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Attenuation of age-related cognitive decline and memory deficits through apomorphine administration

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Abstract: Oxidative stress, stemming from heightened production of reactive oxygen species and free radicals, significantly contributes to the aging process. Apomorphine emerges as a pivotal medication for managing Alzheimer's, Parkinson's, and other age-related conditions. This study aims to explore the memory-enhancing and neuroprotective properties of apomorphine, utilizing male Albino Wistar rats aged 4 and 24 months as subjects. Rats were intraperitoneally injected with apomorphine for 6 days. Decreased glutathione peroxidase, superoxide dismutase and catalase activities with increased lipid peroxidation were observed in the brain and plasma samples of aged rats, which were reversed upon apomorphine administration. Superoxide dismutase (SOD) and AChE activities were significantly decreased along with a decline in short-term- and long-term memory of aged rats, which was reverted by apomorphine. Furthermore, a notable reduction in biogenic amines and metabolite levels in the brains of aged rats was reversed in aged rats treated with apomorphine. The findings indicate a significant restoration of memory impairment and oxidative stress in aged rats by apomorphine. Overall, our data suggests that apomorphine, at a dosage of 1mg/kg, holds promise as a potential therapeutic intervention for dementia and associated disorders in elderly patients.

Keywords: Apomorphine, age, acetylcholine esterase, glutathione peroxidise, catalase, superoxide dismutase, lipid peroxidation.

INTRODUCTION

Several studies have underscored the significance of oxidative stress in dementia and cognitive decline (Bao *et al*., 2024; Hoyos *et al*., 2022; Franzoni *et al*., 2021; Sienes Bailo *et al*., 2022; Ton *et al*., 2020). Specifically, the expression of a highly polymerogenic variant of neuroserpin proteins, implicated in severe dementia known as Familial Encephalopathy with Neuroserpin Inclusion Bodies (FENIB), has been linked to upregulation of antioxidant genes and apoptotic neural cell death when oxidative stress defenses are compromised (Plascencia-Villa and Perry, 2023). Nano-antioxidants like resveratrol-loaded solid lipid nanoparticles (R-SLNs) show promise in enhancing antioxidant delivery for the prevention and treatment of neurodegenerative diseases driven by oxidative stress (Ashok *et al*., 2022). Furthermore, over the past 22 years, there has been a significant increase in new dementia diagnoses among hypertensive individuals, with Alzheimer's disease emerging as the predominant subtype in this population. Notably, the gender gap in dementia incidence among hypertensive individuals has narrowed, and disparities across different socioeconomic categories have also decreased in recent years (Adesuyan *et al*., 2023). Neurological disorders, encompassing conditions such as Alzheimer's disease, motor neuron disease, and Parkinson's disease, pose significant challenges to longevity and quality of life. Their pathogenesis is intricately linked to oxidative stress, highlighting the critical role of antioxidant interventions in their management (Houldsworth, 2024).

Preclinical investigations suggest that apomorphine may mitigate neuronal injury by reducing oxidative stress, a primary contributor to neurodegeneration (Eastman *et al*., 2020). Moreover, apomorphine demonstrates anti-inflammatory properties, which could attenuate neuroinflammatory responses implicated in various neurodegenerative conditions (Giri *et al*., 2024). Additionally, evidence indicates that apomorphine enhances neuroplasticity, promoting the brain's ability to adapt and repair itself (Fresnoza *et al*., 2021). Through its modulation of these interconnected pathways, apomorphine presents a multifaceted approach to neuroprotection, underscoring its therapeutic potential for mitigating neuronal damage in neurodegenerative diseases (Kamboj *et al*., 2024). While preliminary studies suggest its promise as a therapeutic agent for Alzheimer's disease, further elucidation of the exact mechanisms underlying apomorphine's neuroprotective effects is warranted (Poudel and Park, 2022; Goyal *et al*., 2022). These ntioxidative properties of apomorphine could reduce the oxidative stress and neuronal damage, promoting a healthy brain environment.

The present study was designed to monitor memory enhancing and neuroprotective effects of apomorphine in aged rats as a natural animal model of dementia. Antioxidant enzymes activities, lipid peroxidation,

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acetylcholine esterase (AChE) activity in brain and plasma were determined, following repetitive administration of apomorphine. Findings may help in extending therapeutics in dementia, Alzheimer's and related disorders.

MATERIALS AND METHODS

Locally bred male Albino Wistar rats (180-200g), sourced from the HEJ Research Institute of Chemistry, Karachi, were individually housed under 12-hour light-dark cycles (22±2ºC). Rats were provided with free access to tap water and standard rodent diet cubes for 7 days prior to the commencement of the experiment to acclimate to their surroundings. All experimental protocols were approved and conducted in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985) and the Institutional Animal Ethics Committee (IAEC); approval no. KU-IBC-07112023.

Drugs and doses

Apomorphine purchased from Sigma, was dissolved in saline and injected intraperitoneally at the dose of 1.0 mg/kg body weight (Ikram et al., 2018). Control animals were injected with saline (1.0 ml/kg body weight).

Experimental protocol

Twelve male Albino Wistar rats, divided into two age groups (4 months as young and 24 months as aged), were randomly allocated into four groups, each consisting of 6 animals: (i) young-saline, (ii) young-apomorphine, (iii) aged-saline and (iv) aged-apomorphine. Baseline activities in the Skinner's box were assessed before initiating the experiment (day 0). Subsequently, rats underwent training in the Morris water maze with a maximum trial duration of 5 minutes. Rats unable to reach the platform within this time frame were manually placed on the platform for 30 seconds. Intraperitoneal injections of saline (1.0ml/kg) or apomorphine (1.0 mg/kg) were administered once daily for 6 days. Activities in the Skinner's box were recorded 10 minutes post-injection daily for a duration of 20 minutes. On day 13, the rats were euthanized, and whole brain as well as plasma samples were collected and stored at -70°C.

Behavioural assessment

Morris water maze (MWM) test

The procedure of the Morris water maze test was essentially same as described before (Ikram and Haleem, 2019). Animals were trained to locate the hidden platform in the water maze 1hr post injection. After training, animals were placed back in their home cages. Learning acquisition and memory retention were monitored as latency to locate the platform. Test for learning acquisition was conducted 2hr post training while memory retention was monitored 12hr post injection. While memory consolidation was also interpreted.

Skinner's box activity

Transparent Perspex cages (26×26×26 cm) with sawdust covered floor were used to monitor activity in familiar environment. Rats were placed individually in these cages to get familiar with the environment. 15 min later the animals were injected with drug or saline. Numbers of cage crossings were counted 5 min post-injection for 10min (Ikram *et al*., 2021).

