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Original Article

Evaluation of histo-toxicity of nimesulide in Black Kites (*Milvus migrans*): a pharmacodynamic study

Avaliação da histotoxicidade da Nimesulida em Milhafre-preto (*Milvus migrans*): um estudo farmacodinâmico

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Abstract

The present experimental work was conducted to elucidate the toxicity of nimesulide at three different doses in black kites (*Milvus migrans*). *M. migrans* is one of the most common raptors near human habitations. The goal of the current investigation was to determine whether nimesulide is similarly hazardous to raptors as was diclofenac sodium and to investigate the acute oral toxicity of nimesulide in these birds. For this study, eight adult male black kites (*M. migrans*) were randomly divided into four groups. *M. migrans* in the control group (n = 02) were not treated with nimesulide. The other three groups were given nimesulide doses. The birds in the first (n = 02) were declared the control group. The second (n = 02), third (n = 02), and fourth groups were administered nimesulide at a low, medium, and high dose of 2, 4, and 6 mg/kg live body weight of bird/day, respectively, for 10 days. Nimesulide-addled birds became listless and despondent, then anorexic. The birds were standing there with their eyes closed and showing no signs of life. There was an increase in saliva production, a slowing of breathing, and dilated pupils. No clinical signs were observed in the control group. No mortality was seen in the control or treated groups. The control group did not show lesions of gout, but black kites intoxicated with nimesulide at 2, 4, and 6 mg/kg live body weight of bird/day showed inflammation, apoptosis, hemorrhage, necrosis, and leukocytic infiltration tissues of the liver, kidney, and heart of black kites (*M. migrans*) treated with different concentrations of nimesulide. The treated groups also showed apoptosis of myofibrils and hyperplasia. The hypertrophy, atrophy, fibrosis, necrosis of skeletal muscles and hemorrhage were prominent in the muscles of black kites (*M. migrans*) intoxicated with nimesulide. All observed histological alterations got worse in a dose-related way. There was no significant difference in AST, ALT, ALP, serum uric acid, but a significant difference was observed in the values of serum urea (p = 0.001) and serum creatinine (p = 0.019).

Keywords: nimesulide, *Milvus migrans*, histological alterations, gout, serum urea, serum creatinine.

Resumo

O presente trabalho experimental foi conduzido para elucidar a toxicidade da Nimesulida em três doses diferentes em milhafres (*Milvus migrans*). *M. migrans* é uma das aves de rapina mais comuns perto de habitações humanas. O objetivo da presente investigação foi determinar se a Nimesulida é igualmente perigosa para as aves de rapina como foi o diclofenaco sódico e investigar a toxicidade oral aguda do fármaco nessas aves. Para este estudo, 8 milhafres machos adultos (*M. migrans*) foram aleatoriamente divididos em 4 grupos. *M. migrans* no grupo controle (n = 2) não foram tratados com Nimesulida. Os outros 3 grupos receberam doses do fármaco. As aves do primeiro grupo (n = 2) foram declaradas o grupo controle. O segundo (n = 2), terceiro (n = 2) e quarto grupos receberam Nimesulida nas doses baixa, média e alta de 2, 4 e 6 mg/kg de peso corporal vivo da ave/dia, respectivamente, por 10 dias. Aves confusas com Nimesulida tornaram-se apáticas e desanimadas, depois anoréxicas. Os pássaros estavam parados com os olhos fechados e sem sinais de vida. Houve um aumento na produção de saliva, lentidão na respiração e pupilas dilatadas. Nenhum sinal clínico foi observado no grupo controle. Nenhuma mortalidade foi observada nos grupos de controle ou tratados. O grupo controle não apresentou lesões de gota, mas os milhafres intoxicados com Nimesulida nas doses de 2, 4 e 6 mg/kg peso vivo da ave/dia apresentaram inflamação, apoptose, hemorragia, necrose e infiltração leucocitária nos tecidos do fígado, rim e coração de milhafre-preto tratados com diferentes concentrações de Nimesulida. Os grupos tratados também apresentaram apoptose de miofibrilas e hiperplasia. A hipertrofia, atrofia, fibrose, necrose da musculatura esquelética e hemorragia foram proeminentes nos músculos de milhafres negros intoxicados com o fármaco. Todas as alterações histológicas observadas pioraram de forma dose-dependente. Não houve diferença significativa em AST, ALT, ALP, ácido úrico sérico, no entanto, foi observada diferença significativa nos valores de ureia sérica (p = 0,001) e creatinina sérica (p = 0,019).

