

Assessment of Sensorimotor Behaviour in Konzo-Induced Rats Using the Irvine, Beattie Bresnahan Forelimb Scale

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Abstract

Konzo is a neurological disorder of selective upper motor neurons. It is an irreversible paralytic disease associated with prolonged consumption of Cassava. It contains cyanogenic glycosides metabolized to hydrogen cyanide, which has been shown by studies to affect the motor neurons of the central nervous system. The Irvine, Beattie Bresnahan (IBB) scale is a recently developed forelimb scale for the assessment of fine control of the forelimb and digits after cervical spinal cord injury such as Konzo. 20 Adult male Wistar rats were assigned to 4 experimental groups (i) control n=5, (ii) konzo-induced group n=5, (iii) induced + Complian n=5 (iv) Induced + Bambara Nut (Okpa). The bitter cassava foods were taken by oral ingestion for a period of 4 weeks. The assessment of the forelimb and digits were done using the Irvine, Beattie Bresnahan (IBB) with specific parameters such as Predominant Elbow Joint movement, Contact Volar Support, and Grasping method. The body weight of the animals was also recorded every week. The data obtained were analyzed using ANOVA. The result obtained showed that there was a significant difference ($p < 0.05$) between the body weight of the animals induced with Konzo and rehabilitated with Complian milk and Bambara nut when compared to the unrehabilitated Konzo-induced group. There were differences in the results of the parameters being tested for the Irvine, Beattie Bresnahan (IBB) scaling. The IBB scale confirmed that there was a high level of cyanide content in the cassava which affected the behavioral attributes of the induced group and it also confirmed that the induced group can be ameliorated with the use of Complian and Bambara Nut (Okpa) which was shown in the parameters being tested such as Predominant Elbow Joint movement, Contact Volar Support, and Grasping method. It was concluded that insufficiently processed bitter cassava is toxic and has neurotoxicity effects on the Spinal Cord especially on the upper motor neurons and IBB scale is capable of measuring gradual improvements in motor forelimb functions in this model and may be a new and effective assessment tool for peripheral nerve injury.

Keywords: Bambara nut; Cyanogenic; Neurotoxicity; Konzo; Bitter Cassava; Irvine, Beattie Bresnahan Forelimb Scale.

INTRODUCTION

Cassava toxicity has been incriminated in the pathogenesis of tropical myeloneuropathies, such as tropical ataxic neuropathy (TAN) and Konzo. TAN is a progressive myeloneuropathy that was first described in Nigeria and is characterized by a progressive onset of ataxia. On the other hand, Konzo is a permanent and clinically distinct upper motor neuron disease of abrupt onset first described in the Democratic Republic of Congo (DRC). The word “Konzo” means tied legs and originated from the Yaka tribe in DRC to designate a fetish used by hunters to weaken legs and catch wild animals (Trolli, 1938; Van der Beken, 1993).

Konzo occurs mainly in children and young women of childbearing age. The paralysis occurs quite suddenly, does not progress over time, and is irreversible. It is associated with the consumption of a monotonous diet of high cyanide (bitter) cassava, by poor rural people in Africa, many of whom suffer from malnutrition.

Specifically, konzo is associated with a high cyanide diet of bitter cassava consumed over a period of several weeks combined with a low intake of protein, particularly a shortfall of essential S-containing amino acids that are needed to detoxify cyanide to thiocyanate in the body (Howlet et al, 1990).

The exact pathogenetic mechanisms of the disease remain unknown. Epidemiological studies consistently show an association between outbreaks of the disease and chronic dietary reliance on insufficiently processed cyanogenic cassava (manioc or tapioca). Biochemical and toxicological studies suggest that the metabolites of linamarin (α -Hydroxyisobutyronitrile β -D-glucopyranoside, the main cassava cyanogen), notably cyanide (mitochondrial toxin), thiocyanate (AMPA chaotropic agent), and cyanate (protein carbamoylating agent) may play an important role in the pathogenesis of Konzo. Experimental data suggest that thiol-redox and protein-folding mechanisms may also be perturbed. Factors of susceptibility including genetics, poor

nutrition, poverty, and dietary cyanogen exposure, or their interactions have been suggested.

