

Histopathological changes induced in liver, kidney, heart and pancreas of rabbits by prolonged oral cyanide exposure

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Abstract: The aim of the present study was to determine the deleterious effects of prolonged oral cyanide insult on various organs and tissues in rabbits. For this purpose, 12 locally bred adult male rabbits were allocated into two groups of 6 viz. control and experimental. Rabbits in control group were offered feed only while the rabbits in experimental group received feed plus potassium cyanide (KCN) at 3 mg/kg body weight orally for a period of 40 days. None of the rabbit in both the groups demonstrated any of the gross changes in any organ on postmortem examination. Liver was normal in size, shape, texture and color. Kidneys were also normal in size and color. Histopathological examination revealed severe hepatocyte vacuolation and degeneration in liver of rabbits in experimental group. There was also excessive congestion in liver and bile duct of rabbits in experimental group. Kidneys of rabbits in experimental group demonstrated severe glomerular and tubular necrosis and congestion. In the tubular epithelial cells, pyknotic nuclei were also present. On the other hand, heart and pancreas of rabbits in both control and experimental group did not show any histopathological change in microscopic structures. In conclusion, prolonged oral cyanide administration could have harmful effects on liver and kidney functions.

Keywords: Cyanide, rabbits, intoxication, liver, kidney, histopathology.

INTRODUCTION

Cyanide (CN⁻) is largely disseminated in the ecosystem and is linked to toxic effects in animals and humans. Cyanide poisoning can occur due to exposure from dietary sources, environmental effluence, work-related exposure, chemical warfare, deliberate ingestion, homicide, and sometimes by using drugs of plant origin like nitroprusside and laetrile (Way *et al.*, 1984; Watts, 1998).

Cyanide is used extensively with an approximate global consumption of about 1.5 million tons per annum. It is used in the synthesis of certain chemicals where there is need to add carbon atom to a molecule. Its massive use is in the production of adiponitrile, precursor of nylon. In gold mining and electroplating industries, sodium and potassium salts of CN⁻ are used. In animal feed mills, CN⁻ is used for the synthesis of methionine and other amino acids. Cyanide is also liberated during burning of nitrogen based compounds like melamine, nylon etc (Cummings, 2004).

Cyanide intake may cause central nervous system (CNS) syndromes and damage to thyroid in animals and humans as well (Soto-Blanco *et al.*, 2005). Sorghum grazed cattle, horses and sheep exhibited ataxia and also urinary incontinence was observed in horses (McKenzie and

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McMicking, 1977; Bradley *et al.*, 1995). Prolonged CN⁻ exposure has also been associated with reduced growth rate in animals (Tewe *et al.*, 1984; Okolie and Osagie, 1999; Soto-Blanco *et al.*, 2001), disturbance in thyroid metabolism (Philbrick *et al.*, 1979; Okolie and Osagie, 1999), lesions in liver, kidneys, lungs (Okolie and Osagie, 1999, 2000) and also CNS pathology (Soto-Blanco *et al.*, 2002a; Soto-Blanco *et al.*, 2002b). This paper describes the histopathological changes induced in different organs of rabbits by prolonged oral cyanide intoxication.

MATERIALS AND METHODS

Study design

A total of 12 adult mixed breed rabbits of approximately same age and body weight supplied by University Diagnostic Laboratory, University of Veterinary and Animal Sciences, Lahore, Pakistan were used for histopathological studies. Experimental protocol was approved by Ethical Review Committee of the University. Rabbits were housed singly in separate clean metal cages and were kept under standard farming practices and were provided with feed (Singh, 2005) for two weeks before the start of the experiment for acclimatization purpose. The rabbits were then divided into two groups viz. control and experimental each comprising of 6 rabbits. Rabbits in control group were given only feed and served as a control negative while rabbits in experimental group were given feed and KCN at 3mg/kg/day orally diluted in

distilled water for a period of 40 days. Feed was given at 90g/kg/day while clean drinking water was provided *ad lib*. The decayed feed leftovers were removed and discarded on regular basis. During the experimental period, rabbits were closely examined for any clinical abnormality.

