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# Cardio-nephrotoxicity mediated by *Echis ocellatus* venom and its amelioration through kaempferol's suppressive effect on oxidative stress, inflammation, and apoptosis expression

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## Abstract

**Background** *Echis ocellatus* venom toxins have the ability to impact multiple organ systems subsequent to envenomation. Kaempferol have been reported to have several therapeutic benefits. In this study, the therapeutic value of kaempferol was investigated in relation to the cardio-nephrotoxicity in rats resulting from *E. ocellatus* envenoming.

**Methods** Fifty male wistar rats were allotted unbiased into five groups ( $n = 10$ ) for this study. Group 1 was the control, while rats in groups 2 to 5 were envenomed with LD<sub>50</sub> of *E. ocellatus* venom (0.22 mg/kg bw; i.p.). Group 2 was not treated after envenomation while groups 3, 4 and 5 were treated with polyvalent antivenom, 4 and 8 mg/kg of kaempferol, respectively.

**Results** *E. ocellatus* envenomation caused considerable reduction in organ weight and relative organ weight in the envenomed untreated rats. The venom induced intense oxidative stress, inflammation, apoptotic damage to the cardiac and renal tissues accompanied with severe histomorphology in the organ tissues of untreated envenomed rats. In contrast, kaempferol treatment post-envenomation attenuated the venom-induced cardio-nephrotoxic responses in a dose dependent effect. Kaempferol substantially ( $p < 0.05$ ) decreased malondialdehyde levels while enhancing reduced glutathione levels and superoxide dismutase and glutathione peroxidase activities in the heart and kidney of envenomed treated rats. Treatment of envenomed rats with kaempferol successfully decreased nitric oxide levels and myeloperoxidase activity. Overexpression of apoptotic caspase 3 and caspase 9 in cardiac and renal tissues were suppressed by kaempferol ( $p < 0.05$ ). The histopathological result supports kaempferol's ameliorative ability by convalescing the severe morphological alterations of cardiac and renal tissues induced by the venom.

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**Conclusion** Findings elucidate the significance of kaempferol as promising agent in the management of cardio-nephrotoxicity resulting from snakebite envenoming.

**Keywords** *Echis ocellatus*, Cardiotoxicity, Envenomation, Kaempferol, Nephrotoxicity

## Introduction

Snake venoms are complex protein combinations with high biological activity and action selectivity [1]. Different components of the venom may have different effects, and potential synergistic actions of the proteins may increase toxicity and make their biological impact complicated [2]. Snake venom proteins can impact multiple organ systems within an organism; however, their primary targets are the circulatory, neurological, cardiovascular, and renal systems [3, 4]. Many cardio-vascular consequences, such as brady- or tachycardia, atrial fibrillation, hypertension, myocardial infarction, cardiac arrest, and hypotension, have been linked to snake envenomation [1]. Tchaou et al. [4]. and Averin and Utkin [3] have reported issues related to the kidney and heart, respectively following snakebite envenomation, indicating that snake venom is a rich source of toxins, considering the variety of consequences.

*Echis ocellatus* is a viper whose venom is made up of hundreds of distinct peptides and proteins, primarily metalloproteinase and phospholipase A2, which are known to target diverse organ systems in the body [5]. While the latter activate prostaglandin and leukotriene metabolism, which plays a central role in the inflammatory response through the metabolic cascade of arachidonic acid, causing diverse organ pathologies, the former hydrolyze the vascular endothelium's basement membrane, promoting blood leakage, the progression of edema and hematoma, low blood pressure, fibrinolysis, thrombosis, and variable tissue damage [4–6]. Studies in animal models have revealed a number of pathological problems in the cardiac and renal organs after *E. ocellatus* envenomation [7, 8].

Studies have demonstrated acute kidney injury in renal tissues [4], and *E. ocellatus* venom toxins have been linked to increased formation of reactive oxygen species (ROS) leading to oxidative stress (OS) and inflammation in cardiac tissues [7]. Imbalance between ROS and antioxidant activity under any pathophysiological circumstances, will result in oxidative stress and increased tissue damage, which is a significant determinant of the development of cardiovascular impairment [9]. Furthermore, ROS has been shown to have a major role in a number of clinical disorders and to be directly implicated in inflammatory reactions that have detrimental effects on cellular physiology [7].

Research has shown that *E. ocellatus* venom toxins caused acute kidney injury in renal tissues [4], via increased production of reactive oxygen species (ROS)

resulting to oxidative stress (OS) and inflammation in cardiac tissues [4, 7]. Also, ROS had been established to be directly involved in inflammatory reactions with damaging effects on cellular physiology and plays a significant role in various pathological conditions [7]. Despite established evidence of *E. ocellatus* venom detrimental effects on cardiac and renal organs, there is limited report on the amelioration of cardio-nephrotoxicity caused by the venom.

Flavonoids are phenolic compounds that are currently receiving interest in drug-discovery investigations [10]. Naturally occurring flavonoid kaempferol is found in a variety of plants and has a wide range of pharmacological effects, including as anti-inflammatory, anti-cancer, and antiapoptotic effects [10, 11]. According to reports, kaempferol effectively neutralizes the toxins in snake venom following envenomation in animal models [12]. There is currently no substantial evidence to support kaempferol's potential ameliorative benefit against venom-induced toxicity to the kidneys and heart. Through an in vivo experiment, this study examined the possible ameliorative effects of kaempferol on the cardio-nephrotoxicity induced by *E. ocellatus* venom.

