

The Effect of *Imperata cylindrica* (L.) P. Beauv. on Myelin and Nissl Substance of *Drosophila melanogaster* transgenic Flies Overexpressing the Paralytic Gene.

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ABSTRACT

Brain anatomical changes, muscular activity as well as changes in learning and memory function that could arise in these flies from use of the herbas an anti-epileptic molecule during acute and chronic control of seizures were the objectives of our study. This indicates that the extract has neuroprotective compounds that ameliorated the neuropathological features in the transgenic flies overexpressing the paralytic gene. There was only relief of symptoms in acute exposure treatments unlike in chronic exposure treatments where the extract and sodium valproate cured the brain cellular biological defects leading to associated improvement in cognitive and electrophysiological properties.

Keywords: Imperata cylindrical, myelin, nissl substance, transgenic flies, paralytic gene

INTRODUCTION

Epilepsy is a neurological disorder related to seizures that have a habit of recurrence without an obvious trigger or cause. The seizures come as a result of simultaneous, excessive firing of nerve signals within the brain. Thus the syndrome is very difficult to treat yet endangering patients and/or indirectly impacting relatives as well [1]. Ion channel disorders (channelopathies) are increasingly observed for causing most types of epilepsy. Channelopathies due to Na⁺ channels are the most common form of channel disorders leading to epilepsy; the example here is the Dravet syndrome [1]. These forms of human epilepsy occur on a large scale and if not managed effectively, the patient suffers the complications but also family members of the patient are faced

with psychosocial, financial and other problems. The freedom to choose a given anticonvulsant is highly restricted by the severe side-effects and due to unresponsiveness of up to 30% of epileptic sufferers to current antiepileptic drugs [2]. One way in which epilepsy arises from channelopathies is by mutation(s) in genes encoding ion channels [3]. One example for such a genetic form of epilepsy is due to Na⁺ channelopathy where genes that code for sodium channel proteins are mutated. The abnormal sodium channels that result tend to open for a prolonged time making neurons hyper-excitabile due to increased concentration of excitation Na⁺ inside the neuronal cell membrane [4].

MATERIALS AND METHODS

Study Design

The study design had several groups of *D. melanogaster* transgenic flies overexpressing the paralytic gene; these were tested in line with each specific objective. Each objective had three repeated independent experiments and each experiment contained three replicas. A maximum of 6 flies were used in each replica; this was done to

ensure quality and reproducibility in results.

Study Location

The study was conducted in the Institute of Biomedical Research (IBR) of KIU- WC; Uganda in Ishaka Municipality, Bushenyi District 350 Km from Kampala, Uganda. Histological techniques were performed at the Central diagnostic Laboratory at the College of Veterinary

Medicine Animal Resources and Bio-security, in the Joint National Animal Disease Center (J-NADIC), Makerere University, Kampala, Uganda.

Study Population

The study used laboratory-crossed *Drosophila melanogaster* flies. These were adults (5-23-days old) *D. melanogaster* bang-sensitive (bss) transgenic flies overexpressing the paralytic gene (GMR-GAL4>UAS-para). Experiments were performed on male flies only to ensure homogeneity of results. Two wild-type fly strains (GMR-GAL4 and UAS-para) were used in the study as control fly strains; these same strains were crossed in the laboratory to obtain the bss transgenic flies overexpressing the paralytic gene (GMR-GAL4>UAS-para) in accordance with a method by [5].

Sampling Techniques

Each objective included single (acute) exposure and prolonged (chronic) exposure experiments. For any test that was performed there were three replicas (6 flies in each replica) which were randomly selected from a given treatment group; each test was repeated 3 times with different groups (three independent tests). All these aspects ensured quality and reproducibility in the results.

Method of Arriving at the Sample Size

To be able to perform a robust statistical analysis, large sample sizes are used [6]. Working with *D. Melanogaster* allows to obtain fairly large sample sizes without incurring big expenses [7; 8]. The usual number of *Drosophila* flies used in each replica in experiments of exposure to drugs or medicinal herbs is 5-10 and the number of replicas per experiment being 3-5 [9]. Therefore, in order to obtain statistically robust results as stated above, 3 replicas each containing 6 flies was exposed to *I. cylindrica* in each of the 3 independent experiments in each condition for each test performed. The test involved 3 groups of flies (negative control- no extract, positive control-sodium valproate and the test-extract). Thus the total number of flies used was $(3 \times 6 \times 3 \times 3) = 64$ per experiments. With a total number of experiments of 6 (3 acute; 1hr, 2hr, 3hr and 3 chronic exposure; day 6, day 12, day 18) for

muscular activity tests or cognitive analysis (learning and memory); thus the final number of flies used for each strain of flies was $64 \times 6 = 384$. Three strains of flies were used in the study (GMR-GAL4, UAS-para and GMR-GAL4>UAS-para); thus the total number of flies used for the three strains of flies (2 wild-types and 1 transgenic strains) was $384 \times 3 = 2916$. For brain anatomical analysis 5 flies were randomly selected for dissection and histomorphological analysis out of the flies in each treatment group.

Inclusion Criteria

The study involved use of male adult bss transgenic flies overexpressing the paralytic gene (GMR-GAL4>UAS-para) flies whose ages ranged from 5-23-days old. Male flies were recruited because they are always homozygous for the para gene responsible for seizures in *Drosophila*; female flies on the other hand can either be homozygous (infected) or heterozygous (carriers) for the paralytic (para) gene.

