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Report

Neuroprotective role of *Baphia macrocalyx* leaf extract in transgenic *Drosophila* model of Parkinson's disease

Background
Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder with an incidence of about 1% in people older than 65 years (Kalia and Lang, 2015). Characteristically, at a diseased state, the *substantia nigra* shows progressive loss of dopaminergic (DA) neurons and the presence of Lewy bodies in surviving neurons (Forno, 1996). It is considered that both genetic susceptibility and environmental factors such as exposure to pesticides contribute to the pathogenesis. Despite several extensive studies, there is presently no cure or treatment that can stop the development of the disease. While the precise pathologic mechanisms remain unclear, mitochondrial dysfunction and oxidative stress have been implicated as critical mediators of neuronal cell death in PD (Gupta et al 2008, Shulman et al 2011).

Since last decade, *Drosophila* has become a tractable model for PD. The insect has strong PD related phenotypes, including loss of DA neurons, protein aggregation, reduced locomotion, mitochondrial dysfunction and problems with reactive oxygen species (Feany and Bender, 2000; Whitworth, 2011). Recently, a study conducted in *Drosophila* model of PD gave insights of molecular pathways affected at early phase of PD development (Stephano et al 2018). Markedly, *Drosophila* has homology among five of six genes associated with PD in human.

The use of natural products as an alternative therapy for different diseases has recently gain attention in many societies. Some biochemical compounds from the natural products are currently in clinical trials as promising neuroprotective agents for neurodegenerative disorders (Seidl & Potashkin 2011; Mahmood et al. 2014). Recently, the leaf extracts of *Baphia macrocalyx* showed high potent antioxidant activity in in vitro models (Bwire , 2015). *Baphia macrocalyx* is a native leguminous plant found in Tanzania and Northern Mozambique. There is no available

information in the literature on the use of *Baphia* in traditional medicine in East Africa. Beside, two species of *Baphia* namely *nitida* and *bancoensis* are used in West Africa for treatment of skin, gastrointestinal, inflammatory, venereal diseases and cure suppurating eyes. (Adeyemi et al., 2006; Yao-Kouassi, 2008). Hence, the present study analyzed the efficacy of *Baphia macrocalyx* in protecting the DA neurons from oxidative state and premature death.

1. Objectives and hypotheses

The current study aimed at investigating the neuroprotective potential of *Baphia macrocalyx* crude leaf extracts in *in vivo* transgenic *Drosophila* model of PD.

The study has attempted to answer the following specific questions;
Do methanolic leaf extract of *Baphia macrocalyx* (MEBM) have potential for reducing the PD symptoms in transgenic *Drosophila* model? Do MEBM have potential of reducing oxidative stress induced by rotenone in the brain of PD model flies?
To answer these specific questions, rotenone-induced Parkinsonism in the DA neurons was analyzed using genetic manipulation, behavioral assay, lipid peroxidation analysis and qRT-PCR analysis. Parkinsonism in *Drosophila* was induced by a neurotoxin, rotenone. The progeny of TH-GAL4 (Tyrosine hydroxylase-GAL4) strain crossed to 20XUAS-mcD8::GFP flies were used in this study. Tyrosine hydroxylase is used in the biosynthesis of dopamine; hence, it has been used as a marker for dopamine expressing neurons.

Results

Effect of MEBM Exposure on Survival of Flies

In order to determine the dosage and effect of MEBM to flies, the survival rate was monitored for 7 days. Thirty adult flies (1-4 days old) both genders were subjected to various concentrations of MEBM (0-1700 mg /ml) mixed in the diet. Three independent experiments were performed in which each concentration has three replicates. The number of alive and dead flies was recorded for seven days consecutively. The data showed that there is no significance difference in number of dead flies treated with MEBM at different concentrations ($F(10,22) = 1.987$, $p=0.086$) compared to the control group (with out MEBM) (Fig. 1). Following this, the concentration of 1000 mg/ml MEBM was chosen for subsequently experiments.

