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Mitigating effect of alpha lipoic acid against hematological and renal impairments induced by Flunixin meglumine in male albino rats

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ABSTRACT

This study investigated the hematological parameters and renal function effects of Flunixin Meglumine (FM) and assessed the potential protective role of Alpha lipoic acid (ALA) in male rats. Hematological studies, kidney function tests, oxidative stress and antioxidant capacities, kidney weights and index weights, and histopathological studies were evaluated on the 4th and 8th weeks of the experiment. The experiment involved administering FM for 14 successive days and ALA for 56 successive days. Seventy-two (72) male rats were randomly divided into six groups (n=12 animals each): Group 1 (control) received saline and distilled water, Group 2 (ALA) received ALA at a dose level 100mg/kg bwt orally, Group 3 (FM-2.5) received Flunixin meglumine at a dose level (2.5mg/kg bwt) subcutaneously, Group 4 (FM-5) received FM subcutaneously, Group 5 (FM-2.5 and ALA) received FM and ALA, and Group 6 (FM-5 and ALA) received FM and ALA. Flunixin meglumine increased WBCs, serum urea and creatinine levels, MDA, some histopathological changes in kidney tissue, and decreased TAC. These alterations were mitigated by addition of alpha lipoic acid. Non-significant changes in Hb (g/dl), PCV%, RBCs and kidney index weight among all treated and control groups. In conclusion, the administration of FM induced hematological and renal impairments in male rats. However, co-administration of ALA effectively mitigated these impairments, suggesting its potential protective role against FM-induced hematological and renal disturbances.

Introduction

Inflammation, while functioning as a defensive mechanism, can give rise to severe pathological conditions. Various agents have been employed for its treatment, but their success is often limited due to significant toxicity and side effects (e.g., glucocorticoids) or moderate therapeutic efficacy (e.g., penicillamine, gold). Non-steroidal anti-inflammatory drugs (NSAIDs), despite being the most commonly prescribed medications worldwide for their anti-inflammatory, antipyretic, anti-thrombotic, and analgesic properties, are primarily hindered by their undesirable side effects, particularly on the gastrointestinal (GI) tract (Kourounakis *et al*., 2000).

Non-steroidal anti-inflammatory drugs (NSAIDs) encompass a diverse group of compounds with common therapeutic actions and side effects. These drugs exhibit potent anti-inflammatory, analgesic, and antipyretic properties, making them widely utilized worldwide. However, NSAIDs are also associated with an increased risk of adverse effects on the gastrointestinal, renal, and cardiovascular systems (Bacchi *et al*., 2012).

NSAIDs pose a significant risk of kidney damage (Lucas *et al*., 2018) This risk encompasses various nephrological complications, including acute kidney injury (AKI) and chronic kidney disease (CKD). These complications manifest as electrolyte imbalances, glomerulonephritis, renal papillary necrosis, fluid retention-induced hypertension, renal tubular acidosis, hyponatremia, and hyperkalemia, among others (Bensman, 2020; Şener and Okşul, 2020). While high doses of NSAIDs have been associated with AKI, long-term use of NSAIDs significantly increases the risk of developing CKD. Notably, NSAIDs rank second only to aminoglycosides as the most common cause of nephrotoxicity and acute renal failure (Ejaz *et al*., 2004).

Flunixin meglumine (FM) is a commonly used NSAID in veterinary medicine. It is a derivative of nicotinic acid and possesses anti-inflammatory, analgesic, and antipyretic properties. FM works by inhibiting prostaglandin synthesis and is employed in the treatment of various animal diseases, such as fever, mastitis, endotoxemia, and lameness (Yazar *et al*., 2007).

Flunixin meglumine is utilized as adjunctive therapy in the management of inflammation, pain, and fever in cattle, sheep, goats, dogs, and horses, particularly in the treatment of sepsis (Hardie *et al*., 1987).

Oxidative stress induced by environmental xenobiotics can lead to an increase in free radical production, causing detrimental effects on various bodily functions (Henkler *et al*., 2010).

