Mitochondria-Related Apoptosis Regulation by Minocycline: A Study on a Transgenic *Drosophila* Model of Alzheimer's Disease

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ABSTRACT: Alzheimer's disease (AD) is a very complicated and multifactorial neurological disorder having limited therapeutic interventions illustrated by the impairment in memory and cognitive function. Several lines of confirmation are stoutly connected with mitochondrial function perturbation as a significant causative factor in AD, while the molecular mechanisms involved in AD pathogenesis are still poorly understood. Minocycline, a well-known antibiotic, has confirmed efficacy against mitochondrial defects and oxidative stress as a neuroprotective effect. In view of this property, we examined the remedial effect of minocycline on AD. To attain insight into the molecular machinery responsible for AD pathogenesis, we preferred the UAS/GAL4 scheme for the development of AD in flies that overexpress the $A\beta42$ protein in the brain of *Drosophila*. The warning signs like the declined lifespan, locomotion deficit and memory loss, impaired mitochondrial membrane potential, and increased caspase 3 expression with mitogen-associated protein kinases linked with AD pathogenesis were examined in the existence of minocycline. Minocycline halted the $A\beta42$-induced symptoms including behavioral changes and altered the mitochondrial membrane potential along with apoptotic factors' protein expression (JNK/p-JNK and caspase 3). Thus, the current study could be functional to find out the role of minocycline in human $A\beta42$-overexpressed transgenic AD flies.
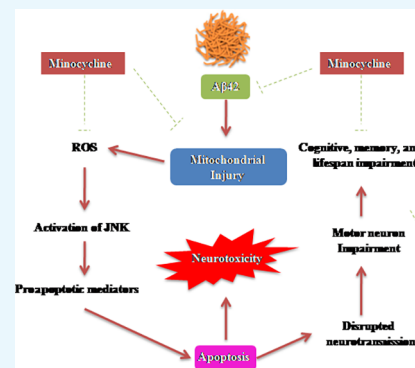
1. INTRODUCTION

Alzheimer's disease (AD) is the most common mental illness affecting millions of people worldwide, caused by extracellular senile plaques (SPs) and intracellular neurofibrillary tangle accumulation in the brain, resulting in the progressive loss of cognitive function and amnesia occurrence.[1] Amyloid-β (Aβ) is the main component of amyloid plaques seen in the neocortex, hippocampus, and other subcortical regions of the brain that are important for cognitive performance in AD. The most prominent forms of amyloid-β peptides, $A\beta40$ and $A\beta42$, are abundantly occurring in the plaques.[2] Previous studies demonstrated $A\beta42$ as a mainly significant player of pathogenesis in AD because of its hydrophobic feature.[3] Alteration in the normal mitochondrial function, calcium overload, impaired axonal trafficking, and microglial activation are seemingly considered as the dire consequences of $A\beta42$ overexpression in AD pathogenesis.[4,5] However, the extent to which these factors contribute to the progression of AD is yet unknown.

A large body of evidence proposed that alteration in the normal mitochondrial function could be highlighted more as a possible cause of AD pathogenesis. It might be the consequence of $A\beta42$ peptides' interaction with components of mitochondria such as proteins or lipids.[1,6] Mitochondria play a vital role in energy production, reactive oxygen species (ROS) production, and cell death.[7] The key early events related with the modification in the normal functioning of electron transport chains in AD involve ATP depletion, excessive ROS generation, and lipid and protein oxidation. The consequences of AD become more severe after the activation of redox-based signaling participated in cell death. Isolated mitochondria demonstrated decreased respiratory capacity when kept in the presence of Aβ, as well as inhibition of numerous important enzymes like pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and cytochrome oxidase. Short-term exposure of cultured rat hippocampus neurons to a sublethal Aβ dose led to quick and severe mitochondrial transport impairment without causing apparent apoptosis.[2]

