IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 6, Issue 4 (Jul. – Aug. 2020), PP 01-12
www.iosrjournals.org

Effect of Ethyl Acetate Extract of *Annona Muricata* (Sour Sop) Leaf on Cyanide Induced Tissue Lesions in Rabbits of the Newzealand Strain

Ukwuonwo Ada Chidimma[1],Ediale Osehahumen Michael[2],
Okolie Ngozi Paulinus[3]

[1](Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Benin city, Nigeria)

[2](Department of Surgery, University of Benin Teaching Hospital, Nigeria)

[3](Department of Biochemistry, Faculty of Life Sciences, University of Benin, Nigeria)

Abstract
Chronic intoxication of cyanide is known to produce some pathologic effects on different tissues, leading to alterations in biochemical parameters. Natural products, especially those derived from plants, have been used to help mankind sustain its health since the dawn of medicine. One such plant with extensive traditional use is Annona muricata. This study was aimed at determining the effects of ethyl acetate extract of Annona muricata leaves on cyanide (CN) induced tissue lesions in rabbits of the New Zealand strain.

Four groups of rabbits,(5 per group) were used for the study. Group A(positive control), received pure grower's mash. Group B(negative control) receivedgrower's mash which had been compounded with 400mg CN/kg of feed. Group Creceived grower's mash which had been compounded with 400mg CN/kg of feed alongside 150mg/kg body weight of ethyl acetate extract ofAnnona muricata leaves and group D receivedgrower's mash which had been compounded with 400mg CN/kg of feedalongside 300mg/kg body weight of ethyl acetate extract ofAnnona muricata leaves. Urinarythiocyanate (SCN) was recorded. The animals were fed for three weeks prior to sacrifice and collection of tissues and blood samples for assays of rhodanese,lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G6PDH), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase(ALP), bilirubin (total, direct and indirect bilirubin), superoxide dismutase (SOD),malondialdehyde (MDA), and catalase.The activities and levels of these biochemical parameters were determined by well established biochemical procedures. Histological analysis of the liver of the different groups of animals was also carried out.

The results showed that exposure of the animals in group B (grower's mash + 400mg CN/kg of feed) to cyanide resulted in a significant reduction in the levels of G6PDH, SOD and catalase and a significant increase in MDA indicating oxidative stress and lipid peroxidation ($p< 0.05$). There was significant increase in serum levels of LDH, ALT, AST, ALP, total and indirect bilirubin and insignificant increase in direct bilirubin and liver AST ($p< 0.05$). Liver ALT reduced but not significantly ($p> 0.05$). All these changes indicated preliminary signs of tissue damage. The histological examination of the liver of group B animals (grower's mash + 400mg CN/kg of feed), indicatedportal congestion, mild periportal infiltrates of inflammatory cells, and focal hepatocyte necrosis. The liver of group C and D animals, treated with the extract, showed heavy periportal infiltrates of lymphocytes, inflammatory cells, andmoderate kupffer cell activation.These results suggest thatsupplementation with ethyl acetate extract of Annona muricata leaves(AMEAE) was effective in mitigating these alterations, indicating that the leaves have some therapeutic and prophylactic effects on cyanide intoxication.

KEYWORDS: *Annona muricata, Ethyl acetate, Cyanide.*

Date of Submission: 15-07-2020 Date of Acceptance: 30-07-2020

I. Introduction

Cyanide is a potent cytotoxic agent found in abundance in nature. This is proven by its presence in several staple food substances in Nigeria like cassava, beans, nuts, and fruit pits. It is notorious for its toxicity (because it is usually destructive to cells and tissues) and having fast lethal action. Other sources of cyanide include industrial materials, such as those used in metal processing, electroplating, rubber and plastic production, insecticide and rodenticide production, chemical synthesis, extraction of gold and silver ores and drugs which release cyanide as part of their waste product of metabolism such as Laetrile and Nitroprusside [1].

Cyanide toxicity is brought about by its high potency as a respiratory poison in all aerobic forms of life. In recent times, chronic cyanide toxicity has become more frequent suggesting that there is an increase in long term exposure to cyanide either through small quantities present in food, or waste products from industries and the environment [2]. Cyanide also encourages production of free radicals by suppressing catalase and SOD activity and inhibiting glucose-6-phosphate dehydrogenase activity which catalyses the first step of the hexose monophosphate shunt.This pathway is responsible for the production of NADPH and glutathione which mops up free radicalsin biological systems [3];[4]. It has been reported that prolonged sublethal cyanide exposure can cause biochemical and histopathological alterations in different species [5];[6]; [7]; [8]. Cyanide is usually converted to thiocyanate in the presence of the enzyme rhodanese or 3-mercaptopyruvate which are sulphurtransferases and this is the main pathway for its detoxification [9]; [10]. Rhodanese ability to catalyze the conversion of thiosulphate to thiocyanate can be inhibited by the presence of Sulphur therefore the use of experimental Sulphur donors can protect against lethal doses of cyanide [11].

Considering the problems that can result from long-term ingestion of low amounts of cyanide, it is important to carefully assess the effects of sublethal doses of cyanide and detremine natural substances that can prevent such tissue damage. This study was therefore carried out to ascertain the biochemical changes that can occur as a result of cyanide poisoning and if and how *Annona muricata* leaf ethyl acetate extract (AMLEAE) can prevent or ameliorate these changes. This study will help to further clarify the pathophysiology of cyanide poisoning and help with diagnosis and palliative care for people who are consistently exposed to cyanide over a very long period.

II. Methodology

Experimental Design

Twenty rabbits weighing approximately 940g-1500g were divided randomly into four groups with five animals each. These animals were allowed to acclimatize to the change in environment and diet for 14 days before the beginning of experiment. The animals were housed in metal cages at room temperature ($25 \pm 1^{o}C$) in a 12 hour light/12 hour dark cycle with 50 ± 5% humidity. Animals received standard laboratory balanced commercial diet (growers mash)*ad libitum*. Group A rabbits received pelleted grower's mash and tap water throughout the experiment and served as the positive control. Rabbits in Groups B, C and D were fed 400mg CN/kg feed , 400mg CN/kg feed + 150mg/kg body weight ethyl acetate extract *Annona muricata* leaves (AMEAE), 400mg CN/kg feed + 300mg/kg body weight ethyl acetate extract *Annona muricata* leaves (AMEAE), and tap water respectively for 21 days.

