

Effects of Hydrogen Cyanide on the Cytoarchitecture of the Pontomedullary Junction of Albino Wistar Rats

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Abstract

The effect of hydrogen cyanide on the cytoarchitecture of the pontomedullary junction was investigated and studied on Albino wister rats. Sixteen albino wister rats were obtained and divided into four group (Group A, B, C, and D). The animals in group A served as the control group which were given normal growers mashed and distal water only. Those in group B (low dosage) received 150mg/kg of aqueous extract of sweet cassava each, morning and evening for three weeks. Those in Group C (medium dosage) received 250mg/kg and Group D (High dosage) were given 400mg/kg of aqueous extract of sweet cassava each, morning and evening for three weeks. The animals were sacrificed after three weeks of administration and the whole brain including the brain stem was harvested. The brain stem of different specimen at the pontomedullary junction specifically were prepared histologically and photographs were taken at magnification of X100 and X400. The photographs obtained showed degeneration and disintegration of neuronal cells bodies, increase interneuronal vacuole and drastic reduction in neuronal fibers in the experimental group as compared to the controlled group. Compared with the controlled, the changes were dose depended. This shows that the chronic ingestion of hydrogen cyanide is deleterious to the pontomedullary nuclei causing adverse effect on the cochlear and vestibular system of an organism.

Keywords: Cassava, Cyanide, Rats, Garri, Pontomedullary.

Introduction

Cassava (*manihotesculentacrantz*) is a perennial shrub grown for its edible tubers, it belongs to the family Euphorbiaceae, cassava is well adapted to poor soils with marginal nutritional status and PH 4 to 9, it is propagated vegetatively with the stem cuttings. It is cultivated extensively in tropical countries where it roots tubers constitute a principal source of dietary carbohydrate. Cassava is the most important root crop in the world and rank second among African stable crops (Nweke *et al.*, 2001). As a carbohydrate source, it ranks first, producing 250×10^3 calories per hectare per day compare to 200×10^3 for maize, 176×10^3 for rice, 114×10^3 for sorghum and 110×10^3 for wheat. Nigeria is among the largest producer of cassava as compared to Malaysia which the first, it almost doubles the production of Indonesia and Thailand (FAO, 2006).

Originally a crop of South America, it was first introduced in to Nigeria (Southern part) during the period of slave trade, proliferated by the Portuguese explorers and colonizers in the sixteenth century. However, its importance to the country got a boost in the late century with more slave returned to their homeland and introduce processing techniques. Over the years it becomes a major economic crop and it has attained the status of largest producer in the world with recorded production of 34 million tones and is a cash crop of great important to the people of Nigeria (Adeniji *et al.*, 2005).

In Nigeria, it is called “Akpu” in Igbo, ‘ege’ in Yoruba, “Rogo” in Hausa and “Iwa” in Efik. Cassava production dominates in the Southern part of the country, both in terms of area covered and number of farmers growing the crop. Planting occurs during four planting seasons in the various geo-ecological zones. The major state of Nigeria which produce cassava are; Anambra, Delta, Edo, Benue, Cross River, Imo, Oyo, Akwa Ibom, and Rivers and to a lesser extent Kwara and Ondo (Nigerian markets, Org. USAID, (2013).

Phytochemical Constituents of Cassava

The phytochemical, nutritive and antinutritive composition of cassava (*maruhotesculenta* Li tubers and leaves were investigated. The presence of alkaloids, flavonoid, tannin cardiac glycoside, anthraquinone, phlobatinins, saponins, and anthrocyanosides in aqueous and ethanolic extracts of raw tubers and leaves were determined. The proximate moisture, fat, fiber, protein, ash, and carbohydrate in raw and boiled cassava tubers and leaves were determined Vitamin A.C and E anti-nutrient such tannin, oxalate, phytate, trypsin inhibitor activity and some minerals were determined and ethanolic extracts of raw cassava tuber contain alkaloid, flavonid, tannin, and reducing sugars and anthrocyanoside, but do not contain cardiac glycosides, anthroquinone, phlobatinnins and saponins. The raw cassava leaves had more presence of alkaloid, flavonoid, tannin, anhraquinone, phlobatinnin, saponin, reducing sugers and anthrocyanosiders, but do not contain cardiac glycoside. Proximate composition of raw and boiled cassava tubers was not significantly different ($p < 0.01$), except in the carbohydrate, fat, and mousture raw cassava leaves contain more crude fibres and protein as compare to the raw and boiled cassava tubers Vitamin A. C and E with minerals such as calcium.



Figure 1a: Cassava tubers.

Source: aglowinvestments.com (2021)

Toxicity of cassava

The major limitations in the consumption of this crop and its products are the low tuber protein contain, rapid tuber perishing ability following harvest, and ins toxicity. Cassava contain cyanogenic glycosides linamarin and lotaustralin in all tissues Cyanogenic glycosides are a group of Nitrile containing plants secondary compound that yield cyanide (cyanogenesis) following their enzymatic breakdown (Poulton, 1990). It is estimated that between and 12,000 plant species produce sequester cyanogenic glycoside including many important crop species such as cassava, sorghum, almond, lima beans (non-domesticated) and white clover Hydrolysis of this glycosides by intestinal or plant derived B glycoside such aslinamarase enzyme produce by D glucose, hydrogen cyanide (HCN)and acetone. The total range of cyanogen's content in cassava from 15 to 400ppm calculated as mgHCN/kg fresh weight of cassava but occasionally, samples are as low as 10mg/kg even though over 200mg/kg, have been reported (Haque et al, 2002).