## Materials and methods

### Procurement of venom and serum antivenom

Lyophilized sample of *E. ocellatus* venom was procured from the serpentarium of the Department of Zoology at the University of Ibadan in Nigeria, and stored in the laboratory at 4 °C. The reference drug used in this study was the EchiTab-Plus ICP polyvalent serum antivenom.

### Procurement of kaempferol

A quantity of kaempferol ( $C_{15}H_{10}O_6$ ) was purchased from a standard chemical company, Sigma-Aldrich®, USA, St Louis, Missouri, USA for this research.

### Ethics consideration

Ethical approval for this study was obtained from University of Ibadan-Animal Care and Use Research Ethics Committee (UI-ACUREC), with authorized number: UI-ACUREC/19/0030. All experimental protocols followed the rules and guidelines established for the care and use of laboratory animals in compliance with the revised ARRIVE guidelines 2.0.

### Experimental animal welfare

Fifty male albino Wistar rats with weight ranging between 100 and 130 g were procured from the Central

Animal Facility of Osun State University, Osogbo, Nigeria. The animals were kept in transparent well-ventilated plastic cages and acclimatized for two weeks at the Department of Zoology Laboratory, Osun State University under the ambient standard conditions ( $25 \pm 2$  °C and relative humidity of  $50 \pm 15\%$ ). The rats were allowed free access to standard rat feed and clean water under 12 h daylight and darkness cycle.

#### Animal model procedures

Fifty male wistar rats were randomly assigned into five groups of ten animals each. The first group received a saline injection as control, whereas groups 2, 3, 4, and 5 were envenomed with *E. ocellatus* venom at a lethal dose ( $LD_{50}$ ) of 0.22 mg/kg-1 intraperitoneally [13]. Group 2 received no treatment post envenomation, while group 3 received 0.2 mg of polyvalent antivenom via intraperitoneal route. Kaempferol at 4 and 8 mg/kg was dissolved separately in 1 ml of saline and injected intraperitoneally into rats in group 4 and 5 respectively. Thirty minutes after the animals were envenomed, the treatment commenced, and it continued for fourteen days in a row. Every experimental rat was monitored for toxic signs of envenomation and mortality.

#### Body weight changes

All the experimental animals were measured pre-venom injection as initial weight and at termination to determine the body weight changes before been sacrificed as terminal weight. Changes in the body weight was calculated using the formula:

$$\text{Body weight gain} = \frac{\text{Terminal weight of rats} - \text{Initial weight of rats}}{\text{Initial weight of rats}} \times 100$$

#### Organ collection

The rats were sacrificed on the last day of the experiment following guides [14]. The heart and kidney of each experimental rat was surgically removed and weighed. The organs were divided into two and the first part was used for biochemical assays while the second was preserved 100% formalin for histopathological studies. The relative organ weight was determined using the formula:

$$\text{Relative organ weight} = \frac{\text{Organ weight}}{\text{Terminal body weight}} \times 100$$

#### Assessments of heart and kidney biochemical analysis

##### Oxidative stress biomarkers

The levels of reduced glutathione (GSH) were carried out following the previous protocol [15]. Glutathione peroxidase (GPX) and Superoxide Dismutase (SOD) activities

were measured using the previous methods of Marklund and Marklund [16], and Rotruck et al. [17], respectively. The levels of lipid peroxidation end product; malondialdehyde (MDA), was determined as described [18].

##### Inflammatory biomarkers

The cardiac and renal nitric oxide (NO) level was measured as described by Green et al. [19], while myeloperoxidase (MPO) activity was assessed by procedures previously described by Granell et al. [20].

##### Apoptosis expression

###### Caspase 3 and caspase 9

Caspase-3 and caspase-9 expression in cardiac and renal tissues were measured using specific enzyme-linked immunosorbent assay (ELISA) kits (CUSABIO, Houston, TX, 77,054, USA). The procedure was done according to the manufacturer's instructions.

##### Histopathological study

Cardiac and renal tissues were examined for morphological defects using conventional techniques of paraffin-wax sectioning and hematoxylin-eosin staining [21].

##### Data analysis

Data obtained from this study were expressed as mean  $\pm$  Standard Error. Significant differences between the control and envenomed groups were tested using one-way Analysis of Variance and Duncan multiple range test of the treatment groups. Analyzed values were considered significant at  $P < 0.05$ . Statistical Package for Social Sciences (SPSS, version 25) software produced by IBM Corp. Ltd. was used for data analysis.

## Results

### Clinical signs of toxicity, mortality, body and organ weight changes

The envenomed animals were shaky, sluggish, restricted in movement within their cages with low appetite for food. Rats envenomed and not treated recorded five mortality while groups 3 and 4 envenomed and treated with antivenom and 4 mg/kg of kaempferol recorded three mortality each (Table 1). The control and envenomed group treated with 8 mg/kg kaempferol recorded no mortality. Likewise, the body weight gain of envenomed untreated rats substantially decreased compared to the envenomed treated rats (Table 2). The heart and kidney weights of envenomed untreated rats were significantly ( $p < 0.05$ ) lower than those of the control and envenomed treated groups, while the experimental rats' testicular somatic index showed a similar trend (Table 2). The envenomed group treated with 8 mg/kg of kaempferol recorded the highest organ and relative organ weight.

