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Original Research Article

Effect of a Phytonutrient-Rich Product and Administration Time on Cyanide-Induced Cardiotoxicity

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ABSTRACT

Exposure to cyanide can cause tachycardia, low heartbeat, and cardiac arrest. Trèvo® is a phytochemical-rich product reported to reduce aging and improve immune system. We investigated the ability of trèvo to mitigate the cardiotoxicity of cyanide in male Wistar rats. Twenty-four animals divided into four groups of six animals per group were used for the experiment. Group I (administered distilled water (orally); group II (administered 5 mg/kg bwt KCN [orally]); group III (administered 5 mg/kg bwt KCN [orally] and 2 mL/kg bwt trèvo [orally] after 5 min of exposure to cyanide); group IV (administered 5 mg/kg bwt KCN [orally] + 2 mL/kg bwt trèvo [orally] after 60 min of exposure to cyanide). Malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), acetylcholinesterase (AChE), cytochrome C oxidase (CCO), as well as p53 were evaluated. KCN caused a significant ($P < 0.05$) decrease in the activities of CCO, CAT, and SOD, raised the level of p53, AChE, and MDA respectively. Trèvo administered immediately after cyanide exposure suppresses the toxic effect of cyanide to various degrees. Histopathological evaluation shows that KCN did not caused any morphological damage to the heart. It can be summarized that trevo has the potential to reverse the biochemical toxicity of cyanide in the heart. There are still more work to ascertain the level of protection offered by trèvo as an antidote against cyanide poison.

Keywords: KCN, Cardiotoxicity, Natural product, Oxidative stress, p53, Cytochrome C oxidase.

Introduction

Humans can be exposed to cyanide through inhalation from fire incidents or ingestion of cyanide containing food or drinks.[1,2] Apart from these two major routes of exposures, humans working close to industries that utilize cyanide as part of the chemicals, such as plastic and foam making industries are also exposed.[3,4] Symptoms of cyanide poison include depression in breathing control, pulseless electrical activity, arrhythmia, and hypotension.[5] Some of the cardiac symptoms of cyanide poisoning includes; dysrhythmia, impaired repolarization, and cardiorespiratory arrest.[6] The sensitivity of the heart to cyanide poison is due to its dependence on ATP, which is majorly produced in the mitochondria through the electron transport system. Cyanide inhibits cytochrome C oxidase, a complex IV enzyme of the mitochondria respiratory system. Inhibition of this enzyme prevents the mitochondria from producing ATP, required by the heart to perform its function effectively, leading to the early symptoms observed in patients exposed to cyanide poison. Also, cyanide induces oxidative stress, which further damages the cardiac system. Approved drugs for treating cyanide poisoning include; hydroxycobalamin,[7] sodium thiosulfate,[1] cobinamide sulfite,[8] and sulfanegen.[9] Some of the mechanisms of action of these drugs includes; redox homeostasis, electron donor, and binding to cyanide to reduce the cyanohemoglobin level in the blood.[10] The toxic effect of cyanide is also time-dependent, that is the physiological alteration of cyanide poison normally occurs within 1 h of exposure, however, under some severe cases it might extend beyond 1 h. Tremor peaks at 15 min and 2 h post-exposure, seizure peaks at 2 h post-exposure, difficulty and labour in breathing peak at 5 min and 60 min, respectively.[11] Thus, there is a strong link between the effectiveness of treatment and duration of exposure. In the search for an antidote against cyanide poison, chemicals such as antioxidants, detoxicants, and biochemical regulators are often tested against cyanide poisoning.[10] While common antidotes, such as methylene blue and sodium thiosulfate are recommended, further search for readily available and accessible antidotes are ongoing.[12] Trèvo® is a product packaged in Oklahoma City, USA. Some of the ingredients reported by the manufacturer to be present in trèvo were sourced from plants such as Mangosteen fruit, bacopa, green tea, grape seed, aloe vera, and turmeric. These plants contain abundant natural antioxidants, some of which are ellagic acid, lycopene, ascorbic acid, α-tocopherol, and β-carotene. These compounds are reported to be responsible for the anti-aging effect of trèvo.[13,14] Some of the reported biological activities of trèvo include hepatoprotective, neuroprotective, and anticancer activities.[13,15,16] However, there have been few scientific reports to support the health claims reported by the manufacturer. We hypothesized that trèvo can mitigate the cardiotoxicity of cyanide through antioxidant action and of cytochrome C oxidase modulation. The aim of this study is to determine the antidotal effect of trèvo on cyanide-induced cardiotoxicity.