It is observed that garri which is a popular cassava food in Nigeria contains as much as 35mgHCN in the average daily consumption of 750g per person. Incomplete processing may result in high cyanide exposure and gives rise to severe disease (Adamolekun, 2011). Cyanide poisoning from high cyagenic cassava is typically associated with insufficient consumption of cysteine and methionine in the diet. Reduced Sulphur are substrate for detoxification of cyanide catalyzed by the enzyme thodanase and B cyanoalanine synthase. Chronic low level cyanide exposure is associated with the development of goiter and tropical ataxic neuropathy a nerve damaging disorder that renders a person unsteady and uncoordinated. Severe cyanide poisoning, particularly during famine, is associated with outbreak of a debilitating, irreversible paralytic disorder called Konzo and in some cases death. The incidence of Konzo and tropical ataxic neuropathy can be as high as 3% in some areas (Ononogbu *et al.*,1980).

Biochemical Effect of Cyanide

However, the rate of reaction of cyanide with reduce enzyme is twice the rate of reaction with oxidize form. It was described that in over 400 Nigerian patients, the essential neurological components of the disease were myelopathy, bilateral optic atrophy, bilateral perceptive deafness and polyneuropathy. The initial and most common symptoms consist of various forms of paresthesia and dysesthesia usually starting in the distal part of the lower limbs. The next most common finding is blurring or loss of vision. Other common symptoms in other of frequency are ataxia, tinnitus, deafness, weakness, and thinning of legs (Osuntokun, 1981). Aqueous extract of starch was not recorded toxic to the pancreas and also possesses hyperglycemic activity (Rusli *et al*, 2015).

Health Benefits of Cassava

Also known as manioc and yoca, cassava is a less expensive food crop, it has high level of carbohydrate content with low glycemic index. Naturally gluten free, it contains saponins to help ease inflammation and balance your gut and minerals that performs many important functions throughout your body. Tapioca also a digestive resistant starch is extracted from cassava root as a flour, it has many food used and is one of the alternatives to wheat and other grains, even for celiac patients, resistant starch is beneficial because, it feed the friendly bacterias and may lower blood glucose level (Boukhers *et al*, 2022).



Figure 1b: Cassava tubers, stems and leaves. **Source:** cassavamatters.org (2019)

Table 1: Some important minerals found in cassava tubers and their functions;

Minerals	Functions
Iron	Helps form two proteins for transporting oxygen to the tissues
Zinc	Helps immune system fight bacteria and in cell growth and division
Calcium	Help form strong bones and teeth
Magnesium	Optimize mitochondrial function and regulates blood sugar level
Potassium	Synthesis proteins and helps breakdown carbohydrate
Manganese	Vital for connective tissues, sex hormones and joints repair.

Organ of Study

The vestibulocochlear nerve is the eight paired cranial nerve. It is comprised of two part; vestibular fibers and cochlear fibers. Both have a purely sensory function. The vestibular and cochlear portion of the vestibulocochlear nerve are functionally discrete and so originate from different nuclei in the brain. Vestibular component, arises from the vestibular nuclei complex in the pons and medulla (pontomedullary junction) has four nuclei, superior, spinal, medial, and lateral nuclei. Cochlear component, arises from the ventral and dorsal cochlear nuclei, situated in the inferior cerebellar peduncle. Both sets of fibers combine in the pons to form the vestibulocochlear nerve. The emerges from the brain at cerebellopontine and exits the cranium via the internal acoustic meatus, the vestibulocochlear nerve splits forming the vestibular system of the inner ear which is responsible for detecting balance (Kryukov, *et al*, 2015).

Equilibrium (Balance)

The vestibular apparatus senses changes in the position of the head in relation to gravity. The vestibular hair cells are located in the otolith organs (sacculle and utricle), where they detect linear movements of the head, as well as in the three semi-circular canals, where they detect rotational movement of the head. The cell body of vestibular nerve are located in the vestibular ganglion which is housed in the outer part of the internal acoustic meatus. Information about the position of the head is used to coordinate balance and T the vestibule ocularreflex. The vestibule - ocular reflex (oculocephalic reflex) allows images on the retina to be stabilised when the head is turning by moving the eyes in opposite direction. It can be demonstrated by holding one finger still at comfortable distance in front of you and twisting your head from side to side whilst staying focus on the finger (Khan *et al*, 2013).

