cited by Glowinski and Iverson.¹⁹ A 10% w/v homogenate of cortex and hippocampus samples were prepared by homogenizing in ice-cooled 0.1M phosphate buffer pH 7.4 using Potter-Elvehjem type homogenizer fitted with Teflon plunger at a speed of 8,000rpm. The homogenates of respective tissue samples were then centrifuged at 10,000rpm for 15 minutes at 4°C for the collection of supernatants which was then stored at 20°C for further estimations.

Estimation of Acetylcholinesterase activity

The level of acetylcholinesterase was determined by Ellman's method.²⁰ Supernatant of tissue homogenate (100µL) was added to a cuvette containing 650µL of 0.1M phosphate buffer of pH 8. DTNB (0.01 M 825µL) was added to the cuvette followed by the addition of 5µL of 0.075M acetylthiocholine iodide. The absorbance change was measured for 4 minutes at 60 seconds interval at 412nm using UV-Visible spectrophotometer and the change in absorbance per minute was calculated. The outcomes were expressed as µmoles of acetylthiocholine iodide hydrolyzed per minute per mg of protein.

Estimation of catalase activity

Catalase activity was determined by method given by Aebi *et al.*, 1984.²¹ 1 ml of the prepared phosphate buffer-H₂O₂ solution was added in the cuvette and the reaction was started by adding 16.6 µL supernatant to the cuvette and recording the time course of absorbance for 1 minute at 240 nm. The catalase activity was expressed as µmoles of hydrogen peroxide decomposed per minute per mg of protein.

Estimation of superoxide dismutase

Superoxide dismutase activity was determined by the procedure given by Bhattacharya *et al.*, 2000.²² 925 µL of carbonate buffer was added in the cuvette to which 25 µL of supernatant was added and the reaction was started by adding 50µL of adrenaline bitartrate to the cuvette and recording the time course of absorbance for 1 minute at 480nm.

Estimation of lipid peroxidation

Malondialdehyde levels were determined by the procedure given by Konings and Drijver, 1979.²³ The procedure involves the mixing of tissue homogenate (100µl) and TBA-TCA-HCl reagent (100µl) followed by heating at 90°C for 15 minutes in a boiling water bath. The absorbance of resultant adduct was measure at 532nm.

Estimation of total protein

Protein present in the tissue samples was estimated by using BCA protein assay kit method given by Thermo Scientific, USA in which bovine serum albumin was used as the standard.

Statistical analysis

Data was statistically analyzed using GraphPad Prism 7.0 software. All the data were expressed as mean \pm SEM. The behavioral assessment data except retention trial data were analyzed by two-way analysis of variance, followed by Bonferroni's post-hoc test. Retention trial and biochemical estimations data were analyzed by using one-way analysis of variance followed by Tukey's post-hoc test. $p < 0.05$ was used statistically significant at 95% confidence interval.

Results

The antioxidants were estimated biochemically and expressed in terms of milligrams of gallic acid equivalent (Table 1). Gas chromatographic analysis was done to determine the medium chain fatty acid content (Table 2).

Table 1. Polyphenol content of VCO was estimated biochemically and expressed in terms of milligrams of gallic acid equivalent (method: TNTH/FOOD/STP/243).

No.	Polyphenol	Results (mg/100g)
1	Ferulic acid	22.1
2	Caffeic acid	4.6
3	p-coumaric acid	4.1
4	Catechin	81.7
5	Quercetin	1.23
6	Gallic acid	20.2

Table 2. Fatty acid composition of VCO by gas chromatographic analysis (method: ISO 5508:1990).

No.	Fatty acid	Results (%)	Specification
1	Caproic acid	0.12	0.0-0.7
2	Caprylic acid	5.01	4.6-10
3	Capric acid	2.23	5-8
4	Lauric acid	47.12	45.1-53.2
5	Myristic acid	16.85	16.8-21
6	Palmitic acid	7.81	7.5-10.2
7	Stearic acid	2.21	2-4
8	Oleic acid	6.02	5-10
9	Linoleic acid	1.06	1-2.5

Body weight

Colchicine group showed no significant difference in body weight between the day (-1) and day 21 as compared to the sham group. None of the treatment groups showed any significant difference in the body weight between the day (-1) and day 21 as compared to the colchicine group (Figure 2a)

Locomotor activity using actophotometer

Colchicine group showed no significant difference in locomotor activity on day 3, 7 and 14 when compared to the sham control group. None of the treatment groups showed a significant difference in the locomotor activity as compared to the colchicine group (Figure 2b).