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Materials and Methods

Chemicals and Reagents
Reduced glutathione, Acetylthiocholine iodide, nicotinamide adenine dinucleotide (NADH) (Sigma-Aldrich, Germany). Trèvo was a product of Trèvo™ LLC, Oklahoma City, USA. Other chemicals were of analytical grade.

Experimental Animals
A total of twenty-four rats weighing between 180-200 g were used for the experiment. They were housed at room temperature in plastic cages and were kept under constant healthy environmental and nutritional conditions. They were fed on rat pellets and water *ad libitum*. All protocols and design were conducted according to the guidelines provided by Basic and Clinical Pharmacology and Toxicology policy for experimental and clinical studies[17] and approved (APN:0418062019) by the committee on animal care and handling, Department of Biochemistry, Faculty of Science, Federal University Otuoke, Bayelsa state, Nigeria.

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 Ilesanmi and Ikpesu,[18] 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 Ilesanmi and Ikpesu [18].

Tissue preparation
The heart was excised, blotted, dried, weighed, and rinsed with ice-cold 1.5% KCl, before homogenizing in 0.1 M phosphate buffer, pH 7.4. The homogenate was centrifuged at 12,000 rpm for 10 min using a cold microcentrifuge (IEC: CENTRA-GP8R, DJB Labcare, UK model to obtain the mitochondria fraction that was used for biochemical assays.

Biochemical Assays
Oxidative Stress
Assay kits for superoxide dismutase (SOD), catalase (CAT), and acetylcholinesterase (AChE) activities as well as malondialdehyde (MDA), and total protein (TP) were all procured from Abcam®.

Cytochrome C Oxidase
Complex IV (cytochrome c oxidase) was determined by following the oxidation of cytochrome c at 550 nm and was expressed as a first-order decay rate constant.[19]

p53
p53 was assayed, using Enzyme-linked Immunosorbent Assay (ELISA) kits, in heart tissue homogenates according to the methods of Yang *et al*.[20]

Histology
Slices of heart tissue were stained with eosin and hematoxylin and observed under an Olympus binocular research microscope (Olympus, New Jersey, USA) and captured 5.0MP Amscope Camera (Amscope Inc., USA) at x400 magnification.

Statistical Analysis
Data were organized, tabulated, and statistically analyzed using 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 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.

Results and Discussion

Effects of trèvo on AChE activity in cyanide-induced cardiotoxicity
The effect of trèvo on AChE activity in rats exposed to cyanide is presented in Figure 1. It was revealed that KCN caused a significant increase in AChE activity, but was reversed after treatment with trèvo at 5 and 60 min post exposure. Comparing the difference between time of trèvo administration showed no significant difference ($P > 0.05$).

Effect of trèvo on SOD and CAT activities in cyanide-induced cardiotoxicity
The effects of trèvo on SOD and CAT activities in cyanide-induced cardiotoxicity are presented in Figures 2 and 3, respectively. KCN caused a significant reduction in the activity of SOD ($P < 0.05$). Upon treatment with trèvo, there was a significant increase in SOD activity as compared to KCN group ($P < 0.05$). However, when the administration time for trèvo was compared, administration of trèvo after 5 minutes post-exposure to KCN had a significant increase in SOD activity as compared to administration of trèvo after 60 minutes post exposure to KCN.
Similar to SOD activity, KCN caused a significant decrease in CAT activity ($P < 0.05$) as compared to the normal control. Treatment with trèvo caused a significant increase in CAT activity when compared to the negative control ($P < 0.05$). Trèvo administered at 5 min post exposure to KCN caused a higher increase in CAT activity as compared to trèvo administered after 60 min post exposure to KCN.

