Ukrainian Journal of Nephrology and Dialysis
Scientific and Practical, Medical Journal

Founders:

- State Institution «Institute of Nephrology NAMS of Ukraine»
- National Kidney Foundation of Ukraine

ISSN 2304-0238;
eISSN 2616-7352

Journal homepage: https://ukrjnd.com.ua

Research Article

doi: 10.31450/ukrjnd.4(72).2021.04

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The beneficial effect of a phytonutrient-rich product against cadmium chloride-induced hepatorenal toxicity

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Citation:

Ilesanmi OB, Lawal RA. The beneficial effect of a phytonutrient-rich product against cadmium chloride-induced hepatorenal toxicity. Ukr J Nephr Dial. 2021;4(72):26-35. doi: 10.31450/ukrjnd.4(72).2021.04.

Abstract. *This study was designed to investigate the hepatorenal protective effects of tr vo, on cadmium-induced renal and hepatic injury in male Wistar rats.*

Methods. Fifteen healthy male Wistar rats were divided into three groups of five rats per group. Group I (control); group II (35mg/kg cadmium chloride (CdCl2); Group III (2 ml/kg tr vo+ CdCl2. The rats were treated with tr vo (2ml/kg orally) and administered CdCl2 3 hrs later. Twenty-four hours after the last administration rats were sacrificed and blood was collected via cardiac puncture and processed for hematological parameters and assessment of urea, creatinine (CREA), and uric acid (UA), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and albumin (ALB). The liver and kidney were excised and processed for markers of oxidative stress.

Results intraperitoneal administration of 35 mg/kg of CdCl2 caused a significant increase in serum concentration of urea, CREA, UA, AST, ALT, while the concentration of ALB was significantly lower (P<0.0001). CdCl2 caused a significant reduction in packed cell volume, hemoglobin while the total white blood cell count, neutrophils, lymphocytes, monocytes, eosinophils, and basophils were increased. Oxidative stress was significantly pronounced in the liver and kidney of rats exposed to CdCl2 as observed in the high concentration of malondialdehyde, decreased concentration of glutathione, the activity of catalase, superoxide dismutase, and glutathione-S-transferase. Pretreatment with tr vo was able to significantly prevent the anemic, oxidative damage, renal and hepatic injury initiated by CdCl2.

Conclusions. The study reveals that tr vo is effective in attenuating cadmium-induced hepatorenal toxicity in male Wistar rats.

Key words: *hepatotoxicity, nephrotoxicity, tr vo, oxidative stress, cadmium.*

Article history:

Received August 27, 2021
Received in revised form October 03, 2021
Accepted October 05, 2021

Conflict of interest statement. The authors declare no competing interest.

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УДК 616.61+616.36]:546.48

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Сприятливий ефект фітонутрієнту у лікуванні кадмій-індукованої гепаторенальної токсичності

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Резюме. *Це дослідження було розроблено для визначення гепаторенального захисного ефекту фітонутрієнту trevo на ураження нирок і печінки, викликане кадмієм, у самців щурів Вістар.*

Методи. П'ятнадцять здорових самців щурів Wistar були розділені на три групи по 5 щурів у групі. I група (контрольна); група II (35 мг/кг хлориду кадмію (CdCl2); група III (2 мл/кг trevo+ CdCl2). Щурам перорально вводили trevo у дозі 2 мл/кг, після чого через 3 години інтраперитонеально вводили CdCl2. Через двадцять чотири години після останнього введення, щури були виведені з експерименту, а кров була зібрана за допомогою пункції серця та оброблена для визначення гематологічних параметрів та оцінки сечовини, креатиніну (CREA) і сечової кислоти (UA), аланінамінотрансферази (ALT), аспартатамінотрансферази (AST), альбуміну (ALB). У печінці та нирках щурів досліджували маркери окисного стресу.

