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Modulating effect of aqueous extract of *telfairia occidentalis* on induced cyanide toxicity in rats

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Summary: The effect of lyophilised aqueous extract of *Telfairia occidentalis* (TO) on induced cyanide toxicity in rats was investigated. Twenty 3-week old male wistar albino rats were randomly distributed into one control and three treatment groups of five rats each: control group (group1), group treated with 3mg/kg body wt of cyanide only (group2), group treated with 3mg/kg body wt. each of cyanide and extract (group3), and a group treated with 3mg/kg Body wt of extract only (group4) were used for the investigation. Cyanide toxicity reduced both food and water intake ($p<0.05$), while the food intake was improved in group3, this effect of the extract on food was not observed on water intake. Cyanide reduced average body weight of rats significantly ($p<0.05$). The reduction effect of cyanide on body weight was countered by *Telfairia occidentalis* extract. The extract did not have an observable effect on rats' body weight. Ocular lesion was observed in 67% of rats in group2 . This ocular effect of cyanide was mitigated significantly by Telfairia occidentalis as only17% of the rats in group3 had ocular lesion. Cyanide toxicity produced nasal discharge in 39% of the rat population in group2 while there was a partial but considerable reduction (21%) in the severity of nasal discharge in group 3. There was no significant difference ($p>0.05$) in the organ/body wt.ratio between the treatments and the control groups for all the organs examined in the study. Biochemical analysis of liver enzymes showed that cyanide (group2) damaged the liver as there was significantly elevated presence ($p<0.05$) of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALP) above those of the control group. The damaging effect of cyanide on the liver was ameliorated by *Telfairia occidentalis* considerably.Histopathological effect of cyanide toxicity on the organs examined included multifocal degeneration and necrosis of the liver, mild kidney congestion and congestion of the brain. These effects were moderated mildly by *Telfairia occidentalis*. Group 4, treated with the vegetable alone had none of the observed histopathology in the organs examined. We concluded that lyophilised aqueous extracts of *Telfairia occidentalis* showed good potential as a safe antidote for cyanide poisoning when administered concomitantly or very shortly after ingestion of sub-lethal dose of cyanide. However, further bioassay guided fractionation and analytical studies are needed to identify the actual chemical compound or molecule in the vegetable responsible for or associated with the observed effects.

Keywords*:* Cyanide toxicity, *Telfairia Occidentalis*, Liver enzymes, Ocular lesion, Nasal discharge

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INTRODUCTION

Cyanides are both man-made and naturally occurring substances. In nature they are found in several plant species (e.g. sorgum, bamboo, cassava etc) as cyanogenic glycosides and are produced in certain bacteria, fungi and algae (ATSDR, 1989). Cyanide is also released into the environment from numerous sources as a result of human activities such as metal and organic chemical finishing, iron and steel production and automobile exhaust (ATSDR, 1989). In general, humans are exposed to gas, liquid and solid forms of cyanide from a broad range of natural, industrial and anthropogenic sources. Other sources of cyanide release into the environment include volatilization from cyanide wastes disposed of in landfills, emissions from municipal solid waste incinerators, biomass burning and fossil fuels combustion (WHO, 2004). Hydrogen cyanide is also a product of tobacco smoke and combustion of some plastics derived from acrylonitriles (ATSDR, 2006). Cyanides are also used as rat poisons and as insecticides to fumigate ships (ATSDR, 1989). Hydrogen cyanide has been used in gas chamber executions (Bokanya *et al* 1994). Several epidemiological studies in cassava eating populations have established an association between cyanide exposure and spastic para paresis, amblyobiaataxia,or tropical ataxia (ATSDR, 2006).Neurological disorders and thyroid abnormalities have been linked with long term consumption of cassava (Ajibade *et al* 2006).Cyanide is found in ambient air as hydrogen cyanide in the stratosphere. It is released into the atmosphere through injury from burning volcanoes, natural biogenic processes from higher plants, bacteria and fungi and to a smaller extent in

