EVALUATION OF *STACHYTERPHETA JAMAICENSIS* ETHANOL EXTRACT ON LIFESPAN, OXIDATIVE STRESS AND NEUROTOXICITY IN TRANSGENIC DROSOPHILA MODEL OF PARKINSON'S DISEASE

ABSTRACT

Background: Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive death of dopaminergic neurons. Stachytarpheta jamaicensis, a medicinal plant, has been shown to possess neuroprotective effects. This study evaluated the effect of S. jamaicensis leaf extract on a transgenic Drosophila model of PD.

Methods: Five groups of Drosophila melanogaster were treated with S. jamaicensis extract: wild control, wild + treatment, transgenic control, transgenic + low dose (50mg/kg), and transgenic + high dose (250mg/kg). Flies were cultured and treated for five days before behavioral, lifespan, and biochemical testing.

Results: S. jamaicensis extract increased lifespan and locomotor activity in flies. biochemical assays revealed reduced acetylcholine esterase activity, oxidative stress markers (malondialdehyde and total oxidized protein), and increased catalase enzyme activity and total thiol levels, $p< 0.05$; $p< 0.01$; $p< 0.005$.

Conclusion:

S. jamaicensis leaf extract exhibited neuroprotective and antioxidant effects in the Drosophila melanogaster model of PD, potentially through modulation of oxidative stress pathways. These findings suggest that S. jamaicensis extract may be a potential therapeutic agent for PD treatment.

Key words: Parkinson's disease, Stachytarpheta jamaicensis, Neuroprotection, Antioxidant, Oxidative stress, Drosophila melanogaster, Transgenic model, Neurodegenerative disorder.

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1. INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) that project to the striatum (0). The clinical diagnosis of PD is based on dopamine deficiency, and alterations in neurotransmitter levels are also known to cause motor and non-motor deficits (0).

The precise pathological mechanism of dopaminergic neuronal degeneration is still unknown, but several factors, including mitochondrial dysfunction, neuroinflammation, excitotoxicity, and abnormal protein aggregation, may contribute to the progression of PD (0). The production of reactive oxygen species (ROS) reduces mitochondrial dysfunction by interfering with the electron transport chain (ETC), resulting in decreased ATP production and nigrostriatal dopaminergic neuronal loss (0).

Medicinal plants have been used as a natural source of bioactive compounds that provide therapeutic benefits and low-cost treatments for various diseases. Starchytapheta jamaicensis, a medicinal plant, has been reported to contain secondary metabolites with various medicinal properties, including analgesic, antidiarrheal, antimicrobial, antioxidant, antihypertensive, antinociceptive, and anti-inflammatory properties (0).

The plant contains several major groups of secondary metabolites, including alkaloids, flavonoids, phenols, steroids, and terpenoids. These bioactive compounds can be found in abundance throughout the plant. Álvarez et al. demonstrated that S. jamaicensis leaf ethyl acetate extract significantly suppresses reactive oxygen species (ROS) production by inhibiting XO and scavenging ROS (0). Hexane extract, on the other hand, has no antioxidant activity.

Studies have extensively demonstrated that S. jamaicensis has antioxidant properties, which could be due to the presence of catechins (a type of flavonoid) in the leaves. Methanolic extract was found to be more effective in total antioxidant activity, DPPH, and FRAP than the other extracts (0). An ethanol leaf extract of S. jamaicensis was also found to improve catalase activity in alloxan-induced diabetic rats (0).

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The discovery of the first missense mutation in the SNCA gene, A53T, in 1997 (0), and the insoluble aggregated -Syn forms as the major component of LBs, a pathological hallmark of Parkinson's disease (0) has led to a new era in PD research (0). Since then, more SNCA pathogenic mutations as well as multiplications of SNCA have been identified as genetic causes of PD.