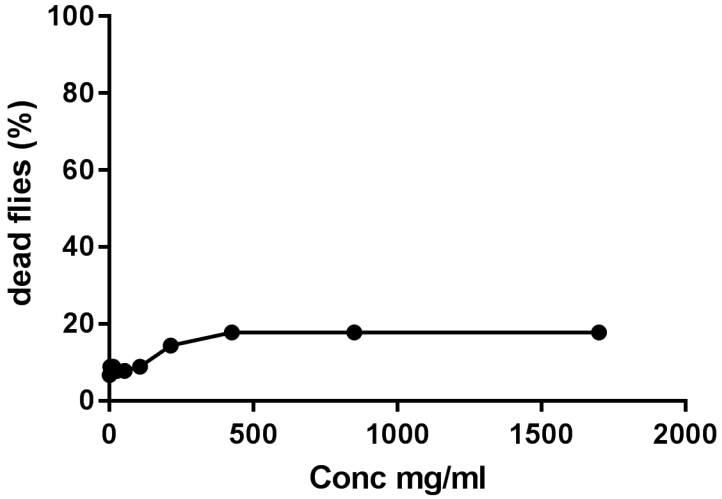

Fig. 1 Effect of MEBM exposure on survival of flies. Data were collected every 24 hrs for each concentration for 7 days. The average numbers of dead flies are expressed in %. The total number of flies (90 per group) for three independent experiments.

Effect of MEBM on survival rate of flies exposed to rotenone

The survival rate determined by counting the number of alive flies every 24 hrs for the period of 7 days as above. Flies exposed to sublethal dose of rotenone (0.5 mM) alone, MEBM (1000 mg/ml) alone, rotenone and MEBM were compared to control flies (normal diet). Total number of flies monitored per group was 180 for three independent experiments (60 flies/each treatment). It was observed that most of the flies were alive at the end of the experiment in all four groups (data not shown).

MEBM rescued the climbing disability induced by rotenone in the *Drosophila* PD model

Flies locomotor ability was tested by negative geotaxis assay (Feany and Bender, 2000) with some minor modifications. Flies from above groups (20 flies/group) were anesthesized with CO_2 and placed into a vertical glass tube (30 cm x 2.5 cm). Given 30 minutes for recovery, flies were gently tapped to the bottom of the glass tube. Flies that crossed a marked line of 12 cm within 10 secs were counted and considered top and those remained below the mark counted as bottom. The procedure was repeated five times at 1-minute interval. One-way ANOVA followed by Tukey's pairwise comparisons showed that the climbing ability behavior was impaired in the flies exposed to retonone, when compared to control flies ($p=0.00019$). This effect was rescued by MEBM treatment $p= 0.00018$. Analysis also showed that there was no significance difference in climbing ability of flies fed with MEBM alone, MEBM and Rotenone compared to control flies ($p= 0.1$; Fig.2).

