Evaluation Of The Protective Effect Of Hydro-Methanolic Extract Of Tiger Nut (*cyperus esculentus l.*) On Pentylenetetrazole Induced Seizures In Mice

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Abstract: Epilepsy is a common neurological disorder with an incidence of 3% in the general population. The prevalence is higher in developing countries where most people still rely on herbal medicine for the management due to cost, side effect and drug interactions associated with orthodox antiepileptics. Tiger nut has been reportedly used in the management of febrile convulsion in children. This study therefore, aims to evaluate the protective effect of tiger nut extract in pentylenetetrazole (PTZ) induced seizures in mice. Twenty-five mice were used for the study, divided into five groups of five mice each. Group I served as the control and received distilled water(1ml/kg) only, group II received 200mg/kg of sodium valproate (SV), an antiepileptic drug, and groups III, IV and V received 500mg/kg, 1000mg/kg and 2000mg/kg of the tigur nut extract respectively, intraperitoneal. Each group received 65mg/kg of PTZ (i.p) for induction of seizures one hour after treatments and the latency to onset (LO) and duration of seizure (DS) were recorded. The results showed that, LO and DS were significantly reduced in the treatment groups compared to the control. The protective effect was greater than SV at highest dose (2000mg/kg) tested. The findings in this study suggest that tiger nut may be beneficial in epilepsy.
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Key words: pentylenetetrazole, seizure, *Cyperus esculentus*, epilepsy

1. Introduction

Epilepsy is one of the most common neurological disorders with an incidence of 3% in the general population (Annegers, 2001) and the most frequent neurological afflictions in men characterized by excessive temporary neuronal discharges resulting in uncontrolled convulsion. It inflicts more than 60 million people worldwide (Nikalje *et al*., 2012). The prevalence is higher in developing countries due to low economic status and limited access to health care (Banarjee *et al 2009*) were most people still rely on herbal medicine for the management because the Conventional anti-epileptic drugs (AEDs) are often associated with serious side effects. Currently available anti-epileptic drugs (AEDs) do not provide cure nor prevent relapse and they are often associated with serious side effects, including teratogenicity, chronic toxicity and adverse effects on cognition and behaviour (Sayyah *et al*., 2011). Consequently, many people living in developing countries still rely on herbal medicine for management of epilepsy. The clinical effectiveness, minimal side effect profile and relatively low costs of herbal drugs are the reasons for their various applications in traditional medicine (Valiathan, 1998). Medicinal plants have contributed considerably to the ethno-therapeutics and drug development all over the world from ancient times to the present day. However, only limited efforts have been made to evaluate the potentials of such plants for their use in modern medicine or to scientifically justify their traditional use in the treatment of central nervous system (CNS) disorders including epilepsy (Yaro *et al*., 2007).

Tiger nut (*Cyperus esculentus (L*) (CP), is an underutilized plant of the family Cyperaceae, which produces rhizomes from the base and tubers that are somewhat spherical (Cortes *et al*., 2005). The plant is not really a nut but a tuber first discovered some 4000 years ago (Lowe and Whitewell, 2000). It has other names like yellow nutsedge, chufa, flatsedge, rush nut, water grass, earth almond, northern nut grass and nut grass (Shilenko *et al*., 1979). It is known in Nigeria as aya in Hausa, ofio in Yoruba and akihausa in Ibo. It grows mainly in the middle belt and northern regions of Nigeria (Okafor *et al*., 2003), where three varieties (black, brown and yellow) are cultivated (Umerie *et al*., 1997). Among these, only two varieties, yellow and brown are readily available in the market. The yellow variety is preferred to all other varieties because of its inherent properties such as bigger size, attractive colour and fleshier body (Belewu and Abodurin, 2006).

Phytochemical screening showed a higher content of alkaloids, sterols and resins than

cyanogenic glycosides, saponins and tannins (Chukwuma *et al.*, 2010). *Cyperus esculentus* has been reported in preventing heart disease, thrombosis and activates blood circulation. It helps in preventing cancer, due to high content of soluble glucose. It was also found to assist in reducing the risk of colon cancer (Adejuyitan *et al*., 2009). The nut is rich in energy content (starch, fat, sugars and protein), mineral (phosphorus, potassium) and vitamins E and C (Belewu and Belewu, 2007). The search for perfect antiepileptic compound with more selective activity and lower toxicity continues to be an area of intensive investigation in medicinal chemistry (Nikalje, *et al.,* 2012).

