Anti-Epileptic Effect of *Imperata Cylindrica* on a *Drosophila Melanogaster* Model of Seizures

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Received: September 21, 2017
Published: October 11, 2017

Abstract

**Background:** Epilepsy is one of the most prevalent non-communicable neurologic conditions and an important cause of disability and mortality in the East African region alone. It is estimated to affect almost 70 million people worldwide. The prevalence of epilepsy in low- and middle-income countries is about twice that of high-income countries (Nabukenya et al., 2014).

**The aim of this study:** was to evaluate the anti-epileptic activity and the mechanism of action of *Imperata cylindrica* extract on bang-sensitive *Drosophila melanogaster*.

**Methodology:** This was an experimental study in which toxicity of the extract was measured by increasing concentration, after which the concentration below the lethal dose were tested for antiepileptic activity followed by side effects and finally the mechanism of action.

**Results:** The extract had reduction in seizures by 25-30% at doses that were generally safe for the fly. *Imperata cylindrica* had minimal toxic effect on *Drosophila*; most importantly it had anticonvulsive activity. There was also minimal side effect of the extract to the general health of the flies.

**Discussion and conclusion:** Though the extract showed some toxicity the extract ameliorated symptoms generated by increased activity of sodium and potassium channels. Therefore, these results show that *Imperata cylindrica* has the potential to ameliorate seizure at safe therapeutic doses, it may also provide a solution to patient who do not respond to the current available AEDs. In future studies for precise determination of the origin of both toxicity and activity, independent analysis of the fractionated active compounds is recommended.

**Keywords:** Imperata cylindrica, epilepsy, seizure, channelopathies, Anti-epileptic Drugs, Bang sensitive

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1. INTRODUCTION

Epilepsy is among one of the most devastating disorders that has a negative impact on the quality of life of patients with epilepsy in third world countries like Uganda, this is irrespective on the availability of anti-epileptic drugs (AEDs) (Nabukenya, Matovu, Wabwire-Mangen, Wanyenze, & Makumbi, 2014) and an
important cause of disability and mortality (Kariuki et al., 2014). It affects over 50-60 million people worldwide (Savage, 2014) with the majority living in low and middle income countries where access to medical treatment is limited (Kamgno, Pion, & Boussinesq, 2003). Among the 60 million people affected worldwide, 10 million live in Africa and 80% are not treated with modern medication (Prevett, 2013). Despite global campaign against epilepsy on improving its management in resource-poor countries, its prevalence in sub-Saharan Africa is higher than in other parts of the world (Prevett, 2013). Risk factors include parasitic diseases and poor antenatal and perinatal care (Mung’ala-Odera et al., 2008). Currently, the available anticonvulsants are effective in reducing the severity and number of seizures. However, approximately 30% of patients continue to have seizures in developing countries, as a result of ineffectiveness, unavailability and high cost of AEDs (Prevett, 2013). Furthermore, the available AEDs on the market have different benefits and side effects, usually the prescription depends on type of seizure, frequency of seizures, lifestyle and age of person (Cavanna, Ali, Rickards, & McCorry, 2010).

In developing countries, plants are used by traditional healers for the treatment of epilepsy, because they are more accessible than pharmaceutical drugs (Kamboj, 2000). Considerable reviews and research for new AEDs has been conducted and, evidence from preclinical research has shown positive activity from plant extracts (Borris, 1996; Agosta, 1997). Therefore, plants have been found to be an important source for the development of alternative and complementary treatment for epilepsy, with several plants exhibiting anticonvulsant activity in different animal models (Grone & Baraban, 2015). The fruit fly Drosophila melanogaster is considered the most useful animal genetic model, because of its high fecundity, short life cycle, and low-cost of maintenance, besides research has shown that 40% of human genes have functional homologs in the fly (Pandey & Nichols, 2011).

