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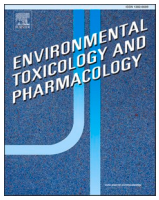

Experimental safety testing confirms that the NSAID nimesulide is toxic to *Gyps* vultures in India

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ABSTRACT

Population declines of *Gyps* vultures throughout South Asia were caused by unintentional poisoning by the NSAID diclofenac, which was subsequently banned. However, other vulture-toxic NSAIDs are available, including nimesulide, which, in experiments carried out in South Africa, was shown to be toxic to *Gyps* vultures. We report on safety-testing of nimesulide carried out on Himalayan Griffons *G. himalayensis*. We gave two vultures a dose of nimesulide by oral gavage at the maximum level of exposure, with two controls dosed with benzyl alcohol. In the two tested birds, plasma nimesulide concentrations peaked after six hours, while serum uric acid concentrations increased steadily up until 24 h post-treatment, after which both birds died, displaying severe visceral gout. The control birds showed no adverse clinical or biochemical signs. We confirm that nimesulide is toxic to *Gyps* vultures. Veterinary use of nimesulide should be banned in all *Gyps* vulture range countries in the region.

1. Introduction

From being some of the most common large raptors in the world (Houston, 1985), three species of *Gyps* vultures endemic to south and south-east Asia were driven to near-extinction, due to unintentional poisoning by the veterinary non-steroidal anti-inflammatory drug (NSAID) diclofenac (Oaks et al., 2004; Shultz et al., 2004; Green et al., 2004; Prakash et al., 2007), and all are now classified as Critically Endangered in the IUCN Red List (BirdLife International, 2021). In India, the population of the worst-affected species, White-rumped Vulture *G. bengalensis* declined by 99.9% between the early 1990s and 2007, while that of Indian *G. indicus* and Slender-billed Vulture *G. tenuirostris* combined declined by 96.8% (Prakash et al., 2007). Vultures ingested diclofenac when they fed on carcasses of domesticated ungulates that had been given the drug shortly before death, a common practice in South Asia. Diclofenac, and other NSAIDs, are commonly given to cattle because of their anti-inflammatory, analgesic and anti-pyretic properties (Bennett and Vila, 2000), to treat several ailments, such as mastitis in lactating females (Taggart et al., 2007). The drug causes kidney failure, elevated plasma uric acid concentrations and death within a few days of ingestion, with severe visceral gout and necrosis of kidney tissues being typical post-mortem signs (Oaks et al., 2004; Swan et al., 2006). Veterinary use of diclofenac was banned in India, Pakistan and Nepal in 2006, and 2010 in Bangladesh (Prakash et al., 2012; Sarowar et al., 2016), which has contributed to the partial recovery of populations in Nepal (Galligan et al., 2020) and the slowing or halting of declines in India and Pakistan (Chaudhry et al., 2012; Prakash et al., 2017).

However, other NSAIDs are widely available that are also toxic to

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vultures (Galligan et al., 2021). These include ketoprofen, (Naidoo et al., 2010), carprofen (Fourie et al., 2015), aceclofenac (Galligan et al., 2016; Chandramohan et al., 2022), and flunixin (Zorilla et al., 2015, Herrero-Villar et al., 2020). Only two NSAIDs have been proven to be safe for *Gyps* vultures at doses likely to be encountered by wild birds: meloxicam (Swan et al., 2006; Swarup et al., 2007) and tolfenamic acid (Chandramohan et al., 2021). In addition, several dead *G. bengalensis* have been found in India with visceral gout and residues of the NSAID nimesulide (Cuthbert et al., 2016; Nambirajan et al., 2021). Subsequently, nimesulide was found to be toxic to *Gyps* vultures (Galligan et al., 2022) in experimental tests on captive vultures. In that study, two African Cape Vultures *G. coprotheres* that were given a dose of nimesulide likely to be encountered by vultures in the wild both died from kidney failure. These birds showed similar symptoms to those that had died of diclofenac poisoning (Oaks et al., 2004), displaying extensive visceral gout (formation of uric acid crystals, especially in and on the kidney and liver) (Galligan et al., 2022). This was accompanied by a 27–79-fold increase in the concentration of uric acid in the blood plasma of the treated birds, which died 27 and 29 h after treatment.

