ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 8| Issue: 4| April 2022|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2022: 8.205 || ISI Value: 1.188

ASSESSEMENT OF ANXIETY AND LOCOMOTIVE ACTIVITY USING ELEVATED PLUS MAZE AND OPEN FIELD TESTS IN A KONZO INDUCED RAT MODEL

David, L.K.[1], Ibeachu, P.C.[2], and Hart, J.S.[3]

[1,2,3]*Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Nigeria*

Corresponding Author: David, L.K, Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Rivers State, Nigeria.

Article DOI: https://doi.org/10.36713/epra9789
DOI No: 10.36713/epra9789

ABSTRACT

The aim of this study was to assess the anxiety and locomotive activity using elevated plus maze and open field tests in a konzo induced rat model. An experimental research method was used in this study. 20 male Wistar rats were used for this study. The twenty (20) albino Wistar rats were randomly selected into four groups of five rats each. Animal feeding was by oral ingestion. Animals were weighed with an electric weighing scale and the weights were recorded. Animals were closely observed for physical manifestations and clinical signs. Group 1 was the control group and the rats were fed with water and feed, group 2 was fed bitter cassava, group 3 was fed with bitter cassava and complain milk, while group 4 was fed with bitter cassava and bambara nut. The study lasted for 4 weeks and during this period, the weight and neurobehavioral assessment was done using weighing balance for weight determination, elevated plus maze and open field test for neurobehavioral assessment. Data was analyzed using Statistical Package for the Social Sciences (SPSS IBM version 23.0) and Microsoft excel 2019 edition. Values were expressed as mean±SD in descriptive statistics. One-way analysis of variance (ANOVA) was used to analyze the difference between the groups followed by least significant difference (LSD) post-hoc test. Confidence interval was set at 95%, and therefore p<0.05 was considered significant. The result shows weekly body weight differences in experimental animals. There was significant weight reduction in the body weight of the Konzo induced group experimental Wistar rats from week 1 to week 4 compared to the significant weight increase observed in the Konzo induced and complan milk fed group Wistar rats. The result from elevated plus maze showed that there was an improvement in anxiety level of Wistar rats fed with complan and bambara nut in terms of the time spent in the sections of the elevated plus maze in week 2 when compared to the week 1. In evaluating the locomotion impairment and anxiety level of wistar rats using Open Field Test, cassava-induced Konzo Wistar rat group, cassava-induced Konzo and complan milk fed group Wistar rats group and cassava-induced Konzo and Bambara nut (Okpa) fed Wistar rats group spent significantly more time in the open field apparatus in week 2 when compared to week 1. The improvement in anxiety and locomotor activity indicates the ameliorative effect of complan milk and Bambara nut (Okpa) and this is due to the protein content of complan milk and bambara nut. Hence a balance diet/protein containing food should be eaten along cassava processed foods.

KEY WORDS: ***Bitter cassava, Konzo, Elevated Plus Maze, Open Field Test, Anxiety, Locomotive Activity***

INTRODUCTION

Konzo is a distinct neurological entity with selective upper motor neuron damage, characterized by an abrupt onset of an irreversible, non-progressive, and symmetrical spastic para/tetraparesis [1]-[8]. The disease is associated with prolonged high dietary cyanogen consumption from insufficiently processed roots of bitter cassava combined with a protein-deficient diet low in sulphur amino acids (SAAs) [1]-[8]. Since its first description by the Italian doctor Trolli eight decades ago in the former Belgian Congo (now the Democratic Republic of Congo [DRC]), epidemics have been reported from many cassava-consuming areas in rural Africa. Up to 1993, the total of reported cases was approximately 3,700 to 4,000 [9]-[11]. Konzo remains a health problem in Africa. Since 1993, the disease has extended beyond its first reported boundaries [12], and the reported number of konzo cases has almost doubled,