Palavras-chave: nimesulida, *Milvus Migrans*, alterações histológicas, gota, ureia sérica, creatinina sérica.

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1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) can be used in a wide variety of clinical treatments in avian medicine. NSAIDs might be a useful therapeutic choice for birds (Degernes et al., 2011). The therapeutic success of NSAIDs deviates between species of animals and even within individuals. This family of medications has detrimental effects on the stomach, intestine, tissues of the kidney, and hematological systems. Nephrotoxicity is the most widespread and harmful effect of NSAIDs detected in birds (Shao et al., 2022).

The action of NSAIDs inhibits the production of prostaglandins, in accordance with Vane's (1971) theory, which has received widespread support from researchers. For instance, salicylate and aspirin are both potent anti-inflammatory drugs, but only aspirin inhibits platelet thromboxane synthesis and platelet aggregation. Because several NSAIDs target cyclooxygenase proteins, the histo-toxicity of NSAIDs on the stomach, gut, respiratory organ, and kidney varies significantly. There is a demand for NSAIDs with fewer side effects (Pereira and Werther, 2007).

Currently, avian species toxicity assessment is restricted to clinical studies that include both the target species and the intended medicine in the specific species (Naidoo and Swan, 2009). There are more facts concerning NSAIDs and an augmented hazard of chronic ailment in birds (Dalili and Kashani, 2018; Xu and Yu, 2020). Nimesulide being a non-steroidal anti-inflammatory drug has analgesic, antipyretic and anti-inflammatory properties (Donati et al., 2016). Nimesulide is an effective COX-2 inhibitor and is consequently assumed to be safer in clinical applications. While various clinical reports do not provide considerable significance to the notion that a specific COX-2 inhibitor does have a positive effect on the digestive system, its intestinal resistance has not been shown to be preferable to that of particular NSAIDs (Swarup et al., 2007).

M. migrans is an inhabitant breeding species that grubs living creatures, primarily small vertebrates, but will also forage dead animals and are positioned at the high rank of food chain as a raptor (Hong et al., 2018). With the deterioration in vulture numbers crosswise Asia, there are increased feeding chances for other scavengers and raptors including black kites (*M. migrans*) (Cuthbert et al., 2006).

Studies have found that, in addition to diclofenac, certain NSAIDs, including aceclofenac, ketoprofen, nimesulide, flunixin, and carprofen, are hazardous to *Gyps* vultures (Nambirajan et al., 2021; Galligan et al., 2022). Nimesulide deposits in tissues with gout symptoms indicated that vultures died from nimesulide poisoning, and symptoms of gout were present in wild, dead white-rumped vultures, similar to diclofenac. Since then, nimesulide has appeared to have harmful consequences like diclofenac (Nambirajan et al., 2021).

The toxicity of nimesulide at different doses in other raptors, such as black kites (*M. migrans*) must be studied. The goal of the current investigation was to determine whether nimesulide is similarly hazardous to black kites (*Milvus migrans*) as described in white-rumped vulture (*Gyps bengalensis*) by Nambirajan et al. (2021) and to determine its acute oral toxicity in *M. migrans* at different doses.

2. Materials and methods

The research conducted in this study was reviewed and given approval by the Ethical Research Committee of the Institute of Zoology, Faculty of Sciences at Bahauddin University, Multan (Pakistan) (Application Number: BZU/Zool. Ethics/23-15).

2.1. Experimental birds

Eight adult male *M. migrans* were taken from their flight aviaries and housed separately in outdoor enclosures with climate control, maintaining a constant temperature and light cycle. The birds were fed in accordance with their regular schedule, and water was readily available. All meat was obtained from reputable vendors who were hand-picked for not using NSAIDS on their livestock. Birds were housed individually in outdoor pens (10–12 feet wide, 10–12 feet long, and 10–12 feet high; constructed of pressure-treated lumber with vinyl-coated wire, and a roof for shade). A bowl containing fresh water was present in each pen. Before the experiment began, all the birds were carefully examined, and their daily food intake was recorded to reduce the study's bias. A homogeneous food provisioning formula was consequently seen throughout the tests. Additionally, a variety of colored leg-rings or bands with numbers were used to identify each bird. After six weeks of acclimatization, birds were handled and weighed every week.