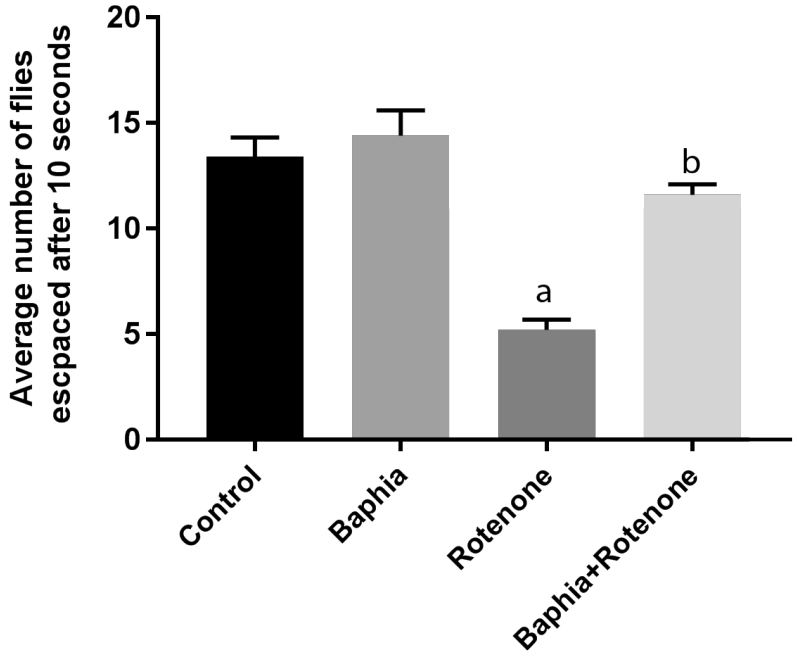

Fig. 2 Effect of *Baphia macrocalyx* on geotaxis response of flies exposed to rotenone for 7 days. The total number of flies (60 per group) for three independent experiments (20 flies/each analysis). The values are the mean of five assays. ([a] significant with respect to control $p < 0.05$; [b] significant with respect to *Baphia* + Rotenone

MEBM has no potential of reducing oxidative stress induced by rotenone in the brain of PD model flies: mRNA expression of SOD, CAT and TH genes

Rotenone acts by inhibiting complex I of the mitochondrial electron transport chain leading to reduced ATP synthesis and electron leakages that produce reactive oxygen species (ROS) like superoxide, subsequently causing oxidative stress and reduced level of glutathione (Duty and Jenner, 2011; Martinez and Greenamyre, 2012). This study sought to seek the expression of endogenous antioxidants enzymes; Superoxide dismutase (Sod), Catalase (Cat) and that of that of dopamine synthesis, Tyrosine hydroxlase (Th) during rotenone induced PD development using qRT-PCR analysis. One-way ANOVA followed by Tukey's pairwise comparisons showed that flies subjected to rotenone had an increase in expression of mRNA of Sod (Fig.3) and Cat (Fig.4) and Th (Fig.5) compared to the control flies but the increase was not significant. However, flies exposed to MEBM and rotenone had a significant increase in mRNA of Sod (Fig.3.), Cat (Fig.4) and Th (Fig.5) compared to control flies $P<0.01$. The significant difference was also observed between rotenone fed flies and MEBM and rotenone fed flies with respect to mRNA expression of Sod. Results also showed that there was a fold change increase in those flies that were fed MEBM alone against rotenone fed flies, $P<0.05$ (Fig. 3). Theses results indicate that combination of rotenone and MEBM synergistically enhance the rotenone toxicity to DA neurons.