At the end of the experiment (21 days), all rabbits were sacrificed using ether as anesthesia. Blood was collected from their hepatic portal vein, and stored in heparinized and non heparinized bottles. The liver and kidney of the rabbits were removed and dissected. The samples were cleaned free of extraneous material, washed with physiological saline and stored at $-10^{o}C$ until subsequent use.
Urine was then collected directly from their bladder, using 10mL syringes.

Biochemical Analysis

Tissue samples were rapidly thawed and homogenized in 10% (w/v) of ice-cold normal saline (pH 7.4) for 5 min, and centrifuged at 3,000×g for 10 min and the supernatant was kept in ice until assayed.

Liver Function Test

The activities of aspartate aminotransferase (EC 2.6.1.1, AST) and alanine aminotransferase (EC 2.6.1.2, ALT) were determined by the colorimetric method of Reitman and Frankel (1957) [12] Activities of lactate dehydrogenase (EC 1.1.1.28, LDH) and alkaline phosphatase (EC 3.1.3.1, ALP) were determined colorimetrically [13]. Colorimetric method based on that described by Jendrassik and Grof (1938)[14] was used for estimating direct and total bilirubin.Rhodanese(thiosulphate: cyanide sulphurtransferase, EC 2.8.1.1) and amount of urine thiocyanate were assayed based on Sorbo (1953) [15]method.

Oxidative Stress and Antioxidant Capacity

Malondialdehyde (MDA) level was estimated by the method of Buege and Aust, (1978) [16].The assay of superoxide dismustatse (SOD) was an indirect method which was based on the inhibitory effect of SOD in the initial rate of epinephrine autooxidation. This is derived from the reaction proposed by Misra and Fridovich (1972) [17].The estimation of catalase was based on the method of Cohen et al(1970) [18]. Glucose-6-phosphate dehydrogenase activity was assayed for colorimetrically (Lohr and Waller, 1974) [19].

Histological Analysis

Histological analysis of the liver was carried out using an Olympus light microscope after the organs were fixed on a microscopic slide and stained using haematoxylin and eosin.

Statistical Analysis

All data were presented as mean ± standard error of mean (SEM). The values obtained were analyzed using analyses of variance (ANOVA) followed by least significance difference (LSD) analyses to evaluate the variations in between the groups and for multiple comparison between different groups (Ogbeibu,2015) [20]. The level of significance was set at $p< 0.05$.

III. Results

Effect of ethyl acetate extract of Annona muricata leaveson urine thiocyanate level

The urinary thiocyanate level in the group given feed only (A) was significantly lower than that of groups B, C, and D. The urinary thiocyanate level of group B (CN+feed) was significantly higher than that of A,C,and D.The urinary thiocyanate level in group C (CN+feed+150mg *A.muricata* extract) and D were significantly higher than that of group A. However they were found to be significantly lower than that of group B. There was no significant difference between group C and group D although the mean value of group D was higher than that of group C.

Table 1: Effect of ethyl acetate extract of Annona muricata leaveson urine thiocyanate levels

Groups	Urine Thiocyanate (µg/ml of urine)
A (Feed only)	60.42 ± 6.69^{a}
B (Feed + CN)	160.93 ± 6.86^{b}
C (Feed + CN + 150mg/kg b.w AMLEAE)	127.78 ± 1.37^{c}
D (Feed + CN + 300mg/kg b.w AMLEAE)	132.77 ± 2.10^{c}

Results are expressed as mean ± standard error of mean (SEM=2).The various alphabets in the table indicate significant differences. Similar alphabets reflect no significant difference ($p>0.05$) between the values while different alphabets reflects significant differences ($p< 0.05$) between the values.

Effect of ethyl acetate extract of Annona muricata leaveson rhodanese activity

Rhodanese activity in group A (feed only), was significantly lower than that of groups B, C, and D. Rhodanese activity in group B (CN+feed), was significantly higher than that of groups A and C. There was no significant difference between rhodanese activity in groups B and D although the mean value of D was lower than that of B.

Rhodanese activity in group C (CN+feed+150mg *A.muricata* extract), was significantly higher than that of group A, but also significantly lower than that of group B (CN+ feed) and D(CN+feed+300mg extract).

Rhodanese activity in group D (CN+feed+300mg *A.muricata* extract), was significantly higher than that of groups A and C. Although it was found to be lower than that of group B, it was not significant.

Table 2: Effect of ethyl acetate extract of *Annona muricata* leaveson rhodanese activity

Groups	Rhodanese activity Units/min/g of liver
A (Feed only)	0.26 ± 0.24^{a}
B (Feed + CN)	1.03 ± 0.00^{b}
C (Feed + CN + 150mg/kg b.w AMLEAE)	0.78 ± 0.06^{c}
D (Feed + CN + 300mg/kg b.w AMLEAE)	0.98 ± 0.03^{b}

Results are expressed as mean ± standard error of mean (SEM=2).The various alphabets in the table indicate significant differences. Similar alphabets reflect no significant difference ($p>0.05$) between the values while different alphabets reflects significant differences ($p< 0.05$) between the values.

Effect of ethyl acetate extract of Annona muricata leaves on serum and liver Aspartate aminotransferase (AST) activity

Serum AST activity in group A (feed only), was significantly lower than that of groups B, C, and D.SerumAST activity in group B (CN+feed), was significantly higher than that of groups A, C, and D.Serum AST activity in group C (CN+feed+150mg *A.muricata* extract) and D (CN+feed+300mg *A.muricata* extract)

were significantly higher than that of group A, but significantly lower than that of group B. There was no significant difference between the Serum AST activity of groups C and D although the mean value of C was slightly greater than that of D.

Group B (CN+feed), had the highest liver AST activity followed by group C (CN+feed+150mg *A.muricata* extract), then group D (CN+feed+300mg *A.muricata* extract). The least activity was seen in group A(feed only) .There was no significant difference between any of the groups.

