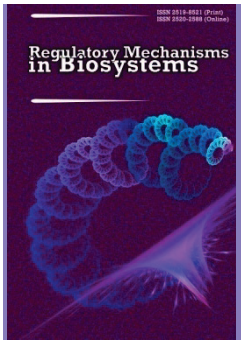

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Long term effect of fluoxetine and memantine on biochemical markers of Alzheimer's disease in scopolamine-induced mice

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Alzheimer's disease is a chronic neurological illness that causes considerable cognitive impairment. However, there is no effective treatment for this disease. Therefore, the current study aimed at investigating the long-term effects of fluoxetine and memantine on biochemical markers of Alzheimer's disease in scopolamine-induced mice. In this study, adult female mice were divided into four equal groups; normal control received distilled water only, the untreated Alzheimer's disease group received scopolamine intraperitoneal IP/SCM for 14 days, following which distilled water was given for six months, the memantine-treated Alzheimer's disease group received IP/SCM for 14 days then memantine hydrochloride for 6 months, the fluoxetine-treated Alzheimer's disease group received IP/SCM for 14 days then fluoxetine hydrochloride for 6 months. The results show that after 2 weeks of induction the mean level of amyloid β and MDA were significantly elevated, while the mean level of BDNF and TAS were significantly reduced in comparison with the normal control group. After 3 months, both treatments (memantine and fluoxetine) caused a highly significant decrease in the mean levels of amyloid β and malondialdehyde as well as an increase in the mean levels of brain derived neurotrophic factor and total antioxidant status in Alzheimer's disease treated mice in comparison with Alzheimer's disease untreated mice. However, after 6 months of treatment, the effects of fluoxetine were more significant than those of memantine. In conclusion, fluoxetine has significant effects on biochemical markers of Alzheimer's disease and these effects are time-dependent as well as more significant than those of memantine, which suggests the potential usefulness of the former in treatment of this disease.

Keywords: amyloid β; antioxidant; brain derived neurotrophic factor; neuronal plasticity; oxidative stress.

Introduction

Alzheimer's disease (AD) is the most prevalent cause of dementia; it affects at least 27 million individuals worldwide and accounts for 60–70% of all dementia cases (Silva et al., 2019). The main clinical manifestations of this condition are cognitive deficits, progressive memory impairment, and behavioral disorders, which greatly decrease patients' quality of life and raise socioeconomic burdens (Ibrahim et al., 2019). The hippocampus and cerebral cortex exhibit the histopathological signs of AD, which include extracellular amyloid beta (Aβ) plaque buildup and intracellular neurofibrillary tangles (NFTs) made of hyperphosphorylated tau (p-tau). However, the pathophysiology of AD is still obscure despite the existence of numerous theories, such as Aβ pathology, tau pathology, mitochondrial dysfunction, oxidative stress, neuroinflammation, and/or neurotransmitter abnormalities (Zhou et al., 2019).

Additionally, recent studies demonstrated an association between the pathophysiology of AD and brain-derived neurotrophic factor (BDNF) down-regulation, which is crucial for neuronal survival and differentiation (Tanila, 2017; Numakawa et al., 2018). As seen *in vivo* and *in vitro* models of acute (stroke, trauma, and seizures) and chronic (Alzheimer's and Parkinson's diseases) neurodegenerative diseases, neurotrophic factors such as nerve growth factor (NGF) and (BDNF) are capable of preventing neuronal death (Sechi et al., 2015).

It is hypothesized that in AD patients, increased presynaptic glutamate release leads to N-Methyl-D-aspartate receptor (NMDAR) over-activation, which overloads postsynaptic neurons with Ca^{2+} and causes excitotoxicity, followed by desensitization and internalization of NMDA/AMPA and resultant synaptic depression and dysfunction (Zhou et al., 2019). Memantine, a non-competitive NMDAR antagonist that has been licensed for the treatment of moderate-to-severe AD to enhance learning and memory, is one of these five medications (Qiao et al., 2021). Memantine has its effects on reducing Aβ deposition, tau hyperphosphorylation, oxidative stress, and increasing BDNF expression (Alawdi et al., 2017; Qiao et al., 2021; Anoush et al., 2022).

