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AQUEOUS LEAF EXTRACT OF *ANNONA MURICATA* AMELIORATES CYANIDE-INDUCED TOXICITY IN NEW ZEALAND RABBITS

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ABSTRACT

In the present study, aqueous leaf extract of *Annona muricata* was evaluated for its healing effect on cyanide- induced toxicity in New Zealand Rabbits. Rabbits weighting between 1.2 - 1.8kg were used in a 28-day study. Group A served as the positive control, while 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* leaf extract (150mg/kg body weight and 300mg/kg body weight, respectively). Group D (negative control) was given only 400 ppm of cyanide salt (KCN) supplemented feed. Lipid profile, total thiol concentration in the liver and kidney homogenates and thiocyanate levels in the urine were estimated. The concentration of thiocyanate in the positive and negative control rabbits were significantly increased ($P < 0.05$) compared to the treatment groups. In the liver and kidney, there was no significant difference ($P > 0.05$) in the concentration of total thiol in the control and treatment groups. However, the concentration was reduced in the negative control (KCN only). These results suggest that aqueous leaf extract of *Annona muricata* may confer protection against cyanide-induced toxicity.

Keywords: *Annona muricata*, Cyanide, Lipid profile, Total thiol, Thiocyanate, Toxicity

INTRODUCTION

Medicinal plants have been used for centuries before the advent of orthodox medicine (Komolafe and Oyelade, 2015). Medicinal plants are important part of natural wealth, serve as important therapeutic agents as well as valuable raw materials for manufacturing numerous traditional and modern medicines (Talalay, 2001). Plants are the richest resources of drugs for traditional system of medicine, modern medicine, nutraceuticals, food supplement, folk medicine, pharmaceutical intermediates and chemical entities for synthetic drug (Hammer *et al.*, 1999). The world Health Organization reported that more than 80% of the world's population relies on traditional medicine for primary healthcare needs (Ammara *et al.*, 2008).

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 (Cliff *et al.*, 1986), tropical ataxic neuropathy (Osuntokun, 1981) and spastic paraparesis (Howlett *et al.*, 1990). 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 mitochondria 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 (Jones *et al.*, 1984; Yen *et al.*, 1995).

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 (Borron and Baud, 1996).

Annona muricata (soursop) is an upright, low-branching tree reaching 8-10 meters (Popenoe *et al.*, 1920). The tree has green, glossy evergreen leaves (Paul *et al.*, 1998). The flowers appear anywhere on the trunk or any branch (Salazar, 1965). It is usually grown from seeds which can be stored for several months before planting. Germination of seeds usually takes three 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 (Encina, 2005). *Annona muricata* is one of the medicinal plants in

Nigeria used in the management of various diseases.

Here, we investigated the effect of aqueous leaf extract of *Annona muricata* on CN⁻ induced toxicity in New Zealand rabbits. The liver, kidney, blood and urine sample were used for this work.

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 (Bendel Feed and Flour Mills Ltd., Ewu, Nigeria) for one week. 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 leaf extract of *Annona muricata* (150mg) and member of the fourth group were also fed with KCN and 300mg daily oral dose of aqueous leaf extract of *A. muricata*.

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.

Body Weight Measurement

The weights of the experimental animals in each group were determined at the beginning and the end of the experiment and shortly before the animals were sacrificed. The average weight gain and loss were estimated from the values obtained from each 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.

Plant Sample Collection

The plant was obtained from a forest area in Benin (Ekehuan) and identified by Dr. Akinnibosun Henry. Voucher specimen was kept in the herbarium with voucher number UBHA356 at the Department of Plant Biology and Biotechnology, 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.

Plant Sample Preparation

Powdered leaves (300g) of *Annona muricata* was soaked in two litres of distilled water for 12h with periodic stirring. After 12h, 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 12h 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, the residue obtained was disposed and the filtrate was concentrated with the aid of a rotary evaporator and freeze-dried with a lyophilizer.

Collection of Blood

Blood samples were drawn from the rabbits' heart into citrate vials using sterile disposable 21-gauge syringe during sacrifice. Dissected pieces of liver and kidney were rinsed, homogenized in cold normal saline solution and used immediately for the assay of total thiol. Urine was used for the evaluation of thiocyanate. Collected blood samples were used for the assay of lipid profile.

