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Effect of curcumin and luteolin on drosophila melanogaster transgenic model of Parkinson's Disease

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ABSTRACT

Background: A neurodegenerative disorder called Parkinson's disease is distinguished by Lewy bodies, overexpression of α-synuclein, and dopaminergic neuron loss. Additionally, tremors, bradykinesia, and muscular rigidity are some of the disease's initial symptoms, and they are all linked to the oxidative stress that pro-oxidant reactive oxygen species create.

Objective: This investigation uses Drosophila melanogaster to assess the effects of curcumin and luteolin on a transgenic Parkinson's disease model. The study has substantial clinical value because the results might lead to the creation of cutting-edge treatments for Parkinson's disease.

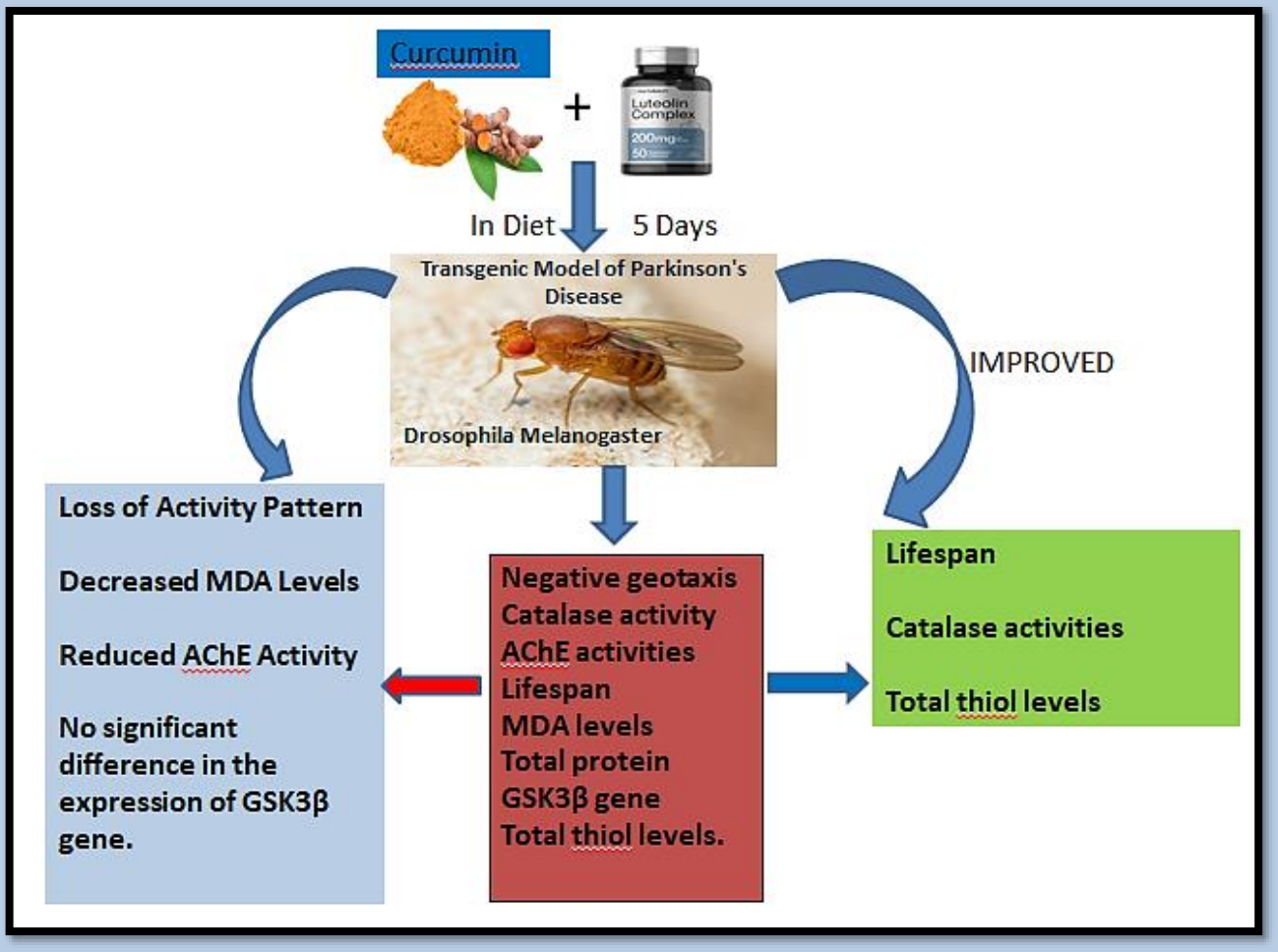
Methods: Both wild-type and transgenic flies were divided into five groups. The transgenic flies were exposed to normal media, 50 mg/kg of curcumin and luteolin, and 100 mg/kg of curcumin and luteolin treatments, while wild-type flies were exposed to normal media and curcumin and luteolin (100 mg/kg) for five days. To assess the effect of curcumin and

