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Investigation of Peanut oil in Colchicine induced model of Dementia and its comparison with Sesame oil

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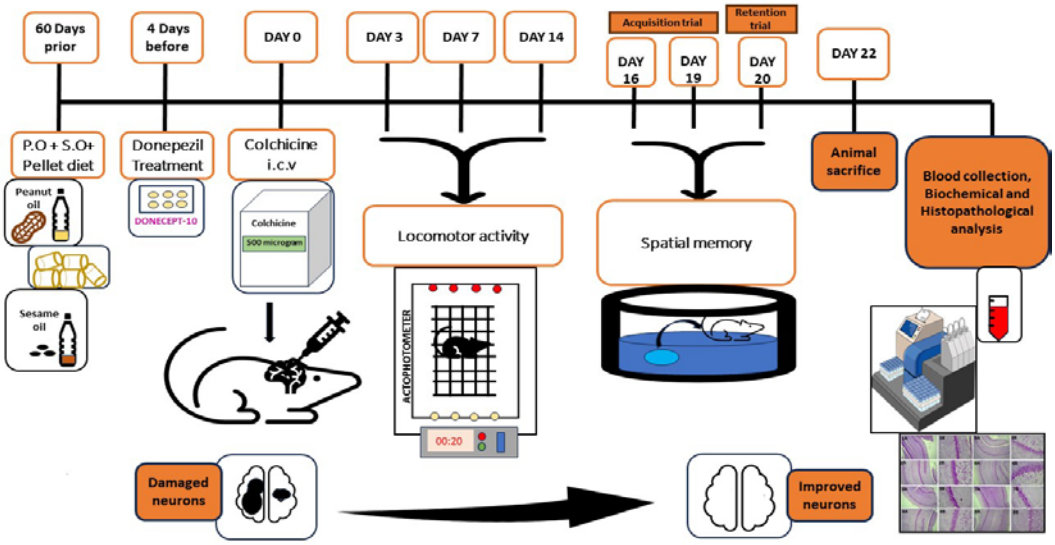
Article

ABSTRACT



Alzheimer's disease (AD) is considered as one of the main reasons for dementia, mainly in geriatric population. Edible oils containing high amount of PUFA provide both energy substrates and integral membrane components that are essential for appropriate neuronal and brain function. Also, edible oils and its constituents like phenol, sterols etc. has rich antioxidant, anti-inflammatory effects playing a key role in the alleviation of the progression of many cardiovascular, central nervous and cancer related diseases. Peanut oil from the seeds of Arachis hypogea has been used for centuries for its medicinal purpose and daily needs. The present study is to evaluate the effect of peanut oil in colchicine induced model of dementia. Post-weaned male and female Wistar rats were fed on diet with peanut oil 100 ml/kg and subjected to intra-cerebroventricular administration of colchicine at dose of 5µl in 5µl of artificial cerebrospinal fluid (ACSF). Donepezil at a dose of 5mg/kg p.o was used as standard. Spatial memory was assessed using Morris water maze. Rats were sacrificed for the isolation of hippocampus and frontal cortex followed by biochemical estimation of acetylcholinesterase (AChE), catalase and superoxide dismutase. Results showed that administration of peanut oil improved spatial memory and significantly reversed the AChE, catalase, and SOD activity. As peanut oil showed improved cholinergic transmission, antioxidant and anti-inflammatory levels in colchicine induced model of dementia, it can be considered as a relevant edible oil in the management of Alzheimer's disease.

Keywords: Donepezil, Frontal cortex, Hippocampus, Acetylcholinesterase, Spatial memory

INTRODUCTION

Alzheimer's disease (AD) is a cataclysmic neurodegenerative disease of the CNS, existing in both familial and sporadic forms and is characterized by cognitive dysfunction and behavioural disability, taking place in presenium and senium.[1] According to WHO report it was estimated that 24.3 million people in the world were affected with dementia in 2001, and this can rise to 42.3 million by 2020 and 81.1 million by 2040.[2] With an alarming rise in the number of AD cases, there is finite medical treatment for managing the occurrence of AD with adverse effects. To decrease the adverse effects and for better treatment options, there is an unmet need to search for alternative sources[3] which can also decrease the incidence of AD.

