Does the renal portal valve exist in a raptor species? A study aimed at further evaluating the mechanism of toxicity of diclofenac in vultures

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Abstract

Diclofenac has been responsible for the deaths of millions of vultures on the Asian subcontinent. While the pathology of toxicity is well described, the mechanism of toxicity remains elusive. However, it was postulated that toxicity could be related to the unique avian renal vascular structure known as the renal portal valve and that that diclofenac altered valve functionality with subsequent renal ischaemia. While plausible, the *valva renalis portalis* has only been described in a small number of other bird species such as the chicken (*Gallus domesticus*), the domestic duck (*Anas platyrhynchos domesticus*) and ostrich (*Struthio camelus*) but not a raptor. The aim of this study was to evaluate the renal anatomy and related vasculature of the Cape griffon vulture (*Gyps coprotheres*) (CGV), a species sensitive to the toxic effects of diclofenac, using gross anatomy, histology and vascular casting. The vasculature of the vulture was found to be almost identical to that of the domestic chicken with the *valva renalis portalis* present in the *v. iliaca externa* between the *v*. *renalis renalis cranialis* and the *v*. *renalis caudalus*. The valve was ring-shaped with finger-like processes and histologically was composed of smooth muscle. The valve was also well vascularized and was associated with a nerve plexus. Based on the findings of this study, the proposed mechanism of toxicity is anatomically possible.

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1 INTRODUCTION

Vultures are large, volant birds which are classically regarded as raptors (family *Raptidae*). Unlike eagles, hawks and similar birds of prey in the family *Accipitridae*, vultures rarely (if ever) rely on predation as their food supply is derived from carrion in the veld (Mundy, Butchart, Ledger, & Piper, 1992). Vultures are divided into two main groups based on their evolutionary lineage and geographical location. The Old World vultures (*Accipitridae*), which are considered to be distant relatives of eagles, are typically found in Asia, Africa and Europe, while the New World vultures (*Cathartidae*), which are considered distant relatives of storks (family *Ciconiidae*), are found across the Americas (Mundy et al., 1992). Both families share an almost global endangered status (Anon, 2011).

Of all the vulture species globally, three have received a large degree of media exposure in the last few years, namely the Oriental white-backed vulture (*Gyps bengalensis*), the long-billed vulture (*Gyps indicus*) and the slender-billed vulture (*Gyps tenuirostris*) (Cuthbert et al., 2011; Green et al., 2007). These three vultures have declined in Eastern Asia by more than 99.9% over the past 15 years (Cuthbert et al., 2011) with the consequence they all are threatened with extinction (Cuthbert et al., 2011; Green et al., 2007). Initially, the reason for their decline was unknown with many proposed aetiologies, such as heavy metal toxicities, West Nile Viral infections and carbamate or organophosphate intoxication (Pain et al., 2003; Satheesan, 2000). However, Oaks et al. (2004) conclusively demonstrated that diclofenac, a non-steroidal anti-inflammatory drug (NSAID), was the cause of the widespread devastation of the vulture population across the Indian subcontinent. Furthermore, it was also demonstrated that the primary food source of these vultures (dead live-stock) was contaminated with varying concentrations of diclofenac (Green et al., 2007). Diclofenac was banned from importation and manufacture on the veterinary market in India in 2006 as a result (Taggart et al., 2007). Another interesting feature in toxicity has been the seeming sensitivity of only the Old World vultures to diclofenac, which in addition to the three vultures from India, includes the Griffon vulture (*G. fulvus*), the African white-backed vulture (*G. africanus*) and the Cape griffon vulture (*G. coprotheres*)(CGV) and not New World vultures (Naidoo, Wolter, Cuthbert, & Duncan, 2009; Rattner et al., 2008; Swan et al., 2006).

As a drug, diclofenac functions in mammals through the inhibition of both the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzyme systems, thereby decreasing concentrations of prostaglandins within the body (Pountos, Georgouli, Bird, & Giannoudis, 2011). Clinically, this NSAID has the beneficial effects of the suppression of pain, fever and inflammation (Burke, Smyth, & FitzGerald, 2005). However, this general inhibition of the prostaglandins within the body can result in numerous side effects as these autocoids are protective of the gastric mucosa (by reducing acid secretion and stimulating mucus production) as well as being protective of renal function (by modulating renal perfusion) (Pountos et al., 2011). As a result, the major side effects associated with the use of the NSAIDs are gastric ulceration and renal necrosis.