Biochemical Assays

Lipid profile analysis

Cholesterol concentration was estimated by the method of Tinder, (1969). Triacylglyceride (TAG) concentration was estimated by the method of Tietz (1986), High Density Lipoprotein-Cholesterol (HDL-

C) by the method of Lopes-Virella, *et al.*, (1997) and Low Density Lipoprotein-Cholesterol (LDL-C) by the method of Friedewald *et al.*, (1972)

Thiocyanate test

Thiocyanate level was assayed by the method of Bowler (1994).

Total thiol analysis

Total thiol concentration was estimated by the method of Thannhauser *et al.*, (1987).

STATISTICAL ANALYSIS

The results were expressed as mean \pm SEM. Data were analyzed using SPSS (20:0) for difference between all the groups. Duncan's multiple range test was used to test for significant differences between the means at $P < 0.05$.

RESULTS

Lipid profile of rabbits with cyanide-induced toxicity

The concentrations of cholesterol and triacylglycerol of the negative control are significantly reduced ($p < 0.05$) compared to the treatment groups.

Table 1: Lipid profile of rabbits with cyanide-induced toxicity treated with aqueous leaf extract of *Annona muricata*

Groups	Cholesterol (mg/dl)	TAG (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	LDL-C (mg/dl)
A (Control)	130.20 \pm 10.02	97.20 \pm 12.18	28.20 \pm 4.67	19.44 \pm 1.82	85.00 \pm 8.24
B (150 mg/kg b.wt.+ KCN)	151.00 \pm 7.60 ^a	84.50 \pm 8.44 ^a	37.25 \pm 2.01 ^a	16.90 \pm 1.08 ^a	109.25 \pm 15.20 ^a
C (300 mg/kg b.wt.+ KCN)	67.00 \pm 5.93 ^{ab}	101.00 \pm 10.81 ^a	27.33 \pm 2.18 ^b	20.20 \pm 1.30 ^a	20.00 \pm 3.46 ^b
D (KCN only)	74.33 \pm 6.85 ^b	70.00 \pm 7.55 ^b	39.00 \pm 3.19 ^a	14.00 \pm 1.60 ^b	21.33 \pm 2.84 ^b

Data are expressed as mean \pm SEM (n = 5).

^b $p < 0.05$ compared to control, B and C groups.

Levels of thiocyanate in the urine of rabbits

The concentration of thiocyanate in the positive and negative control rabbits were significantly increased ($p < 0.05$) compared to the treatment groups (Table 2).

Table 2: Levels of thiocyanate in the urine of rabbits with cyanide-induced toxicity treated with aqueous leaf extract of *Annona muricata*

Group	SCN (mmol/l)
A (Control)	211.04±6.58
B (150mg/kg body wt.+KCN)	192.00±8.09 ^a
C (300mg/kg body wt.+KCN)	171.08±9.30 ^a
D (KCN Only)	265.25±11.70 ^b

Data are expressed as mean ± SEM (n = 5).

^b $p < 0.05$ compared to control, B and C groups.

Concentrations of total thiol in the kidney and liver of rabbits

There was no significant difference ($p > 0.05$) in the concentration of total thiol in the control and treatment groups. However, the concentration was reduced in the negative control (KCN only), as shown in Table 3.

Table 3: Concentrations of total thiol in the kidney and liver of rabbits with cyanide-induced toxicity treated with aqueous leaf extract of *Annona muricata*

Groups	Liver total thiols (mol/dl)	Kidney total thiols (mol/dl)
A (Control)	0.57±0.12 ^a	0.37±0.05
B (150mg/kg body wt.+KCN)	0.50±0.07 ^a	0.28±0.03 ^a
C (300mg/kg body wt.+KCN)	0.54±0.03 ^a	0.33±0.03 ^a
D (KCN Only)	0.27±0.09 ^b	0.15±0.05 ^b

Data are expressed as mean ± SEM (n = 5).

^b $p < 0.05$ compared to control, B and C groups.

DISCUSSION

Medicinal values of plants have assumed important dimensions in the past few decades (Anjali and Sheetal, 2013). Plants are important sources of potential compounds for the development of new

therapeutic agents (Sahoo *et al.*, 2009). These non-nutrients plant chemical compounds or phyto-constituents are responsible for protecting the plant against microbial infections or infestations by pests (Doughari, 2012). The diverse groups of

secondary metabolites produced by plants have antioxidant potential. Antioxidants block the action of free radicals which have been implicated in the pathogenesis of many diseases and in the ageing process (Aruoma, 2003).