Imperata cylindrica (Iridaceae), also known as ‘Obiya lele’ by the Luo community in Kenya, is a grassy perennial plant of the family Iridaceae, native to moist, intermediate temperate zones in tropical Africa and equatorial regions including Indonesia, India, and South Eastern States of US, China and most countries in Africa (Yoon, Lee, Sung, & Kim, 2006). The roots of I. cylindrica has traditionally been used in the treatment for venereal diseases, as a diuretic; oral treatments, as a pain-killer, paralysis, epileptic fits, convulsions, spasm and stomach problems (Liu, Zhang, Yang, Chou & Wang, 2014). Previous work by (Mohamed et al., 2009) I. cylindrica has shown the presence of compounds like saponins, lignans, tannin, cardiac glycosides, alkaloids, flavonoids, 2-(2-Phenylethyl) chromones and reducing sugars. 2-(2-Phenylethyl) chromones has significant neuro-protective activity against glutamate-induced neurotoxicity (Yoon et al., 2006), lignans (graminones A and B) with inhibitory activity on the contraction of the rabbit aorta. The water extract of I. cylindrica has also been documented to have anti-inflammatory effects on mice (Liu et al., 2014). The present studies examine the anti-epileptic activity and mechanism of action I. cylindrica, as an herbal option for the treatment of epilepsy, using bang-sensitive D. melanogaster as an alternative potential source of AEDs.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Materials

The plant roots were harvested from the river banks of River Yalla in Kisumu County, Western Kenya placed in dry moisturized container. The Department of botany, Mbarara University of Science and Technology identified the plant as I. cylindrica Var Africa, collection number: Fredrick Otieno 001. The plant roots were stored at 4°C at Kampala International University Western Campus in the Institute of Biomedical Research Laboratory until extraction process started. The roots were washed with tap water and rinsed in distilled water later dried in an oven at 35 – 40°C for 3 days. The dried roots were pulverized using a mortar and pestle, to obtain a powdered mixture, which was then stored in airtight glass containers, protected from sunlight until analysis. Crude extract was prepared as described by (Jayalakshmi, Patra, Lal,
& Ghosh, 2010), by soaking 10g of powdered sample in 100 mL of 70% Methanol for 12 hours, after which the extract was then filtered using filter paper.

### 2.2 Experimental Flies

Pharmacologically modified and mutant (para$^{\text{bss}}$) D. melanogaster were used in the study. The pharmacologically modified flies obtained through starving the wild type flies for 14-16 hour, before single exposure to 0.2 mg/mL of Pentylentetrazole to block sodium channel and sotalol 0.3mg/mL and a beta blocker 0.2mg/mL to produce flies with hyper active and hypoactive potassium channels, respectively (Kamb, Iverson, & Tanouye, 1987). The mutant flies were amplified from the existing stocks in the laboratory by allowing mating of the adult flies and, allowed to hatch for 72 hours maintained on standard cornmeal food at room temperature. The adult flies were then transferred to fresh vials to allow the larvae to grow. This was continued until the necessary number of flies for a given experiment was achieved. The separation of flies into vials during the experiment was done by anaesthetizing flies using ether and allowing them to rest in the vials for 30 minutes, to prevent the effect of the anesthetizing agent on refractory period. All procedures and techniques used in this study were in accordance with the Care and Use of Laboratory Animals of the Uganda National Council for Science and Technology and, approval was sought from the postgraduate Ethics and research committee of Kampala International University Western Campus.

#### 2.2.1 Determination of Anti-Convulsive Effect of Imperata cylindrica

The 3–5 day-old adult para bss flies fed on cornmeal food containing 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% mg/mL of the extract were tested for response to mechanical shock as described by (Reynolds et al., 2004). Replicates Five flies were transferred into testing vial by slight anaesthetization using ether, allowed to rest for 10 minutes to eliminate inconsistencies due to refractory period. Mechanical shock was delivered by vortexing flies at high speed for 10s. The number of flies recovered, the Mean recovery times (MRTs) and the number of flies undergoing paralysis or seizure was recorded at 15s intervals (Reynolds et al., 2004).