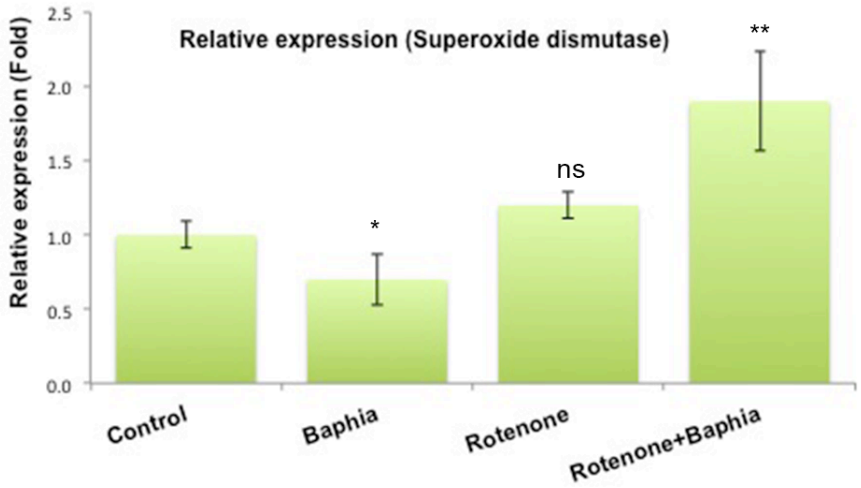

Fig. 3: qRT-PCR relative gene expression of superoxide (Sod). Results are expressed as mean ±SEM. The analysis was done from 70 heads (per group) in each experiment for three independent experiments and quantification was done in duplicate. ^{ns} not significant ($p>0.05$) with respect to control group, *significant difference ($p<0.05$) with respect to *Baphia*, **significance different ($p<0.01$) between control group and *Baphia* and rotenone group; One-way ANOVA followed by Tukey's pairwise comparisons test.

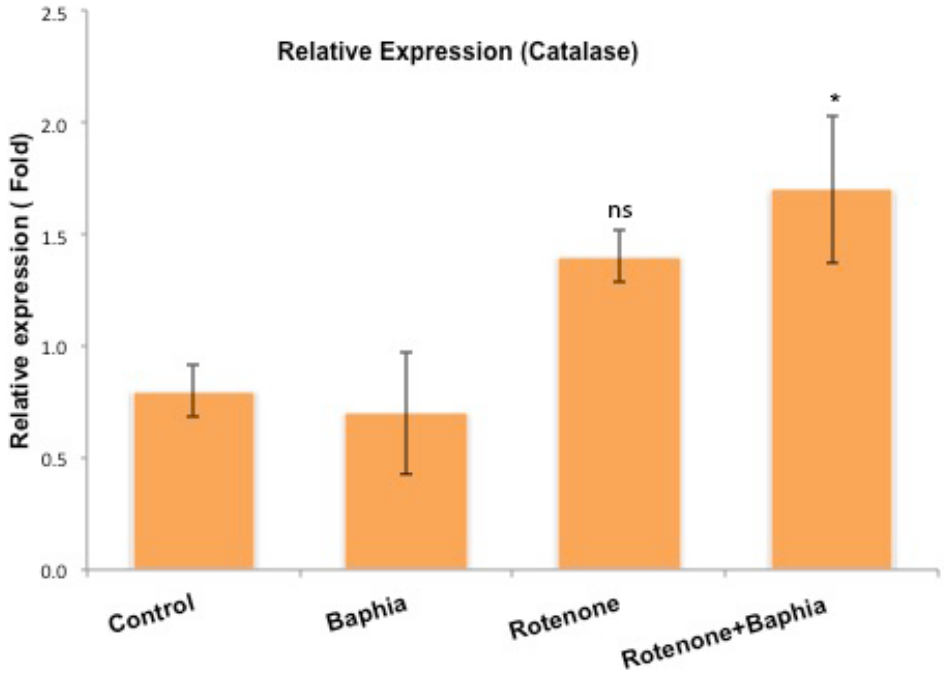

Fig. 4: qRT-PCR relative gene expression of Catalase (Cat Results are expressed as mean ±SEM. The analysis was done from 70 heads (per group) in each experiment for three independent experiments and quantification was done in duplicate. ^{ns} not significant ($p>0.05$) with respect to control group, *significance different ($p<0.05$) between control group and *Baphia* and rotenone group; One-way ANOVA followed by Tukey's pairwise comparisons test