Despite the vultures exposed to diclofenac displaying visceral gout and acute renal failure at death, the pathophysiology behind this phenomenon remains unknown (Oaks et al., 2004). Meteyer, Rideout, Gilbert, Shivaprasad, and Oaks (2005) presented histopathological evidence of acute necrosis of the proximal convoluted tubules in the kidney with the

absence of glomerular lesions in early mortality in conjunction with minimal inflammatory changes. It was suggested that the toxicity results from ischaemic renal necrosis through the inhibition of prostaglandin synthesis (cyclooxygenase inhibition) with subsequent opening of the *valva renalis portalis* and shunting of venous blood away from the cranial lobe of the kidney (Meteyer et al., 2005). Despite the proposed theory being plausible, it is based on the anatomy and physiology of other avian species such as the chicken and ostrich. As a first step in further evaluating this proposed mechanism of toxicity, the circulatory system of vultures needs to be evaluated. For this study, we evaluate the anatomy of the renal circulation of the Cape griffon vulture (*Gyps coprotheres*) (CGV), one the Old World species known to be sensitive to the effects of diclofenac.

2 MATERIALS AND METHODS

In all cases, the bird carcasses were provided by VulPro, a vulture conservation organization. The use of the carcasses was approved by the Animal Use and Care Committee (Animal Ethics Committee) of the University of Pretoria (SOP043-12) in accordance with the South African standard for the care and use of animals in research. Research to work on a CITES protected species was approval was obtained from South African Nature Conservation (TOPS permit 07137). For the formalin fixation, birds (*n* = 5) were euthanized using pentobarbital at 1ml/kg of a 100mg/ml solution via a cannula placed in the *vena metatarsalis dorsalis*. All animals used in this study were collected opportunistically, and represent injured birds for which euthanasia was recommended by the treating veterinarian at the Onderstepoort Veterinary Academic Hospital of the University of Pretoria, South Africa. For the evaluation of the gross anatomy, three of the euthanized birds were embalmed through a catheter placed into the left ventricle. For histological evaluation, the four kidneys from the remaining two birds were immediately dissected free of the underlying structures following perfusion and placed in 10% buffered formalin. Sections between the *v. iliaca externa* and the *v. iliaca communis*, and related structures were paraffin-fixed and stained with standard H&E stains and evaluated by light microscopy (Olympus BX63).

For the vascular casting, dead birds (*n* = 3) that were previously frozen were defrosted in a water bath overnight at room temperature. The carcasses were infused with coloured latex for arterial and venous casts. For the arterial sample, arterial cannulation via the left ventricle and *aorta* (Siller & Hindle, 1969) was performed and coloured (red) latex forcefully injected. For venous casting, 18G Teflon catheters (Jelco) were inserted into each *V. iliaca externa* under a surgical dissection microscope (Zeis) (Akester, 1967) and coloured (blue) latex injected into each vein. The basic arrangement of the major blood vessels relevant to the vascularization of the kidneys was subsequently described, following the careful dissection of the tissue away from the hardened latex, under a surgical microscope.

3 RESULTS

The major blood vessels are presented in Figure 1 for the dissected latex cast and as line diagrams in Figures 2 and 3. At the level of the last thoracic to first lumbar vertebra (at the point that the *vv. iliacae communii* combine to form the *vena cava caudalis*), the abdominal *aorta descendens* gave off the *aa. renalis cranialis* which supplied the *division renalis*

cranialis. The hind limb of the bird was supplied with arterial blood by the *a. iliaca externa* and by the *a. ischiadica externa*. The *a. iliaca externa* arose from the *aorta descendens* on the *facies ventralis* of the synsacrum between the *divisio renales mediales* (at the junction of *divisio renales mediales* and *caudales*) and leaves the pelvic cavity above the hip joint and branches into the *a. circumflexa femoris, a. glutea* and the *a. pelvina*. The *aa. ischiadicae ext*ernae take their origin from the *aorta descendens* at the middle axis of the *division renalis media*. They traverse the *renalis sin*. and *dext*., respectively, in the groove between the *divisio renales mediales* and *caudales* and leave the pelvic cavity through the *foramen ischiadicum*. While still within the kidney, the *a. ischiadica* gives rise to the *aa. renales media* and *caudales* on each side. The *aorta descendens* continued as the *aorta abdominus* before terminating as the *a. sacralis*. The arterial blood supply of the vulture kidney followed closely the path described for the chicken (Figure 2), with the exception that the *a. spermatica int*. and *a. testicularis* were not visible, most likely as result of the birds being immature or the birds being in the quiescent phase of their reproductive cycle being seasonal breeders.

