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ORIGINAL STUDY

Hydromethanolic Extract of *Ipomoea Cairica* Leaf Extract Ameliorate the Oxidative Activity of Cadmium Chloride in the Hearts and Testes of Male Wistar Rats

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Abstract

Background: *Ipomoea cairica* (L.) sweet Convolvulaceae also known as morning glory or 'Railway creeper' is a traditional herb that has been used in treating various diseases throughout the world. Some of the reported pharmacological activities include antioxidant, anti-inflammatory, and antiviral. Cadmium is a toxic metal that can cause both hearts and testicular damage and its major mechanism is oxidative stress. **Objective:** Therefore, this study was done to investigate the effect of *I. cairica* leaf extract (ICE) against cadmium chloride-induced hearts and testicular damage in Wistar rats. **Materials and Methods:** Twenty male Wistar rats were divided into 4 groups of 5 animals each. Group I: Control (administered distilled water), Group II (administered 35 mg/kg CdCl₂ intraperitoneally), group III (orally administered 100 mg/kg ICE and CdCl₂), group IV (orally administered 100 mg/kg, *I. cairica* and CdCl₂). Animals were sacrificed via cervical dislocation 24 h after the last administration. The hearts and testes were collected and processed for biochemical assays. Biochemical assays evaluated were Malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and Glutathione-S-transferase (GST). **Results:** Results showed that Cd exposure significantly increases the level of MDA, decreased the concentration of GSH (non-enzymatic antioxidant), and decreased the activity of CAT, SOD, and GST (enzymatic antioxidants). Treatment with ICE prevented the alteration of redox status by CdCl₂, as observed in the significant decrease in MDA concentration, and increase in GSH concentration when compared with the animals in the untreated groups. In addition, treatment with *I. cairica* also caused a significant increase in the activity of CAT, SOD, and GST as compared to the untreated group ($P < 0.05$). **Conclusions:** In conclusion, *I. cairica* extract was protective against cadmium-induced oxidative damage in rat hearts and testes. This activity can be linked to the presence of antioxidant phytochemicals present in the plant.

Keywords: Cadmium, *Ipomoea cairica*, Hearts, Testes, Oxidative stress, Antioxidant

1. Introduction

Cadmium (Cd) is an example of a toxic heavy metal similar to other poisonous chemicals from the industries, which is a serious threat to human life [1]. The Accumulation of Cd in the environment is as a result of human activities such as the use of fossil fuel, metal ore combustion, and waste burning. Industrial uses of Cd include the production of nickel-cadmium

rechargeable batteries, the protection of iron and steel against corrosion, and as a phosphate fertilizer. The exposure to Cd can be due to the consumption of fruits, vegetables, and grains grown on soil with high Cd content which is caused by leaked sewage sludge into the soil [2]. There have been several reports on the connection between Cd exposure and male infertility [3–5]. The mechanism of Cd toxic effect on the male reproductive system is mainly due to oxidative stress

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[6,7]. Exposure to Cd has been linked to increased generation of reactive oxygen species (ROS), and oxidation of lipids, proteins, and DNA as a result of Cd's ability to disrupt calcium homeostasis. In addition, Cd exposure has been linked to alteration in the immune system, and antioxidant defense systems, such as superoxide dismutase and catalase. Some of the toxic effect of Cd on the testes includes destruction in the scrotum size, while another investigation reported an increase in the weight of the scrotum. Cd has also been reported to destroy the seminal vesicle, sperm cells, and epididymis. Both humans and rodents are vulnerable to Cd toxicity. Cd also induces apoptosis through the activation of caspase-9 and the release of cytochrome C. Accumulation of Cd has been reported in hearts tissue. Since, Cd toxicity has been linked to oxidative stress, therefore it can be assumed that the cardiotoxicity of Cd can be due to oxidative stress.

The use of natural plants/herbs has been improved due to the belief that herbs are safer product than synthetic drugs, thus, there have been an increase in the use of plants for therapeutic purpose globally. This has led to more than 70% of the world population relying on traditional herbs for the cure/treatment of different types of diseases according to [8]. The efficacy of most plants is due to the presence of antioxidant-rich phytochemicals with pharmacological activities, which are often evaluated based on their traditional use. *Ipomoea cairica* (Convolvulaceae) is also known as morning glory, railroad creeper, coast morning glory, five-fingered morning glory, mile-a-minute is a perennial plant that is majorly found in Asian countries and also African countries [9]. *I. cairica* contains some chemical constituents like ecdysteroids, a steroidal glycoside, aromatic acids, triterpenes, amino acids, organic acids, some minerals, and vitamins. *I. cairica* is medically used because of its pharmacological properties such as antioxidant activity, anti-inflammatory properties, and antiviral properties [10]. The aim of this investigation is to evaluate the effect of *I. cairica* leaf extract on antioxidant status in the hearts and testes of male Wistar rats following cadmium exposure.