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Peanut or ground nut (*Arachis hypogea*) are grown as legumes which are widely grown in the tropical and subtropical regions comprising monounsaturated fatty acids and PUFA.[4] Peanuts are grown worldwide, first originated from Peru country, and are recognised as functional food because of the presence of resveratrol, monounsaturated fatty acids, polyunsaturated fatty acids, flavanols, phytosterols and phenolic acids. Peanuts contain high amounts of amino acid-arginine and are rich in fibres, vitamins, minerals which are helpful in the prevention of various diseases like cancer, diabetes, gall stones and cardiovascular related problems.[2] Many studies proved that regular intake of PUFA is beneficial for mild to moderate form of Alzheimer's disease as they are membrane lipids which help by maintaining the structure and function of neuronal membrane and their associated membrane bound proteins and their complexes.[5] PUFA plays a very prominent role for the neuronal membrane functioning by getting incorporated into the neuronal membrane thereby decreasing the cholesterol fraction, thus increasing the membrane fluidity which in turn elevates the receptor affinity in the synapse and thereby revamp neurotransmission.[6] Membrane fluidity is also important to maintain the synaptic structures.[7] Studies suggest that high intake of PUFA by dietary oils may alleviate the frequency of AD. Intake of dietary sesamol oil can effectively ameliorate cerebral ischemia induced oxidative damage [8] and literature has reported that dietary sesame oil is an effective antioxidant[9] and has a neuroprotective effect. Various studies on natural sources and edible oils have been reported to show neuroprotective activity.[10–12] Oral administration of caffeic acid investigates how it might counteract colchicine's negative effects, which include cognitive impairment and oxidative stress, by having neuroprotective characteristics[13] The potential mechanisms behind the neuroprotective effects of clove oil have been studied on cognitive function which offers insightful information on clove oil's medicinal potential for preventing cognitive decline brought on by colchicine-induced neurotoxicity.[14] Another study focussed on lowering the levels of acetylcholinesterase and malondialdehyde and raising the levels of catalase and superoxide dismutase using Virgin coconut oil, reversing the antagonistic effects brought on by colchicine.[15] The present study was undertaken to observe the effect of dietary peanut oil against colchicine induced model of dementia and to compare its effect with sesame oil.

MATERIALS AND METHODS

Animals: Post weaned male and female Wistar rats were procured from Central Animal Research Facility (CARF) of Manipal Academy of Higher Education, Manipal for the experimental study. Animals were acclimatized with oil fed diet instead of normal pellet diet, and study was approved by Institutional Ethics Committee, Kasturba Medical College, Manipal Academy of Higher Education, Manipal (IAEC/KMC/78/2018), with the rules followed under the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental design: Animals were divided into groups containing six animals each. After acclimatization, the animals were randomized into groups and treatment was assigned. Peanut and sesame oils along with the pellet diet were mixed in required amounts (200ml/kg of SO & PO was mixed in 1 Kg diet),[8] administered 60 days prior to the experiment and was continued till the end of the experiment. Animals were pre-treated with standard donepezil for four days at a dose of 5mg/kg.[16] Acquisition trial was performed for four days followed by retention trial from the 5th day. For the memory impairment colchicine was administered ICV on day 0 by using 15µg in 5µl of ACSF.[17] Saline was given for control group at 10mg/kg.

Stereotaxic surgery was first employed by Horsley and Clarke in rodent models in mid-1900.[16] Stereotaxic surgery is a method, where the coordinates are set from the software utilising the rat brain atlas. Point to be noted is that bregma which is located on the rat skull (seen after incision of the skin) is noted as standard reference or starting point for the Hamilton syringe which moves along the anterior, posterior or dorso-ventral regions based on the coordinates which are set on the software. The method was also applied in humans by Spiegel and Wycis in the year 1947.