luteolin on Drosophila transgenic model of PD, we conducted experimental tests including negative geotaxis assay, catalase activities, AChE activities, fly lifespan, MDA levels, total protein, GSK3β gene expression, and total thiol levels.

Results: The transgenic flies with and without treatment expressed similar expression of the GSK3β gene, as well as a dose-dependent delay in the pattern of activity decrease, increased lifespan, higher catalase activities, increased total thiol levels, decreased MDA levels, and reduced AChE activity.

Conclusion: These findings suggest that the combination of curcumin and luteolin could serve as a supplementary approach in the treatment of Parkinson's disease.

Keywords: Parkinson's Disease, Neurodegenerative, Lewy Bodies, Drosophila Melanogaster, Transgenic, Curcumin, Luteolin.



INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra of the brain, leading to motor symptoms such as tremors, bradykinesia, and postural instability. The disease has been reported to be the second most common neurodegenerative disease after Alzheimer's disease [1-2]. While the exact cause of PD remains elusive, accumulating evidence suggests that oxidative stress, mitochondrial dysfunction, and protein aggregation play critical roles in the pathogenesis of the disease [3]. Many studies have identified the development of Lewy bodies that are associated with the incidence of the disease. Lewy bodies are intracytoplasmic inclusion bodies which have now been identified as an indicator of PD [4]. They are also associated with the over-expression of α-synuclein and loss of dopaminergic neurons in the basal ganglia [5]. They are more prevalent in men than in women [5-6].

The major setback in the treatment of PD is the inability to determine the biomarker responsible for the progressiveness of the neurodegenerative disease. The development of Lewy bodies has been attributed to the action of reactive oxygen species and reactive nitrogen species which produce the superoxide radical anions, hydroxyl ions and hydrogen peroxide ions [7] which are responsible for the development of the oxidative stress [4, 8]. Moreover, hypersecretion of free radicals may cause oxidative damage to lipids, proteins, and DNA bases. Oxidative stress has been identified as the cause of neuronal loss which is linked to cognitive decline [9-10]. As current treatments for PD provide only symptomatic relief and do not halt disease progression, there is a pressing need to explore novel therapeutic approaches. Moreover, dietary antioxidants are now of interest to researchers based on their ability to resolve the oxidative burden of PD [10]. The potential of dietary antioxidants to reduce the oxidative stress and to stall or prevent the aggregation and fibrillation of α-synuclein is valuable to their use as a therapeutic agent in PD treatment [11].

Curcumin and luteolin are natural polyphenolic compounds with potent antioxidant, anti-inflammatory, and neuroprotective properties. Both compounds have demonstrated promising effects in various neurodegenerative disease models [12-13]. Curcumin, a diferuloyl-methane derived from the rhizome of Curcuma longa, is the active component of the spice turmeric [14] which has been extensively studied for its ability to modulate multiple signaling pathways involved in neuroprotection. The antioxidant effect of curcumin has been reported in rotenone-induced Drosophila models of PD where it reduced oxidative burden and the degeneration of dopamine neuron while increasing its lifespan and locomotion capacity [15-16]. There has been report of curcumin protecting the dopamine neurons in the substantia nigra and the dopamine levels in the brain in 6-OHDA rat model [17]. Similarly, luteolin, a flavonoid found in various fruits and vegetables, has shown anti-inflammatory effects and the ability to attenuate oxidative stress in neurodegenerative conditions [18]. Luteolin has been reported to cause the improvement of cognitive impairment in mice and rats [19-20].

