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Full Length Research Article

AFRAMOMUM MELEGUETA: A STIMULATOR OF LIVER FUNCTION ENZYMES AND A DOWN-REGULATOR OF CYANIDE-MEDIATED OXIDATIVE INJURIES IN RATS

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ABSTRACT
Despite the risk of cyanide poisoning a suitable antidote that can be administered at a reasonable quantity to a large number of individuals is not yet available. This study was carried out to determine the possible hepatoprotective effect of ethanolic extract of *Aframomum melegueta* seed against cyanide-induced liver injury and its possible antidote effect. Thirty male rats divided into five groups were used for this study. Group 1, received neither cyanide nor the seed extract (Normal control), Groups 2-5 were administered cyanide orally by gavage in form of KCN at a concentration of 4 mg/kg body weight according to body weights as follows; Group 2, received cyanide only (positive control). Group 3 received cyanide and sodium thiosulphate (500 mg/kg body weight) a standard cyanide antidote. (Standard control). Group 4 and 5 were co-treated with cyanide and different doses of the extract three times weekly for the duration of the experiment. Co-treatment with *A. melegueta* restored the weight loss and the activities of AST, ALT. ALP to levels compared to that obtained in the normal control. In addition co-treatment with *A. melegueta* modulated the cyanide mediated depletion of the antioxidant capacities of the rats that were exposed to cyanide and the increasing lipid peroxidation profile. The results indicated that ethanolic extract of *A.melegueta* seed ameliorated cyanide-induced hepatoxicity in rats through their free radical- scavenging mechanisms.

Keywords: (Cyanide, Hepatotoxicity, Antioxidants *Aframomum melegueta,* sodium thiosulphate)

INTRODUCTION

Cyanide (CN) refers to both the anion CN and the combined form of hydrogen cyanide (HCN). Plants and animals synthesize CN as a component of cyanogenic glycosides to provide a source of nitrogen and for self-defense (Moller, 2010; Petrikovics *et al*., 2015). CN intoxication can result from several ways; this includes consumption of poorly processed cyanogenic plants (e.g., cassava roots, yams, sorghum, maize), consumption of amygdalin (a cyanogenic glycoside present in fruits such as apricot cherries) and metabolism of cyanogenic chemicals such as nitriles and halides (Petrikovics *et al*., 2015). Acute cyanide exposure results from smokes from burning of certain substances, structural fires, cigarettes, and steel and gold extraction companies. Cyanide has also been used as chemical warfare agents during the first and Second World War and as well as poison for self and others. It is highly toxic and acts rapidly as a metabolic poison, by binding and inhibiting cytochrome a_3, thereby disrupting the electron transport chain of aerobic respiration resulting in histotoxic hypoxia (Moller, 2010). Despite the risk of cyanide poisoning a suitable antidote that can be administered at a suitable quantity to a large number of individuals is not available (Morningstar *et al*, 2019). This has necessitated the search for possible plant antidote for cyanide (Morningstar *et al*, 2019) poisoning,

Aframomum melegueta belongs to the ginger family (Zingiberaceae) and is commonly known as grains of paradise or alligator pepper (Nwaehujor *et al*., 2014). In Nigeria, it is called *Oseoji* by the igbos, *Ataare* by the Yoruba's and *Cittáá* by the Hausa's (Odugbem, 2008). The plant is a perennial deciduous herb native to the tropics and grows most times in swampy habitats of the West African coast with a leafy stem that may be up to 1.5 m high. It produces trumpet-shaped, purple-colored flowers which grow into 5 to 7 long pods each having about 300 reddish-brown seeds (Odugbemi, 2008;Ajaiyieoba, 1999)

