DRUG DISCOVERY

Ameliorative effect of a multi-nutrient-rich product against cyanide induced hepato-renotoxicity

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ABSTRACT

The present study was designed to investigate the protective effects of trevo, on potassium cyanide-induced hepato- and nephrotoxicity in male Wistar rats. Rats received Trévo (2 ml) after cyanide (0.5 mg/kg) exposure. Twenty-four hours after last administration rats were sacrificed and blood, liver and kidney tissues collected for haematological (neutrophils, lymphocytes, monocytes, eosinophils, basophils, white blood cells (WBC) and parked cell volume (PCV)), biochemical and histological assays. Our results shows that trevo significantly reverse the increased activity of alanine aminotransferase (ALT) and alkaline phosphatase (ALP) induced by cyanide while it had no significant effect on aspartate aminotransferase (AST) induced increased ($P<0.05$) at the various time of trevo administration. Trevo also significantly decrease the concentration of urea as compared to cyanide group while it caused an insignificant effect in creatinine concentration in comparison to cyanide only group. The alteration in all the hematological parameters analysed caused by cyanide was significantly reversed by treatment with trevo ($P<0.05$) at the various time of administration. Histology showed that cyanide caused pathological lesion in the hepatocyte as observed with increased bile deposition and fatty droplets and reduction in hepatocyte density which was reversed by treatment with trevo. In the nephrocyte, cyanide caused no pathological lesion in the kidney tissue, neither did administration of trevo had any pathological effect. The reversal of cyanide-induced hepato- and nephrotoxicity by trevo, shows that it can be administered as an antidote against cyanide exposure, especially when administered immediately after cyanide poison.

Keywords: cyanide; phyto-nutrient; hepatoprotective; nephroprotective; histology

1. INTRODUCTION

The uses of cyanide in industries, presence in some foods and environmental contaminants makes it one of the chemicals human are often exposed to [1]. Some of its industrial uses include - mining of gold, synthesis of organic compounds, electroplating and buffing [2]. Cyanide is a highly toxic compound, and its toxicity is often initiated via alteration in oxygen metabolism through cytochrome C oxidase inhibition. There are several approved drugs for treating cyanide poison, however, their availability and side effects are some of the limitation [3,4]. These have led to continuous search for drugs that are effective, low or zero toxicity and readily available for the treatment of cyanide poison. The liver is one of the biggest organs in the human and it is involved in various metabolic processes. It is involved in the catabolism and anabolism of important nutrients for physiological processes, drugs and other chemicals are metabolized and processed for excretion in the liver, prevents the accumulation of toxic chemicals in the body through detoxification and elimination from the body [5,6]. The role of kidney in filtering and excretion of drug and toxic compounds makes it one of the organs prone to injury from chemical exposure [7]. Apart from the above functions, the kidney also plays important role in homeostasis processes, such as electrolyte balance, blood pressure, water and metabolite concentration [8]. The role of kidney in excretion, filtering and excretion of drug and toxic compounds, makes it a potential target for drug and chemical toxicity [9]. Nature has provided human and animals with plethora of plants that can be used to treat various infections and poison. Investigations have shown that more than 80% of human population depends on these plants for survival [10]. With studies confirming the therapeutic effects of some of these plants in the management and treatment of several ailments, scientists were able to identify the active phytochemicals present in the plants and developed them into various nutraceutical and pharmaceutical products [11]. These group of phytochemicals are generally safe and work by acting as antioxidant, anti-inflammatory and improving the host-defense system and they include, polyphenols and flavonoids. Trévo is an America product that contains several bioactive phytochemicals, vitamins and minerals that are uniformly mixed to give a standard phytonutrient-rich product [12]. [13] reported that some of the health benefits of trevo includes improving cardiovascular, immune and digestive systems as well as regulating cholesterol, blood sugar, pH level. Some investigations has reported to possess hepatoprotective and cardioprotective properties which can be linked to the presence of various phytochemicals such as phenolic acid, coumarin, flavonoids, stilbenes, alkaloids, tannins and lignans (trevo, 2020). The toxicity of cyanide via alteration in oxygen metabolism makes it a chemical that can damage most of the organs in the body [14] as they all requires oxygen for efficient performance.