2. Materials and methods

2.1. Chemicals and reagents

Cadmium chloride, Chlorodinitrobenzene, reduced glutathione, trichloroacetic acid, Chlorodinitrobenzene, thiobarbituric acid (TBA), nicotinamide adenine dinucleotide (NADH) were obtained from

Sigma Aldrich. All other chemicals and solvents were of analytical grade.

2.2. Extraction of plant materials

The leaves of *I. cairica* were collected from the town of Nembe in Bayelsa state, Nigeria. It was identified by a plant taxonomist from University of Benin. The leaves were dried in an open room till a constant weight was obtained. The dried leaves were blended to obtain a fine powder and 500 g from it was soaked in 2.5L of 80% methanol. The mixture was stirred regularly for 48 h, before filtering using a Whatman filter paper to obtain a filtrate that was subjected to rotary evaporation and lyophilization to obtain a dried sample.

2.3. Experimental design

Rats weighing between 180 and 200g were obtained from the central animal house, University of Benin. Twenty male wiser rats were randomly divided into four groups of five animals each and kept in plastic cage, maintained on a 12-h light/dark cycle. The rats were supplied with water and food ad libitum. Group I (administered distilled water intraperitoneally); group II (administered 35 mg/kg of CdCl₂ intraperitoneally) [11]; group III (orally administered 100 mg/kg *I. cairica* leaf extract (ICE) for five consecutive days before a single dose administration of CdCl₂); group IV (orally administered 250 mg/kg of ICE for 5 consecutive days before intraperitoneal administration of CdCl₂).

2.4. Processing of the hearts and testes

Animals were observed for any physiological changes during the course of the experiment, animals were sacrificed 24 h after the last administration by cervical dislocation. The hearts and testes were dissected from the rats, weighed, rinsed and homogenized in a homogenizing buffer (0.1M phosphate, pH = 7.4). The post mitochondria fraction (PMF) were obtained by centrifuging the homogenate using a cold ultracentrifuge set at 12,500g for 15 min at 4 °C.

3. Biochemical assay

3.1. Estimation of malondialdehyde level in hearts and testes

The method described by Varshney and Kale [12], was used in measuring the amount of malondialdehyde generated as a biomarker of lipid

peroxidation. Briefly, 0.4 ml of sample was mixed with 1.6 mL of Tris-KCl buffer to which 0.5 ml of 30% TCA was added. This was followed by the addition of 0.5 ml of TBA and the mixture was boiled at 80 °C. After cooling and centrifugation, absorbance of the clear supernatant was measured at 532 nm. Lipid peroxidation was calculated using a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$.

3.2. Estimation of antioxidants in hearts and testes

3.2.1. Estimation of reduced glutathione concentration

The method of Jollow et al. [13], was used in measuring the concentration of GSH in the hearts and testes, briefly 0.2 ml of sample was added to 1.8 mL distilled water and 3 mL 4% sulphosalicylic acid and centrifuged at 3000 g. The supernatant was added to 0.4 mg/mL DTNB in 0.1 mol/l phosphate buffer. The absorbance of the reaction mixture was read at 412 nm.

3.2.2. Determination of catalase activity

The method described by Aebi [14], was used to determine the activity of catalase. Briefly, the assay mixture contained 2 ml of H_2O_2 solution (800 μmol) and 2.5 ml of phosphate buffer in a test tube. 0.5 ml of properly diluted enzyme preparation was rapidly mixed with the reaction mixture by a gentle swirling motion. The reaction was run at room temperature. A 1 ml portion of the reaction mixture was withdrawn and blown into 2 ml dichromate/acetic acid reagent at 60s intervals. Addition of the reagent instantaneously produced an unstable blue precipitate of perchromic acid. Subsequent heating for 10 min in a boiling water bath changed the colour of the solution to stable green due to formation of chromic acetate. After cooling at room temperature, the optical density was measured with a spectrophotometer at 570 nm. The catalase contents of the enzyme preparation were expressed in term of Kat.f.