Previous studies concluded that AD and depression coexist in the same patient in a synergistic manner (Mdawa et al., 2020). Also, it has been shown that in the median and dorsal raphe nuclei of AD patients, there are substantial declines in 5-hydroxytryptamine (5-HT) levels, together with abundant localization of Aβ deposits. Moreover, cortical 5-HT levels showed an inverse correlation with the amount of NFTs, suggesting that the development of the disease could be linked to serotonergic system dysfunction (Ibrahim et al., 2019). In addition, the 5-HT1, 5-HT4, 5-HT6, and 5-HT7 receptor classes are of special interest for cognitive enhancement (Mdawa et al., 2020). According to data from pre-clinical investigations, particular ligands for these subtypes of receptors seem to promote neurogenesis and neuronal plasticity as well as reduce amyloid accumulation in the brain in addition to increasing cholinergic neurotransmission (Shahidi et al., 2018). These earlier studies have shown that the serotonergic pathway is significantly impaired in the AD brain, and it is speculated that the anti-depressant selective serotonin reuptake inhibitors could offer a new targeted treatment for cognitive diseases like AD (Ibrahim et al., 2019). Taken together, the current study aimed to investigate the possible long-term effects of fluoxetine in comparison with memantine on biochemical biomarkers (such as amyloid β deposition, BDNF level, and oxidative stress) in scopolamine-induced Alzheimer's disease mice.

Methods

The present study received approval from the Research Ethics Committee at the Department of Pharmacology/College of Medicine, University of Baghdad, Baghdad, Iraq, with reference number (Pharmacomed) in UVB 23.13. This experimental study was conducted at the College of Veterinary Medicine, University of Kirkuk, and the Department of Pharmacology, College of Medicine, University of Baghdad, during the period from 1st, July 2022 to 1st, June 2023.

The current study involved one hundred and forty adult female mice (4–8 weeks old) weighing (20–25 g),which were purchased from the Al-Razi Center, Ministry of Industry and Minerals, Baghdad, Iraq. The mice were kept in the experimental area for 2 weeks for the habituation phase and housed as ten mice per cage at an appropriate temperature (25 °C) and humidity (30 ± 10%), with a standard 12-hour light/dark cycle and free access to water and standard food (high protein feed and milk powder).

At the beginning, twenty adult mice were included in a pilot study to detect the effective dose and duration of treatment for scopolamine intraperitoneal IP/SCM to induce Alzheimer disease (AD) in adult mice. The results of the pilot study showed that IP/SCM (1 mg/kg) for 14 days induced AD effectively. Then, one hundred and twenty adult mice were divided into 4 equal groups; the first group was the normal group, which received only distilled water during the total period of the study and was considered the control group, the second one was the induction untreated Alzheimer group, which received IP/SCM (1 mg/kg) for 14 days only; after that distilled water was given for the next 6 months, the third group was treated with IP/SCM (1 mg/kg) for 14 days; then, memantine hydrochloride (2.6 mg/kg/day orally) was given for the next 6 months; and the fourth group was treated with IP/SCM (1 mg/kg) for 14 days; after that, fluoxetine hydrochloride (2.6 mg/kg/day orally) was given for the next 6 months. The doses of both drugs (fluoxetine hydrochloride and memantine hydrochloride) employed were based upon the human dose after conversion to that of mice, according to Paget & Barnes (1964) conversion tables.

Mice were anesthetized and scarified in order to isolate brain tissue for further homogenization and ELISA analyses of biochemical markers such as BDNF, TAS, MDA, and 1-42 β-amyloid peptide (YLbiont®, Shanghai). This procedure was repeated 3 and 6 months after induction.

Computer feeding is conducted by the prepared computer program SigmaPlot, version 11 (Systat Software, Inc., 2008). Data were analyzed using unpaired t-tests, and ANOVA (Analysis of Variance) was used to identify the variation in the different variables in relation to the duration of the study. Tukey's post hoc test was used to identify groups responsible for statistical difference through comparison. All values expressed as mean ± standard deviation (x ± SD) and P value < 0.05 was considered significant (Kirkwood, 1988).