2.2. Drug used and formulation of drug

The black kites (n = 8) were divided into four equal groups, with two birds in each group. The first group served as the control, and the other groups (second, third, and fourth) were treated with different doses of nimesulide. After preparation of a fresh solution or suspension of each dose of nimesulide (Nimaran, Novartis Pharma (PAK) LTD), it was orally administered daily at 24-hour intervals into the crops of *M. migrans* of groups 2, 3, and 4 through a plastic tube attached to a syringe for 10 consecutive days at dose rates of 2, 4, and 6 mg/kg body weight of birds as recorded on the corresponding days of the trial. Information on the efficacy and optimal dosage in avian species is scarce (Thompson, 2008). The dose selection of nimesulide in this experiment was based on directions of previous studies (Reddy et al., 2006) and the dose used in this experiment was 20% of the dose of nimesulide proposed by Shafi et al. (2015) in chickens and 50% of the dose of nimesulide proposed by Candelario-Jalil et al. (2004) in rats. This approach of using previous studies to determine an appropriate dose is common in toxicology studies, as it allows for comparison between studies and helps ensure that the dose used is safe and effective.

2.3. Observations

2.3.1. Clinical parameters

For the duration of the trial, all birds were observed twice daily after treatment for changes in their physical appearance, drinking, and feeding. Anomalies in behavioral patterns, such as depressive symptoms, adjustments to body posture, movement, and gaze, as well as morbidity and death, were observed and documented.

2.3.2. Blood sampling

Blood samples were obtained from each bird's wing vein using a disposable syringe with a capacity of two mL. Blood samples were taken from live birds before treatment, on the fifth day of the trial, and at the end of the trial. A total of 1.5 mL of blood was extracted from each bird. The blood from the syringe was transferred to clean, dry 3 mL glass tubes, allowing the blood to clot. Following a 10-minute centrifugation at 2500 rpm at room temperature to remove serum from clotted blood, serum was extracted in serum-specific cups and stored at -20Ċ for analysis of the serum's biochemistry. The clinical chemistry analyzer, Micro Lab 300 (Merck, Germany), was used to quantify AST enzyme, ALT enzyme, ALP enzyme, serum urea, serum uric acid, and serum creatinine parameters from serum samples taken from the birds The serum biochemical variables were computed using commercially available diagnostic kits according to the manufacturer's instructions.

2.3.3. Histopathology

Birds that perished during the experiment underwent thorough post-mortem examinations, and any gross lesions were noted. The organs were subjected to histopathology. At the end of the study period, all living birds were weighed, incised, and necropsied to look for any noticeable pathological deviations. After the isolation of the liver, kidneys, and heart, representative samples from the liver, kidney, heart, and muscle were collected in 10% neutral buffered formalin for histological investigation, processed by routine paraffin embedding technique, and sections of 5 µm thickness were cut and stained. The photographs of selected areas on slides were taken with a digital camera (Electron eyepiece, YJEYE 01) connected to a light microscope (XSZ 107 BN, Made in China) and observed on 200X.

3. Results

3.1. Clinical parameters

Throughout the entire study period, the intoxicated birds of the experimental groups (second, third, and fourth) experienced anorexia-related depression. The pretentious birds hoisted themselves motionless with both eyes fastened. The birds in the experimental groups showed hyperactive salivation with a reduced respiratory rate; their pupils were widened, and they showed a staggering gait. The intoxicated birds vomited suddenly. The marked clinical signs observed in *M. migrans* in the experimental groups included huddling, dullness, appearing listless, and laboured breathing. The birds were reluctant to move and stood with ruffled feathers and a cyanosed comb. The severity of the observed clinical signs was dose-related.

3.2. Mortality

No mortality was seen in birds of the control group or birds treated with different doses (2, 4, and 6 mg/kg body weight of the bird on the test day) of nimesulide for 10 days.

3.3. Biochemical studies

There was no significant difference in AST enzyme, ALT enzyme, ALP enzyme, serum uric acid, but a significant difference was observed in the values of serum urea and serum creatinine. (Table 1).