Oxidative parameters

Determination of lipid peroxidation

The procedure for estimation of lipid peroxidation was performed as described previously (Chow and Tappel, 1972) with slight modifications. An aliquot of 100µL plasma and 2mL of 0.375% TBA in 15% TCA were thoroughly mixed in test tubes. This mixture was placed in boiling water for 20 min and allowed to cool in ice-caged water at 4°C. After centrifugation at 11,100rpm for 10 min (4°C), the resulting clear supernatant of light pink colour (250µL) was collected and transferred to 96-well micro plate. The absorbance of the supernatant was recorded at 532nm in absorbance reader. The amount of TBA reactants was calculated using molar extinction coefficient of malondialdehyde (1.56×105). Results were expressed as µmoles of MDA per Liter of rat plasma.

Determination of AchE activity

AChE activity of plasma was determined according to the method of Ellman *et al*., (1961) with slight modifications. Exactly 31µL of the plasma was added to a well containing 20µL of phosphate buffer (pH 8.0, 0.1M). 8µL of the DTNB reagent (10mM) was added to the well. The resulting mixture was given a shake duration of 15sec by placing the 96-well micro plate in the absorbance reader. Absorbance was measured at 450nm; when this had stopped increasing (approx. after 22min), the basal reading was recorded. 1.5µL of the substrate ATC (75 mM), was added to start the reaction and changes in absorbances were recorded at time zero and after 10 min at 25°C. AChE activity was expressed as µmoles of thiocholine produced per minute per milliliter of rat plasma.

Determination of superoxide dismutase (SOD) activity

SOD activity of plasma was estimated by the method of Chidambara-Murthy *et al*., (2002). Exactly 50µL of the plasma was added to a well containing 100µL of sodium carbonate (50mM). 100µL of the NBT reagent (24µM), and 20µL EDTA (0.1mM) were added to the well. The resulting mixture was given a shake duration of 15sec by placing the 96-well microplate in the absorbance reader. 40µL hydroxylamine hydrochloride (1mM) was added to start the reaction and changes in absorbances were recorded at time zero and after 5min at 560nm at 25°C. A reagent blank without plasma (containing only sodium carbonate, NBT, EDTA and hydroxylamine hydrochloride) was run along the samples. Units of SOD

activity were expressed as the amount of enzyme (mmol per min) required to inhibit the reduction of NBT by 50 %. The specific activity was expressed as units per milliliter of rat plasma.

Determination of catalase (CAT) activity

Catalase activity of plasma was estimated as described previously (Sinha 1972). The assay mixture contained 0.4 mL of H2O2 solution (0.2 M) and 1mL of sodium phosphate buffer (0.01 M, pH 7.4) in a test tube. 100µL of plasma was rapidly mixed with the reaction mixture by a gentle swirling motion. The tubes containing reaction mixture were incubated at 37°C for 1.5 min. In order to stop the reaction, the mixture was blown into 2mL of dichromate/acetic acid (5%). Tubes were placed in boiling water (100°C) for 15 min and allowed to cool in ice-caged water at 4°C. The resulting mixture (250µL) was collected and transferred to 96-well micro plate. The absorbance of the supernatant was recorded at 570 nm in absorbance reader. An appropriate control was run along each plasma sample and its reaction was immediately stopped at 0 min. CAT activity was expressed as the µmoles of H2O2 consumed per milliliter of rat plasma.

Determination of Glutathione Peroxidase (GSH-Px) Activity

Glutathione peroxidase activity of plasma was estimated as described previously (Crisol *et al*., 2012). A mixture containing 30µL of sodium phosphate buffer (0.1 M, pH 7.4), 20µL of glutathione (2 mM), 30µL of plasma, 10µL of sodium azide (10mM), and 10µL of hydrogen peroxide solution (1mM) in a 2mL microcentrifuge tube was incubated for 15 min at 37°C. The reaction was stopped by vigorously injecting a total of 50µL 5 % TCA. After centrifugation at 8,325 rpm for 5 min (4°C), the resulting supernatant (25µL) was collected and transferred to 96-well micro plate. 50µL of sodium phosphate buffer (0.1 M, pH 7.4) and 175µL of DTNB (1 mM) were added to supernatant. The mixture was given a shake duration of 10 s by placing the 96-well microplate in the absorbance reader. The absorbance was measured at 420 nm. An appropriate control was run along each plasma sample and its reaction was immediately stopped at 0 min. GSH-Px activity was expressed as the µmoles of GSH converted to GSSG per min per milliliter of rat plasma.

Dissection of rat whole brain

Dissection procedure was essentially same as described earlier (Ikram et al., 2021). After decapitation, fresh brain was washed with ice-cold saline and stored at -70ºC.

STATISTICAL ANALYSIS

Results are given as means±SD. Analysis of the data was performed by two-way- or three-way ANOVA, wherever applicable, using SPSS ver 19. Post hoc comparisons were done by Tukey's test. Values of $p<0.05$ were considered statistically significant.

RESULTS

Fig. 1 shows effects of apomorphine on Morris water maze test, in young and aged rats. Analysis of the data by two-way ANOVA on learning acquisition (fig. 1a) showed significant effects of age (df= 1,20; F= 30.21; p= 0.0001), apomorphine (df= 1,20; F= 15.97; p= 0.0001) as well as interaction between the two (df= 1,20; F= 45.76; p= 0.0001). Post hoc analysis by Tukey's test showed that aged rats took more time ($p<0.01$) to reach platform, as compared to saline treated rats. While, aged-apomorphine rats decreased this time taken ($p<0.01$) to reach platform. fig. 1b shows effects of apomorphine in memory consolidation in young and aged rats. Analysis of the data by two-way ANOVA showed significant effects of age (df= 1,20; F= 21.34; p= 0.0001) apomorphine (df= 1,20; F= 27.93; p= 0.0001) as well as interaction between the two (df= 1,20; F= 45.67; p= 0.0001). Post hoc analysis by Tukey's test showed that aged rats took more time ($p<0.01$) to reach platform, as compared to young. While, aged-apomorphine injected rats decreased this time taken ($p<0.01$) to reach platform. fig. 1c shows effects of apomorphine in memory retention, following repeated administration of age. Analysis of the data by two-way ANOVA showed significant effects of age (df= 1,20; F= 19.78; p= 0.0001), apomorphine (df= 1,20; F= 38.54; p= 0.0001) as well as interaction between the two (df= 1,20; F= 20.59; p= 0.0001). Post hoc analysis by Tukey's test showed that aged rats took more time ($p<0.01$) to reach platform, as compared to young rats. While, aged-apomorphine injected rats decreased this time taken ($p<0.01$) to reach platform.

Fig. 2 shows effects of apomorphine on Skinner's box activity, in young and aged rats. Analysis of the data by three-way ANOVA showed significant effects of apomorphine (df= 1,140; F= 4786.70; p=0.0001), age (df= 1,140; F= 456.98; p= 0.0001) and repeated administration (df= 6,140; F= 2351.76; p= 0.0001). Interactions of apomorphine*repeated administration (df= 6,140; F= 234.47; p=0.0001), age*repeated administration (df= 6,140; F= 289.25; p=0.0001), apomorphine*age (df= 1,140; F= 344.74; p=0.0001) and age* apomorphine* repeated administration (df= 6,140; F= 276.28; p=0.0001) were all significant. Post hoc analysis by Tukey's test showed significant increase ($p<0.05$) in number of cage crossings in young rats injected with apomorphine from day3 till day 6. While number of cage crossings in aged-apomorphine rats was also increased ($p<0.01$) from day3 till day 6 but was comparable with young-apomorphine treated rats. fig. 3 shows effects of apomorphine on lipid peroxidation and acetylcholine esterase activity in young and aged rats.