3.2.3. Determination of superoxide dismutase activity

The method of Misra and Fridovich [15] Briefly, Sample (1 ml) was diluted in 9 ml of distilled water to make a 1 in 10 dilution. An aliquot of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction was started by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of water. The increase in absorbance at 480 nm was monitored every 30s for 150s. 1 unit of SOD activity was given as the amount of SOD necessary to cause 50%

inhibition of the oxidation of adrenaline to adrenochrome during 1 min.

3.2.4. Glutathione transferase (GST) assay

The method of Habig et al. [16], was used to determine the activity of GST in the hearts and testes of male wistar rats.

3.3. Statistical analysis

Results were analyzed statistically using GraphPad Prism, 6.01 software. One-way ANOVA was used to compare values between groups while Duncan's multiple range test was used as a descriptive. All values were expressed as the mean \pm standard deviation of five animals per group. $P < 0.05$ was considered statistically significant.

3.4. Ethical consideration

All of the rats used in this study 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 ethics committee on Animal Research and Treatment (ART) of the Federal University Otuoke, Nigeria (Code: ART2021009).

4. Results

4.1. Protective effect of ICE against cadmium-induced oxidative stress in the hearts and testes of male wistar rats

4.1.1. Effect on MDA in hearts and testes of male wistar rats

Fig. 1(A and B) shows the effect of ICE on the concentration of MDA in the hearts and testes. It shows that CdCl_2 significantly increase the concentration of MDA $P < 0.05$ as compared to the control group. It also showed that treatment of rats with 100- and 250 mg/kg of ICE significantly prevent the production of MDA as compared to the untreated group ($P < 0.05$).

4.1.2. Effect on GSH

The exposure of rats to CdCl_2 caused a significant decrease in the concentration of GSH as compared to the control ($P < 0.05$) in both the hearts and testes as shown in Fig. 2 (A and B) respectively. Pretreatment with 100 and 250 mg/kg of ICE was able to prevent CdCl_2 -induced depletion of GSH in the hearts and testes of male Wistar rats as observed in the significant increase in the concentration of GSH as compared to the untreated group ($P < 0.05$) in both the hearts and testes. The Fig. 2 also showed that in the hearts, there

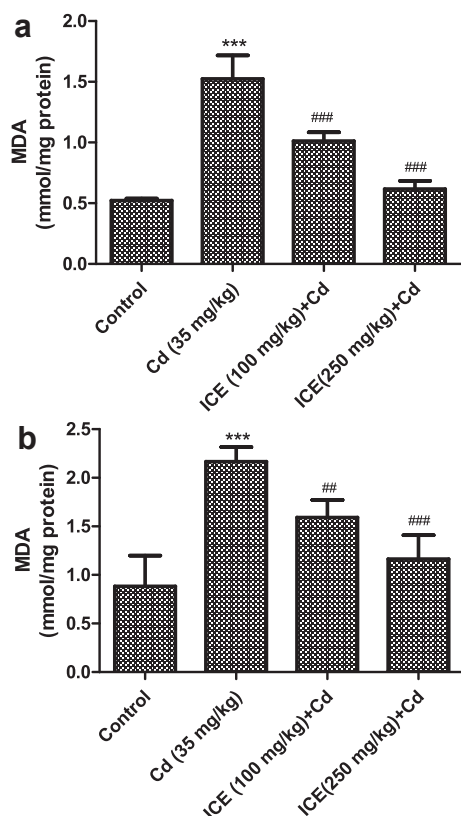


Fig. 1. a: The concentration of lipid peroxides product - malondialdehyde (MDA), in the heart of male rats after pretreatment with *Ipomoea cairica* leaf extract (ICE) following exposure to cadmium (35 mg/kg) via intraperitoneal administration. Data are shown as mean \pm standard deviation (SD) for 5 animals. Statistically significant differences: *Control group vs Cd; # Cd vs treatment. b: The concentration of lipid peroxides product - malondialdehyde (MDA), in the testes of male rats after pretreatment with *Ipomoea cairica* leaf extract (ICE) following exposure to cadmium (35 mg/kg) via intraperitoneal administration. Data are shown as mean \pm standard deviation (SD) for 5 animals. Statistically significant differences: * Control group vs Cd; # Cd vs treatment.

was no significant difference between 100 and 250 mg/kg, however, in the testes, 250 mg/kg of ICE was more effective than 100 mg/kg of ICE.

