Research Report By: Dr. Amos Olalekan Abolaji
Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria

Research Topic: Protective Mechanism of Resveratrol Against Neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-Induced Parkinsonism in Drosophila melanogaster.

1. Background Information

Dr. Abolaji Amos was privileged to be awarded $3,500 by the Committee for Aid and Education in Neurochemistry (CAEN) CATEGORY 1B (2016) Grant to investigate the Protective Mechanism of Resveratrol Against Neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-Induced Parkinsonism in Drosophila melanogaster. The research was carried out in the Drosophila laboratory, Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Oyo State, Nigeria. The research was successfully carried out. Two M.Sc. students carried out their projects with this research under the supervision of Dr. Abolaji.

Parkinson’s Disease (PD) is a common progressive neurodegenerative disease that affects the dopaminergic neurons. Apart from genetic factors, exposure to environmental toxins also contribute to the pathogenesis of PD. For instance, the treatment of primates with environmental toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) resulted in development of clinical features of PD. In addition, conventional drugs against PD only provide symptomatic relief with copious side effects. Thus, the search for affordable and effective phytochemicals to treat PD is ongoing.

Phytochemicals such as resveratrol and curcumin have been shown to protect against PD. Truly, resveratrol, a phytoalexin polyphenol, found in grapes and other plants, has the ability to cross the blood brain barrier just like L-3,4-dihydroxyphenylalanine (Abolaji et al., 2018).

Although, resveratrol has been reported to protect against PD, in-depth studies on the biochemical mechanisms of protection using simple model organisms such as Drosophila melanogaster are scarce in the literature. Therefore, we used D. melanogaster with a huge bioinformatics database and short lifespan, to evaluate the biochemical changes following treatment with MPTP and the protective role of resveratrol.

2. Execution of Project

2.1. Chemicals

1 methyl, 4 phenyl, 1,2,3,6 tetrahydropyridine (MPTP) and resveratrol were procured from A K Scientific, 30023 Ahern Ave, Union City, CA 94587, united State of America at a percentage purity of 95%.

2.2. Drosophila melanogaster stock and culture

D. melanogaster (Harwich strain) used as a model in this study was obtained from the National Species Stock Center (Bowling Green, OH, USA).
2.3. MPTP exposure and survival rate analyses

*D. melanogaster* (both genders) of 1 to 3 days old were divided into six groups of 50 flies each: control, MPTP (250 µM, 500 µM, 1000 µM, 2000 µM, and 3000 µM) prepared in absolute ethanol. In order to determine the doses of MPTP and the duration of exposure, survival assays were carried out involving three independent treatments with each containing five replicates of 50 flies/vial. Based on these data, two doses of MPTP (250 µM and 500 µM) were selected for the study.

2.4. Resveratrol exposure and survival rate analyses

*D. melanogaster* (both genders) of 1 to 3 days old were divided into different groups of 50 flies/vial: 2 sets of control (with and without ethanol final concentration of 2.5%), resveratrol (7.5, 15, 30, 60, and 120 mg/kg diet) prepared in absolute ethanol. To determine the doses of resveratrol and the duration of exposure, a longevity assay was carried out and data were analyzed and plotted as percentage of live flies. Thereafter, two doses of resveratrol (30 and 60 mg/kg diet) were selected to investigate the rescue role of resveratrol on MPTP-induced toxicity as shown below:

**Research Design**

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Control (Vehicle)</td>
</tr>
<tr>
<td>Group 2</td>
<td>MPTP (250 µM)</td>
</tr>
<tr>
<td>Group 3</td>
<td>MPTP (500 µM)</td>
</tr>
<tr>
<td>Group 4</td>
<td>Resveratrol (30 mg/kg diet)</td>
</tr>
<tr>
<td>Group 5</td>
<td>Resveratrol (60 mg/kg diet)</td>
</tr>
<tr>
<td>Group 6</td>
<td>MPTP (250 µM) + Resveratrol (30 mg/kg diet)</td>
</tr>
<tr>
<td>Group 7</td>
<td>MPTP (500 µM) + Resveratrol (60 mg/kg diet)</td>
</tr>
<tr>
<td>Group 8</td>
<td>MPTP (250 µM) + Resveratrol (60 mg/kg diet)</td>
</tr>
<tr>
<td>Group 9</td>
<td>MPTP (500 µM) + Resveratrol (30 mg/kg diet)</td>
</tr>
</tbody>
</table>

2.5. Negative geotaxis

Locomotor performance of MPTP-treated and control flies were evaluated.

