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Synergistic Therapeutic Potential of Curcumin and Quercetin Co-Administration in Alzheimer's disease: Insights from Transgenic *Drosophila melanogaster* Models

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Abstract

Glycogen Synthase Kinase 3 beta (GSK-3β) activation promotes intracellular neurofibrillary tangles (NFTs) and extracellular amyloid beta plaques formation which are the major hallmarks of Alzheimer's disease (AD). Oxidative stress and mitochondria dysfunction have been implicated in the perturbation of GSK-3β. Curcumin and quercetin are phytochemicals with antioxidant and anti-inflammatory properties. This study was aimed at investigating the neuroprotective effects of curcumin and quercetin on AD models of *Drosophila melanogaster*. Flies were grouped into five (5) groups containing one hundred and fifty flies per group. First group (wild control) received distilled water; second group (Wild treated) received 200 mg/kg of curcumin and quercetin; third group (transgenic) received distilled water; fourth group (transgenic+LD) received 50 mg/kg of curcumin and quercetin; fifth group (transgenic + HD) received 200 mg/kg of curcumin and quercetin for duration of five days. Survival assay, climbing assay, catalase, total thiol, acetylcholinesterase, and malondialdehyde level were investigated at the end of treatment duration. Additionally a molecular docking analysis was performed using PyRx and curcumin and quercetin were docked against the implicated protein GSK-3β and this showed higher binding affinities than the standard. This study showed that curcumin and quercetin improved locomotor activity and survival rate and increased total thiols levels and catalase activity and decreased MDA and AChE activities. Overall curcumin and quercetin ameliorated AD in *D. melanogaster*. Therefore, co-administration can be regarded as a possible therapeutic agent for AD.

Keywords: *Alzheimer's disease, Curcumin, Quercetin, GSK-3β, Oxidative stress and Drosophila melanogaster*

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Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common form of dementia, characterized by brain atrophy, neuronal death, and a decline in cognitive, behavioral, and social abilities. This deterioration profoundly impairs an individual's ability to function independently. Globally, AD accounts for 60% to 70% of the estimated 50 million cases of dementia, affecting approximately 5.8 million individuals in the United States aged 65 and above. The initial symptoms of AD often include forgetfulness of recent events or discussions. Despite its widespread prevalence, there is no cure or treatment capable of halting the pathological processes underlying the disease (Liu *et al*., 2019). The hallmark features of AD pathogenesis include extracellular amyloid-β (Aβ) plaques, intracellular neurofibrillary tangles (NFTs), glial activation, and neuronal loss. These are accompanied by cerebrovascular amyloidosis, inflammation, and substantial synaptic alterations (Olanrewaju *et al*., 2018; Guo *et al*., 2020). A central molecular player in AD pathology is glycogen synthase kinase 3β (GSK3β), a kinase implicated in the genesis of Aβ plaques and NFTs (Lauretti *et al*., 2020). NFTs arise from the hyperphosphorylation of tau, a microtubule-associated protein, while Aβ plaques originate from the proteolytic cleavage of amyloid precursor protein (APP) (Xiong *et al*., 2011). Moreover, oxidative stress exacerbates AD pathology

by perturbing the Akt signaling pathway, which modulates GSK3β activity. This suggests that substances with potent antioxidative properties could serve as putative therapeutic agents.

Among promising candidates, curcumin, a polyphenol derived from *Curcuma longa*, has demonstrated therapeutic potential in AD. Curcumin enhances cognitive function by scavenging free radicals, mitigating inflammatory responses, and inhibiting the activity of lipophilic agents (Mishra *et al*., 2008). Another potent compound is quercetin, a flavonoid found in various fruits, vegetables, and teas. Quercetin exhibits antioxidative, anti-inflammatory, and anticancer properties, as shown in both in vitro and in vivo studies (Angélica *et al*., 2015). While quercetin has been associated with toxicity at high concentrations, co-administration with curcumin could counterbalance this effect, maximizing therapeutic potential. This study investigates the therapeutic efficacy of co-administering curcumin and quercetin in an AD-like model using *Drosophila melanogaster*. The fruit fly, *Drosophila melanogaster*, has been a foundational model organism in genetic and behavioral research for over a century, owing to its genetic overlap with humans -approximately 60% of its genome is conserved. It is particularly valuable in studying neurodegenerative diseases, including AD, due to its short life cycle, genetic tractability, and the availability of mutants. The *Drosophila* genome contains the gene shaggy (sgg), encoding a protein homologous to human GSK3β, which is central to AD pathology. Specific brain regions in *Drosophila*, such as mushroom bodies and the central complex, are integral to studying learning, memory, and motor control. These structures serve as proxies for human brain regions affected by AD. Overexpression of GSK3β in the fly's nervous system has been linked to age-related neurodegeneration and cognitive impairments, underscoring its relevance for modeling AD pathogenesis. The availability of genetic tools and well-characterized mutants in *Drosophila* facilitates high-throughput investigations into the molecular mechanisms underlying AD and testing of therapeutic compounds.

GSK3β is a serine/threonine kinase that plays a dual role in AD pathology by promoting both tau hyperphosphorylation and Aβ production. Hyperactivation of GSK3β destabilizes the neuronal cytoskeleton, disrupts synaptic function, and accelerates cognitive decline. Dysregulated signaling pathways, such as PI3K/Akt and Wnt/β-catenin, further enhance GSK3β activity, creating a feedback loop that perpetuates neurodegeneration. Inflammation, driven by activated microglia, exacerbates the neurotoxic environment in AD. Microglial activation results in the release of pro-inflammatory cytokines, further activating GSK3β and amplifying neuronal damage. Studies using *Drosophila* models have shown that modulating GSK3β activity can mitigate synaptic impairments and cognitive dysfunction, highlighting its potential as a therapeutic target. The interplay between Aβ plaques, tau tangles, and GSK3β underscores a complex network of pathological interactions driving AD progression. Current pharmacological interventions for AD, including acetylcholinesterase inhibitors and NMDA receptor antagonists, offer symptomatic relief but do not address the underlying disease mechanisms. Antioxidants such as curcumin and quercetin have emerged as promising candidates due to their neuroprotective properties. Curcumin reduces Aβ plaque formation, inhibits tau hyperphosphorylation, and combats oxidative stress through its potent free radical scavenging activity. Additionally, it modulates microglial activity and inhibits acetylcholinesterase, further enhancing its therapeutic potential (Mishra *et al*., 2008).