The Irvine, Beattie Bresnahan (IBB) scale is a recently developed forelimb scale for the assessment of fine control of the forelimb and digits after cervical spinal cord injury (SCI). Several experimental models of cervical spinal cord injury (SCI) have been developed recently to assess the consequences of damage to this level of the spinal cord (Pearse et al., 2005; Gensel et al., 2006; Anderson et al., 2009), as the majority of human SCI occur here. Behavioral deficits include loss of forelimb function due to damage to the white matter affecting both descending motor and ascending sensory systems, and to the gray matter containing the segmental circuitry for processing sensory input and motor output for the forelimb. This is the basis for the study; hence, this study aims to assess the sensorimotor behaviour in a konzo-induced rat using the Irvine, Beattie Bresnahan forelimb scale.

METHODOLOGY

Animal Procurement

Twenty (20) Wister rats weighing between 150-200g were acquired from the Animal House of the Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt for this research work. The animals had free access to water and were fed *ad libitum* with standard feed (broiler finisher – Guinean feed). Two weeks before the start of the test, the animals were acclimated.

Plant Collection and Identification

The cassava plants were gotten at the Ministry of Agricultural Development Program, Rumuodomaya, Port Harcourt, Rivers State, and were identified by the Agriculture Department of the University of Port Harcourt, Choba, Rivers State.

Processing of Bitter Cassava Root

Fresh cassava roots were uprooted from the farm, and immediately after harvesting the outer layer (skin or cortex) was removed with a knife to expose the white inner layer. Then the roots were sliced into smaller sizes like chips and allowed to sundry for 3 consecutive days. The dry cassava pieces were manually grinded into powder form using a grinding machine and served to the experimental animal as cassava chow.

Induction of Konzo Disease in Rats

After two weeks of acclimatization, 15 rats were fed with the processed bitter cassava flour constantly to induce the Konzo disease and its effects. The oral feeding method used represents the real scenario of food being consumed by ingesting through the mouth, passing through the esophagus into the stomach following the alimentary canal, contacting the necessary visceral organs along

with their associated fluids such as saliva in the mouth and gastric juices including any contributions provided via sublingual or buccal absorption during digestion.

Experimental Design

The experimental method as described by David et al. (2022) was used in this study. Twenty (20) albino Wistar rats were used for this study. Out of the 20 rats, 15 were induced with Konzo disease for two weeks and then randomly selected into three (3) groups of five (5) animals each (groups 2, 3, and 4), while the remaining 5 rats without Konzo disease served as control (group 1).

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

Table 1. Experimental Design.

Group	Identity	No. of Rats	Treatment
Group 1	Control	5	2ml Normal Saline
Group 2	Induced Control	5	Bitter Cassava only
Group 3	Complan	5	Bitter Cassava + Complan Milk
Group 4	Bambara Nut	5	Bitter Cassava + Bambara Nut

Determination of Body Weight

The animals were weighed daily with an electric weighing scale (SF-400C) and the body weight of the rats was recorded.

Sensorimotor Behaviour Assessment

The forelimb function was assessed using a special method, which is IBB (Irvine, Beattis and Bresnahan Test). The procedure is as explained; Rats were given pieces of cereal in their home cage twice daily beginning as soon as they entered the lab. Forelimb function was assessed while rats were eating cereal as described previously (Irvine et al., 2010). Briefly, rats were individually placed in a Plexiglas cylinder (diameter = 20 cm; height = 46 cm) or in their home cage and they were given donut-shaped pieces of cereal that were of a consistent size and shape prior to the initiation of eating. Rats were not scored when eating cereal pieces that were broken prior to the initiation of testing. Each trial was recorded to allow slow-motion HD playback and evaluation of forelimb use. Videos of animals eating the cereal were evaluated using a standardized scoring sheet to record observations of forelimb behaviors, including joint position, object support, wrist and digit movement, and grasping method used while consuming both cereal shapes.