Alpha-lipoic acid (ALA), also known as thiotic acid, is a naturally occurring cofactor synthesized in the mitochondria of plants and animal tissues (Thaakur and Himabindhu, 2009). ALA has garnered attention for its protective effects against pathological conditions resulting from oxidative stress (Li *et al*., 2012; Wang *et al*., 2013). Alpha lipoic acid acts as a free radical scavenger and directly affects the recycling of other cellular antioxidants. ALA has demonstrated effectiveness in various oxidative stress models, including ischemia-reperfusion, diabetes, cataract formation, neurodegeneration, and radiation injury (Bilska and Wlodek, 2005; Bulmuş *et al*., 2013). The objective of this study was to examine the impact of administering flunixin meglumine, either alone or in combination with alpha lipoic acid as a protective agent, on hematological parameters,

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renal function levels, oxidative status, and histopathological changes.

Materials and methods

Ethical approval

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of ICLAS-2015. All procedures and experiments were accepted by the local ethics committee of animal use from the Faculty of Veterinary Medicine, Alexandria University-Institutional Animal Care and Use Committee (AU-IACUC).

Animals and experimental design

A total of 72 male rats (Rattus norvegicus) with an average body weight of)180±20 g) and approximately 150 days old were obtained from the Medical Research Institute, Alexandria University, Egypt, for utilization in this study. The rats were individually housed in plastic cages under controlled environmental conditions, maintained at a temperature of 23.0±2.0°C, relative humidity of 55%, and subjected to a 12-hour light/dark cycle. Standard laboratory food and water were provided ad-libitum throughout the experiment. Prior to the commencement of the study, a two-week acclimation period was implemented, during which the rats were kept under consistent hygienic and environmental conditions. These conditions were maintained throughout the entire experimental period. The rats were divided into six-matched groups, with 12 rats in each group, as follows: Group 1 (control) received subcutaneous administration of saline at a dose of 2 ml/kg body weight daily for 14 successive days, along with oral administration of distilled water (2 ml/kg body weight) by stomach tube once daily for 56 successive days, serving as a vehicle for alpha lipoic acid (ALA). Group 2 (ALA) received oral administration of ALA at a dose of 100 mg/kg body weight (Pari & Murugavel, 2004; Khalaf *et al*., 2017) orally by stomach tube once daily for 56 successive days, concomitant with subcutaneous administration of saline (2 ml/kg body weight) daily for 14 successive days. Group 3 (FM-2.5) rats were subcutaneously injected with Flunixin meglumine (obtained from Shering-Plough Animal Health Co, USA) at a dose of 2.5 mg/kg body weight once daily for 14 successive days according to Erpek *et al.* (2006); Tubbs *et al*. (2011), along with oral administration of saline (2 ml/kg body weight) once daily for 56 successive days. Group 4 (FM-5) rats were subcutaneously injected with Flunixin meglumine at a dose of 5 mg/kg body weight (Paksoy and KIRBAŞ, 2017) once daily for 14 successive days, concomitant with oral administration of saline (2 ml/kg body weight) once daily for 56 successive days. In Group 5 (FM-2.5 and ALA), rats were subjected to subcutaneous administration of Flunixin meglumine as in Group 3. Simultaneously, they received alpha lipoic acid (ALA) as in Group 2. Finally, in Group 6 (FM-5 and ALA), rats received subcutaneous injections of Flunixin meglumine as in Group 4. Simultaneously, they were orally administered alpha lipoic acid (ALA) as Group 2.

Collection of blood

After 4 and 8 weeks of the experiment, rats were individually weighed and sacrificed. Two blood samples were collected from each animal. The first blood sample was collected on EDTA for hematological studies. The second blood sample was collected without anticoagulant in clean centrifuge tubes, left for 2 hours at room temperature in slope position to clot and then centrifuged at 3000 r.p.m for 15 minutes to separate serum. Clear serum samples were stored at -20°C until used for kidney function analysis.

Tissue sampling

After macroscopical examination, kidneys were dissected out from each rat and weighed. The dissected organs index weight was calculated as I.W.= [organ weight (g) ÷ body weight (g)]×100 (Matousek, 1969). After that, kidneys were made into two parts, one part from each rat of all groups was snap frozen by liquid nitrogen and stored at −80°C for biochemical analysis of oxidative stress and the other parts were stored in 10% formalin/saline for histopathological examination.

Hematological studies

The hematological parameters were measured automatically by using Mindray® BC-2800 Auto Hematology Analyzer with three-part differentiation of WBC according to the method obtained by Buttarello and Plebani (2008).

