# THE EFFECTS OF AQUEOUS EXTRACTS OF RIPE AND UNRIPE PEPPER FRUIT (*Dennettia tripetala*) ON SOME BIOCHEMICAL PARAMETERS IN RATS EXPOSED TO CYANIDE: POSSIBLE ANTIDOTE

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**Abstract:** This study was carried out to determine the effect of ripe pepper fruit (RPF) and Unripe pepper fruit (UPF) extract on some biochemical parameters in rats so as to ascertain their possible antidotal effect. Twenty male rats were divided into 5 groups of 4 rats as follows: Group 1: normal control (not exposed to CN), Group 2: cyanide control (exposed to CN alone), Group 3: cyanide + ripe pepper fruit extract (RPF), Group 4: cyanide + unripe pepper fruit (UPF) and Group 5: cyanide + sodium thiosulphate. 500 mg/kg b.w of both ripe and unripe pepper fruit extracts and 1000 mg/kg b.w. of sodium thiosulphate were given by gavages to the respective rats three times a week. While KCN was given to the cyanide exposed rats at a concentration of 9.0 mgCN in their drinking water for 4 weeks. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea, uric acid, creatinine, and liver and kidney lipid peroxidation (MDA), superoxide dismutase (SOD) and catalase determined using standard biochemical procedures. The results indicated a significant increase ($P<0.05$) in AST, ALT, ALP, urea, creatinine, uric acid, liver and kidney MDA in all rats exposed to cyanide when compared with the control group. However a significant decrease ($P>0.05$) was observed in all these parameters in the groups treated with both the RPF and UPF extract. In conclusion, although the Ripe pepper fruit aqueous extract and the unripe extract were able to ameliorate the effect of cyanide in the rats, the Unripe Pepper fruit extract can be substituted as a better antidote to cyanide poisoning.

**Keywords:** Cyanide, pepper fruit, ast, alt, alp, urea, Mda

## Introduction

The toxicity of cyanide to both man and animals has been well established (Kadiri and Asagba, 2017). Despite this knowledge, it is still consumed daily in varying amounts in our food diets (Nwaichi *et al*., 2013; Guede *et al*., 2013). Cyanide causes poisoning, either by inhalation of cyanide containing gas or dust containing solid or liquid cyanide. It is also present as cyanogenic compounds in some food samples such as cassava, where it is consumed in various forms such as meal forms, flour and even in the form of chips and pellets. It is also found in lima beans, sorghums and almonds (Orjiekwe, 2013; Nunn *et al.*, 2011; Ogundele *et al.*, 2010). Cassava is also usually used to compound animal feeds as a source of carbohydrate and fiber in recent times due to high cost of cereals particularly in the developing countries (Sri *et al*, 2018).

*Dennettia tripetala (*Pepper fruit) plant is usually found in the eastern and southern part of Nigeria. It's fruits ripe or unripe have been shown to be highly nutritious (Iseghohi, 2015). It contain carbohydrate, protein, and fibres and are also rich in vitamins such as vitamin A and vitamin C as well as in minerals such as calcium, magnesium, potassium, sodium, phosphorus and sulphur (Osuagwu and Eme, 2013; Iseghohi, 2015). Pepper fruit also contains essential oils such as oleoresins. In addition, studies have also shown that it is rich in flavonoids, saponins, phenols and tannins (Iseghohi, 2015; Anosike *et al.*, 2016; Akpakpan, 2017). Traditionally, this fruit has been used to cure ailments such as cough, diabetes and fever. This in turn could be due to its high content of antioxidants.

Compounds containing cyanide ions (CN-) are rapidly acting poisons that interfere with mitochondrial oxygen utilization. It goes into the tissues and binds to the target site within seconds resulting in death. Several studies have reported that high intake of cyanide can also lead to organ damage such as the liver and kidney as well as other neurological disorders (Adekanye, 2013). There is therefore the need to search for natural antidotes that can be easily assessable to people living in regions where cassava and other cyanogenic glycosides are being consumed on a daily basis in order to ameliorate the effect of cyanide toxicity in the absence of the synthetic antidotes

## Materials and Methods

### *Experimental design*

A total of 20 rats were purchased from Emmanuel laboratory animal house in Abraka Delta state weighing between 100 to 150 g were used for the study. The rats were acclimatized in the laboratory for seven days. They were then divided into 5 groups of 4 rats per group. The grouping were as follows: Group 1: normal control (not exposed to CN), Group 2: cyanide control (exposed to CN alone), Group 3: cyanide + ripe pepper fruit extract (RPF), Group 4: cyanide + unripe pepper fruit (UPF) and Group 5: cyanide + sodium – thiosulphate. 500 mg/kg b.w of both ripe and unripe pepper fruit extracts and 1000 mg/kg b.w. of sodium thiosulphate were given by gavages to the respective rats three times a week. While KCN was given to the cyanide exposed rats at a concentration of 9.0 $mgCN^{-1}$ /kg b.w in their drinking water for 4 weeks.