Embryology of the vestibular system

In embryology the otic placode is a thickening of the ectoderm on the outer surface of a developing embryo from which the ear develops. The ear, both the vestibular system and the auditory system, develop from the otic placode at the beginning of third week of development. During the fourth week, otic placode invaginate into mesenchyme adjacent to rhombencephalon to form the otic pit, which then pinches off from the surface ectoderm to form the otic vesicles. The central expanded utricular region, which form the utricle and the three semicircular ducts which arise from utricular diverticula that appear in the seven week (Beisel et al, 2005). A ventral conical saccular region which form the sacculle and the cochlear duct, which coils to about 2.5 spiral and elongates in the 5th week with the spiral organ of corti differentiating in the 7 week the ductus reunion joining the sacculle and the cochlear

Histology of the vestibular system

In the vestibular system, there two types of hair cells;

Type I hair cells, Piriform in shape with a rounded base and thin neck, surrounded by an afferent chalic and a few efferent fibers

Type II hair cells, Cylindrical in shape, with afferent and efferent bowton nerve endings, they synapse basally. Both types of vestibular hair cells have one cilium called Kinocilium.

Cristae Ampullaris, each crista is lined with the epithelium containing sensory hair cells and supporting epithelia cells.

The stereocilia and kinocilia of each hair cells are embedded in a gelatinous cupula that projects into the lumen of amp and is surrounded by endolymph, during turning movement the endolymph tends to lay behind because of it inertia, thus swaying the cupula and bending the sensory hairs that lie within an d generating nerve impulses.

Macula sacculle and Macula utricle; like the cristae ampullaris the macula are innervated sensory thickenings of the epithelium.

The macula is oriented at right angles to each other so that when a person is standing, the macula utricle is in a horizontal plane and the macula saccule is in a vertical plane. The stereocilia and kinocilia of each hair cell are embedded in the gelatinous otolithic membrane, upon which crystalline particles called otoconia (Otoliths) lies. Again the stereocilia are bent by gravity in the stationary individual or linear movement in the moving individuals as the otolithic membrane drags on the stereocilia (Sakka, *et al.*, 2004).

Methodology

The materials used for this experiment include 16 adult Albino Wistar rats, four wooden cages with iron netting, sawdust, animal feed (vital feed, growers mash), water containers and gloves. Other materials used in this experiment are found in the table below:

Table 2: Materials used in this experiment

Dissecting set and board	Xylene	Hot plate
Weighing balance	Paraffin wax	Distal water
Syringes	Embedding mould	Filter paper
10% formal saline	DPX (Distrene, plasticizer, xylene)	Rotatory microtome
Cotton wool and chloroform	Haematoxylin and Eosin stain	Water bath
Graded alcohol (70%, 95%, and 100%)	Cover slips	Oven
Glass slides and slide rack	Light microscope	Grinder, blender and filter

Acclimatization of animals

Sixteenth (16) mixed gender adult wistar rats were purchase from the animal house of Faculty of Basic Medical Sciences University of Calabar, where they are acclimatized for three weeks in wooden cages. A grower feed was used for feeding the animals and with containers fill with water. The sawdust used for bedding was replaced regularly to maintain stable standard environmental condition, with proper ventilation and approximate room temperature of 28 to 32 degree celcius to prevent infections and disease.

Weight of animals before administration

The weights of the animals were taken individually and are placed in groups before administration. It is represented as;

Table 3: Weight of animals before administration

Cage A (Control)	Cage B (Low Dose)	Cage C (Medium Dose)	Cage D (High Dose)
290g	170g	180g	200g

200g	160g	190g	200g
150g	170g	175g	210g
130g	165g	180g	215g

Preparation of Extract

Sweet cassava tubers of 3kg were obtained, peeled and crushed Extract was then dried and properly filtered to get a solute which was kept in a clean dry container for preservation.

Calculation and administration

The LD50 (Lethal Dose) for dietary cyanide present in cassava in rats is found to be through oral administration. Based on this, I graded my doses into 400mg/kg(High dose),250mg/kg(medium dose) and 150mg/kg(Low dose)

Cage D High Dose (400mg/kg)

Rat 1 weighing 200g will take $400 \times 200/1000$ -80mg of HCN

Rat 2 weighing 200g will take $400 \times 200/1000$ -80mg of HCN

Rat 3 weighing 210g will take $400 \times 210/1000$ -84mg of HCN

Rat 4 weighing 215 will take $400 \times 215/1000$ 86mg of HCN

Summation of the doses of each rat

$80+80+84+86=330$ mg of HCN

In grams $330/1000$ -0.33g (which is used for measurement)

To get the volume of the solution for each rat in group D

Dissolve total HCN consumed by group D in a certain volume of distilled water

0.33g of HCN in 2mls of water

$2/330$ mg -0.006061

Rat 1: 0.006061×80 -0.48ml

Rat 2: 0.006061×800 .48ml

Rat 3: 0.006061×84 -0.50ml

Rat 4: 0.006061×86 -0.52ml

Table 4: Summary showing the volume of hydrogen cyanide administered to each rat in Group B

	Weight	HCN	Volume
Rat 1	200g	80mg	0.48ml

Rat 2	200g	80mg	0.48ml
Rat 3	210g	84mg	0.50ml
Rat 4	215g	86mg	0.52ml

HIGH DOSE (400mg/kg)