Postmortem examination

At the end of day 40, rabbits from each group were euthanized by cervical dislocation to perform postmortem examination. Liver, kidneys, intestine, stomach, lungs, heart, brain and other tissues were observed for any gross change.

For histopathological study different organs were excised quickly and rinsed in cold physiological saline (0.9%). Slices of no more than 5 mm thickness from liver, kidney, heart and pancreas were fixed in 10% buffered formalin solution (Okolie and Osagie, 1999).

Histopathological studies

Tissue samples of liver, kidney, heart and pancreas were fixed in 10% buffered formalin and were processed for histopathological examination. A routine method of dehydration in ascending series of ethanol, clearing with xylene and embedding in paraffin was used. Sections of 5 µm thickness were sliced using microtome and were stained with Hematoxylin and Eosin stain (Bancroft and Gamble, 2007).

RESULTS

None of the rabbit in control and experimental groups demonstrated any of the gross changes in any organ. Liver was normal in size, shape, texture and color. Kidneys were also normal in size and color. Lungs and brain were also normal in color and consistency. Heart, intestine, pancreas and other organs also showed normal gross structures.

Liver of rabbits in control group, showed normal morphological pattern of all histological structure. A normal lobular structure of liver with hepatocytes having centrally placed nuclei and normal cytoplasm was observed. No deviation of morphology of portal triads and sinusoidal spaces from the normal pattern was noticed. Similarly, a normal histological pattern having clear glomerular spaces and normal tubular structure was observed in kidneys of rabbits in control group. Likewise, heart of rabbits in control group did not show any histopathological change in microscopic structures. Pancreatic tissue of rabbits in control group also demonstrated normal histological pattern of various structures.

On the other hand, severe hepatocyte vacuolation and degeneration was present in liver of rabbits in experimental group (Plate 2). There was also excessive congestion of liver (Plate 3) and bile duct hyperplasia of rabbits in experimental group (Plate 1). Kidneys of rabbits

in experimental group demonstrated severe glomerular and tubular necrosis and congestion (Plate 4). In the tubular epithelial cells, pyknotic nuclei were also present (Plate 5). Whereas, heart of rabbits in experimental group did not show any histopathological change in microscopic structures (Plate 6). Likewise, the pancreatic tissue of rabbits in experimental group also demonstrated normal histological pattern of different microscopic structures.

DISCUSSION

Severe degenerative changes in liver of rabbits in experimental group were observed compared to control group which showed normal morphological patterns. Damage to the liver, characterized by periportal hepatocellular vacuolation, has been observed in dogs fed on cassava (Kamalu, 1993). The same type of histopathological lesion was observed in liver of KCN treated rabbits (Okolie and Osagie, 1999) and rats (Sousa *et al.*, 2002), while goats exhibited only minimal liver degenerative changes (Soto-Blanco *et al.*, 2005, 2008). Focal areas of congestion and necrosis were observed in the liver of CNĬ fed rabbits (Okolie and Osagie, 1999). Alterations in AST activity in KCN treated rats were observed, which could be consequence of hepatic lesion (Sousa *et al.*, 2002). Similarly, degenerative liver necrosis and loss of the typical pattern of hepatocytes were reported in rainbow trout exposed to CNĬ (Dixon and Leduc, 1981). According to Schmidt (1978), increased serum ALT activity was a consistent indicator of damage to hepatocytes. In addition, elevation in serum ALP activity has been associated with focal degeneration of liver following chloroquine treatment (Ngaha *et al.*, 1989) and halothane anesthesia (Watanabe *et al.*, 1988). Results of histopathology analysis indicated that the amount of CNĬ ingested may have exceeded the CNĬ detoxification ability of the rhodanese system, resulting in chronic CNĬ toxicosis in CNĬ treated rabbits (Okolie and Osagie, 1999). This conclusion is supported by degenerative changes exhibited in CNĬ treated rabbits in this study.

