African Journal of Cellular Pathology 7:11-16 (2016)
The Official Journal of the Society for Cellular Pathology Scientists of Nigeria
ISSN 2449 - 0776
www.ajcpath.com

COGNITIVE-ENHANCING AND NEUROTHERAPEUTIC PROSPECTS OF VISCUM ALBUM IN EXPERIMENTAL MODEL OF ALZHEIMER'S DISEASE

Oremosu Ademola[1] Edem Edem[1,2], Dosunmu Olufunke[1], Kunlere Oladunni[1]

1. Department of Anatomy, College of Medicine, University of Lagos, Lagos, Nigeria.
2. Department of Anatomy, Afe Babalola University, Ado Ekiti, Ekiti, Nigeria.

Corresponding Author: Edem Edem
Email: edemekpenyongedem@gmail.com

Abstract

Aim: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterised by multiple cognitive deficits, behavioural disorders, mood changes and neurohistological alterations including neurofibrillary degeneration, and development of plaques and tangles of fibrous proteins in the brain. Novel natural antioxidants from plants like Viscum album may offer an effective and safe means of bolstering the body's defence against free radicals and thereby provide protection against Alzheimer's like problems. The present study was designed to investigate the neuroprotective and cognitive enhancing prospects of Viscum album in Alzheimer's disease Model.

Methods: Aluminium chloride (150mg/kg) was administered daily for 21 days significantly; the 'Y' Maze and Novel Object Recognition (NOR) Tests were employed to assess neurobehavioral indices before scarification.

Result: Chronic treatment with Viscum album extract (100mg/kg, orally) for a period of 21days, simultaneously with aluminium chloride administration; and also beginning 10days after $AlCl_3$ administration, significantly attenuated aluminium chloride-induced memory impairment and oxidative damage. Thus, the present study indicates protective and therapeutic prospects of Viscum album against $AlCl_3$ induced cognitive impairment and associated neurodegeneration compared to the control mice ($p < 0.05$).

Conclusion: This novel study demonstrates that Viscum album has neuroprotective, therapeutic, as well as cognitive-enhancing potentials in ameliorating Aluminium Chloride-induced neuropathology.

Key Words: Alzheimer's disease, Viscum album, Neurodegeneration.

INTRODUCTION

Alzheimer's disease (AD), a progressive neurodegenerative disorder characterized by multiple cognitive deficits, is often accompanied by behavioural disorders, by memory loss, language deterioration, poor judgment and impaired visuospatial skill. The prevalence of Alzheimer's disease worldwide is markedly increased and becomes one of the biggest challenges for most societies throughout the world (Cruz & Tsai, 2004). These pathologies are evident in specific, vulnerable brain areas and the hippocampus is one of the earliest to be affected (Braak et al., 1993). At present, no effective treatment completely cures Alzheimer's disease. Most of the treatment can only slow down the progression of the disease. Moreover, most drugs used nowadays are usually targeted at the cholinergic system by suppressing acetyl cholinesterase (AChE) activity. Unfortunately, they usually produce undesired side effects (Zarotsky et al., 2003; Woodruff-Pak et al., 2001; Bickel et al., 1991). They become ineffective with time; thus resulting in further deteriorated cognitive ability of the patient. Viscum album (mistletoe), an idiosyncratic plant surrounded by age-old traditions of ancient cult and magic rites has gained widespread research (Babara & Peter, 1991). The primary chemical constituents have been found to vary according to the host plant but typically include glycoprotein, polypeptides (Viscotoxin), flavonoids, flavonol agylcones,

lectins, triterpenes, saponins, caffeic acid, lignans, choline derivatives related to acetylcholine, vitamin C, histamine, resins, thionins, cardionolids and phenolic compounds (Lyu et al., 2000; Wollenweber et al., 2000; Edlund et al., 2000). A decoction of the leaves of mistletoe is traditionally used in the treatment of a number of ailments like nervousness, diabetes, cancer, epilepsy, including hypertension (Fernandez et al., 1998; Deliorman et al., 2002). It has been observed that methods used by traditionalists in early diagnosis lack scientific basis (Oyebola, 1980). To assess a spectrum of learning and memory functions a battery of tests is needed. These tests are selected to assess behavior and memory. With the increase in the prevalence and incidence of AD and its attendant impact on public health system, series of therapeutic measures have been adopted for its management, yet the quest for further trials is unending. Thus, based on mistletoe's nervine effects and its reputation to serve as brain tonic in traditional folklore and naturopathy, extracts of Viscum album is investigated in this study to assess its potential in mitigating neurodegeneration and memory impairment, characteristic of AD.

