Niger. J. Physiol. Sci. 39 (December 2024): 223 – 232
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Full length Research Article

Bromelain Improved Cognitive & Mood Behaviors, Oxido-Inflammatory Indices and Cholinergic Transmission in Scopolamine-Induced Neurotoxicity in Male Wistar Rats.

Bayo-Olugbami A.A.[1], Ukpabio P.[2], Babalola K.M.[3], Benson I.O.[4], Owoyele B.V.[5]

[1]*Neuroscience unit, Department of Physiology, Osun State University, Osogbo. Osogbo, Nigeria*
[2]*Department of Physiology, Adeleke University, Ede. Osun State, Nigeria*
[3]*Department of Anatomy, Federal University of Health Sciences, Ila-Orangun. Osun State, Nigeria*
[4]*Department of Anatomy, Osun State University, Osogbo. Osun State, Nigeria*
[5]*Neuroscience & Inflammation unit, Department of Physiology, University of Ilorin, Ilorin, Nigeria*

Summary: Alzheimer's disease (AD) is associated with pathophysiological and psychological disturbances including cognitive decline, depression, anxiety and motor imbalance. Conventional drugs for managing AD do not address associated non-cognitive co-morbidities. Hence, the need to investigate alternative therapies especially from plants. The neuroprotective benefits of bromelain have been identified, but its impacts on scopolamine-induced neurotoxicity is yet to be elucidated. Twenty-Five male rats were separated randomly into 5 groups: Control (normal saline; 1ml/kg); Scopolamine (i.p; 1mg/kg); Bromelain (50mg/kg); Scopolamine + Bromelain; Scopolamine + Donepezil (reference drug, 1mg/kg). Neurobehavioral paradigms (novel object recognition, elevated plus maze, forced swimming and open field tests were assessed, followed by biochemical assay of Malondialdehyde, MDA; Super oxide dismutase, SOD; Acetylcholinesterase, AChE; Nitric oxide, NO; Total protein & Interleukin 1β, IL-1β in the Prefrontal cortex (PFC). Data were analyzed using one-way ANOVA (Tukey's posthoc.). Values with $p<0.05$ were considered significant. Scopolamine reduced memory index ($P<0.01$) (cognitive function), ambulatory & rearing activities ($P<0.05$) (motor behavior), open arm duration ($P<0.001$) (anxiety-like behavior) & increased immobility time ($P<0.05$) (depressive-like behavior). Exposure to scopolamine also led to significant reduction in the prefrontal cortical level of SOD ($P<0.05$) while increasing MDA ($P<0.05$), acetylcholinesterase ($P<0.01$) and IL-1β ($P<0.05$). However, levels of NO and total protein were not significantly altered. In contrast, intervention with bromelain or donepezil significantly reversed most of the behavioral and biochemical alterations induced by scopolamine. Bromelain compared favorably with donepezil in improving memory decline and other non-cognitive dysfunctions associated with scopolamine exposure. This could have resulted from modulation of oxidative stress, inflammation and cholinergic transmission.

Keywords*: Bromelain; Scopolamine; Oxidative stress; Neurotoxicity; Cognition; Mood*.

**Authors for correspondence: adedamola.bayo-olugbami@uniosun.edu.ng , Tel: +234-8132794032*

Manuscript received- April 2024; Accepted: November 2024

DOI: https://doi.org/10.54548/njps.v39i2.8

INTRODUCTION

Cognitive dysfunction which characterizes Alzheimer's disease (AD) is caused by multiple factors including deposition of amyloid plaques, oxidative stress, neuro-inflammation and cholinergic dysfunction (Ayinla *et al*., 2020). Apart from cognitive decline, AD is associated with pathophysiological and psychological disturbances including motor imbalance, depression and anxiety, usually referred to as behavioral and psychological symptoms of dementia (BPSD) (Brechin *et al*., 2013). Up to 75% of patients with dementia experience these non-cognitive symptoms, which provide a significant challenge for patients and their carers, and are often difficult to manage. Conventional drugs for managing dementia do not address associated non-cognitive co-morbidities. Antipsychotic drugs have been widely used to treat non-cognitive symptoms of dementia. However, data emerged linking the use of some antipsychotic drugs with an increased risk of stroke and death in patients with dementia (Kales *et al*., 2012). Consequently, there has been a world-wide drive to seek appropriate non-pharmacologic interventions like diet modification and exercise (Livingston *et al*., 2005; Fernando *et al*. 2015). Hence the need to investigate alternative therapies especially from plants that can provide holistic protection or treatment.

Scopolamine is a non-selective post-synaptic muscarinic receptor blocker. It causes cognitive impairments in rodents and humans by decreasing the effectiveness of acetylcholine in the CNS (Kim *et al*., 2013). Though scopolamine is not a classical model for inducing experimental AD in preclinical

models, however, it presents one of the best approaches for understanding the mechanism of AD and other types of dementia; and also widely used as a valid pharmacological model for investigating and screening potential anti-amnesic molecules (Blokland, 2005; Haider *et al*., 2016).

The use of natural products in the prevention and treatment of diseases have gained significant attention. Bromelain is a group of sulfur-containing proteolytic enzymes present in pineapple (Ananas comosus), a tropical plant widely cultivated worldwide (Chaisakdanugull *et al*., 2007). It is made up of several proteinases, flavonoids and endopeptidases (Tochi *et al*., 2008) with many uses as natural anti-inflammatory, anti-oxidant, antithrombotic, and fibrinolytic agent. Because of its history of safety and little to no side effects, it has gained universal acceptance as a phytotherapeutic agent (Chakraborty et., 2021).

Bromelain reportedly reduced MDA and increased the concentrations of SOD, CAT, GPx in a rat model of bisphenol A-induced oxidative stress and testicular damage. Bromelain has antioxidant activities through free radical scavenging and up-regulation of enzymatic and non-enzymatic antioxidants (Agarwal *et al*., 2016). Another study demonstrated the beneficial effects of bromelain in reducing neurotoxicity through antioxidant and AChE-inhibitory effects (Bist *et al*., 2021). Bromelain elicits anti-inflammatory response by reducing PGE2 and COX-2 synthesis (Bhui *et al*., 2009). It acts as an antioxidant by stimulating antioxidant enzyme secretion of catalase, superoxide dismutase, and reduced glutathione via increased concentration of nuclear factors (NrF-1 and NrF-2) (Bakare & Owoyele, 2020). Many pharmacological benefits of bromelain have been identified, but its impacts on scopolamine-induced neurotoxicity is yet to be elucidated.

In a rat model of AlCl3-induced toxicity, treatment with bromelain ameliorated the effects of AlCl3 by restoring locomotion and exploratory activity, improving cognitive functions, and inducing anxiolytic effects. Further, the effects of bromelain were comparable to donepezil therapy (Eraky *et al*., 2023). Similarly, it was shown to reduce anxiety-like behavior in a rat model of neuropathic pain (NP) where it decreased the cortical levels of IL-1β, IL-6 and PGE2. It also reduced NF-κB, IL-1β, IL-6, TNF-α, PGE2, and nitrate concentrations as well as the expression of iNOS in the sciatic nerve. Hence, the anti-nociceptive and anxiolytic effects of bromelain in the sciatic nerve ligation model of NP was ascribed to its ability to reduce nitrosative and inflammatory activities (Bakare & Owoyele, 2021).