Kidneys of rabbits in the experimental group demonstrated severe glomerular and tubular necrosis. Pyknotic nuclei were present in tubular epithelial cells, whereas a normal histological pattern was observed in kidneys of control group rabbits. These finding were consistent with those of Okolie and Osagie (1999) who also reported serum levels of urea and creatinine as well as urinary thiocyanate significantly higher in CNĬ treated rabbits compared to control values. Okolie and Osagie (1999) also found tubular and glomerular necrosis of kidney after long-term CNĬ administration to rabbits. Nephrosis has been observed in cassava fed dogs (Kamalu, 1993) and rats (Ononogbu and Emole, 1978), as well as humans (Clark, 1936). In rats fed KCN, histological analysis revealed alterations in kidney tissue, characterized by congestion and cytoplasmic vacuolation of epithelial cells of proximal tubules (Sousa *et al.*, 2002).

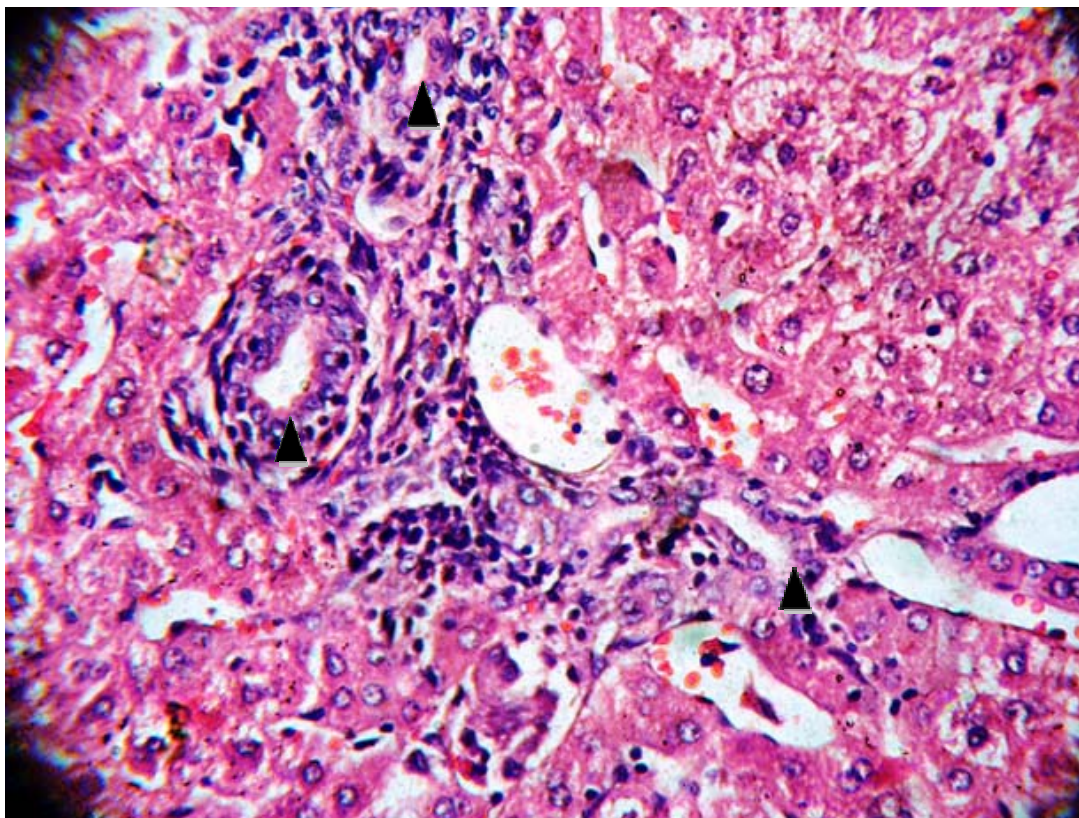


Plate 1: Section of the liver from rabbit in Experimental group showing bile duct hyperplasia (H & E staining 400X).