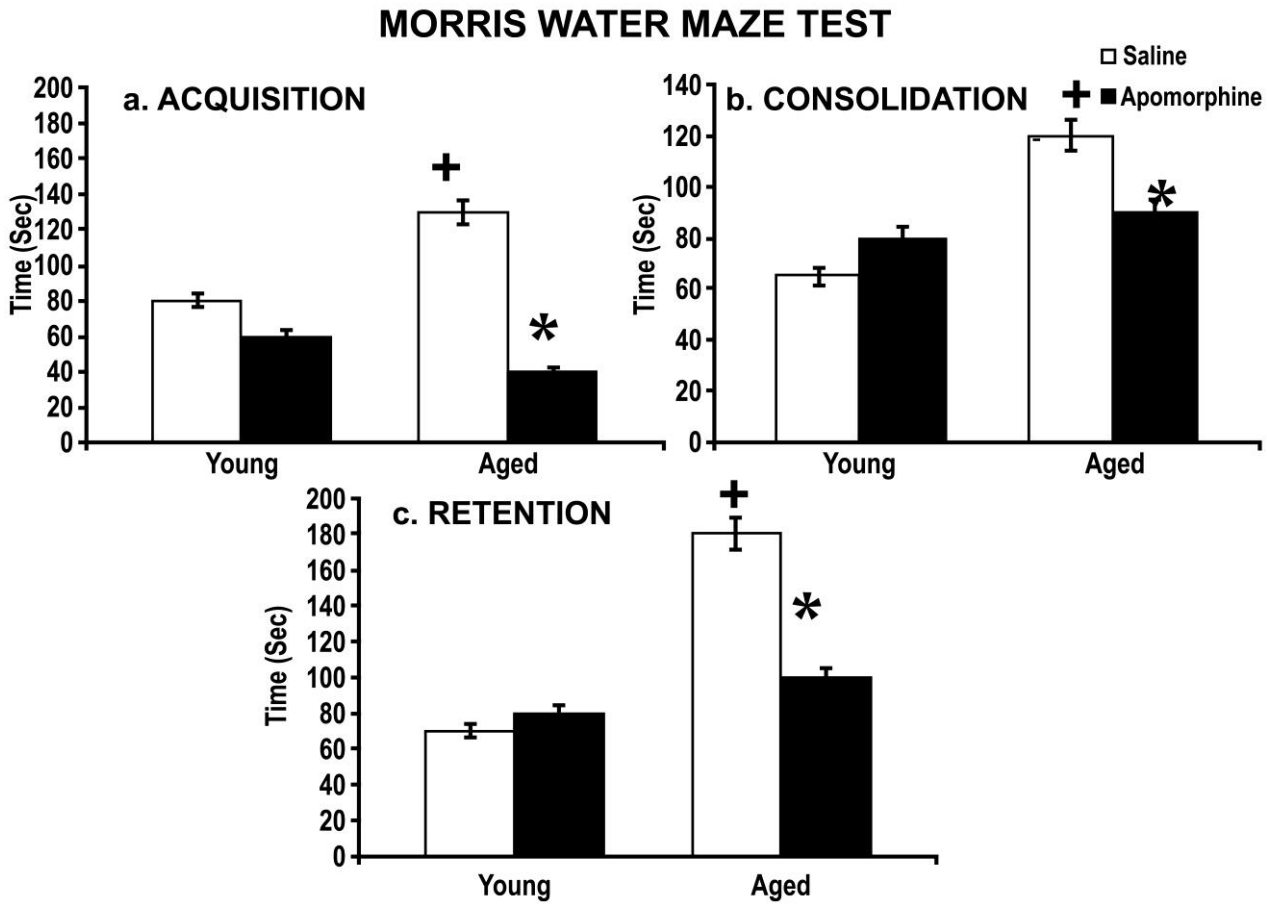

Fig. 1: Effects of apomorphine on Morris water maze test, in aged and young rats. Significant differences by Tukey's test: *p<0.01 as compared to respective saline injected controls; +p<0.01 as compared to respective young rats, following two-way ANOVA.

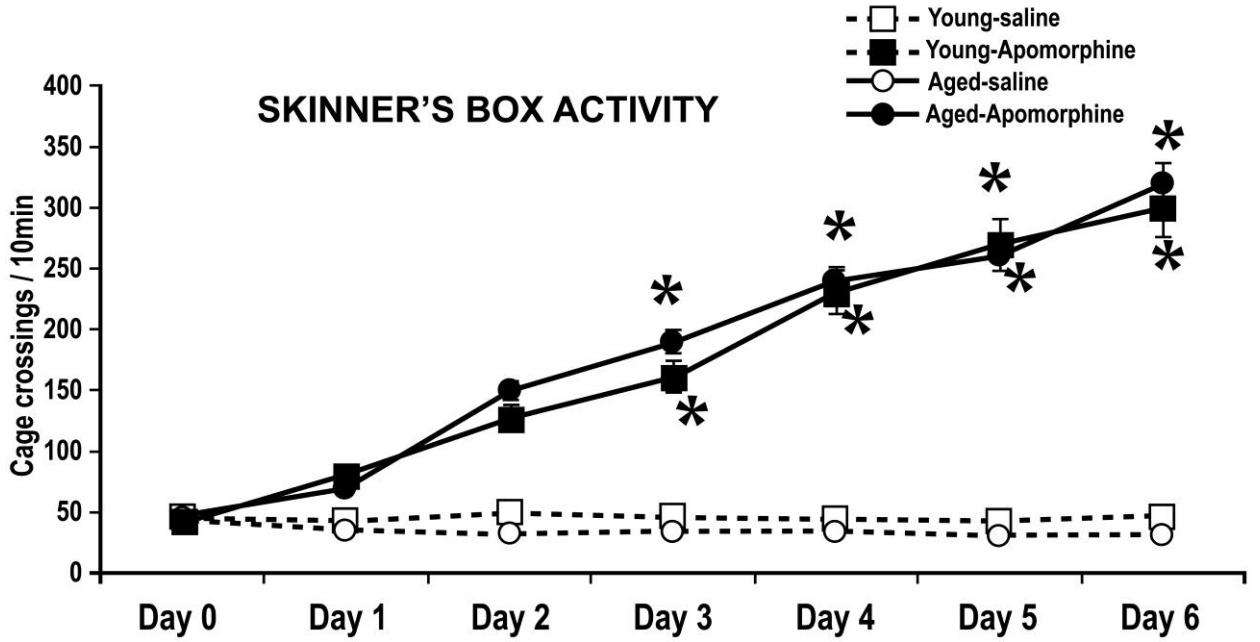

Fig. 2: Effects of apomorphine on Skinner's box activity of aged and young rats. Significant differences by Tukey's test: *p<0.01 as compared to respective saline injected controls following three-way ANOVA.