In recent years, Drosophila melanogaster has emerged as a valuable model organism for studying PD due to its genetic tractability, well-conserved dopaminergic system, and amenability to high-throughput screening. Transgenic Drosophila models expressing human alpha-synuclein, a key protein involved in PD pathology, recapitulate many aspects of the disease, making them suitable for investigating potential therapeutic interventions [21].

Despite the promising preclinical data on the neuroprotective effects of curcumin and luteolin, there is a paucity of research focusing on their potential therapeutic benefits in PD models. Therefore, the present study aims to evaluate the effects of curcumin and luteolin in a Drosophila melanogaster transgenic model of PD. We hypothesize that these compounds will attenuate neurodegeneration and motor impairments by targeting oxidative stress, mitochondrial dysfunction, and protein aggregation pathways. This investigation holds significant clinical relevance, as the findings could pave the way for the development of novel therapeutic strategies for PD. Moreover, the use of Drosophila as a model organism allows for cost-effective and rapid screening of potential compounds, providing a valuable platform for identifying new drug candidates for further evaluation in mammalian models and eventually in human clinical trials.

MATERIALS AND METHODS

Drosophila culturing: Drosophila melanogaster (Harwich strain) of both genders (1–3 days old) were cultured on a cornmeal medium containing 1% w/v brewer's yeast, 2% w/v sucrose, 1% w/v powdered milk, 1% w/v agar and 0.08% v/w nipagin at constant temperature and humidity (22–24 °C; 60–70% relative humidity) under 12 h dark/light cycle conditions, at the Eureka Drosophila Laboratory, Department of Anatomy, Benjamin Carson School of Medical Sciences, Babcock University Ilishan-Remo, Nigeria. The flies were obtained directly from Department of Biochemistry, College of Medicine, University of Ibadan, Oyo State, Nigeria. The flies were originally obtained from the Federal University of Santa Maria, Brazil.

Experimental Design

Group	1	2	3	4	5
Types of Flies	Wild	Wild	Transgenic	Transgenic	Transgenic
No of Vials	3	3	3	3	3
Treatment Groups	Normal media	Curcumin 100mg/kg +Luteolin 100mg/kg	Normal media	Curcumin 50mg/kg +luteolin 50mg/kg	Curcumin 100mg/kg +Luteolin 100mg/kg
Time Frame (Days)	5	5	5	5	5

Negative geotaxis behavioral study: This test was used to assess the locomotor activity of flies, as previously mentioned by Abolaji et al. [22]. Empty glass vials (10.5 cm by 2.5 cm) contained ten flies. Eight centimeters (8 cm) above the vial's bottom, a horizontal line was drawn. Both the control and treatment groups were assessed at random after the flies had acclimated for 10 min at room temperature. The process entailed carefully tapping the flies into the vials' bottoms. After 6 seconds of ascending, the number of flies above the 8 cm mark of the vial was counted. All behavioral research was conducted at 25 °C with normal illumination.

Determination of survival rate: As previously mentioned by Farombi et al. (2018), the survival rate was calculated [23]. The flies underwent the same procedures and circumstances as their parents. Fly mortality was tracked on a daily basis, and data were examined using the Kaplan Meier survival test.

Preparation of samples for biochemical assays: All flies were anaesthetized in CO_2, weighed, homogenized in 0.1 M potassium phosphate buffer of pH 7.4 (1:10 (flies/volume (µL)), and centrifuged at 4,000g for 10 min at 4 °C. The supernatants obtained were used to estimate biochemical assays: Total thiol (T-SH), Total Protein (TP), Catalase (CAT), Malondihyde (MDA), nitric oxide (nitrate and nitrate) level, acetylcholinesterase (AChE) activities as GSK3β.

Determination of biochemical parameters: Protein concentration was measured in accordance with Lowry, Rosebrough, Farr, and Randall's instructions [24]. The Ellman method was used to assess the total thiol content [25]. Using the thiobarbituric acid (TBA) test established by Ohkawa, the MDA content was assessed by detecting the thiobarbituric acid reactive compounds (TBARS) [26]. The method developed by Aebi (1984) was used to measure catalase activity [27]. Using the technique described by Ellman, Courtney, Andres, and Feathers-Stone, acetylcholinesterase activity was assessed [28].