Effect of trèvo on MDA levels in cyanide-induced cardiotoxicity
The Effect of trèvo on MDA levels in cyanide-induced cardiotoxicity is presented in Figure 4 below. KCN caused a significant increase in MDA concentration as compared to the normal control ($P < 0.05$). Treatment with trèvo at different time of post exposure to cyanide showed a significant decrease in MDA concentration as compared to negative control ($P < 0.05$). Comparing the concentration of MDA at 5 min and 60 min post exposure shows that treatment with trèvo after 5 min caused a higher decrease in MDA concentration as compared to trèvo administered 60 min post exposure to KCN.

Effect of trèvo on p53 levels in cyanide-induced cardiotoxicity in rats
The effect of trèvo following cyanide exposure on the concentration of p53 in the heart is presented in Figure 5 below. The concentration of p53 was significantly increased by KCN when compared to the normal control ($P < 0.05$). Treatment with trèvo caused a significant reduction in concentration of p53 as compared to the negative control ($P < 0.05$). However, the late administration of trèvo to the rats was not as effective as early administration ($P < 0.05$).

Effects of trèvo on cytochrome C oxidase activity in cyanide-induced cardiotoxicity in rats
The effect of trèvo on CCO activity in cyanide-induced cardiotoxicity in rats is presented in Figure 6 below. It was observed that KCN caused a significant decrease in CCO activity as compared to normal control ($P < 0.05$). Treatment with trèvo significantly increased the activity of CCO as compared to the negative control ($P < 0.05$). However, administration of trèvo 60 min post-exposure to KCN could not reverse KCN-induced inhibition of CCO activity.

Histology
Figure 7 shows the typical histomorphological manifestation of the cardiac myocytes. I. The myocytes appear striated and branching to join adjacent myocytes. The staining characteristic and nucleic histomanifestation are normal with no deducible pathological alterations. II: vertical sections of cardiomyocytes with striated muscular fibers, intact and typically stained nuclei. The cellular density and distribution are characteristically normal. III: appears characteristically normal with no deducible histopathological alteration. IV: The staining characteristic and nucleic histomorphology are normal with no deducible pathological alterations.

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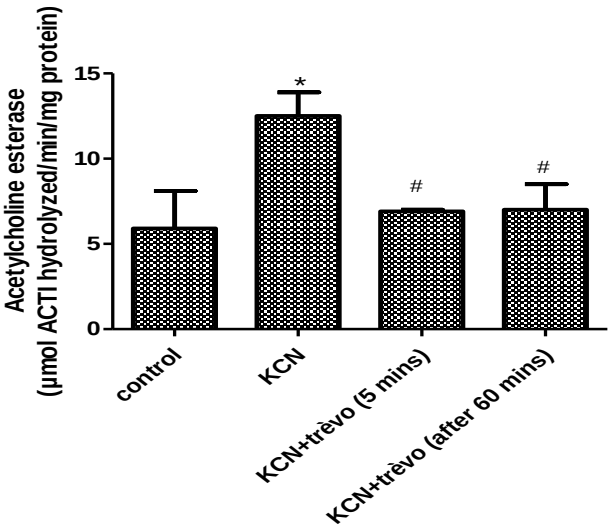

Figure 1: Effect of trèvo on acetylcholinesterase activity in the rat heart following oral administration of 5 mg/kg of potassium cyanide (KCN).
Results are expressed as mean ± SD (n = 6). *Significantly different at P < 0.05 (control vs KCN), #significantly different at P < 0.05 (KCN vs treatment group).

