

ECO-DAMAGE BY THE PHARMACEUTICAL PRODUCT DICLOFENAC A REVIEW

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ABSTRACT: The vultures have great significance for human well-being from the ecological and religious viewpoints. These scavengers are eco-cleaner and play a vital role in environmental health by consuming carcasses of dead animals and thus reducing the risk of disease. The population of three vulture species Oriental White-backed Vulture (*Gyps bengalensis*), Slender billed Vulture (*Gyps tenuirostris*), Long billed Vulture (*Gyps indicus*), had declined drastically during last 15 years. Because of the evidence of widespread and rapid population decline, all three vulture species were listed by IUCN, the World Conservation Union, in 2000 as 'Critically Endangered', which is the highest category of endangerment. The veterinary use of Diclofenac Na, a Non-steroidal Anti-inflammatory Drugs (NSAID) has been linked to the catastrophic decline in South Asian vulture population (Above 95% since 1993). Vultures are exposed to diclofenac when scavenging on carcass of animal treated with diclofenac. These birds had died due to visceral gout/renal failure caused by diclofenac residue present in dead animals. A dose of 0.8mg.kg^{-1} was highly toxic to vulture. The estimated LD 50 was $0.1\text{-}0.22\text{ mg kg}^{-1}$. All concerned must act by doing level best to protect and conserve the vulture in order to prevent their extinction and conserve ecosystem.

Keywords: Vulture, Ecosystem, Diclofenac, Relay Toxicity, Veterinary,

INTRODUCTION

A freely available and frequently used Non-Steroidal Anti-Inflammatory Drug (NSAID) diclofenac has been reported as the first clear case of major ecological damage by pharmaceutical product. The background is that the population of three vulture species Oriental White-backed Vulture (*Gyps bengalensis*), Slender billed Vulture (*Gyps tenuirostris*), Long billed Vulture (*Gyps indicus*), declined drastically. Decline of vulture populations was first recorded at the Keoladeo Ghana National Park, Rajasthan in India during mid 1980's to mid 1990's (Prakash, 1999).

Vultures have great significance for human well-being from the ecological, social and cultural viewpoints especially in Pakistan, India Bangladesh, Nepal and South Africa. These scavengers are eco-cleaner and play a vital role in environmental health by consuming carcasses of dead animals and thus reducing the risk of livestock born diseases. The decline in vultures had also affected the traditional custom of the Parsis of placing their dead in the 'Towers of Silence' for vultures to feed upon. (Mackenzie 2000; Prakash *et al.*, 2003; Shultz *et al.*, 2004).

Removal of a major scavenger from the ecosystem

will affect the equilibrium between populations of other scavenging species. In the absence of carcass disposing mechanisms, vulture declines may lead to an increase in the number of putrefying animal carcasses in the country side. In the absence of any alternative mode of disposal of animal carcasses, they continue to be disposed off in the open and with increasing numbers of feral dogs, there is increased risk of spread of rabies, and livestock borne diseases like anthrax (Prakash *et al.*, 2003).

The present review paper briefly describes linkage of a pharmaceutical product to the catastrophic decline in vulture population.

Vultures: There are nine species of vultures in the Indian subcontinent. These are the *Neophron percnopterus* (Egyptian Vulture), *Sarcogyps calvus* (Red Headed Vulture), *Gyps fulvus* (Indian Griffon Vulture), *Gyps himalayensis* (Himalayan Griffon), *Aegypius monachus* (Cinereous Vulture) and *Gypaetus barbatus* (Bearded Vulture) *Gyps bengalensis* (Oriental White-backed Vulture), *Gyps tenuirostris* (Slender billed Vulture), *Gyps indicus* (Long billed Vulture) (Ali and Ripley, 1983).

Populations of last three vulture species have undergone catastrophic declines. Populations have

decreased by at least 97% in India over the last 12 years and 92% in five years in Pakistan (Virani, 2006). Current rate of annual decline in Nepal is estimated to be 40% and the rate of decline within a decade is estimated at 90 to 95% (Nepal Country Report, 2006). In Bangladesh, the *Gyps bengalensis* is threatened and *Gyps indicus* and *Gyps fulvus* are now rare (Bangladesh Country Report 2006). Populations of *Gyps bengalensis* and *Gyps tenuirostris* in South-East Asia (Cambodia, India, Laos PDR, Myanmar, Nepal, Pakistan and Vietnam) are low, but declines are thought to have been historical and slower, rather than recent and rapid. World population size is not known for any of these species. Vulture numbers continue to decline at around 40% a year, placing these three species on the brink of extinction (Prakash, 1999; Oaks *et al.*, 2004; Rondeau and Thiollay, 2004).

