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Curcumin and *Quercetin* Ameliorates Reserpine-Induced Neurotoxicity in *Drosophila melanogaster* Models

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ABSTRACT: Reserpine has reportedly been used to induce neurotoxicity in animal models, including *Drosophila melanogaster*, and has been reported to cause oxidative stress, mitochondrial dysfunction, and neuroinflammation, while *Curcumin* and *quercetin* have demonstrated anti-inflammatory and antioxidant properties. The study investigated the effect of co-administration of *curcumin* and *quercetin* on the lifespan and exploratory/locomotory activities of *Drosophila melanogaster* flies; examined the muscular integrity of the *Drosophila melanogaster* larvae, and evaluated the anti-oxidant effect of co-administered *curcumin* and *quercetin* on oxidative stress following reserpine- induced neurotoxicity. The flies were distributed into four groups: Group 1 received normal corn meal diet, Group 2 received 100mg/kg of *curcumin* and *quercetin*, Group 3 received 5mg/kg of reserpine, while Group 4 received 5mg/kg of reserpine and 100mg/kg of *curcumin* and *quercetin* for 7 days. The antioxidant status, lipid peroxidation, acetylcholinesterase activity, and locomotor behavior of the flies were evaluated. Reserpine exposure caused significant reduction in antioxidant status, locomotor behavior impairment, and increased lipid peroxidation/acetylcholinesterase activity in the toxicant group. However, *curcumin* and *quercetin* improved significantly, the antioxidant status and behavioral deficits, and also decreased lipid peroxidation/acetylcholinesterase activities in the treated groups. *Curcumin* and *quercetin* co-administration showed potential therapeutic and antioxidant benefits against reserpine-induced neurotoxicity and cognitive impairment.

Keywords: *Curcumin*, *quercetin*, Reserpine, Neurotoxicity, *Drosophila melanogaster*.

Introduction

The degenerative nature of neurological illnesses, including depression, Parkinson's disease, Alzheimer's disease, and Huntington's disease, along with their restricted treatment options, significantly poses a heavy burden on healthcare systems worldwide (Wang and Xu, 2020). According to recent reports, reserpine-induced neurotoxicity in animal models, including *Drosophila melanogaster* has caused oxidative stress, mitochondrial dysfunction, and neuroinflammation (Adeola *et al.*, 2022; Chakraborty *et al.*, 2017; Kim *et al.*, 2014). Oxidative stress occurs in response to reactive oxygen species (ROS) and the antioxidant defense system imbalance (Quinlan *et al.*, 2013).

The fruit fly (*Drosophila melanogaster*), has been widely utilized as a model organism for the study of numerous biological processes, including neurotoxicity (Stephenson and Metcalfe, 2013), as it offers several advantages including its short lifespan, rapid generation period, simple nervous system, genetic manipulability,

well-characterized immune system, and the ease and affordability of culturing (Moulin *et al.,* 2020) which makes it an excellent model for studying neuroinflammation.

Quite a number of human disorders, such as Parkinson's disease, Huntington's disease, spinocerebellar ataxia, and Alzheimer's disease, have been studied using *Drosophila melanogaster* as a genetic model (Margret *et al.,* 2020) due to its genomic compatibility with human's. A study comparing the genomes of *Drosophila melanogaster* and humans was published in March 2000 by the National Human Genome Research Institute. It was discovered that over 60 % of genes are conserved between the two species. 50 % of fly protein sequences have mammalian homologs, and 75 % of known human disease genes have a recognizable match in the genome of *Drosophila melanogaster* (Rubin and Lewis, 2000).

Curcumin and *quercetin* have been shown to demonstrate anti-inflammatory and antioxidant properties, and also regulate mitochondrial function (Farghali, *et al.*, 2015; Jana and Modi, 2017). It should be noted that this study did not consider the individual effects of c*urcumin* and *quercetin*, as their individual effects have been studied extensively, according to literature. This study therefore seeks to investigate the synergistic effect of co-administration of both compounds, in the management of reserpine-induced neurotoxicity, as against treating neurotoxicity with each of the compounds, which is similar to previous work done by Alessio *et al*. (2022); Wang and Xu, (2020).