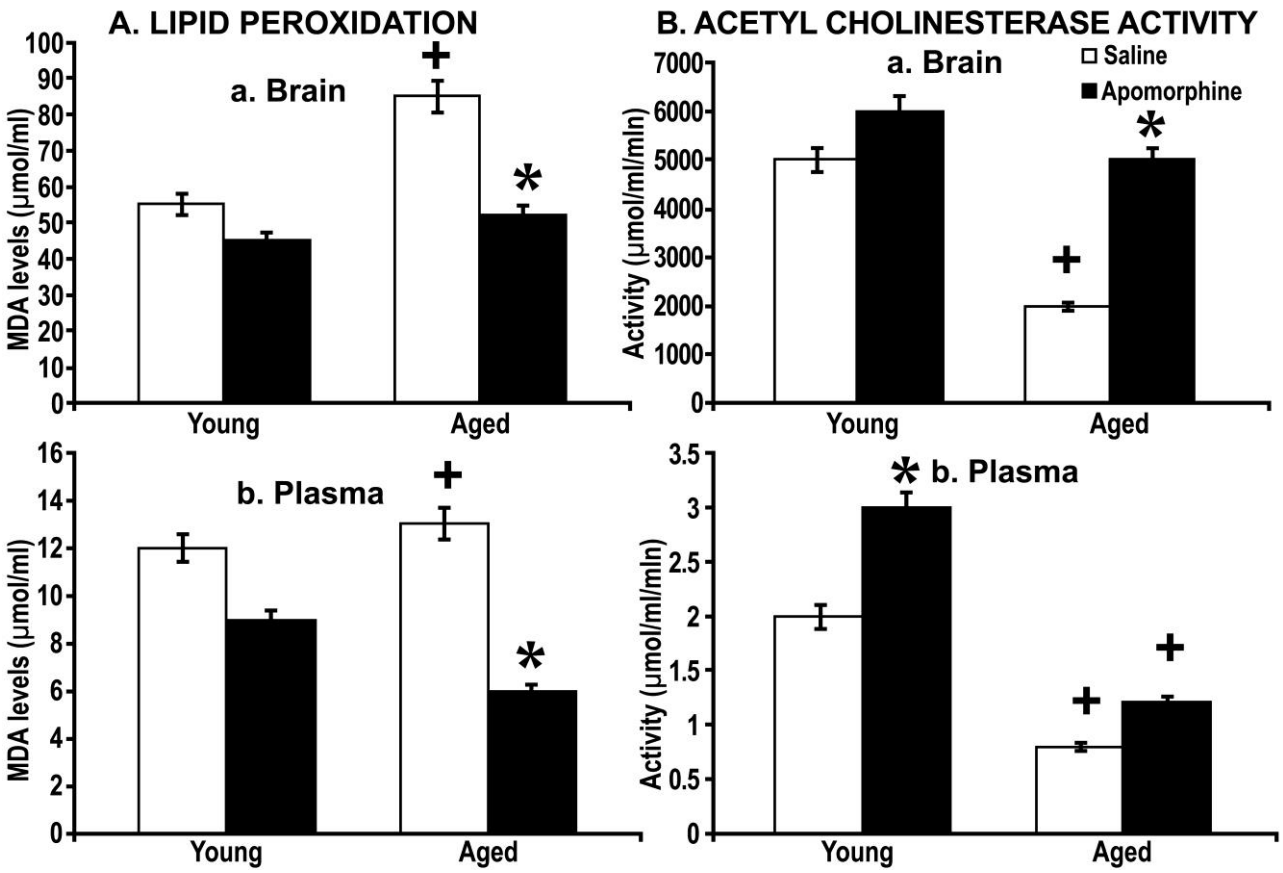

Fig. 3: Effects of apomorphine on lipid peroxidaiton and acetylcholine esterase activity of aged and young rats. Significant differences by Tukey's test: *p<0.01 as compared to respective saline injected controls; +p<0.01 as compared to young rats, following two-way ANOVA.

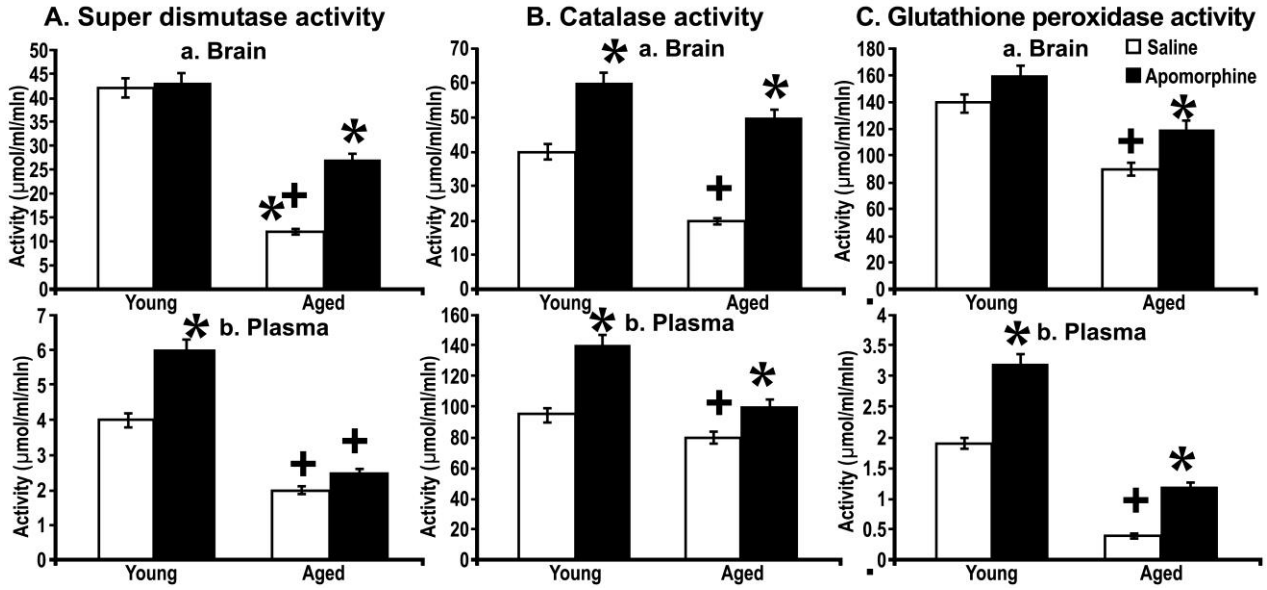

Fig. 4: Effects of apomorphine on superoxide dismutase-, catalase-, and glutathione peroxidase activity of aged and young rats. Significant differences by Tukey's test: *p<0.01 as compared to respective saline injected controls; +p<0.01 as compared to young rats, following two-way ANOVA.