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 8| Issue: 4| April 2022|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2022: 8.205 || ISI Value: 1.188

reaching a total of 6,788. Cassava is linked to a variety of neurological illnesses, including myeloneuropathy and konzo. Cassava use, for example, has been linked to epilepsy in Africa [13], as well as behavioral and emotional difficulties in Kenyan youngsters [14]. The paralytic disease konzo, which is little known in the West, has caused polio-like symptoms in thousands of the poorest individuals in the Democratic Republic of Congo (DRC) and other African countries [2]-[6]. Inadequately handled cassava, which naturally contains cyanide, can result in irreversible leg paralysis. Konzo forces its victims to rely on others for a living and causes them to crawl in the ground or use homemade crutches [1]. Due to a spinal cord condition, Konzo is an epidemic paralytic disease that inhibits movement. Several studies have been conducted in order to find a treatment for this disability; however, while no treatment has been discovered, it has been demonstrated that affected individuals gain greatly from rehabilitation and the use of appropriate walking aids [11]. Further research on locomotive assessment utilizing an elevated plus maze and open field test in a konzo induced rat is being conducted in light of this.

METHODOLOGY

Research Design: 20 male Wistar rats weighing between 200g to 250g used for this research work were acquired from the animal house of the Department of Anatomy. The twenty (20) albino Wistar rats were randomly selected into four (4) groups of five (5) rats each. The animals were kept in two separate cages (5 in a cage). Animals were allowed to acclimatize for 3 weeks in their cages, with pellet animal feed and water. After the three weeks of acclimatization, the rats where feed with bitter cassava (cassava induced Konzo group). Animal feeding was by oral ingestion. Animals were weighed with an electric weighing scale and the weights were recorded. Animals were closely observed for physical manifestations and clinical signs. Group 1 was the control group and the rats were fed with water and feed, group 2 was fed bitter cassava, group 3 was fed with bitter cassava and complain milk, while group 4 was fed with bitter cassava and bambara nut. The study lasted for 4 weeks and during this period, the weight and neurobehavioral assessment was done using weighing balance for weight determination, elevated plus maze and open field test for neurobehavioral assessment.

Plant Collection and Identification: The bitter cassava roots were collected from the Ministry of Agriculture, Agricultural Development Programme and were identified in the Faculty of Agricultural Science, University of Port Harcourt, Rivers State.

Inducing the rats with Konzo Disease: After two weeks of acclimatization, 15 Wistar rats were allowed to feed freely on inappropriately processed bitter cassava for the period of 4 weeks

Rehabilitation Group: After period of Konzo disease induction, the rehabilitation group (group 3 and group 4) were completely stopped from consuming the bitter cassava and replaced by feed + Complan for group 3 and Bambara nut (Okpa) for group 4. Mode of feeding was by oral ingestion.

PROCESSING OF BITTER CASSAVA

Procedure

1. Roots were cleaned in water to remove any soil clinging to them
2. Peel off the cassava roots were removed using a clean knife
3. The roots were cut into smaller pieces of chips (2-5cm thickness) to enhance the drying process and also to reduce the cyanide content of the cassava roots. Chopping of cassava roots was done in an open area with good ventilation so as to avoid sickness caused by the release of chemical from cassava.
4. Drying cassava chips: the pieces of cassava were placed on a metal tray and dried under direct sunlight for 1-2 days, by being placed on the concrete floor or rocky surfaces as rocks becomes very hot and will enable the chips to dry quickly. During drying, the cassava chips were turned over within every few hours to enable uniform drying. Drying was dependent on the weather; on a very sunny day drying will take up to ten hours (10hrs). minimal period of drying was used to ensure the cassava chips retain cyanide content and prevent mould growth
5. Grinding of dried cassava chips were done using a grinding machine, the chips were grounded smoothly until the powdered form was attained. This was done to ease digestion of the cassava by the rats.
6. Cassava induction: dried powdered cassava was weighed to measure 86g per 1kg of rats following the food restriction strategy by the IACUC and PA, USA. The normal rat chow in the food dishes of the rat cages was then replaced with the powdered cassava. The cassava powder was consumed adequately by the rats.