particulate matter (Cirenone and Zellner,1983; Jaramillo *et al* 1989; Okafor and Maduagwu 2000).Cyanide in form of hydrogen cyanide, sodium cyanide, potassium cyanide, calcium cyanide or copper(1) cyanide have been detected in surface water (WHO,2004;HazDat,2005; ATSDR,1997; Bedding *et al* 1982).Cyanides have been identified in the soil of hazardous waste sites, in the USA(HazDat 2005;Shifrinf *et al,* 1996).Many edible plants contain cyanogenic glycosides whose concentration can vary widely as a result of genetic and environmental factors(Ermans *et al* 1980,JECFA 1993).Elevated blood cyanide level has been implicated in smoke-inhalation injury and death(Baud *et al,* 1991). In the human body, cyanide is detoxified mainly by enzymatic conversion to the much less toxic thiocyanate (SCN) by sulphur containing dietary amino acids, cysteine and methionine (Okigbo, 1999).

Telfairia occidentalis is a tropical vine grown in many tropical regions including Nigeria, Caribbean and Latin America (Aiyeloja and Bello, 2006). The plant has been found to be rich in protein, the nutritional interest in some vegetable species as possible detoxifiers of cyanide stems from their rich essential amino acid, vitamin and mineral contents. In addition, vegetables are cheap and abundant sources of protein in poor developing countries of the tropics (Aletor and Adeogun, 1995; Fasuyi and Aletor, 2005). Leafy vegetables are important items of diet in many Nigerian homes. These vegetables are harvested at all stages of growth and fed as processed, semi-processed or fresh to man. *Telfairia occidentalis* has protein (21-37%CP), ash (14%), fat (13%) and fibre (13%) (Akoroda, 1990). The amino acid contents compare favourably with those of important legumes, while the vitamin, mineral and carotene contents make the leaves potentially useful as food supplements (Asiegbu, 1987). However, the plant contains considerable amounts of anti-nutrients such as phytic acid, tannin and saponin which could have some hazardous health effects on its consumers (Ladeji *et al,* 1995). The richness of the leaves in iron (Fe), it is used to cure anaemia (Ajibade *et al,* 2006). *Telfairia occidentalis* is rich in sulphur containing amino acid methionine and cystein (Aiyeloja and Bello, 2006). In this study therefore we investigated the potential of *Telfairia occidentalis* on induced cyanide toxicity in rats as a model for human cyanide toxicity.

MATERIALS AND METHODS

Preparation of vegetable

One kilogram of *Telfairia occidentalis* (vegetable) was purchased from Olodo agricultural farm in Ibadan. The vegetable was identified and authenticated by botanists at the Botany Department, University of Ibadan, and the Horticulture Research Institute based in Ibadan.

The vegetable was blended in warring blender and the paste was sieved through prewashed white cloth in distilled water. The sieved liquid was then filtered through filter paper (Whatman #1) to obtain a clear aqueous extract of the vegetable. The extract was lyophilised (Edward Freeze-dryer, MODULYO) to obtain a dry powder. Protein concentration in the lyophilised extract of the vegetable was determined by the method of Lowry *et al,* (1951) using bovine serum albumin (BSA) fraction V as standard protein.

Animals

Three- weeks old wistar albino rats were obtained from the animal breeding house of the department of Pharmacy of the University of Ibadan and transferred to the experimental laboratory of the Institute for Advanced Medical Research and Training of the College of Medicine, University of Ibadan

The rats were maintained on commercial rat chow and water *ad libitum* until the weight range of between 160g and 280g was attained. They were subsequently distributed randomly into 1 control and three (3) experimental groups as follows: Group1-no treatment (control), Group2-given 3mg/kg body wt KCN; Group3 given 3mg/kg body wt each of both KCN and extract; Group 4 –given 3mg/kg body wt extract only. All treatments were administered orally (by gavage) and daily. All animals were maintained on commercial rat feed and water *ad-libitum* for the entire period of the experiment (14 days).

Physical observations were made on the animals in the respective groups on day zero (when treatment commenced) and subsequently on daily basis. Before treatment was administered on any day, feed, water intake and body weight were measured. This is in addition to noting and enumerating physiological parameters, e.g. eye and fur colour, nasal discharge and ocular lesion.