**Table 1** Mortality recorded following envenomation

Groups	Envenomation	Day 1	Day 2	Day 3	Day 14	Mortality (%)
1	-	-	-	-	-	0.00
2	-	3	1	1	-	50.00
3	-	2	1	-	-	30.00
4	-	2	-	1	-	30.00
5	-	-	-	-	-	0.00

Number of rats per group ( $n=10$ )

#### Legend

Group 1: Control (Injected with saline only), Group 2: Venom control (injected with *E. ocellatus* venom and not treated), Group 3: Injected with *E. ocellatus* venom and treated with antivenom (0.2 ml), Group 4: Injected with *E. ocellatus* venom and treated with kaempferol (4 mg/kg<sup>-1</sup>), Group 5: Injected with *E. ocellatus* venom and treated with kaempferol (8 mg/kg<sup>-1</sup>)

**Table 2** Organ weight and relative organ index of the experimental animals

Groups	Body weight gain (g)	Heart weight (g)	Heart index (g)	Kidney weight (g)	Kidney index (%)
1	1.12 ± 0.01 <sup>a</sup>	1.53 ± 0.08 <sup>a</sup>	0.68 ± 0.04 <sup>a</sup>	2.66 ± 0.12 <sup>a</sup>	0.84 ± 0.01 <sup>a</sup>
2	0.14 ± 0.02 <sup>d</sup>	1.12 ± 0.01 <sup>d</sup>	0.32 ± 0.01 <sup>d</sup>	1.94 ± 0.07 <sup>e</sup>	0.44 ± 0.00 <sup>d</sup>
3	0.53 ± 0.01 <sup>c</sup>	1.27 ± 0.10 <sup>c</sup>	0.47 ± 0.02 <sup>c</sup>	2.32 ± 0.04 <sup>d</sup>	0.53 ± 0.01 <sup>c</sup>
4	0.73 ± 0.10 <sup>b</sup>	1.34 ± 0.02 <sup>b</sup>	0.56 ± 0.02 <sup>b</sup>	2.41 ± 0.12 <sup>c</sup>	0.73 ± 0.11 <sup>b</sup>
5	0.89 ± 0.12 <sup>a</sup>	1.45 ± 0.06 <sup>a</sup>	0.60 ± 0.10 <sup>b</sup>	2.60 ± 0.10 <sup>b</sup>	0.82 ± 0.12 <sup>a</sup>

Data are represented as mean ± SE ( $n=5$ ). Mean with different lower-case letter represent significant difference among the groups at  $p < 0.05$  using DMRT

#### Legend

Group 1: Control (Injected with saline only), Group 2: Venom control (injected with *E. ocellatus* venom and not treated), Group 3: Injected with *E. ocellatus* venom and treated with antivenom (0.2 ml), Group 4: Injected with *E. ocellatus* venom and treated with kaempferol (4 mg/kg<sup>-1</sup>), Group 5: Injected with *E. ocellatus* venom and treated with kaempferol (8 mg/kg<sup>-1</sup>)

## Oxidative stress profiles of the heart and kidney

### Malondialdehyde (MDA) levels

The MDA levels in the heart and kidney of envenomed untreated rats significantly ( $p < 0.05$ ) increased in contrast with the control and envenomed treated groups. The substantial levels of MDA as observed in the untreated envenomed rats was significantly decreased ( $p < 0.05$ ) after treatment with 8 mg/kg of kaempferol compared to other envenomed treated groups (Fig. 1A).

### Reduced glutathione (GSH) levels

The reduced GSH levels significantly ( $P < 0.05$ ) decreased in the group envenomed with *E. ocellatus* venom and not treated post envenomation compared to the control. However, treatment with antivenom and varying doses of kaempferol significantly ( $P < 0.05$ ) increased the GSH levels of the envenomed treated groups. The envenomed group treated with 8 mg/kg of kaempferol showed substantial increase compared to the envenomed groups treated with 4 mg/kg of kaempferol and antivenom, respectively (Fig. 1B).

### Superoxide dismutase (SOD) activity

The SOD activity in the heart and kidney of envenomed untreated rats was more significantly intense relative to the control. However, treatment with antivenom and different concentrations of kaempferol significantly ( $p < 0.05$ ) enhanced the activity of SOD in the organs compared to the envenomed untreated group. In

comparison, the envenomed group treated with 8 mg/kg of kaempferol recorded the highest SOD activity compared to other envenomed treated groups (Fig. 1C).

### Glutathione peroxidase (GPX) activity

The *E. ocellatus* venom caused substantial ( $p < 0.05$ ) decline in the GPX activity of the heart and kidney of untreated rats in contrast with the control. However, different doses of kaempferol enhanced the activity of GPX in the envenomed treated groups compared to groups treated with antivenom (Fig. 1D).

