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Effects of Aqueous Extract of *Annona muricata* Leaves on Cyanide – induced Toxicity in New Zealand Rabbits

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Abstract
Aqueous extract from Annona muricata leaves was investigated for its ameliorative effect on cyanide- induced toxicity in New Zealand Rabbits of weights 1200g-1800g. Four groups of rabbits (four per group) were used in a 28 day study. Group A served as the experimental control, groups B and C were given 400 ppm of cyanide salt (KCN) incorporated in the feed; this was done by dissolving the salt in water and then mixed with the feed in a ratio of 10:1 in addition to daily oral administration of Annona muricata extract (150mg/kg body weight and 300mg/kg body weight respectively). Group D was given only 400 ppm of cyanide salt (KCN) supplemented feed. Oxidative stress was evaluated by liver and kidney superoxide dismutase, catalase, and malondialdehyde. Administration of the aqueous extract of Annona muricata leaves for 21days significantly (P<0.05) ameliorated the changes in the biochemical parameters induced by cyanide when compared to the cyanide treated group. There was significant increase in the activity of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST); while alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities in the liver were not altered significantly when compared to the control but significantly different(P>0.05) from the untreated group. However, the activities of catalase (CAT) and superoxide dismutase were significantly reduced (P<0.05) in both the liver and the kidney. Increasing the dose of the extract (from 150mg/kg body weight to 300mg/kg body weight) resulted in significant decrease (P>0.05) in catalase activity but increase in MDA concentration. The concentrations of total protein as well as MDA of the liver were improved to levels that were not significantly different from the control. This suggested that aqueous extract of Annona muricata leaves might have a protective action against cyanide-induced hepatotoxicity arising from its bioactive constituents
Keywords: *Annona muricata*, cyanide, antioxidants, liver function enzymes, hepatotoxicity

Introduction
Medicinal plants are plants that generally contain constituents that have been found useful for the treatment and management of both animal and human diseases. Cyanide is one of the lethal chemical agents that can be spread as a result of accidental exposure, and by chemical warfare. Chronic cyanide intoxication has been linked to the pathogenesis of goiter ([1]), tropical ataxic neuropathy ([2]) and spastic paraparesis ([3]). The routes of human exposure of cyanide may be either dietary or environment. The mechanism of cyanide toxicity involves the binding of ferric ion in mitochondrial cytochrome oxidase preventing electron transport in the cytochrome system and bringing oxidative phosphorylation and ATP production to a halt. The toxicity of cyanide in aerobic organisms arises from inhibition of cytochrome oxidase, the terminal electron acceptor in the respiratory chain, thus cyanide block electron transport, mitochondrial oxygen utilization and cellular respiration ([4, 5]).
Once ingested, cyanide is detoxified by enzymatic conversion to a less toxic renally excreted metabolite thiocyanate. A small amount of cyanide is also detoxified by vitamin B12 precursor, hydroxycobalamin. This chelating agent binds cyanide to form non-toxic cyanocobalamin ([6]).
The soursop is an upright, low-branching tree reaching 8-10 meters ([7]). The tree has green, glossy evergreen leaves ([8]). The flowers appear anywhere on the trunk or any branch ([9]). It is usually grown from seeds which can be stored for several months before planting. Germination of seeds usually takes 3 weeks, but under suboptimal conditions can be delayed for up to 2-3 months. Alternatively, propagation of genotypes and for the elimination of viral and disease infection, with the exception of a few cultivars, clonal propagation of the Annona species by rootstocks or cultivars of known agronomic potential could eliminate tree to tree variability in growth and productivity. However, the seedling rootstocks are highly variable in vigor and disease resistance and consequently scion growth and productivity are also variable. They are considered as minor tropical fruits due to strict environmental requirements for tree planting and the short post-harvest life of their fruits ([10]).
Here, we investigated the ameliorating effect of aqueous extract of *Annona muricata* leaves on cyanide-induced toxicity in New Zealand rabbits.

