https://doi.org/10.52973/rcfcv-e34428 Revista Científica, FCV-LUZ / Vol. XXXIV, rcfcv-e34428

Effect of Meloxicam and Flunixin Meglumine on some Kidney parameters in Geriatric male rats

Efecto del meloxicam y el Flunixin meglumina sobre algunos parámetros renales en ratas macho geriátricas

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ABSTRACT

Advanced age, called geriatrics, negatively affects the aging-related physiological changes on bodysystems. non-steroidal anti-inflammatory drugs (NSAIDs) used in geriatric pets or humans have negative effects on the kidneys. For this purpose, , the effects of Meloxicam and Flunixin Meglumine, which are NSAIDs frequently used in Veterinary Medicine, on the liver and kidney structure and function in geriatric male rats were evaluated. Twenty-four male geriatric rats (30-36 month old) and twenty-four 3-month-old young male Wistar albino rats were used in the study. Six groups were created, with eight rats in each group: young control (YC), young Meloxicam (YM), young Flunixin Meglubine (YFM), geriatric control (GC), geriatric meloxicam (GM), geriatric Flunixin Meglubine (GFM). Control groups (YC and GC) received an intraperitoneal injection of saline using the same volume as in the othergroups. Meloxicam was administered at 5.8 mg·kg^{-1} to the YM and GM groups, and Flunixin Meglumine at 2.5 mg·kg^{-1} intraperitoneally to the YFM and GFM groups once a day for 5 days. Neutrophil Gelatinase-Associated Lipocalin (NGAL), Cystatin C (Cyc-c), Kidney Injury Molecule-1 (KIM-1), Interleukin-18 (IL-18), Urea, Creatinine (Crea), Albumin (Alb), and Total Protein (TP) levels, were determined in sera and urine samples. Serum NGAL, Cys-C, and KIM-1 levels in the GC group were found to be significantly higher than those in the YC group (P<0.05). Administration of both NSAIDs caused an increase in serum Cyc-c and NGAL levels in both young and geriatric rats (P<0.05). Since both Meloxicam and Flunixin Meglumine administration caused an increase in NGAL and Cys-c levels in young and geriatric rats, adjusting the drug dose and frequency of administration by evaluating the pretreatment renal function should be considered as a preventive measure.

Key words: Geriatric rat; Meloxicam; Flunixin Meglumine; KIM-1; NGAL; CYS-C; kidney

RESUMEN

La edad avanzada, llamada geriatría, tiene efectos negativos sobre los cambios fisiológicos que se producen con el envejecimiento en los sistemas. Se sabe que los fármacos no esteroides utilizados en edades geriátricas tienen efectos negativos sobre los riñones. Para ello, en este estudio; se evaluaron los efectos del Meloxicam y el Flunixin Meglumina, fármacos no esteroides frecuentemente preferidos en Medicina Vveterinaria, sobre los riñones de ratas geriátricas. En el estudio se utilizaron 24 ratas geriátricas macho y 24 ratas albinas Wistar macho jóvenes de 3 meses de edad. Se crearon 6 grupos, con 8 ratas en cada grupo: control joven (YC), Meloxicam joven (YM), Flunixin Meglubina joven (YFM), control geriátrico (GC), Meloxicam geriátrico (GM) y Flunixino Meglubina geriátrico (GFM). No se adminsitro ningun farmaco a los grupo YC y GC. Se administraron 5,8 mg·kg^{-1} de meloxicam a los grupos YM y GM y 2,5 mg·kg^{-1} de flunixima meglubina por vía intraperitoneal a los grupos YFM y GFM durante 5 días. Lipocalina asociada a gelatinasa de neutrófilos (NGAL), cistatina C (Cyc-c), molécula de lesión renal-1 (KIM-1) e interleucina-18 (IL-18), urea, creatinina (Crea), albúmina (Alb) y proteína total (TP), que son marcadores de daño renal a partir de las muestras de suero y orina obtenidas, se midieron. Se encontró que los niveles séricos de NGAL, Cys-C y KIM-1 en el grupo GC fueronsignificativamente más altos que los del grupo YC (P<0,05). La administración de ambos fármacos provocó un aumento de los niveles séricos de Cyc-c y NGAL tanto en ratas jóvenes como geriátricas (P<0,05). Dado que tanto la administración de meloxicam como de flunixina meglubina provocan un aumento de los niveles de NGAL y Cys-c en ratas jóvenes y geriátricas, se puede considerar como medida preventiva ajustar la dosis del fármaco y el tiempo de aplicación mediante la evaluación de las funciones renales iniciales antes de iniciar la terapia.