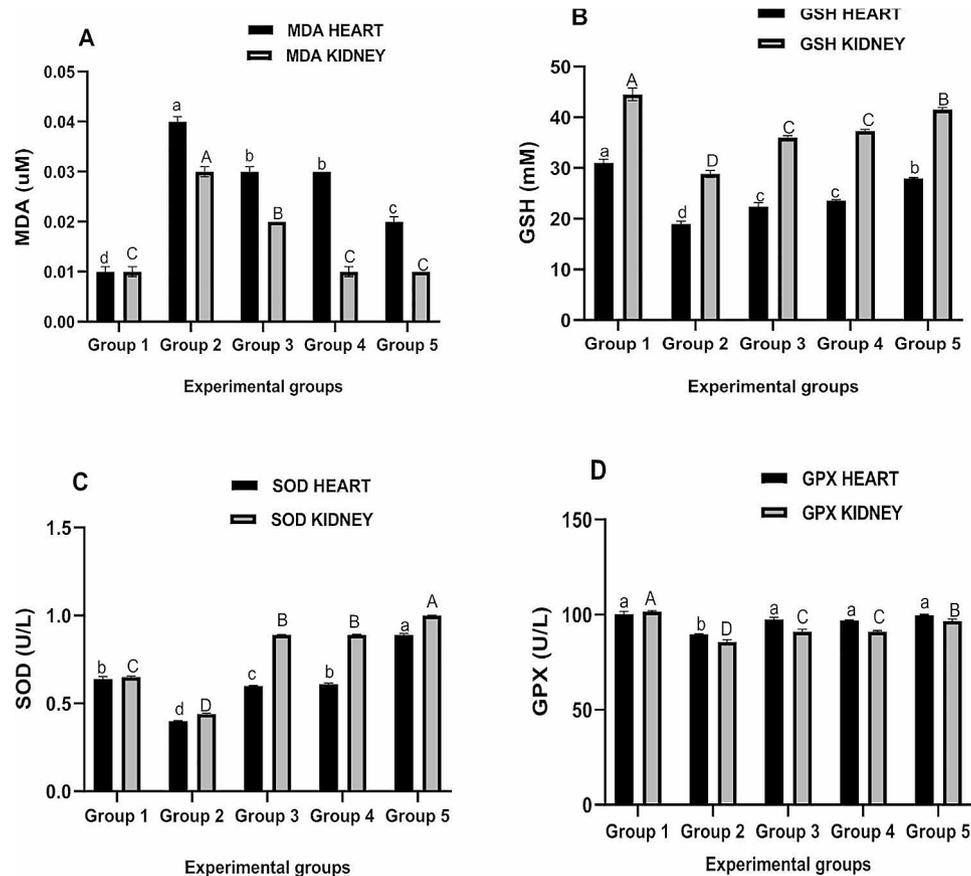
## Inflammatory profiles of the heart and kidney

### Nitric oxide (NO) levels

The NO levels were more intense in the heart and kidney of the envenomed untreated group compared to the control and envenomed treated rats. However, treatment of envenomed rats with antivenom and different doses of kaempferol significantly ( $p < 0.05$ ) repressed the NO levels. Group treated with 8 mg/kg of kaempferol showed significant decline compared to other treated groups (Fig. 2A).

### Myeloperoxidase (MPO) activity

*E. ocellatus* venom significantly ( $p < 0.05$ ) increased the MPO activity in the heart and kidney of envenomed untreated rats compared to the control and envenomed treated groups. However, treatment with kaempferol caused a significant ( $p < 0.05$ ) dose dependent decrease



**Fig. 1** Effects of kaempferol on oxidative stress biomarkers and antioxidant enzymes in the cardiac and renal organs of envenomed treated rats Group 1: Control, Group 2: Envenomed untreated, Group 3: Envenomed and treated with antivenom, Group 4: Envenomed and treated with kaempferol (4 mg/kg<sup>-1</sup>), Group 5: Envenomed and treated with kaempferol (8 mg/kg<sup>-1</sup>). Data are represented as mean  $\pm$  SE ( $n=5$ ). There was a statistically significant difference between the control and treatment groups at  $p < 0.05$ . Bar with different lower-case letter represent significant difference in stress profile of the heart among the groups at  $p < 0.05$  using DMRT. Bar with different upper-case letter represent significant difference in stress profile of the kidney among the groups at  $p < 0.05$  using DMRT. MDA: Malondialdehyde, GSH: Glutathione, SOD: Superoxide dismutase, GPX: Glutathione Peroxidase

in the MPO activities and the highest reduction was recorded in envenomed group treated with 8 mg/kg of kaempferol (Fig. 2B).

### Effect of kaempferol on the apoptosis markers

#### Caspase 3 expression

Casp-3 was substantially expressed in the heart and kidney of the envenomed untreated group relative to the control and envenomed treated rats (Fig. 3A). However, kaempferol and antivenom suppressed the expression of casp-3 in groups envenomed and treated. The suppressive effect of kaempferol was more intense in envenomed group treated with 8 mg/kg of kaempferol.

#### Caspase 9 expression

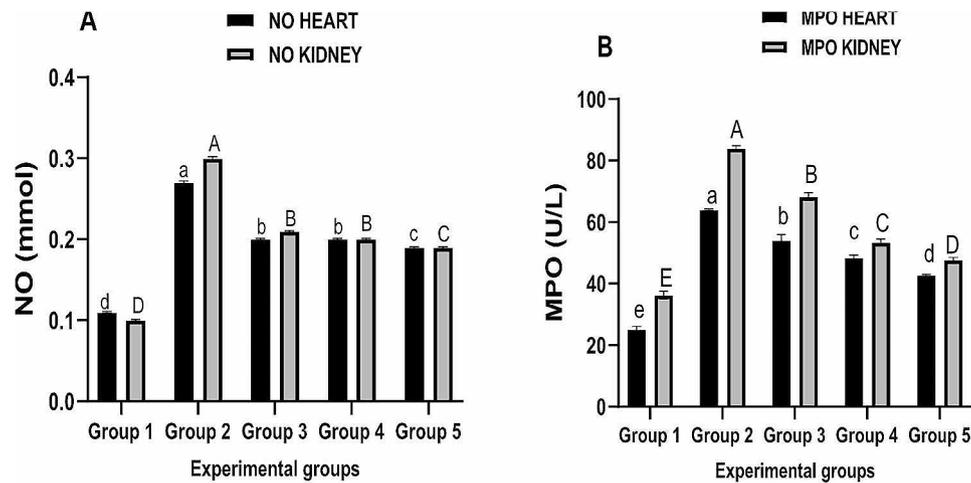
*E. ocellatus* venom caused significant expression of casp-9 in the cardiac and renal tissues of envenomed untreated rats in comparison with the control and

envenomed treated groups (Fig. 3B). However, kaempferol recorded a substantial ( $p < 0.05$ ) dose dependent reduction in the casp-9 expression in heart and kidney of envenomed treated groups. Furthermore, envenomed group treated with 8 mg/kg of kaempferol recorded the highest reduction of casp-9 expression in the heart and kidney tissues (Fig. 3B).