Spatial memory assessment using Morris water maze

Acquisition trial

a. Swimming speed

None of the treatment groups showed any significant difference in swimming speed from day 1 to 4 as compared to the colchicine group (Figure 3a).

b. Escape latency

Time taken to escape into the hidden platform was significantly decreased on day 4 of the VCO group as compared to day 1 of the same group (Figure 3b).

c. Path efficiency

A significant increase in path efficiency was observed on

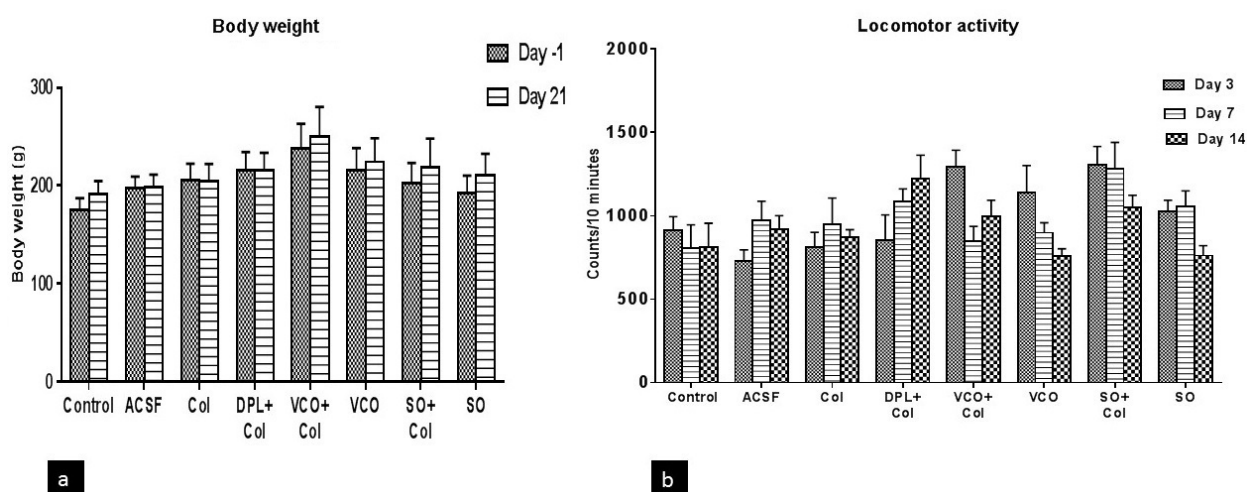


Figure 2. Effect of Colchicine (Col) and Colchicine + treatments (DPL- Donepezil, VCO – VCO, SO – SO) on; (a) Body weight of animals before and after the intracerebroventricular administration of Colchicine. (b) Locomotor activity of animals on day 3, 7 and 14. Values are expressed as mean \pm SEM (n=6). Statistical analysis was done by using two-way ANOVA followed by Bonferroni's post hoc test.

day 4 of VCO as compared to the day 1 of the same group. There was a significant increase in path efficiency on day 4 of VCO + colchicine group when compared to day 1 of the same group (Figure 3c).

d. Target quadrant latency

Time taken to reach the target quadrant was significantly decreased on day 4 of the VCO group as compared to day 1 of the same group (Figure 3d).

Retention trial

a. Swimming speed

There was a significant increase in swimming speed in the donepezil + colchicine group as compared to the colchicine group (Figure 4a).

b. Path efficiency

There was a significant increase in path efficiency in donepezil + colchicine group and VCO + colchicine group as compared to the colchicine group on the retention trial (Figure 4b)

c. Time spent in the target quadrant

There was a significant decrease in the time spent in target quadrant in the colchicine group as compared to the ACSF treated group. A significant increase in the time spent in target quadrant was observed in the donepezil + colchicine and VCO + colchicine groups as compared to the colchicine group (Figure 4c).

d. Target quadrant latency

There was a significant increase in the latency to find the target quadrant in the colchicine group as compared to the ACSF treated group. A significant decrease in the latency to reach the target quadrant was observed in VCO + colchicine group as compared to the colchicine group (Figure 4d)

e. Total zone entries

There was a significant decrease in total zone entries in the disease control group as compared to the ACSF treated group. A significant increase in total zone entries was observed in the donepezil + colchicine and VCO + colchicine groups as compared to the colchicine group (Figure 4e)

f. Escape latency

There was a significant increase in the escape latency of animals in the colchicine group as compared to the ACSF treated group. A significant decrease in the escape latency was observed in donepezil + colchicine, VCO + colchicine and SO + colchicine groups when compared to the colchicine group (Figure 4f)

Biochemical tests

Serum cholesterol estimation

Chronic administration of VCO and SO for 81 days showed no significant difference in the serum cholesterol levels as compared to normal control group (Figure 5).