Using the conventional Gal4/USA expression system, Feany and Bender created -Syn transgenic Drosophila models by overexpressing either wild-type or familial mutants A53T and A30P of human -Syn (0). These models replicate the key features of Parkinson's disease (PD): adult-onset loss of DA neurons, filamentous intra-neuronal inclusions containing -Syn, and locomotor dysfunction (0).

However, Parkinson's disease is a complex neurodegenerative disorder that requires further research into its underlying mechanisms and potential therapeutic targets. Starchytapheta jamaicensis has been shown to possess antioxidant properties and may have potential therapeutic benefits for the treatment of Parkinson's disease. Further studies are needed to investigate the efficacy and safety of S. jamaicensis as a potential treatment for Parkinson's disease.

In this study, we investigate the potential therapeutic benefits of S. jamaicensis leaf extracts in Parkinson's disease using Drosophila melanogaster as a model system. The Drosophila model is widely used to study the pathogenesis of Parkinson's disease due to its genetic tractability and conservation of key molecular mechanisms with humans (0). Our study aims to explore the potential therapeutic benefits of S. jamaicensis leaf extracts in reducing oxidative stress and neurodegeneration in Drosophila melanogaster models of Parkinson's disease.

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2. MATERIALS AND METHODS

2.1 Reagents

Analytical grade substances were used in this study. Acetylcholine iodide, DTNB, GSH, CDNB, sodium dihydrogen phosphate, potassium hydrogen phosphate, Randox protein kit, and EDTA were purchased from Sigma Aldrich and Chidex Surgical Suppliers Limited.

2.2 Plant Collection and Extraction

S. jamaicensis leaf was collected from the University of Calabar, Nigeria. The leaf was air-dried for 7 days, then pounded and extracted in 90% ethanol for 72 hours. The extract was filtered and freeze-dried, and the percentage total yield was calculated using the formula: % extraction yield = (W2 - W1) / W0 × 100.

2.3 Drosophila Melanogaster Model

Transgenic fly lines expressing wild-type human synuclein (h-αS) under UAS control in neurons were obtained from the Bloomington Drosophila Stock Center. When crossed with GAL4-elav.L females, the males expressed human alpha synuclein in neurons.

2.4 7-days LC50 and 28-days Survival Assay

The 7-day LC50 of the plant extract was tested using the ingestion method of exposure. Mortality readings were taken every 24 hours for 7 days. A survival study was performed using the same method, with mortality readings taken at 28 days.

2.5 Seven(7)-days Treatment of D. melanogaster with S. jamaicensis

Short exposure of D. melanogaster was conducted using the ingestion method of exposure. Treatment duration was determined from the survival study. Fifty flies were treated with low dose (LD) or high dose (HD) of the plant extract for 7 days. Mortality readings were taken every 24 hours.

2.6 Drosophila Culture and Treatment

Flies were cultured on standard Drosophila food containing agar, corn meal, sugar, and yeast. The PD flies were exposed separately to different doses of crude extract (Sigma Aldrich, CAS 458-37-7) and mixed in culture medium.

2.7 Lifespan Determination

Newly enclosed male flies (control and PD) were placed in culture tubes containing 100, 200, and 250μM of plant extract mixed in diet.

Flies were switched to a new diet every third day, and the number of dead flies was counted every three days until the last one died.

2.8 Negative Geotaxis (measured by % of climbing activity)

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Negative geotaxis was performed by applying the technique described by Abolaji et al. (0). Ten flies from each group were counted every day for seven days.

2.9 Acetylcholinesterase Activity

AChE activity was assessed using the Ellman method with minor adjustments. Protein content was employed to adjust the results as data were compared to the blank and sample blank.

2.10 Estimation of Protein Carbonyl Content

The protein carbonyl content was estimated using the method described by Hawkins et al. (0). The brain homogenate was diluted to a protein concentration of approx 1 mg/ml. About 250 µL of each diluted homogenate was taken in an eppendorf centrifuge tube separately.

