



EFFECT OF *ABELMOSCHUS ESCULENTUS* (OKRA) ON SOME INTESTINAL ATPASES AND PLASMA LIPID PROFILES OF RABBITS EXPOSED TO POTASSIUM CYANIDE

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ABSTRACT

This study was carried out to investigate the effect of *Abelmoschus esculenta*, a rich source of viscous fibre, on plasma lipids and activities of some intestinal ATPases in rabbits exposed to potassium cyanide. Two groups of New Zealand White rabbits (4 per group) received mash + 400ppm cyanide, or mash + 400ppm cyanide + 40% *A. esculentus* for 4 weeks. Members of a third group (control) were fed pure mash. At the end of 4 weeks, the rabbits were sacrificed by cardiac puncture under chloroform anaesthesia, and sections of the colon, duodenum and ileum were dissected out, rinsed in cold physiological saline, and assayed for activities of $\text{Na}^+\text{-K}^+\text{-ATPase}$ and $\text{Ca}^{2+}\text{-ATPase}$. In addition, plasma total triglycerides, total cholesterol and HDL-cholesterol were determined. The results showed that cyanide significantly inhibited colon, ileum and duodenum activities of $\text{Na}^+\text{-K}^+\text{-ATPase}$ and $\text{Ca}^{2+}\text{-ATPase}$, relative to controls ($p < 0.05$). However, the cyanide-induced decreases in $\text{Na}^+\text{-K}^+\text{-ATPase}$ and $\text{Ca}^{2+}\text{-ATPase}$ were reversed in the group that received *A. esculentus* in addition to cyanide. Moreover, the cyanide treatment brought about significant elevation of plasma triglycerides levels ($p < 0.05$), but plasma total cholesterol and HDL cholesterol were not significantly affected. The hypertriglyceridemia due to cyanide was significantly reduced in the group that received cyanide and *A. esculentus* ($p < 0.05$). These results strongly suggest that *A. esculentus* has potential for mitigating some of the metabolic consequences of cyanide exposure, such as inhibition of ATPases and hyperlipidemia. This is considered crucial, especially for population routinely exposed to dietary cyanide through cassava and legumes.

KEYWORDS: *Abelmoschus esculenta*, Cyanide, Intestinal ATPases, Plasma triglycerides

INTRODUCTION

Cyanide is a rapidly acting respiratory poison in all aerobic organisms. It is a potent inhibitor of cytochrome oxidase, the terminal electron acceptor in the respiratory chain, which inhibition blocks oxygen utilisation and ATP production in the mitochondria^{1,2}. Humans are exposed to cyanide through dietary and environmental sources³. In Nigeria and many topical countries, cassava (*Manihot esculenta* Crantz) and legumes constitute very important staple food sources. Unfortunately these essential sources of calories contain cyanogenic glycosides^{4, 5}. Studies have shown that processing does not entirely eliminate cyanide from cassava and legumes^{5, 6}. Thus populations for whom cassava is a staple source of calories are chronically exposed to cyanide. Acute cyanide poisoning results in death due to respiratory failure⁷, while chronic exposure to cyanide has been linked to pathogenesis of goitre⁸, epidemic spastic paraparesis^{9, 10} and tropical ataxic neuropathy¹¹. Studies in our laboratory and investigations elsewhere have revealed that cyanide inhibited the activities of some rabbit tissue ATPases^{12, 13}. These membrane-bound enzymes regulate trans-membrane active transport of ions and solutes, utilising energy derived from ATP hydrolysis. Trans-membrane gradients of sodium and potassium ions are maintained by $\text{Na}^+\text{-K}^+\text{-ATPase}$, which simultaneously drives secondary transport of glucose and amino acids¹⁴. $\text{Ca}^{2+}\text{-ATPase}$ maintains low concentrations of intracellular calcium ions. Decreases in $\text{Na}^+\text{-K}^+\text{-ATPase}$ and $\text{Ca}^{2+}\text{-ATPase}$ are associated with impaired cellular glucose uptake^{15, 16}. $\text{Na}^+\text{-K}^+\text{-ATPase}$ powers the symport transport of sodium ions and glucose¹⁷. Thus cyanide-impaired trans-membrane ion transport has negative consequences for solute transport. Since processing cannot completely remove cyanide from cyanophoric foods, studies aimed at elucidating ways of

mitigating the toxic effects of dietary cyanide are important for minimising adverse health effects of the poison.

Dietary fibre consists of plant components resistant to hydrolysis by the alimentary canal of humans¹⁸. It is a diverse group of polymers which include cellulose, pectin, lignin and inulin. The importance of dietary fibre in human health is underscored by results of epidemiological investigations which link low fibre intake to prevalence of coronary heart disease, colorectal cancer and atherosclerosis^{19 - 24}. It has been reported that high fibre diets increase the activities of intestinal mucosal ATPases²⁵. In addition, the influence of dietary fibre on plasma lipids has been investigated extensively in many animal models, with results showing that it exerts a lipid-lowering effect^{26, 27}.