4.1.3. Effect of CAT and SOD in the hearts and testes

Figures 3a, b, 4a, and 4b showed the effect of CdCl₂ and ICE on CAT activity in the hearts and testes, as well as SOD activity in the hearts and testes respectively.

Exposure of the rats to CdCl₂ caused a significant decrease in the activity of catalase in the hearts and testes as compared to the control ($P < 0.05$ and 0.001). Pretreatment with 100 and 250 mg/kg of ICE caused a significant increase in the activity of CAT in the hearts and testes as compared to the untreated group ($P < 0.05$). It also showed that pretreatment with 250 mg/kg of ICE was more effective than 100 mg/kg of ICE in both organs.

The Fig. 4a and b showed that CdCl₂ caused a significant decrease in the activity of SOD in the hearts and testes as compared to the control ($P < 0.05$). Pretreatment with 100 and 250 mg/kg of ICE caused a significant increase in SOD activities in both hearts and testes as compared to the untreated group ($P < 0.05$). Concerning the hearts, Fig. 4a showed that 100 mg/kg of ICE was more effective than 250 mg/kg of ICE in increasing the activity of SOD in the hearts. However, there was no significant difference between 100 and 250 mg/kg of ICE in increasing the activity of SOD in the testes (Fig. 4b).

4.1.4. Effect on GST activity on the hearts and testes

Fig. 5 (A and B) shows the effect of ICE and CdCl₂ on the activity of GST in the hearts and testes of male wistar rats. It reveals that CdCl₂ significantly

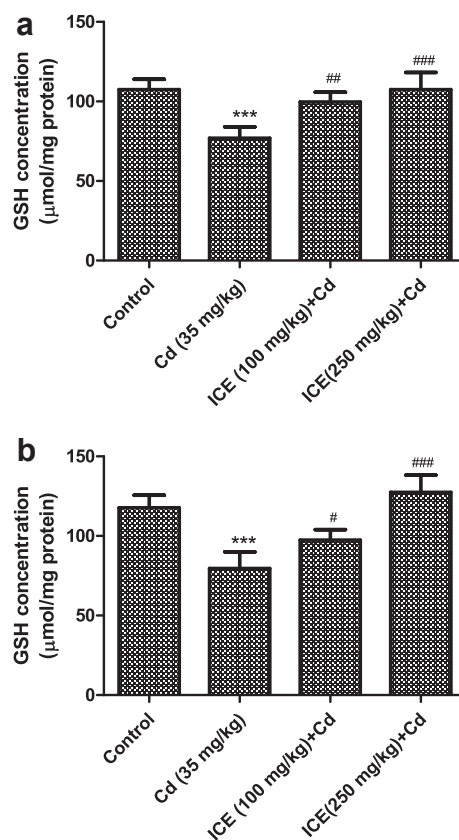


Fig. 2. a: The concentration of non-enzymatic antioxidant-reduced glutathione (GSH) in the heart of male rats after pretreatment with *Ipomoea cairica* leaf extract (ICE), followed by exposure to cadmium (35 mg/kg) via intraperitoneal administration. Data are shown as mean \pm standard deviation (SD) for 5 animals. Statistically significant differences: * Control group vs Cd; # Cd vs treatment. b: The concentration of non-enzymatic antioxidant-reduced glutathione (GSH) in the testes of male rats after pretreatment with *Ipomoea cairica* leaf extract (ICE), followed by exposure to cadmium (35 mg/kg) via intraperitoneal administration. Data were shown as mean \pm standard deviation (SD) for 5 animals. Statistically significant differences: * Control group vs Cd; # Cd vs treatment.