Palabras clave: Rata geriátrica; Meloxicam; Flunixin Meglumina; KIM-1; NGAL; CYS-C; riñón

Received: 30/03/2024 Accepted: 12/06/2024 Published: 04/09/2024

INTRODUCTION

Aging refers to the natural and progressive life stages that continue through development, adulthood, and finally at old age. Although it is often misunderstood, aging is not a pathological process but involves normal changes over time that occur throughout the life of every living organism [1]. The World Health Organization (WHO) defines 49–59 years of age as middle age, 60–74 years of age as elderly, and 75 years of age and above as the oldest old [2]. Due to the wide variety of species and breeds of animals, there is no specific age that can be defined as geriatric. However, animals that reach 75% of their lifespan are defined as geriatric [2]. When the average human lifespan is calculated as 80 years and the rat lifespan is 3 years, 13.8 rat days are reported as 1 human year [3]. WHO considers people over the age of 60 to be elderly. In line with this equation, rats aged 27 months and above are considered elderly (geriatric) [4, 5].

With aging, several clinical and pathological changes occur in the kidneys, such as a decrease in kweight, sclerogenic changes in the glomeruli, infiltration of chronic inflammatory cells, thickening of the intracranial vascular intima, and cells and fibrosis in the stroma, and these changes lead to a decrease in renal functions as age progresses [5]. Elderly kidneys lose their capacity to retain sodium and water and concentrate urine due to exposure to harmful processes. The process of aging is linked to interstitial fibrosis and the development of glomerulosclerosis [6].

Some pet owners delay seeing their geriatric animal to the Veterinarian for treatment because they fear that the veterinarian may detect a serious illness and recommend euthanasia. In fact, many diseases in older animals can be treated. Even without treatment, many things can be done to improve the quality of life of animals [7].

Nonsteroidal anti-inflammatory drugs, used in both veterinary medicine and human medicine, are frequently used to control inflammation. NSAIDs hinder the production of prostaglandins by inhibiting cyclooxygenase, leading to advantageous outcomes such as decreased inflammation and discomfort [8]. Flunixin megluminine and Meloxicam are among the non-steroidal anti-inflammatory drugs. Meloxicam is a COX2-preferring NSAID, blocking both the anti-inflammatory and protective actions of COX1 and COX2. The suppression of prostaglandins by NSAIDs may result in reduced blood flow to the kidneys, decreased glomerular filtration rate (GFR), accumulation of nitrogenous waste products in the blood (azotemia), retention of salt and water, and the development of systemic hypertension [9, 10].

Nephrotoxicity is defined as rapid deterioration in kidney functions due to the toxic effects of drugs and chemicals. Some drugs can affect renal function in one or more ways [11]. Traditional markers of nephrotoxicity and renal dysfunction are serum urea and creatinine. These markers are not specific because they have low sensitivity in detecting early renal damage. Therefore, more sensitive and specific markers are needed to predict renal damage early. KIM-1 and Cys-C have been reported to be the main proteins reflecting renal glomerular and/or tubular damage during nephrotoxicity [12]. NSAIDs cause upregulation of KIM-1 due to ischemia-reperfusion injury [13]. NGAL binds to granulocytes. Therefore, it is associated with nephrotoxicity as it is responsible for inflammation during renal ischemia and renal damage [14]. In addition, interleukins such as IL-18 play important and integral roles in renal tubular injury and repair, thus are considered biomarkers of renal injury during drug-induced nephrotoxicity [15]. High IL-18 levels are associated with renal tubular atrophy and interstitial fibrosis. Additionally, elevated urinary IL-18 correlates with acute kidney injury and drug-induced nephrotoxicity [16]. These markers can be detected in both urine and blood to evaluate drug-induced nephrotoxicity. Therefore, measuring these markers can help predict kidney damage, providing an important picture of disease progression and clinical outcomes.

MATERIALS AND METHODS

This study received permission from Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee with the decision dated 06/02/2020 and numbered 2020/01. The study was carried out at Van Yüzüncü Yıl University Experimental Medicine Application and Research Center.

Wistar albino rats (*Rattus norvegicus*) were used in this study. Twenty-four geriatric rats (30–36 month old) and twenty-four 3-month-old male rats were used. They were divided into 6 groups, with 8 rats in each group. Meloxicam group was administered to rats at a dose of 5.8 mg·kg^{-1} [17] and flunixin meglumine was administered at 2.5 mg·kg^{-1} [18, 19].