Abelmoschus esculentus (also known as okra) is a tropical flowering plant whose greenish, mucilaginous fruits are used as vegetable in many cultures. It originated from Ethiopia, but enjoys so much popularity that it is now cultivated in commercial scale in many countries of Asia, South America and Africa²⁸. Okra is a good source of viscous fibre, as well as vitamins and minerals^{29, 30}. It has been reported that *Abelmoschus esculentus* possesses hypolipidemic properties^{31, 32}. However, not much is known about its effect on intestinal ATPases, especially in the presence of cyanide intoxication.

The aim of this study was to investigate the effect of *Abelmoschus esculentus* administration on levels of some intestinal ATPases and plasma lipoproteins in rabbits exposed to dietary potassium cyanide.

MATERIALS AND METHODS

Abelmoschus esculentus

Freshly-harvested seeds of *Abelmoschus esculentus* (okra) were purchased from a local market in Benin City, Nigeria. They were washed in tap water, sliced into thin, mucilaginous sections which were then spread out to sun-dry in open trays for 7 days. The dry material was ground to a powder and kept in a dry glass jar prior to use.

Experimental animals and treatment

New Zealand White rabbits of both sexes were obtained from a breeder in Aduwawa rabbit farm, Benin City. The animals were housed in clean, disinfected rabbit hutches placed in a well ventilated room and acclimatised for 3 weeks to feed (finisher mash, product of Bendel Feed & Flour Mills Ltd, Ewu, Edo State, Nigeria) and laboratory environment before commencement of the study. Subsequently, they were randomly assigned to 3 groups (4 rabbits per group). Members of each group were housed in the same hutch. One group was given finisher mash containing 40% *Abelmoschus esculentus* flour and 400ppm inorganic cyanide (potassium cyanide, KCN), while members of the second group received finisher mash containing 400ppm inorganic cyanide only. Rabbits in the third group were fed pure finisher mash only, and served as control. To prepare 400ppm cyanide-supplemented feed, 2g of KCN was dissolved in 20 ml of tap water and added with thorough mixing, to 1000g of feed in a plastic bowl. This was prepared fresh every day prior to presentation. Clean drinking water and feed were provided *ad libitum*, while stale feed remnants were discarded daily. The study was conducted at room temperature (25°C) and 12-hr light/dark cycle. Animal treatment was in strict compliance with Guidelines and Specifications on Experimental Animal Care³³. The feeding experiment lasted 4 weeks, after which the rabbits were anaesthetized in chloroform and the thoracic and abdominal regions dissected open to expose the gastrointestinal tracts. Blood was obtained from each animal via cardiac puncture with a 5-ml hypodermic syringe, and quickly transferred into EDTA sample bottles. Following centrifugation at 5000g for 5 min, the plasma samples were deep-frozen at -4 °C, and analysed within a few hours. The colon, duodenum and ileum were carefully dissected out and flushed with ice-cold physiological saline. Sections of these tissues were used immediately in the preparation of tissue homogenates for intestinal ATPase assays.

Preparation of tissue homogenates

For each tissue (ileum, colon and duodenum), 1g was weighed and homogenised for 10 min with acid-washed sand in 30 ml of ice-cold 20 mM Tris-HCl buffer, pH 7.4, containing 0.25M sucrose and 0.5mM EDTA. The homogenates were centrifuged for 5 min at 5000g, and the clear supernatant used for assay of Na⁺-K⁺-ATPase and Ca²⁺-ATPase.

Enzyme assays

Na⁺-K⁺-ATPase activity was assayed according to the method of Heskett *et al.*,³⁴ by estimating the amount of inorganic phosphate liberated after incubation of the tissue extracts with ATP. The assay was carried out at room temperature (25 °C) in 0.05M Tris-HCl buffer, pH 7.4 containing 1.5mM NaCl, 1 mM EDTA, 0.2M disodium ATP and 0.25 mM sucrose. Enzyme reaction was initiated by extract addition, and stopped 30 min later by addition of 10% trichloroacetic acid, TCA. The resultant precipitate was removed by centrifugation, and inorganic phosphate, Pi was estimated in the supernatant fraction in a colorimetric reaction with ammonium molybdate³⁵. Corrections for endogenous inorganic phosphate were made with assays in the absence of ATP. Enzyme activity was expressed as mg Pi liberated/min/g fresh weight of tissue.

The assay of Ca²⁺-ATPase followed the same protocol as Na⁺-K⁺-ATPase except that the assay medium used was 160 mM Tris-HCl buffer, pH 7.4 containing 30mM MgCl₂, 5mM CaCl₂, 1mM EDTA and 0.3mM disodium ATP.