Quercetin, a flavonoid found in grapes, onions, and green tea, exhibits antioxidative, anti-inflammatory, and anti-carcinogenic properties. It has been shown to improve cognitive function and reduce tau phosphorylation in AD models (Angélica *et al*., 2015). However, high doses of quercetin can induce toxicity, limiting its clinical utility. Co-administration with curcumin could counteract this toxicity, allowing for the synergistic effects of these compounds to be harnessed. Such combination therapies hold promise in mitigating AD pathology by targeting multiple disease pathways simultaneously. Oxidative stress, a hallmark of AD, results from an imbalance between ROS production and antioxidant defenses. The brain's high oxygen consumption and lipid-rich environment make it particularly vulnerable to oxidative damage. ROS-mediated lipid peroxidation disrupts cellular membranes and exacerbates neurodegeneration. Elevated levels of oxidative biomarkers, such as malondialdehyde (MDA) and reduced total thiol concentrations, have been observed in AD patients. Curcumin and quercetin mitigate oxidative stress through complementary mechanisms. Curcumin scavenges ROS and enhances the activity of endogenous antioxidant enzymes, while quercetin inhibits lipid peroxidation and chelates metal ions. These antioxidative effects protect neurons from oxidative damage, preserving synaptic function and cognitive performance. The co-administration of these compounds could provide a robust defense against oxidative stress, addressing a key pathological feature of AD.

This study is specifically designed to investigate the therapeutic effects of co-administration of curcumin and quercetin against the manifestations of Alzheimer's disease. By leveraging the *Drosophila melanogaster* model, this research seeks to evaluate the potential synergistic benefits of these compounds in mitigating key pathological hallmarks of AD, such as amyloid-β plaques, tau hyperphosphorylation, oxidative stress, and chronic neuroinflammation. The study will also explore how the combination of these antioxidants can counteract the limitations associated with individual administration, such as quercetin's toxicity at high doses. These findings are expected to provide valuable insights into the development of more effective therapeutic strategies for combating Alzheimer's disease and improving patient outcomes.

Materials and Methods

2.1 Materials

Electric cooker, Pot, Turning stick, Bowl, Spoon, Sensitive scale, Petri dishes, Spatula, Funnel, Conical flask, Tape, Tissue roll, Trays, Vials, Markers, Foam corks, Eppendorf tubes, Test tubes, Rack, Tips, Mortar, Pestle, Small paint brush, Refrigerator, Centrifuge, Spectrometer, Jars, Micropipette.

2.2 Chemical and Reagents

Curcumin and Quercetin were obtained and administered at different doses to the standard cornmeal medium for larvae rearing and used to supplement fresh yeast paste for adult feeding in order to observe their effects and was provided by the Eureka drosophila Laboratory. Distilled water, Corn meal, Yeast, Nipagin, Agar Agar, Phosphate Buffer saline, Ethanol, Thiobarbituric acid (TBA), Dithionitrobenzoic acid (DTNB), Carbon dioxide were also provided by Eureka Laboratory.

2.3 Ethical Approval

This study was approved by the Babcock University Ethics commission (BUHREC) with the BUHREC number 995/21

2.4 *Drosophila melanogaster* stock and culture

Drosophila melanogaster models of both genders were cultured on a cornmeal medium which consists of corn meal, yeast, agar agar, and nipagin mixed with ethanol at a constant temperature and humidity (21-25 C; 60-70% relative humidity) under 12 hours dark/light cycle conditions at the Eureka Drosophila Laboratory, in the department of Anatomy, Babcock University, Ogun State, Nigeria. The Wild flies were obtained from Abolaji's Lab in the University of Ibadan, Nigeria while the transgenic flies were obtained from Russell's Lab in the University of Cambridge, United Kingdom.

2.5 Meal Preparation

Table 1 presents cornmeal preparation.

Table1: Cornmeal medium measurements

S/N	Ingredients	Full meal	Half meal	Quarter meal
1.	Distilled Water	1000ml	500ml	250ml
2.	Corn meal	60g	30g	15g
3.	Yeast	7g	3.5g	1.75g
4.	Agar Agar	7.5g	3.75g	1.9g
5.	Nipagin	1g	0.5g	0.25g

2.6 Treatments of *Drosophila melanogaster* with Curcumin and Quercetin

Five groups of 3 vials each containing 50 flies and cultured for 5 days for biochemical assays and 7 days for survival assay. Flies were exposed to doses of 50 mg/kg and 200 mg/kg diet of Curcumin, as well as 50 and 200 mg/kg diet of Quercetin for 5 days as indicated below:

Group 1. Wild type (Control)
Group 2. Wild type (Treatment) 200 mg/kg of Quercetin and Curcumin (High dose)
Group 3. Transgenic type (Control)
Group 4. Transgenic type (Treatment) 50 mg/kg of Quercetin and Curcumin (Low dose)
Group 5. Transgenic type (Treatment) 200 mg/kg of Quercetin and Curcumin (high dose)

2.7 Negative Geotaxis Assay

This was conducted on 10 flies from each group. This was done to determine the locomotor activity of the flies. 10 flies of both genders were selected from each of the control groups and were anesthetized, they were put in different vials which have already been labelled of diameter 1.5cm and length of 15cm. In each of the vials, a 6cm mark was made and the number of flies that were able to regain consciousness and fly above the mark within 10 seconds was recorded and was used to calculate the climbing activity. This procedure was done 3 times for each group.