Результати. Інтраперитонеальне введення 35 мг/кг CdCl2 призводило до статистично значущого підвищення концентрацій сечовини, CREA, UA, AST, ALT в сироватці крові, тоді як концентрація ALB була значно нижчою (P<0,0001). CdCl2 спричиняв значне зменшення гематокриту, гемоглобіну, тоді як загальна кількість лейкоцитів, нейтрофілів, лімфоцитів, моноцитів, еозинофілів та базофілів збільшувалась. Окислювальний стрес був значно виражений у печінці та нирках щурів, підданих впливу CdCl2, що проявлялось високою концентрацією малонового діальдегіду, зниженням концентрації глутатіону, активності каталази, супероксиддисмутази та глутатіон-S-трансферази. Попереднє пероральне введення фітонутрієнту trevo було здатно значно запобігти анемії, окислювальному пошкодженню, ураженню нирок і печінки, ініційованих CdCl2.

Висновки. Дослідження показує, що trevo э ефективним у зменшенns спричиненої кадмієм гепаторенальної токсичності у самців щурів лінії Вістар.

Ключові слова: *гепатотоксичність, нефротоксичність, трево®, окислювальний стрес, кадмій.*

Introduction. The rate at which human is exposed to heavy metals is becoming a global challenge. Despite various attempts by the regulating agencies to reduce their usage and exposure, the amount of toxic heavy metals in the environment has not decreased. The industrial use of cadmium has made it one of the toxic heavy metals humans are exposed to. Underdeveloped countries such as Nigeria are dumpsites for the disposal of used Cd-made batteries and industrial wastes. They leached into soil and water and were absorbed by the plant, aqueous and terrestrial organisms [1-4]. The non-biodegradable nature of Cd increases its life span in the environment leading to long-term accumulation in both the environment and living organisms. There is no known benefit of Cd in the body, however, reports have shown that Cd disrupts the normal metabolic activities in the body, targeting organs such as the kidney and liver for damage. The kidney is the most affected organ of Cd poison as more than 50 % of Cd amassed in the body domicile in the kidney [5]. Accumulation of Cd in the kidney often leads to the inefficiency of the kidney in performing filtration and reabsorption. While in the liver, cadmium exposure causes malignancies and alteration of the hematopoietic system [6, 7]. Most investigations on Cd nephrotoxicity concluded that oxidative stress plays a significant role in the mechanism by which Cd causes renal injury. Cadmium exposure increases reactive oxygen species (ROS) and reactive nitrogen species (RNS) production and suppresses the activity of the antioxidant defense system [8-10]. Medicinal plants and their bioactive components have been investigated against various models of Cd toxicity [11]. Bekheet et al., [12] reported the protective effect of venom isolated from Buthus occitanus against cadmium toxicity. Deevika, et al., [13] and El-Sharaky et al., [14] reported the protective effect of curcumin and selenium against cd toxicity respectively, Ognjanovi et al., [15] (Vit E and Co enQ10), Renugadevi and Prabu, [16], naringenin. One of the supplement phytochemical drinks that are gaining popularity in Nigeria is trevo. It is a phytonutrient supplement produced in the USA. Some of the phytochemicals in 'trevo' include such as pomegranate, ellagic acid, Amalaki, ascorbic acid, Vit E, and polyunsaturated fatty acids. These phytochemi-

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cals are generally safe and of health benefit, even when consumed at a high dose. It is claimed by the manufacturer to improve human health and well-being. There are limited scientific reports on various pharmacological benefits of the product. Though we earlier reported the protective effect of the plant against acetaminophen and cyanide [17-20]. This study aims to investigate the protective effect of trevo against Cd nephrotoxicity.

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

Ethical Considerations. All the rats used for this experiment were healthy and treated according to the guidelines of the Helsinki Declaration of 1975 for the care and use of laboratory animals. The experimental design was approved by the ethical committee on animal research and treatment (ART) of the Federal University Otuoke, Nigeria. The approval code was ART2021005.

Study period and location. In specific terms, the experiment was conducted in the animal house of the Department of Biochemistry, Faculty of Science, Federal University Otuoke from February to June 2021.

Experimental Design. Fifteen 75-day-old male Wistar rats weighing 170±10g were purchased from the Central Animal House, University of Benin, Edo State, Nigeria were used for this experiment. The animals were housed in well-ventilated cages and provided water and food *ad libitum*, and acclimatized for seven days before the start of the experiment. Animals were pretreated with 2 ml/kg of trevo, 3 hrs later, they were administered 35 mg/kg of $CdCl_2$. Male Wistar rats were randomly divided into 3 groups of 5 rats per group as follows:

Group 1: administered vehicle (distilled water)

Group 2: administered cadmium chloride ($CdCl_2$) (35 mg/kg) intraperitoneally

Group 3: administered 2 ml/kg of trevo before intraperitoneal administration of $CdCl_2$

Blood Collection. Blood was collected via cardiac puncture into two different serum bottles. The first serum bottle was coated with ethylene diamine tetraacetic acid and blood collected in this bottle was used for evaluating hematological parameters, while the second bottle is plain and blood collected in this bottle was used for biochemical assays.