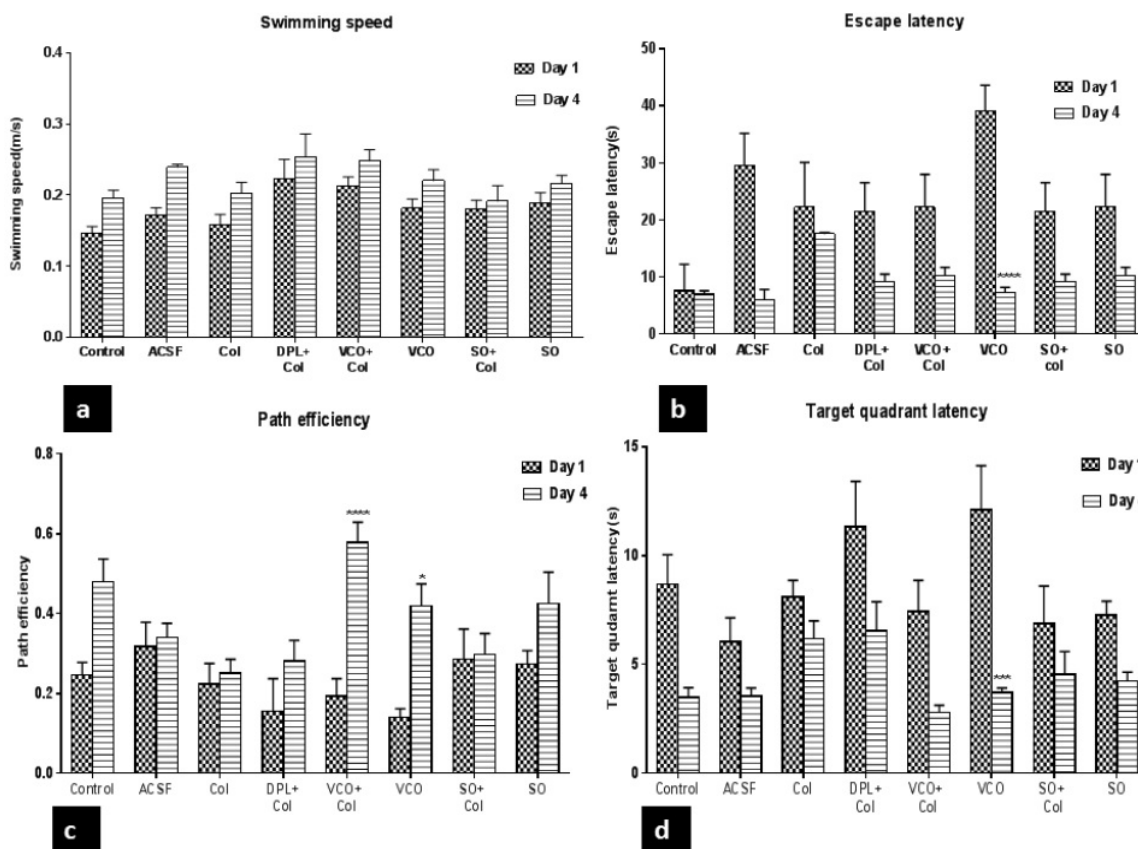


Figure 3. Effect of Colchicine and Colchicine + treatments (DPL- Donepezil, VCO – VCO, SO – SO) on; (a) Swimming speed (b) Escape latency (c) Path efficiency (d) Target quadrant latency of animals during acquisition trials in Morris water maze test. Values are expressed as mean±SEM (n=6). Statistical analysis was done by using two-way ANOVA followed by Bonferroni's post hoc test. *p<0.05 as compared to day 1 of the same group. ****p< 0.0001 as compared to day 1 of the same group.