Curcumin is one of the widely studied agents for therapeutic effects, recently, particularly in the area of nervous system injuries (Alessio and Angelo, 2022). Oxidative stress is a major factor in the pathogenesis of numerous diseases, including cancer, diabetes, cardiovascular diseases, neural cell injury, and hypoxia. Meanwhile, many studies have demonstrated *curcumin*'s abilities as a free radical scavenger, reducing agent, DNA damage inhibitor and strongly support the biological classification of *curcumin* as both a pro-oxidant and antioxidant (Jovanovic *et al.*, 2021), while *quercetin* has been shown to be beneficial in mitigating cardiovascular diseases, diabetes, inflammation, asthma, viral infections, and cancers (Shabbir *et al.*, 2021).

However, the potential ameliorative effects of *curcumin* and *quercetin* co-administration, on Reserpine-induced neurotoxicity in *Drosophila melanogaster* has not been extensively investigated, hence, this study. The results of this study provide valuable insights into the potential therapeutic use of *curcumin* and *quercetin* in the treatment of neurotoxicity.

Materials and methods

Drosophila melanogaster stock and culture: D. Melanogaster of both genders (male and female) was cultured on a cornmeal medium containing 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 h dark/light cycle conditions at the Eureka drosophila laboratory, in the Department of Anatomy, Benjamin Carson (Snr.) College of Health and Medical Science, Babccock University, Ogun State, Nigeria. The Oregon k model drosophila was gotten from Dr. Amos Abolaji's laboratory in the Department of Biochemistry in Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

Meal preparation (full meal): Preparation of corn meal was done according to Abolaji *et al*. (2014). 800 ml of distilled water was added to a pot and allowed to boil on an electric cooker. A sensitive scale was used to measure 7 g of yeast which was added to boiling water, and the mixture was stirred for 5 min. Using the sensitive scale again 7.5 g of agar-agar was measured and gently added to the boiling mixture, and this was stirred for 3 min. 200 ml of distilled water was used to mix 60 g of cornmeal in a mixing bowl after which it was added to the still boiling mixture and was stirred for 3 min. 1 cap full of ethanol was mixed with 1 g of Nipagin in a Petri dish and this was carefully combined. The cornmeal mixture was brought down from the electric cooker and allowed to cool for 2-3 min. The Nipagin mixture was added to the cornmeal mixture after it was allowed to cool and this was stirred.

Experimental design: The method of regimen exposure [*quercetin* (Q) and *curcumin* (C)] was via ingestion method. Two hundred (n=200) *D. melanogaster* (wild flies) of both sexes were counted, induced with carbon dioxide anesthesia and distributed into four groups. Group 1 (Wild Control) received normal diet, Group 2 (Wild Treated) was treated with 100 mg/kg of *curcumin* and *quercetin*; Group 3 (Reserpine Untreated) was treated with 5 mg/kg of reserpine, and Group 4 (Reserpine Treated) received 5 mg/kg of reserpine + 100mg/kg of *curcumin* and *quercetin* (Govind *et al*., 2024). Standard laboratory protocols were observed according to Babcock University's Health Research Ethical Committee (BUHREC), Babcock University, Ilishan –Remo, Ogun State, Nigeria.

Behaviour assays

Larva crawling: 50-100 ml of 20 % sucrose solution was added to the vials containing the larvae to facilitate their collection, and they were allowed to sit for 10 min. This process made the larvae to float to the top of the solution and the third-stage larvae were carefully selected and transferred to a Petri dish, using a brush. The Petri dish was placed on a graph book, while the recording of larval crawling behavior was captured using a high-resolution camera capable of capturing larval movement with sufficient temporal and spatial resolution.

Recording and observation of the larvae was set at duration of 5 minutes per group, to capture a representative sample of crawling behavior (Feany *et al*., 2000).

Negative geotaxis

Survival assay: The survival assay was carried out for the duration of fourteen (14) days. The numbers of dead flies were recorded first thing in the morning everyday throughout the duration of the experiment in all experiment groups. Flies were transferred in new vials containing new meal and treatments at the end of the first seven (7) days to avoid emergence of new flies from lavas that were laid during the first 7 days. A total of 10 flies from each group were utilized in this study to assess the locomotive activity of the flies. 10 flies of both genders were selected from each group and subjected to CO_2 anesthesia. Subsequently, they were individually placed in labeled vials with a diameter of 1.5 cm and a length of 15 cm. A mark was made at the 6 cm position within each vial, and the number of flies that regained consciousness and successfully flew above this mark within a 10-sec timeframe was documented. This process was repeated three times for each group, and the climbing activity was computed using the recorded values (Abolaji *et al.*, 2014; Feany *et al*., 2000).