2.8 Survival Assay

30 flies of both genders were subdued to a similar diet for each group. This was done for 7 days and the daily mortality of flies was recorded.

2.9 Preparation of Samples for Biochemical Assays

All flies were anaesthetized with CO2 and homogenized using 0.1 M phosphate buffered saline of pH 7.4 (1:10 (flies/volume (µL)), and centrifuged at 13,000 rpm for 5 minutes at 4°C. The supernatants were collected and used to estimate biochemical assays for catalase activity, total thiol levels, acetylcholinesterase activity and malondialdehyde.

1. Total thiol levels:

510 µL of 0.1 M phosphate buffer (pH 7.4), 20 µL of sample, 35 µL of 1 mM dithionitrobenzoic acid (DTNB), and 35 µL of distilled water make up the reaction mixture. The absorbance was measured at 412 nm after 30 minutes of incubation at room temperature.

2. Catalase activity

The clearance of H_2O_2 at 240 nm at 25 °C was monitored using a reaction mixture containing 1.8 mL potassium phosphate buffer, pH 7.0, 180 mL 300 mM H_2O_2, and 20 mL sample (1:50 dilution). Using a UV–visible spectrophotometer (Shimadzu), the decrease in H_2O_2 was measured at 240 nm for 2 minutes (10 s intervals) and expressed as mmol of H_2O_2 consumed/min/mg of protein.

3. Malondialdehyde (MDA)

With 500 l of phosphate buffer saline (1 M, pH 7.4) and 500 l of trichloroacetic acid (TCA), 100 l of the sample was pipetted into each of the test tubes. The resultant mixture was then incubated at ambient temperature for 2 minutes before being centrifuged at 13000 rpm. 1000 (l) of 0.33percent thiobarbituric acid (TBA) was added to the supernatant, and the combination was then incubated for 1 hour. The absorbance of the pink-colored product was measured at 532 nm.

4. Acetylcholinesterase activity:

The reaction was observed in a 0.1 M potassium phosphate buffer with a pH of 7.4, 1 mM 5, 5'-dithionitrobenzoic acid (DTNB), and 0.8 mM acetylthiocholine as the initiator. At 412 nm, the reaction was measured for 2 minutes (30 sec intervals). The activity of the enzyme was calculated as µmol of acetylthiocholine hydrolyzed/minute/mg protein.

2.10 In-Silico Methodology

Ligands and protein mining: The structure data file (SDF) format for Quercetin and Curcumin were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) with PubChem ID 5280343 and 969516 respectively. The 3D crystal structures for GSK-3 Beta complexed with Indirubin-3'-monoxime (PDB ID: 1Q41) were obtained from the RCSB protein data bank (PDB) (http://www.rcsb.org/pdb/home/home.do).

Ligands and protein preparation: The 3D structure of the protein targets were uploaded onto Pymol in order to prepare for docking and it was converted into PDBQT format using the PyRx software. The ligands were also imported onto the PyRx accordingly and converted to PDBQT format using Open Babel.

Molecular Docking: Molecular docking analysis was performed to further investigate the anti-oxidative capacity of Curcumin and Quercetin by comparing their inhibitory capabilities to that of the standard ligand (ANP) against the implicated protein. Each ligand was docked using the Autodock Vina option based on scoring function in the PyRx workspace. The 3D structures of the protein–ligand complexes and ligand–protein interactions were visualized using the Pymol and Discovery Studio 2017.

2.11 Statistical Analysis

Analysis was carried out using a one-way analysis of variance (ANOVA) - ordinary, to determine significant differences among multiple groups under various treatments, succeeded by Tukey's post-hoc test. Survival data was however analyzed using the Bonferroni analysis method, and the log rank test was used to make comparisons between groups, utilizing the GraphPad Prism 7.0 software. P values less than 0.05 ($p < 0.05$) was considered statistically significant. All the data were reported as the Mean ± Standard Error of Mean (SEM).

3. Results

3.1 Negative Geotaxis Assay

The negative geotaxis assay (Figure 1) shows a significant decrease of locomotory activity in the transgenic (Sgg) group when compared to the wild treated (Wild+Q+C) group, Low dose (Sgg+50 mg/kg) and High dose (Sgg+200 mg/kg) and no significance when compared to the Wild. (*p<0.05, **p<0.01, ***p<0.005).

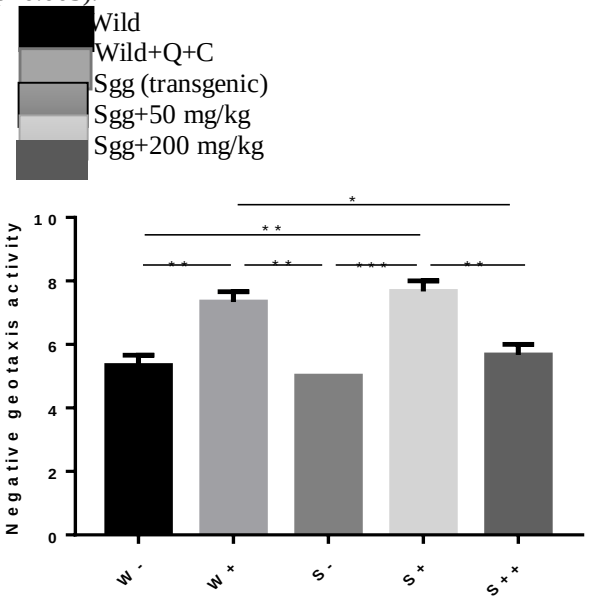

Figure 1: Represents locomotory activity of *Drosophila melanogaster* across experimental groups.