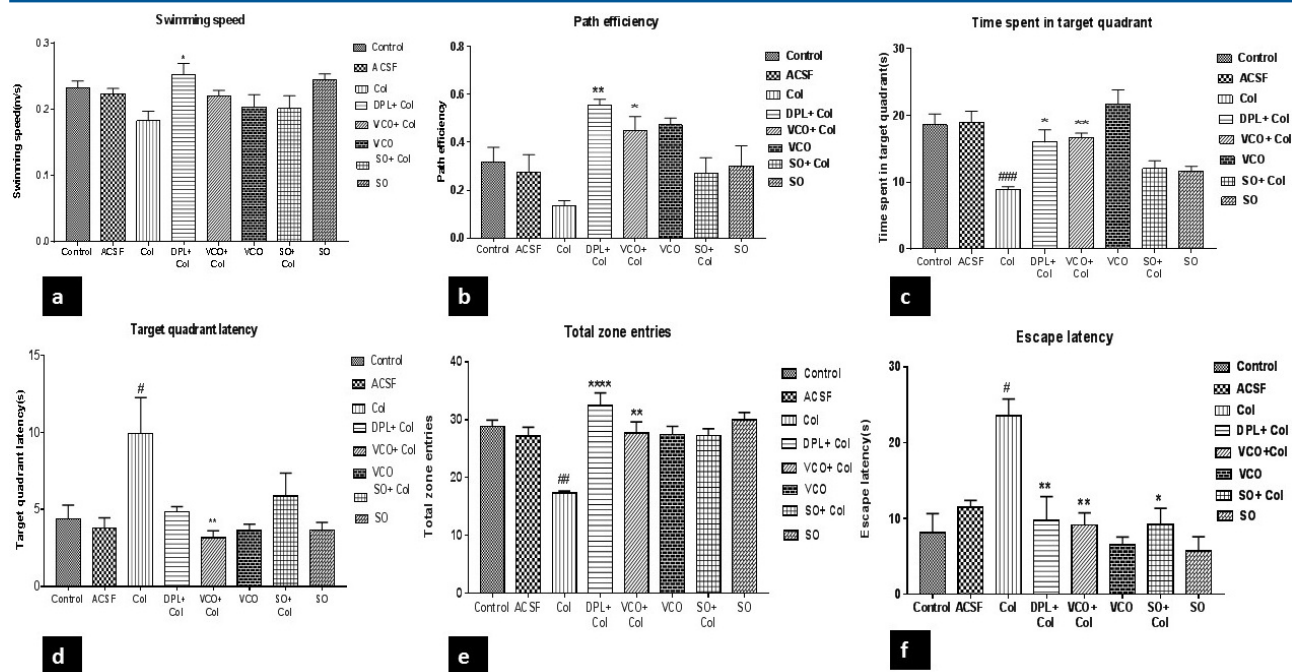


Figure 4. Effect of Colchicine (Col) and Colchicine + treatments (DPL- Donepezil, VCO – VCO, SO – SO) on:(a) Swimming speed (b) Path efficiency (c) Time spent in target quadrant (d) Target quadrant latency (e) Total zone entries (f) Escape latency of animals during retention trial in Morris water maze test. Values are expressed as mean±SEM (n=6). Statistical analysis was done by using one-way ANOVA followed by Tukey's multiple comparison tests. #p<0.05 as compared to ACSF treated group. ##P<0.01 as compared to ACSF treated group. ###P<0.001 as compared to ACSF treated group. *P<0.05 as compared to colchicine group. **P<0.01 as compared to colchicine group. ****P<0.0001 as compared to colchicine group.

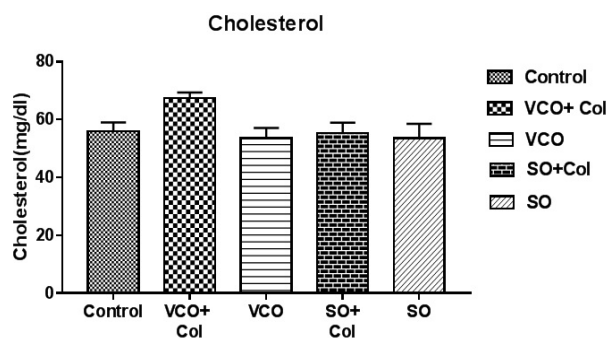


Figure 5. Effect of oil enriched diet on the cholesterol levels of the treatment groups (VCO – VCO, SO – SO, Col- Colchicine) and that of the control group. Values are expressed as mean±SEM (n=6). Statistical analysis was done by using one-way ANOVA followed by Tukey's multiple comparison tests.

Acetylcholinesterase activity

There was a significant increase in acetylcholinesterase level in the colchicine group as compared to the ACSF treated group in the hippocampus and frontal cortex areas. A significant decrease in acetylcholinesterase activity was observed in donepezil + colchicine and VCO + colchicine treated groups as compared to the colchicine group (Figure 6).

Estimation of catalase activity

There was a significant decrease in the catalase level in the colchicine group as compared to the ACSF treated group in the hippocampus (Figure 7a) and frontal cortex

(Figure 8a). A significant increase in the catalase level was observed in the VCO + colchicine group when compared to the colchicine group.

Estimation of superoxide dismutase activity

Enzymatic activity of superoxide dismutase significantly decreased in the colchicine group as compared to the ACSF treated group in the hippocampus (Figure 7b) and frontal cortex (Figure 8b). None of the treatment groups showed any significant change in superoxide dismutase levels when compared to the colchicine group in the hippocampus whereas the superoxide dismutase levels significantly increased in the VCO + colchicine group when compared to the colchicine group in the frontal cortex. A significant decrease in the superoxide dismutase level was also observed in SO + colchicine group when compared to the VCO + colchicine group in the frontal cortex.