Table 3: Effect of ethyl acetate extract of *Annona muricata* leaves on serum and liver Aspartate aminotransferase (AST) activity

Groups	Serum AST activity (U/l)	Liver AST activity (U/l)
A (Feed only)	17.21±1.74[a]	53.55±2.80[a]
B (Feed + CN)	34.54±1.07[b]	60.83±1.33[a]
C (Feed + CN + 150mg/kg b.w AMLEAE)	25.26±1.69[c]	56.50±3.55[a]
D (Feed + CN + 300mg/kg b.w AMLEAE)	25.10±1.05[c]	55.94±1.40[a]

Results are expressed as mean ± standard error of mean (SEM=2).The various alphabets in the table indicate significant differences. Similar alphabets reflect no significant difference (p>0.05) between the values while different alphabets reflects significant differences (p< 0.05) between the values.

Effect of ethyl acetate extract of Annona muricata leaveson serum and liver Alanine aminotransferase (ALT) activity

Serum ALT activity in group B (CN+feed), is significantly higher than that of groups A, C and D. Serum ALT activity in group C (CN+feed+150mg *A.muricata* extract) was significantly lower than that of groups A, B, and D.

The activity of A (feed only) was significantly lower than B but significantly higher than C. Group A activity was also found to be higher than D, but it was not significant.

Group D activity (CN+feed+300mg *A.muricata* extract), was found to be lower than A, but it was insignificant. It was significantly lower than B and significantly higher than C (CN+feed+150mg *A.muricata* extract). For liver ALT, there was no significant difference between group A (CN+feed), and the other groups. Group B (negative control) was significantly higher than group C (CN+feed+150mg *A.muricata* extract) and D (CN+feed+300mg *A.muricata* extract). There was no significant difference between group C and D although the ALT activity of group C was more than that of D.

Table 4: Effect of ethyl acetate extract of *Annona muricata* leaveson serum and liver Alanine amino transferase (ALT) activity

Groups	Serum ALT activity (U/l)	Liver ALT activity (U/l)
A (Feed only)	25.61±0.76[a]	1.66[ab]
B (Feed + CN)	34.58±1.60[b]	1.72[a]
C (Feed + CN + 150mg/kg b.w AMLEAE)	14.26±1.52[c]	1.62[b]
D (Feed + CN + 300mg/kg b.w AMLEAE)	22.10±1.89[a]	1.60[b]

Results are expressed as mean ± standard error of mean (SEM=2). The various alphabets in the table indicate significant differences. Similar alphabets reflect no significant difference (p>0.05) between the values while different alphabets reflects significant differences (p< 0.05) between the values.

Effect of ethyl acetate extract of Annona muricata leaveson Alkaline phosphatase (ALP) activity

There was a significant increase in the activity of ALP in group B (CN+feed), when compared to that of group A (feed only), C (CN+feed+150mg *A.muricata* extract), and D (CN+feed+300mg *A.muricata* extract). There was no significant difference (p>0.05) between groups C and D though the mean value of C was higher. The ALP activity of group A (positive control) was significantly lower than that of groups B, C and D).

Table 5: Effect of ethyl acetate extract of *Annona muricata* leaveson Alkaline phosphatase (ALP) activity

Groups	ALP activity (IU/L)
A (Feed only)	5.32±0.11[a]
B (Feed + CN)	8.31±0.40[b]
C (Feed + CN + 150mg/kg b.w AMLEAE)	6.69±0.10[c]
D (Feed + CN + 300mg/kg b.w AMLEAE)	6.55±0.24[c]

Results are expressed as mean ± standard error of mean (SEM=2). The various alphabets in the table indicate significant differences. Similar alphabets reflect no significant difference (p>0.05) between the values while different alphabets reflects significant differences (p< 0.05) between the values.

E*ffect of ethyl acetate extract of Annona muricata leaveson Superoxide dismutase (SOD) activity*

SOD activity in group B (CN+feed) was significantly lower than that of groups A, (CN+feed) C, (CN+feed+150mg *A.muricata* extract) and D (CN+feed+300mg *A.muricata* extract). The activity of superoxide dismutase (SOD) was highest in group C. Although no significant difference was observed between group A, C and D the activity of SOD in group A was lowest, followed by group C as shown by their mean values.

Table 6: Effect of ethyl acetate Eextract of *Annona muricata* leaveson Superoxide dismutase (SOD) activity

Groups	SOD activity (Units/g of tissue)
A (Feed only)	52.72±7.71[a]
B (Feed + CN)	37.64±1.90[b]
C (Feed + CN + 150mg/kg b.w AMLEAE)	63.33±1.36[a]
D (Feed + CN + 300mg/kg b.w AMLEAE)	60.42±1.53 [a]

Results are expressed as mean ± standard error of mean (SEM=2). The various alphabets in the table indicate significant differences. Similar alphabets reflect no significant difference (p>0.05) between the values while different alphabets reflects significant differences (p< 0.05) between the values.

Effect of ethyl acetate extract of Annona muricata leaveson Catalase activity

The activity of catalase in group B (CN+feed) was significantly lower than that of group A (feed only), C (CN+feed+150mg *A.muricata* extract),and D (CN+feed+300mg *A.muricata* extract). There was no significant difference between group A, C, and D although the highest activity was seen in group D. There was slightly less activity in group C as compared to group A.

Table 7: Effect of ethyl acetate extract of *Annona muricata* leaveson Catalase activity

Groups	Catalase activity (Units/g of tissue) × 10^{-5}
A (Feed only)	5.73±1.24[a]
B (Feed + CN)	2.02±0.45[b]
C (Feed + CN + 150mg/kg b.w AMLEAE)	5.64±0.93[a]
D (Feed + CN + 300mg/kg b.w AMLEAE)	7.56±0.98[a]

Results are expressed as mean ± standard error of mean (SEM=2). The various alphabets in the table indicate significant differences. Similar alphabets reflect no significant difference (p>0.05) between the values while different alphabets reflects significant differences (p< 0.05) between the values. All values are x 10^{-5}.

