Journal of Cell and Animal Biology Vol. 4(9), pp. 131-136, September 2010
Available online at http://www.academicjournals.org/JCAB
ISSN 1996-0867 ©2010 Academic Journals

Full Length Research Paper

Phosphatase profile in *Manihot escculenta* induced neurotoxicity; role in neuronal degeneration in the brain of adult Wistar rats

O. M. Ogundele[1]*, E. A. Caxton-Martins[2], O. K. Ghazal[3] and O. R. Jimoh[2]

[1]Trinitron Biotech LTD, Science and Technology Complex, Abuja, Nigeria.
[2]Department of Anatomy, University of Ilorin, Kwara State, Nigeria.
[3]Unilorin Stem Cell Research Laboratory, Ilorin, Kwara State, Nigeria.

Accepted 24 August, 2010

As a general trend, a change in cell activity and morphology is usually depicted as biochemical differentiation occurring structural differentiation, cell migration and even cell death. In cassava induced neurotoxicity, several substance has been identified to be naturally occurring in cassava and Cyanogenic glycosides or other phytotoxins which has been found to have neurotoxic effects; Scopoletin, afflatoxin and CYANIDE as described by Osuntokun (1981) and Ernesto et al. (2002). These substances elicit toxicity by accumulation over a period of time, or exposure to high concentrations from environmental contamination of water, food substance and sometimes occupational exposure. Cassava has been found to be neurotoxic as its cyanide component is capable of inducing oxidative stress by blocking cytochrome c oxidase (CcOX) and inhibition of other metalloenzymes. In this study we investigated the profile of acid phosphates (ACP) and alkaline phosphatase (ALP) in the brain tissue of adult wistar rats treated with varying dose of cassava diet for a period of 60 days. ACP serves as a biochemical marker for lysosomal activity while ALP indicates membrane transport and integrity in the neuronal architecture. The brain tissue were excised and homogenized in 0.25 M sucrose (Sigma: β-D-Fructofuranosyl-α-D-Glycopyranoside) and centrifuged in Multifuge 3SR+ by ThermoScientific. The supernatant was obtained and assayed for ACP and ALP change in optical density per minute.

Key words: Alkaline phosphatase, acid phosphatase, neurodegeneration, membrane, lysosomes, cassava, cyanide, cytochrome c oxidase.

INTRODUCTION

Cassava (*Manihot escculenta)* is a major food crop in the tropics and sub-tropics as it serves as a source of cheap calorie food (Osuntokun, 1981; Oke, 1979; De la cruz et al., 2009; Ernesto et al., 2002). In cassava endemic regions of Uganda, Tanzania and Niger, various neurological disorders have been reported to include Tropical Ataxic Neuropathy (TAN), spastic endemic paraparesis (Konzo), gradual loss of vision and other symptoms resembling those observed in parkinsonism (Osuntokun, 1981; El-Ghawabi et al., 2005; Soler-Martin et al., 2010). Cassava however contains several cyanogenic glycosides (Mathangi et al., 2000; Lee et al., 2009); In the animal system, the major defense of the body against cyanide is the enzyme rhodanese (Tor-Agbidye et al., 1999) which is capable of converting cyanide (CN^-) to thiocyanate (SCN), the major form in which cyanide is being excreted. This reaction will occur in the presence of the S-group present in thiosulphate and S-containing amino acid (SAA) like cysteine and tyrosine, cysteine has been found to react with free cyanide to generate 2-Iminothiazoldine-4-Carboxylic acid which could be found in saliva of cyanide intoxicated animals (Mathangi and Namasivayam, 2000).

Thus, animals fed on low protein diets will elicit more toxic effects compared to those fed on protein diets especially those containing SAA (Mathangi et al., 2000).