Biochemical analysis

Serum urea and creatinine levels were measured in serum. The measurements were conducted following the instructions provided by the manufacturer of the respective assay kits that were purchased from Biodiagnostic chemical Co., Egypt.

Oxidative stress and antioxidants assays

Oxidative stress and antioxidant activity in kidney tissues were assessed by measuring malondialdehyde (MDA) levels and total antioxidant capacity (TAC). Tissue samples were homogenized in chilled Tris-HCl buffer (pH 7.4) and subsequently centrifuged at 12,000 × g for 30 minutes at 4°C. The resulting supernatant was spectrophotometrically analyzed using diagnostic kits obtained from Biodiagnostic Co., Egypt. The content of lipid peroxidation (MDA) in renal tissues was determined by reacting the samples with thiobarbituric acid in an acidic medium. The reaction was carried out at a temperature of 95°C for 30 minutes to form a thiobarbituric acid-reactive product. The absorbance of the resulting pink color was measured at 532 nm. Lipid peroxidation was quantified as nmol MDA/gm wet tissue using the extension coefficient of MDA (32.54), following the method described by Ohkawa *et al.* (1979). Total antioxidant capacity in the renal tissues was measured spectrophotometrically, based on the method described by Koracevic *et al.* (2001). The method involved the reaction of antioxidants in the tissue samples with a fixed amount of externally supplied hydrogen peroxide (H_2O_2). The antioxidants in the samples eliminated a specific quantity of the provided hydrogen peroxide. The remaining H_2O_2 was determined colorimetrically through an enzymatic reaction that converted 3,5-dichloro-2-hydroxybenzensulphonate to a colored product.

Histopathological studies

Small fresh specimen from kidneys were collected and rapidly fixed in formalin solution 10%. The fixed specimens were processed using conventional paraffin- embedding techniques. Paraffin blocks were cut by microtome into 5 microns thick sections and stain with Haematoxylin and Eosin (H&E) for light microscopic examination according to the method described by Harries (1989).

Statistical analysis

The obtained data were statistically analyzed by using one-way analysis of variance using SPSS, 25. Means were compared by Tukey's test was performed to evaluate variations between groups when a significant difference was detected. The significant effect was set up at ($P<0.05$). Data were presented as mean±standard errors (SE) (Steel and Torrie, 1980).

Results

Hematological findings

Our study investigated the effects of Flunixin meglumine (FM) and Alpha lipoic acid (ALA) on hematological parameters in male rats. On the 4th and 8th weeks of the experiment, Flunixin meglumine at a dose level of 2.5 and 5 mg/kg bwt significantly increased the WBCs count in comparison to the control group. The therapeutic dose of FM significantly raised WBCs count, albeit not to the same extent as FM5. In contrast, the level was lower in the FM-2.5 + ALA and FM-5 + ALA combination groups than it was in the treatment groups. Additionally, compared to the control group, on the 8th week, WBCs count increased significantly in FM-5 treated group (Fig. 1). On the other hand, the level was lower in the FM-5 + ALA combination groups than it was in the FM-5 treatment group. There were non-significant alterations observed in PCV% (Fig. 2), Hb (g/dl) and RBCs (Table 1) count on both the 4th and 8th weeks of the experiment.

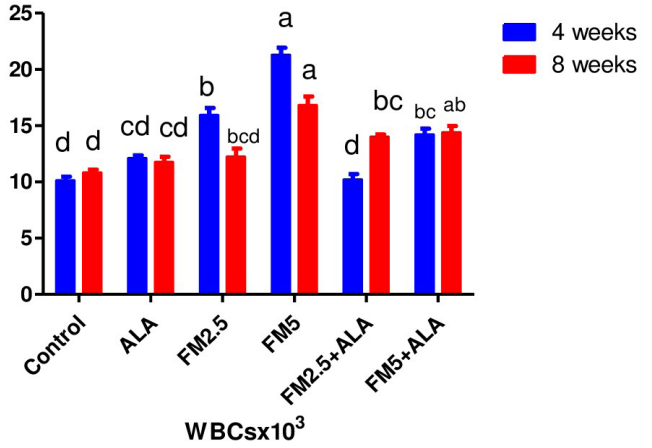

Fig. 1. Effect of Flunixin meglumine (FM) and alpha lipoic acid (ALA) on WBCs count on the 4th and 8th weeks of the experiment. Values are expressed as the means±S.E.M. (n = 6).

