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Ameliorative Effect of Ethanolic Leaf Extracts of Dichrostachys Glomerata on Post Seizure Visuo-Spartial and Cognitive Memory and Learning Function in Pentelenetetrazol Kindled Mice

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Abstract

According In addition to the recurrent seizures, epilepsy is also associated with post-seizure impairment in cognitive and visuo-spartial memory function. Pentylenetetrazole (PTZ) kindling is a well-established animal model in simulation of clinical epilepsy. This present study was designed to comparatively assess the effects of diazepam (DZP) and ethanol leaf-extract of Dichrostachys glomerata (DG) on learning and memory in pentylenetetrazol induced epileptic mice. Twenty eight male Swiss white mice were randomly grouped into four (n=7). Group 1 served as control and was given 0.9% normal saline (IP). Group 2 served as untreated epileptic control group and was administered(IP) 60mg/kg of pentylenetetrazol (PTZ) while the other two groups (3 and 4) served as epileptic mice groups treated with DZP (1mg/kg) and DG (4.5mg/kg) respectively prior to induction of epilepsy. The anti-epileptic effects of both agents on cognitive and visuo-spatial memory status on the animals were assessed using the Novel object recognition task (NORT) and the Morris water maze (MWM) respectively. The results obtained during the anti-epileptic study showed that seizure activities was completely abolished in the DZP treated epileptic mice group and significantly lower in the DG treated epileptic mice group compared to the PTZ group. In the NORT, the index of habituation was significantly higher in the PTZ group compared to control but however significantly lower in the DZP and DG treated epileptic mice groups compared to the PTZ during the short-term memory test. Also the index of discrimination was consistently lower in the PTZ group compared to control but significantly higher in the DZP and DG treated groups compared to PTZ group in both short-term and long-term memory test. This indicated that cognitive memory impaired in the epileptic mice groups was improved following treatment with both DZP and DG. In the MWM, there was no significant difference in the swim latencies amongst the animal groups during the acquisition and reversal training. On the probe trial day, there was no significant difference in the mean south west quadrant duration in the PTZ group compared to control, however this was significantly higher in the DZP and DG treated epileptic mice groups compared to the PTZ group. There was also a significant decrease in annulus reversal crosses in the PTZ group compared to control but a significant increase in the DZP and DG treated epileptic mice groups compared to the PTZ group. Also the swim latencies on the visible platform day increased significantly in the PTZ group compared to control but significantly decreased in the DZP and PTZ treated epileptic mice groups compared to the PTZ group. Therefore, the results suggests that both diazepam and ethanol leaf-extract of Dichrostachys glomerata improved cognitive and visuo-spatial memory and learning that was impaired following pentylenetetrazol induced epilepsy in mice. However, diazepam was more potent and more efficient than the plant leaf- extract.

Introduction

Epilepsy is one amongst the most common serious chronic neurological disorders (Hirtz et al., 2007). It is a chronic neurological disorder that is characterized by seizures that occur due to uncontrolled excessive neuronal discharge in part or whole of the central neurons system with resultant uncontrolled involuntary muscular contractions (Riss et al., 2008).

It is a prevalent neurological disorder that affects an estimated 50 million people worldwide, accounting for 0.5% of disease burden with nearly 80% occurring in developed countries (WHO, 2019) and onset of new cases occurring most frequently in infants and in the elderly (Holmes et al., 2008). Although defined by the presence of recurrent seizures, also associated with epilepsy are neurobehavioral disorders including abnormalities in social adaptive behaviour as well as impaired cognitive memory function (Lancet Neurology, 2008).

The main stay treatment for epilepsy is through the use of anticonvulsant medications -some standard ones including Benzodiazepine, Betazenol, dizocilpine, ketamine etc.

Despite the availability of many synthetic medications, the increasing burden of neurological disorders prompts indiscriminate local utilization of plants/herbs and their extracts in the treatment of mental health disorders. Prescriptions used in the treatment of epileptic seizures amongst the Hausa / Fulani tribe in northern Nigeria include traditional herbs like: securidaca longipedunculata (family polygalaceae), mitragyna internis (family Rubiaceae) and certis integrafolia (Muazu & Kaita, 2008). Sadly however, there are relatively scanty available scientific proves (though experimentation) or publications about

the therapeutic benefits, appropriate dosing as well as possible side effects that may be associated with use of these traditional plant/herbs.

Dichrostachys glomerata (the plant of study) have been claimed to have antiepileptic properties and so is being used traditionally by some natives in Nigeria to manage or treat epilepsy. However, since there are relatively scanty or no demonstrable scientifically proven experiments or publications showing the antiepileptic and/or post-seizure effect of this plant, it is most appropriate therefore, that this research study attempts to investigate (through scientific experimentation) the ability of this plant (Dichrostachys glomerata) to reduce epileptic seizures as well as relieve the neurobehavioral disorder of impaired cognitive memory function that is associated with epilepsy. This research study was aimed therefore at: There are associated with epilepsy many post- seizure neuro-behavioral disorders lik increased pain perception, impaired motor co-ordination, increased anxiety etc. However, this research study was limited to investigating the anti-epileptic of the plant Dichrostachys glomerata on memory and learning alone.

Materials and method

Materials

The following materials was used for this study: sample bottles, electric oven, refrigerator, electric blender, stirrer, Wattman Filter paper, plastic funnel, spatula, disposable syringes (1ml, 2ml, 5ml, 10ml and 20ml), stop watches, masking tapes, measuring cylinders, animal cages, weighing balance, feeding troughs, water bottles, conical flasks, 50g pentylenetetrazol (PTZ), methylated spirit (40% ethyl alcohol), cotton wool, diazepam, distilled water, ethanol, 0.9% Normal saline, the Morris Water Maze set up, the Open Field Maze (OFM), paper towels, white cardboard paper, plastic objects of different sizes etc. The above materials were gotten from the Department of Physiology, College of Medical Sciences, University of Calabar. Some of them however, was personally acquired.

Methods

After Plants Materials

The leaves of the plant Dichrostachys glomerata were obtained from Afikpo North Local Government of Ebonyi State, Nigeria. Taxonomic identification and authentication was done by the Chief Herbarium Officer of the Department of Botany University of Calabar, Calabar. After identification, the leaves was cleared of debris, sun dried for 3 days and then grounded to powder.