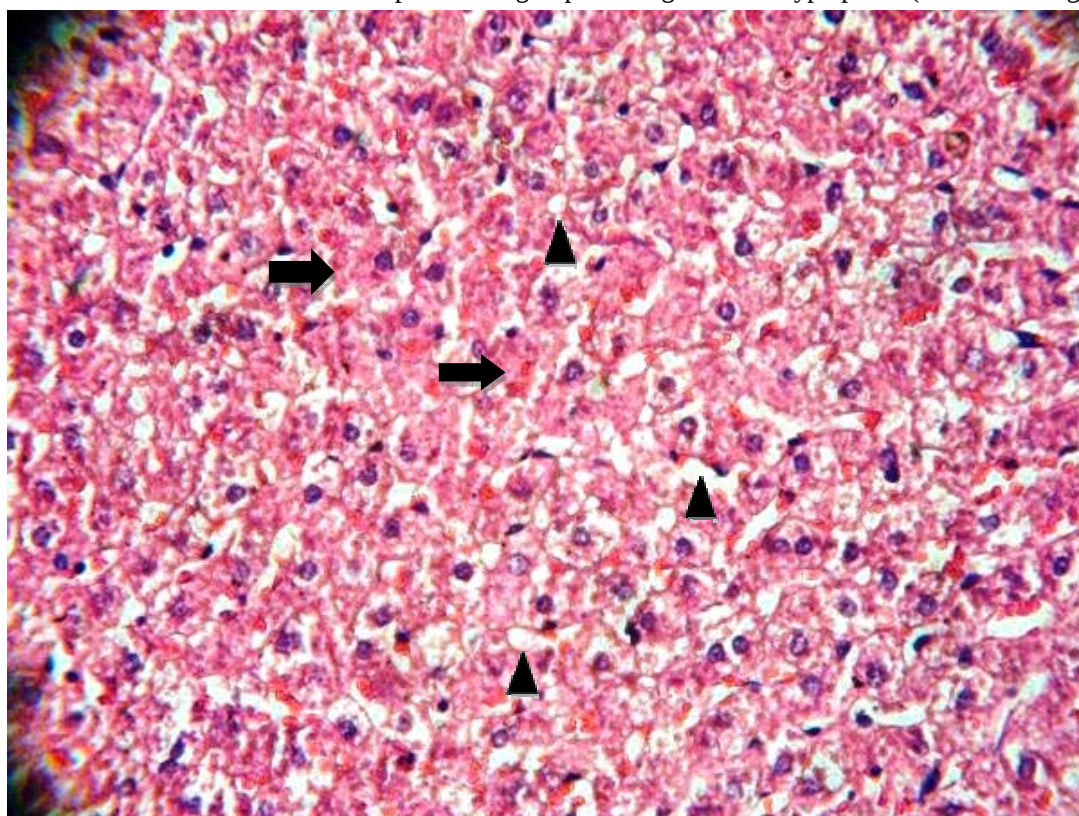


Plate 2: Section of the liver from rabbit in experimental group showing congestion (arrows) and vacuolation (arrow heads) (H & E staining 200X).