Results

Effects of scopolamine on the brain of mice. The current study indicated that there was a highly significant elevation in the mean level of amyloid β (P < 0.001) and MDA (P = 0.003), while there was a significant reduction in the mean level of BDNF (P = 0.006) and TAS (P < 0.001) in the AD induction group in comparison with the normal control group (Table 1).

Table 1
Comparison of mean levels of biochemical markers between AD induction groups and normal group at zero time (x ± SD, n = 6)

Marker	Normal group at zero time	Induction group after 2 weeks
BDNF, ng/mL	3.06 ± 0.97	0.69 ± 0.14*
Amyloid *β*, ug/L	9.93 ± 0.57	57.23 ± 8.55**
TAS, pg/mL	35.2 ± 3.7	10.1 ± 2.9**
MDA, nmol/mL	0.621 ± 0.018	2.764 ± 0.344*

Note: * – P < 0.01, ** – P < 0.001 between the control and induction groups by unpaired t-tests, SD – standard deviation, BDNF – brain derived neurotrophic factor, TAS – total antioxidant status, MDA – malondialdehyde.

The effect of 3-month treatment with fluoxetine or memantine on AD brain tissue of mice. Table 2 showed that there was a statistically significant elevation in the mean level of amyloid β and MDA (P < 0.001) and a reduction in the mean level of BDNF and TAS (P < 0.001) in the AD induction group when compared to those of the control group after 3 months.

In addition, the treatment with fluoxetine and memantine for 3 months caused a highly significant (P < 0.001) decrease in the mean levels of amyloid β and MDA, yet an increase in the mean levels of TAS (P < 0.001). The BDNF level was also statistically higher (P < 0.01) in the fluoxetine-treated group but not in the memantine-treated group in comparison with the AD induction group after 3 months, but there was a statistically significant variation (P < 0.05) in the mean level of each of the biochemical markers between the normal control group and the fluoxetine-, and memantine-treated groups.

Furthermore, there were no statistically significant differences in the mean levels of each of the biochemical markers between the fluoxetine-treated and memantine-treated groups after 3 months of therapy.

Table 2
Comparison of the effects of a 3-month treatment with memantine and fluoxetine individually on biochemical markers among the four studied groups (x ± SD, n = 6)

Marker	Normal group after 3 months	Induction group after 3 months	Memantine treated group after 3 months	Fluoxetine treated group after 3 months
BDNF, ng/mL	4.09 ± 1.13^{a}	0.62 ± 0.21^{b}	1.42 ± 0.35^{c}	1.94 ± 0.45^{c}
Amyloid *β*, µg/L	12.2 ± 1.7^{a}	61.8 ± 10.1^{b}	29.5 ± 3.5^{c}	30.7 ± 6.6^{c}
TAS, pg/mL	37.378 ± 1.020^{a}	11.3 ± 1.0^{b}	24.5 ± 6.1^{c}	23.5 ± 2.3^{c}
MDA, nmol/mL	0.613 ± 0.05^{a}	2.56 ± 0.38^{b}	1.43 ± 0.36^{c}	1.17 ± 0.32^{c}

Note: the use of ANOVA with Tukey's post hoc test gives a specific significance value for the differences; comparison in terms of one item in a row and different letters indicate statistical samples that are significantly different from each other; BDNF – brain derived neurotrophic factor, TAS – total antioxidant status, MDA – malondialdehyde.

The effect of 6-month treatment with fluoxetine and memantine individually on AD brain tissue. Table 3, showed that there was a statistically highly significant elevation in the mean level of amyloid β and MDA (P < 0.001) and a reduction in the mean level of BDNF and TAS (P < 0.001) in the AD induction group when compared to that of the normal control group after 6 months.

In addition, treatment with fluoxetine and memantine for 6 months caused a highly significant (P < 0.001) decrease in the mean level of amyloid β, and MDA while increasing the mean level of BDNF and TAS (P < 0.001) in comparison with the AD induction group after 6 months.