Cyanide Analysis

The samples for analysis of the Cyanide in the bitter cassava were prepared as follows:

- 10g of the sample was mixed with 50ml of water in a corked conical flask.
- Allowed to stand for 24hrs to extract the residual cyanoglucosides in the samples.
- The mixture was subsequently filtered to obtain the soluble extract containing cyanoglucosides
- The same procedure as with standard KCN solutions was followed to determine the free cyanide concentration (as HCN equivalent) in the sample filtrate.
- The absorbance of the sample solution was equally measured at 510nm wavelength against a blank devoid of KCN solution.
- Cyanide levels of the test samples were evaluated from the standard calibration curve by extrapolation.

Ethical Clearance

The experimental animals were acquired from the animal house of the Department of Pharmacology 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 metal cages and at room temperature.

Statistical Analysis

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

RESULTS

Table 2. Effect of Konzo disease, Complan milk and Bambara Nut (Okpa) on the Body Weight (Grams) of Wistar rats.

Group	Baseline Weight	Week 1	Week 2	Week 3	Week 4	Mean Weight
Group 1	263.01 \pm 5.13	263.00 \pm 5.87	265.02 \pm 6.10	260.10 \pm 6.32	273.05 \pm 6.75	265.25 \pm 2.78 ^b
Group 2	242.30 \pm 5.17	175.70 \pm 5.16	181.10 \pm 4.80	171.80 \pm 5.15	172.40 \pm 5.90	175.25 \pm 2.13 ^a
Group 3	175.25 \pm 4.87	194.10 \pm 4.21	192.50 \pm 4.11	223.10 \pm 8.23	230.10 \pm 8.40	209.95 \pm 9.72 ^{a,b}
Group 4	175.25 \pm 5.10	188.30 \pm 5.87	190.05 \pm 7.02	200.60 \pm 7.50	208.90 \pm 7.11	196.95 \pm 4.82 ^{a,b}

The results are expressed as mean \pm SEM. Level of significance values are ^a $p < 0.05$ when compared with group 1 (control), ^b $p < 0.05$ when compared with Group 2 (konzo induced).

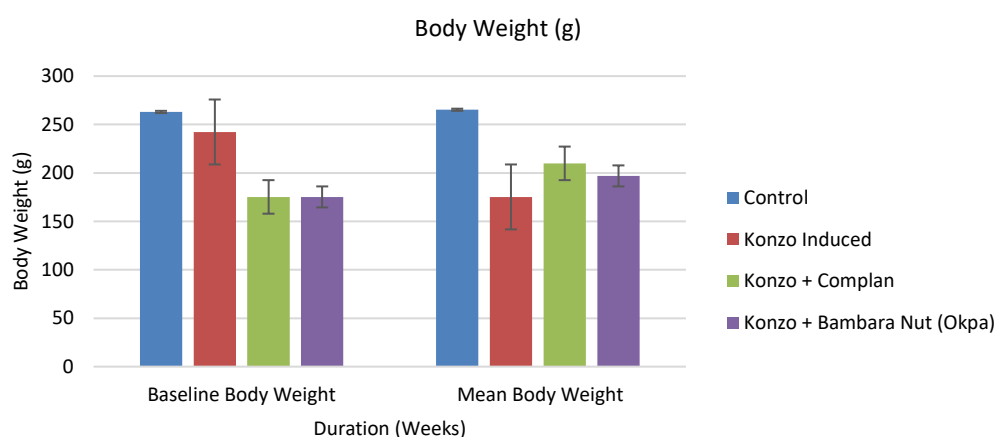


Figure 1. Comparison of Baseline Body Weight with Final Mean Body weight of Wistar rats.

Table 3. Irvine, Beatties and Bresnahan Forelimb Scale.