Procedure: All animals except control groups i.e., normal control group, peanut oil + diet and sesame oil + diet were subjected to anaesthesia with thiopentone sodium (20 mg/kg) before conducting surgery. Fur was trimmed; alternatively, a small incision was made with scalpel blade to locate the bregma. Rat was placed on the frame of stereotaxic apparatus and made stable with the help of ear bars and by fixing the upper tooth to a holder. Precautions like cleaning the instrument and skull driller with 70 percent v/v of ethanol before starting the experiment was done. The following coordinates were set- 0.8 posterior to bregma, 1.8 lateral to sagittal suture and 3.6 dorsoventrally, then skull was drilled. Colchicine and ACSF were injected according to the groups. After injecting, a two-minute waiting time was allowed for the proper diffusion of drug into the brain.[17] After completion, sutures are done, and betadine solution was applied on rat's head to avoid microbial infection. Special care was taken post-operatively viz., accommodation, hygiene, food, and water.

BEHAVIORAL ASSESSMENT

Locomotor activity: Locomotor activity was assessed by actophotometer which emits an IR beam where the counting is noted by interference of the animal in the path of IR beam. A time lapse of 600 seconds was set, and the number of recordings were noted. After completion of the experiment, animals were kept for habituation period of five minutes, the activity was performed on 3rd, 7th and 14th consecutive days.[18]

Morris Water Maze: Spatial memory was assessed by Morris water maze test where the animal uses spatial cues to locate the platform. The apparatus consists of a large circular water tank with a diameter of 110 cm. Water was filled till the platform sinks and the water level was maintained as 2cm from above the platform. Appropriate temperature was maintained at 27^{0}C. The tank was divided into four quadrants namely A, B, C, D respectively and the platform was placed in D quadrant.[19] The total set up was recorded by the camera and the results were analysed by Anymaze software.

Acquisition trial: Animals were trained to locate the platform for four days by keeping each animal in three different quadrants. Precautions of light and sound disturbances were taken before starting the experiment. Rat was placed gently by holding its tail in

water and was allowed to swim for a period of 60 seconds to locate the platform. After reaching the platform, rat was enabled to sit on the platform for 30 seconds. On the first day of acquisition trial as the rodents were naïve, they did not locate the platform even after 60 seconds, therefore rats were trained to locate the platform. Different parameters were examined such as escape latency, D quadrant latency, D time and D entries respectively under acquisition trial. Precautions were taken to remove any foreign dust particles like husk, fur, and faecal matter from the water in each trial because they can act as interference for the rat to locate the platform.

Retention trial: Retention trial was performed on day five by removing the platform. Rat was placed in the quadrant B and allowed to swim for 60 seconds and the number of entries in the D quadrant were noted. Parameters such as island entries, escape latency, D latency, D time and D entries were analysed by Anymaze software.

Biochemical estimations: Blood samples were collected by retro-orbital puncture method. Rats were sacrificed with anaesthetic agent followed by cervical dislocation and brain samples were collected (hippocampus and frontal cortex) in micro centrifuge tubes. Phosphate buffer saline (pH 7.4) was added, according to the weight of the tissue and stored at -20^{0}C. Hippocampus and frontal cortex were homogenised with ice cold 0.1M phosphate buffer (pH 7.4) at an rpm of 8000, the tissue homogenate was stored at -20^{0}C.

Estimation of acetylcholinesterase activity: Acetylcholinesterase activity was performed by Ellman's method. Reaction mixture containing 2.6 ml phosphate buffer with a pH of 8, and 0.4ml of supernatant was prepared. Ellman's reagent (20ul of Acetylthiocholine iodide and 100ul of 5,5'-dithio-bis-2-nitrobenzoic acid) was added to it. Using a UV-Visible spectrophotometer at 412 nm, change in absorbance was measured for a period of 4min at an interval of 60 sec and the per minute change in absorbance was calculated.[20]

Estimation of Antioxidant enzymes

Catalase (CAT) activity: Catalase activity was determined according to Aebi et al., 1984. By using UV spectrophotometric method, the assay was performed, i.e., change in absorbance was measured which was due to the hydrogen peroxide degradation. It was designated by µmole/min/mg protein.[21]

Superoxide dismutase (SOD) activity: Superoxide dismutase was determined as described by Saggu et al.,1989. It was based upon the inhibition of adrenaline to adrenochrome by SOD. It was indicated as units (U) of SOD activity/mg protein [22].

Statistical Analysis: Results were analysed by one-way and two-way ANOVA followed by Tukey's multiple comparison test with a p value of <0.05 and 0.001.