3.2 Survival Assay

The survival assay (Figure 2) showed higher mortality in the transgenic group, which was mitigated by curcumin and quercetin treatment. When compared to the other groups, the number of dead flies in the transgenic group increased significantly and progressively over the course of the trial. Treatment with low and high doses of Curcumin and Quercetin decreased transgenic flies' mortality and increased the number of flies that survived to the end of the experiment.

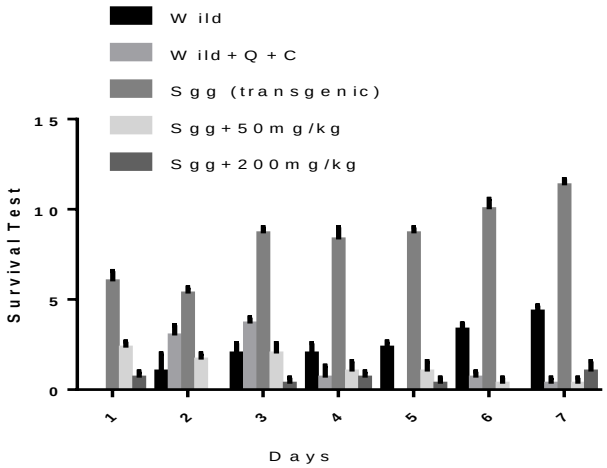

Figure 2: The mortality rate of flies across experimental groups.

3.3 Total Thiol Level

The data from this result (Figure 3) shows a significant increase of total thiol levels in the transgenic (Sgg) group when compared to the Low dose (Sgg+50 mg/kg) and a significance decrease when compared to the Wild. (*p<0.05, **p<0.01, ***p<0.005).

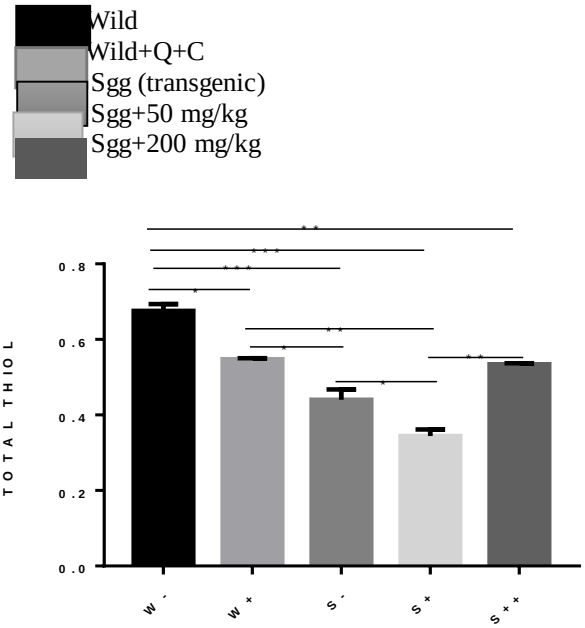

Figure 3: Represents Total Thiol levels of *Drosophila melanogaster* across experimental group.

3.4 Catalase Activity

The data from this result (Figure 4) shows a significant decrease of catalase activity in the transgenic (Sgg) group when compared to the wild, wild treated (Wild+Q+C) group and High dose (Sgg+200 mg/kg). (*p<0.05, ***p<0.005, ****p<0.001)

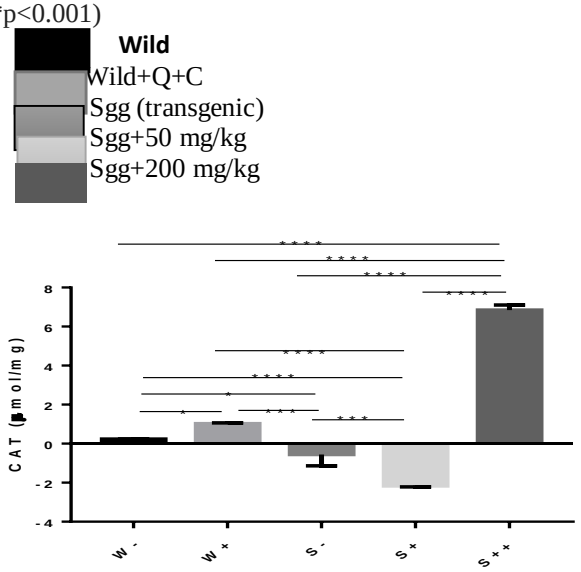

Figure 4: Represents catalase activity of *Drosophila melanogaster* across experimental groups.

3.5 Malondialdehyde Level

The data from this result (Figure 5) shows a significant increase of malondialdehyde level in the transgenic (Sgg) group when compared to all other groups. (*p<0.05, **p<0.01, ***p<0.005).

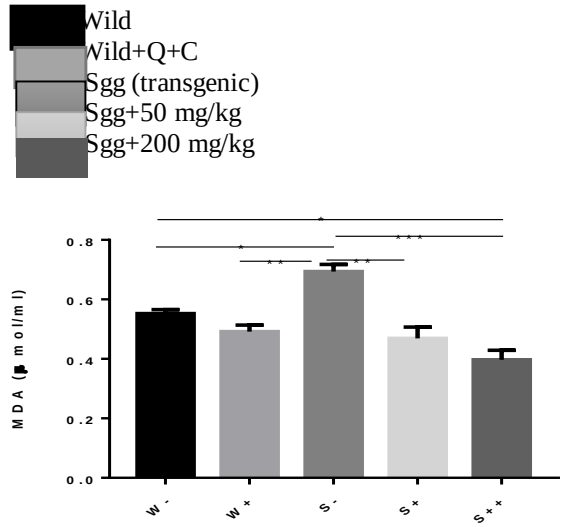

Figure 5: Lipid peroxidation marked by *malondialdehyde* level in the brain lysate of *Drosophila melanogaster* across experimental groups.

