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Cyanide-Induced Metabolic Stress- The role of *Aframomum sceptrum* Aqueous Extract (ASAE)

Atinaya, D.U., Ichipi-Ifukor, P.C. *, Okpoghono, J. and George B.O.

Department of Biochemistry, Faculty of Science Delta State University, P.M.B 1, Abraka, Nigeria.
Author for Correspondence*: +234-703-826-8448/patychykyresearch@hotmail.com

Abstract

This study explored the ability of *Aframomum sceptrum* aqueous extract (ASAE) to mitigate the virulence of cyanide poisoning. Thirty (30) male albino rats all eight weeks old weighing between 100g and 160g included the subjects for this study. The rats were divided into six (6) groups of 5 rats per group and treated for 28 days. The six groups included; the normal control, cyanide control, and treatment with 10mg/Kg⁻¹ and 20mg/Kg⁻¹ weight ASAE post-cyanide administration (9mg/Kg⁻¹ KCN) respectively. There was remarkable drop (p<0.05) in PCV, Hb, and TP, ALB, levels in serum and liver of cyanide intoxicated rats. Also observed were rising (p<0.05) levels of liver marker enzymes (AST, ALT, and ALP) in serum and liver tissue and decreased serum/tissue enzymatic antioxidants (SOD, CAT and GPx) linked to cyanide. Also maintained in the untreated rats relative to control were notable drop (p<0.05) in Glutathione (GSH) and rising malondialdehyde (MDA) levels. ASAE treatment at doses of 10mg/Kg⁻¹ and 20mg/Kg⁻¹ body weight improved all biochemical parameters and reversed tissue structural changes to near normality. The study suggests that ASAE may hold levels of potency for managing cyanide virulence.

Keywords: Antidote, *Aframomum sceptrum*, Cyanide, Metabolic stress and toxicity

Introduction

Cyanide is a potent cytotoxic agent found in so many places and it is known for its quick detrimental action and toxic effect (Ghods and

Baghshani, 2013). As a consequence of infusion of certain therapeutic drugs, smoke inhalation, industrial accidents, or terrorism, consumption of cyanide-containing foods, cyanide exposure can take place (Alcorta, 2004; Greenfield *et al.*, 2002). The toxic effect of cyanide is as a result of its increased potency as a poison of respiration in all forms of life that is aerobic (Ghods and Baghshani, 2013; Barley, 1997; Okoh, 1992). Apart from acute cyanide intoxication, in recent years, chronic toxic effect has frequently been reported and it is said that chronic dietary, industrial and environmental sources are the most widespread problems emanating from cyanide (Kadiri and Asagba, 2015; Katsumata *et al.*, 2013; Barillo, 2009). In the cases of cyanide intoxication several key metabolic pathways are affected thus leading to the depletion of neurotransmitters (Persson *et al.*, 2013; Rieders, 1971). Enzymatic and non-enzymatic antioxidants necessary for buffering the system and also contributes to the shortfall in oxygenated blood and increase in metabolic stress (Nelson and Cox, 2000; Way, 1984).

Fortunately, the body itself has its own set of defense against ravaging free radicals which leads to the cells production of special antioxidant enzymes that contributes to quenching oxidative radicals generated during metabolic stress (Beyer, *et al.*, 2018; Yang, *et al.*, 2017; Katar, *et al.*, 2014; Biller, 2007; Muller, *et al.*, 2007; Menvielle-Bourg, 2005; Ho, *et al.*, 2004; Gaetani *et al.*, 1996). When these antioxidants are highly depleted, several other key enzymes and tissue proteins are also affected. For example, the transaminases;

aspartate (AST) and alanine (ALT) transaminases are said to be raised in the serum during acute liver damage (Walker and Lorimer, 2004; Sorbi *et al.*, 1999) while AST is also raised during myocardial infarction and diseases associated with the skeletal muscle. It is an established marker for cyanide induced myocardial anomalies (Okpoghono *et al.*, 2018; Fortin, *et al.*, 2010; Ghouri *et al.*, 2010; Vozarova *et al.*, 2002; Schindhelm *et al.*, 2002; Pratt *et al.*, 2000).