- Group 1. (n: 8) Young Control group (YC); No medication was applied, only 0.3 ml physiological saline IP was applied for 5 days.
- Group 2 (n: 8) Geriatric control group (GC); No medication was applied, only 0.3 ml physiological saline IP was applied for 5 days.
- Group 3 (n: 8) Young Meloxicam group (YM); 5.8 mg·kg^{-1} Meloxicam IP was administered for 5 days
- Group 4 (n: 8) Geriatric Meloxicam group (GM); 5.8 mg·kg^{-1} Meloxicam IP was administered for 5 days
- Group 5 (n: 8) Young Flunixin meglubine group (YFM); 2.5 mg·kg^{-1} Flunixin meglubin IP was administered for 5 days
- Group 6 (n: 8) Geriatric Flunixin meglubin group (GFM); 2.5 mg·kg^{-1} Flunixin meglubin IP was administered for 5 days

After the last drug administration, the rats were placed in metabolic cages (Tecniplast®) and 24-h urine samples were collected. After urine samples were taken, the rats were euthanized using xylazine 10 mg·kg^{-1} IP (2% Rompun® Bayer) and Ketamine (HCl) (10% Alfamine® Atafen) 75 mg·kg^{-1} IP injectable anesthetics, and the animals were sacrificed by high volume blood collection under anesthesia. The blood taken was centrifuged (Nuve, NF 800R, Turkey) at 4000 G for 10 min and the serum was separated. Serum and urine samples were stored (Dt, Fydl-268, Turkey) at -40 °C until the day of study. Kidney damage markers NGAL (Catalog No: E0762Ra, BT LAB), KIM-1 (Catalog No: E0549Ra, BT LAB), Cyc-c (Catalog No: E0145Ra, BT LAB) and IL-18 (Catalog No. E0117Ra, BT LAB) were obtained from serum and urine samples levels were determined with species-specific ELISA kits. Additionally, urea, cretainine, albumin and total protein levels were measured from blood and urine samples (Abott, Architect ci16200, Germany).

Histopathological Analysis

Liver and kidney tissue samples were fixed in 10% buffered formalin for 48–72 h and then trimmed and processed for routine histopathological examination. Tissue samples were embedded in paraffin for serial sectioning. Longitudinal 4–5 μm sections were stained with hematoxylin and eosin (HE) and examined under a light microscope (Leica DMRB, Germany); images were also taken via the attached camera (Basler Ace, Germany)

The data were expressed as mean ± standard deviation (SD). The data were compared using one-way analysis of variance (ANOVA), followed by the TUKEY multiple comparison test in order to compare the differences in each treatment. The differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The physiological activities of the young rats were normal and no clinically adverse events were observed. In geriatric rats; fur shedding was observed, their physiological activities were relatively slower compared to young rats.

Serum Cys-C, KIM-1 and NGAL levels in the GC, GM and GFM groups were significantly higher compared to the YC, YM and YFM ($P<0.05$). Serum IL-18 levels did not show significant differences across the groups ($P>0.05$)(TABLE I). The Cys-C level was considerably greater in the GM group compared to the GC group ($P<0.05$) but comparable to the GFM group ($P>0.05$). Cystatin-C levels in the YM and YFM groups were considerably elevated compared to the YC group ($P<0.05$)(TABLE I). The blood Cys-C level in the GC group was substantially greater than that in both the YC group and the medication groups ($P<0.05$).

There was no significant difference between KIM-1 levels in the GC, GM, GFM groups ($P>0.05$). The serum KIM-1 level was found to be significantly lower in the YC group compared to the YM and YFM groups. ($P<0.05$).

Serum NGAL levels in the GC group were found to be significantly lower than the GM and GFM groups. Serum NGAL levels in the GM group were found to be significantly higher than those in the GFM group. Serum NGAL levels in the YC group were found to be significantly lower compared to the YM and YFM groups. Serum IL-18 levels of the GC, GM and GFM groups were no significant difference ($P>0.05$). There was no significant difference in IL-18 levels between young groups ($P<0.05$).

There was no significant difference between serum urea levels in all groups. Serum creatine levels in the GM and aged GFM were found to be significantly higher than all other groups ($P<0.05$).

Although the serum total protein level of the GM group was higher than all groups, there was a significant difference only in the YM group. The albumin level of the GFM group was found to be significantly higher compared to the YC, YM, YFM and GC groups.

Urinary Cys-C levels of YFM, GFM and GM were significantly higher than the YC group ($P<0.05$), although it was also higher than the GC group, this elevation was not significant ($P>0.05$)(TABLE II). Urinary KIM-1 levels of all geriatric groups were found to be significantly higher than the YC group. Urinary NGAL level in the GFM group was found to be significantly higher than the YC group ($P<0.05$)(TABLE II).

There was no significant difference between urine IL-18, Urea, creatinine and protein levels in all groups ($P>0.05$).