2. MATERIALS AND METHODS

Chemicals and Reagents

All reagent kits were purchased from Sigma-Aldrich, Germany. Trèvo was a product of TrèvoTM LLC, Oklahoma City, USA. Other chemicals were of analytical grade.

Experimental Animals

All the animals used for the experiment were in accordance with the protocol provided by Basic and Clinical Pharmacology and Toxicology policy for experimental and clinical studies [15] and approved (APN:0418062019) by the departmental committee on animal ethics (Department of Biochemistry, Faculty of Science, Federal University Otuoke, Bayelsa state, Nigeria). Animals with average weight of 200 g were used for the experiment. They were placed in a well-regulated room that is alternated between 12 light and dark cycle. They were fed on rat pellets and water ad libitum.

Experimental Protocol

Twenty-four animals divided into four groups of six rats each as follows: [I] Normal control (administered distilled water); [II] negative control (administered a single dose of KCN): [III] treatment group (administered KCN and Trèvo (5 min later)) and [IV] treatment group 2 (administered KCN and trèvo (60 min later)). Animals were sacrificed 24 h after administration of trèvo via mild anaesthetic using chloral hydrate. KCN was administered at 5 mg/kg per body weight (bwt), which according to [16] induced moderate to severe toxicity with little lethality. The effective dose of trèvo (2 mL/kg bwt) was based on an unpublished pilot study. Administration of trèvo was based on method described by [16]. Prior to sacrifice, the rats were denied food overnight. Unconsciousness was induced by exposing them to chloral hydrate and the blood collected through cardiac puncture into serum bottles for biochemical and hematological assays.

Hematological Analysis

White blood cell (WBC) count and differential count (Eosinophil, monocyte, lymphocyte, neutrophil and PCV) were assayed by an electronic automate Coulter MAXM (Beckman Coulter, Inc., Fullerton, CA).

Serum Biochemistry

Serum Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea and creatinine kit were assayed spectrophotometrically using commercial kits purchased from Bio Diagnostic Co., according to the manufacturer's protocol.

Histology

Histopathological assay: Histopathological examination of the kidney and liver isolated from experiment rats were carried out by fixing them in 10% formalin solution, prior to preparation for staining and assessing under a microscope with x100 magnification. Slides were captured with a camera attached to the microscope and then analyzed for histopathology according to the method described by [17].

Statistical Analysis

Data were organized, tabulated, and statistically analyzed using the SPSS Inc. Released 2007. SPSS for Windows, Version 16.0. (SPSS Inc., Chicago, USA). For quantitative data, the mean and SD were calculated and were expressed as mean ± standard deviation and were analyzed using Analysis of Variance (ANOVA). For comparison of means of more than two groups, the F-test was used. Statistical significance was taken at a P value of less than 0.05.

3. RESULTS AND DISCUSSION

The effect of trèvo and cyanide on hematological parameters is presented in table 1. The results showed that cyanide caused a significant increase in neutrophil, lymphocyte, monocyte , eosinophil, basophil and white blood cell (WBC) count as compared to the unexposed rats ($P<0.05$). While there was a decrease in PCV count, though not significant at $P<0.05$. The table shows that early administration of trevo caused a significant reversal of the effect of KCN on the hematological parameters. Hematology test is done to determine the health status of the host. There is a strong correlation between cyanide increase in the blood with respect to high WBC and anemic condition [18]. A decrease in RBC is an indication of anemic and inability of the host to utilize oxygen efficiently, while an increase in WBC reflects infection and the host response to combat it [19] in the work of [20]. Our results supported the reported toxicity of cyanide through its binding to hemoglobin, thereby causing low concentration of RBC [21, 22]. Phytochemical rich plant and products have being shown to be effective in combating the damaging effect of toxicants including cyanide [23], which are often measured by the level of different blood parameters (This test have been shown to be a good determinant of homeostatis status as a reflection of healthy state of human). They have been shown to improve and boost the immune system and increase RBC [24]. As observed in our result, trèvo was able to increase WBC volume as well as decrease WBC and other hematology parameters measured. The protective effect of trevo might be as a result of trevo reacting with cyanide, thereby preventing or limiting their destruction of RBC and stressing the host immune system. Another possibility is the potential of trevo to increase RBC production and aid the body in attacking radicals that can cause oxidative stress in the body.