3.6 Acetylcholinesterase Activity

The data from this result shows a significant decrease of Acetylcholinesterase activity in the transgenic (Sgg) group when compared to the Low dose (Sgg+50 mg/kg) and High dose (Sgg+200 mg/kg). (*p<0.05, **p<0.01, ***p<0.005, ****p<0.001).

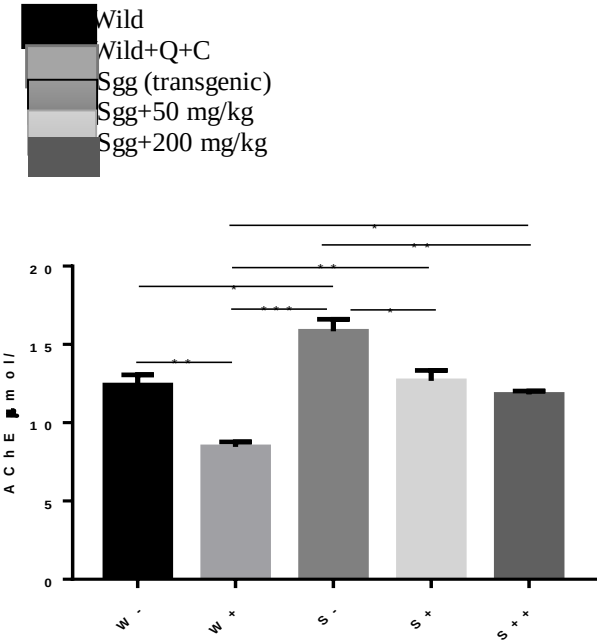

Figure 6: Represents Acetylcholinesterase activity of *Drosophila melanogaster* across experimental groups.

3.7 Molecular Docking Analysis

Molecular docking analysis (Tables 2 & 3, Figures 7 & 8) demonstrated strong binding affinities of quercetin and curcumin, supporting their potential therapeutic role.

According to Table2, the docking scores of Quercetin (−7.7 kcal/mol) and Curcumin (−7.1kcal/mol) were higher than that of the standard (ANP) (−6.6 kcal/mol) in H. sapiens.
According to Table 3, Quercetin and curcumin m*et a*ll the parameters of Lipinski rule It indicated that the quercetin and curcumin had good absorbability and bioavailability as a drug

Table 2. The Binding Affinities of the Ligands

Ligands	PubChem CID	Binding Affinity ΔG energy (Kcal/mol)
Quercetin	5280343	-7.7
Curcumin	969516	-7.1
ANP	-	-6.6

Table 3: Molecular Properties (Drug likeness) of Ligands compounds

Ligands	Molecular Weight (g/mol)	Acceptor of H-bond	Donor of H bond	LogP	Topological Polar Surface Area TPSA (Å2)
Lipinski rule of five	≤ 500	≤ 10	≤ 5	< 5	< 140
Quercetin	302.237	7	5	1.4902	127.45
Curcumin	368.384	6	2	2.949	93.06

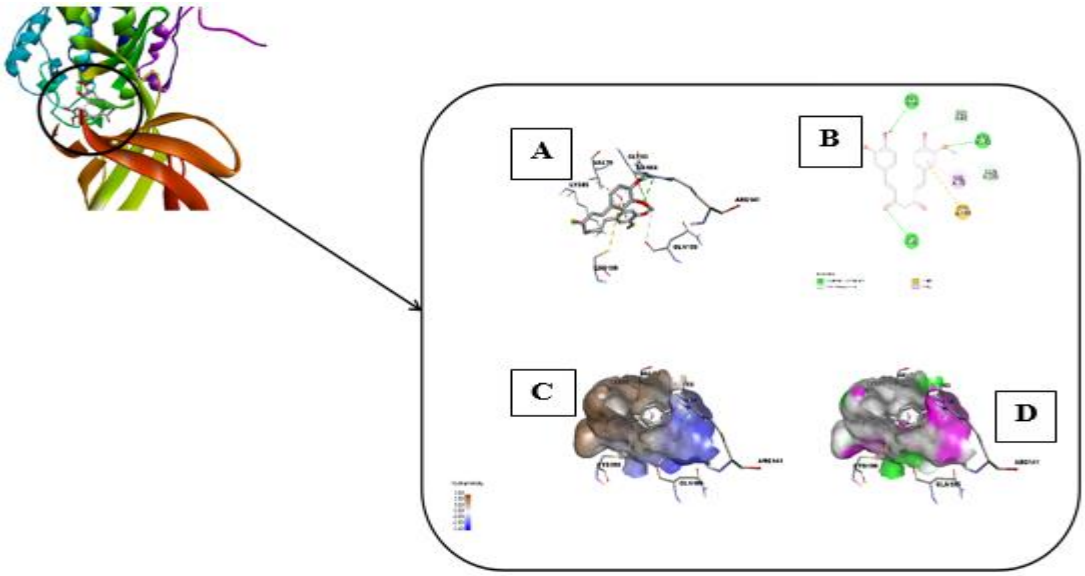

Figure 7: Complex ligand interaction (Curcumin binding to the protein) (A) 3D view of binding interaction (B) 2D view of binding interaction (C) Hydrogen bond (acceptors and donors) (D) Hydrophobic regions.