In this study, we investigated if scopolamine mechanistic model of studying dementia could show non-cognitive (anxiety- and depressive like) symptoms of dementia in addition to the classical cognitive impairment; and to determine the neuroprotective impact(s) of bromelain in comparison to donepezil (a reference drug) on both cognitive and non-cognitive symptoms that may be associated with scopolamine-induced neurotoxicity and its mechanism(s) of action.

MATERIALS AND METHODS

Experimental Animals: Twenty-five adult male rats with an average weight of 115 ± 5.1 g were obtained from the central animal house, University of Ibadan. The rats were kept in the animal facility of the Faculty of Basic Medical Sciences, Adeleke University, Ede, Nigeria. They were kept in polypropylene plastic cages with wood shavings as bedding at a room temperature of (27-30°C) with 12 h light/dark cycle. Rats had access to standard rodent pellet food and water ad libitum. Acclimatization was for 7 days. Approval for this study was provided by the Adeleke University Animal Care and Use Ethical Review Committee (Ethical approval number: AUERC/FBMS/20) which complies with the National Institutes of Health (NIH) Guideline for the Care and Use of Laboratory Animals.

Drug/chemical preparations and dosages: The drugs and chemicals used were of analytical grade. Scopolamine and donepezil were products of Macleods Pharma (Billingham, UK). Bromelain powder was produced by KAN Phytochemicals (Rai, India). The doses of bromelain (50 mg/kg) (Bakare & Owoyele, 2020; Bakare & Owoyele, 2021), scopolamine (1mg/kg) (Ayinla *et al*., 2019; Ayinla *et al*., 2020; Oladun *et al*., 2023), donepezil (1mg/kg) (Filarowska *et al*., 2016; Eraky *et al*., 2023) were selected according to findings from preliminary and previous reports. Saline (0.9%) was used to dissolve bromelain, scopolamine and donepezil and were administered at a volume of 5ml/kg of individual animal weight.

Experimental design: Twenty-five male Wistar rats were divided into five groups. Group one (control) received 5ml/kg of normal saline. Group 2 received 1mg/kg of scopolamine. Group 3 received 50mg/kg of Bromelain. Group 4 received both scopolamine and Bromelain. Group 5 received scopolamine and donepezil (Reference drug). The experiment lasted for 15 days. Bromelain or donepezil was administered for 14 days, with concurrent administration of scopolamine in the last 7 days. During co-administration, bromelain or donepezil was given 30 minutes before scopolamine. Behavioral assessments for motor function using horizontal bar test and open field test; cognitive function using novel object recognition test; anxiety using elevated plus maze test and open field test; and depression using forced swimming test were carried out on the last two days of the experiment. Rats were sacrificed on day 15 for biochemical processing. Cortical assessment of SOD, MDA, NO, AChE, total protein and IL-1b were quantified spectrophotometrically.

Animal grouping:

Control group: Normal saline at 5ml/kg orally for 14 days

Scopolamine group: 1mg/kg of Scopolamine (i.p.) in the last 7 days (Mostafa *et al*., 2021; Bayo-Olugbami *et al*., 2024).

Bromelain group: 50mg/kg of Bromelain orally for 14 days.

Bromelain + Scopolamine group: 50mg/kg of Bromelain (oral) for (14) days + concurrent administration of 1mg/kg of Scopolamine (i.p.) in the last 7 days

Scopolamine + Donepezil group: 1mg/kg of Donepezil (oral) for 14 days + concurrent administration of 1mg/kg of Scopolamine (i.p.) in the last 7 days.

Behavioral Assessment: Behavioral tests were carried out on the last two days of the experiment. Animals were evaluated for behavioral phenotypes in the following order: (a) open field & horizontal bar tests, for motor or exploratory assessment (b) novel object recognition test for assessment of cognitive function (c) elevated plus maze test for assessment of anxiety-like behavior and (d) forced swimming test for assessment of depressive-like behavior. All behavioral tests were carried out between 9:00 a.m.-1:00 p.m. each day by trained observers who were blind to the treatment groups.

Open field test: This was used to assess locomotion and exploratory activities of rats. An open wooden box, measuring 100 x 100 x 50 cm was used. The rat was placed at the centre square. A high resolution video recorder (Logitech C920 HD) kept at a safe distance to cover the entire field was used in recording the activities of each rat in the box for a duration of 6 minutes. The number of lines crossed (horizontal exploration), rearing (vertical exploration) and frequency of centre exploration were later scored by an independent investigator. The number of visit to the centre of the open field maze and fecal boli voided are another good indicator of anxiety-like behavior. This is done by estimating the frequency of visit to the central zone of the open field maze and the number of fecal pellets voided in the process of exploring the maze within 6mins. An increased central visit or reduced peripheral/wall zone exploration (thigmotaxis) and decreased fecal output are indicative of anxiolytic (reduced anxiety) propensity. Olfactory cue was reduced by removing rat's litters and cleaning the test field with 70% ethanol after each experiment.

Horizontal bar test: A horizontal bar 38 cm long, 3 cm in diameter, and 50 cm above the bench surface was used in a modified technique (Deacon, 2013), with a wooden support column at each end. Each rat was grasped by the tail, slanted backwards about 20 cm, and immediately raised to hold the centre point of the horizontal bar with its forepaws while the tail was released. The time taken by each rat to move through the bar was recorded in seconds.

Novel Object recognition test

Novel Object Recognition (NOR): This test assesses non-spatial short term memory in animals as originally described by Bevins and Besheer, (2006) but with slight modification by Bayo-Olugbami *et al*. (2020). These tests rely on the fact that rodents generally will preferentially explore a novel object over a previously experienced object. A 45cm by 50cm opaque box was used. Rats were allowed to explore two identical objects placed 5cm apart from each other and from the walls of the box for 10 minutes (training section) in trial 1 (T1). An inter trial period of about 30 minutes was allowed after which each rat was returned to the test field for another 6 minutes in T2, during which one of the old objects was replaced with a new object (test session). Exploration was scored positive when the nose or vibrissae is about 2cm from the object while sitting on the object was excluded. A rat not used in the experiment was allowed to explore the arena prior to acclimatization, training, and testing so that the field would have a familiar odor to the first rat to be tested each day. The placement of the objects was changed frequently while the box arena and objects used were thoroughly wiped with 70% ethanol after each test before introducing the next rat in order to reduce olfactory cues. The session was video recorded and later evaluated. The memory index was calculated as: (Time spent exploring new object/ total time spent exploring both objects) × 100

Elevated plus maze test: Elevated plus maze test (EPMT) is commonly used to assess anxiety-like behavior as previously described by Lister (1987). The rats were individually placed in the central zone facing one of the open arms. Video camera mounted above the maze connected to a computer was used to monitor and score the exploratory behavior during 5mins experimental period. The parameter measured was the duration of open arm entry. An entry was scored when the four paws of the animals were completely inside any of the arms. An increased open arm entry or exploration reflects a lesser tendency for anxiety behavior.

Forced swimming test: It was performed as originally described by Porsolt and co-workers (Porsolt *et al*., 1978). The apparatus consists of a clear plexiglass cylinder (20cm by 12cm) filled to a 15cm depth with water. Rats were pre-exposed to swimming environment for five minutes a day prior to the test. Thirty minutes after treatment, animals were gently placed inside the cylinder and allowed to swim for 6 minutes. Duration of Immobility was assessed during the last five minutes using an automated stopwatch. The rats were assumed immobile when they floated and made only movements necessary to keep their heads above water. Higher immobility time indicates depressive-like behavior (Yankelevitch-Yahav *et al*., 2015).