Blood collection and plasma biochemical analysis

On day 15 of the experiment, blood samples were collected into capillary tubes from the rats using the ocular puncture method. The blood samples were collected in Lithium heparinized bottles and centrifuged at 1000rpm for ten minutes using MSE Table centrifuge at room temperature. Plasma was aspirated into universal bottles for Liver function test analysis by assaying for alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Reitman and Frankel, 1957) using automated analyzer. The packed cell volume (PCV) was also estimated.

Organ pathology

Immediately after the collection of blood samples, each rat was terminated by cervical dislocation, dissected through the sternum and *linea alba*. The liver, kidney and brain were harvested, weighed and placed into labelled bottles containing 10% phosphate-buffered formalin for 24hrs for proper fixation.Thereafter, the organs were sectioned and each section was embedded in paraffin, prepared and stained with haematoxylin and eosin (H&E) for histopathological changes using light microscope.

Statistical analysis

Data obtained were presented as Mean ± SD, comparison of the groups were done using ANOVA (SAS 1987) and student's t test. P <0.05 was considered statistically significant.

RESULTS

There was a significant difference (P< 0.05) in water intake between the control group and the group treated with 3mg/kg body weight of cyanide as shown in Table 1. The reduction in water intake was persistent even in the group treated with the equivalent combination (3mg/kg body weight) of cyanide and lyophilised aqueous extract of *Telfairia occidentalis*. The vegetable alone did not have a significant effect on water intake as there was no difference (P>0.05) when compared with the control.

The average food intake by the group treated with cyanide alone was significantly (P<0.05) less than that of the control group, signifying that cyanide toxicity reduced the appetite of the rats in the group. The food intake of the control group and the group treated with a combination of equivalent concentrations of cyanide and the vegetable extract were comparable with no significant difference (P>0.05) between them. This result indicates that *Telfairia occidentalis* probably annulled the reduction of food intake caused by cyanide toxicity. The appetite of the group fed with the vegetable extract alone was not affected as there was no significant difference between the food intake by the group and that of the control group (p> 0.05)

When compared with the control, cyanide reduced the body weight of the treated rats significantly (P<0.05). The effect of cyanide on body weight of treated rats was not reversed by the vegetable as there was no significant difference (P>0.05) between the average body weight of rats treated with cyanide only and the group treated with the combination of equivalent concentrations of cyanide and the vegetable. Also the average body weight of rats treated with extract only was comparable with that of the control group (P>0.05). While 67.1% of rats treated with cyanide alone had ocular lesion, the group treated with equivalent concentrations of cyanide and extract had 17.1% with ocular lesion. The group treated with extract only had 1.4% with ocular lesion but the control group had none.

Table 1:

Water (ml), Food (g) intake and Body weight (g) (n=14)

Group	Water intake	Food intake	Body wt.
A	24.0±4.6	31.1± 9.7	238.86±30.4
B	18.2±8.8	20.1 ±9.8	188.43±15.9
C	17.0±9.7	26.2±9.8	196.57±34.9
D	24.1±3.4	26.4±10.6	231.14±38.4

Values are mean ± SD, A=Control, B=Cyanide only, C= Cyanide + Extract, D= Extract only

Table 2:

Organ/ body weight Index (OBI)

Group	Brain	Kidney	Liver
A	0.007	0.005	0.200
B	0.009	0.007	0.027
C	0.008	0.006	0.025
D	0.007	0.006	0.021

n=5 (P>0.05) in each treatment against the control, A=Control, B=Cyanide only, C= Cyanide + Extract, D= Extract only

Table 3:

Biochemical Parameters: Liver Enzymes and PCV (u/L) n=5

Group	ALT	ALP	AST	PCV (%)
A	36.4±23.8	29.6±17.4	32.6±24.4	71.2±3.1
B	46.4±25.7	106.2±40.0	123.2±32.6	71.6±1.9
C	29.8±18.3	27.6±24.5	27.6±24.5	67.2±3.6
D	41.8±24.5	40.4±32.7	30.6±10.8	72.6±1.5