2. Materials and Methods

Drugs:

Petylenetetrazole (PTZ) used to induce seizure, sodium valproate (SV) a standard antiepileptic drug, distilled water.

Preparation of Extract:

Fresh nuts of *Cyperus esculentus* were obtained from Samaru market, Zaria in Kaduna state. The fresh nuts were authenticated in the Herbarium Section of the Department of Biological Science, Ahmadu Bello University Zaria by comparing the existing specimen (No 348). The hydromethanolic extraction was carried out according to the modified method of Su *et al.*, (2011).

Acute Toxicity Study

The median lethal dose (LD_{50}) was estimated as described by Lorke (1983). In the initial phase, 3 groups of three animals each were treated with the hydro-methanolic extract of the plant at doses of 10, 100, 1000 mg/kg body weight through oral administration and observed for 24 hours for signs of toxicity In the second phase, 4 groups of one animal each were given the methanolic extract at doses of 1200, 1600, 2900 and 5000mg/kg through oral administration based on the outcome of the first phase. The median lethal dose (LD_{50}) was determine as the geometric means of the highest non lethal dose (for which the animal survived) and the lowest lethal dose.

Animals

Twenty-five Swiss Albino Mice weighing 18-22 grams were obtained from the Animal House of Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria. The animals were housed in laboratory cages and provided with vital feed (protein 14%, carbohydrate 35%, fats 7%, produced by Grand Cereals, Jos, Plateau state) and water, *ad libitum.* They were allowed to acclimatize to the laboratory condition under twelve (12) hours light/darkness cycle for 3 weeks before the commencement of the experiment. They were assigned into five groups of five animals each. Group I served as the negative control group (i.e. the untreated group) and they were given 1ml/mice of distilled water through oral administration followed by PTZ, one hour later through subcutaneous route. Group II served as the positive control group and were pretreated with 200mg/kg of sodium valproate through oral administration. Group III, IV and V orally received 500mg/kg, 1000mg/kg and 2000mg/kg of extract respectively, after which they were administered with pentylenetetrazole (65mg/kg) to induce the seizure. Latency of onset (LO) and duration of seizures (DS) were observed over 24 hours after 30 minutes of induction of the seizure.

Statistical Analysis

All data were expressed as mean ± SEM. Data were analysed using one-way Analysis of Variance (ANOVA) followed by Dunnett's test. Results were considered significant at $p < 0.05$.

3. Results:

The extraction of 600g of the fine powder of *Cyperus esculentus* yielded 53.72 grams. On day one (Fig 1), the latency of seizure onset in the positive control group (group two)was not significant in relation to the negative control group (group one) but the duration of seizure in the positive control group was significance when compared with the negative control group. In the experimental groups, the onset of seizures in group three which received low dose of extract (500mg/kg) showed no significance when compared with negative control as well as the positive control but the duration of seizures were significant only in relation to the positive control. The onset and duration of seizures in group four which received a medium dose of the extract (1000mg/kg) were all significant (longer onset and shorter duration of seizures) when compared with both negative and positive control groups. Thus, the medium dose delays the onset and decrease the duration of seizure as compared to the positive and negative control groups. However, in group five which received a high dose of the extract (2000mg/kg), the onset of seizures were not significant in relation to both negative and positive control groups but the duration of seizures were significant when compared with the negative control group only.