Effect of ethyl acetate extract of Annona muricata leaveson malondialdehyde (MDA) activity

The level of malondialdehyde (MDA) in group B (CN+feed), was significantly higher than that of group A (feed only) C, (CN+feed+150mg *A.muricata* extract), and D (CN+feed+300mg *A.muricata* extract). No significant difference was observed between group A, C and D. Group D had the highest mean value while group A had the least.

Table 8: Effect of ethyl acetate extract of *Annona muricata* leaveson malondialdehyde (MDA) activity

Groups	MDA activity (moles/mg of tissue) × 10^{-6}
A (Feed only)	2.08±0.35[a]
B (Feed + CN)	3.72±0.17[b]
C (Feed + CN + 150mg/kg b.w AMLEAE)	2.45±0.25[a]
D (Feed + CN + 300mg/kg b.w AMLEAE)	2.74±0.19[a]

Results are expressed as mean ± standard error of mean (SEM=2). The various alphabets in the table indicate significant differences. Similar alphabets reflect no significant difference (p>0.05) between the values while different alphabets reflects significant differences (p< 0.05) between the values.

Effect of ethyl acetate extract of Annona muricata leaveson total, direct and indirect bilirubin levels

The level of total serum bilirubin in group B (CN+feed), was significantly higher than that of group A (feed only) C and D. No significant difference was observed between group A, C (CN+feed+150mg *A.muricata* extract) and D (CN+feed+300mg *A.muricata* extract), although the level of total bilirubin in group C was highest when compared to A and D closely followed by D and finally A.

The level of direct bilirubin in group B (CN+feed), was significantly higher than that of group A (feed only). No significant difference was observed between group B and group C (CN+feed+150mg *A.muricata* extract), although the level of conjugated bilirubin in group C was slightly lower than that of B. No significant difference was observed between group D (CN+feed+300mg *A.muricata* extract) and all other groups (A, B and C), although the level of conjugated bilirubin was higher compared to group A but lower than that of groups B and C.

The indirect bilirubin levels in group B (CN+feed), was found to be significantly higher than that in groups A (feed only), C (CN+feed+150mg *A.muricata* extract), and D (CN+feed+300mg *A.muricata* extract). There was no significant difference between groups A, C, and D. however, group C level was more than that of group A and group A was greater than that of group D.

Table 9: Effect of ethyl acetate extract of *Annona muricata* leaves on total, direct and indirect bilirubin levels

Groups	Total bilirubin (mg/dL)	Direct bilirubin (mg/dL)	Indirect bilirubin (mg/dL)
A (Feed only)	0.16±0.02[a]	0.07±0.01[a]	0.085±0.01[a]
B (Feed + CN)	0.72±0.26[b]	0.32±0.11[b]	0.406±0.16[b]
C (Feed + CN + 150mg/kg b.w AMLEAE)	0.24±0.05[a]	0.29±0.09[b]	0.144±0.05[a]
D (Feed + CN + 300mg/kg b.w AMLEAE)	0.19±0.06[a]	0.11±0.02[ab]	0.078±0.04[a]

Results are expressed as mean ± standard error of mean (SEM=2). The various alphabets in the table indicate significant differences. Similar alphabets reflect no significant difference (p>0.05) between the values while different alphabets reflects significant differences (p< 0.05) between the values.

Effect of ethyl acetate extract of Annona muricata leaveson Glucose-6-phosphate dehydrogenase (G6PDH) activity

G6PDH activity in group B (CN+feed), was significantly lower than that of C (CN+feed+150mg *A.muricata* extract) and D (CN+feed+300mg *A.muricata* extract) and very significantly lower than that of A (feed only). There was no significant difference between the G6PDH activity of group C and group D though the activity of D was higher than that of C. Groups C and D were significantly lower than A.

Table 10: Effect of ethyl acetate extract of *Annona muricata* leaveson Glucose-6-phosphate dehydrogenase (G6PDH) activity

Groups	G6PDH activity (mU/10^9)
A (Feed only)	1285.70±38.51[a]
B (Feed + CN)	58.80±3.04[b]
C (Feed + CN + 150mg/kg b.w AMLEAE)	171.42±4.50[c]
D (Feed + CN + 300mg/kg b.w AMLEAE)	206.35±12.85[c]

Results are expressed as mean ± standard error of mean (SEM=2). The various alphabets in the table indicate significant differences. Similar alphabets reflect no significant difference (p>0.05) between the values while different alphabets reflects significant differences (p< 0.05) between the values. All values are /10^9.

Effect of Annona muricata leaf ethyl acetate extracton Lactate dehydrogenase LDH activity

LDH levels in group A (feed only), was found to be significantly lower than that of group B (CN + feed). It was also found to be higher than the levels found in group C (CN+feed+150mg *A.muricata* extract) and D (CN+feed+300mg *A.muricata* extract) but it was not significant. Group B was significantly higher than groups A, C and D. There was no significant difference between groups C and D but the mean value of C was higher than that of D.

Table 11: Effect of *Annona muricata* leaf ethyl acetate extracton Lactate dehydrogenase LDH activity

Groups	**LDH (U/L)**
A (Feed only)	5.41±46.10[a]
B (Feed + CN)	7.64±66.87[b]
C (Feed + CN + 150mg/kg b.w AMLEAE)	5.21±33.34[a]
D (Feed + CN + 300mg/kg b.w AMLEAE)	5.00±40.33[a]

Results are expressed as mean ± standard error of mean (SEM=2). The various alphabets in the table indicate significant differences. Similar alphabets reflect no significant difference (p>0.05) between the values while different alphabets reflects significant differences (p< 0.05) between the values.