Activation of mitogen-activated protein kinase (MAPK) signaling components is reported in various neuronal conditions.[8,9] Mitochondrial distress resulted in activation of c-Jun N-terminal kinases (JNK), which are identified as family members of MAPKs. Also, JNK activation is related with elevated levels of senile plaques and neurofibrillary tangles in

experimental models of AD, according to a study employing a mouse model of AD that comprises the Swedish APP mutation and a mutant presenilin-1.[8,10] Increased JNK phosphorylation promotes the cleavage of procaspase 3 protein in neurological illnesses including Parkinson's disease (PD) and AD.[10,11] In this point of view, therapeutic agents having potential to slow down the perturbation function of mitochondria may increase knowledge in the search of effective remedies in AD pathogenesis. Recently, antibiotics have drawn keen attention because of showing antioxidative efficacy in other neurological disease conditions.[12] This idea is also supported by previous studies that suggest that the antioxidant nature of antibiotics such as minocycline has potential to slow the rate of alteration in the normal mitochondrial function and cognitive function in an animal model of various neurodegenerative diseases including AD.[12−14]

Minocycline is a lipophilic, broad-spectrum, semisynthetic antibiotic, which can easily cross the blood−brain barrier. In addition to its antibiotic nature, it has been reported to have neuroprotective effects on various neurodegenerative diseases including AD by limiting the inflammation and oxidative stress.[15] In a PD rat model, it demonstrated neuroprotective effects by blocking the release of cyt c from mitochondria to the cytosol and caspase 3 activation.[16] Minocycline suppressed the activation of microglia and expression of interleukins like IL-6, IL-12, and TNF in in vitro as well as in vivo models of Huntington's disease.[17] It has the ability to scavenge the formation of ROS/RNS, stimulate the activity of sodium dismutase (SOD), catalase, glutathione peroxidase (GPx), and glutathione S-transferase (GST) antioxidant enzymes, enhance the electron transport chain efficiency, thereby limiting leakage of electrons, and promotes ATP synthesis in various neurological disorders such as PD and AD. Minocycline decreases the expression of JNK, ERK, and caspase 3 proteins in HD and PD models of rats.[18] Minocycline's pharmacological profile has been confirmed in preclinical trials to be of interest in the treatment of AD. In 2004, the first report described the beneficial effects of minocycline in an experimental model of AD induced by i.c.v. injection of μ-p75-saporin in mice.[12] Also, previous studies suggested that minocycline has potential to prevent the formation of the Aβ monomer to an oligomer in ex vivo and in vivo models of AD. Available studies suggested that minocycline prevents the death of neurons by inhibiting the formation of pores in mitochondria in various neurodegenerative diseases.[19,20] Based on these previous findings, the present study aims to find out whether minocycline can preserve the integrity of mitochondria and helps to maintain the neuron functions and survival in a transgenic *Drosophila* AD model. *Drosophila* is a well-known genetic model in the area of AD research to explore the potential targets and screening of a pharmacological agent in the search of effective drugs against AD.[21] In this study, the effect of minocycline on specific human Aβ42 overexpression in the central nervous system of *Drosophila* was investigated. Here, we present that supplementation of minocycline mitigates the reduction in the lifespan, locomotion deficit, altered mitochondrial function, and activation of apoptosis. Minocycline showed potential to reduce the Aβ42 accumulation.

2. RESULTS

2.1. Minocycline Treatment Improves the Motor Illness in Aβ42-Overexpressed Flies.
A negative geotaxis assay demonstrated a late-onset locomotion deficit in Aβ42-

overexpressed transgenic flies. The severity of Aβ42 aggregation in flies' brain tissue correlates well with the phenotype of locomotory performance decline. After the 30th day, transgenic AD flies showed a significant reduction [$F_{(4,20)}$, $p < 0.001$, $q = 16.32$] in locomotion as compared to control flies (Figure 1A). However, 870 μM minocycline exposure of
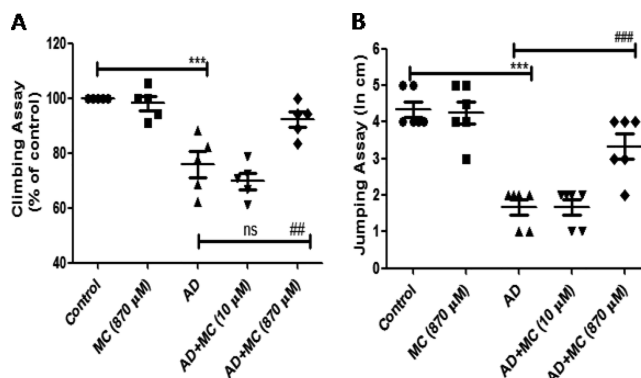
Figure 1. Effect of minocycline on (A) climbing and (B) jumping of AD flies. Minocycline-treated AD flies were compared to unexposed control and exposed positive control flies for 30 days. Data are depicted here as the mean ± SE for six assays ($n = 6$), and significance is described as ***$p < 0.001$ vs the unexposed control and ##$p < 0.01$ and ###$p < 0.001$ vs untreated AD flies.