Estimation of lipid peroxidation

Colchicine treated group showed a significant increase in malondialdehyde (MDA) level as compared to the ACSF treated group in the hippocampus (Figure 7c) and frontal cortex (Figure 8c). Significant reduction in MDA level was observed in donepezil+ colchicine, VCO + colchicine and SO + colchicine groups as compared to colchicine group.

Discussion

Sporadic dementia of Alzheimer's type is the most common form of AD associated with the microtubular dysfunction which interferes in cell's transportation and thereby the

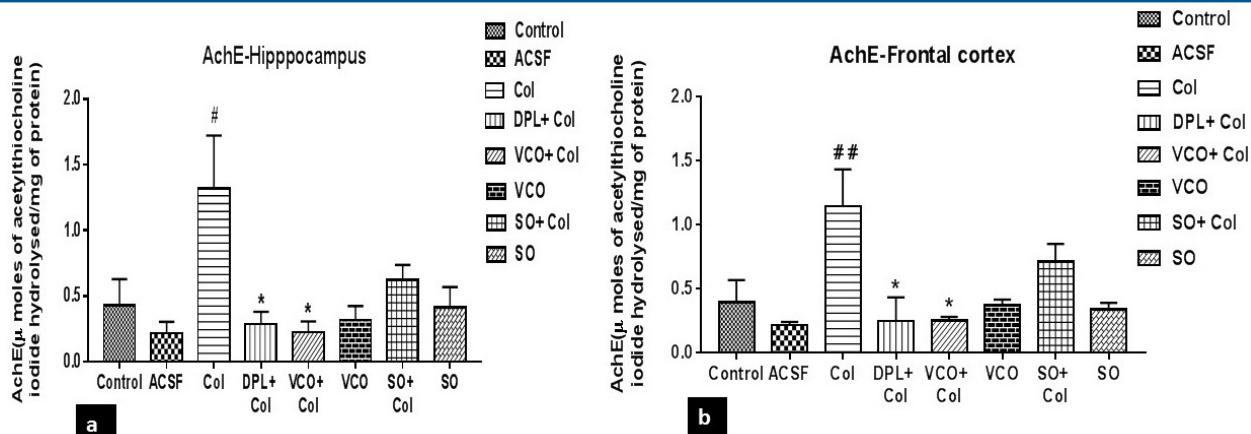


Figure 6. Effect of Colchicine (Col) and Colchicine + treatments (DPL- Donepezil, VCO – VCO, SO – SO) on acetylcholinesterase activity in the; (a) Hippocampus (b) Frontal cortex of rats. Values are expressed as mean±SEM (n=6). Statistical analysis was done by using one-way ANOVA followed by Tukey's multiple comparison test. [#]P<0.05 as compared to ACSF treated group. ^{##}P<0.01 as compared to ACSF treated group ^{*}P<0.05 as compared to colchicine group.