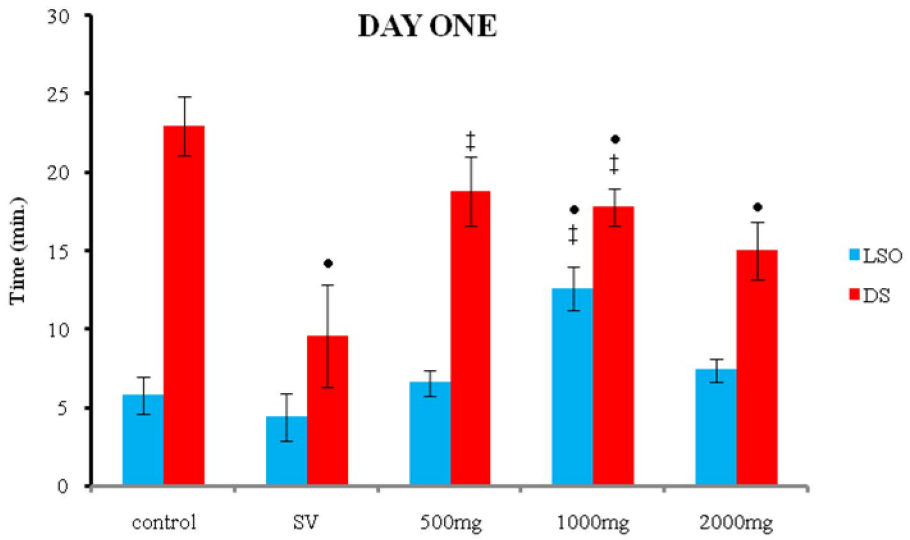

Fig. 1 A graph showing the onset and duration of seizures on the first day.
SV= Sodium valproate, LSO= Latency of seizure onset, DS=Duration of seizure.
● $p<0.05$ vs. control group and ‡ $p<0.05$ vs. SV.

On day two (Fig. 2), group two which received the standard drug were significantly higher and lower in both the onset and duration of seizure respectively as compared with group one. The onset of seizures in the experimental groups were significantly higher when compared with the positive control in group two which received standard antiepileptic drug (sodium valproate), but they were not significant when compared with the negative control in group one which receive only PTZ. The duration of seizures were significantly higher in the experimental groups when compared with the negative control, but they were not significant when compared with the positive control.

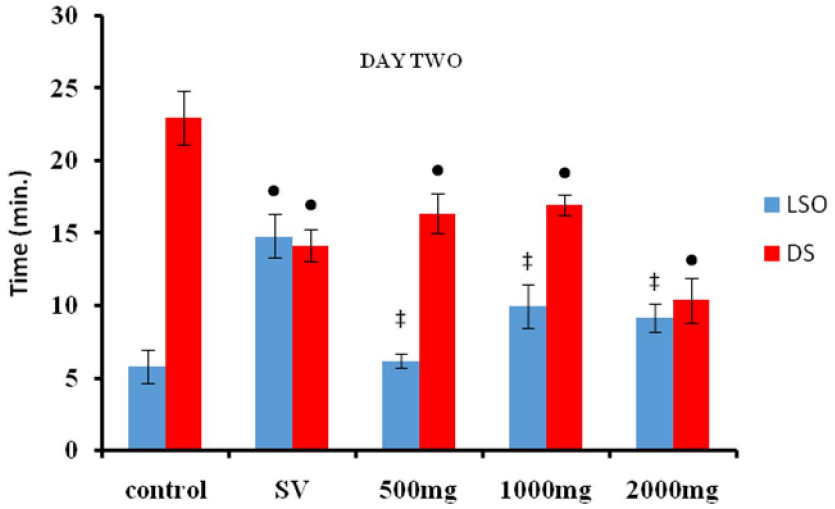

Fig. 2. A graph showing the onset and duration of seizure on the second day
SV= Sodium valproate, LSO= Latency of seizure onset, DS=Duration of seizure.
● $p<0.05$ vs. control group and ‡ $p<0.05$ vs. SV.

On day three (Fig.3), the latency of seizure onset showed no significance in the positive control group which received sodium valproate when compared with the negative control group (group one), but the

duration of seizures were significant in relation to the negative control. The onset of seizures in all the experimental groups showed no significance when compared with both negative and positive control groups but the duration of seizures were significantly lower in the respective groups when compared with the negative control group (Group one).

