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BETA-GLUCOGALLIN PREVENTS SCOPOLAMINE-INDUCED AMNESIA *VIA* CHOLINERGIC MODULATION AND ANTI-OXIDATIVE EFFECTS IN MICE

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ABSTRACT: Background: Beta-glucogallin (BGG) is a tannin precursor naturally found in a variety of plants such as gooseberry, raspberry, amla, *etc.* BGG was reported to be a potential therapeutic agent in managing a variety of diseases. This investigation was intended to assess whether BGG alleviates amnesia induced by scopolamine in mice. We also investigated possible mechanisms associated with the cholinergic system and antioxidant effects. **Materials and Methods:** Two doses of BGG (5 & 10 mg/kg) were tested in the exteroceptive and interoceptive amnesia model: Morris Water Maze test, Elevated plus-maze model, scopolamine-induced amnesia and assessment of motor coordination activity. The assessment of in vitro antioxidant activity by DPPH method and molecular docking analysis was also performed. **Results:** Pre-treatment with BGG at doses 5 and 10 mg/kg have shown significant anti-amnesia activity in the behavioral models in a dose-dependent manner.BGG at a lower dose did not impact the motor coordination activity of mice; however, at a higher dose, the results did not show a major impact, though the results look statistically significant compared with the control group. The results also showed that the radical scavenging activity of BGG increased with increasing concentration. **Conclusion:** This investigation indicates that BGG exerts potent anti-amnesic effects *via* cholinergic modulation and antioxidant activity, thus providing evidence for its potential as a cognitive enhancer for amnesia.

INTRODUCTION: Today, deprived memory, sluggish recall, and lesser retention are widespread concerns worldwide. Alzheimer's disease (AD) is the most common neurodegenerative disease, which affects memory and the brain, and hence it is considered the leading cause of dementia in the aged population [1, 2].

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The prevention or treatment of amnesia is thus an urgent issue to address. As many countries are transforming into aging societies, an increasing number of individuals in elderly populations suffers from memory loss and amnesia: worldwide cost of care and medicine for amnesia was reported to be 600 billion dollars in the last decade and has been increasing annually [1].

Synthetic drugs are useful for managing AD and many other chronic illnesses; still, there are side effects [3]. Consequently, the attention of researchers has inclined toward phytochemicals as promising therapeutic agents. Many are anti-inflammatory, antioxidant and possess anticholinesterase activities

with minimal side effects. Furthermore, herbs regulate mitochondrial stress, apoptotic factors, free radical scavenging systems, and neurotrophic factors [4]. The changes in the neurotransmitter acetylcholine (ACh) levels, which is released *via* cholinergic projections to the brain areas from the basal forebrain, have been reported to affect cognitive function and have been implicated in memory loss. Therapeutic interventions aiming to alleviate cognitive impairments have thus targeted ACh regulation and degradation; acetylcholine esterase (AChE) inhibitors blocking ACh hydrolysis have been administered to treat amnesia as a decrease of ACh level in the brain is responsible primarily for impaired cognition of AD [4].

Oxidative stress refers to the physiological condition at which the capacity of the endogenous antioxidant system fails to cope with the damaging effects of free radicals [6]. Strong experimental evidence has been established about the oxidative stress theory of AD pathogenesis, where oxidative damage plays a major role in neurological degeneration [2]. However, these free radicals have short half-lives and adverse reactions, including hepatotoxicity and nausea [7]. By contrast, medicinal herbs and plants exhibit fewer side effects and drug interactions; identifying effective alternative medicines could thus yield valuable contributions to the treatment of amnesia [8].

Traditional medicine still contributes significantly to the treatment regimen in developing nations [9]. India has a rich history of ayurvedic medicines, which have provided effective treatment for numerous disease-like conditions using plants, plant parts, and plant-derived compounds [9]. Beta-glucogallin (BGG) is an important tannin precursor naturally found in a variety of plants such as gooseberry (fruits of *Embellica officinalis*), raspberry, amla fruit extracts, and date palms (β-D-glucogallin present in fruits of *Phoenix dactylifera* L.var.), *etc* [10, 12]. BGG was reported to be a potential therapeutic agent in managing a variety of diseases, including diabetic complications such as diabetic cataracts, prevention of cataract development and progression, retinal degradation in diabetic eyes, hyperglycemia, and inflammatory diseases and associated stress [10, 15]. It was found to possess hepatoprotective, anti-hyperlipidemic, nephroprotective, cardioprotective, and significant photoprotective efficacy against UV-induced cytotoxicity and enhanced melanogenesis. β-D-glucogallin was reported to possess antioxidant, anticancer, antibacterial, antimutagenic, and antiprotozoal activities [10, 15]. This investigation was intended to assess the anti-amnesic modulating potential of BGG, so we evaluated the memory-enhancing effects of BGG on scopolamine-treated mice using the Morris water maze test. We subsequently investigated the levels of AChE, the brain. To elucidate any anti-oxidative activities, we also ascertained the effects of BGG on reactive oxygen species. We, all the authors, declare no conflict of interest. We thank Dr. Atul R. Chopade from Rajarambapu College of Pharmacy, Kasegaon, and Dr. Prafulla Chaudhary from Bharati Vidyapeeth College of Pharmacy, Kolhapur, for their support and guidance in conducting interoceptive/exteroceptive studies and molecular modeling, respectively.

MATERIAL AND METHODS:

Animals Used: Swiss albino mice of either sex were used in the present study. Young mice 2-3 months old, weighing approximately 20g, and aged mice, 12-15-month-old mice, weighing approximately 30 g, were utilized. Mice were grouped as 6/cage and housed in the standard laboratory conditions of light (12 h each of dark and light cycles) and temperature. Food and water provided *ad libitum*.

The animals were kept at controlled room temperature (24 ± 2 ºC) and humidity (55 ± 5%). The experiments were carried out from 9 am to 3 pm. Animals were fasted (of food but not water) for 12 h before the set of experimental trials. The acclimatization of mice to the laboratory environment was ensured (*i.e.,* housed for at least 10 days before the first set of trials). The experimental protocols were developed as per the ethical principles/ guidelines and are approved by institutional animal ethical committee (constituted for the purpose of control and supervision of experimental animals by the ministry of Environmental and Forests, Government of India, New Delhi) and were followed during the conduct per the guidance stated above. The approved protocol number was RCP/18 19/P-19.

Test samples and Chemical Agents: The BGG [(2S, 3R, 4S, 5S, 6R)-3, 4, 5-trihydroxy-6-(hydroxymethyl) oxan-2-yl] 3, 4, 5-trihydroxybenzoate (Product code: G012; Lot. no.: T18D239) was purchased from Natural Remedies Pvt. Ltd., Bangalore. The manufacturer determined the purity of BGG by HPLC area normalization and was certified as 95.4%. Sodium chloride (NaCl), potassium bromide (KBr) (all from Loba chemicals), and Scopolamine (Sco) of Alcon Inc were used in this investigation. Diazepam of Ranbaxy laboratories and Piracetam (Pir) of Dr. Reddy's Laboratories Ltd were purchased from a local medical shop.