### *Biochemical analysis*

#### *Determination of AST and ALT activity*

The enzymes were assayed for according to the method of Reitman and Frankel (1951).

Their activity is expressed in Unit/ L

#### *Determination of alkaline phosphatase (ALP) activity*

ALP was assayed for using the method of Anino and Giese (1976). The enzyme activity is expressed in micromoles p-nitrophenol/min/mg /protein

#### *Assay for urea*

Urea was determined according to Urease-Berthelot method (Weatherburn, 1967).

#### *Assay for creatinine*

Creatinine was determined using the method of Bartels and Bohmer (1952).

#### *Assay for uric acid*

Uric acid was determined according to the method of Tietz, 1995**.** It is oxidized by uricase to allantoin with the formation of hydrogen peroxide. In the presence of peroxidase (POD), a mixture of N-ethyl-Nsulphopropyl-m-anisidine (ADPS) and

4-aminoantipyrine (4AA) is oxidized by hydrogen peroxide to form quinoneimine dye proportional to the uric acid concentration of uric acid in the sample.

***Determination of catalase activity***

The method of Deisseroth, A., D and Ounce, A. L. (1970) was adopted for the assay of liver and kidney catalase activity spectrophotometrically

***Determination of superoxide dismutase (SOD) activity***

The activity of SOD in the kidney and liver was determined using the method Misra and Fridovich (1972); 1 unit of the enzyme is equal to the amount the enzyme needed for 50 percent inhibition of epinephrine.

***Determination of lipid peroxidation***

Lipid peroxidation was determined by the method of Gutteridge and Wilkins (1982). 1.56 x '$10^5$M/cm was the value used as molar extinction coefficient and it was expressed in terms of rnalondialdehyde (MDA) units per gramme tissue with a unit representing I umol of MDA

**Results and Discussion**

This study was carried out to determine the effects of RPF and UPF extract on the liver and kidney enzymes and some oxidative parameters in rats so as to ascertain its possible antidotal effect.

Amino aspartate transaminase (AST) and Alanine amino transaminase (ALT) are known markers of liver and kidney function. They also play important roles in the metabolism of carbohydrates and proteins (Kadiri, 2018; Kadiri and Asagba, 2015; Baghshani and Ghodsi, 2013). The result of AST in this present study (Table 1) indicates a significant increase in all the Groups exposed to cyanide when compared to the control group. However, a significant decrease in AST was observed in Group 3 and 4 rats given ripe and unripe pepper fruit extract when compared with Group 2 bird not treated with the extract. In addition to this there was no significant difference in the AST value in Group 4 birds given unripe pepper fruit extract (UPF) when compared with Group 5 given a known cyanide antidote (sodium thiosulphate). Similarly, the result from ALT indicates a significant increase in all the Groups exposed to cyanide when compared with the control. In addition as was observed in the AST analysis, a significant decrease in ALT was also observed In Group 3 and 4 rats given ripe and unripe pepper fruit extract when compared with Group 2 bird not treated with the extract. Again, there was no significant difference in the ALT value in Group 4 birds given unripe pepper fruit extract (UPF) when compared with Group 5 rats given the known antidote.

The significant increase in AST and ALT activities is an indication of liver and kidney damage. This confirms the work of Elsaid and Elkomy (2006) in rats and Manzano *et al.* (2007) in pigs. However, in this present study, both the RPF and the URPF extract was able to significantly reduce the AST and ALT activities in all the birds given the pepper fruit extract. This signifies that it was probably able to ameliorate the effect of cyanide on the organs by detoxifying the cyanide to a less toxic compound thiocyanate due to the sulphur content of the fruit.

Alkaline phosphatase (ALP) is involved in the hydrolysis of many monoester substrates. The result from this present study indicates a significant increase ALP activity in all the groups given cyanide when compared with the control. A significant decrease in ALP was however observed in the Groups given the RPF and URP extract when compared with the control. This in agreement with the works of Kadiri and Asagba (2017), Okolie and Osagie (2017) that cyanide significantly increases ALP values in birds and in rabbit.