3.4. Histopathological changes

No histological changes in specimens of kidney, liver, heart and muscles were observed in birds of control group (Figure 1-4). Apoptosis, severe inflammatory cells infiltrating the glomerulus, hemorrhage, and Bowman's space are all visible in the histological image of a black kite's (*M. migrans*) kidney treated with nimesulide (2 mg/kg body weight of the bird on test day). A black kite's (*M. migrans*) kidney treated with nimesulide (4 mg/kg body weight of the bird on test day) showed necrosis of the glomerulus cell and some inflammatory cells infiltrating the glomerulus and Bowman's capsule in the microscopic image. The kidney of a black kite (*M. migrans*) given nimesulide (6 mg/kg of the bird's body weight per day) showed that the architecture of the glomerulus and Bowman's capsule space was not clear, and that there was infiltration, necrosis, and apoptosis in the cells of the glomerulus and Bowman's capsule (Figure 1). The tissue section of the liver of black kites (*M. migrans*) intoxicated with nimesulide at a rate of 2 mg/kg and 4 mg/kg body weight of the bird showed apoptosis, necrosis, and infiltration of the hepatocytes. The histological image of the liver of black kites (*M. migrans*) orally administered by nimesulide (6 mg/kg body weight of the bird on the respective day of experiment) showed hepatocyte disarray, enlargement of the sinusoidal, necrosis, and apoptosis (Figure 2). Microscopically, the hearts of the intoxicated birds revealed apoptosis of myofibril cells and infiltration and hyperplasia of myofibril cells. The above-mentioned symptoms were common in all three experimental groups of birds, but the severity of toxicity was related to the dose of nimesulide (Figure 3). The histological image of skeletal muscle of black kites (*M. migrans*) treated by nimesulide (2, 4 and 6 mg/kg body weight of the bird on the respective day experiment) showed fibrosis on skeletal muscle, hypertrophy of skeletal muscles, prominent nuclei, atrophy of skeletal muscle fibres, and loss of striations (Figure 4).

Table 1 Estimated AST, ALT, ALP, Serum urea, Serum uric acid and Serum creatinine concentrations on different days of experiment in black kites administered orally with three different doses of nimesulide on different days of the experiment (Mean±SE) by Duncan multiple tests in two-way ANOVA.

Parameters	Groups & Dosage (mg/kg body weight of body)	Sampling Days			P Value
		1st day	5th Day	10th Day	
AST (U/L)	1st (Control)	407.00±196.00	290.00±12.00	364.50±15.50	0.574
	2nd (2 mg/kg)	270.00±143.00	466.50±58.50	366.00±145.00	
	3rd (4 mg/kg)	158.50±55.50	395.00±58.00	335.00±104.00	
	4th (6 mg/kg)	288.00±87.00	524.50±46.50	418.50±115.50	
ALT(U/L)	1st (Control)	162.00±5.00	576.50±412.50	114.00±34.00	0.506
	2nd (2 mg/kg)	60.00±7.00	118.50±3.50	91.00±26.00	
	3rd (4 mg/kg)	183.00±95.00	197.00±75.00	176.50±62.50	
	4th (6 mg/kg)	82.50±24.50	110.00±±33.00	136.50±13.50	
ALP (U/L)	1st (Control)	30.00±1.00	91.50±36.50	63.50±1.50	0.305
	2nd (2 mg/kg)	64.00±9.00	74.00±34.00	62.00±35.00	
	3rd (4 mg/kg)	43.50±7.50	111.00±11.00	40.00±18.00	
	4th (6 mg/kg)	78.50±19.50	49.00±2.00	47.50±19.50	
Serum Urea (mg/dl)	1st (Control)	5.50 ± 1.50^{cd}	10.50 ± 0.50^{ab}	12.50 ± 1.50^{a}	0.001
	2nd (2 mg/kg)	10.50 ± 0.50^{ab}	0.65 ± 0.05^{e}	3.50 ± 0.50^{de}	
	3rd (4 mg/kg)	8.50 ± 0.50^{bc}	5.00 ± 1.00^{cd}	3.50 ± 1.50^{de}	
	4th (6 mg/kg)	8.00 ± 1.00^{bc}	5.25 ± 0.75^{cd}	5.00 ± 2.00^{cd}	
Serum Uric Acid (mg/dl)	1st (Control)	22.85±6.45	15.25±0.45	11.10±2.20	0.254
	2nd (2 mg/kg)	17.55±5.35	17.40±3.80	15.20±0.10	
	3rd (4 mg/kg)	10.10±4.20	16.50±2.50	13.80±0.10	
	4th (6 mg/kg)	10.65±0.95	22.35±7.25	20.10±6.00	
Serum Creatinine (mg/dl)	1st (Control)	0.30 ± 0.10^{bcde}	0.30 ± 0.00^{bcde}	0.35 ± 0.05^{bcd}	0.019
	2nd (2 mg/kg)	0.50 ± 0.10^{ab}	0.25 ± 0.05^{cde}	0.10 ± 0.00^{e}	
	3rd (4 mg/kg)	0.65 ± 0.05^{a}	0.15 ± 0.05^{de}	0.15 ± 0.05^{de}	
	4th (6 mg/kg)	0.45 ± 0.05^{abc}	0.15 ± 0.05^{de}	0.20 ± 0.10^{de}	