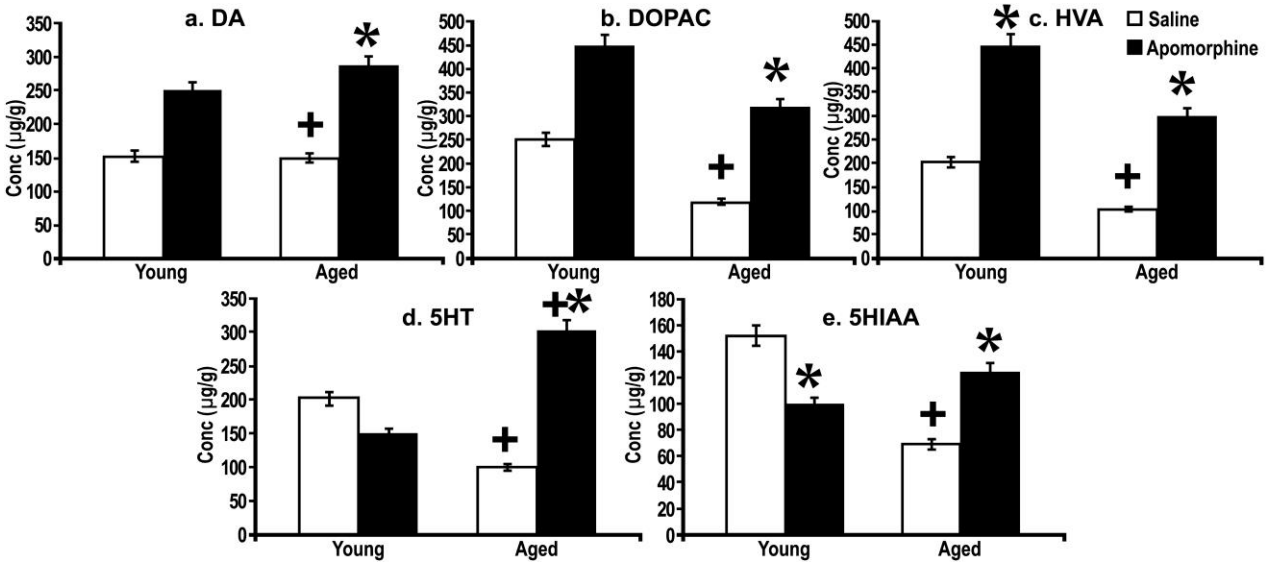

Fig. 5: Effects of apomorphine on biogenic amines and metabolites in hippocampus of aged and young rats. Significant differences by Tukey's test: *p<0.01 as compared to respective saline injected controls; +p<0.01 as compared to young rats, following two-way ANOVA.

Analysis of the data on lipid per oxidation (fig. 3A) by two-way ANOVA showed significant effects of age (df= 1,20; F= 32.67; p= 0.0001) apomorphine (df= 1,20; F= 29.35; p= 0.0001) as well as interaction between the two (df= 1,20; F= 41.07; p= 0.0001) on brain lipid peroxidation. While in plasma samples, effects of age (df= 1,20; F= 74.53; p= 0.0001) apomorphine (df= 1,20; F= 38.76; p= 0.0001) as well as interaction between the two (df= 1,20; F= 69.83; p= 0.0001) were also significant. Post hoc analysis by Tukey's test showed that aged-saline injected rats showed increased (p<0.01) lipid peroxidation in both brain and plasma samples which was decreased (p<0.01) in age-apomorphine injected rats.

Analysis of the data on acetyl cholinesterase activity (fig. 3B) by two-way ANOVA showed significant effects of age (df= 1,20; F= 64.43; p= 0.0001) apomorphine (df= 1,20; F= 63.14; p= 0.0001) as well as interaction between the two (df= 1,20; F= 63.21; p= 0.0001) on brain lipid peroxidation . While in plasma samples, effects of age (df= 1,20; F= 75.28; p= 0.0001) apomorphine (df= 1,20; F= 64.83; p= 0.0001) as well as interaction between the two (df= 1,20; F= 40.98; p= 0.0001) were all significant. Post hoc analysis by Tukey's test showed that aged-saline injected rats showed decreased (p<0.01) acetyl cholinesterase activity in both brain and plasma samples which was attenuated (p<0.01) in aged-apomorphine injected rats.

Fig. 4 shows effects of apomorphine on superoxide dismutase-, catalase-, and glutathione peroxidase activity, in young and aged rats. Analysis of the data on superoxide dismutase activity (fig. 4A) by two-way ANOVA showed significant effects of age (df= 1,20; F= 42.98; p= 0.0001) apomorphine (df= 1,20; F= 38.76; p= 0.0001) as well as interaction between the two (df= 1,20; F= 39.29; p= 0.0001) on brain superoxide dismutase activity. While in plasma samples, effects of age (df= 1,20; F= 74.93; p= 0.0001) apomorphine (df= 1,20; F= 109.27; p= 0.0001) as well as interaction between the two (df= 1,20; F= 83.90; p= 0.0001) were all significant. Post hoc analysis by Tukey's test showed that apomorphine treated rats showed increased (p<0.01) superoxide dismutase activity in brain but not in plasma samples of young rats and the same was decreased (p<0.01) in both brain and plasma samples of aged-saline treated rats. While superoxide dismutase activity was increased (p<0.01) in aged-apomorphine injected rats.

Analysis of the data on catalase activity (fig. 4B) by two-way ANOVA showed significant effects of age (df= 1,20; F= 72.98; p= 0.0001) apomorphine (df= 1,20; F= 102.85; p= 0.0001) as well as interaction between the two (df= 1,20; F= 92.73; p= 0.0001) on catalase activity. While in plasma samples, effects of age (df= 1,20; F= 91.73; p= 0.0001) apomorphine (df= 1,20; F= 83.21; p= 0.0001) as well as interaction between the two (df= 1,20; F= 82.54; p= 0.0001) were also significant. Post hoc analysis by Tukey's test showed that young-apomorphine treated rats showed increased (p<0.01) catalase activity in both brain and plasma samples and the same was decreased (p<0.01) in aged-saline treated rats in brain but not in plasma samples. While catalase activity was also increased (p<0.01) in aged-apomorphine injected rats treated rats in both brain and plasma samples, as compared to aged-saline injected rats.

Analysis of the data on glutathione peroxidase activity (fig. 4C) by two-way ANOVA showed significant effects of age (df= 1,20; F= 39.43; p= 0.0001) apomorphine (df=

1,20; F= 92.65; p= 0.0001) as well as interaction between the two (df= 1,20; F= 48.57; p= 0.0001) on glutathione peroxidase activity. While in plasma samples, effects of age (df= 1,20; F= 82.35; p= 0.0001) apomorphine (df= 1,20; F= 68.92; p= 0.0001) as well as interaction between the two (df= 1,20; F= 73.20; p= 0.0001) were also significant. Post hoc analysis by Tukey's test showed that youg-apomorphine treated rats showed increased ($p<0.01$) glutathione peroxidase activity in plasma but not brain samples and the same was decreased ($p<0.01$) in brain and plasma samples of aged-saline injected rats. While glutathione peroxidase activity was also increased ($p<0.01$) in both brain and plasma samples of aged-apomorphine injected rats, as compared to aged-saline injected rats.