Here, we report on a study, experimentally testing the toxicity of nimesulide on a near-threatened species of *Gyps* vulture, Himalayan Griffon, *G. himalayensis*, which breeds in mountainous areas of India and neighbouring countries, is a common winter visitor to the north Indian plains and is sensitive to diclofenac nephrotoxicity (Das et al., 2010). We followed the protocol of Galligan et al. (2022), giving the same dose (per kilogram body weight) of nimesulide, which was based on a vulture's maximum likely exposure to the drug in the wild, estimated from a pharmacokinetic study of the drug in cattle.

2. Methods

2.1. Trial animals: housing and management

The experiments were carried out at the Vulture Conservation Breeding Centre at Pinjore, Haryana, India in February 2022. Fifteen wild *G. himalayensis* were trapped on 18th and 25th February in a large (27 × 5 × 9 m) baited walk-in cage trap. They were transferred to a four-compartment purpose-built aviary, each compartment being 6 × 6 × 5 m, and holding a maximum of four vultures. A health check was performed on the birds on 26th February, involving an external examination of body weight, condition and plumage quality, and monitoring of body temperature. Birds were kept in the aviary for three weeks to acclimatize, before the experiment commenced on 11th March. They were fed with 5 kg of goat meat, sourced to be free of NSAIDs, on two days per week, and provided with water ad libitum. Samples were taken for haematological and serum analysis (Supplementary Information, Appendix B, Table S1). Surviving birds were released back to the wild on 20th April.

2.2. Treatment and study design for oral gavage experiments

Two birds were randomly chosen to be the test subjects and another two acted as controls. Although the sample size is small, it has previously been shown to be adequate for ascertaining that a NSAID is toxic (Cuthbert et al., 2006), the major advantage being not requiring the death of several individuals of an endangered species (Fourie et al., 2015). The two test subjects were both juveniles, less than one year old; one of the control birds was also a juvenile, the other was in its second year. Before the experiment, we weighed each vulture (treatment group, weight = 8.0 kg for both birds; control group, weight = 8.0 kg and 8.8 kg), and a baseline blood sample taken. We used a commercial injectable brand of veterinary nimesulide (Nimovet, Indian Immunologicals Limited, Hyderabad, India), which is widely available and was purchased from a local pharmacy. The product had a stated nimesulide concentration of 100 mg l^{-1}. We administered a 1.50 ml aqueous solution of 1% benzyl alcohol and 10% ethanol, which is the carrier solution of nimesulide in the Nimovet formulation, to the control birds by gavage.

We calculated the maximum likely meal weight (*M*) using estimates of the mean daily energy use (*DEU*) of individual vultures. We calculated DEU from the body weight (W = 8.0 kg) of the vulture, $DEU = 668.4 * W^{0.622}$. Meal weight was calculated, as $M = 3 * (DEU / 5160)$, where 5160 kJ kg^{-1} is the energy assimilated by a vulture (Galligan et al., 2022), the figure was multiplied by 3 to reflect that the maximum meal size of *Gyps* vultures is typically about three times the amount of food required per day (Swan et al. (2006a). This gave a maximum likely meal of 1.4 kg for the two vultures in the present study. A pharmacokinetics and tissue residue experiment in cattle (Galligan et al., 2022) identified the tissue with the highest mean concentration of nimesulide as the injection site muscle at 134.08 mg kg^{-1} (*Rmax*), with the mean weight of that muscle being 1.11 kg (*Vmax*) (Galligan et al., 2022). The next highest concentration was found in the non-injection site muscle, at 0.945 mg kg^{-1} (*Rnext*). As *M* was greater than *Vmax*, Maximum Level of Exposure (*MLE*) for an individual vulture was calculated as $MLE = Rmax*Vmax + Rnext*(M\text{-}Vmax)$. Based on Galligan et al. (2022), we calculated the dose for individual vultures in mg kg^{-1} (*D1*) as $D1 = MLE/W$ and ml (*D2*) as $D2 = (MLE*W)/U$, where *U* was the concentration of nimesulide in the brand used (Nimovet; 100 mg ml^{-1}). We calculated an MLE of 149.1 mg kg^{-1}, a D1 of 18.64 mg kg^{-1} and a D2 of 1.50 ml for both vultures. Further details can be found in Galligan et al. (2022).

2.3. Blood sampling

During blood sampling, birds were restrained by an experienced vulture-handler, securely holding the neck and legs of the bird, close to the person's body so that it could not flap its wings. We collected blood samples (4–5 ml) from each bird, by direct veno-puncture from the tarsal vein, at 0 h (i.e., just before treatment), and at 2, 6, 12 and 24 h after treatment, and serum and plasma separated. A maximum total of 25 ml of blood was taken per bird, which falls well within the ethical limits (i.e. 1% of body weight, or 80 ml) for birds weighing at least 8 kg, and is aligned with all other vulture studies where similar volumes have been collected for pharmacokinetic evaluation. Serum tubes with clot activator and heparin-coated plasma tubes were used for collecting blood.

