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RESEARCH ARTICLE

Effects of Aqueous Extract of Xylopia aethiopica and Vitamin E on Hepatic and Oxidative Enzyme Markers in Rats Exposed to Cyanide Toxicity

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Abstract

This research was conducted to study the effects of aqueous extract of *Xylopia aethiopica* and vitamin E supplementation on hepatic and oxidative enzyme markers in rats exposed to cyanide toxicity. A total of 30 male albino rats weighing between 100 – 160g were used for the study. Rats were divided into 6 groups of 5 rats per group as follows: normal control, cyanide control, cyanide plus *Xylopia aethiopica* seed extract (XSE), cyanide plus *Xylopia aethiopica* peels extract (XPE), cyanide plus *Xylopia aethiopica* whole extract (XWE), and cyanide plus vitamin E. The cyanide treated groups received XSE, XPE and XWE at 50mg/kg and vitamin E at 100 mg/kg three times per week. The cyanide group were given potassium cyanide (KCN) solution at concentration of 9.0 mg/kg in the drinking tap water daily. The experiment extended for 4 weeks. The activities of alanine aminotransferase (ALT), aspartate aminotranferase (AST), superoxide dismutase (SOD), catalase (CAT), and concentrations of reduced glutathione (GSH) and malondialdhyde (MDA) were measured in serum and liver. The results showed that the cyanide control rats had a significant ($p < 0.05$) increase in AST, ALT in the serum, MDA in the liver and decrease SOD and CAT in the liver when compared to the normal control respectively. Treatment of cyanide-intoxicated rats with the XSE, XPE, XWE and vitamin E showed significant decrease in the activities of AST, ALT in the serum, MDA level in the liver and increase SOD and CAT activities in the liver when compared with the rats given cyanide only. Overall, the different aqueous extract made from *Xylopia aethiopica* exhibited significant protective effect in ameliorating cyanide poisoning in rats as compared to vitamin E, a known potent antioxidant. Thus, *Xylopia aethiopica* may be considered as a potential antidote against cyanide – mediated toxicity.

Introduction

Cyanide is a ubiquitous and potent cytotoxic agent known for its rapid lethal action and toxicity (Ghodsi and Baghshani, 2013). The toxicity of cyanide is a consequence of its high potency as a respiratory poison in all aerobic forms of life (Ghodsi and Baghshani, 2013). In addition to acute cyanide intoxication, chronic toxicity has frequently been reported in recent years, and it is suggested that the most widespread problems arising from cyanide are from chronic dietary, industrial and environmental sources (Barillo, 2009).

Cloves of the plant *Xylopia aethiopica*, a member of the custard apple family, *Annonaceae*, are used as a spice in various traditional dishes of Western and Central Africa. In Delta State the southern part of Nigeria, it is called locally "Uda" by the Ukwani people. The plant's fruit is an essential ingredient in preparation of local soups to help new mothers in breastfeeding. It remains an important item of local trade throughout Africa as a spice, and in food flavouring and for medicine (Uwakwe *et al*., 2008). Studies have shown that *Xylopia aethiopica* dried fruit contains antioxidant compounds (Karioti *et al*., 2004).

Reduced glutathione (GSH), an essential component of the GSH peroxidase system, and Vitamin E are probably the most important nonenzymatic antioxidants and participate in a wide range of cellular functions. Superoxide dismutase (SOD) catalyses the dismutation of superoxide anion radicals (O_2•) to hydrogen peroxide (H_2O_2), while catalase convert H_2O_2 into a molecule of oxygen and water (Eboh, 2014). Lipid peroxidation is regarded as one of the basic mechanisms of cellular damage caused by free radicals. Free radicals react with lipids causing peroxidation, and result in the release of products such as malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and hydroxyl radicals. An increase in lipid peroxides indicates damage to cell membranes, inhibition of several important enzymes, reduced cellular function and cell death (Pompella *et al*., 1991).The aim of study was to investigate the effects of vitamin E supplementation and *Xylopia aethiopica* aqueous extracts in rats exposed to cyanide.