All three vulture species were listed by IUCN, the World Conservation Union, in 2000 as 'Critically Endangered', which is the highest category of endangerment. The IUCN Red List 2008, indicated that extinction crisis of vultures had escalated. The IUCN Red List is the global standard when it comes to measuring species loss. The information contained in it must be taken seriously by all the concerned for doing their level best to protect and conserve the world's birds.

Current status of vulture population: Recent repeat surveys have been carried out by Bombay Natural History Society, (BNHS), with the support from Peregrine Fund USA and UK Government's Darwin Initiative. The scientists counted vultures in northern and central India between March and June 2006. Results of a major road transect survey in India revealed the shocking news that numbers of Oriental white-backed vultures have decreased by more than 99.9% in the past 15 years and that this species continues to decline at over 40% each year. The other two threatened species (long-billed and slender-billed vultures) have decreased by close to 97% over the same period. The study followed four previous counts undertaken during 1991-93, 2000, 2002 and 2003. In the early 1990s hundreds if not thousands of vultures were sighted each day. In 2007 recording a single vulture was note-worthy. The scientists believed that numbers of oriental white-backed vultures in India could now be down to 11,000 from tens of millions in the 1980s. Populations of long-billed and slender-billed vultures have dropped to around 45,000 and 1,000 birds respectively (Prakash *et al.*, 2007).

United Nation Environment Programme (UNEP) along with other international agencies, had celebrated World Migratory Bird Day on 10 – 11 May 2008 in more than 50 countries including Pakistan. However, the global celebrations were being overshadowed by above reports of Bombay Natural History Society, (BNHS) which had indicated that the numbers of migratory birds including vultures in Asia would be extinct in the wild.

INVESTIGATIONS ON CAUSE OF DEATH AND DECLINE IN VULTURE POPULATION

The cause of the vulture deaths in southern Asia remained unknown for many years but lot of work was done to reach a scientific explanation.

Signs and symptoms: The birds in all the region often had appeared sick and lethargic. Many had shown prolonged severe neck drooping, before collapsing—sometimes from their perches.' Neck drooping' was first observed in Keoladeo National Park, where birds would exhibit this behavior for protracted periods over several weeks before collapsing and falling out of trees, at the point of, or just prior to death (Prakash, 1999). It was an important behavior to monitor, as it was the only visible behavioral indication that birds were ill.

Various theories on the cause of death: Various theories were proposed which included (1) lack of food due to improved hygiene and disposal of carcasses (2) air force/aviation intervention to reduce vulture air-strikes (3) pesticides (4) direct or secondary poisoning (5) human persecution (6) environmental factors (such as global warming) (7) an infectious disease. All the hypotheses except infectious disease were rejected due to absence of a scientific correlation. The "infectious disease hypothesis" was viewed by many as the most likely explanation for the observed mortalities. This is because: high mortalities have been observed in all age classes; the decline has been observed over a relatively large geographical area during a short time; there appears to be no shortage of food as animal carcasses are left uneaten; there are few records of mass poisoning events; the deaths and declines are largely restricted to the *Gyps* and *Pseudogyps* genera; sick and dead vultures have been found with the same clinical signs throughout southern Asia, and initial necropsies and histo-pathological analyses have identified a possible infectious disease agent, including an adenovirus which was isolated from

vulture faeces. (Anon, 1999; Cunningham, 2000; Satheesan, 2001; Rondeau and Thiollay, 2004). However, signs and symptoms of sick and dead birds indicated probability of an epidemic disease. If so, this is likely to be an agent to which the population is native, e.g. an introduced agent or from which the species were previously isolated (Prakash *et al.*, 2003).