Table 1. Effect of trèvo on hematology following cyanide poisoning

Group	Neutrophils ($x10^3/mm^3$)	Lymphocytes ($x10^3/mm^3$)	Monocytes ($x10^3/mm^3$)	Eosinophils ($x10^3/mm^3$)	Basophils ($x10^3/mm^3$)	WBC ($x10^3/mm^3$)	PCV (%)
Control	4.93±0.23	3.74±0.45	0.33±0.014	0.22±0.017	0.00±0.00	9.00±1.58	32±1.22
KCN	5.98±0.35*	5.89±0.58*	0.83±0.52*	0.42±0.05*	0.9±0.1*	10.8±1.33*	29.50±1.05
KCN+trevo (5 min)	5.2±0.34#	3.83±0.31#	0.60±0.07#	0.33±0.03#	0.4±0.02#	9.64±0.78#	32.2±1.92
KCN+ trevo (60 min)	5.37±0.50#	3.91±0.41#	0.84±0.04	0.36±0.05#	0.7±0.10	9.5±0.88#	29.5±2.58

Values are expressed as Mean±SD (n=6). * $P<0.05$ (control vs KCN), # $P<0.05$ (KCN vs trèvo)

Hepatoprotective effect of trevo against cyanide toxicity

Figure 1 and 2 shows the effect of trevo on serum level of AST, ALT and ALP respectively following KCN intoxication in male wistar. There was a significant increase in AST concentration of animals administered. KCN only as compared to the animals in the control group ($P<0.05$). The figure also showed that administration of trevo after 5 minutes of exposure to KCN caused a significant decrease in serum level of AST when compared to the group administered only KCN. Administration of trevo after 1hr of KCN intoxication also decrease creatinine concentration, however it was not significant ($P>0.05$). Figure 1 also showed that cyanide did not have a significant effect on serum level of ALT ($P>0.05$). With respect to ALP, it was observed that KCN caused a significant

increase in ALP activity in the serum of animals administered KCN only as compared to the animals in the control group (P<0.05). Figure 2 also showed that administration of trevo after 5 minutes and 1h of exposure to KCN cause a significant decrease in serum activity of ALP, when compared to the group administered only KCN (P<0.05).

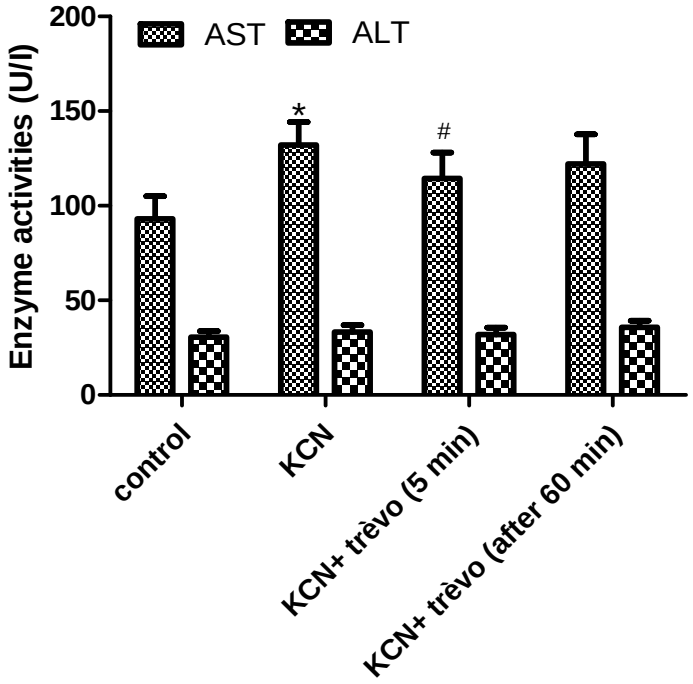

Figure 1. Effect of trèvo on serum activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) following KCN intoxication in male Wistar. Results are expressed as mean±SD (n=6). * P<0.05 (control vs KCN), # P<0.05 (KCN vs trèvo)