Plant Extraction

The dried and powered leaf of DG (360g) was suspended 1728ml in ethanol. The suspension was then agitated with an electric blender for an about 10 minutes and allowed in a refrigerator (40c) for 24 hours after which it was filtered with a Wattman No4 filter paper. The ethanolic filtrate was then concentrated using a rotary evaporator at 400C. This concentrates was then allowed in a water bath at 370C for complete dryness yielding of crude extract of 33.14g. The yield was re-constituted to an appropriate concentration in distilled water before administration.

Experimental Animals

A total number of 28 male CD-1 mice weighing between 18-36g was purchased from the animal house of the Department of Pharmacology, Faculty of Basic Medical Sciences, University of Calabar, Calabar. The animals where then housed in standard animal cages to acclimatized for at-least 10 days during which they were exposed to or fed with rodent laboratory chow and clean tap water.

Experimental Design

The twenty eight (28) male CD-1 mice where be randomly grouped into four (4) groups of seven each(n=7) as follows:

- Group One=control group- Received 0.9% normal saline only (intraperitoneally)
- Group Two=PTZ group- Treated with PTZ only (intraperitoneally)
- Group Three=PTZ and DG Group- where treated with PTZ and ethanolic extract of DG (intraperitoneally)
- Group Four=PTZ and Diazepam group- were treated with PTZ and diazepam (used as reference drug)

Drug Preparation and Treatment

All drugs where prepared by dissolving in 0.9% normal saline and administered intraperitoneally at an administration rate of 0.1 ml/10g body weight. The ethanolic extract of DG was re-constituted to a stock concentration of 10mg/ml from where a dose of 4.5mg/kg (predetermined by an acute toxicity test) was then administered at a rate of 0.1ml/10g body weight. Powdered PTZ was then re-constituted (by dissolving in 0.9% normal saline) to a stock concentration of 10mg/ml from where a pre-determined dose of 60mg/kg was administered at the rate of 0.1mg/10g body weight intraperitoneally. Also diazepam (the reference drug) was re-constituted to a stock concentration of 5mg/ml from where a dose of 1.0mg/kg body weight was administered intra-peritoneally (IP) at the same rate of 0.1ml/10mg.

All the animals were treated 5 days before commencement of behavioural studies and three days between study (i.e., on the day 4 to 6 of the Morris Water Maze).

Induction of Epilepsy

Animals in group two, three and four then received (via intra-peritoneal injection and at the rate of 0.1ml/10g body weight) 0.9% normal saline, ethanolic extract of DG and 1mg/kg of diazepam respectively. After 30 minutes interval, seizure activities was induced in these set of animals using an IP injection of 60mg/kg of PTZ (also at the rate of 0.1ml/10g body weight).

The following seizure activities was observed and recorded manually immediately after the injection of PTZ: onset of jerk, frequency of jerk, latency to clonic jerks, and frequency of clonic

jerks as well as duration of jerks. The results of the anti-epileptic study will be discussed in the chapter that follows.

Behavioral Assay

The neurobehavioral study was conducted in the neurobehavioral laboratory of the Faculty of Basic Medical Sciences University of Calabar. Cognitive memory function status as well as visuo-spatial memory and learning status for all the animals where assessed using the Novel Object Recognition Task (NORT) and the Morris Water Maze test respectively.

The Novel Object Recognition Task (NORT) was originally developed for rats as a test of declarative memory, after it was discovered that rats will spend more time exploring new or novel object than familiar ones. It has since been validated as a test of recognition memory in mice (Hashemi-Firouzi et al; 2015). The Morris Water Maze (MWM) Consist of a circular pool filled with opaque water. Rats or mice are trained to use extra-maze visual cues to locate an escape platform hidden just below the surface of the opaque water. The hidden-platform version of the MWM is a test of visuo-spatial learning and memory, performance of which is impaired by hippocampal lesions. It has been used extensively to study differences in spatial learning in mice (Joanne R. et al; 2010)

Statistical Analysis

Data collected was analyzed using the Statviewsv5.0.1 (SAS Instrument for windows or Mac) and the results were presented in mean standard error of mean (SEM). Statistical significance between the control and the treated groups were analyzed using the one way Analysis of variance (ANOVA) where $p>0.05$ was considered statistically significant.

Neurobebavioural Assay

Novel Object Recognition Task (Nort)

The objects recognition task was conducted in a neuro-behavioural test apparatus called the open to field (OF) box (38x38cm), which is also used to assess exploratory behaviour.

The floor and three walls of the open field are made of 2cm thick plywood that has been painted white. The fourth wall is made of clear Plexiglas so that the mice can be observed from the front of the apparatus as well as from the top. Blue lines painted on the floor divides the open field into forty-nine 5×5cm squares and these lines are used to assess locomotor activity. The center squares (15×15cm) is formed from the four inner squares and this square is highlighted with a black marker. A sheet of Plexiglas covers the floor.

Four (4) plastic objects of various shapes (2×2×2cm) were used as object- two of them being similar in size, colour, and shape, and the others being different in shape, size and colour(i.e Q1 and Q2 being identical and Q3 being different from Q1, Q2, and Q4).

During the first trial (acquisition) objects Q1 and Q2 are placed in the squares of the OFM box at locations being diagonally separated from each other. The animals where then introduced into the maze and allowed to explore the maze and thus the objects for 5 minutes. All objects and the apparatus were cleaned with 70% ethyl alcohol to eliminate olfactory cues or stimuli before the next animal was introduced into the maze. The same procedure was repeated until all the animals had received the first trial.

After a 30 mins interval, all the animals received a second trial (recognition or test trial). But this time one the objects (Q1) was replaced with a novel object (Q3) and the same procedure as in trial1 was repeated for all the animals. The animals were then returned to their home cages. After 24 hours, the animals received a third trial. Again one of the objects (Q2) was replaced with another novel object (Q4) and the same procedure repeated for all the animals.

The behaviours that where scored using during three trials of the NORT includes:

- Line crossing: frequency with which the animals crossed the grid lines with all four paws.
- Rearing: Frequency with which the animals stand on its hind legs in the maze.
- Rearing against the wall: Frequency with which the animals stand on its hind legs against the wall of the open field.
- Stretch Attend posture: Frequency with which the animals demonstrated forward elongation of the head and shoulders followed by retraction to the original position.
- Grooming: Frequency and duration of time the animals spent licking of scratching itself while stationary.
- Approach to each object: Directing the nose to the object at a distance < 1cm and/or touching it with the nose.
- Time spent with each object: Sniffing or climbing the objects.