AD flies for 30 days showed a significant enhancement in climbing behavior ($p < 0.01$, $q = 12.97$) as compared to transgenic AD flies. There was no significant effect seen with a dose of 10 μM minocycline exposure of AD flies for 30 days in comparison to transgenic AD flies.

Analyzing the reduced jumping performance in transgenic AD flies revealed the severity of Aβ42 in the brain. As shown in Figure 1B, a significant reduction was observed in transgenic AD flies [$F_{(4,20)}$, $p < 0.001$, $q = 25.69$] when compared to control flies. However, after 30 days of exposure to 870 μM minocycline, transgenic AD flies showed a significant mitigating effect on jumping behavior with reference to unexposed transgenic AD flies ($p < 0.05$, $q = 6.39$). Like the climbing assay, there was no significant effect seen with 10 μM minocycline exposure in the jumping assay as well.

2.2. Minocycline Attenuates the Decline in Survival of Aβ42-Overexpressed Flies.
We conducted the survival experiment in all of the groups to further corroborate our findings. When compared to control flies, the survival of transgenic AD flies was significantly reduced ($F_{(4,20)}$, $p < 0.01$) (Figure 2). Therefore, we exposed transgenic AD flies to an 870 μM concentration of minocycline, based on the feeding schedule. When transgenic AD flies were exposed to minocycline, their lifespan was dramatically increased compared to control flies. While 75% of transgenic AD flies survived after 30 days of being treated with minocycline, only 50% survival of transgenic AD flies was observed in minocycline-unexposed flies.

2.3. Minocycline Treatment Resulted in a Considerable Reduction in Aβ42 Protein Levels in AD Flies.
Western blotting was used to confirm the efficiency of minocycline in lowering the Aβ42 protein level in transgenic AD flies (Figure 3). When comparing the AD flies to the control flies, there was a significant increase in Aβ42 protein expression [$F_{(4,20)}$, $p < 0.001$, $q = 17.57$]. However, supplementing Aβ42-overexpressed AD flies with an 870 μM
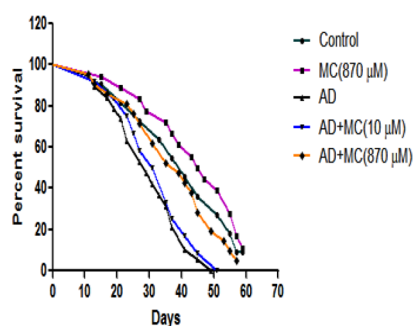
Figure 2. Effect of minocycline on the survival of transgenic AD flies expressing Aβ42. On alternate days, the minocycline-containing meal vials were replaced. Data are presented here as the mean ± SE, and significance is described as *$p < 0.05$ vs the unexposed control.
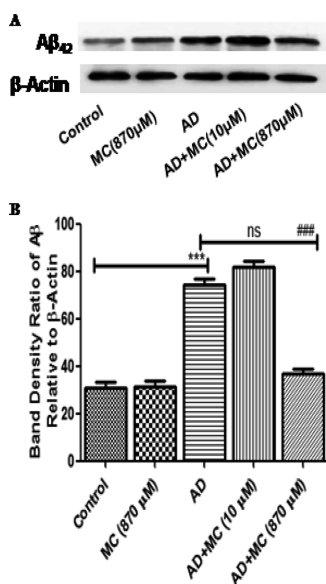

Figure 3. Effect of minocycline on Aβ42 expression in AD flies' brain tissue. Aβ42 expression was determined by Western blotting (A), and the densitometric data shown are after normalization using β-actin as a loading control (B). Data are presented here as the mean ± SD for three assays ($n = 3$), and significance is described as ***$p < 0.001$ vs the unexposed control and ###$p < 0.001$ vs unexposed AD flies.

dosage of minocycline dramatically reduced the Aβ42 protein expression when compared to untreated AD flies ($p < 0.001$, $q = 15.09$). No statistically significant difference was observed in the levels of Aβ42 in control and minocycline-exposed AD flies.