MATERIALS AND METHODS

Animals: All experiments were designed in strict adherence to the guidelines of the Afe Babalola University's Animal Ethics Committee. 25 healthy male mice weighing between 35 – 40g were used for this research. They were housed in well-ventilated plastic cages, kept and maintained under laboratory conditions of temperature, humidity and light. At the time of procurement, they weighed between 20 – 25g. The mice were allowed to acclimatize for a period of two weeks and were fed Growers Mash. They were also given tap water at pleasure using water bottles. At the end of two weeks, the mice were weighed and randomly assigned to five different groups, namely: Control, Extract, Alzheimer's Model, Neuroprotection and Therapeutics.

Collection and Preparation of aqueous extract from the leaves of Viscum album (Mistletoe): Fresh, healthy leaves of Viscum album (from orange tree) after identification were shade dried and ground with the help of an electrical grinder to get a free flowing powder. 20g of the coarse powder was dissolved in 20ml of distilled water and left for 24 hours. The mixture was then kept aside for 30mins to allow to infuse. It was then filtered using cheese cloth. The filtrate was kept in electrical oven until it became pasty.

Treatment: The aqueous extract of leaves of Viscumalbum was given orally at the dose of One Hundred milligramme per kilogramme body weight (100mg/kg) for 21 consecutive days.

Preparation of Alzheimer's Disease Model: Induction of Alzheimer's disease was achieved by the oral administration of a high dose of Aluminium Chloride ($AlCl_3$) at one hundred and fifty milligrammes per kilogramme body weight (150mg/kg). The induction was done orally using syringe and canula.

Experimental Design: The animals were randomly divided into five groups of five animals each. The animals' average weight stood at 35g. Namely - **Control Group:** Here, animals received distilled water throughout the course of the experiment; **Extract Group:** Each mouse was administered 100mg/kg of Viscum album daily for 21 days; **A.D Model Group:** Each animal was given 150mg/kg $AlCl_3$ daily for 21 days; **Neuroprotection Group:** 150mg/kg of $AlCl_3$ and 100mg/kg of Viscum album were administered to each of the animals simultaneously daily for 21 days; **Therapeutic Group:** Mice in this Group received 150mg/kg of $AlCl_3$ each for 10 days, and later each of them was administered 100mg/kg of Viscum album for the remaining 11 days.

Behavioural Assessment

After the last administration the mice were subjected to memory test using Novel Object Recognition Test (NOR) and 'Y' Maze Test.

Novel Object Recognition (NOR) Test

The mice were subjected to NOR Test using a 75cm x 50cm x 30 cm transparent box. The mice were acclimatized to the box and the test room 3 days before the test. On the test day the mice were exposed to two identical objects to acclimatize with for three minutes which is termed trial 1 (T1). The mice were then put inside a cage with food and water. One hour later i.e. inter trial interval (ITI), the mice were put back inside the box with one of the object replaced by a novel one for three minutes. The time used in rearing on the old (old time) and new object (new time) was recorded. Animal rearing was measured when the nose of the animal is less than 2cm from the object while sitting on the object is not considered (Li et al., 2013).

'Y' Maze Test

This is done to check the spatial working memory of the mice. A "Y" Maze of 75cm x 25cm x 15cm was constructed. The mice were placed facing the edge and were to make their arm decision. The duration for the test was ten minutes. The percentage alternation was recorded. Visiting the three arms consecutively was termed right decision (right) and visiting one arm twice in three alternation was termed wrong decision (wrong). Percentage alternation was calculated for the mice. % alternation =? The place was cleaned with ethanol soaked cotton wool before placing the next animal.

Sacrification

The animals were sacrificed after 21days by cervical dislocation and the hippocampus was isolated for histological analysis.

Statistical Analyses

Data were analysed using One-way ANOVA with the aid of GraphPad Prism V.5.0 software. The level of statistical significance was put at 95% ($P<0.05$).

RESULTS

NOR Test: For the NOR Test, there was no statistically significant difference ($P>0.05$) between the time spent on old object (O) and new object (N) in Alzheimer's Model Group animals, indicating that there is no memory retention; but the Neuroprotection, Therapeutic, Extract and Control Groups showed statistically significant difference ($P<0.05$).