Note: Values in column at an experimental day followed by different superscript letters are significantly different ($p<0.05$).

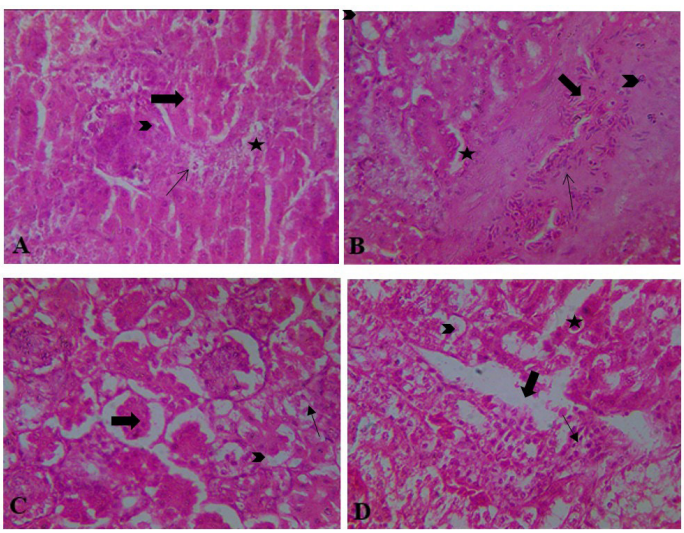

Figure 1. A: Histological image of *M. migrans*'s kidney from the control group showed visible glomerular (thick arrow), intact space of the cup shaped Bowmans capsule (thin arrow), integral capsule (arrow head), and normal interstitial tissue (star). **B**: Histologic image of the kidney of *M. migrans* treated by nimesulide (2 mg/kg body weight of the bird on each test day) showed visibility of apoptosis in single cells (thick arrow), infiltration in the cells of the glomerulus (thin arrow), noticeable hemorrhage (arrow head), and Bowman's space is prominent (star). **C**: A section of kidney tissue from *M. migrans* treated by nimesulide (4 mg/kg body weight of the bird on each test day) showed the glomerulus is prominent (thick arrow), necrosis of single glomerulus cells (thin arrow), infiltration in the glomerulus, and Bowman's capsule (arrow head). **D**: Histological image of *M. migrans*'s kidney treated by nimesulide (6 mg/kg body weight of the bird on each test day) showed the architecture of the glomerulus and Bowman's capsule space was not prominent (thick arrow), infiltration occurred in the glomerulus and Bowman's capsule (thin arrow), necrosis (arrow head), and apoptosis (star).