Polymerase Chain Reaction: Since the RNA was separated from the fragment or gene, visual methods based on size and charge can be used to detect and identify gene sequences.

Statistical analysis: The information is displayed as Mean SEM. GraphPad Prism5.0 was used to conduct the analysis. ANOVA, or one-way analysis of variance, was used to see whether there were any significant differences between numerous groups receiving different treatments. However, Kaplan-Meier analysis was employed to assess the survival data, and the log rank test was utilized to compare groups. The cutoff for statistical significance was p 0.05.

RESULTS

Effect of Curcumin and Luteolin on the lifespan of the flies: The lifespan of the wild-type and transgenic flies treated with the curcumin and luteolin had higher survival rate (or a decreased mortality rate) in comparison with untreated transgenic and wild-type flies. Also, the mortality rate of the untreated transgenic group increased compared to other groups.

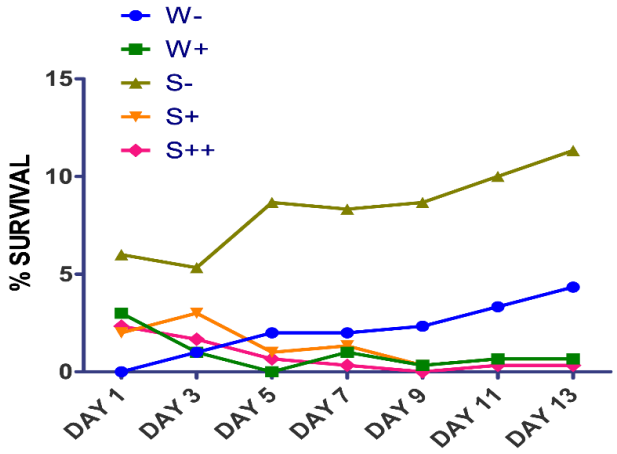


Fig 1: Displaying the survival rate as determined by tally of dead flies over the course of the 14-day trial.

Effect of Curcumin and Luteolin on Negative Geotaxis Assay: Curcumin and luteolin treatment resulted in significant ($p < 0.001$) improvement in locomotion of the transgenic flies compared with untreated transgenic flies. The transgenic flies treated with luteolin, and curcumin had a significantly ($p<0.001$) increased locomotion compared to the untreated wild-type flies. There was no significant difference in the locomotive activities of the untreated wild-type flies when compared with the untreated transgenic flies ($p>0.05$).

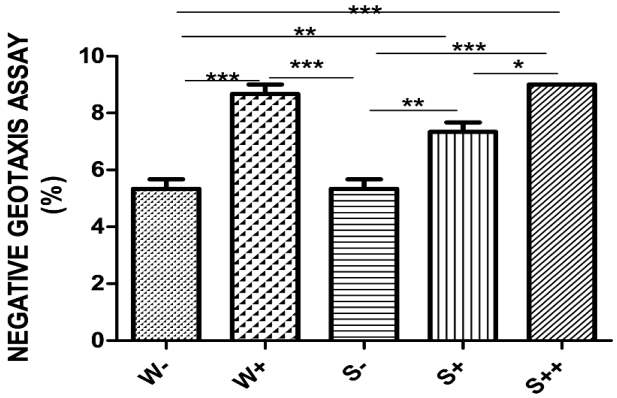


Fig 2: Locomotory activity of *Drosophila melanogaster* across experimental groups ascertained by negative geotaxis assay. (*=p<0.05, **=p<0.1, ***=p<0.005).

Effect of Curcumin and Luteolin on Total Protein: Curcumin and luteolin significantly increased the total protein levels of the treated (with high dose of curcumin and luteolin) transgenic *Drosophila melanogaster* compared to all other groups (with p value less than 0.001). Also, the total protein levels of the treated wild-type group presented a significant increase ($p<0.001$) in the comparison with the untreated wild-type *Drosophila melanogaster*. Curcumin and luteolin significantly increased the total thiol levels ($p<0.05$) of the treated (with high dose of curcumin and luteolin) transgenic *Drosophila melanogaster* in comparison with the untreated transgenic and wild-type *Drosophila melanogaster* flies.