OPEN FIELD ACTIVITY TEST

Before testing

1. Acclimation: subjects in home cage were placed in testing room for at least 1hr before testing to minimize effects of stress on behavior during testing.
2. Subject training: none required.

Testing procedures

- Animal was placed in corner of arena and allowed to move freely for 10min while being monitored by automated tracking system. Trial will begin once animal is placed at the center of the arena and is able to touch the four corners of the arena and will end when defined duration has elapsed. The defined duration is 5 minutes
- Animal was returned to home cage and number of fecal pellets was recorded.
- Arena was cleaned between each trial.

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 8| Issue: 4| April 2022|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2022: 8.205 || ISI Value: 1.188

(a) (b)

Figure 1: (a) Open Field Apparatus (before testing); (b) Open Field Apparatus (During Testing)

Elevated Plus Maze

The elevated plus maze test is one of the most widely used behavioral assays to evaluate anxiety related behavior in rodent. The elevated maze plus is made of four arms to enclosed and two open arms. This was used to test the anxiety of the mouse. The rodent was placed at the intercession of the four arms of the elevated plus maze and their behavior is typically recorded for 5mins. This was based upon the early studies by montgomory that revealed that rats demonstrated the most robust avoidance response in the first five minutes after placement in the elevated open alleys. The behavior that was typically recorded was the time the rodent spent in the open and closed arms. Behavior in this task (i.e. activity in the open arm reflects a conflict between the rodents preference for protected areas (closed arms) and their innate motivation to explore novel environment.

Figure 2: Elevated Plus Maze

Method of Data Analysis

Data was analyzed using Statistical Package for the Social Sciences (SPSS IBM version 23.0) and Microsoft excel 2019 edition. Values were expressed as mean ± SD in descriptive statistics. One-way analysis of variance (ANOVA) was used to analyze the difference between the groups followed by least significant difference (LSD) post-hoc test. Confidence interval was set at 95%, and therefore $p<0.05$ was considered significant.

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 8| Issue: 4| April 2022|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2022: 8.205 || ISI Value: 1.188

RESULTS

The results are presented in tables and bar charts as shown below;