Table 5: Summary showing the volume of hydrogen cyanide administered to each rat in Group C

	Weight	HCN	Volume
Rat 1	180g	45mg	0.49ml
Rat 2	190g	47.5mg	0.52ml
Rat 3	175g	43.75mg	0.48ml
Rat 4	180g	45mg	0.49ml

MEDIUM DOSE (250mg/kg)

Table 6: Summary showing the volume of hydrogen cyanide administered to each rat in Group D

	Weight	HCN	Volume
Rat 1	170g	25.5mg	0.51ml
Rat 2	160g	24mg	0.48ml
Rat 3	170g	25.5mg	0.51ml
Rat 4	165g	24.75mg	0.49ml

LOW DOSE (150mg/kg)

Termination of Administration

The administration lasted for 3 weeks. A day after the last administration, the animals were placed in a closed plastic bucket containing cotton wool soaked with chloroform. After which the brain was carefully removed and placed in a 10% formal saline.

Tissue Processing

The whole brain was fixed in 10% formal saline. This process hardens and preserves the tissue, ensuring that their original shapes and structures are retained as close to life as possible. The course of processing the tissues were taken through the following stages,

Dehydration

The tissue was transferred from 10% formal saline into an ascending grade of alcohol (Two changes of 70%, 95% and 100% for two hours each) in order to remove water from the tissue.

Clearing

The tissue was then cleared of alcohol using xylene. This is to avoid distortion of the tissue. The dehydrated tissue was placed in xylene, two changes each for one hour.

Infiltration

The tissue was transferred from xylene to molten paraffin wax in an oven at a temperature of about 60 degree Celsius, two changes one hour each. The tissue was transferred to the mould by holding it gently with a warm smooth up forcep. The process helps remove the clearing agent from the tissue.

Embedding

Fresh molten paraffin wax and metal embedding molds were used. After embedding, blocks obtained were cast into wooden blocks with the aid of a lead spatula.

Sectioning

A rotator was used to make sections of the tissue. The ribbon of the section was gently lowered into the surface of a warm bath. The floated section was mounted on a slide and allowed to dry before staining.

Staining; Hematoxylin and Eosin technique

The staining process was carried out using troughs and racks. The slide with the tissues on them were flooded with xylene to dewax for about 5-10 minutes. The section was then passed through absolute alcohol for 3 minutes to clear xylene. The sections were hydrated through descending grade of alcohol from 95% to 70% alcohol for 3 minutes in each grade and then water for 3 minutes. The sections were then stained in hematoxylin for 15 minutes. The differentiation of the sections was done using acid alcohol for one second. This was done very quickly so as to avoid washing away of section. They were subject under running tap water for 30 minutes to blue (bluing). The sections were counterstain in 10% alcohol. They were emerged in eosin for 5 minutes and then dehydrated in ascending grades of alcohol 70%, 95% and absolute alcohol, section was then cleared in xylene for 3 minutes and mounted with DPX and then cover slip. The slides were left dry before viewing under micrographing. The slides were carefully studied under low and high magnification of the light microscope. Comparisons were made with the slides of the control group for detection of histological changes.

Result

Different sections of the pontomedullary junction were viewed after histological preparation of the control and experimental group of animals. The result and observation obtained are discussed based on each group. The result is limited to the comparison between histological appearance of the control group and that of the experimental groups. Also, the weight of the animals were observed and taken into consideration before and after administration. The photomicrographs obtained are all in the same magnification (X100 and X400) showing histopathological changes.

Histological Observation

Control Group (Plate 1): The control group was given distilled water. Histological section of the pontomedullary junction from this group presented normal histological features, starting with prominent round to oval neuronal cell bodies with interwoven neurofibrils. The neuronal cells have deeply stained nuclei with coarse chromatin pattern and moderate cytoplasm. The neurofibrils are compact and wavy. Having two layers, the first upper layer neurons are fewer as compared to the lower (inner) layer neurons which are much in number.

Low Dose, Group B (Plate 2): Section from this group shows prominent round to oval neuronal cell bodies with interwoven neurofibrils. The neuronal cells are of various sizes and shape with deeply stained nuclei having coarse chromatin pattern and moderate granular cytoplasm. The neurofibrils are abundant and wavy. Continuous reduction of number of neurons with moderate interneuronal vacuole.

Medium Dose, Group C (Plate 3): Section shows prominent round to oval neuronal cell bodies with interwoven neurofibrils. The neuronal cells are scanty and are of various sizes and shapes with deeply stained nuclei having coarse chromatin pattern and moderate granular cytoplasm. The neurofibrils are abundant and wavy. Continuous reduction of number of neurons with moderate interneuronal vacuole.

High Dose, Group D (Plate 4): Section shows prominent round to oval neuronal cell bodies with interwoven neurofibrils. The neuronal cells are scanty and are at various sizes and shape with deeply stained nuclei having coarse chromatin pattern and moderate granular cytoplasm. The neurofibrils are lesser in appearance. There is a drastic reduction in number of neurons with lots of interneuronal vacuole.