2.4. Minocycline Exposure Attenuates the AchE Activity in AD Flies. Acetylcholinesterase (AchE) activity is a well-known neurotoxicity biomarker that is required for synaptic termination of nerve impulses via acetylcholine metabolism. There was a significant increase ($F_{(4,20)}$, $p < 0.01$, $q = 18.66$) found in AchE activity of the AD flies when compared to the control group (Figure 4). Minocycline administration at an 870 μM concentration significantly ($p < 0.001$, $q = 8.47$) reduced the activity of AchE in AD flies when compared to the unexposed AD flies.

2.5. Minocycline Treatment Improves the Mitochondrial Membrane Potential (ψm) in AD Flies. Mitochondria have been identified as a potential target of Aβ42 under AD circumstances, which has been confirmed by measuring the mitochondrial membrane potential. As shown in Figure 5, a
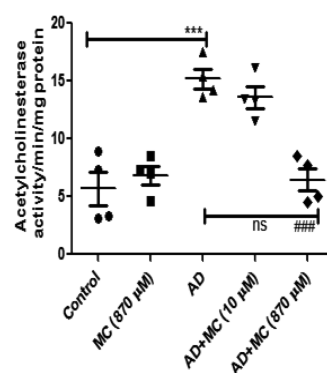
Figure 4. Effect of minocycline on the level of AchE in the brain tissue of Alzheimer's disease flies. A spectrophotometer was used to determine the level of AchE. Data are presented here as the mean ± SE, and significance is described as ***$p < 0.001$ vs the unexposed control and ###$p < 0.001$ vs unexposed AD flies.
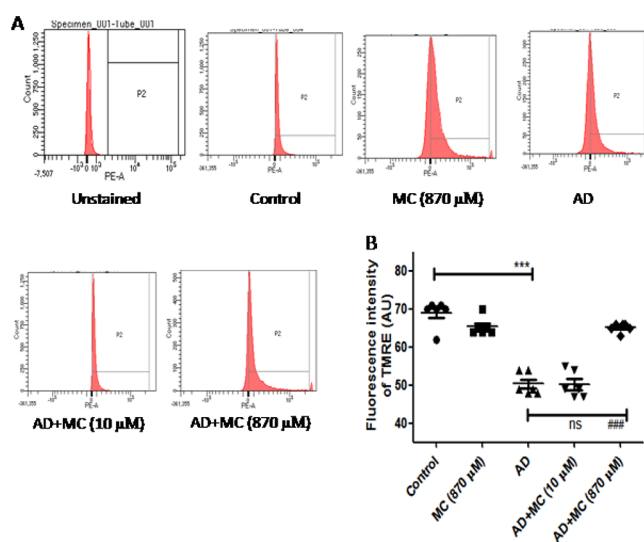

Figure 5. Effect of minocycline on MMP in the mitochondria of AD flies' brain tissue. For MMP analysis, isolated mitochondria were treated with the TMRE dye. Flow cytometry was used to measure MMP. (A) Pictorial representation of TMRE expression. (B) Fluorescence intensity of TMRE. Data are presented here as the mean ± SE, and significance is described as ***$p < 0.001$ vs the unexposed control and ###$p < 0.001$ vs unexposed AD flies.

significant decrease was observed in the mitochondrial membrane potential (MMP) of transgenic AD flies [$F_{(4,20)}$, $p < 0.001$, $q = 16.32$] as compared to the control group. However, transgenic AD flies exposed to 870 μM minocycline revealed a significant reduction in the mitochondrial membrane potential ($p < 0.001$, $q = 12.97$) with reference to unexposed transgenic AD flies. In transgenic AD flies subjected to a low dose of minocycline (10 μM), no statistically significant differences were seen when compared to AD flies.