TABLE I
Serum Cyc-c, KIM-1, NGAL, IL-18, Urea, Crea, TP, Alb and glucose levels in Old and Young rats administered Meloxicam and Flunixin meglumine

Parameters	Young Control	Young Meloxicam	Young Flunixin meglumine	Geriatric control	Geriatric Meloxicam	Geriatric Flunixin meglumine
Cyc-c (ng·mL^{-1})	5.71 ± 0.46^{d}	7.02 ± 0.81^{c}	7.30 ± 0.67^{c}	8.52 ± 0.67^{b}	9.59 ± 1.24^{a}	9.34 ± 0.94 ^{ab}
KIM-1 (pg·mL^{-1})	1.90 ± 0.39^{c}	2.63 ± 0.46^{b}	2.58 ± 0.62^{b}	3.52 ± 0.61^{a}	3.71 ± 0.61^{a}	3.74 ± 0.55^{a}
NGAL(ng·mL^{-1})	13.72 ± 1.35^{e}	16.61 ± 1.74^{d}	17.18 ± 2.90^{d}	23.76 ± 3.45^{c}	32.60 ± 2.81^{a}	28.79 ± 3.51^{b}
IL-18 (pg·mL^{-1})	71.957 ± 6.80^{a}	74.71 ± 5.73^{a}	75.69 ± 7.50^{a}	78.00 ± 7.80^{a}	80.263 ± 7.78^{a}	79.17 ± 7.57^{a}
BUN (mg·dL^{-1})	35.49 ± 5.18^{a}	35.74 ± 6.11^{a}	36.07 ± 4.74^{a}	38.64 ± 5.73^{a}	39.92 ± 3.38^{a}	40.65 ± 3.32^{a}
Crea (mg·dL^{-1})	0.68 ± 0.25^{b}	0.68 ± 0.11^{b}	0.70 ± 0.12^{b}	0.78 ± 0.10^{ab}	0.90 ± 0.09^{a}	0.89 ± 0.11^{a}
TP (g·L^{-1})	56.78 ± 4.06^{ab}	54.85 ± 4.85^{b}	57.90 ± 5.48^{ab}	57.27 ± 5.97^{ab}	60.85 ± 5.05^{a}	59.90 ± 5.35^{ab}
Alb (g·L^{-1})	30.90 ± 1.99^{b}	30.78 ± 1.95^{b}	31.72 ± 3.60^{b}	31.41 ± 3.65^{ab}	33.55 ± 3.05^{ab}	36.10 ± 2.90^{a}

^{a,b}: Different letters in each row indicate statistical significance, $P<0.05$. Cys-c: Cystatin C. KIM-1: Kidney Injury Molecule-1. NGAL: Neutrophil Gelatinase-Associated Lipocalin. IL-18: Interleukin-18. BUN: Blood urea nitrogen. Crea: Creatinine. TP: Total Proteine. Alb: Albumine

TABLE II
Cyc-c, KIM-1, NGAL, IL-18, Urea, Crea and Protein levels in urine samples of old and young rats

Parameters	Young Control	Young Meloxicam	Young Flunixin meglumine	Geriatric control	Geriatric Meloxicam	Geriatric Flunixin meglumine
Cyc-c (ng·mL^{-1})	9.20 ± 2.41^{b}	12.01 ± 2.52^{ab}	13.11 ± 2.63^{a}	11.70 ± 2.38^{ab}	12.55 ± 3.35^{a}	13.87 ± 3.06^{a}
KIM-1 (pg·mL^{-1})	1.60 ± 0.40^{c}	2.07 ± 0.46^{bc}	2.69 ± 0.56^{ab}	2.88 ± 0.42^{a}	3.19 ± 0.58^{a}	2.99 ± 0.47^{a}
NGAL(ng·mL^{-1})	33.06 ± 5.58^{c}	39.86 ± 5.87^{abc}	43.86 ± 6.83^{ab}	33.71 ± 8.06^{bc}	40.52 ± 5.68^{abc}	45.31 ± 8.73^{c}
IL-18 (pg·mL^{-1})	54.93 ± 6.23^{a}	57.19 ± 4.69^{a}	57.52 ± 5.70^{a}	59.28 ± 5.93^{a}	61.00 ± 5.91^{a}	60.17 ± 5.75^{a}
BUN (mg·dL^{-1})	1198.23 ± 117.72^{a}	1219.31 ± 148.80^{a}	1212.70 ± 153.38^{a}	1275.28 ± 189.24^{a}	1317.48 ± 111.55^{a}	1341.69 ± 109.85^{a}
Crea (mg·dL^{-1})	32.73 ± 7.18^{a}	32.36 ± 4.88^{a}	33.10 ± 4.10^{a}	33.97 ± 3.52^{a}	38.34 ± 3.56^{a}	37.36 ± 3.99^{a}
Protein (mg·L^{-1})	99.82 ± 7.42^{a}	98.45 ± 6.83^{a}	104.99 ± 9.51^{a}	100.27 ± 10.13^{a}	107.86 ± 7.98^{a}	106.84 ± 11.19^{a}
Protein/Cre	3.212 ± 0.87^{a}	3.092 ± 0.46^{a}	3.202 ± 0.44^{a}	2.992 ± 0.50^{a}	2.832 ± 0.30^{a}	2.892 ± 0.46^{a}

