EFFECT OF *SANTALUM ALBUM* LINN ON MEMORY ENHANCING ACTIVITY ON MICE

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ABSTRACT

The plant *Santalum album* Linn. is commonly known as Chandana (Family: Santalaceae) and whole plant is having medicinal active constituents, such as Carbohydrates, Triterpenoids, Volatile oils, α and β santalol etc are responsible for its activity. The aqueous extract of *Santalum album* on memory enhancing activity was evaluated by using experimental model such as Amyloid-β peptide deposition in mice. The mice were treated with graded dose of *Santalum album* extract. The dose grading from 100mg/Kg to 500mg/Kg were administered orally to the mice and Donepezil was used as standard drug 0.5ml for the group I and VII i.e., dementia mice treated with Donepezil. The aqueous extract of *Santalum album* increase the level of Acetyl cholinesterase helpful in the brain for storing the memory, decreased levels of GSH , due to excess release of Glutathione may cause the excitotoxity and decreased the formation of reactive oxygen species due to its anti oxidant activity. Hence, it is concluded that, aqueous extract of *Santalum album* increases Memory Enhancing Property in mice.

KEY WORDS: *Santalum album*, memory enhancing activity, Amyloid-β peptide, dementia mice.

1.INTRODUCTION

Dementia is a disorder which is caused due to loss of neurons in the brain or due to dehydration of acetylcholine in the nerve junction region or due to accumulation of NFT's in the neurons (Cummings, 2004; Wisniewski, 1985). Hormone replacement therapy is generally prescribed for preventing and treating osteoporosis. A decrease in the concentration level of estrogen also causes a neuro–degenerative effect which might increases the risk of Alzheimers disease (Pangnani and Henderson , 1996). Amyloid-β-specific antibodies are used for the clearing the Amyloid-β deposition in the brain. Potential therapies that decreases Amyloid deposition in the brain by stimulating immune system. (Mononego and Weiner, 2003). Currently available drugs for treating dementia have been associated with number of side effects. Treatment of dementia involves acetylcholinesterase inhibitors (Wilinkson, 2004; Giacobini, 2000). Antioxidants such as Donepezil, Rivastigmin (Engelhart, 2002; Greenberg, 2000). The consumption of synthetic drugs leads to common cold, muscle cramps, diarrhea, vomiting, nausea and some metabolic disorders.

Medicinal plants are used for various research purposes (Kirtikar, 1993). It has been reported that traditional system has immune potential against various diseases (Duke and Ayensu, 1985). More than 13,000 plants have been studied for various pharmacological properties (Bown 1995; Raskin, 2002). Herbal treatment for memory loss has no side effects and is local available (Howes and Houghton, 2003). They are effective in reducing the oxidation property in animals (Williams, 2004). So we had taken the one of the well known traditional drug *Santalum album* Linn. as a part for my project to increase the memory and cognitive function (Howea, 2003). It was described in one of the Siddha system that *Santalum album* was having the property to increase the memory in brain and also in the Ayurvedic formulation S*mruthi leha* (drugs which increases the memory) also described that *Santalum album* has the property to decrease dementia in older people.

The reason for choosing this drug is because that the *Santalum album* has having the antioxidant activity which decreases the neuron apoptosis (self degeneration of neurons due to free radical oxygen species) (Burdock and Carabin, 2008). Due to the activity of antioxidant property we had selected the *Santalum album.* The present study deals with the "Memory Enhancing Property of *Santalum album* in Mice".

2. MATERIALS AND METHODS

Plant material and Preparation of extraction

The fully grown plants are uprooted and the bark from roots and stems is removed along with little sap

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wood. The wood is yellowish or pale red in colour. It is very much dense, hard, and heavy and splits easily. The wood is cut into small pieces and powder and packed well in Soxhelet apparatus and was subjected to continuous hot extraction with water for 18 hrs. The extract was filtered while hot and the resultant extract was distilled in vaccum under reduced pressure in order to remove the solvent completely. It was dried and kept in a desiccators till experimentation.

Experimental animals

Albino Wistar mice weighing between 30-50 gm were used. The animals were allow acclimatizing to the environment for 7 days and supplied with a standard pellet diet (Lipton India Ltd., Mumbai, India) and water *ad libitum*. The congenial temperature 30± 2°c and 12 hrs light and dark cycles were maintained. Institutional Animal Ethics Committee approved the experimental protocol; animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Chemicals

Amyloid – β – protein fragment 25 – 35 which was obtained from SIGMA – ACORICH, INC USA.

Acute toxicity studies

The acute toxicity study was carried according to the limit test described by the OPPTS guidelines (http://www.epa.gov/oppts/home/guideline.htm). Briefly, a test dose of 50 mg/kg and 500 mg/kg were given to the mice. The extract was found to be safe at the dose of 500 mg/kg, p.o. Hence, the mice were treated with graded dose of *Santalum album* extract. The dose grading from 100mg/Kg to 500mg/Kg were administered orally to the mice were selected (Kulakarni, 1993).