In addition, there was high statistical variation in the mean level of BDNF and TAS in the normal control group when compared with 6 months of therapy with fluoxetine or memantine. Moreover, the level of amyloid β and MDA in the memantine-treated group was significantly higher than the normal control group after 6 months, while the level of amyloid β and MDA in the fluoxetine-treated group after 6 months was comparable (non-significant differences) to the normal control group.

Furthermore, there was a statistically significant high mean level of BDNF and TAS in the fluoxetine-treated group when compared with the memantine-treated group after 6 months of therapy. However, the mean level of Amyloid β was statistically lower in the fluoxetine-treated group than the memantine-treated group, while there were non-significant differences in MDA levels between the fluoxetine and memantine-treated groups after 6 months of therapy.

Table 3
Comparison of biochemical markers among the four studied groups after 6 months of treatment (x ± SD, n = 6)

Marker	Normal group after 6 months	Induction group after 6 months	Memantine treated group after 6 months	Fluoxetine treated group after 6 months
BDNF, ng/mL	4.03 ± 1.13^{a}	0.64 ± 0.16^{b}	1.57 ± 0.44^{c}	2.55 ± 0.80^{d}
Amyloid β, µg/L	11.7 ± 1.8^{a}	49.1 ± 11.3^{b}	30.5 ± 4.3^{c}	16.2 ± 3.9^{ad}
TAS, pg/mL	41.9 ± 11.0^{a}	9.5 ± 3.1^{b}	20.9 ± 2.7^{c}	29.6 ± 3.6^{d}
MDA, nmol/mL	0.59 ± 0.12^{a}	3.01 ± 0.76^{b}	1.33 ± 0.19^{c}	0.85 ± 0.27^{ac}

Note: see Table 2.

Discussion

Effects of scopolamine on brain of mice. According to the current study, SCM injections increased brain Aβ deposition and oxidative stress while decreasing BDNF levels. There are very few studies that assess the effect of SCM on Aβ concentration. For example, Aykac et al. (2019) found that administering SCM (1 mg/kg) IP for 14 days dramatically raised MDA levels, lowered glutathione (GSH) levels, decreased BDNF expression, and lowered short- and long-term memory. In addition, Anand et al. (2022) concluded that SCM single injection (2 mg/kg) increased oxidative stress in the hippocampus by increasing thiobarbituric acid-reactive substances, which reflect lipid peroxidation, and decreased GSH as well as catalase (CAT) levels.

However, a study done by Ban et al. (2020) found that CAT activity was unaffected after SCM injection in mice compared to control mice. Additionally, Lee et al. (2010) found that SCM significantly increased superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) antioxidant enzyme activities.

Despite that, it is well-known that the non-selective muscarinic acetylcholine receptor antagonist scopolamine (SCM) prevents cholinergic signals from traveling through the brain, resulting in dysregulation of cholinergic activity and increased activity of acetylcholinesterase in the hippocampus, which interferes with mouse learning and memory functions (Maurer & Williams, 2017; Lee et al., 2018; Yadang et al., 2020). Moreover, there is a relationship between Aβ and oxidative stress, because prooxidants elevated Aβ formation and Aβ generates oxidative stress (Resende et al., 2008; Janssen et al., 2016).

The effect of 3-month treatment with fluoxetine and memantine individually in AD brain tissue. Data obtained from the present study demonstrated beneficial effects of the administration of fluoxetine and memantine for 3 months by inhibiting neuronal and synaptic loss, reducing lipid peroxidation, as well as increasing neuronal plasticity and neurogenesis.

A study by Jadhav & Kulkarni (2023), indicated that $AlCl_3$-induced AD rats treated with memantine for 42 days showed improvement in memory and cognitive function. Also, histological analysis showed mild Aβ-deposition, mild neuronal degeneration in the hippocampus, and increased expression of BDNF. However, the study found that memantine produced non-significant changes in antioxidant enzyme activity and MDA levels. Also, previous studies concluded that APP/PS1 mice treated with memantine for 4 weeks displayed improved spatial memory that tended to be normal, increased density of spines related to synaptic function, and increased antioxidant enzyme activity with a decrease in MDA level (Martinez-Coria et al., 2010; Alawdi et al., 2017; Qiao et al., 2021).