The index of discrimination here is the different in time spent exploring the objects. It is believed animals with good visuo-spatial memory will spend more time exploring new or novel objects than they would for familiar ones. The results that were obtained will be presented and discussed in the chapters that follow.

Morris Water Maze

The Morris Water Maze that was used for this study was one that is modified for mice (Joseph, 2008). The Morris Water Maze (MWM) was made out of the circular pool that measured 110cm diameter and 20cm in-depth. The pool was filled to the depth of 14cm (0.5cm over the escape platform) with room temperature tap water. The water was made opaque by addition of grounded non-toxic chalk.

The pool was divided into four quadrants: northwest, southwest, northeast and southeast. Boundaries of these quadrants (North, East, South, and West) were marked on the edges of the pole using a masking tape.

An escape platform made of a cylinder (13.5cmx9cm in diameter) filled with cement (to make firm) was suspended and hidden 0.5cm beneath the pool. The pool was left 24 hours before testing so as to attain a room temperature (22 ± 1 C). The Morris Water Maze is an experimental test protocol that lasted for eight days as follows:

- Day 1: Acquisition day 1
- Day 2: Acquisition day 2
- Day 3: Acquisition day 3
- Day 4: Reversal day 1
- Day 5: Reversal day 2
- Day 6: Reversal day 3
- Day 7: Probe trail
- Day 8: Visible platform day

The acquisition and reversal training were done with the escape platform hidden 0.5cm below the opaque water(in the Northeast quadrant during acquisition training and in the Southwest quadrant during the reversal training).

During the acquisition training (Day 1 -3), the platform was placed (and hidden 0.5cm below) in the center of the Northeast quadrant. Each mouse received four (4) trial per day from different start locations (north, south, east, west). In each trial, each mouse was given a maximum of 60 secs, to locate the hidden platform. If the animals located and climbed the platform within the allotted time,it was then allowed at least 10 secs on the platform to view extra maze cues after which it was removed from the pool using a small container and the swim latency (i.e the time it took the animal to locate and climb the escape platform) was recorded.

If the animals could not locate the platform after the allotted 60 secs, then it is directed to the platform using the small container and allowed 10 secs before it was then taken out of the pool. It is important that all the animals be removed from the pool only after they may have climbed the escape platform so to let the animals associate climbing of the platform with escape from the pool.

When the animal is removed from the pool, it was usually placed in an holding cage where their body was dried using paper towel before being returned to their home cages. Also care was taken so as prevent repetition of starting locations sequences on back-to- back test days. This is usually done using a pre- determined Latin square design shown in the figure below.

During the reversal training (day4-6), the location of the escape platform was changed to the southwest quadrant, the mice again assigned appropriate start locations and the same procedure as in the acquisition training was repeated. On the probe trial day (day 7), visuo-spatial memory status of all the animals were assessed. On this day, the platform was taken out of the pool.

All the animals received only one trial from any one of the four start locations (from the North Pole) and allowed to explore the maze for 60secs. Here the quadrant duration (i.e. the amount of time the animals spent on each quadrant) was recorded. Also frequency of annulus crosses(i.e.the number of time the animals crosses the particular location where the platform was placed during the acquisition and reversal training) was recorded. At the completion of the trail the animals where then scoped out of the maze using the small container and placed in its appropriate holding cage to dry and then returned to their home cages. It is believed that animals with a good visuo-spatial memory will spend more time in the quadrants where the escape platform was located.

On the visible platform day (day 8), the platform is placed in a new quadrant or location (North West quadrant) but this time made visible through the attachment of a colorful detachable flag to the top of the platform. The same procedures as in acquisition and reversal training were repeated as each of animal received and completed four trials.

Table: chart showing the different start locations or points during the m..w.m test

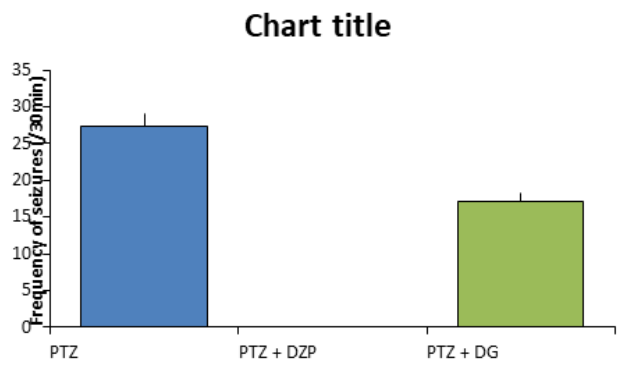
DAY	POINT 1	POINT 2	POINT 3	POINT 4
1	North	South	East	West
2	South	East	West	North
3	East	West	North	South
	West	North	South	East
5	South	West	East	North
6	North	East	West	South
8	East	South	North	West

Results

Otitis Anti-Epileptic Test Results

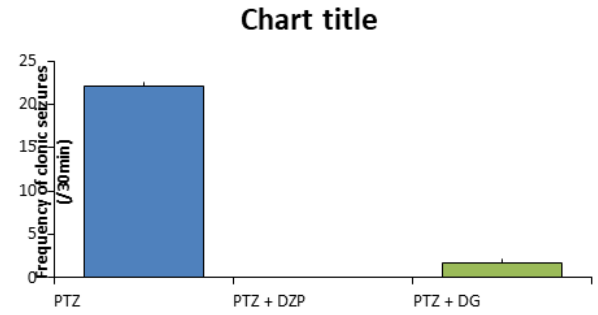
Comparison of frequency of seizures in the anti-epileptic test following treatment with pentylenetetrazol (PTZ) alone (to induce epilepsy), PTZ + Diazepam (DZP) and PTZ + ethanol leaf extract of Dichrostachys glomerata (DG).