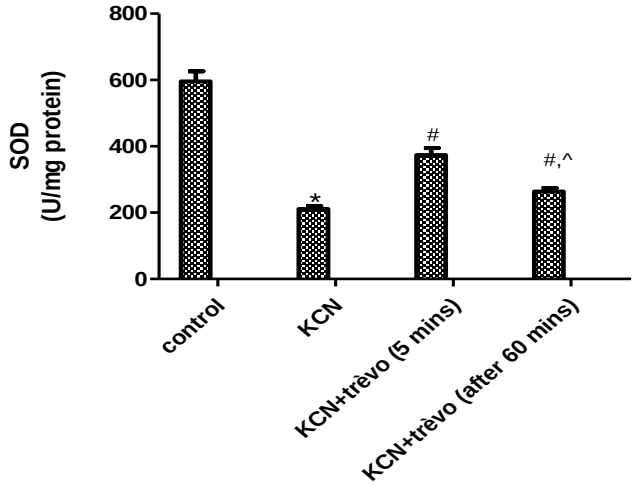

Figure 2: Reversal of cyanide-induced decrease in Superoxide dismutase activity by trèvo in rat heart.
Results are expressed as mean ± SD (n = 6). *Significantly different at P < 0.05 (control vs KCN), #significantly different at P < 0.05 (KCN vs treatment group), ^significantly different at P < 0.05 (trèvo 5 min vs trèvo 60 min).

Mechanism of cyanide poisoning is due to the inhibition of cytochrome C oxidase (CCO), an enzyme of the mitochondria respiratory chain.[20] This deprives the cell of its full compliments of ATP, resulting in cell dependence on the anaerobic source of ATP.[22,23] This affects various cardiac functions such as centrality and arrhythmia.[1,23] In this study, the ability of trèvo to ameliorate the cardiotoxicity of cyanide and the effect of the time of administration was evaluated. Our results showed that trèvo reversed the cardiac poison of cyanide through its antioxidant, antiapoptotic, anticholinesterase and increase cytochrome C oxidase activity. Also, the early administration of trèvo was more effective than the late administration.

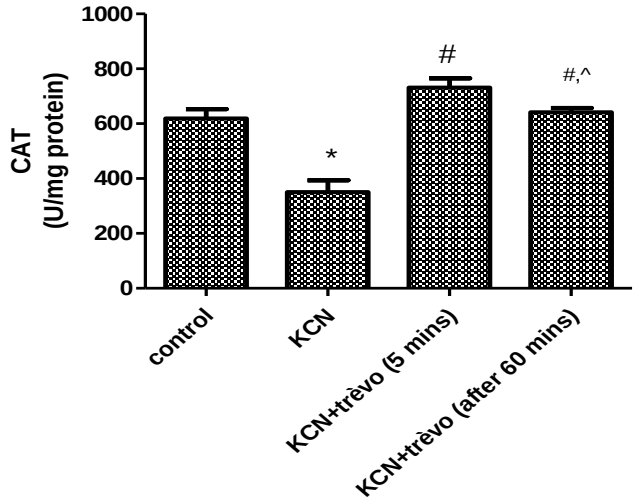

Figure 3: Effect of trèvo in cyanide-induced decrease in catalase activity in rat heart.
Results are expressed as mean ± SD (n = 6). *Significantly different at P<0.05 (control vs KCN), #significantly different at P < 0.05 (KCN vs treatment group), ^significantly different at P < 0.05 (trèvo 5 min vs trèvo 60 min).

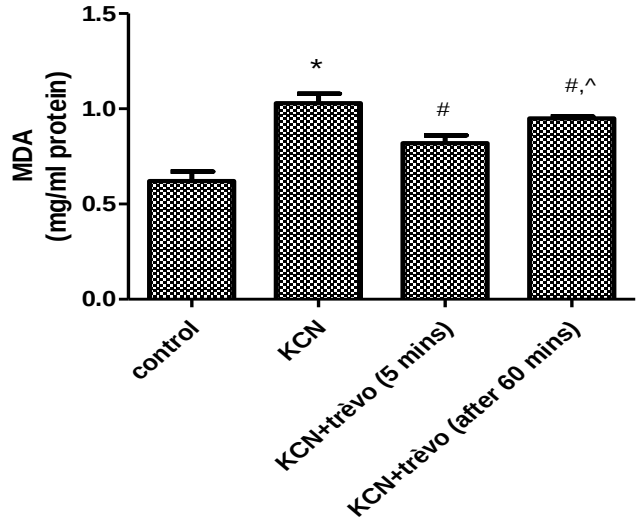

Figure 4: Effect of trèvo on cyanide-induced generation of Malonedialdehyde (MDA) in heart tissue.
Results are expressed as mean ± S.D (n = 6). *Significantly different at P < 0.05 (control vs KCN), # significantly different at P < 0.05 (KCN vs treatment group), ^ significantly different at P < 0.05 (trèvo 5 min vs trèvo 60 min).