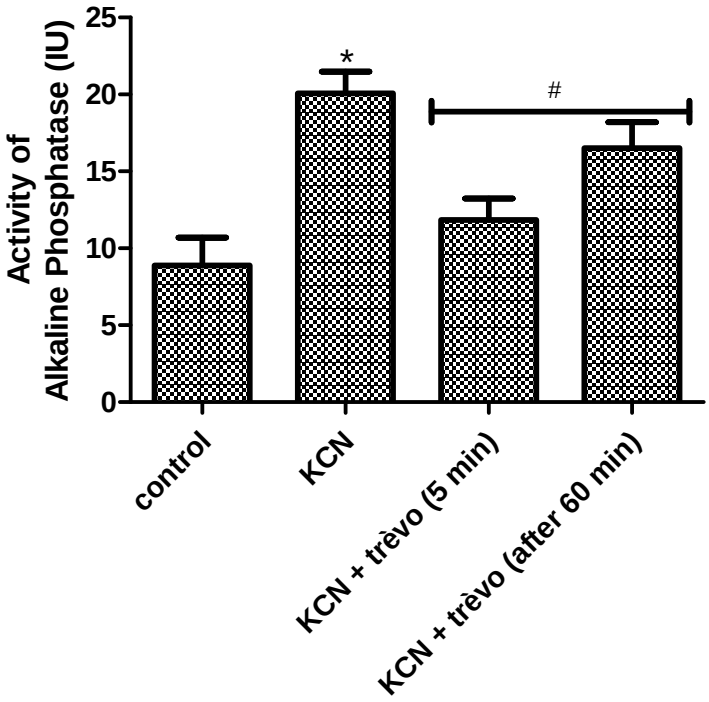

Figure 2. Effect of trèvo on serum activity of alkaline phosphatase following KCN intoxication in male Wistar. Results are expressed as mean±SD (n=6). * P<0.05 (control vs KCN), # P<0.05 (KCN vs trèvo).

Administration of trevo significantly reverse the serum markers of renal toxicity induced by KCN. Figure 3 shows the effect of trevo on serum level of creatinine and urea following KCN intoxication in male wistar. There was a significant increase in creatinine concentration of animals administered KCN only as compared to the animals in the control group (P<0.05). The figure also showed that administration of trevo after 5 minutes of exposure to KCN caused a significant decrease in serum level of creatinine when compared to the group administered only KCN. Administration of trevo after 1hr of KCN intoxication also decrease creatinine concentration, however it was not significant (P>0.05). With respect to urea, it was observed that KCN caused a significant increase in urea concentration of animals administered KCN only as compared to the animals in the control group (P<0.05). The figure also showed that administration of trevo after 5 minutes and 1h of exposure to KCN cause a significant decrease in serum level of urea, when compared to the group administered only KCN (P<0.05). However, comparing the time of administration, early administration of trevo significantly decrease urea concentration as compared to late administration of trevo (P<0.05).

