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Effect of *Parinari excelsa* on Some Haematological and Parameters of Renal Function in Cyanide Toxicity- Induced Rats.Kadiri, Helen Ejiro¹Department of Biochemistry, Delta State University, Abraka, Nigeria¹.

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Abstract

The aim of this study is to determine the effect of *Parinari excelsa* (*P. excelsa*) on haematological and nephrological indices in rats induced with Cyanide toxicity. A total of 30 male rats were used for this study. The rats were divided into six groups of five rats each as follows: Group 1 (Normal control rats): Rats received distilled water in place of extract. Group 2: (cyanide control) received 4mg CN/kg body weight (bw) in form of Potassium cyanide. Group 3: received 4mg CN/kg b.w and treated with 500mg/kg bw of sodium thiosulphate by gavage. Group 4: received 4mg/kg bw of KCN and treated with 100mg/kg of aqueous extract of *P. excelsa* by gavage. Group 5: received 4mg/kg b.w of CN and treated with 250mg/kg bw of aqueous extract of *Parinari excelsa* by gavage. Group 6: received 4mg CN/kg b.w of and treated with 500mg/kg of aqueous extract of *Parinari excelsa* by gavage. The experiment was for 21 days. Biochemical Parameters were determined using standard procedures. The results indicate a significant reduction in PCV, Hb blood platelets, and catalase and an increase in Lymphocytes, Neutrophil, Urea Creatinine, calcium, Potassium, chloride, sodium and lipid peroxidation in all Group 2 rats when compared with Group 1. In addition, a significant increase was indicated in Group 2 rats when compared with Normal control. Aqueous extract of *P. excelsa* however, brought a reversal in all these parameters investigated in the rats in a dose dependent manner. In conclusion the result of this study indicates that *P. excelsa* is able to improve haematocrit parameters and has nephroprotective properties due its rich iron

composition and its antioxidant properties.

Keywords: Cyanide, Nephrology, Haematology, Oxidative stress

Introduction

Cyanide is well known as a serious environmental pollutant which is extremely toxic to most forms of life due of its inhibitory action on a some key enzymes. Cytochrome c oxidase enzyme is one of the main enzymes inhibited by cyanide where it prevents cellular oxygen utilization and ATP production. Cyanide poisoning can result from various sources, from exposure to hydrocyanic acid and cyanide salts to ingestion of plants containing cyanogenic compounds. Cyanide toxicity has been implicated to alter haematological parameters in humans in cat fish and rats Cyanide has also been shown in earlier studies to cause damage to the kidney. It accumulates in different cells of the body by binding to either enzymes or metalloproteins. Hence it is a potent cytotoxic agent that kills cells by inhibiting cytochrome oxidase of the electron transport chain.

Parinari excelsa Sab. belongs to the family Chrysobalanaceae. It is a woody herb found in West and Eastern African Forest. It is used traditionally for treatment of diarrhoea as an antihelminthic remedy, as a remedy for malaria. It is also used in the treatment of anaemia and is taken as a tonic by pregnant women during pregnancy. Phytochemical studies on the aqueous and ethanolic extracts on the seed shows that it contains alkaloids, flavonoids, saponins, cardiac glycosides, tannins, phenolic

compounds and anthraquinones with the aqueous extract being a better solvent than the ethanolic extract . The seed has also been found to be rich in iron, sodium and Potassium.

Blood act as a pathological reflector of the status of exposed animals to toxicant and other health conditions . Haematological studies are useful in the diagnosis of many diseases as well as in the investigation of the extent of damage to blood . They are also good indicators of the physiological status of animals . Current FDA-approved cyanide countermeasures are constrained by a requirement for intravenous administration, this leads to a severe limitation of their usefulness in a mass-exposure cyanide emergency. There is therefore need for alternatives to these countermeasures. The aim of this study was to investigate the effect of *Parinari Excelsa* (Umilo) on Haematological and Nephrological Indices in Rats Induced with Cyanide Toxicity.