Cyanide in cassava is released either as free cyanide or HCN, the most reactive form of cyanide is CN but the state depends on the pH, salinity and temperature of the

*Corresponding author. E-mail: mikealslaw@hotmail.com. Tel: +2347031022702.

medium (Chen et al., 2003; Lee et al., 2009). At pH 7.0, 99% of cyanide will exist as HCN, at pH 11 about 99% of cyanide will exist as CN while an equilibrium has been observed for pH range of 9.0 - 9.3 (Li et al., 2000). The neurotoxic effects of cassava can not be attributed to a single substance as it contains several substances capable of generating neurotoxicity (Ernesto et al., 2002; Denison et al., 2009; Dorea, 2003). The cyanide released for cassava generates oxidative stress by inhibiting Cytochrome c oxidase (CcOX) a terminal enzyme in the electron transport chain, Cyanide also inhibit energy production by binding to the three states of the Binuclear centre heme a3-CuB formed by combination of CcOX to molecular oxygen released form water, this will thus prevent production of ATP at complex IV (Bonfoco et al., 1995; Bathachanya and Tulsawani, 2008).

The cellular mechanism of this inhibition is associated with generation of heat, leakage of proton into then mitochondria matrix and conversion of 20% of molecular oxygen to ROS (reactive oxygen species) this includes superoxide ions and are measured in the as cytoplasm as superoxide dismutase (SOD) (Bove et al., 2005; Nelson, 2006). ROS reacts with accumulated NO at complex I and III to generate RNS, NO are naturally occurring endogenous modulators of cellular activity but if present in high levels could trigger toxic pathways or cell death, the mode of cell death observed in neurons for different toxicology experiments have been found to correspond with the level of accumulated NO and ROS (Gruetter et al., 2001; Lee et al., 2009). Several models have been used to describe the mode of cell death in neurons, Isom and Way (1984) reported that elevated levels of cerebral calcium initiate a caspase system in cell death while secondary autophagic bodies of lysosomes have been found in other experiments by Osuntokun (1981), Li et al. (2000) and Dorea et al. (2003). Although they measured lysosomal activity using β-glucoronidase as indicator, this results has been found not to be weight (Gunasekar et al., 1996) dependent and also variations has been found in different brain regions this however explains the models involving region specific cytotoxic pathways in the brain Isom et al., 1999; Bathachanya and Tulsawani, 2008).

The study describes the role of acid and alkaline phosphatase in the various cellular changes observed in the cells for various dose of treatment and the adopted mode of cell death observed in the cells of the brain.

MATERIALS AND METHODS

ALP assay kits (Sigma, Germany) and ACP Assay kit (Sigma, Germany), Sucrose (sigma Aldrich, Germany).

Tissue preparation

The occipital region, superior colliculus and lateral geniculate body of adult Wistar rats fed with 2.5, 10, 20 and 30 g of cassava per animal/ day orally for 60 days alongside a control group treated with 0.25 M sucrose. The tissues were homogenized in 0.25 M sucrose at 4°C and then centrifuged at 10,000 rpm for 20 min using multifuge 3SR+; the supernatant was collected and assayed using the substrate technique using the spectrophotometer (Jenway, 5550) (Enulat et al., 2010). The data was analyzed in SPSS 15.0 software to determine the analysis of variance.

Alkaline phosphatase

The working reagent is composed of magnesium chloride 0.625 mMol/L, alkaline phosphate 2 ml, p-nitrophenyl phosphate 50 mMol/L pH7.8. The reagent was linear up to 700 µl. A blank working reagent was used in a couvette and was discarded since the absorbance has exceeded 405 nm. 1000 µl of the working solution was mixed with 20 µl of the sample, the solution was incubated at 37°C for 30 min and the absorbance was measured at 60, 120 and 180 s respectively, after each 60 s the solution was mixed to check for change in optical density (ΔOD/min) using a dilution factor of 1:50, the ALP activity was then expressed as ΔOD/MinX2750 (Fishman and Baker, 1998; Wintola et al., 2010).

