Anticonvulsant Activity of *Elaesis guineensis* (Jacq) Oil in Pentylenetetrazol-Induced Seizure in *Drosophila melanogaster*

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

*Elaesis guineensis*, a plant whose oil extract (palm kernel oil) is medicinal, is reported to treat a wide range of disorders, including seizures. However, the anticonvulsant activity of this oil extract has not been exhaustively studied. This study aimed at evaluating the anticonvulsant activity of *Elaesis guineensis* oil in pentylenetetrazol-induced seizure in *Drosophila melanogaster* (fruit-fly). Pentylenetetrazol (50 mg/5 g diet) was used to induce seizure in *Drosophila melanogaster*. Flies were exposed to different concentrations (0.5-5%) of the oil and phenytoin for 28 days in a survival assay to determine the safety in the fruit flies. Five replicate of fifty flies each were exposed to diet containing the LC₅₀ of phenytoin and other groups were exposed to different concentrations of the extract for 7 days. Seizure was then induced with Pentylenetetrazol. The Trikinetic system was used to monitor activity and the DAMSystem3 data collection program to collect, process and store data. The results showed that the extract increased the latency of seizures and improved survival in the flies and suggest that the extract possesses anticonvulsant properties.

Keywords: *Elaesis guineensis*; *drosophila melanogaster*; pentylenetetrazol; trikinetic system.

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1. INTRODUCTION

Epilepsy is a central nervous system (neurological) disorder characterized by abnormal brain activity, resulting in seizures or periods of unusual behavior, sensations, and sometimes loss of awareness. Epilepsy can affect people of both sexes, all races, ethnic backgrounds and ages.

Convulsion is a medical condition where body muscles contract and relax rapidly and repeatedly causing uncontrolled shaking of the body. Because epileptic seizures typically include convulsion, the term convulsion is sometimes used as a synonym for seizure.

Anticonvulsants, also known as antiepileptic drugs or anti-seizure drugs are a diverse group of pharmacological agents used in the treatment of seizure. Anticonvulsants are also interestingly being used in the treatment of bipolar disorder [1] and border line personality disorder since many seems to act as mood stabilizers and for the treatment of neuropathic pain [2].

Anticonvulsants suppress the excessive rapid firing of neurons during seizures. It also prevents the spread of the seizures within the brain. Conventional anticonvulsant drug may block sodium channels, enhance GABA function or block calcium channels. By blocking sodium and calcium channels, antiepileptic drugs reduce the release of excitatory glutamate whose release is considered to be elevated in epilepsy but also that of GABA [3]. *Elaesis guineensis* is a species of palm commonly called African oil palm or macaw-fat. This plant is claimed to be used as a traditional medicine for the treatment of convulsive disorders. Researchers agree that it is capable of curing certain illnesses and that its oil is highly medicinal. The oil has many health benefits including antioxidant effect, helps soften the skin, help control blood pressure, tackles body odor, improves vision, increases hair growth, delays aging, treat cough, alleviate constipation and of importance in this research use as remedy for convolution mostly in children [4]. In a previous study, the effect of straight chain fatty acids on seizures induced by picrotoxin and pentyleneetrazol in mice was evaluated [5].

Pentyleneetrazol, also called PTZ, is a drug formerly used as a circulatory and respiratory stimulant. High doses cause convulsions, as discovered by the Hungarian-American neurologist and psychiatrist Ladislas J. Meduna in 1934. This drug acts as a convulsion inducer in experimental animal models.

*Drosophila* are flies, belonging to the family Drosophilidae, whose members are often called "small fruit flies" or (less frequently) pomace flies, vinegar flies, or wine flies, a reference to the characteristic of many species to linger around overripe or rotting fruit. One species of Drosophila in particular, *D. melanogaster*, has been heavily used in research in genetics and is a common model organism in developmental biology [6].

Pentyleneetrazol-induced seizure has the following bio-markers in *Drosophila melanogaster* namely, brief leg twitches followed by failure to maintain standing posture with leg shaking, abdominal muscle contraction, wing flapping and scissoring and proboscis extensions [7].

TriKinetics systems use infrared beams to detect and quantify animal movement over time. In a typical experiment, test subjects are placed into transparent tubes with a supply of food, and as they move back and forth, their locomotor activity is recorded. Such daily activity records were first used to characterize the circadian rhythm of drosophila, and since then have been used to measure sleep, longevity, social interaction, geotaxis, phototaxis, learning, and drug response in various species of flies, mosquitoes, bees, spiders, ants, moths, cockroaches, wasps, beetles, zooplankton, and fish.