### Histological evaluation of the cardiac and renal tissues

#### The heart

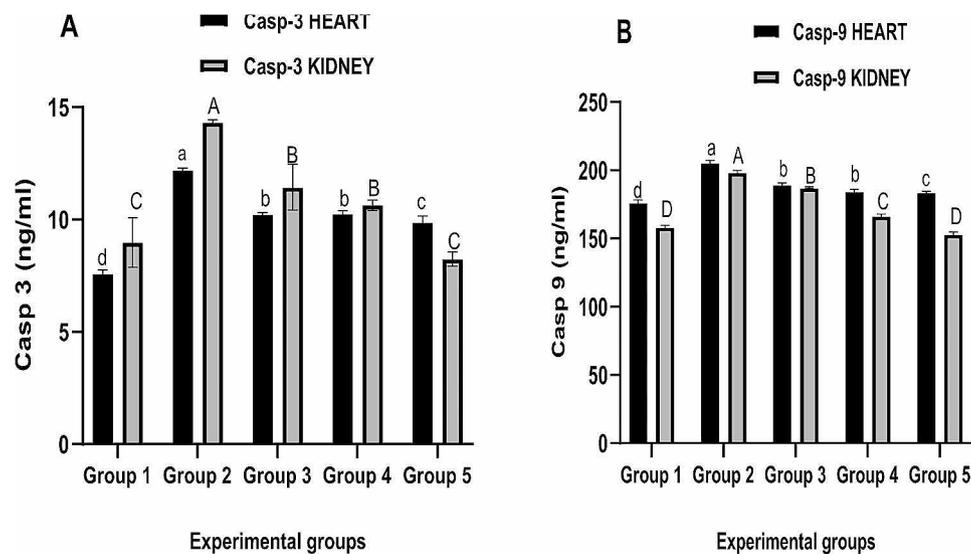
The cardiomyocytes of the control appeared normal and compact with no structural deformities observed (Fig. 4, plate 1), whereas *E. ocellatus* venom highly disarranged the cardiomyocytes with areas of muscle fiber separation and pyknotic nuclei of the cardiac muscle cells indicating cell degeneration (Fig. 4, plate 2). The envenomed group treated with antivenom showed compacted cardiac muscle cells with area of enlarged and congested capillaries



**Fig. 2** Effect of kaempferol on inflammatory biomarkers in the cardiac and renal organs of envenomed treated rats

Group 1: Control, Group 2: Envenomed untreated, Group 3: Envenomed and treated with antivenom, Group 4: Envenomed and treated with kaempferol ( $4 \text{ mg/kg}^{-1}$ ), Group 5: Envenomed and treated with kaempferol ( $8 \text{ mg/kg}^{-1}$ ). Data are represented as mean  $\pm$  SE ( $n=5$ ). There was a statistically significant difference between the control and treatment groups at  $p < 0.05$ . Bar with different lower-case letter represent significant difference in inflammatory biomarkers of the heart among the groups at  $p < 0.05$  using DMRT. Bar with different upper-case letter represent significant difference in inflammatory biomarkers of the kidney among the groups at  $p < 0.05$  using DMRT