The plant is rich in secondary metabolites such as modified gingerols, paradols, shogaols, and diarylhepanoid: these metabolites are responsible for the hot spicy taste of the seeds (Ajaiyieoba, 1999). The seeds of *A. melegueta* are known to be rich in carbohydrates, crude fiber, and bulk minerals (Echo *et al.*, 2012). In Ethno medicine, the seed has been used in the treatment of stomach pain, snakebite, diarrhea, cardiovascular diseases, diabetes, and inflammation. Previous reports have shown that *A. melegueta* seeds possess highly potent anti-inflammatory and antinociceptive properties (Ajaiyieoba, 1999). Extensive *in vitro* and *in vivo* studies carried out on the ethanolic extract of *A.melegueta* seed evidently showed its anti-inflammatory properties against pro-inflammatory seed. (Ilic *et al*., 2014; Oyinloye *et al*., 2016) .To the best of our knowledge, there has been no scientific report to show the hepato-protective effect of ethanolic extract of the seed against cyanide induced liver injury and its possible antidote effect. Therefore this study was carried out to determine the possible hepatoprotective effect of ethanolic extract of *A.melegueta* seed against cyanide induced liver injury and its possible antidote effect.

MATERIALS AND METHODS

Plant materials

Dry fruit of *Aframomum melegueta* was bought from Abraka, Delta State. It was duly identified and authenticated by Dr.Akinnibosun a taxonomist from the University of Benin, Edo State, Nigeria (Voucher number UBH-A471).

Plant material and extraction

The seeds were carefully selected from the clove before grinding in a Molineux electric blender in to a powdery form and stored in an air-tight container before extraction. (Okochi *et al*., 1999). Fifty grams of the powder were soaked in 200 ml of ethanol and allowed

to stand for 24 h (Okoro *et al*., 2019). After which, the mixture was filtered through a muslin cloth and Whatman No. 1. Filter paper. It was then concentrated at reduced pressure and low temperature using a rotary evaporator. The resultant extract was kept at a temperature of 4 °C before use. The dried extract was then suspended in ethanol to prepare the two concentrations (100 and 200 mg/(ml)) that were used in this study (Okoro *et al*., 2019)

Ethical approval
Ethical approval for the use of the experimental animals was obtained from Faculty of Science, Delta state University, Abraka, Nigeria. The approval no. is REC/FOS/21/03. All animals were handled according to the care and use of laboratory animals of the national research council (NRC, 2011).

Animals
Male Wister rats were obtained from the animal house of the College of Medicine, Delta State University Abraka, Nigeria. They were housed in well-aerated cages at a temperature of between 22-30^{o}C. (12 h light and 12 h dark). All procedures were carried out in accordance with the guidelines of the institutional Committee for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985)

Protocol design
Male *Wister* rats (n= 35, 120 ± 8 g) were used for this study and randomly divided into seven groups (n=5).Group 1 which served as the normal control received neither cyanide nor *A melegueta* fruit extract, Group 2 served as the positive control and was given cyanide only. Group 3(standard control) received cyanide and sodium thiosulphate (500 mg / Kg body weight) a standard cyanide antidote, Group 4 and 5 received cyanide and 100 and 200 mg of *A melegueta* seed extract respectively. Groups 2-5 were administered cyanide orally by gavage in form of KCN at a concentration of 4 mg/kg body weight.(A sub-lethal dose) (Satpute *et al*., 2010). The dose of the plant extract administered to Groups 4 and 5 was in the range of the safe dose as reported by Illic *et al*., (2014). All the rats were sacrificed after 21 days using previous protocol (Satpute *et al*., 2010).

Tissue sampling and biochemical analysis
The serum and liver was collected and alanine aminotransferases (ALT), aspartate aminotransferase (AST) activities determined according to the method described by Reitman (1957), Alkaline phosphatase (ALP) was assayed according to the protocol of klinischeChemie (1972). Total protein concentration (TPC) was determined according to the method of Tietz (1995) and estimation of Protein content was by the method of Lowry *et al*., (1951). The protocol of Doumas *et al*., (1971) was used to determine serum albumin (ALB). The protocol described by Aebi (1974) was used for the assay of Liver catalase (CAT) activity. Superoxide dismutase (SOD) activity was measured using the method of Kakkar and Viswanathan (1984). Malondialdehyde (MDA level was measured according to the protocol of Buege and Aust (1951)

Statistical analysis
Data from all analyses were collected and analyzed statistically and expressed as the mean ±SD using the one-way analysis of variance (ANOVA) and Bonferonni Post –Hoc Test (SPSS version 18). Results were considered to be statistically significant at P < 0.05.

