

Sodium 3-phenylpropanoate alleviate oxidative stress and iron-induced testicular toxicity in Wistar rats

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ABSTRACT

There are various derivatives of cinnamic acid with pharmacological significance. Sodium 3-phenylpropanoate (KAD 1), a derivative of cinnamic acid, has also been synthesized and it is important to investigate its effects on iron-induced testicular injury in an *ex vivo* study. Evaluations were done on KAD 1's l, l-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, ferric-reducing antioxidant power, and iron chelating potential. Through the *ex vivo* incubation of tissue supernatant and 0.1 mM FeSO₄ for 30 minutes at 37°C and various concentrations of KAD 1, oxidative testicular damage induced was treated. The scavenging property of KAD 1 increases significantly ($p < 0.05$) as the concentration increases when compared with the standard quercetin. The malondialdehyde, catalase, ATPase, as well as ENTPDase activities, were reduced when testicular damage was induced ($p < 0.05$). A significant rise in glutathione level was observed. Therefore, KAD 1 has the potential to treat and protect against oxidative testicular toxicity, as revealed by its capacity to control nucleotide hydrolysis and reduce oxidative stress. Thus, KAD 1 may be a suitable potent modality, which can help treat testicular injury.

INTRODUCTION

Due to its potential effects on health, oxidative stress (OS) has recently attracted a lot of attention. Reactive oxygen species (ROS) concentrations perpetually and continuously rise

in oxidative stress (OS) [1]. Many diseases, including cancer, neurological disorders, hypertension, arteriosclerosis, diabetes, and infertility, are associated with OS [2,3]. Male infertility has been identified as one of the end products of OS in about 50% of the identified cases [4]. The reproductive system in males, especially the tissues in the testes, is prone to attacks from the invasion of OS because of the increased level of cell division that occurs as well as the demand placed on oxygen demand from the mitochondria [5–7]. The exposure of the sperm cells and the tissues in the testes to oxidative attack is made possible because of the increased content of unsaturated fatty acid, especially when the integrity of the antioxidant is been put at risk [8–11]. Excessive

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Fe^{2+} in the system leads to testicular damage and hence reduced fertility rate [12]. Numerous animal studies have been reported involving elevated Fe^{2+} levels and testicular toxicity which has led to OS in the testes of the animals [12–15]. The morphological metamorphosis that occurs in the epididymis and sperm cells arises from the interaction that occurs between the iron and proteins, lipids as well as molecules in the DNA which, most times, results in infertility [16,17].

Plants have been identified and proven to be rich sources of phytochemicals, which are rich sources of antioxidants, preventing OS produced from exposure to Fe^{2+} [12]. The interest in herbal remedies has unexpectedly resurfaced as a result of the drive to comprehend and record the knowledge of ancient therapeutic systems [18,19]. Recently, due to some circumstances, interest in the study of natural product chemistry has increased. Only through studying the pharmacology of secondary metabolites from medicinal plants can the many therapeutic demands for bioactive molecules with few or no side effects be addressed [20]. Therefore, there is a pressing need to improve the methods for detecting biologically active natural products as well as those for isolating, purifying, characterizing, and altering the structural makeup of these active components [18,21].

The principal antioxidants found in medicinal plants are phenolics and flavonoids [3,22,23]. The diphenyl propane moiety, which consists of two distinctive aromatic rings linked by three carbon atoms and typically forms an oxygenated heterocycle, is responsible for their collective structural function [24–26]. Anthocyanidins, isoflavones, flavanones, and flavonols (or catechins) are the different types of flavonoids depending on the type of heterocycle involved [27,28]. Benzoic acid and cinnamic acid analogs are examples of phenolic acids [29–31]. Cinnamic acid and its derivatives are known for their various benefits because of their application in the management of different forms of diseases [32]. There are various derivatives of cinnamic acid, having a chemical composition of an acrylic acid group on an aromatic carboxylic acid [33–36]. Sodium 3-phenylpropanoate (KAD 1) a derivative of cinnamic acid has also been synthesized and it is important to investigate its effects in iron-induced testicular injury in an *ex vivo* study.