The frequency of seizures in the PTZ-induced epileptic mice (PTZ), PTZ- induced epileptic mice treated with diazepam(PTZ + DZP), and PTZ-induced epileptic mice treated with ethanol extract of D. glomerata(PTZ+DG) groups were 2.79±1.71, 0.00±0.00, 17.17±1.17 respectively. The result shows that frequency of seizures for the PTZ+DZP group was significantly ($p<0.001$) lower compared to control. Also the frequency of seizures for the PTZ+DG group was significantly ($p<0.001$) lower compared to control but significantly ($p<0.05$) higher compared to PTZ+DZP group significant at $p< 0.001$ compared to control; b – significant at $p<0.05$ vs PTZ + DZP



Comparison of frequency of clonic seizures in the anti-epileptic test following treatment with pentylenetetrazol (PTZ)alone (to induce epilepsy), PTZ + Diazepam (DZP) and PTZ + ethanol leaf extract of Dichrostachys glomerata (DG).

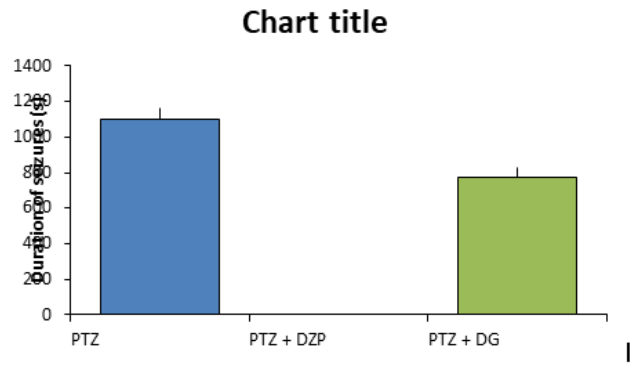
The frequency of clonic seizures in the PTZ-induced epileptic mice (PTZ), PTZ- induced epileptic mice treated with diazepam(PTZ + DZP), and PTZ-induced epileptic mice treated with ethanol extract of D. glomerata(PTZ+DG) groups were 1102.66±62.73, 0.00±0.00, 776.85±47.87 respectively. The result shows that frequency of clonic seizures for the PTZ+DZP group was significantly ($p<0.001$) lower compared to control. Also the frequency of clonic seizures for the PTZ+DG group was significantly ($p<0.001$) lower compared to control but significantly ($p<0.05$) higher compared to PTZ+DZP group as shown below. Significant at $p< 0.001$ compared to control; b – significant at $p<0.05$ vs PTZ + DZP



Comparison of duration of seizures in the anti-epileptic test following treatment with pentylenetetrazol (PTZ) alone (to induce epilepsy), PTZ + Diazepam (DZP) and PTZ + ethanol leaf extract of Dichrostachys glomerata (DG).

The duration of seizures in the PTZ-induced epileptic mice (PTZ), PTZ- induced epileptic mice treated with diazepam(PTZ + DZP), and PTZ-induced epileptic mice treated with ethanol extract of D. glomerata(PTZ+DG) groups were 22.14±0.40, 0.00±0.00, 1.67±0.42 respectively.

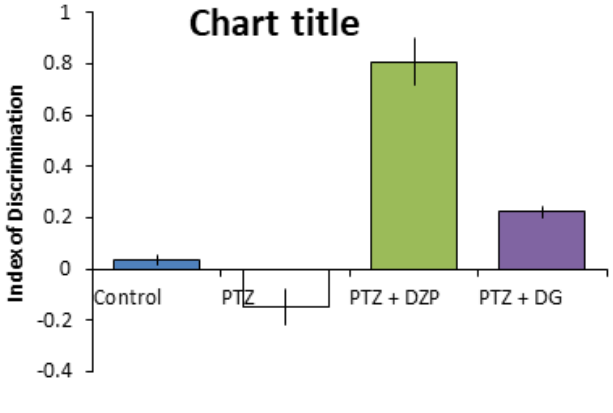
The result below shows that duration of seizures for the PTZ +DZP group was significantly ($p<0.001$) lower compared to control. Also the duration of seizures for the PTZ+DG group was significantly ($p<0.001$) lower compared to control but significantly ($p<0.05$) higher compared to PTZ+DZP group significant at $p< 0.001$ compared to control; b – significant at $p<0.05$ vs PTZ + DZP



Novel Object Recogniton Task (Short Term) Results
Comparison of the index of discrimination during the short term memory test in the novel object recognition task in (PTZ)-induced epileptic mice treated with diazepam or ethanol leaf-extract of Dichrostachys glomerata.

The index of discrimination(ID) in the control, PTZ-induced epileptic mice(PTZ), PTZ- induced epileptic mice treated with diazepam(PTZ + DZP), and PTZ-induced epileptic mice treated with ethanol extract of D. glomerata(PTZ+DG) groups were 0.03±0.02, -0.15±0.07, 0.81±0.09, 0.22±0.21 respectively.

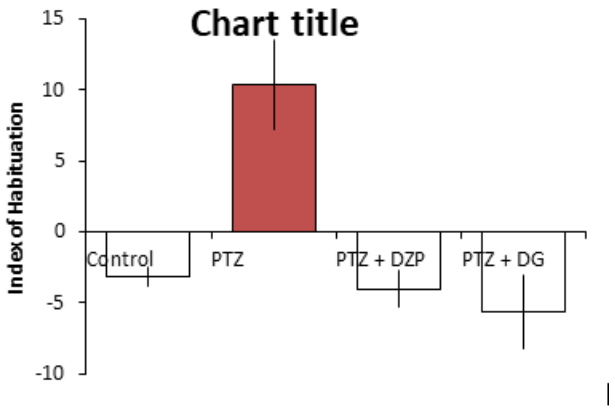
The result show that the ID for the PTZ group was significantly ($p<0.01$) lower compared to control. The ID for PTZ+DZP group was significantly ($p<0.01$) higher compared to control and also significantly ($p<0.001$) higher compared to PTZ group. Also the ID for the PTZ+DG group was significantly ($p<0.01$) higher compared to control and significantly ($p<0.05$) higher compared to PTZ group as shown in the figure below Significant at $p< 0.001$ vs control; ** - significant ant $p< 0.01$ vs control; b – significant at $p< 0.05$ vs PTZ group.



Comparison of the index of habituation during the short term memory test in the novel object recognition task in pentylenetatrazol (PTZ)-induced epileptic mice treated with diazepam or ethanol leaf-extract of Dichrostachys glomerata.