Hematological Parameters. Packed cell volume (PCV), hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), and differentials leucocyte count, were determined by the method described by Jain, 1993.

Serum Preparation. The blood collected into plain bottles was centrifuged at 3000 rpm for 10 min to separate the serum from the plasma. The serum was stored at -20°C for evaluation of liver and renal function test.

Biomarkers of liver function. The serum concentration of aspartate aminotransferase, alanine aminotransferase, and albumin were determined following the instruction from the kit manual.

Biomarkers of kidney function. The serum concentration of urea, creatinine, and uric acid were determined following the instruction from the kit manual.

Processing of the liver. 24 h after last administration, animals were sacrificed via cervical dislocation, and the liver excised, rinsed, and homogenized in a phosphate buffer saline (0.1M, pH 7.4) to obtain a 10% w/v homogenate. The homogenate was centrifuged at 15000 rpm for 10 min with the temperature set at 4°C to obtain a clear supernatant that was used for biochemical assays.

Processing of the kidneys. 24 h after last administration, animals were sacrificed via cervical dislocation, and the kidney excised, rinsed, and homogenized in a phosphate buffer saline (0.1M, pH 7.4) to obtain a 10 % w/v homogenate. The homogenate was centrifuged at 15000 rpm for 10 min with the temperature set at 4°C to obtain a clear supernatant that was used for biochemical assays.

Estimation of oxidants. The level of oxidative stress was determined by measuring the amount of malondialdehyde (MDA) formed from lipid peroxidation (LPO) in the kidney tissue according to the method of [21].

Estimation of antioxidants in kidney tissues. The concentration of glutathione (GSH) was measured according to [22]. Catalase (CAT) activity was determined as described by [23]. The activity of Superoxide dismutase (SOD) was measured as described by [24].

GST Assay. The activity of GST was assessed as described by [25]. The flowchart of the study methodology is presented in Figure 1.

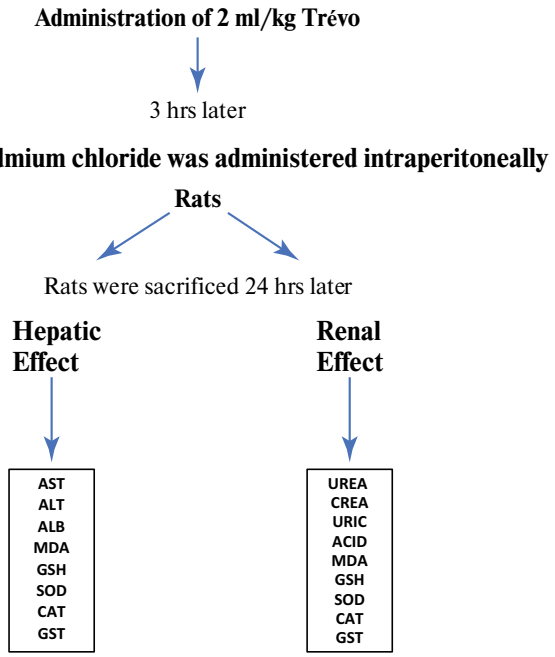

Fig. 1. Flowchart of the study methodology.

Statistical analysis. All grouped data were statistically performed with Prism (GraphPad Prism, 6.01) software. Differences among groups were evaluated by one-way analysis of variance followed by Duncan's multiple range tests. All values were expressed as the mean ±standard deviation of five animals per group.

Results. ***Effect of CdCl2 and pretreatment with trevo on hematological parameters.*** Table 1 shows that CdCl2 caused a significant decrease in packed cell volume (PCV), and hemoglobin (Hb) as compared to the control ($p<0.05$), while the level of white blood cells (WBC), neutrophils (Neu), lymphocytes (lymph), monocytes (mon), eosinophil (eos), and basophils (Bas) significantly increased when compared with the control. Pretreatment with 2ml/kg of trevo was able to prevent the anemic and elevation of the inflammation induced by CdCl2 as observed in the significant increase in PCV and Hb level as compared to the untreated group. In addition, the level of other blood parameters (WBC, Neu, lymph, mon, eos, and Bas) was significantly decreased as compared to the untreated group ($p<0.05$).