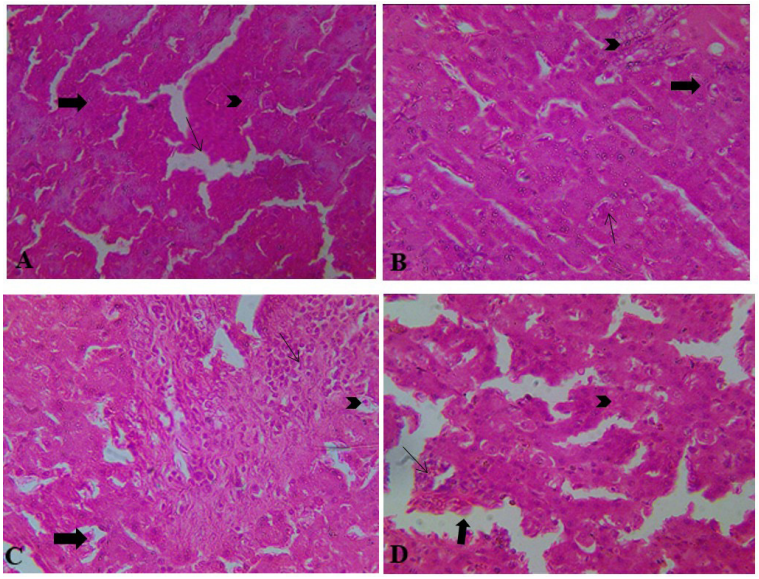

Figure 2. A: Histological image of the liver of *M. migrans* in the control group: columnar sheets in hepatocytes (thick arrow), visibility of the sinusoid (thin arrow), complete parenchyma (arrow head). **B**: Histological image of *M. migrans* liver intoxicated by nimesulide (2 mg/kg body weight of the bird on each test day) showed necrosis of single hepatocytes (thick arrow), apoptosis of single hepatocytes visible (thin arrow), and some inflammatory cells infiltrated in the hepatocytes (arrow head). **C**: Histological image of hepatocytic tissues of *M. migrans* treated by nimesulide (4 mg/kg body weight of the bird on each test day) showed apoptosis of single hepatocytes (thick arrow), infiltration (thin arrow), and necrosis (arrow head). **D:** Histological image of the liver of *M. migrans* treated by nimesulide (6 mg/kg body weight of the bird on each test day) showed a larger sinusoid (thick arrow), apoptosis of single hepatocytes (thin arrow), and infiltration in the whole hepatocyte's cells (arrow head).

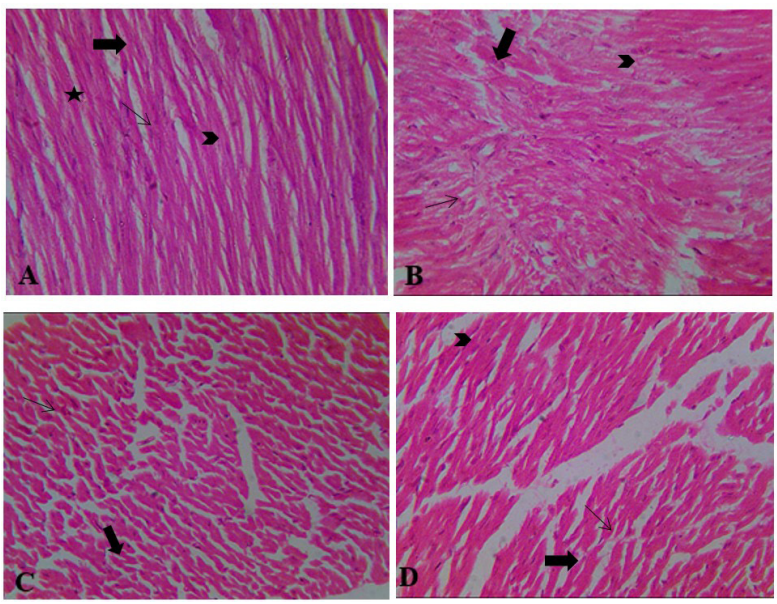

Figure 3. A: Histological image of the heart of *M. migrans* from the control group showed normal architecture of the heart (thick arrow), prominent nuclei (thin arrow), spindle shaped fibres (arrow head), and a prominent intercalated disc (star). **B**: Histological image of tissues of the heart of *M. migrans* intoxicated by nimesulide (2 mg/kg body weight of the bird on each test day) exhibited apoptosis of myofibril cells (thick arrow), myofibril cell damage (thin arrow), and some inflammatory cells infiltrated (arrow head). **C**: Histological image of tissues of the heart of *M. migrans* intoxicated by nimesulide (4 mg/kg body weight of the bird on each test day) showed hyperplasia of myofibril cells (thick arrow), and inflammatory cells infiltrated the myofibril cells (thin arrow). **D**: Image of tissues of heart of *M. migrans* treated by nimesulide (6 mg/kg body weight of the bird on each test day) exhibited necrosis (thick arrow), hyperplasia of heart muscle (thin arrow), some nuclei existing in the myofibril cell (arrow head).