Hepatotoxicity and pathological injury have been found to occur in liver. Anti-hepatotoxic substances are the remedies to reverse this effect. The results from this study show that the plant *Annona muricata* has moderate anti-hepatotoxic and anti-hyperlipidemic ability.

Generation of free radicals in the body beyond antioxidant 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 (Sosa *et al.*, 2013).

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. Cyanide also inhibits superoxide dismutase, catalase, thus triggering oxidative stress by the generation of reactive oxygen species. 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 (Nwanna and Oboh, 2007).

In this study, aqueous leaf extract of *Annona muricata* was evaluated for its healing effect on cyanide-induced toxicity in New Zealand Rabbits

In the liver, the total thiol level of group B rabbits was not significantly different ($p>0.05$) from control, although there was slight reduction in the concentration. Those of group C had a value relatively closer to control (group A) indicating the healing effect of the plant leaf extract on hepatotoxicity. The group without the plant extract (D) had a concentration significantly different ($P<0.05$) from control group and those of groups B and C. This probably indicates oxidative stress due to decrease in the concentration of total thiol. In the kidney, similar result was also observed. The level of total thiol in the control group was not significantly different ($P>0.05$) from those of groups B and C but significantly different ($P<0.05$) from group D. This may suggest that the plant extract has protective effect against KCN renal-toxicity.

The fall in total thiol concentration in group D is indicative for the rise in the concentration of thiocyanate in table 2. Cyanide readily displaces hydrogen ion from sulphhydryl group to form thiocyanate causing a fall in the level of -SH in the blood. This can be further elucidated by the action of the enzyme Rhodanese (thiosulfate cyanide sulfurtransferase), ubiquitous enzyme presents in man (Mueller *et al.*, 2001). Rhodanese catalyzes the cyanide-dependent cleavage of thiosulfate to form thiocyanate and sulfite. In spite of extensive studies, the true biological role of this enzyme is still an enigma. It is believed that rhodanese is

involved in cyanide detoxification (Nandi *et al.*, 2004).

The concentration of thiocyanate in group D was significantly different ($P < 0.05$) from control and those administered 150mg/kg body weight and 300mg/kg body weight *Annona muricata* leaf extract. Thiocyanate concentration in groups B and C was not significantly different from control group. There was an increase in the concentration of thiocyanate in the urine of rabbits in group D when compared to control group, groups B and C. This agrees with the report of Okolie and Asonye, (2004). They noted that cyanide-fed rabbits had significant higher thiocyanate concentration when compared to rabbits in the control group ($P < 0.5$). Also, work done by Ralph, (1940), indicated that the administration of cyanide resulted in a greater increase in thiocyanate concentration in serum of rabbits than in dogs. The recovery of cyanide as urinary thiocyanate was rapid in rabbits when compared to dogs (Ralph *et al.*, 1940).

Administration of 150mg/kg body weight resulted in an increase in serum total cholesterol, high density lipoproteins cholesterol (HDL-C) and low-density lipoproteins cholesterol (LDL-C) but decrease in Triacylglycerol (TAG). Invariably, treatment with 300mg/kg body weight of *Annona muricata* leaf extract resulted in a decrease in serum total cholesterol, HDL-C and LDL-C but an increase in TAG. Previous work done (Adeyemi *et al.*, 2008) on rat showed significant decrease ($P < 0.5$) in total cholesterol, HDL-C and LDL-C respectively. *Annona muricata* leaf extract had anti-

hyperlipidemic effect and may reduce lipid peroxidation. (Adeyemi *et al.*, 2008). Phytochemical screening (Qualitative) of *Annona muricata* leaves showed the presence of flavonoid, saponins, tannins and alkaloids (Usunobun and Okolie, 2015). All these active ingredients may contribute to the ameliorating properties of the plant extract. Further study on this extract may involve subjecting it to gas chromatography-mass spectrometry (GC-MS) to identify biologically active compounds that could be responsible for the observed effect.

CONCLUSION

The result of this preliminary study suggests the plant leaf extract may have anti-hyperlipidemic effect and protective potential on the liver and kidney in cyanide induced toxicity in rabbits. Notably, 300mg/kg body weight of the plant leaf extract showed more ameliorative effect than 150mg/kg body weight.

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