Biochemical assay

Preparation of samples for biochemical assay: The remaining flies from each vial of every group were sedated using carbon dioxide and crushed in their respective and already labeled Eppendorf tubes which contained 100 microliters of phosphate buffer solution. 900 microliters of the phosphate buffer solution was added to the Eppendorf tubes after the flies have been crushed. All flies were homogenized using 0.1 m phosphate buffer solution and centrifuged for 5 min at 4 °C and 13,000 rpm. After being moved to fresh Eppendorf tubes, the supernatants were utilized to calculate biochemical assays for total MDA, acetylcholinesterase, catalase, protein level and total thiol (Shen *et al*., 2020).

Determination of catalase activity: Catalase activity was determined by the method of Mahmoud (2018). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0), 300 mM H_2O_2 and sample (1:50 dilution). The loss in absorbance of H_2O_2 was monitored for 2 min at 240 nm and thereafter used to calculate catalase activity expressed as micromol of H_2O_2 consumed per minute per milligram of protein.

Determination of acetylcholinesterase activity: The activity of acetylcholinesterase was determined with the procedure outlined by Franz *et al.* (2012). The reaction was carried out in 0.1 M potassium phosphate buffer of pH 7.4, 1 mM DTNB and 0.8 mM acetylthiocholine, the initiator. The reaction was monitored for 2 min (30 seconds interval) at 412 nm. The enzyme activity was estimated as micromol of acetylthiocholine hydrolyzed per minute per mg protein.

Determination of MDA activity: In glacial acetic acid, a standard stock solution of MDA (1 mM) was produced. MDA (31.35 milligram) was weighed correctly and dissolved in 100 mL solvent. Different concentrations of 0.1, 0.2, 0.4, 0.6, and 0.8 mM were produced from the stock solution. In the concentration range of 0.1 to 1.0 mM, the calibration curve was created (Varshney and Kale, 1990).

Determination of total thiol levels: Total thiol content was determined according to the method described by Jakob (2009). The reaction mixture contained 510 microliters of 0.1 M phosphate buffer (pH 7.4), 20 microliters of sample, 35 µL of 1 mM DTNB and 35 µL of distilled water. Incubation was carried out at room temperature for 30 min. The absorbance was measured at 412 nm.

Determination of protein concentration: The concentration of protein was determined using Lowry's method as described by Waterborg and Matthews (1984), with minor modifications.

Statistical analysis: The data are presented as the mean +/- SEM. Analysis was carried out using one-way ANOVA analysis of variance, to access significant differences among multiple groups under various treatments, followed by Turkey posthoc test, using the prism 7.0 software. The turkey test was used to analyze the survival data, with a statistical significance set at $p<0.05$.

Results

Behavioural assay

Climbing assay result: Figure 1 shows a reduction in the locomotor activity of the reserpine un-treated group (RUT) flies when compared to the wild control, while an increase in the locomotors activity of the treated groups (RT) was observed when compared to wild treated group (RT) (*=$p<0.05$, **=$p<0.01$, ***=$p<0.005$, ****=$p<0.001$). WC = Wild Control, WT = Wild Treated, RUT = Reserpine Untreated, RT = Reserpine Treated

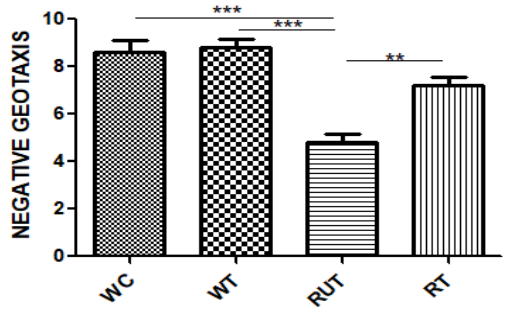

Figure 1: Locomotor activity of *Drosophila melanogaster* across the experimental groups assessed using negative geotaxis activity.