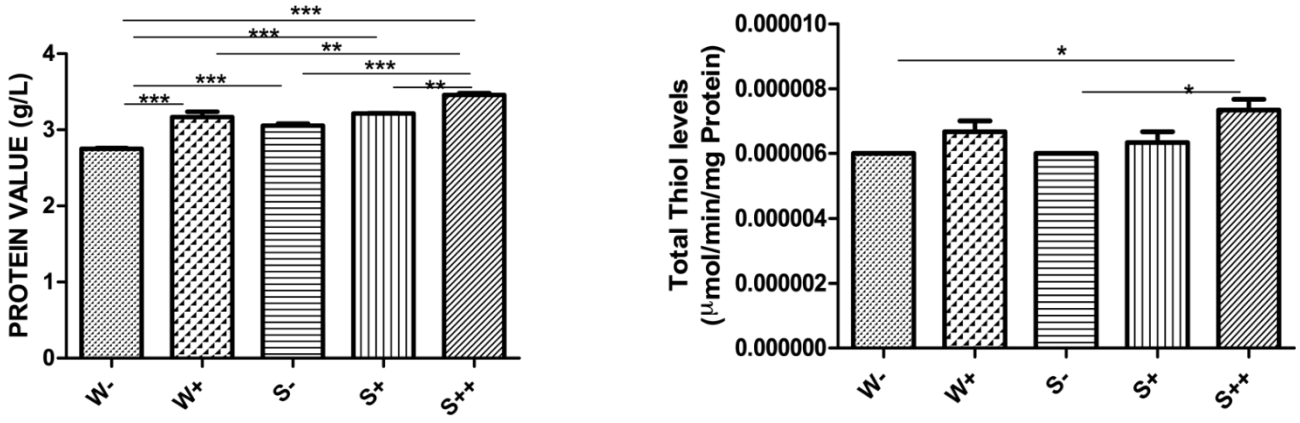


Fig 3: Total protein and Total Thiol levels of *Drosophila melanogaster* determined across experimental groups. (** = p<0.01, *** = p<0.005)

Effect of Curcumin and Luteolin on Catalase MDA Activities: In treated (with high and low doses of curcumin and luteolin) transgenic *Drosophila melanogaster* flies compared to untreated transgenic *Drosophila melanogaster* flies, catalase activity was significantly increased (p0.001). In compared to the untreated wild-type *Drosophila melanogaster* flies, there was a substantial increase (p<0.001) in the catalase activity of the treated wild-type *Drosophila melanogaster* flies. Comparing transgenic *Drosophila melanogaster* groups (S-, S+, and S++) to the wild-type *Drosophila melanogaster* flies, curcumin and luteolin generally reduced catalase activity.

MDA levels was significantly lower (p<0.01) in the treated (with high dose of curcumin and luteolin) transgenic *Drosophila melanogaster* flies when compared with wild-type untreated, transgenic untreated and the treated (with low dose of curcumin and luteolin) transgenic *Drosophila melanogaster* flies. Curcumin and luteolin significantly decreased the MDA levels in the treated (with low dose of curcumin and luteolin) transgenic *Drosophila melanogaster* flies compared with untreated transgenic *Drosophila melanogaster* flies. However, MDA level of the treated (with low and high dose of curcumin and luteolin) transgenic *Drosophila melanogaster* flies was significantly higher (p<0.001) compared to the treated wild-type group. Untreated transgenic *Drosophila melanogaster* flies also had a significantly higher (*=p<0.05) MDA levels compared to the untreated and treated wild-type *Drosophila melanogaster* flies.