2.6. Minocycline Supplementation Reduces Mitochondrial Mediated Apoptosis in AD Flies through Modulating the Expression of Apoptotic Proteins. In Aβ42-overexpressed AD, the modulatory effect of minocycline on mitochondrial mediated apoptosis was validated. Apoptosis has been linked to increased expression of JNK, p-JNK, and cleaved caspase 3 proteins. To compare the levels of JNK, p-JNK, and cleaved caspase 3 proteins in control and transgenic AD flies, we used the Western blotting technique to quantify
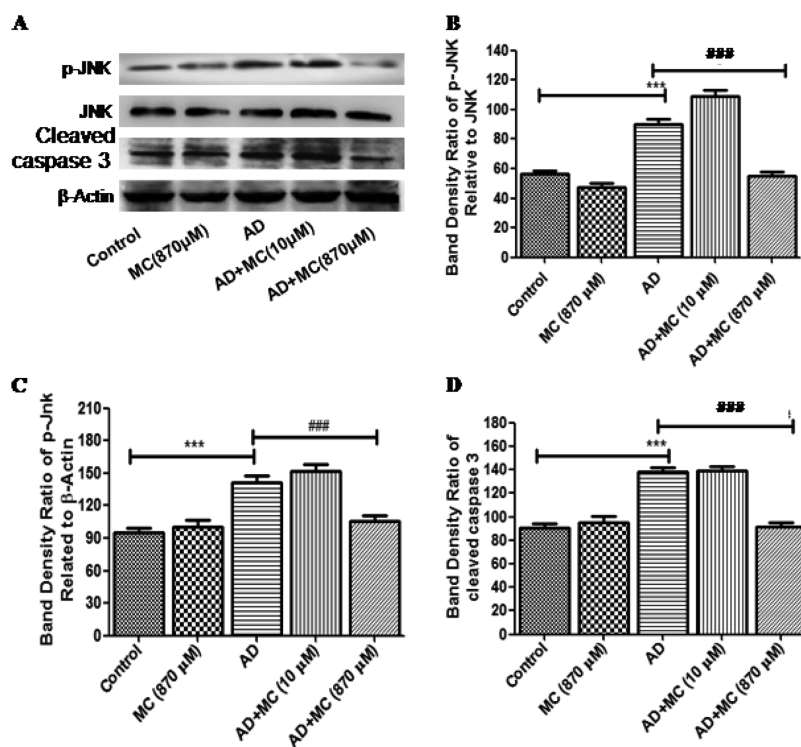
Figure 6. Effects of minocycline on the expression of JNK, p-JNK, and cleaved caspase 3 proteins in AD flies' brain tissue. Western blotting was used to look for JNK, p-JNK, and cleaved caspase 3 expression (A−D). Densitometric data presented are after normalization with the loading control JNK and β-actin for p-JNK (A−C) and for cleaved caspase 3 (A,D). Data are presented here as the mean ± SD, and significance is described as ***$p < 0.001$ vs the unexposed control and ###$p < 0.001$ vs unexposed AD flies.

their expression. As shown in Figure 6, the levels of p-JNK ($F_{(4,20)}$, $p < 0.001$, $q = 12.42$) and cleaved caspase 3 proteins ($p < 0.001$, $q = 27.94$) in the brain were significantly increased in transgenic AD flies as compared to the control group. However, minocycline at a dose of 870 μM significantly normalized the level of p-JNK ($p < 0.001$, $q = 12.85$) and cleaved caspase 3 proteins ($p < 0.001$, $q = 26.80$) in AD flies with reference to unexposed AD flies. However, no significant effect was seen with the low dose (10 μM) of minocycline when compared to the untreated AD flies.

3. DISCUSSION

Clinically, AD is characterized by neurobehavioral alterations, memory decline, and degradation of neurons. Despite the availability of numerous medications and treatments, the disease's severity has yet to be managed. As a result, different drugs (drug repurposing) are becoming popular as alternative treatments for AD. Therefore, the current study was to look at how minocycline affected behavioral and mitochondrial dysfunction in transgenic AD flies.

