Vulture population decline, Diclofenac and avian gout

Populations of the *Gyps* vultures of southern Asian countries have been declining precipitously during the recent past, especially in the western parts of its distributional range. A linkage between the common non-steroidal anti-inflammatory drug (NSAID) 'Diclofenac' and the mortality of these vultures was established recently from Pakistan[1]. However, any conclusive evidence on Diclofenac-poisoning is still lacking from the Indian region, where the degree of decline has been severe. Post-mortem examinations of vulture carcasses revealed visceral gout (deposition of uric acid crystals in the tissues) as the cause of mortality in most of the dead birds. The visceral gout condition could be induced by a variety of potential factors, mostly associated with environmental degradation and climate changes. It appears quite likely that many such factors together have made a cumulative contribution towards the observed population decline of *Gyps* vultures during the recent years, which has pushed these scavenging sky-lords to near extinction. However, because of the scarcity of studies available on the impact of these factors on vultures, the factors would remain speculative until confirmed through empirical studies. Our aim is to provoke and direct further research in this direction. It is also intended to highlight the need for examining many of the pharmaceuticals and personal-care products from the point of view of their ecological impacts.

The Oriental white-backed vultures (OWBV; *Gyps bengalensis*), also known as white-rumped vultures, are distributed in southern Asia from Indus Valley of Pakistan in the west to Thai–Malay peninsula in the east (Figure 1) and have historically been the commonest vulture species of the area. The OWBVs were once so common in South Asian countries, that their abundant presence high in the skies[2] even posed a serious threat to smooth air traffic[3]. In the changed scenario, these once-common species have become rare and have made their way to the latest *Red Data Book* under the critically endangered category. According to the *Red Data Book*, 'it (OWBV) has suffered an extremely rapid population decline, particularly across the Indian subcontinent, probably as a result of disease compounded by poisoning, pesticide use and changes in the processing of dead livestock'[4].

Populations of all the three *Gyps* vultures of the area, namely the white-backed vulture (*Gyps bengalensis*; Figure 2), Indian vulture or long-billed vulture (*Gyps indicus indicus*) and slender-billed vulture (*Gyps indicus tenuirostris*) have declined drastically to below-sustainable limits throughout their distributional range. Considering the current pace of decline, the extinction of these species from the face of the earth appears all too imminent. Although populations of OWBVs have been declining moderately in the eastern parts of its distributional range from early last century itself, this slow decline could be attributed to habitat alterations and changes in food availability[5]. The OWBV populations in the western parts of its range remained healthy until early last decade and showed a sudden and sweeping decline during the nineties. As early as 1993 itself the population decline and even local extinction of this species were reported from certain pockets of the country[6], and it was only during recent times that this cataclysmic population decline received serious attention from conservationists and the general scientific community of the country[7–14]. The magnitude of decline and frequent sightings of dead vultures indicated that the enhanced mortality rate due to unknown reasons is causing the population decline. Further, investigations revealed characteristic symptoms of visceral gout in most dead birds, which was diagnosed as the cause of mortality.

The ecology and population dynamics of the OWBVs are poorly known. It is unfortunate that except a doctoral thesis[15] and a few related publications reported from the Gir forest, Gujarat[15–17], there is not much literature available in the public domain of the country on ecological aspects of these birds, crucially important in the ecosystem as efficient scavengers. Most of the documented information about them is in the form of grey literature as occasional short notes based on opportunistic observations. Many of such articles discuss various ecological and behavioural aspects of the species such as feeding[18–23], breeding[24–28] and interspecific interactions[17,29]. However, these isolated fragments of information are of little use for devising a crisis management plan to save the species, which would demand a comprehensive information base.

Once the population decline came to the limelight, various possible causes such as pesticide contamination, mass killing by people (poisoning/shooting) and a contagious viral disease[30] were examined by ornithologists[11]. However, none of these