RESULTS

Locomotor activity: Locomotor activity was performed on 3, 7 and 14th day of treatment. Treatment groups showed a significant increase in the locomotor activity when compared to disease control group (Fig 1)

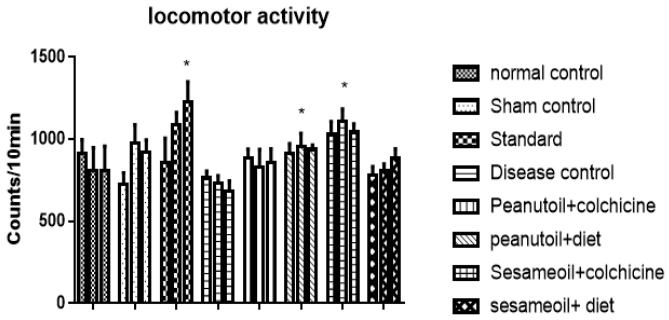
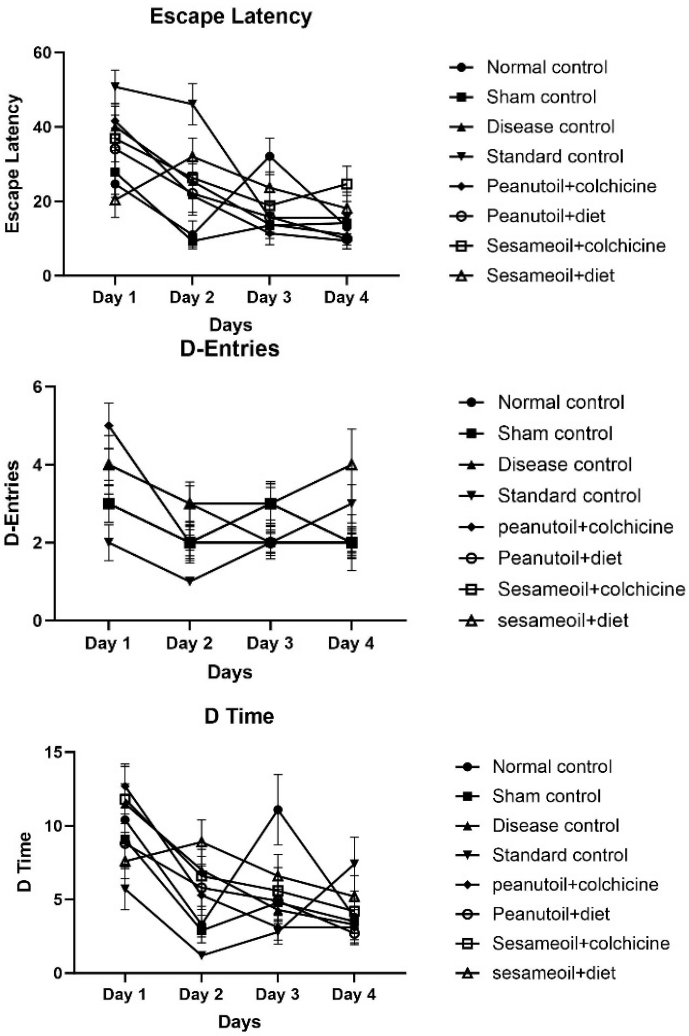


Figure 1: Effect of groups on 3rd, 7th and 14th day of treatment

Morris water Maze: Treatment groups showed improvement in different parameters such as escape latency, D latency, D entries, D time after four days of acquisition trial (Fig 2).