Effects of BGG on Escape Latency in Young Mice Using Morris Water Maze Test and Scopolamine-Induced Amnesia [16, 17]: The Morris Water Maze (MWM) is a widely used test that evaluates the drug's potential in improving learning and memory. This model throws light on the phytochemicals' ability to enhance cognitive functions impaired by scopolamine, thereby emphasizing its utility in cognitive disorders involving dementia. The MWM consists of a large water tank [48 × 28 × 18] cm filled with water, which is made opaque by adding milk. Water helps to eliminate olfactory obstructions and provides an even unvarying environment within the maze. A [7 × 7] cm rectangular escape platform is constructed of water-resistant material (plexiglass in this study) that allows experimental animals to stay on top when submerged. The platform is 10 cm in height, and water is filled so that it is submerged 2 cm below the level of the water surface. The water temperature is maintained at 26 ± 1 ºC.

To assess spatial memory, a young mouse with a head pointing towards the side of the pool was released and the time taken (escape latency [EL]) to reach the submerged platform was noted. With previous exposure to this set up, the time the mouse is taking to find a hidden platform using only available external cues utilized to quantify the spatial memory. For acclimatization, the mice were permitted to swim for 90s before the hidden platform training began. Then the platform is positioned in the middle of the target quadrant of the pool, and the animals are released into the pool from the opposite quadrant. Each mouse was given 90s to reach the platform. If the animal fails to locate the platform in 90s, then the animal is guided to the platform by the researcher. Then the mouse is allowed to remain on the platform for 20s to rest. Again, the mouse was released from the same place, and the time for reaching the submerged platform was recorded. Likewise, a total of 4 trials were conducted in a row, in a day and the average time to reach the submerged platform was recorded, keeping similar experimental conditions.

Standard drug (Piracetam 200 mg/kg) and BGG in two doses, *i.e.*, 05 and 10 mg/kg, were administered *i.p.* and after 60 min, all the groups were exposed to the training schedule. This procedure was repeated at 24 h intervals for three more days until each subject acquired a minimum time interval to reach the submerged platform in the pool. On the fourth day, all groups were administered scopolamine (1 mg/kg, *i.p.*) 30 min later, they were treated with BGG, and after 60 min, they were tested for spatial memory. Latency to reach the platform in seconds (mean values) was calculated on days 1, 2, 3, 4, and 5 Day 2 is the day from which animals were treated with the drug. The mice assessed again on the fifth day for spatial memory to check the ability of BGG to restore scopolamine-induced amnesia (retention trial).

Effects of BGG on Transfer Latency in Young Versus Aged Mice Using Elevated Plus Maze Apparatus and with Scopolamine-Induced Amnesia [18]: Both young and aged mice were utilized in this behavioral model. The BGG (two doses, *i.e.,* 05 and 10 mg/kg) was administered intraperitoneally for eight consecutive days to mice of both age groups (*i.e.,* aged and young). On the eighth day, scopolamine 1 mg/kg was given intraperitoneally post 60 minutes of the last dose of BGG to induce amnesia in young mice. Post 45 min of scopolamine treatment, animals were permitted to the training session on elevated plus maze (EPM) apparatus. The transfer latency (TL), *i.e.,* moved from the open arm into any one of the closed arms of EPM apparatus recorded. The TLs recoded on the eighth day presented as results of the acquisition trail. On the ninth day (*i.e.,* after 24 h), the mice were assessed again on EPM to record retention of memory (Retention trail). Piracetam (200 mg/kg, *i.p.*) was used as the reference standard and was injected for eight consecutive days, and procedures outlined in the above

paragraph are followed. Similarly, animals in the control group received normal saline for 8 consecutive days. The EPM apparatus as described by Pellow *et al.*, and for mice as specified by Lister was utilized in this study. The EPM test apparatus is comprised of enclosed arms sized [37 × 5 × 12] cm and open arms sized [37 × 5] cm and a 12 cm high wall linked to 2 closed and 2 open arms and the wall is placed so that the same arms were opposite to each other and all four arms are linked to each other by a [5 × 5] cm of the central square. The wooden apparatus was elevated to 25 cm above the floor. Each mouse was placed individually in the central square with the head pointing towards the open arm and TL recorded for 5 min. Each mouse was utilized only once, and every test was conducted during the scheduled time, *i.e.*, per time and other conditions specified above in Section 2.1. After each test was carried out, the EPM apparatus was cleaned using hydrogen peroxide. The rationale behind utilizing EPM apparatus included the fear-provoking nature of the open arms and feeling of relative safety towards closed arms and assessing the memory retention of animals to prefer closed arms over open arms. The EPM test is a widely utilized behavioral animal model for assessing memory and learning in rodents.

Assessment of Motor Coordination [18]: The complex system of motor coordination involves a specific pattern of walking, balancing, and strength of muscle. It is a well-established fact that sedatives (such as benzodiazepines, barbiturates, *etc.*) and other molecules/drugs that interfere with balancing or ambulatory activities or weaken muscles have demonstrated impaired performance in the tests conducted Rota-rod apparatus. Hence, the Rota-rod apparatus is used popularly to estimate the potential impact of test drugs on the motor coordination of rodents. The Rota-rod apparatus (model - K19616-2 Inco, Ambala) is comprised of a central bar (with a constant speed of 22 rpm) subdivided into 3 compartments by disks. A day (24 h) before actual testing, mice were selected. The animals that failed to remain on the central bar for 150 seconds in two consecutive trials were excluded. Selected mice treated with BGG in two doses, *i.e.,* 05 and 10 mg/kg or standard drug (Diazepam 2 mg/kg) or vehicle as per the group and tests carried out 30 min post-treatment. The outcome measure included the time when mice remained on the revolving bar. The cut-off time for each test was 150 sec.

Estimation of Acetylcholine [ACh] Levels in Brain by Quantifying Cholinesterase Inhibition [19]: After completing the Morris Water maze test, the mice from each group were sacrificed by cervical dislocation. The whole brain was immediately removed and chilled in ice-cold phosphate buffer. After washing in ice-cold phosphate buffer, it was homogenized in 5 ml of phosphate buffer in a Glass Teflon homogenizer. The brain homogenate is then evaluated for enzyme activity.

Standard Curve of ACh: Aliquots of 0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 ml of ACh in buffer added to different test tubes. Phosphate buffer was added to each tube to give a volume of 1 ml. 2 ml of Alkali was added to each tube by shaking vigorously. After not less than a minute, 1 ml of HCL solution (pH of 1.2 ± 0.2) and 1 ml of $FeCl_3$ solution were added. The absorbance of the colour in each tube read at 540 nm. The control tube (with 0 ml of Ach) was used to adjust the zero reading of the instrument. The plot of millimoles of Ach *vs.* absorbance obtained.