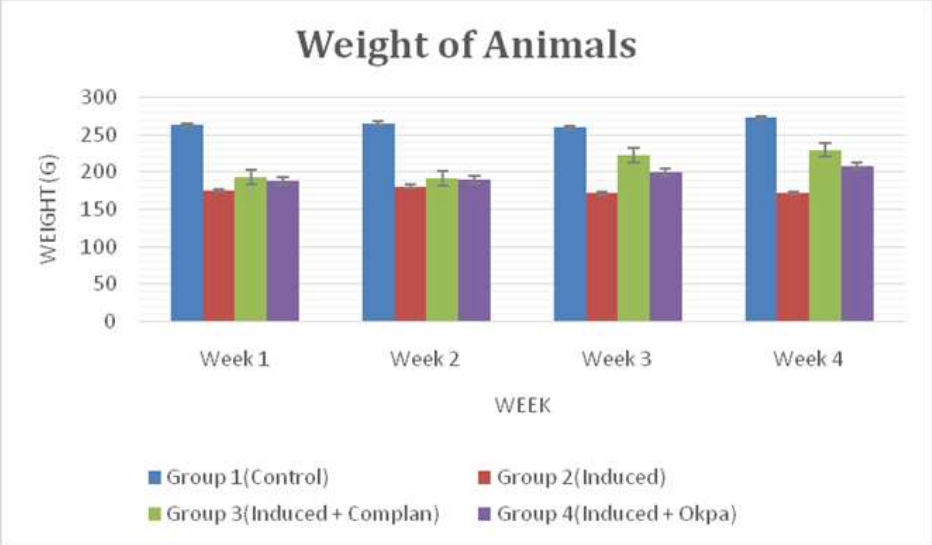
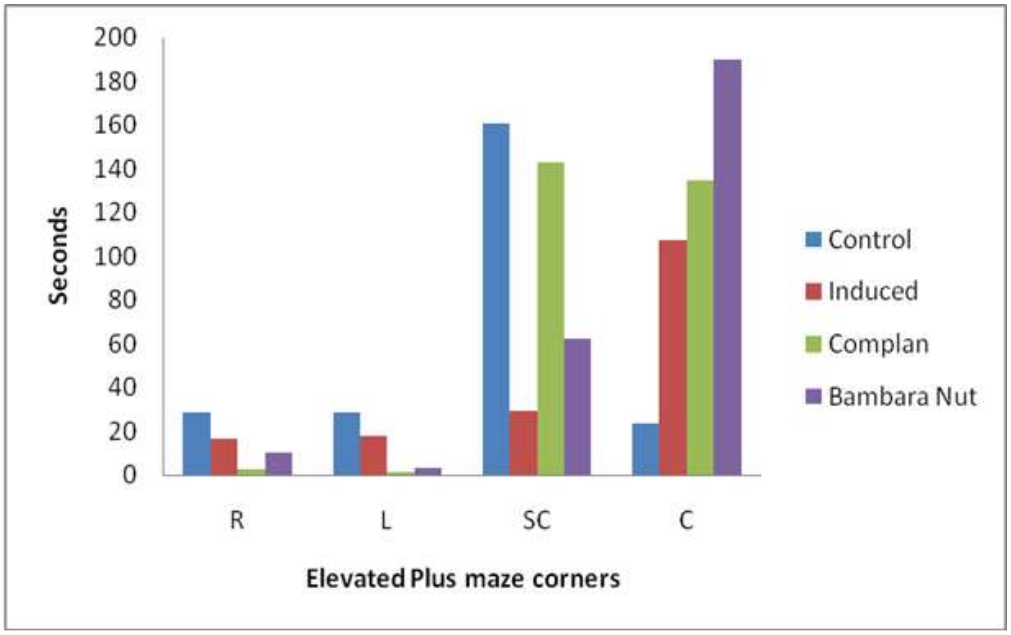


Figure 3: Weight of Animals

Table 1: Evaluation of anxiety using elevated plus maze test in Konzo induced disease in Wistar rats

	Week 1				Week 2			
Group	**R (Sec)**	**L (Sec)**	**SC (Sec)**	**C (Sec)**	**R (Sec)**	**L (Sec)**	**SC (Sec)**	**C (Sec)**
Group 1	28.80±7.51	28.87±11.11	160.73±30.51	23.73±10.85	30.60±7.50	36.40±7.42	91.67±16.76	106.27±20.89
Group 2	16.53±4.26	18.07±8.59	29.13±8.52	107.00±31.69	3.53±1.68	10.93±3.20	139.73±33.03	67.73±24.04
Group 3	2.47±1.38	1.53±1.53	143.00±38.20	134.40±37.61	7.07±2.81	11.00±4.66	163.47±32.32	91.27±31.20
Group 4	10.33±4.23	3.40±1.82	62.20±26.20	189.60±32.64	2.87±1.80	15.33±8.52	190.33±29.85	86.53±31.93

Note: Group 1: Control, Group 2: Induced, Group 3: Induced + Complan, Group 4: Induced + Bambara Nut (Okpa), R = Right Corner; L = Left; SC = Semi Closed; C = Closed



ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 8| Issue: 4| April 2022|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2022: 8.205 || ISI Value: 1.188

Figure 4: Comparison chart of anxiety level in test rats in Week 1 from Elevated Plus Maze test