Materials and Methods

Plant Material

The Spice was bought from Eku market in Delta State and was duly authenticated and assigned a voucher number (UBH_p436) by Dr. H. A. Akinnibonsu of Botany Department of University of Benin, Benin City Nigeria. The seeds were dried in the laboratory under shade and blended using Panasonic blender model MX-J120P. Water extraction was then carried out by the method of El-Mahmood (2009).

Induction of Cyanide intoxication.

Rats were acclimatized for 7 days in the laboratory, after which the test rats were given 4mg/kg body weight of cyanide in form of Potassium cyanide (KCN) by gavage three times a week for 21 days.

Table 1: Experimental Design

Groups	Number of rats	Treatment
Group 1. Normal control (NC)	5	Distilled water
Group 2. Cyanide control (CC)	5	Cyanide only minus plant extract
Group 3. Standard control (SC)	5	Cyanide +500mg/kg b.w. of sodium thiosulphate only
Group 4. Cyanide (C-100)	5	Cyanide +100mg/kg b.w. of aqueous extract of <i>P. excelsa</i>
Group 5. Cyanide (C-250)	5	Cyanide +250mg/kg b.w. of aqueous extract of <i>P. excelsa</i>
Group 6. Cyanide (C-500)	5	Cyanide +500mg/kg b.w. of aqueous extract of <i>P. excelsa</i>

Experimental animals

Thirty (30) healthy Wistar rats were obtained from the animal house of the School of Basic Medical Studies, Delta State University Abraka. The rats used weighed between 120-150 g and were housed in well- ventilated cages provided by the Animal House. All animals had access to water ad libitum and were exposed to 12hrs light dark cycle. The approximate temperature was 27

± 2°C with a relative humidity of 60-70 %. The Laboratory animals were handled in accordance with the laboratory practice contained in the revised NIH publication number 85-93 (1985). The animals were randomly assigned into seven groups of five rats each (n=5) and the doses administered by gavage for 21days as follows: Group 1 (Normal control rats): Rats received distilled water in place of extract. Group 2:

(cyanide control) received 4mg CN/kg body weight (bw) in form of Potassium cyanide. Group 3: received 4mg CN/kg b.w and treated with 500mg/kg bw of sodium thiosulphate by gavage. Group 4: received 4mg/kg bw of KCN and treated with 100mg/kg of aqueous extract of *P. excelsa* by gavage. Group 5: received 4mg/kg b.w of CN and treated with 250mg/kg of aqueous extract of *Parinari excels* by gavage. Group 6: received 4mg CN/kg b.w of and treated with 500mg/kg of aqueous extract of *Parinari excels* by gavage.

Collection of samples and Analysis

The animals were sacrificed under cervical dislocation. Blood samples of the animals were collected via cardiac puncture in relevant containers for haematological and biochemical analysis. All analyses were carried out within 24 hours of blood collection. At the end of the exposure time, the rats were sacrificed under chloroform sedation and blood collected into reagent bottles. The haematological markers were determined by standard procedures described by . The method of were used for the

estimation of superoxide dismutase (SOD) activity. One unit of the enzyme activity represents the amount of the enzyme required for 50% inhibition of epinephrine in 1 min. lipid peroxidation (LPO) level was determined by the method of . Creatinine and Urea activity was determined spectrophotometrically by the method of and respectively. While the sodium, potassium and calcium were according to procedures of kits supplied by TECO diagnostics, U.S.A.

Statistical Analysis

All the data are expressed as mean ± standard error of mean SEM. Statistical comparisons was performed by one-way analysis of variance (ANOVA) followed by Fishers least significant difference (LSD). The SPSS software (version 20) was used in the statistical analysis using multiple comparison tests. A p-value of less than 0.05 ($p < 0.05$) was considered significant.