The Habituation index(HI) in the control, PTZ-induced epileptic mice(PTZ), PTZ- induced epileptic mice treated with diazepam(PTZ + DZP), and PTZ-induced epileptic mice treated with ethanol extract of D. glomerata(PTZ+DG) groups were -3.14±0.68, 10.38±3.17, -4.02±1.31, -5.65±2.63 respectively.

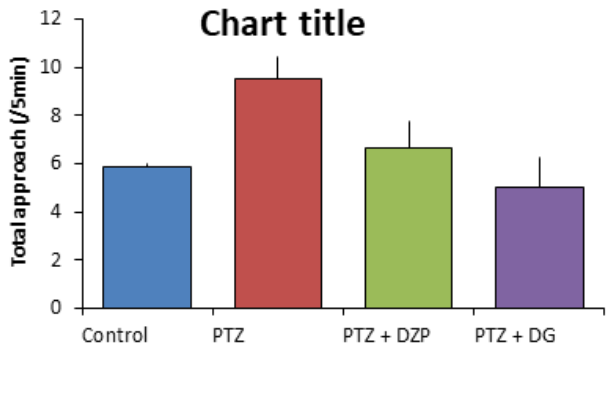
The result show that the HI for the PTZ group was significantly ($p<0.001$) higher compared to control. The HI for PTZ+DZP group was significantly ($p<0.05$) lower compared to PTZ group and also HI for the PTZ+DG group was significantly ($p<0.05$) lower compared to the PTZ group. This is clearly shown in the figure below Significant at $p< 0.001$ vs control; b – significant at $p< 0.05$ vs PTZ group.



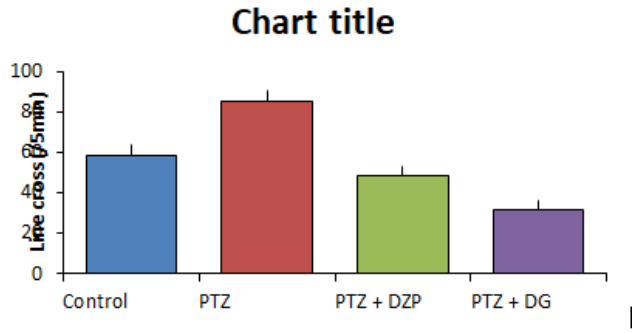
Comparison of the total number of approaches during the short term memory test in the novel object recognition task in pentylenetatrazol (PTZ)-induced epileptic mice treated with diazepam or ethanol leaf-extract of Dichrostachys glomerata.

The total number of approaches in the control, PTZ-induced epileptic mice(PTZ), PTZ- induced epileptic mice treated with diazepam(PTZ + DZP), and PTZ-induced epileptic mice treated with ethanol extract of D. glomerata(PTZ+DG) groups were -58.86±0.14, 9.50±0.95, 6.67±1.08, 5.00±1.29 respectively.

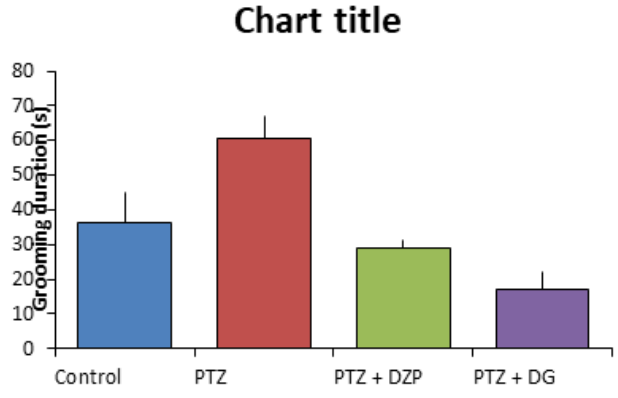
The result show that the total number of approach (tAP) for the PTZ group was significantly ($p<0.01$) higher compared to control. The tAP for PTZ+DZP group was significantly ($p<0.05$) lower compared to PTZ group. Also tAP for the PTZ+DG group was significantly ($p<0.05$) lower compared to the PTZ group as shown in the figure below significant at $p< 0.01$ vs control; b – significant at $p< 0.05$ vs PTZ group.



Comparison of the frequency of line crosses during the short term memory test in the novel object recognition task in pentylenetatrazol (PTZ)-induced epileptic mice treated with diazepam or ethanol leaf-extract of Dichrostachys glomerata. The frequency of line crosses in the control, PTZ-induced epileptic mice(PTZ), PTZ- induced epileptic mice treated with diazepam(PTZ + DZP), and PTZ-induced epileptic mice treated with ethanol extract of D. glomerata(PTZ+DG) groups were 58.26±5.55, 85.33±4.75, 48.33±4.72, 31.42±4.58 respectively. The result show that the frequency of line crosses for the PTZ group was significantly ($p<0.001$) higher compared to control. The line crosses for PTZ+DZP group was significantly ($p<0.05$) lower compared to control and also significantly ($p<0.05$) lower compared to PTZ group. Also the frequency of line crosses for the PTZ+DG group was significantly ($p<0.01$) lower compared to control and also significantly ($p<0.05$) lower compared to PTZ group. Significant at $p< 0.001$ vs control; ** - Significant at $p< 0.01$ vs control; * - significant ant $p< 0.05$ vs control; b – significant at $p< 0.05$ vs PTZ group.



Comparison of the duration of grooming during the short term memory test in the novel object recognition task in pentylenetatrazol (PTZ)-induced epileptic mice treated with diazepam or ethanol leaf-extract of Dichrostachys glomerata. The duration of grooming, PTZ-induced epileptic mice(PTZ), PTZ-induced epileptic mice treated with diazepam(PTZ + DZP), and PTZ-induced epileptic mice treated with ethanol extract of D. glomerata(PTZ+DG) groups were 36.17±8.87, 60.36±6.70, 28.99±2.34, 19.64±5.19 respectively. The result show that the grooming duration for the PTZ group was significantly ($p<0.05$) higher compared to control. The grooming duration for PTZ+DZP group was significantly ($p<0.05$) lower compared to PTZ group. Also the grooming duration for the PTZ+DG group was significantly ($p<0.05$) lower compared to the PTZ group. significant ant $p< 0.05$ vs control; b – significant at $p< 0.05$ vs PTZ group.