Animal sacrifice, sample collection and preparation of tissue for biochemical assays: After behavioral evaluations, rats were anaesthetized with ketamine (50mg/kg)/Xylazine (10mg/kg) cocktail, subjected to trans-cardiac perfusion using 50ml 0.1 M PBS (pH 7.4). Rat was decapitated and the excised brain (PFC) was rinsed in cold isotonic saline. PFC was isolated and homogenized over ice in 0.1 M cold sodium phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 rpm, 4oC for 10 min. The supernatant obtained was made into aliquots for determination of markers of oxidative stress, inflammation, and acetylcholinesterase activity.

Determination of oxidative stress markers: The superoxide dismutase (SOD): SOD activity was determined according to the method of Sun *et al*. (1988). The principle of the method is based on the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine–xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the lysate after 1.0 ml ethanol–chloroform mixture (5/3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate.

The Malondialdehyde (MDA): MDA, one of the by-products of peroxidation of polyunsaturated fatty acid was used to determine the oxidative stress levels in the prefrontal

cortex. This assay was performed according to the method of Ohkawa *et al*. (1979) in which MDA reacted with Thiobarbituric acid (TBA) in an acidic medium at 100°C to produce a pink/red-coloured product (Thiobarbituric acid reactive substances) extracted with butanol and measured using a spectrophometer at an absorbance of 520-535nm.

NO: Nitric oxide was assayed according to a previous method by Tracey *et al*. (1995) using Griess reagent system with a few modifications (Sun *et al*., 2003). 0.1% w/v NED solution (naphthyl ethylene diamine di-hydrochloride) was used. The reaction mixture containing 50 µl supernatant and 50µl PBS were incubated at 25°C for 15 min. Then 50 µl of sulphanilamide solution (1% sulphanilamide in 5% phosphoric acid) was added. After 5 min, the absorbance was measured at a wavelength of 540 nm against the corresponding blank solutions. Sodium nitrite was used as a standard sample.

Total protein: The total protein was determined according to the method of Bradford (1976) using Pierce Coomassie Protein assay kit, a commercially ready to use kit procured from Sigma, USA. When mixed with the protein solution, the acidic Coomasie –dye reagent changed color from brown to blue in proportion to the amount of protein present in the sample. Absorbance was read with a spectrophotometer at 595nm.

Determination of cholinergic marker: Estimation of acetyl-cholinesterase activity: AChE was quantified in the prefrontal cortex using the Ellman method (Ellman *et al*., 1961). In this test, the Ellman's reagent, 5, 5'- dithiobis (2-nitrobenzoate), (DTNB) was used to assay for free thiol groups. The phosphate buffer (2.7 mL) and 0.1 mL of DTNB were added to the homogenate and allowed to stand for 5 min. Following the addition of 0.1 mL of newly made acetylthiocholine iodide (pH 8), the absorbance was read at 412 nm.

Determination of Inflammatory marker: The concentration of IL-1b in the PFC was determined using enzyme-linked immunosorbent assay kits (Nanjing Mornmed Medical, Nanjing City, Jiangsu province, China) following the manufacturers' guidelines. All the measurements were done at room temperature using microplate reader with 450 nm filter (Micro READ 1000, Belgium). The concentrations of IL-1b was extrapolated from the standard curve and expressed as pg/mg protein.

Statistical Analysis: GraphPad Prism version 8.0 was used for all statistical analyses. All data were expressed as Mean±SEM. Differences among the groups were analyzed by one-way ANOVA using Tukey's post hoc for multiple comparison. P value < 0.05 was considered to be statistically significant.

RESULTS

Effects of bromelain on behavioral assessment in scopolamine-induced neurotoxicity: We investigated the impacts of scopolamine on cognitive and non-cognitive co-morbidities like motor, anxiety- and depressive-like behaviors using appropriate protocols; and also determined the neuroprotective effects of bromelain on such deficits.

Effects of bromelain on locomotion and exploration in the open field test (OFT) following scopolamine exposure:

Number of lines crossed: As shown in Fig 1a, rats in Scopolamine (SCOP) group had significantly lower total number of line crossed while exploring the maze compared to control ($p<0.05$ vs control group), indicating an impaired locomotor and exploratory activity. Number of lines crossed was markedly increased in Bromelain (BROM) only group compared with control ($p<0.01$ vs control group). Conversely, only BROM group significantly increased number of lines crossed when compared to SCOP group ($p<0.01$ vs SCOP group). SCOP+BROM group showed reduction in number of lines crossed compared with BROM only group ($p<0.01$). Horizontal exploration was also reduced in the SCOP+DONE group compared with SCOP+BROM group ($p<0.05$).

Rearing frequency: In Fig 1b, vertical exploration depicted by rearing frequency was significantly lower in the SCOP ($P<0.001$) and SCOP+DONE ($p<0.01$) groups when compared with control. However, BROM only ($p<0.01$) and SCOP+BROM ($p<0.05$) groups showed a marked increase in rearing frequency compared with SCOP group.

Time spent on bar: In Fig 1c, rats in SCOP group spent longer time moving through the bar compared with control ($p<0.05$). However, the groups that received BROM only ($p<0.01$), SCOP+BROM and SCOP+DONE ($p<0.05$) spent lesser time on bar which implies improved motor activity.

Centre exploration: As shown in Fig 1d, the time spent at the centre of the maze (an indicator of anxiety) was significantly lower in the SCOP group compared with control ($p<0.001$). In contrast, BROM only ($p<0.001$), SCOP+BROM and SCOP+DONE ($p<0.05$) showed marked increase in the number of times rats visited the centre of the maze.

Number of fecal boli: This is also used to estimate anxiety-like behavior. As shown in Fig 1e, rats in the SCOP group had significant increase in the number of fecal pellets when compared with control group ($p<0.05$), implying anxiety-like propensity. Only BROM group markedly reduced the number of fecal pellets compared with SCOP group ($p<0.05$).

Effects of bromelain on cognitive function in the novel object recognition test (NORT) following scopolamine exposure: Fig 2 shows that exposure to SCOP caused a significant reduction in memory index compared with control ($p<0.01$). This shows that the rats spent less time exploring the novel one, indicating a low novelty preference. Conversely, the groups that received BROM only ($p<0.05$), SCOP+BROM ($p<0.05$) and SCOP+DONE ($p<0.001$) had increased memory index compared with SCOP only group which implies an improvement in memory function.