Materials and Methods

Experimental animals

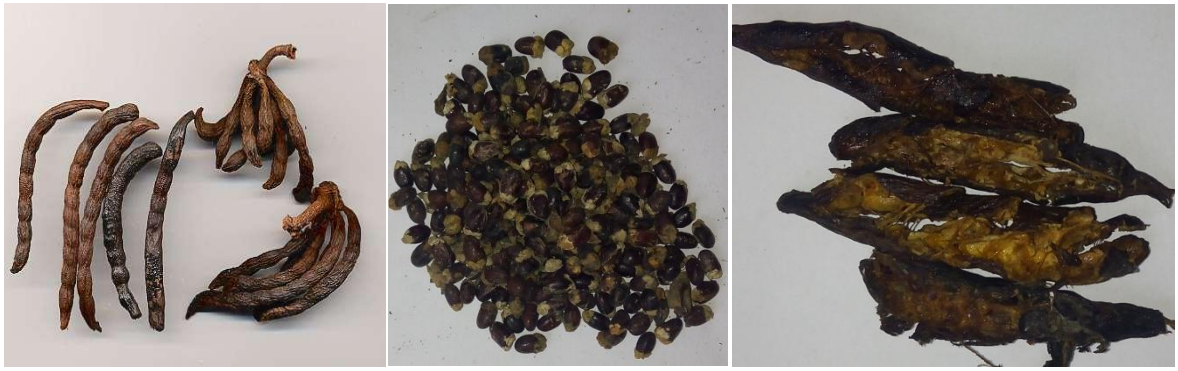
Eight weeks old male albino rats weighing between 100g and 160g were obtained from the animal house Delta State university Abraka. The rats were fed on growers mash and were given water *ad libitum*. The rats were housed in cages constructed with wood and wire gauze under control condition of 12h light/ 12 dark cycle.

Ethical recommendations

Ethical approval was sought from the Research Ethics Committee of the College of Health Sciences, Delta State University, Abraka, Delta State.

Spice (*Xylopia aethiopica)*

The spice *Xylopia aethiopica* were obtained from Abraka main market, Delta State. The spice was identified at the Department of Botany, Delta State University, Abraka.



a b c

a = *Xylopia aethiopica* whole pods, b= *Xylopia aethiopica* seeds, c= *Xylopia aethiopica* peels **(**pods without the seeds)

Preparation of *Xylopia aethiopica* extract

The *Xylopia aethiopica* seeds, peels, and whole pods were sun-dried to obtain a constant weight for two weeks, then ground to fine powder using Warren blender. Twenty-five grams (25g) of the ground spice labelled seeds, peels, and whole pods was soaked in 100ml of distilled water boiled for 5mins. This was shaken for 10min and allowed to cool then filtered. The extract was then concentrated using rotary evaporator at 40 – 50^0C under reduced pressure. The extracts were stored frozen in a deep freezer until required.

Preparation of cyanide solution

Potassium cyanide (KCN) solution was prepared at a concentration 0.09g/L in tap water; rats received KCN solution at the concentration of 9.0 mg/kg every day. The amount of KCN administered in the drinking water was adjusted to the body weight. The stability of KCN in the drinking water is stable for at least four days after preparation.

Preparation of vitamin E

Vitamin E was prepared according to the method described by Iribhogbe et al. (2013). The Vitamin E capsule used in this study was purchased from Humphrey Pharmacy Abraka, Delta State. Zero point two milliliters (0.2 ml) of vitamin E was dissolved in 0.2 ml of Tween 80 and 9.6 ml distilled water in a ratio of 0.2:0.2:9.6 making up a total volume of 10 ml. The dose administered to the rats was 100 mg kg^{-1}. The oral administration of the extracts and vitamin E was carried out using intragastric tube, three times per week for 4 weeks. On the last day the rats were allowed to fast overnight and sacrified by cervical decapitation.

Experimental design

A total of thirty (30) rats were used for the study. The rats were divided into 6 groups of 5 rats per group as follows:
Group 1 - Normal control
Group 2 - Cyanide control
Group 3 - Cyanide + 50mg/kg b.wt of *Xylopia aethiopica* seeds extract (XSE)
Group 4 - Cyanide + 50mg/kg b.wt of *Xylopia aethiopica* peels extract (XPE)
Group 5 - Cyanide + 50mg/kg b.wt of *Xylopia aethiopica* whole pod extract (XWE)
Group 6 - Cyanide + vitamin E

Preparation of serum

Blood samples were collected into plain tubes and serum was separated by centrifugation at 1000xg for 15mins. The serum was stored in the freezer until required.

