Comparative study of rivastigmine and galantamine on the transgenic Drosophila model of Alzheimer's disease

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ABSTRACT

Alzheimer's Disease (AD) is characterized as a progressive neurodegenerative disease most commonly associated with memory deficits and cognitive decline. The formation of amyloid plaques and neurofibrillary tangles are important pathological markers of AD. The accumulation of amyloid plaques and neurofibrillary tangles leads to the loss of neurons including the cholinergic neurons thus decreasing the levels of acetylcholine (a neurotransmitter). To reduce the AD symptoms cholinesterase inhibitors are widely used to decrease the hydrolysis of acetylcholine released from presynaptic neurons. In the present study we have studied the effect of rivastigmine and galantamine (commonly used cholinesterase inhibitors) on the transgenic Drosophila model of AD expressing human Aβ-42 in the neurons. The effect of similar doses of rivastigmine and galantamine (i.e. 0.1,1 and 10 mM) was studied on the climbing ability, lifespan, oxidative stress markers, caspase 9 and 3, acetylcholinesterase activity and on the formation of Aβ-42 aggregates. The results suggest that the rivastigmine is more potent in reducing the oxidative stress and improving climbing ability of AD flies. Both the drugs were found to be effective in increasing the lifespan of AD flies. Galantamine was found to be a more potent inhibitor of acetylcholinesterase compared to rivastigmine. Galantamine prevents the formation of Aβ-42 aggregates more effectively compared to rivastigmine.

1. Introduction

Alzheimer's Disease (AD) is a progressive, degenerative brain disorder which results in cognitive as well as behavior decline and ultimately leads to death (Lockhart et al., 2009). During the progression of AD there is a profound loss of neurons including the cholinergic neurons leading to decline in the levels of acetylcholine (García-Ayllón et al., 2011). Acetylcholinesterase breaks down acetylcholine into acetic acid and choline. In this context the inhibitors of acetylcholinesterase are effective in maintaining the proper levels of acetylcholine and thus reducing the symptoms of AD (Marucci et al., 2021). For the treatment of mild to moderate AD symptoms, cholinesterase inhibitors such as donepezil, rivastigmine and galantamine are used and for the treatment of moderate to severe AD symptoms N-methyl-D-aspartate antagonist memantine is prescribed (Bond et al., 2012). The efficacy of these drugs has been studied in a number of clinical studies (Li et al., 2019; Onor et al., 2007) and in various experimental models (Van Dam et al., 2005; Bezerra da Silva et al., 2016; Mohamed et al., 2015). Kröger et al. (2015) has reported that the use of rivastigmine and galantamine is associated with an increased risk of cardiac events. The systematic review by Clegg et al. (2002) suggest that the donepezil, rivastigmine and galantamine were beneficial in treating various stages of AD, but the implications of the use of donepezil, rivastigmine and galantamine were unclear. No clear evidence exists to date which one of these drugs is more efficacious (Hansen et al., 2008). The comparative study by Aguglia et al. (2004) and Spencer and Noble (1998) showed that there was no statistical difference between the three drugs at three months of treatment. Galantamine bromide, is a tertiary alkaloid. It is not only a reversible competitive inhibitor of acetylcholinesterase, but also act as an allosteric modulator of nicotinic acetylcholine receptors (Zarotsky et al., 2003). Rivastigmine is a carbamylating, long-acting reversible and non-competitive carba-mate acetylcholinesterase inhibitor (Spencer and Noble, 1998). Oxidative stress has been proposed as one of the factors that plays a vital role in the pathogenesis various neurodegenerative disorders such as Amyotrophic Lateral Sclerosis (ALS), Parkinson's disease (PD) and AD (Niedzielska et al., 2016). It has been reported to cause the death of neuronal cells not only in transgenic mouse model for AD but also in AD patients (Butterfield Allan, 2002; Resende et al., 2008).

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Drosophila melanogaster due to the fact that 70% of disease-associated human genes have a fly homolog is the best suited invertebrate model to study the pathogenesis of neurodegenerative disorders (Bier, 2005; Lenz et al., 2013). The genome of Drosophila is well defined and its culture also offer low cost maintenance due to which this model provides good platform to study the pathology of several diseases. The brain of Drosophila is easily accessible which allows easy image capturing and quantification of amyloid plaque deposition in transgenic models along with the study of various cognitive parameters (Uras et al., 2021). The newly synthesized acetylcholinesterase inhibitor XJP-1, resulted in a significant improvement of AD symptoms and reduction of amyloid plaques by reducing the amyloid aggregation in transgenic Drosophila model of AD (Uras et al., 2021). XJP-1, also improves the climbing ability and life span of the transgenic flies expressing Aβ-42 (Uras et al., 2021).