RESULTS
The result in Table 1 shows the body weight gain and organ/ body weight ratio of the liver of rats co-treated with cyanide and *A. melegueta* extract. The result shows a significant decrease in body weight and a elevation in the organ/ body weight ratio of the liver in Groups 2 rats intoxicated with cyanide without treatment when compared with the normal control. However treatment with the plant extract was able to reverse this trend as was indicated in Groups 4 and 5 rats co-treated with 100 and 200mg of the extract when compared with Group 2 not treated in a dose dependent manner. Therefore the study reveals that the extract is able to ameliorate the negative effect of cyanide intoxication on body weight gain and organ/ body weight ratio of the liver rats, although this parameter did not return to normal level, as the observed weight loss remained evident.

Table 1: Effect of *A. melegueta* extract on body weight gain and organ / body weight ratio for liver of cyanide intoxicated rats.

Groups	Body weight gain % change	liver weight/ body weight % change
1.	12.82±4.14^{a}	2.52±0.32^{a}
2.	2.84±5.45^{b}	4.57±0.76.^{b}
3.	10.70±1.19^{ac}	2.35±0.24^{a}
4.	8.45±1.38^{c}	2.87±0.14^{a}
5.	9.18±1.48^{c}	2.72±0.24^{a}

Values are presented as mean ± standard deviation. (n=5). Values with different superscript letter in the same column differ significantly at p< 0.05.

Effects of Cyanide intoxication and *Aframomum melegueta* extract on the levels of serum transaminases, (AST, ALT) alkaline phosphatase, (ALP) total protein and albumin in rats is indicated in Table 2. Oral administration of cyanide to animals resulted in hepatotoxicity as was indicated in the significant increase (P < 0.05) in AST, ALT, ALP and significant decrease (P > 0.05) in total protein and albumin (group 2) when compared with group 1 rats. Treatment with the ethanolic extract of *A.melegueta* seeds however, was able to ameliorate the effects of cyanide toxicity in a dose-dependent manner (Groups 4 and 5). In addition the plant extract modulated the elevated cyanide effects on activities of ALT and ALP in the rats better than the standard cyanide antidote. (Group 3).

Table 2: Effects of *Aframomum melegueta* extract on the levels of serum transaminases, Alkaline Phosphatase, Total Protein and albumin in cyanide intoxicated rats.

GROUPS/ Parameters	AST(U/L)	ALT(U/L)	ALP(U/L)	Total Protein (mg /dl)	Albumin (g /dl)
1.	39.06±0.67^{a}	10.25±0.86^{a}	377.81± 5.21^{a}	48.82±0.45^{a}	24.96±0.37^{a}
2.	100.80±1.93^{b}	47.63±0.85^{b}	795.43± 19.37^{b}	26.08±0.84^{b}	11.18±0.91^{b}
3.	61.87± 0.57^{c}	21.31±1.18^{c}	595.45± 12.71^{c}	38.25±1.03^{c}	19.82± 0.39^{c}
4.	60.25±1.04^{c}	18.81±1.43^{c}	593.09±9.60^{c}	41.66±4.78^{c}	19.02±1.52^{c}
5.	51.31±0.55^{d}	11.75±0.64^{a}	533.53±2.14^{d}	39.61±0.92^{c}	20.73±1.48^{c}

Values are presented as mean ± standard deviation. (n=5). Values with different superscript letter in the same column differ significantly at P< 0.05.

Table 3 shows the effects of cyanide intoxification and *Aframomum melegueta* extract on levels of MDA and on the activities of SOD

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and CAT in the liver of rats . A remarkable increase (P <0.05) in MDA and a concomitant decrease in SOD and catalase activities were indicated in the cyanide induced rats (Groups 2) when compared with the normal control. However treatment with different doses of the ethanolic extract of *A. melegueta* seed significantly(P>0.05) mitigated the elevated MDA levels, as was observed in Groups 4 and 5, and also modulated the cyanide mediated depletion of the antioxidant capacities of the rats, due to cyanide exposure. Again the ameliorating effect of the plant extract on the rats was better than the standard (Group 3).

Table 3: Effects of *Aframomum melegueta* extract on levels of lipid peroxidation (MDA), Superoxide dismutase (SOD) and Catalase (CAT) in the liver of cyanide intoxicated rats.