Fig. 5 shows effects of apomorphine on biogenic amines and metabolites in hippocampus of young and aged rats. Analysis of the data on dopamine levels (fig. 5a) by two-way ANOVA showed significant effects of age (df= 1,20; F= 87.35; p= 0.0001) apomorphine (df= 1,20; F=93.65; p= 0.0001) as well as interaction between the two (df= 1,20; F= 72.19; p= 0.0001) on dopamine levels. Analysis of the data on DOPAC levels (fig. 5b) by two-way ANOVA showed significant effects of age (df= 1,20; F= 30.29; p= 0.0001) apomorphine (df= 1,20; F= 47.91; p= 0.0001) as well as interaction between the two (df= 1,20; F= 91.64; p= 0.0001) on DOPAC levels. Analysis of the data on HVA levels (fig. 5c) by two-way ANOVA showed significant effects of age (df= 1,20; F= 65.34; p= 0.0001) apomorphine (df= 1,20; F= 57.83; p= 0.0001) as well as interaction between the two (df= 1,20; F= 72.19; p= 0.0001) on HVA levels. Post hoc analysis by Tukey's test showed that apomorphine increased ($p<0.01$) the levels of dopamine, DOPAC and HVA in young rats. While in aged rats (saline injected), there was a decrease ($p<0.01$) in the levels of dopamine, DOPAC and HVA. These effects of age were attenuated in aged-apomorphine injected rats which showed elevated ($p<0.01$) dopamine, DOPAC and HVA levels.

Analysis of the data on 5HT levels (fig. 5d) by two-way ANOVA showed significant effects of age (df= 1,20; F= 42.09; p= 0.0001) apomorphine (df= 1,20; F= 68.32; p= 0.0001) as well as interaction between the two (df= 1,20; F= 91.20; p= 0.0001) on 5HT levels. Analysis of the data on 5HIAA levels (fig. 5e) by two-way ANOVA showed significant effects of age (df= 1,20; F= 86.39; p= 0.0001) apomorphine (df= 1,20; F= 73.20; p= 0.0001) as well as interaction between the two (df= 1,20; F= 65.49; p= 0.0001) on 5HIAA levels. Post hoc analysis by Tukey's test showed that apomorphine decreased ($p<0.01$) the levels of 5HT and 5HIAA in young rats. While aged rats showed a decrease ($p<0.01$) in the levels of 5HT and 5HIAA. These effects were attenuated in aged-apomorphine injected rats which showed elevated ($p<0.01$) 5HT as compared to both aged-saline and young-apomorphine injected rats, while levels of 5HIAA were elevated ($p<0.01$) as compared to young-apomorphine injected rats.

DISCUSSION

Current research is dedicated to identifying therapeutic approaches for managing dementia in patients who exhibit resistance to conventional treatment methods. In some instances, patients may present with treatment-resistant dementia alongside other psychiatric conditions such as depression or display treatment-resistant symptoms associated with Alzheimer's disease (Reuben *et al*., 2024). Studies conducted on a triple transgenic Alzheimer's disease mouse model (3xTg-AD) have demonstrated that apomorphine treatment reduces intraneuronal Aβ and p-tau levels, suggesting its potential as a novel therapeutic agent for Alzheimer's disease (Roda *et al*., 2020). Additionally, apomorphine has shown promise in alleviating cognitive impairments associated with Parkinson's disease and related disorders. Its effects on amyloid deposition in nondemented Parkinson's disease cases suggest a potential therapeutic avenue (Wanger *et al*., 2023). Consistent with these findings, our investigation revealed that apomorphine administration significantly mitigated age-related memory impairments, as evidenced by improvements in acquisition and retention in the Morris water maze test. This effect could be attributed to enhanced cholinergic function in apomorphine-treated rats, as evidenced by a positive correlation between increased acetylcholine esterase activity and memory retention. Furthermore, the antioxidant properties of apomorphine, stemming from its ability to scavenge reactive oxygen species and inhibit lipid peroxidation, may play a crucial role in mitigating oxidative stress-induced neuronal damage. Understanding these mechanisms sheds light on the therapeutic potential of apomorphine in preserving neuronal integrity and function in neurodegenerative disorders (Ramli *et al*., 2020).

Dementia involves disruptions in neurotransmitter mechanisms, with alterations in the dopaminergic system frequently associated with both cognitive and non-cognitive symptoms of Alzheimer's disease (AD). However, further research is necessary to elucidate the role of dopaminergic system dysfunction in AD. In a study conducted on Tg2576 mice, which overexpress mutated human amyloid precursor protein, dopaminergic neuron loss in the ventral tegmental area (VTA) was observed. This loss was correlated with deficits in food reward processing, memory performance and CA1 synaptic plasticity, highlighting the significance of dopamine in memory deficits and dysfunction in reward processing (Osorio-Gómez, 2022). Additionally, besides linking the frontal cortex with the striatal dopaminergic system, the dorsolateral frontostriatal circuit plays a

critical role in memory recall, attention, and planning (Zhou *et al*., 2024). In our study, aged rats exhibited reduced acetylcholine content, as evidenced by decreased acetylcholine esterase activity, which correlated with observed memory impairments. However, treatment with apomorphine reversed these memory impairments associated with aging, suggesting that enhanced dopaminergic function and improved acetylcholine esterase activity may contribute to the memory-improving effects of apomorphine. Previous reports have indicated that animals with damaged dopaminergic neurons exhibit memory deterioration and non-cognitive symptoms, closely resembling early AD both histopathologically and behaviorally (Chen and Zhang, 2022).