There is also a reasonable similarity between the central nervous systems of flies and humans with both consisting of neurons, glia and utilizes the same neurotransmitters (Lenz et al., 2013). At present number of transgenic Drosophila models are available to study the various aspects of neurodegenerative diseases such as PD, AD and Huntington’s Disease (HD) (Marsh and Thompson, 2006; Bilan and Benini, 2005). Among these transgenic models, one model express human Aβ-42 under GAL4-UAS system in the brain of flies. Such flies exhibit diffuse amyloid deposits, age dependent loss in climbing ability, memory, olfaction and neurodegeneration (Lu and Vogel, 2009; Neg et al., 2018; Ali et al., 2019). Since AD is associated with the decline motor activity, memory loss, deposition of Aβ-42 and increase in oxidative stress hence, we decided to study the effect of rivastigmine and galantamine on lifespan, climbing ability and oxidative stress markers. Immunohistochemistry was also performed on the brain sections to study the effect of rivastigmine and galantamine on the expression of Aβ-42 aggregates.

2. Materials and methods

Drosophila stocks. Transgenic fly lines expressing wild-type human Abeta42\textsuperscript{w}[118]P[w+]mC = UASAPP.Abeta42.B \(m^{26a}\) under UAS control and GAL4\textsuperscript{w}P[w+]mC = GAL4-elavL3 were obtained from Bloomington Drosophila Stock Center (Indiana University, Bloomington, IN). When the males of UAS (Upstream Activation Sequence)-APP.Abeta42.B strains were crossed with the females of GAL4-elav. L (vice-versa) the progeny express human Aβ-42 in the neurons and the flies are referred as AD flies (Prubing et al., 2013).

Drosophila culture and crosses. The flies were cultured on standard Drosophila food containing agar, corn meal, sugar and yeast at 25 °C (24 ± 1) (Siddique et al., 2014). Crosses were set up as described in our earlier published work (Ali et al., 2011). The AD flies were allowed to feed separately on different doses of rivastigmine and galantamine mixed in the diet for 30 days. The doses were selected according to the method described by Fatima et al. (2017) (Fatima et al., 2017) The LD 50 for both Rivastigmine and galantamine was calculated. For rivastigmine and galantamine the LD 50 was 50 mM and 60 mM, respectively. The highest tested dose was kept less than 1/4th of the LD 50. In order to get a comparative account of the drugs the doses were kept similar for both the drugs i.e., 0.1, 1 and 10 mM.

Drosophila life span determination. Newly eclosed flies from each treated as well as control groups were placed in the culture tubes (10 flies per tube; 3 replicates/treatment) containing desired concentration of the drugs. The flies were transferred to a new diet at every 3rd day containing desired concentration of the drugs till the last one died (Long et al., 2019).

Drosophila climbing assay. The climbing assay was performed as described by Pendleton et al. (2002). Ten flies were placed in an empty glass vial (10.5 cm × 2.5 cm). A horizontal line was drawn 8 cm above the bottom of the vial. After the flies had acclimated for 10 min at room temperature, both controls and treated groups were assayed at random to a total of 10 trials for each. The mean values were calculated and then averaged a group mean and standard error were obtained.

Preparation of homogenate for biochemical assays. Fly heads from each group were isolated (100 heads/group; five replicates/group) and the homogenate was prepared in 0.1 M phosphate buffer for the biochemical assays. Before performing the biochemical assays, the protein content in the homogenate was estimated by the method of Lowry (Waterborg and Matthews, 1994).

Estimation of glutathione (GSH) content. GSH content was estimated colorimetrically using Ellman’s reagent (DTNB) according to the procedure described by Jollow et al. (1974). The assay mixture consisted of 550 μl of 0.1 M phosphate buffer, 100 μl of supernatant and 100 μl of DTNB. The OD was read at 412 nm and the results were expressed as μmol of GSH/gram tissue.