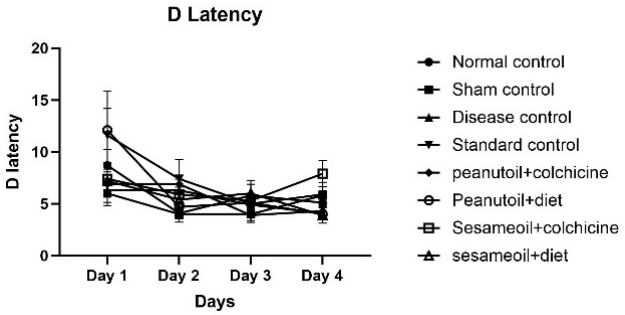
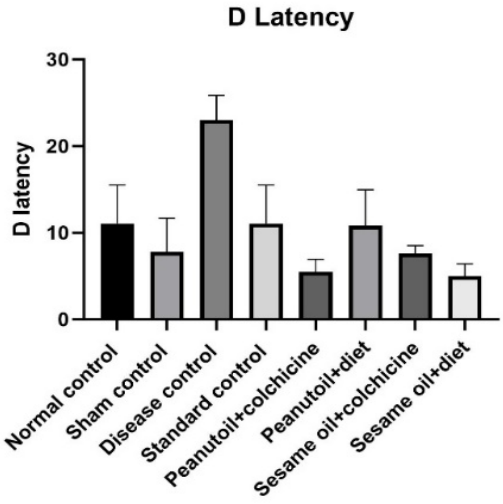
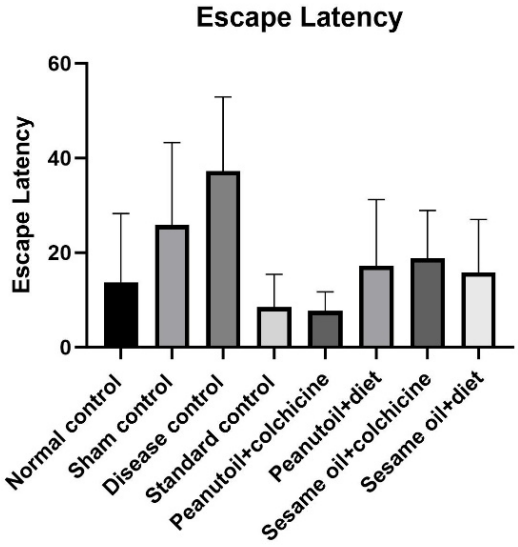
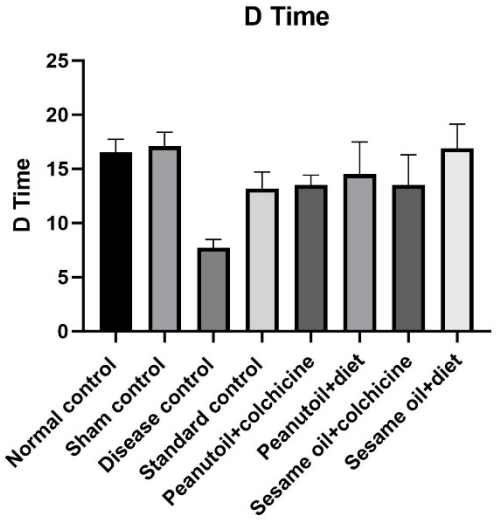


Figure 2: Effect of peanut oil with diet and sesame oil with diet on spatial memory in colchicine induced model of dementia demonstrated as D entry, Escape latency, D time, D latency, after acquisition trial.

Retention trial: It was performed on 5th day and showed significant improvement in the above-mentioned parameters when compared to disease control. The animals treated with peanut oil with diet and sesame oil with diet showed increase in D time when compared to other group of animals.







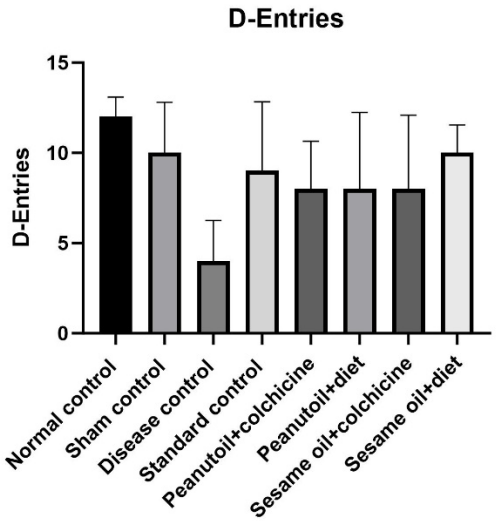


Figure 3: Effect of peanut oil with diet and sesame oil with diet on spatial memory in colchicine induced model of dementia demonstrated as D time, D entry, Escape latency, D latency, after retention trial.