Acid phosphatase

ACP activity was determined using the substrate method described for ALP, an additional dye. The working reagent was freshly prepared and its composed of Acid phosphate10 mMol/L, fast red 6 mMol/L, the reagent was linear up to 150 µl. one tablet of acid phosphate was dissolved in freshly prepared citrate buffer pH 5.2, this reagent was found to be stable for 2 days at 2 - 8°C; 100 µl of the working solution was added to 10µl of the titrate solution (fast red) (Nachilas et al., 1989) then 100 µl of the sample was added, the mixture was then vortexed and incubated at 37°C for 6 min. Change in OD was measured at intervals of 60 s for 180 s. Activity was measured as a factor of ΔOD/MinX750 (Baker, 1998; Enulat et al., 2010; Volbracht et al., 2009).

RESULTS

In Group 1 which is a high dose treatment group, ALP activity followed a sinusoidal pattern with the activity at the 60 s being 0.862, it increases at 120 s 0.891 and then falls at 180 s, Group 4 showed a rise in ALP at 60 s an decrease from this initial value was observed at 120 and at 180 s ALP activity increased but did not get to value observed at 60 s, group 2 showed a decline in ALP (0.636, 0.576, 0.519). Group 3 showed ALP activity below the levels observed in the control (60 s: group 3 (0.016) and group 5/control (0.238). The high dose treatment groups (Group 1, 2 and 3) showed an irregular pattern in ALP activity. Comparing the factors for each of the groups, only Group 3 which received a moderate dose showed ALP activity below that of the control, also the activity observed in Group 4, low dose group is higher than the factor in Group 2, which suggests that the activity of ALP in a model system is dose dependent in such a way that extreme doses triggers increase in membrane activity and synthesis rather than moderate does which generate similar effects but below those elicited by the extreme doses (Table 1: Group 1 and 5). ACP activity in Group 1 is similar to ALP activity characterized by a rise then a fall at

Table 1. ALP activity.

Group \ Seconds	60	120	180	Average	Factor
1	0.862	0.891	0.808	0.854	2,348.25
2	0.636	0.576	0.519	0.577	1,586.75
3	0.016	0.013	0.086	0.115	316.25
4	0.796	0.667	0.676	0.713	1,960.75
5	0.238	0.236	0.273	0.249	684.75

Table 2. ACP activity.

Group \ Seconds	60	120	180	Average	Factor
1	0.279	0.500	0.309	0.309	272.75
2	0.555	0.364	0.291	0.403	302.25
3	0.350	0.301	0.295	0.315	236.25
4	0.373	0.388	0.327	0.363	272.15
5	0.460	0.475	0.533	0.491	368.25

180 s, Group 2 and 3 (Table 2) shows a decrease from 60 - 180 s while Group 4 has similar pattern to Group 1 again this further re-affirms the hypothesis that phosphatase activity are elicited by extremes doses rather than moderate doses as described in this study for ALP.

All groups gave a factor below the control value (368.25) and the level of activity in Group 1 and 4 are almost equal (272.75 and 272.15), respectively. We can, however, deduce from these result that; 1. Extreme exposure to cassava causes neurotoxicity by altering the activity across and synthesis of membranes (rise in ALP) and suppressing lysosomal activity as seen in decrease in ACP factor; 2. Moderate exposure initiate degeneration by increasing the level of ALP to a point 25% less that Extreme dose treatment and having less suppressing effects on the ACP activity compared to the extreme dose group which was found to have a greater ACP suppressing activity, thus, characterized by an apoptosis-necrosis continuum. Rise in ALP indicates necrotic activity while partial suppression of lysosomal activity; increased activity compared to the extreme dose groups (302.25) indicates tendency of physiological cell death rather than programmed cell death. these two models suggests that extreme doses (high or low) have a greater tendency of inducing cell death by necrosis while moderate doses induces cell death by partial apoptosis and Necrosis (Figures 1 - 3).