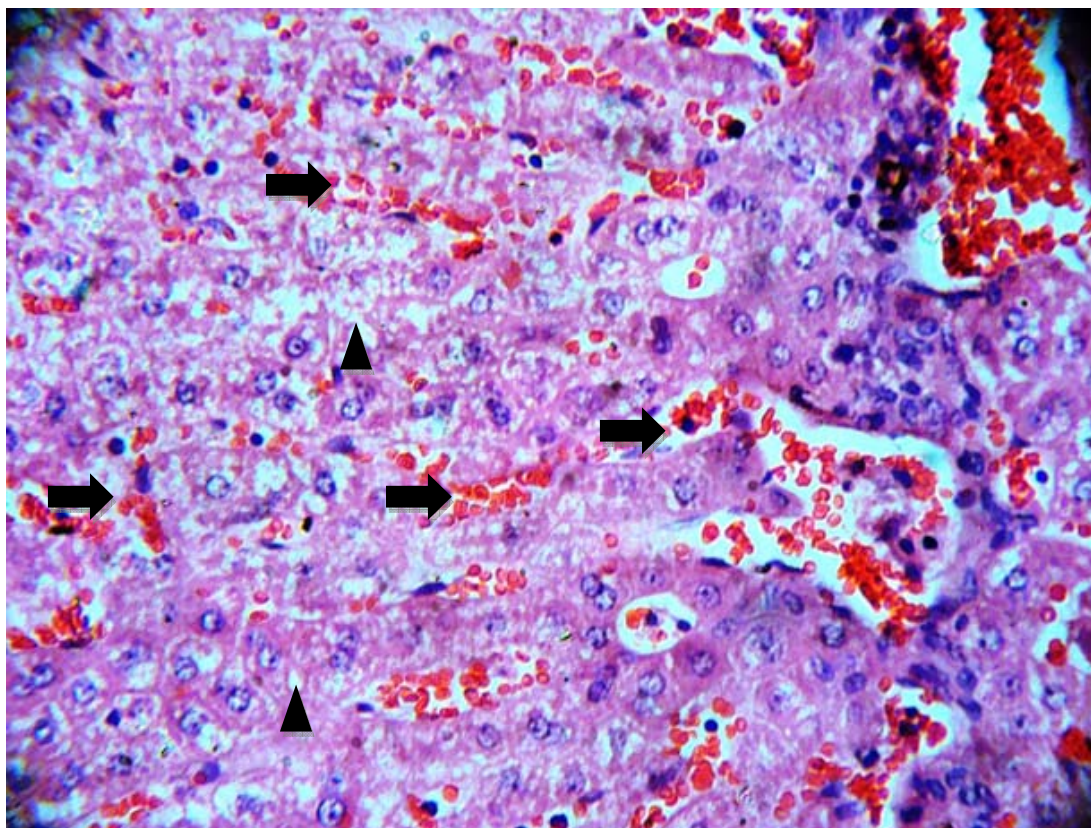


Plate 3: Section of the liver from rabbit in experimental group showing congestion (arrows) along with foci of necrosis (arrow heads) (H & E staining 400X).

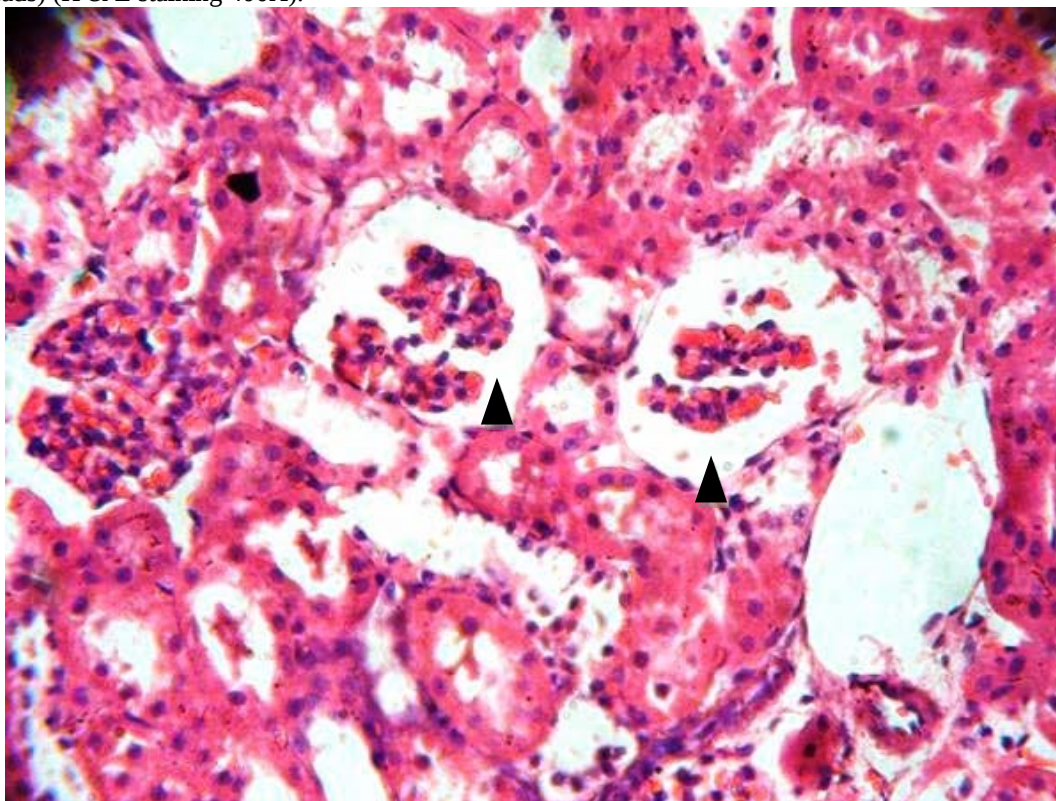


Plate 4: Section of the kidney from rabbit in experimental group showing degenerative changes in glomeruli (H & E staining 600X).