Table 1

The effect of trevo on blood hematology parameters of rats exposed to cadmium

	PCV (%)	HB(d)	TOTAL WBC count	NEUT	LYM	MONO	EOSINO	BASO
Control	48.3±4.77	15.8±1.90	5.4±0.30	49.7±2.63	33.2±3.98	5.9±0.69	2.0±0.15	0.6±0.08
Cd (35 mg/kg)	39.7±3.47*	13.2±1.59*	12.6±1.46*	57.8±5.47*	33.0±3.58*	12.0±1.12*	3.0±0.10*	0.8±0.10*
Trevo (2 ml/kg)+Cd	47.4±4.77#	16.1±1.15#	9.2±0.95#	51.0±5.85#	40.9±2.65#	7.3±0.58#	2.2±0.01#	0.0±0.00#

Data are shown as mean ± standard deviation (SD) for 5 animals. Statistically significant ifferences: $p<0.05$ (*)=Control group vs Cd; $p<0.05$ (#)=Cd vs 2 ml/kg trevo

Effect of $CdCl_2$ and pretreatment with trevo in serum markers of hepatotoxicity. Table 2, shows that exposure of rats to cadmium caused a significant increase in the activity of AST and ALT, a significant decrease in the level of ALB and TP level as compared to the control group ($p<0.05$).

Table 2

Hepatoprotective effect of trevo following cadmium exposure based on serum activities of ALT and AST and concentration of total protein and albumin in control and all experimental groups

GROUP	AST (U/l)	ALT (U/l)	TP (mg/dl)	ALB (mg/dl)
control	12.7±1.51	7.9±0.97	101.6±15.99	5.7±0.52
$CdCl_2$ (35 mg/kg)	22.4±2.62*	24.0±1.35*	74.0±4.92*	3.3±0.62*
trevo (2 ml/kg)+Cd	16.5±0.71#	20.5±1.12#	88.6±6.60#	4.3±0.66#

Data are shown as mean ± standard deviation (SD) for 5 animals. Statistically significant differences: $p<0.05$ (*) = Control group vs Cd; $p<0.05$ (#) = Cd vs 2 ml/kg trevo

Pretreatment of the rat with 2 ml/kg of trevo reduced the hepatotoxic effect of CdCl2 as observed in the significant decrease in the serum activity of AST and ALT, a significant increase in the concentration of TP, and ALB when compared to the untreated group ($p<0.05$).

Effect of trevo and $CdCl_2$ on serum markers of renal damage. As presented in Table 3, the effect of trevo and $CdCl_2$ on serum urea, creatinine, and uric acid level. Exposure of the rats to the $CdCl_2$ causes a significant increase in the serum level of urea, uric acid. and creatinine as compared to the control ($p>0.05$). Pretreatment of the rats with 2 ml/kg of trevo significantly prevents $CdCl_2$ induced renal damage as observed in the significant decrease in the serum level of urea, CREA and uric acid as compared to the control ($p<0.05$).

Table 3

Effect of pretreatment of tr*e*vo and $CdCl_2$ on serum level of urea, uric acid, and creatinine following $CdCl_2$ exposure

GROUP	UREA (mg/dl)	URIC ACID (mg/dl)	CRT (mg/dl)
Control	5.7±0.51	150.0±13.70	25.4±1.73
$CdCl_2$(35 mg/kg)	11.7±1.35*	377.2±32.51*	65.0±6.57*
trevo(2ml/kg)+Cd	6.9±0.55#	218.0±28.67#	25.7±1.41#

Data are shown as mean ± standard deviation (SD) for 5 animals. Statistically significant differences: p<0.05 (*) = Control group vs Cd; p<0.05 (#)=Cd vs 2 ml/kg trevo