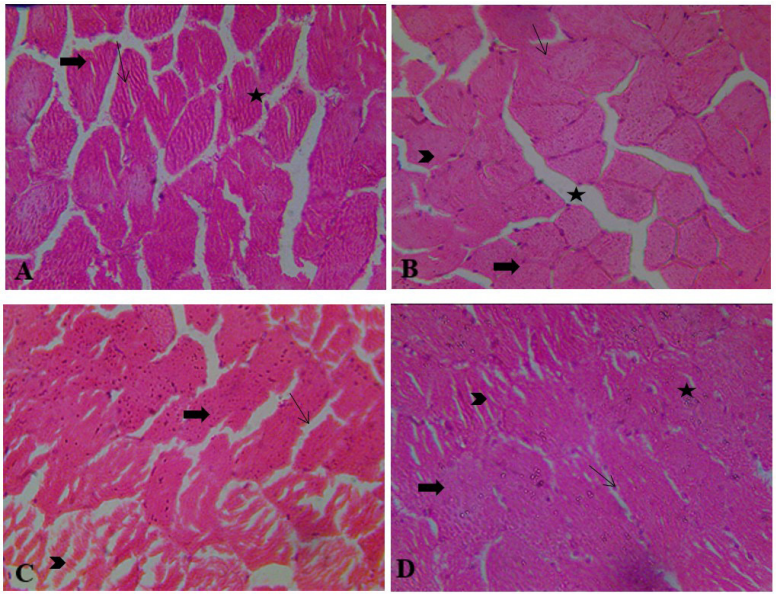

Figure 4. A: Histological image of muscles of *M. migrans* from the control group showed transverse muscle fibres (thick arrow), striations (thin arrow), and peripheral muscle (star). **B**: Histological image of muscles of *M. migrans* treated by nimesulide (2 mg/kg body weight of the bird on each test day) exhibited fibrosis on skeletal muscles visible (thick arrow), hypertrophy of skeletal muscles and striations absent (thin arrow), nuclei are prominent in some skeletal muscles (arrow head), and the transverse cut between muscle fibres is larger than normal (star). **C**: Histological images of muscles of *M. migrans* administered by nimesulide (4 mg/kg body weight of the bird on each test day) showed fibrosis and no striations in skeletal muscle fibres (thick arrow), nuclei destroyed (thin arrow), and atrophy of skeletal muscle fibres (arrow head). **D**: Histological image of muscles of *M. migrans* administered by nimesulide (6 mg/kg body weight of the bird on each test day) showed hypertrophy of skeletal muscle (thick arrow), prominent nuclei (thin arrow), atrophy of skeletal muscle (arrow head), and fibrosis (star).

4. Discussion

The investigated birds in this study showed depression, appeared listless, had anorexia, had a reduced respiratory rate, vomited, and huddled, and no mortality was seen in birds during the present evaluation of the toxicity of nimesulide in black kites (*M. migrans*). These findings are in line with the studies of Mori et al. (2000), Reddy et al. (2006), and Kawoosa and Khan (2020). The levels of AST, ALT, and alkaline phosphatase were non-significant in the present study as compared to the control. The AST levels were found to rise in cases of hepatic and muscular injury, and both measures serve as useful tools for monitoring muscle and liver impairment (Lumeij, 1999). Alanine aminotransferase (ALT) is present in the liver's cytosol and mitochondria. The basic function of ALT is to speed up the transportation of amino groups from 2-oxoglutarate to L-alanine. It is utilized as a vector for living abnormalities or liver dysfunction (Sulochana and Ramakrishna, 2016). The surge in alkaline phosphatase could be attributed to an increase in osteoblastic activity, impaired liver function, or bile flow obstruction (Shafi et al., 2012). However, according to Harr (2002) and Simaraks et al. (2004), the alkaline phosphatase ratio stayed normal, as previously indicated in several bird species. The results of the present investigation regarding AST, ALT, and alkaline phosphatase are parallel with the studies of Mori et al. (2000) and Shafi et al. (2015). The values of serum creatinine and serum urea showed a significant difference in the results of the current study. Creatine exists mainly in vertebrates, where it participates in metabolic actions within cells before being catabolized to creatinine in the muscle and eliminated by the kidneys (Balsom et al., 1995). The results of creatinine and urea in the present study are opposite to the comparative studies in birds using nimesulide and diclofenac sodium by Reddy et al. (2006) and in line with Shafi et al. (2015).