2.4. Extraction and measurement of nimesulide in vulture plasma

We extracted nimesulide from vulture plasma and tissue samples using a dilution method and measured concentrations of nimesulide in the acetonitrile using liquid chromatography tandem mass spectrometry (LC-MS/MS; Sciex API 6500 +™) with electro spray ionization (ESI) and multiple reaction monitoring (MRM) in negative ionization mode.

Dimethyl sulfoxide (DMSO, 2.0 ml) was added to 2.12 mg of nimesulide in a 2.0 ml micro-centrifuge tube, mixed well and sonicated; 2 ml DMSO was added to 2.32 mg of nimesulide (D-5), which was used as an internal standard stock. Analyte working calibration standards (0.5–500 ng ml^{-1}) and quality control samples (QC) of nimesulide were prepared in DMSO. An internal working standard solution was prepared by diluting 0.15 ml of the internal standard stock solution to 50 ml with acetonitrile to provide a concentration of 3.0 μg ml^{-1}. This solution was mixed well and stored at 2–8 °C. Calibration standards and quality control samples of nimesulide were prepared by spiking 47.5 μl of blank vulture plasma with 2.5 μl of the analyte working solution. Study samples (20 μl) were transferred to Eppendorf tubes and 10 μl of the internal working standard solution added. Samples were quenched with 200 μl of acetonitrile, vortexed and centrifuged at 14,000 rpm for 5 min at 4 °C. 150 μl of supernatant was transferred to 1 ml vials for analysis by LC-MS/MS.

Further details of the methods for the extraction and measurement of nimesulide can be found in Appendix A of the Supplementary Information.

2.5. Measurement of serum constituents

We analysed serum samples to estimate the concentration of the following biochemical analytes, changes in concentrations of which are often a symptom of kidney failure: uric acid, creatinine, urea, total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), sodium and potassium. Concentrations of serum biochemical analytes were measured using commercially available measurement kits (Coral Clinical Systems, Tulip Diagnostics) using a GENESYS 10UV spectrophotometer (Thermo Scientific).

Comparison of changes in analyte concentrations between test and control subjects was carried out using a generalized linear model, with the time since treatment, whether birds were test or control subjects (status), and their interaction, as predictor variables. Differences were assessed based on the significance of the time*status interaction. All modelling was performed in R v4.2.2 (R Development Core Team, 2023).

2.6. Pharmacokinetic evaluation

Pharmacokinetic parameters for vulture plasma samples were ascertained by non-compartmental modelling in Kinetica 5.1 (Thermo 2012). Following Galligan et al. (2022), the maximum plasma concentration (C_{max}) and the time to reach it (T_{max}) were read from the plasma concentration versus time profile. The last quantifiable time point C_{last} and the linear trapezoidal rule was used to calculate the area under the curve (AUC_{last}) and the area under the moment curve ($AUMC_{last}$) as ($AUC_{last} = \Sigma([T_{last}\text{-}T_{last}\text{-}1]^* [C_{last} + C_{last}\text{-}1] / 2)$) and ($AUMC_{last} = \Sigma([T_{last}\text{-}T_{last}\text{-}1]^*[T_{last}^*C_{last} + T_{last}\text{-}1 ^*C_{last}\text{-}1] / 2)$), respectively. The elimination rate constant (λ) was calculated by ordinary least squares regression of the terminal three points of the curve after natural logarithmic transformation; and subsequently, the half-life of elimination (T_{half}) was calculated as ln (2) / λ. The mean residence time (MRT) was calculated as $AUMC_{last} / AUC_{last}$ and the area under the curve to infinity (AUC_{inf}) was calculated as $AUC_{last} + C_{last} / \lambda$. The apparent volume of distribution (Vz / F) was calculated as dose / (AUC_{last}*λ), the apparent volume of distribution at steady state (Vss / F) was calculated as (dose*MRT) / AUC_{last} and the apparent clearance (Cl / F) was calculated as dose / AUC_{last}; and for nimesulide administered intravenously, the actual volume of distribution (Vz), actual volume of distribution at steady state (Vss) and actual clearance (Cl) were calculated by first finding the fraction of absorption (F) and dividing this by the apparent measures of these parameters. F was calculated as a bird's extravascular AUC_{inf} divided by the pooled AUC_{inf} from the intramuscular profile. All parameters are presented as geometric means with standard deviations.