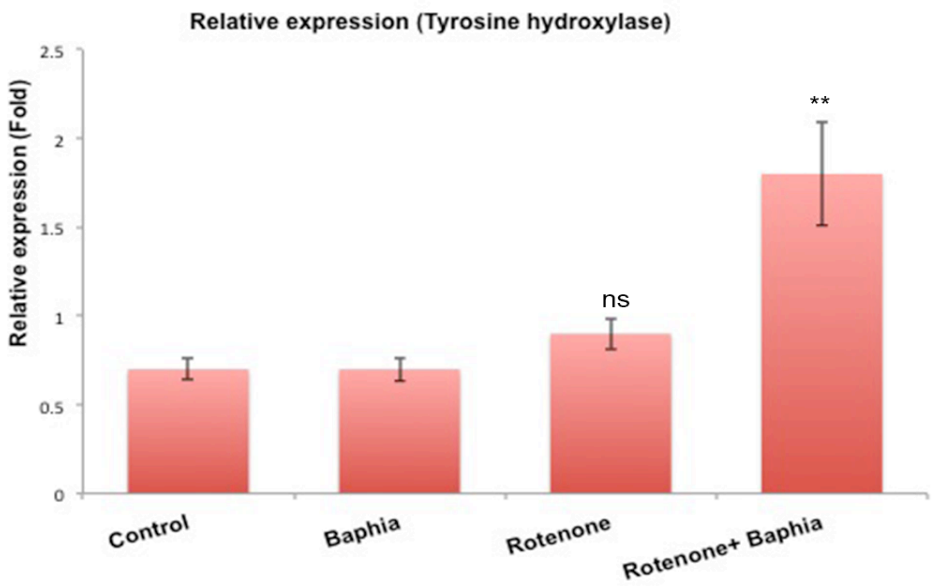

Fig. 5: qRT-PCR relative gene expression of Tyrosine hydroxylase (Th). Results are expressed as mean ±SEM. The analysis was done from 70 heads (per group) in each experiment for three independent experiments and quantification was done in duplicate. ^{ns} not significant ($p>0.05$) with respect to control group, **significance different ($p<0.01$) between control group and *Baphia* and rotenone group; One-way ANOVA followed by Tukey's pairwise comparisons test

MEBM has no potential of reducing oxidative stress induced by rotenone in the brain of PD model flies: lipid peroxidation assay

Lipid peroxidation is one of the forms that have been used to measure the level of oxidative stress in cell metabolism (Olanow, 1992). In this study lipid peroxidation was done as described previously by (Siddique et al 2012, 2014). Figure. 6 shows the standard curve for Malondialdehyde (MDA) estimation. Figure. 7 shows the mean absorbance values. One-way ANOVA followed by Tukey's pairwise comparisons showed that flies treated with rotenone alone and those that were treated with rotenone and MEBM had significant increase in OD values compared to control flies $p<0.01$. However, there was no significant difference between flies that were exposed to MEBM alone and control flies. Concomitantly, this result shows that rotenone and MEBM synergistically enhance toxicity to DA neurons.