Determination of Cholinesterase Inhibition: Three types of test tubes were prepared: Tube 1: served as control that contained 1 ml of buffer (instead of ACh solution) and other reagents. Tube 2: served as sample or S that contained 1 ml of ACh solution, 0.1 ml of homogenate, and was kept for incubation at 37 °C ± 1 °C for 1 h. Tube 3: served as STD or S60 that contained 1 ml of ACh solution and 0.1 ml of homogenate was added after the addition of Alkhyd, which itself was added after incubation at 37 ºC ± 1 ºC for 1 hr. After the incubation period, 2ml of Alkhyd was added by shaking vigorously to tubes 1 and 2. After not less than a minute, 1 ml of HCL solution (pH of 1.2 ± 0.2) and 1 ml of $FeCl_3$ were added to all three test tubes. The resultant mixtures were centrifuged, and the absorbance of the supernatant read at 540 nm. Note: S60 was used to correct the determination of non-enzymatic hydrolysis of ACh since the homogenate was added after incubation. The

control tube (with 1 ml of buffer) was used to adjust the zero of the instrument.

Assessment of Antioxidant Activity Using DPPH [20]: DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. Due to its odd electron, the methanolic solution of DPPH shows a strong absorption band at 517 nm. DPPH radicals react with suitable reducing agents, and then electrons become paired off, and the solution loses colour stoichiometrically with the number of electrons taken up. Such reactivity has been widely used to test the ability of compounds /plant extracts to act as free radical scavengers. DPPH radicals can be reduced by the decrease in absorbance at 517 nm. Ascorbic acid was used as a reference compound. To the methanolic solution of DPPH, an equal volume of test compound or ascorbic acid was added at different concentrations. An equal volume of methanol was added to the control also. The above mixtures were kept for incubation at room temperature for about 20 min. Then absorbance was recorded at 517 nm, and the percentage inhibition was calculated by using the formula mentioned below

Scavenging activity (%) = Absorbance of control - Absorbance of test × 100 / Absorbance of control

Docking Methodology [18, 21, 26]: Identification of the possible mode of action of the BGG for the anti-Alzheimer potential docking simulations was performed with the crystal structure of acetylcholine esterase complexed with anti-Alzheimer drug E2020 (1EVE), Structure of Beta-Secretase Complexed with Inhibitor (1FKN), Binary complex structure of human tau protein kinase I with AMPPNP(1J1B), the Crystal structure of mouse acetylcholine esterase in the apo form (1J06), the Crystal structure of human butyrylcholinesterase (1P0I), Human Monoamine Oxidase B in complex with Deprenyl (2BYB), Torpedo californica acetylcholine esterase in complex with 20mM thiocholine (2C5G). Crystal structures of all these selected targets were downloaded from the free protein database www.rcsb.org. The protein structure was refined in the biopredicta module via the removal of the water molecule and restoring native hydrogens. BGG structure was downloaded from the https://pubchem.ncbi.nlm.nih.gov/ in SDF format and developed in the V life engine, and optimized via the application of the MMFF.

RESULTS:

Assessing Improvement in Cognitive Functions Using Morris Water Maze Test: Lowering of escape latency is an indication of improvement in learning and spatial memory. Pre-treatment with BGG (5 mg/kg and 10 mg/kg) significantly decreased the escape latency to reach the platform on both 4th and 5th days in comparison with the results of the scopolamine group.

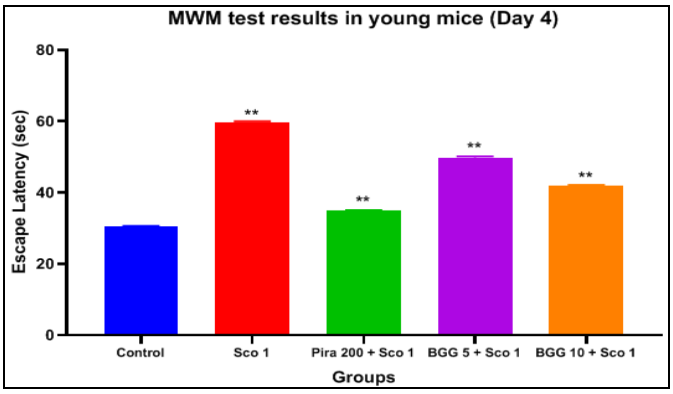

FIG. 1: MORRIS WATER MAZE TASK EFFECTS OBSERVED IN YOUNG MICE OF BETA-GLUCOGALLIN IN SCOPOLAMINE-INDUCED AMNESIA ON DAY 4. Values are expressed as Mean ± SEM. (N = 6), **P < 0.01, when compared to normal control group by one-way ANOVA followed by DUNNETT'S test.

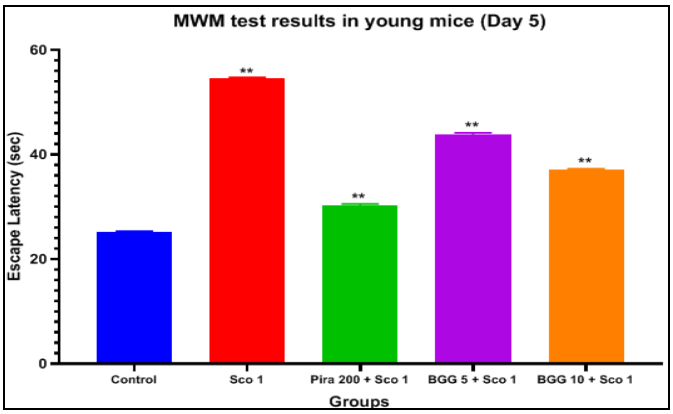

FIG. 2: MORRIS WATER MAZE TASK EFFECTS OBSERVED IN YOUNG MICE OF BETA-GLUCOGALLIN IN SCOPOLAMINE-INDUCED AMNESIA ON DAY 5. Values are expressed as Mean ± SEM. (N = 6), **P < 0.01, when compared to normal control group by one-way ANOVA followed by DUNNETT'S test.