DISCUSSION

The role of phosphatase is completely opposite to that of kinase and phosphorylase, which add phosphate groups to proteins by the help of energy-supplying molecules ATP (adenosine triphosphate) (Baker, 1998). The addition of a phosphate group can set off a protein-protein interaction. This also can activate or deactivate the function of an enzyme. Phosphatase is an important constituent of many biological processes involving genetic transduction because it can regulate the proteins to which they are attached (Fishman and Baker, 1998; Bathacharya and Tulsawani, 2008; Wintola et al., 2010). The alkaline phosphatases are determined by at least three gene loci, which can be sharply distinguished one from another by their sensitivity to inhibition with various amino acids and peptides and by thermostability (Becker et al., 2000). Alkaline phosphatase is present in the brains of guinea pig, rat, mouse, hamster, squirrel, rabbit, cat, sheep, cow, tamarin, baboon, and man. The gene locus coding for alkaline phosphatase in all these brains is the liver/ bone/kidney locus, as indicated by thermostability studies and by inhibition studies with L-phenylalanine, L-homoarginine, and L-phenylalanylglycylglycine. The average brain alkaline Phosphatase activity is about 35% of the average for the livers and only 7.2 and 4.4% of the average kidney and placental activities, respectively (Ven-Watson and Ridgway, 2007).

During growth and development, brain alkaline Phosphatase activity decreases in the mammals studied. The amount of change is tissue- and species-dependent. Phosphatases are enzymes that act as catalysts in the hydrolysis of organic phosphoric acid. A few classes of phosphatase enzymes are found to be involved with many common physiological disorder (Van-Watson and Ridgway, 2007; Soler-Martin et al., 2009; El-Ghawabi and De Filipo, 2005). This fact indicates that phosphatases control many fundamental processes in cellular

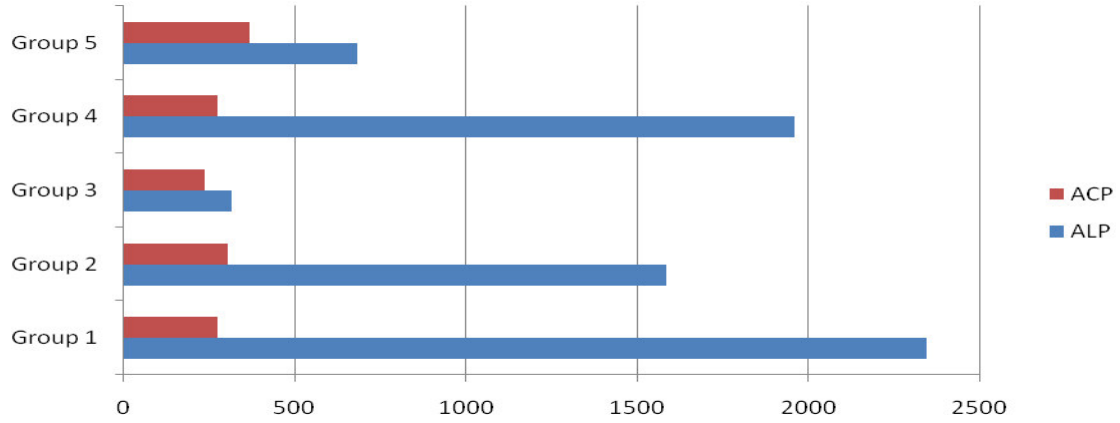

Figure 1. Curve demonstrating varying activity levels for ALP and ACP for each of the groups and withdrawal effects from group 6 - 8.

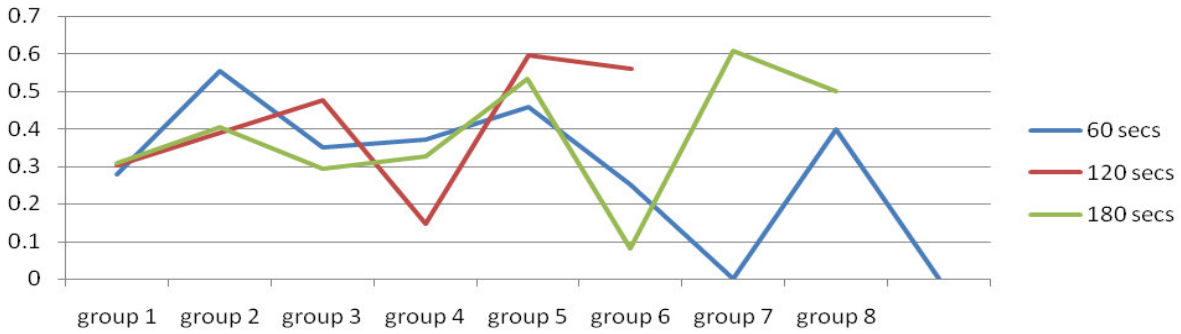

Figure 2. Curve demonstrating varying activity levels for ACP for each of the groups and withdrawal effects from group 6 - 8.