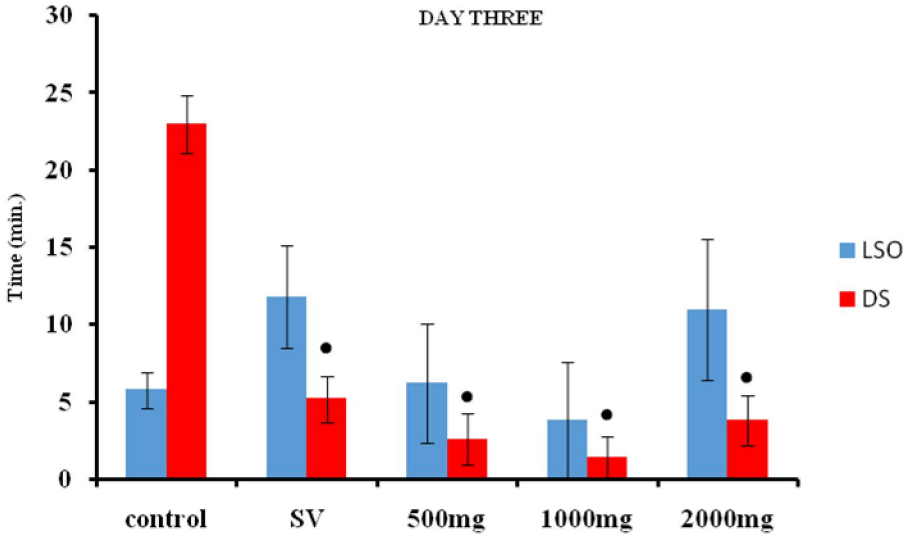

Fig. 3. A graph showing the onset and duration of seizure on the third day.
SV= Sodium valproate, LSO= Latency of seizure onset, DS=Duration of seizure.
● $p<0.05$ vs. control group and ‡ $p<0.05$ vs. SV.

4. Discussion

The present study investigated the antiepileptic activity of hydro-methanolic extract of tiger nut (*Cyperus esculentus L.*) against pentylenetetrazole induced seizure in mice. In PTZ induced seizure, the methanolic extract of tiger nut showed significant increase in the latency of seizure onset and a reduction in the duration of seizure as compared to the negative control as well as the positive control. PTZ may be exerting its convulsant effects by inhibiting the activity of gamma amino butyric acid (GABA) at $GABA_A$ receptors (De Sarro, *et al*., 1999). Gamma amino butyric acid is the major inhibitory neurotransmitter which is implicated in epilepsy. The enhancement and inhibition of the neurotransmission of GABA will attenuate and enhance convulsion, respectively (Gale *et al*., 1992). Sodium valproate, the standard antiepileptic drug protected the animals against the convulsion induced by PTZ by inhibiting GABA transaminase, an enzyme which degrade GABA, thereby, prolonging the activity of GABA. (Brodie *et al,* 2011).

Since the methanolic extract of *Cyperus esculentus* nuts has exhibited significant anticonvulsant activity, it is possible that it may be interfering with gabaergic mechanism(s) to exert its anticonvulsant effect.

Phytochemical investigation of methanolic extract of *Cyperus esculentus* revealed the presence of alkaloids, sterols, resins, vitamins, fats and carbohydrates with a trace quantity of tannins and saponins. The alkaloids are known to possess various actions on central nervous system. Some alkaloids have stimulatory effect (e.g. caffeine, nicotine etc.) while some class of alkaloids have depressive effect on the central nervous system (e.g. morphine, codeine etc.) (Avallone *et al*., 2000). Yazdi, *et al*. (2012) suggested that alkaloids present in the leaves of *Glycyrrhiza glabra* var. *glandulifera* showed some anticonvulsant activity. Plant sterols were also reported to have an anti-aging and neuroprotective effect functions. (Sun, *et al*., 2014). Resins from asafoetida plant was also shown to possess antiepileptic activity. (Prashant, *et al*., 2014). The presence of vitamin C in tiger nut extract may also contribute to its anticonvulsive activity, this agrees with a research conducted by Misael *et al,* (2015). Vitamin C helps to prevent brain oxidative stress and act synergistically with progesterone in the brain. Researches have demonstrated that tannins and saponins have some antiepileptic effects (Luciana *et al*., 2010). Hence, the presence of alkaloids, sterols, resins, vitamins, fats, tannins and saponins in methanolic extract of *Cyperus esculentus* could be attributed for the observed significant anticonvulsant

activity. The mechanism by which this components act is still controversial. Advancement in understanding pathophysiology of epilepsies in terms of cellular physiology and genetics would allow for more judicious therapeutic approaches to this complex neurological disorder (White and Loscher, 2014; Jacob *et al.,* 2009).