**Table 1: Changes in AST, ALT and ALP activities in rats exposed to cyanide and treated with ripe, unripe and leaf of pepper fruit (*Dennettia tripetala*) extracts as possible antidote**

| Groups | AST (U/L) | ALT(U/L) | ALP (µm/p-nitrophenol/ min/mg /protein) |
|---|---|---|---|
| Group 1: Normal Control | 40.25 ± 0.65$^{a}$ | 36.38 ± 4.50$^{a}$ | 60.34±3.16 $^{a}$ |
| Group 2: Cyanide control | 75.25 ± 3.86$^{b}$ | 68.25 ± 1.85$^{b}$ | 102.35 ± 1.73 $^{b}$ |
| Group 3: Cyanide + ripe pepper fruit (RPF) extract | 61.25± 1.85$^{c}$ | 60.25± 4.13$^{c}$ | 82.34 ± 2.52 $^{c}$ |
| Group 4: Cyanide + unripe pepper fruit (UPF) extract | 54.38± 3.28 $^{d}$ | 53.13± 2.66 $^{d}$ | 74.43± 3.39$^{d}$ |
| Group 5: Cyanide + Sodium –thiosulphate | 50.11± 5.01$^{d}$ | 48.13± 6.61$^{e,d}$ | 71.22± 1.59$^{d}$ |

Values are given as mean ± standard deviation, n = 4. Values not sharing a common superscript letter in the same column differ significantly at (P < 0.05)

**Table 2: Changes in the levels of urea, uric acid and creatinine concentrations in rats exposed to cyanide and treated with ripe, unripe and leaf of pepper fruit (*Dennettia tripetala*) extracts as possible antidote**

| Groups | Urea mg/dl | Uric acid mg/dl | Creatinine mg/dl |
|---|---|---|---|
| Group 1: Normal Control | 20.47 ±4.08$^{a}$ | 4.35 ±1.44$^{a}$ | 1.05 ± 0.42$^{a}$ |
| Group 2: Cyanide control | 45.37± 4.19 $^{b}$ | 15.30± 4.17 $^{b}$ | 7.17 ± 1.52 $^{b}$ |
| Group 3: Cyanide + ripe pepper fruit (RPF) extract | 32.38± 1.64$^{c}$ | 14.34± 0.29$^{b}$ | 6.47± 0.60 $^{b}$ |
| Group 4: Cyanide + unripe pepper fruit (UPF) extract | 27.30 ± 1.71$^{d}$ | 10.49 ± 0.81$^{c}$ | 4.56 ± 1.72$^{c}$ |
| Group 5: Cyanide + Sodium –thiosulphate | 23.18± 2.36 $^{ad}$ | 9.45± 0.31 $^{c}$ | 4.45± 0.80$^{c}$ |

Values are given as mean ± standard deviation, n = 4.Values not sharing a common superscript letter in the same column differ significantly at (P < 0.05).

**Table 3: Changes in the level of liver and kidney MDA in rats exposed to cyanide, treated with ripe, unripe and leaf of pepper fruit (*Dennettia tripetala*) extracts as possible antidote**

| Groups | LIVER MDA (unit/g of wet tissue) | KIDNEY MDA (unit/g of wet tissue) |
|---|---|---|
| Group 1: Normal Control | 1.62 ± 0.75$^{a}$ | 0.41 ± 0.16$^{a}$ |
| Group 2: Cyanide control | 9.17 ± 0.75$^{b}$ | 7.16 ± 1.51$^{b}$ |
| Group 3: Cyanide + ripe pepper fruit (RPF) extract | 7.35± 1.82$^{c}$ | 5.46± 1.66$^{b}$ |
| Group 4: Cyanide + unripe pepper fruit (UPF) extract | 5.45± 0.58 $^{d}$ | 3.24± 0.90$^{c}$ |
| Group 6: Cyanide + Sodium –thiosulphate | 4.42± 0.77$^{cd}$ | 2.13± 0.48$^{c,d}$ |

Values are given as mean ± standard deviation, n = 4. Values not sharing a common superscript letter in the same column differ significantly at (P < 0.05)

**Table 4: Changes in the level of liver and kidney SOD and Catalase in rats exposed to cyanide, treated with ripe, unripe and leaf of pepper fruit (*Dennettia tripetala*) extracts as possible antidote**

| Groups | LIVER SOD | KIDNEY SOD | LIVER CATALASE | KIDNEY CATALASE |
|---|---|---|---|---|
| Group1: Normal Control | 51.37 ±1.08 [a] | 45.37 ± 3.89 [a] | 41.32± 0.78 [a] | 38.27±2.33 [a] |
| Group2: Cyanide control | 33.43 ± 3.30 [b] | 28.31 ± 6.64 [b] | 24.52 ±3.20 [b] | 20.19 ± 4.10 [b] |
| Group3: Cyanide + ripe pepper fruit (RPF) extract | 39.23 ± 1.70 [c] | 33.35 ± 1.43 [c] | 30.47 ±4.33 [c] | 26.28 ± 3.43 [c] |
| Group4: Cyanide + unripe pepper fruit (UPF) extract | 40.43 ±4.00 [c] | 34.22 ± 3.34 [c] | 32.29 ±1.47 [c] | 28.20± 1.82 [c] |
| Group5: Cyanide + Sodium –thiosulphate | 41.24 ± 0.82 [c] | 35.38 ± 4.00 [c] | 34.40 ±3.17 [c] | 30.46± 1.43 [cd] |