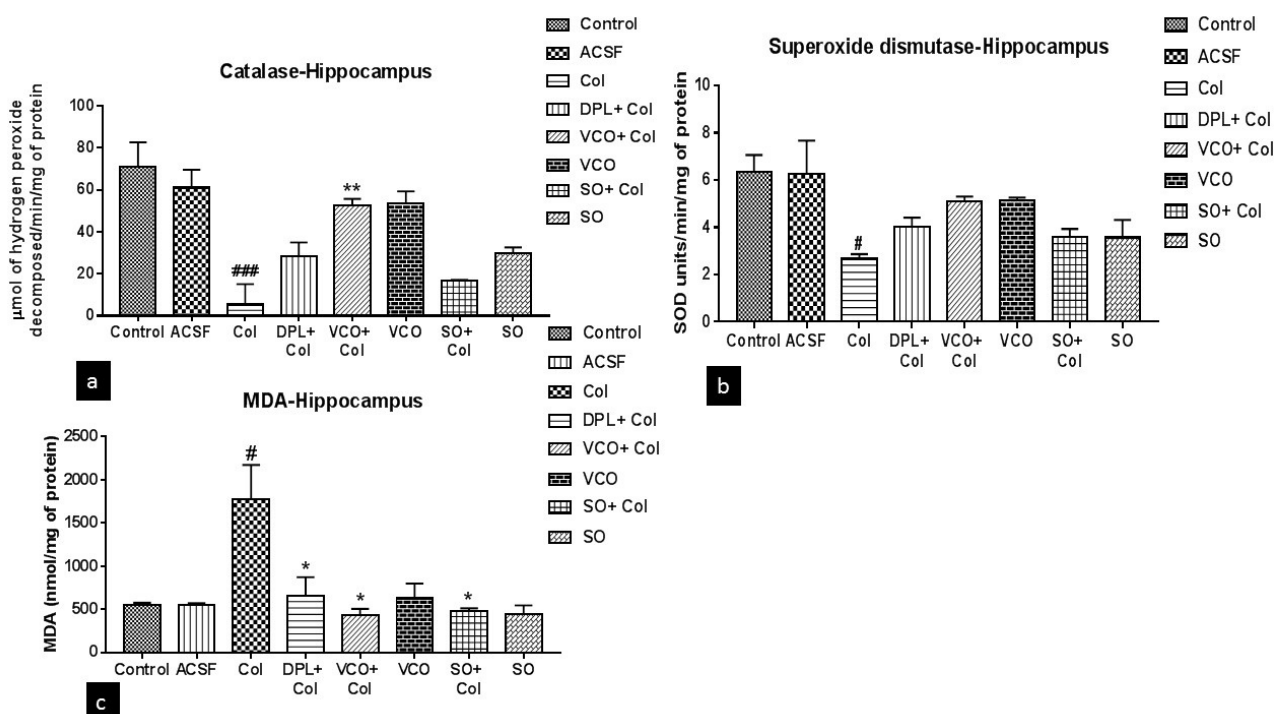


Figure 7. Effect of Colchicine (Col) and Colchicine + treatments (DPL- Donepezil, VCO – VCO, SO – SO) on (a) Catalase (b) Superoxide dismutase (C) Malondialdehyde levels in the hippocampus of rats. Values are expressed as mean±SEM (n=6). Statistical analysis was done by using one-way ANOVA followed by Tukey's multiple comparison tests. [#]P<0.05 as compared to ACSF treated group. ^{###}P<0.001 as compared to ACSF treated group. ^{**}P<0.01 as compared to colchicine group. ^{*}P<0.05 as compared to colchicine group.

cells cannot function properly and eventually results in the cell death with the appearance of neurofibrillary tangles. Colchicine is an agent that disrupts microtubulin integrity which results in the marked destruction of hippocampal granule cells, loss of cholinergic neurons and causes an elevation in free radical generation leading to extensive oxidative damage which finally results in cognitive impairment.²⁴

In recent years, there is increasing evidence which supports the role of diet and nutrition in improving the symptoms of AD.²⁵ Different dietary oils like canola oil, rice bran oil and olive oil were found to be beneficial in aluminum chloride

induced dementia in Wistar rats.²⁶ Cerebral glucose uptake and metabolism deteriorate in AD and this feature occurs early in the disease and may contribute to cognitive decline.²⁷ VCO contains MCFAs which are easily absorbed and metabolized in the liver to convert to ketone bodies. VCO is rich in polyphenols like p-coumaric acid, ferulic acid, caffeic acid, and catechins.²⁸ These polyphenols are natural antioxidants which provide protective effects in AD through a wide variety of pharmacological actions like preventing the oxidative stress resulting from the oxidation of biomolecules, inhibiting different types of inflammatory response and influencing the intracellular