^{a,b,c}: Different letters in each row indicate statistical significance, $P<0.05$. Cys-c: Cystatin C. KIM-1: Kidney Injury Molecule-1. NGAL: Neutrophil Gelatinase-Associated Lipocalin. IL-18: Interleukin-18. BUN: Blood urea nitrogen. Crea: Creatinine

Histopathological Examination

Liver

In the examination of tissue sections of YC (FIG. 1 A) and GC (FIG. 1 B) rats, the normal histological structure of the liver was observed. In the examination of liver sections of YM group (FIG. 1C) rats, a few necrotic hepatocytes were found in the parenchyma, but their appearance was similar to the YC. A significant increase in the number of necrotic hepatocytes in the liver parenchyma was detected in the GM group (FIG. 1D) rats. In the examination of liver sections of YFM (FIG. 1E) and GFM group (FIG. 1F) rats, the presence of necrotic hepatocytes in a density similar to the GM group was detected in both groups, while congestion was also present in the vena portae and sinusoids in the GM group rats. While pyknosis, karyorrhexis and karyolysis were observed in the nuclei of necrotic hepatocytes, their cytoplasms had an eosinophilic appearance.

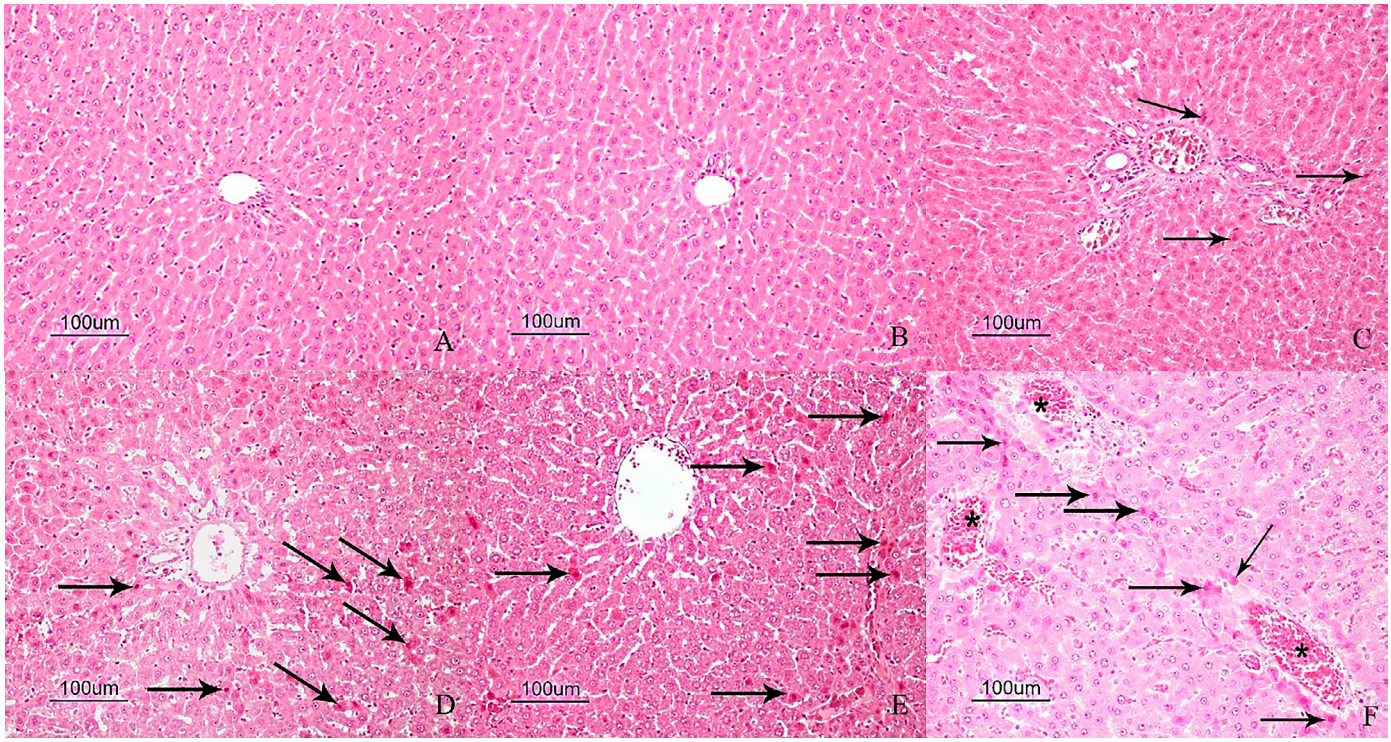

FIGURE 1. A) YC; The normal histological appearance of the liver is observed. B) GC; The normal histological appearance of the liver is observed. C) YM group; The presence of necrotic hepatocytes is observed in the parenchyma of the liver. D) GM group; The presence of necrotic hepatocytes is observed in the liver parenchyma (arrows). E) YFM group; The presence of necrotic hepatocytes is observed in the liver parenchyma (arrows). F) GFM group; The presence of necrotic hepatocytes (arrows) in the liver parenchyma and congestion (stars) in the portal veins are observed H & E

Kidney