Preparation of tissue homogenate

Zero point five gram (0.5g) of wet liver tissue was homogenized in 4.5ml of normal saline and was separated by centrifugation at 1000xg for 15mins. The tissues homogenate supernatant were stored in the freezer until required.

Biochemical analysis

The biochemical investigations in serum samples were carried out using commercially available kits as supplied by TECO Diagnostic, Anahein, USA. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were determined by method of Reitman and Frankel (1957). Liver reduced glutathione was determined using the method of Ellman (1959). The activity of superoxide dismutase (SOD) was assayed using the method of Misra and Fridovich (1972). Catalase activity was assayed (CAT) using the method of Kaplan et al. (1972). Lipid peroxidation was determined using the method of Buege and Aust (1978).

Statistical analysis

The data obtained was expressed as mean ±SD and analyzed using analysis of variance (ANOVA) and the group means were compared by Duncan's multiple range test (DMRT).

Results

Table 1: Activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of rats exposed to cyanide

Groups	Serum AST(u/l)	Serum ALT(u/l)
1	30.14 ±1.64 [a]	20.32 ±0.79 [a]
2	50.10 ±1.42 [b]	41.38 ±1.39 [b]
3	44.20 ± 1.34 [c]	38.24 ±1.71 [c]
4	43.20± 1.33 [c]	34.48 ±0.95 [c]
5	40.41 ± 0.22 [c]	27.08 ±0.49 [c]
6	32.54 ±0.45 [a]	25.44 ±1.79 [c]

Values are given as mean ± S.D, n = 5. Means not sharing a common superscript letter on same column differ significantly at $p < 0.05$.

Table 1 shows the effect of aqueous extract of *Xylopia aethiopica* seeds, peels, whole pods and vitamin E on the activities of serum alanine and aspartate aminotransferases in rats exposed to cyanide. Serum ALT and AST activities significantly ($p < 0.05$) increase in cyanide control rats as compared to all other groups. Rats exposed to cyanide but treated with 50 mg/kg b.wt of *Xylopia aethiopica* seeds, peels, whole pods (groups 3, 4 & 5) and vitamin E (group 6) exhibited a significant decrease in activities of serum ALT and AST as compared with the rats given cyanide only (group 2). Normal control rats (group 1) have the lowest ALT and AST activities.

Table 2: Activities of superoxide dismutase (SOD) and catalase (CAT) and levels of reduced glutathione (GSH) and malondialdehyde (MDA) in liver of rats exposed to cyanide

Groups	SOD (units/g of tissue)	CAT (units/g of tissue)	GSH (units/g of tissue)	MDA (units/g of tissue)
1	81.55± 1.54 [a]	42.00 ±1.58 [a]	11.30 ± 0.76 [a]	0.42 ± 0.06 [a]
2	33.26 ±0.22 [b]	21.37 ±1.63 [b]	6.31 ±0.21 [a]	1.51± 0.05 [b]
3	40.42 ±1.63 [c]	25.17± 0.63 [b]	7.39 ± 0.45 [a]	1.01 ± 0.06 [b]
4	42.45 ±0.36 [c]	26.51 ± 1.53 [b]	8.28 ±0.87 [a]	0.93 ±0.14 [b]
5	50.32 ±1.15 [d]	35.43 ±1.68 [c]	9.39 ± 0.64 [a]	0.97 ±0.15 [b]
6	65.31 ±2.90 [e]	40.66 ±0.66 [a]	10.54± 1.08 [a]	0.53 ± 0.22 [a]

Values are given as mean ± S.D, n = 5. Means not sharing a common superscript letter on the same column differ significantly at $p < 0.05$.