The present study demonstrated that the hydro-methanolic extract of tiger nut has protective effect against PTZ-induced seizures in mice.

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References

1. Adejuyitan, J.A., Otunola, E.T., Akande, E.A., Bolarinwa, I.F. and Oladokun, F.M. (2009). Some Physicochemical Properties of Flour Obtained from Fermentation of Tigernut (Cyperus esculentus) Sourced from a Market in Ogbomosho. *Nigeria. African. Journal of Food Science*. 3: 51-55.
2. Almashhadania, A.M. and Al-Essawe, M.A. (2010). The Effect of Cyperus esculentus on Sperm Function Parameters in Prepubertal Mice as a Good Model for Human. *Journal of Baghdad for Science*. 7: 389-393.
3. Annegers, J.F. (2001). The Epidimiology of Epilepsy. In Wyllre, Ed. *The Treatment of Epilepsy*: Principle and Practice. 3rd ed. Philadelphia: *Lippincott Williams and Watking*. Pp 131-138.
4. Avallone, R., Zanoli, P. (2000). *Biochemistry and Pharmacology*. 1:1387-94.
5. Banarjee, P.N., Filippi, D., Allen, H.W. (2009). The descriptive epidemiology of epilepsy. A review. *Epilepsy. Res*. 85:31-45.
6. Belewu, M.A. and Abodurin, A.O. (2006). Preparation of Kuunu from Unexploited Rich Food Source: Tigernut (Cyperus esculentus L). *Pak. Journal of Nutrition*. 7: 109-111.
7. Belewu, M.A. and Belewu, K.Y. (2007). Comparative physicochemical evaluation of tigernut, soybean and coconut milk sources. *International Journal of Agriculture and Biology*. Vol 5: Pp. 785-787.
8. Brodie, M. J., Covanis, A. and Gil-Nagel, A. (2011). Antiepileptic drug therapy: does mechanism of action matter? *Epilepsy Behaviour*. 21 (6): 331-41.
9. Chukuma, R.E., Njoku, O. and Onongbu, I.C. (2010). The Phytochemical Composition and Some Biochemical Effects of Nigerian Tiger nut *(Cyperus esculentus L.*) Tuber. *Pakistan Journal of Nutrition* 9 (7): 709-715.
10. Cortes, C., M. Estere, A. Frigola and F. Torregrosa (2005). Quality characteristics of Horchata (a Spanish vegetable beverage) treated with pulsed electcric field during shelf life. *Food Chemistry*. 91 (12): 319-315.
11. De Sarro, A., Cecchetti, V., Fravolini, V., Naccari, F., Tabarrini, O. and De Sarro, G. (1999). Effects of novel 6-desfluoroquinolones and classic quinolones on pentylenetetrazole-induced seizures in mice. *Antimicrobial Agents and Chemotherapy*. 43: 1729–1736.
12. Gale, K. (1992). GABA and epilepsy: basic concepts from preclinical research. *Epilepsia*. 33: 3–12.
13. Jacob, M.P; Lablanc, G.G; Brooks-Kayal, A; Jensen, F.E; Lowestein, D.D; Noebels, J.L; Spencer, D.D; Swan J.W. (2009). Curing epilepsy: progress and future directions. *Epilepsy Behaviour 14 (3):438-445*.
14. Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*. 54: 275-287.
15. Lowe, D.B. and Whitewell, T. (2000). Yellow nutsedge (*Cyperus esculentus*) management and tuber reduction in Bermuda grass turf with selected herbicide programs. *Weed Technology*. 14:72-76.
16. Luciana, C.B.F. and Carlos, C.C. (2010). Anticonvulsant activity of extracts of *Plectranthus barbatus* leaves in mice. *Evidence-Based Complementary and Alternative Medicine* 1 (1): 54-55.
17. Misael, G., Razo-Tuarez, L., Sauer-Ramires, J.L., Gonzalez-Trujano, M.E., Salgado-Ceballos, H. and Oronzco-Suarez, S. (2015). Anticonvulsive effect of vitamin c on pentylenetetrazole-induced seizures in immature rats. *Pharmacology Biochemistry and Behavior*. 97(2) 267-272.
18. Nikalje, A.G., Altamash, A. and Ghodke, M.S. (2012). Herbal Anticonvulsant Agents. A Brief Review. *International Journal of Research in Pharmacy and Science*. 2. (3): 1-13.
19. Okafor, J.N.C., Mordi, J.I., Ozumba, A.U., Solomon, H.M. and Olatunji, I. (2003). Preliminary studies on the Characterization of contaminants in Tigernut (yellow variety). *In*