Estimation of glutathione-S-transferase (GST) activity. The GST activity was determined by the method of Habig et al. (1974). The reaction mixture consisted of 500 μl of 0.1M phosphate buffer, 150 μl of 10 mM CDNB, 200 μl of 10 mM reduced glutathione, and 50 μl of supernatant. The OD was taken at 340 nm and the enzyme activity was expressed as μmol of CDNB conjugates formed/min/mg protein.

Lipid peroxidation assay. Lipid peroxidation was measured according to the method described by Ohkawa et al. (1979). 5 μl of 10 μM butylhydroxy tolouene (BHT), 200 μl of 0.67% thiobarbituric acid (TBA), 600 μl of 1% orthophosphoric acid (OPA), 105 μl of distilled water and 90 μl of the sample were taken in an eppendorf, vortexed and kept in the water bath at 90 °C for 45 min. OD was read at 535 nm and the results were expressed in μmol of TBARS formed/60 min/g tissue.

Estimation of protein carbonyl content (PCC). The PCC was estimated according to the protocol described by Hawkins et al. (2009). The homogenate was diluted to a protein concentration of approx 1 mg/ml. About, 250 μl of diluted homogenate was taken in eppendorf. To it, 250 μl of 10 M 2,4-dinitrophenyl hydrazine (dissolved in 2.5 M HCl) was added, vortexed and kept in dark for 20 min. About 125 μl of 50% (w/v) trichloroacetic acid (TCA) was added, mixed thoroughly and incubated at −20 °C for 15 min. The tubes were then centrifuged at 4 °C for 10 min at 8200g. The supernatant was discarded and the pellet obtained was washed twice by ice cold ethanol:ethyl acetate (1:1). Finally, the pellets were re-dissolved in 1 ml of 6 M guanidine hydrochloride and the absorbance was read at 370 nm.

Determination of catalase (CAT) activity. The catalase activity was estimated according to the method of Beers and Sizer (1952) by kinetic method where rate of dismutation of H₂O₂ to water and molecular oxygen is proportional to the concentration of catalase in the sample. The reaction mixture consisted of 650 μl of 0.1 M phosphate buffer, 333 μl of H₂O₂ (0.05M) and 17 μl of sample. A decrease in OD was measured for 2 min, at 30 s intervals at 240 nm. The activity of catalase was calculated and expressed as μmol of H₂O₂ consumed/min/mg protein.

Determination of superoxide dismutase activity (SOD) activity. The SOD activity was estimated according to the method of Marklund and Marklund (1974). The reaction mixture consisted of 17 μl of sample and 950 μl of 0.1M phosphate buffer. The reaction was initiated by adding pyrogallol. An increase in OD was noted at 420 nm for 3 min at 30 s interval and the results were expressed as units/mg protein.

Caspase-3 (Drice) and Caspase-9 (Dronc) activities. The assay was performed according to the manufacturer protocol (Bio-Vision, CA, USA). The assay was based on spectrophotometric detection of the chromophore p-nitroanilido (pNA) obtained after specific action of caspase-3 and caspase-9 on tetrapeptide substrates, DEVD-pNA and LEHD-pNA, respectively. The assay mixture (50 μl of homogenate and 50 μl of chilnin buffer) was reacted on ice for 10 min. After 1reaction, 50 μl of 2X reaction buffer (containing 10 mM DTT) with 200 μM substrate (DEVD-pNA for Drice, and IETD-pNA for Dronc) was added and incubated at 37 °C for 1.5 h. The reaction was quantified at 405 nm.

Acetylcholinesterase (AChE) activity. Acetylcholinesterase activity was determined by the method described by Ellman et al. (1961). It is based on the principle that AChE hydrolyses the acetylthiocholine to produce thiocholine and acetate. The thiocholine in turn reduces the Dithiobis-nitrobenzoate liberating nitrobenzoate, which absorbs light at
412 nm. The assay is based on measurement of the change in absorbance at 412 nm. The experiment was initiated with the reaction mixture consisting of 100 μl of the sample, 650 μl of 0.1 M phosphate buffer and 100 μl of 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB). To the mixture 10 μl of acetylthiocholine was added and the change in OD was recorded at every 1 min interval for 3 min.