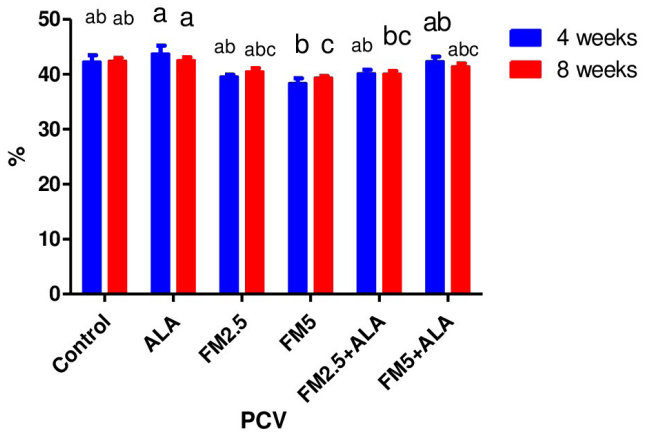

Fig. 2. Effect of Flunixin meglumine (FM) and alpha lipoic acid (ALA) on PCV% on the 4th and 8th weeks of the experiment. Values are expressed as the means±S.E.M. (n = 6).

Biochemical findings

Our study found a significant increase in serum urea levels in Flunixin meglumine treated groups at both dose levels on the 4th and 8th weeks. The therapeutic dose of FM (FM-2.5) significantly raised serum urea level, albeit not to the same extent as FM-5 (Fig. 2). There were no significant changes in serum creatinine levels among all the treated groups compared to the control group on the 4th week. However, on the 8th week, significant increases in serum creatinine levels were observed in rats treated with FM at doses of 5 mg/kg bwt and 2.5 mg/kg bwt. In contrast, the level of serum urea and creatinine were lower in the FM-2.5 + ALA and FM-5 + ALA combination groups than it was in the treatment groups (Fig. 4).

Tissue oxidative stress and total antioxidant capacity level

The obtained results demonstrated that high-dose FM (5 mg/kg bwt) significantly increased MDA levels compared to the control group on both the 4th and 8th weeks. However, the combination of FM-5 with ALA showed lower MDA levels than FM-5 treatment alone. Similarly, the

Table 1. Effect of Flunixin meglumine (FM) and alpha lipoic acid (ALA) on Hb (g/dl) and RBCs (×10⁶) on the 4th and 8th weeks of the experiment.

Parameter	Period	Control	ALA	FM 2.5	FM 5	FM2.5+ALA	FM5+ALA
Hb (g/dl)	4 Wk.	14.08±0.41^{ab}	14.58±0.50^{a}	13.19±0.12^{ab}	12.58±0.42^{b}	13.23±0.29^{ab}	14.12±0.29^{ab}
	8 Wk.	14.23±0.18^{a}	14.41±0.36^{a}	13.82±0.27^{a}	13.36±0.19^{a}	13.33±0.21^{a}	13.85±0.46^{a}
RBCs×10⁶/µl	4 Wk.	4.69±0.14^{ab}	4.85±0.17^{a}	4.39±0.04^{ab}	4.16±0.16^{b}	4.42±0.10^{ab}	4.70±.09^{a}
	8 Wk.	4.26±0.07^{a}	4.29±0.08^{a}	4.31±0.12^{a}	4.14±0.07^{a}	4.02±0.03^{a}	4.23±0.18^{a}

*Values are expressed as mean±S.E., *Means carrying different letters within the same raw are significantly different (p≤0.05).
ALA: Alpha lipoic acid; FM2.5: Flunixin meglumine treated group at a dose level 2.5 mg/kg bwt; FM5: Flunixin meglumine treated group at a dose level 5 mg/kg bwt.; FM2.5+ALA: Flunixin meglumine treated group at a dose level 2.5 mg/kg bwt. In combination with alpha lipoic acid; FM5+ALA: Flunixin meglumine treated group at a dose level 5 mg/kg bwt. In combination with alpha lipoic acid.

Table 2. Effect of Flunixin meglumine (FM) and alpha lipoic acid (ALA) on kidney weight and index weight (g/100 g. Bwt.) on the 4th and 8th weeks of the experiment.