Memantine blocks the NMDAR and does not leave it in the presence of tonic pathological over-activation of the receptor, while permitting the transmission of transient physiological signals important for memory and learning processes because the NMDAR plays a crucial role in the synaptic transmission and synaptic plasticity thought to underlie learning and memory (Liu et al., 2019). Furthermore, memantine blocks NMDA and 5-HT3 receptors simultaneously. These combined antagonistic activities have beneficial synergistic effects that are linked to treatment safety and effectiveness in AD by improving mental capacity (Parsons et al., 2008; Shelat et al., 2008; Bordji et al., 2010; Liu et al., 2019).

Regarding the impact of fluoxetine therapy for 3 months on AD, recent studies (Abu-Elfotuh et al., 2021; Abu-Elfotuh et al., 2022) observed that 4–5 weeks' treatment with fluoxetine of $AlCl_3$-induced AD rats improved learning and memory performance, decreased oxidative stress by elevation of SOD, total antioxidant capacity (TAC) level, and decreased MDA level, as well as reduced levels of Aβ and tau protein while increasing BDNF level in comparison with AD rats.

Moreover, it was concluded that 3xTg-AD mice treated with fluoxetine for 4 months showed improved short- and long-term memory, decreased levels of Aβ, enhanced APP's non-amyloidogenic process, and increased levels of BDNF in the hippocampus when compared to 3xTg-AD mice (Qiao et al., 2015; Jin et al., 2017; Huang et al., 2018).

Fluoxetine acts by increasing extracellular serotonin (5-HT) levels in different brain regions, such as the frontal cortex, striatum, diencephalon, or hippocampus (Perez-Caballero et al., 2014). This resulted in the G protein-coupled receptors being activated, adenyl cyclase being stimulated, and ultimately the cAMP cascade being up regulated. As a result of this cascade, cAMP-related element-binding protein (CREB) and BDNF expression levels rise, cAMP-dependent protein kinase (PKA) levels rise, and synaptic plasticity is enhanced (Aboukhatwa et al., 2010). Fluoxetine stimulates CREB phosphorylation, which controls the expression of BDNF, a downstream gene of CREB (Jin et al., 2017). In addition, acute and long-term fluoxetine therapies, increase the phosphorylation of the BDNF receptor, tropomyosin-related kinase B (TrkB) (Perez-Caballero et al., 2014). BDNF was bound up with neural survival, development, maturation, differentiation, and mature neural functioning. Therefore, stimulation of the CREB/pCREB/BDNF signaling pathway was thought to be the primary molecular mechanism of fluoxetine's therapeutic effects on improving cognition and memory function in AD (Jin et al., 2017).

Additionally, fluoxetine inhibits proinflammatory gene expression, which leads to inhibition of reactive oxygen species (ROS) production and enhanced antioxidant activity (Karmakar & La, 2021). As there is a correlation between Aβ level and oxidative stress so, this effect may decrease Aβ (Resende et al., 2008).

The effect of 6-month treatment with fluoxetine and memantine individually in AD brain tissue. In this study, treatment with fluoxetine and memantine for 6 months produced an antioxidant effect, decreased lipid peroxidation, inhibited synaptic and neuronal loss, as well as enhanced neurogenesis and synaptic plasticity.

It has been stated that long-term administration of memantine (10 and 20 mg/kg/day for 6 months) to Tg2576 AD mice was associated with a significant decrease in Aβ plaque deposition, increased synaptic density, and a lowered appearance of degenerating axons (Dong et al., 2008, Al-Hussaniy et al., 2023). However, there is a previous study that showed that 10 months' administration of fluoxetine for 3xTg AD mice produced no improvement in spatial learning and memory, as well as no change in the level of BDNF, Aβ accumulation, tau hyperphosphorylation, and neurogenesis.