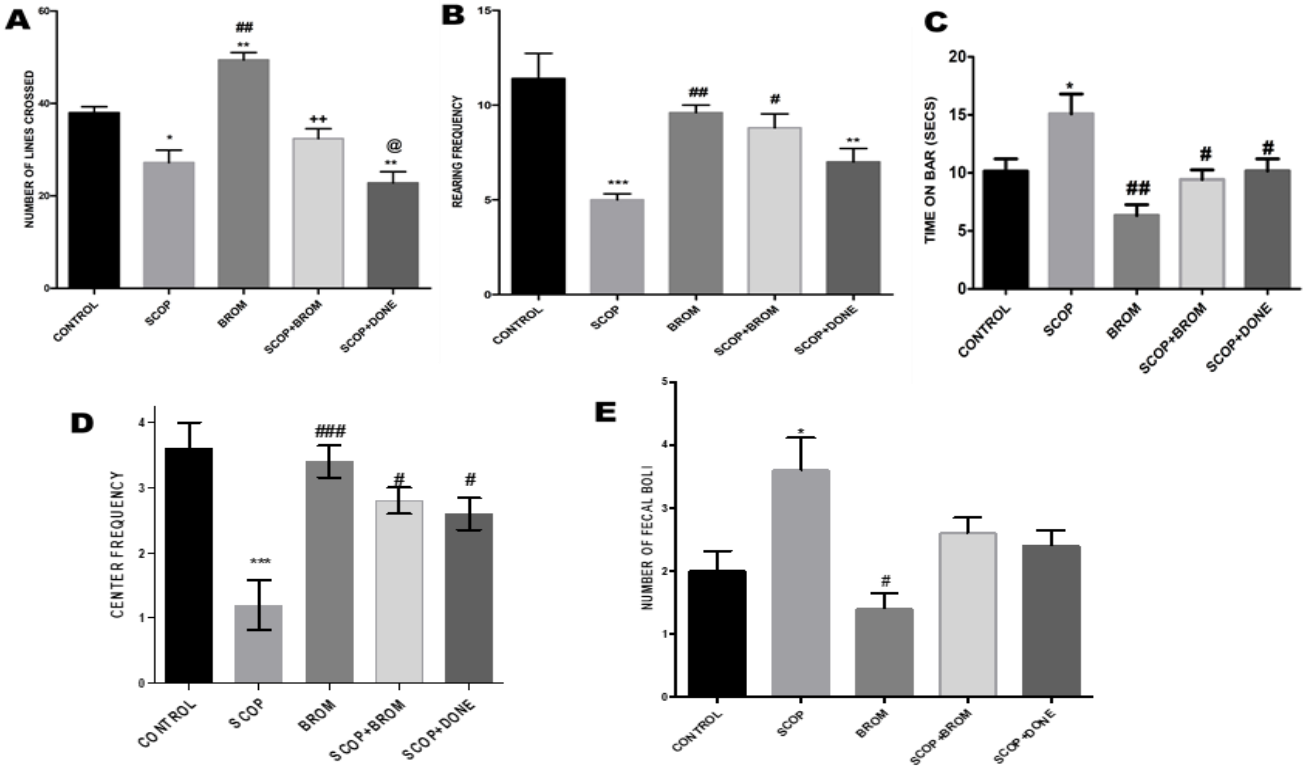

Fig 1: Effects of bromelain on the number of lines crossed (A), rearing frequency (B), time on bar (C), centre exploration (D) & number of fecal boli (E) in scopolamine induced neurotoxicity in rats.
Values are expressed as mean ± SEM (n=5).
*P<0.05 vs control, **P< 0.01 vs control, ***P< 0.001 vs control; $^{\#}$P<0.05 vs Scopolamine, $^{\#\#}$P<0.01 vs Scopolamine, $^{\#\#\#}$P<0.001 vs Scopolamine; $^{@}$P<0.05 vs Scop+Brom; $^{++}$P<0.01 vs Bromelain. SCOP: Scopolamine, BROM: Bromelain, DONE: Donepezil

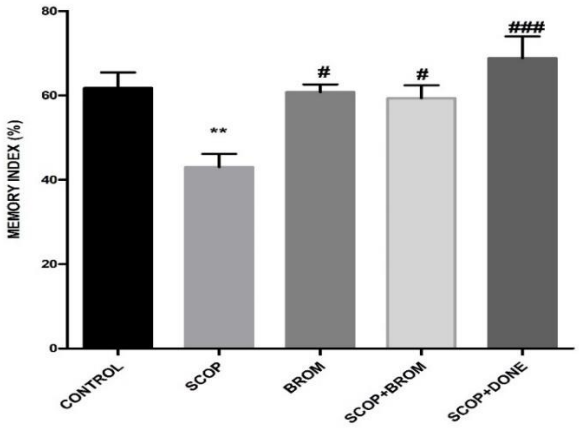

Figure 2:
Effects of bromelain on memory index in scopolamine induced neurotoxicity in rats**.**
Values are expressed as mean ± SEM (n=5).
**P< 0.01 vs control; $^{\#}$P<0.05 vs Scopolamine, $^{\#\#\#}$P<0.001 vs Scopolamine. SCOP: Scopolamine, BROM: Bromelain, DONE: Donepezil

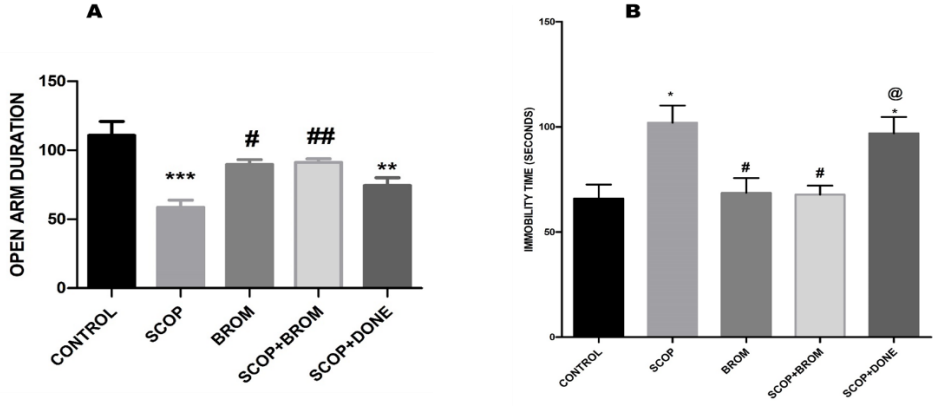

Fig 3: Effects of bromelain on open arm duration in EPM test (A) and immobility time in FST (B) in scopolamine-induced neurotoxicity in rats.
Values are expressed as mean ± SEM (n=5). *P<0.05 vs control, **P< 0.01 vs control, ***P< 0.001 vs control; #P<0.05 vs Scopolamine, ##P<0.01 vs Scopolamine; @P<0.05 vs Scop+Brom. SCOP: Scopolamine, BROM: Bromelain, DONE: Donepezil, EPM: elevated plus maze, FST: forced swimming test**.**

Effects of bromelain on mood (anxiety- and depression-like behavior) in the elevated plus maze and forced swimming test following scopolamine exposure: Time spent in the open arm: In Fig 3a, rats in the SCOP group ($p<0.001$) and SCOP+DONE ($p<0.01$) spent significantly lower time in the open arm of EPM compared with control rats. However, only BROM ($p<0.05$) and SCOP+BROM ($p<0.01$) groups showed significant increase in the time spent in open arm which indicates anxiolytic propensity. However, donepezil showed no anxiolytic property.

Immobility time: In Fig 3b, there was a significant increase in the immobility time in SCOP rats compared to control ($p<0.05$). This depicts depressive-like action. Administration of BROM only and SCOP+BROM ($p<0.05$) reduced the depressive-like symptom as depicted by a lower immobility time compared with SCOP untreated group. In addition, the immobility time in the SCOP+DONE group was also markedly higher compared to SCOP+BROM group ($p<0.05$). This shows that bromelain reduced depression but donepezil did not show anti-depressive propensity in scopolamine-induced neurotoxicity in rats.

Effects of bromelain on prefrontal cortical levels of oxidative stress, cholinergic and inflammatory markers following scopolamine exposure:

SOD: cortical SOD (an anti-oxidant enzyme) level was significantly lower in SCOP group compared to control ($p<0.05$). In contrast, rats in BROM, SCOP+BROM and SCOP+DONE ($p<0.01$) groups all had a marked increase in the level of SOD when compared with SCOP only, indicating an upregulation in the level of the anti-oxidant enzyme (Fig 4a).