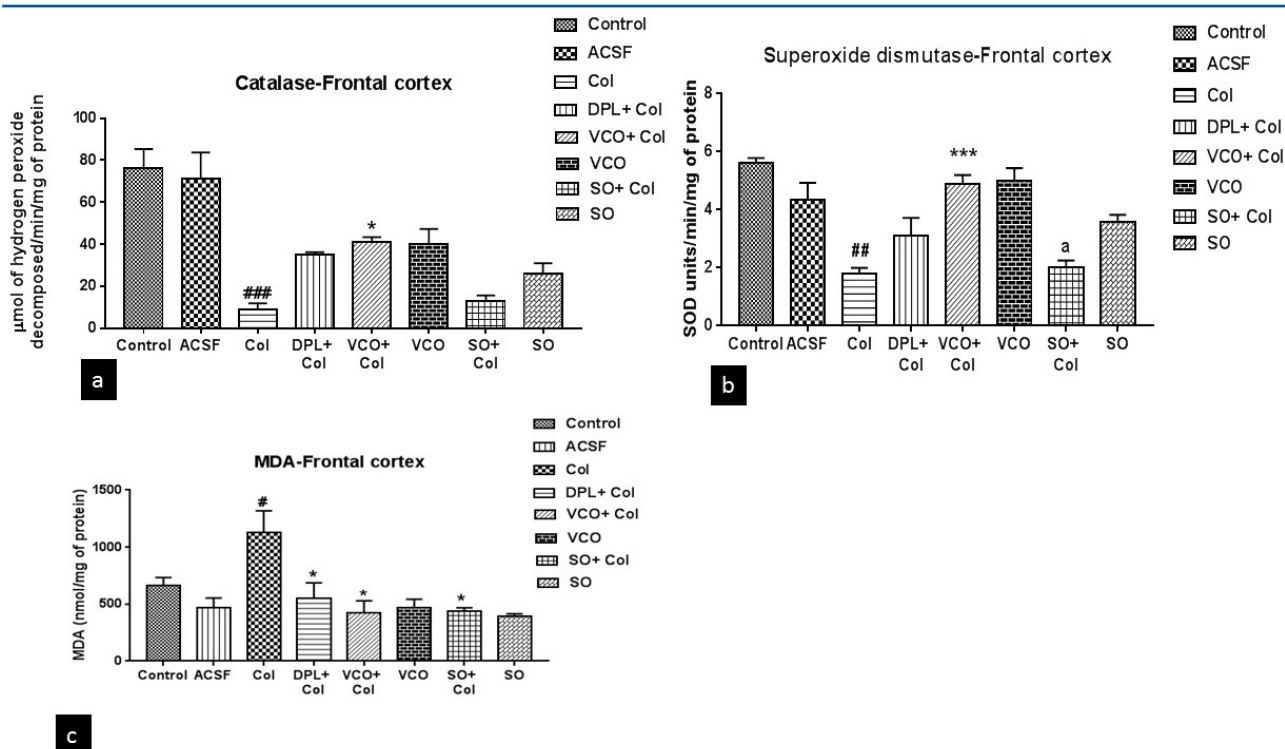


Figure 8. Effect of Colchicine (Col) and Colchicine + treatments (DPL- Donepezil, VCO – VCO, SO – SO) on (a) Catalase (b) Superoxide dismutase (C) Malondialdehyde levels in the frontal cortex of rats. Values are expressed as mean±SEM (n=6). Statistical analysis was done by using one-way ANOVA followed by Tukey's multiple comparison tests. #P<0.05 as compared to ACSF treated group. ##P<0.01 as compared to ACSF treated group. ###P<0.001 as compared to ACSF treated group. *P<0.05 as compared to colchicine group. ***P<0.001 as compared to colchicine group. a=p<0.05 as compared to VCO + colchicine group.

signaling pathways.²⁹ Also, recent study have shown that VCO showed neuroprotective actions in amyloid beta induced toxicity on high fat diet fed rats by normalizing neuroinflammatory pathways.³⁰

SO is an important part of dietary regimen which is rich in polyunsaturated fatty acids. Recent studies suggest that elevated intake of PUFA might be useful to AD.³¹ Sunflower is rich in omega-6 fatty acids and also contains omega -3 fatty acids in variable amount. Omega -3 fatty acids like α -linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid have anti-inflammatory effects and neuroprotective actions which may be beneficial to AD.³² Current study evaluated the neuroprotective potential of dietary oils like VCO and SO-enriched through diet in alleviating cognitive dysfunction induced by i.c.v administration of colchicine.

The gas chromatographic analysis showed that VCO contains 54.48% of medium chain fatty acids (C6-C12).

The polyphenol analysis of VCO revealed that it contains 22.1 mg/100g ferulic acid, 4.6mg/100g caffeic acid, 4.1mg/100g p-coumaric acid, 81.7 mg/100 g catechin and 1.23 mg/100g quercetin expressed in terms of milligrams of gallic acid equivalent.

Brain areas like frontal cortex and hippocampus were chosen to assess the effects of i.c.v administration of colchicine because these regions play a significant role in learning and memory.^{33,34}

The control group animals which were administered with normal saline did not influence parameters such as

body weight, spatial learning and memory and different biochemical parameters that were estimated.

Intracerebroventricular administration of colchicine and the VCO and SO-enriched diet does not produce any significant changes in the mean scores of body weight and locomotor activity. This signifies surgery had no negative effects in affecting the locomotion of the animals. Non-alteration of locomotor activity after colchicine administration is already supported from previous studies.^{35,36}

In the Morris water maze test, spatial learning and memory were mainly assessed by acquisition and retention trials. Acquisition trial was performed by training the animals to find the visual platform placed in the target quadrant which helps to evaluate the reference memory and retention trial was performed by removing the visual platform and assessed by the searching behavior of the animals to find the platform which helps to evaluate the spatial memory. During the 4 days of acquisition trial, different parameters like target quadrant latency, escape latency, path efficiency and swimming speed of the animals were assessed. Rats fed with VCO without colchicine induction produced a significant change in path efficiency, escape latency (time is taken to reach the platform) and target quadrant latency (time taken to reach the target quadrant) when day 1 and day 4 are compared. This may be indicating the quick learning behavior of the VCO group animals. Even though the time period to reach the target quadrant and locate the platform was reduced in all other groups, no significant

difference was observed.