Lava crawling assay result: Figure 2(a-c): show a significant decrease in muscular integrity of fly's lava in reserpine intoxicated group (RUT) when compared to other experimental groups. Treatment with both *quercetin* and *curcumin* (RT) improved significantly the muscular integrity of the flies lavae as there was an increase in the number of lava contraction, distance traveled, distance/contraction ratio when compared to the reserpine intoxicated group (*=p<0.05, **=p<0.01, ***=p<0.001). WC = Wild Control, WT = Wild Treated, RUT = Reserpine Untreated, RT = Reserpine Treated

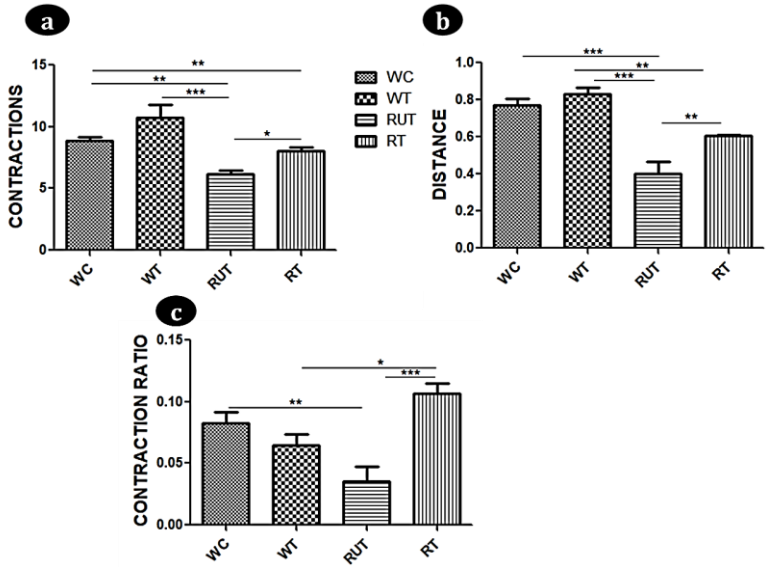

Figure 2(a-c): Effect of *curcumin* and *quercetin* on muscular integrity of *Drosophila melanogaster* with reserpine-induced neurotoxicity investigated with drosophila lava crawling assay.

Survival assay result: Figure 3 shows an increase in the mortality rate of the reserpine induced groups compared to other groups. There was a reduced mortality rate in the treated groups which suggests an increased lifespan for the flies. (*=p<0.05, **=p<0.01, ***=p<0.005, ****=p<0.001). WC = Wild Control, WT = Wild Treated, RUT = Reserpine Untreated, RT = Reserpine Treated

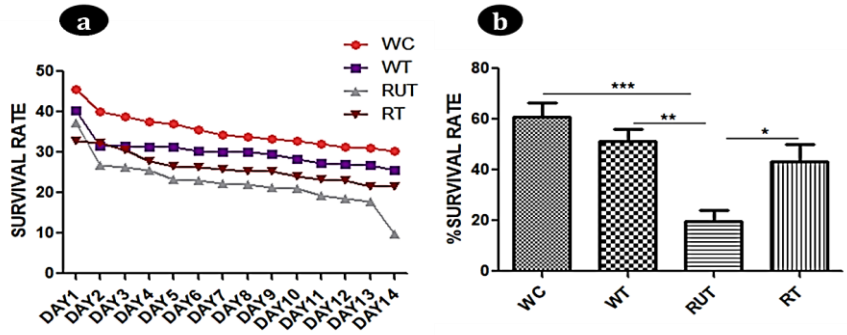

Figure 3: Survival rate across experimental groups (using number of dead flies per day for the period of 14 days

Oxidative stress markers

Acetylcholinesterase, malondialdehyde and catalase assay results: Figure 4 shows that MDA level significantly increased, while the activities of acetylcholinesterase, catalase and total thiol significantly reduced in the reserpine Un-treated group (RUT), as compared to other treated groups. However, a significant reduction in MDA level and increased acetylcholinesterase, catalase and total thiol activity levels were observed in the quercetin and curcumin treated groups (WT, RT) (*=p<0.05, **=p<0.01, ***=p<0.005, ****=p<0.001). WC = Wild Control, WT = Wild Treated, RUT = Reserpine Untreated, RT = Reserpine Treated