Exclusion Criteria

The study excluded female flies. This was done to ensure that only flies which were homozygous (males) for the para gene were included in the study and those which might have been carriers for the gene (females) were eliminated; this ensured uniformity in the results since a single sex of flies was used. The study excluded fly larvae or flies that were too young or too old since these would interfere with cognitive abilities; larvae as well as flies younger than 5 days or older than 23 days were eliminated in the study.

Collection of *Imperata cylindrica* Samples

The plant materials were harvested from the banks of Rwizi River located in Mbarara Municipality, Mbarara district, Western Uganda. This area is located 295 Km by road from Kampala. A sample of the plant, roots and leaves were placed in a dry moisturized container and transported by road to the department of botany, Mbarara University of Science and Technology for botanical identification by Dr. Olet Eunice (Botanist). The plant was identified as *Imperata cylindrica* (L.) P. Beauv., Collection number: Fred Kalanzi #001. The plant roots from

which the extract was obtained were stored in a fridge at Kampala International University, Western Campus in the Institute of Biomedical Research Laboratory for preservation.

Processing of *Imperata cylindrica*

Samples

The roots of the plant were appropriately washed with tap water and then cleaned in distilled water; the rinsed roots were dried up in an oven at a temperature of 40 °C for 24 hours. For each dry sample of the plant roots grinding was done using a metallic mortar and pestle to obtain fine powders.

Preparation of the Methanolic Extract of *Imperata cylindrica*

The methanolic extract of each plant sample was prepared by soaking 10g of powdered sample in 100ml of 70% methanol in an air-tight conical flask. The flask(s) were put on an electric shaker overnight to allow for complete mixing of methanol and powder after which filtration was done using whattman filter papers. The filtrates were concentrated using a rotary evaporator with temperature regulated at 53°C to evaporate off methanol. The remaining dry concentrated extracts were weighed and stored in an air-tight sample bottle wrapped in aluminium foils and refrigerated at 5°C for use when required. When the extract was needed, a given amount of the dry concentrated extract was weighed and reconstituted using distilled water in concentrations of 0.8g/ml and 0.025g/ml for single and prolonged exposure experiments respectively. The remaining powder, that was not soaked in methanol was weighed, stored in a dry place and soaked whenever required [10].

Fly Stock

The control fly stocks used in this study included two wild-type *D. melanogaster* flies (GMR-GAL4 and UAS-para). In UAS-para (Upstream Activating Sequences-para) flies the para gene responsible for the bss phenotype is kept silenced and it is only triggered when crossed with an activator gene kept in GMR-GAL4 (glass multiple reporter-GAL4) system; therefore UAS-para flies are regarded as 'silencer' and GMR-GAL4 are regarded as 'activator' wild-type flies [5]. To

obtain transgenic bss fly strains overexpressing the para gene for use in the study, female virgin UAS-para flies were crossed with male GMR-GAL4 flies in a method similar to that used by [5]. The larvae obtained from the cross were isolated from the adult flies to ensure that progeny is not mixed with the parents. Then these larvae were raised to obtain first generation adult GMR-GAL4>UAS-para flies. The resultant F1 progeny were sexed to isolate the males from the females; the male flies were kept as the bss transgenic flies overexpressing the para gene. These transgenic flies were kept as the test stock flies for the study.

Fly Handling

Only male flies of the transgenic bss *D. melanogaster* flies overexpressing the para gene were used in this study. Separate tests were performed on GMR-GAL4>UAS-para flies to which a 'single exposure' to the extract was done and to GMR-GAL4>UAS-para flies to which 'prolonged exposure' to the extract was done. Two wild-type flies (GMR-GAL4 and UAS-para) were used in the study as control flies; these same wild-type flies were crossed by us in the laboratory to obtain the transgenic flies used in the study. The wild-type fly stocks were provided by Bloomington *Drosophila* Stock Center (BDSC), Indiana in U.S.A with help from IBR, KIU-WC. Once obtained from BDSC, the fly stocks were housed in *Drosophila* Stock Center in IBR Laboratory at KIU-WC for amplification of new stock and for performing crosses. The *D. melanogaster* flies were raised on a standard cornmeal at room temperature (25°C) with natural day/night light cycle. After crossing of the two adult wild-type strains to obtain transgenic larvae, the adult crossed flies (parents) were separated from the larvae to allow larvae to grow and hatch separately into progeny. In order to amplify these transgenic flies, we had to perform further similar crosses between the two wild-type fly strains to raise more F1 progeny as our tests were performed in the F1 progeny (the transgenic flies). This process was continued until the necessary number of transgenic flies for a given experiment was achieved. In the separation of flies

into vials during particular experiments, the flies would be anaesthetized using ethyl-ether and the flies were allowed to rest in the vials for 30 minutes to prevent the effect of the anesthetizing agent on refractory period. Then the resultant F1 transgenic male progeny (GMRGAL4>UAS-para) were separated from females through the process of 'sexing' under a light microscope. The males were kept as the test transgenic stock flies for use in the study [5].