GROUPS/ Parameters	MDA(nmol/mg protein)	SOD(U/mg protein)	CAT(U/mg protein)
1.	1.34±0.23[a]	30.07±1.02[a]	106.28±1.30[a]
2.	9.84±0.53[b]	13.95±0.75[b]	57.75±1.27[b]
3.	2.92±0.77[c]	18.85±1.16[c]	70.35±1.61[c]
4.	4.01±0.32[c]	20.83±0.94[c]	71.05±0.86[c]
5.	2.20 ± 0.60[c]	27.77±0.88[a]	78.68± 1.96[d]

Values are presented as mean ± standard deviation. (n=5). Values with different superscript letter in the same column differ significantly at p< 0.05.

Histology of the liver

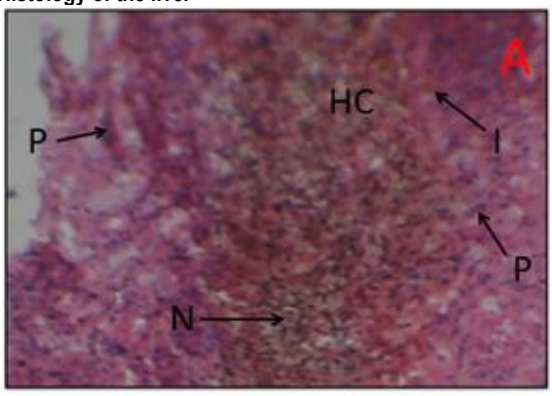

Plate I: Photomicrograph A of rat liver section of cyanide only (Group 2) showing severe degenerated hepatic cell (HC), necrosis (N) multi pyknotic nuclei (P). (H&E stain x 200)

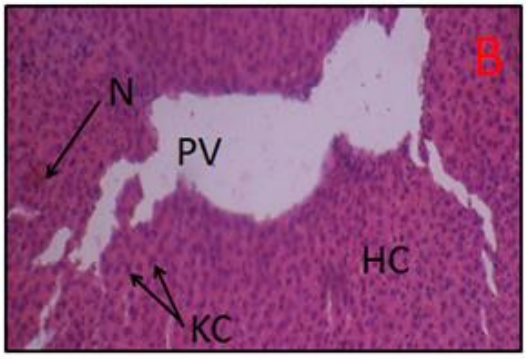

Plate II: Group 3= Photomicrograph B of rat liver section of cyanide plus sodium thiosulphate (Group 3) showing regeneration of hepatic cell (HC) kupffer cells (KC), portal vein (PV)and mild necrosis (N). (H&E stain x 200)

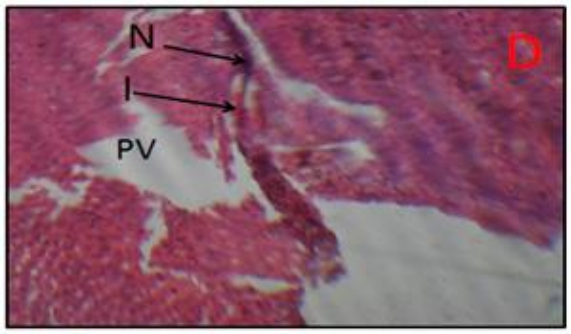

Plate III: Photomicrograph D of rat liver section of cyanide plus 100 mg/kg b. wt. ethanol extract of *A. melegueta* seed (Group 4) showing improvement portal vein (PV)and mild necrosis (N). (H&E stain x 200)

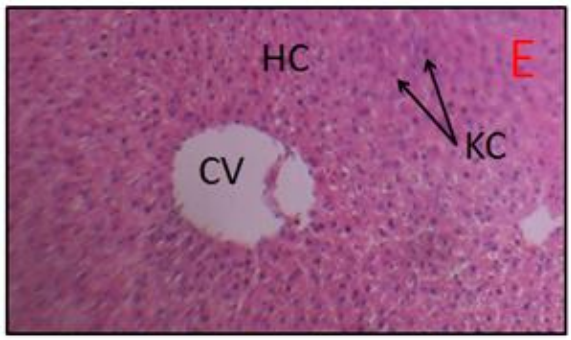

Plate IV: Photomicrograph E of rat liver section of cyanide plus 200 mg/kg b. wt. ethanol extract of *A. melegueta* seed (Group 5) showing normal hepatic cell (HC), central vein (CV), kupffer cell (KC), no inflammation and necrosis. (H&E stain x 200)