physiology. Phosphatases also can avert genetic changes. They are degenerated in response to DNA damage, thus preventing chromosomal abnormalities. Phosphatases also enhance the progression of cell cycle, in the brain, Phosphatase are found in multiple compartments of neuralgia and neuronal and play an important role in various neuronal functions (that is, pre-and-post synapses and gene expression). These functions are also important for maintaining the coordinated action of signaling cascades (Beckner et al., 2000).

As a general observation increase in size of the vacuolar spaces in the tissue conformed with an increase in activity of ACP (Figure 3) such that different regions of the brain reacts differently at the same treatment dose (Figure 3), in 3A (primary cortex) shows the presence of spaces around the cells while fibrous layer has become predominant in 3B (lateral geniculate body), while in 3C, presence of reduced metachromasia in the cells coupled with an increased cell diameter. After staining with Cresyl fast violet .This however, explains the variation in the cytotoxic pathway adopted by different brain regions. This was also similar to the findings of Solomonson et al. (1981) although they went further to examine these parameters at higher doses of cassava diet over a period of 6 months but found out that the effects are too deleterious and irreversible on a long term basis. However, Osuntokun (1981) reported a rise in the level of β-Glucoronidase at low dose treatment as an indicator of lysosomal activity which was also found to be proportional to sulphur excretion. The pattern of change in the activity of the phosphatase enzymes is in a zigzag manner (Figure 1:ALP) and (Figure 2: ACP) as it explains the feedback mechanisms involved in the cytotoxic pathways (Isom et al., 1999), higher dose causes cell death and a decrease in the enzyme activity while lower doses will inhibit the ALP causing membrane malfunction and influx of the calcium ions (Di Filipo et al., 2008), internal build up of ROS and NO will activate caspase cascades which affects apoptotic pathways at moderate doses or stimulate necrosis at higher doses (Phrabakharan et al., 2007).

Available reports of toxicological studies lack information on the level of intake of cyanogenic glycosides or on the amount of hydrogen cyanide potentially released. No long-term toxicity or carcinogenicity studies were available. However, *in vitro* and *in vivo* genotoxicity were negative. Teratogenic and adverse reproductive effects attributable to linamarin (cassava) and hydrogen cyanide