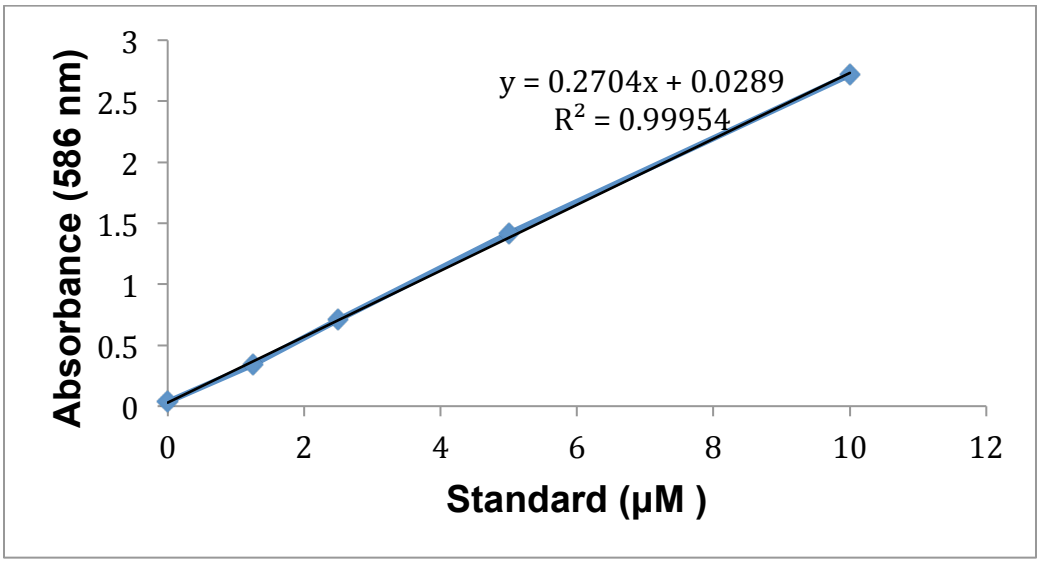

Fig. 6: MDA standard graph for the evaluation of lipid peroxidation in the brains of rotenone *Drosophila* model of PD.

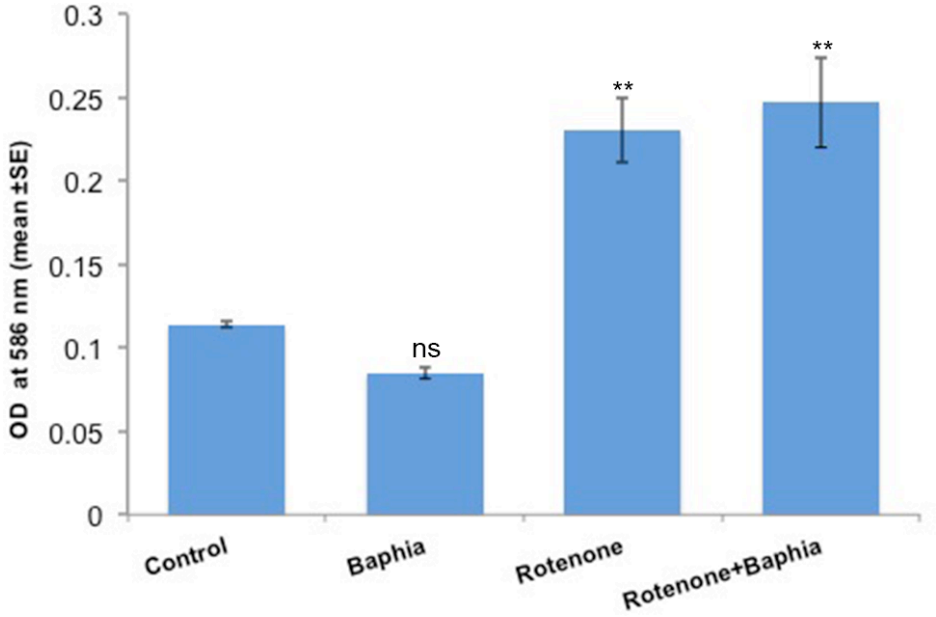

Fig. 7: Effect of MEBM on lipid peroxidation quantified in the brains of rotenone *Drosophila* model of PD. Ten (10) brains (per group) in each experiment for three independent experiments. **Significance different ($p<0.01$) between control group and rotenone and rotenone and *Baphia,* respectively. ns not significant ($p>0.05$) between control group and *Baphia* alone. One-way ANOVA followed by Tukey's pairwise comparisons test

Conclusion

Collectively, this study shows that MEBM has a potential to rescue the functional impairments of dopaminergic cells probably through neurotransmission mechanisms. On other hand at the molecular and biochemical levels MEBM enhanced the toxicity effect induced by rotenone. Thus, in order to be certain with

the protective role of MEBM against DA neurons extraction of pure products is recommended.

Purchases

The supported grant was used to cover the costs of chemicals, reagents, laboratory consumables and rearing of the *Drosophila.*

Acknowledgement

I would like to express my sincere thank to Prof. Caroline Rae (the former Chairperson) and the Committee for Aid and Education in Neurochemistry (CAEN) for grant given to me and also for the extension period due to medical reasons. The grant given did not only support the current study but also the remaining reagents and consumables assist 3 undergraduates students and partly one M.Sc. student to whom I am a supervisor. The work is in the preparation for publication and the ISN will be acknowledged.

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