Materials and Methods
Animals and feeding
Four groups of New Zealand White rabbits (four per group) aged about two months were used for this experiment. They were housed in clean disinfected metal hutches and initially acclimatized on growers mash

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(Bendel Feed & Flour Mills Ltd., Ewu, Nigeria) for one week. Subsequently, members of each group were housed singly. One group received growers mash only, while another group was fed growers mash and 400ppm inorganic cyanide (Potassium cyanide). Members of the third group were fed with KCN alongside with daily oral dose of aqueous extract of *Annona muricata l*eaves (150mg) and member of the fourth group were also fed with KCN and 300mg daily oral dose of aqueous extract of *Annona muricata* leaves.

The jelly-like syrup was administered orally once daily to the rabbits through sterile disposable syringes. Prior to presentation, the feeds were mixed with water in the ratio of 10:1 (w/v) to attain an acceptable texture. Each rabbit was fed at the rate of 55g feed/day. Clean drinking water was liberally provided while stale feed remnants were daily weighed and discarded prior to fresh feed presentations. All animals were weighed weekly. After three weeks, feeding was terminated.

Plant Sample Preparation

The plant was identified by an expert in the Department of Botany, University of Benin, Benin City.

The leaves of this plant were air-dried in the laboratory at the Department of Biochemistry, University of Benin, Benin City. The leaves were later pulverized to powdery form in Pharmacognosis laboratory at the Faculty of Pharmacy, University of Benin.

300g of the powdered leaves of *Annona muricata* was soaked in 2liters of water for 12hours with periodic stirring of the mixture. After 12hours, the mixture was filtered with fine cheese cloth, the residue was discarded and the filtrate was used to soak another 300g of powdered leaves of same plant above, allowed to stand for another 12hours with continuous stirring, thereafter, the mixture was again filtered. The residue was discarded and the filtrate was used again to soak another 300g of the powdered leaves of *Annona muricata* and allowed to stand for 12hours with continuous stirring, then was filtered and the filtrate was concentrated with the aid of the rotary evaporator. The concentrates were then further dried in the desiccator with frequent changing of the self-indicating silica gel. The concentrates were then weighed and used as experiment sample.

Body Weight Measurement

The weights of the experimental animals in each group were determined at the beginning and the end of each week and shortly before the animals were sacrificed. The average weight gained and lost were estimated from the values obtained per group.

Determination of Feed Intake

The amount of feed consumed per rabbit per day was estimated by determining the difference between weight of feed given and feed leftover.

Collection of Blood

Blood samples were drawn from the rabbits' heart into citrate vials using sterile disposable 21-guage syringe during sacrifice. The plasma obtained after centrifuging the blood at 3000rpm for 10mins was used to assay for the activities of the liver enzymes. The liver, heart and kidneys were harvested for histopathological examination. Dissected pieces of the various organs were rinsed and homogenized in cold normal saline solution and used immediately to assay for SOD catalase and MDA respectively.

Biochemical Assays

Activities of SOD, Catalase, and Concentration of MDA

Malondialdehyde (MDA) level was estimated by the method of Buege and Aust [11] . Catalase activity (CAT) was estimated by the method of Cohen *et al* [12]. Superoxide dismutase (SOD) was also assayed by the method of Misra and Fridovich,[13] .

Liver function tests

Aspartate amino transferase (AST) and Alanine amino transferase activities were estimated by the method of Reitman and Frankel, [14]. Alkaline phosphatase and Lactase dehydrogenase activities were estimated using appropriate reagent kits (Randox, U.K).

Statistical Analysis

The results were expressed as mean ±SEM. Analysis of variance was used to test for difference between all the groups. Duncan's multiple range tests was used to test for significant differences between the means at $P<0.05$.

Results

Table 1: Effect of aqueous extract of *Annona muricata* leaves treatments on weights of rabbits exposed to cyanide -induced toxicity

	Weight (g)			
GROUPS	Week 1	Week 4	Wt. Change	%Wt. Change
A (Control)	1200±20.15	1410±15.92	210±5.94 [a]	17.50±3.01[a]
B (150mg/kg body wt. +KCN)	1400±16.02	1512±12.82	112±6.05[b]	8.00112±1.05[b]
C (300mg/kg body wt.+KCN)	1300±25.19	1450±9.05	150±7.95[a]	11.54±2.61[a]
D (KCN Only)	1800±35.72	1641±26.19	159±5.93[c]	-8.83±2.83[c]

Data are expressed as Mean ± SEM; n=4
Within each column, means superscripts with different letters are significantly different at P<0.05 compared to control group.