HISTOLOGY RESULTS

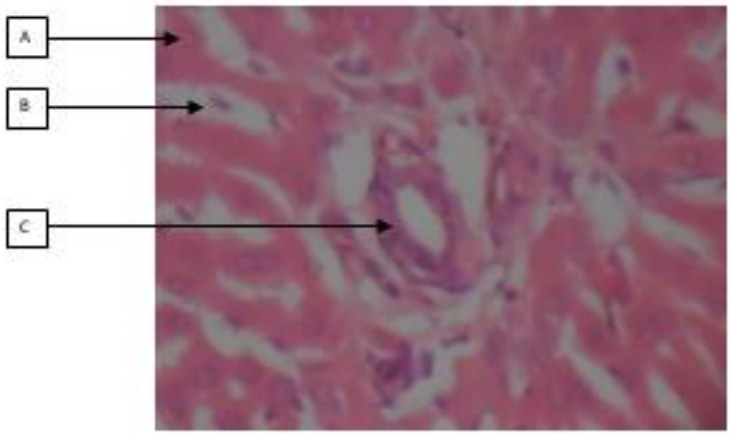


Plate 1: Photomicrograph of liver tissue from positive control (feed only) rabbit, showing normal features. (A) Hepatocytes, (B) Sinusoids and (C) bile duct (H&E x 100)

This is usually characteristic of a healthy liver. No signs of inflammation or injury. The hepatocytes , sinusoids, bile ducts and every other aspect of the liver tissue shown above is normal.

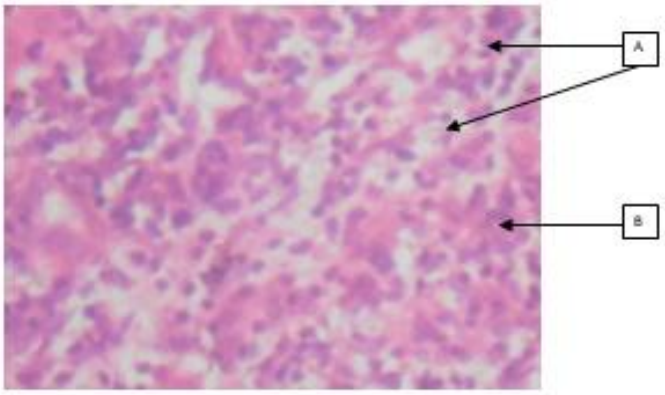


Plate 2: Photomicrograph of liver tissue from negative control (CN + feed) rabbit, showing; (A) focal hepatocyte necrosis and (B) heavy periportal infiltrates of inflammatory cells (H&E X100)

Introduction of cyanide to the feed of the animals led to liver cell death (hepatocyte necrosis) as shown above. There was also an increase in inflammation as shown by the heavy infiltration of inflammatory cells.

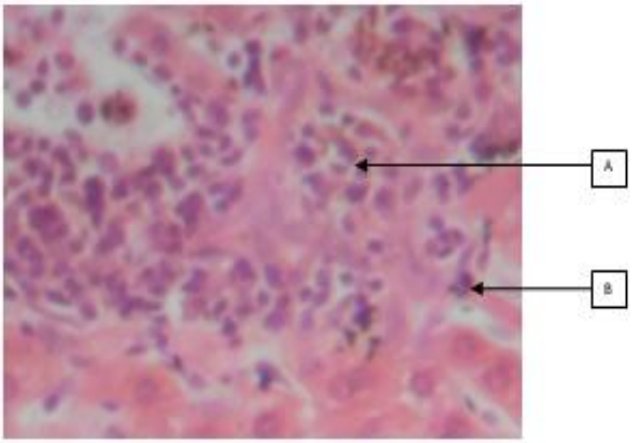


Plate 3: Photomicrograph of liver tissue from rabbits, given CN + feed + 150mg ethyl acetate extract of *Annona muricata* leaf showing; (A) heavy periportal infiltrates of lymphocytes and (B) moderate kupffer cell activation (H&E X100)

On introduction of the extract it can be seen that inflammatory response has been activated attempting to restore normalcy.

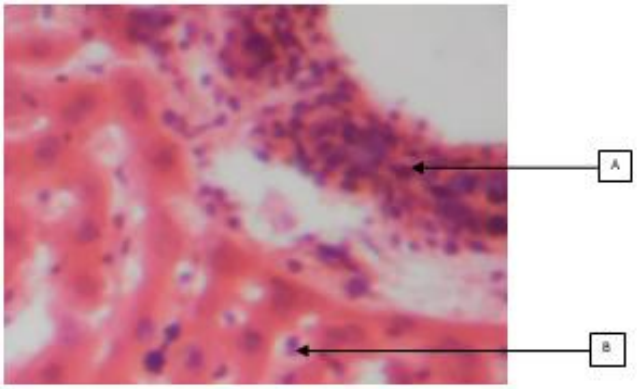


Plate 4: Photomicrograph of liver tissue from rabbits, given CN + feed + 300mg ethyl acetate extract of *Annona muricata* leaf showing; (A) heavy periportal infiltrates of inflammatory cells and (B) moderate kupffer cell activation (H&E X100)

Increasing the concentration of the extract to 300mg had the same effect as the extract concentration at 150mg.

IV. Discussion

Medicinal plants are always at the forefront where treatment of diseases is concerned. This research work has investigated the effect of *Annona muricata* leaf ethyl acetate extract (AMLEAE) on cyanide induced tissue lesions in rabbits by accessing liver function (viz ALP, AST, ALT) biomarkers of oxidative stress i.e. antioxidant enzymes (viz.,SOD, catalase) as well as its effects on lipid peroxidation (MDA) and energy metabolism (G6PDH and LDH).

Four groups of rabbits were used (5 per group). Group 1 (positive control) was given normal pelleted growers mash for 21 days after a two week acclimatization period of being given the same food. Group 2 (negative control) was given normal pelleted growers mash for the two weeks acclimatization period then they were given growers mash along with 50g potassium cyanide (KCN) for 21 days. Group 3 was given the same diet as group 2 along with 150mg per kg body weight of AMLEAE for 21 days. Group 4 was given the same diet as group 2 along with 300mg per kg body weight of AMLEAE for 21 days. Cyanide was incorporated into the rabbit feed to induce tissue lesions. The early events of cyanide toxicity were monitored through the assessment of urinary thiocyanate because it is usually present in the urine during the break down of cyanide.

Increased lipid peroxidation weakens the membrane by decreasing its fluidity and changing the shape and function of the enzymes and receptors that are usually found in them. Oxidative stress arises from both endogenous and exogenous sources. Despite antioxidant defense mechanisms (antioxidant enzymes especially) cell damage from oxygen free radicals (OFR) is ubiquitous.