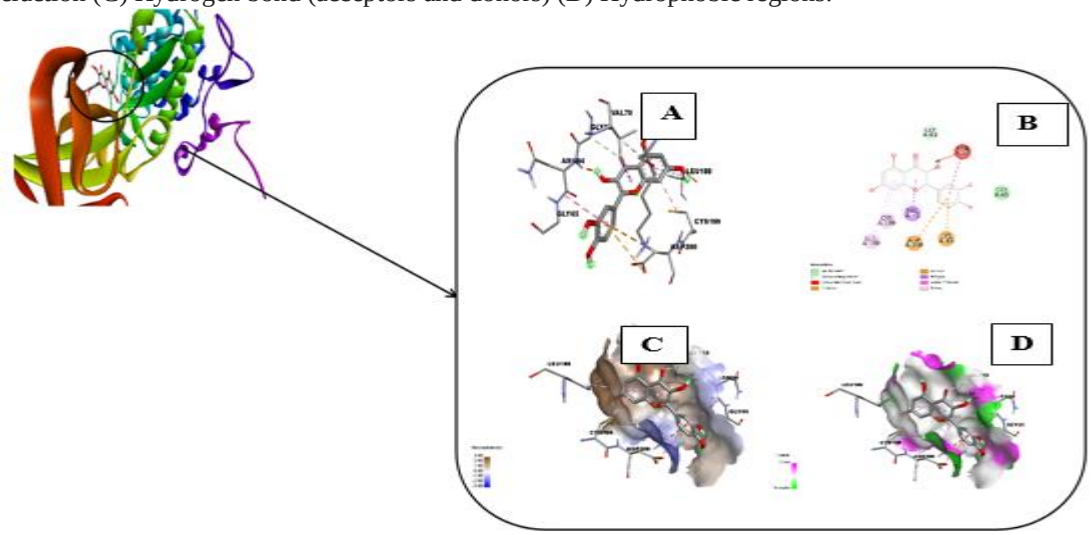

Figure 8: Complex ligand interaction (Quercetin binding to the protein) (A) 3D view of binding interaction (B) 2D view of binding interaction (C) Hydrogen bond (acceptors and donors) (D) Hydrophobic regions.

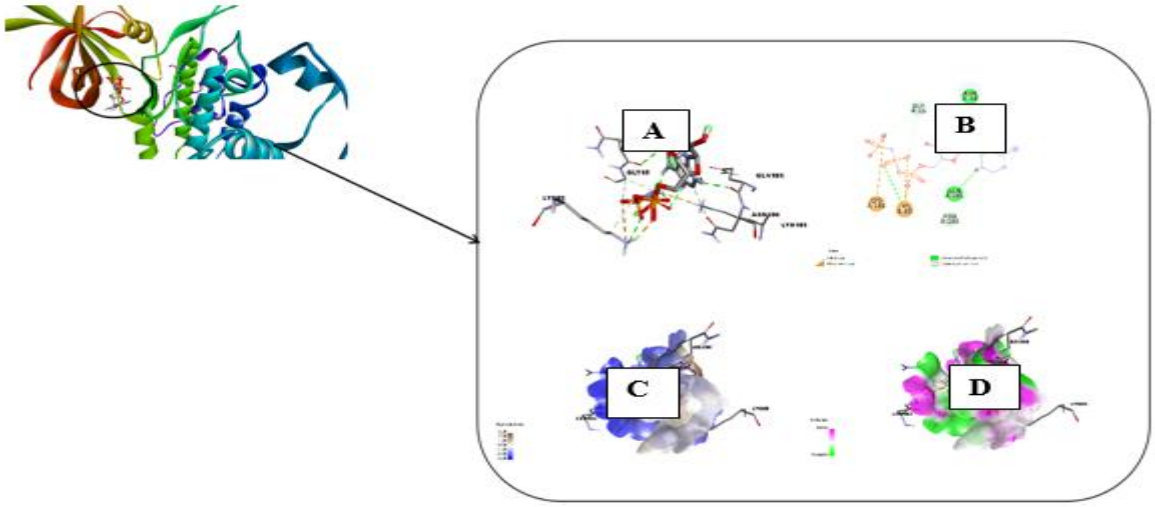

Figure 9: Complex ligand interaction (ANP binding to the protein) (A) 3D view of binding interaction (B) 2D view of binding interaction C) Hydrogen bond (acceptors and donor) (D) Hydrophobic regions.

4. Discussion

Stress is a condition that has been known to induce alteration in various physiological responses and may lead to pathological states. Studies have shown that different paradigms of stress can greatly affect memory and learning process and intensified fear memory in mice. These effects has been linked to an outcome of a complex interaction of stress and altered activity of different mechanisms such as decrease in central neurotransmitters, neurohormonal factors, neurotrophic factors, increase in free radical generation and oxidative damage in the central nerve system (Xia *et al.*, 2017). The mechanism of cytotoxic and/or neurotoxic substances are thought to be mainly due to the oxidative stress involved in the production of reactive oxygen species (ROS), that include superoxide anion, hydrogen peroxide, superoxide radical and hydroxyl radical and the degree of oxidative damage is depended on the balance between the oxidative stress and the efficiency of the antioxidant mechanism that found in the majority of cells (Godoy *et al*., 2018). Generally, brain tissues are highly susceptible to attacks of free radicals due to its highly unsaturated lipid content especially in the axonal layer in the hippocampus than the cortical region (Olajide *et al.*, 2015). Curcumin and Quercetin has been established to possess therapeutic effects via antioxidant, anti-inflammatory, anti-diabetic, anticancer anti-inhibition, inhibition of acetylcholinesterase and it restores tau hyperphosphorylation (Aggarwal *et al.*, 2007; Salehi *et al.*, 2020). In comparison to rodents, Drosophila melanogaster is a suitable organism due to its short lifetime, rapid generation period, and simple nervous system, as well as the ease and low cost of culture (Tolwinski, 2017; Moulin *et al*., 2020).

This study shows the effect of curcumin and quercetin on the brains of *Drosophila melanogaster* with Alzheimer's disease based on survival assay, Negative geotaxis assay, and biochemical assay. It compares the brains with the disease, the brains without and those that have been treated.