Table 2: Liver function tests for rabbits with cyanide -induced toxicity treated with aqueous extract of *Annona muricata* leaves.

GROUPS	ALT (U/l)	AST (U/l)	ALP (U/l)	LDH (U/l)
A (Control)	36.20±1.82[a]	21.51±0.36[a]	12.20±1.20[a]	90.20±2.20[a]
B (150mg/kg body Wt. +KCN)	52.00±2.14[b]	38.33±0.56[b]	12.75±1.75[a]	91.75±4.11[a]
C (300mg/kg body Wt.+KCN)	29.00±0.37[a]	21.33±0.12[a]	12.00±1.53[a]	91.66±0.66[a]
D (KCN Only)	60.00±3.57[b]	45.50±2.33[b]	8.33±0.33[a]	93.33±2.90[a]

Data are expressed as mean ± SEM; n=4
Within each column, means superscripts with different letters are significantly different at P<0.05 compared to control group.

The activities of ALT, and AST of group D are significantly increased compared to the other groups (i.e. A, B and C). ALT and AST activities of group B are significantly increased compared to those of groups A and C, but reduced (P<0.05) compared to group D. There are no significant differences in the activities of ALP and LDH across the groups.
Treated groups with the extract showed significant reduction in the alteration induced by KCN in a dose dependent manner. The activity of ALP was normalized by 150mg/kg body weight and 300mg/kg body weight of the plant extract.

Table 3a: Activities of SOD, catalase, and concentration of MDA in the liver of rabbits with cyanide-induced toxicity treated with aqueous extract of *Annona muricata* leaves.

Groups	SOD×10^{-2} (Unit/g wet tissue)	Catalase (Activity/min)	MDA × 10^{-6} (Unit /g-tissue)
A (Control)	4.13±0.56[a]	177.00±9.46[a]	4.85±0.62[a]
B (150mg/kg body Wt.+KCN)	3.08±0.73[b]	180.00±11.47[a]	3.18±0.74[b]
C (300mg/kg body Wt.+KCN)	5.93±0.36[a]	82.96±7.73[b]	4.34±0.55[a]
D (KCN Only)	3.00±0.20[b]	38.27±2.55[c]	6.33±0.91[c]

Data are expressed as Mean±SEM; n=4.
Within each column, means superscripts with different letters are significantly different at $P<0.05$ compared to the control.

The activities of SOD and catalase of group D are significantly reduced ($P<0.05$) compared to those of control (group A).The concentration of MDA of group D is significantly increased compared to those of the other groups.($P<0.05$)

Table 3b: Activities of SOD, catalase, and concentration of MDA in the kidney of rabbits with cyanide-induced toxicity treated with aqueous extract of *Annona muricata* leaves.

Groups	SOD×10^{-2} (Unit/g wet tissue)	Catalase (Activity/min)	MDA × 10^{-6} (Unit /g-tissue)
A (Control)	4.76±0.28[a]	100.50±6.23[a]	2.90±0.60[a]
B (150mg/kg body Wt.+KCN)	3.99±0.54[a]	76.14±3.80[a]	3.57±0.02[a]
C (300mg/kg body Wt.+KCN)	6.35.±0.58[b]	68.02±2.63[a]	4.15±0.76[b]
D (KCN Only)	2.31±0.40[c]	47.40±2.33[b]	6.13±1.77[c]

Data are expressed as Mean±SEM; n=4.
Within each column, means superscripts with different letters are significantly different at P<0.05 compared to the control.

The activities of SOD and catalase of group D are significantly reduced (P<0.05) compared to those of control (group A).The concentration of MDA of group D is significantly increased compared to those of the other groups.