Effect of $CdCl_2$ and trevo on markers of oxidative stress in the liver and kidney. Figure 1 shows the effect of $CdCl_2$ and pretreatment with trevo on the concentration of MDA in the kidney (Fig. 1A) and liver (Fig 1B). $CdCl_2$ caused a significant increase in MDA level in the liver (p<0.0001) and kidney (p<0.05) when compared to the control. Pretreatment with 2 ml/kg of trevo was able to prevent the oxidative stress induced by $CdCl_2$ as observed in the low level of MDA in the liver (p<0.05) and kidney (p<0.05) when compared to the untreated group

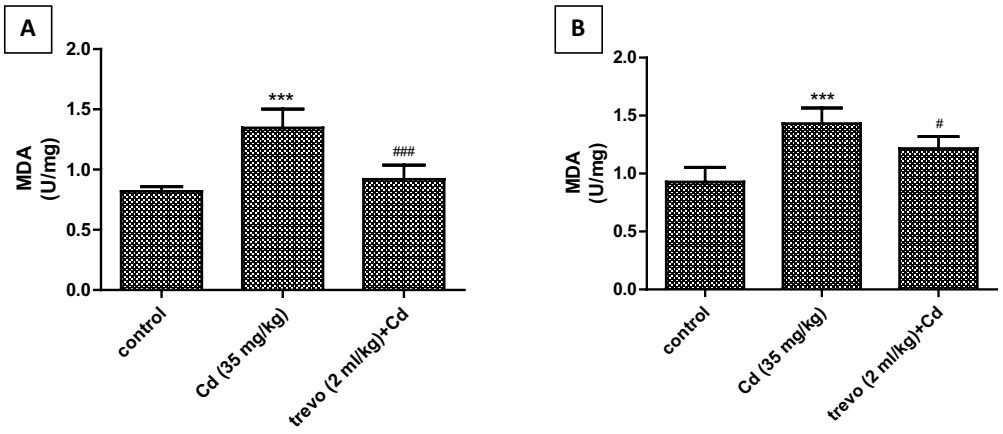

Fig. 1. The concentration of MDA in the kidney (A) and the liver (B) of male rats after pretreatment with trevo following exposure to cadmium (35 mg/kg) via intraperitoneal administration. Data are shown as mean ± standard deviation (SD) for 5 animals. Statistically significant differences: p<0.001 (***)=Control group vs Cd; p<0.001 (###)=Cd vs 2 ml/kg trevo; (#) = Cd vs 2 ml/kg trevo

Moreover, $CdCl_2$ caused a significant decrease in the level of GSH in the liver (p<0.001) and kidney (p<0.001) as compared to the control. Pretreatment with trevo was able to prevent the depletion of GSH by $CdCl_2$ as observed in the significant increase in GSH concentration in both the liver (p<0.01) and kidney (p<0.01) as compared to the untreated group (Fig. 2).

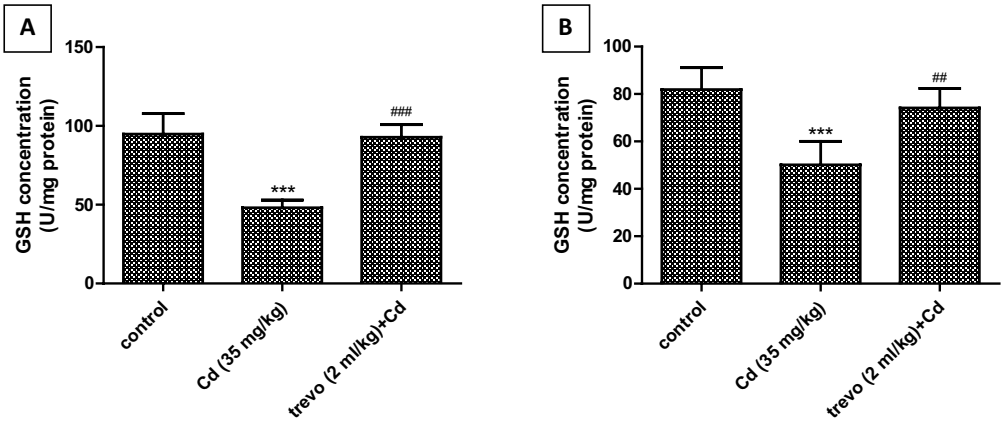

Fig. 2. The concentration of GSH in the kidney (A) and the liver (B) of male rats after pretreatment with trevo, followed by exposure to cadmium (35 mg/kg) via intraperitoneal administration. Data are shown as mean ± standard deviation (SD) for 5 animals. Statistically significant differences: *** p<0.0001=Control group vs Cd; ### p<0.0001=Cd vs 2 ml/kg trevo; p<0.01 (##) = Cd vs 2 ml/kg trevo

Figure 3 show the effect of $CdCl_2$ and pretreatment with trevo on CAT activity in the kidney (A) and liver (B) tissues in male Wistar rats.