Preparation of Fly Food Containing *Imperata cylindrica* Extract

A measured concentration of the extract or standard anti-epileptic drug was dropped in liquidified food at a temperature of 40°C; homogenous distribution of the extract was enabled by transferring the extract into food in a vial or petri-dish with a pipette and then stirring with the pipette for thorough mixing. Solvent food colour was added and mixed with the food-extract mixture; the solvent food colour was used to check if the extract had distributed evenly. The solvent food colour was also used to see if flies had eaten the food containing the extract. Solvent food colours with dissimilar colours were assigned to variable concentrations of the extract and standard anti-epileptic drug in the food to avoid errors [11].

Feeding Flies on Food Containing *Imperata cylindrica* Extract and the Standard Antiepileptic Drug

For acute (single) exposure studies, the flies were starved for 15 hours to induce appetite; the flies were then fed on food containing 0.0, 0.8g/ml of the extract and 0.3mg/ml of the standard anti-epileptic drug (sodium valproate) prepared in a petri-dish; a filter paper was dipped in the mixture of food and the extract or standard anti-epileptic drug. The filter paper was lowered inside a vial containing the starving flies and left in place for 1, 2 and 3 hours for the flies to feed. The outcomes were tested in line with the objectives. For prolonged exposure studies, the flies were continuously fed on food containing 0.0g/ml, 0.025g/ml of the extract and 0.15mg/ml of standard anti-epileptic drug (sodium valproate) prepared in vials. The flies were fed for

6, 12 and 18 days before the outcomes were tested in line with the objectives [1].

Solvent Food Colour Exclusion Test

To ensure that the flies had taken the extract mixed with the food, solvent food colour with dissimilar colours were mixed with food and each colour was corresponding to a particular concentration of the extractor standard anti-epileptic drug in the food. To confirm whether the flies had eaten the food with the extractor the standard anti-epileptic drug, the flies in each group were transferred from a vial, anaesthetised with ethyl-ether and then their abdomens were observed under a light microscope for colour in line with each specific colour of the solvent food colour. If a give colour corresponding to that of the food colour in the food or filter paper was seen in the abdomen of a fly, it meant that that fly had eaten the food with the extract or standard anti-epileptic drug. Absence of a colour of interest meant that the flies had not eaten the food with the extract or standard anti-epileptic drug. Only flies that had eaten food with the extract (positive for solvent food colour test) were included in the study [11].

Determination of Seizure Characteristics for F1GMR-GAL4>UAS-para Transgenic Flies Used in the Study

In UAS-para *D. melanogaster* flies, the para gene is kept silenced and it is only overexpressed or over-activated when crossed with an activator gene kept in GMR-GAL4 fly system; therefore UAS-para flies are regarded as 'silencers' and GMR-GAL4 are regarded as 'activators' [5]. In determination of seizures characteristics undergone by the transgenic flies for use in the study, wild-type female virgin UAS-paraflies were crossed with wild-type male GMR-GAL4 flies. The larvae obtained were isolated from adult flies to ensure that progeny is not mixed with parents. Then the resultant progeny (GMR-GAL4>UAS-para) were sexed to isolate the males from females and the males were kept as stock for the study. To determine if the transgenic flies raised through crossing reproduced the phenotype of the established epileptic *D. melanogaster* model for seizures

(parabss) and to determine the extent of the neuropathology, the stocked flies were tested for convulsions. Here 50 independent transgenic flies were selected from the stock. Each fly was transferred into a cylindrical testing vial by slight anaesthetization using ether. The fly in the testing vial was then allowed to rest for 30 minutes to minimise the effect of anaesthetising agent on the refractory period. Then mechanical stimulation was subjected by vortexing each fly at high speed for 10 seconds. Each fly underwent seizure phases including shaking of body, paralysis (fly lying on its back), rapid tensing and relaxation of body and then the fly recovered. Convulsion time (CT) was taken as the time taken by the fly while shaking. Paralysis is the second phase after a seizure; paralysis time (PT) was taken as the time between seizure and recovery. Recovery was taken as standing and normal mobility or flight of flies; mean recovery time (MRT) was taken as the average time it takes any individual fly that exhibits bang-sensitive behaviour to recover. All experiments were performed at room temperature (22- 25oC) unless stated otherwise. Then mean, median, SD for CT, PT and MRT were calculated. Frequency graphs were plotted to determine if the seizure properties of the flies are normally distributed to represent the whole population [12].

Determination of Activity of *Imperata cylindrica* Extract

The activity of the plant was determined by using the working concentrations that were established by Oginga, 2016, and the change in CT, PT and MRT before and after exposure to different concentrations of the extract was noted. To do this, 50 independent GMR-GAL4>UAS-paraflyies were exposed to relatively safe doses of the plant extract as established in Oginga's study. In acute exposure treatments the safe dose range was established by Oginga as being below 1.0g/ml; therefore doses of 0.0, 0.6, 0.8 and 1.0g/ml of the extract were used to determine the effect of single exposure (2hr-exposure) on CT, PT and MRT. In prolonged exposure treatments (12-day exposure) the safe dose range was established by Oginga,

2016 as being below 0.1g/ml; therefore we used doses of 0.0, 0.0125, 0.025 and 0.05 g/ml to determine the effect of prolonged exposure on CT, PT and MRT. All experiments were performed at room temperature (22- 25oC) unless stated otherwise [9].