Values are means ±SD, A=Control, B=Cyanide only, C= Cyanide + Extract, D= Extract only, ALT= Alanine Transaminase, ALP= Alkaline Phosphatase, AST= Aspartate Transaminase, PCV= Packed cell Volume

Table 4:

Histopathology Analysis

Group	Liver	Kidney	Brain
A	No visible lesion	Mild congestion of blood vessels	No visible lesion
B	Multifocal degeneration and necrosis of the liver with loss of hepatic cords of the liver	Mild kidney congestion	Congestion of the brain
C	Focal hepatic degeneration and necrosis of the liver	Loss of tubular epithelial cells of the proximal tubules	No visible lesion
D	No visible lesion	No visible lesion	No visible lesion

A=Control, B=Cyanide only, C= Cyanide + Extract, D= Extract only

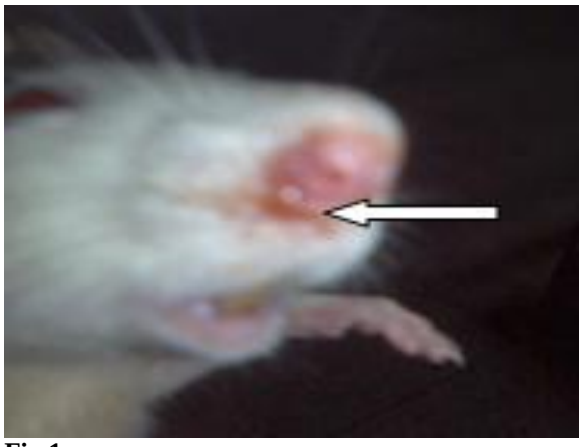

Fig 1.
Shows a rat with nasal lesion after being treated with 3mgKCN/kg body wt./day

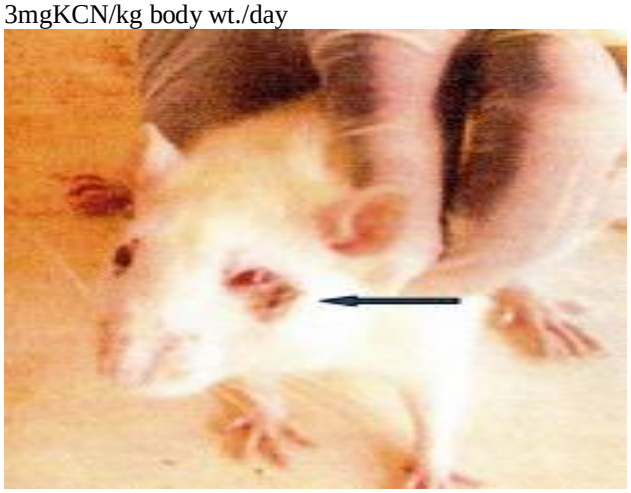

Fig 2.
Shows a rat with ocular lesion after being treated with 3mgKCN/kg body wt./day

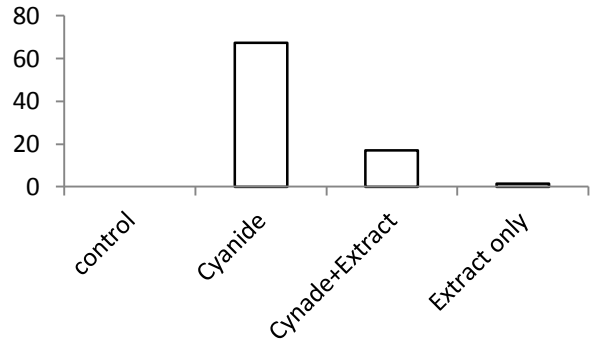

Fig 3.
Frequency of ocular lesion in groups of control, cyanide only, cyanide+extract and Extract only n=70 (number of observations)

While 39% of rats treated with cyanide alone had nasal discharge, 21% in the group treated with equivalent concentrations of cyanide plus extract had nasal discharge there was no nasal discharge in the group treated with extract only and the control group. There was no significant difference (P>0.05) in the organ/Body weight Index (OBI) between each treatment against the control (Table 2) for all the organs examined.