Bombay Natural History Society (BNHS) and Indian Veterinary Research Institute, Bareilly (IVRI) carried investigations on the hypothesis of epidemic disease in 2000. Visceral gout, an accumulation of uric acid within tissues and on the surfaces of internal organs was observed in 85% of dead vultures found. Death was caused by renal failure, which is known to occur as a result of metabolic, infectious or toxic disease. Visceral gout was observed in approximately 85% of dead adult and sub-adult birds collected in Pakistan (Oaks, 2006). Analysis of samples of dead vultures during 2000-2002 showed 28 cases of Avian Gout, while 17 samples did not exhibit Avian Gout. Microbiological studies; Virus isolation, Electron Microscopy, Molecular Biology, Bacteriology, and Transmissibility all gave negative results. Histopathology revealed that in all cases there was severe renal tubule damage, which became acute in 1-4 days, and minimal inflammation, indicating a toxic cause. However, toxicological studies did not indicate heavy metal poisoning, toxic residues of organophosphates, or organo chlorines. Accordingly, no conclusive evidence of epidemic disease was found (Green, *et al.*, 2004).

Diclofenac as the probable cause: NSAIDs, diclofenac, were marketed as analgesic and anti inflammatory drug for veterinary use in the subcontinent after 1985. Cheap, effective and multiple formulations of diclofenac were widely available in Pakistan, India, and Nepal which were used commonly to treat livestock for pain, fever and inflammation. It was suggested that probably, introduction of diclofenac was a new risk factor in the environment, to which vultures could plausibly be exposed through consumption of carcasses of livestock treated with the drug.

The WWF Pakistan had referred the problem to Department of Pharmacology and Toxicology, Faculty of Biosciences, UVAS, Lahore in 2004 to evaluate toxicity of diclofenac sodium. The work was started in April 2004. Broiler chicken were used as a surrogate avian species for carrying out various toxicological studies at experimental level. Different dosage levels of diclofenac were

tried. Study showed that diclofenac sodium was toxic. Sign and symptom of morbidity were similar to those which were reported for vulture i.e. neck drooping, lethargy, visceral gout and renal failure as a consequence of uric acid deposit. High mortality (60-80%) was observed at therapeutic dosage level (Personal communication).

An organized research was carried in Pakistan by Ornithological Society of Pakistan in collaboration with several organizations, including Peregrine Fund USA, the Bombay Natural History Society (BNHS,) and Royal Society for Protection of Birds, UK (RSPB). The study sites were established in the Kasur, Khanewal, Muzaffargarh and Layyah Districts of Pakistan to measure mortality at over 2,400 active nest sites. Between 2000 and 2003, high annual adult and sub adult mortality (5-86%) and resulting population declines (34-95%) were associated with renal failure and visceral gout. The vulture had consumed carcasses of animals treated with diclofenac. The scientific link between the diclofenac and dramatic fall in the raptor numbers was established. Vultures succumb to kidney failure and visceral gout when they eat a dead animal that has been treated with diclofenac (Oaks *et al.*, 2004; Taggart *et al.*, 2007). Although visceral gout had been reported previously from vultures in India (Mishra, 2002; Cunningham *et al.*, 2003), but it was never linked to diclofenac poisoning. The experiments in South Africa had shown that captive vultures are highly susceptible to diclofenac, and are killed by kidney failure within a short time of feeding on the carcass of an animal treated with the normal veterinary dose. Residue analysis in kidney samples showed a perfect 100% correlation between gout and diclofenac residues. Further, vultures fed either diclofenac or treated buffalo carcasses, died of gout. They had the exact same pathology and tissue residues as the wild cases, the frequency of mortality being dose dependent, a dose of 0.8 mg kg⁻¹ of diclofenac was highly toxic for which estimated LD50 was 0.1-0.2 mg kg⁻¹ (Oaks, 2006). It is added that normal therapeutic anti inflammatory dose for human is 3-4 mg kg⁻¹.