proceeding of 27th annual Nigeria Institute of food science and technology (NIFEST) conference. 13 (17): 210-211.
20. Oladele, A.K. and Aina J.O. (2007). Chemical Composition and Functional Properties of Flour Produced from Two Varieties of Tigernut (Cyperus esculentus). *African Journal of Biotechnology*. 6: 2473-2476.
21. Osonoe, K., Mori, N., Suzuki, K., Osonoe, M., (1994). Antiepieptic effects of inhibitors of nitric oxide synthase examined in pentylenetetrazone induced seizures in rats. Brain Research 663:338-340.
22. Prashant, P.S., Divakar, K., Goli, D. and Mishra, A. (2014). Anxiolytic and Antiepileptic effects of asafoetida oleo-gum resin. *World journal of pharmaceutical research*. 3(6):793-802.
23. Sayyah, M., Khodaparast, A., Yazdi, A. and Sardari, S., (2011). Screening of the anticonvulsant activity of some plants from Fabaceae family in experimental seizure models in mice. Daru. (19):4 (301—305).
24. Shilenko, M.P., Kalacheva, G.S., Lisovski, G.N. and Trubachev, I.N. (1979). *Cyperus esculentus* L. as a source of vegetable oil in a closed life support system. KOSM Biology. *Aviakosm Medicine.* (13): 70-74.
25. Sun, Y., Lin, Y., Cao, X., Xiang, L. and Qi, J. (2014). Sterols from *Mytilidae* show anti-aging and neuroprotective effects via anti-oxidative activity. *International journal of molecular science*. 15: 21660-21673.
26. Su, S., Wang, T., Chen, T., Duan, J., Yu, L. and Tang, Y. (2011). Cytotoxicity activity of extracts and compounds from *Commiphora myrrha* resin against human gynecologic cancer cells. *Journal of Medicinal Plants Research* 5 (8): 1382-1389.
27. Umerie S. C., Okafor P. E. and Uka A. S. (1997). Evaluation of Tubers and Oil of Cyperus esculentus. *Biresour. Technol*. 61: 171-173.
28. Valiathan, M.S. (1998). Healing Plants. *Current Science*. Vol. 75. No.10, 11: Pp 1122- 1126.
29. White, H.S., Loscher, W. (2014). Searching for the ideal antiepileptogenic agent in experimental models: single treatment versus combinatorial treatment stratergies. *Neurotherapeutics 11 (2):373-384.*
30. Yaro, A.H., Anuka, J.A., Salawu, O.A. and Magaji, M.G. (2007). Anticonvulsant activities of methanol extract of Chrysanthellum Indicum Linn. Vatke in mice and chicks. *Nigerian Journal of Pharmaceutical sciences*. 6 (2): 21-26.
31. Yazdi, A., Sardari, S., Sayyah, M. and Ezzati, M.H. (2012). Evaluation of the anticonvulsant activity of the leaves of *Glycyrrhiza glabra* var. *glandulifera* grown in Iran, as a possible renewable source for anticonvulsant compounds. *Iranian journal of pharmaceutical research.* 10 (1): 75-82.

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