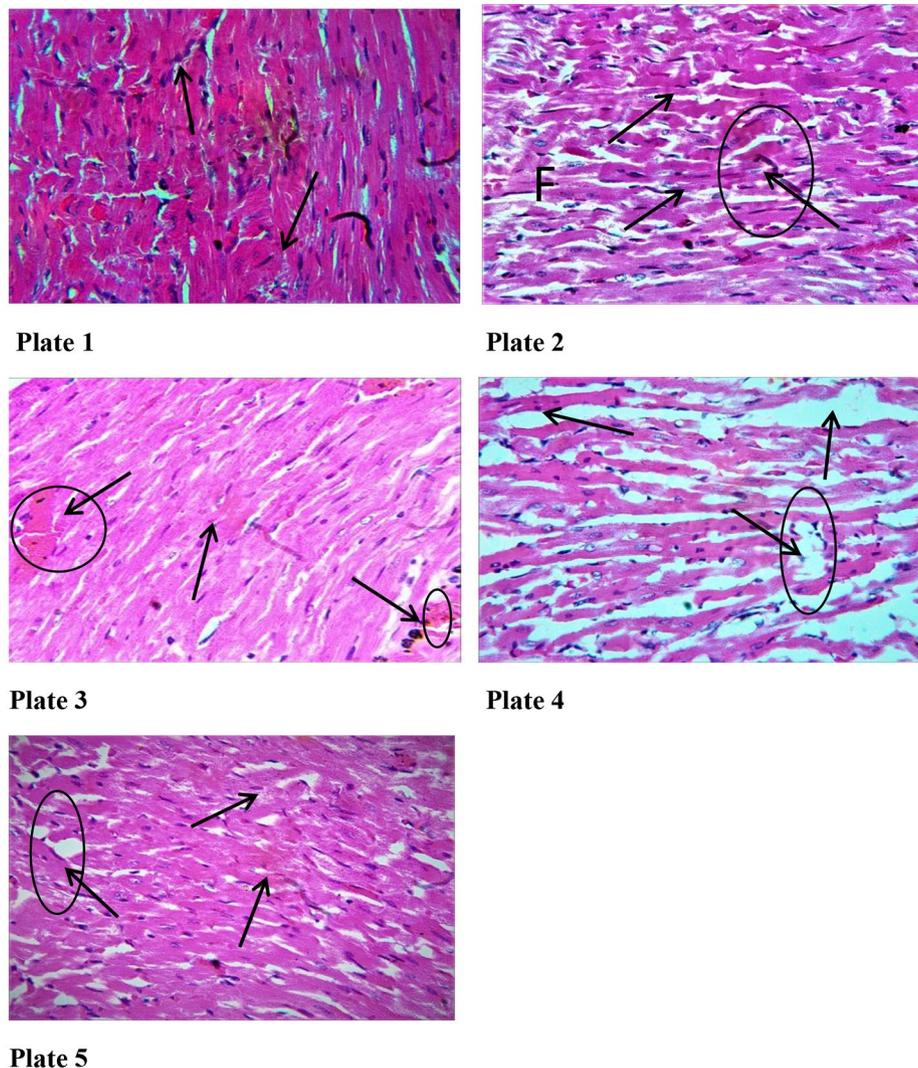
NO: Nitric Oxide, MPO: Myeloperoxidase



**Fig. 3** Effect of kaempferol on caspase 3 and 9 expression in the cardiac and renal organs of envenomed treated rats

Group 1: Control, Group 2: Envenomed untreated, Group 3: Envenomed and treated with antivenom, Group 4: Envenomed and treated with kaempferol ( $4 \text{ mg/kg}^{-1}$ ), Group 5: Envenomed and treated with kaempferol ( $8 \text{ mg/kg}^{-1}$ ). Data are represented as mean  $\pm$  SE ( $n=5$ ). There was a statistically significant difference between the control and treatment groups at  $p < 0.05$ . Bar with different lower-case letter represent significant difference in apoptosis marker of the heart among the groups at  $p < 0.05$  using DMRT. Bar with different upper-case letter represent significant difference in apoptosis marker of the kidney among the groups at  $p < 0.05$  using DMRT

Casp-3: Caspase 3, Casp-9: Caspase 9



#### Hematoxylin and Eosin (H&E); Magnification: 400×

**Fig. 4** Histology of the cardiac tissues

**Plate 1 (control):** The cardiomyocytes appeared normal and no observable lesion was noticed. **Plate 2 (venom control):** revealed highly disarranged cardiomyocytes with areas of muscle fiber separation (CT) and pyknotic nuclei of the cardiac muscle cells indicating cell degeneration. **Plate 3 (venom/antivenom):** shows compacted cardiac muscle cells with area of enlarged and congested capillaries. **Plate 4 (venom/4 mg/kg kaempferol):** moderately distorted cardiac muscle cells with indistinct branching. **Plate 5 (venom/8 mg/kg kaempferol):** Highly vascularized connective tissue spaces with mild area of fiber degeneration

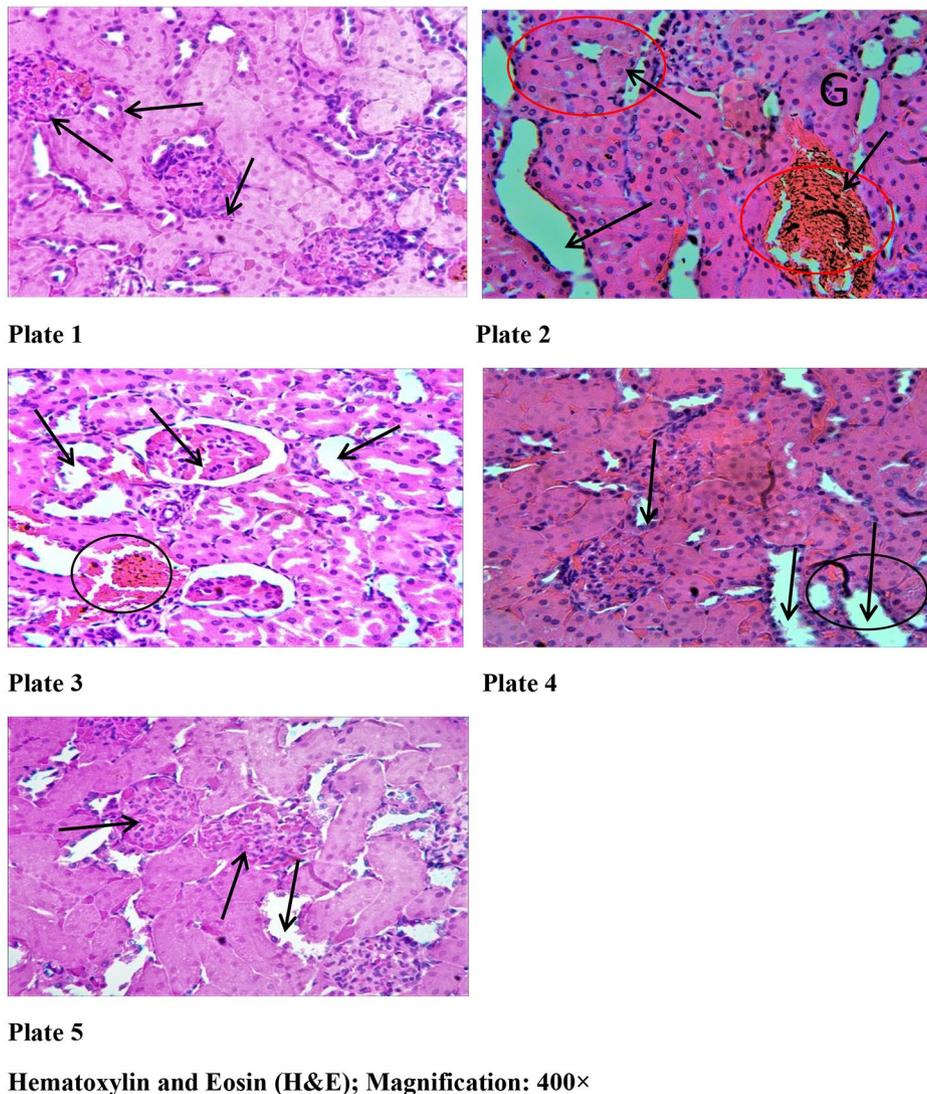
Hematoxylin and Eosin (H&E); Magnification: 400×