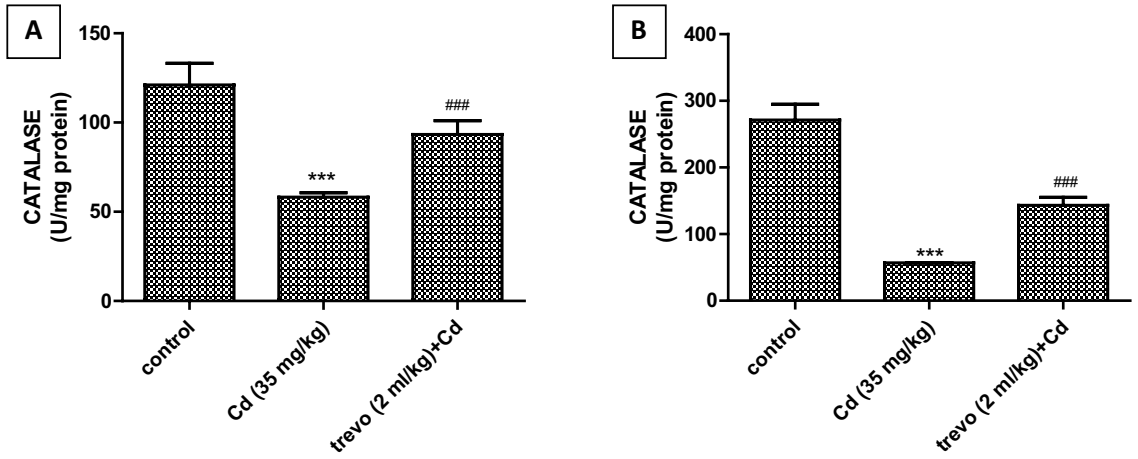

Fig. 3. The catalase activity in the kidney (A) and the liver (B) of male rats after pretreatment with trevo followed by exposure to cadmium (35 mg/kg) via intraperitoneal administration. Data are shown as mean ± standard deviation (SD) for 5 animals. Statistically significant differences: $p<0.001$ (***) = Control group vs Cd; $p<0.001$ ($^{###}$) = Cd vs 2 ml/kg trevo

$CdCl_2$ caused a significant decrease in catalase activity in the liver ($p<0.001$) and kidney ($p<0.01$) as compared to the control ($p<0.001$). Pretreatment with 2 ml/kg of trevo was able to prevent a Cd-induced decrease in the activity of CAT in both the liver ($p<0.001$) and kidney ($p<0.05$) as compared to the untreated group ($p<0.001$ and 0.001).

In addition, $CdCl_2$ significantly inhibited the activity of SOD in the liver and kidney as compared to the control (Fig. 4A, 4B). Pretreatment of the rats with trevo significantly prevent the inhibition of SOD by $CdCl_2$ as observed in the increased activity of SOD in both tissues as compared to the untreated groups.