Parameter	Period	Control	ALA	FM 2.5	FM 5	FM2.5+ALA	FM5+ALA
Kidney weight (g)	4 Wk.	1.20±0.03^{a}	1.28±0.07^{a}	1.26±0.06^{a}	1.36±0.02^{a}	1.36±0.12^{a}	1.28±0.03^{a}
	8 Wk.	1.26±0.05^{a}	1.30±0.03^{a}	1.3200±0.04^{a}	1.3400±0.07^{a}	1.32±0.07^{a}	1.36±0.06^{a}
Kidney index weight (g/100 g. Bwt.)	4 Wk.	0.56±0.02^{a}	0.57±0.03^{a}	0.54±0.03^{a}	0.56±0.02^{a}	0.58±0.04^{a}	0.59±0.01^{a}
	8 Wk.	0.53±0.01^{a}	0.54±0.01^{a}	0.51±0.02^{a}	0.56±0.02^{a}	0.54±0.02^{a}	0.59±0.04^{a}

*Values are expressed as mean±S.E., *Means carrying different letters within the same raw are significantly different (p≤0.05).
ALA: Alpha lipoic acid; FM2.5: Flunixin meglumine treated group at a dose level 2.5 mg/kg bwt; FM5: Flunixin meglumine treated group at a dose level 5 mg/kg bwt.; FM2.5+ALA: Flunixin meglumine treated group at a dose level 2.5 mg/kg bwt. In combination with alpha lipoic acid; FM5+ALA: Flunixin meglumine treated group at a dose level 5 mg/kg bwt. In combination with alpha lipoic acid.

therapeutic dose of FM resulted in elevated MDA levels compared to the control group, although not to the same extent as FM-5 (Fig. 5). In contrast, the FM-2.5 + ALA combination group exhibited lower MDA levels than the FM-2.5 treatment group. Moreover, FM treatment led to significant decreases in renal TAC levels, while ALA treatment alone significantly increased TAC levels. The combined treatment of FM with ALA showed intermediate effects on TAC levels (Fig. 6).

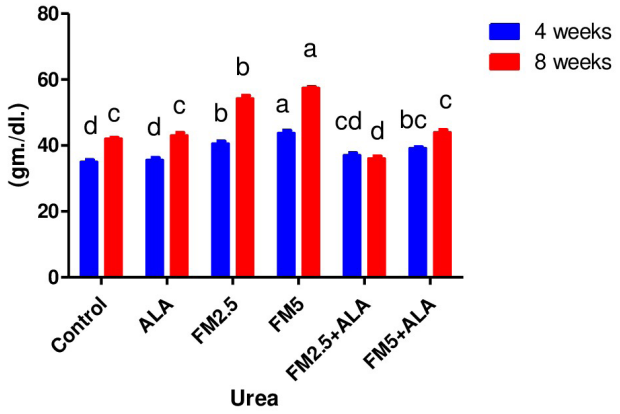

Fig. 3. Effect of Flunixin meglumine (FM) and alpha lipoic acid (ALA) on serum urea level on the 4th and 8th weeks of the experiment. Values are expressed as the means±S.E.M. (n = 6).

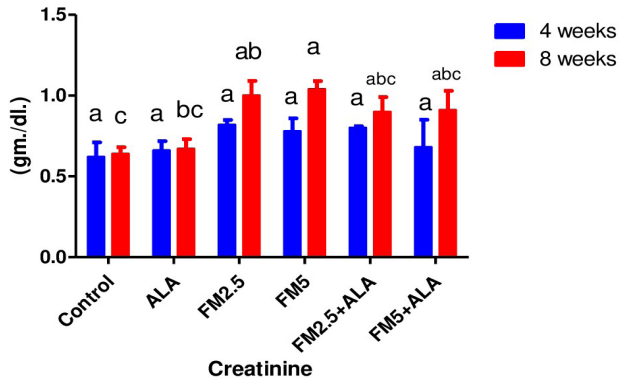

Fig. 4. Effect of Flunixin meglumine (FM) and alpha lipoic acid (ALA) on serum creatinine level on the 4th and 8th weeks of the experiment. Values are expressed as the means±S.E.M. (n = 6).

