

Effects of Soya Beans and Brown Beans in Degenerative Changes Expressed in Cortical Neun Immunoreactivity in a Casava-Induced Konzo Disease Rat Model

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Abstract

Background: Konzo is related to a habit of long-term consumption of cassava; it is a chronic disease with quite different symptoms from acute cassava poisoning. The ameliorative effect of soya beans and brown beans in degenerative changes expressed in cortical NeuN immunoreactivity in a konzo disease rat model was investigated in the present study. **Materials and Methods:** 30 male wistar rats weighing 200-250g were assigned to Group 1 (Control, n=5) and was fed on animal pellet, whereas Group 2 (Protein, n=5) was administered with protein food (Soya beans+Brown bean). Bitter cassava flour was provided to Group 3 (cassava-induced Konzo, n=15). Protein (Soya beans+Brown bean) and bitter cassava flour were administered to Group 4 (protein treatment group, n=5). The Cerebrum (primary motor cortex) region was harvested through transcardiac perfusion for histological and immunohistochemical staining. Image J was used to quantify the neurons in the motor cortex. We examined general structures in the primary motor cortex with hematoxylin and eosin (H&E) stain. **Result and Discussion:** H&E demonstration showed distinct pyramidal neurons with large round nucleus, and prominent nucleoli in the Control group. Interspersed between the pyramidal neurons were numerous glia cells. Protein (Soya beans+Brown bean) group, Cassava group, and Cassava + Protein group also showed intact histology of the cortex compared to control usig H&E stain. NeuN stains analyzed immunoreactive neurons. The rats fed on cassava showed significant decrease in the NeuN immunoreactive neurons at $***[p<.001]$ compared to the Control and Protein administered group, whereas Cassava + Protein groups showed decrease NeuN immunoreactive neurons at $**p<0.1$ compared to Protein group(Soya beans+Brown bean). **Conclusions:** This study has provided an empirical data on the ameliorative effect of soya beans and brown beans supplement on Cassava-induced konzo disease animal model. This data will be very useful to Anatomists, konzo researchers and neuroscientist.

Keywords: Bitter cassava, Soya beans, Brown beans, Konzo, Cortical NeuN.

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INTRODUCTION

A bilateral, often symmetrical lesion at particular regions of the brain and/or spinal cord are the two most common symptoms of dietary metabolic disorders of the nervous system [1]. Numerous plant-based diets contain substances that may be neurotoxic to people, although their presence in varying concentrations or when combined with other diets can reduce the risk of negative health effects [2]. One such diet, cassava, provides a fundamental carbohydrate diet for more than half a billion people worldwide. It is a key stable staple in several regions of the world, including Africa. Cassava is tropical Africa's third-largest source of carbohydrates after rice and maize [3].

However, consumption of cassava has been linked to konzo.

Clinically, Konzo is a neurological condition that affects the higher motor neurons [4]. Compared to adult males, prevalence is higher in females and youngsters. While a prior high intake of toxic cassava products for several weeks appears to be required for konzo development. One striking feature of the disease is that it manifests abruptly within days or even hours. This strongly suggest that the degeneration of a specific set of neurons is occurring in an acute time frame.

Unknown pathogenic processes and causative agent(s) underlie konzo. Significant levels of cyanide, acetone cyanohydrin, and linamarin are present in inadequately processed cassava flour, and exposure to cyanide and linamarin is known to cause the acute phases of konzo [5]. However, konzo is almost always related to a habit of long-term consumption of cassava; it is a chronic disease with quite different symptoms from acute cassava poisoning [6, 4]. In spite of the fact that, Konzo disease is a challenge in Africa, there is scarcity of literatures on the effects of Brown Beans and Soya Beans on Cassava-induced Konzo disease rat model. This is the driving force behind this research. This research work seeks to determine the effects of soya beans and brown beans supplement on Cassava-induced konzo disease animal model using histological and immunohistochemical technique.

MATERIALS AND METHOD

Animals

Thirty (30) male Wistar rats weighing between 200 and 250 grams were used for the study. The animals were each housed in a standard metal cage. The animals were allowed full access to distilled water and a commercial pellet food for three (3) weeks while being acclimated in a typical laboratory setting with 12-hour light/dark cycles. For the benefit of the animals, the temperature in the room was kept under control. On top of carpet remnants, a layer of coarse sawdust had been sprayed to act as padding for the cage's floor. To keep hygiene standards up and to remove waste droppings, the coarse sawdust was changed every day.