#### 2.2.2 Determination of Effect of the Extract on Locomotion of bang sensitive Flies

The functionality of the muscles of the flies was tested using the climbing assay, which tests negative geotaxis and the general health of the flies(Nichols, Becnel, & Pandey, 2012b; Nichols, Becnel, & Pandey, 2012a) on the fourth day. Ten flies were anaesthetized using ether then transferred to cylindrical vials left to rest for 10 minutes to minimize the effect of the anaesthetizing agent on refractory period. Using a cotton wool, all the flies were pushed to the bottom of the vial and once the timer was set, the cotton was released and the number of flies at half the vial in 5 minutes was recorded. For accuracy and reduction of errors, each vial was focused using a USB microscope and images were taken through the 5 minutes, the number of flies halfway to the top of the vial at 40 seconds was counted (Nichols et al., 2012a).

#### 2.2.3 Mechanism of Action of Imperata cylindrica

Pharmacological modified flies were grouped and fed on food containing 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% mg/mL of the extract in the food. Phenotypic behavior and locomotion changes were monitored via climbing assay to ascertain the mechanism of action. The extract was observed for its effects on the phenotype before doing electrophysiological recording.
2.3 Data Analysis and Presentation

Three replicates were performed in each experiment and MRT, mean survival, frequency of wing biting, number of flies, effective dose, convulsion time and paralysis time were recorded. The data was recorded in tables and then plotted in graphs and exposed to descriptive statistics for general analysis and detection of outliers.

3. DISCUSSION

Drosophila has long been used for genetic, biochemistry and developmental biology (Hales, Korey, Larracuente, & Roberts, 2015). It has shown great potential for drug screening and toxicity studies (Abolaji, Kamdem, Farombi, & Rocha, 2013). The purpose of toxicity studies is to obtain information on the safety of chemical compounds. Flies exposed to concentrations above 1.5g/ml showed increased lethality and the LD50 was found to be 1.2g/ml. The doses below the LD50 did not induce tissue damage but a dose of 1g/ml also increased lethality. The safe dose range is, therefore below 1g/ml.

Given the weight of flies tested and that they can consume between 20-100µg a day and twice this amount whenstarved for 14-17hours (Markow, 2015), the safe range dose, which in acute exposure is below 0.8g/ml, corresponds to 16g/kg when projected to a human adult. Available AEDs are consumed over a long period of time and, this was projected in the chronic exposure to detect effect of the extract on longevity of the flies. In chronic exposure the safe range dose was below 0.1g/ml, which in the context of human consumption would correspond to a range of 3.5g/kg for an average human adult. The Luo community who currently use the remedy to treat convulsions give a handful of the paste obtained from the roots (approximately 70g) to their patients. They are, therefore, working within the safe range dose. The lowest safe dose was found to be 5 times bigger than the therapeutic dose, and therefore, this gives room for optimization. It is worth noting that the available AEDs have lower doses in human compared to the extrapolated doses of the extract from drosophila to human. For instance, Gabapentin one of the mostly prescribed AED is used at 10-15mg/kg, similarly other new drugs like lamotrigine have equally lower doses of 0.6g/kg. Though, this shouldn’t be surprising because most of these drugs are purified compounds unlike this extract that was crude. Possibly fractionation of crude extract could lead bioactive compounds with precise lower doses compared to this.

In summary, the LD50 was found to be 1.2g/ml. Doses below 0.8 g/ml were found to be safe in acute exposure, while in chronic exposure concentrations below 0.1g/ml were found to be safe and did not induce tissue damage. For precise conclusion on the toxicity independent analysis of the fractionated active compounds may give us an insight on the toxic and non toxic compounds. The concentrations below the LD50 were used in acute exposure of the parabss flies, while concentrations below 0.1gm/ml were tested for activity in chronic exposure. In testing for the activity of the extract on convulsion, three phases of seizure were measured the parabss fly, majorly the convulsion time, paralysis time and recovery time. The extract had reduction on the three parameters by a mean of 30% in single exposure whereas by 26% in chronic exposure.