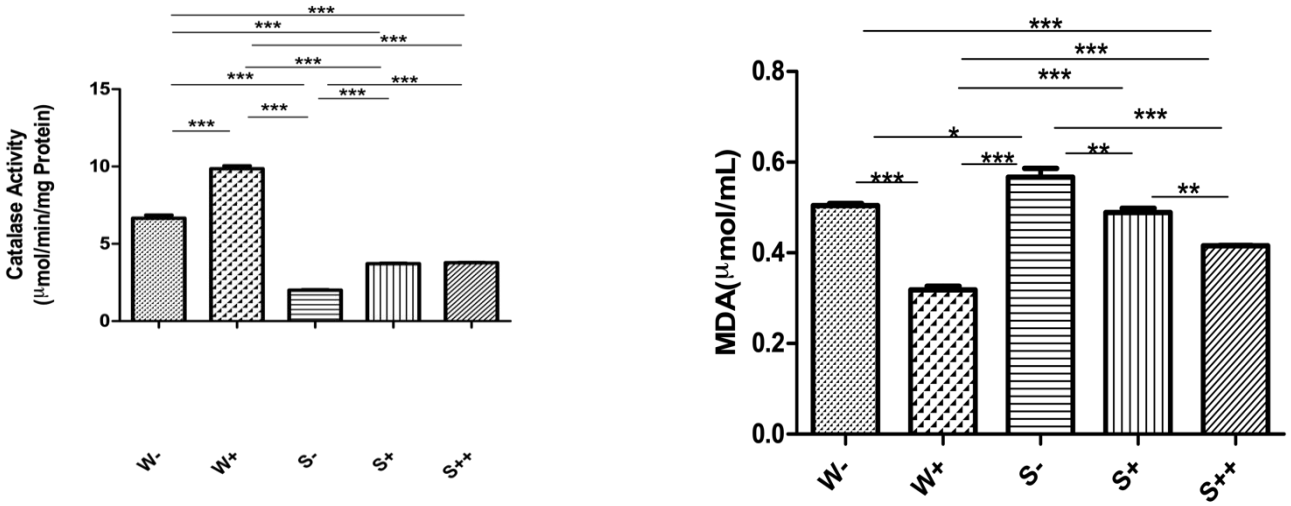


Fig 4: The effect of curcumin and luteolin on antioxidant status (*=p<0.05, **=p<0.01, ***=p<0.005).

Effects of Curcumin and Luteolin on AChE Activities: The acetylcholinesterase activity of the treated transgenic Drosophila melanogaster flies was considerably reduced (p<0.05) by curcumin and luteolin treatment compared to the untreated transgenic flies. When acetylcholinesterase activity was examined between the transgenic and wild-type Drosophila melanogaster flies, there was a substantial decrease (p<0.001). When treated wild-type Drosophila melanogaster flies were compared to untreated wild-type Drosophila melanogaster flies, there was no discernible variation in the acetylcholinesterase activity (p>0.05).

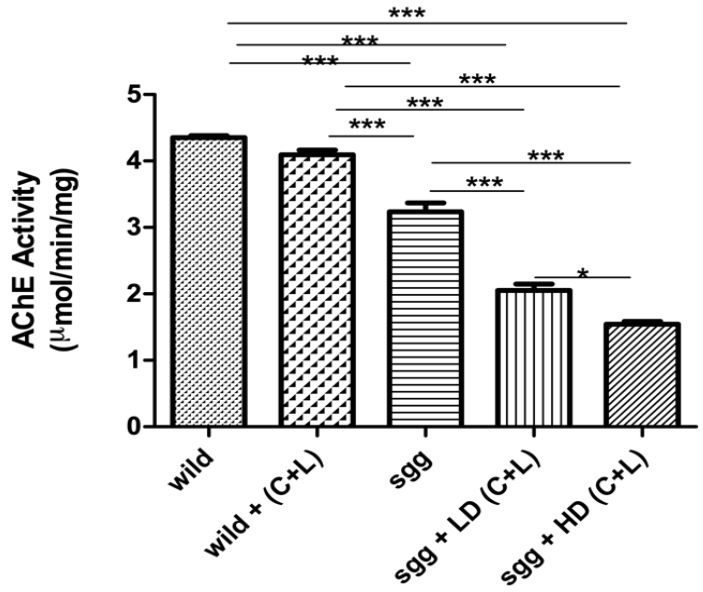


Fig 5: Acetylcholinesterase activity of *Drosophila melanogaster* determined across experimental groups**.** (*=p<0.05, ***=p<0.005).

Effects of Curcumin and Luteolin on the Relative GSK3β Gene Expression: Treatment of curcumin and luteolin in the transgenic *Drosophila melanogaster* flies resulted in a non-significant decrease (p>0.05) in the relative expression of GSK3β when compared with the untreated transgenic *Drosophila melanogaster* flies. There was also a non-substantial decrease in the relative expression of GSK3β gene in the treated wild-type flies in comparison to the untreated wild-type flies (p>0.05).