2.11 Total Thiol Determination

Total thiol determination was evaluated using the Ellman approach. The protocol used by Ololade et al. (0) was followed for the production of the assay reaction mixture and absorbance measurement.

2.12 Catalase Activity

Catalase activity was measured using Aebi's approach (0). H2O2 clearance was monitored at 240 nm and 25°C while 10 L of the sample was mixed with solution A.

2.13 Malondialdehyde (MDA)

MDA was estimated using the method described by Hawkins et al. (0). The pink-colored product's absorbance was measured at 532 nm after it had cooled.

STATISTICAL ANALYSIS

One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used to find statistically significant differences between numerous groups receiving different treatments. Kaplan-Meier analysis method was performed to examine survival data, and log rank test was employed to compare groups. Statistical significance was defined as a P value of less than 0.05, 0.01 and 0.005 ($p < 0.05$, $p < 0.01$, $p < 0.005$).

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3. RESULT AND DISCUSSION

3.1 Results

3.1.1 Survival Assay

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 plant extract decreased transgenic flies' mortality and increased the number of flies that survived to the end of the experiment. This suggests the effect of S. jamaicensis leaf extract on toxicity in Transgenic Drosophila model of Parkinson's disease (PD) flies as seen in (Fig. 1).

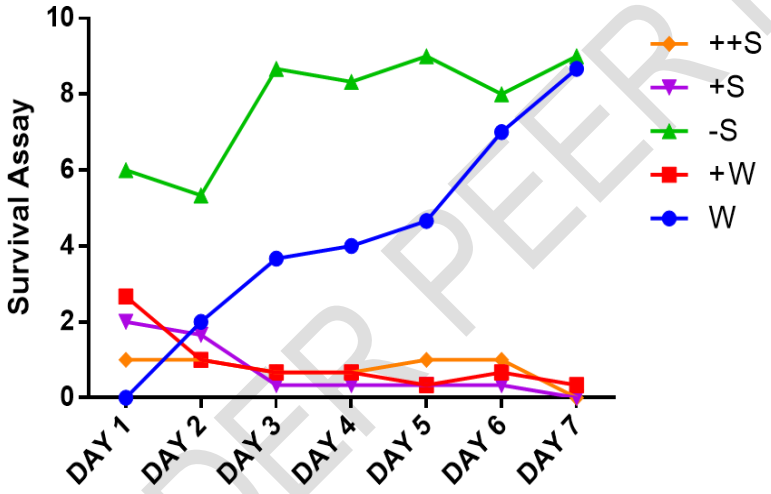

Figure 1: Mortality rate of flies treated with different doses of S. jamaicensis leaf extract, per day for the duration of 7 days.

Transgenic (Sgg) group: Significant from wild treated (Wild+ plant extract), Low dose (LD), and high dose group not Significant from wild group.

Wild (W), wild treated (+W), transgenic only group (-S), low dose (+S) high dose (++S).

3.1.2 Negative Geotaxis Assay

The data from this result shows a significant decrease of locomotory activity in the transgenic (Sgg) group when compared to the wild treated (Wild+ plant extract) group, Low dose (LD)

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(Sgg+100mg/kg) and High dose (HD) (Sgg+250mg/kg) and no significance when compared to the Wild. (*p<0.05, **p<0.01, ***p<0.005). This suggests the Effect of S. jamaicensis leaf extract on Impaired motor function or mobility in Transgenic Drosophila model of Parkinson's disease (PD) flies as shown in (Fig. 2).