Data Analysis and Statistical Measures

Photomicrographs taken with the camera attached to the microscope were analysed and myelin was shown by a blue colour and nissl substance was shown by magenta (violet) colour with Klüver LFB stain. Weak LFB stain (light blue patchy areas) indicated abnormalities in myelin sheath (demyelination) of nerve tracts; weak cresyl echt violet stain (light violet) indicated abnormalities in nissl substance [13]. Data was presented in form micrographs. Brain sections with particular defects in general brain morphology, myelin sheath and nissl substance were analysed using 'Microsoft Excel Windows 2010' and graphically represented as stacked column bar graphs to compare the percentage that each defect contributes to a total across categories.

Proper Handling of Flies

The fly strains and reagents used in the study were acquired by using the stipulated proper channels to get them (ordered from Bloomington Drosophila Stock Center) and ethics were followed to ensure that the flies were handled well and not intentionally injured or harmed. All procedures and techniques used in this study were in accordance with the care and use of laboratory animals from the Uganda National Council of Research and approval was sought from the Postgraduate Ethics and Research Committee (PGERC) and Institutional Review and Ethics Committee (IREC) of KIU-WC. Less invasive techniques were employed to avoid causing pain on the flies; inflicting pain to live flies was minimized by sacrificing the flies under ether anaesthesia. Flies were provided with necessary requirements for their survival such as food, water and favorable environment.

RESULTS

Myelin and Nissl Substance

In acute (single) exposure treatments, flies that do not overexpress paralytic gene and those that overexpress paralytic gene (GMR-GAL4>UAS-para) had no significant lesion in myelin sheath of nerve fibers and no significant

abnormality in nissl substance after 1, 2 and 3 hours of ingesting a standard fly food with no extract (negative control dose). Thus overexpression of paralytic gene showed no significant lesion in myelin sheath or in nissl substance (NSL; Figures 1 and 2).

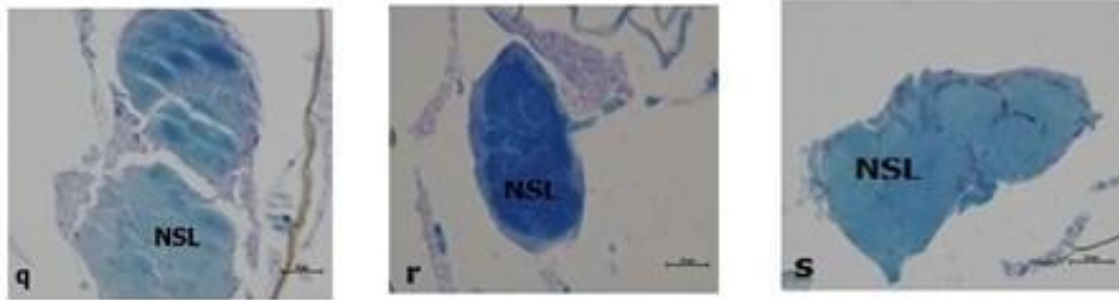


Figure 1. Brain sections showing defects in myelin sheath (A) and in nissl substance (B) of flies at 0.0g/ml of *Imperata cylindrica* extract.

This data was from five *Drosophila melanogaster* flies in each treatment group; a total of 15 sections were analysed from each group; NSL- no significant lesion; MD- mild demyelination.



Figure 2. Klüver LFB micrographs (TS) from brain of GMR-GAL4 (q), UAS-para (r) and GMR-GAL4>UAS-para (s) flies treated with 0.0g/ml of extract.

This data was from five *Drosophila melanogaster* flies in each treatment group. Three brain sections were obtained from each of those flies. Myelin was shown by a blue colour and nissl substance was shown by magenta (violet) colour with Klüver LFB stain. (q), (r) and (s) show good, normal intensity stain with no significant lesion in myelin sheath of nerve fibers and no significant abnormality in nissl substance (NSL) in the brains of GMR-GAL4, UAS-para and GMR-GAL4>UAS-para flies respectively, x40; findings are similar to that after 1,

2 or 3 hours of feeding the flies with the standard fly food with no extract. In acute (single) exposure treatments, subjecting GMR-GAL4 flies to 0.8g/ml of extract for 1, 2 and 3 hours caused none significant lesion in myelin sheath and in nissl substance. In acute (single) exposure treatments, after exposing GMR-GAL4 flies to 0.3mg/ml of the standard antiepileptic drug (sodium valproate) for 1, 2 and 3 hours we observed no significant lesion in myelin sheath and in nissl substance.