Discussion

Aqueous extract from *Annona muricata leaves* was investigated for its ameliorative effect on cyanide- induced toxicity in New Zealand Rabbits.The results from this study have shown that the plant *Annona muricata* has moderate anti-hepatotoxic ability.

Generation of free radicals in the body beyond antioxidants capacity leads to oxidative stress which has been implicated in diseases such as cancer, cardiovascular disease, aging and several other chronic diseases because of their ability to induce oxidative damage to biomolecules such as lipid, DNA and protein ([15]).

These free radicals can also cause the rupture of hepatocytes, hence the leakage of intracellular components and enzymes into the blood. Against these damages, the organisms have important antioxidant defense such as superoxide dismutase (SOD) and catalase. SOD plays a role in detoxifying superoxide anions which otherwise damage cell membranes and molecules ([16]).

Cyanide-induced toxicity has to do with the inhibition of cytochrome c oxidase, a key enzyme in the electron transport chain. The mechanism of toxicity involves the binding of ferric ion in the mitochondrial cytochrome c oxidase, preventing electron transport in the cytochrome system and bringing oxidative phosphorylation to a halt ([16]).

Cyanide also inhibits superoxide dismutase, catalase, thus triggering oxidative stress by generating reactive oxygen species (ROS). ROS scavengers known as antioxidants are widely distributed in medicinal plants and they include plants-derived bioactive phytochemicals such as flavonoids, tannins, alkaloids, polyphenols etc. They are major contributor to anti-hepatotoxic capability of most medicinal plants ([17]).

SOD activity leads to the production of hydrogen peroxide which reacts with iron to generate hydroxyl radicals by fenton reaction which is thought to be the most toxic oxygen molecules in vivo ([18]).

MDA is known to be a reliable marker of lipoperoxidation and oxidative stress. High level of MDA means excessive generation of reactive oxygen species and a decrease in the effectiveness of antioxidant defense such as catalase ([19]). Catalase is among the enzymes that help the cell to handle hydrogen peroxide though should be noted that the enzyme is not the major route of hydrogen peroxide catabolism ([20]).

In the current study, the activity of SOD in the liver tissue of the group with KCN only was observed to be 27% decrease compared to control (P<0.05), while SOD activity in the kidney tissue of the group with KCN alone showed more than 50% reduction compared to control (P<0.05).The decrease in SOD activity which indicates oxidative stress is an index to identify the free radicals-induced injuries. SOD activity in the liver and kidney tissues of group with KCN treated with 150mg/kg body weight of *Annona muricata* leaves extract was enhanced

when compared to control. The group given 300mg/kg body weight of same extract, alongside KCN had significant increase in SOD activity.

There was significant decrease ($P<0.05$) in catalase activity in the liver and kidney of KCN-induced rabbits compared to control, and this indicates damage to the liver and kidney of the untreated rabbits. The extract treated rabbits showed high level of catalase activity indicating the antioxidative properties of the plant extract.

MDA concentration in the tissues of liver and kidney of rabbits with KCN only, showed significant increase compared to control ($P<0.05$).

While the level of MDA in tissues of liver and kidney of the groups treated with 150mg/kg body weight and 300mg/kg body weight of extract (*Annona muricata* leaves) plus KCN were significantly improved when compared to control. These observations may be as a result of the anti-oxidative properties of *Annona muricata* leaves. Previous study ([21]) indicated that New Zealand Rabbits exposed to cyanide revealed lower activities of superoxide dismutase (SOD) and catalase, and an increase in MDA concentration in the lens, which aligns with the present study.

Phytochemical screening (Qualitative) of *Annona murica*ta leaves showed the presence of flavonoid, Saponins, tannins and alkaloids ([22]). All these active ingredients seem to contribute to the ameliorating properties of the plant extract.

Conclusion

This preliminary study was to investigate the effect of aqueous extract of *Annona muricata* on cyanide-induced toxicity in rabbits .The overall results suggest that the plant extract may have ameliorative effect on cyanide-induced toxicity in rabbits. Notably, 300mg/kg body weight of the plant extract showed more ameliorating effect on cyanide-induced toxicity than 150mg/kg body weight of the plant extract.

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