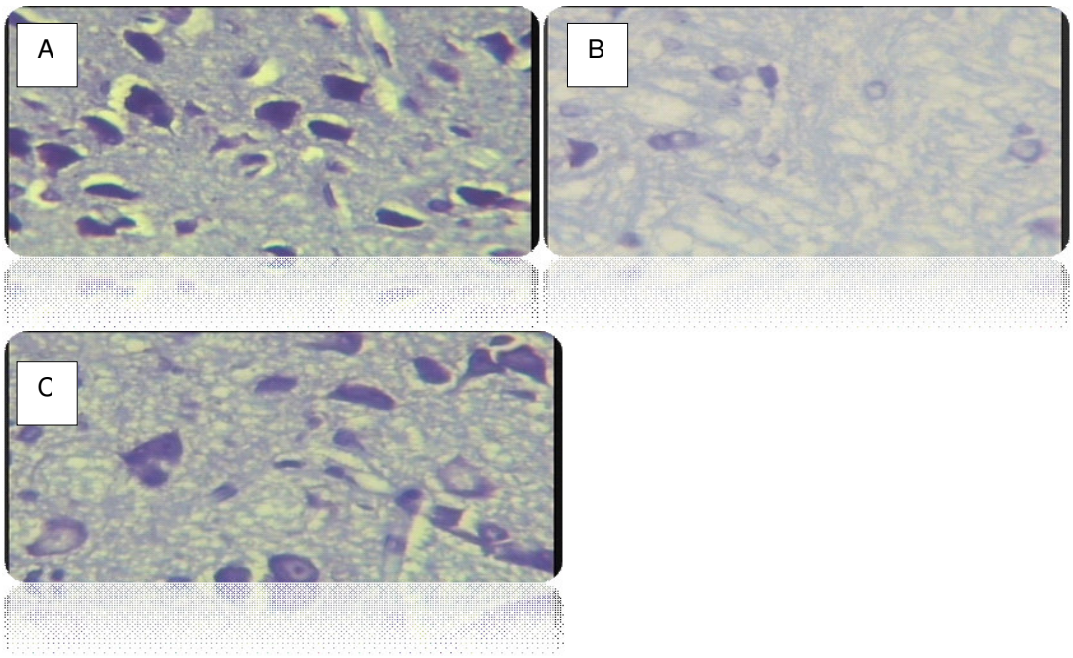

Figure 3. Structure of different parts of the brain A- Cortex, B- Lateral geniculate body and C- Superior colliculus showing different cellular changes to the same dose of treatment, this explains the variation in the cytotoxic pathway adopted by different brain regions.

were seen only at doses that also caused maternal toxicity (Ernesto et al., 2002). The toxic effects of cyanide on the thyroid (via its metabolite thiocyanate) depend on the iodine status of the test animals, as indicated earlier. On the basis of epidemiological observations, associations have been made between chronic exposure to cyanogenic glycosides and diseases such as spastic paraparesis, tropical ataxic neuropathy, and goiter. However, these observations were confounded by nutritional deficiencies, and causal relationships have not been definitely established (Osuntokun, 1981; Oke, 1979; Mathangi and Namasivayam, 2000).

Traditional users of foods containing cyanogenic glycosides usually have a basic understanding of the treatment required to render them safe for consumption. However, some products are sold commercially and are consumed by people who may not be familiar with such procedures. The EPA (Environmental protection Agency, USA) recommended that guidelines be developed to provide reliable and sensitive methods for the analysis of these foodstuffs for hydrogen cyanide releasable from cyanogenic glycosides, in order to ensure that amounts in foods as consumed do not present a hazard. Because of a lack of quantitative toxicological and epidemiological information, a safe level of intake of cyanogenic glycosides could not be estimated. However, it was concluded that a level of up to 10 mg/kg hydrogen cyanide in the Codex Standard for Cassava Flour (Varone et al., 2008) is not associated with acute toxicity (Table 3).

Conclusion

Toxicity of cassava has been found to be initiated as function of membrane malfunction and lysosomal activity thus causing degeneration of the neurons of the brain, although the effect is dose dependent as matter of general effects such that extreme doses causing cell death by necrosis and moderate doses causing cell death by apoptosis-necrosis continuum rather than solely apoptosis or necrosis, the effect is how ever non-specific and of irregular pattern. The pattern adopted in each of the cytotoxic pathways will determine the mode of cell death adopted by the neurons.

ACKNOWLEDGEMENTS

Trinitron Biotech LTD, Mr. Gerry Nash, Rosita Menezes, Helen Odedairo, Dr. Arise Olusanya.

FOOTNOTES

Ogundele, Olalekan Michael (B.Sc, M.Sc); Reasearch and Development Department, Trinitron Biotech JV, LTD. Science and Technology Complex, Abuja.

Prof E.A Caxton-Martins (FASN) Department of Cell Biology, University of Ilorin, Nigeria.

Dr.O.R Jimoh (FMSN): Department of Cell Biology, University of Ilorin, Nigeria.

Dr O.K. Ghazal (B.Sc, MB.ChB, M.Sc): Department of Cell Biology, University of Ilorin, Nigeria

REFERENCES

Baker JR, HEW H, Fishman WH (1998). The use of a chioral hydrate formaldehyde fixative solution in enzyme histocheunistry. J. Histochem. Cytochern., 6: 244-250.