The decreased escape latency on the 5th day signifies retention of memory. The results of a higher dose of BGG (10 mg/kg) reflected a marked decrease in the escape latencies on the 4th and 5th

day, which were comparable to that of piracetam 200mg/kg group results. Thus, the results signify improved memory and the learning of animals treated with BGG. See **Fig. 1 & 2.**

Assessment of Anti-Alzheimer Activity in Young and Aging Animals Using Elevated Plus Maze (EPM) Test with Scopolamine-Induced Amnesia: Lowering of TL is an indication of improvement in memory. The decreased TL on 9th day signifies retention of memory. BGG (5 mg/kg and 10 mg/kg) administered orally for 8 consecutive days have resulted in remarkably decreased TL on 8th and 9th day in both young and aged, compared to scopolamine groups on EPM test apparatus details are shown in **Fig. 3, 6.** Young Mice show a remarkable reduction in TL on 9th day, signifying retention memory **Fig. 4.**

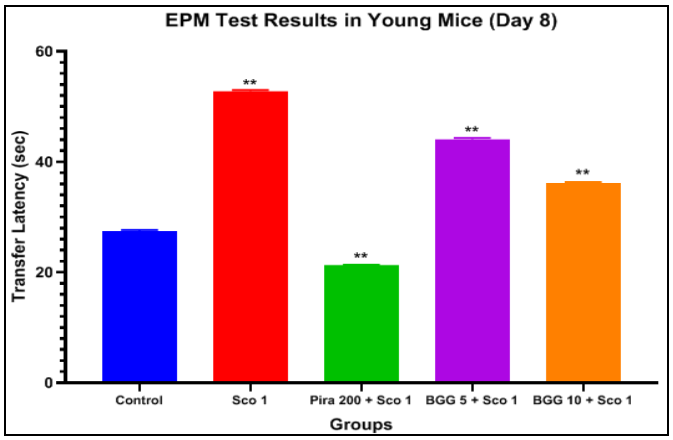

FIG. 3: ELEVATED PLUS-MAZE TASK EFFECTS OBSERVED IN YOUNG MICE OF BETA-GLUCOGALLIN IN SCOPOLAMINE-INDUCED AMNESIA ON DAY 8. Values are in mean ± SEM (n=6): ** denotes p < 0.01as compared to the control group of young mice. (One-way ANOVA followed by Dunnett's test).

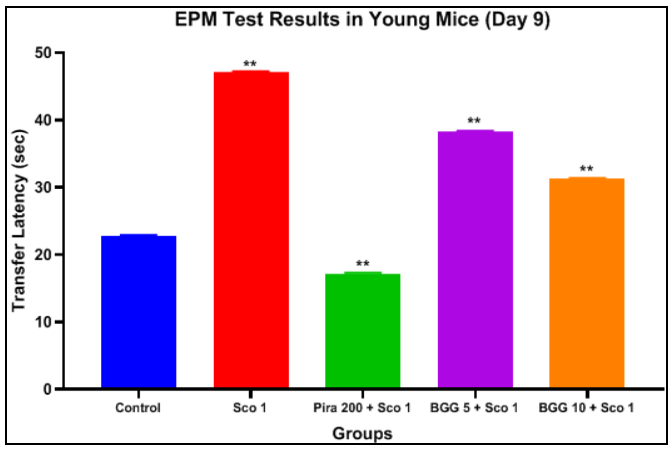

FIG. 4: ELEVATED PLUS-MAZE TASK EFFECTS OBSERVED IN YOUNG MICE OF BETA-GLUCOGALLIN IN SCOPOLAMINE-INDUCED AMNESIA ON DAY 9. Values are in mean ± SEM (n=6): ** denotes p < 0.01 as compared to control group of young mice. (One-way ANOVA followed by Dunnett's test).

Results of a high dose of BGG (10 mg/kg) reflect a major decrease in TL on the 8th and 9th days, which were comparable to the results of piracetam 200 mg/kg. Also, it was observed that BGG 10 mg/kg i.p. successfully reversed memory deficits induced by scopolamine.

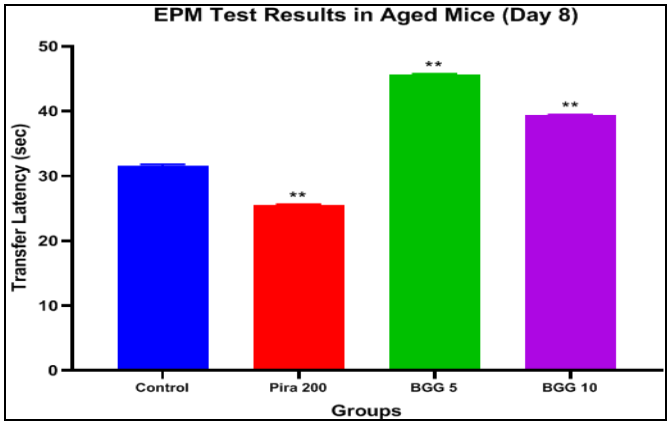

FIG. 5: ELEVATED PLUS-MAZE TASK EFFECTS OBSERVED IN AGED MICE OF BETA-GLUCOGALLIN IN SCOPOLAMINE-INDUCED AMNESIA ON DAY 8. Values are in mean ± SEM (n=6): ** denotes p < 0.01 as compared to the control group of young mice. (One-way ANOVA followed by Dunnett's test).

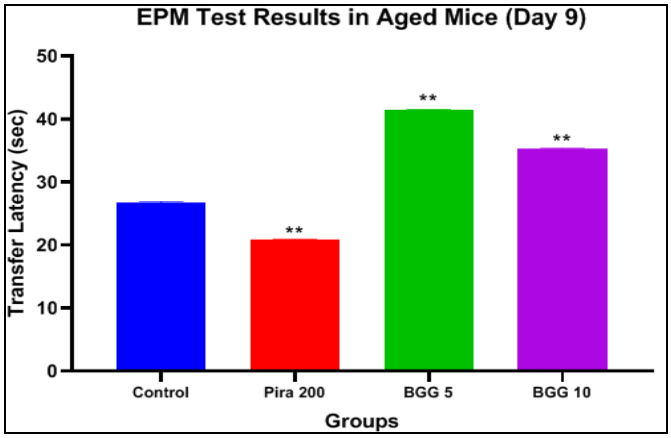

FIG. 6: ELEVATED PLUS-MAZE TASK EFFECTS OBSERVED IN AGED MICE OF BETA-GLUCOGALLIN IN SCOPOLAMINE-INDUCED AMNESIA ON DAY 9. Values are in mean ± SEM (n=6): ** denotes p < 0.01 as compared to the control group of young mice. (One-way ANOVA followed by Dunnett's test)

The results of aged mice demonstrate high TL on the 8th and on 9th day when compared with TL values of young mice, which signifies impairment of memory and learning abilities in aged mice. Pretreatment with standard drug, *i.e.*, piracetam 200 mg/kg for 8 consecutive days, resulted in decreased

TL on 8th day and on 9th day as compared to control, which validates our model **Fig. 5** & **6.** Treatment with scopolamine (1 mg/kg) resulted in a significant increase in TL in young mice on 8th and 9th day as compared to control, which signifies memory impairment. Lastly, the results also noted improved memory and the learning of aged animals compared to scopolamine-treated young mice as demonstrated by a noteworthy decrease in TL when subjected to elevated plus maze tests.

Effect of BGG on Motor Coordination: The BGG at a lower dose did not impact motor coordination activity of mice shown by results of BGG 5 mg/kg compared with the results for the control group see **Fig. 7.** Also, at a higher dose (BGG 10 mg/kg) the results did not show a major impact on motor coordination activity of animals, though the results look statistically significant compared with the control group. The result signifies BGG may have lower sedative potential.