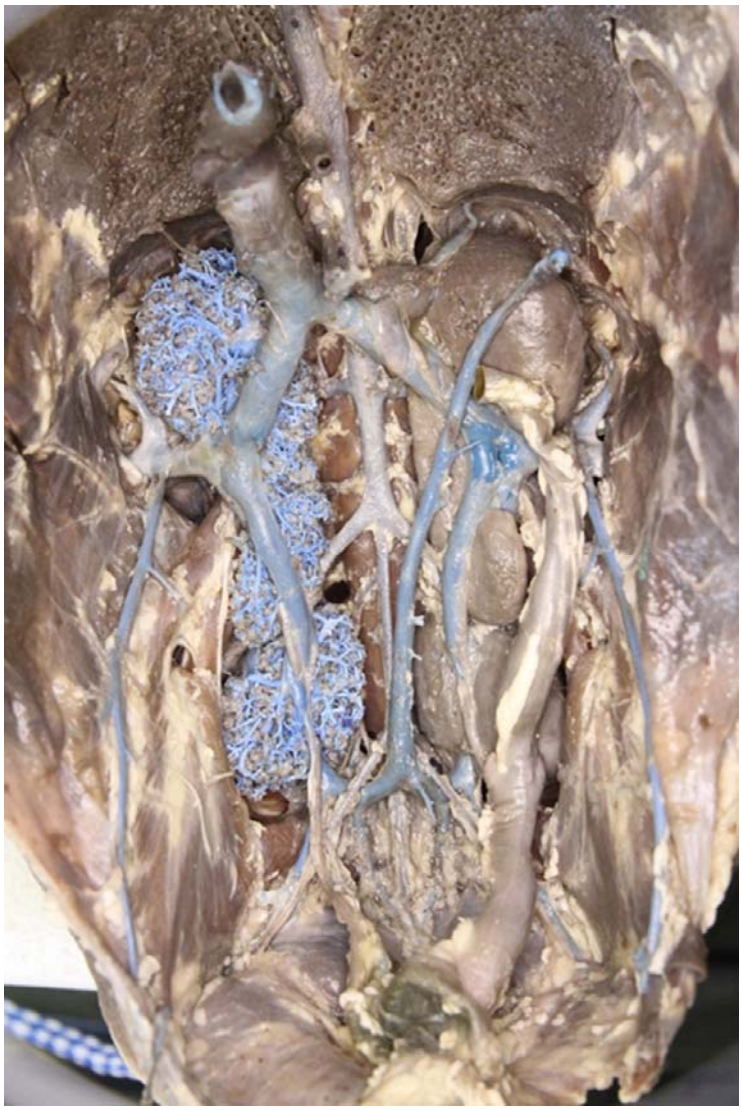

FIGURE 1. Venous casting of the right kidney with parenchyma removed next to the intact kidney