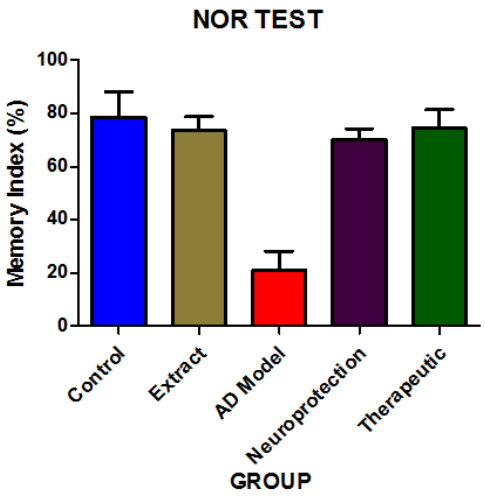


Fig. 1. Graph Showing the Effect of Viscum album (100mg/kg) on Memory Index in aluminium chloride treated mice. Values are the means ± SEM.p < 0.05 as compared to Control Group; p < 0.05 as compared to AD Model Group; p < 0.05 as compared to Therapeutic Group (repeated measures one-way ANOVA followed by Tukey's test for multiple comparisons)

'Y' Maze: Animals in the Neuroprotection and Therapeutic Groups showed statistically significant increase ($P<0.05$) in the memory index compared to the Alzheimer's Model Group. There is statistically significant increase ($P<0.05$) in the right decisions (R) of Neuroprotection, Therapeutic, Extract and Control Groups than the wrong decision (W), meaning there is retention of spatial memory in the Neuroprotection, Therapeutic, Extract and Control Groups. There is statistically significant difference ($P>0.05$) in the percentage alternation of the groups.

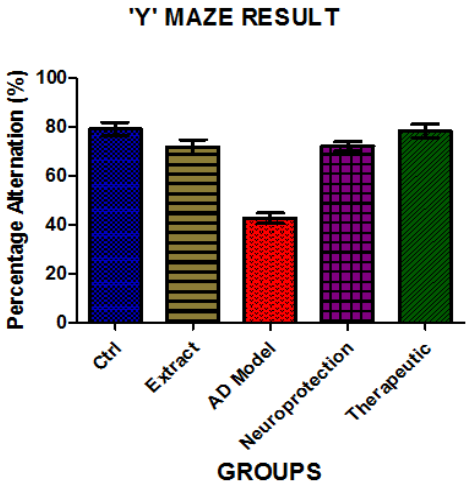
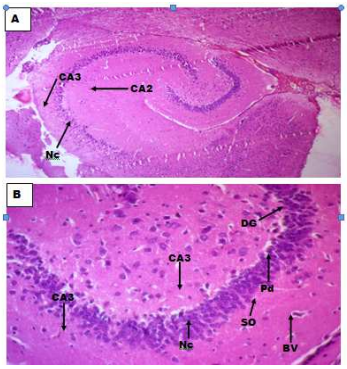
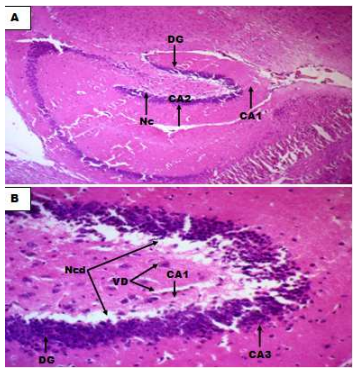


Fig. 2. Graph Showing the Effect of Viscum album (100mg/kg) on Percentage Alternations in aluminium chloride treated mice. Values are the means ± SEM.p < 0.05 as compared to Control Group; p < 0.05 as compared to AD Model Group; p < 0.05 as compared to Therapeutic Group (repeated measures one-way ANOVA followed by Tukey's test for multiple comparisons)

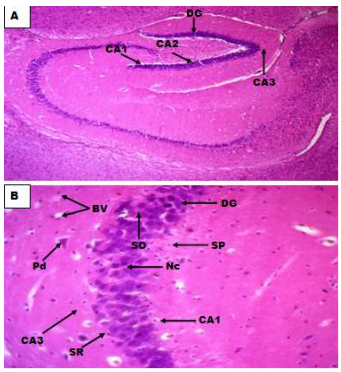
Histological studies: Haematoxylin and Eosin (H&E) staining technique revealed massive cytoarchitectural alterations like; vascular degeneration, neuronal cell degeneration with decrease in neuronal cell density, vacuolation and cellular fragmentation of the CA3 and dentate gyrus, which are the neuropathological changes characteristic of Alzheimer's disease, in the AD Model Group. Hippocampal cytoarchitecture of the Control Group shows that there are normal neuronal cells within distinct layers of strata orien (SO), pyramidal (SP) and radiatum (SR) containing cornu ammonis (CA1-3), the dentate gyrus is intact and well stained, no cellular abnormality seen. Viscum album administration shows moderated neuropathological changes and varying degree of cellular restorations. The extract reveals active recovery effect and restoration of the neuronal cells, and improvement in decreased neuronal density and population, brought by the neurotoxicity of $AlCl_3$.