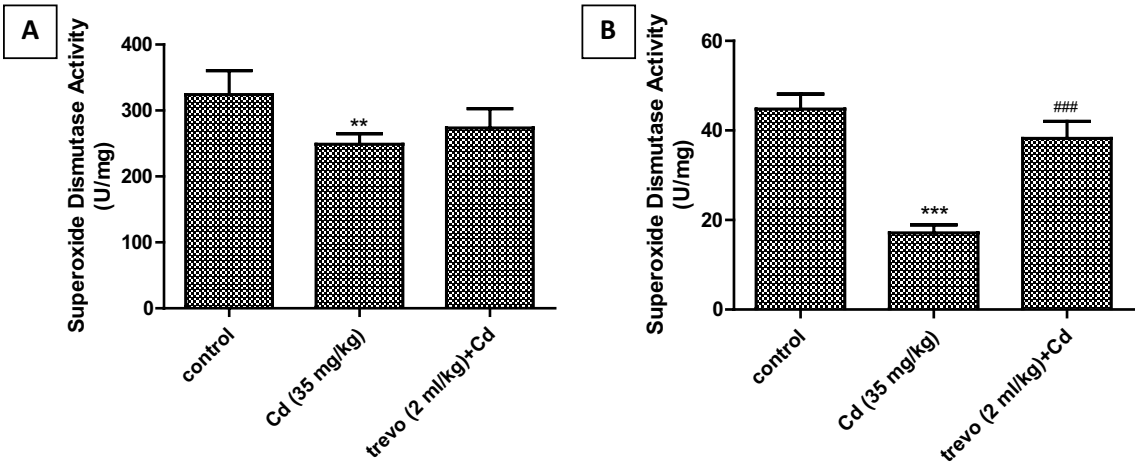

Fig. 4. The superoxide dismutase activity in the kidney (A) and the liver (B) tissues of male Wistar rats after pretreatment with trevo followed by exposure to cadmium (35 mg/kg) via intraperitoneal administration. Data are shown as mean ± standard deviation (SD) for 5 animals. Statistically significant differences: $p<0.01$ (**) = Control group vs Cd; $p<0.001$ (***) = Control group vs Cd; $p<0.001$ ($^{###}$) = Cd vs 2 ml/kg trevo

Effect of pretreatment with trevo and $CdCl_2$ on GST activity in the liver and kidney tissues of male Wistar rats. $CdCl_2$ causes a significant reduction in the activity of GST in the liver ($p<0.001$) and kidney ($p<0.05$) as compared to the control. Pretreatment with trevo was able to significantly increase the activity of GST in the liver ($p<0.001$) and kidney ($p<0.001$) as compared to the untreated group (Fig. 5).

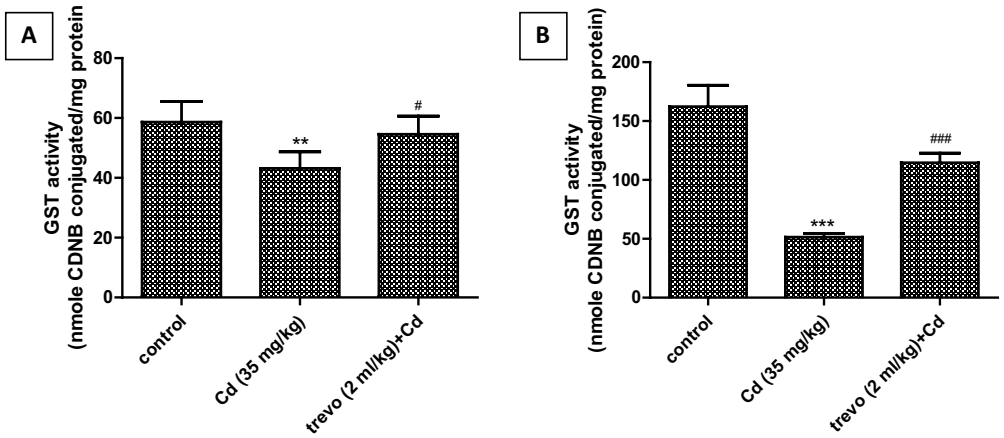

Fig. 5. GST activity in the kidney tissue (A) and hepatocytes (B) of male rats after pretreatment with trevo before exposure to cadmium (35 mg/Kg) via intraperitoneal administration. Data are shown as mean ± standard deviation (SD) for 5 animals. Statistically significant differences: $p<0.01$ (**) = Control group vs Cd; $p<0.05$ (#) = Cd vs 2 ml/kg trevo; $p<0.001$ (***) = Control group vs Cd; $p<0.001$ (###) = Cd vs 2 ml/kg trevo