Sgg (transgenic)

Sgg+100mg/kg

Sgg++250mg/kg

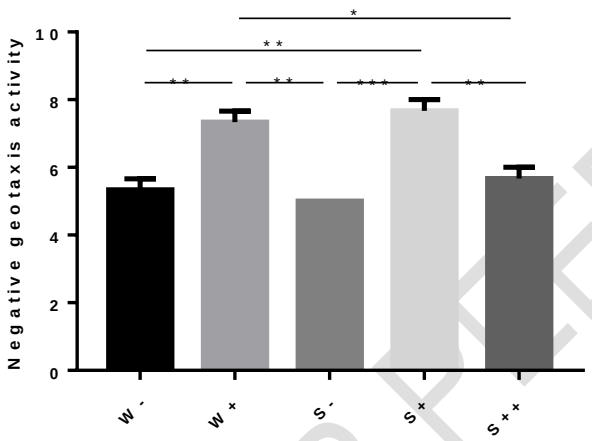

Fig. 2: Locomotory activity in transgenic Drosophila model of Parkinson's disease (PD) flies treated with different doses of S. jamaicensis leaf extract.

Transgenic (Sgg) group: Significant from wild treated (Wild+ plant extract), Low dose (LD), and High dose group, and not Significant from Wild group. Data are presented as mean ± SEM (n=6). *p<0.05; **p<0.01; and ***p<0.005 compared the wild treated (Wild+ plant extract), transgenic (Sgg-), low dose and wild.

Wild (W-), wild treated (W+), transgenic only group (Sgg-), low dose (Sgg+) high 13428dose (Sgg++).

3.1.3 Protein Status

3.1.3.1. Total Protein Level

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The data from this result shows a significant increase of total protein level in the transgenic (Sgg+250mg/kg) group when compared to the wild treated (Wild+plant extract), transgenic (Sgg-) and Low dose (LD) (Sgg+100mg/kg) groups and no significance when compared to the Wild group. (**p<0.01; ***p<0.005). This suggests the Effect of S. jamaicensis leaf extract on protein damage or degradation caused by oxidative stress in Transgenic Drosophila model of Parkinson's disease (PD) flies and seen in (Fig. 3).

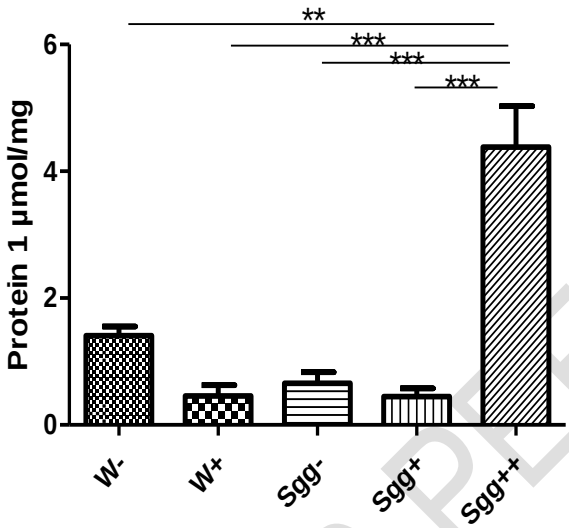

Figure 3: Total protein levels in transgenic Drosophila model of Parkinson's disease (PD) flies treated with different doses of S. jamaicensis leaf extract.

transgenic (Sgg+250mg/kg) group: Significant from wild treated (Wild+ plant extract) transgenic (Sgg-), Low dose (LD) group and not Significant from Wild group. Data are presented as mean ± SEM (n=6). *p<0.05; **p<0.01; and ***p<0.005 compared the wild treated (Wild+ plant extract), transgenic (Sgg-), low dose and wild.

Wild (W-), wild treated (W+), transgenic only group (Sgg-), low dose (Sgg+) high dose (Sgg++). 134

3.1.4 Lipid Peroxidation

The data from this result shows a significant increase of malondialdehyde level in the

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transgenic (Sgg) group when compared to all other groups. (*p<0.05, **p<0.01, ***p<0.005). This suggests the effect of S. jamaicensis leaf extract on lipid peroxidation and oxidative damage to cell membranes in Transgenic Drosophila model of Parkinson's disease (PD) flies as seen in (Fig. 4).