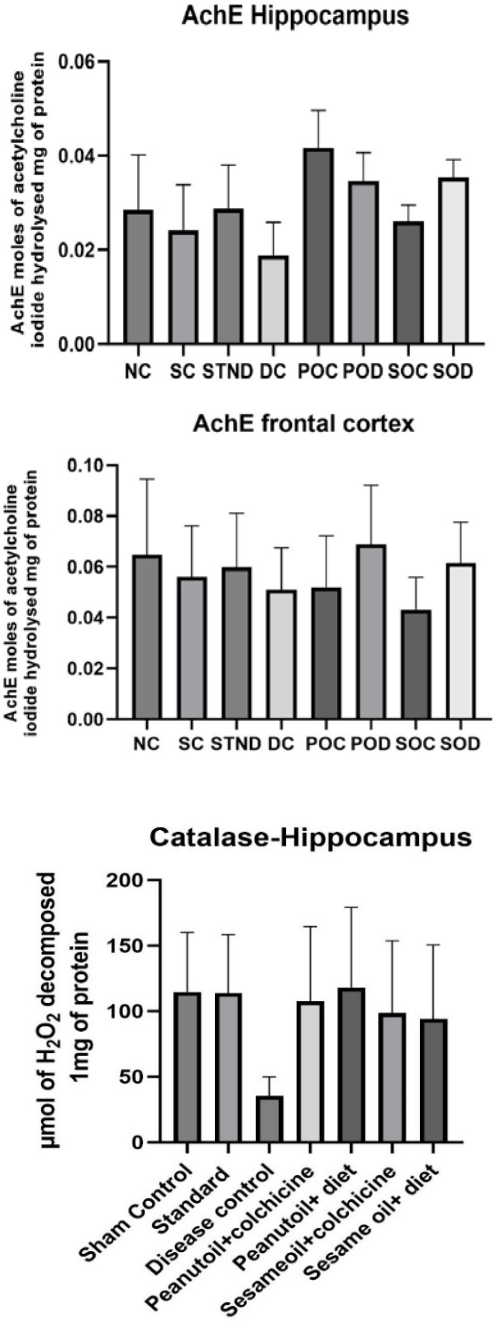
Escape latency, D time, D entries: Colchicine administration caused memory impairment. Standard group (donepezil 5mg/kg) and oil fed diet rats has shown decrease in the escape latency when compared to disease control group, acquisition and retention trial has shown that D time increased in treatment groups, treatment groups has shown significant increase in the D entries when compared to disease control group (Figure 3).

Effect of treatment groups on antioxidant markers in Frontal cortex and Hippocampus

Catalase activity: A decrease in the brain catalase levels was seen in colchicine group of animals. Treatment with dietary peanut oil showed protective effect when compared with sham control, peanut oil with colchicine and sesame oil with colchicine group of animals and a slight improvement in catalase levels in the frontal cortex and hippocampus was observed when compared with sesame oil with diet (Fig 4).

Acetylcholinesterase (AchE) activity: AchE activity was significantly increased in the frontal cortex and hippocampus in colchicine induced animals. Administration of dietary peanut oil and sesame oil produced marked reduction in the levels of AchE activity in the frontal cortex and hippocampus (Fig 4).

SOD activity: A decrease in the brain SOD levels was seen in colchicine treated animals. Treatment with dietary peanut oil showed an increased SOD effect when compared with sham control. Peanut oil with colchicine and sesame oil with colchicine group of animals however it did not show any marked improvement in SOD activity in the frontal cortex and hippocampus when compared with sesame oil with diet (Fig 4).