Group	PEJP	CVS	GM
Group 1 (Control)	Flexed	None	Normal
Group 2 (Induced)	Partially flexed	Nearly Always	Abnormal
Group 3 (Induced + Complan)	Flexed	Some	Normal
Group 4 (Induced + Bambara Nut)	Flexed	Some	Normal

Keys: PEJP = Predominant Elbow Joint Position; CVS = Contact Volar Support; GM= Grasping Method

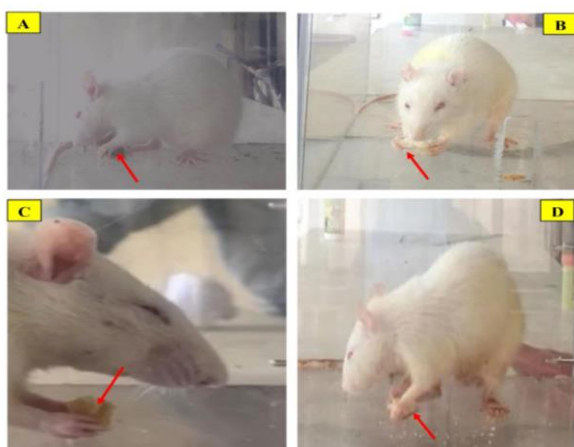


Figure 2. Contact Volar Support: A – Control (Fed with normal feeds – finishers), B – Fed with bitter cassava, C - fed with bitter cassava then treated with Complan, D - Fed with bitter cassava then treated with Bambara Nut

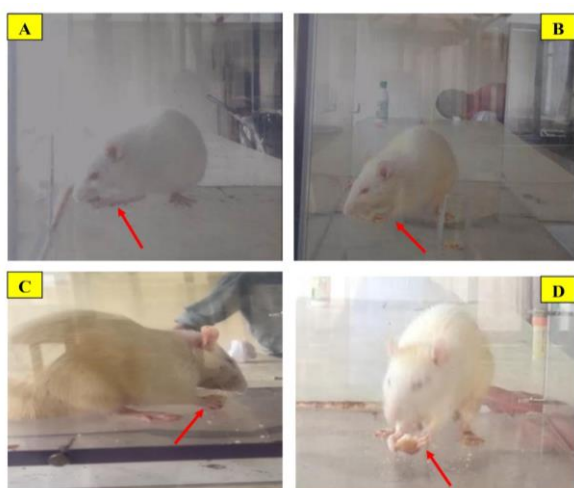


Figure 3. Predominant Elbow Joint movement: A – Control (Fed with normal feeds – finishers), B – Fed with bitter cassava, C - fed with bitter cassava then treated with Complan, D - Fed with bitter cassava then treated with Bambara Nut

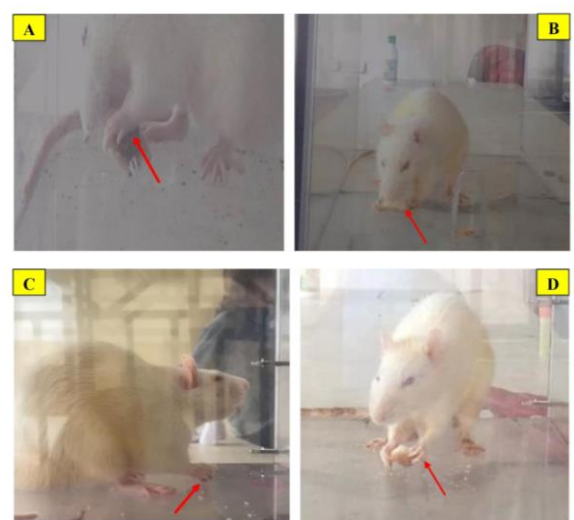


Figure 4. Grasping Method: A – Control (Fed with normal feeds – finishers), B – Fed with bitter cassava, C - fed with bitter cassava then treated with Complan, D - Fed with bitter cassava then treated with Bambara Nut

DISCUSSION

The effect of bitter cassava has been studied extensively and its neurotoxicity was being studied with the use of experimental animal models to evaluate the effects of diverse substances on motor function and their relationship with CNS disorders. (Gerhart et al., 1982; Kulig et al., 1985; Fowler et al., 1990). The weight of the rats was taken and measured accurately from the beginning of the week till the end of the experiment at a significant value of $p < 0.05$. It was observed that there was a significant difference between the mean body weight of the control group 265.25 ± 2.78 g and the mean body weight of the induced group (175.25 ± 2.13 g). The difference between the weight of the induced was different from that of the induced as a result of the intake of the bitter cassava which has caused a neurotoxic effect on the experimental models. This agrees with the findings of David et al. (2022) and Enefa et al. (2020).