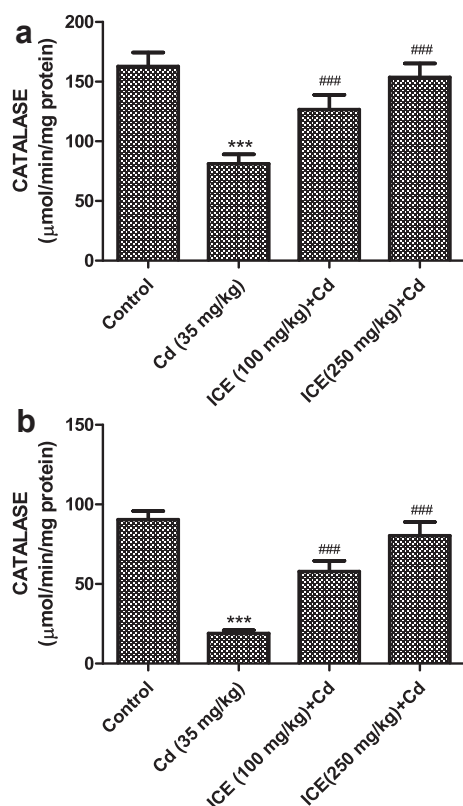


Fig. 3. a: The catalase activity in the heart of male rats after pretreatment with *Ipomoea cairica* leaf extract (ICE) followed by exposure to cadmium (35 mg/kg) via intraperitoneal administration. Data are shown as mean \pm standard deviation (SD) for 5 animals. Statistically significant differences: * Control group vs Cd; # Cd vs treatment. b: The catalase activity in the testes of male rats after pretreatment with *Ipomoea cairica* leaf extract (ICE) followed by exposure to cadmium (35 mg/kg) via intraperitoneal administration. Data are shown as mean \pm standard deviation (SD) for 5 animals. Statistically significant differences: * Control group vs Cd; # Cd vs treatment.

decrease the activity of GST as compared to the control group ($P < 0.05$). administration of 100 mg/kg- and 250 mg/kg of ICE was able to significantly prevent the inhibition of GST by CdCl₂ ($P < 0.05$) in the hearts and testes of male wistar rats.

The figures also showed that pretreatment with 250 mg/kg was more effective in increasing the activity of GST as compared to 100 mg/kg in both the hearts and testes.

5. Discussion

This experiment investigated the antioxidant effect of *I. cairica* leaf extract (ICE) in the hearts and testes of male Wistar rats following cadmium (Cd) exposure. Administration of cadmium at 35 mg/kg resulted in increased lipid peroxidation, decreased concentration of glutathione (GSH), decreased activity of catalase (CAT), superoxide dismutase (SOD), and glutathione transferase (GST) in the

hearts and testes. Pretreatment with ICE was able to prevent the oxidative damage of testes by cadmium as observed in decreased concentration of malondialdehyde (MDA), increased concentration of GSH, and increased activity of CAT, SOD, and GST. MDA is a biomarker for accessing oxidative stress. Increased concentration of MDA is an indication of oxidative stress and is linked to tissue damage, thus the observed increase in MDA in rats exposed to cadmium might indicate testicular injury. This observation was similar to the report of [17,18]. The oxidative effect of Cd was also manifested in the decreased concentration of GSH. This can be due to the increased generation of reactive oxygen species that overburden the concentration of GSH and formation of Cd–GSH complex in the testes [6,19] to reduce the oxidative effect of Cd [20]. Some investigators reported that the decreased

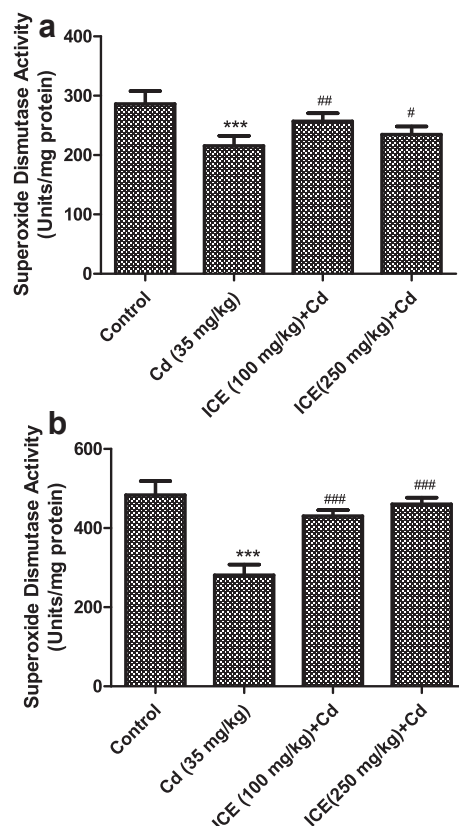


Fig. 4. a: The superoxide dismutase activity in the heart of male Wistar rats after pretreatment with *Ipomoea cairica* leaf extract (ICE) followed by exposure to cadmium (35 mg/kg) via intraperitoneal administration. Data are shown as mean \pm standard deviation (SD) for 5 animals. Statistically significant differences: * Control group vs Cd; # Cd vs treatment. b: The superoxide dismutase activity in the testes of male Wistar rats after pretreatment with *Ipomoea cairica* leaf extract (ICE) followed by exposure to cadmium (35 mg/kg) via intraperitoneal administration. Data are shown as mean \pm standard deviation (SD) for 5 animals. Statistically significant differences: * Control group vs Cd; # Cd vs treatment.