MATERIALS AND METHODS

Materials and reagents chemicals

KAD 1 was obtained from the derivatization of cinnamic acid (CAS 140-10-3; Merck, Germany) from our previous study [37], while quercetin (CAS 117-39-5) was obtained from Santa Cruz Biotechnology, Heidelberg, Germany. All substances, including solvents, were of analytical grade. A spectrophotometer (Spectra Max Plus, Molecular Devices, CA, USA) was used to measure all absorbances.

In vitro antioxidant activity

KAD 1's iron chelating activity was assessed using the standard procedure [38], and antioxidant potential via 1,1-diphenyl-2-picrylhydrazyl (DPPH) was evaluated following the procedure described by Ruan *et al.* [39]. The ferric-reducing antioxidant power (FRAP) experiment was done following the guidelines provided by Benzie and Strain [40].

Ex vivo studies

Experimental rats and organ preparation

We obtained healthy male Wistar rats (10–12 weeks old) from the Animal House, Bowen University, Iwo, Nigeria, weighing 250–300 g each. At room temperature (20°C–25°C), all animals were kept in cages with a 12/12-hour light-dark schedule. Softwood shavings were used as bedding inside the cages to absorb animal waste, and it was replaced frequently. Throughout this study, they had unrestricted access to water and constant food pellets (provided by Ladokun Feeds Nig. Ltd.). Before beginning the experimental methods, animals underwent a minimum of 1 week of acclimatization. The Guide for the Care and Use of Laboratory Animals' guidelines were followed when conducting the exercise. All relevant national, institutional, and/or foreign regulations for the handling and use of animals were adhered to. In addition, the experiment was approved by the Institutional animal Ethics Committee at Bowen University, Iwo (BUI/BCH/2022/0002). Ten rats were euthanized with halothane, as described by Slott *et al.* [41], after being fasted the night before, and their testicles were then excised and homogenized in 1% Triton X-100 in 50 mM phosphate buffer. At a temperature of 40°C, the homogenate was centrifuged at 15,000 rpm. The supernatants were obtained in plain tubes for *ex vivo* research and were stored at –40°C.

Testicular injury induction

The technique described by Ojo *et al.* [35,36] was used on injured testicles *ex vivo* with a few minor modifications. In essence, 100 μl of 0.1 mM FeSO_4 and varied KAD 1 concentrations (30, 60, 120, and 240 g/ml) were added to 200 μl of the organ supernatant. The samples were used for biochemical examinations after being incubated for 30 minutes at 37°C. The positive control used reaction mixtures with only the organ supernatant, and the negative control used reaction mixtures with only the tissue supernatant and FeSO_4 .

Measurement of antioxidant activities

Level of glutathione (GSH)

600 ml of the tissue lysates were deproteinized with 10% trichloroacetic acid, according to Salau *et al.* [42]. The mixture was then centrifuged for 10 minutes at 3,500 rpm. 100 ml of the Ellman reagent and 500 ml of the sample were put into a clean test tube. After incubation for 5 minutes at 25°C, the absorbance was measured at 415 nm. The GSH was used as a reference.

Catalase (CAT) activity

The CAT activity analysis for KAD 1 was evaluated using the method described by Ojo *et al.* [35,36] with a minor modification. Several KAD 1 concentrations were present in 20 ml of tissue samples, and 780 μl of 50 mM phosphate buffer was added to those samples. Hence, the absorbance was measured at 240 nm for 3 minutes at intervals of 1 minute after adding 300 μl of 2 M H_2O_2 .

Level of lipid peroxidation

KAD 1's ability to prevent lipid peroxidation was evaluated using the procedure outlined by Ojo *et al.* [35,36]. 100

μ l of tissue lysates with variable concentrations of KAD 1 were introduced progressively to 375 μ l of 20% acetic acid, 1,000 μ l of 0.25% thiobarbituric acid, and 100 μ l of 8.1% sodium dodecyl sulphate (SDS). The reaction mixture was warmed up for 60 minutes at 95°C (in a water bath). After the mixture had cooled to room temperature, the absorbance at 532 nm was measured.