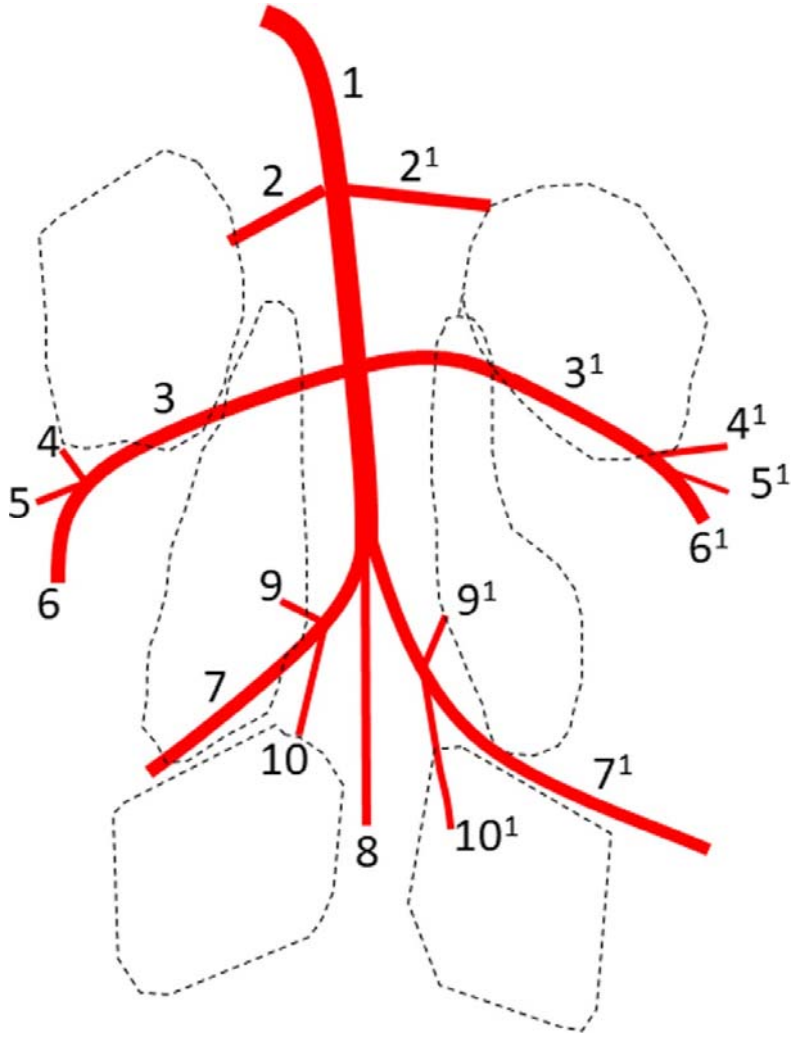

FIGURE 2. Arterial supply in the region of the kidneys. 1—aorta descendens dext. & sin.; 2,2[1]—a renalis cranialis dext. & sin.; 3,31—a. iliaca ext. dext. and sin; 4,4[1], 5,5[1] & 6,6[1]—branches of the a iliaca externa; 7,7[1]—a ischiadica ext. dext. & sin.; 8,8[1]–aorta abdominalis; 9,9[1]—a renalis media dext. & sin., 10,10[1]—a renalis caudalis ext. & sin