However, no previous research has been done to assess the effect of long-term treatment with fluoxetine and memantine on oxidative stress in AD mice. The latter is necessary to achieve a therapeutic impact as it elevates 5-HT levels over time in the diencephalon, striatum, hippocampus, and frontal cortex but does not affect the levels of cortical noradrenaline and dopamine. These effects might be explained by the desensitization of terminal 5-HT1B auto-receptors, and of raphe somatodendritic and 5-HT1A auto-receptors, whose activation produces negative feedback on 5-HT release. Also, downregulation of these receptors increases expression of CREB in the hippocampus. Moreover, serotonin and its metabolites (N-acetyl serotonin and melatonin) are known to decrease the generation of nitric oxide in human macrophage cells due to their capacity to scavenge ROS (Perez-Caballero et al., 2014; Karmakar & La, 2021).

Conclusion

Fluoxetine and memantine may produce neuroprotection and enhance neurogenesis with synaptic plasticity as well as decrease neuronal loss through their antioxidant ability, decreasing lipid peroxidation, increasing

BDNF level in mice with scopolamine-induced AD with comparable efficacy after 3 months of treatment. Furthermore, the efficacy of fluoxetine is time-dependent as treatment for 6 months with fluoxetine can increase its efficacy over that of memantine.

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The authors declare no conflict of interest.

References

Aboukhatwa, M., Dosanjh, L., & Luo, Y. (2010). Antidepressants are a rational complementary therapy for the treatment of Alzheimer's disease. Molecular Neurodegeneration, 5(1), 10.

Abu-Elfotuh, K., Al-Najjar, A. H., Mohammed, A. A., Aboutaleb, A. S., & Badawi, G. A. (2022). Fluoxetine ameliorates Alzheimer's disease progression and prevents the exacerbation of cardiovascular dysfunction of socially isolated depressed rats through activation of Nrf2/HO-1 and hindering TLR4/NLRP3 inflammasome signaling pathway. International Immunopharmacology, 104, 108488.

Abu-Elfotuh, K., Ragab, G. M., Salahuddin, A., Jamil, L., & Abd Al Haleem, E. N. (2021). Attenuative effects of fluoxetine and *Triticum aestivum* against aluminum-induced Alzheimer's disease in rats: The possible consequences on heaptotoxicity and nephrotoxicity. Molecules, 26(21), 6752.

Alawdi, S. H., El-Denshary, E. S., Safar, M. M., Eidi, H., David, M. O., & Abdel-Wahhab, M. A. (2017). Neuroprotective effect of nanodiamond in Alzheimer's disease rat model: A pivotal role for modulating NF-κB and STAT3 signaling. Molecular Neurobiology, 54, 1906–1918.

Al-Hussaniy, H. A., Alkhafaje, Z., Altamimi, Z. S., Oraibi, A. I., Abdalhassan, A. H., Abdulhamza, H. M., & Al-Zobaidy, M. J. (2023). Memantine and its role in parkinsonism, seizure, depression, migraine headache, and Alzheimer's disease. Pharmacia, 70(2), 291–297.

Anand, A., Khurana, N., Ali, N., AlAsmari, A. F., Alharbi, M., Waseem, M., & Sharma, N. (2022). Ameliorative effect of vanillin on scopolamine-induced dementia-like cognitive impairment in a mouse model. Frontiers in Neuroscience, 16, 1005972.

Anoush, M., Pourmansouri, Z., Javadi, R., GhorbanPour, B., Sharafi, A., Mohamadpour, H., Anarkooli, I. J., & Andalib, S. (2022). Clavulanic acid: A novel potential agent in prevention and treatment of scopolamine-induced Alzheimer's disease. ACS Omega, 7(16), 13861–13869.

Aykac, A., Ozbeyli, D., Uncu, M., Ertaş, B., Kılınc, O., Şen, A., Orun, O., & Sener, G. (2019). Evaluation of the protective effect of *Myrtus communis* in scopolamine-induced Alzheimer model through cholinergic receptors. Gene, 689, 194–201.

Ban, J. Y., Park, H. K., & Kim, S. K. (2020). Effect of glycyrrhizic acid on scopolamine-induced cognitive impairment in mice. International Neurourology Journal, 24(Suppl 1), S48.