Purinergic activity

Na/K⁺ ATPase enzyme activity

To measure the Na⁺/K⁺ ATPase activity, the procedure as described in Erukainure *et al.* [43] was slightly modified. In 1.3 ml of 0.1 M Tris-HCl buffer, 200 μ l of the organ lysate with various KAD 1 concentrations, 200 μ l of 5 mM KCl, and 40 ml of 50 mM ATP were added. After being vigorously agitated for 30 minutes at 37°C, the reaction mixture was added to 1 μ l of distilled water, as well as 1 ml of 1.25% ammonium molybdate. After that, 1 ml of 9% ascorbic acid was added to the solution, which was then left at room temperature for 30 minutes. At 660 nm, the absorbance was measured.

E-NTPDase enzyme activity

Ojo *et al.* [35,36] procedure, with some minor modifications, was used. 400 μ l of a reaction mixture made up of 1.5 mM CaCl₂, 5 mM KCl, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 45 mM Tris-HCl, 225 mM sucrose, and 10 mM glucose was added to 40 μ l of tissue lysates containing different doses of KAD 1. After that, the mixtures were incubated for a further 10 minutes at 37°C. The solution was then kept at 37°C using an electric-powered shaker after 40 μ l of 50 mM adenosine triphosphate (ATP) was added. 400 μ l of 10% trichloroacetic acid (TCA) was added to the mixture to halt the process. After an ice-based incubation period of 10 minutes, the absorbance at 600 nm was measured.

Data analysis

Software called Graphpad Prism 9.0.1 was used to analyze the data. The descriptive data were represented by the mean standard deviation ($\pm$ SD). One-way ANOVA and Tukey's post hoc analysis, at a significance level of $p < 0.05$ was used to compare the mean.

RESULTS

Antioxidant activities

Figure 1 shows the DPPH scavenging radical ability of KAD 1. It is seen that the scavenging property increases significantly ($p < 0.05$) as the concentration increases when compared with the standard quercetin, which also had the same trend. KAD 1 displayed the capacity to mop up DPPH radicals (Figure 1), while Figure 2 displays the FRAP of KAD 1 and it was observed that the compound possesses a certain level of FRAP potential in a dose-dependent manner as compared to the standard that shows a higher quality in a dose-dependent mode significantly. From the highlight of KAD 1 Fe²⁺ chelating potential displayed in Figure 3, a trend was observed in a concentration-dependent mode significantly when compared to the standard control, quercetin. The reduced GSH level is shown in Figure 4. The antioxidant CAT result is displayed in Figure 5 with the 30

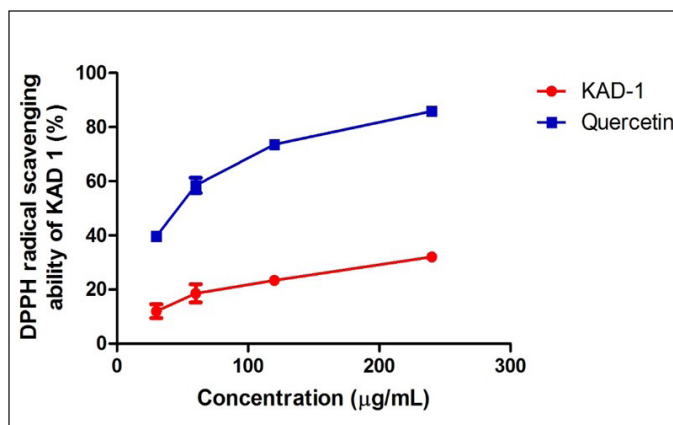


Figure 1. DPPH scavenging ability of KAD 1. Data are expressed as mean $\pm$ SD ($n = 3$).