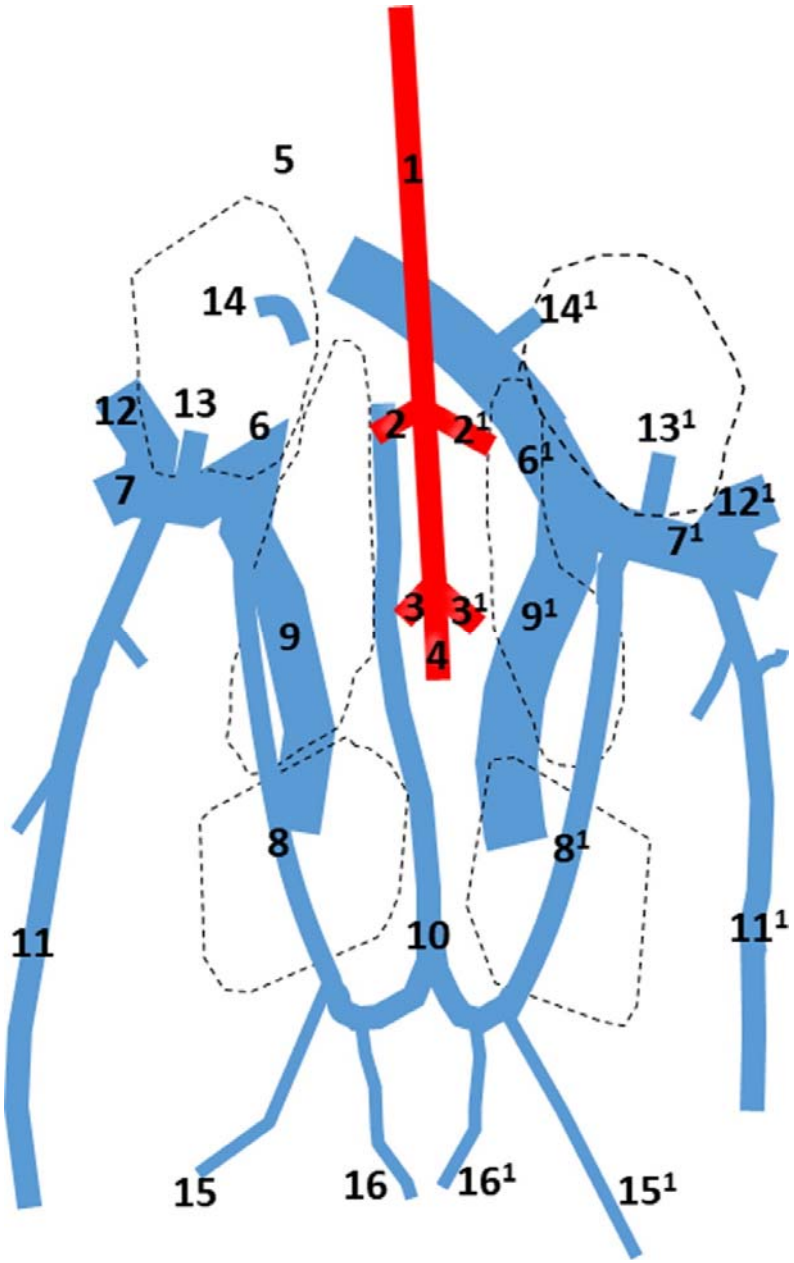

FIGURE 3. Veins of the vulture kidney, including the renal portal system. 1—aorta descendens; 2,2[1]—a. iliaca ext. dext. & sin; 3,3[1]—a ischiadica ext. dext. & sin.; 4,4[1]—aorta abdominalis; 5,5[1]—vena cava caudalis; 6,6[1]—v iliaca communis dext. & sin.; 7,7[1]—v iliaca externa dext. & sin.; 8,8[1]—v renalis portalis caudalis dext. & sin.; 9,9[1]—v renalis caudalis dext. & sin.; 10,10[1]—v mesenterica caudalis dext. & sin.; 11,11[1]—v pelvina dext. and sin.; 12,121—v circumflex femora dext. & sin.; 13,131v—renalis portalis cranialis dext. and sin.; 14,14[1]—v renalis cranialis dext. & sin.; 15,15[1]—v ischiatica dext. & sin.; and 16,16[1]—v iliaca interna dext. & sin

The major veins of the vulture kidney are annotated in Figure 3. The *v. iliaca externa* from the hind leg entered the abdominal cavity cranial to the *os pelvis* and on entering the kidney gave off the *v. renalis portalis cranialis* which entered the parenchyma of the *divisio renalis cranialis*, before dividing to form the *v. iliaca communis* and the *v. renalis portalis caudalis* which enters in the parenchyma of the *divisio renalis medialis and cadualis*. The *v. renalis portalis cranialis* was much smaller in diameter than the *v. renalis portalis caudalis*. The *v.*

renalis portalis caudalis varied in its exit from the *v. iliaca communis*, exiting slightly caudo-dorsal (under) to the *v*. *renalis caudalis* or slightly cranio-medial (adjacent) to it. The latter was a major difference on the left side, where the *v. renalis portalis caudalis* always exited from the *v. iliaca communis* caudo-laterally to the *v. renalis* caudalis. Cranially, the *vv. iliacae communii sin*. and *dext*. unite to form the *vena cava caudalis*. At their caudal ends, the two *vv. renales portales caudales* form a circumflex bilaterally before merging on the midline to form an *anastomosis interiliaca* to which the *v. mesenterica caudalis* (also referred to as the *v*. *coccygeomesenterica*) joins. Also joining the *anastomosis interiliaca* are the *v. iliaca interna* and *v. ischiatica*. These portal vessels form the renal portal venous ring spanning the *facies ventralis*s of both kidneys.

The *valva renalis portalis* was located in the venous lumen at the junction of the *v*. *iliaca communis*, *v. renalis caudalis* and *v. iliaca externa*. The valve was ring-shaped with the apex appearing wider than its base (Figure 4). The apex of the valve was characterized by finger-like processes. The valve itself was also hollow. Histologically, on cross section, the valve was made up predominantly of smooth muscle and was lined by a layer of endothelial cells (Figure 5). The valve was both vascularized and well innervated. Two prominent nerve plexus were present next to the valve (Figure 6).