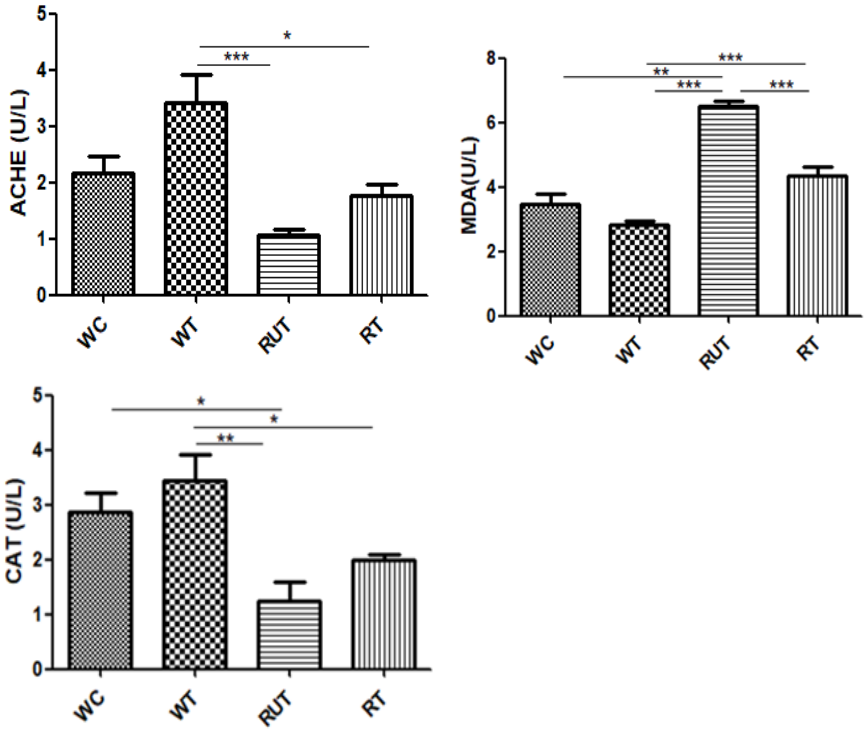

Figure 4: Effects of *quercetine + curcumin* on the level of acetylcholinesterase (ACHE), malondialdehyde (MDA) and catalase (CAT) in *Drosophila melanogaster* models of reserpine-induced neurotoxicity.

Total thiol assay result: Figure 5 revealed that total thiol levels significantly reduced in the reserpine untreated group (RUT) when compared to other groups. However, quercetin and curcumin treatment (WT, RT), significantly enhanced the total thiol level (RT) (*=p<0.05, **=p<0.01, ***=p<0.005, ****=p<0.001). WC = Wild Control, WT = Wild Treated, RUT = Reserpine Untreated, RT = Reserpine Treated

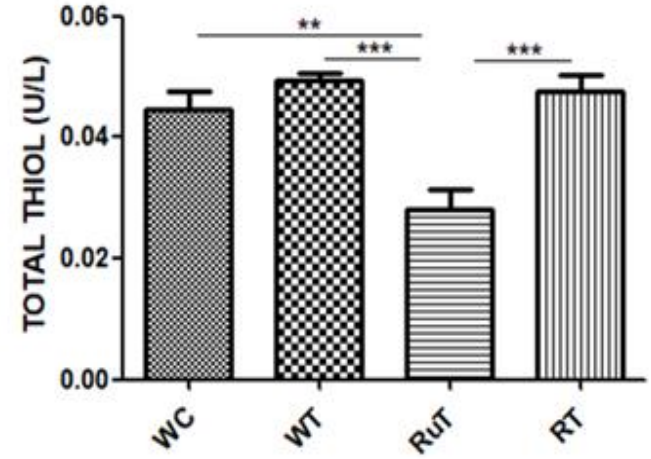

Figure 5: Effects of *quercetine + curcumin* on brain homogenate total thiol levels in *Drosophila melanogaster* models of reserpine-induced toxicity.

Total protein value: Figure 6 showed no significant difference in total protein levels across all experimental groups.

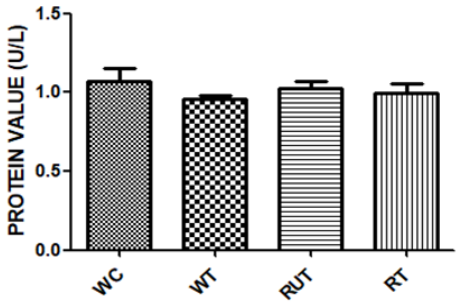

Figure 6: Effects of *quercetine + curcumin* on brain homogenate total protein levels in *Drosophila melanogaster* models of reserpine-induced toxicity.