Estimation of plasma lipoproteins

Total plasma triglycerides were estimated after enzymatic hydrolysis with lipases according to the method of Tietz³⁶, as outlined in Randox assay kits. The assay is based on formation of an indicator quinoneimine from the reaction between 4-aminophenazone, 4-chlorophenol and H₂O₂ in the presence of peroxidase. Total cholesterol was assayed by the method of Trinder³⁷. This entails enzymatic hydrolysis of cholesteryl esters, and oxidation of the free cholesterol to liberate H₂O₂, which reacts with 4-aminophenazone in the presence of phenol and peroxidase to form quinoneimine. The assay was carried out with Randox kits according to manufacturer's instructions. HDL cholesterol assay was done with Randox kits according to the method of Lopez-Virella *et al.*,³⁸ after precipitation of LDL + VLDL with phosphotungstic acid in the presence of Mg ions.

STATISTICS

Data were expressed as Mean ± SEM of four replicates, and analysed for statistical differences using ANOVA. P values < 0.05 were taken as statistically significant.

RESULTS

Table 1 shows the activities of Na⁺-K⁺-ATPase in the ileum, colon and duodenum of rabbits in the three groups. KCN administration led to significant decreases in Na⁺-K⁺-ATPase levels in the three tissues when compared with corresponding values for control (p < 0.05). However, incorporation of 40% *A. esculentus* significantly reversed the cyanide-induced decreases in the tissue activities of the enzyme.

Table 1: Na⁺-K⁺-ATPase activities in the colon, ileum and duodenum of rabbits given cyanide, and rabbits given 400ppm cyanide plus 40% *A. esculentus* (mg Pi/min/g fresh wt)

Group	Ileum	Duodenum	Colon
Control	0.026 ± 0.007 ^a	0.064 ± 0.005 ^a	0.126 ± 0.024 ^a
KCN	0.014 ± 0.004 ^b	0.018 ± 0.005 ^b	0.053 ± 0.008 ^b
KCN + <i>A. esculentus</i>	0.025 ± 0.007 ^a	0.066 ± 0.002 ^a	0.076 ± 0.025 ^c

Data are expressed as Mean ± SEM; n=4. For each tissue, values that bear different superscripts amongst the groups differ significantly (p < 0.05).

Changes in activities of Ca^{2+} -ATPase in the colon, ileum and duodenum are depicted in Table 2. There were significant decreases in the colon, ileum and duodenum activities of the enzyme in the cyanide-treated group relative to the control group ($p < 0.05$). In contrast, Ca^{2+} -ATPase activities in the

colon, ileum and duodenum of the group that received *A. esculentus* and cyanide were significantly higher than corresponding activities for the group that received cyanide only.

Table 2: Ca^{2+} -ATPase activities in the colon, ileum and duodenum of rabbits given cyanide, and those given 400ppm cyanide plus 40% *A. esculentus* (mg Pi/min/g fresh wt)

Group	Ileum	Duodenum	Colon
Control	0.171 ± 0.070^a	0.060 ± 0.005^a	0.149 ± 0.024^a
KCN	0.053 ± 0.011^b	0.029 ± 0.016^b	0.080 ± 0.011^b
KCN + <i>A. esculentus</i>	0.062 ± 0.008^c	0.048 ± 0.016^c	0.099 ± 0.015^c

Data are expressed as Mean \pm SEM (n=4). For each tissue, values that have different superscripts amongst the groups differ significantly ($p < 0.05$).

The effect of *A. esculentus* on plasma lipoprotein profiles of rabbits treated with cyanide is shown in Figure 1. Cyanide brought about significant increase in plasma total triglycerides ($p < 0.05$), but the increases in total plasma cholesterol and HDL cholesterol were not statistically significant ($p > 0.05$). The

cyanide-induced increase in plasma total triglycerides was significantly reduced by *A. esculentus* treatment. However the changes in plasma total cholesterol and HDL cholesterol due to *A. esculentus* were not statistically significant ($p > 0.05$).