Values are given as mean ± standard deviation, n = 4.Values not sharing a common superscript letter in the same column differ significantly at ($P < 0.05$)

Creatinine, Urea and Uric acid levels in the serum are usually used as markers of kidney function. The results in Table 3 showed that all groups exposed to cyanide had significantly higher ($P<0.05$) serum urea, uric acid and creatinine activities when compared with the normal control. A significant decrease in serum urea was however observed in Group 3 and 4 rats given the RPF and UPF extract respectively when compared with the cyanide control. However serum uric acid and creatinine levels were only significantly lower in the Group 4 rats given UPF extract when compared with the cyanide control. Also there was no significant difference in the urea, uric acid and creatinine levels in the groups given UPF extract when compared with the Group 5 rats given the known antidote.

One major function of the kidney is that it clears metabolites from the blood (Guyton and Hall, 2006). Agents with nephro-toxicity effects may therefore lead to a rise in serum urea, uric acid and creatinine levels in the serum. In addition cyanide is a rapid-acting mitochondrial toxicant that inhibits cytochrome oxidase, thereby blocking the flow of electrons through complex IV which prevent oxidative metabolism and enhance ROS generation at complex III (Chen *et al*., 2003).The damaging effects of cyanide on the tissue and organ may be mediated by cyanide ion (being a nucleophile) and its inhibition of mitochondria respiratory chain thus producing free radical such as superoxide anion. Elsaid and Elkomy (2006) also reported a rise in serum urea and uric acid level in cyanide rats when compared to the normal control.

The effect of ripe, unripe and leaf of pepper fruit (*D. tripetala*) extracts on lipid peroxidation as indicated by the MDA Level was shown in Table 3. The results indicates that all cyanide treated (Group 2, 3 and 4) had a significant higher ($P <0.05$) liver and kidney MDA level as compared to normal control. Previous studies by Gunasekar *et al*., 1996 and Mills *et al*., 1996 indicates that cyanide induced oxidative stress by increasing reactive oxygen species and nitric oxide in rats.

This present study also indicates a significant decrease ($P>0.05$) in the liver and kidney MDA levels in rats exposed to cyanide treated with RPF and UPF extract when compared with the untreated cyanide group. However, there was no significant difference ($P>0.05$) in the liver and kidney MDA of cyanide rats treated with UPF extract (Groups 4) when compared with Group 5 treated with sodium thiosulphate. The decreased MDA levels in the liver and kidney of rats treated with RPF and URP extract indicates the ability of the extract to scavenge the radicals generated from cyanide exposure.

The effect of ripe, unripe pepper fruit (*D. tripetala*) extracts on some antioxidants was also studied in this work (Table 4). The result indicates that all cyanide treated Group 2, 3 and 4 had a significant lower ($P < 0.05$) liver and kidney catalase and SOD activities when compared to normal control. This is in agreement Ardelt *et al*. (1989). However, there was a significant increase in the liver and kidney catalase and SOD activity in rats exposed to cyanide treated with RPF and UPF, extract (Group 3 and 4) when compared with the cyanide control . Also there was no significant difference in the liver and kidney catalase and SOD of rats in Groups 3 and 4 treated with pepper fruits extract when compared with each other. No significant difference was also observed in the groups treated with pepper fruit extracts (Groups 3 and 4) when compared with sodium thiosulphate (Group 5) a known cyanide antidote.

Studies have indicated that the medicinal properties of pepper fruit may be due to its secondary metabolites such as alkaloids, flavonoids, tannins and terpenoids (Akpakpan 2017; Anosike *et al.*, 2016; Iseghohi, 2015; Lewis and Ausubel, 2006) Pharmacological research reports have also shown that secondary metabolites in plants exert a wide range of antioxidant activities on physiological system (Kumar *et al.*, 2010). Thus the antioxidant effect of the flavonoids and vitamins such as thiamine, ascorbic acid and riboflavin in the pepper fruits extract is reflected in this present study.

This present study also shows that unripe pepper fruit (URP) extract has higher antioxidant activity when compared with the Ripe pepper fruit (RTP) extract, despite its lower total phenol content (Adedayo *et al.*, 2010) . Therefore this study confirms that the physiological changes that accompanies ripening of pepper fruits that brings about changes in pigment although increases the total phenol in Pepper fruit it decreases the antioxidant properties of the fruit (Adedayo *et al*., 2010 ; Iseghohi, 2015).

In conclusion, although the Ripe pepper fruit aqueous extract and the unripe extract were able to ameliorate the effect of cyanide in the rats, the Unripe Pepper fruit extract can be substituted as a better antidote to cyanide poisoning.

**Conflict of Interest**

Author declares that there is no conflict of interest.

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