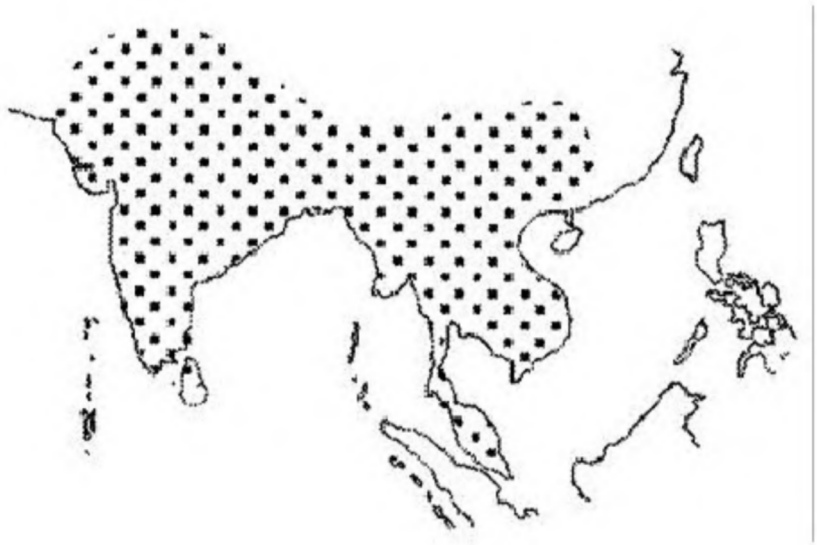

Figure 1. Distribution of Oriental white-backed vultures in Asia.

hypothesized causes could be proven beyond reasonable doubts. Organochlorine pesticides may be ruled out as a cause, because of lesser impact observed on natality, while organophosphate pesticides may also be spared as they are not known to cause visceral gout.

The only major headway in solving the mystery of vulture population decline was made recently by Oaks and his team at the Peregrine Foundation, when they successfully established that the residues of Diclofenac, a popular painkiller drug, caused decline in population[1] of OWBVs in Pakistan. It is probably the first ever report on the lethal impact of a therapeutic substance on wildlife and has attracted serious scientific attention. While Diclofenac may well be among the possible causes behind the decline of the Indian populations of OWBVs as well, it is yet to be proven that the Indian vultures were also exposed to the drug residues in sufficient enough magnitudes to effect mass mortality. Role of other possible factors cannot be completely ruled out and it would take detailed field and laboratory investigations to evaluate the role of other likely yet unexplored factors, such as a possible lethal mutation in the purine metabolism (because of increased exposure to potential mutagenic radiations and chemicals), impacts of recent climate change[31] or changing habits and habitat conditions of the vultures.

The potentially serious environmental implications of pharmaceuticals and many other personal-care products are often neglected[32]. Although some of the common therapeutic compounds have known lethal impacts on other taxa (Table 1), in most therapeutics the impacts on non-target species are practically unknown. Until its role in the decline of vulture populations were revealed, any harmful effects of Diclofenac on the environment were practically unheard of. Once its lethal toxicity to vultures was exposed, the common NSAID Diclofenac has been in the eye of a storm. NSAIDs are known to act in the body by inhibiting the synthesis of prostaglandins (substances found in body tissues produced in response to trauma) and are widely used in palliative care for symptomatic relief from pain and inflammation. While physiological responses to NSAID compounds are known to vary widely from species to species[33], certain responses such as renal complications and gastrointestinal disorders are common and similar across taxonomic groups. However, the exact mode of action of NSAIDs in the body or in the environment is not yet completely known.

Diclofenac is an arylacetic acid derivative (Figure 3) and is one of the commonly used painkillers, mostly available in the form of Diclofenac sodium. Diclofenac has proven anti-inflammatory, analgesic and antipyretic properties. It is widely used all over the world in palliative care, especially as a pain reliever. In USA alone, NSAIDs worth US$ 2 billion are sold out every year. NSAIDs are cheap and freely available over-the-counter, without proper medical prescription and are generally considered safe. It has resulted in the over-use of these therapeutic compounds and often needlessly so.

Diclofenac is absorbed quickly and its half-life period in the mammalian body is short (few hours). Under fasting condition, diclofenac is completely absorbed from the gastrointestinal tract. Metabolism of Diclofenac in humans is partitioned between acyl glucuronidation and phenyl hydroxylation[34]. It is eliminated from the body through subsequent urinary (~ 65%) and biliary (~ 35%) excretion of glucuronide and the sulfate conjugates of the metabolites. Little or no unchanged and unconjugated drug is excreted out of the human body. The elimination half-life values for Diclofenac metabolites are shorter than those for the parent drug. The degree of accumulation and activities of Diclofenac metabolites are largely unknown.

Figure 2. White-backed vultures.