Albumins such as that found in the serum are made up of a family of water dissolvable proteins and are relatively dispersible in dense salt solutions which may be destroyed if heated (Ghodsi and Baghshani, 2013). Albumin and globulin are synthesized in liver and are abundantly distributed, thus, a short fall in the sum total proteins may suggest dysfunction in the kidney, liver or poor digestion/absorption of consumed proteins. Lipid peroxidation which is often reported as Malondialdehyde (MDA) concentrations in the tissue due to its' end to end decomposition of arachidonic acid and larger PUFAs, through enzymatic or non-enzymatic (ROS/RNS generating activities) processes as a result of tissue antioxidant depletion (Marrocco *et al.*, 2017; Zarkovic *et al.*, 2013; Ramalingam and Kim, 2012) Dietary antioxidant is a substance (commonly found in foods) that greatly reduces the detrimental effects of "reactive species", such as reactive oxygen and nitrogen molecules, that hampers normal physiological activities on a cellular level in humans (Aruoma, 1994).

Spices have been acknowledged not only to have properties that make food more pleasant but also important preservative and antioxidant properties (Shobana, and Naidu, 2000). Spices have been used for medicinal purposes as well as food additives over centuries (Wilson and Demmig-Adams, 2007). *Aframomum sceptrum*, a well-known local spice, is consumed in South-South Nigeria, has been analyzed for its chemical and antioxidant composition (George *et al.*, 2013). The concentrations of bioactive metabolites like flavonoids, phenols, tannins alkaloids and saponins have been reported to account for its antioxidant activity and this

affirms the use of *A. sceptrum* in the management of various ailments and various contaminants (Ogbeke *et al.*, 2016). It was against this backdrop that this study focused on the evaluation of the effect of *Aframomum sceptrum* aqueous extract (ASAE) as an antidote for cyanide-induced metabolic stress in rats.

Materials and methods

Ethical approval.

Ethical approval was sought and approved from the Delta State University Abraka, Faculty of Science research ethics committee and all procedures conformed to the guidelines for animal researches stated by Animal and Research Ethics in 2009.

Plant material purchase and preparation

A. sceptrum (Atiko) purchase was done at Abraka main market which followed identification at Botany Department of the Delta State University Abraka. Further authentication in comparison with earlier identified specie of *A. sceptrum* with Fhi no. 109938 at the Forestry Research Institute of Nigeria (FRIN) Ibadan was also done. The *A. sceptrum* seeds were sun-dried until a constant weight was attained after two weeks followed by grinding into a very smooth powder with the aid of a warren blender. The ground seeds comprising (25g) were soaked in 100ml of distilled water and boiled for five minutes (5 mins) as previously described by George *et al.* (2012). This was followed by constant shaking for ten minutes (10 mins) and allowed to cool. Following cooling, the extract was filtered using Whatman filter paper and later concentrated with a rotary evaporator at a temperature range of 40-50°C under a reduced pressure. The collected concentrate was stored at a constant temperature of -200C until it was required.

Preparation of Cyanide solution

Potassium cyanide (KCN) solution administered orally was at a concentration of 9mg/Kg in drinking water. This was prepared by dissolving 0.09g of KCN in 1liter of water. The choice of the administered KCN dose was based on a similar dose administration by Sousa *et al.* (2002) via drinking water that produced deleterious effects over 15 days. KCN administration was adjusted

according to body weight and calculated water consumption levels after fresh solution of cyanide has been prepared. The KCN stability in drinking water is at least four (4) days after preparation.

Experimental design and treatment. Thirty (30) male albino rats of eight weeks weighing between 100g and 160g were purchased from the animal house of Department of Anatomy, Delta State University Abraka. The rats were fed on growers mash and given water ad libitum. The rats were housed in cages constructed of wood and wire gauze under controlled condition of 12 hours light and 12 hours dark cycle and a temperature range of 25-30°C. The rats were divided into six (6) groups of 5 rats per group and treated for 28 days as follows:

Group A: Normal control: Rats in this group received grower mash and tap water daily throughout the experiment.

Group B: Cyanide control: Animals in this group were given KCN solution at concentration 9.0mg/kg bodyweight and growers mash.

Group C: Cyanide + ASAE Dose 1: The rats in this group were given KCN solution and 10mg/kg of ASE plus growers mash.

Group D: Cyanide + ASAE Dose 2: Animals in this group were given KCN solution and 20mg/kg of ASE plus growers mash.

Group E: Normal + ASAE Dose 1: The rats in this group received normal tap water and 10mg/kg of ASE plus growers mash.

Group F: Normal + ASAE Dose 2: Animals in this group were given tap water and 20mg/kg of ASE plus growers mash.