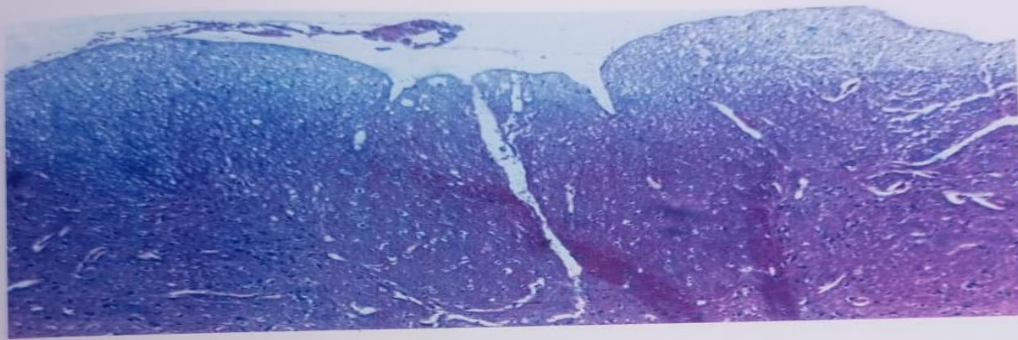
Plate 1 and 2: Control Group (Group A) Photomicrograph of the pontomedullary Junction showing normal histological appearance stained with H&E (X100 and X400).

NF - Neurofibrils

NCB - Neuronal Cell Bodies

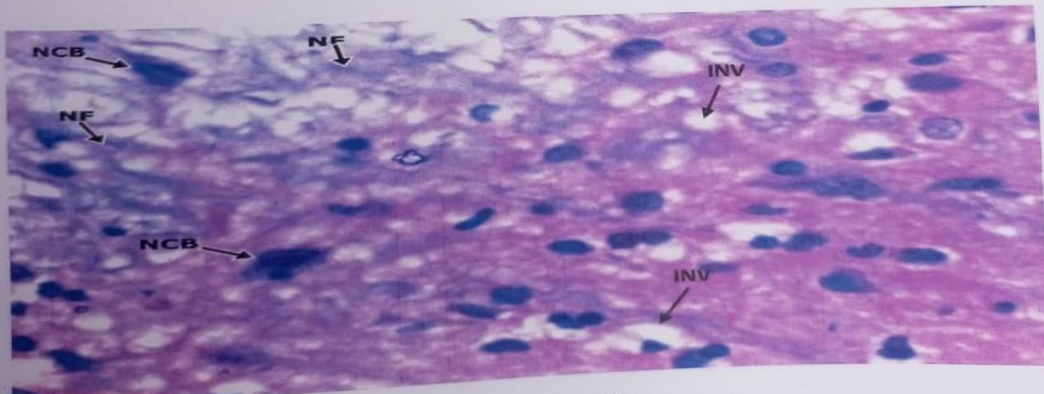
INV - Interneuronal Vacuole

PLATE 1



CONTROL PONTOMEDULLARY JUNCTION X100

PLATE 2



CONTROL PONTOMEDULLARY JUNCTION X 400

Plate 3 and 4: Low Dose (Group B)

Photomicrograph of the pontomedullary junction of Wistar rats given 150mg/kg of aqueous extract of sweet cassava. Stained with H and E (X100 and X400) showing radial degeneration and disintegration of Neuronal Cell Bodies, increasingly showing in interneuronal vacuole.