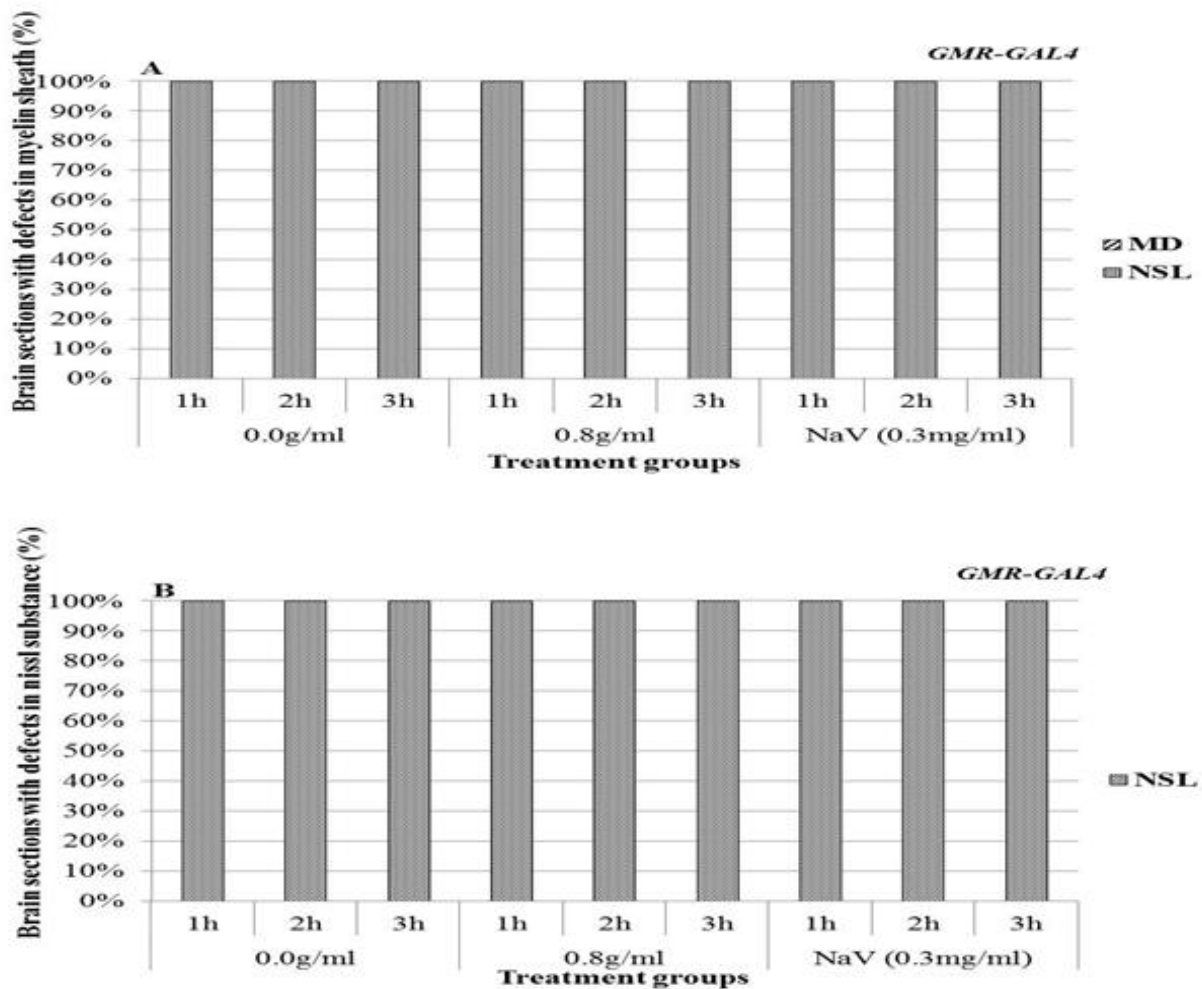


Figure 3. Brain sections showing defects in myelin sheath (A) and in nissl substance (B) of GMR-GAL4 flies in various treatment groups.

This data was from five *Drosophila melanogaster* flies in each treatment group; a total of 15 sections were analysed from each group; NSL- no significant lesion; MD- mild demyelination. In acute (single) exposure treatments, subjecting UAS-para flies to 0.8g/ml of extract for 1, 2 and 3 hours caused none significant lesion in myelin sheath and in nissl substance. However there was one exception to this general observation which appeared in UAS-para flies; we

noted a low myelin density and weak Luxol Fast Blue stain (demyelination) but with no abnormality in nissl substance after 2 hours of exposing these flies to 0.8g/ml of the extract. In acute (single) exposure treatments, after exposing UAS-para flies to 0.3mg/ml of the standard anti-epileptic drug (sodium valproate) for 1, 2 and 3 hours we observed no significant lesion in myelin sheath and in nissl substance.