Becker NH, Goldfischer S, Shin W, Novikoff AB (2000). The localization of enzyme activities in the rat brain. J. Riophys. Biochem. Cytol., 8: (149-663, 19(10).

Bhattacharya R, Tulsawani R (2008). *In vitro* and *in vivo* evaluation of various carbonyl compounds against cyanide toxicity with particular reference to alpha-ketoglutaric acid. Drug Chem Toxicol., 31(1): 149-61.

Blomgren K, Leist M, Groc L (2007). Pathological apoptosis in the developing brain. Apoptosis Review, 12(5): 993-1010.

Bonfoco E, Krainc D, Ankarcrona M, Nicotera P, Lipton SA (1995). Apoptosis and necrosis: two distinct events induced respectively by mild and intense insults with NMDA or nitric oxide/superoxide in control cell cultures. Proc. Natl. Acad. Sci. USA, 92: 7162-7166.

Bove J, Prou D, Perier C, Przedborski S (2005). Toxininducedmodels of Parkinson's disease. NeuroRx., 2: 484-494.

Chen Kk, Rose Cl, Clowes G (2003). Comparative values of several antidotes in cyanide poisoning. Am. J. Med Sci., 188: 767.

de Haro L (2009). Disulfiram-like syndrome after hydrogen cyanamide professional skin exposure: two case reports in France. J Agromed. 14(3): 382-384.

de la Cruz Cosme C, Medialdea Natera P, Romero Acebal M (2009). Amyotrophic lateral sclerosis syndrome-plus and consumption of cassava (Manihot) . Is this a new presentation of the neurotoxic motor-neuron syndrome? Neurologia., 24(5): 342-343.

Denison TA, Koch CF, Shapiro IM, Schwartz Z, Boyan BD (2009). Inorganic phosphate modulates responsiveness to 24,25(OH)2D3 in chondrogenic ATDC5 cells. J. Cell. Biochem., 1; 107(1):155-62.

Di Filippo M, Tambasco N, Muzi G, Balucani C, Saggese E, Parnetti L, Calabresi P, Rossi A (2008). Parkinsonism and cognitive impairment following chronic exposure to potassium cyanide. Mov. Disord. 15;23(3): 468-470.

Dorea JG (2003). Fish are central in the diet of Amazonian riparians: should we worry about their mercury concentrations? Review, Environ. Res. 92(3): 232-244.

El-Ghawabi G, De Flipe J (2005). A correlative electron microscopic studies of basket cells. Neuro. Sci., 17: 991-1009.

Ennulat D, Magid-Slav M, Rehm S, Tatsuoka KS (2010). Diagnostic performance of traditional hepatobiliary biomarkers of drug-induced liver injury in the rat. Toxicol. Sci. 13. [Epub ahead of print].

EPA (1990). Summary Review of Health Effects Associated with Hydrogen Cyanide, Health Issue Assessment Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment Office of Research and Development, US Environmental Protection Agency Research Triangle Park, North Carolina, USA 1023-1029, 1955.

Ernesto M, Cardosso AP, Nicala D, Mirone E, Massasa F, Cliff J, Haque MR (2002). Persistent Konzo and Cyanogenic toxicity from cassava in Northern Mozambique. Acta. Trop., 82(3): 357-362.

Fishman WH, Baker JR (1956). Cellular localization of ı-glucuromsidase in rat tissues. J. Histochem. Cytocheın., 4: 570-587.

Hour SJ (1998). In General Cytochernical Methods, Ed. J. F. Daniehhi, Academic Press, Inc. New York.

Gamper N, Li Y, Shapiro MS. Structural requirements for differential sensitivity of KCNQ K+ channels to modulation by Ca2+/calmodulin. Mol Biol Cell. 2005 Aug; Epub 2005 May 18, 16(8): 3538-3551.

Gruetter R, Seaquist ER, Ugurbil K (2001). Am. J. Physiol., 281: E100–E112.