2.6 Preparation of sample for biochemical assays and histological investigation

Fifty (50) flies (of both genders) were exposed to final doses of MPTP (250 and 500 µM) and resveratrol (30 and 60 mg/kg diet) as indicated above in the diet for 3 days. Then, flies were anaesthetised in ice, weighed, and homogenised in 0.1 M phosphate buffer (pH 7.0), and centrifuged at 4000g for 10 minutes at 4°C in a Thermo scientific Sorval Micro 17R centrifuge. Thereafter, supernatants were separated from pellets into labelled Eppendorf tubes, for the determination of acetylcholinesterase (AChE), glutathione-S-transferase (GST) and catalase (CAT) activities as well as total thiol, hydrogen peroxide and nitric oxide levels and cell viability. A one-way ANOVA was used to determine the significant differences among multiple groups with differences with a *p*-value < 0.05 considered statistically significant. Another set of flies were similarly treated and used for histological study.

3. Project Outcome

3.1. Summary of results: The results indicated that resveratrol extended lifespan and reversed MPTP-induced toxicity in *D. melanogaster* by substantially improving the level of total thiol and activities of antioxidant enzymes. Overall, the MPTP-induced oxidative stress was due to an imbalance between ROS and antioxidant system in the...
flies. However, resveratrol protected against MPTP-induced toxicity by reducing mortality, increasing acetylcholinesterase (AChE) activity, reducing inflammatory marker and increasing antioxidant status and cell viability in *D. melanogaster*. The protective role of resveratrol can be partly linked with its free radical scavenging and antioxidant actions. In addition, resveratrol showed no obvious adverse effect in flies (Abolaji et al., 2018).

![Figure 1](image1.png)

**Figure 1. The Structures of resveratrol and MPTP**

![Figure 2](image2.png)

**Figure 2:** Impacts of MPTP and resveratrol on the survival rates of *D. melanogaster*: (A) lifespan extending role of resveratrol in *D. melanogaster*, and (B) MPTP Survival curve showing reduced survival rate of *D. melanogaster* exposed to MPTP (Abolaji et al. 2018).
Figure 3. The histology of *D. melanogaster* brains treated with MPTP and resveratrol. The control (A) and resveratrol groups (B and C) indicated even distribution of neurons (periphery) around the central nerve fibres (white matter). Lesions were observed in the brains of MPTP-exposed flies (D and E, dotted arrow), and (arrows); *D. melanogaster* co-exposed to (F) MPTP (250 μM) and resveratrol (30 mg/kg diet) and (G) MPTP (500 μM) and resveratrol (60 mg/kg diet) indicated even distribution of neurons (arrows); in (H) MPTP (250 μM) and resveratrol (60 mg/kg diet) and (I) MPTP (500 μM) and resveratrol (30 mg/kg diet) indicated slight eosinophilia of nerve fibres (dotted arrow). Magnification: X400. MPTP: 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine. (Abolaji et al., 2018).

The full data and other details can be obtained via this link: [https://www.ncbi.nlm.nih.gov/pubmed/29935183](https://www.ncbi.nlm.nih.gov/pubmed/29935183)
3.2. Publication
From this study, we published one paper in Biochemical and Biophysical Research Communications (impact factor 2.559) in which ISN was acknowledged as indicated below:


3.3. Presentation at a Major Conference
We presented the results at the 6th UNIBADAN Conference of Biomedical Research, 11-14 July, 2018 at the Conference Centre, University of Ibadan, Ibadan, Nigeria.