In the examination of tissue sections of YC (FIG. 2A) and GC (FIG. 2B) rats, the normal histological structure of the kidney was observed. In the examination of kidney sections of YM group (FIG. 2C) rats, congestion was observed in the glomerular capillaries. Sclerosis was detected in some glomeruli in the kidney sections of GM group (FIG. 2D) rats. It was observed that these shrank and adhesion occurred as a result of the disappearance of Bowman's space. In the examination of kidney sections of YFM (FIG. 2E) and GFM group (FIG. 2F) rats, there were degeneration, necrosis and glomerular atrophy in the mesangial cells. In the glomeruli where these changes were observed, the glomerular ball and Bowman's space were enlarged. However, it was determined that these changes occurred more in the GF group rats.

Monitoring the medications used by the geriatric population is very important for their health and well-being. This population uses nonsteroidal anti-inflammatory drugs for many conditions. Even if these medications are taken correctly, they can be harmful due to the normal changes that come with aging. Despite the elderly population could mantain a normal kidney and liver function, they have lower drug metabolism and elimination rates than younger adults [20]. As people age, kidney function declines, regardless of disease. geriatric individuals are more susceptible to renal failure because of inherent physiological changes associated with aging [21]. It has been reported that the initial stages of chronic renal failure are more likely to occur in patients aged 65 and over who take NSAIDs for 2 months [20]. NSAID-related kidney damage mostly occurs due to reduced glomerular filtration rate and disrupted hemodynamics, resulting in nephron ischemia. Increased risk of injury is associated with high-dose or prolonged exposure to NSAIDs, volume depletion, renal vasoconstriction, and poor autoregulation. Therefore, geriatric individuals are more susceptible to NSAID-associated kidney damage [22]. NSAIDs can both reduce prostaglandin production and lead to deterioration of renal function under conditions where effective circulating volume is reduced, this rate being as high as 13% in patients living in geriatric care homes [23]. For this purpose, in