The DAMSystem3 data collection program uploads periodically from each activity monitor and saves its data in the respective raw monitor file on the hard drive. These simple files will each contain all of the raw experimental data for a single monitor, and may include multiple data types, errors, collection gaps, etc. The File Scan program then scans and corrects errors and select particular data types and date/time ranges to produce, processed or ‘good’ data files. These files are then ready for input into a text editor, spreadsheet, or any number of available analysis programs. Note that once collected, the raw monitor files may be moved to another computer for storage, processing, and analysis.

2. METHODS

2.1 Maintenance of Fly

*Drosophila melanogaster* Oregon R strain, was maintained in clean culture bottles with an air
pore cork in the fly laboratory at (22 ± 1 °C) in Standard Drosophila Medium (SDM) which was prepared using agar-agar, maize powder, yeast, and sucrose etc prepared using standard methods.

2.2 Collection & Identification

Fresh palm kernel was collected from Gwantu market in Kaduna State, Nigeria. Authentication and identification of the plant material was carried out at the department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria.

2.3 Extraction

The hot extraction method was used to extract the oil. The palm nuts were de-shelled and properly dried and then heated in a dried pot until the oil started coming out. A continuous stirring is required to ensure proper extraction of the oil. The oil after extraction was drained and allowed to cool.

2.4 Experiment Design

Five groups of fifty flies each in replicates of five were used and each group given a different treatment as follows:

Group one (1) served as the positive control and was fed on SDM only;
Group two (2) was fed on SDM incorporated with 0.5 % oil (extract);
Group three (3) was fed on SDM incorporated with 1.0 % oil (extract);
Group four (4) was fed on SDM incorporated with 2.0 % oil (extract);
Group Five (5) was fed on SDM incorporated with 0.5 % oil (extract).

All flies were exposed to the oil for 7 days.

Five replicates of fifty flies each were exposed to diet containing phenytoin for 7 days. After this, seizure was induced with Pentylentetrazol and the Trikinetic system was used to monitor its activity.

Anticonvulsant activity was determined using the TriKinetics system which uses infrared beams to detect and quantify animal movement over time, treated flies were placed into transparent tubes with a supply of food, and as they move back and forth, their locomotor activity is recorded. Differences in the movement were observed between flies in different treatment groups.

Seven groups of flies were used for determination of anticonvulsant activity. Each group was made up of five replicates containing fifty flies each. Some flies fed on normal diet, some fed on the various concentrations of the oil extract and others on phenytoin for 5 days and then exposure to PTZ.

The treatment groups were as follows:

Group one served as the positive control feeding on the normal diet;
Group two fed on diet but was induced using PTZ;
Group three fed on diet containing 2 mg of phenytoin;
Group four fed on diet containing 0.5 % oil extract;
Group five fed on diet containing 1 % oil extract;
Group six fed on diet containing 2 % oil extract;
Group seven fed on diet containing 5 % oil extract.

After this exposure to the different groups as seen above, flies where then exposed to PTZ to induce seizure and the Trikinetic system was used to monitor its activity.

3. RESULTS

Percentage survival ranged from 27-42 %. The highest survival was recorded in the control while the least survival proportion was recorded in the 1 mg of phenytoin (Fig 1). Comparing the survival curves, Log-Rank (Mantel-Cox) test P-value was 0.0030, indicating a statistically significant difference (P < 0.05). This result shows that flies exposed to 2 mg Phenytin survived best with a 42 % survival.

Survival proportion ranged from 10.5 - 33 %. The highest survival proportion was recorded in the control while the least survival proportion was recorded in the 5 % of palm kernel oil extract. 0.5 % Phenytoin showed a survival of 33 % which is comparable to control. Comparing the survival curves, Log-Rank (Mantel-Cox) test P-value was 0.0001, indicating a statistically significant difference (P < 0.05).

Survival proportion ranged from 69.8 – 85.2 %. The highest percentage survival was recorded in the group that were exposed to 5 % palm kernel oil extract while the least survival proportion was recorded in the 0.5 % of palm kernel oil extract. Groups that were exposed to 2 % and 5 % extract survived better than the
control which recorded a 78.5% survival. Comparing the survival curves, Log-Rank (Mantel-Cox) test P-value was 0.0017, indicating a statistically significant difference (P < 0.05).

![Fig. 1. 28-day survival assay of *Drosophila melanogaster* exposed to Phenytoin](image1)

Fig. 1. 28-day survival assay of *Drosophila melanogaster* exposed to Phenytoin

![Fig. 2. 28-day survival of *Drosophila melanogaster* exposed to palm kernel oil](image2)

Fig. 2. 28-day survival of *Drosophila melanogaster* exposed to palm kernel oil

![Fig. 3. 5-day Survival Assay of *Drosophila melanogaster* exposed to palm kernel oil](image3)

Fig. 3. 5-day Survival Assay of *Drosophila melanogaster* exposed to palm kernel oil
Fig. 4. Locomotor activity count of flies exposed to Pentylenetetrazol, Phenytoin and various concentrations of oil extract

*In groups 3-7, flies were first pretreated as shown above and then exposed to PTZ to induce seizures.