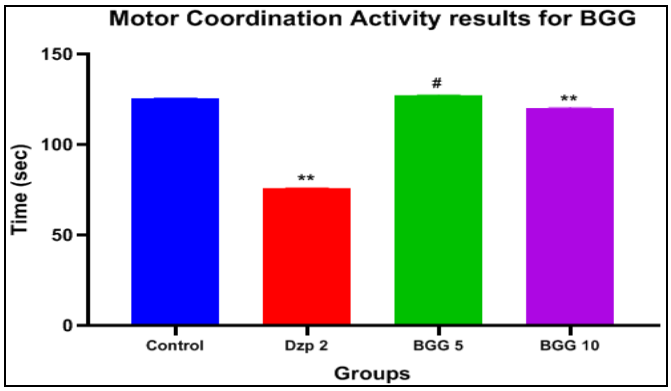

FIG. 7: EFFECTS OF BETA-GLUCOGALLIN ON MOTOR COORDINATION PERFORMANCE. Values are in mean ± SEM (n=6): ** denotes p < 0.01 and # denotes insignificant results (i.e., p>0.05), when compared to control group of young mice. (One-way ANOVA followed by Dunnett's test).

Effect of BGG on Acetylcholine [ACh] Levels in Mouse Brain: Pre-treatment with BGG exhibited significant protective effect against ACh breakdown as indicated by an increase in the amount of UN hydrolyzed ACh and decrease in the amount of hydrolyzed ACh **Fig. 8.** Increased levels of unhydrolyzed acetylcholine levels in the brain homogenate of treated animals indicate acetylcholinesterase inhibitory activity of BGG.

Antioxidant Activity: The BGG at both doses (5 mg/kg and 10 mg/kg) was found to present significantly in vitro antioxidant activity by the DPPH method **Fig. 9.** Results showed that the radical scavenging activity of BGG increased with increasing concentration. Ascorbic acid was found to be more potent. The IC_{50} value of standard ascorbic acid was found to be 75.22. At the same time, the IC_{50} values BGG 5 mg/kg and 10 mg/kg were calculated to be 117.97 and 109.24, respectively.

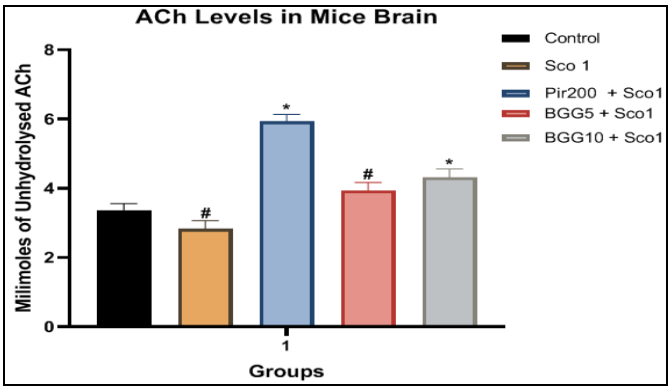

FIG. 8: EFFECTS OF BETA-GLUCOGALLIN ON ACETYLCHOLINE LEVELS IN MOUSE BRAIN. Values are in mean ± SEM (n=6): * denotes p < 0.05 and # denotes insignificant results (i.e., p > 0.05), when compared to control group of young mice. (One-way ANOVA followed by Dunnett's test).

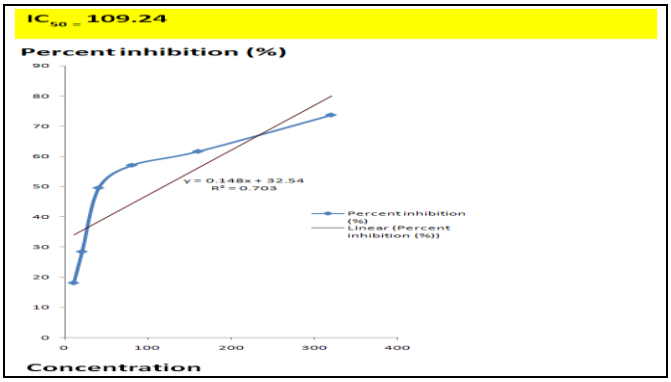

FIG. 9: ANTIOXIDANT ACTIVITY OF BETA-GLUCOGALLIN

Docking Study Results: Docking analysis utilized to analyze the possible mode mechanism of action BGG to exhibit the anti-alzheimer's potential. The details are summarized in **Table 1.** Docking analysis was performed using three dimensional structure of the anti-alzheimer drug, e2020 (aricept), complexed with its target acetyl cholinesterase (1EVE) with resolution of 2.50 Å, structure of Beta-secretase complexed with inhibitor (1FKN) with resolution of 1.90 Å, complex binary structure of human tau protein kinase I with AMPPNP(1J1B) with a resolution of 1.80 Å, the crystal structure of mouse acetylcholine

esterase in the apo form (1J06) with a resolution of 2.35 Å, the crystal structure of human butyrylcholinesterase (1P0I) with a resolution of 2.00 Å and human monoamine oxidase B in complex with deprenyl (2BYB) with a resolution of 2.20 Å. BGG interacted with acetylcholine esterase (1EVE) *via* hydrogen bond formation with PHE288, aromatic interaction with TRP279 and showed a total docking score of -72.61. BGG interacted with the Beta-secretase target (1FKN) via hydrogen bond formation with ARG235, aromatic interaction with PHE7 and showed a total docking score of -55.69. BGG interacted with human tau protein kinase I targeted (1J1B) *via* the formation of a hydrogen bond with SER66 and LYS771 and showed a total docking score of -49.69. BGG interacted with the crystal structure of mouse acetylcholine esterase in the apo form (1J06) via the formation of a hydrogen bond with HIS393, aromatic interaction with HIS393, and showed a total docking score of -40.88. BGG interacted with the crystal structure of human butyrylcholinesterase (1P0I) with a docking score of -61.16 and showed hydrogen bond formation with ILE69; GLN71and aromatic interaction with TYR332. BGG interacted with Human Monoamine Oxidase B(2BYB) target with a docking score of -65.10 and showed the formation of aromatic interaction with TRP119. The picture of the best docking poses is depicted in **Fig. 10, 15.**