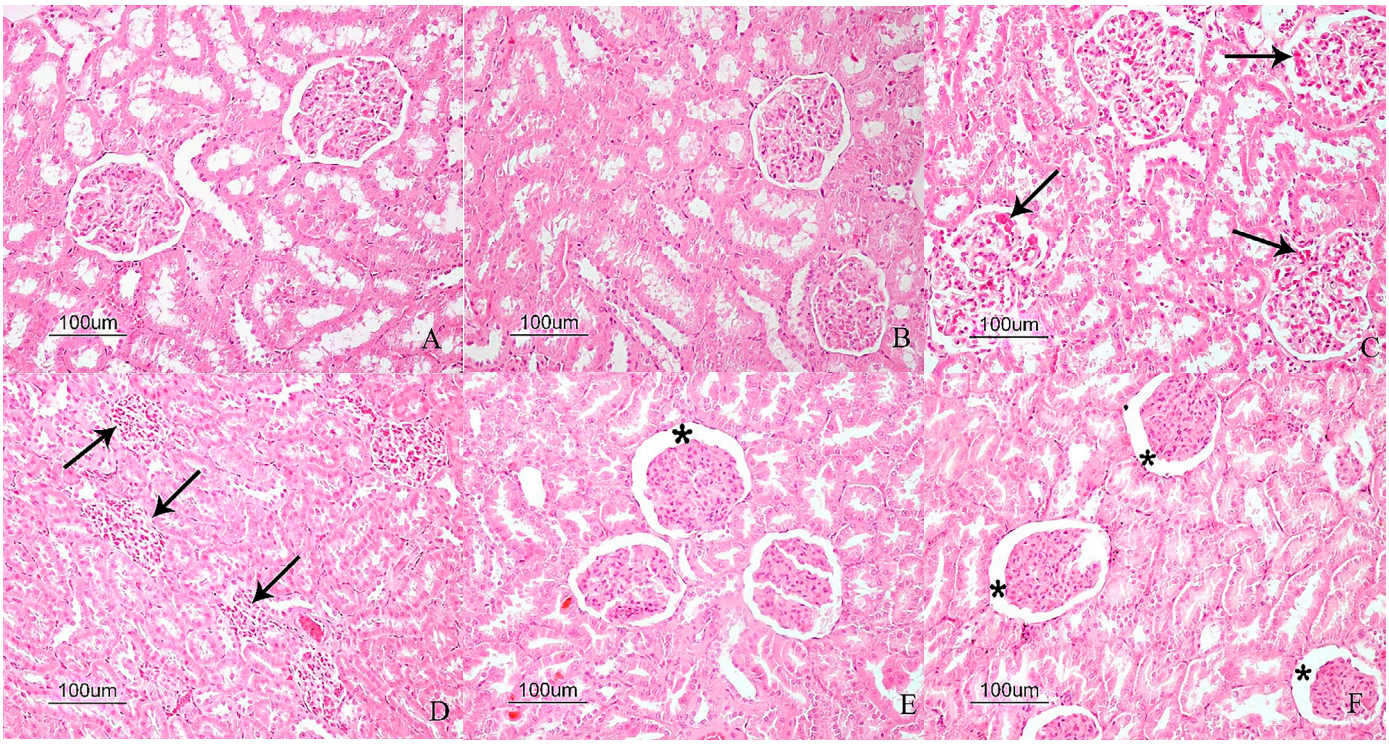

FIGURE 2. A) YC; The normal histological appearance of the kidney is observed. B) GC; The normal histological appearance of the kidney is observed. C) YM group; Congestion is observed in the glomerular capillaries. D) GM group; Glomerular sclerosis is observed (arrows). E) YFM group; Expansion of Bowman's space is observed (asterisk). F) GFM group; Expansion of Bowman's space is observed (stars) H & E

this study, we used the NSAIDs meloxicam and fluniski to reduce the levels of renal markers NGAL, KIM-1, CYS-c and IL-18 in aged rats. Serum Cyc-c, KIM-1, NGAL levels were found to be statistically significant in the comparison between the young people who were not administered medication and the elderly group who were not administered medication (TABLE I). This indicates that there are some changes in the kidney tissue with aging and that it loses its normal physiological structure.

The kidney typically maintains a consistent glomerular filtration rate by controlling intraglomerular pressure, mostly influenced by renal prostaglandins [24]. NSAIDs, which impact prostaglandins, may influence intra-glomerular pressure and result in glomerular dysfunction. Prostaglandin-metabolizing enzymes like COX-1 and COX-2 are mainly found in renal tissues. Both selective and non-selective COX inhibitors may cause severe kidney adverse effects, resulting in acute and chronic renal failure [24].

NGAL is a small protein found in the bloodstream that is significantly affected by many different diseases and is a valuable indicator of numerous health issues. NGAL is a top indicator of acute renal damage [25]. Plasma and urine levels of NGAL significantly increase in inflammation, ischemia, and nephrotoxic states [26]. NGAL has been proposed to have a crucial function in renal disorders [27]. NGAL levels rise quickly within hours after acute tubular necrosis and then decrease to baseline levels within days as injured tubules regenerate. NGAL is particularly sensitive in diagnosing early nephrotoxicity and acute kidney damage. Animal studies have shown that the genes that codify NGAL are highly upregulated in kidney damaged [28]. It has been reported that chronic NSAID exposure significantly increases urinary NGAL levels in adults with spondyarthritis. It has been stated that NGAL may be a determinant in the initial step of NSAID-mediated kidney damage. It has been reported that urinary NGAL levels continue to increase in patients taking multiple NSAID doses, but decrease when drug use is not repeated [29]. In the presented study, serum NGAL levels of old rats were found to be significantly higher than young rats ($P<0.05$) (TABLE I). Serum NGAL levels of geriatric rats treated with meloxicam were found to be significantly higher than both the geriatric control group and the aged flunixin meglumine administered groups. The urinary NGAL level of old rats treated with flunixin meglumine was found to be higher than all groups, but it was significantly higher only than the young control. Administration of NSAIDs meloxicam and flunixin meglumine to old rats may have caused kidney damage and increased serum NGAL levels.