ASAE treatment and animal sacrifice. The two doses of ASAE (10mgKg⁻¹ and 20mgKg⁻¹) body weight were respectively administered using an intragastric tube daily at 7am for the first dose, while a repeat dose of ASAE was done at 1pm and 7pm throughout the experiment for four (4) weeks. On the 29th day, the rats were allowed to fast overnight and sacrificed by cervical decapitation. Blood samples were collected

immediately by cardiac puncture into sterile plain tubes and labeled. The Liver tissue were excised into labeled containers under cold conditions.

Sample preparation, haematological and biochemical analysis

Sample preparation for biochemical assay. Serum collection was collected after centrifuging the clotted blood at 3000xg and stored in the refrigerator at 4°C. The collected liver tissues were homogenized in a pre-chilled mortar and pestle in cold normal saline solution. Liver homogenates were also centrifuged at 5000xg for 10 minutes and the supernatants collected and stored in the refrigerator at 4°C until required.

Determination of Packed Cell Volume (PCV). Method of Jones (1961) was used to determine PCV which was based on the proportion of whole blood occupied by red cells.

Determination of haemoglobin. Determination of haemoglobin was by Tietz (1976) which was done with the aid of a commercially available haemoglobin kit (TECO Diagnostics, U.S).

Determination of serum and liver total protein and albumin. Serum and liver total protein/albumin determination employed the use of a commercially available total protein kit (Randox Laboratory Ltd, United Kingdom) as described by the manufacturer. While total protein assay principle followed the methods of Tietz (1975). Albumin was assayed using the method of Doumas *et al.* (1971).

Assay for aspartate and alanine aminotransferases (AST) and (ALT). The estimation of the transferase enzymes was based on the methods of Rietman and Frankel (1957). However, the use of a commercial assay kit (Randox Laboratories, United Kingdom)

Assay for alkaline phosphatase (ALP) activity. Assay for ALP activity was determined using TECO Diagnostics kit, U.S.A.

Assay for superoxide dismutase (SOD) activity.

Assay for activities of SOD in serum and liver was based on the method described by Misra and Fredorich (1972). Its principle was based on the ability of the enzyme to inhibit the initial rate of epinephrine auto-oxidation.

Assay of catalase (CAT) activity. This was determined by a method described by Kaplan (1972). The assay principle was based on the ability of catalase to decompose hydrogen peroxide in the presence of metabolic oxidative stress.

Assay for glutathione peroxidase (GPx) activity. Determination of this was by method of Khan *et al.* (2009). The principle was based on the monitoring the disappearance of β -NADPH which initiates the transfer of H to an oxidized glutathione molecule during oxidative transduction.

Determination of serum and tissue reduced glutathione (GSH). Serum and tissue reduced glutathione assay was done with method of Beulter *et al.* (1962).

Determination of lipid peroxidation. Based on the principle of quantitative determination of malondialdehyde (MDA) formed from the breakdown of polyunsaturated fatty acid (PUFA) by thiobarbitoric acid reactive substance (TBARS), the assay was done using the method of Gutteridge and Wilkins (1982).

Histopathology of the liver

Tissue examination was carried out by a method previously described by Tulswani *et al.* (2005). The rinsed harvested tissue was cut into small sizes and fixed in a 10% formol saline. After 48 hours, the fixed tissue was processed with the aid of an automatic tissue processor for embedding using paraffin wax. At the end of the embedding process, tissues were cut into sections of 5 μ m using a microtome followed by haematoxylin and eosin (H and E) staining. The stained tissues were viewed with a light microscope and then photographed.

Statistical analysis

Analysis of biochemical parameter data was done using analysis of variance (ANOVA). Using the Statistical Package for The Social Sciences Version 21 (SPSS 21). Group mean comparison employed the Tukey Multiple Range Test (TMRT) which followed the expression of all values in mean \pm S.D.

Results

Effect of ASAE on cyanide Induced changes on PCV and Hb.

The results in Figure 1 shows the result of Haematocrit (PCV) and Haemoglobin (Hb) levels in rats exposed to cyanide. PCV and Hb values of Group B (cyanide control) was significantly ($p < 0.05$) lower than all other groups. No observed significance in Groups E and F when compared with group A (Normal control) but there were increase when comparing group C and D with cyanide control (group B).