TABLE 1: SUMMARIZED DETAILS OF MOLECULAR DOCKING INTERACTIONS OF BGG AND SIMILAR MOLECULAR TANNINS

Protein Target characteristics and PDB ID	Molecule Name	Types of interaction		Docking Score
		Hydrogen Bond	Aromatic	
Crystal structure of mouse acetylcholinesterase PDB id- **1JO6**	Beta-Glucogallin	HIS393	HIS393	-40.88
	1-O-galloyl-d-glucopyranose	GLN527	HIS393	-46.82
	Ginnalin C	PHE330; PHE288	TRP279	-45.14
Crystal structure of human butyryl cholinesterase PDB id- 1P0I	Beta-Glucogallin	ILE69; GLN71	TYR332	-61.16
	1-O-galloyl-d-glucopyranose	GLN71; SER72; PRO285	-	-55.85
	Ginnalin C	ILE69; GLN71	TYR332	-54.22
Human Monoamine Oxidase B PDB id- 2BYB	Beta-Glucogallin	-	TRP119	-65.10
	1-O-galloyl-d-glucopyranose	-	TRP119	-60.38
	Ginnalin C	TRP119	LEU164;PHE168	-66.68
Three dimensional structure of acetylcholinesterase. PDB id- 1EVE	Beta-Glucogallin	PHE288	TRP279	-72.61
	1-O-galloyl-d-glucopyranose	PHE288	TRP279	-65.42
	Ginnalin C	PHE330; PHE288	TRP279	-62.41
Beta-Secretase PDB id- 1FKN	Beta-Glucogallin	ARG235	PHE7	-55.69
	1-O-galloyl-d-glucopyranose	ARG235	PHE7	-51.17
	Ginnalin C	ARG289; TRP279; LEU263	-	-54.87
Human tau protein kinase I PDB id- 1J1B	Beta-Glucogallin	SER66; LYS771	-	-49.69
	1-O-galloyl-d-glucopyranose	ASN64; PHE67	-	-33.96
	Ginnalin C	LYS771	-	-50.58

TABLE 2: *IN-SILICO* BBB PERMEABILITY AND DRUG LIKELINESS PREDICTIONS OF BGG BY HTTPS://MOLSOFT.COM/.

Molecule	MW	#H-bond acceptors	#H-bond donors	MolPSA	MolVol	Drug likeliness score	BBB score
BGG	332.26 g/mol	10	7*	142.30 A^2	275.47 A^3	0.81	1.78#

*As per Lipinski's RO5 the number of hydrogen bond donors should be > 5 # The BBB Score: 6-High,0-Low (DOI: 10.1021/acs.jmedchem.9b01220)

***In-silico* Analysis of BGG:** *In-silico* studies were performed for predictions of Molecular Properties and Drug-likeness of BGG by Web Molecular Editor v1.5.1 (http://www. http://www.molsoft.com/mprop/). It predicts an overall drug-likeness score using and Molsoft's chemical fingerprints. The training set for this mode consisted of 5K of marketed drugs from WDI (positives), 10K of carefully selected non-drug compounds. H1NMR for BGG is depicted in **Fig.**

7. While **Fig. 8** shows C13 NMR for BGG. The Drug-likeness model score plot of BGG for its antianxiety potential is depicted in **Fig. 9.** The summarized details of *in-silico* predictions of Molecular Properties and Drug-likeness of BGG are given in **Tables 2** and **3.**

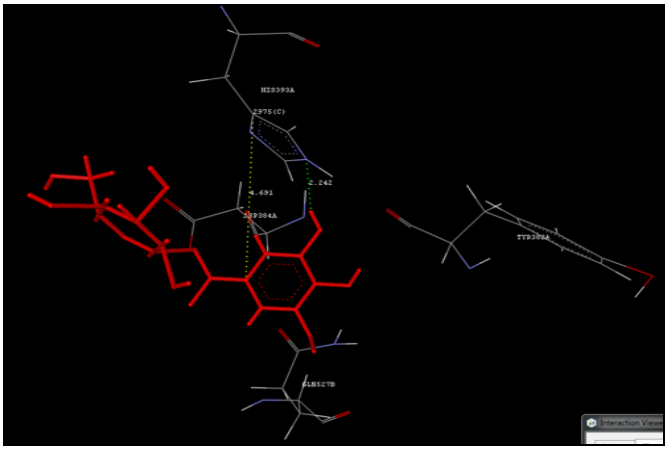

FIG. 10: BETA-GLUCOGALLIN INTERACTED WITH 3D STRUCTURE OF THE ANTI-ALZHEIMER DRUG, E2020 (ARICEPT), COMPLEXED WITH ITS TARGET ACETYLCHOLINESTERASE (1EVE)

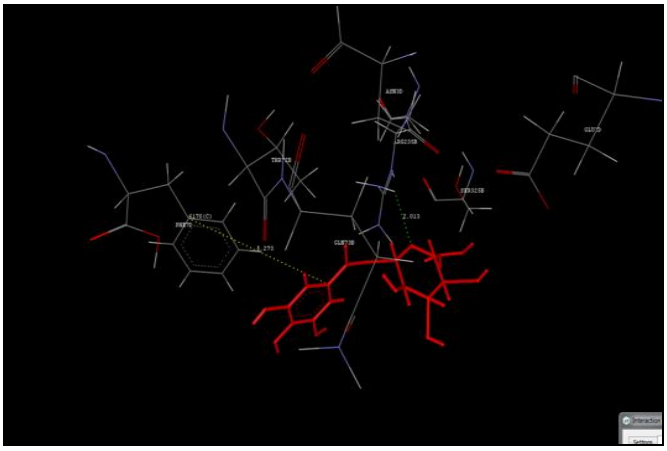

FIG. 11: BETA-GLUCOGALLIN INTERACTED WITH BETA-SECRETASE COMPLEXED WITH INHIBITOR (1FKN)

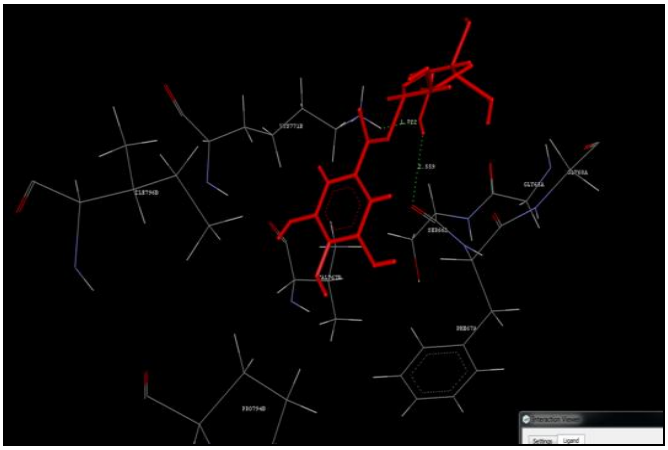

FIG. 12: BETA-GLUCOGALLIN INTERACTED WITH HUMAN TAU PROTEIN KINASE I (1J1B)