Immunohistochemistry. The fly heads were isolated and the parafﬁn sections were prepared according to the procedure described by Palladino et al. (2002). The sections were deparafﬁnized and rehydrated. The slides were blocked in 8% Bovine Serum Albumin (BSA) for 2.5 h. Then the slides were washed with phosphate buffer saline (pH 7.2) containing 2% BSA for 5 min. For immunohistochemistry, after washing the slides were incubated with primary antibody (Rabbit monoclonal Aβ42 antibody, Merck; 1:200 dilutions) in a humidified chamber for 12 h at 4 °C. The slides were then washed with PBS containing 2% BSA for 5 min and incubated with secondary antibody (Goat anti-Rabbit IgG, alkaline phosphatase conjugate, Merck, USA) at room temperature for 2 h. The final wash was given by PBS containing 2% BSA for 5 min 5-Bromo-4-chloro-3-indolyl phosphate-Nitro blue tetrazolium chloride (BCIP-NBT) was used as a chromogenic substrate which interacts with secondary antibody to produce blue coloured product. The slides were then mounted in DPX and observed under the microscope. The Aβ42 aggregates were quantiﬁed in terms of Aβ-positive cells using Image-J software.

Statistical analysis. The data was analyzed by one-way analysis of variance (ANOVA) followed by post hoc Tukey test using GraphPad Prism software [version 5.0]. The level of signiﬁcance was kept at p < 0.05. The results were expressed as mean ± SEM.

3. Results

3.1. Climbing assay

The AD flies showed a signiﬁcant decrease of 2.61-fold in the climbing ability compared to control ﬂies (Fig. 1; p < 0.05). The AD flies exposed to 0.1, 1 and 10 mM of rivastigmine showed a signiﬁcant increase of 1.27, 1.49 and 1.74 folds, respectively, in the climbing ability compared to AD ﬂies (Fig. 1; p < 0.05).

3.2. Life span

The results obtained for the life span is shown in Fig. 2. The analysis on the 3rd day reveal no signiﬁcant difference between AD ﬂies, AD ﬂies exposed to rivastigmine as well as galantamine and control ﬂies (F = 0; df = 23; p < 0.05). The analysis on the 12th day also reveal the similar
The AD flies exposed to 1 and 10 mM of rivastigmine showed a dose dependent significant increase of 1.18 and 1.28 folds, respectively, in the PC content compared to control flies (p < 0.05). The AD flies exposed to 0.1, 1 and 10 mM of galantamine showed a dose dependent decrease of 1.13 and 1.15 folds, respectively, in TBARS compared to control flies (p < 0.05). No significant increase in GSH content was observed in AD flies exposed to 0.1 mM of galantamine did not showed significant decrease in the activity of GST compared to unexposed AD flies (Fig. 3b).

3.3. GSH content

The AD flies showed a significant decrease of 1.72-fold in GSH content compared to control flies (Fig. 3a; p < 0.05). The AD flies exposed to 1 and 10 mM of rivastigmine showed a dose dependent significant increase of 1.16 and 1.37 folds, respectively, in GSH content compared to unexposed AD flies (p < 0.05). No significant increase in GSH content was observed in AD flies exposed to 0.1 mM of rivastigmine and galantamine compared to unexposed AD flies (Fig. 3a).

3.4. GST activity

The AD flies showed a significant increase of 1.92-fold in the activity of GST compared to control flies (Fig. 3b; p < 0.05). The AD flies exposed to 0.1, 1 and 10 mM of rivastigmine showed a dose dependent decrease of 1.18, 1.38 and 1.57 folds, respectively, in the activity of GST compared to unexposed AD flies (Fig. 3b; p < 0.05). The AD flies exposed to 1 and 10 mM of galantamine showed a dose dependent decrease of 1.16 and 1.25 folds, respectively, in the activity of GST compared to unexposed AD flies (p < 0.05). The AD flies exposed to 0.1 mM of galantamine did not showed significant decrease in the activity of GST compared to unexposed AD flies (Fig. 3b).

3.5. TBARS

The AD flies showed a significant increase of 4.12-fold in TBARS compared to control flies (Fig. 3c; p < 0.05). A significant dose dependent decrease of 1.26, 1.44 and 1.69 folds in TBARS was observed in AD flies exposed to 0.1, 1 and 10 mM of rivastigmine, respectively, compared to unexposed AD flies (p < 0.05). The AD flies exposed to 1 and 10 mM of galantamine showed a significant decrease of 1.24 and 1.53 folds, respectively, in TBARS compared to unexposed AD flies (p < 0.05). The AD flies exposed to 0.1 mM of galantamine did not showed significant decrease in TBARS compared to unexposed AD flies (Fig. 3c; p < 0.05).