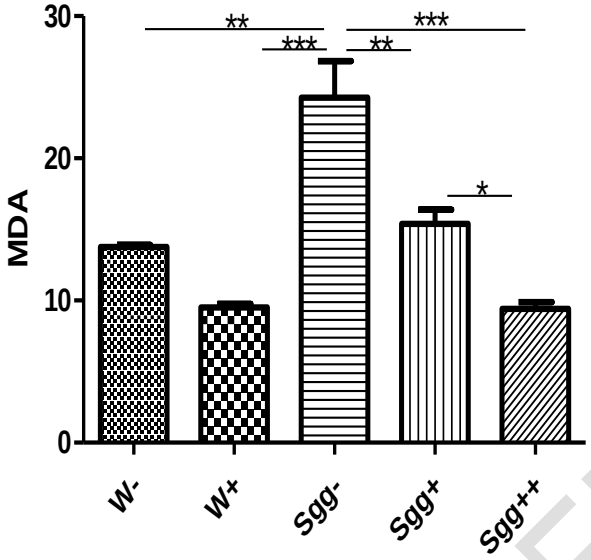

Figure 4: Malondialdehyde (MDA) levels in transgenic Drosophila model of Parkinson's disease (PD) flies treated with different doses of S. jamaicensis leaf extract.

Transgenic (only) group: Significant from wild, wild treated (Wild+ plant extract) Low dose (LD) and High dose. Data are presented as mean ± SEM (n=6). *p<0.05; **p<0.01; and ***p<0.005 compared the wild, wild treated (Wild+ plant extract), low dose and high dose groups.

Wild (W-), wild treated (W+), transgenic only group (Sgg-), low dose (Sgg+) high dose (Sgg++).

3.1.5 Oxidative Stress Parameters

3.1.5.1 Total Thiol Level

The data from this result shows a significant increase of total thiol levels in the transgenic high dose (HD) (Sgg++250mg/kg) group when compared to the Low dose (LD) (Sgg+100mg/kg) and a significance decrease when compared to the Wild. (*p<0.05, **p<0.01, ***p<0.005, ****p<0.001). This suggests the Effect of S. jamaicensis leaf extract

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on oxidative stress in Transgenic Drosophila model of 13428Parkinson's disease (PD) flies as seen in (Fig. 5).

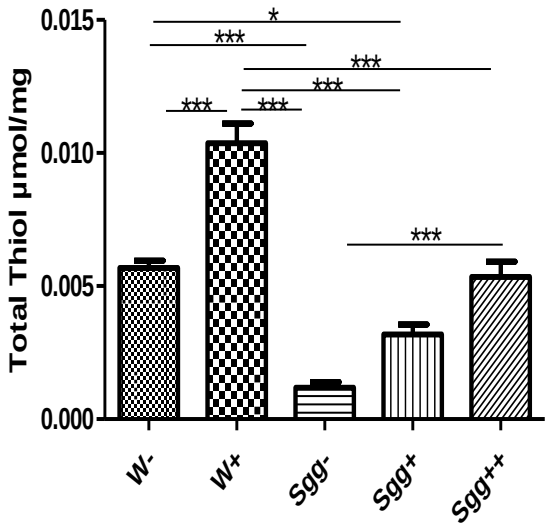

Figure 5: Total Thiol Level in transgenic Drosophila model of Parkinson's disease (PD) flies treated with different doses of S. jamaicensis leaf extract.

Transgenic high dose (HD) (Sgg++250mg/kg) group: Significant from Low dose (LD) group. Data are presented as mean ± SEM (n=6). *p<0.05; **p<0.01; and ***p<0.005 compared with the wild, wild treated (Wild+ plant extract), and low dose groups.

Transgenic high dose (HD) (Sgg++250mg/kg) group: Significant from Wild group . 134Data are presented as mean ± SEM (n=6). *p<0.05; **p<0.01; and ***p<0.005 compared with the wild, wild treated (Wild+ plant extract), and low dose groups.