Plant Collection and Identification

The roots of the bitter cassava, soya beans and brown beans were obtained from the Department of Crop and Soil Sciences, Faculty of Agriculture, University of Port Harcourt.

Bitter Cassava Root Processing

The farm's fresh cassava roots were uprooted. The cortex was scraped using a cutter shortly after harvesting to reveal the whitish interior layer. The cassava roots were then chopped into tiny pieces, similar to pommes frites, and sun-dried for three days. Cassava chow was made from pommes frites cassava that were grounded into powdered form using a grounding machine and fed to the animals used for the experiment [1].

Processing of Protein Food Supplement

For this study, a protein dietary supplement consisting of a mixture of soya bean and brown bean was served. Brown beans and soya beans were ground together in a grounding machine into a powdered form that was offered as protein diet nutrient to the "protein (soya beans and brown beans) group" and "cassava + protein (soya beans and brown beans) treatment group" animals, respectively

Experimental Design

The experimental animals were randomly divided into four groups.

Table 1: Animal groups

Group	Group Name/No. of Rats	Treatment
Group 1	Negative control group (n=5)	Were fed on pellet animal feed and water
Group 2	Protein group (n=5)	Were fed with protein food (Soya beans and brown beans) and water.
Group 3	Positive control group (n=15)	Were fed with bitter cassava flour (Konzo induced)
Group 4	The rehabilitation/protein treated group (n=5)	Were fed with bitter cassava flour for 3 weeks (Konzo illness induction period) and then with soya beans and brown beans for two weeks.

Histology

Prior to transcardiac perfusion, the animals were put to sleep in a desiccator with diethyl Ether inhalation. The abdomen and thoracic regions were visible through incisions. After the rat was completely exposed, 10% formal saline solutions were used to do transcardiac perfusion. Formaldehyde was injected into the ventricle of the heart at a 10% dosage. The cerebrum was taken out and put in a 10% formal saline solution to preserve them. The primary motor cortex was excised by 5 microns. The tissue was kept in formalin for 48 hours. The tissues were processed for microscopy.

H&E staining

The tissue was stained for 5 to 10 minutes in a hematoxylin solution, and then rinsed briefly in water. It was then differentiated in 1% acid alcohol with constant agitation for 10–15 seconds, cleaned in running water

for five minutes, stained with 1% aqueous eosin solution for five minutes, cleaned in running water for 30 seconds, dehydrated in 1% alcohol, cleared in xylene, and mounted.

NeuN staining

ImmPRESSTM HRP Anti-Rabbit IgG (Peroxidase) Polymer Reagent, produced in horse, was incubated on sections for 30 minutes. Colour was created using a DAB Peroxidase (HRP) Substrate Kit from Vector® Labs in the USA). Using the ImmunoRatio plugin for Image J, which divides and calculates the percentage of DAB (positive immunoreactivity), NeuN immunoreactivity was measured (Ijomone *et al.*, 2015; Erukainure *et al.*, 2019) and the data obtained were statistically analyzed.

Ethical Consideration

According to the University of Port Harcourt animal care and Research ethical committee, the ethical use of animals in research was approved. The ethical number is UPH/CEREMAD/REC/MM87/037.

Statistical analysis

The data was examined using Graph Pad Prism (version 8.0) and Microsoft Excel (2016 edition). Values were presented in descriptive statistics as Mean \pm SEM. A Tukey post-hoc multiple comparison test was used after a one-way analysis of variance (ANOVA) to evaluate whether there was a significant difference between the groups. A result of $p \leq 0.05$ was considered significant since the confidence interval was set at 95%.

RESULT

The phytochemical screening of the soya beans and brown beans is shown in table 2 and 3. It showed positive results for flavonoids, Isoflavones and tannins.

Table 2: Phytochemical Analysis of Soya Beans

Sample	Qualitative screening
Flavonoids	++
Isoflavones	+++
Tannin	++

Table 3: Phytochemical Analysis of Brown Beans

Sample	Qualitative screening
Flavonoids	+++
Isoflavones	++
Tannin	+++

Hematoxylin and Eosin (H&E)

Figure 1 shows the histological result in the general control group and the treatment groups. It was observed that, the result showed distinct pyramidal neurons with large round nucleus, and prominent nucleoli in the General Control group. Interspersed between the pyramidal neurons are numerous glia cells. The results in the treatment groups; Protein(Soya beans+Brown bean), Cassava, and Cassava + Protein(Soya beans+Brown bean) groups also showed intact histology of the cortex when compared to the General control group.