The SHINY-R -SOFTWARE recorded the hourly activity count of the flies indicating how the flies were protected due to pre-treatment of the extract and standard drug (Phenytoin).

Fig 4 above showed normal activity in the control group (group 1), while an increase in the rate of seizure in flies not pre-treated was observed (group two, represented by the yellow spikes), which led to death before 300 minutes. Flies in group 3 (the green spikes) were protected by pre-treatment with phenytoin, though the flies still convulsed (as seen by the number and frequency of the spikes). Phenytoin’s protective effect did not last long compared to flies treated with the oil extract (Fig. 4). Flies pretreated with 0.5 % of the oil extract before exposure to PTZ were protected against excessive seizure (as seen in the blue spikes) up to 200 minutes, after which an increase in the rate of seizure was recorded. However, the flies in this group were still protected for over 500 minutes. Group 5 flies (represented by purple spikes) were pretreated with 1% of the oil extract before administration of PTZ showed similar protective effect to flies in group 4. Flies in group 6 (red spikes) which had 2 % of the oil extract before PTZ showed a marked and higher protective rate compared to the other groups. Group 7 flies (as shown by light blue spikes) which had 5 % of the oil extract and PTZ were protected until 400 minutes.

This result indicates that the extract had seizure protective effect compared to the standard anti-seizure drug (phenytoin).

The concentration of acetylcholinesterase (Mmol/min/mg protein) was assayed and the highest concentration was recorded in 0.5% of the oil extract. ANOVA P-value was 0.0011, which is significant.

The highest concentration of total thiol (umol/mg protein) was recorded in flies exposed to 0.5 % of the palm kernel oil.

The highest concentration of Glutathione-S-Transferase (umol/min/mg protein) as recorded in 0.5 % of the oil extract. The ANOVA P-value is 0.5100 which is not significant.

4. DISCUSSION

The 28-day survival assay (Fig 2) performed in the presence of the extract showed similar levels of survival at the lowest concentration, compared to the control. This is an indication of the safety of the oil.

Locomotor Activity Count (Fig 4) of flies pretreated with phenytoin and different concentrations of the extract before seizure...
induction, as recorded by the SHINY-R SOFTWARE, indicates that the flies were protected and the protection seen with the extract is similar to that seen in the reference drug, phenytoin. In flies exposed to 0.5 % of the oil extract, there was protection from excessive seizure and the flies survived for the longest period (up to 600 minutes).

Fig. 5. Acetylcholinesterase assay of flies exposed to Phenytoin and different concentrations of palm kernel oil

Fig. 6. Total Thiol Assay of Flies Exposed to Phenytoin and different concentrations of palm kernel oil

Fig. 7. Glutathione-S-Transferase assay of flies exposed to Phenytoin and different concentrations of palm kernel oil
Biochemical assays performed on flies treated with various concentrations of the extract showed that levels of acetylcholinesterase decreased with increasing extract concentration. This clearly indicates the extract’s ability to decrease seizures induced by PTZ. The extracts’ protective activity was observed with increase in levels of Glutathione-S-Transferase, an endogenous antioxidant. The level was significantly higher than that of the standard drug, phenytoin and points to the extract’s ability to reverse injury or damage caused by PTZ.

The highest concentration of total thiol was also seen in 0.5 % (Fig 6), same was seen in the Glutathione-S-Transferase assay. These all support the results obtained from the survival assay that shows better safety with lower doses. It also shows that the extract has anticonvulsant properties.

The increase in the rate of seizure seen in group two is indicative of the induction of seizures when treated with Pentylenetetrazol alone. Activity of flies in treated with Phenytoin shows protective effects of Phenytoin which is our standard drug, the death of the flies about the 400th minute also suggests it is a short acting anticonvulsant, this supports the report by Singh et al. [8], which concluded that the flavonoid rich fraction of Glycyrrhiza glabra in combination with phenytoin reduces seizure severity and improve cognitive functions in PTZ-kindled mice. The oil extract showed protective effects at different doses with differences in duration of protection with differences in dose. The highest protection was seen in flies treated with 2 % of the oil extract which showed a marked and higher protective rate compared to the other groups. These indicate that the extract has seizure protective effect comparable to the standard drug (phenytoin).

5. CONCLUSION

Findings from this present research showed that the Eleasis guineensis oil possesses anticonvulsant activity (protective activity) against Pentylenetetrazol-induced seizures with the highest activity seen at 2 % and could serve as an alternative anticonvulsant.

DISCLAIMER

The materials used for this research are commonly used products in our area of research and country. There is no conflict of interest between the authors and producers of the materials because we do not intend to use them other than for research and the advancement of knowledge.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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