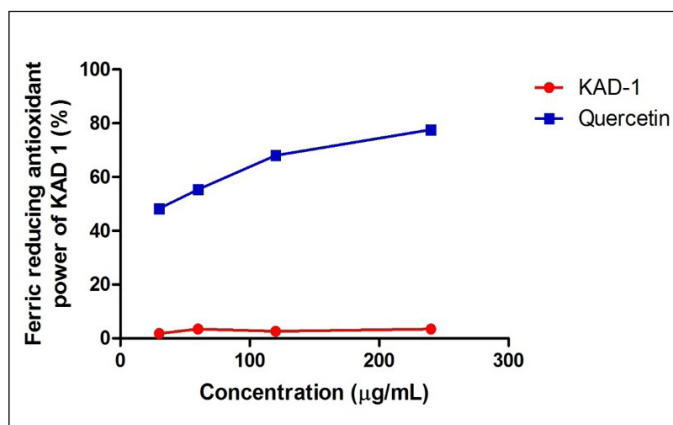


Figure 2. KAD 1 impact on FRAP. Data are expressed as mean $\pm$ SD ($n = 3$).

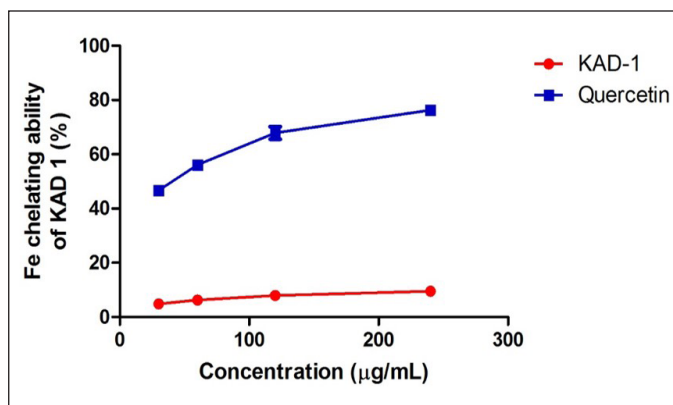


Figure 3. Iron chelating ability of KAD 1. Data are expressed as mean $\pm$ SD ($n = 3$).

mg/ml group having the highest CAT potential when compared to the other groups. Figure 6 showed that lipid peroxidation was induced in negative control with Fe²⁺. Furthermore, the level of malondialdehyde (MDA) in the testicular toxicity damage with KAD 1 reduced the lipid peroxidation activity considerably in a dose-dependent mode.

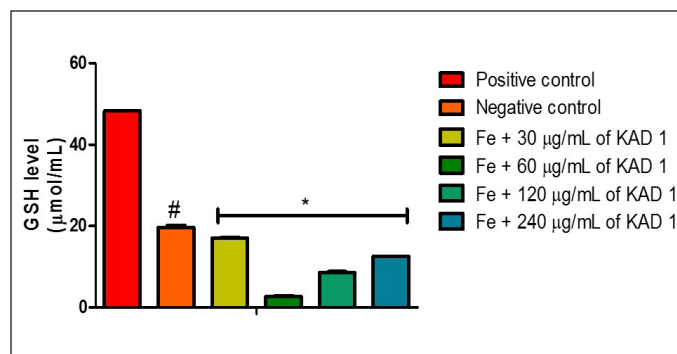


Figure 4. KAD 1's impact on GSH levels in iron-mediated oxidative testicular damage. Data are expressed as mean $\pm$ SD ($n = 3$), while * and # are statistically significant ($p < 0.05$) when compared to positive and negative controls, respectively.

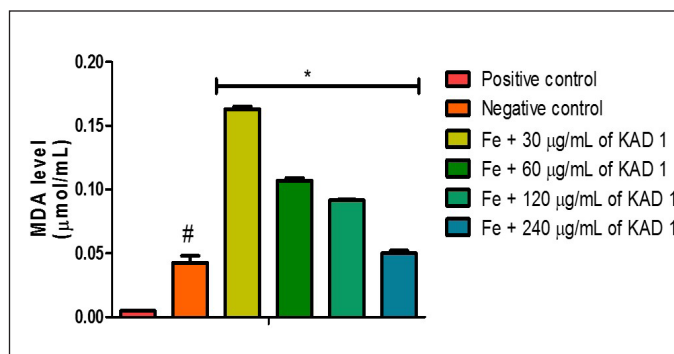


Figure 6. Impact of KAD 1 on MDA concentration in iron-induced testicular oxidative injury. Data are expressed as mean $\pm$ SD ($n = 3$), while * and # are statistically significant ($p < 0.05$) when compared to positive and negative controls, respectively.