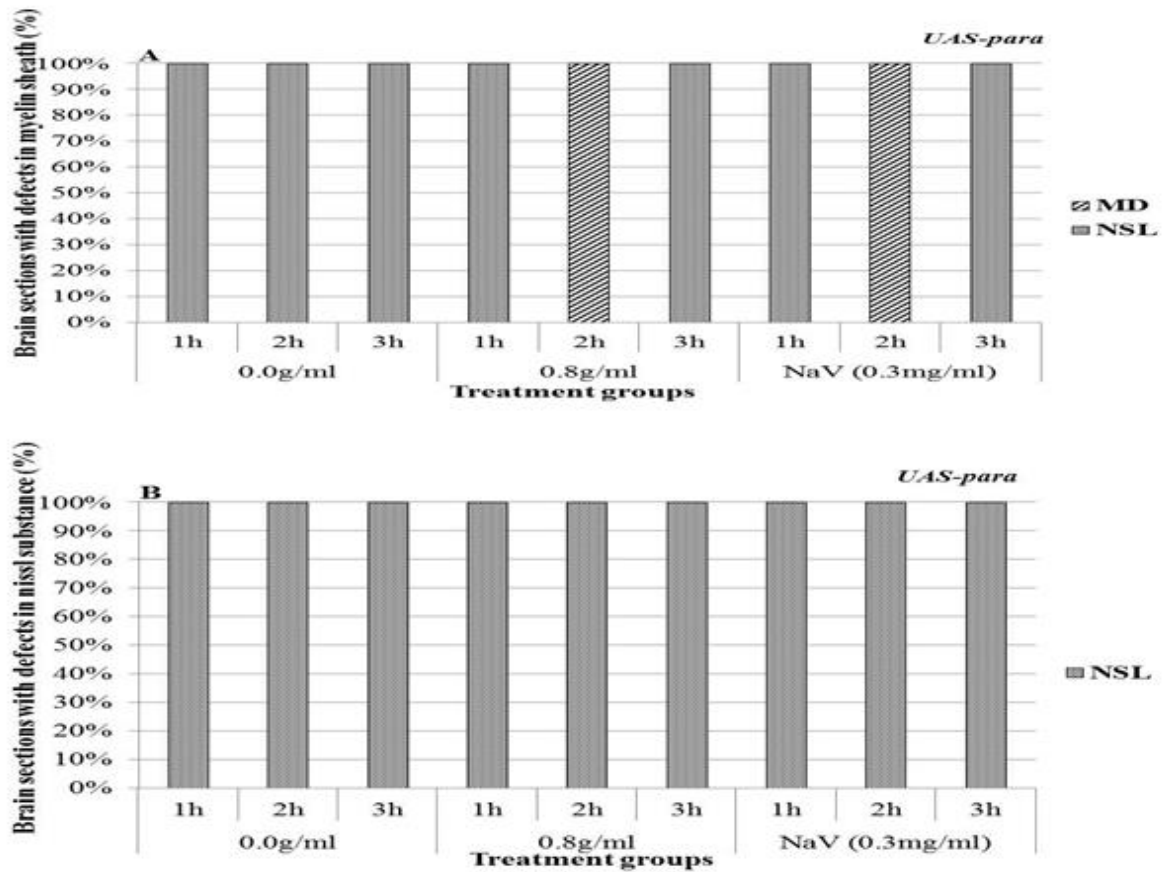


Figure 4. Brain sections showing defects in myelin sheath (A) and in nissl substance (B) of UAS-para flies in various treatment groups.

This data was from five *Drosophila melanogaster* flies in each treatment group; a total of 15 sections were analysed from each group; NSL- no significant lesion; MD- mild demyelination. In acute exposure treatments, subjecting flies that overexpress paralytic gene GMR-GAL4>UAS-para) to 0.8g/ml of the extract for 1, 2 and 3 hours caused no significant lesion in myelin sheath and in nissl substance. In acute (single) exposure

treatments, after exposing flies that overexpress paralytic gene to 0.3mg/ml of the standard anti-epileptic drug (sodium valproate) for 1 or 2 hours we observed no significant lesion in myelin sheath neurons and in nissl substance. But after 3 hours of the exposure, a low myelin density and weak Luxol Fast Blue stain (mild demyelination; MD) was observed but with no abnormality in brain parenchyma of nissl substance).