2.7. The effect of nimesulide treatment on the concentrations of serum metabolites

We extracted metabolites of nimesulide from the serum of treated and control-group Himalayan Griffons, which were analysed using liquid chromatography mass spectrometry (LC-MS/MS). Further details can be found in the Supplementary Information, Appendix C.

2.8. Post-mortem examination of vultures

Post-mortem examination was carried out on any vulture that died during the safety testing experiment. We performed a necropsy, which focussed principally on the viscera because visceral gout is a typical finding in *Gyps* vultures killed by NSAID poisoning (Oaks et al., 2004; Swan et al., 2006; Naidoo et al., 2010; Zorilla et al., 2014; Cuthbert et al., 2016; Nambirajan et al., 2021). Tissue samples were collected during the necropsy, fixed in 10% buffered formalin, embedded in paraffin wax and cut at 4 μm. After mounting, the sections were stained with Haematoxylin and Eosin (H&E), and silver nitrate / hydroquinone (De Galantha staining) to confirm the presence of urate crystals in the visceral organs.

2.9. Animal ethics

Permission to carry out experiments on vultures was granted by the Government of Haryana Forest Department, the Indian Veterinary Research Institute (IVRI) and the RSPB's Animal Ethics Committee (EAC2016–02).

3. Results

3.1. Concentration of nimesulide in tissues

Pharmacokinetic profiles (Fig. 1) differed slightly between the two birds treated with nimesulide. Whereas nimesulide was rapidly absorbed up until 6 h after dosing in bird X36, concentrations of the drug in the plasma of bird X33 fell between 2 and 6 h. Subsequently, elimination of nimesulide occurred at a similar rate in both birds up until their deaths after 26 h. The rate of elimination was slower than the rate of absorption. Nimesulide concentration in the plasma of the two birds assigned to the control group was zero.

3.2. Analysing samples for clinical pathology

Both treated vultures showed increases in serum uric acid concentrations (Fig. 2). Uric acid levels in the treated birds increased steadily until the birds died 26 h post-treatment. The concentrations just prior to death were 43–75-fold greater than the pre-treatment concentrations. Uric acid levels in control birds remained low throughout the experiment (Fig. 2).

There were no significant differences between treated and control vultures in the change in concentration of any of the other serum analytes, possibly due to small samples sizes (Supplementary Information, Table S1, Figs. S1-S8). However, there was a tendency for greater increases in the concentrations of serum potassium (Fig. S7), aspartate aminotransferase (AST; Fig. S4) and creatinine (Fig. S1).

3.3. Changes in the concentration of endogenous serum metabolites

A total of 158 compounds were extracted from the serum of treated and control-group *G. himalayensis*. Of these, concentrations of 47 metabolites significantly increased after treatment with nimesulide compared to the control group, 33 significantly declined, and for the remaining 78 metabolites there was no change (Supplementary Information, Appendix C, Tables S2, S3 and S4).

Most of the metabolites were unrelated to nimesulide toxicity, with likely exposure through water or food, with decreases in concentration likely due to decreased appetite. There were increases in several metabolites that are known to be water soluble and urinary excreted. There was also an absence of hydroxy-nimesulide, which is an important metabolite of nimesulide, and an increase in the concentration of pentoxifylline, both of which may be related to cytochrome metabolism.

3.4. Pharmacokinetic evaluation

Pharmacokinetic profiles (Fig. 1) and parameters (Table 1) were similar between the two vultures treated with nimesulide. Vulture X36 showed rapid absorption in the first 6 h, followed by a slower elimination until 24 h; whereas X33, after rapid absorption in the first 2 h, concentrations plateaued until 24 h (Fig. 1). X36 had a much higher C_{max} than X33, with a lower T_{max} (Table 1), suggesting a more complete and rapid absorption, which was evident from other parameters, such as a shorter mean residence time (Table 1).