The activities of SOD, CAT and levels of GSH and MDA in liver of experimental rats exposed to cyanide were presented in table 2. A significant decrease in liver SOD activity was observed in cyanide control rats (group 2) as compared with all other groups (1, 3, 4, 5 & 6). The results in table 2 also indicated that the observed increase in

liver SOD activities in rats treated with *Xylopia aethiopica* and were lower ($p < 0.05$) as compared with the normal control rats (group 1). Same trend was observed for liver catalase activity, although, values obtained were comparable ($p > 0.05$) for groups 2, 3 & 4. Treatment with vitamin E normalizes liver catalase activities. The levels of reduced glutathione were comparable ($p > 0.05$) in all groups according to table 2, with the cyanide control rats expressing the lowest values.

Concentration of MDA did not differ significantly among compared groups (i.e. groups 2, 3, 4 & 5) of rats. However, MDA levels were relatively lower in rats exposed to cyanide but treated with either aqueous spice extract or vitamin E. Normal control (group 1) showed a significant ($p < 0.05$) decrease in MDA value as compared to 2, 3, 4 & 5.

Discussion

Cyanide like other potent cytotoxic agents affects liver cell integrity. However, if the liver is damaged or injured, the liver enzymes spill into the blood causing elevated hepatic enzymes levels (Naik and Panda, 2007). Significant ($p < 0.05$) increase in the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in cyanide control group (table 1) as compared with normal control (group 1) from this study could indicate that the integrity of the hepatocyte may have been compromised by exposure to cyanide. ALT is regarded as the standard clinical biomarker of hepatotoxicity since it is primarily found in the liver (Amacher, 2002). Serum levels of both AST and ALT become elevated whenever disease processes affect liver cell integrity. Treatment with aqueous extract of *Xylopia aethiopica* seeds (XSE), peels (XPE), whole pod (XWE) and vitamin E decreases both serum ALT and AST activities. This indicates that the spice (*Xylopia aethiopica*) and vitamin E may have some curative effect on the already compromised hepatocyte as a result of cyanide exposure.

Many toxicants (or their metabolites) may exert toxicity related to oxidative stress – which is caused by an alteration in the intracellular prooxidant to antoxidant ratio in favour of prooxidants (Sies, 1985). Thus, to combat the deleterious effect of oxidant stress, enzymatic and non – enzymatic antioxidants are immediately employed by the body system to quench the effect of the free radicals produced. The superoxide dismutase – catalase (SOD – CAT) system provides the first defense against oxygen toxicity. Results from this study indicates a significant reduction in the activities of SOD, CAT and in levels of reduced glutathione (GSH) in the liver of the cyanide control (group 2) as compared with all other groups (including normal control and treatment groups). This observation could be as a result of increase utilization of these endogenous antioxidants to curb the excess production of free radicals due to cyanide toxicity on rat's hepatocytes. The administration of aqueous extract of *Xylopia aethiopica* seeds, peels, whole pod and vitamin E to cyanide poisoned rats lead to increase activities of SOD, CAT and GSH concentration in rat's liver. Results also show that liver SOD activity is relatively higher than the corresponding CAT activity for each group. This could be as a consequent of the inhibitory effect of excess production of SOD on CAT activity (Kono and Fridovich, 1982).

Manlondialdhyde (MDA) level – a biomarker for lipid peroxidation was relatively higher in cyanide control rats (group 2) as compared to all other groups. The high level of MDA could be the resultant effect of increased utilization of antioxidant enzymes in cyanide poisoned rats (group 2). The reduced level of MDA observed in treated cyanide groups (groups 3, 4, 5, & 6) probably indicates the protective effect of *Xylopia aethiopica* and vitamin E respectively. The above results agrees with the findings of Okpashi *et al.* (2014) who reported the *Xylopia aethiopica* extract significantly decrease lipid peroxidation and increase activities of endogenous antioxidants such as SOD, CAT and GSH concentration.

Overall, the different aqueous extract made from *Xylopia aethiopica* exhibited significant protective effect in ameliorating cyanide poisoning in rats. This property compared favourably with vitamin E, a known potent antioxidant. Better performance was observed with the aqueous extract of *Xylopia aethiopica* whole pod as compared with aqueous extract of *Xylopia aethiopica* seeds and peels. Thus, *Xylopia aethiopica* may be considered as a potential antidote against cyanide – mediated toxicity. Hence, studies are required to elucidate the molecular basis of the ameliorative properties of *Xylopia aethiopica* in cyanide poisoning.

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