Effect on Glucose 6 Phosphate Dehydrogenase (G6PDH)

G6PDH is an enzyme that has been used as a direct indicator of oxidative stress . G6PDH catalyses the conversion of glucose-6-phosphate into ribose sugar. This represents the diversion from early stages of glycolysis to the pentose phosphate pathway (PPP) .G6PDH shunts glucose-6-phosphate (G6P) into RNA/DNA formation as against formation of high energy pyruvate. High levels of G6PDH will indicate a diversion of G6P into RNA production and reduction in the G6P meant for pyruvate formation; which will imply the neuronal metabolic system favouring repair of the genetic materials against energy requirements of the neuron. While a reduction in G6PDH observed during oxidative stress means a reduction in the rate at which G6P is converted into ribose sugar (DNA precursor), thus, the system favours energy production over the repair of genetic materials . The glucose-6-phosphate dehydrogenase levels of group B animals (CN + Feed only) was significantly lower than that of those in group A (Feed only) which is indicative of oxidative damage [21]. This enzyme is found in the pentose phosphate pathway, a pathway that supplies NADPH (nicotinamide adenine dinucleotide phosphate) to cells like red blood cells and tissues involved in biosynthesis of fatty acids like the liver. The NADPH helps maintain glutathione levels. Glutathione in turn prevents oxidative damage in the cells [21]. G6PDH reduces NADP+ to NADPH and oxidizes glucose-6-phosphate to 6-phospho-gluconolactone [22].

A similar study by Isom et al., in 1975 showed the effect of cyanide on the metabolic degradation of glucose in the mouse. In that study, glucose in the untreated animals was metabolized via three pathways: Embden-Meyerhof-Parnas pathway and tricarboxylic acid cycle (EMP-TCA), pentose phosphate pathway and glucuronate pathway. Administration of 5 mg/kg of potassium cyanide, a sub-lethal dose, increased catabolism of carbohydrates by the pentose phosphate pathway with a decline in utilization of the EMP-TCA cycle and glucuronate pathway. These results suggest that chronic exposure to cyanide may induce increase in G6PDH levels since the ppp was favoured thus increasing utilization of the enzyme. The glucose 6 phosphate dehydrogenase activity of group C animals (CN+ feed+ 150mg AMLEAE) and group D animals (CN+ feed+ 300mg AMLEAE) was also significantly higher than that of group B animals. This shows that the extract at both doses ameliorated the effect of the cyanide.

Effect on Lactate Dehydrogenase (LDH)

Lactate dehydrogenase (LDH) is expressed extensively in body tissues, such as blood cells and heart muscle. Because it is released during tissue damage, it is a marker of common injuries and disease. It converts pyruvate, the final product of glycolysis, to lactate when oxygen is absent or in short supply, and it performs the reverse reaction during the Cori cycle in the liver.The results showed that there was significant increase in LDH activity in the presence of cyanide.This is in keeping with previous studies [5]; [3] indicating increased LDH activity due to cyanide intoxication. AMLEAE significantly reduced this increase at both doses. Lactate dehydrogenase (LDH) catalyzes the conversion of lactate to pyruvic acid in a reversible reaction converting NAD+ to NADH in the process. LDH is seen as a marker of tissue injury because it is released during tissue damage [23].

Effect on Bilirubin

The total bilirubin level was significantly increased in the presence of cyanide. (0.16mg/dl -0,72mg.dl) (p<0.05). However, administration of AMLEAE improved the conjugating capacity of the liver by decreasing the total bilirubin level (from 0.72mg/dl -0.24 at 150 mg and 0.72mg/dl – 0.19mg/dl at 300mg) Similar trends were observed in indirect bilirubin levels. Direct bilirubin levels showed no significant decrease after administration of both concentrations of AMlEAE. Bilirubin is a breakdown product of the haeme component of the haemoglobin molecule. AMLEAE was able to lower bilirubin levels probably because of the presence of glucosides in the extract which might be converted to glucorunic acid for conjugating with bilirubin for excretion. It could also be suggested that AMLEAE activated the Constitutive Andostane Receptor (CAR), a key regulator in the bilirubin clearance pathway increasing the activity of glucuronyl transferases, synthesis of ligandin, (a transporter of bilirubin), and increasing its transport to the liver for conjugation . Also, AMLEAE could inhibit the activity of haem oxygenase, the rate limiting enzyme of the bilirubin pathway, reducing total serum bilirubin in treated animals.

Effect on Serum and Liver Alanine Transaminase, Aspartate Transaminase (ALT ,AST) and Serum Alkaline Phosphatase (ALP) Activities

Serum ALT AST, and ALP levels were increased followingcyanide exposure. This was similar to results gotten in a 2003 study by Okolie and Irosanya where serum ALP and ALT increased with great significance following cyanide consumption by rabbits. On administration of AMLEAE ALT AST and ALP levels significantly reduced at both concentrations proving that AMLEAE has hepatoprotective effects

ALT levels in the liver were found to increase following cyanide intoxication, though not significantly. They reduced significantly on administration of AMLEAE at both concentrations. AST levels in the liver were higher on administration of cyanide but not significantly. AMLEAE reduced these levels at both concentrations although not significantly. ALT and AST arefound in the liver. But while ALT is more specific for the liver AST can also be found in skeletal muscle, pancreas, erythrocytes, kidneys and heart. The ratio of ALT to AST is useful in diagnosing liver problems. Mild liver diseases increase the ratio of cytoplasmic ALT to AST (>1) while more severe diseases reduce the ratio (<1) [24].

ALP works best at a pH of 10 it is usually inactive in the blood.it is a marker for bone and most especially liver disease.The primary importance of measuring ALP is to check the possibility of liver disease. Since the mucosal cells that line the bile system of the liver are the source of alkaline phosphatase, the free flow of bile through the liver and down into the biliary tract and gallbladder are responsible for maintaining the proper level of this enzyme in the blood. When the liver, bile ducts or gallbladder system are not functioning properly or are blocked, this enzyme is not excreted through the bile and is released into the blood stream. Thus serum ALP is a measure of the integrity of the hepatobiliary system and the flow of bile into the small intestine [24].