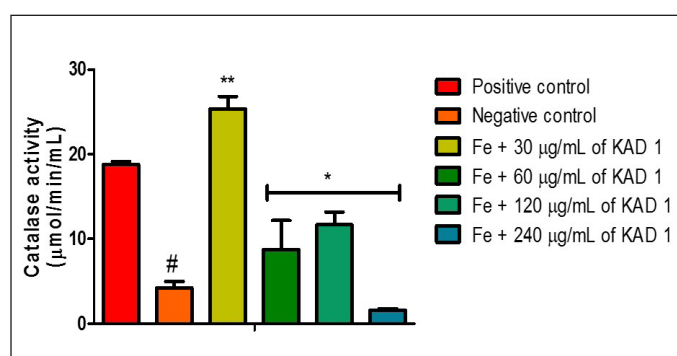


Figure 5. KAD 1's impact on CAT enzyme activity in iron-mediated oxidative testicular injury. Data are expressed as mean $\pm$ SD ($n = 3$), while *, **, and # are statistically significant (* # at $p < 0.05$ and ** at $p < 0.01$) when compared to positive and negative controls, respectively.

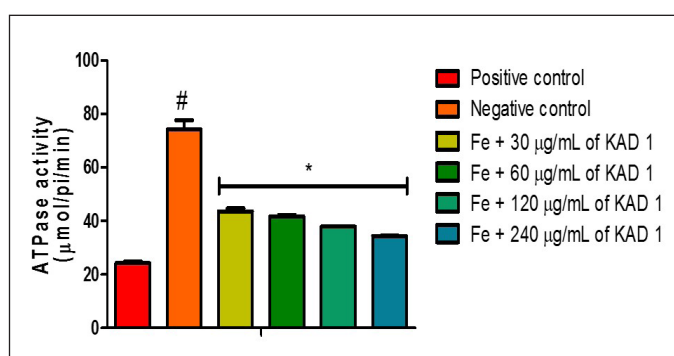


Figure 7. Impact of KAD 1 on ATPase activity in iron-mediated oxidative testicular injury. Data are expressed as mean $\pm$ SD ($n = 3$), while * and # are statistically significant ($p < 0.05$) when compared to positive and negative controls, respectively.

Purinergic function

The impact of KAD 1 on ATPase activity is represented in Figure 7. This outcome reveals that the treated groups differ significantly ($p < 0.05$) from the negative control. The difference observed is the reduction in activity as the concentration of KAD 1 increases. ENTPDase activity is shown in Figure 8. The activity is shown to increase as compared to the negative control. Nevertheless, the group treated with 120 mg/ml shows to have the highest activity compared to other treatment groups.

DISCUSSION

Excessive generation of ROS is a major contributor to man sterility, and prostrate impotence [44]. Previous studies and research reported the importance of phytochemicals that act as antioxidants, which are lethal to the toxic ROS in the male reproductive organ [45–47]. Fe^{2+} triggers the production of radicals (such as hydroxyl and hydroperoxyl) through the contact it has with H_2O_2 that is manufactured in the mitochondria of the Fenton reaction respiratory pathway. The occurrence of these dangerous lethal chemicals in the cells could bring about a series of reactions that would then produce more ROS in the system [48]. Thereafter the increased level of

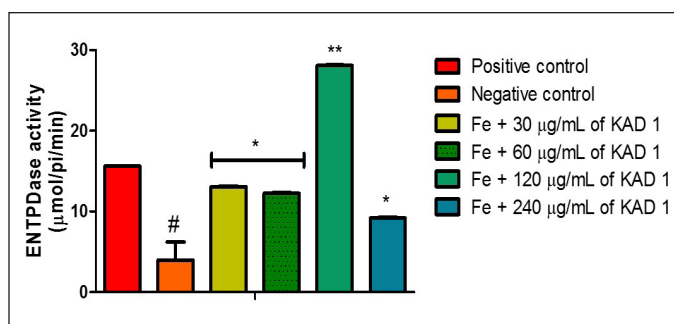


Figure 8. Impact of KAD 1 on ENTPDase activity in iron-mediated oxidative testicular damage. Data are expressed as mean $\pm$ SD ($n = 3$), while * and # are statistically significant (* # at $p < 0.05$ and ** at $p < 0.01$) when compared to positive and negative controls, respectively.