(Fig. 4, plate 3). However, a moderately distorted cardiac muscle cells with indistinct branching were observed in cardiac tissue of the envenomed group treated with 4 mg/kg of kaempferol (Fig. 4, plate 4). The envenomed group treated with 8 mg/kg revealed a highly vascularized connective tissue spaces with mild area of fiber degeneration (Fig. 4, plate 5).

#### **The kidney**

The kidney of the control rats showed intact cuboidal epithelium of renal tubules with normal glomeruli

appearance (Fig. 5, plate 1). Meanwhile, glomeruli of the envenomed untreated rats appear highly congested with indistinct tuft in the loops and aberrant mesangial regions. Metaplasia of the capsular epithelium (squamous cell metaplasia) is seen in the ringed region (circle) (Fig. 5, plate 2). The envenomed group treated with antivenom showed a mild peritubular congestion (Fig. 5, plate 3), while the envenomed groups treated with 4 mg/kg of kaempferol revealed a moderate peritubular congestion and hypertrophied cells (Fig. 5, plate 4). Also, group envenomed and treated with 8 mg/kg of kaempferol



**Fig. 5** Histology of the renal tissues

**Plate 1 (control):** showed intact cuboidal epithelium of renal tubules with normal glomeruli appearance. **Plate 2 (venom control):** glomeruli appear highly congested with indistinct tuft in the loops and aberrant mesangial regions. Metaplasia of the capsular epithelium (squamous cell metaplasia) is seen in the ringed region (circle). **Plate 3 (venom/antivenom):** Mild peritubular congestion, **Plate 4 (venom/4 mg/kg kaempferol):** showed moderate peritubular congestion and hypertrophied cells. **Plate 5 (venom/8 mg/kg kaempferol):** showed mild peritubular congestion with cortical features similar in appearance to the control  
Hematoxylin and Eosin (H&E); Magnification: 400×.

showed mild peritubular congestion with cortical features similar in appearance to the control Fig. 5, plate 5).

## Discussion

The kidneys and heart are important organs that are vulnerable targets for attacks by toxins in snake venom via overwhelming their defense systems, leading to cardio-nephrotoxicity following snakebite envenomation. Viper venoms are known to cause organ toxicity after envenomation due to the presence of snake venom metalloproteinase (SVMPs) and phospholipase A2 (PLA2) enzymes that alters morphological arrangement of organ tissues

post envenomation [1]. SVMPs is abundant in *E. ocellatus* venom and can damage the nephron of the kidney by breaking extracellular matrix proteins of the membrane, perforating it, inhibiting cell adhesion, destroying membrane receptors and activating mediators of inflammatory response and apoptosis [4]. Likewise, PLA2s could hydrolyze the lipid layer of organ cells [4].

In this study, *E. ocellatus* venom caused substantial reduction in organs and relative organs weight in the envenomed untreated rats which is an evidence of organ toxicity through direct interactions of the venom toxins with the organs as previously reported [7, 22]. In

contrast, kaempferol a plant-derived flavonoid compound significantly increased the organ and relative organ weights as observed in the envenomed treated rats which affirmed the ability of the bioactive compound to alleviate the detrimental effect of snake venom toxins as earlier established [12].

The primary cause of venom-induced pathogenesis is considered to be oxidative stress, which is caused by the production of reactive oxygen species (ROS) through increased lipid peroxidation. There is growing evidence that renal damage can be accelerated by increased lipid peroxidation [23]. Numerous studies have demonstrated that the mechanism underlying renal dysfunction involves lipoprotein binding with the glomerular basement membrane's glycosaminoglycan, tubulointerstitial damage brought on by intraluminal apoprotein precipitation, cholesterol deposition, altered cortical fatty acid metabolism, accumulation of excess lipids by mesangial macrophages, and increased glomerular pressure [24]. In this study, *E. ocellatus* venom caused significant elevation of MDA (lipid peroxidation end-product) in the heart and kidney of the envenomed untreated rats indicating venom-induced oxidative stress in the organs which affirmed the venom can induce oxidative stress in vital organs after envenoming which corroborated previous study [7].

Superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione levels (GSH) are components of the enzymatic and non-enzymic antioxidant defense mechanism that shields cells from ROS damage and lipid peroxidation. These antioxidant levels will decline in any biological system where the formation of reactive oxygen species increases significantly [25]. In the present investigation, in envenomed untreated rats, the activity of antioxidants such as SOD, GSH, and GPx significantly decreased. This shows that the increase in ROS caused by lipid peroxidation may have overwhelmed the body's natural antioxidant defenses, inhibiting it and causing oxidative stress in the kidneys and heart. This observation has been previously reported in *E. ocellatus* envenomed rats [7]. Kaempferol protects the heart and kidney by reducing lipid peroxidation product (malondialdehyde) and significantly elevated antioxidant enzymes to a considerable level in envenomed treated rats which substantiated the antioxidant property of kaempferol against venom induced toxicities as earlier reported [12].