Discussion. Cd is one of the heavy metals linked to renal and hepatic damage, exposure to it can be acute or chronic [26, 27]. Antioxidant compounds are generally known to prevent the toxic effect of cadmium poison [28, 29]. Trevo is an antioxidant-rich product with various pharmacological activities. The aim is to investigate the nephro- and hepatoprotective effect of trevo on cadmium-induced acute renal and hepatic injury in male Wistar rats. Urea is a common biomarker used in the diagnosis of kidney function. An elevated level of urea is an indicator of renal damage [30, 31]. In our result, Cd injection at the dose of 35mg/kg caused a significant increase in serum urea concentration, indicative of renal damage. An increase in blood urea is associated with renal injury associated with the leakage of urea, which indicates nephrotoxicity. This observation is similar to the result of [11, 12]. Creatinine is an excretory product of nitrogen metabolism in the kidney. An increased level of creatinine in the blood is an indicator of kidney injury. In the experiment, the result shows that Cd exposure caused an elevation in serum creatinine level. One of the effects of Cd exposure to humans is a rise in creatinine concentration [32]. This observation might be due to ineffective reabsorption of creatinine and a decline in the glomerular filtration function of the kidney [33, 34, 35]. Uric acid is a product of purine nucleotide catabolism, its concentration increases in the blood of patients suffering from kidney disease [36]. The observed increase in the concentration of uric acid in the blood due to Cd exposure also confirms the nephrotoxic effect of the metal. The neuroprotective effect of trevo against cyanide toxicity was reported by [37]. This beneficial effect of trevo can be due to its multiple antioxidant phytochemicals, which reverse the toxic effect of Cd in the kidney. Some of the phytochemicals in trevo are confirmed to possess nephroprotective activities. This includes ellagic acid, lycopene, ascorbic acid, tocopherol, and carotene, extracted from green tea, grape seed, aloe vera, bacopa, and turmeric [38]. Concerning the hepatotoxic effect of Cd, our result reveals that administration of CdCl2 caused significant damage to the liver as observed in the high concentration of AST and ALT, with a concomitant reduction in albumin concentration. Trevo was hepatoprotective activity against Cd-induced poisoning as observed in the low serum concentration of ALT and AST, and increased albumin concentration. The increased concentration of ALT and AST is due to damage to the hepatic membrane by Cd, causing increased permeability of the enzymes to the blood, while the decrease in ALB can be due to the possibility of Cd binding to albumin as it was reported to have an affinity for some proteins [39-42]. Thus, pretreatment with trevo prevented the binding of Cd to albumin and the leakage of the enzymes from the hepatocytes. One of the confirmed mechanisms by which Cd exerts its toxicity is oxidative stress [43, 44]. In our study, acute administration of Cd at 35 mg/kg induced oxidative stress as observed in the increase in MDA concentration with a concomitant decrease in GSH, CAT, SOD, and GST in the liver and kidney of male Wistar exposed rats. This observation shows that Cd might induce renal damage by altering the redox status in the rats [45, 46]. The increased MDA concentration can be due to the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which often initiate lipid peroxidation and production of MDA [47]. MDA is one of the markers of oxidative stress, high level of MDA is often due to oxidative stress. The increased ROS and RNS overloaded the animal's antioxidant defense system and were often marked by low concentrations of GSH (nonenzymatic antioxidant), CAT and SOD (enzymatic antioxidant), and GST (catalyze the conjugation of toxic compounds with GSH for excretion from the body). GSH deradicalized the ROS and RNS, by acting as an electron donor, thereby preventing the ROS and RNS from reacting with functional biomolecules. SOD and CAT work in a coordinated manner to catalyze the conversion of superoxide anion (the most reactive radicals) to water. Thus the multiple effects of

Cd in inducing oxidative stress were confirmed in our experiment and were in support of the result. Trevo showed its rich antioxidant components as observed in the decreased concentration of MDA, increased concentration of GSH, the activity of CAT and SOD.

Conclusion. Our investigation confirmed the ability of trevo to prevent hepatic and renal damage induced by cadmium. Administering a dose of 2 ml/kg BW of trevo was able to restore the homeostasis of blood parameters, improve the antioxidant system, and maintain liver and kidney integrity. Further work can be done to investigate the protective effect of trevo on long-term exposure to cadmium.

Conflict of Interest Statement. The authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used products in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but the advancement of knowledge.

Authors' Contributions.

***Omotayo Babatunde Ilesanmi*:** conceptualization, experimental design, analysis and manuscript preparation;

Ridwan Abiodun Lawal: the reviewing and submission of the final manuscript.

Acknowledgment: The authors will like to appreciate all the undergraduate project students that assisted in the completion of this project.

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