In the present study, rats treated with apomorphine demonstrated a notable increase in hippocampal dopaminergic concentration, alongside significantly enhanced memory performance as assessed in the Morris water maze test. Conversely, the group of aged rats treated with apomorphine exhibited heightened acetylcholine esterase activity and displayed improved memory performance compared to aged rats injected with saline. Previous research suggests that novelty-associated memory enhancement relies on the persistence of synaptic plasticity, mediated by dopaminergic signaling through D1/D5 receptors in the hippocampus (Tse *et al*., 2023). This enhancement is hypothesized to occur through the subiculum-accumbens-pallidum-ventral tegmental area-hippocampus pathway (Cowan *et al*., 2021). Additionally, memory impairment has been associated with a decline in cortical dopaminergic function, suggesting a role for dopaminergic dysregulation in memory deficits. Adjusting dopamine signaling may therefore offer a potential avenue for improving memory impairment (Dahl *et al*., 2023). Furthermore, it has been proposed that apomorphine-enhanced memory re-consolidation serves as a cue during drug conditioning (Carey, 2020). The resulting behavioral sensitization is also influenced by improved memory functions in these rats. Administration of a single dose of apomorphine during the psychostimulant memory re-consolidation phase has been shown to reverse psychostimulant conditioning, leading to the reversal of psychostimulant-induced sensitization and conditioned inhibition. However, for drug memory substitution, the memory re-consolidation period is crucial, as sensitized responses were unaffected by apomorphine treatment 15 minutes after the trial (Barak and Goltseker, 2021). Immediate post-trial apomorphine treatment, on the other hand, abolished sensitization and induced a conditioned hypoactivity response, highlighting the potential utility of apomorphine in inhibiting dopaminergic activity during addictive drug memory re-consolidation. In addition to preclinical studies, clinical trials and epidemiological data collectively underscore the promising efficacy of apomorphine in dementia treatment, indicating its potential to alleviate cognitive decline and improve quality of life in affected individuals (Metta *et al*., 2023). These findings provide valuable insights into the therapeutic landscape of dementia management and position apomorphine as a promising candidate for further investigation and implementation in clinical practice.

Oxidative stress plays a significant role in the development and progression of dementia and age-related cognitive impairments. This concept is supported by studies conducted in mice models such as APP23 mice, carrying the APP KM670/671NL mutation, and triple transgenic mice, harboring PS1 M146 V, Tau P301L and APP KM670/671NL mutations. These studies have demonstrated that oxidative stress markers are expressed in the early stages of Alzheimer's disease (AD), preceding the formation of amyloid deposits (Bornemann and Staufenbiel, 2000). Comparisons of hippocampi from AD patients have revealed decreased expression of ApoE protein, leading to heightened oxidative stress on lipids, as evidenced by increased levels of thiobarbituric acid reactive substances (TBARS) (Ramassamy *et al*., 2001). In addition to its antioxidant properties, apomorphine also has the ability to inhibit the aggregation of amyloid β-protein (Aβ42), a key contributor to Alzheimer's disease (Gallego Villarejo *et al*., 2022). It has been proposed that the progressive decline in cognitive function in dementia is associated with a decrease in the expression of antioxidant enzymes such as SOD, CAT, and GPx (Sidiropoulou *et al*., 2023; Vural *et al*., 2023). Consistent with these findings, our results demonstrate increased lipid peroxidation and decreased antioxidant enzyme activities in aged rats injected with saline. However, in the aged-apomorphine group, reduced lipid peroxidation accompanied by normalized activities of antioxidant enzymes were observed, suggesting attenuation of age-related oxidative stress in rats treated with apomorphine. The beneficial effects of apomorphine have garnered attention due to repeated evidence indicating its ability to improve cognitive performance.

CONCLUSION

In conclusion, the findings of this study suggest a potential role for apomorphine in mitigating age-related learning and memory deficits. Through its neuroprotective properties, apomorphine shows promise in preserving cognitive function in aging individuals. These results underscore the importance of further research into the therapeutic potential of apomorphine in combating age-related cognitive decline, offering hope for novel interventions to enhance cognitive health in the elderly population.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Adesuyan M, Jani YH, Alsugeir D, Howard R, Wong ICK, Wei L and Brauer R (2023). Trends in the incidence of dementia in people with hypertension in the UK 2000 to 2021. Alzheimers Dement (Amst). **15**(3): e12466.

Ashok A, Andrabi SS, Mansoor S, Kuang Y, Kwon BK, and Labhasetwar V (2022). Antioxidant therapy in oxidative stress-induced neurodegenerative diseases: role of nanoparticle-based drug delivery systems in clinical translation. *Antioxidants* (Basel), **11**(2): 408.

Bao QN, Xia MZ, Xiong J, Liu YW, Li YQ, Zhang XY, Chen ZH, Yao J, Wu KX, Zhong WQ, Xu SJ, Yin ZH, and Liang FR (2024). The effect of acupuncture on oxidative stress in animal models of vascular dementia: A systematic review and meta-analysis. *Syst. Rev.*, **13**(1): 59.

Barak S and Goltseker K (2021). Targeting the reconsolidation of licit drug memories to prevent relapse: Focus on alcohol and nicotine. *Int. J. Mol. Sci.*, **22**(8): 4090.

Bornemann KD and Staufenbiel M (2000). Transgenic mouse models of Alzheimer's disease. *Ann N Y Acad Sci.*, **908**: 260-266.

Carey RJ (2020). Drugs and memory: Evidence that drug effects can become associated with contextual cues by being paired post-trial with consolidation/re-consolidation. *Mini review. Pharmacol. Biochem. Behav.*, **192**: 172911.

Chen ZY and Zhang Y (2022). Animal models of Alzheimer's disease: Applications, evaluation and perspectives. *Zool. Res.*, **43**(6): 1026-1040.

Chidambara-Murthy KN, Jayaprakasha GK, and Singh RP (2002). Studies on antioxidant activity of pomegranate (Punica granatum) peel extract using in vivo models. *J. Agric. Food Chem.*, **50**: 4791-4795.

Chow CK, and Tappel AL (1971). An enzymatic protective mechanism against lipid peroxidation damage to lungs of ozone-exposed rats. *Lipids.*, **7**: 518-524.

Cowan ET, Fain M, O'Shea I, Ellman LM and Murty VP (2021). VTA and anterior hippocampus target dissociable neocortical networks for post-novelty enhancements. *J. Neurosci.*, **41**(38): 8040-8050.

Crisol L, Matorras R, Aspichueta F, Expósito A, Hernández ML, Ruiz-Larrea MB, Mendoza R and Ruiz-Sanz JI (2012). Glutathione peroxidase activity in seminal plasma and its relationship to classical sperm parameters and *in vitro* fertilization-intracytoplasmic sperm injection outcome. *Fertil. Steril.*, **97**(4): 852-857.

Dahl MJ, Kulesza A, Werkle-Bergner M, and Mather M (2023). Declining locus coeruleus-dopaminergic and noradrenergic modulation of long-term memory in aging and Alzheimer's disease. *Neurosci. Biobehav. Rev.*, **153**: 105358.

Eastman CL, D'Ambrosio R and Ganesh T (2020). Modulating neuroinflammation and oxidative stress to prevent epilepsy and improve outcomes after traumatic brain injury. *Neuropharmacology*, **172**: 107907.

Franzoni F, Scarfò G, Guidotti S, Fusi J, Asomov M and Pruneti C (2021). Oxidative stress and cognitive decline: The neuroprotective role of natural antioxidants. *Front. Neurosci.*, **15**: 729757.

Fresnoza SM, Batsikadze G, Müller LE, Rost C, Chamoun M, Paulus W, Kuo MF and Nitsche MA (2021). Inhibitory effect of apomorphine on focal and nonfocal plasticity in the human motor cortex. *Pharmaceutics*, **13**(5): 718.

Giri PM, Banerjee A, Ghosal A and Layek B (2024). Neuroinflammation in neurodegenerative disorders: current knowledge and therapeutic implications. *Int. J. Mol. Sci.*, **25**(7): 3995.