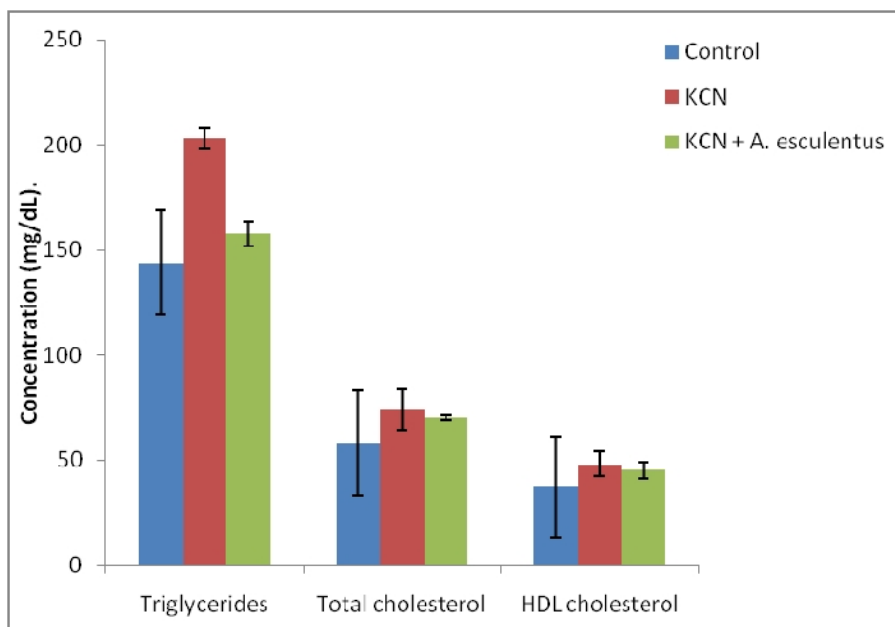


Figure 1: Effect of *Abelmoschus esculentus* supplementation on plasma lipids of rabbits exposed to cyanide

DISCUSSION

The significant reduction in Na^+ - K^+ -ATPase and Ca^{2+} -ATPase activities brought about by cyanide is in line with the metabolic consequences of cyanide exposure. Similar cyanide-induced decreases have been previously reported in kidney, liver and intestinal tissues of rabbits¹². Earlier studies revealed significant decreases in ATPase activities in rats given linamarin, a cyanogenic glucoside³⁹; and in Na^+ - K^+ -ATPase, Mg^{2+} -ATPase and Ca^{2+} -ATPase activities in gill, liver and muscle of carp (*Catla catla*)¹³. The inhibition of cytochrome oxidase and oxidative phosphorylation is the basis of the toxic effects of cyanide⁴⁰. The resultant shortfall in ATP levels has a negative impact on various cellular processes that require ATP, including ATPases, as well as protein synthesis and maintenance of membrane integrity⁴¹. In addition, cyanide specifically inhibits superoxide dismutase and catalase, two important antioxidant enzymes needed for neutralising the damaging effects of free

radicals⁴². The inhibition of these enzymes imposes oxidative stress and increases the rate of lipid peroxidation in cell membranes, leading to disruption of membrane integrity. Thus cyanide poisoning seriously impairs cellular functions in aerobic tissues. In this study, incorporation of *A. esculentus* in the cyanide feed led to reversal of the cyanide-induced decreases in Na^+ - K^+ -ATPase and Ca^{2+} -ATPase activities in the various tissues. This reversal suggests that the toxic effect of cyanide on the ion pumps might be mitigated by consumption of this popular viscous fibre. To the best of our knowledge, there are no reports in the literature on effect of *A. esculentus* on intestinal ATPase activities. However, it has been established that dikanut (*Irvingia gabonensis*), another type of viscous fibre, significantly increased the activities of erythrocyte membrane ATPases and normalised fasting blood glucose levels in diabetic patients¹⁶. This is in agreement with the *A. esculentus*-induced increases seen in the activities of Na^+ - K^+ -ATPase and Ca^{2+} -ATPase.

The plasma total triglyceride levels of the rabbits given cyanide alone was significantly higher than that of control group. A similar increase in blood triglyceride levels has been reported in a study on cassava workers⁴³. A few studies have shown that *A. esculentus* possesses hypolipidemic properties^{31, 44}. These reports are consistent with the triglyceride-lowering effect observed in the present investigation. However the observed changes in plasma total cholesterol and HDL cholesterol due to the fibre were not significant. One of the ways by which fibre reduces plasma lipids is through binding to, and removal of bile acids⁴⁵. An *in vitro* study has shown that *A. esculentus* binds to bile acids⁴⁴. This might be responsible for the observed significant reduction in plasma triglycerides.

Fibres are known to decrease absorption of nutrients and potential toxins through their bulking effects. It is possible that the fibre-induced increases in the ATPases and the reduction in triglycerides may in part, be due to reduction in the amount of cyanide absorbed by the rabbits that received *A. esculentus* in addition to cyanide.

CONCLUSION

This study has demonstrated that *A. esculentus* incorporation in the feed of cyanide-treated rabbits brought about reversal of cyanide-induced significant decreases in intestinal Na⁺-K⁺-ATPase and Ca²⁺-ATPase activities. It also resulted in significant reduction in plasma total triglycerides. In view of the well-established toxic effects of cyanide in all aerobic tissues, these results suggest that regular consumption of *A. esculentus* would be of health benefit, especially for populations that depend on cyanophoric food crops such as cassava and legumes for their caloric needs.

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