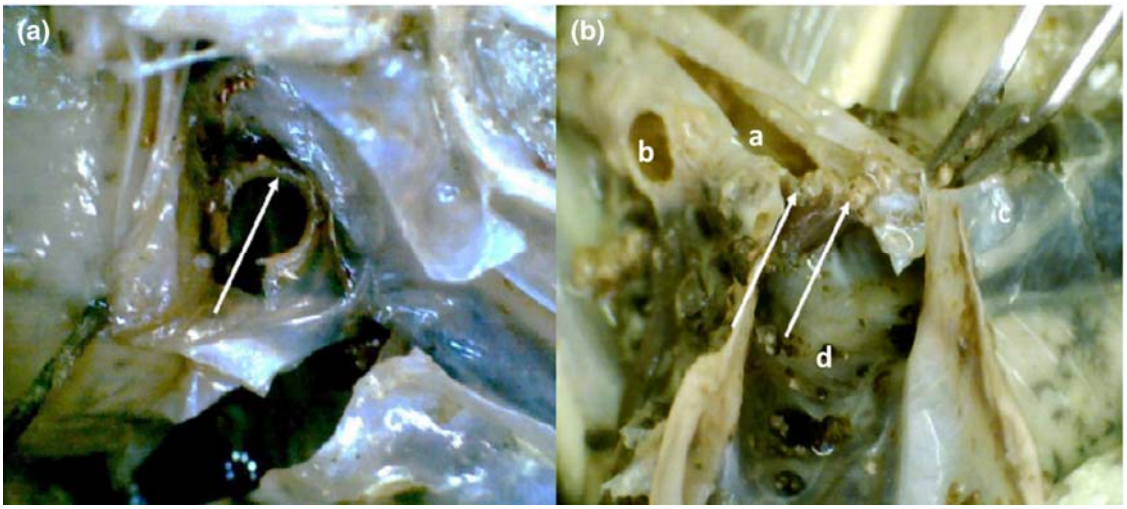

FIGURE 4. The valva renalis portalis in the v. iliaca communis with panel A showing the ring-like appearance of the valve and panel B showing the finger-like projections (Arrows) at the apical surface into the lumen of the v. iliaca communis. a—v iliaca externa; b—v renalis portalis cranialis point of exit; c—v renalis caudalis; and d —v iliaca communis

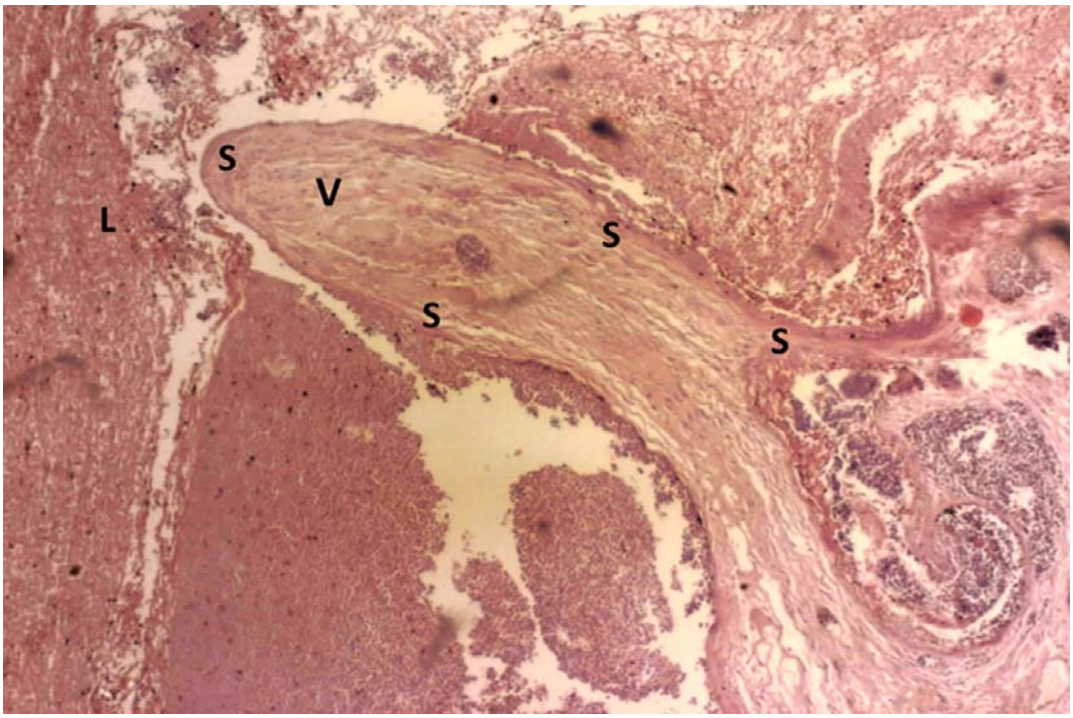

FIGURE 5. Cross section of the well-vascularized valva renalis portalis (V). L—lumen of vessel. The smooth muscle fibres that comprise the majority of the valve are clearly evident (S). Magnification 40×