ROS tends to endanger the antioxidant capacity of the system which ultimately leads to the production of radicals [49,50].

KAD 1 showed its antioxidant potential, displaying its potential to scavenge free radicals produced in the system. FRAP as well as Fe chelating was also measured in KAD 1 and found that it can reduce ferric ions. Previous reports have stated the danger observed in the testes after exposure to Fe^{2+} , which has led to the

continuous reduction of CAT and GSH. Furthermore, exposure to Fe^{2+} also leads to lipid peroxidation, causing damage to the testicles [51,52]. The results obtained from this research show a depleted CAT and GSH in the negative control, which suggests that OS has occurred in the testes and this could be ascribed to the iron induction that took place through the Fenton, as well as Haber Weiss reactions [53]. Increased levels of MDA observed in the negative control when compared to the positive control, and treated groups could be a pointer to the fact that lipid peroxidation has occurred in the testicular tissues. The MDA level in treated groups reduces as the concentration of KAD 1 increases, which also suggests the potency of KAD 1 on the testicular tissue. OS in the testes is injurious to fertility in men [35,36].

Purinergic enzymes are vital in the secretion of fluids and protein in the testicles [12,54]. From the result obtained from ATPase activity, the untreated group experienced an increased activity as against the control and treated groups. The elevated levels signify that there could be an imbalance of the acid/base content in the testicular tissue owing to the attack that reduced the ATP levels [35,36]. The ATPases that are present on the surface of the membrane in the testes are responsible for the creation of the ideal pH condition, necessary for smooth sperm cell maturation as well as storage [12]. In cases of a prostrate, an increased level of ATPase is known to boost motility, as well as an invasion [55]. Additionally, the survival of the sperm cells as well as their functionality critically depends on the maintenance of the pH range [56]. Furthermore, the KAD 1 potential to reverse this action could suggest the potential of the KAD 1. The reduction in the activity of ENTDPase following treatment with KAD 1 indicates that there could be increased metabolism of glucose in the tissues of the testes, resulting in an elevated level of ATP in the testes observed in a similar study [12].

Although in antimicrobial studies, metal complexes displayed better activity than the starting materials. Recently, alkali earth metal's complexes of a polyphenolic compound, cichoric acid, possessed significant antimicrobial activity than free compound [57,58]. However, their biological activity is mostly affected by the chelation process [59]. This is the first time investigating the potential of KAD 1 in ameliorating iron-induced testicular injury. Although we did not investigate the effect of chelation on KAD 1 activity on iron-induced testicular toxicity, the chelating process might be a factor responsible for the poor activity of KAD 1 at 60 mg/ml and above.

CONCLUSION

Overall, the results of this study point to a potential application of KAD 1 in the prevention of OS testis injury. The ability of KAD 1 to regulate nucleotide hydrolysis and reduce OS suggests that it has the potential to protect against oxidative testis toxicity. Thus, KAD 1 may be a suitable potent modality, which can help treat testicular injury.

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AUTHOR CONTRIBUTIONS

Each author gave their approval for the final version to be published, agreed to submit the paper to the specified journal,

and made substantial contributions to the conceptualization, design, data collecting, analysis, and interpretation of the research. Each author promised to be accountable for every aspect of the work and contributed to its creation or critically revised the book for key intellectual substance. All of the writers are eligible to be authors per the requirements/guidelines of the International Council of Medical Journal Editors (ICMJE).

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICAL APPROVAL

Rats were cared for in compliance with procedures endorsed by the institutional animal ethics committee at Bowen University, Iwo (BUI). Before the start of the trial, the approval was obtained (Approval Number: BUI/BCH/2022/0002).

DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

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