Another viewpoint: A toxicological study on diclofenac was undertaken in turkey vultures (*Cathartes aura*) as an initial step in examining sensitivity of New World scavenging birds. Two trials were conducted entailing oral gavage of diclofenac at doses ranging from 0.08 to 25 mg/kg body weight. Birds were observed for 7 day, blood samples were collected for plasma chemistry (pre-

dose and 12, 24 and 48 h, and 7 d post-dose), and selected individuals were necropsied. Diclofenac failed to evoke overt signs of toxicity, visceral gout, renal necrosis, or elevate plasma uric acid at concentrations greater than 100 times the estimated median lethal dose reported for Gyps vultures. For turkey vultures receiving 8 or 25 mgkg⁻¹ the plasma half-life of diclofenac was estimated to be 6 h, and it was apparently cleared after several days as no residues were detectable in liver or kidney at necropsy. Differential sensitivity among avian species is a hallmark of cyclooxygenase-2 inhibitors, and despite the tolerance of turkey vultures to diclofenac, additional studies in related scavenging species seem warranted (Reddy, *et al* 2006).

PLAN OF ACTION TO PROTECT AND CONSERVE VULTURE POPULATION

There were innumerable voices all over the world for putting ban on the veterinary use of the diclofenac. An internationally agreed action plan in response to the vulture conservation crisis had concluded that it was essential to control the veterinary use of diclofenac so as to remove it as a contaminant of the food of wild vultures. Alternative cheap and effective but safe veterinary drug must be identified.

Ban on Diclofenac for veterinary use: Ministry of Health (MOH), Government of Pakistan Islamabad was provided details regarding environmental disaster emerging from relay toxicity of diclofenac which was linked with catastrophic decline in population of vulture in Pakistan. The Ministry of Environment had requested MOH to look into the possibility for banning the use of diclofenac on priority basis. The issue was discussed in detail in the 199th meeting of the Drug Registration Board Ministry of Health, Islamabad which was held on 23rd & 24th August, 2006. After listening to all stakeholders and keeping in view the published data /research carried out, Registration Board had de-registered all the drugs of veterinary diclofenac product in Pakistan. The drug had also been banned in India.

Ban is ineffective: The ban on veterinary use of diclofenac is not effective as indicated by free availability of veterinary formulations in India and Pakistan. Furthermore, diclofenac formulated for humans is being used to treat livestock. This news was flashed by the Royal Society for the Protection of Birds, Zoological Society of London and Bird Life International on websites in April,

2008. Conservationists say that banning the retail sale of the veterinary drug diclofenac and constructing captive breeding centres is the only way to save the birds.

Meloxicam, an alternative to diclofenac: The control or elimination of veterinary drugs harmful to vultures was likely to be impossible without first identifying alternative veterinary drugs that are cheap and effective but safe for vultures and other scavenging species. Meloxicam another NSAID has been found as safe alternate to diclofenac sodium. It has similar effectiveness but better safety profile for use on animals (Naidoo *et al.*, 2008; Swan *et al.*, 2006; Gross 2006).

A success story in Nepal: A local scale project led by Bird Conservation Nepal has led to localized increases in numbers of nesting birds around one of Nepal's few remaining breeding colonies. The in-situ conservation work has focused on removing stock of diclofenac from vets and pharmacies and swapping this for the vulture safe drug meloxicam across the whole Nawalparasi district of Nepal where the vulture colony is located, as well as providing drug free feeding resources and vulture education and advocacy to local people in the area (Cuthbert, 2008).

CONCLUSION AND RECOMMENDATION

The swift and widespread decline in Asian vulture populations has been conclusively linked to the use of the Non-Steroidal Anti-Inflammatory Drug diclofenac in dead animals. Vultures succumb to kidney failure and visceral gout when they eat a dead animal that has been treated with diclofenac. Relay toxicity of diclofenac is the first clear case of major ecological damage by pharmaceutical product.

The ecological, social, religious and cultural significance of vultures may be summed up as: scavenging on animal carcasses and thereby helping keep the environment clean; and the disposal of dead bodies as per the religious practices of the Parsi community. An absence of vultures could result in the spread of diseases, some of which could affect humans.

The key steps necessary to save vultures from extinction are: effective and swift elimination of the veterinary drug diclofenac from the retail sale and captive breeding of vultures.

The governments need to encourage pharmaceutical companies to produce the quality

meloxicam formulations at low cost. The reducing its cost, would be of great value in ensuring vultures survival..

The public awareness campaigns always produce good results. The will and collaborative actions are required for vulture conservations/protection in order to ensure balance in ecosystem.

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