Wild (W-), wild treated (W+), transgenic only group (Sgg-), low dose (Sgg+) high dose (Sgg++).

3.1.5.2 Total Catalase Concentration

The data from this result shows a significant decrease of catalase concentration in the transgenic (Sgg+100mg/kg) group when compared to the wild, wild treated (Wild+ plant extract) group and High dose (HD) (Sgg++250mg/kg). (*p<0.05, ***p<0.005). This suggests the Effect of S. jamaicensis leaf extract on alterations in cellular defenses against oxidative stress in Transgenic Drosophila model of Parkinson's disease (PD) flies as seen in (Fig. 6).

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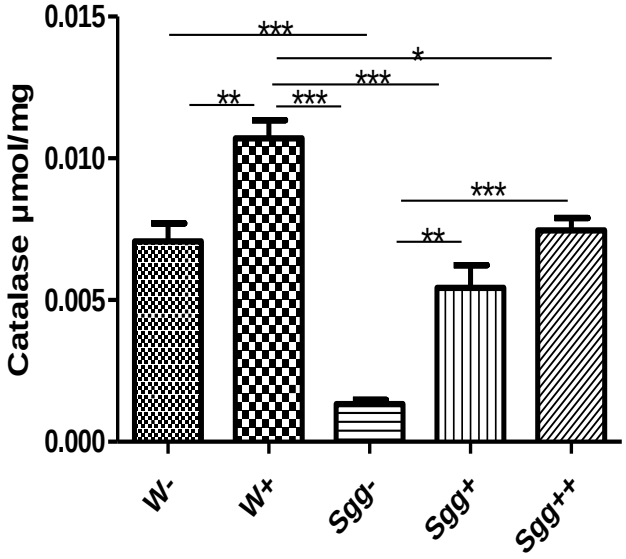

Figure 6: Catalase concentration in transgenic Drosophila model of Parkinson's disease (PD) flies treated with different doses of S. jamaicensis leaf extract.

transgenic (Sgg+100mg/kg) group: Significant from wild, wild treated (Wild+ plant extract), transgenic (only) group and High dose. Data are presented as mean ± SEM (n=6). *p<0.05; **p<0.01; and ***p<0.005 compared the wild, wild treated (Wild+ plant extract), and high dose groups.

Wild (W-), wild treated (W+), transgenic only group (Sgg-), low dose (Sgg+) high dose (Sgg++).

3.1.6 Antioxidant Enzyme Activity

The data from this result shows a significant decrease of Acetylcholinesterase activity level in the transgenic (Sgg) group when compared to the *wild, wild treated (Wild+ plant extract),* Low dose (LD) (Sgg+50mg/kg) and High dose (LD) (Sgg+200mg/kg). (*p<0.05, **p<0.01, ***p<0.005). This suggests the Effect of S. jamaicensis leaf extract on acetylcholinesterase activity oxidative stress in Transgenic Drosophila model of Parkinson's disease (PD) flies as seen in (Fig. 7).

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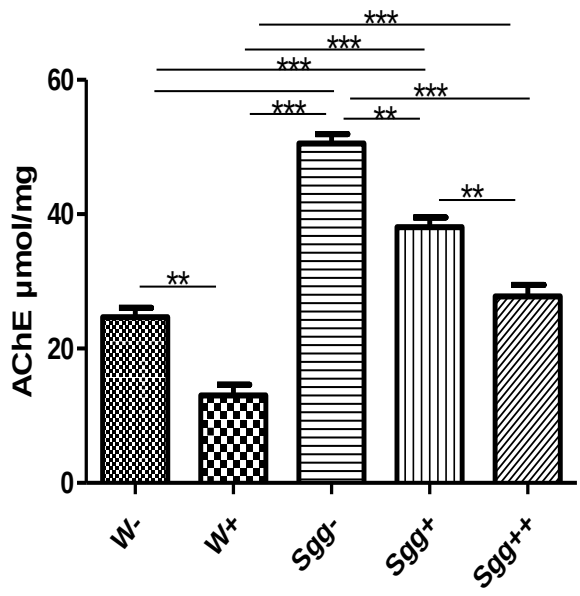

Figure 7: Acetylcholinesterase (AChE) concentration in transgenic Drosophila model of Parkinson's disease (PD) flies treated with different doses of S. jamaicensis leaf extract (low dose 50 and high dose 200 mg/kg).