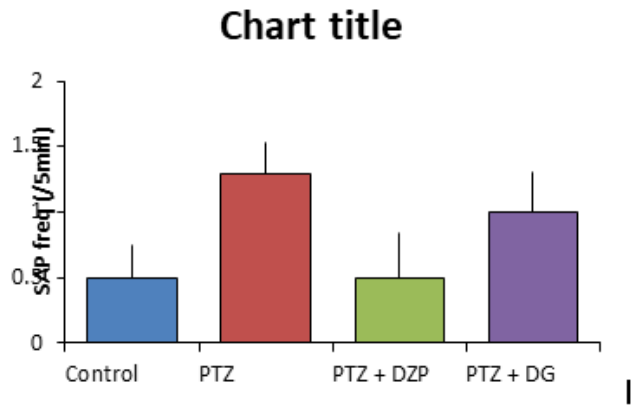
Comparison of the frequency of line crosses during the long term memory test in the novel object recognition task in pentylenetetrazol (PTZ)-induced epileptic mice treated with diazepam or ethanol leaf-extract of Dichrostachys glomerata.

The frequency of line crosses for the control, pentylenetetrazol induced epileptic mice (PTZ), pentylenetetrazol induced epileptic mice treated with diazepam (PTZ+DZP), and pentylenetetrazol induced epileptic mice treated with ethanol leaf extract of Dichrostachys glomerata (PTZ+DG)

groups were 30.86±4.36, 58.83±7.93, 55.17±10.78, 44.86±2.53 respectively. The result shows that the frequency of line crosses for the PTZ and PTZ +DZP groups are significantly higher ($p<0.01$) compared to control. Also that of the PTZ+DG group is also significantly higher ($p<0.05$) compared to control. Significant at $p< 0.01$ vs control; * - significant ant $p< 0.05$ vs control

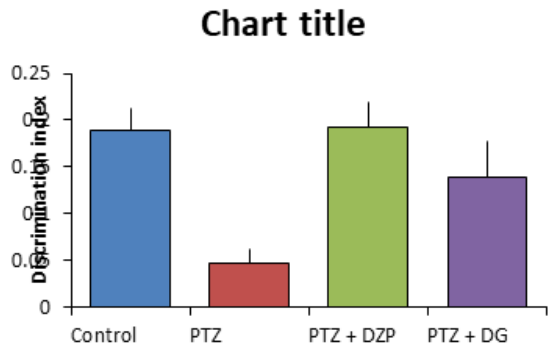
Comparison of the index of discrimination during the short term memory test in the novel object recognition task in (PTZ)-induced epileptic mice treated with diazepam or ethanol leaf-extract of Dichrostachys glomerata. The index of discrimination(ID) in the control, PTZ-induced epileptic mice(PTZ), PTZ- induced epileptic mice treated with diazepam(PTZ + DZP), and PTZ-induced epileptic mice treated with ethanol extract of D. glomerata(PTZ+DG) groups were 0.03±0.02, -0.15±0.07, 0.81±0.09, 0.22±0.21 respectively.

The result show that the ID for the PTZ group was significantly ($p<0.01$) lower compared to control. The ID for PTZ+DZP group was significantly ($p<0.01$) higher compared to control and also significantly ($p<0.001$) higher compared to PTZ group. Also the ID for the PTZ+DG group was significantly ($p<0.01$) higher compared to control and significantly ($p<0.05$) higher compared to PTZ group as shown in the figure below.



Comparison of the index of habituation during the short term memory test in the novel object recognition task in pentylenetatrazol (PTZ)-induced epileptic mice treated with diazepam or ethanol leaf-extract of Dichrostachys glomerata. The Habituation index(HI) in the control, PTZ-induced epileptic mice(PTZ), PTZ- induced epileptic mice treated with diazepam(PTZ + DZP), and PTZ-induced epileptic mice treated with ethanol extract of D. glomerata(PTZ+DG) groups were -3.14±0.68, 10.38±3.17, -4.02±1.31, -5.65±2.63 respectively.

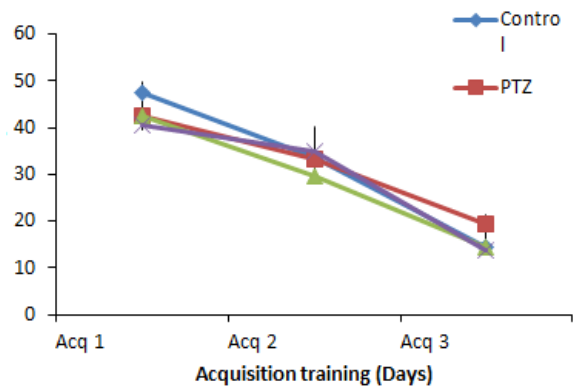
The result show that the HI for the PTZ group was significantly ($p<0.001$) higher compared to control. The HI for PTZ+DZP group was significantly ($p<0.05$) lower compared to PTZ group and also HI for the PTZ+DG group was significantly ($p<0.05$) lower compared to the PTZ group. This is clearly shown in the figure below NS – Not significant vs control; *** - Significant at $p< 0.01$ vs control;



Comparison of the swim latencies during the acquisition training of the Morris water maze task in pentylenetatrazol (PTZ)-induced epileptic mice treated with diazepam or ethanol leaf-extract of Dichrostachys glomerata.

The figure below shows the learning curves for the swim latencies during the acquisition training (Day 1-3) of the Morris water maze task in pentylenetatrazol (PTZ)-induced epileptic mice treated with diazepam or ethanol leaf-extract of Dichrostachys glomerata.

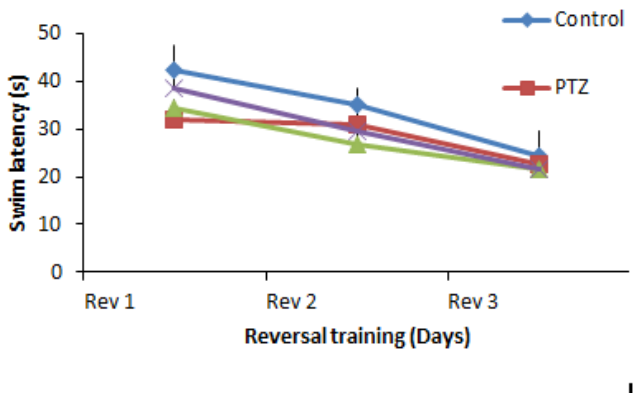
The result shows no significant difference in the trend of the learning curves and thus no significant difference in swim latencies between the groups and the control.