Results

Table 1 Effect of *P. excelsa* extract on body weight gain and relative organ weight for kidney cyanide intoxicated rats

Groups	Relative Organ Weight	Body Weight Gain
1. Normal control (NC)	0.70±0.02	15.65±6.14 ^a
2. Cyanide control (CC)	0.68±0.03 ^a	-1.74±5.76 ^b
3. Standard control (SC)	0.86±0.07 ^a	7.30±1.19 ^c
4. Cyanide (C-100)	0.53±0.06 ^b	8.65±2.38 ^c
5. Cyanide (C-250)	0.75±0.04 ^a	9.78±1.88 ^c
6. Cyanide (C-500)	0.55±0.12 ^b	13.53±1.12 ^a

Values are expressed as mean ± SEM. Values not sharing a common superscript along each column differ significantly at $p < 0.05$

Table 2: The Effect of extract of *Parinari excelsa* seed on the Haematological parameters of cyanide intoxicated Wistar rats

Haematological Parameters						
Groups	PCV (%)	Hb (g/dl)	RBC (mm ³)	Platelet (mm ³)	Neutrophil (%)	Lymphocyte (%)
1. Normal control (NC)	40.75±1.89 ^a	13.41±0.64 ^a	4.35±0.33 ^a	136.59±6.24 ^a	30.00±4.08 ^a	21.87±0.33 ^a
2. Cyanide control (CC)	36.00±1.23 ^b	11.00±0.40 ^b	3.52±0.20 ^a	129.05±4.08 ^b	45.00±0.00 ^b	26.00±0.41 ^b
3. Standard control (SC)	40.25±0.63 ^a	13.49±0.21 ^a	4.23±0.11 ^a	134.17±2.10 ^a	37.50±2.50 ^a	23.61±0.28 ^a
4. Cyanide (C-100)	39.50±1.04 ^a	13.17±0.35 ^a	4.10±0.17 ^a	131.66±3.47 ^a	37.50±2.50 ^a	23.92±0.85 ^a
5. Cyanide (C-250)	41.75±1.18 ^a	13.92±0.39 ^a	4.60±0.17 ^a	139.17±3.94 ^a	40.00±5.00 ^a	22.98±0.34 ^a
6. Cyanide (C-500)	41.50±1.19 ^a	13.83±0.40 ^a	4.43±0.20 ^a	138.33±3.97 ^a	41.25±1.25 ^a	22.00±0.31 ^a

Values are expressed as mean ± SEM. Values not sharing a common superscript along each column differ significantly at $p < 0.05$

Table 3: The Effect of extract of *Parinari excelsa* seed on some Renal parameters of cyanide intoxicated Wistar rats

Renal Parameters						
Groups	Urea	Creatinine	Sodium	Chloride	Potassium	Calcium
Group 1: Normal Control (NC)	19.65±0.70 ^a	0.79±0.02 ^a	133.80±0.90 ^a	73.80±0.90 ^c	4.95±0.17 ^a	5.48±0.14 ^a
Group 2: Cyanide Control (CC)	51.18±1.82 ^b	2.05±0.05 ^b	143.50±1.94 ^b	98.68±3.00 ^b	7.95±0.25 ^b	2.43±0.20 ^b
Group 3: Standard Control (SC)	34.48±1.38 ^c	1.07±0.27 ^c	137.75±1.03 ^c	80.45±2.10 ^c	6.03±0.23 ^c	4.50±0.50 ^c
Group 4: Cyanide (C-100)	44.58±2.13 ^d	1.50±0.11 ^d	140.75±2.10 ^b	92.60±2.34 ^d	7.35±0.23 ^d	3.23±0.09 ^d
Group 5: Cyanide (C-250)	42.95±2.39 ^d	1.48±0.07 ^d	140.00±1.78 ^b	84.65±1.85 ^c	6.78±0.14 ^d	3.40±0.08 ^d
Group 6: Cyanide (C-500)	37.37±1.04 ^c	1.08±0.13 ^c	139.25±1.65 ^c	78.18±1.44 ^a	6.90±0.33 ^d	3.48±0.17 ^d

Values are expressed as mean \pm SEM. Values not sharing a common superscript along each column differ significantly at $p < 0.05$.