Table 1. Therapeutics used as biocides

Drug	Medical use	Biocide use
4-Aminopyridine	In multiple sclerosis	Avicide
Warfarin	Anticoagulant	Rodenticide
Triclosan	Gingivitis (toothpaste)	General biocide
Acetaminophen	Analgesic	Kills brown tree-snake (Indonesia)
Caffeine	Beverage, stimulant	Kills coqui frogs and slugs (Hawaii)
Diclofenac	Analgesic, Antipyretic	Kills vultures (Pakistan)

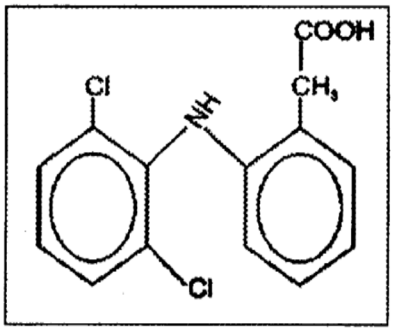

Figure 3. Structure of Diclofenac molecule (The 'OH' of carboxyl group is replaced with NaO in the common therapeutic form called Diclofenac sodium).

Toxic side effects of Diclofenac have been demonstrated in various animals. While minor upper gastrointestinal problems such as dyspepsia are common during Diclofenac administration, severe pathologic conditions such as peptic ulceration, gastrointestinal bleeding, hepatotoxicity (nausea, fatigue, lethargy, pruritus (intense itching sensation), jaundice, 'flu-like' symptoms, etc.), renal papillary necrosis, and renal failure are reported in patients subjected to long-term administration of the drug. Cardio-protective effect is a positive side effect revealed recently. Hepato-toxicity[35,36] and even acute immune haemolytic anaemia[37], renal dysfunction[38] and acute renal failure[39] are reported in mammals. However, in humans it is generally considered that the benefits are sufficient enough to offset the potential risks.

Gout is a disorder of metabolism that allows uric acid to accumulate in the blood and tissues. Uric acid is the end-product of purine (nucleic acid component of DNA) metabolism and is produced normally by the body during tissue remodelling and breakdown. Gout in birds is caused by renal failure and is analogous to hyperuricemia (accumulation of urea) in humans. When kidneys fail to remove the uric acid efficiently from the blood, tissues become supersaturated with uric acid, resulting in urate salt precipitation as crystals. A cellular reaction to uric-acid crystal deposition causes gout. These crystals are less soluble under acid conditions and any condition predisposing to acidosis also precipitates urate crystals. These crystals stimulate phagocytosis by neutrophils and initiate the inflammatory cascade. Interleukin-1 and tumour necrosis factor-α are known to be involved in the inflammatory cascade.

Visceral gout had been the prominent abnormality reported in most of the post-mortem reports of vultures. Hence it is normally assumed that whatever the responsible factor might be, it is in some way predisposing the vultures to a gouty condition. Gout has been reported from various animal taxa such as snakes, lizards, crocodiles, birds and mammals[33,40–44]. Presence of red meat in the diet is considered to be a major contributing factor for susceptibility to gout. Interestingly, among the dinosaurs, evidence for gout has been found in the fossils of the carnivorous Tyrannosaurus, a red meat-eater[40]. This, along with high levels of uric acid in raptors, might have contributed to predisposing vultures to increased risk of gout formation compared to other birds. No treatment for visceral gout is immediately known. Hence it is felt essential that immediate attention be given to explore the treatment of visceral gout in birds and to identify the possible causative factors that are in operation. It appears that along with Diclofenac, a host of other factors might also have contributed to the development of gout in vultures that ultimately led to OWBV mortality in such great magnitude.

Causes of gout can be divided into two major categories: increased synthesis of uric acid and decreased clearance of uric acid by the kidneys. High intake of purine-rich foods such as red meat and poultry (especially the offal foods like liver, kidney, heart, tripe, etc.) is an important causative factor that enhances uric acid synthesis in the body. Apart from the Diclofenac residues, which is only a latest addition on the list, many other factors are already known to cause the physiological condition of gout. Major among them are renal impairment, dietary excess of purines, fasting-induced ketosis, inborn errors of metabolism, lead poisoning (high lead levels are already reported as the cause of mortality in one of the vultures from Pakistan[1]), hyperproliferative skin disorders (e.g.- psoriasis), haemolytic and pernicious anaemia, radiation exposure, gut sterilization by antibiotics and various medications[45], such as caffeine, corticosteroids, cytotoxic drugs dopamine, nicotinic acid, salicylates, low dose sulfinpyrazone and vitamins B-12 and C.