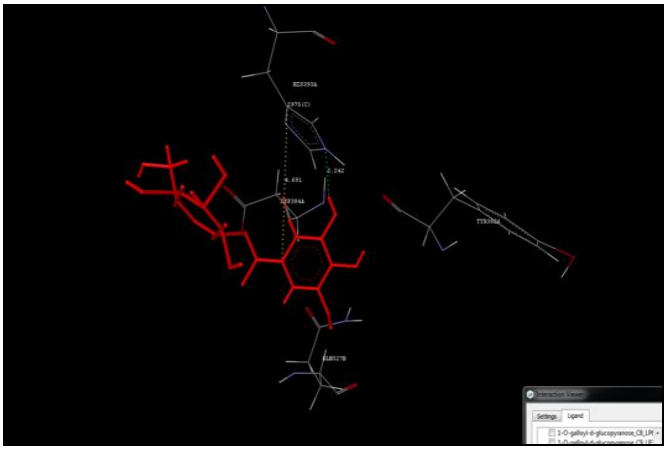

FIG. 13: BETA-GLUCOGALLIN INTERACTED WITH CRYSTAL STRUCTURE OF MOUSE ACETYLCHOLINESTERASE IN THE APO FORM

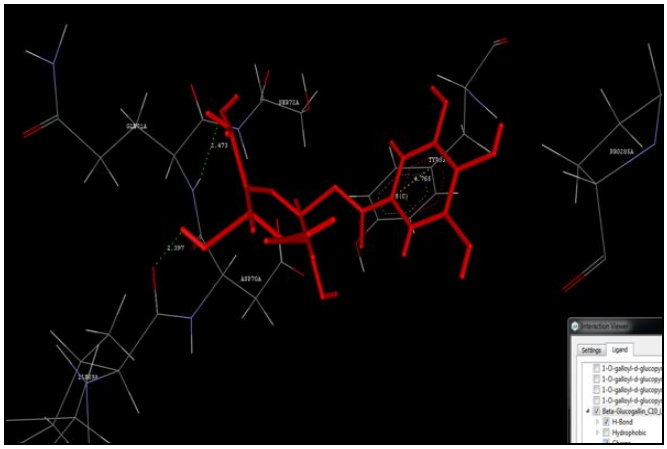

FIG. 14: BETA-GLUCOGALLIN INTERACTED WITH CRYSTAL STRUCTURE OF HUMAN BUTYRYL CHOLINESTERASE (1P0I)

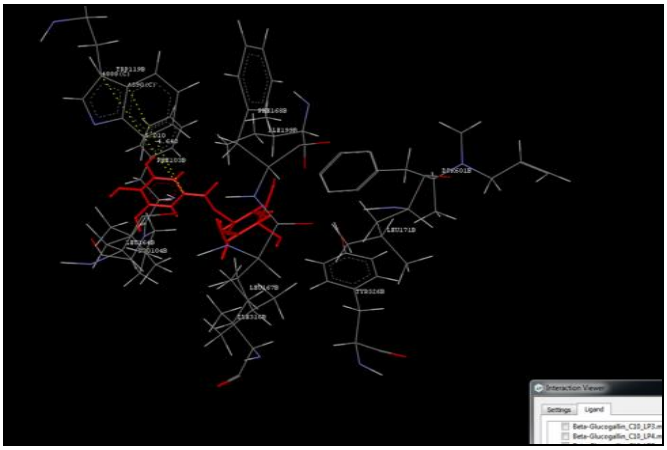

FIG. 15: BETA-GLUCOGALLIN INTERACTED WITH HUMAN MONOAMINE OXIDASE B (2BYB)

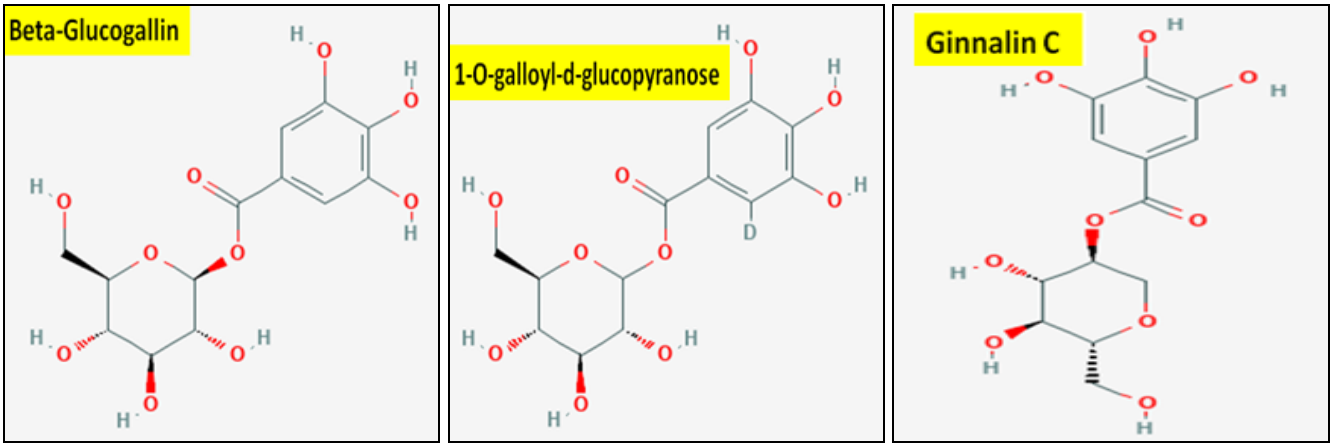

FIG. 16: MOLECULAR STRUCTURES BETA-GLUCOGALLIN AND STRUCTURALLY SIMILAR COMPOUNDS

TABLE 3: SUMMARIZED DETAILS OF PHYSICO-CHEMICAL PROPERTIES OF BGG

Parameters	Description
Chemical name	[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] 3,4,5-trihydroxybenzoate
Chemical Formula	$C_{13}H_{16}O_{10}$
Exact Mass	332.074
Molecular Weight [g/mol]	332.26
m/z:	332.074 (100.0%), 333.078 (14.1%), 334.079 (2.1%)
Elemental Analysis	C, 46.99; H, 4.85; O, 48.15
Boiling Point [K]	1220.34
Melting Point [K]	899.95
Critical Temp [K]	1041.18
Critical Pres [Bar]	63.9
Critical Vol [cm3/mol]	755.5
Gibbs Energy [kJ/mol]	-1243.03
Log P	-1.42
MR [cm3/mol]	71.48
Henry's Law	25.34
Heat of Form [kJ/mol]	-1692.29
tPSA	177.14
CLogP	-1.17352
CMR	7.1714

DISCUSSION: BGG is supposed to be naturally generated by esterification of gallic acid and glucose as an essential step in the biosynthesis of an ellagitannin or a gallotannin. Molecular structures of Beta-glucogallin and structurally similar compounds are given in **Fig 16**. However, it was synthesized for the first time in 1918 by Emil Fisher. 10 BGG is considered a selective and relatively potent inhibitor of aldose reductase AKR1B1 at IC_{50} of 17 µM, *in-vitro* [11, 12]. Lens organ culture activities with BGG showed that it also effectively inhibits sorbitol accumulation by 73% (IC_{50} of 30 µM) in transgenic mice under hyperglycemic conditions, *i.e.*, acting as a ROS (reactive oxygen species) – scavenger or free radical scavenger. BGG also reported low cytotoxicity in Raw 264.7 murine macrophages. Treatment with BGG was found to attenuate the LPS-induced activation of JNK and p38 and that of LPS-induced uveitis in mice eyes. BGG was reported to have a urinary half-life of 31 h [10, 15].