Cys-C, an important member of the cysteine protease inhibitor superfamily, is a soluble, non-glycosylated secretory protein that is constantly produced by nucleated cells in the body and is widely found in body fluids [30]. Cys-C passes through the blood into the glomerulus and is fully reabsorbed and broken down in the proximal renal tubules. Tubular injury leads to impaired cystatin C reabsorption in the proximal tubule. Therefore, Cys-C is considered to have the potential to detect both glomerular and proximal kidney damage [31]. Additionally, Cys-c is not affected by muscle mass like creatinine and detects GFR changes more accurately in older people [22]. In the case of renal tubular damage, Cys-C reabsorption decreases, and its serum level increases in the early stages of damage. Serum Cys-C level may rise to high levels with decreased glomerular filtration rate and may, therefore, identify mild renal failure [32]. In the presented study, serum and urine Cys-C levels of rats administered meloxicam and flunixin meglumine were found to be significantly higher than

the young control group. Serum Cys-c levels of both control and drug-treated old rats were found to be significantly higher than young control and drug-treated young rats.

KIM-1 is a transmembrane proximal tubular protein and is expressed in response to kidney injury [33]. KIM-1 is thought to be one of the very specific and sensitive biomarkers whose urine levels increase during the day in experimental models of toxic molecules or ischemic kidney injury [34]. In a study conducted on elderly people aged 70-79 with preserved physical functions, no significant difference was found between urinary KIM-1 levels in NSAID users and non-users [22]. Serum KIM-1 levels in all old rat groups were found to be significantly higher than in all young rat groups. Although the serum KIM-1 level of old rats administered meloxicam and flunixin meglumine was higher than the old control group, this increase was not significant. Although the serum KIM-1 levels of young rats administered meloxicam and flunixin meglumine were higher than the young control, this increase was not significant. Drug administration in both old and young rats may increase serum KIM-1 levels by causing renal tubular damage.

Interleukin-18 (IL-18) is a proinflammatory cytokine produced from proximal tubular cells and has been proven to play an important role in acute kidney injury and is a mediator of tubular damage [35, 36]. It was found that kidney IL-18 content increased significantly in Potassium dichromate-induced acute kidney injury in rats [35]. IL-18 participates in the pathogenesis of many renal diseases such as renal ischemic reperfusion injury, allograft rejection, autoimmune disease, and obstructive uropathy [11]. In a study conducted in elderly people with preserved physical functions who used and did not use NSAIDs, it was found that the urinary IL-18 level in those who used NSAIDs was 10% lower compared to those who did not use it [22]. Although both serum and urine levels of IL-18 were high in the geriatric groups, this elevation was not significant.

Urea and creatinine serve as biochemical parameters of kidney damage, therefore, an increase in these parameters may indicate kidney disorders [37]. Keratin, a non-protein nitrogen compound, is produced in muscle during keratin-phosphocreatine metabolism and secreted by filtration from the glomeruli [38]. Like urea, creatinine secretion is affected by the glomerular filtration rate, as a result, any change that lowers the glomerular filtration rate (GFR) will cause the serum creatinine level to rise [39]. Serum urea and creatinine levels in rats treated with diclofenac, an NSAID, were significantly higher than the control group [40]. It has been stated that NSAIDs cause a decrease in GFR, and as a result, serum urea and keratin levels may increase [40]. In a study conducted on dogs, it was reported that serum urea and creatinine levels did not change when blood samples taken on the fifth and tenth days of meloxicam application were compared for 10 days [41]. In the presented study, serum urea and creatinine levels in the geriatric control group were higher than the young control and the young rats treated with the drugs, but this increase was not significant. Serum urea and creatinine levels of old rats administered meloxicam and flunixin meglumine were higher than the geriatric control but were not significant.

CONCLUSION

In humans or animals entering the geriatric period with aging, deteriorations occur in the normal physiological and histological structure of the kidney . Renal function should be monitored during the use of NSAIDs such as meloxicam and flunixin meglumine. It may be useful to monitor KIM-1, NGAL, Cyc-c and IL-18 levels in people taking type of drugs, especially in the elderly. If the level of these markers increases, it may indicate kidney damage, and the use of the drug may be re-evaluated. Therefore, during the use of meloxicam and flunixin meglumine, attention should be paid to the dose and duration of use to reduce the risk of kidney injury or damage.

ACKNOWLEDGEMENTS

This study was supported by Van Yüzüncü Yıl University Scientific Research Project Coordination Unit as project number TSA-2020-8947.

Conflict of interest

The authors declare no conflicts of interest.

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