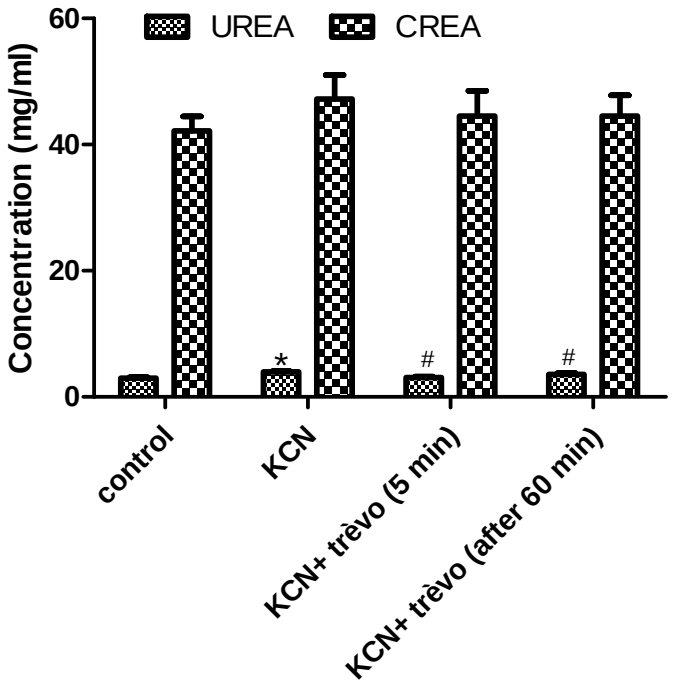

Figure 3. Effect of trèvo on serum level of urea and creatinine following KCN intoxication in male Wistar. Results are expressed as mean±SD (n=6). * P<0.05 (control vs KCN), # P<0.05 (KCN vs trèvo).

As observed in various publications, liver damage are often marked by an increase in the AST, ALT and ALP levels in the serum [25, 26]. In most cases, the liver injury is often signified by increase in at least two of the marker enzymes. In our experiments, cyanide caused a significant increase in AST and ALP while it has no significant effect on ALT level. [2] showed that cyanide increase the level of ALT and ALP, which was contrary to our results, while [21] reported a significant increase in all the enzymes. The different in the results might as a result of several factors, such as dosage, route and duration of administration [27, 28, 29]. Kidney is another organ that is also susceptible to cyanide toxicity [30]. An elevated level of urea and creatinine are the major indicators of renal damage [31, 32]. In our result, cyanide induced significant increase in urea and creatinine, in tandem with other report on nephrotoxicity of cyanide [33, 2]. In rats with normal renal function, urea and creatinine are filtered from the blood and excreted from the body, thus the observed increase in blood urea and creatinine are prove of renal damage as a result of cyanide exposure [34]. Kidney and liver related disorders have being linked to anemic condition [35, 36]. This is linked to low or deficiency of erythropoietin and iron required for the production of RBC. Phytochemical rich supplements have been shown to be effective in the management of kidney related disorders [31]. Report also shows that phytochemical rich supplements are also effective in combating toxic effects of chemicals on the kidney [25, 37, 38, 39, 40]. Trevo showed its hepatoprotective and nephroprotective effect against elevated markers of liver and kidney-induced cyanide toxicity. The therapeutic effect might not be unconnected to the presence of bioactive phytochemicals such as ellagic acid, lycopene, ascorbic acid, tocopherol, carotene which were extracted from green tea, grape seed, aloe vera, bacopa and turmeric [41]. All these phytochemicals have both hepatoprotective and nephroprotective properties [42-47].