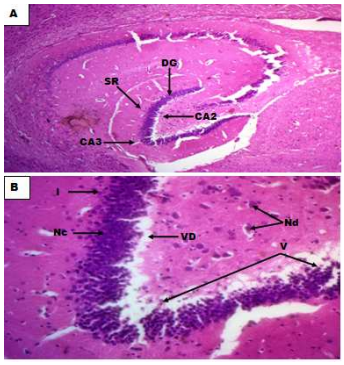
Photomicrographs of Control hippocampus at magnification A (x100) and B (x400) stained with H and E Technique revealed normal area neuronal cells within distinct layers of stratum orien ***(SO)****, pyramidal* ***(SP)*** *and radiatum* ***(SR)*** *containing cornu ammonis (CA1-3), the dentate gyrus is intact and well stained, no cellular abnormality seen.*



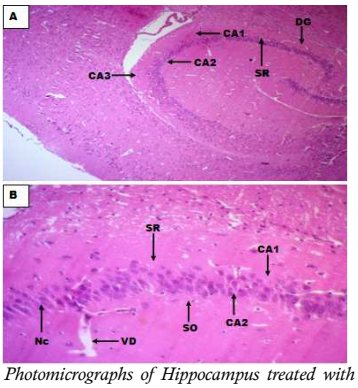
Photomicrographs of Hippocampus treated with 150mg/kg of $AlCl_3$ alone for 21days at magnification A(x100) and B(x400) stained with H and E Technique revealed vascular degeneration, neuronal cell degeneration with decrease in neuronal cell density, vacuolation and cellular fragmentation of the CA3 and dentate gyrus.



Photomicrographs of Hippocampus treated with 100mg/kg of ***Viscum album*** *alone for 21days at magnification C (x100) and D(x400) stained with H and E technique revealed distinct cellular profile CA1-3 neuronal density and prominent pyramidal cells with minimal cellular distortion.*



Photomicrographs of Hippocampus treated with ***150mg/kg of $AlCl_3$ + 100mg/kg of Viscum album*** *simultaneously for 21days at magnification C(x100) and D(x400) stained with H and E technique revealed reactive neuronal cell undergoing recovery from cellular damage.*



Photomicrographs of Hippocampus treated with ***150mg/kg of AlCl3 for 10days and later treated 100mg/kg of Viscum albumfor 11 days*** *at magnification C(x100) and D(x400) stained with H and E technique revealed active recovery effect and restoration of the neuronal cells, decrease in neuronal density and population.*

DISCUSSION

The adult brain is extremely vulnerable to various insults. The recent discovery of neural progenitors in adult mammals, however, raises the possibility of repairing damaged tissues by recruiting their latent regenerative potential. Nakatomi and Kuriu (2002) in their work, Regeneration of Hippocampal Neurons after Ischemic Brain Injury by Recruitment of Endogenous Neural Progenitor Cells, show that activation of endogenous progenitors leads to massive regeneration of hippocampal pyramidal neurons after ischemic injury. Endogenous progenitors proliferate in response to ischemia and subsequently migrate into the hippocampus to regenerate new neurons. Intraventricular infusion of growth factor markedly augments these responses, thereby increasing the number of newborn neurons. The studies suggest that regenerated neurons are integrated into existing brain circuitry and contribute to ameliorating neurological deficit (Nakatomi & Kuriu, 2002). Oxidative damage occasioned by aluminium produced the neuropathological alterations shown above (Mattson et al., 2004). In the present study, the effects of chronic exposure to aluminium were investigated to describe the associated behavioural and brain modifications. Neurodegenerative disease is characterized by progressive pathological changes in the brain that translate into clinical signs of decline in cognitive abilities (memory), functional abilities, mood, behaviour, and finally physical changes. The pathological changes in the Alzheimer's brain include deterioration and loss of neurons (nerve cells) leading to brain atrophy (Exley, 2005). Antioxidants cooperate with the body enzymes to protect the brain from free radical damage (Xu et al., 2006). Viscum album (Mistletoe) is a semi-parasitic plant with apparent broad spectrum of therapeutic actions (Hutt et al., 2001; Eno et al., 2004). Following its wide use and abundance in Britain, it is commonly known as "European