Comparison of the swim latencies during the reversal training of the Morris water maze task in pentylenetatrazol (PTZ)-induced epileptic mice treated with diazepam or ethanol leaf-extract of Dichrostachys glomerata.

The figure shows the learning curves for the swim latencies during the reversal training(Day 4-6) of the Morris water maze task in pentylenetatrazol (PTZ)-induced epileptic mice treated with diazepam or ethanol leaf-extract of Dichrostachys glomerata.

The result shows no significant difference in the trend of the learning curves and thus no significant difference in swim latencies between the groups and the control. However, there is a characteristic minimal reduction in swim latencies in all the groups.

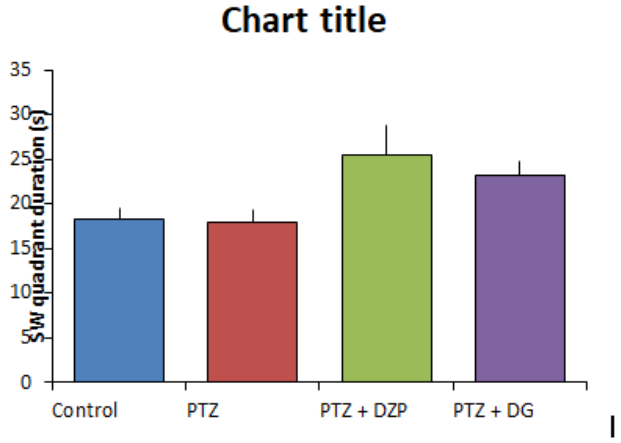


Comparison of the South West (retention) quadrant on the probe trial of the Morris water maze task in pentylenetatrazol (PTZ)-induced epileptic mice treated with diazepam or ethanol leaf-extract of Dichrostachys glomerata.

The south West (retention) quadrant duration during the probe trial for the control, pentylenetatrazol induced epileptic mice (PTZ), pentylenetatrazol induced epileptic mice treated with diazepam (PTZ+DZP), and pentylenetatrazol induced epileptic mice treated with ethanol leaf extract of Dichrostachys glomerata (PTZ+DG) groups were 18.26±1.31, 17.92±1.46, 25.50±3.35, 23.17±1.60 respectively.

The result shows that the south West (retention) quadrant duration for the PTZ group is not significant to control. That of the PTZ+DZP is however significantly higher (p>0.05) compared to control and also significantly higher (p<0.05) compared to PTZ group. It also shows that that of the PTZ+DG group is significantly higher (p<0.05) compared to control and also significantly higher (p<0.05) compared with the PTZ group.

NS – Not significant vs control; * - significant ant p< 0.05 vs control; b – significant at p< 0.05 vs PTZ group.

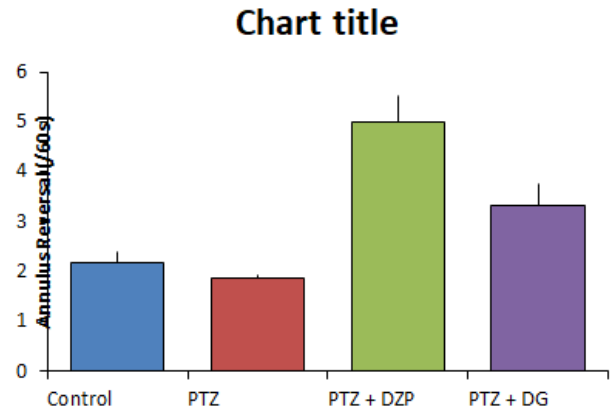


Comparison of annulus reversal crossings during the probe trial of the Morris water maze task in pentylenetatrazol (PTZ)-induced epileptic mice treated with diazepam or ethanol leaf-extract of Dichrostachys glomerata.

The annulus reversal crossings during the probe trial for the control, pentylenetatrazol induced epileptic mice (PTZ), pentylenetatrazol induced epileptic mice treated with diazepam (PTZ+DZP), and pentylenetatrazol induced epileptic mice treated with ethanol leaf extract of Dichrostachys glomerata (PTZ+DG) groups were 2.17±0.23, 1,86±0.08, 5.00±0.52, 3.33±0.42 respectively.

The result shows that the annulus reversal crossings for the PTZ group is significantly lower (p<0.01) compared to control. That of the PTZ+DZP is however significantly higher (p>0.05) compared to control and also significantly higher (p<0.05) compared to PTZ group. It also shows that that of the PTZ+DG group is significantly higher (p<0.05) compared to control and also significantly higher (p<0.05) compared with the PTZ group as shown in the figure below.

NS – Not significant vs control; ** - significant ant p< 0.01vs control; * - significant ant p< 0.05 vs control; b – significant at p< 0.05 vs PTZ group.

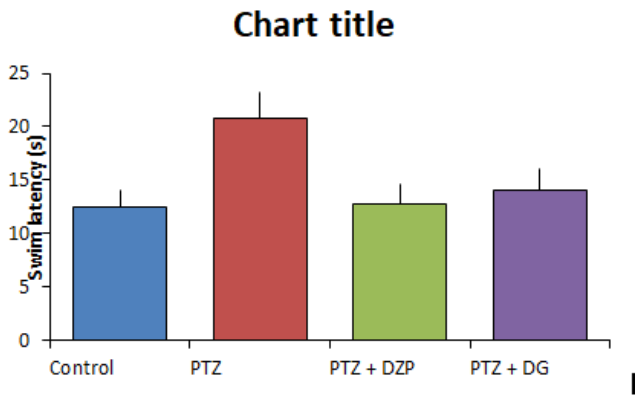


Comparison of swim latencies during the visible platform task of the Morris water maze task in pentylenetatrazol (PTZ)-induced epileptic mice treated with diazepam or ethanol leaf-extract of Dichrostachys glomerata.

The swim latencies during the visible platform task for the control, pentylenetatrazol induced epileptic mice (PTZ), pentylenetatrazol induced epileptic mice treated with diazepam (PTZ+DZP), and pentylenetatrazol induced epileptic mice treated with ethanol leaf extract of Dichrostachys glomerata (PTZ+DG) groups were 12.47±1.66, 20.82±2.40, 12.76±1.93, 14.00±1.10 respectively.