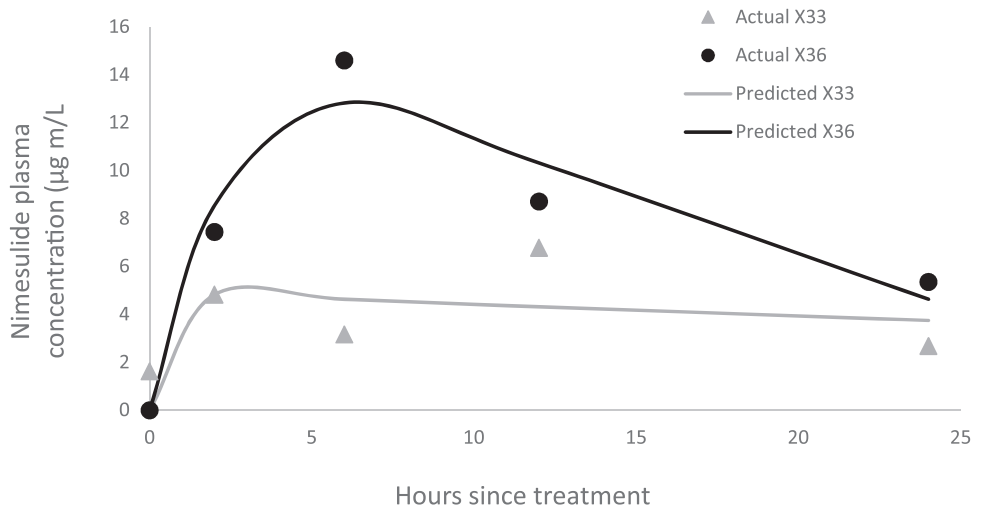

Fig. 1. Pharmacokinetic profiles for the two Himalayan Griffons *Gyps himalayensis* treated with nimesulide at 18.64 mg kg^{-1} by gavage.