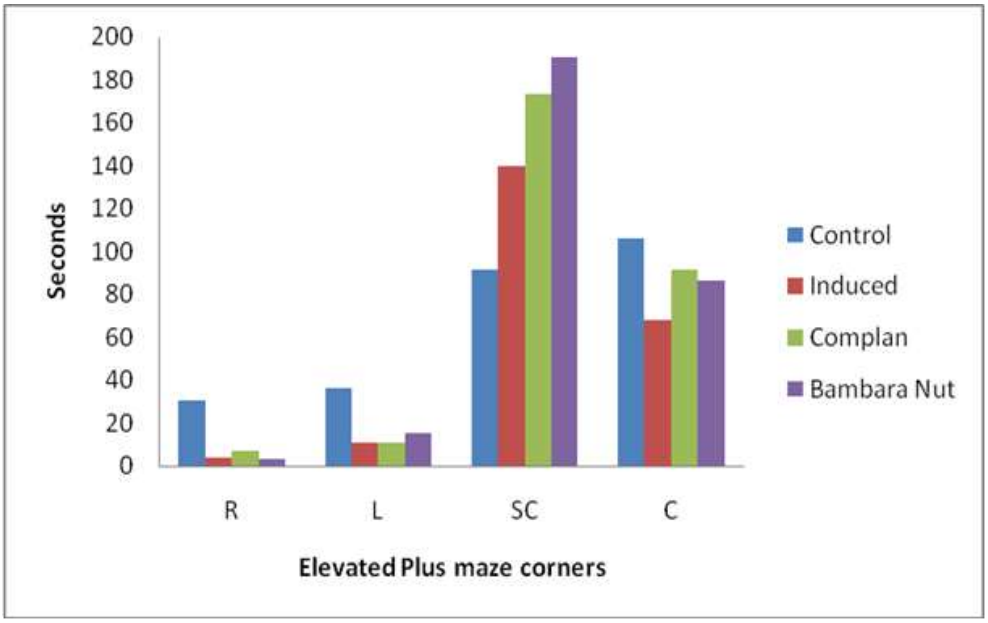


Figure 5: Comparison chart of anxiety level in test rats in Week 2 from Elevated Plus Maze test

Table 2: Result of Locomotion Activity level/impairment, anxiety and willingness to explore in Wistar rats using Open Field Test

Group	Week 1	Week 2	Week 3	Mean (Sec)
Group 1 (Control)	208.33±20.46	177.00±17.95	165.33±18.93	183.55±12.84
Group 2 (Induced)	173.20±27.36	247.80±16.92	239.07±22.18	220.02±23.55*
Group 3 (Induced + Complan)	270.33±12.17	277.87±12.72	248.93±16.58	265.71±8.67*
Group 4 (Induced + Bambara Nut)	234.67±20.22	269.73±12.74	278.20±9.48	260.87±13.33*

*Significant at $p<0.05$. The results are expressed as mean ± SEM per group and the respective control group. Level of significance values are $P<0.05$.

DISCUSSION

Weight Assessment in Konzo induced rats

The result shows weekly body weight differences in experimental animals. There was significant weight reduction in the body weight of the Konzo induced group experimental Wistar rats from week 1 to week 4 compared to the significant weight increase observed in the Konzo induced and complan milk fed group Wistar rats with a mean weight as shown in figure 3. There was also a significant weight increase observed in the Konzo induced and Bambara nut (Okpa) fed group Wistar rats when compared with the konzo induced group. There was a mean weight loss observed from the cassava-induced Konzo Wistar rats compared to the mean weight loss in cassava-induced Konzo and complan milk fed group Wistar rats and cassava-induced Konzo and Bambara nut (Okpa) fed group Wistar rats. A weight gain was observed in the cassava-induced Konzo and complan milk fed group Wistar rats and cassava-induced Konzo and Bambara nut (Okpa) fed group Wistar rats when compared with the cassava-induced Konzo group Wistar rats which indicated the ameliorative effect of complan milk and bambara nut (nut). This is in line with the observation made by Enefa *et al.* [15] and David *et al.* [16] that did a neurobiohavioral and ameliorative study on the effect of complan milk and bamabara nut on bitter cassava induced toxicity on Wistar rat and obtained a similar significant difference in the weight of induced rats compared to the control group. Weight gain was observed in the bitter cassava-induced and complan milk fed group Wistar rats and bitter cassava-induced and Bambara nut (Okpa) fed group when compared with the bitter cassava-induced group indicating a possible ameliorative effect of complan milk and bambara nut (okpa) on the weight of the Wistar rats fed with complain milk and bambara nut. This weight gain observed could be due to the high protein contained in complain milk and bambara nut. This finding agrees with the study by David *et al.* [16] who established in his study that complan milk and bambara nut has ameliorative effect in Wistar rats induced with konzo disease.