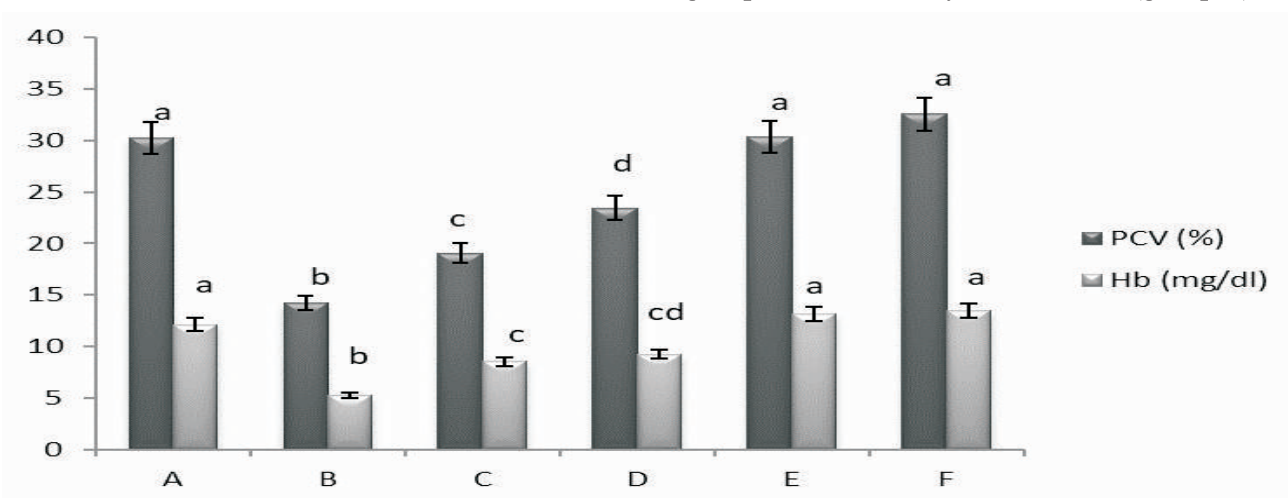


Figure 1: Effect of *A. sceptrum* on PCV and Haemoglobin Levels of Rats Exposed to Cyanide

Key:

Group A= Normal Control, **Group B**= Cyanide Control, **Group C** = Cyanide + 10 mg /kg b. wt. *A. sceptrum*, **Group D** = Cyanide + 20 mg /kg b. wt. *A. sceptrum*, **Group E** = Normal + 10 mg /kg b. wt. *A. sceptrum*, **Group F** = Normal + 20 mg /kg b. wt. *A. sceptrum*

Effect of ASAE on cyanide mediated alteration of liver function markers in serum and liver tissue.

Tables 1 and 2 indicates that ALT, AST and ALP activities in untreated rats exposed to cyanide (group B) increased matched to control while levels of serum Albumin and total protein were reduced. Both doses of *A. sceptrum* however reversed the observed trend in the metabolites (groups C and D) towards control values. No significant change occurred in serum ALT, AST and ALP activities and total protein and albumin levels in groups E and F (ASAE only) when compared with group A respectively but changed significantly compared to group B.

Table 1: The effect of *A. sceptrum* on liver Function Parameters in the Serum of Rats exposed to cyanide.

Parameters	A*	B	C	D	E	F
AST(UL ⁻¹)	30.14±1.64 ^a	50.10±4.42 ^b	40.68±3.52 ^c	35.76±1.59 ^{ac}	29.26±2.34 ^{ad}	28.66±3.81 ^a
ALT(UL ⁻¹)	20.28±0.79 ^a	41.38±2.40 ^b	33.16±2.54 ^c	27.54±2.17 ^d	19.68±0.80 ^a	18.84±0.63 ^a
ALP(UL ⁻¹)	110.02±14.58 ^a	160.26±3.86 ^b	141.92±7.90 ^c	121.88±4.22 ^{ad}	106.56±4.06 ^{ad}	100.26±9.90 ^a
Total Protein (gdL ⁻¹)	14.66±7.64 ^a	7.32±1.33 ^b	13.69±0.78 ^{ab}	14.82±1.11 ^a	15.66±1.88 ^a	16.76±2.31 ^a
Albumin (gdL ⁻¹)	9.48±1.46 ^a	2.30±0.72 ^b	4.82±0.81 ^c	6.36±1.11 ^c	9.17±0.42 ^a	10.36±1.31 ^a

All values are expressed as Mean ± SD, n=5. Values sharing different letters of the alphabet as superscript on the same row are significantly different (p ≤ 0.05). Superscript a indicates that there is no significant difference from Group A; b indicates there is a significant difference from Group A but not with Group B; c indicates significant difference from all other groups but not from Group C; d indicates a significant difference from other groups but not from Group D. *See Figure 1 footnote for interpretation of abbreviations

Table 2: The effect of *A. sceptrum* on liver Function Parameters in the Liver of rats exposed to cyanide.