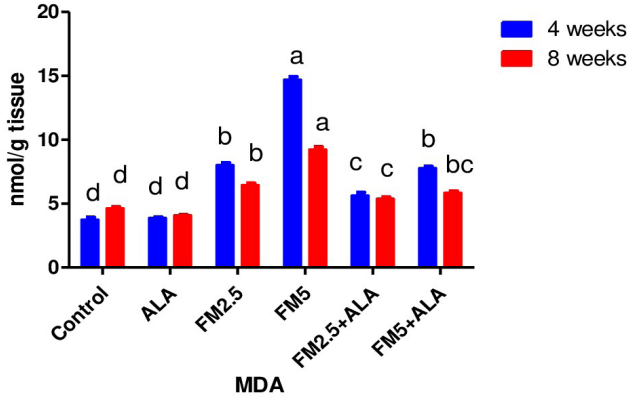

Fig. 5. Effect of Flunixin meglumine (FM) and alpha lipoic acid (ALA) on renal MDA level on the 4th and 8th weeks of the experiment. Values are expressed as the means±S.E.M. (n = 6).

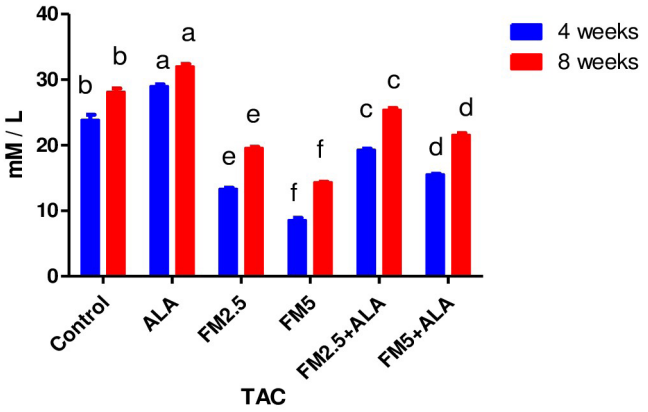

Fig. 6. Effect of Flunixin meglumine (FM) and alpha lipoic acid (ALA) on renal TAC level on the 4th and 8th weeks of the experiment. Values are expressed as the means±S.E.M. (n = 6).

Renal relative index weight

The results of our study revealed there were non-significant changes ($p>0.05$) in organ weight and index weight of kidney among all the treated groups compared to the control group on the 4th and 8th weeks of the experiment (Table 2).

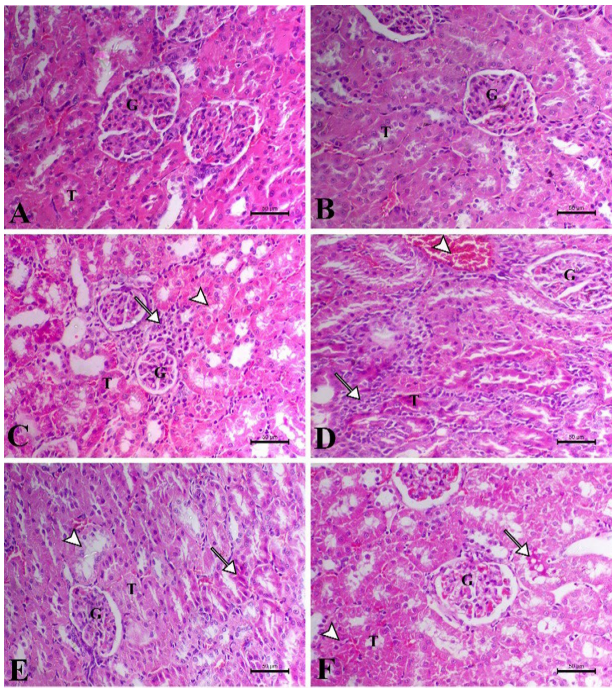

Fig.7. Photomicrographs of renal section of control and ALA groups on the 4th week of the experiment (A and B respectively) showing normal renal parenchyma (G indicates glomeruli) and (T indicates renal tubules). C. Flunixin meglumine treated group at a dose level of 2.5 mg/kg bwt on the 4th week showing marked eosinophilic degenerative changes (arrowed) associated with periglomerular and peritubular mononuclear inflammatory cells consisted of macrophages and lymphocytes (arrow). D. Flunixin meglumine treated group at a dose level of 5 mg/kg bwt on the 4th weeks of the experiment showing marked interstitial nephritis associated with tubular necrosis (T), congestion of renal capillaries (arrowhead) and marked initial inflammatory cells infiltration. E. Flunixin meglumine treated group at a dose level of 2.5 mg/kg bwt in combination with ALA on the 4th week of the experiment showing local eosinophilic degenerative change (arrow) and vacuolization of renal tubular epithelium (arrowhead). F. Flunixin meglumine treated group at a dose level of 5 mg/kg bwt in combination with ALA showing moderate degree of eosinophilic and vacuolar degenerative changes with presence of protein-rich filtrates within the lumen of the renal tubules. , H&E, X40, bar = 200 µm.