3.4. Training Opportunity Provided by the Grant
The grant provided opportunity to train and supervise two M.Sc. students (Adeola Adedara and Moji Adie) whose dissertations were based on this project. In addition, several other postgraduate and undergraduate students have carried out their projects with the research resources provided by this grant.

3.5. Benefits to the Host Institution
2017 ICGEB-DrosAfrica Drosophila workshop.
The materials, chemicals, reagents and equipment (spectrophotometer, pH meter) provided by this grant were also used during the 2017 ICGEB-DrosAfrica Workshop that took place for two weeks in the Drosophila laboratory in July 2017. Thirty participants from Nigeria, Uganda, Rwanda and Ghana as well as 8 resource senior scientists were present during the workshop. This workshop was supported by ICGEB, DrosAfrica, University of Ibadan, Company of Biologists and Cambridge Africa Alborada Research Funds. In addition, the research resources of our Drosophila laboratory have been improved.
4. Other Grants Received
In addition to the ISN grant, we received the following grants:


ii. Cambridge-Africa ALBORADA Research Funds: Abolaji A.O. and Weil T.T. (2016). This was used to purchase a new refrigerated centrifuge.

iii. Company of Biologists: With the support of DrosAfrica, 6 dissecting microscopes were purchased due to the funds from Company of Biologists.

vi. International Centre for Genetic Engineering and Biotechnology (ICGEB) - CRP 2018 Grant Award: The results obtained from this study were used as preliminary data to apply for the 2018 ICGEB-CRP Grant. The Drosophila laboratory will be supported by ICGEB from 2019-2021 to continue the study with Parkinson’s Disease Models in Drosophila using resveratrol as a rescue agent.

5. Fellowship Award
2018 Africa Oxford Initiative Fellowship Award (Visiting Scholar): This was awarded to Dr. Amos Abolaji to carry out further training on D. melanogaster at the Department of Biochemistry (Prof. Ilan Davis’ laboratory), University of Oxford, UK, from August to September 2018

6. Invitation as Speaker

Conclusion
This study has added to the body of literature on the protective role of resveratrol in MPTP-induced Parkinsonism. The data also served as preliminary data to apply for the 2018 ICGEB-CRP Grant award.

Acknowledgements
1. We would like to thank the International Society of Neurochemistry, Committee for Aid and Education in Neurochemistry (CAEN) for the award of this grant that enabled us to carry out this project.

2. We thank the Vice Chancellor, University of Ibadan, Professor Abel Olayinka for the provision of laboratory benches and 5 KVA inverter that provided uninterrupted supply of electricity in the course of this project.

3. We thank Professor James Olopade for his advice in the course of this project.

4. We thank DrosAfrica, Company of Biologists, Cambridge Africa Alborada Research Funds UK and ICGEB Italy, for the support to the our Drosophila laboratory.
A. ITEMS PURCHASED WITH THE ISN-CAEN FUNDS

1. New Spectrophotometer for biochemical assays (One)
2. New pH meter (One)
3. New Weighing Balance (One)
4. New Micropipettes (Two)
5. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP, 2 grams)
6. Resveratrol (10 grams)
7. *Drosophila* vials and bottles (50)
8. Agar-agar (500 grams)
9. Nipagin (Diet preservative, 200 grams)
10. Materials, reagents and chemicals (for biochemical assays and histology)

B. Other Equipment Used (Provided by Other Funders)

1. Refrigerated Centrifuge (Cambridge Africa Alborada Research Funds, UK)
2. Dissecting microscopes (Company of Biologists, UK)
3. CO₂ system (DrosAfrica, UK)

Reported By: Dr. Amos Olalekan Abolaji

Signature: 

Date: 30 December, 2018
A. Pictures

2017 U.I.-DrosAfrica-ICGEB
Drosophila Workshop

- 30 participants from Nigeria, Ghana, Rwanda and Uganda
- 8 Seasoned scientists from Spain, UK, USA, Uganda and Italy.

B. Postgraduate students supervised by Dr. Amos Abolaji

Adeola Adedara (M.Sc., 2018)  Moji Adie (M.Sc., 2018)

C. Dr. Abolaji