NF - Neuronal fibers
NCB - Neuronal Cell Bodies
INV - Interneuronal Vacuole

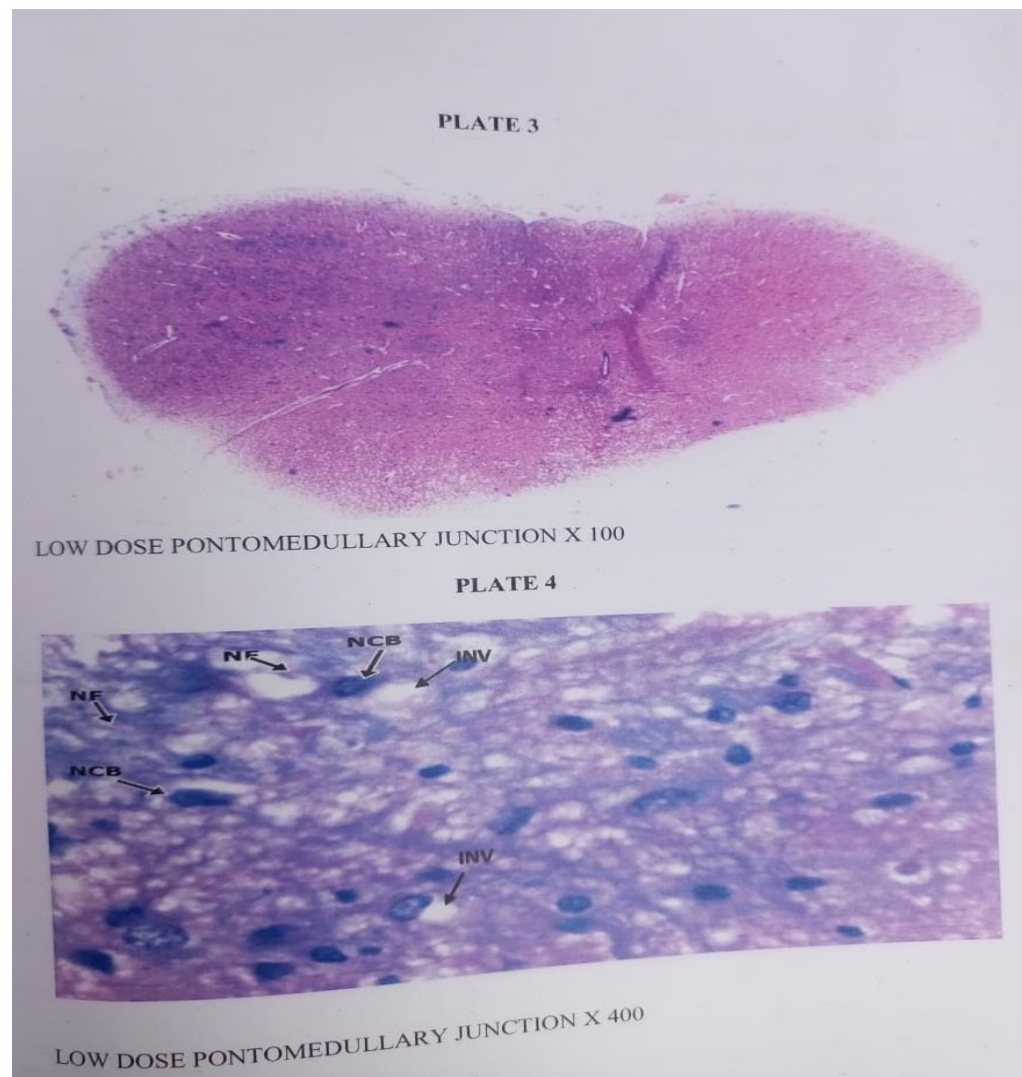


Plate 5 and 6: Medium Dose (Group C)

Photomicrographs of pontomedullary junction of Wistar rat given 250mg/kg of aqueous extract of sweet cassava. Stained with H&E (X100 and X400) showing disintegration of cell bodies, decrease in neuronal fibers and rapid increase of interneuronal vacuoles.