Parameters	Experimental Groups					
	A*	B	C	D	E	F
AST(u/l)	44.22±2.84 ^{ad}	60.30±4.29 ^b	50.88±5.04 ^c	45.48±2.67 ^{ad}	42.58±3.31 ^d	41.54±0.98 ^d
ALT(u/l)	35.40±3.64 ^a	56.20±4.61 ^b	48.20±5.21 ^c	42.58±1.87 ^{ac}	32.86±3.78 ^a	30.76±3.78 ^a
ALP(u/l)	130.89±2.13 ^a	180.50±7.82 ^b	168.68±1.95 ^{cd}	159.62±2.62 ^d	127.82±5.43 ^a	126.04±4.99 ^a
Total Protein (gdL ⁻¹)	17.86±7.04 ^a	10.52±1.63 ^b	16.09±0.98 ^a	17.02±1.21 ^a	18.36±2.08 ^a	19.46±2.97 ^a
Albumin (gdL ⁻¹)	11.88±0.86 ^a	5.56±1.72 ^b	6.02±0.91 ^b	9.58±2.08 ^c	12.07±0.82 ^a	13.06±1.03 ^a

All values are expressed as Mean ± SD, n=5. Values sharing different letters of the alphabet as superscript on the same row are significantly different (p ≤ 0.05). superscript a indicates that there is no significant difference from Group A; b indicates there is a significant difference from group A but not with Group B; c indicates significant difference from all other groups but not from Group C; d indicates a significant difference from other groups but not from Group D. *See Figure 1 footnote for interpretation of abbreviations

Effect of ASAE on cyanide induced changes in serum and liver antioxidants and oxidative stress indicators.

Results in Tables 3 and 4 showed that there were heightening in serum and liver lipid peroxidation (MDA) levels and concomitant drop in CAT, SOD, GPx activities and GSH levels of rats that received only cyanide (group B) compared to all experimental groups. Administration of 10mgKg⁻¹ and 20mgKg⁻¹ body weight of *A. sceptrum* significantly reversed the trend in the direction of normal control (group A). The rats not exposed to cyanide receiving treatment with 10mg/kg body weight of *A. sceptrum* (group E) and 20mg/kg body weight of *A. sceptrum* (group F) showed no significant difference in serum and liver MDA levels, CAT, SOD, GPx activities and GSH levels when compared with group A.

Table 3: Effect of *A. sceptrum* on Levels of Oxidative Stress indicators in the Serum of rats exposed to cyanide.

	MDA μmolml^{-1}	CAT Units ml^{-1}	SOD Units ml^{-1}	GPx μmolmg^{-1} protein	GSH μmolmg^{-1} protein
A*	0.53±0.08 ^a	38.33±2.22 ^a	74.80±3.88 ^a	9.72±2.06 ^a	7.41±2.44 ^a
B	1.67±0.34 ^b	15.30±5.05 ^b	26.56±4.48 ^b	3.53±1.60 ^b	0.91±0.22 ^b
C	1.43±0.43 ^{bc}	23.94±2.55 ^c	40.78±1.19 ^c	5.40±0.98 ^b	4.86±0.17 ^c
D	1.28±0.24 ^c	34.40±3.71 ^a	51.99±4.76 ^d	8.69±1.06 ^c	6.14±0.66 ^{ac}
E	0.48±0.07 ^a	39.46±3.68 ^a	74.68±3.19 ^a	8.20±1.04 ^a	7.75±0.58 ^a
F	0.49±0.05 ^a	40.07±3.98 ^a	76.52±3.60 ^a	7.84±0.65 ^a	8.33±0.57 ^a

All values are expressed as Mean ± SD, n=5. Values sharing different letters of the alphabet as superscript on the same column are significantly different ($p \leq 0.05$). superscript a indicates that there is no significant difference from Group A; b indicates there is a significant difference from a but not with Group B; c indicates significant difference from all other groups but not from Group C; d indicates a significant difference from other groups but not from Group D. *See Figure 1 footnote for interpretation of abbreviations.