The 10 mg/kg IP dose was chosen by ophthalmology researchers as it should give approximately 30 micromolar *in-vivo* concentrations of BGG if the compound is evenly distributed throughout the aqueous volume of the animal. This is almost 2x the IC_{50} concentration measured against AR activity. So, we decided to go with a lower dose (IC50) and the dose used previously [12]. In detail, this investigation assessed in detail the anti-amnesic activity of two doses of BGG, *viz.* 5 mg/kg and 10 mg/kg in mice, by utilizing exteroceptive and interoceptive behavioral rodent models *viz* MWM test, EPM test, and scopolamine-induced amnesia. The in-vitro antioxidant activity and the molecular docking studies helped to see a path forward. The study was planned and performed with an assumption to evaluate the effects of BGG on multiple targets. The study's promising results indicate its potential use in AD management as an adjuvant to the currently used drugs owing to its actions on multiple targets.

Anti-amnesic and Anti-dementia Activities of BGG:

Morris Water Maze Task Effects: Further, the anti-Alzheimer potential of both doses of BGG was evaluated using the Morris water maze test. The application of scopolamine to block muscarinic

acetylcholine receptors causing cognitive deficit is a representative model used to evaluate the anti-dementia activity of herbal extracts and phytochemicals. The Morris water maze model has been extensively used to investigate the neurological mechanisms underlying spatial navigation to influence special cognitive processes. The same model can also be used to test working memory by changing the hidden platform from one quadrant to another quadrant [27, 28]. Pre-treatment with BGG at doses 5 mg/kg and 10 mg/kg remarkably ($P<0.05$) reduced the time required reaching the platform post scopolamine treatment. It improved basal as well as the scopolamine-impaired performance with respect to acquisition and retention of memory. These results signifying possible anti-AD-like activity of BGG may have been mediating via cholinergic pathway [29, 30].

Elevated Plus-Maze Task Effects: The EPM test is an established rodent model for evoking an approach-avoidance conflict and assessing the memory retention of animals to prefer closed arms over open arms [31]. It was noted by researchers that memory processes *viz* acquisition, retention, especially the long-term spatial memory in rodents, can also be studied by utilizing EPM apparatus [31]. We treated mice with scopolamine and demonstrated that it increased TL in the EPM test, which would validate our paradigm as an effective measurement of learning and memory in mice. In this study, two doses of BGG (5 and 10 mg/kg), separately, produced a significant effect in a dose-dependent manner compared to the Piracetam group. Also, results indicated that BGG did not impair motor coordination in the rodents at these dose levels. A study of exteroceptive and interoceptive behavioral models (scopolamine and aging-induced amnesia) using EPM in mice reveals that BGG treated mice possess anti-Alzheimer-like activities [31]. It was noted that the reduction of spontaneous motor activity could be related to the calmness/sedative effect. In this investigation, BGG showed no impact on motor coordination in mice.

Molecular Docking Studies: The molecular docking studies also support the anti-dementia-like, anti-Alzheimer-like effects of BGG, possibly attributable to its hydrogen/hydrophobic interactions with the target proteins like acetylcholinesterase at the molecular level in the brain. BGG was additionally assessed with other molecularly similar three tannins such as 1-O-galloyl-d-glucopyranose and Ginnalin C see figure. Additionally, molecular docking interactions of BGG and other three compounds were studied with six important targets of AD, such as 1EVE is a three-dimensional structure of the anti-Alzheimer drug, e2020 (aricept), complexed with its target acetylcholine esterase, 1J06 a crystal structure of mouse acetylcholinesterase in the apo form, 1P0I a crystal structure of human butyrylcholinesterase, 1FKN a structure of Beta-Secretase complexed with inhibitor, 1J1B a binary complex structure of human tau protein kinase I, 2Z5× a crystal structure of Human Monoamine Oxidase A with Harmine and 2BYB a human Monoamine Oxidase B complex with Deprenyl [21, 26]. Summarized details of molecular docking interactions of BGG and similar molecular tannins are summarized in **Table 1.** Thus, the docking results of present study suggest significance of BGG in the management of Alzheimer's disease.

For the first time, we report the inhibition mechanism of AChE by the BGG molecule for the hydrolysis of acetylcholine (ACh) with docking and simulations with a human acetylcholine esterase (hAChE) crystal structure. However, abnormal cerebrospinal fluid biomarkers of AD patients, namely, Aβ, Tau protein phosphorylation, and neurofibrillary tangle, could not be produced by administration of scopolamine [29, 31]. Therefore, the beneficial effects of BGG on other approximate models could be further studied, such as the knockout mouse model or secretase transgenic mouse models.

Antioxidant Activity: Increased oxidative stress causes cell damage in protein, lipid, and DNA oxidations. Elevated ROS levels are also associated with increased amyloid deposition and senile plaques, a hallmark of the AD brain [6, 31]. If enhanced ROS exceeds the basal level of cellular protective mechanisms, oxidative damage and cell death will result.

The BCG demonstrated potent free radical scavenging properties particularly expected to play a vital role in reducing oxidative stress, and this may explain their use in traditional medicine for

improvement of AD and/or ageing-related diseases [31, 32]. A previous study has reported that levels of AChE splice variants were increased under conditions of oxidative stress [30]. We, therefore, analyzed the correlation between AChE activity and oxidative markers and observed a significant correlation between AChE activity and ROS; our findings agree with those of a previous study [31]. These results, therefore, suggest a possible interaction between scopolamine-induced cholinergic modulation and oxidative stress. However, further research is needed to clarify the mechanism underlying the interaction. The present study has limitations, such as; restricted our investigation to spatial learning and memory. The effect of BGG on other types of learning and memory (*e.g.*, object recognition memory and fear memory) remains unknown. Also, we were not able to study which channel(s) or receptor(s) are involved in cholinergic transmission after the administration BGG [2, 4]. Therefore further studies are therefore necessary to explore the anti-amnesic effects of BGG using a transgenic animal model of AD.

CONCLUSION: The present study was conducted to evaluate the antiamnesic activity of Beta-glucogallin in a scopolamine-induced model of memory impairment. Our data demonstrated that Beta-glucogallin administration ameliorated cognitive deficits measured by performance in water maze tasks. Beta-glucogallin treatment reduced AChE, thus elevating ACh levels in scopolamine-induced mice. Furthermore, it decreases ROS levels in evince of the anti-oxidative capability of Beta-glucogallin treatment. The results indicate that the underlying mechanism of learning and memory improvement may involve modulations of the cholinergic system and the reduction of oxidative stress. These findings thus provide evidence for the potential of Beta-glucogallin as a natural, alternative treatment for amnesia.

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CONFLICTS OF INTEREST- We, all the authors, declare no conflict of interest.

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