NF - Neuronal fibers

NCB - Neuronal Cell Bodies

INV - Interneuronal Vacuole

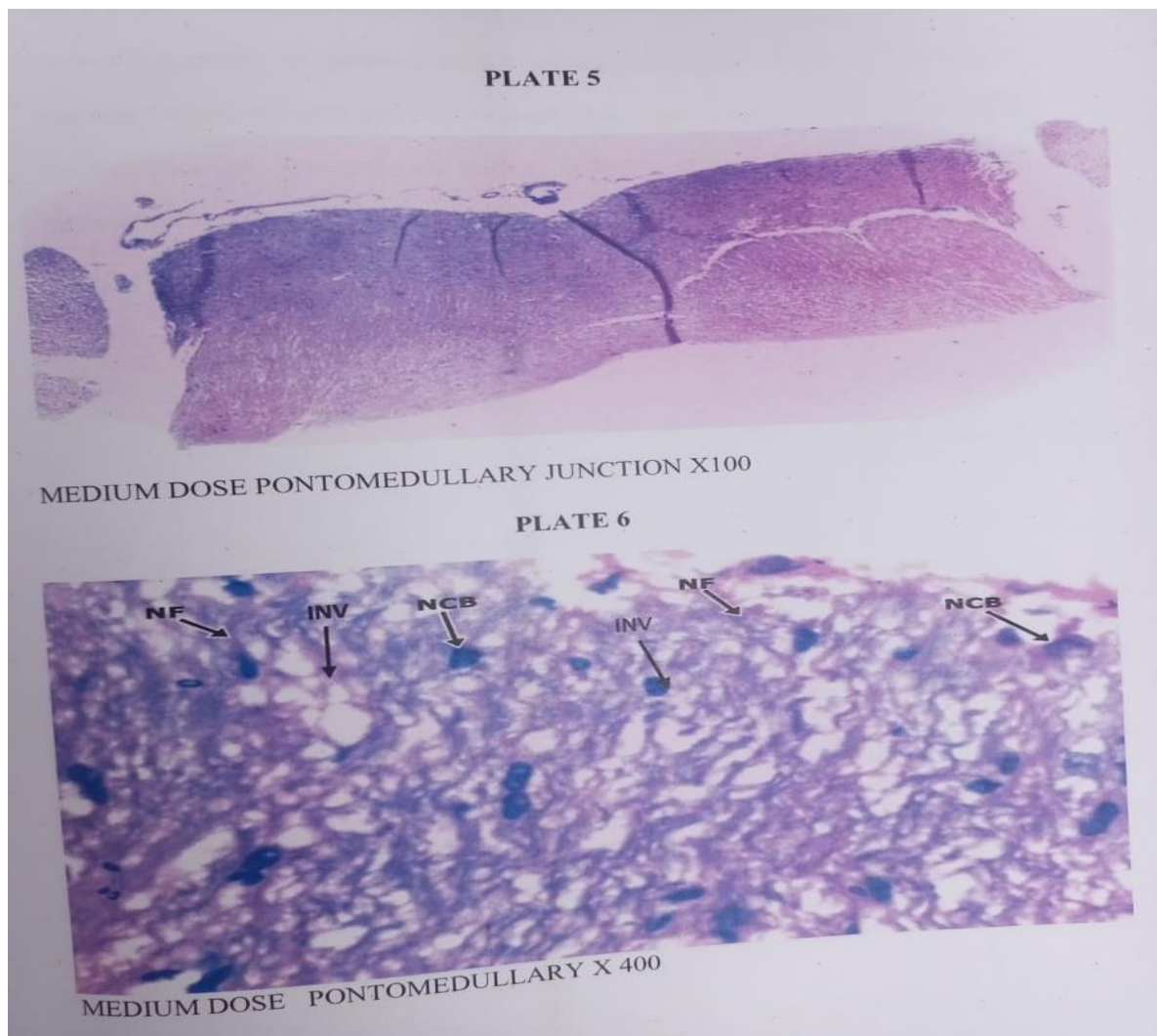
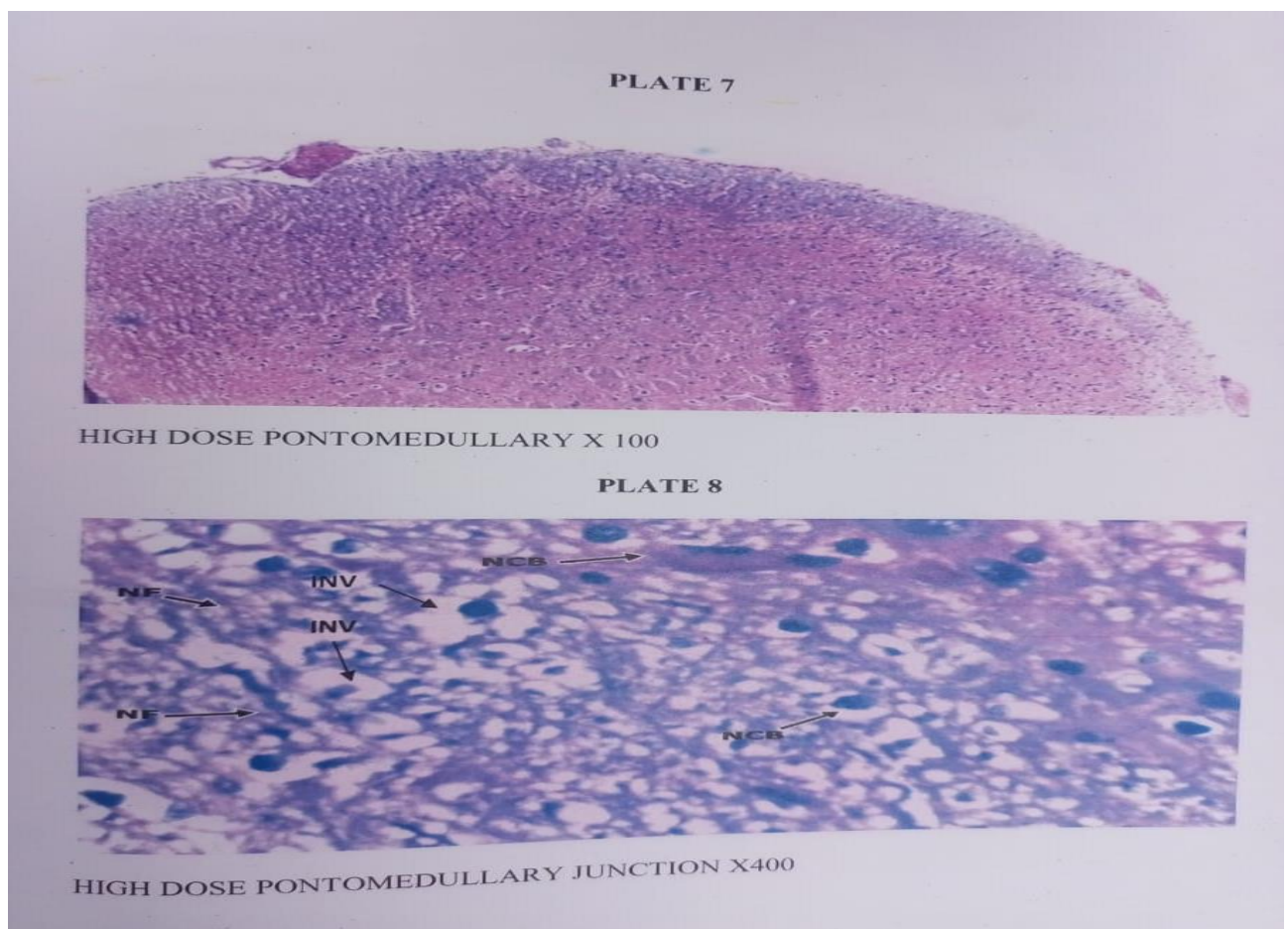


Plate 7 and 8: High Dose (Group D)

Photomicrographs of pontomedullary junction of wistar rats given 400mg/kg of aqueous extract of sweet cassava. Stained with H&E (X100 and X400) Showing drastic degeneration of neuronal cell bodies, decrease in neuronal fibers and increase in interneuronal vacuoles.