MDA, a marker of lipid peroxidation was markedly reduced in the SCOP group ($p<0.05$) when compared with control. The groups that received BROM ($p<0.01$), SCOP+BROM ($p<0.01$) and SCOP+DONE ($p<0.001$) had a marked reduction in the level of MDA when compared with SCOP (Fig 4b).

NO: In Fig 4c, nitric oxide, a marker of oxidative stress was empirically increased in the SCOP group but significantly lower in BROM only group ($p<0.05$) when compared to control. However, BROM ($p<0.01$) and SCOP+BROM ($p<0.05$) groups had a significant reduction in NO compared with SCOP untreated rats. In addition, SCOP+DONE group showed marked reduction in the level of NO compared to BROM only group ($p<0.05$).

Total protein: Fig 4d shows an insignificant reduction in the level of total protein in the SCOP exposed rats when compared with control group. Only BROM group was able to increase TP level significantly compared with untreated SCOP rats ($p<0.05$). Similarly, intervention with bromelain and donepezil following scopolamine exposure also showed empirical increase in the level of total protein.

AChE: Prefrontal cortex level of AChE was markedly increased in SCOP exposed rats compared with control ($p<0.01$). The groups that received BROM ($p<0.05$), SCOP+BROM and SCOP+DONE ($p<0.001$) had significantly lower level of AChE compared with SCOP rats. This might be interpreted to mean that cholinergic activity was preserved as a result of reduced acetylcholinesterase activities (Fig 4e).

IL-1β: As shown in Fig 4f, the level of IL-1β was markedly increased in SCOP rats compared with control ($p<0.05$). Both BROM ($p<0.01$) and SCOP+BROM ($p<0.05$) groups showed a marked decline in the level of IL-1β when compared with SCOP only.

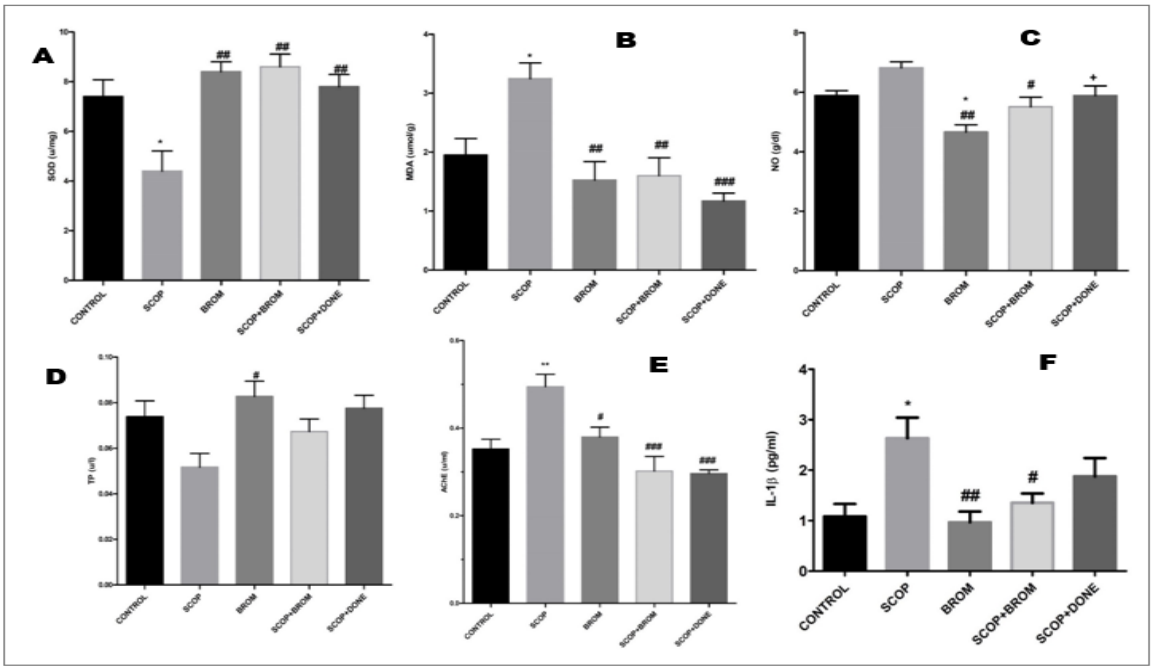

Figure 4:
Effects of bromelain on prefrontal cortical levels of SOD (A), MDA (B), NO (C), Total protein (D), AChE (E) & IL-1β (F) in scopolamine induced neurotoxicity in rats.
Values are expressed as mean ± SEM (n=5).
$^{*}P<0.05$ vs control, $^{**}P< 0.01$ vs control; $^{\#}P<0.05$ vs Scopolamine, $^{\#\#}P<0.01$ vs Scopolamine, $^{\#\#\#}P<0.001$ vs Scopolamine; $^{+}P<0.05$ vs Bromelain. SCOP: Scopolamine, BROM: Bromelain, DONE: Donepezil, SOD: Superoxide dismutase, MDA: Malondialdehyde, NO: Nitric oxide, AChE; Acetylcholinesterase, IL-1β: Interleukin-1β

DISCUSSION

Oxidative stress, neuro-inflammation and cholinergic dysfunction have been implicated in the pathomechanism of Alzheimer's disease (AD). AD is not just a cognitive disorder, but also associated with pathophysiological and psychological disturbances including motor imbalance, depression and anxiety which are often difficult to manage (Brechin *et al*., 2013). Drugs for managing dementia do not relieve the non-cognitive co-morbidities while the antipsychotic drugs used to treat non-cognitive symptoms of dementia put patients at the risk of stroke and sudden death (Kales *et al*., 2012). Non-pharmacologic interventions like alternative therapies from plant sources may be beneficial as there is increasing interest in the use of natural products which have the advantage of fewer side effects. As such, the present study investigated the neuroprotective effects of bromelain on cognitive, motor and mood (anxiety and depression) imbalance that may be associated with scopolamine induced neurotoxicity and its mechanism(s) of action.