Experimental induction of memory loss in animal models

β-amyloid is protein having a high molecular weight which causes the damage of the neurons in brain i.e., causes the memory loss (Schinechel, 1993). Memory loss was induced to group I, II, III, IV, V and VII animals by intra cranio ventricular route through a hamilton micro liter syringe and the dose is 10 micro liters per each animal.

Experimental design

The mice were divided into 7 groups comprising of 6 animals in each group.

Group I : Positive control mice received 0.5 ml of Donapezil through oral route.

Group II : Negative control mice received 0.5 ml of the solvent through oral route.

Group III : Dementia mice treated with *Santalum album* extract 2 ml is given orally.

Group IV : Dementia mice treated with *Santalum album* extract 4 ml is given orally.

Group V : Dementia mice treated with *Santalum album* extract 6 ml is given orally.

Group VI : 0.5 ml of solvent without any induction with β amyloid.

Group VII: In this group a combined dose of 0.5 ml of Donpezil and 0.4 ml of medicine dose of *Santalum album* is given orally.

Experimental procedure:

Male albino mice weighing around 30-50 gm which are one to two month old were taken for the study (Saloke, 1994). The mice were treated with graded dose of *Santalum album* extract. The dose grading from 50mg/Kg to 400mg/Kg were orally administered to the mice. The standard Donepezil was also administered 0.5ml for the group I and VII i.e., dementia mice treated with Donepezil. The treatment was carried out for 30 days and the Acetlycholinesterase is calculated by taking the brain tissue. A normal group was also maintained with normal water group VI. At the end of the experimental period, the animals were sacrificed by applying intra-peritoneal thiopentone (thiosol / Na+). The brain was dissected out and cleaned with ice-cold saline, blotted dry and immediately transferred to the ice chamber. Various oxidative stress and toxicity related biochemical parameters were estimated. The Animal Ethics Committee of the Institution approved the procedures (Gours, 2005).

Estimation of Biochemical Parameters:

Estimation of Ach levels

The quantitative measurement of acetyl cholinesterase levels in brain was performed according to the method of Ellman (1961). The assay mixture contained 0.05 ml of supernatant, 3 ml of 0.01m sodium phosphate buffer (pH 8), 0.10 ml of acetyl thiocholine iodied and 0.01ml of DTNB. The change in absorbance was measured immediately at 412nm using Perkin ellman lambla spectrophotometer. Results were calculated using molar extinction coefficient of chromophore (1.36 X 104 μm^{-1}/ cm^{-1}) and expressed as percentage of control.

Estimation of Glutathione levels:

GSH in stratium, cortex and hippocampus was estimated according to the method described by Ellman (1961). Results were calculated using molar extinctions coefficient of chromophore (1.36X 104 $\mu m^{-1}/ cm^{-1}$) and expressed as percentage of control. Total glutathione analysis was done by the method of Zahler and Cleland (1968). The method is based on the reduction with dithioerythritol and determination of the resulting monothiols with 5, 5^1 dithiobis 2-nitro benzoic acid (DTNB) (Ellman reagent) in the presence of arsenite. Oxidized glutathione were quantified by subtracting the value of glutathione reduced from total glutathione. GST was assayed by the method (Habig, 1974). It catalyses the formation of the glutathione conjugates of 1-chloro-2,4-dinitrobenzene (CDNB) which absorb maximum at 340nm and have an extinction coefficient of 9.6 $\mu m^{-1}/ cm^{-1}$. Protein estimation was done by burettes method using bovine serum albumin as standards (Granall, 1949) Table 1.

Estimation of total antioxidant activity:

The Antioxidant activity was estimated by spectrophotometric method (Koracevic, 2001).Two milliliter of blood samples were collected in EDTA bottles. Plasma was separated immediately by centrifugation and anti oxidant activity is measured. This method is based on the principle that the standardized solution of iron EDTA complex reacts with hydrogen peroxide by a Fenton type of reaction, leading to the formation of hydroxyl radicals. This reactive oxygen species degrades benzoate, resulting in the release of TBARS. Antioxidants from the added plasma cause the suppression of production of TBARS. The reaction is measured spectrophotometrically at 532nm. The inhibition of the colour developed is defined as Antioxidant activity.

Statistical analysis

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Bonferroni's comparison test.

3.RESULTS

Acetyl cholinesterase levels

During memory loss the Ach levels are drastically decreased which results from decreased in cognitive function. Amyloid -β induced memory loss which causes damage in the neuronal junction and there is a decreased in the levels of Acetlycholinesterase levels in brain. Administration of aqueous extract of *Santalum album* to mildly induced memory loss mice shows increased levels in the Acetlycholinesterase.

Glutathione levels

In the Amyloid-β induced memory loss mice contains a high amount of the Glutathione levels. But after administration of the aqueous extract of *Santalum album* for a period of 30 days causes decreased levels of GSH (Glutathione stimulating hormone) (Tabel 1).