The present study demonstrates for the first time that minocycline attenuates Aβ42 overexpression in a genetic model of AD in *Drosophila*. Minocycline reduced the Aβ42 level and mitochondrial dysfunction in AD-like *Drosophila*. In our previous study, we found that examining the Aβ42 overexpression in a *Drosophila* model offers an excellent platform for understanding AD pathology. The results of the study reflected that mitochondrial dysfunction and JNK activation have a key role in the progression of AD.[22] Hence, to further explore this concept, the present study was designed with minocycline, an antibiotic, which has been demonstrated to affect the mitochondrial function and MAPK signaling.[23] It

has been reported to diminish microgliosis and reduce caspase protease expression following spinal cord injury in mice.[15] Minocycline exerts promising neuroprotective effects against PD. Cankaya et al., in their review discussed numerous in vitro and in vivo studies promoting minocycline as a neuroprotective agent with its well-known effect on various neurodegenerative disease pathological pathways.[13] It has also been studied that minocycline resists oxidative stress and extends the lifespan of *Drosophila* by forkhead box O (FOXO).[24] Based on these findings, in the current study, we chose the similar humanoid Aβ42 peptide to overexpress Aβ42 in the *Drosophila* brain to initiate amyloidogenesis and confirm the neuroprotective efficacy of minocycline against the AD consequences through behavioral and survival assays, mitochondrial function changes, and neuronal loss in the presence and absence of minocycline.

AD is a progressive neurological disease defined by age-related memory loss and impairment of many cognitive processes. Extracellular Aβ plaques and intracellular neurofibrillary tangles are the two most common pathology hallmarks of AD. Losses of neurons, synapses, and synaptic function, as well as mitochondrial alterations and inflammatory responses, are all linked to AD. Neuronal loss could account for 20−30% of the brain weight reduction seen in AD. Synaptic loss, synaptic damage, and mitochondrial oxidative injury have all been identified as early steps in the course of AD.[4] Aβ overexpression causes diffused Aβ accumulation, progressive locomotor impairment, early death, and learning disabilities. It was previously confirmed that neurological deficit at the organism level in AD was proven by analysis of behavioral alteration.[24,25] Therefore, we assessed the climbing assay, jumping assay, and survival assay in the present study. In the

PD model, minocycline helps in the healing of perturbation in rotarod and gait patterns.[16] Also, in other neurological disease conditions, minocycline treatment improved motor neuron-associated deficits. In consensus with previous findings, our results also reflected that treatment with minocycline for 30 days greatly reduced the deterioration in climbing and jumping behavior of AD flies when compared to the AD alone group. Our results reflected a drop in survival of Aβ42-overexpressed transgenic flies in comparison to normal flies. However, addition of an 870 μM dosage of minocycline to the diet mitigated the deleterious effect of Aβ42 overexpression. Long-term minocycline treatment has also been shown to improve the survival rate of aged *Drosophila*.[26] Related findings have also been examined in the rat and mouse model systems after minocycline supplementation.[27,28] The availability of the neurotransmitter, which plays a vital role in the control of the neuronal system, also affects motor neuron activity.[29] Acetylcholine is a critical neurotransmitter for memory and learning consolidation, which is impaired in AD patients.[30] The presence of acetylcholine is regulated by the AchE enzyme. Increased AchE activity is related with less availability of acetylcholine.[31] Keeping this in mind, we have checked the AchE enzyme activity, and a significant increase was found in AD flies when compared to normal flies. This would suggest that minocycline might have attenuated behavioral impairments via acetylcholine regulation. This result is associated with dementia encountered by AD patients.

Mitochondrial dysfunction plays a key role in neuronal degeneration and AD progression. Mitochondria that have been damaged are less bioenergetically effective, resulting in structural and functional repercussions for AD neurons.[32] Cumulative results of previous studies suggested that alteration of the mitochondrial function plays a significant role in AD pathogenesis.[33] The major episodes studied in AD pathogenesis are energy failure, excessive production of ROS, membrane potential alteration, and cell death, all of which are associated to mitochondrial function variation.[34] In addition, compromised enzyme activity of the tricarboxylic acid (TCA) cycle, mitophagy, and impaired dynamics of mitochondria have been also reported in AD pathogenesis.[35] It was established in the mouse model that minocycline supplementation has potential to preserve the integrity of the mitochondrial membrane potential.[36] In this context, we have examined the effect of minocycline on the mitochondrial membrane potential for the verification of minocycline as a neuroprotective agent on mitochondrial mediated AD pathogenesis. Our flow cytometric result analysis of TMRE proved that minocycline significantly turns the altered mitochondrial function toward normal in transgenic AD flies in terms of increased fluorescence intensity when compared to the AD alone group.