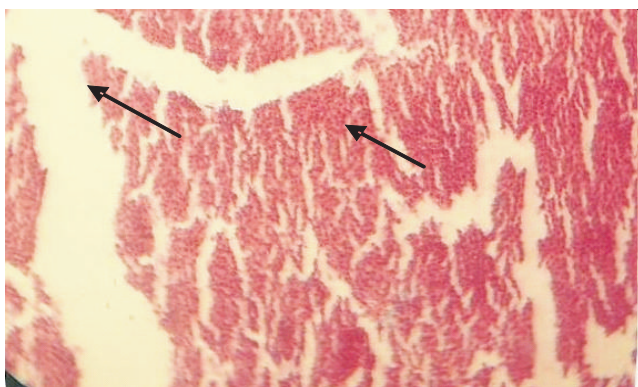
Table 4: Effect of *A. sceptrum* on Levels of Oxidative Stress indicators in the Liver of rats exposed to cyanide

	MDA μmolml^{-1}	CAT Units $\text{g}^{-1}\text{tissue}$	SOD Units g^{-1} ¹ tissue	GPx μmolmg^{-1} protein	GSH μmolmg^{-1} protein
A*	0.57±0.12 ^a	45.43±2.88 ^a	85.33±7.73 ^a	11.27±3.66 ^a	17.43±3.46 ^a
B	2.15±0.78 ^b	25.38±3.62 ^b	37.40±5.03 ^b	4.35±1.96 ^b	9.18±2.89 ^b
C	1.83±0.52 ^b	34.64±3.83 ^c	48.04±2.35 ^c	7.04±1.12 ^b	12.36±1.65 ^{bc}
D	1.53±0.12 ^b	42.48±1.82 ^a	54.50±1.27 ^c	8.96±0.96 ^c	14.65±1.09 ^{ac}
E	0.52±0.97 ^a	47.16±1.84 ^a	86.20±3.83 ^a	12.02±0.65 ^a	18.08±0.93 ^a
F	0.49±0.03 ^a	48.48±1.29 ^a	87.76±2.17 ^a	12.48±0.52 ^a	18.28±1.01 ^a

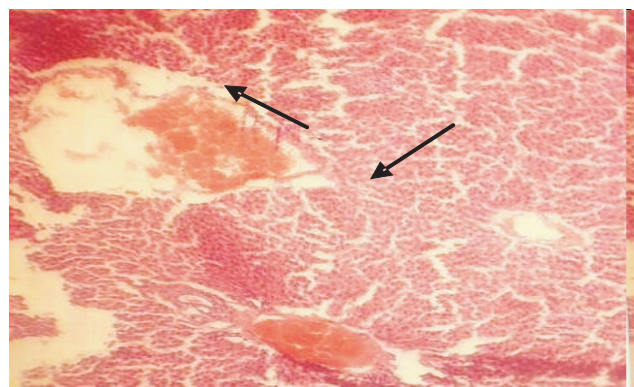
All values are expressed as Mean \pm SD, n=5. Values sharing different letters of the alphabet as superscript on the same column are significantly different ($p \leq 0.05$). Superscript a indicates that there is no significant difference from Group A; b indicates there is a significant difference from a but not with Group B; c indicates significant difference from all other groups but not from Group C. *See Figure 1 footnote for interpretation of abbreviations

Effect of ASAE on liver histology.

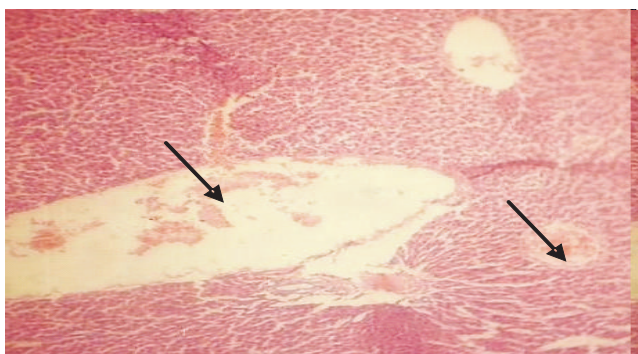
There were cyanide mediated ballooning and necrosis of hepatic cells in rats that received only cyanide (group B). Ballooning and necrosis were reversed by administration of both doses of ASAE (Figures 2C and 2D) while ASAE only administration (Figures 2E and 2D) did not indicate any histoarchitectural abnormality.