- NF - Neuronal fibers
NCB - Neuronal Cell Bodies
INV - Interneuronal Vacuole



Discussion of Findings

Cassava (manibotesculitacrantz) and its products have been widely studied and reported to have been beneficial, though sometimes with toxic effects, most of these effects are due to presence of some non-nutrient factors in cassava. The medicinal properties of the constituent phytochemicals have been documented by a number of researchers (Ezeanyika *et al.*, 1999).

In this study, the results showed that after 21 days of ingestion of the aqueous extract from sweet cassava tuber' in doses control, low dose (150mg/kg). medium dose (250mg/kg), high dose (400mg/kg), there are effects on the histological appearance of the neurons present in the pontomedullary junction, with marked cytoplasmic disintegration of neurons and increased interneuronal vacuoles, which is caused by the effect of oral intake of hydrogen cyanide for 21 days, there was drastic cytoplasmic disintegration of neurons present in the pontomedullary junction, increase in interneuronal vacuoles in the experimental groups.

The controlled group (cage A), shows normal neuronal cell bodies and processes but with increase in doses from low dose, medium dose to high dose, the cells began to disintegrate leading to loss of weight. The neuronal cell bodies and fibers which are compact and wavy in the control group became scanty in the experimental group. Hydrogen cyanide is a toxic substance present in sweet cassava do cause pathogenic neuropathy in the brain of Albino Wister rats and is dose dependent The effect increases from Low dose (group B) which showed gradual degeneration of neuronal cell bodies, Medium dose showed moderate degeneration of neuronal cell bodies and increase in interneuronal vacuoles, High dose showed drastic disintegration of neuronal cell bodies, neuronal fibres and increase in interneuronal vacuoles.

Neurological disorders such as ataxic neuropathy and cretinism have been associated directly with the intake of cyanogenic glycoside-contaminated diets. There are grounds to suspect that cyanogenic glycoside-contaminated causes chronic cyanide toxicity in the tropical regions of the world according to Osuntokun, 1981. Although correlation between dietary cyanogen-contaminants and diseases such as (TAN) Tropical Ataxic Neuropathy, goiter, cretinism and mental mechanism of action of cyanide and thiocyanate on the cellular level is not well understood. Therefore, with this study it is suspected that the degeneration of the neuronal cell bodies can lead to these neurological disorders. Comparing the research work of Osuntokun, 1981 to this study carried out to access the safety profile of the methanol and chloroform extracts of garri using Albino Wistar rats revealed no death at 2.5g/kg body weight. This finding would imply that garri extracts are relatively not life threatening at that dose. This is expected since garri is the major source of dietary carbohydrate in tropical countries such as Nigeria.

It also reveals that the activity of the liver function enzymes Alanine Aminotransferases (ALT) and aspartate aminotransferase (AST) increased significantly after 21 days administration of chloroform and methanol extracts of garri when compared with those of the control. This suggests that the chloroform and methanol extracts of garri are capable of causing hepatocellular injury. This study shows that the doses administered (400mg/kg for high dose,

250mg/kg for medium doses and 150mg/kg for low dose) causes disintegration of the neurons present in the pontomedullary junction of Albino Wistar rats which contains the nerve root or nuclei of cranial nerve VIII thereby causing impairment in the functional anatomy of cochlear and vestibular system which is responsible for coordinating equilibrium (balance) and hearing in an organism.

Summary

This study demonstrates that hydrogen cyanide in sweet cassava tubers produces adverse effect which is dependent on dosage and time of administration.

In this research 16 wistar rats were bought and assigned to 4 groups in different cages using alphabetical label of group A,B,C and D. Group D. There were given 0.48ml for rat one and two, 0.50ml for rat three and rat four 0.52ml of aqueous extract from 400mg/kg of cassava which contained 0.33g of HCN. Using histological technique, the tissue which was processed and viewed under light microscope in the laboratory, showed disintegration and degeneration of neurons of the pontomedullary junction starting with group B(low dose), group C(medium dose), to group D(high dose), as compared to the Group A (Control Dose).

Conclusion

This study reveals that hydrogen cyanide consumption from poorly processed cassava tubers has harmful effect on the histological features of the brain stem, thereby affecting the vestibulocochlear nuclei which control the hearing and balancing of an organism in any posture.

Recommendations

1. Further research should be carried out to note the effect on the structure of the hair cells (maculae vascularis), semicircular ducts, and the hearing center in the cerebrum.
2. The duration of the administration of the extract should be prolong in further studies.
3. Other extracts of cassava tubers like (ethanol, chloroform e.tc) should be used in other further studies.
4. Other studies should be carried out to investigate the effect on cranial nerve II
5. Further research should be carried out to note the effect of hydrogen cyanide on cranial nerve III

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