3.6. Protein carbonyl content

The AD flies showed a significant increase of 3.91-fold in the PC content compared to control flies (Fig. 3d; p < 0.05). The AD flies exposed to 0.1, 1 and 10 mM of rivastigmine showed a dose dependent significant decrease of 1.20, 1.51 and 1.68 folds, respectively, in the PC content compared to unexposed AD flies (p < 0.05). The AD flies exposed to 1 and 10 mM of galantamine showed a dose dependent significant decrease of 1.18 and 1.28 folds, respectively, in the PC content compared to control flies.
Effect of Rivastigmine (R) and Galantamine (G) on the Caspase-9 activity

Fig. 4.

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3.7. Catalase activity

The AD flies showed a significant increase of 2.89-fold in the activity of catalase compared to control flies (Fig. 3a; p < 0.05). The AD flies exposed to 0.1, 1 and 10 mM of rivastigmine showed a significant decrease of 1.25, 1.41 and 1.72 folds, respectively, in the activity of catalase compared to unexposed AD flies (p < 0.05). The AD flies exposed to 10 mM of galantamine showed a significant decrease of 1.30-fold, in the activity of catalase compared to unexposed AD flies (p < 0.05). The AD flies exposed to 0.1 and 1 mM of galantamine did not showed significant decrease in the activity of catalase compared to unexposed AD flies (Fig. 3e; p < 0.05).

3.8. SOD activity

The AD flies showed a significant increase of 2.98-fold in the activity of SOD compared to control flies (Fig. 3f; p < 0.05). The AD flies exposed to 0.1, 1 and 10 mM of rivastigmine showed a significant decrease of 1.12, 1.41 and 1.72 folds, respectively, in the activity of SOD compared to unexposed AD flies (p < 0.05). The AD flies exposed to 1 and 10 mM of galantamine showed significant decrease of 1.23 and 1.28 folds, respectively, in the SOD activity compared to unexposed AD flies (p < 0.05). The AD flies exposed to 0.1 mM of galantamine did not showed significant decrease in the SOD activity compared to unexposed AD flies (Fig. 3f; p < 0.05).

3.9. Caspase-9 activity

The AD flies showed a significant increase of 3.53-fold in Caspase-9 activity compared to control flies (Fig. 4a; p < 0.05). The AD flies exposed to 0.1, 1 and 10 mM of rivastigmine showed a significant decrease of 1.21, 1.41 and 1.65 folds, respectively, in Caspase-9 activity compared to unexposed AD flies (p < 0.05). The AD flies exposed to 1 and 10 mM of galantamine showed a significant decrease of 1.19 and 1.27 folds, respectively in Caspase-9 activity compared to unexposed AD flies (p < 0.05). The AD flies exposed to 0.1 mM of galantamine did not showed significant decrease in caspase-9 activity compared to unexposed AD flies (Fig. 4a; p < 0.05).

3.10. Caspase-3 activity

The AD flies showed a significant increase of 3.43-fold in the Caspase-3 activity compared to control flies (Fig. 4b; p < 0.05). The AD flies exposed to 0.1, 1 and 10 mM of rivastigmine showed a significant decrease of 1.14, 1.39 and 1.67 folds, respectively, in Caspase-3 activity compared to unexposed AD flies (p < 0.05). The AD flies exposed to 1 and 10 mM of galantamine showed a significant decrease of 1.14 and 1.18 folds, respectively, in Caspase-3 activity compared to unexposed AD flies (p < 0.05). The AD flies exposed to 0.1 mM of galantamine did not showed significant decrease in caspase-3 activity compared to unexposed AD flies (Fig. 4b; p < 0.05).