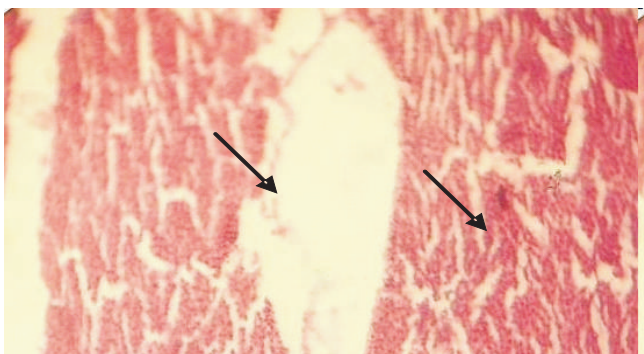
A = Normal control showing hepatic cells and broad central vein



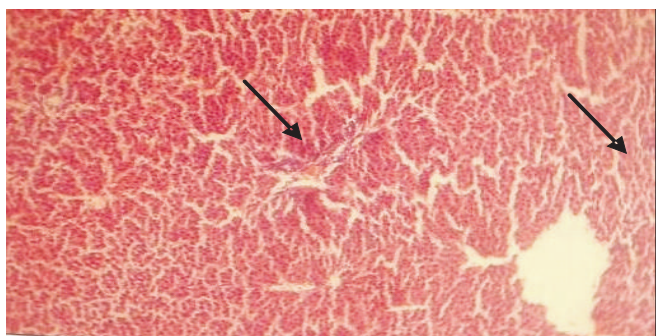
B = Cyanide control showing ballooning degradation and severe hepatic necrosis



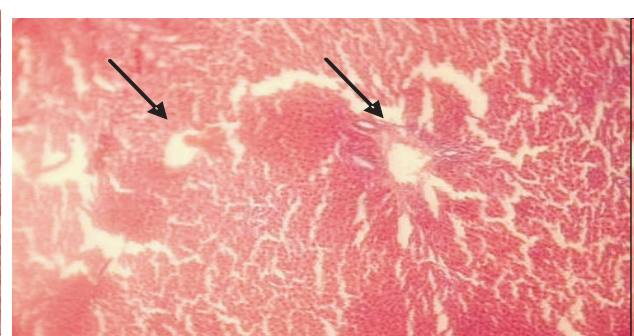
C = Cyanide + 10mg/kg body weight of A. sceptrum, showing reduction in hepatic necrosis and ballooning degradation



D = Cyanide + 20mg/kg body weight of A. sceptrum, showing broad central vein and hepatic cell degradation



E = Normal + 10mg/kg body weight of A. sceptrum, showing hepatic cells prominent nucleus, no hepatic necrosis



F = Normal + 20mg/kg body weight of A. sceptrum, showing preserved nucleus, hepatic cells and no hepatic necrosis

Discussion

Environmental cyanide, has been noted for its association with many deadly incidents in living organisms, especially humans via food consumption, drugs and non-conventional means (Abraham *et al.*, 2016; Baud *et al.*, 2002). Cyanide concentrates in erythrocytes through binding to methemoglobin (Tylleskar *et al.*, 1991). Subacute cyanide exposures in rats have been previously reported to produce changes using several biochemical pathological indices (Tylleskar *et al.*, 1991). This study investigated the potency of ASAE as an antidote for cyanide virulence via utilization of several antioxidant and oxidative metabolic stress indices.

The significant decrease in the PCV and Hb level in cyanide control rats relative to normal control and other groups could be traced to the significant increased destruction of erythrocytes due to cyanide (Dede *et al.*, 2002). Information in literature indicated the antioxidants protect animals from noxious chemicals (Fariss, 1991). Thus, treatment with ASAE was observed to have reduced the noxious effects impacted by cyanide on haematological parameters and may be said to possess anti-anaemic potentials. The justification of this claim is that ASAE therapy restored haematological parameters (PCV and Hb) near control values with the 20mg/kg body weight dose being more potent than the 10mg/kg body weight dose. Reduction in the protein levels of serum and liver observed in Tables 2 and 3 may be indicative of possible hepato-toxic anomalies of cyanide exposure in rats that is reminiscent of previously reported pathological and biochemical findings in several animal and human models of cyanide insult (Dhas *et al.*, 2011; Tulswani *et al.*, 2005; Okolie and Iroanya, 2003). These reductions however were mitigated by ASAE in comparison to the non-treated group (cyanide control).