Intracerebroventricular administration of colchicine was known to decrease the performance of animals in the Morris water maze test and in an elevated plus maze test.³⁷ In retention trial of Morris water maze test, administration of colchicine resulted in a significant decrease in spatial memory as indicated by increased target quadrant latency, increased escape latency, decreased time spent in target quadrant and decreased total zone entries. The swimming speed of treatment groups showed no significant difference from that of colchicine administered rats suggesting the surgery had no effect on the impairment of motor activity which is backed by the results from actophotometer study. Only VCO group reversed the effects of colchicine significantly in all the above parameters. This suggests the beneficial effect of VCO in antagonizing the spatial memory decline caused by colchicine. SO group showed a significant decrease only in escape latency. Rats fed with VCO and SO without colchicine induction showed no significant difference in any of these parameters when compared to normal pellet diet rats.

The serum cholesterol values showed no significant difference amongst all the treatment groups and control group animals suggesting that enrichment of these oils in the diet does not alter the cholesterol levels greatly and these oils may be beneficial in preventing the altering levels of cholesterol which is usually seen in patients with AD.^{38,39} Loss of cholinergic neurons in late AD implies the significance of the cholinergic system in learning and memory.⁴⁰ Acetylcholinesterase enzyme depletes the levels of acetylcholine and terminates cholinergic activity. Administration of colchicine caused a significant elevation in acetylcholinesterase activity by the destruction of the cholinergic pathway in the hippocampus and frontal cortex areas of the brain and is in accordance to the previous reports.^{41,42} VCO group was found to significantly decrease the acetylcholinesterase levels showing its attenuating effect on cognitive dysfunction which could be by the mechanism of ketone bodies that provide precursors of acetylcholine during cerebral hypometabolism. Donepezil, standard AChE inhibitor showed a significant decrease in acetylcholinesterase activity.

Oxidation of biomolecules can cause oxidative stress which may hamper the intracellular signaling pathways.⁴³ A significant increase in malondialdehyde level and a decrease in the levels of endogenous antioxidant enzymes like catalase and superoxide dismutase(SOD) was observed in the frontal cortex and hippocampus areas in colchicine treated group indicating that significant oxidative stress was caused by colchicine in the brain and is supported by the previous reports.^{44,45} Treatment with VCO reversed the colchicine associated oxidative stress by elevating the levels of SOD in the frontal cortex and catalase in both hippocampus and frontal cortex. VCO with colchicine produced a significant increase in the SOD levels as compared to SO with colchicine in the frontal cortex. This suggest that the MCFAs and the polyphenol content in

VCO enhanced the levels of SOD compared to the PUFA in the SO and this aids the VCO to antagonize the effects of colchicine. Treatment with VCO and SO decreased the malondialdehyde levels both in frontal cortex and hippocampus. This indicates the free-radical scavenging activity of these dietary oils by reversing the effects of colchicine.

Rats fed with VCO and SO-enriched diet without colchicine showed no significant difference in any of the oxidative parameters as well as in acetylcholinesterase activity when compared to the normal diet fed rats. This was contradicting to the study conducted where the normal rats were given VCO orally for 31 days and found to be neuroprotective when compared to the control animals.⁴⁶ Thus, the neuroprotective effect of a diet enriched VCO against colchicine-induced cognitive dysfunction in rats is by reducing oxidative stress and reducing the acetylcholinesterase level in hippocampus and cortex regions. Although SO has polyunsaturated fatty acids which are already said to have beneficial effects in AD, the overall result shows that MCFAs in VCO as a better dietary oil over SO in ameliorating the deficits associated with the cognitive dysfunction.

Conclusion

Sunflower oil and VCO showed changes in the level of various pathophysiological markers associated with the colchicine induced dementia. VCO reversed the different biochemical changes induced by colchicine by decreasing the acetylcholinesterase levels and reducing the oxidative stress. The MCFAs and polyphenols present in VCO showed better neuroprotective effects compared to the polyunsaturated fatty acids in the SO.

Even though the results showed the neuroprotective potential of VCO, more studies are required to establish the involvement of other biomarkers especially the ketone bodies that can be influenced by VCO in the disease progression.

Ethical issues

The experimental protocol entitled 'Evaluation of VCO and SO enriched diet against colchicine induced dementia' (IAEC No.-IAEC/KMC/77/2018) was approved by the Institutional Animal Ethics Committee, Kasturba Medical College, Manipal Academy of Higher Education and was performed out in accordance with the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

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Conflict of interests

The authors claim that there is no conflict of interest.

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