Most of pathogenesis of epilepsy in human is channel related. Only channelopathy-related mutations account for 90% for human seizures and most of them affect the sodium channel in the membrane of neurons (Meisler & Kearney, 2005). Ionic channels are important in maintenance of resting membrane potential. For seizures to occur in human, just like in flies, there is usually a trigger, which disrupts the normal resting membrane potential. In flies the trigger is usually a bang after which the phases that were tested above follows. In humans, the trigger can be flash of light, sound or memory. Human seizures have different phases though generally divided into aura, ictus and post-ictal state. The trigger often occurs in the aura. Human seizures are longer and there are different variations due to the complexity of the mammalian
system, though the effect of the extract across all phases of the fly seizures suggests that it can be effective in reducing the recovery time of human seizures further studies should be done for proper extrapolation.

The extract induced significant reduction in the three parameters that were measured when the flies were exposed to concentrations of extract below the toxicity range. Imperata cylindrica, therefore, shows potential as anti-epileptic treatment. Even though the extract showed potential therapeutic effect measuring of any existing side effect is important. Similarly, some of the existing AEDs great efficacy against seizure but on the other hand they also have side effects and reduce the health related quality of life of epileptic patients. It is, therefore, necessary to ensure that beneficial effects of this anti-convulsion remedy are not accompanied by detrimental effect for the general health of these patients.

The climbing assay was used to test for the effect of the extract on the general wellbeing of the flies exposed to the extract on both acute and chronic treatments. The extract had minimal side effects on Drosophila on chronic exposure. Even though higher doses showed a greater reduction in mean recovery time, these doses also reduced the climbing activity of the flies. It’s good to note that the flies also showed reduction in hyperactivity in almost the entire treatment group.

Despite the effectiveness of the available AEDs towards management of seizures there are gross side effects to the patients. One of the major side contributors to the side effect is the practice of poly pharmacy in management of epilepsy (Tolou-Ghamari, 2012). The drugs used in suppressing seizure have potential side effects, including cognitive and affective changes or altered sleep and appetite. This is majorly, because most of the available therapies act via ionic channel that regulate impulse transmission. To the F1 generation, the AEDs have several side effects like teratogenic effect that leads to congenital anomalies, fetal hydantoin syndrome and Tetra-logy of fallot (Loring & Meador, 2004).

These results show that the effective doses of the extract may have slight side effects on the general well being of the organism and more studies need to be carried out to identify the active principles and, if possible, isolate them from the components causing the side effects. Our last objective was to determine the mechanism of action of the extract. In this study the hypothesis was that the extract could act in blocking the sodium channel. Study done to illicit the interaction between the sodium channel and potassium channel showed that para hypo-morphs suppresses shaker activity due to hyperactivity of potassium channels, whereas increased activity of para enhances that phenotype (Parker, Padilla, Du, Dong, & Tanouye, 2011). Our predictions were, therefore, an improvement of flies with increased activity Na+ channels and also flies with reduced activity of K+ channel, while worsening of flies with reduced activity of Na+ channel and flies with increased K+ activity. The results did not match these predictions. The extract ameliorated seizures and electromyogram recording of the parabss flies and worsened the shaking of the pharmacologically modified flies with blocked Na+ channel. On the other hand, the complexity of the seizure leaves the door open for compounds that can suppress seizures through a variety of mechanism. These include regulation of neurotransmission via the resting potential in neurons, ionic uptake in astrocytes or permeability of the blood brain barrier. The extract did not act as specific Na+ channel or K+ channel. It could be acting as general channel blocker just like other AEDs available in the market e.g. sodium valproate or Gabapentine. It could also be acting via as chelating agent reducing the amount of free ions. Other possible mechanisms are that it is acting on channels that regulate the resting potential like the Cl- channel or the Na+/K+ pump.
4. RESULTS