Effect on Superoxide Dismutase (SOD), Catalase (CAT) and Malondialdehyde (MDA)

SOD and catalase levels were significantly reduced on administration of cyanide. Superoxide dismutase is an enzyme that drives the conversion of the superoxide (O_2^-) free radical into either molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). Superoxide is a by-product of oxygen metabolism and, uncontrolled, can cause cell damage (Hayyan *et al.*,2016). Thus, SOD is an important antioxidant in living cells exposed to oxygen. This study revealed that cyanide caused cell damage by producing free radicals while suppressing the action of SOD. Another study carried out on mirror carp showed that when the SOD activity in the fish exposed to 0.5 mg/L of cyanide for twenty four hours was compared to the control group, a significant decrease (p<0.05) was observed in the brain tissues confirming that cyanide caused SOD levels to reduce leading to oxidative stress [25].

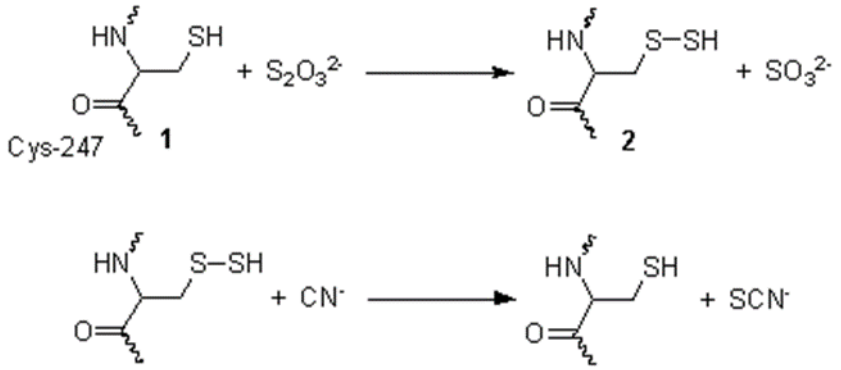
Catalase is found in most living organisms exposed to oxygen. It catalyzes the break down of hydrogen peroxide to water and oxygen.. By so doing ,it protects cells from oxidative stress. This study arrived at the conclusion that catalase action was disrupted by cyanide availability in the biological system. Another study showed that CAT activity in the brain tissues of Mirror carp exposed to 0.5 mg/L of cyanide for 24 h was decreased significantly(p<0.05) compared to the control group [25].On administration of AMLEAE at both concentrations, both enzymatic activities were significantly increased. This shows that AMLEAE has antioxidant properties and was able to counter the effects of cyanide.

MDA significantly increased when cyanide was given but was significantly reduced on administration of AMLEAE at both concentrations. This shows that AMLEAE prevents lipid peroxidation and undue cell death. Increased levels of MDA is indicative of oxidative stress and lipid peroxidation. It is important to note that the antioxidant system of the body defends against the ROS produced in the body. In summary Malondialdehyde increases when there is lipid peroxidation, superoxide dismutases (SOD) help the body to remove the superoxide radicals by converting it to hydrogen peroxide (H_2O_2) or oxygen and catalase further catalyzes the conversion of hydrogen peroxide to water and oxygen. If the production of FRs increases beyond a

certain level in the body of the organism, the defensive enzyme systems fail and the condition leads to oxidative stress. Oxidative stress inturn may lead to increase in the free calcium ions and iron within the cells in mammals and this rise in intracellular free Ca^{2+} can result in DNA damage by endonuclease activation . Severe oxidative stress can result in cell damage and death .

Effect on Rhodanese

Rhodanese, is a mitochondrial enzyme that detoxifies cyanide (CN−) by converting it to thiocyanate (SCN−) .This reaction takes place in two steps. In the first step, thiosulfate is reduced by the thiol group on cysteine-247, to form a persulfide and a sulfite. In the second step, the persulfide reacts with cyanide to produce thiocyanate, re-generating the cysteine thiol.



Rhodanese levels were significantly higher in the animals given cyanide ($p<0.05$) (0.26 - 1.03units/ml) and these levels significantly reduced on administration of 150mg of AMLEAE (1.03 – 0.78units/ml). The reduction observed at 300mg AMLEAE was insignificant.

Effect on urine thiocyanate levels

Urinary thiocyanate levels were significantly increased significantly ($p<0.05$) on exposure to cyanide proving there was indeed cyanide toxicity. Previous studies showed that cyanide,is usually converted to thiocyanate (SCN) by a thiosulphate sulphurtransferase, in the mitochondria of the liver. This is in keeping with the results of this study. These levels however, reduced significantly on administration of both concentrations of AMLEAE indicating that the extract indeed ameliorated the toxic effects of cyanide. This is in agreement with a similar study that compared the toxic effects of organic cyanide in cassava and similar levels of inorganic cyanide in potassium cyanide in white rabbits [26].

V. Conclusion

The results of the this study showed that cyanide exposure caused a significant increase in the plasma levels of AST,ALT,ALP, and Bilirubin, caused lipid peroxidation and oxidative stress,increased glucose metabolism and reduced production of reducing equivalents and AMLEAE supplementation was effective in mitigating resultant alterations. These results imply that *Annona muricata* leaves have both therapeutic and prophylactic effects on cyanide intoxication. However, with respect to the fact that the metabolism of cyanide and its main metabolite, thiocyanate, is species-linked, and toxicokinetic parameters of cyanide compounds vary in different species further research might be needed using other species to elucidate the molecular basis of the ameliorative properties of *Annona muricata* leaves in cyanide poisoning.

Acknowledgement

I would like to thank Professor N. P. Okolie, Professor E.K.I. Omogbai and Dr. G.I. Eze whose inputs were invaluable to the success of this paper.

References

[1]. **Ellenhorn, M. J**. (1997). Cyanide poisoning. In: Ellenhorn's medical toxicology: diagnosis and treatment of human poisoning. Ellenhorn, M. J., Schonwald, S., Ordog, G., Wasserberger, J. (eds) Baltimore:Williams& Wilkins. Pp 1474–84.

[2]. **Mathangi, D. C**., Namasivayam, A. (2000). Effect of chronic cyanide intoxication on memory in albino rats. *Food Chemistry and Toxicology* .**38**:51–55.