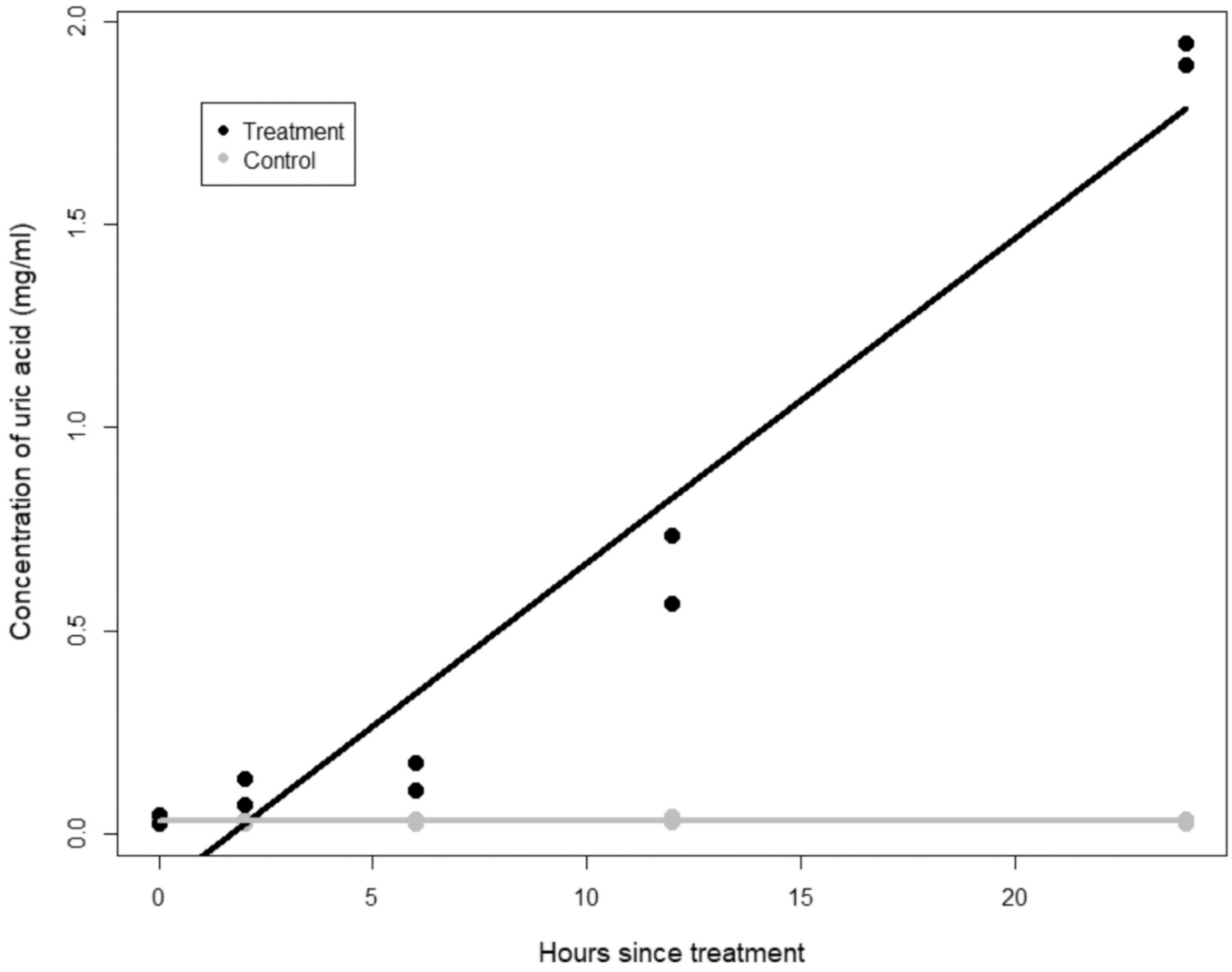

Fig. 2. Change over time in plasma uric acid concentrations in two vultures treated with nimesulide (T) at 18.64 mg kg^{-1} by gavage, in comparison to two vultures assigned to a control group (C) and given a 1.50 ml solution of benzyl alcohol / ethanol (the carrier solution for nimesulide in the Nimovet formulation).

Table 1
Pharmacokinetic parameters for the two vultures *Gyps himalayensis* (X33 and X36), treated with nimesulide at 18.64 mg kg^{-1} by gavage.

		Vulture			
Parameter	**Units**	**X33**	**X36**	**Mean**	**SD**
C_{max}	μg ml^{-1}	6.78	14.6	9.95	5.53
T_{max}	h	12	6	8.49	4.24
AUC_{last}	μg ml*h	109.07	205.87	149.85	68.45
AUC_{tot}	μg ml*h	278.24	302.22	289.98	16.96
λ	1/h	0.019	0.053	0.032	0.024
$AUMC_{last}$	μg ml* $(h)^2$	1242.24	2195.16	1651.3	673.82
T_{half}	h	36.70	12.96	21.806	16.79
MRT	h	51.25	20.87	32.707	21.48
Clearance	1/h*kg	0.067	0.062	0.064	0.004
V_z	1/kg	3.547	1.153	2.02	1.69
V_{ss}	1/kg	3.433	1.287	2.10	1.52

3.5. Post-mortem results for treated G. himalayensis

Post-mortem analysis was carried out on the two *G. himalayensis* that died following oral gavage treatment with nimesulide. Systematic necropsy examination of both birds revealed deposition of chalky white urate crystals on the visceral organs (severe visceral gout; Fig. 3, a), caused by kidney failure. Staining confirmed the presence of urate crystals (tophi) in the visceral organs, i.e., kidney and liver (Fig. 3, b-d).

4. Discussion

The results of our study confirm that nimesulide is toxic to *Gyps* vultures. Both birds treated with the drug died 26 h after treatment, had elevated plasma levels of uric acid and showed post-mortem signs of severe visceral gout. These post-mortem outcomes have previously been recognised as indicating NSAID nephrotoxicity (Oaks et al., 2004; Swan et al., 2006; Naidoo et al., 2010; Zorilla et al., 2014; Cuthbert et al., 2016; Nambirajan et al., 2021). In contrast, the two birds in the control group, which were given a dose of benzyl alcohol (the carrier solution of nimesulide), survived and showed no adverse symptoms. Several dead wild *Gyps bengalensis* in India have previously been found to have residues of nimesulide co-occurring with visceral gout and other symptoms of kidney failure in two independent studies (Cuthbert et al., 2016; Nambirajan et al., 2021). The toxicity of nimesulide to *Gyps* vultures was also established by safety testing experiments carried out on an African species, *G. coprotheres* (Galligan et al., 2022). Our study shows that nimesulide caused the deaths of two *G. himalayensis*, showing the same symptoms of high serum uric acid levels and post-mortem visceral gout. As far as we know, this is the only such experimental test of the toxicity of nimesulide conducted on an Asian species of *Gyps* vulture. These findings further highlight the need for veterinary use of nimesulide to be banned in India and other countries in Asia that are home to critically endangered species of vultures.

The pharmacokinetics of nimesulide in the two Himalayan Griffons in this study were similar to that found in Cape Vultures in South Africa (Galligan et al., 2022). In the current study, the half-life of elimination (T_{half}) was 12 and 36 h, compared to 22 and 8.98 h in Cape Griffons. The level of exposure (*AUC*) was also very similar in the two studies, as were the pharmacokinetic profiles (Fig. 1). However, X36 had a much higher C_{max} than X33, whereas in the South African study, this was similar in both birds (Galligan et al., 2022). This higher C_{max} was associated with a longer T_{max}, indicating more rapid absorption. We cannot be sure of the cause of these differences, but they are often linked to feeding or gastric emptying; in vultures, it may have to do with the emptying of the crop, also.