Discussion

In this study, we aimed to investigate the effects of Flunixin meglumine treatment at two different dose levels (2.5 mg/kg bwt and 5 mg/kg bwt) administered subcutaneously for 14 consecutive days. Additionally, we examined the combination of Flunixin meglumine with Alpha lipoic acid, which was administered orally at a dose of 100 mg/kg bwt using a stomach tube for 56 consecutive days, as a protective measure for the

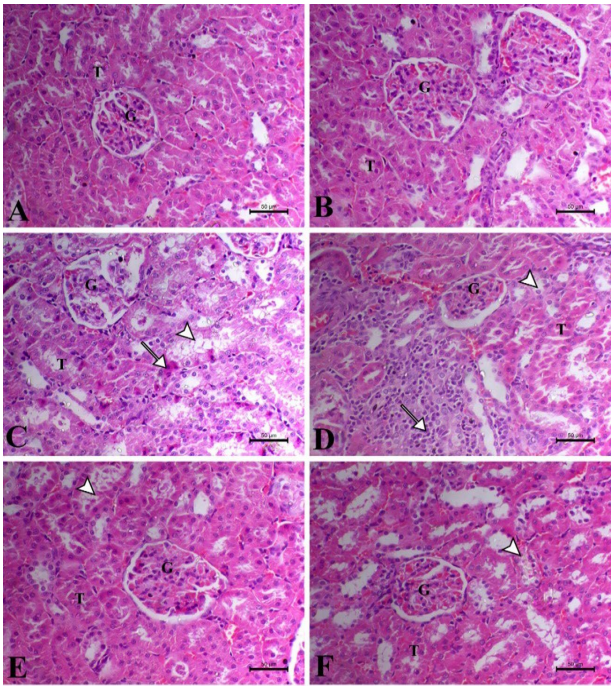

Fig. 8. Photomicrographs of renal section of control and ALA groups on the 8th week of the experiment (A and B respectively) showing normal renal parenchyma (G indicates glomeruli) and (T indicates renal tubules). C. Flunixin meglumine treated group at a dose level of 2.5 mg/kg bwt showing vacuolation and apoptosis of the renal tubular epithelium (arrowhead and arrow respectively). D. Flunixin meglumine treated group at a dose level of 5 mg/kg bwt showing interstitial nephritis associated with infiltration of inflammatory cells and regeneration of tubular epithelium. E. Flunixin meglumine treated group at a dose level of 2.5 mg/kg bwt in combination with ALA showing degeneration within the tubular epithelium and mostly within normal. F. Flunixin meglumine treated group at a dose level of 5 mg/kg bwt in combination with ALA showing mild vacuolation of renal tubular epithelium, H&E, X40, bar = 200 µm.

kidney and blood in male rats. Previous studies have demonstrated the adverse effects of Flunixin meglumine on the kidney function in various species (Luna *et al*., 2007; Mohammed *et al*., 2022). Furthermore, it was demonstrated by Sarvaiya *et al.* (2022) that flunixin meglumine had a slight impact on the hematological parameters and renal function.

Alpha lipoic acid (ALA) is a ubiquitous compound found in both eukaryotic and prokaryotic cells. It can be synthesized endogenously in human cells and serves as a natural cofactor for mitochondrial enzymes. ALA is present in a wide range of foods, with notable concentrations in spinach, yeast extract, kidney, and liver (Kenny *et al*., 2013). Known for its ability to scavenge free radicals, ALA is believed to possess beneficial properties. As a result, it has been employed in the prevention and treatment of conditions associated with oxidative stress (Byun *et al*., 2014) and/or inflammation-related pathologies (Shay *et al*., 2009).

The present study revealed that male rats treated with single daily administration of Flunixin Meglumine (FM) at two different dose levels (therapeutic dose of 2.5 mg/kg bwt and double therapeutic dose of 5 mg/kg b.wt. s/c. for 14 successive days) showed insignificant changes in hemoglobin (Hb) concentration, packed cell volume (PCV) values, and red blood cell (RBCs.) count on the 4th and 8th weeks of the experiment as compared to the control rats.