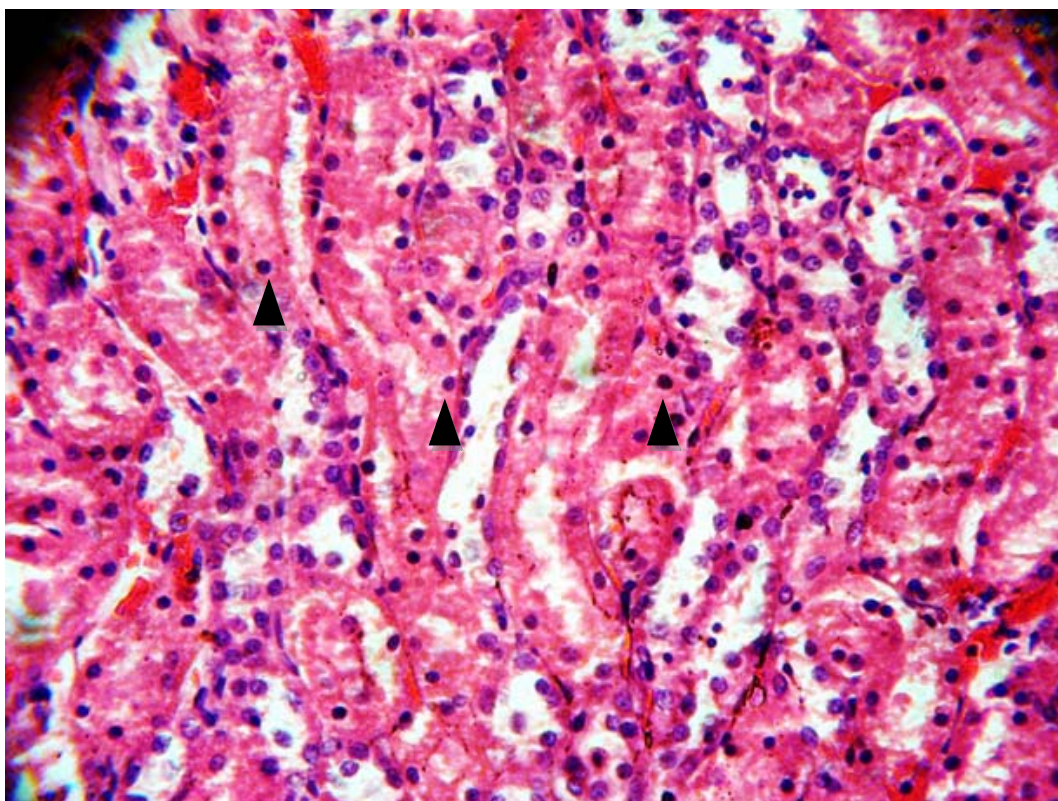


Plate 5: Section of the kidney from rabbit experimental group showing pyknotic nuclei (arrow heads) in tubular epithelial cells (H & E staining 600X).