The result shows that the of swim latencies during the visible platform task for the PTZ group is significantly higher (p<0.01) compared to control. That of the PTZ+DZP is however significantly lower (p>0.05) compared to PTZ. It also shows that that of the PTZ+DG group is significantly lower (p<0.05) with the PTZ group as shown in the figure below.

Significant at p< 0.01vs control; b – significant atp< 0.05 vs PTZ group.



Discussion

This study was designed to study the effect of the ethanol leaf extract of Dichrostachys glomerata on memory and learning in Pentelenetetrazol kindled epileptic mice. Epilepsy was induced in the mice using an intraperitoneal injection of pentylenetetrazol (60mg/kg). The effect of the extract on cognitive memory status in the epileptic mice was investigated using the Novel object recognition task (NORT) while the effect on visuo-spatial learning and memory was studied using the Morris water maze (MWM). The anti-epileptic activities recorded during the anti-epileptic study were frequency of seizures, duration of seizures and frequency of clonic (rapid) seizures.

The results obtained from the anti-epileptic test showed that the animals that were treated with diazepam (1.0mg/kg) before induction of epilepsy showed no seizure activity. However, seizure activities in the animals that were treated with ethanol leaf extract of Dichrostachys glomerata (4.5mg/kg) was found to be significantly lower when compared to the epileptic mice group (those that received no treatment prior to epilepsy induction).

The exact mechanism of action of the anti-epileptic effect of the ethanol leaf extract of Dichrostachys glomerata is still unclear. However possible explanations of the anti-epileptic effect of the ethanol leaf extract of the plant can be traced to its high flavonoids and tannins (phytochemicals) contents which has been reported to have sedative or depressive effects on the central nervous system (CNS). This argument is in line with the findings of Anna et al; (2011) who reported in their study the CNS depressant effects of quercetin and penta-o-ethylquercetin (flavonoid compounds). Also Cho, S., et al (2012) in their epileptic study, reported that phlorotannin(a tannin compound) showed a significant anticonvulsive effect on picrotoxin induced epileptic mice through positive allosteric modulation of GABA(A)-BZD receptors (like diazepam).

The novel object recognition task (NORT) was originally developed to test recognition memory in mice following the discovering that rats spends more time investigating or exploring a new objects than a familiar one (Hashemi-Firouzi et al; 2015). The results obtained during the short term recognition memory test showed that the index of habituation (i.e. time spent exploring objects in trial 1 – time spent exploring objects in trial 2) was significantly higher in the PTZ group compared to the control. However it was found to be significantly lower (more negative) in PTZ+DZP and PTZ+DG groups compared to control. This implies therefore that the epileptic mice group (PTZ group) had no preference for the new or novel object in the retention trial thus indicating impairment in cognitive memory function.

The index of discrimination (i.e time spent exploring new objects- time spent exploring familiar object/ time spent exploring novel object + time spent exploring familiar object) during the short term trial was seen to be significantly lower in the PTZ group compared to control but however significantly in both PTZ+DZP and PTZ+DG groups compared to both control and PTZ groups. During the long term retention trial, the index of discrimination in the PTZ group was significantly lower compared to control. There was no significant difference in the PTZ+DZP and PTZ+DG groups compared to control, however both were significantly higher compared to the PTZ group.

Normally a higher index of discrimination but lower index of habituation depicts good cognitive memory status(and vice versa), thus the results indicates an impaired cognitive memory in the PTZ mice group but on the contrary an improved or enhanced cognitive memory status in the epileptic mice groups treated with diazepam and ethanol leaf extract of DG. Therefore, the ethanol leaf extract of DG improved cognitive memory in PTZ-induced epileptic mice.

Visuo-spatial memory status was assessed in the mice using the morris water maze(MWM). Here the mice were trained to use extra maze cues to locate an escape platform hidden in the different quadrants of the maze. The results obtained during the Acquisition and Reversal trials showed that there was no significant difference in swim latencies amongst all the mice groups. The trend of the learning curves in all the groups indicates a normal decrease in swim latencies following days of training in the acquisition and reversal trials.

On the probe trial day, there was no significant difference in the mean South West (SW) quadrant duration in the PTZ group compared to control. However, the mean SW quadrant duration in the PTZ+DZP and PTZ+DG groups were significantly higher compared to the PTZ group. Also the annulus crosses (the frequency at which the mice crosses the exact location in the quadrant were the hidden escape platform was placed) was significantly lower in the PTZ group compared to control but significantly higher in the PTZ+DZP and PTZ+DG groups respectively(with PTZ+DZP being higher) compared to the PTZ group.

It is usually expected that mice with good visuo-spatial memory will spend more time in quadrants where the platform was located during the acquisition and reversal training and also make more crosses over the exact spots where the escape platform was placed.

On the visible platform trial day, the mean swim latency in the PTZ group was seen to be significantly higher compared to control but significantly lower in the PTZ+DZP and PTZ+DG groups compared to control. The results thus shows that the visuo-spatial memory that was impaired in the PTZ group was

improved in the epileptic mice groups treated with DZP an ethanol extract of DG.

Conclusion

In whole, this research study results suggest that the ethanol leaf extract of Dichrostachys glomerata improves cognitive and visuo-spatial memory that is impaired in PTZ-induced epilepsy in mice. This study findings thus suggests that the plant of study can in the near future serve as a raw material for use in the production of anti-epileptic medications.

The precise or exact underlying mechanism of the anti-epileptic effects of the ethanol leaf extract of DG observed in this study remains unclear. However, two possible explanations proceed as follows. First, the pharmacological effect of PTZ appears to be mediated through a specific interaction with the GABA-gated chloride ionophore leading to a decrease in the biochemical indices of central GABAergic function (M. Ganzella et al., 2011;Anna et al,. 2011)). On the contrary, the pharmacological action of diazepam enhances the effect of GABA (the major brain inhibitory neurotransmitter) leading ultimately to CNS depression (Riss et al., 2008).It is possible therefore that the anti-epileptic effects of ethanol leaf extract of DG operates via a mechanism similar to that of diazepam. The second possibility may be due its high flavonoids and tannins (pyhtochemicals) constituents which have been found to have CNS depressant effects as mentioned earlier. However, these are only assumed possibilities, therefore i strongly recommend that further studies be conducted to unravel the exact mechanism of action of this anti-epileptic agent.

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