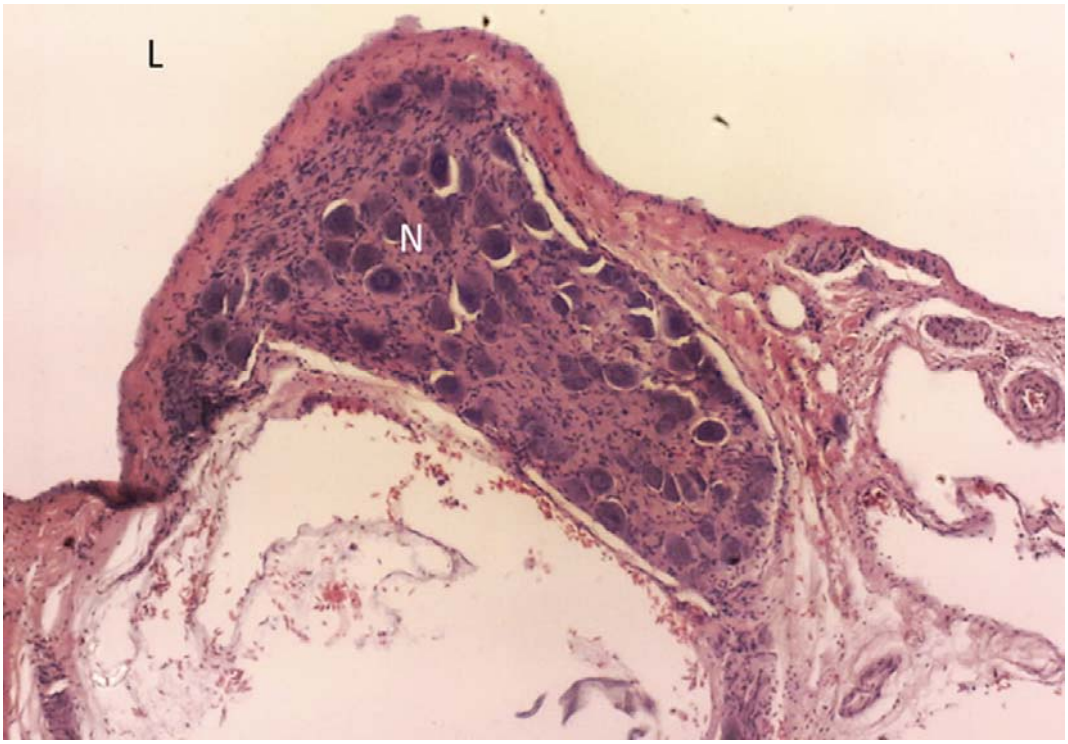

FIGURE 6. Cross section of one of the two nerve plexus (N) that was associated with valva renalis portalis, adjacent to the vessel lumen (L)

Venous draining of the kidney was provided by the *v. renalis cranialis*, which while not evident from the *facies ventralis*, from venous casting, was seen to transverses within the parenchyma of the kidney. The two veins exited the *divisio renalis cranialis* on their most medial surface to join the *v iliaca communis* before their union to form the *vena cava caudalis*. Draining of the *divisio renalis media and caudalis* was facilitated by the *v renalis caudalis*. This vein exited the cranial boundary of the *divisio renalis caudalis* and transversed within the *divisio renalis media*, before joining the *v iliaca communis*, medial to the *valva renalis portalis*. The *v renalis caudalis* was much larger than the *v renalis cranialis*.

On the microscopic level, the glomeruli of the CGV kidney was supplied by arteriolar blood which entered as expected via the afferent arteriole and exited via the efferent arteriole. Hereafter, the blood supply to the rest of the nephron was supplied by a vascular plexus. While the plexus could not be followed, the casting indicated that they were supplied by both venous and arterial blood.

4 DISCUSSION

The following study was undertaken to ascertain whether the renal portal valve is present in an old vulture species. Following the devastating effect of diclofenac on the Asian subcontinent, Meteyer et al. (2005) speculated that toxicity could be linked to the renal portal circulation of birds and the renal portal valve. The latter is a rather controversial structure with its presence being described in only a few bird species like the chicken and duck. More so, there has been much debate as to whether the structure has any major physiology effect. For this study, as a first step in ascertaining the importance of the valve in toxicity, we mapped the blood supply to and from the vulture kidney, as well as determining of the renal portal valve was an anatomical structure in the vulture. We also compare our results to the chicken, as this species has been shown to contain the valve. More importantly, this species has been shown to be susceptible to diclofenac.

The vulture kidney, in an expected manner, received mixed arterial and venous blood supply. For the cranial lobe, arterial blood was supplied directly of the abdominal aorta by the *aa. renalis cranialis,* while the other two lobes received their blood supply from the *aa. renales media* and *caudales* which arose from the *a. ischiadica* after it branched off the abdominal aorta. The arterial blood supply of the vulture kidney followed closely the path described for the chicken, with the exception that the *a. spermatica int*. and *a. testicularis* were not visible, most likely as result of the birds being immature or the birds being in the quiescent phase of their reproductive cycle being seasonal breeders. The kidney also received a venous blood supply via the *v. iliaca externa* from the hind leg; the *v. mesenterica caudalis* (also referred to as the *v*. *coccygeomesenterica*) from the caudal mesentery; and the *v. iliaca interna* and *v. ischiatica* from the pelvis. Together these venous vessels gave rise to the renal portal venous ring spanning the ventral surface of both kidneys, as seen in other avian species. With the exception of some structural difference, the ventral topography of the vulture vasculature was identical to that described for the chicken. At the microscopic level, only the glomeruli received full arteriolar blood, while the rest of the parenchyma appeared to receive a mixed venous-arterial blood supply from the dual venous and arterial supply.