Transgenic (only) (Sgg-) group: Significant from wild, wild treated (Wild+ plant extract) Low dose (LD) and High dose. Data are presented as mean ± SEM (n=6). *p<0.05; **p<0.01; and ***p<0.005 compared to the wild, wild treated (Wild+ plant extract), low dose and high dose groups.

Wild (W-), wild treated (W+), transgenic only group (Sgg-), low dose (Sgg+) high dose (Sgg++).

3.2 Discussion

The results of this study demonstrate that the S. jamaicensis extract exhibits a significant impact on the survival rate of D. melanogaster. The 7-day LC50 value of 353 mg extract/10 g diet highlights the extract's toxicity, which is consistent with previous research that has identified various plant extracts as sources of toxic compounds affecting insect physiology (0). Moreover, the significant reduction in the 28-day survival ratio of the treated flies compared to the control group ($P < 0.05$) underscores the detrimental effects of prolonged exposure to the extract. These findings suggest that the extract exerts a time-dependent negative effect on the viability of D. melanogaster, pointing to its potential toxicity.

In addition, we investigated the impact of the plant extract on AChE and climbing activities in D. melanogaster. AChE activity remains a prime marker of neurotoxicity, as its inhibition

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may indicate poor locomotive operations and common toxicity (0). Our results showed that S. jamaicensis leaf extract did not significantly inhibit AChE and climbing activities when compared to control ($P > 0.05$). This suggests that the extract does not interfere with the antioxidant system of fruit flies.

Furthermore, our study demonstrated that the plant extract exhibits antioxidant activities in vivo, as shown by a significant increase in catalase (CAT) activities and whole thiol content compared to the control group. This suggests that the plant extract does not interfere with the antioxidant system of fruit flies, which is crucial for combating free radicals and maintaining cellular integrity (0).

The findings suggest that S. jamaicensis may have potential as a treatment for conditions related to oxidative stress-induced neuronal imbalance. The observed effects in fruit flies are particularly noteworthy due to the conservation of genes and biochemical pathways related to oxidative stress between fruit flies and humans (0). This implies that the beneficial effects observed in fruit flies could potentially translate to humans.

4. CONCLUSION

In conclusion, our study demonstrates that the ethanol extract of S. jamaicensis leaf shows a trend towards increasing total thiol and catalase levels in D. melanogaster, although these changes were not statistically significant ($P > 0.05$). Despite this, the extract significantly enhances the survival rate, young fly emergence rate, and climbing activities (acetylcholinesterase and negative geotaxis) of adult D. melanogaster treated with the extract.

Overall, these findings suggest that S. jamaicensis may have potential as a treatment for conditions related to oxidative stress-induced neuronal imbalance. Further research is needed to elucidate the specific mechanisms involved and to determine the potential therapeutic implications of these findings.

ETHICAL APPROVAL

This study was conducted in accordance with the guidelines of the College of medical sciences ethical committee, university of calabar, which has approved the use of Drosophila

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melanogaster in research. The protocols were reviewed and approved and the ethical approval number was obtained **202ANA1023**. The flies were handled and maintained in accordance with the guidelines for animal care and welfare, and all efforts were made to minimize their stress and discomfort.

An approval for the experimental protocol of this study with registration number 190ANA3023 was obtained from the Faculty Animal Research Ethics committee, Faculty of Basic Medical Sciences, University of Calabar, Cross River state, Nigeria.

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