Discussion

Curcumin and quercetin administration improves exploratory and locomotor activities in Drosophila Melanogaster models of reserpine-induced neurotoxicity: The data obtained from the investigation of cellular and neuropathological changes in the brain of *Drosophila melanogaster* using negative geotaxis (behavioral) tests (Fig 1) demonstrate that locomotor and exploratory activities significantly increased in the groups treated with *Curcumin* and *Quercetin* (WT, RT). Conversely, flies exposed to the toxicant exhibited a substantial decline in locomotor and exploratory activities compared to the other experimental groups. These results align with earlier studies reporting notable enhancements in exploratory and locomotor activities following *curcumin* and *quercetin* treatment in various animal models of neurotoxicity (Abolaji *et al*., 2020; Adedayo *et al.*, 2022).
In addition, the effect of *curcumin* and *quercetin* on the muscular integrity of *Drosophila melanogaster* in the presence of reserpine-induced neurotoxicity was evaluated using the drosophila larva crawling assay. The results showed that the muscular integrity of the fly larvae in the reserpine-intoxicated group significantly reduced, and was signified by the decrease in number of lava contraction (**a**), distance traveled (**b**), and distance/contraction ratio (**c**) of Fig. 2, when compared to the other experimental groups, whereas, treatment with both quercetin and curcumin (RT) significantly improved the muscular integrity of the treated fly larvae when compared to the reserpine un-treated group (RUT). These findings align with previous studies that have reported the neuroprotective properties of *curcumin* and *quercetin*, (Smith *et al.*, 2019; Patel *et al*., 2017; Nabavi *et al.*, 2015; Jagetia and Aggarwal, 2007) demonstrated that treatment with *curcumin* improved muscle strength and function in experimental rat which modeled neurotoxicity caused by a different neurotoxin, and Lee *et al.* (2020) reported same in the investigation on the effects of *quercetin* on muscle integrity in a zebrafish model of neurotoxicity. These potential protective effects of curcumin and quercetin may be attributed to their antioxidative and anti-inflammatory properties, which could help preserve muscle function and prevent deterioration.
Effect of curcumin and quercetin on flies' longevity and survival rate in reserpine model of neurotoxicity: Survival rate of the fruit flies was assessed using the number of deceased flies per day, over a 14-day period, across the experimental groups. The results indicate a higher mortality rate in the reserpine un-treated groups (RUT) as compared to others, as earlier reported in a study by Smith *et al.* (2019), that reserpine administration in Drosophila led to a significant increase in mortality rate, which may be attributed to its ability to deplete neurotransmitters, disrupt cellular processes, and induce oxidative stress (Chen *et al.*, 2017). Conversely, the *curcumin* and *quercetin* treated groups exhibited a decrease in mortality rate (Fig. 2) which is consistent with previous investigations on potential therapeutic agents, where Zhang *et al.* (2020) demonstrated that treatment with *quercetin* extended the lifespan of Drosophila by modulating oxidative stress and improving mitochondrial function.
Curcumin and quercetin administration mitigated reserpine-induced neurotoxicity and oxidative stress in experimental D. melanogaster models: The results of the oxidative markers (Fig 5) show a significant increased MDA level and a significant decreased AChE and CAT activity level, in the reserpine Un-treated group (RUT) as compared to other treated groups, indicative of excitotoxicity and oxidative stress. However, a significant reduction in MDA level and increased AChE and CAT activity levels were observed in the quercetin and curcumin treated groups (WT, RT).
Decreased AChE activity level in the quercetin and curcumin treated groups (WT, RT) according to AChE bar chart (Fig. 5) is consistent with previous studies that reported significant reduction in AChE activity of brain

homogenates in *Drosophila melanogaster* models of aluminum chloride-induced neurotoxicity following treatment with quercetin and curcumin (Oyetayo *et al*., 2020; Ademosun *et al.,* 2015). The ability of *curcumin* and *quercetin* to reduce AChE activity may be due to their antioxidative properties, which help maintain proper ATP production and ion channel conductivity, ensuring proper neuronal function.