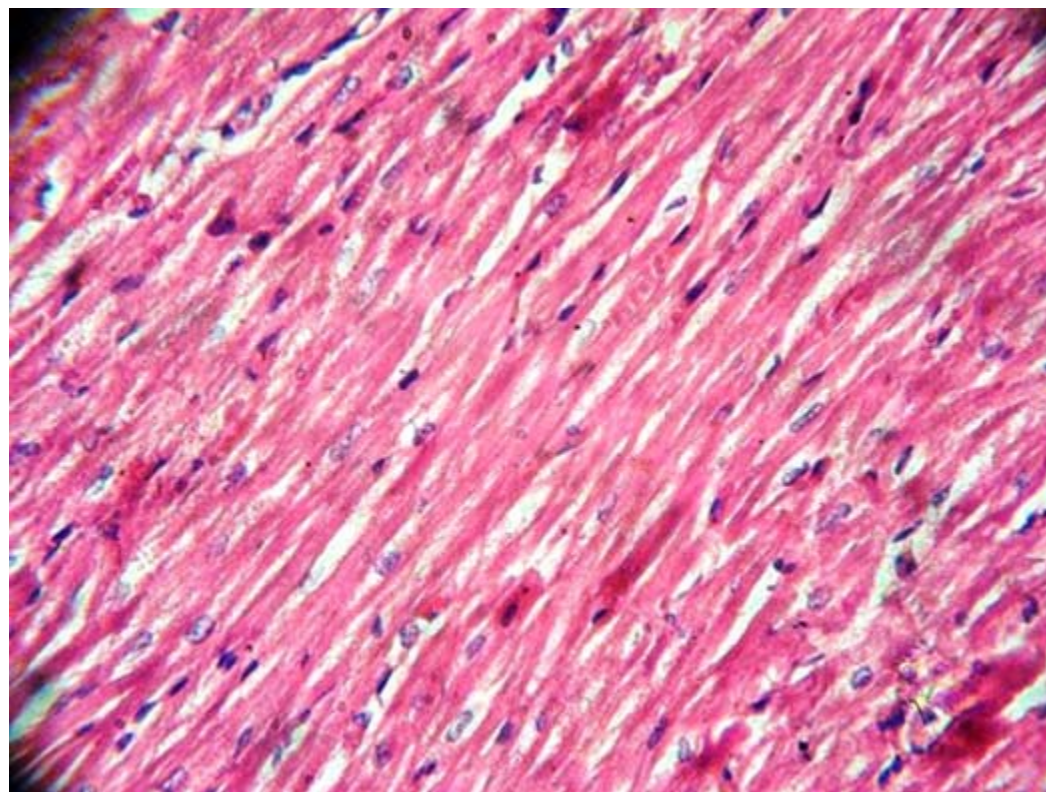


Plate 6: Section of the heart from rabbit in experimental group showing normal histological architecture (H & E staining 400X).

Humans chronically exposed to CN⁻ in the form of cassava consumption exhibited disorders of glucose metabolism and fibrocalculous pancreatic diabetes or malnutrition-related diabetes (McMillan and Geevarghese, 1979; Kamalu, 1995; Petersen, 2002). In the present study, histological examination of the pancreas revealed no microscopic lesions. These data were in agreement with the findings of others (Kamalu, 1991; Akanji and Famuyiwa, 1993; Okolie and Osagie, 2000; Soto-Blanco *et al.*, 2001, 2002a, 2005, 2008). These results also suggested that CN⁻ do not have a diabetogenic effect in rabbits. The pancreato-toxic effect of CN⁻ occurs mainly in malnourished animals (Morrison *et al.*, 2006). According to Yessoufou *et al.* (2006), cassava fed rats did not exhibit diabetes, but found cassava a factor that aggravated diabetes, if associated with insufficient methionine.

The results of the present study differ from other studies that showed histological evidence of islet cell hyperplasia in rats fed for 16 weeks on pure cassava (Izokun-Etiobhio, 1989). The reason may be that cassava is low in protein and lacks sulfur amino acids, which are required for CN⁻ detoxification (Cliff *et al.*, 1985). In the present study, rabbits were fed a balanced diet. Tropical malnutrition diabetes has been reported to affect adolescents on a protein deficient diet (McMillan and Geevarghese, 1979). Thus due to insufficient sulfur amino acids, CN⁻ may have toxic effects on the pancreas, leading to diabetes (Okolie and Osagie, 2000). Kamalu (1995) suggested that not CN⁻ but linamarin (major cyanogenic glycoside of cassava) inactivate Na⁺, K⁺ ATPase in pancreas to produce diabetes.

Heart of rabbits in both CN⁻ and control group did not show histopathological changes under microscopic examination. Okolie and Osagie (2000) also reported no pathological changes to heart tissue from a CN⁻ group in relation to controls.

In conclusion, prolonged oral cyanide exposure has deleterious effects on liver and kidneys while it does not have damaging effect on pancreas and heart of rabbits.

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