Antioxidant levels

These Reactive Oxygen species (ROS) attack many molecules such as membrane lipids and DNA. These Reactive Oxygen species accumulate and neurons become more susceptible to excitotoxity damage. The aqueous extract of *Santalum album* has decreased the formation of reactive oxygen species, because the *Santalum album* is having the capacity of anti oxidant activity.

Table 1:- Effect on Glutathione levels in different groups of mice

Group	Protein (g/dl)	Glu.Reductase (µg/ mg protein)	Glu.peroxidase (µg of Glutathione consumed^{-1}/mg protein)	SOD (μ/mg^{-1} protein)	CAT ($\mu/Sec/mg^{-1}$ protein)
I	4.25	31.51	24.26	18.26	2.32
II	7.23	28.59	28.46	24.65	4.63
III	6.23	29.38	31.3	22.09	5.23
IV	5.45	28.98	29.59	20.32	4.25
V	5.26	26.56	26.49	17.38	3.85
VI	9.1	24.53	33.28	21.67	6.25
VII	4.6	23.14	25.64	16.5	3.75

DISCUSSION

The results of the present study suggest that *Santalum album* extract affects memory enhancing property as shown by its effect in the Amyloid-β peptide deposition method, Acetylcholiesterase levels, Protein level and Glutathione level and anti oxidative property. It was described in one of the Siddha system that *Santalum album* was having the property to increase the memory in brain and also in the Ayurvedic formulation S*mruthi leha* (drugs which increases the memory) also described that *Santalum album* has the property to decrease dementia in older people (Patel and Prince, 2001). The α-santalol is a very potent antagonist of dopamine D_2 and serotonin 5-HT_2 a receptor binding, the effect being similar to that of the anti-psychotic agents Chloropromazine and risperidone, used to treat schizophrenia. Acetlycholinesterase is one of the important neurotransmitter which is helpful in the brain for storing the memory.

During memory loss the Acetlycholinesterase levels are drastically decreased which results from decreased in cognitive function. Amyloid -β induced memory loss which causes damage in the neuronal junction and there is a decreased in the levels of Acetlycholinesterase levels in brain (Feng, 2004). Administration of aqueous extract of *Santalum album* to mildly induced memory loss mice shows an increased level in the Acetlycholinesterase. In amyloid-β induced memory loss mice contains a high amount of the Glutathione levels. It has been reported that Sandalwood oil result in decreases in hepatic Glutathione-S-Transferase level and acid soluble Sulphyldryl level helps in Cancer treatment (Banerjee, 1993). But after administration of the aqueous extract of *Santalum album* for a period of 30 days. There is a significant decreased level of GSH. Excess release of Glutathione may cause the excitotoxity due to high intra cellular concentration of calcium, which causes neuronal dysfunction and death (Anil and Sankar Rao, 2001). The presence of glutathione in leaves of *Santalum album* L. has been reported (Kuttan., 1974). The aqueous extract of *Santalum album* decreases intra cellular calcium level of GSH and prevents from neuronal dysfunction.

The brain derives nearly all the energy from mitochondrial oxidative phosphorylation, which generates ATP at same time as reducing molecular oxygen to water. Under certain conditions reactive oxygen species are produced as side–products of this process (Sagara, 1998). These Reactive Oxygen species (ROS) attack many molecules such as membrane lipids and DNA. These ROS accumulate and neurons become more susceptible to excitotoxity damage (Parihar and Hemnani, 2003). It has been reported that Amyloid beta-peptide, plays a central role which causes neuronal dysfunction by release of reactive oxygen species (Butterfield, 2001).The aqueous extract of *Santalum album* showed significant decrease the formation of reactive oxygen species, because the *Santalum album* is having the capacity of anti oxidant activity.

The present data is insufficient to predict the exact constituent(s) responsible for memory enhancing property and further work has to be carried out to determine the constituent(s) responsible for the effect. The exact constituent responsible for the activity is not known. However, the above results due to the presence of biologically active constituents such as Santalol, (90%), Exo-norbicycloekasantolol, β-santalic, teresantalic, nortricycloekasantalic, bicycloekosantalic, β-Santalol, trans β-santalol, α-santalic acid isolated along with 11-keto-dihydro-α-santalic acid and along the trace elements of *Santalum album* extract exhibits the memory enhancing in the amyloid-β induced mice. The above results due to the presence of biologically active compounds along the trace elements of *Santalum album* extract exhibits the memory enhancing in the amyloid-β induced mice.

4.CONCLUSION

In conclusion, the aqueous extract of *Santalum album* has shown a significant memory enhancing property in experimental animals. This confirms the utility use of the plant in Siddha system to increase the memory in brain and supports the claims memory enhancing property of title plant. The plant could be subjected to further studies, which may lead to possibility of isolating the active principle from the plant.

5.ACKNOWLEDGEMENTS

The authors are thankful to Mrs. Anita Prasad, Chairman and Mrs. Archana Swamy, Principal, Gautham College of Pharmacy for providing facilities to carry out this work.

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