4.1 Toxicity

4.1.1 Single Exposure to the Extract

Wild type flies were exposed to several concentrations of the extract for 1.5 hours to determine the lethal dose (LD50), this was a dose that was supposed to produce 50% lethality on the flies exposed to the extract. In the initial experiment concentrations above 1.5g/ml resulted in greatly reduced survival (p-value < 0.05; t-test Fig. 1A) and increased lethality by more than 70%. In order to more precisely determine the LD50, concentrations below 1.2g/ml were used. LD50 was found to be 1.2g/ml this dose produced 50% lethality (fig 1B). 1g/ml and 1.2g/ml showed significant effect on the percentage of flies that were alive (p=0.00026 and p=0.00014 respectively in t-test). Doses below 1gm/ml produced less than 20% lethality.

![Acute Toxicity Study for Determination of Lethal Dose](image)

**Fig. 1: Acute Toxicity Study for Determination of Lethal Dose**  
Percentage of surviving wild type flies (w^1118^) exposed to a single dose of several concentrations of extract. Means of three experiments with three replicas of 10 flies each are represented. Error bars are Standard deviation. Wild type flies (w^1118^) exposed to several concentration. A) Concentration above 1.5g/ml resulted in increased lethality. B) Concentrations above 1mg/ml reduce survival and LD50 was found to be 1.2g/ml.

4.1.2 Chronic Exposure to the Extract

Most of the available AEDs exert their effect over a long period of treatment. To model this situation, flies were fed on food containing concentrations of extract below the LD50 determined in the single exposure experiment.

A) Mean Percentage Survival

Mean percentage survival was the average number of flies that were alive in a given treatment group after every 24-hour from the first day of exposure until death. Wild type flies (w^1118^) fed on different concentrations of extract showed dose-dependent shortening of survival (Fig.2). Concentrations over 0.1g/ml resulted in reduced survival p<0.05. Only 0.1g/ml showed no significant increase in lethality (p-value = 0.44; Student T-test. Flies exposed to concentration above 0.1gm/ml showed reduction in mean percentage survival.
**Fig. 2: Effect of Chronic Exposure to Extract on Longevity**

Negative log of the percentage of wild type flies (w1118) alive after every 24 hours exposed to decreasing concentrations below the lethal dose during their entire lives against days was plotted above. Means of three experiments with three replicas of 10 flies each are represented. Concentrations above 0.1g/ml showed significant difference with respect to the control (p-value<0.05).

**B) Mean Lifespan in Chronic Exposure**

Analysis of the mean lifespan of the same flies above was done (Fig.3). Unlike mean percentage of survival the mean life span violin describes the average number of days that each fly in a particular treatment group lived before its death, basically the violin represents the density, the higher the density the bigger the polygon at that specific moment. The shorter the polygon the shorter the life span and vice versa. Concentrations over 0.5g/ml reduced lifespan by half moreover all concentrations with the exception of 0.1g/ml showed significant difference with the control (p-value<0.05; Student T-Test). A concentration of 0.1g/ml resulted in a mild reduction of lifespan of 5 days, which was not significant when analyzed using the Student T-Test.

**Fig. 3: Effect of Chronic Exposure of the Extract on Average Life Span**

Violin plot of average lifespan of wild type flies (w1118) exposed to chronic exposure of the extract against days. This data was from three experiments with three replicas in each experiment, each replica had 10 flies. The area of the violins reflects the number of flies which died in a given day. White circles show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the inter-quartile range from the 25th and 75th percentiles; polygons
4.2 seizure effect

4.2.1 Convulsion Time

Para<sup>bos</sup> mutant flies, showed an anticonvulsive activity (p>0.05) on single exposure of the extract. Upon increase in concentration showed p>0.01

(Fig. 4). The 0.6gm/mL showed slight significant effect in convulsion reduction (p=0.027; t-test) while 0.8gm/mL and 1gm/mL showed significant reduction in convulsion time. Though 1.0gm/mL showed the highest significance compared to the other treatment groups (p=0.00007; T-test).