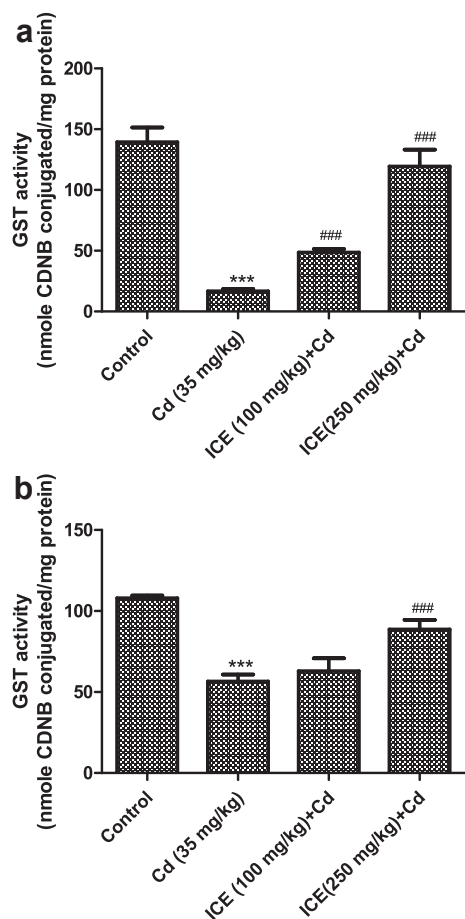


Fig. 5. a: The glutathione-S-transferase (GST) activity in the heart of male rats after pretreatment with *Ipomoea cairica* leaf extract (ICE) before exposure to cadmium (35 mg/kg) via intraperitoneal administration. Data are shown as mean \pm standard deviation (SD) for 5 animals. Statistically significant differences: * Control group vs Cd; #Cd vs treatment CDNB = 1-Chloro-2,4-dinitrobenzene. b: The glutathione-S-transferase (GST) activity in the testis of male rats after pretreatment with *Ipomoea cairica* leaf extract (ICE) before exposure to cadmium (35 mg/kg) via intraperitoneal administration. Data are shown as mean \pm standard deviation (SD) for 5 animals. Statistically significant differences: * Control group vs Cd; # Cd vs treatment CDNB = 1-Chloro-2,4-dinitrobenzene.

concentration of GSH by Cd might be linked to the inhibition of enzymes involved in GSH homeostasis, which includes gamma-glutamyl transferase and glutathione reductase, both involved in the restoration of the reduced form of GSH following oxidative activity [21,22]. In this study, there was a significant decrease in CAT activity compared to the control group. CAT and SOD are involved in the clearance of ROS produced following oxidative activity in the cell [23,24]. Toxic chemicals either inhibit or suppress the activities of these enzymes, thereby increasing the concentration of ROS in the cell and aggravating cellular damage. The results show that Cd-induced decreased activity of SOD

and CAT, supporting the outcome from other researchers [6,25]. The experiment also showed that Cd decreased the activity of GST, which might involve inhibiting or suppressing the activity of the enzyme. GST is a drug metabolism enzyme involved in the clearance of toxic compounds from the body. Another possible cause of low GST activity can be due to decreased concentration of GSH, a substrate required for the efficient function of GST [26]. The protective activity of ICE against Cd-induced testicular damage can be linked to the presence of beneficial phytochemicals present in the plant. These phytochemicals have been reported to possess antioxidant activities such as metal chelating, radical scavenging, and termination of lipid peroxidation [27]. Some of the phytochemicals identified in the plants include polyketides, terpenoids, steroids, shikimides, flavonoids, xanthone, and alkaloids [28]. *I. cairica* is a medicinal plant [9]. The methanolic extract of *I. cairica* possesses a good antioxidant potential because of its phytochemical constituents [28].

Conclusion

In conclusion, the results show that *I. cairica* is protective against cadmium-induced cardiac and testicular damage. These protection can be linked to the antioxidant compounds present in *I. cairica*, which might be responsible for the plant to counter the oxidative stress activities of cadmium chloride.

Significance statement

This study provides scientific proof of the medicinal importance of *I. cairica*, one of the plants that is abundant and underutilized in this part of the world. Further work is ongoing to elucidate the phytochemicals responsible for the pharmacological activities.

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