That nimesulide should be found to be as toxic to Himalayan Griffons

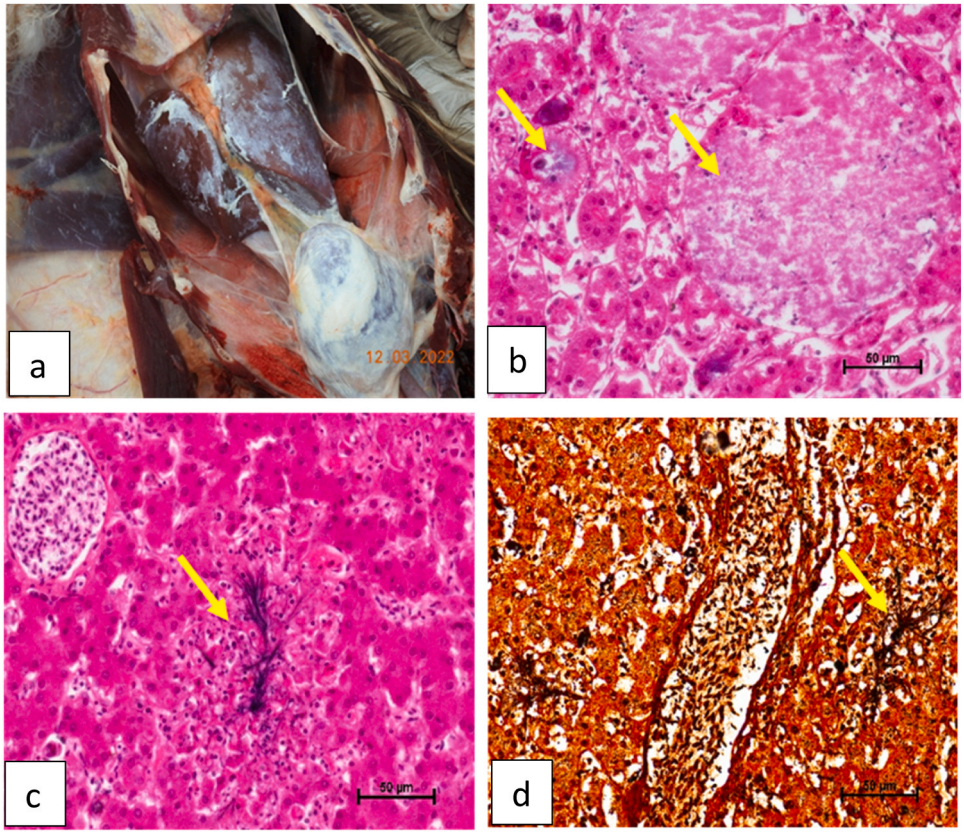

Fig. 3. (a) Post-mortem image showing deposition of chalky white urate crystals on visceral organs. Histopathological images of (b) liver and (c) kidney tissues showing deposition of urate crystals, highlighted by Haematoxylin and Eosin (H&E) staining, and (d) silver nitrate / hydroquinone (De Galantha) staining (400 x magnification).

as it was to Cape Griffons is not surprising given the sensitivity to NSAID toxicity shown by all species of *Gyps* vultures that have been tested. Although some unrelated species of scavenging birds appear to be tolerant of the effects of diclofenac, e.g., Turkey Vulture *Cathartes aura* (Rattner et al., 2008) and Pied Crow *Corvus albus* (Naidoo et al., 2011), individuals of all five *Gyps* species on which diclofenac was experimentally tested, i.e., *G. bengalensis* (Oaks et al., 2004), Eurasian Griffon *G. fulvus* and African White-backed Vulture *G. africanus* (Swan et al., 2006), *G. coprotheres* (Naidoo et al., 2009) and *G. himalayensis* (Das et al., 2011)), all died showing the same typical symptoms of kidney failure, elevated uric acid levels and visceral gout. Additionally, carcasses of a sixth species, Indian Vulture, *Gyps indicus*, were regularly found throughout South Asia with the same symptoms (Shultz et al., 2004). It is thus likely that all species of *Gyps* vultures have the same sensitivity to NSAID toxicity.