Table 4: The Effect of extract of *Parinari excelsa* seed on lipid peroxidation, Superoxide dismutase (SOD), and Catalase activity in the kidney cyanide intoxicated Wister rats

Groups/Parameters	MDA	SOD	Catalase
1. Normal control (NC)	0.29 \pm 0.04 ^a	35.19 \pm 0.09 ^a	25.79 \pm 0.43 ^a
2. Cyanide control (CC)	0.72 \pm 0.32 ^b	35.15 \pm 0.16 ^a	24.40 \pm 0.23 ^b
3. Standard control (SC)	0.32 \pm 0.01 ^a	35.28 \pm 0.18 ^a	32.04 \pm 0.34 ^c
4. Cyanide (C-100)	0.44 \pm 0.14 ^c	35.32 \pm 0.37 ^a	29.98 \pm 0.12 ^c
5. Cyanide (C-250)	0.61 \pm 0.14 ^d	35.10 \pm 0.54 ^a	31.05 \pm 1.90 ^c
6. Cyanide (C-500)	0.55 \pm 0.12 ^c	35.15 \pm 0.37 ^a	30.60 \pm 0.11 ^c

Values are expressed as mean \pm SEM. Values not sharing a common superscript along each column differ significantly at $p < 0.05$

Result

Effect of *P. excelsa* on body weight gain and organ weight ratio on cyanide intoxicated rats was observed in Table 1. The result indicates a negative body weight gain among Group 2 rats intoxicated with cyanide without treatment when compared with control. However, treatment with different concentrations of the aqueous and ethanolic extract (Groups 4, 5, 6 and 7) indicated no significant difference when compared with Group 1 control rats and Group 3 rats given a standard antidote of cyanide poisoning. Cyanide induced body weight loss was ameliorated in a dose dependent manner by the aqueous extract. Table 2 shows the effect of aqueous extract *P. excelsa* on haematological parameters in cyanide intoxicated rats. A significant reduction in PCV, Hb and blood platelets was indicated in Group 2 rats exposed to cyanide only, however, a significant increase was indicated in percentage Lymphocytes and Neutrophil in Group 2 rats when compared with normal control.

Treatment with different concentrations of aqueous extract of *P. excelsa* was able to reverse

this Haematological condition in the cyanide intoxicated rats resulting in the significant increase in the HB, PCV and platelets. The result indicates that aqueous extract of *P. excelsa* was able to improve haematological parameters in rats but not in a dose- dependent manner.

Table 3 indicates the effect of aqueous extract *P. excelsa* on the serum Urea, Creatinine and Serum electrolytes (Sodium, Chloride, Potassium and Calcium). Serum Urea and Creatinine was significantly higher in all test groups exposed to Cyanide when compared with normal control. Treatment with 100mg, 250mg and 500mg of *P. excelsa* however significantly reduced the Serum Urea and Creatinine in the rats when compared with Cyanide control. A dose of 500mg/kg bw of *P. excelsa* however significantly reduced Urea and Creatinine activity comparable to the standard cyanide Group (Group 3) when compared to 100mg and 200mg/kg extract. The results indicate that aqueous extract *P. excelsa* was able to ameliorate Urea and Creatinine activity in rats in a dose-dependent manner.

Results on the serum electrolytes in Table 3 indicates a significantly higher serum sodium in all test rats when compared with Normal control, However treatment with 500mg/kg bw of extract significantly reduced serum sodium in rats comparable with the Standard control. (Group 3).

Serum Chloride was significantly increased in all Test Groups exposed to Cyanide except Group 6 rats treated with 500mg/kg extract.