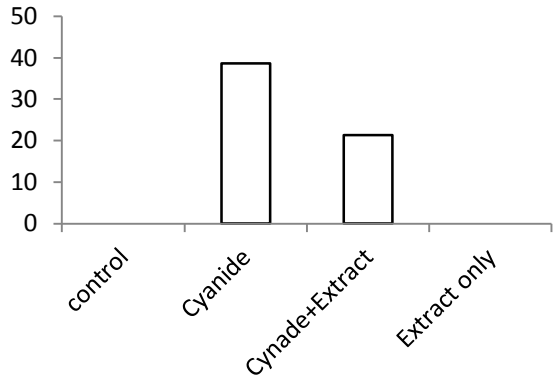

Fig 4.
Frequency of nasal discharge in groups of control, cyanide only, cyanide+extract, and extract only n=70(number of observations).

The activities of liver enzymes (AST, ALP ALT) in the group treated with cyanide only were significantly higher (P<0.05) than those in the control group (Table 3). This signifies that cyanide toxicity caused liver damage. The reduced values of the activities of the enzymes in group 3 and group 4 indicated that *Telfairia occidentalis* had an ameliorative effect on the damage caused by cyanide toxicity.

Cyanide toxicity caused multifocal degeneration and liver necrosis, mild congestion of the kidney as well as congestion in the brain as shown in Table 4. These effects of organ damage due to cyanide toxicity were ameliorated by *Telfairia occidentalis* as indicated by the histophathological observations in group 3. Observation in group 4 showed that the vegetable may be protective against organ damage.

DISCUSSION

Data in this study is compatible with literature on the potential health hazards inherent in the exposure to and ingestion of cyanide or cyanide containing compounds. We also show the possible ameliorative effect(s) of a plant used as vegetable in communities of Nigeria where plants containing toxic amounts of cyanogenic glycosides form the staple. Oral administration of 100 mg/kg KCN or more has been shown to result in decreased water and food consumption by rats and mice. This suggests poor palatability (Tulsawani *et al*, 2005).Our study shows that Potassium cyanide solution fed to rats by gavage at 3mg/kg body weight decreased food and water intake significantly (P<0.05) when compared with the control group. Thus, cyanide toxicity, even at this concentration and method of ingestion, may cause reduced appetite or palatability in animals. The effect

of reduced food intake due to cyanide toxicity was reversed in the group fed simultaneously with equal concentrations of cyanide and extract of *Telfairia occidentalis*.The vegetable alone did not have a significant effect on both food and water intake when compared with the control group (P>0.05). A reduced weight gain and decreased thyroid activity have been observed in male rats given 30mgKCN/kg body weight in the diet for11.5 months (Philbrick *et al*, 1979) .This study has shown a reduction in weight gain and indeed weight loss in rats given 3mg KCN/kg body weight by gavage for 14days. The study also shows that weight loss due to cyanide toxicity was reversed by *Telfairia occidentalis* as shown in the group treated with equivalent concentrations of cyanide and the vegetable. This observation is understandable as it is possible that the vegetable restored the appetite of the rats for food. The vegetable alone maintained the appetite of the animals.

Previous studies have shown that histopathological lesions due to cyanide toxicity include demyelination of the optic nerve tracts and the corpus callosum, swelling of astrocytes and myelin damage in rats injected with sodium cyanide in doses sufficient to keep the rats comatose for 225-260 minutes (Lessel and Kuwabara, 1974). In rats however, the corpus callosum appears to be more sensitive than the optic nerves, whereas in humans, optic damage is frequently the only central nervous system lesion (Way, 1982).The neurologic lesions observed in cyanide toxicity are thought to be the result of cyanide induced histotoxic anoxia (IPCS, 2000). Other studies have implicated cyanide as the etiologic agent in human neuropathies, including Nigerian nutritional neuropathy, tobacco amblyopia and Leber's optical atrophy (Towill *et al* 1978). Also the syndrome of tropical ataxic neuropathy have been shown to include bilateral optic atrophy, nerve deafness, sensory spinal ataxia, weekness of the legs and numbness of feet (Osuntokun, 1968).Our study shows that rats fed with 3mgKCN/kg body weight by gavage for 14days could elicit clinical and neurological signs of cyanide toxicity. It has also shown that the neurologic effects induced by cyanide toxicity could be reduced or ameliorated by aqueous extract of *Telfairia occidentalis.*