NeuN in the Motor Cortex

Figure 2 and 3 shows the immunohistochemical result in the general control group and the treatment groups. It was observed that, the immunohistochemical localization of NeuN shows distinct NeuN expression in the motor cortex. The Cassava group showed less NeuN immunoreactive neurons than Control, Beans (Bown+Soya) and Cassava + Beans group.

One way ANOVA showed significant decrease in the NeuN immunoreactivity in Cassava [23.47 \pm 2.496] group compared to the Control [55.08 \pm 3.471; $p < .001$], Beans [67.73 \pm 2.221; $p < .001$] and Cassava + Beans [49.38 \pm 4.999; $p < .01$] groups. Similarly, NeuN immunoreactivity was lower in the Cassava + Protein [49.38 \pm 4.999] group compared to Beans group [67.73 \pm 2.221; $p < .01$].

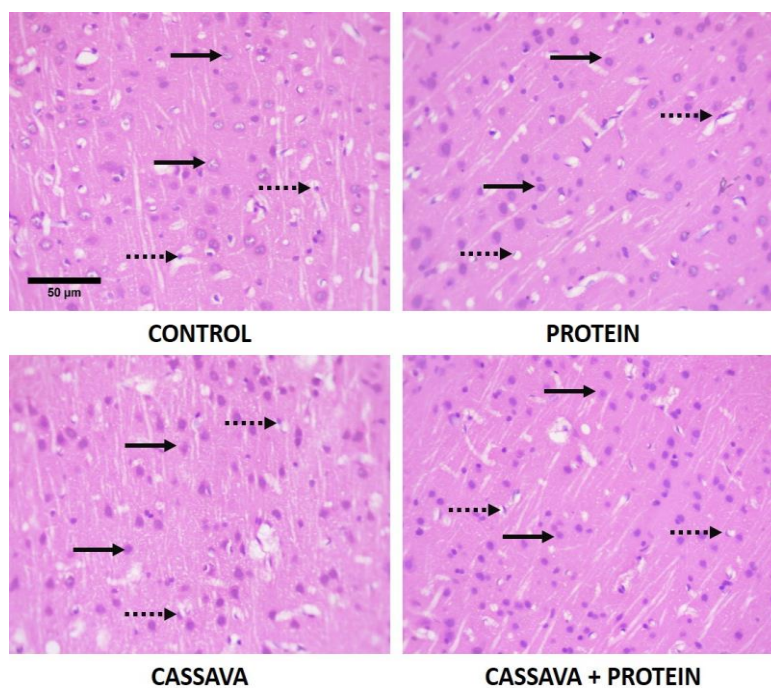


Fig 1: Histological changes in the motor cortex of experimental groups. H&E; Magnification = x400. Black arrows – pyramidal neurons; dashed arrows – glia cells.(Protein- Brown Beans+Soya Beans)