In this study, intervention with bromelain ameliorated scopolamine-induced neurotoxicity by improving cognitive function, exploratory activities and inducing anxiolytic and anti-depressive effects. Furthermore, the effects of bromelain favorably compare with donepezil intervention. This corroborates a recent study wherein bromelain showed similar behavioral profile as ours, though in an aluminum chloride model of AD. However, while the mechanisms underlying their observation were modulations of TXNIP/ P-IRS-1/PI3K pathway and AChE inhibition (Eraky *et al*., 2023), we attribute our results to modulation of oxido-nitrosative stress, cholinergic transmission and inflammation. We observed anxiety- and depression-like symptoms in rats exposed to scopolamine. Anxiety and depression are common comorbid emotional deficits associated with dementia which adversely affect the patient's quality of life (Sinoff & Werner, 2003). Anxiety-like behavior is an early sign of AD and can predict future cognitive deterioration in elderly individuals (Donovan *et al*., 2018). Depression is also a predictor of preclinical cognitive deterioration in AD and is positively correlated with the risk of developing dementia (Byers & Yaffe, 2011). The use of open field, elevated plus maze and forced swimming tests in the analysis of anxiety-like and depressive behavior in rodents are well documented for screening potential anti-depressant and anxiolytic agents (Kremer *et al*., 2020). Our study design is a little deviation from most studies which often use scopolamine to model only cognitive deficit (Imran *et al*., 2020; Ayinla *et al*., 2019; Oladun *et al*., 2023; Ayinla *et al*., 2020), with the exception of very few studies that showed emotional disturbance following scopolamine exposure (Jafarian *et al*., 2019; Cheon *et al*., 2021). Anxiety and depression are common in dementia including Alzheimer's disease. A single or repeated administration of scopolamine to rodents at a dose that can induce cognitive impairment can in turn, induce anxiety- and depressive-like behaviors (Aydin *et al*., 2016; Rahmati *et al*., 2017) primarily by acting as a muscarinic acetylcholine receptor antagonist, disrupting the cholinergic system in the brain, which is crucial for cognitive function and mood regulation. Scopolamine blocks muscarinic acetylcholine receptors, disrupting the normal signaling pathways involve in learning, memory and mood regulation (Popovic *et al*., 2015). However, pretreatment with 50mg/kg dose of bromelain effectively prevented the anxiety-like and depressive-like behavior induced by scopolamine exposure suggesting that bromelain could be used as a prophylactic agent to manage mood disorder. This is in tandem with the report of Bakare &Owoyele (2021) wherein, a similar dose of bromelain reportedly attenuated anxiety and depressive-like comorbidity in a rat model of neuropathic pain. An in-vitro study reported that bromelain promoted the degradation of amyloid β1–42 monomers and soluble aggregates in the cerebrospinal fluid from patients with AD (Sancesario *et al*., 2018). Being an in-vitro study, there were no behavioral evidence to further validate this report. We also showed that bromelain improved memory index in the novel object recognition test (NORT) which further affirms earlier studies that reported that juice and ethanolic extract of pineapple significantly restored object recognition ability in mice treated with scopolamine. Similarly, peel extract of pineapple significantly attenuated high fat diet-induced reduction in correct alternation in Y-maze test and discrimination index in NORT (Ajayi *et al*., 2021). These findings suggest that pineapple has a protective role against cognitive disorders (Momtazi-borojeni *et al*., 2017). Similarly, bromelain was observed to be beneficial in healthy animals compared with control group. The translational implication of this finding is that bromelain could be consumed by healthy individuals or in non-pathological states to prevent the impacts of disorders associated with neuro-inflammation and oxidative stress. This observation corroborates the findings of (Bakare & Owoyele, (2021) and Bayo-Olugbami *et al*. (2024).

In addition to anxiety, the open field and horizontal bar tests showed that exploratory activity, rearing and movement on bar were decreased following scopolamine exposure. The state of anxiety is a typical reactionary response to dementia (Sinoff & Werner, 2003). The degree of anxiety is conventionally linked with the state of alertness during exploration in a novel environment (Ferdman *et al*., 2007). Therefore, the suppressed rearing and exploration is thought to have resulted from a feeling of apprehension, resulting from anxiety (Adu *et al*., 2022).

Acetylcholine is the major neurotransmitter that acts on widely distributed cholinergic receptors in the brain, and appropriate cholinergic neurotransmission is fundamental to learning and the formation of memory (Donovan *et al*., 2014). AChE hydrolyzes acetylcholine, thus diminishing cholinergic neurotransmission. Hence, the assessment of the in vitro and ex vivo activity of AChE proves valuable to assess cholinergic activity in the brain and can be correlated with cognitive function. The non-selective anti-muscarinic drug, scopolamine, passes through the blood–brain barrier (BBB) and mimics the memory impairment symptoms of AD and aging in rats by impairing cholinergic neuronal transmission (Lee *et al*., 2011). In our behavioral studies, scopolamine-induced memory deficit as depicted by reduced recognition index is a pointer to the fact that rats failed to show any preference for novelty in the NORT. On the contrary, rats treated with bromelain or donepezil, explored the novel object significantly. In furtherance, biochemical evaluation of the brain (PFC) also revealed that scopolamine-induced elevated AChE activity was reduced

by intervention with bromelain or donepezil, suggesting that inhibition of AChE might be one of the possible mechanisms behind this memory improvement. This agrees with the submission of Eraky *et al*. (2023) but opposes the findings of Chaudhary *et al.* (2018) which reported an increase in AChE level by bromelain following dichlovors toxicity. Consistent with our results, bromelain was previously shown to decrease hippocampal AChE levels in a mouse model of AD (Kumar *et al*., 2022). Donepezil is a selective, reversible, and centrally acting AChE inhibitor approved for mild to severe AD. Choline esterase inhibitors are the first class of drugs approved in the US and Europe for symptomatic relief of AD (Szeto & Lewis, 2016) and act by increasing brain ACh levels by preventing its degradation. Improving cholinergic neurotransmission remains a common therapeutic strategy for providing symptomatic relief in mild and moderate stages of AD (Marucci *et al*., 2021).

An imbalance in the antioxidant-oxidant defense mechanism leads to neurological disturbance and neuronal death (Yoshikawa and Naito, 2002). This imbalance was investigated in the current study by estimating the levels of SOD, MDA & NO, which are indices of oxidative stress. Exposure to scopolamine builds oxidative stress in the brain as evident from reduced SOD, increased NO and lipid peroxidation-marked by elevated MDA. This confirms the findings of Ayinla *et al*. (2019) and Ayinla *et al*. (2020). The brain is known to have high concentration of iron which may be involved in the generation of hydroxyl radicals by iron catalysis. It is rich in oxidizable fatty acids and comparatively low antioxidant resistance, which makes the organ more susceptible to oxidative stress (Chaudhary *et al*., 2018). Overproduction of ROS deteriorates cellular components of neurons, leading to oxidative stress, inflammation and consequently, increased risk of anxiety, depression and altered learning process. Moreover, the pathogenesis of a range of neurological diseases has a strong association with inflammation and oxidative stress (Salim, 2014). In the present study, treatment with bromelain decreased the levels of MDA and NO while it increased SOD following scopolamine exposure. Habashi *et al*. (2012) reported that bromelain significantly reduced the production of nitric oxide in the rat primary microglia. TBARS and PCC level which are markers of oxidative stress were also decreased by concomitant exposure of dichlorvos and bromelain in another study (Chaudhary *et al*., 2018). Bromelain is able to diminish oxidative stress by minimizing ROS production and halting protein oxidation. It therefore shows that bromelain exerts its protective action by altering the molecular cascades involving SOD, MDA and NO. This action against oxidative stress can be ascribed to the presence of cysteine, an amino acid with known antioxidant properties. Cysteine is also an important precursor in the production of antioxidant-glutathione, which protects cells from toxins. Thus, the cysteine in bromelain could be responsible for combating the oxidative stress (Piste, 2013).

Many studies have shown that sustained inflammation plays an important role in the progression of neurodegenerative disorders. Upregulation of central and peripheral pro-inflammatory cytokines have been implicated as neurobiological molecules that mediate the emotional comorbidity of dementia (Koprich *et al*., 2008). IL-1β receptors are located all over the brain, especially in the PFC, a brain region involved in cognitive, anxiogenic and depressive phenotypes (Lotrich, 2015). Our study shows that scopolamine promotes the elevation of central inflammatory mediator, IL-1β while treatment with bromelain inhibited it. This further confirms the anti-inflammatory property of bromelain as earlier reported (Adu *et al*., 2020; Bakare & Owoyele, 2021; Ajayi *et al*., 2021). Furthermore, our finding suggests that IL-1β might be responsible for the anxiety and depression associated with scopolamine induced neurotoxicity. Therefore, we suggest that the anti-anxio-depressive-like effects of bromelain may be due in part to its inhibitory effect on central IL-1β.