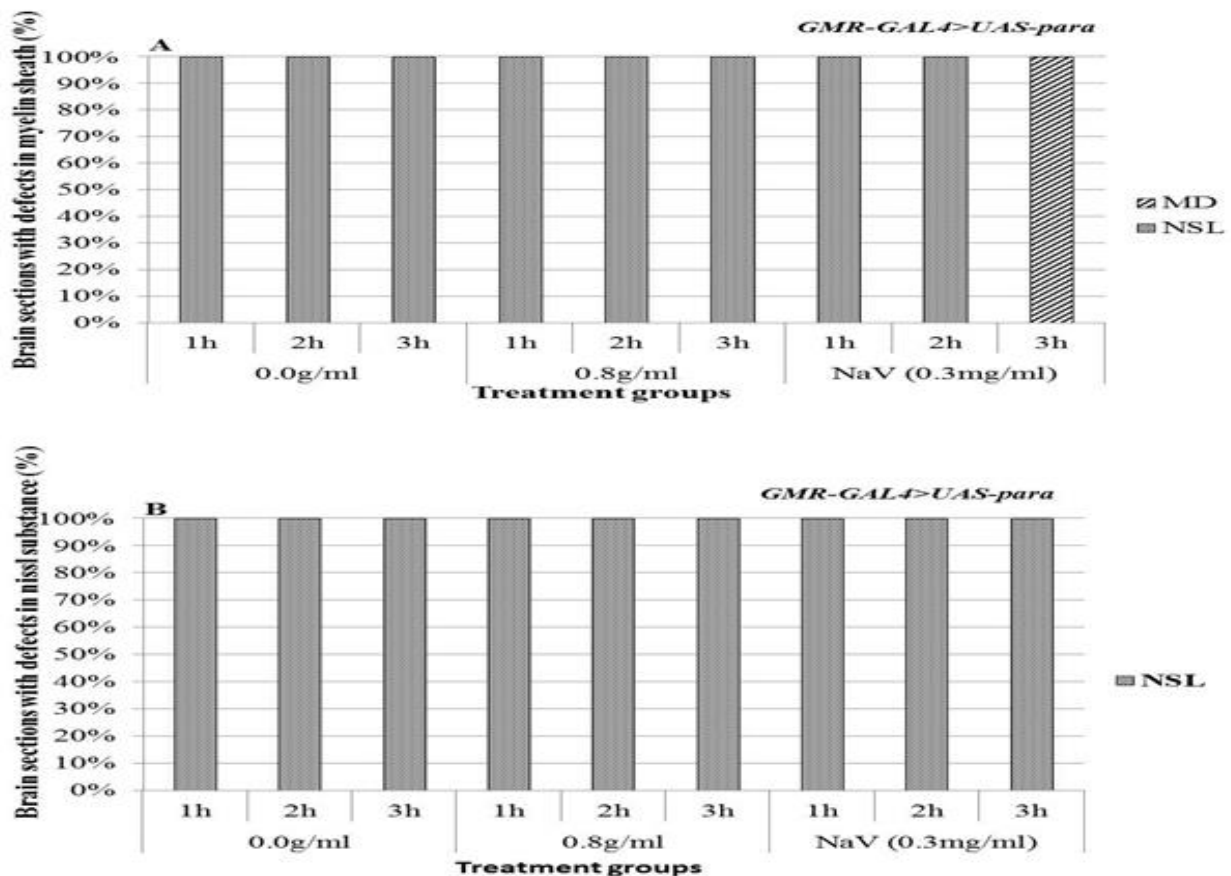


Figure 5. Brain sections showing defects in myelin sheath (A) and in nissl substance (B) of *GMR-GAL4>UAS-para* flies in various treatment group .

This data was from five *Drosophila melanogaster* flies in each treatment group; a total of 15 sections were

analysed from each group; NSL- no significant lesion; MD- mild demyelination.

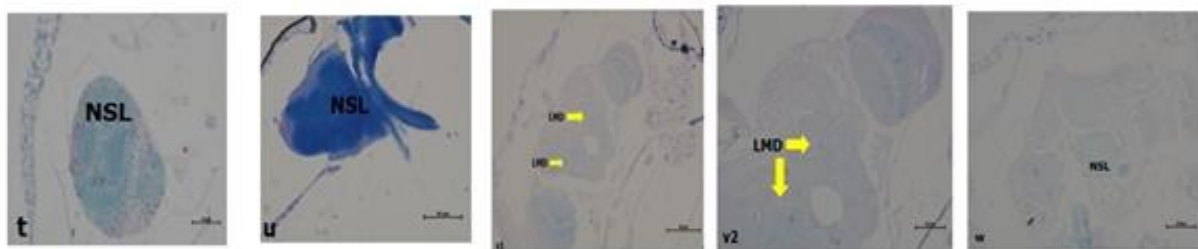


Figure 6. Klüver LFB micrographs (TS) from brain of *GMR-GAL4* (t), *UAS-para* (u, v1 and v2) and *GMR-GAL4>UAS-para* (w) flies that were treated with 0.8g/ml of the extract.

This data was from five *Drosophila melanogaster* flies in each treatment group. Five brain sections were obtained from each of those flies. Myelin was shown by a blue colour and nissl

substance was shown by magenta (violet) colour with Klüver LFB stain. Yellow arrows display various focal points of weak LFB stain (t), (u) and (w) show good, normal intensity stain with

no significant lesion in myelin sheath of nerve fibers and no significant abnormality in nissl substance (NSL) in the brain of GMR-GAL4, UAS-para and GMR-GAL4>UAS-para flies respectively, x40; findings were similar to that after 1, 2 or 3 hours of feeding the flies on 0.8g/ml of the extract, except in UAS-para flies where we noted a low myelin density (LMD) and weak LFB stain

(demyelination) but with normal brain parenchyma or normal nissl substance (NN) shown in (v1), x20 after 2 hours of exposure to 0.8g/ml of the extract. (v2) is a higher magnification showing low myelin density (LMD) and weak LFB stain (demyelination) but with normal brain parenchyma or normal nissl substance (NN) of UAS-para flies, x40 after 2 hours of exposure to 0.8g/ml of the extract.

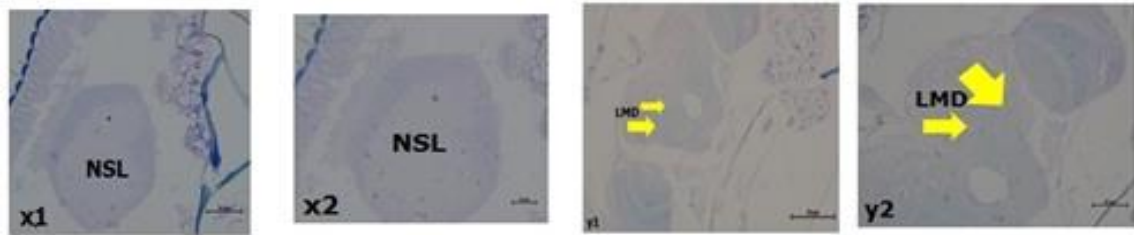


Figure 7. Klüver LFB micrographs (TS) from brain of GMR-GAL4>UAS-para flies that were treated with 0.3mg/ml of sodium valproate