Goyal A, Verma A, Dubey N, Raghav J and Agrawal A (2022). Naringenin: A prospective therapeutic agent for Alzheimer's and Parkinson's disease. *J. Food Biochem.*, **46**(12): e14415.

Grunblatt E, Mandel S, Maor G and Youdim MB (2001). Effects of R- and S-apomorphine on MPTP-induced nigro-striatal dopamine neuronal loss. *J. Neurochem.*, **77**: 146-156.

Houldsworth A (2024). Role of oxidative stress in neurodegenerative disorders: A review of reactive oxygen species and prevention by antioxidants. *Brain Commun.*, **6**(1): fcad356.

Hoyos CM, Stephen Colagiuri, Turner A, Ireland C, Naismith SL and Duffy SL (2022). Brain oxidative stress and cognitive function in older adults with diabetes and pre-diabetes who are at risk for dementia. *Diabetes Res. Clin. Pract.*, **184**: 109178.

Ikram H and Haleem DJ (2019). Repeated treatment with a low dose of reserpine as a progressive model of Parkinson's dementia. *Pak. J. Pharm. Sci.*, **32**(2): 555-562.

Ikram H, Sheikh SA, Haleem DJ, Ganau M and Choudhry AM (2021). Dose related acute behavioral and neurochemical profile of pioglitazone. *Pak. J. Pharm. Sci.*, **34**(2): 615-620.

Ikram H, Zakir R and Haleem DJ (2018). Effects of single administration of apomorphine on memory and monoamine metabolism: A dose related study. *Pak. J. Pharm. Sci.*, **31**(2): 439–445.

Kamboj S, Sharma P, Kamboj R, Kamboj S, Hari Om, Girija, Guarve K, Dutt R, Verma I, Dua K and Rani N (2024). Exploring the therapeutic potential of phytoconstituents for addressing neurodegenerative disorders. *Cent. Nerv. Syst. Agents Med. Chem.*, **24**(2): 129-144.

Kassubek J, Factor SA, Balaguer E, Schwarz J, Chaudhuri KR, Isaacson SH, Wu S, Denecke Muhr C and Kulisevsky J (2024). Long-term safety, tolerability and efficacy of apomorphine sublingual film in patients with Parkinson's disease complicated by OFF episodes: A phase 3, open-label study. *J. Neurol.,* **271**(6): 3554-3570.

Metta V, Dhamija RK, Batzu L, Mrudula R, Kumar NSS, S A, Falup-Pecurariu C, Rodriguez-Blazquez C, Goyal V, LKP, Bhattacharya K, Kumar S, Chaudhuri KR, and Borgohain R (2023). Safety and tolerability of long-term apomorphine infusion in advanced Parkinson's disease: An Indian multi-center (APO-IND) experience. *Sci. Rep.,* **13**(1): 18681.

Osorio-Gómez D, Guzmán-Ramos K and Bermúdez-Rattoni F (2022). Dopamine activity on the perceptual salience for recognition memory. *Front. Behav. Neurosci.,* **16**: 963739.

Plascencia-Villa G and Perry G (2023). Roles of oxidative stress in synaptic dysfunction and neuronal cell death in Alzheimer's disease. *Antioxidants* (Basel). **12**(8): 1628.

Poudel P and Park S (2022). Recent advances in the treatment of Alzheimer's disease using nanoparticle-based drug delivery systems. *Pharmaceutics,* **14**(4): 835.

Ramassamy C, Krzywkowski P, Averill D, Lussier-Cacan S, Theroux L, Christen Y, Davignon J and Poirier J (2001). Impact of apoE deficiency on oxidative insults and antioxidant levels in the brain. *Brain Res. Mol. Brain Res.,* **86**(1-2): 76-83.

Ramli NZ, Yahaya MF, Tooyama I and Damanhuri HA (2020). A mechanistic evaluation of antioxidant nutraceuticals on their potential against age-associated neurodegenerative diseases. *Antioxidants* (Basel), **9**(10): 1019.

Reuben DB, Kremen S and Maust DT (2024). Dementia prevention and treatment: A narrative review. *JAMA Intern. Med.,* **184**(5):563-572.

Roda AR, Esquerda-Canals G, Martí-Clúa J and Villegas S (2020). Cognitive impairment in the 3xTg-AD mouse model of Alzheimer's disease is affected by aβ-immuno therapy and cognitive stimulation. *Pharmaceutics,* **12**(10): 944.

Sidiropoulou GA, Metaxas A and Kourti M (2023). Natural antioxidants that act against Alzheimer's disease through modulation of the NRF2 pathway: A focus on their molecular mechanisms of action. Front Endocrinol (Lausanne). **14**: 1217730.

Sienes Bailo P, Llorente Martín E, Calmarza P, Montolio Breva S, Bravo Gómez A, Pozo Giráldez A, Sánchez-Pascuala Callau JJ, Vaquer Santamaría JM, Dayaldasani Khialani A, Cerdá Micó C, Camps Andreu J, Sáez Tormo G and Fort Gallifa I (2022). The role of oxidative stress in neurodegenerative diseases and potential antioxidant therapies. *Adv. Lab Med.,* **3**(4): 342-360.

Sinha AK (1972). Colorimetric assay of catalase. *Anal Biochem.,* **47**: 389-394.

Ton AMM, Campagnaro BP, Alves GA, Aires R, Côco LZ, Arpini CM, Guerra E Oliveira T, Campos-Toimil M, Meyrelles SS, Pereira TMC and Vasquez EC (2020). Oxidative Stress and Dementia in Alzheimer's Patients: Effects of Synbiotic Supplementation. *Oxid. Med. Cell Longev.,* **2020**: 2638703.

Tse D, Privitera L, Norton AC, Gobbo F, Spooner P, Takeuchi T, Martin SJ and Morris RGM (2023). Cell-type-specific optogenetic stimulation of the locus coeruleus induces slow-onset potentiation and enhances everyday memory in rats. *Proc. Natl. Acad. Sci. USA.,* **120**(46): e2307275120.

Vural H, Demirin H, Kara Y, Eren I and Delibas N (2010) Alterations of plasma magnesium, copper, zinc, iron and selenium concentrations and some related erythrocyte antioxidant enzyme activities in patients with Alzheimer's disease. *J. Trace Elem Med Biol.,* **24**: 169-173.

Wagner MJ, Daniel CP, Plaisance CJ, Borne GE, Ahmadzadeh S, Shekoohi S, and Kaye AD (2023). Apomorphine for Parkinson's disease: Pharmacologic and clinical considerations. *Expert Opin Emerg Drugs,* **28**(4): 275-281.

Zhou Z, Yan Y, Gu H, Sun R, Liao Z, Xue K and Tang C (2024). Dopamine in the prefrontal cortex plays multiple roles in the executive function of patients with Parkinson's disease. *Neural. Regen. Res.,* **19**(8): 1759-1767.