Bordji, K., Becerril-Ortega, J., Nicole, O., & Buisson, A. (2010). Activation of extrasynaptic, but not synaptic, NMDA receptors modifies amyloid precursor protein expression pattern and increases amyloid-β production. Journal of Neuroscience, 30(47), 15927–15942.

Dong, H., Yuede, C. M., Coughlan, C., Lewis, B., & Csernansky, J. G. (2008). Effects of memantine on neuronal structure and conditioned fear in the Tg2576 mouse model of Alzheimer's disease. Neuropsychopharmacology, 33(13), 3226–3236.

Huang, M., Liang, Y., Chen, H., Xu, B., Chai, C., & Xing, P. (2018). The role of fluoxetine in activating Wnt/β-catenin signaling and repressing β-amyloid production in an Alzheimer mouse model. Frontiers in Aging Neuroscience, 10, 164.

Ibrahim, W. W., Abdelkader, N. F., Ismail, H. M., & Khattab, M. M. (2019). Escitalopram ameliorates cognitive impairment in D-galactose-injected ovariectomized rats: Modulation of JNK, GSK-3β, and ERK signalling pathways. Scientific Reports, 9(1), 10056.

Jadhav, R., & Kulkarni, Y. A. (2023). Neuroprotective effect of quercetin and memantine against $AlCl_3$-induced neurotoxicity in albino wistar rats. Molecules, 28(1), 417.

Janssen, L., Keppens, C., De Deyn, P. P., & Van Dam, D. (2016). Late age increase in soluble amyloid-beta levels in the APP23 mouse model despite steady-state levels of amyloid-beta-producing proteins. Biochimica et Biophysica Acta – Molecular Basis of Disease, 1862(1), 105–112.

Jin, L., Gao, L. F., Sun, D. S., Wu, H., Wang, Q., Ke, D., Lei, H., Wang, J., & Liu, G. P. (2017). Long-term ameliorative effects of the antidepressant fluoxetine exposure on cognitive deficits in 3× TgAD mice. Molecular Neurobiology, 54, 4160–4171.

Karmakar, S., & Lal, G. (2021). Role of serotonin receptor signaling in cancer cells and anti-tumor immunity. Theranostics, 11(11), 5296.

Kirkwood, B. R. (1988). Essential of medical statistics. Blackwell Scientific Publications, Oxford.

Lee, J. C., Park, J. H., Ahn, J. H., Park, J., Kim, I. H., Cho, J. H., Shin, B. N., Lee, T. K., Kim, H., Song, M., & Choi, S. Y. (2018). Effects of chronic scopolamine treatment on cognitive impairment and neurofilament expression in the mouse hippocampus. Molecular Medicine Reports, 17(1), 1625–1632.

Lee, M. R., Yun, B. S., Park, S. Y., Ly, S. Y., Kim, S. N., Han, B. H., & Sung, C. K. (2010). Anti-amnesic effect of Chong-Myung-Tang on scopolamine-induced memory impairments in mice. Journal of Ethnopharmacology, 132(1), 70–74.

Liu, J., Chang, L., Song, Y., Li, H., & Wu, Y. (2019). The role of NMDA receptors in Alzheimer's disease. Frontiers in Neuroscience, 13, 425433.

Martinez-Coria, H., Green, K. N., Billings, L. M., Kitazawa, M., Albrecht, M., Rammes, G., Parsons, C. G., Gupta, S., Banerjee, P., & LaFerla, F. M. (2010). Memantine improves cognition and reduces Alzheimer's-like neuropathology in transgenic mice. The American Journal of Pathology, 176(2), 870–880.

Maurer, S. V., & Williams, C. L. (2017). The cholinergic system modulates memory and hippocampal plasticity via its interactions with non-neuronal cells. Frontiers in Immunology, 8, 299560.

Mdawar, B., Ghossoub, E., & Khoury, R. (2020). Selective serotonin reuptake inhibitors and Alzheimer's disease. Neural Regeneration Research, 15(1), 41–46.

Numakawa, T., Odaka, H., & Adachi, N. (2018). Actions of brain-derived neurotrophin factor in the neurogenesis and neuronal function, and its involvement in the pathophysiology of brain diseases. International Journal of Molecular Sciences, 19(11), 3650.