Cyanide-induced oxidative stress occurs through the inhibition of CCO, an enzyme that catalyzes the reduction of oxygen to water with the concomitant production of ATP.[24,25] This singular act leads to partial oxidation of oxygen leading to the formation of superoxide anion and hydrogen peroxide.[26-29] This highly reactive species oxidize functional lipids and proteins, leading to cell injury and death. One of the key regulators of cell death is the tumor suppressor protein, p53,[30] this was also evaluated to check if cyanide poison can exert its cell death through p53. Our results showed that cyanide inhibits CCO, which could have been responsible for the observed increase in MDA concentration and expression of p53.[31]

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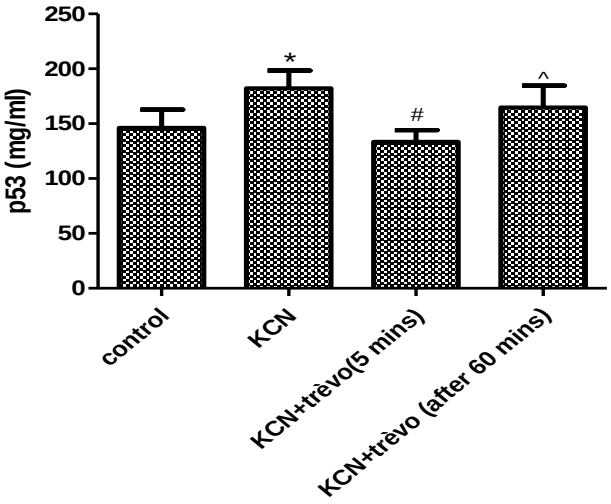

Figure 5: Effect of trèvo on cardiac p53 following oral administration of 5 mg/kg of potassium cyanide.
Results are expressed as mean ± SD (n = 6). *Significantly different at $P < 0.05$ (control vs KCN), #significantly different at $P < 0.05$ (KCN vs treatment group), ^significantly different at $P < 0.05$ (trèvo 5 min vs trèvo 60 min).

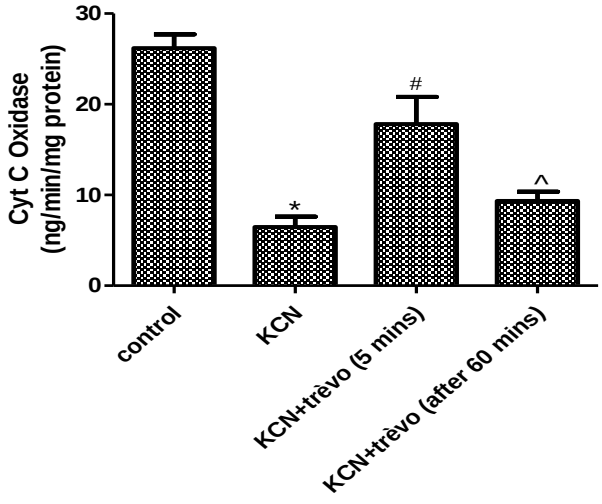

Figure 6: Effect of trèvo on cardiac cytochrome C oxidase activity following oral administration of 5 mg/kg potassium cyanide (KCN).
Results are expressed as mean ± SD (n = 6). *Significantly different at $P < 0.05$ (control vs KCN), #significantly different at $P < 0.05$ (KCN vs treatment group), ^significantly different at $P < 0.05$ (trèvo 5 min vs trèvo 60 min).