3.11. Acetylcholinesterase activity

The AD flies showed no significant decrease in acetylcholinesterase activity compared to control flies (Fig. 4c; p < 0.05). The AD flies exposed to 0.1, 1 and 10 mM of rivastigmine showed a significant dose dependent decrease of 1.15, 1.36 and 1.42 folds, respectively, in acetylcholinesterase activity compared to unexposed AD flies (p < 0.05). The AD flies showed no significant decrease in acetylcholinesterase activity compared to control flies (Fig. 4c; p < 0.05). The AD flies exposed to 0.1, 1 and 10 mM of rivastigmine showed a significant dose dependent decrease of 1.15, 1.36 and 1.42 folds, respectively, in acetylcholinesterase activity compared to unexposed AD flies (p < 0.05). The AD flies exposed to 0.1, 1 and 10 mM of galantamine showed a significant decrease of 1.14, 1.39 and 1.65 folds, respectively, in acetylcholinesterase activity compared to unexposed AD flies (p < 0.05). The AD flies exposed to 0.1, 1 and 10 mM of galantamine showed a significant decrease of 1.19 and 1.27 folds, respectively, in acetylcholinesterase activity compared to unexposed AD flies (p < 0.05). The AD flies exposed to 0.1 mM of galantamine did not showed significant decrease in acetylcholinesterase activity compared to unexposed AD flies (p < 0.05).
exposed to 0.1, 1 and 10 mM of galantamine showed a significant dose dependent decrease of 1.46, 1.66 and 1.99 folds, respectively, in acetylcholinesterase activity compared to unexposed AD flies (Fig. 4c; p < 0.05).

3.12. Immunohistochemistry

The results obtained for immunohistochemistry are shown in Fig. 5(a–i). The AD flies showed a marked age dependent increase in the Aβ-42 aggregates compared to control flies (Fig. 5 a & b). The AD flies exposed to 0.1, 1 and 10 mM of rivastigmine showed a dose dependent significant decrease of 1.26, 1.42 and 1.56 folds, respectively, in the Aβ-42 aggregates compared to the unexposed AD flies (Fig. 5i). The AD flies exposed to 0.1, 1 and 10 mM of galantamine also showed a significant dose dependent decrease of 1.34, 1.45 and 1.49 folds, respectively, in the Aβ-42 aggregates compared to the unexposed AD flies (Fig. 5i). The aggregates were quantified by using Image-J software.

4. Discussion

The results of the present study reveal that both the drugs i.e. rivastigmine and galantamine are effective in reducing the AD symptoms being mimicked in the transgenic Drosophila. Rivastigmine was found to be more effective in reducing the oxidative stress and improving the
climbing ability of AD flies compared to galantamine. Both the drugs were found to be almost equally effective in improving the life span of AD flies. Rivastigmine was found to be more effective in reducing the activity of Caspase-3 and 9, compared to galantamine, implying that rivastigmine is a more potent anti-apoptotic agent compared to galantamine. Galantamine inhibited the activity of acetylcholinesterase more effectively compared to rivastigmine thus exhibiting a more potent cholinesterase inhibitor compared to rivastigmine. The results obtained for immuno-histochemistry reveals that both the drugs also prevent the formation of Aβ-42 aggregates in a dose dependent manner. Galantamine was found to be more effective in preventing the formation of Aβ-42 at lower doses.

The use of Drosophila and the negative geotaxis assay provides an inexpensive and reliable method to screen candidate drugs for phenotype rescue (Malabattula et al., 2015). In our present study both drugs significantly delayed the loss of climbing ability of AD flies but the effect was more significant in AD flies exposed to rivastigmine. Concerning their effect on the life span of AD flies both the drugs were effective in increasing the life span of AD flies, but the effect was more prominent in the AD flies exposed to rivastigmine. This may be due to the reduction in oxidative stress. Oxidative stress results from an imbalance in the production of ROS and antioxidative defense system which is responsible for the removal of ROS (Harman, 1981). High levels of protein oxidation, lipids peroxidation, DNA oxidation and the formation of toxic substances such as ketones, aldehyde, peroxides have been implicated not only in brain but also in cerebrospinal fluid, blood and urine of AD patients (Lovell and Markesbery, 2007). The important part of anti-oxidant defense system is GSH which donates electrons to ROS for proper scavenging (Praticó et al., 2000). The use of anti-oxidants has also been reported to reduce the oxidative stress in AD patients as well as in experimental models of AD (Feng and Wang, 2012). GSH, a tripeptide protects cells against oxidation. The antioxidative function of GSH is closely associated with its function in providing reducing environment to the cell with the help of NADPH (Meister, 1995). Glutathione in its reduced form is the most powerful intracellular anti-oxidant. Low levels of GSH indicates the increased level of oxidative stress (Exner et al., 2000). In our present study the AD flies show the low levels of GSH content, but the AD flies exposed to rivastigmine and galantamine showed increased levels of GSH content compared to unexposed AD flies. Rivastigmine was more effective in increasing the levels of reduced GSH compared to Galantamine. GST is involved in the process of detoxification via conjugation of reduced GSH (Nebert and Vasiliov, 2004). It can act on a wide variety of endogenous as well as xenobiotic substrates (Strange et al., 2001). The levels of GST have been reported to alter in several pathological diseases such as AD, PD and cancer (Kumar et al., 2017). In our study the AD flies showed increased levels of GST activity compared to control flies. The AD flies exposed to rivastigmine and galantamine showed reduction in the activity of GST compared to galantamine. Our earlier studies with the same strain of Dugesia tigrina showed an increase in GST activity compared to galantamine. Both the drugs showed a slight decrease in the activity of acetylcholinesterase compared to control flies. Both the drugs i.e. rivastigmine and galantamine were effective in reducing the activity of acetylcholinesterase, but galantamine was more effective in reducing the activity of acetylcholinesterase, hence the galantamine is more effective inhibitor of acetylcholinesterase compared to rivastigmine. The study on Dugesia tigrina for the inhibition of acetylcholinesterase activity, galantamine showed high inhibitory effect compared to donepezil, tacrine and rivastigmine (Bezerra da Silva et al., 2016). Rivastigmine and galantamine are well known acetylcholinesterase inhibitors. The comparative study of these both inhibitors at the same dose was studied on some human AD patients exhibiting features, such as reduced lifespan, locomotor defects and increased oxidative stress (Li et al., 2019; Onor et al., 2007; Takeda et al., 2006).