The renal portal valve was present in the vulture at the junction of the *v. iliaca communis*, *v. renalis caudalis* and *v. iliaca externa* as described by Akester (1964) in the chicken including being a hollow structure with finger-like projections. The valve was made up predominantly of smooth muscle and was well innervated. The latter agreed with the description of Akester and Mann (1969), who described the renal portal valve as being the only intravascular structure in any vertebrate species to be comprised mainly of smooth muscle and to have a rich nerve supply comprising of a double innervation viz. adrenergic and cholinergic. Gilbert (1961) described considerable innervation to the basal two-thirds of the valve with large trunks in the basal third giving off fine bundles to the middle third. He also described the apical third of being comprised of epithelioid cells and collagen fibres. Akester and Mann (1969), however, described a more uniform distribution of smooth muscle throughout the valve without large nerve trunks within the basal third of the valve, which is more consistent with the findings of this study.

With both the renal portal circulation and its associated valve being identified in this Old World vulture species, its potential role in toxicity needs to be evaluated. As a first step, the physiological role of the valve needs to be evaluated, which in itself has been a debate since its first description in the literature. Some authors have suggested that the valve is relatively redundant and plays no major role in the renal functioning (Sturkie, Dirner, & Gister, 1978). Other authors have speculated that the valve may allow more blood to enter into the caudal vena cava much quicker in times of stress, thereby allowing for increased preload and subsequently increase cardiac output. A more plausible theory put forward suggests that the valve is actually protective of the cranial lobe (Gilbert, 1961), and under times of increased activity, when the muscular pump of the hind quarters are more active, the valve prevents the volume overload of the cranial lobe, by shunting some of the blood away from the cranial lobe into the caudal vena cava and *vice versa* increasing blood supply to the kidney when there is reduced supply from the hindquarters. As a result, it would appear that the renal portal system is important in supplying blood, nutrients and oxygen to the cranial lobe of the kidney.

Based on the blood supply to the kidney and the vena cava, the theory put forward by Meteyer can be more critically evaluated. Firstly, we can confirm that the kidney of the vulture does receive mixed venous and arterial blood, with the former entering from the hind quarters via three veins, and the latter entering via the aortic blood supply. Furthermore, the results indicate that the glomerulus is supplied predominantly by oxygenated arterial blood, while the nephron is supplied by mixed venous and arterial blood of lower oxygen saturation via a plexus. Based on this distribution, it is not surprising that ischaemia at the level of the kidney would first result in the destruction at the level of the nephron, as seen with zone 3 necrosis in the liver which is also supported by a portal blood supply. We would also postulate that since the proximal convoluted tubules are more metabolically active than the rest of the tubules, that this area would be first affected once again as per zonal necrosis in the liver.

The valve itself was composed mainly of smooth muscle and was well innervated. If the tissue is reliant on the COX system to mediate opening or closing, it is certainly possible that the changes in COX functionality could influence the functioning of the valve. As such, we believe that, based on the anatomy of the vasculature of the CGV kidney, ischaemic necrosis

could potentially play a role. The question that needs to be answered, due to the direct link to the arterial supply, is whether the any restriction to the venous supply would not automatically result in a compensatory increase in arterial blood supply. Also of importance would be to establish which receptors play a role in the functionality of the valve. Evidence in the literature thus far confirms the importance of histamine, cholinesterase and adrenergic innervation, with no further studies being undertaken on the importance of the prostaglandins. Nonetheless, the prostaglandins most likely do play as a role as limited studies on the chicken reproductive tract have shown that the prostaglandins are important in adrenaline-mediated muscle contractility. Limited studies on the renal veins, using isolated organ baths, have also shown the importance of the prostaglandins in maintaining venous tones (Naidoo & Swan, 2009).

Based on the findings of this study, the proposed mechanism of toxicity of Meteyer et al., (2005) is anatomically possible. The similarity of the chicken and vulture in their vascular structure may also explain previous finding that the chicken could serve as a physiological model of the study of the pathophysiology of diclofenac's toxicity. With the *valva renalis portalis* being identified in the Cape griffon vulture, further studies should focus on the importance of the valve in toxicity.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

AUTHORS CONTRIBUTION

LH, VN, ND and HG conceived the study. LH and VN participated in sample and data analysis. KW and VN contributed substantial materials, resources or funding. HG and ND were master degree supervisors to LH. All authors contributed to the write-up of the publication.

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