Taken together, to the best of our knowledge, this study is the first to demonstrate cognitive and non-cognitive effects of scopolamine in rat and the neuroprotective impacts of bromelain. Hence, we suggest bromelain as a potential natural source of functional food component that may be useful in preventing cognitive and neuropsychiatric disorders. However, we identify with the limitation of our study as we could not compare the observed anxiolytic and anti-depressive effect of bromelain with a reference drug used in managing anxiety and depression.

In conclusion, Bromelain compared favorably with donepezil in improving memory decline and other non-cognitive alterations associated with scopolamine exposure. This could have resulted from modulation of oxidative stress, cholinergic transmission and inflammation. Since bromelain is reportedly safe, its regular consumption is encouraged as a protective protocol against cognitive decline associated with neurodegenerative disorders and mood deficiencies.

REFERENCES

Adu T, Adu O & Mabandla M (2022). Effects of Bromelain on Noradrenaline Release During Anxiety-Like Behaviour Following Intra-Medial Forebrain Bundle 6-OHDA Injection in Rat Model of Parkinsonism.*Surg Res*, 4(2):1-7

Agarwal S, Chaudhary B, Bist R (2016). Bacoside A and bromelain relieve dichlorvos induced changes in oxidative responses in mice serum, *Chem. Biol. Interact*. 254: 173–178.

Ajayi AM, John KA, Emmanuel IB, Chidebe EO, Adedapo DAA (2021). High-fat diet-induced memory impairment and anxiety-like behavior in rats attenuated by peel extract of Ananas comosus fruit via atheroprotective, antioxidant and anti-inflammatory actions. *Metabolism Open* 9:100077

Aydin E, Hritcu L, Dogan G, Hayta S, Bagci E (2016). The effects of inhaled Pimpinella Peregrine Essential Oil on Scopolamine-induced Memory Impairment, Anxiety and depression in Laboratory Rats. *Mol Neurobiol*. 53 (9):6557-6567

Ayinla M T, Asuku A O, Bayo-Olugbami A, Ayeni O A, Abiola A A and Owoyele B V (2020). Melatonin and Vitamin C modulate cholinergic neurotransmission and oxidative stress in scopolamine-induced rat model of memory impairment *J. Afr. Ass. Physiol. Sci*. 8 (1): 50-58

Ayinla, M. T., Uthman, Y., Bayo-Olugbami, A., Oyewole, A. L. and Owoyele, B. V (2019). Effects of Ethylacetate Leaf Extracts of Ocimum gratissimum and Momordica charantia on Memory in Scopolamine-induced Dementia Rats. *Nigerian Journal of Biochemistry and Molecular Biology*; 34(1&2): 28-37

Bakare A.O & Owoyele B.V (2021). Bromelain reduced pro-inflammatory mediators as a common pathway that mediate antinociceptive and anti-anxiety effects in sciatic nerve ligated Wistar rats. *Scientific Reports* 11:289. doi.org/10.1038/s41598-020-79421-9

Bakare, A. O. & Owoyele, B. V (2020). Antinociceptive and neuroprotective effects of bromelain in chronic constriction injury-induced neuropathic pain in Wistar rats. *Korean J. Pain* 33, 13–22

Bayo-Olugbami A, Nafiu AB, Amin A, Ogundele OM, Lee CC & Owoyele BV (2020). Vitamin D attenuated 6-OHDA-induced behavioural deficits, dopamine dysmetabolism, oxidative stress, and neuro-inflammation in mice, *Nutritional Neuroscience*, DOI:10.1080/1028415X.2020.1815331

Bayo-Olugbami, A.A., Muritala, A.K., Abdur-Rahman, H., Bakare, A.O., Arogundade, T.T, Atere, T.G. and Owoyele, B.V. (2024). Bromelain administration ameliorates neurobehavioural deficits mediated by cadmium neurotoxicity via oxido-nitrosative stress, cholinergic and neuro-inflammatory modulations in male Wistar rats. Nig. J. Neurosci. 15(2). https://doi.org/10.47081/njn2024 .15.2/001

Bevins RA and Besheer J (2006). Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study recognition memory. *Nat Protoc*. 1: 1306–11.

Bhui K, Prasad S, George J, Shukla Y (2009). Bromelain inhibits COX-2 expression by blocking the activation of MAPK regulated NF-kappa B against skin tumor-initiation triggering mitochondrial death pathway. *Cancer Lett;* 282, 167–176.

Bist R, Chaudhary B, Bhatt D K (2021). Defensive proclivity of bacoside A and bromelain against oxidative stress and AChE gene expression induced by dichlorvos in the brain of Mus musculus, *Sci. Rep*. 11 (1): 3668.

Blokland, A. (2005). Scopolamine-induced deficits in cognitive performance: A review of animal studies. *Scopolamine Review*. 1: 1-76.

Bradford M.M (1976). A rapid sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 72:248-254

Brechin D *et al*. (2013). Alternatives to antipsychotic medication: psychological approaches in managing psychological and behavioural distress in people with dementia. Leicester: *British Psychological Society*,

Byers AL & Yaffe K (2011). Depression and risk of developing dementia, Nat. Rev. Neurol. 7 (6):323–331.

Chaisakdanugull C, Theerakulkait C, Wrolstad R E (2007) Pineapple juice and its fractions in enzymatic browning inhibition of banana. *J. Agric. Food Chem.* 55 (10): 4252–4257.

Chakraborty AJ, Mitra S, Tallei TE, Tareq AM, Nainu F, Cicia D, Dhama K, Emran TB, Simal-Gandara J, Capasso R (2021). Bromelain a Potential Bioactive Compound: A Comprehensive Overview from a Pharmacological Perspective. *Life* 11, 317.

Chaudhary B & Agarwal S, R (2018). Invulnerability of bromelain against oxidative degeneration and cholinergic deficits imposed by dichlorvos in mice brains. doi.org/10.1007/s11515-018-1479-1

Cheon SY, Koo BN, Kim SY, Kam EH, Nam J & Kim EJ (2021). Scopolamine promotes neuroinflammation and delirium-like neuropsychiatric disorder in mice. *Sci Rep*. 11:8376

Donovan NJ, Amariglio RE, Zoller AS, Rudel RK, Gomez-Isla T, Blacker D, *et al* (2014). Subjective cognitive concerns and neuropsychiatric predictors of progression to the early clinical stages of Alzheimer disease. *Am J Geriatr Psychiatry*. 22:1642–51. doi: 10.1016/j.jagp.2014.02.007

Donovan NJ, Locascio JJ, Marshall GA, Gatchel J, Hanseeuw BJ, Rentz DM, Johnson KA, Sperling RA (2018). Longitudinal association of amyloid beta and anxious-depressive symptoms in cognitively normal older adults, *Am. J. Psychiatry* 175 (6):530–537.

Ellman, G.L., Courtney, K.D., Andres, V. and Featherstone, R.M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharm*. 7(2):88-90.

Eraky S M, Ramadan N M, Abo El-Magd N F (2023). Ameliorative effects of bromelain on aluminum-induced Alzheimer's disease in rats through modulation of TXNIP pathway. *International Journal of Biological Macromolecules* 227 (2023) 1119–1131. https://doi.org/10.1016/j.ijbiomac.2022.11.291

Ferdman N, Murmu R, Bock J, *et al* (2007). Weaning age, social isolation, and gender, interact to determine adult explorative and social behavior, and dendritic and spine morphology in prefrontal cortex of rats. *Behavioural brain researc*h, 180: 174-182.