The major route of metabolism and elimination for hydrogen cyanide and cyanides has been reported (Williams, 1959; Ansell and Lewis, 1970) to be its detoxification in the liver by mitochondrial enzyme rhodanese which catalyzes the transfer of the sulphane sulphur of thiosulphate to the cyanide ion to form thiocyanate. While rhodanese is present in the mitochondria of all tissues, there is tissue and specie variability in its distribution (Aminlari *et al,* 1994). The highest concentrations of rhodanese are found in the liver, kidney, brain and muscle, but the supply of thiosulphate is limited (Aminlari *et al,* 1994). Rhodanese is present in rat nasal mucosa tissues, particularly in the olfactory region at a 7-fold higher concentration than in the liver (Dahl, 1989). In animal studies, cyanides have produced neuropathies, lesions, ferotoxicity and teratogenic effects including encephaly and encephalocele (Frakes *et al* 1986a; Doherty *et al,* 1982; Tewe and Maner, 1981). This study reports that cyanide (3mgKCN/kg body weight) in rats induced neurological signs of nasal lesion which was reduced or ameliorated by aqueous extracts of *Telfairia occidentalis*. It may be explained through the observed nasal discharge that cyanide, at the administered concentration, may have challenged the olfactory lobe for enhanced production of rhodanese enzymes for neutralization of cyanide toxicity or induced pathology of the frontal lobe of the brain. It is noteworthy then that *Telfairia occidentalis* mitigated this challenge or effect and returned the tissue to its normal or basal activity. Sub-acute exposure of male rats to 7mgKCN/kg body weight administered orally has been shown not to produce significant change in parameters like organ body weight index (OBI), haematology, levels of urea, creatinine, Aspartate Aminotransferase (AST),Triiodothyroxine (T3) and Tetraiodothyroxine (T4). It however produced various histological changes in the brain, heart and kidney (Tulsawani *et al,* 2005). Histological and biochemical parameters in this study showed evidence of liver damage induced by treatment of rats with 3mgKCN/kg body weight. These indices of liver damage by cyanide were moderated or ameliorated by the treatment of rats with aqueous extract of *Telfairia occidentalis*.

The major mechanism for removing cyanide from the body is through enzymatic conversion by the mitochondrial enzyme rhodanese (thiosulphate-cyanide sulphur transferase EC2.8.1.1) to thiocyanate. The enzymatic conversion of cyanide to thiocyanate requires a source of sulphane sulphur which is usually offered by thiosulphate or other biological compounds containing sulphane sulphur, like polythionates, thiosulphonates and persulphides. It is presumed that the sulphane sulphur binds first with the serum albumin to yield sulphane sulphur albumin complex which eventually reacts with the cyanide to form thiocyanate (Wesley *et al,* 1983). *Telfairia occidentalis* is a rich source of sulphur containing amino acids and this may be partly responsible for the various ameliorative effects shown by this vegetable on cyanide toxicity. It is also possible that such effects can be caused by any other molecule involving a different biochemical pathway from that of thiocyanate formation.

In conclusion, this study has shown the potential of *Telfairia occidentalis* as an antidote candidate for cyanide toxicity when administered as treatment regimen particularly if taken concomitantly with cyanide containing food(s) or shortly after ingestion of sub-lethal dose of cyanide. This potential also unveils its possible use as a treatment regimen even in complex clinical situations where diagnosis is rapid and presumptive. However, further bioassay guided fractionation and analytical studies need to be carried out to identify the actual chemical component or molecule in the vegetable responsible or associated with the therapeutic or prophylactic potential in the plant candidate.

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