Free radicals (otherwise known as reactive oxygen species (ROS), have been long known to directly damage lipids. In organisms, free radicals initiate this lipid peroxidation process. Malondialdehyde (MDA) is a by-product of lipid peroxidation in cells, and increase in free radicals cause overproduction of MDA, and this is often used as a measure of oxidative stress and antioxidant activity status (Chen *et al*., 2019; Gawel *et al*., 2004). The MDA bar chart, (Fig 5) shows that MDA level increased significantly in the reserpine Un-treated group (RUT). This result agrees with previous studies that reported decreased MDA expression in rats treated with curcumin and quercetin following doxorubicin and cyclophosphamide-induced toxicity (Alizadeh and Kheirouri, 2019; Kocahan *et al.,* 2017). *Curcumin* and *quercetin*'s ability to ameliorate MDA levels following oxidative stress could be attributed to their antioxidant and free radical scavenging properties. Catalase initiates the breakdown of hydrogen peroxide into water and oxygen, thus preventing the accumulation of free radicals that can lead to cell damage (Fang *et al.,* 2021; Nandi *et al*., 2019).

Catalase is found in practically every aerobic organism, and its activity is essential for protecting cellular organelles and tissues from oxidative stress. Catalase bar chart, (Fig 5) shows relative increase in catalase activity in the treated groups, relative to the RUT group. This shows that *curcumin* and *quercetin* can elevate catalase activity, which is consistent with Kom *et al.* (2019) and Ayodele *et al.* (2018) and) who reported an increase in catalase activity following treatment with *curcumin* and *quercetin* in *Drosophila melanogaster* models and in the brain of mice, respectively. The ability of *curcumin* and *quercetin* to improve catalase expression may be attributable to their free radical scavenging ability and antioxidant properties.

Curcumin and quercetin treatment could improve ATP production and reverse alterations in protein synthesis: Thiols are the most prevalent organic compounds with a sulfhydryl group (-SH) that are found in the body. Total thiols include both intracellular and extracellular thiols, which can be found in the body as free oxidised or reduced glutathione or as thiols attached to proteins (Prakash *et al*., 2009). Thiols are essential for the metabolism of proteins in the organism and are the first antioxidants to be ingested when oxidative stress occurs. They have also been demonstrated to play significant roles in enzymatic activities, apoptosis, detoxification, and antioxidant defense in the body (Kükürt *et al*., 2021).

Total thiol results (Fig. 5) reveal that the toxicant group (RUT) showed a significant reduction in total thiol levels relative to the control groups. However, treatment with both curcumin and quercetin significantly improved the expression of total thiol levels in the treated groups (WT, RT), especially the reserpine treated group (RT), relative to the reserpine untreated (RUT) group. This result is consistent with previous studies conducted by Adeyemi *et al*. (2020), which reported an increase in total thiol levels following treatment with *quercetin* and *curcumin* in 3-NP-induced rodent models of Huntington disease and bacterial isolates, respectively. The decrease in total thiol levels in the toxicant group may be due to the mutation of the GSK-3β gene, while the ability of *curcumin* and *quercetin* to improve total thiol levels may be due to their ability to inhibit GSK-3β activity and prevent free radical generation.

Total protein result shows an observable decrease in protein level in the reserpine untreated, when compared other treated groups. However, a slight increase in the total protein levels in the wild group was observed when compared to other experimental groups, though the observations were not statistically significant. The result of the total protein analysis is consistent with the findings of Meshkibaf *et al*. (2019) and Azza *et al*. (2017), which reported an increase in protein levels following treatment with *quercetin* and *curcumin* in Cd-induced hepatorenal damage and Freund's adjuvant inflammation-induced male rats, respectively. The observed decrease in protein levels in the toxicant group may be due to alterations in mitochondrial function, resulting in inhibition of ATP production, DNA damage, and inhibition of protein synthesis.

Conclusion and recommendation

Conclusively, the results from this study showed that reserpine induced neurotoxic damages to the wild flies (*D. melanogaster*), as expressed in the significant reduction of locomotor and exploratory activities, decreased muscular integrity of the fly larvae, significant increase in flies mortality rate, increased MDA levels with significant reduction of AChE levels, which was a significant indication of oxidative stress, as well as disruption of enzyme levels. *Curcumin* and *quercetin* intervention at 100 mg/kg was able to ameliorate the damages induced, by significantly increasing locomotor and exploratory activities, increasing muscular integrity of the fly larvae, significantly decreasing flies mortality rate and reversing oxidative stress by reducing lipid peroxidation and improving the antioxidant system. The combination of these natural antioxidants may have a synergistic

effect, further increasing their protective effects against neuroinflammation and neurotoxicty, paving the way for future drug development; and making them a potential option for the prevention and management of neurodegenerative disorders.

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