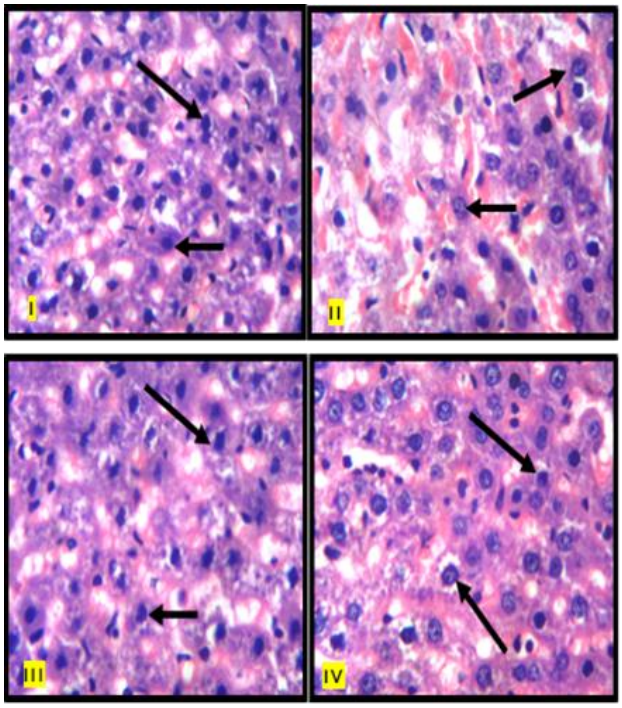

Figure 4. I: The histology of liver sample in the control group, it showed no histomorphology alteration. II: The histology of liver sample in rats intoxicated with potassium cyanide alone, it showed the hepatocyte with increased bile deposition and fatty droplets. Hepatocyte density is reduced. These suggest pathological alterations. III; The histology of liver sample in the group of animals intoxicated with potassium cyanide, followed by trevo administration after 5minutes. It showed normal histomorphology of liver tissue presenting with typical cellular density and cellular distribution. The Nuclei of hepatocytes are distinctively stained and properly disposed within their respective cytoplasm. There are no histopathological alterations in the histological presentation of these tissues. IV: The histology showed that that the hepatocyte appears characteristically normal with typical cellular density and staining intensity.

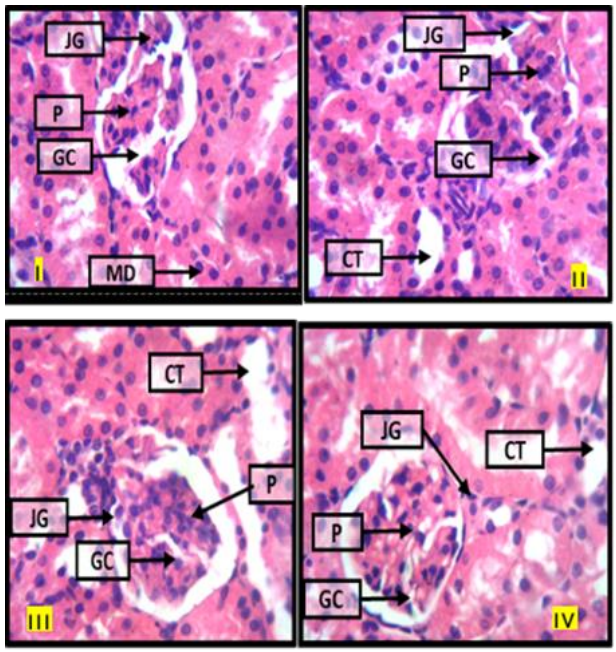

Figure 5. I: The histology showed normal renal corpuscle with typical cellular delineation, distribution, density and staining intensity. The urinary space appears characteristically normally with no vacuolations or cellular erosion. No apparent histopathological alteration. II: The histology showed normal renal corpuscle with typical cellular delineation, distribution, density and staining intensity. The urinary space appears characteristically normally with no vacuolations or cellular erosion. No apparent histopathological alteration. III: The histology showed normal renal corpuscle with typical cellular delineation, distribution, density

and staining intensity. The urinary space appears characteristically normally with no vacuolations or cellular erosion. No apparent histopathological alteration. IV: The histology showed normal renal corpuscle with typical cellular delineation, distribution, density and staining intensity. The urinary space appears characteristically normally with no vacuolations or cellular erosion. No apparent histopathological alteration. I (Control); II (KCN); III (KCN+ Trevo [after 5 min]); IV (KCN+ Trevo [after 60 min]); JG (Juxya-glomerular Cell); P (Podocyte); GC (Glomerular capillary); MD (Macula dense cell); CT (Convoluted tubules).

Histopathological examinations also confirmed the biochemical and hematological parameters obtained from the experiment. This corroborated the report of [48], who reported the degeneration of liver and kidney tissues as a result of cyanide exposure. However treatment with trevo caused a significant reduction in tissue degeneration.

4. CONCLUSION

In summary, our results showed that cyanide induce significant alteration in hematological parameters as well as biochemical changes in both liver and kidney functions which was supported with histological analysis. Trevo was able to reverse the assault of cyanide on the tissues. These protective effects might be linked to the presence of various bioactive phytochemicals and phytonutrients present in it, which have been reported to possess antioxidant, anti-inflammatory, chelating and restorative activities. Further work can be done to investigate the effect of the supplement on long-term exposure to cyanide poison.

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O.B.I. and A.M.B. conducted the research and prepare the manuscript.

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Conflict of Interest:
The authors declare that there are no conflicts of interests.

Peer-review:
External peer-review was done through double-blind method.

Data and materials availability:
All data associated with this study are present in the paper.

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