Changes in liver function biomarkers are in consonance with an earlier study by Okolie and Osagie (2003) who stated that sublethal cyanide poisoning has the ability to increase serum AST activity in rats. Similar results were found in previous studies (Elsaid and Elkomy, 2006; Chandra *et al.*, 1980; Hyl'nezak *et al.*, 1980) that indicated a noticeable rise of ALP and the transferases in rats drinking water contaminated

with cyanide. It is an established truth that these enzymes have very high liver activities, hence they are often used as significant marker enzymes for status of liver integrity and damage especially in cases where they rise above normal limits in the serum. Based on the reported decline and stability of the levels of liver proteins and enzyme activities after dosing with ASAE, it may be suggested that ASAE is an effective antidote for cyanide noxiousness and has the capability of providing some level of protection against cyanide induced liver damage. A significant observation that was observed is stability in the levels of total protein, albumin and eventual insignificant rise of the liver enzymes in rats treated with only the plant extracts compared to control. This gives credence to an earlier report on the ability of plant materials to confer protection on hepatocellular damage during cyanide intoxication (Kadiri, 2017).

There are many documents in the literature about cyanide and antioxidant enzymes systems in different cells and animal tissues which has established that SOD catalyses dismutation of several superoxide radicals to hydrogen peroxide and eventually converted to water by GPx or by catalase (Nikoli-Kokic, 2010; Weydert and Cullen, 2010). Based on this results, prolonged sublethal cyanide administration caused significant depletion in the mean value of serum and liver antioxidant enzyme, activities as compared to control group (Tables 3 and 4) and is somewhat similar with the previous reports indicating the reduction of SOD and catalase activities in some tissues of cyanide intoxicated rats and rabbits (Okolie and Osobase, 2005). The decreased SOD activity may be attributed to irreversible inactivation of this enzyme by its product, H₂O₂, due to cyanide-induced increase in superoxide anion generation (Nikoli-Kokic, 2010) or directly to its irreversible inhibition by cyanide (Okolie and Osobase, 2005). Conversely, higher activities of serum and liver SOD, CAT and liver GPx in cyanide intoxicated rats which were later treated with ASAEs might be a compensatory response to enhanced ROS formation and may supply more protection against free radical-mediated oxidative damages. GSH helps in the removal of peroxides of hydrogen by the GPx-catalysed reaction (Konukoglu, 1998). Reports indicate cyanide poisoning depreciates GSH in



liver, brain and blood of rats (Mathangi *et al.*, 2011). Also, acute insult of lethal dose of cyanide reportedly led to a downturn in liver and serum GSH levels of cyanide intoxicated rats in comparison with the normal controls (Table 3). This is in tandem with the previous reports (Mathangi *et al.*, 2011; Okafor *et al.*, 2006; Bhattacharya *et al.*, 2002) which observed a significant cutback in erythrocytic GSH assemblage in cyanide treated rats.

As shown by the results, enhancement of MDA values was perceived in cyanide-intoxicated rats. In line with these submissions, Mathangiet *al* (2011) showed that 90 days cyanide exposure to rats heightened lipid peroxidation in liver and brain cells. The trend reversibility of heightening MDA levels by *A. sceptrum* comparable to control groups is in agreement with previous investigation conducted by George *et al.* (2012). The administration of *A sceptrum* tend to normalized liver MDA level (George *et al.*, 2013; George and Osioma 2011). The observed reversal of the altered tissue ultra-structure evidenced by reduced tissue necrosis and ballooning in cyanide exposed rats by 10mgKg⁻¹ and 20mgKg⁻¹ of *A. sceptrum* extracts (Fig. 2) may be due to possible hepatoprotective properties of the rich antioxidants and active constituents such as flavonoids, and quinones (Erukainure, *et al.*, 2011; George *et al.*, 2011).

Conclusion

The mechanistic modulation of the reactive oxygen generating system by ASAE at 10mg/kg and 20mg/kg weight dose and its buffering capacity for the enhancement of the detoxifying and defense enzyme systems makes it a potential standard remedy of cyanide noxiousness.

Conflict of interest: The authors have declared no conflicts of interest.

Funding source: There was no specific funding received for this research.

Data Statement: Data for this study is available in the Department of Biochemistry, Delta State University Abraka where the work was carried out as part requirement and evaluation for postgraduate study progress and could be released under the written permission of the research supervisor.

Abbreviations

ASAE, *Aframomum sceptrum* aqueous extract; PCV, Packed cell volume; HB, Haemoglobin, TP, Total protein; ALB, Albumin; ALT, Alanine amino transferase, AST, Aspartate amino transferase; SOD, superoxide dismutase; GSH: Reduced glutathione; CAT, Catalase; GPx, Glutathione peroxidase; MDA: Malondialdehyde.

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