mistletoe". In Nigeria, dried mistletoe leaves are being sold in sachets to be taken as tea in order to provide preventive therapy (Eno et al., 2004). A decoction of the leaves of mistletoe is traditionally used in the treatment of a number of ailments like nervousness, diabetes, cancer, epilepsy, including hypertension (Fernandez et al., 1998; Duke, 1985). Also it is a potent antioxidant with low or no side effects; it increases antioxidant enzymes, hypoglycemic, antibacterial and antifungal properties. Viscum album has been claimed to be antidiabetic (Obatomi et al., 1999), immunomodulatory (Solar et al., 1998), bacteriostatic (Fulder, 1998), antihypertensive and reduces cholesterol level (Nkanu et al., 2002) and therapeutic values for many other ailments. In this investigation, the effect of Viscum album with over load of aluminum chloride to mice led to reduction of neurotoxicity and Alzheimer's disease appeared as shrunken decreasing of pyramidal cells. These brain moderation changes brought about by Viscum album against aluminum chloride administration were due to reducing oxidative damage which contribute to disease pathogenesis and were in accordance with the aim of this study which is to prove the protective effect of Viscum album against neurotoxicity, learning and memory. There is no animal model available at the moment that can mimic all the cognitive, behavioral and histopathological abnormalities observed in patient with Alzheimer disease (Yamada & Nabeshima, 2000). The present histopathological study of chronic exposure shows obvious difference between the treated poisoned groups (Neuroptotective and Therapeutic Groups) and the poisoned, aluminium neurotoxicity (Alzheimer's Disease Model Group). Mice were poisoned with aluminium chloride ($AlCl_3$) orally at 150mg/kg/day, another group poisoned with the same manner but treated with Viscum album orally (100mg/kg/day) for 21days. The animals of the Control Group received drinking water only during this period; yet another group (Extract Group) received Viscum album (100mg/kg) only for the 21days. Results showed that Viscum album moderated the aluminium effect on exploratory activity/curiosity of treated intoxicated animals (as shown in the NOR Test). The assessment of animal memory using different types of mazes has been used in neurosciences (Exley, 2005). Based on these results, it seems likely that an enhancement in exploratory behavior and general neurocognition in treated poisoned mice during the 'Y' Maze and Novel Object Recognition Tests may reflect the less anxiety response of an animal to an unfamiliar environment. These results show the willingness to explore new environments ('Y' Maze) and the capacity to recognize new objects [Novel Object Recognition (NOR) Test]. The above observations were important for the evaluation of the cognitive tests since impaired physiological functions or changed motor performance may conflict with learning and memory tests (Borsini & Meli, 1998). Most learning paradigms that require configural associations require a fully functioning hippocampus; however learning paradigms that can be solved using only elemental associations can be solved without input from this structure (Yamada & Nabeshima, 2000).

Conclusion

Viscum album has great neuroprotective, therapeutic, as well as cognitive enhancing potentials in Aluminium Chloride-induced Alzheimer's disease model. However, further cellular and molecular studies are required to fully understand the effect and mechanisms of action of Viscum album on neurotoxicity associated with dementia in different experimental systems.

REFERENCES

Babara I, Peter T (1992). The family herbal healing, Young, A. B (ed). Art Press Rochaster Vermont. P. 80

Bickel U, Thomsen T, Weber W, Fischer JP, Bachus R, et al (1991). Pharmacokinetics of galanthamine in humans and corresponding cholinesterase inhibition. Clin. Pharmacol. Ther., 50:420-428.

Borsini F, Meli A (1998). Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology. 94: 147-160.

Braak H, Braak E, Bohl J (1993). Staging of Alzheimer-realted cortical destruction. Eur. Neurol. 33, 403-408.

Cruz JC, Tsai LH (2004). Cdk5 deregulation in the pathogenesis of Alzheimer's disease. Trends Mol. Med. 10, 452-458.

Deliorman D, Calis T, Ergun F, Dogan B, Buharalioghu C, Kanzik K (2002). Studies on the vascular effects of the fractions and phenolic compounds isolated from Viscum album . J Ethnopharmacol. 72:323-39.