[3]. **Okolie, N. P**., Iroanya, C. U. (2003). Some histologic and biochemical evidence for mitigation of cyanide-induced tissue lesions by antioxidant vitamin administration in rabbits. *Food Chemistry and Toxicology.***41**:463–469.

[4]. **Okolie, N. P**., Asonye, C. C. (2004). Mitigation of cataractogenic potential of cyanide by antioxidant vitamin administration. *Journal of Medicine and Biomedical Research*.**3**:48–52.

[5]. **Okolie, N. P**., Osagie, A. U. (2000). Differential effects of chronic cyanide intoxication on heart, lung and pancreatic tissues. *Food Chemistry and Toxicology* .**38**:543-548.

[6]. **Sousa, A. B**., Soto-Blanco, B., Guerra, J. L., Kimura, E. T., and Gorniak, S. L. (2002). Does prolonged oral exposure to cyanide promote hepatotoxicity and nephrotoxicity? *Toxicology*. **174**:87-95.

[7]. **Soto-Blanco, B.,** Marioka, P.C., Gorniak, S. L. (2000). Effects of longterm low-dose cyanide administration to rats. *Ecotoxicology and Environmental Safety*.**53**:37-41.

[8]. **Tulsawani, R. K**., Debnath, M., Pant, S. C., Kumar, O. M., Prakash, A. O., Vijayaraghavan, R., and Bhattacharya, R. (2005). Effect of sub-acute oral cyanide administration in rats: Protective efficacy of alpha-ketoglutarate and sodium thiosulfate. *Chemistry and Biology Interaction***156**:1–12.

[9]. **Megarbane, B.** (2003) . Antidotal treatment of cyanide poisoning. *Journal of Chinese Medical Association*. **66**:193–203.

[10]. **Nagahara, N**., Li, Q., Sawada, N. (2003). Do antidotes for acute cyanide poisoning act on mercaptopyruvate sulfurtransferase to facilitate detoxification? *Current Drug Targets for Immune Endocrinology and Metabolic Disorders***.3**:198–204.

[11]. **Baskin, S.I.,** Porter, D.W., Rockwood, G.A., Romano, J. A. Jr., Patel, H. C., Kiser, R. C., Cook, C. M., and Ternay, A. L. Jr. (1999). In vitro and in vivo comparison of sulfur donors as antidotes to acute cyanide intoxication. *Journal of Applied Toxicology*.**19**:173-183.

[12]. **Reitman, S., and Frankel, S. A. (1957). Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, 25: 56–62.**

[13]. **Burtis, C. A.** and Ashwood, E. R. (1994). *Tietz Textbook of Clinical Chemistry*, 2nd ed. Saunders: Philadelphia. Pp 625–1081.

[14]. **Jendrassik, L.,** and Grof, P. (1938). *Biochemische Zeitschrift*,**297**:81.

[15]. **Sorbo, B. H.** (1953). Crystalline rhodanese: the enzyme catalyzed reaction. *ActaChemica Scandinavia*,**7**: 1137–1145.

[16]. **Buege, J. A.** and Aust, S. D. (1978). Microsomal Lipid Peroxidation. In: *Methods in Enzymology.* Biomembranes.Fleicher S, and Packer, L, (Eds). Academic Press, London. **52**: 302-309.

[17]. **Misra, H. P.,** and Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*, **247**: 3170

[18]. **Cohen, G.,** Dembiec, D., and Marcus, J. (1970). Measurement of catalase activity in tissue extract. *Analytical Biochemistry*,**34**: 30-38.

[19]. **Lohr, G. W.,** and Waller, H. D. (1974). Glucose-6-phosphate Dehydrogenase. *Methods of Enzymatic Analysis.* 3rd ed. Varlag Chemie, Wehnheim**.** Pp. 636.

[20]. **Ogbeibu, A. E.** (2015). *Biostatistic: A Practical Approach to Research and Data Handling*.2nd ed. Mindex Publishing Company, Lagos, Nigeria. Pp: 17-22.

[21]. **Thomas, D.,** Cherest, H., and Surdin-Kerjan, Y. (1991). Identification of the structural gene for glucose-6-phosphate dehydrogenase in yeast. Inactivation leads to a nutritional requirement for organic sulfur. *The European Molecular Biology Organisation Journal*. **10**:547–553.

[22]. **Aster, J**., Kumar, V., Robbins, S.L., Abbas, A.K., Fausto, N., and Cotran, R.S. (2010). Robbins and Cotran *Pathologic Basis of Disease*. Saunders/Elsevier. Pp.33340–33341.

[23]. **Irani, K.,** Xia, Y., Zweier, J.L., Sollott, S.J., Der, C.J., Fearon, E.R., Sundaresan, M., Finkel, T., and Goldschmidt-Clermont, P.J. (1997). Mitogenic signaling mediated by oxidants in Ras-transformed fibroblasts. *Science***.275**: 1649–1652.

[24]. **Wang, C.S.**, Chang, T.T., Yao, W.J., Wang, S.T., and Chou, P.(2012).Impact of increasing alanine aminotransferase levels within normal range on incident diabetes. *Journal of the Formosan Medical Association.* **111**(4): 201–8.

[25]. **Mustafa, K.,** Yusuf, S., Kazım, U., Muhammet, D.,Sayit, A., İrfan, Y., and Halil I.K. (2015).Effect of Sodium Cyanide on Antioxidant Enzyme Activities and Lipid Peroxidation in Some Tissues of Mirror Carp (*Cyprinus carpio)*.*Pakistani Journal of Zoology*. **47**:1777-1782.

[26]. **Okolie, N.P.**, and Uanseoje, S.O. (2013). A comparative study of the toxic effects of prolonged intake of cassava-borne organic cyanide and inorganic cyanide in some rabbit tissues. *Journal of Pharmaceutical and Scientific Innovation.* **2**: 65-69.

Ukwuonwo Ada Chidimma, et. al. "Effect of Ethyl Acetate Extract of Annona Muricata (Sour Sop) Leaf on Cyanide Induced Tissue Lesions In Rabbits Of The Newzealand Strain." *IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB),* 6(4), (2020): pp. 01-12.