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 8| Issue: 4| April 2022|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2022: 8.205 || ISI Value: 1.188

Evaluation of anxiety using elevated plus maze test in Konzo induced disease in Wistar rats

The result from elevated plus maze as presented in table 1 shows that there was no positive improvement in anxiety level of Wistar rats in the time spent in the R and L sections of the elevated plus maze in the experimental groups when compared to the control group in week 1 (Fig. 4.2 and 4.3). Although, there was a slight improvement in the SC and C section (in terms of time spent) when compared to the control group in week 1. The result from elevated plus maze as presented in table 1 shows that there was positive improvement in anxiety level of Wistar rats in terms of the time spent in the R, L, SC and C sections of the elevated plus maze in the experimental groups when compared to group 2 and control group in week 2. This indicates a positive reduction in anxiety level in week 2 compared to week 1 as observed with the elevated plus maze apparatus. This positive improvement in anxiety level in week 2 is due to the ameliorative effect of protein nature of complain milk and bambara nut which is not keenly expressed in the group induced with bitter cassava only. This finding conforms to the study of David *et al.* [16].

Locomotion Activity level/impairment, anxiety and willingness to explore in Wistar rats using Open Field Test

In evaluating the locomotion impairment and anxiety level of wistar rats using Open Field Test, cassava-induced Konzo Wistar rat group, cassava-induced Konzo and complan milk fed group Wistar rats group and cassava-induced Konzo and Bambara nut (Okpa) fed Wistar rats group spent significantly more time with a mean time of 220.02±23.55, 265.71±8.67 and 260.87±13.33 respectively when compared to the control group mean time of 183.55±12.84. Also, a significant improvement in locomotive activity and anxiety level was observed in cassava-induced Konzo and complan milk fed group Wistar rats group and cassava-induced Konzo and Bambara nut (Okpa) fed Wistar rats group when compared with cassava-induced Konzo Wistar rat group indicating the ameliorative effect of complain milk and Bambara nut (Okpa). The improved locomotive activity observed in this study also conforms to the study by Enefa *et al*. [15].

CONCLUSION

The anxiety level and locomotor impairment in the experimental group caused by bitter cassava showed that improperly processed bitter cassava is toxic and has neurotoxic effects on the brain (particularly the central nervous system and higher motor neurons). According to this study, the harmful effect of bitter cassava can be reduced by eating proteins or eating a well-balanced diet that includes milk and bambara nuts (Okpa). Following the findings of this study can help to lessen the toxicity of bitter cassava consumption, while nutritional deficiency can likely increase the toxicity of various processed bitter cassava roots.

RECOMMENDATION

It is recommended that the findings from this study be used to enlighten rural dwellers who consume different variety of bitter cassava processed roots on the need to also balance their diet while consuming varieties of processed cassava roots. Also, further studies to corroborate the findings of this study are recommended.

ACKNOWLEDGEMENTS

The authors wish to acknowledge and appreciate the input of participants and the research assistants who took part in the study.

ETHICAL CLEARANCE

The experimental animals were obtained from the animal house of the department of Anatomy in the Faculty of Basic Medical Sciences. All procedures carried out during this research were done in accordance with the guiding principles of research involving animals as recommended by the Research Ethics Committee of the University of Port Harcourt. Animals were kept in standard cages and at normal room temperature.

CONFLICT OF INTEREST

The author reported no conflict of interest and no funding was received for this study.