Fernando W.M.A.D.B., Martins I.J., Goozee K.G., Brennan C.S., Jayasena V & Martins R.N. (2015) The role of dietary coconut for the prevention and treatment of Alzheimer's disease: Potential mechanisms of action. *Br J Nutr*. 114:1–14.

Filarowska J, Silberring J, Kotlinska J H (2016). Cholinesterase inhibitors, donepezil and rivastigmine, attenuate spatial memory and cognitive flexibility impairment induced by acute ethanol in the Barnes maze task in rats, Naunyn Schmiedeberg's Arch. *Pharmacol*. 389 (10): 1059–1071.

Habashi S A, Moghimi A, Sabouni F, Majd S A (2012). Inhibition of NO production in LPS-stimulated primary rat microglial cells by Bromelain. *J of Cell and Mol Res*, 3(2): 57–65

Haider, S., Tabassum, S., Perveen, T. (2016). Scopolamine-induced greater alterations in neurochemical profile and increased oxidative stress demonstrated a better model of dementia: a comparative study. *Brain Research Bulletin* 127: 234–247.

Imran I, Javaid S, Waheed A, Rasool MF, Majeed A, Samad N, Saeed H, Alqahtani F, Ahmed MM and Alaqil FA (2021) Grewia asiatica Berry Juice Diminishes Anxiety, Depression, and Scopolamine-Induced Learning and Memory Impairment in Behavioral Experimental Animal Models. *Front. Nutr*. 7:587367. doi: 10.3389/fnut.2020.587367

Jafarian S, Ling KH, Hassan Z, Perimal-Lewis L, Sulaiman MR, Perimal EK (2019). Effects of Zerumbone on scopolamine-induced memory impairment and anxiety-like behaviours in rats. *Alzheimers Dement*. 5:637-643

Kales HC *et al*. (2012). Risk of mortality among individual antipsychotics in patients with dementia. *Am J Psychiatry*; 169: 71-9.

Kim EJ, Jung IH, Jeong JJ, Kim NJ, Kim DH (2013). Ginsenosides Rg5 and Rh3 protect scopolamine-induced memory deficits in mice. *Journal of Ethnopharmacology* 146: 294-299.

Koprich JB, Reske-Nielsen C, Mithal P, Isacson O (2008). Neuroinflammation mediated by IL-1β increases susceptibility of dopamine neurons to degeneration in an animal model of Parkinson's disease, *J. Neuroinflamm*. 5:8–14.

Kremer M, Becker LJ, Barrot M & Yalcin I (2020). How to study anxiety and depression in rodent models of chronic pain? *Eur. J. Neurosci*. 2020, 1–35.

Kumar R, Kumar R, Sharma N, Khurana N, Singh SK, Satija S, Mehta M, Vyas M (2022). Pharmacological evaluation of bromelain in mouse model of Alzheimer's disease, *NeuroToxicology* 90:19–34.

Lee B, Shim I, Lee H, Hahm DH (2011). Rehmannia glutinosa ameliorates scopolamine-induced learning and memory impairment in rats. *J Microbiol Biotechnol*.21:874–83

Lister RG (1987). The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology* 92:180e5.

Livingston G *et al*., (2005). Systematic review of psychological approaches to the management of neuropsychiatric symptoms of dementia. *Am J Psychiatry*; 162: 1996-2021.

Lotrich FE (2015). Inflammatory cytokine-associated depression. *Brain Res*, 1617:113e25.

Marucci G, Buccioni M, Ben DD, Lambertucci C, Volpini R, Amenta F (2021). Efficacy of acetylcholinesterase inhibitors in Alzheimer's disease, *Neuropharmacology* 190:108352.

Momtazi-borojeni AA, Sadeghi-Aliabadi H, Rabbani M, Ghannadi A & Abdollahi E (2017). Cognitive enhancing of pineapple extract and juice in scopolamine-induced amnesia in mice. *Research in Pharmaceutical Sciences*, 12(3): 257-264.

Mostafa, N.M.; Mostafa, A.M.; Ashour, M.L.; Elhady, S.S (2021). Neuroprotective Effects of Black Pepper Cold-Pressed Oil on Scopolamine-Induced Oxidative Stress and Memory Impairment in Rats. *Antioxidants*, 10:1993. https://doi.org/10.3390/ antiox10121993

Ohkawa, H., Ohishi, N., Yagi, K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry*. 95(2):351-8.

Oladun B T, Oyerinde A, Oyadeyi A S, Badmus H A, Onasanwo A S (2023). Effect of Cocos nucifera L. Water on Scopolamine-Induced Memory Impairment in the Wistar Rat. *Nig J Neurosci* 14(1): 17-25

Piste P. (2013) Cysteine–Master Antioxidant. *Int. J. Pharmaceut. Chem. Bio. Sci*. 3, 143–149.

Popovic M, Gimenez de Bejar V, Popovic N, Caballero-Bleda M (2015). Time course of scopolamine effect on memory consolidation and forgetting in rats. *Neurobiol Learn Mem*. 118: 49-54

Porsolt, R. D., Anton, G., Blavet, N., & Jalfre, M. (1978). Behavioural despair in rats: a new model sensitive to antidepressant treatments. *European Journal of Pharmacology*, 47(4), 379–391. doi.org/10.1016/0014-2999(78)90118-8

Rahmati B, Kiasalari Z, Roghani M, Khalili M, Ansari F (2017). Antidepressant and anxiolytic activity of Lavandula Officinalis aerial parts hydroalcoholic extract in scopolamine-treated rats. *Pharm Biol*, 55(1): 958-965

Salim S (2014). Oxidative stress and psychological disorders. *Curr Neuropharmacol*. 12:140–7

Sancesario GM, Nuccetelli M, Cerri A, Zegeer J, Severini C, Ciotti MT, Pieri M, Martorana A, Caltagirone C, Nistico R, Bernardini S (2018). Bromelain degrades Aβ1-42 monomers and soluble aggregates: an in vitro study in cerebrospinal fluid of Alzheimer's disease patients, *Curr. Alzheimer Res*. 15 (7):628–636.

Sinoff G & Werner P (2003). Anxiety disorder and accompanying subjective memory loss in the elderly as a predictor of future cognitive decline, *Int. J. Geriatr. Psychiatry* 18 (10):951–959.

Szeto JY & Lewis SJ (2016). Current treatment options for Alzheimer's disease and Parkinson's disease dementia, *Curr. Neuropharmacol.* 14 (4):326–338.

Tochi B.N, Wang Z, Xu S.Y, Zhang W (2008). Therapeutic application of pineapple protease (Bromelain): A review. *Pakistan J. Nutr*. 7, 513–520.

Tracey, W.R., J. Tse, and G. Carter (1995) Lipopolysaccharide-induced changes in plasma nitrite and nitrate concentrations in rats and mice: pharmacological evaluation of nitric oxide synthase inhibitors. *J Pharmacol Exp Ther*. 272(3): 1011-5.

Yankelevitch-Yahav R, Franko M, Huly A, Doron R. (2015). The forced swim test as a model of depressive-like behavior. *J Vis Exp*. e52587.

Yoshikawa T & Naito Y (2002). What Is Oxidative Stress? *J of the Japan Med Association*, 45(7): 271–2.