Some nutritional approaches were used to ameliorate the effect of the bitter cassava for a period of 4 weeks after the initial four weeks of being induced with Konzo disease. The nutritional approach used was Complan and Bambara nut (Okpa). There was a significant difference between the mean body weight of the ameliorated group and that of the induced. The Complan group had a mean body weight of 209.95 ± 9.72 g while the Bambara nut group has a mean body weight of 196.95 ± 4.82 g. This shows that the effect of the bitter cassava on the experimental models was ameliorated as there was a significant increase in the weight of the induced group after being fed with Complan and Bambara nut (Okpa). This is also similar to the findings of David et al. (2022) and Enefa et al. (2020).

The IBB Forelimb Scale provided a sensitive measurement scale for detecting recovery of both proximal and distal forelimb function, including digit movements, during a naturally occurring behavior. This behavioral test was developed and tested in rats with spinal cord injury by Irvine et al. (2010). This method evaluated the overall integrity of rodent forelimb functionality during a food intake task, measuring how rats grasp a cereal piece. The IBB scale was used to test just 3 parameters which include predominant elbow joint movement, contact Volar support, and grasping method.

During the assessment of contact volar support, the scale was rated as ‘None’, ‘Some’, and ‘Almost Always’. The rat was assessed for its ability to use the volar (palmar) surface of the impaired forepaw to stabilize the cereal and, in doing so, maintain a position to aid eating. In Figure 2, group A being the control group, no volar support by the forelimb during eating ($< 5\%$ of the time). But in group 2, being the induced group that was affected by the cyanide content of the bitter cassava, the Volar support of the object occurred nearly always or always during eating ($> 95\%$ of the time). This is because the Spinal Cord had been affected which in turn relates to

how the rats had to use their Volar for support. The Ameliorated group which is group 3 (Complan) and group 4 (Bambara nut) had the support of the volar but did not occur always. This shows that the Konzo disease was ameliorated which helped to reduce the use of volar for support in the case of the induced group.

The original scale rated the Predominant Elbow Joint Position as “extended, partially flexed, or fully flexed.” Discrimination between partially and fully flexed appeared to be problematic, and perhaps irrelevant in more recovered animals. Looking at Figure 3, the rat models were assessed for the most common position (more than 50% of the time). The elbow joint movement for group A was found to be flexed. Group B was partially flexed as a result of the cyanide content of the bitter cassava affecting the spinal cord. After the induced were ameliorated, the Elbow joint movement was flexed fully.

The grasping method was rated to be ‘Normal’ and ‘Abnormal’ according to Figure 4, the experimental rat models were assessed for the most common (more than 50% of the time) grasping technique used during the eating phase. The rat models of group 1 were found to be normal with consistent use of the grasping method used prior to injury to support and control the cereal piece during the eating phase. Group 2 being induced was abnormal because they consistently used an alternative method of grasping to the method used prior to the injury to support and control the cereal piece during the eating phase. They were able to support their grasping with their volar. This is a result of the spinal cord injury that occurred as a result of the intake of bitter cassava which had a high percentage of cyanide content. The ameliorated group was found to be normal in their grasping method.

According to Speck et al. (2014), the IBB scale is capable of measuring gradual improvements in motor forelimb functions in this model and may be a new and effective assessment tool for peripheral nerve injury. This was seen in this study as the induced group was being ameliorated with Complan and Bambara nut and there was gradual improvement in the motor forelimb function using the three parameters, Predominant Elbow Joint Movement, Contact Volar Support, Grasping method.

CONCLUSION

This study has shown that insufficiently processed bitter cassava is toxic and has neurotoxicity effects on the Spinal Cord especially on the upper motor neurons. A balanced diet with sufficient nutrients such as Complan

and Bambara Nut (Okpa), can ameliorate the effects of konzo disease as there was no effect on motor neurons of the spinal cord of the animals rehabilitated with Complan and Bambara Nut (Okpa).

Competing Interests: The authors declare that there are no competing interests.

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