Hashem MA, Mohamed MH (2009). Haemato-biochemical and pathological studies on aflatoxicosis and treatment of broiler chicks in Egypt. Vet Ital. Apr-Jun; 45(2): 323-337

Isom GE, Gunasekar PG, Borowitz JL (1999). Cyanide and neurodegenerative disease. In *Chemicals and Neurodegenerative Disease* (S. C. Bondy, Ed.), pp. 101–129. Prominent Press, Scottsdale, AZ.

Isom GE, Way JL (1984). Effects of oxygen on the antagonism of cyanide intoxication: Cytochrome oxidase *in vitro*. Toxicol. Appl. Pharmacol., 74: 57-62.

JB, John S, Flora MV, Nenad Š, Pasko R. Notch regulates cell fate and dendrite morphology of newborn neurons in the postnatal dentate gyrus PNAS 2007 104 (51) 20558-20563.

Li R, Sonik A, Stindl R, Rasnick, D, Duesberg P (2000). Aneuploidy vs. gene mutation hypothesis of cancer: Recent study claims mutation but is found to support aneuploidy. Proc. Natl. Acad. Sci. U.S.A., 97: 3236–3241.

Mathangi DC, Namasivayam A (2000). Neurochemical and behavioural correlates in cassava-induced neurotoxicity in rats. Neurotox Res., 2(1): 29-35.

Mathangi DC, Namasivayam A (2000). Effects of Chronic cyanide intoxification on memory in albino rats. Food Chem. Toxicol., 38: 51-55.

Nachlas MM, Seligman AM (1989). The histochemical demonstration of esterase. J., Vat. Cancer Inst., 9: 415-425.

Nelson L (2006). Acute cyanide toxicity: mechanisms and manifestations Review. J. Emerg. Nurs. Aug; 32(4 Suppl): S8-11.

Oke OL (1979). Some aspects of the role of cyanogenic glycosides in nutrition. Wld. Rev. Nutr. Diet, 33: 70-103.

Osuntokun BO (1981).Cassava in diet, chronic cyanide intoxification and neuropathy in Nigerians. World Rev. Nutr. Dietetics, 36: 259-339.

Soler-Martín C, Riera J, Seoane A, Cutillas B, Ambrosio S, Boadas-Vaello P, Llorens J (2009). The targets of acetone cyanohydrin neurotoxicity in the rat are not the ones expected in an animal model of konzo. Neurotoxicol Teratol. 2010 Mar-Apr; 32(2): 289-294.

Solomonson LP (1981). Cyanide as a metabolic inhibitor. In Cyanide in Biology, Vennes L and, B., Conn, E.E., Knowles, C.J., Wesley, J and Wissing, F. Academic Press, London, New York, Toronto, pp. 11-18.

Tor-Agbidye J, Palmer VS, Spencer PS, Craig AM, Blythe LL, Sabri MI (1999). Sodium cyanate alters glutathione homeostasis in rodent brain: relationship to neurodegenerative diseases in protein-deficient malnourished populations in Africa. Brain Res., 820(1-2): 12-19.

Varone JC, Warren TN, Jutras K, Molis J, Dorsey J (2008). Report of the investigation committee into the cyanide poisonings of Providence firefighters. New Solut., 18(1): 87-101.

Venn-Watson SK, Ridgway SH (2007). Big brains and blood glucose: common ground for diabetes mellitus in humans and healthy dolphins. Comp Med., 57(4): 390-395.

Volbracht C, Penzkofer S, Mansson D, Christensen KV, Fog K, Schildknecht S, Leist M, Nielsen J (2009). Measurement of cellular beta-site of APP cleaving enzyme 1 activity and its modulation in neuronal assay systems. Anal. Biochem. Apr 15; 387(2): 208-220.

Wintola OA, Sunmonu TO, Afolayan A (2010). Toxicological evaluation of aqueous extract of Aloe ferox Mill. in loperamide-induced constipated rats. Hum. Exp. Toxicol. May 24. [Epub ahead of print].