Effect of Curcumin and Quercetin on Survival and Behavioral Changes in Transgenic Ad Model

The use of neurobehavioral studies in risk assessment lies in the fact that behaviour can be regarded as the net output of the memory, motor, locomotor and cognitive functions occurring in the nervous system and the measurement of behavioural outcomes in neurodegeneration versus behavioural outcomes in therapeutic targets represents an important means of evaluating treatment effectiveness, and methodologies for behavioural instrumentation are evolving to facilitate drug development in this important neuro–therapeutic target area (Nehru and Bhalla, 2007). As a correlative test for cellular and neuropathological changes in the brain of *Drosophila melanogaster* in this study, negative geotaxis (behavioural) test was used to evaluate the locomotory and exploratory activities of flies across all experimental groups. Data from this study (Fig 1) shows a significant increase of locomotory and exploratory activity in the flies treated with Curcumin and Quercetin. Locomotory and exploratory activities in the transgenic group show a significant decline when compared across experimental groups. Result from this study agrees with that of a previous study that documented a significant decrease in the exploratory and locomotory activity in the rat model of AD. The underlying mechanisms of these behavioural deficits might be due to the mutation of GSK-3β which a gene involves metabolism as a result the generation of free radical and inhibition of ATP production. However, co-administration of curcumin and quercetin with AD significantly improves the exploratory and locomotory activities. The above results is in agreement with that of previous studies that documented a significant improvement of exploratory and locomotory activities following curcumin and quercetin treatment in different animal model of neurodegeneration (Oyetayo *et al*., 2020; Abolaji *et al*., 2020; Adedayo *et al.*, 2022).

The life span of *Drosophila melanogaster* was determined by carrying out survival assay for the duration for seven (7) days. The result (Fig 2) shows a significant increase in mortality rate of the transgenic group when compared to other experimental groups. However, treatment with quercetin and curcumin significantly decreased the death rate of flies thereby increasing the life span of flies. This finding agrees with the previous reports that Curcumin and Quercetin increased the lifespan and survival of *D. melanogaster* (Chen *et al*., 2018). The possible underlying mechanisms of curcumin and quercetin in improving the life span of flies might be due to their ability to mop out free radicals by improving health status owing to their antioxidative properties.

Effect of Curcumin and Quercetin Oxidative Stress Marks

Oxidative stress is an imbalance between the production/accumulation of free radicals (ROS and RNS) and the ability of the antioxidative system to detoxify these reactive products (Pizzino *et al*., 2017).

Exposure to reactive oxygen intermediates such as superoxide anion (O_2-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH), and nitric oxide (NO) can damage proteins, nucleic acids, and cell membranes, resulting in oxidative stress while the antioxidant defence mechanisms in the cells help to neutralize ROS. In this present study the following antioxidative markers were investigated which include; Total thiol, CAT, MDA.

Effect of Courcumin and Quercetin on Total Thiol Level

Thiols are chemical molecules with a sulfhydryl group. Thiols are the most abundant antioxidants in the body, accounting for the majority of total antioxidants, and they play an important role in defence against reactive oxygen species. Total thiols are made up of both intracellular and extracellular thiols, which can exist as free oxidized or reduced glutathione or as thiols attached to proteins (Prakash *et al.*, 2009). Thiols are one of the body's basic defense mechanisms against oxidative damage, and they are crucial components in protein metabolism in the organism. They are also the first antioxidants to be consumed in the event of oxidative stress and have been shown to have important roles in enzymatic activities, apoptosis, detoxification, and antioxidant defense in the body. Thiols regulate intracellular redox metabolism and protect keratinocytes from oxidative stress (Sertac *et al.*, 2019; Kükürt *et al.*, 2021).

The results of this study (Fig 3) show a significant increase of total thiol level in the wild control when compared across the

experimental groups. Total thiol level in the transgenic group was significantly reduced when compared the control groups. Treatment with both curcumin and quercetin significantly increases the expression of total thiol when compared to the transgenic group. This results is in agreement with both Sandhir and Mehrotra (2013) and Adeyemi *et al.* (2020) who reported an increase in total thiol level following treatment of Quercetin and curcumin in 3-NP-induced rodent model of Huntington Disease and bacteria isolates respectively. The decrease in total thiol level in the transgenic group might be due to the mutation of the GSK-3β gene while the ability of curcumin and quercetin to improve total thiol level might be due to its ability inhibit GSK-3β activity and preventing free radical generation.

Effect of Curcumin and Quercetin on Catalase Activity

One of the most important antioxidant enzymes is catalase. It's found in practically every aerobic organism. Catalases are a well-known family of ROS defense enzymes that catalyze the breakdown of H_2O_2. In a two-step procedure, catalase breaks down two hydrogen peroxide molecules into one molecule of oxygen and two molecules of water (Nandi *et al.*, 2019; Fang *et al.*, 2021). Catalase avoids the accumulation of peroxide, which is continuously created by multiple metabolic reactions, and protects cellular organelles and tissues from damage

The results of this study (Fig 4) show a slight significant increase of catalase activity in the wild control group compared to the transgenic group. The catalase activity in the high dose group was significantly higher when compared to other experimental groups. This result shows curcumin and quercetin elevated catalase activity and is in agreement with both Ayodele *et al*. (2018) and Kom *et al*. (2019) who reported an increase in catalase activity following treatment of Curcumin and Quercetin in *Drosophila melanogaster* models and inhibits neuroinflammation in brain of mice. Increased catalase lowers the risk of oxidative stress-mediated toxicity and the ability of curcumin and quercetin in improving catalase expression might be due to their free radical scavenging ability and antioxidant properties.