However, there is still a need for a better understanding of what other species are sensitive to NSAID toxicity. In south Asia, populations of two other species of vulture – Red-headed Vulture *Sarcogyps calvus* and Egyptian Vulture *Neophron percnopterus* – underwent declines of similar magnitude, and over a similar time period (although with a slight time-lag), to those of species of *Gyps* vulture (Cuthbert et al., 2006). They have also shown signs of recovery since the ban on veterinary use of diclofenac (Galligan et al., 2014). Therefore, it is quite possible that both these species are also sensitive to diclofenac toxicity, although no individuals of either species have been found with symptoms of NSAID toxicity, nor has experimental safety testing been carried out on these species. Other studies have, however, widened the range of scavenging species that are affected by NSAIDs, including Steppe Eagle *Aquila nipalensis* in India (Sharma et al., 2014). Furthermore, a nestling Cinereous Vulture *Aegypius monachus* in Spain recently became the first-known avian victim of diclofenac poisoning outside of Asia (Herrero-Villar et al., 2021). Also, experimental work has been carried out to investigate the toxicity of both diclofenac and nimesulide in Black Kites *Milvus migrans* (Farooq and Khan, 2023a, 2023b), common scavengers in Asia. Whereas being given diclofenac at relatively high doses resulted in the deaths of four out of six kites (Farooq and Khan, 2023b), no birds died after being given nimesulide, although they did show the typical signs of nephrotoxicity (Farooq and Khan, 2023a).

Ideally, one would be able to assess the toxicity of NSAIDs to vultures without the need to dose any animals, and therefore without the subsequent mortalities, for instance using *ex vivo* liver cell cultures. However, efforts to do this have not been successful (Adawaren et al., 2018), partly due to the inability to extract functional enzyme from cultured liver cells.

Changes in the concentrations of many metabolites in vultures treated with nimesulide (Supplementary Information, Appendix C), suggests inhibition of metabolic pathways and excretory processes in vultures. Although these results are preliminary due to the small sample size, in future, with larger sample sizes, we may be able to use metabolomics to identify the biomarkers of NSAID toxicity. Also, by comparing metabolic differences between vulture-toxic and vulture-safe NSAIDs, such methods could be used as a non-lethal alternative to current safety testing experiments.

A particularly important finding of the metabolomic analysis was the absence of hydroxy-nimesulide, which is an important metabolite of nimesulide. This suggests that there may be a problem with cytochrome metabolism, i.e. the absence or reduced functionality of the enzyme CYP2C9/2C19 (Adawaren et al. pre-print), which in humans is involved in NSAID metabolism (EMEA, 2004). This may also explain the increase in concentrations of pentoxifylline following treatment with nimesulide, although a decrease in renal blood supply, another effect of diclofenac (and perhaps nimesulide) (Nethathe et al., 2021). Such a reduction in renal excretion and blood supply may also explain the increases in concentrations of several water-soluble metabolites that are renally excreted.

Nimesulide is increasingly being sold in pharmacies for the treatment of cattle in both India and Nepal (Galligan et al., 2021). Although in India figures vary by state, up to 37.3% of pharmacies sampled within the State of Haryana in 2017 offered the drug; while in Nepal, up to 13.7% of pharmacies in the western Terai in 2016 did so (Galligan et al., 2021). Given the evidence of the toxicity of nimesulide to vultures from several studies, there is an urgent need for the drug to be banned in India. This will not only benefit vultures in India, but across the wider South Asian region, too.

CRediT authorship contribution statement

Karikalan Mathesh: Formal analysis, Investigation, Data curation, Writing – review & editing. **Kesavan Manickam:** Formal analysis, Investigation. **John Mallord:** Writing – original draft, Writing – review & editing, Project administration. **K. Mahendran:** Formal analysis, Investigation. **Asok Kumar M:** Formal analysis, Investigation. **Krishna Chutia:** Formal analysis, Investigation. **Debasish Saikia:** Formal analysis, Investigation. **Beena V:** Formal analysis, Investigation. **Sree Lakshmi P:** Formal analysis, Investigation. **Nikita Prakash:** Resources, Project administration. **Rohan Shringarpure:** Formal analysis, Investigation. **Abhijit M. Pawde:** Formal analysis, Investigation. **Rhys Green:** Conceptualization, Methodology, Writing – review & editing. **Vinny Naidoo:** Formal analysis, Writing – review & editing. **Vibhu Prakash:** Conceptualization, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.etap.2023.104284.

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