Another factor that was probably overlooked in the study of vultures from Pakistan[1] is the effect of Diclofenac metabolites. Although *in vitro* studies in rat hepatocytes have shown that the reactive Diclofenac metabolite such as acyl glucuronide is less cytotoxic than the drug itself in mammals, it need not necessarily be true in vultures. Hence the toxicity of mammalian metabolites of Diclofenac and other NSAIDs also needs to be screened.

Apart from the above-mentioned possible contributory factors, deteriorating health, affecting the general immunity of the birds, might have also assisted in ushering them into their gouty graves. Vultures had wide home ranges of more than 100 km radius, and before finding the waste dumps of urban areas as convenient 'fast food restaurants', the OWBVs had to forage over vast areas of land for finding carcasses. It is thus quite likely that the vultures too have become far more sedentary in their habits compared to earlier times. Mass congregations in places such as solid-waste dump yards have exposed them to various noxious chemicals and pollutants, which would have taken a heavy toll on their health and immunity levels and rendered them increasingly susceptible to environmental stress and contagious diseases. The solid wastes in the country are not segregated and contain a variety of insidious and recalcitrant polluting chemicals. It may be noted that lesions reported from the carcasses of Indian OWBVs have indicated the role of an undiagnosed disease factor as well[30,46]. Such ecological factors along with other inconclusive hypotheses would have added on to the overall population decline.

The lacunae existing in the ecological and behavioural information on our common birds is a major lesson to be learned during the recent episode of vulture population decline. For instance, the normal behaviour of 'head drooping' during the hot summer days was often mistakenly identified as a disease symptom. (It is actually a natural behaviour of these birds possibly to avoid direct sunlight into their eyes). Unfortunately, the situation is not different with most other urban birds such as house crows and sparrows. Serious ecological studies and long-term population monitoring programmes need to be targeted on the urban bird populations. It would serve as an early warning system to facilitate effective precautionary, mitigatory and remedial measures to be taken up well in advance, before the populations cross critical limits as happened with *Gyps* vultures.

There are multiple factors, which possibly have played a role in the drastic population decline of the Oriental white-backed vultures of southern Asia. Prudent management actions duly considering all such potential factors only can save them from the risk of extinction. In the absence of a detailed diagnostic investigation and management strategy specific to the Indian conditions, activities such as captive breeding and release may not yield the expected results. Although the role of Diclofenac residues in the OWBV mortality from the Indian region is yet to be confirmed experimentally, a substitution of Diclofenac with other NSAID compounds may be advocated as an immediate (and desperate) measure to

put breaks on the decline of the vulture population. However, care should be taken while replacing Diclofenac with other NSAIDs, because the replacement drugs may not be environmentally safe either, and their impacts are unknown. Research into the ecological impact of all major therapeutics, especially the common and widely used ones, should be undertaken urgently to facilitate the policy makers to regulate these compounds with potentially hazardous environmental impacts. The exact physiological mechanism through which Diclofenac acts in three species of only a single genus (*Gyps*), appears mysterious and highly intriguing. The metabolic pathways through which Diclofenac poisoning causes renal complications, gout and consequent mortality in only *Gyps* vultures is yet to be elucidated. As of now, it appears premature to conclude that the Diclofenac residue is the universal causative agent behind the decline of vulture population.

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Development of a novel lyophilization protocol for preservation of mushroom mycelial cultures

The maintenance and production of reliable pure culture spawn with desirable quality is the key operation and the first critical stage in the success of mushroom cultivation. Maintenance of vigour and genetic characteristics of a pure strain in the form of a culture is the main objective of culture preservation. Besides this, strain improvement of cultivated mushrooms demands a well-planned system of maintenance, preservation and availability of genetic diversity[1]. Mushroom culture repositories/gene banks play a vital role in supply of pure and authentic cultures to most of the mushroom spawn-producing units. There are various methods of maintenance and preservation of mushroom cultures and a good culture collection section adopts more than one method to preserve them. Mushrooms might be of academic, medicinal or horticultural importance. Mushroom strains having industrial importance are patented and preserved, although availability of such strains becomes restricted[2]. If no degenerative changes were to take place during preparation or maintenance of mush-