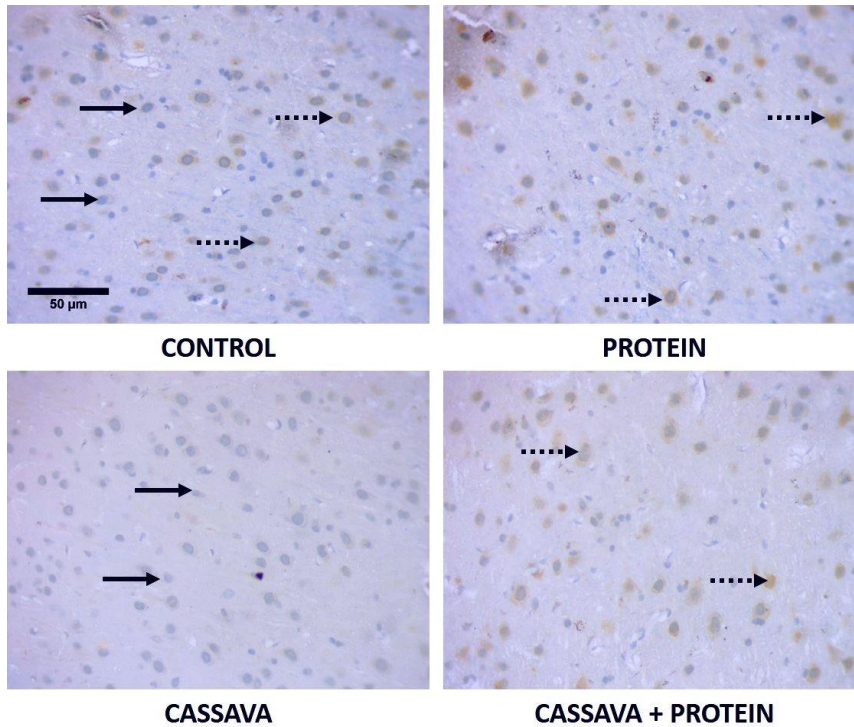


Fig 2: Immunohistochemical changes in the cortex of experimental groups. NeuN; Magnification = x400. Black arrows – pyramidal neurons; dashed arrows – NeuN expressing neurons. (Protein- Brown Beans+Soya Beans)

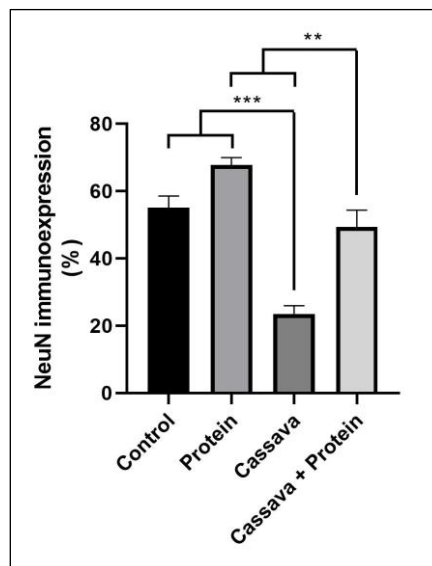


Fig 3: Shows the percentage NeuN immunoeexpression in the motor cortex of control and treatment groups. Bars are Mean±SEM of N=5/group. Cassava group showed significant decrease (*)p<.001 compared to control and protein groups. Cassava + protein group showed significant decrease (**p<.01) compared to protein group. (Protein is: Brown Beans+Soya Beans)**

DISCUSSION

The neurological disorder konzo, which only affects higher motor neurons, is characterized by clinical spastic paresis [4]. Growth retardation and neurological disease brought on by central nervous system tissue damage are common warning indications of extended exposure to cyanide from inadequately cooked cassava roots. The body's cyanide metabolism

and excretion are aided by Flavonoid and Isoflavones, which are present in soya beans and brown beans. The present study duplicated Konzo disease and associated signs in an animal laboratory model to evaluate the neurotoxic impact on the motor neurons and skeletal muscle functioning and how it can be alleviated with a protein-based diet. Given that muscles are the key biological machinery for locomotion and other motor activities, many studies have examined muscular

alteration as a result of cassava-induced Konzo [7-9]. However, in this study we studied the ultrastructural morphology of the motor cortex. First, this study demonstrated cortical histology with H&E which indicate no diagnostic histopathology features. We suggest that the H&E staining technique is rudimentary and as such could not demonstrate specific tissue change. However, upon demonstration with NeuN immunohistochemistry, cassava diet reduced the number of neuronal population in the motor cortex which is a characteristic of neurodegeneration [10]. This neurodegenerative features was recused in rats that were co-fed protein diet (Brown Beans+Soya Beans).

The motor cortex is critical for proper motor-associated functions including locomotion and muscle coordination as well as executive functions [11]. Given the prior motor abnormalities seen in rats exposed to cassava during gait tests, the considerable neuronal death in the motor is not surprising. Additionally, the better motor functions seen in rats co-fed with a protein-rich diet are consistent with the motor cortex's ameliorated degeneration.

CONCLUSION

This study has provided an empirical data on the ameliorative effect of soya beans and brown beans supplement on cassava-induced konzo disease rat model. The ameliorative effect could be as a result of the presence of flavonoids, tannins and Isoflavonoid contents in soya beans and brown beans This data will be very useful to Anatomists, konzo researchers and Neuroscientist.

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Conflict of interest: No conflict of interest.

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