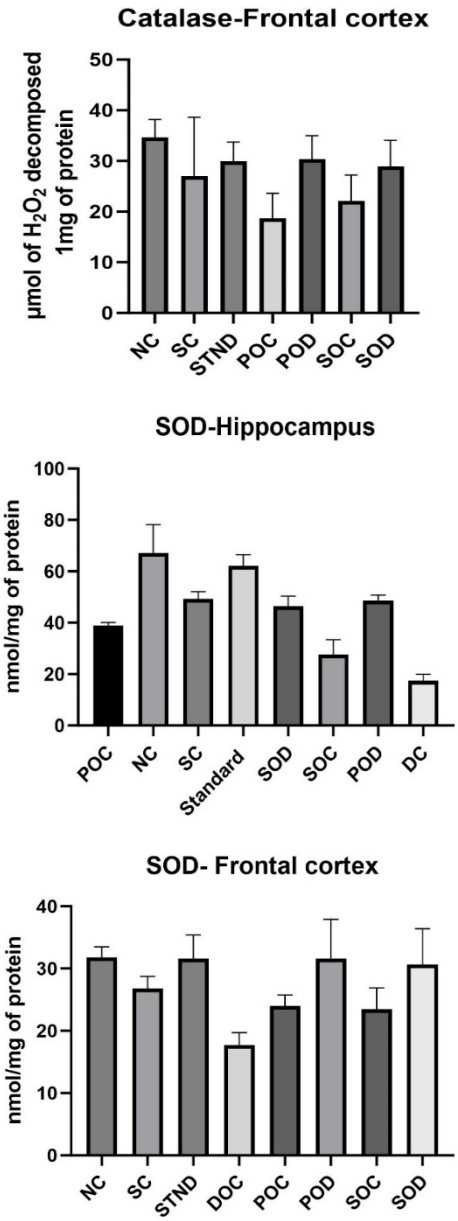


Figure 4: Effect of peanut oil with diet and sesame oil with diet on Acetylcholinesterase activity, SOD levels and catalase activity in hippocampus and frontal cortex of colchicine induced dementia rats

Histopathological studies were performed using rat brain to analyse the damaged portions and normal status of the neuronal cells. Figure 5.1A and 5.1B showed healthy neurons in the hippocampal area. While 5.2A and 5.2B represent the sham control animal brain showing the normal brain function. Figure 5.3A and 5.3B represent the damaged neural cells in the hippocampal area treated with the colchicine. Figure 5.4A and 5.4B represents the normal healthy brain in the hippocampal area with the standard drug (donepezil 5mg/kg). Figure 5.5A and 5.5B represents the peanut oil + colchicine group showing improved neuronal activity. Figure 5.6A and 5.6B represents the peanut oil + diet group showing normal healthy neuronal activity. Figure 5.7A and 5.7B represents the sesame oil + colchicine group showing normal

neurons. Figure 5.8A and 5.8B represents sesame oil + diet group healthy brain with improved neuronal function. After comparing the figures of both the groups (diet & standard), the healthy brain was found in the diet groups than in standard groups.

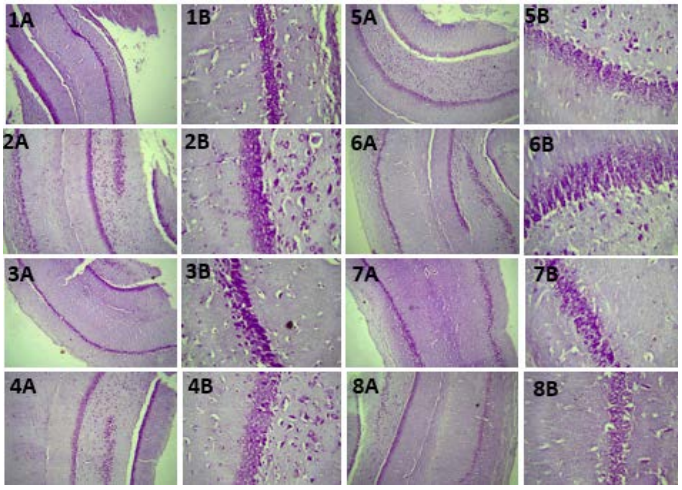


Figure 5: Histopathological studies of rat brain to analyse the damaged portions and normal status of the neuronal cells

DISCUSSION

Alzheimer's disease (AD) is considered as one of the main reasons for dementia in the elderly population. Edible oils containing high amount of PUFA provide components which are essential for appropriate neuronal and brain function.[5] Studies suggest that the use of olive oil, rice bran oil, canola oil,[23] sesame oil decrease the incidence of AD**.** To envisage the neuro-protective effect of peanut oil in colchicine induced model of dementia, the present study was taken up. In this study, investigation of the activity of peanut oil in colchicine induced model of dementia and then comparing its effect with sesame oil was performed and it was found that peanut oil like other oils can also serve as a dietary source which can decrease the incidence of AD.