Inflammation is a major factor in the pathophysiology of various systems, including the dysfunction of organs [26]. In envenomed rats, *E. ocellatus* venom has been linked to an inflammatory reaction. Through an increase in MPO activity and NO level, the venom in this study produced inflammation in the kidney and heart of envenomed untreated rats. Reports abound that in envenomed patients, significant organ

inflammation caused by snakebite envenomation has played a role in the cause of mortality [4]. However, kaempferol treatment substantially suppressed the inflammatory biomarkers in the envenomed treated groups. Kaempferol's anti-inflammatory properties have been demonstrated by toxicants-mediated reductions in inflammatory biomarkers [10].

The process of apoptosis, which is linked to excessive formation of reactive oxygen species (ROS) and plays a critical role in mitochondrial function, is enhanced in experimental cardiac illnesses [27, 28]. According to Brentnall et al. [29], caspase 3 and caspase 9 are significant indicators that show apoptosis in organ tissues. The caspase cascade involves the release of cytochrome c from the intermembrane gap into the cytosol, where it binds Apaf-1 to create the apoptosome and activates caspase-9. This caspase can remodel mitochondria and enhance generation of reactive oxygen species (ROS) by cleaving Bid into tBid [29, 30]. In a state of activeness, caspase-9 can directly cleave and activate caspase-3 which is the effector caspase necessary for efficient cell death. Effector caspases are the ones that cause DNA fragmentation, cell shrinkage, and membrane blebbing, which are characteristics of the breakdown phase of apoptosis [31].

The venom in this study increased the number of apoptotic cells in the heart and kidney of envenomed untreated rats via elevating apoptosis indicators. Conversely, kaempferol decreases the number of apoptotic cells in the heart and kidney of the envenomed treated rats by decreasing the expression of the key protease effectors caspase 3 and caspase 9. Kaempferol has been shown to have anti-apoptotic effects in a number of cardiac model studies [26, 32]. Studies have established the anti-apoptotic mechanism of kaempferol via inhibition of the release of cytochrome c into the cytosol [27], thereby preventing the formation of cytochrome c-apoptotic protease activating factor 1 complex that is responsible for activation of caspase activity [33].

A hallmark of snake venom PLA2 found in the *E. ocellatus* venom is the changing of the composition and physiological state of membrane lipids due to peroxidative damage. Animal cell membranes and cytoskeletons depend on phospholipids as structural elements [24]. Significant changes in cellular structure and function can result from direct interactions between toxicants and other xenobiotics, or indirect interactions caused by modifications in the synthesis and metabolism of phospholipids [24]. This study demonstrated that the heart and kidney of envenomed untreated groups had growing patterns of lipid peroxidation, which resulted to organ damage. This effect was substantiated with additional damaging evidence in histological structures of the cardiac and renal

tissues of envenomed untreated rats. The relative magnitude and degree of necrosis in the cardiac tissue of the envenomed untreated rats which revealed highly disarranged cardiomyocytes with areas of muscle fiber separation and pyknotic nuclei of the cardiac muscle cells indicating cell degeneration.

The kidney is an organ which filters all blood of an organism and it is quite important since it is an effective organ in eliminating toxins. The fact that the cytotoxic components of the venom are filtered through the glomerulus and tubules causes disorders in these structures [34]. In this study, the renal histological structural of the envenomed untreated rats display various pathological modification with the glomeruli appeared highly congested with indistinct tuft in the loops and aberrant mesangial regions. These alterations were exemplified by renal tubular damage, indicating tubular necrosis. However, kaempferol ameliorated the observed structural damages with very mild tissue necrosis observed in heart and kidney of envenomed treated rats suggesting that kaempferol is an outstanding flavonoid to treat venom-induced cardiac and renal toxicity.

Plants produces flavonoids as secondary metabolites for self-defense, and kaempferol is a naturally occurring flavonoid that may be found in many different types of plants. The precise mechanism of kaempferol alleviating toxicities induced by *E. ocellatus* venom in cardiac and renal organs of envenomed treated rats may not be ascertain in this study. Studies have reported the anti-inflammatory, antiapoptotic, anti-oxidative properties of kaempferol in vital organs, with possible mechanism via suppression of oxidative stress, inflammatory and apoptosis responses [35–37]. In addition, kaempferol is reported to have excellent anti-inflammatory and strong antioxidant activities via scavenging excessive ROS and preserving the intrinsic antioxidant enzyme activities at normal levels [38–40].

## Conclusion

The present investigation showed that kaempferol could potentially reduce the cardio-nephrotoxic effects of *E. ocellatus* venom in an animal model. In envenomed treated rats, kaempferol substantially reduced the expression of apoptosis, oxidative stress, and inflammatory biomarkers in the cardiac and renal organs. The results validate kaempferol's potential as a viable option for treating clinical cardiac and renal problems following snake envenomation. The results provided a baseline for further investigation on the potential of kaempferol in medication development and clinical application for cardio-nephrotoxicity in envenomed snakebite patients.

## Acknowledgements

Not applicable.

## Author contributions

BSA and JOO conceptualized and designed the study under the guidance of AOA. BSA, BPD, and JOO executed the in vivo antivenom study, laboratory and data analysis. BSA, AKM, and AOA were responsible for drafting and editing the manuscript. The final manuscript was reviewed and approved by all authors.

## Funding

Author did not receive any funding for this research.

## Data availability

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

### Ethical approval

Ethical approval for this study was obtained from University of Ibadan-Animal Care and Use Research Ethics Committee (UI-ACUREC), with authorized number: UI-ACUREC/19/0030.

### Competing interests

The authors declare no competing interests.

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Received: 15 February 2024 / Accepted: 12 June 2024

Published online: 19 June 2024

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