This data was from five *Drosophila melanogaster* flies in each treatment group. Five brain sections were obtained from each of those flies. Myelin was shown by a blue colour and nissl substance was shown by magenta (violet) colour with Klüver LFB stain. Yellow arrows display various focal points of weak LFB stain. (x1) shows good, normal intensity stain with no significant lesion in myelin sheath of nerve fibers and no significant abnormality in nissl substance (NSL) in the brain of GMR-GAL4>UAS-para flies, x40 after 1 or 2 hours of ingestion with the standard anti-epileptic drug; findings were similar after 1 or 2 hours of feeding the flies on 0.3mg/ml of standard anti-epileptic drug. (x2) shows a higher magnification with good,

normal intensity stain having no significant lesion in myelin sheath of nerve fibers and no significant abnormality in nissl substance (NSL) in the brain of GMR-GAL4>UAS-para, x60 after 1 or 2 hours of ingesting the standard anti-epileptic drug. (y1) shows no significant lesion in parenchymal appearance (NN) but a low intensity LFB stain (low myelin density; LMD), x20 after 3 hours of feeding the flies with 0.3mg/ml of the standard anti-epileptic drug. (y2) shows a higher magnification with no significant lesion in parenchymal appearance (NN) but a low intensity LFB stain (low myelin density; LMD), x40 after 3 hours of feeding the flies with 0.3mg/ml of the standard anti-epileptic drug.

DISCUSSION

Myelin and Nissl Substance

In acute exposure treatments, the flies that do not overexpress the para gene and those that overexpress the para gene had no significant lesion in both myelin sheath of nerve fibers and nissl substance before giving the herb. In prolonged exposure treatments, before giving any drugs flies that do not overexpress the para gene showed no

significant lesion in myelin sheath and nissl substance but we observed mild demyelination in the flies overexpressing the para gene. Thus overexpression of the para gene led to mild demyelination in nerve fibers after several days that was aggravated with time. The observation of normal myelin sheath and nissl substance in neurons of wild-type flies concurs with [14]

where their study revealed no abnormality in myelin sheath and nissl substance in the brain tissue of wild-type *D. melanogaster* flies with immunohistochemistry of brain tissue. The observation in the transgenic flies where we saw no abnormality in myelin and nissl substance in the younger flies (5-days old; acute phases) and demyelination in older flies (11-23 days old; chronic phases) suggests a progressive demyelinating disorder aggravated with time and age of due to overexpression of para gene as seen in parabss flies [15]. The progressive demyelination of nerve fibers seen in the current study could propose that there is reduction in nerve tracts and myelin density and therefore interferes with conduction of impulses [16; 17] as well as impacting on electrical insulation of neurons. This interference in physiological functioning of the neurons might be the cause of the progressive defects in muscular and cognitive activity seen in our transgenic models of epilepsy.

In general, treatment of the flies that do not overexpress the para gene with therapeutic doses of

I. cylindrica and sodium valproate in both single and prolonged exposure treatments were not toxic in myelin sheath and nissl due to intact homeostatic control mechanisms in these flies [18] or due normal cellular properties in brain cells of these flies [19]. However, there was one exception to this general observation where we observed demyelination in brain tissue of UAS-para flies after 2 hours of exposing these flies to 0.8g/ml of the extract and to 0.3mg/ml of sodium valproate. The cause for this toxicity needs further investigation before we can recommend the herb as a reliable antiepileptic molecule. Similarly, treatment of the transgenic flies overexpressing para gene with 0.8g/ml of the extract and 0.3mg/ml of sodium valproate (acute exposure treatments) was not toxic on myelin sheath and nissl substance except in one case

CONCLUSION

Overexpression of paralytic gene leads to progressive neurodegeneration and demyelination being aggravated with time; *Imperata cylindrica* and sodium

where 0.3mg/ml of sodium valproate caused demyelination after 3 hours of treatment. This toxicity in myelin sheath seen in transgenic flies treated with sodium valproate for 3 hours is similar to that observed in UAS-para treated with the extract for 2 hours and could be suggestive of presence of toxic compound (s) in some antiepileptic molecules [20]. These speculations require further investigations before we recommend use of the extract as an anti-epileptic molecule. Prolonged (chronic) treatment of the transgenic flies with 0.025g/ml of the extract and 0.15mg/ml of sodium valproate was able to improve the progressive demyelination present in these flies. This is an indicator of neuroprotective (curative or myelin regeneration) potential of these two anti-epileptic molecules a finding in contradiction with [21] where they found a reduction in myelination and myelin activities in rat brains after treating the epileptic rats with Vigabatrin (antiepileptic agent). However, other researchers determined the neuroprotective effects of antiepileptic drugs as being neuroprotective, neutral or detrimental on the morphology of the CNS [22]. The myelin regenerative potential of the extract and sodium valproate could have led to an increase in number of nerve tracts [23]. This coupled to the increase in myelin sheath might have improved the conduction of impulses in the transgenic flies and as a result modulated the electrophysiological, muscular activity as well as improving the general coordination in the learning and memory pathways located in mushroom bodies [24,25,26]. There could also be a possibility that the myelin regeneration proposed here following prolonged treatment could be one reason for the improvement in life-span and in resting membrane potential observed in muscles as well as the mild negative geotaxis of the mutant fly models for seizures (parabss) observed in previous studies by Oginga in 2016.

valproate ameliorate the progressive histomorphological defects in flies overexpressing paralytic gene and this cellular curative effect is seen in

prolonged exposure treatments where it increases with time. *Drosophila* extract seems to be relieving symptoms in acute exposure treatments and curative by improving brain cell biology which in

Ssempijja *et al* turn ameliorates muscular basal membrane potential, learning and memory defects following prolonged exposure treatments.

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