Colchicine which is a cytotoxic agent binds irreversibly to dimers of tubulin and thereby inhibits the addition of the molecules of tubulin to the fast-growing ends, leading to the inhibition of assembly of microtubules and thus microtubule polymerization is disrupted.[17,24] It destroys the hippocampal granule cells, septi-hippocampal pathways, mossy fibres and induces degeneration of neurofibrils[25,26] thus leading to the loss of cholinergic neurons thereby presynaptic cholinergic parameters will be decreased resulting in the reduction of acetylcholinesterase activity and also choline acetyl transferase. All the above combined activities result in loss of memory and decreases the ability of learning.[17]

In the present study, colchicine is administered intra-cerebroventricularly (ICV) leading to the loss of memory which was confirmed by the results of locomotor activity. ICV model is considered as a relevant model for understanding sporadic form of AD. Pre-treatment was done with peanut oil with diet and sesame oil with diet for 60 days, which was continued till the end of the study. Standard donepezil was given four days prior to the experiment.

Gradual decrease in the locomotor activity was observed in the colchicine induced memory impairment in male and female Wistar rats. Treatment groups with peanut oil and sesame oil diet (200ml/kg) before and after the induction of memory impairment had shown positive effect and reversed the effect of colchicine induced sporadic form of dementia. A period of 10 min (600 seconds) was utilised to check the locomotor activity of animals in the actophotometer.[18]

In acquisition trial various parameters were assessed like escape latency, D time, and D latency and D entries. Treatment with donepezil four days prior to the induction of colchicine showed remarkable enhancement in the escape latency, D time, D latency and island entries as detected in Morris water maze test. Colchicine induced animals showed memory deficit when compared to the peanut and sesame oil fed rats. Peanut oil fed rats showed positive results in the behavioural parameters like decrease in the escape latency, D latency and increased the D time in the water maze test compared to sesame oil fed rats. Treatment groups with donepezil, dietary oil fed rats have shown decrease in the oxidative stress levels when compared with diseased control rats.

On the 5th day of retention trial, various parameters were assessed like escape latency, D time, and D latency and D entries and island entries. Escape latency is defined as the duration of time taken by the animal to locate the hidden platform. Induction of colchicine through ICV route characteristically increased escape latency in Wistar rats as compared to sham control animals. Escape latency, D time was increased when compared with other treatment groups. D entries are the total number of crossings made by the disease control and treatment groups into the D quadrant. No significant difference was observed in the number of D entries when compared with the disease control animals. Also, treatment groups showed increase in the D entries and island entries when compared with the disease control group.

Biochemical estimations such as catalase, acetylcholinesterase and super oxide dismutase were assessed. Estimation of catalase & super oxide dismutase showed significant decrease in disease control group when compared with the normal control groups and were increased in both dietary peanut oil and dietary sesame oil groups.[27] Acetyl choline serves as a functional and important neurotransmitter in the CNS which was decreased by the enzymatic activity of cholinesterase. Colchicine induced rats have shown increased acetylcholinesterase activity and it was decreased in dietary peanut oil and dietary sesame oil treated groups.

Peanut oil group was compared with sesame oil group which showed better protective effect against colchicine induced model of dementia, which was marked by improved locomotor activity and improved brain antioxidant levels.

CONCLUSION

Administration of peanut oil fed diet remarkably enhanced the spatial memory function in colchicine induced memory impairment. Also, the monounsaturated fatty acids and PUFA contents in the oils showed improved cholinergic transmission, antioxidant and anti-inflammatory levels in colchicine induced model of dementia. Hence, peanut oil can be considered as a relevant edible oil in the management of Alzheimer's disease.

Declarations

Ethics Approval: Animal studies has been approved by Institutional Animal Ethics Committee, Kasturba Medical College, Manipal Academy of Higher Education, Manipal (IAEC/KMC/78/2018)

Consent for publication: All the authors have read and approved the final version of the manuscript submitted.

Competing Interest: The authors declare no conflict of interest.

Author Contribution: Conceptualization R.S., N.S., Investigation N.S., J.J., F.B., Original draft preparation N.S., J.J., F.B., Review and Editing R.S., F.B., S.K., Supervision R.S.

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