DISCUSSION

Cyanide is a hepatotoxicity chemical that is primarily metabolized in the liver (David and Kartheek 2016). Although, to this present moment, chemical antidote therapy, through the use of "Amyl nitrile" and "Sodium thiosulphate," still remains the main therapeutic intervention for CN poisoining (Ghodsi and Baghshani 2013), there is therefore need to the search for other natural sources that are cheap and easily accessible, hence the need to source for some natural therapies.

The results from this present study shows significant increase (P<0.05) in ALT and AST in the serum of cyanide intoxicated rats indicating hepatotoxicity (Table 1 and 2). This result is consistent with several studies that have been done previously by Kadiri and Asagba (2019) on birds and Gotardo *et al*., (2015) on sows. The significant increase (P<0.05) in ALP also indicated in this study is in agreement with earlier findings where a significant increase in ALP was indicated in the tissues of the birds (Kadiri and Asagba 2015) and rats (Kadiri, 2017) following chronic cyanide exposure. The therapeutic effects of *A.melegueta* extract that was recorded in this study in the group's co treated with cyanide and the plant extract can be attributed to the presence of phytochemicals such as flavonoids and phenols in the plant (Mahmoud, 2019). These phytochemicals possess myriads of pharmacological activities including hepato protective roles (Nwozo and Oyinloye 2011; Mahmoud, 2019).

The significant increase in total protein and albumin in the serum of cyanide intoxicated rats is also an indication of hepatic impairment (Kadiri *et al*., 2020). Cyanide probably reduces the synthesis of protein by compromising the endoplasmic reticulum. Administration of *A. melegueta* gave a reversal effect. This is in agreement with similar studies carried out by Oyinloye *et al*., (2016) on the protective effect of *A. melegueta* on cadmium toxicity in rats. Cyanide has been established to induce oxidative stress in organisms, by increasing the production of free radicals (Mediavilla *et al*., 2017; Kadiri *et al*., 2020). This is consistent with this study, as was indicated by the significant increase ($P<0.05$) in the level of lipid peroxidation (MDA) and the concomitant decrease in the activity of SOD and CAT in the negative control rats when compared with the normal control. Again *A. melegueta* was able to mitigate the effect of this oxidative stress as was indicated by the significant decrease ($P>0.05$) in MDA levels and increase in the activities of catalase and SOD in the liver of rats treated with different doses of the extract. This ameliorative effect is probably due to the presence of flavonoids in the ethanolic extract. Flavonoids are known for their chelating property and their ability to protect against free radical attacks (Asagba *et al*., 2019). Studies have also shown that flavonoids can induce the activity and expression of enzymes such as SOD and CAT, hence they have the potential to produce long lasting effects on cellular function, which could be highly beneficial to cells exposed to chronic oxidative stress (Myhrstad *et al*., 2002; Okoro and Kadiri 2019). Histological examinations on the liver in this study are also in agreement with the hepatic damage induced by cyanide as revealed by the significant increase in lipid peroxidation and the corresponding decrease in the activities of SOD and catalase observed in the group 2 rats (Fig.1). Studies from Fulda *et al*. (2010), show that cells respond histopathologically to toxic insult by the following processes degeneration, proliferation, inflammation, and repair. Some of which was revealed in the liver section of the positive control rats (Plate I). This result is in agreement with previous studies by kadiri and Asagba, (2019) and David and Kartheek, (2016) who reported that cyanide induces pathological aberrations in the liver of birds and the liver of fresh water fishes following exposure to sub-lethal concentrations. However, treatment with different doses of the extract (Plate III and IV) showed hepatocytes with marked improvement.

In conclusion administration of oral ethanolic extract of *A. melegueta* seed on male Wister rats provides significant protection against cyanide induced hepatotoxicity in a dose dependent manner by acting as an antidote to cyanide poisoning through its free radical scavenging mechanism which could be linked to the bioactive constituents earlier reported to be present in the ethanolic extract.

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Author Disclosure Statement
The authors declare that this work does not have any conflict of interest.

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