The neuropil in the insect brain is suggested to be responsible for the coordination on between neuronal information and function (Kahsai and Winther, 2011). A number of neurotransmitters and neuromodulators have been reported in the Drosophila brain such as acetylcholine, Y-a-mino butyric acid (GABA), glutamate, dopamine, serotonin and octopamine (Kahsai and Winther, 2011). From the study of Kahsai and Winther (Kahsai and Winther, 2011) it has been suggested that acetylcholine and glutamate are important primary neurotransmitters of central complex and in combination with neuropeptides play an important role in controlling learning, courtship and locomotor in Drosophila. The impairment of glutamate (GABA) glutamine cycle leads to motor deficit and shortens life span (Mazaud et al., 2019). The primary excitatory neurotransmitter in CNS and the sensory neurons in Drosophila is acetylcholine (Lee and O'Dowd, 1999), but it is not present in...
neuromuscular junction as in vertebrate. Despite of having such differences acetylcholine in Drosophila regulates jumping, climbing ability and motion (Hou et al., 2003; Takemura et al., 2013).

Acetylcholinesterase inhibitors have been reported to increase the plasma levels of Aβ42 in AD patients (Conti et al., 2010). In brain of AD patients, a reduction in the deposition of Aβ was found who were undergoing with the therapy of cholinesterase inhibitors (Ballard et al., 2007). The results obtained for the Aβ-42 aggregates by performing immunohistochemistry on brain sections also supports the reduction in the formation of Aβ-42 aggregates in the AD flies exposed to rivastigmine and galantamine. The study on human neuroblastoma cell line SH-SY5Y reveals a U-shaped neuroprotective curve for galantamine and donepezil against okadaic acid toxicity (Arias et al., 2005). Maximum protection was achieved at 0.3 μM galantamine, 1 μM of donepezil and 3 μM rivastigmine (Arias et al., 2005). The maximum protection against apoptosis induced by Aβ25-35 in SH-SY5Y cells was also observed at the same concentrations (Arias et al., 2005). The retrospective comparative analysis of donepezil, rivastigmine and galantamine for the treatment of dementia associated with AD showed donepezil as a more persistent drug compared to rivastigmine and galantamine (Sicras and Rejas-Gutierrez, 2004).

5. Conclusion

It is concluded from the results (Fig. 6) obtained from our present study that rivastigmine exerts the protective effect mainly by reducing the oxidative stress but on the other hand it is also a cholinesterase inhibitor. Galantamine is more potent cholinesterase inhibitor compared to rivastigmine.

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**Ethical statement**

Not Required.

**Data availability**

All data is presented in the manuscript.

**CRediT authorship contribution statement**

Yasir Hasan Siddique: Participated in research design and Wrote manuscript, Conducted experiments, Performed data analysis. Falaq Naz: Conducted experiments, Performed data analysis. Rahul: Conducted experiments, Performed data analysis. Himanshi Varshney: Conducted experiments.

**Declaration of competing interest**

The authors declare that they have no known competing financial
interests or personal relationships that could have appeared to influence the work reported in this paper.

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