The kidney sections of black kites (*M. migrans*) treated by a low dose of nimesulide (2 mg/kg body weight of the bird on the respective day) showed apoptosis, severe inflammatory cells infiltrated in the glomerulus, hemorrhage was visible, and Bowman's space is prominent. The microscopic image of a black kite's (*M. migrans*) kidney treated by a medium dose of nimesulide (4 mg/kg body weight of the bird on the respective day of the test) exhibited necrosis of the glomerulus cell and some inflammatory cells infiltrated in the glomerulus and Bowman's capsule. The histological image of a black kite's (*M. migrans*) kidney treated by a high dose of nimesulide (6 mg/kg body weight of the bird on each test day) showed the architecture of the glomerulus and Bowman's capsule space was not prominent, severe inflammatory infiltration occurred in the glomerulus and Bowman's capsule, necrosis occurred, and apoptosis occurred in the current investigation. Birds faced histo-toxicity in the functional structure (nephron) of the kidney as a side effect of certain NSAIDs (diclofenac, carprofen, flunixin, ibuprofen, and phenylbutazone; nimesulide) (Brater et al., 2001; Meteyer et al., 2005). The same results were presented by researchers studying NSAIDs and birds (Harris, 2006; Swan et al., 2006; Cuthbert et al., 2007; Naidoo et al., 2007; Hussain et al., 2008; Zollinger et al., 2011; Sinclair et al., 2012; Zorrilla et al., 2015).

The tissue section of the liver of black kites (*M. migrans*) intoxicated with nimesulide at doses of 2 mg/kg and 4 mg/kg body weight of the bird on the respective days of the experiment, showed the apoptosis, necrosis of hepatocytes, as well as some inflammatory cells infiltrating the hepatocytes. The histological image of the liver of black kites (*M. migrans*) treated by nimesulide (6 mg/kg body weight of the bird on the respective day of experiment) showed hepatocyte disarray, sinusoidal enlargement, apoptosis, and necrosis at the high dose of nimesulide in the current study.

The birds intoxicated with nimesulide (2, 4, and 6 mg/kg body weight of the bird on each test day) showed fibrosis on skeletal muscle, hypertrophy of skeletal muscles, prominent nuclei, atrophy of skeletal muscle fibres, and loss of striations, and the hearts of the intoxicated birds revealed apoptosis of myofibril cells, infiltration, and hyperplasia of myofibril cells. The above-mentioned symptoms were common in all three experimental groups of birds, but the severity of toxicity was related to the dose of nimesulide. The current study found that the histo-toxicity of the studied organs (kidney, liver, heart, and skeletal muscles) of *M. migrans* was related to the dose of nimesulide for 10 days. The histo-toxicity in different organs in the current study is in accordance with previous reports (Akter and Sarker, 2015; Saran et al., 2016; Nambirajan et al., 2021; Galligan et al., 2022). Since there are large differences in the toxicity of NSAIDs among different species of birds (Cuthbert et al., 2006), the safety of nimesulide for domestic fowl need not be designated for *M. migrans*.

5. Conclusion

Grounded on the results of the study, it can be concluded that the use of nimesulide at the dosage used in *M. migrans* caused some abnormalities in the physiology of the vital organs (kidney, liver, and heart) of black kites (*M. migrans*). The biochemical results of this study regarding serum urea and creatinine showed that nimesulide at doses of 2, 4, and 6 mg/kg body weight of birds on the respective day of the experiment for 10 days is significant. Urea and creatinine are waste products produced during protein metabolism, and both of these waste products are carried to the kidney for filtration. The measured values of urea and creatinine are indicators of kidney function. If the kidney is not functioning properly, the levels of urea and creatinine in the blood can increase, leading to health problems. Therefore, the levels of urea and creatinine are indicative of this study detecting kidney disease in nimesulide-treated birds. However, it is important to note that further studies may be needed to investigate the long-term effects of different doses and administration methods of the medication, as well as potential interactions with other medications or medical conditions. It is also crucial to consider individual differences in response to the medication, such as age, gender, and genetic factors.

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