4.2.2 Paralysis Time

Paralysis is the 2nd phase after convulsion, all the concentrations showed great significant reduction on the paralysis p>0.01. All the concentration reduced the paralysis time (p=0.000072; T-test).

Fig. 5: Effect of Single Exposure to extract on the Paralysis Time of para<sup>bos</sup> flies.

The figure represents the paralysis time of Para<sup>bos</sup> flies after exposure to the extract. Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the inter-quartile range from the 25th and 75th percentiles. Outliers are represented by dots. crosses represent sample means. n = 91, 77, 79, 81 sample points. Concentration showed significant reduction on paralysis time (*: P <0.05; **: P <0.01).
Fig. 6: Effect of Single Exposure to extract on the Convulsion Time of para^{bss} mutant flies.

Para^{bss} mutant flies exposed to extract and tested for the effect on convulsion time. Convulsion time recorded in para^{bss} flies in second (y-axis) exposed to different concentration of the extract. Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the inter-quartile range from the 25th and 75th percentiles, crosses represent sample means. Outliers are represented by dots. n = 78, 77, 67, 79 sample points. Higher concentration produced significant reduction on convulsion time (*: P-value < 0.05; **: P value < 0.01). Boxes represent the distribution while the line at the center is the median.

4.2.3 Mean Recovery Time

There was significant reduction on the recovery time. The MRT on chronic exposure to the extract, 0.025gm/mL showed no statistically significant reduction in recovery time (p=0.9998; t-test) on day 3 of exposure.

Figure 6: Effect of single Exposure to Recovery Time in para^{bss} Drosophila.

Para^{bss} exposed to extract and MRT was measured. Box plot of the mean recovery time recorded in seconds (y-axis) against different concentrations. Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the inter-quartile range from the 25th and 75th percentiles, outliers are represented by dots; crosses represent sample means. n = 77, 73, 87, 66 sample points. All the concentration showed significant reduction on the mean recovery time (*: P-value < 0.05; **: P-value < 0.01).
4.3 Negative Geotaxis

Geotaxis was used to the general well being of para\textsuperscript{bss} and on single exposure to the extract, there was no significant difference between the climbing time taken between 0.6g/mL and the control, though flies fed on 0.8mg/mL and 1gm/mL of extract showed significant effect compared to the control ($p=0.0006; T$-test).

![Fig. 7: Effect of Single Exposure of the flies on Negative Geotaxis.](image)

The para\textsuperscript{bss} flies on single exposure of the extract. This data was from three experiments with three replicas in each experiment, each replica had 10 flies. Error bars represent the standard deviation. The average number of flies per height (y-axis) is plotted against different concentration. There is slight significance between the control and higher concentration (*: $P$ value <0.05; **: $P$ value <0.01).

Concentrations that had significant difference with the control showed significance in the number of larvae that showed tissue damage, on staining with trypan blue. Staining was seen majorly on the mid-gut (data not shown). On chronic exposure to the extract, there was no significant difference with control on 0.25-0.05g/mL ($p=0.84$, $p=0.06$; T-test). Though slight significance with 0.1gm/mL ($p=0.0002$; T-test). Despite not finding a between the control and the treatment groups ($p>0.05$), there was mild staining with trypan blue in the mid-gut of the flies that were chronically exposed to the extract, indicating mild tissue damage (data not shown).

![Fig. 8: Effect of Chronic Exposure to the Extract on Negative Geotaxis](image)

The para\textsuperscript{bss} flies on chronic exposure of the extract. This data was from three experiments with three replicas in each experiment, each replica had 10 flies. Error bars represent the standard deviation. A) Mean number of flies per height (y-axis) was plotted against different concentration. No significant difference of the flies per height compared to the control (*: $P$-value <0.05; **: $P$-value <0.01). B) Equally the mean number of flies in chronic exposure per height was not significantly different from the 3\textsuperscript{rd} and 6\textsuperscript{th} day of exposure.
Mechanism of Action of the Extract

The phenotypic results of pharmacologically modified flies that have blocked sodium channel showed increased activity of potassium channel and blocked potassium channel were used.