Furthermore, all the above consequences have been linked with the promotion of apoptotic mechanisms. The activation of the JNK signaling pathway is intimately linked to apoptosis. JNK is a member of the MAPK family. It has been reported previously that Aβ might activate the JNK signaling pathway, increasing the level of p-JNK and colocalizing p-JNK and Aβ expression in postmortem samples of the brain of AD patients. Indeed, Aβ peptides have been shown to activate JNK in vitro, with p-JNK increasing after treatment with Aβ in primary cultures of the cortex and the hippocampus of C57BL/6 mice, primary cell cultures of the cortex from Wistar rats, and SH-SY5Y neuroblastoma cells.[37] The apoptotic effect on Aβ-induced neurons was considerably decreased in JNK3 knockout mice.[38] Additionally, our previous study demonstrated that the activation of JNK participated in the degeneration of neuronal cells in the *Drosophila* model of AD.[22] In this context, the expression of JNK/p-JNK and cleaved caspase 3 proteins was examined in the presence and absence of minocycline. A significant decrease in the expression of p-JNK and cleaved caspase 3 proteins in transgenic AD flies as compared to the AD alone group confirms the antiapoptotic nature of minocycline. Similar studies reported that the administration of minocycline in a mouse model of AD reduced the neuronal loss through the inhibition of JNK activation.[39,40] These observations suggest that minocycline improves the mitochondrial function and attenuates apoptosis, which might be by inhibiting the JNK-mediated neuronal loss and slowing down the related behavioral deficit paradigm linked with AD pathogenesis. However, more detailed studies are required in the future to explore the neuroprotective efficacy of minocycline against AD symptoms. Taken together, it is advocated that minocycline could be employed as an effective neuroprotective agent for the cure of AD.

4. MATERIALS AND METHODS

4.1. Fly Strain. The Bloomington *Drosophila* Stock Center provided transgenic fly lines that express wild-type human Aβ42 under UAS control in neuron w[1118];P{w[+mc] =UAS-APP.Aβ42.B}m26a and GAL4"w[*];P{w[+mc] =GAL4-elavL}"3 (Indiana University, Bloomington, IN, USA). Virgin females of GAL4-elav.L were crossed with males of these strains (and vice versa), and the progenies were expressed as human Aβ42 in the fly brain.[22]

4.2. Rearing of Flies and Exposure to the Drug. At 24 ± 1 °C, the flies were cultivated on a conventional *Drosophila* feed, which included agar, maize, sugar, and yeast. For healthy growth, additional yeast suspensions were administered. At final concentrations of 10 and 870 μM, minocycline was mixed in the food and administered to the flies.[26] A control of Elav-gal4 was used.[22]

4.3. Behavioral Assays. *4.3.1. Climbing Assay.* A total of 20 male flies were used in the climbing assays, which were placed in the first chamber, taped to the bottom, and given 20 s to climb a distance of 10 cm. Those flies that successfully climbed 10 cm or more in 20 s were moved to a different chamber, where both groups of flies were given another chance to climb the 10 cm distance. This technique was carried out five times. The total number of flies in each chamber was counted after five trials.[22,25]

4.3.2. Jumping Assay. This assay was carried out using both control and drug-exposed flies, according to a previously published report. In an empty plastic vial, a single fly was placed and given a one-minute rest period. The fly tapped at the bottom after resting, and its jumping behavior was compared to the marks on the vial. Each fly was given five jump attempts at 1 min intervals, and the jumping activity was calculated as the average height of each fly's jumps. The data were displayed as a centimeter-long fly jump.[22,25]

4.4. Survival Assay. Two-days-old males were transferred to Pyrex culture 9.6 9100 mm glass vials with 1 mL of the test food and cotton stoppers. The flies were housed in vials in groups of five. Daily, fresh protectant solutions were made in normal maize meals at various concentrations. The dead flies were tallied, and survivors were moved to freshly prepared

food at the same time every day. Three replicates of each treatment and control were done.[22,25]