Paget, G. E., & Barnes, J. M. (1964). Toxicity tests. In: Lawrence, D. R., & Bacharach, A. L. (Eds.). Evaluation of drug activities pharmacometrics. Vol. 1. Academic Press, New York.

Parsons, C. G., Rammes, G., & Danysz, W. (2008). Pharmacodynamics of memantine: An update. Current Neuropharmacology, 6(1), 55–78.

Perez-Caballero, L., Torres-Sanchez, S., Bravo, L., Mico, J. A., & Berrocoso, E. (2014). Fluoxetine: A case history of its discovery and preclinical development. Expert Opinion on Drug Discovery, 9(5), 567–578.

Qiao, J., Wang, J., Wang, H., Zhang, Y., Zhu, S., Adilijiang, A., Guo, H., Zhang, R., Guo, W., Luo, G., Qiu, Y., Xu, H., Kong, J., Huang, Q., & Li, X. M. (2016). Regulation of astrocyte pathology by fluoxetine prevents the deterioration of Alzheimer phenotypes in an APP/PS 1 mouse model. Glia, 64(2), 240–254.

Qiao, O., Zhang, X., Zhang, Y., Ji, H., Li, Z., Han, X., Wang, W., Li, X., Wang, J., Liu, C., & Gao, W. (2021). Cerebralcare Granule® enhances memantine hydrochloride efficacy in APP/PS1 mice by ameliorating amyloid pathology and cognitive functions. Chinese Medicine, 16, 47.

Resende, R., Moreira, P. I., Proença, T., Deshpande, A., Busciglio, J., Pereira, C., & Oliveira, C. R. (2008). Brain oxidative stress in a triple-transgenic mouse model of Alzheimer disease. Free Radical Biology and Medicine, 44(12), 2051–2057.

Sechi, S., Chiavolelli, F., Spissu, N., Di Cerbo, A., Canello, S., Guidetti, G., Fiore, F., & Cocco, R. (2015). An antioxidant dietary supplement improves brain-derived neurotrophic factor levels in serum of aged dogs: Preliminary results. Journal of Veterinary Medicine, 2015, 412501.

Shahidi, S., Asl, S. S., Komaki, A., & Hashemi-Firouzi, N. (2018). The effect of chronic stimulation of serotonin receptor type 7 on recognition, passive avoidance memory, hippocampal long-term potentiation, and neuronal apoptosis in the amyloid β protein treated rat. Psychopharmacology, 235, 1513–1525.

Shelat, P. B., Chalimoniuk, M., Wang, J. H., Strosznajder, J. B., Lee, J. C., Sun, A. Y., Simonyi, A., & Sun, G. Y. (2008). Amyloid beta peptide and NMDA induce ROS from NADPH oxidase and AA release from cytosolic phospholipase A2 in cortical neurons. Journal of Neurochemistry, 106(1), 45–55.

Silva, M. V. F., Loures, C. D. M. G., Alves, L. C. V., de Souza, L. C., Borges, K. B. G., & Carvalho, M. D. G. (2019). Alzheimer's disease: Risk factors and potentially protective measures. Journal of Biomedical Science, 26, 33.

Tanila, H. (2017). The role of BDNF in Alzheimer's disease. Neurobiology of Disease, 97, 114–118.

Yadang, F. S. A., Nguezeye, Y., Kom, C. W., Betote, P. H. D., Mamat, A., Tchokouaha, L. R. Y., Taiwé, G. S., Agbor, G. A., & Bum, E. N. (2020). Scopolamine-induced memory impairment in mice: Neuroprotective effects of *Carissa edulis* (Forssk.) Valh (Apocynaceae) aqueous extract. International Journal of Alzheimer's Disease, 2020, 6372059.

Zhou, X., Wang, L., Xiao, W., Su, Z., Zheng, C., Zhang, Z., Wang, Y., Xu, B., Yang, X., & Hoi, M. P. M. (2019). Memantine improves cognitive function and alters hippocampal and cortical proteome in triple transgenic mouse model of Alzheimer's disease. Experimental Neurobiology, 28(3), 390.