REFERENCES

1. *Cliff J, Lundquist P, Mårtensson J, Rosling H, Sörbo B. (1985). Association of high cyanide and low sulphur intake in cassava-induced spastic paraparesis. Lancet; 326:1211–1213.*
2. *Howlett WP, Brubaker GR, Mlingi N, Rosling H. (1990). Konzo, an epidemic upper motor neuron disease studied in Tanzania. Brain; 113:223–235.*
3. *Tylleskär T, Banea M, Bikangi N, Fresco L, Persson LA, Rosling H. (1991). Epidemiological evidence from Zaire for a dietary etiology of konzo, an upper motor neuron disease. Bull World Health Organ. 1991; 69:581–589.*
4. *Tylleskär T, Banea M, Bikangi N, Cooke RD, Poulter NH, Rosling H. (1992). Cassava cyanogens and konzo, an upper motoneuron disease found in Africa. Lancet; 339:208–211.*
5. *Tylleskär T, Howlett PW, Aquilonius SM, Stålberg E, Rosling H, et al. (1993). Konzo: a distinct disease entity with selective upper motor neuron damage. J Neurol Neurosurg Psychiatry; 56:638–643.*
6. *Tylleskär T. (1994). Studies on a paralytic disease in Africa. Acta Universitatis Upsaliensis. Uppsala; The causation of konzo. pp. 39–41.*
7. *Banea-Mayambu JP, Tylleskär T, Gitebo N, Matadi N, Gebre-Medhin M, Rosling H. (1997). Geographical and seasonal association between linamarin and cyanide exposure from cassava and the upper motor neurone disease konzo in former Zaire. Trop Med Int Health; 12:1143–1151.*
8. *Tshala-Katumbay D, Banea M, Nunga M, Edebol K, Tylleskär T, et al. (2001). Neuroepidemiology of konzo – a spastic para/tetraparesis of acute onset in a new area of the Democratic Republic of Congo. Afr J Neurol Sci.; 20:8–13.*

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 8| Issue: 4| April 2022|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2022: 8.205 || ISI Value: 1.188

9. *Rosling H, Tylleskär T. Konzo. In: Shakir RA, Newman PK, Posner CM, (1995). Tropical neurology. London: Saunders; pp. 353–364.*
10. *WHO (1996). Konzo, a distinct type of upper motor neuron disease. Wkly Epidemiol Rec.; 71:225–232.*
11. *Cliff J. (2010). CCDN News No. 15; 2010. Konzo count.4*
12. *Ciglenecki I, Eyema R, Kabanda F, Taafo F, Mekaoui H, et al. (2011). Konzo outbreak among refugees from Central African Republic in Eastern region, Cameroon. Food Chem Toxicol.; 49:579–582.*
13. *Ngugi AK, Bottomley C, Kleinschmidt I, et al.(2013). Prevalence of active convulsive epilepsy in sub-Saharan Africa and associated risk factors: Cross-sectional and case-control studies. Lancet Neurol.; 12:253–63.*
14. *Kariuki SM. Abubakar A. Kombe M. et al. (2007). Burden, risk factors, and comorbidities of behavioural and emotional problems in Kenyan children: a population-based study. Lancet Psych. 2017; 4: 136-145.*
15. *Stella Enefa, Chikwuogwo W. Paul, Lekpa K. David (2020). Model of Konzo Disease: Reviewing the Effect of Bitter Cassava Neurotoxicity on the Motor Neurons of Cassava-Induced Konzo Disease on Wistar Rats. Saudi J Med.; 5(11): 336-348.*
16. *David, L. K., Idung, V. H., & Uahomo, P. O. (2022). Neurobehavioral and Ameliorative Effect of Complan Milk and Bambara Nut on Rats Fed With Bitter Cassava – A Nutritional Approach. International Neuropsychiatric Disease Journal, 17(1):7-17. https://doi.org/10.9734/indj/2022/v17i130190*