Duke JA (1985). Mistletoe. In: Handbook of Medicinal Herbs. CRC Press: Florida, pp: 512-513

Edlund U, Hensel A, Frose D, Pfuller U, Scheffler A (2000). Polysaccharides from fresh Viscum album L. berry extract and their Interaction with Viscum album aggl. I. Arzneimi Helforschung. 50:645-51.

Eno AE, Ibokette UE, Ofem OE, Umoh FB, Nkanu E, Azah N, et al (2004). The effect of a Nigerian species Viscum album (Mistletoe) leaf extract on the blood pressure of normotensive and DOCA-induced hypertension rats. Nig. J. Physiol. Sc. 19: 33-40.

Exley C (2005). The aluminium-amyloid cascade hypothesis and Alzheimer disease. Subcell Biochem. 38: 225-234.

Fernandez T, Wagner M, Varela B, Ricco R, Hajos S, Gurni A (1998). Study of an Argentine Mistletoe, the hemiparasite. Ligaria cuneifolia (R. et. R). Tiegh (Loranthaceae). J Ethnopharmacol. 62:25-34.

Fulder S (1998). Complementary medicine. Clin. Pharmac. and Herbal Med. 4: 12-2

Hirofumi N., Toshihiko K., Shigeo O., Shin-ichi Y, Osamu H, Nobutaka K (2002). Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. Cell. 110(4):429-41.

Hutt N, Kopferschmitt-kubler M, Cabalion J, Purohit A, Alt M, Pauli G (2001). Anaphylactic reactions after therapeutic injection of mistletoe (Viscum album L.). Allergal Immunopathol. (Med.). 29:201-203.

Li W, Yu J, Liu Y, Huang X, Abumaria N, Zhu Y, et al (2003). Elevation of Brain Magnesium Prevents and Reverses Cognitive Deficits and Synaptic Loss in Alzheimer's Disease Mouse Model. The Journal of Neuroscience. 33(19):8423–8441

Lyu S, Park S, Cheng B, Park W (2000). Comparative study of Korean (Viscum album var. coloratum) and European mistletoe (Viscum album L.) Arch Pharmacol Res. 23:590-8.

Mattson M, Duan W, Wan R, Guo Z (2004). Prophylactic activation of neuroprotective stress response pathways by dietary and behavioural manipulations, NeuroPx. 1(1):111-6.

Nakatomi H, Kuriu T, Okabe S, Yamamoto S, Hatano O, Kawahara N (2002). Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. Cell. 110(4):429-41.

Nkanu RE, Umoh FB, Ofem DT, Eno AE (2002). The effect of aqueous extract from the leaves of Viscum album on blood glucose level and lipid profile in male Wistar rats. Nig. J. physiol. Sci. 17 (1-2): 108-112.

Obatomi DK, Bikomo EO, Temple VJ (1999). Antidiabetic properties of African mistletoe in streptozotocin induced diabetic rats. J. Ethnopharmacol. 43: 13-17.

Oyebola DO (1980). Definition, classification and description of muscle in clinical medicine. (Vol. 7) Center, D. (ed). Edinburgh, Churchill Livingstone. pp. 280 – 282.

Pamplona-Roger CH (1999). Medicinal Plants: Encyclopedia of Medicinal Plants. Sateliz Publication, Spain. pp: 5-247.

Solar MH, Stoeva S, Voelter W (1998). Complete amino acid sequence of the beta chain of mistletoe lectin. Biochem. Biophys. Res Com. 246: 596-601.

Sweeney JA, Wetzler S, Stokes P, Kocsis, J (1989). Cognitive functioning in depression. J. Clin. Psychol., 45: 836-42.

Wollenweber E, Wieland A, Haask A (2000). Epicutical wax and flavonol aglycones of the European Mistletoe. Viscum album L. Z. Naturforsch. 55:311-7.

Woodruff-Pak DS, Vogel III RW, Wenk GL (2001). Galanthamine: Effect on nicotinic receptor binding, acetylcholinesterease inhibition, and learning. Proc. Natl. Acad. Sci. 98: 2089-2094.

Xu Y, Ku B, Tie L, Yao H, Jiang W (2006). Curcumin reverses the effects of chronic stress on behavior, the HPA axis, BDNF expression and phosphorylation of CREB. Brain Res. 1122: 56-64.

Yamada K, Nabeshima T (2000). Animal models of Alzheimer's disease and evaluation of anti-dementia drugs. Pharmacol Ther 88: 93-113.

Zarotsky V, Sramek JJ, Cutler NR (2003). Galanthamine hydrobromide: an agent for Alzheimer's disease. Am J Health-System Pharmacist. 60: 446-452.