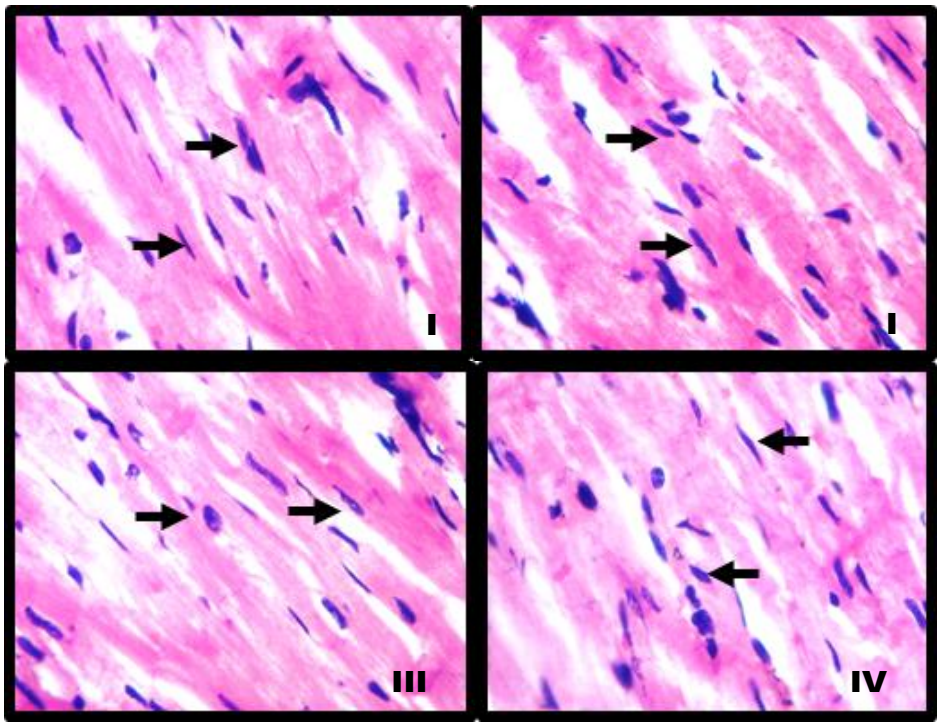

Figure 7: Representative photomicrographs of the heart wall of experimental animals (x400) revealing the myocytes. **I** (Control); **II** (KCN); **III** (KCN + trèvo (after 5 min); **IV** (KCN + trèvo (after 60 min).

Cyanide has also been reported to inhibit antioxidant enzymes and molecules such as catalase and superoxide dismutase, working in a two-way mechanism to aggravate the oxidative stress condition.[32-34] In line with this report, our results also showed the inhibition of CAT and SOD activity by cyanide in the heart. Trèvo has been reported to improve the health status of the heart, prevent cardiovascular-related diseases through its antioxidant, immune boosting, and invigorating phytonutrients. While the presence of some of these phytonutrients have not been scientifically proven, the hepatoprotective, neuroprotective, and other therapeutic effects have been reported.[15,16] Our results showed that trèvo, through its antioxidant, hormone boosting, suppression of p53, and stability of mitochondria function was able to reverse the biochemical poison of cyanide in the heart. Antioxidants such as catalase and superoxide dismutase were

increased, p53 was decreased, and AChE and MDA were decreased. All these biochemical activities showed that trèvo has the potential to be used as an antidote against cyanide poisoning. This antidote effect of trèvo might be linked to the abundance of various bioactive phytochemicals present in the product (as reported on its label). Some of these chemicals have been reported to be cardioprotective.

Histological parameters showed that the heart of rats in the control group showed normal heart tissue with normal epicardial and myocardial layer, the cardiac muscles generally are normal. There was no pathological lesion seen in the heart tissue. Exposure to KCN had no observable pathological alteration to the heart tissue, this might be as a result of acute dose, which has been reported not to have much effect on organ morphology.[35] This antidote effect of trèvo might be linked to the abundance of various bioactive phytochemicals present in the product (as reported on its label). Some of these compounds have been reported to be cardioprotective.[36-39] Though the mechanism by which trèvo reverses the toxic effect of cyanide on biochemical parameters is not well understood, we can deduce based on other reports on some of the antidotes against cyanide, that trèvo might neutralize the toxic effect of cyanide by binding to it or enhance its clearance from the animals.[1,7,9] Also, some of the natural products present in trèvo such as vitamins, flavonoids, and polyphenols can improve the antioxidant state of the animals in countering the oxidative stress induced by cyanide.

Conclusion

From the results, it can be summarized that early administration of trèvo, which is rich in bioactive compounds can be a new therapy against toxic chemicals such as cyanide, through its antioxidant, anticholinesterase and improvement in the activity of CCO in the heart.

Conflict of interest

The authors declare no conflicting interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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