![Image of graphs showing mechanism of action](image)

**Figure 9:** Mechanism of Action of the Extract.

A) This data was from three experiments with three replicas in each experiment, each replica had 10 flies. Pharmacological modified flies (with blocked Na+) showed no significant difference with the control as shown below. Mean number of flies per height (y-axis) was plotted against single exposed treatment flies to the extract. Error bars represent the standard deviation. B) Pharmacological flies that mimicked Shaker fly mainly was observed by the number of leg shakes per minute (y-axis) was plotted against concentration. The pharmacological modified flies (with increased k+) had significant reduction on the leg shaking compared to the control (*: P-value<0.05; **: P-value <0.01). C) The flies that had blocked K+ had no significant effect between the control and the treatment p-value>0.05.

4.4 Electrophysiological Recording on Flies

Resting membrane potential was recorded for parabss flies fed on different bioactive substances. This was compared with wild type flies (w1118). The experiment was repeated 3 times, with 3 replicas average of the spikes/milliseconds, amplitude and trough was recorded from 5 flies in each replica.

![Image of electrophysiological recordings](image)

**Fig.10:** Effect of Extract on Electrophysiological Recording.

A) Electro-myogram recording in bs flies millivolts (y-axis) per second, average recording showed that the bs flies had 13spikes/millisecond and a resting membrane potential 0.3975 millivolts. B) From w1118 showed an average of 10spikes/millisecond and RMP of 0.397millivolts. C) Recorded results from flies with increased K+ showed
10 spikes/millisecond and 0.396 millivolts. D) Recorded results on flies with reduced K+ activity showed 8 spikes/millisecond and RMP of 0.3965 millivolts.

There was significant reduction on the spikes per millisecond in the parabss flies after treatment. The shaker flies that had increased K+ activity showed no significant difference in spikes per millisecond with the treatment though the RMP of the control was higher than the treatment. The shaker flies with reduced activity of the K+ had significant reduction in the number of spike per millisecond with slight reduction on the resting membrane potential.

5. CONCLUSIONS
The results of this study show that the LD50 for acute exposure to Imperata cylindrica in Drosophila is 1.2g/ml, being the safe range below 1g/ml. For chronic exposure to food containing the extract, the safe dose was determined to be below 0.1g/ml. Both acute treatment and chronic exposure to safe doses had anti-convulsive activity in parabss flies hence Imperata cylindrica has anti-convulsive activity. The extract has mild effect on negative geotaxis (locomotion) of the flies. The extract ameliorated seizures induced by increased sodium channel activity and shaking induced by increased potassium activity. Therefore, the activity of the extract does not fit with the proposed hypothesis of acting as a specific sodium blocker.

6. RECOMMENDATIONS
In this study, the complete crude extract was used to study the anti-epileptic activity of the plant. For precise determination of the origin of both toxicity and activity, independent analysis of the fractionated active compounds is recommended. The use of more mutants or model flies for other channels and proteins involved in the maintenance of the resting potential rather than pharmacologically modified flies shall help discriminating the mechanism of action. Finally, further analysis of isolated bioactive compounds and protein bio assay can also help in finding the mechanism of action of the extract. The present study has shown that doses equivalent to the portions currently used by the community to treat convulsions are safe. An effort will be made to bring this information back to the users. Future studies should be performed to optimize the use of the plant as a traditional remedy and protect their interest and intellectual property.

7. REFERENCE


Tolou-Ghamari, Z. (2012). Antiepileptic drugs (AEDs) polypharmacy could lead to buried pharmacokinetic


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