Effect of Curcumin and Quercetin on MDA Activity

Cell, tissue, and organ harm induced by oxidative stress is one of the implications of uncontrolled oxidative stress. High amounts of free radicals, also known as reactive oxygen species (ROS), have long been known to cause direct damage to lipids. Lipid peroxidation is a process in which oxidants such as free radicals or nonradical species attack lipids with carbon-carbon double bonds, particularly polyunsaturated fatty acids (PUFAs) that involve hydrogen abstraction from a carbon, with oxygen insertion resulting in lipid peroxyl radicals and hydroperoxides (Ayala *et al.*, 2014). In an organism, free radicals initiate this lipid peroxidation process. Malondialdehyde (MDA) is a byproduct of the peroxidation of polyunsaturated fatty acids in cells that have been associated with ageing. Overproduction of MDA is caused by an increase in free radicals. The level of malondialdehyde is often used as a measure of oxidative stress and antioxidant status (Chen *et al*., 2019).

The results of this study (Fig 5) show a significant increase in transgenic group MDA level which indicates increased free radical production resulting in oxidative stress and subsequent lipid peroxidation. However, there was a significant decrease in the MDA level in the groups treated with both curcumin and quercetin which collaborate curcumin and quercetin ability to improve the antioxidative system seen in this study. This results agrees with previous studies that reported decreased MDA expression in rats treated with curcumin and quercetin following doxorubicin and cyclophosphamide induced toxicity (Kochan *et al*., 2017; Alizadeh and Kheirouri 2019). The ability of Curcumin and Quercetin to ameliorate the level of MDA following Oxidative stress is due to their antioxidant and free radical scavenging properties.

Effect of Curcumin and Quercetin on Ache Activity

The AChE enzyme has received special attention in the study of Alzheimer's disease because it plays a key role in learning, memory, and cognitive functioning. Cholinergic neurons are positive markers for the evolution of memory and related disorders affecting acetylcholine, and decreased cholinergic transmission was observed in a variety of neurological conditions linked with cognitive impairment. There have been reports of both increased and decreased AChE activity with cognitive impairment and behavioral changes. Acetylcholinesterase (AChE) is a cholinergic enzyme that is found largely in postsynaptic neuromuscular junctions. It degrades or hydrolyses acetylcholine (ACh), a naturally occurring neurotransmitter, into acetic acid and choline almost instantly. AChE's main function is to stop neuronal transmission and signalling between synapses, preventing ACh from spreading and activating neighbouring receptors (Maria *et al*., 2013; Trang and Khandhar *et al*., 2021).

The results of this study (Fig 6) show a significant increase in the AChE expression in the transgenic group when compared to other experimental groups. However, there was a significant decrease of AChE expression in the treated groups with quercetin and curcumin. This result indicates that curcumin and quercetin reduced AChE activity and agrees with both Adesmosun *et al*. (2015) and Oyetayo *et al*. (2020) who reported a decrease in AChE activity following treatment of Quercetin and Curcumin in Rat's brain homogenates and *Drosophila melanogaster* model of aluminum chloride-induced neurotoxicity respectively. The ability of Curcumin and Quercetin to ameliorate AChE activity might be due to its effect against oxidative stress, thereby ensuring proper ATP production which ensures the proper functioning of gated ions channels and therefore proper conductivity.

Molecular Docking of Curcumin and Quercetin against Implicated Protein

Glycogen synthase kinase 3 (GSK3) is a glycogen metabolism-related serine/threonine protein kinase. GSK3 is encoded by two related genes, GSK3α and GSK3β. Because of its predominance in the majority of cells and tissues, GSK3β is the primary focus and the best defined isoform in this study. Diabetes, neuronal dysfunction, Alzheimer's disease, schizophrenia, Parkinson's disease, and cancer are all caused by dysregulation of GSK-3β expression (Jacobs *et al.*, 2012 Choi *et al.*, 2020).

The pharmaceutical method used in this study for AD treatment was to reduce GSK-3β expression, which in turn inhibits BACE 1 expression. All the binding parameters of Curcumin, Quercetin and ANP (standard) were docked into the binding pocket of GSK-3β and their GSK-3β antagonistic characteristics were compared. Quercetin was shown to have a greater binding affinity of -7.7 Kcal/mol than curcumin and the standard ligand, which had -7.1 Kcal/mol and -6.6 Kcal/mol, respectively. (Table 2). Quercetin and Curcumin passed the parameters of the Lipinski rule of five. (Table 3). The binding affinities are as a result of weak intermolecular forces formed between the amino acid residues that make up the active site and our ligands as the intermolecular forces bring stability to the complex formed between ligands and protein. An analysis of the docked complex of Curcumin, Quercetin and ANP with GSK3β reveals several significant interactions of these ligands within the active site of GSK3β. Visual renderings of these interactions constructed in Discovery Studio are shown in Figures 7, 8 and 9. Several hydrogen-bonding interactions are observed between active site residues and the phenolic and side chain OH groups of Curcumin, Quercetin and ANP.

5.2 Conclusion and Recommendation

In this study Curcumin and Quercetin exhibited neuroprotection against oxidative stress by decreasing MDA and increasing Total thiol and Catalase expression. Curcumin and Quercetin exerted protection against elevated AChE activity and locomotor impairment and decreased BACE 1 activity. Further investigations using In-silico study showed Curcumin and Quercetin inhibitory effects against GSK-3β. The findings from this study have provided a lead on possible co-administration of Curcumin and Quercetin as a neuroprotective agent against neurodegenerative diseases therefore more studies should be caried out to further understand these phytochemicals' neuroprotective properties.

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Data Availability

The data supporting the current study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the manuscript and its contents.

Authors' Contributions

OOO Conceptualized the project idea, performed all experiments; EDD and AJO wrote and proofread the manuscript. All authors read and approved the manuscript.

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