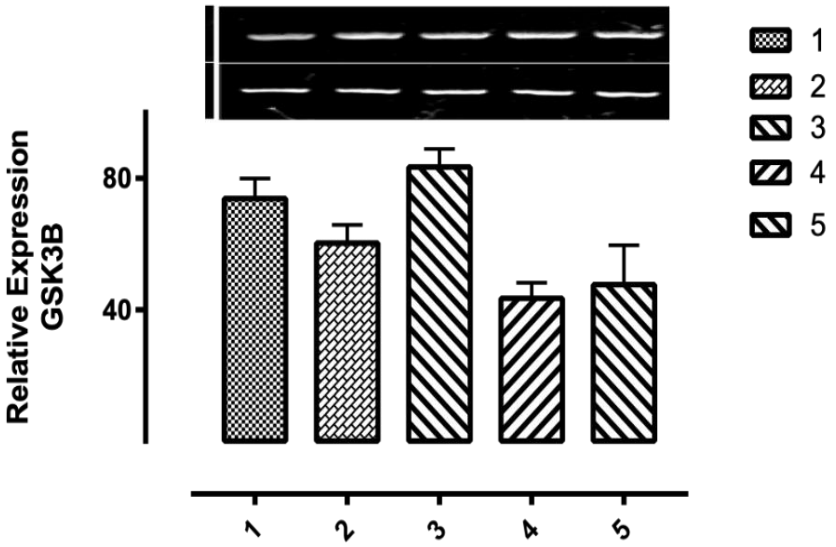


Fig 6: Bar graph showing relative expression of GSK3β gene across groups through RNA isolation of tissue samples using RNA.

DISCUSSION

Effect of Curcumin and Luteolin on Locomotive Activities: The negative geotaxis assay, which measures movement against gravity, is commonly used to assess motor function [29]. In this study, curcumin and luteolin treatment resulted in concentration-dependent improvement in locomotion in the transgenic flies, consistent with previous research [10, 29]. The effect of curcumin was suggested to slow the degeneration of dopaminergic neurons, leading to improved locomotion [29]. According to Nguyen et al. (2018) [29], a Drosophila model with the knockdown of dUCH is a homolog of human UCH-L1 and there is a submission that the locomotion improvement by curcumin may be due to protection against the negative effects of dUCH knockdown on the locomotive abilities of the flies [29]. Both curcumin and luteolin demonstrated neuroprotective actions and improved locomotive abilities.

Effect of Curcumin and Luteolin on Lifespan: Oxidative stress contributes to reduced lifespan and accelerated aging through alterations of cellular compositions [30-31]. Oxidative stress affects various cellular components, including DNA, proteins, and lipids [32-33]. PD is characterized by dopaminergic neuron loss, attributed to oxidative stress [34]. In this study, curcumin and luteolin treatment extended the lifespan of both wild-type and transgenic flies, with fewer deaths in the treated groups compared to untreated transgenic flies. Curcumin and luteolin's antioxidant effects were suggested to reduce oxidative stress and promote longevity [35-36].

Effect of Curcumin and Luteolin on Total Protein and Total Thiol: Curcumin and luteolin treatment resulted in increased total protein levels in both wild-type and transgenic flies compared to their untreated counterparts. Additionally, the treated transgenic flies showed higher total thiol levels. These findings indicate that curcumin and luteolin may positively influence protein levels and the redox cell balance, potentially mitigating oxidative stress [37].

Effect of Curcumin and Luteolin on Catalase Activity: PD is associated with ROS including H_2O_2 and other superoxides which are responsible for further progression of neurodegenerative diseases. The natural tendency of antioxidants is to intervene while acting roles of protection by scavenging the destructive ROS accompanying the disease [38]. Curcumin and luteolin, due to their antioxidant properties can enable catalase activities [39]. Curcumin and luteolin showed increased catalase activity among the treated transgenic Drosophila melanogaster compared to the untreated transgenic Drosophila melanogaster flies in this study. Catalase, a heme-containing enzyme has been reported to act on H_2O_2 causing the dismutation of H_2O_2 to molecular oxygen and water. By enhancing catalase activity, curcumin and luteolin can scavenge ROS and protect against neurodegeneration [40].