4.5. Assay of Oxidative Stress. *4.5.1. Estimation of Acetylcholinesterase (AchE) Activity.* Estimation of the AchE activity was performed in the reaction mixture consisted of 100 μL of the sample, 650 μL of 0.1 M phosphate buffer, and 100 μL of DTNB (dithionitrobenzoic acid; 5,5′-dithiobis(2-nitrobenzoic acid)). Then, 10 μL of acetylthiocholine was added, and the change in the OD at 412 nm was noted at a 3 min interval as described previously by Beg et al.[25]

4.5.2. Isolation of Mitochondria. Differential centrifugation was used to isolate the mitochondria from the brain. In a nutshell, the heads of the flies were homogenized in an ice-cold isolation buffer containing 250 mM sucrose, 10 mM HEPES (4-(2-hydroxyehtyl)-1-piperazineethanesulfonic acid), 1 mM EGTA (ethylene glycol tetraacetic acid), and 0.1% fat-free BSA (bovine serum albumin) adjusted to pH 7.4 with Tris and then centrifuged at 1000g for 5 min at 4 °C. The supernatant was taken and centrifuged for 10 min at 4 °C at 10,000g. The pellets were then resuspended and washed twice in a washing medium comprising 250 mM sucrose, 10 mM HEPES, and 0.1 mM EGTA buffer adjusted to pH 7.4 with Tris. Finally, the pellet was resuspended in a 0.1% fat-free BSA suspension medium with 250 mM sucrose and 10 mM HEPES and adjusted to pH 7.4 with Tris.[22]

4.5.3. Assessment of the Mitochondrial Membrane Potential (ψm). Flow cytometry was used to quantify the mitochondrial membrane potential using the membrane-permeable fluorescent dye TMRE (tetramethylrhodamine ethyl ester). In a reaction solution (pH 7.0) containing 50 mM sucrose, 20 mM MOPS (3-(N-morpholino)propane sulfonic acid), 10 mM Tris, 0.5 mM Mg^{2+}, and 5 mM succinate, isolated mitochondria were treated with the TMRE dye. Excitation at 488 nm and emission at 590 nm were used to examine samples after 10 min of incubation at 37 °C. Amounts of arbitrary fluorescence units per milligram of protein were calculated.[22]

4.6. Western Blotting. Fly heads (100) were homogenized in RIPA (radioimmunoprecipitation assay) buffer (50 mM Tris-HCl, pH 8.0, 0.5% sodium deoxycholate, 1% Triton X-100, and 150 mM NaCl) containing 1% SDS (sodium dodecyl sulfate) for consecutive extractions. The protein (30 mg) was separated on a 4−20% gradient Tris-HCl gel and then transferred to PVDF (polyvinylidene fluoride) or nitrocellulose membranes (Bio-Rad). The transferred membrane was then blocked using nonfat milk powder. For blocking nonspecific antibody binding, the membrane was incubated in TBST buffer (10 mM Tris-HCl, 150 mM NaCl, and 0.1% Tween 20, pH 7.4) containing 5% nonfat milk. After blocking, primary antibodies against JNK, p-JNK, cleaved caspase 3, and actin were used to probe the membrane (1:1000; Santa Cruz, USA). Horseradish peroxidase-conjugated secondary antibodies (1:2000) were utilized for immunodetection. Finally, using a Femto reagent (Thermo Fisher Scientific, Rockford, USA) on a ChemiDoc system, proteins were visualized (Bio-Rad, CA, USA).[22]

4.7. Statistical Analysis. Statistical analysis was performed using GraphPad Prism software (version 5.0). ANOVA followed by Tukey's test was performed to compare significant differences between different groups. Values of $p < 0.05$ were considered significant.[22]

5. CONCLUSIONS

In conclusion, minocycline exhibited neuroprotective effects against Aβ-mediated mitochondrial dysfunction and apoptosis. Additionally, minocycline reduced Aβ accumulation and attenuated behavioral impairment in the Aβ-overexpressed AD transgenic model of *Drosophila*. These observations speculate on the fact that minocycline may abrogate AD-like manifestations perhaps via the JNK/caspase 3-mediated pathway in Aβ-induced AD-like symptoms in flies. The current study has addressed novel aspects of minocycline in neuroprotection by investigating its role as a modulator of mitochondrial dysfunction. Hence, minocycline could be a promising therapeutic approach in the treatment of AD. However, future research in this area will attempt to decipher its possible role and mechanism of action.

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Notes

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