Serum Potassium and Calcium were also significantly increased in all test Groups. However, treatment with all the different doses of the extract significantly reduced serum Potassium and Calcium in rats in these Groups when compared with Cyanide Control. The results indicate that aqueous extract *P. excelsa* was able to ameliorate serum electrolytes in rats in a dose-dependent manner.

Results in Table 4 shows the Effect of extract of *P. excelsa* seed on lipid peroxidation level, Superoxide dismutase (SOD), and Catalase activity in the kidney cyanide intoxicated Wistar rats.

Lipid peroxidation level was significantly increased in Cyanide control rats when compared with Normal Control, However treatment with the different doses of the extract significantly reduced LPO levels but not in a dose-dependent manner. The activity of Catalase was significantly reduced in Cyanide control rats, Again, treatment with aqueous extract of *P. excelsa* seed significantly increased Catalase activity in treated rats when compared with Cyanide control.

Discussion

The ameliorating effect of *P. excelsa* on nephrological and haematological parameters was investigated in this study. No mortality was recorded throughout the duration of this study. A significant decrease in body weight in Cyanide control was indicated in this study rats when compared with Normal control. Our finding is in agreement with the works of . The weight loss could be attributed to loss in appetite leading to loss in muscle and adipose tissue resulting from excessive breakdown of tissue protein and fatty

acids occasioned by cyanide toxicity . However, treatment with *P. excelsa* extract reversed the weight loss in a dose dependent manner

Haematological parameters are good indicators of the physiological status of animals . The significant decrease in PCV, Hb and platelets observed in this study is in agreement with earlier studies on the haematotoxic effect of cyanide on humans in rats and in fish . Lymphocytes are white blood cells that help fight diseases. An increase is therefore an indication of inflammation occasioned by the oxidative stress induced by cyanide. This correlates with earlier studies in human by . Treatment with aqueous extract of *P. excelsa* was able to improve the haematological indices in the rat particularly at high dose probably due to the rich iron content of the herb .

Nephrotoxic effect is usually employed in the estimation of specific and reliable biomarkers such as urea and creatinine . Urea which is one of the major products of protein metabolism is filtered by the glomerulus, and passively excreted in to the urine at a high concentration. Hence Serum urea level is often a useful indicator of renal function . Creatinine is an end product of muscle breakdown which is constantly removed by the kidneys. Creatinine levels in serum is also an indicator of renal function. Increase in serum level of creatinine is therefore a signal of the abnormal functioning of the kidneys (Lesely and Levey, 2005). In this present study the level of Urea, Creatinine, sodium, chloride, potassium and calcium was significantly increased in Cyanide control (CC) rats when compared with Normal control. This is an indication that there is damage to the kidney from cyanide intoxication. This correlates with earlier studies by . Studies indicate that cyanide accumulates in different cells of the body by binding to either enzymes or metalloproteins. Hence it is a potent cytotoxic agent that kills cells by inhibiting cytochrome oxidase of the electron transport chain . The ameliorating effect of cyanide toxicity particularly at high dose is an indication of the nephroprotective effect of the herb. This can be attributed to the antioxidant properties that have been detected in the herb. Earlier studies have

indicated that reactive oxygen species is likely to be involved in the disruption of Glomerular filtration rate.

This study indicated a correlation between oxidative stress and nephrotoxicity in different experimental models. In this present study, there was an elevation of MDA level (a known marker of lipid peroxidation) in all cyanide intoxicated rats and a significant reduction in catalase activity in CC rats when compared with Normal control indicating renal damage this is consistence with earlier studies on Cyanide . Again aqueous extract of *P. excelsa* was able to scavenge the hydroxyl radical and super oxide anion and modulate lipid peroxidation due to its antioxidant properties.

Conclusion

In conclusion the result of this study indicates that *P. excelsa* is able to improve haematocrit parameters and has nephroprotective properties due its rich iron composition and its antioxidant properties

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