Effect of Curcumin and Luteolin on MDA Levels: Malondialdehyde (MDA) is a marker of lipid peroxidation and oxidative stress. In transgenic flies, the levels of ROS can be deduced or is an indication of the MDA, therefore, the reduction of MDA is a signal of the effect of the antioxidant effect [15, 41]. Curcumin and luteolin treatment significantly decreased MDA levels in transgenic flies, suggesting their ability to counteract oxidative damage [42-43]. This anti-oxidative property of curcumin and luteolin is crucial in the reversal of oxidant effects leading to dose-dependent decrease in the MDA levels or lipid peroxidation of curcumin and luteolin [42-43].

Effect of Curcumin and Luteolin on Acetylcholinesterase (AChE) Activity: Acetylcholinesterase gene expression

has been found to be linked with lifespan in the Drosophila melanogaster model [44]. Moreover, the presence of acetylcholinesterase is important to the progression of neurodegenerative diseases. High expression of acetylcholinesterase is an indication of oxidative stress [45]. In this study, transgenic *drosophila* flies treated with curcumin and luteolin showed a substantial decrease in the activity of AchE when compared with the untreated transgenic drosophila group. In the same vein, there was an overall decrease in the activity level of AchE in the transgenic treated group compared to the wild group. The presence of increased level of AChE suggests elevated oxidative stress in the concerned group, which in this study, is the transgenic untreated group, while the decrease in the level of AChE in the treated transgenic group suggests that the co-administration of curcumin and luteolin exerted ameliorative effects in the transgenic flies. This can be because of the anti-oxidative properties of curcumin and luteolin because its antioxidants have been reported to inhibit the acetylcholinesterase activity thereby preventing the breakdown of acetylcholine to acetate and choline which is responsible for cholinergic transmission [46-47]. The use of antioxidants has been suggested for the management or prevention of aging [48]. AChE activity is also associated with cognitive function and aging.

Based on the result from this study, curcumin and luteolin treatment led to a significant decrease in AChE activity in transgenic flies, thereby potentially improving cognitive and locomotive abilities.

Effect of Curcumin and Luteolin on GSK3β Gene Expression: Glycogen synthase kinase-3β (GSK3β) is implicated in neurodegenerative diseases. Activation and overexpression of GSK3β is a signal of neurodegenerative disease involved in the accumulation of tau protein and Aβ in AD and α-synuclein protein in PD [49]. Although, the effect of curcumin has been reported to be associated with its actions on the GSK3β pathways [50], curcumin and luteolin treatment in our study did not cause a significant difference in the GSK3β gene expression in the transgenic flies. Therefore, more studies may be needed to further understand the dynamic effects of curcumin and luteolin on the GSK3β pathway in neurodegenerative diseases [51].

CONCLUSION

Curcumin and luteolin showed promising effects in the Drosophila melanogaster transgenic model of Parkinson's disease. They improved locomotive performance, extended lifespan, increased total protein and total thiol levels, enhanced catalase activity, and reduced MDA and AChE activity. The combination of curcumin and luteolin may therefore hold potential as an adjunct therapy for Parkinson's disease. However, further investigations are recommended to fully explore their therapeutic value in managing PD.

Abbreviations:

6-OHDA: 6-hydroxydopamine;
AChE: Acetylcholinesterase;
ANOVA: Analysis of Variance; CATL Catalase
CO2 – Carbon dioxide
DNA – Deoxyribonucleic Acid
dUCH – drosophila Ubiquitin Carboxyl-terminase Hydrolase
GSK-3β – Glycogen Synthase Kinase-3β
H202 – Hydrogen Peroxide
MDA – Malondialdehyde
PD – Parkinson Disease
ROS – Reactive Oxygen Specie
SEM – Standard Error of Mean
TBA – Thiobarbituric Acid
TP – Total protein
UCH-L1 - Ubiquitin Carboxyl-terminase Hydrolase 1

Competing Interest: The authors have no competing interest to declare.

Author's Contribution: Dr. Olanrewaju J and Mofolorunso A participated in the conceptualization, design and writing of the manuscript. Arietarhire L helped in the statistical analysis and drafting of the manuscript. Okwute P, Soremekun O, Adeleke S and Afolabi T also contributed to the writing and editing of the manuscript.

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