These findings are compatible with the results obtained by Mohammed *et al.* (2022) in sheep. They also reported insignificant differences in hematological parameters after administration of flunixin at a dose of 2.5 mg/kg b.wt. and silymarin at a dose of 200 mg/kg b.wt. for seven consecutive days.

Similarly, Bouasla *et al.* (2014) observed no significant effect of aluminium chloride on hematological parameters in rats treated with Alpha lipoic acid (ALA) at a dose of 35 mg/kg b.wt. orally once daily for three weeks. ALA supplementation protected against the significant decrease in RBC count, hemoglobin concentration, mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) induced by aluminium chloride. Additionally, ALA administration ameliorated the significant increase in white blood cell (WBC) count compared to the aluminium chloride-intoxicated untreated rats. Furthermore, our results were consistent with those obtained by Luna *et al.* (2007) in dogs. They found no changes in complete blood count (CBC) values and hemoglobin levels in dogs treated with flunixin meglumine at a dose of 1 mg/kg for three days, with four-day intervals. While, our findings are incompatible with those obtained by Carrick *et al.* (1989). They reported that all groups treated with daily intravenous administration of flunixin meglumine at dosages of 0.55, 1.1, 2.2, and 6.6 mg/kg for five days were studied in neonatal foals, and that on day six, the packed cell volume (PCV) decreased significantly ($p<0.05$) to its lowest value.

The obtained results showed that there was a significant ($p<0.05$) increase in W.B.Cs. count in rates treated with single daily administration of Flunixin Meglumine (FM) at a dose level of 5 mg/kg b.wt. s/c. for 14 successive days) on the 4th and 8th weeks of the experiment compared to the control group. The elevation in the overall count of white blood cells could be associated with the mechanism of action of flunixin. Flunixin functions by inhibiting cyclooxygenase, thereby reducing the synthesis of prostaglandins from arachidonic acid, the primary substrate. Consequently, the availability of arachidonic acid as a substrate for the lipooxygenase enzyme increases, leading to heightened production of leukotrienes. It is well-established that leukotrienes act as potent chemical attractants, stimulating the movement of white blood cells (Paino *et al*., 2005). These findings are compatible with those obtained by Mohammed *et al.* (2022), who reported a significant increase in W.B.Cs. count in groups treated with silymarin at a dose of 200 mg/kg b.wt. orally and flunixin at a dose of 2.5 mg/kg b.wt. intraperitoneally. Additionally, the group treated with both silymarin and flunixin concurrently exhibited a significant increase in WBC count compared to the control group.

The results of our study revealed statistically significant increases ($p<0.05$) in serum urea levels on the 4th and 8th weeks of the experiment, as well as a significant increase in serum creatinine levels on the 8th week, in rats treated with Flunixin meglumine (FM) at both dose levels (2.5 and 5 mg/kg b.wt.) compared to the control group. Notably, male rats treated with FM at a dose of 5 mg/kg b.wt. exhibited the most notable elevation in serum urea levels compared to the control group. Furthermore, our study showed that a photomicrograph of the kidney of treated groups with Flunixin meglumine marked eosinophilic degenerative changes associated with periglomerular and peritubular mononuclear inflammatory cells consisted of macrophages and lymphocytes and marked interstitial nephritis associated with tubular necrosis in flunixin meglumine treated groups. Flunixin meglumine is a non-selective blocker of both cyclooxygenase-1 (COX-1) and COX-2 enzymes, and it is approved for the treatment of various inflammatory and noninflammatory conditions in humans and animals (Jarolmasjed *et al*., 2015). The conversion of arachidonic acid into prostanoids, including prostaglandins (PGs), prostacyclines, and thromboxane, is catalyzed by COX enzymes. Prostanoids play crucial roles in controlling various functions in the gastrointestinal, cardiovascular, urogenital, and nervous systems, as well as immunity and inflammation (Kirkby *et al*., 2016). The functions of COX-1 involve the regulation of renal hemodynamics and glomerular filtration, while COX-2 is responsible for water and salt elimination (Weir, 2002). Creatinine, along with urea, serves as an indicator of renal activity, with the creatinine test being more sensitive than urea (Vasudevan & Sreekumari, 2007). Inhibition of COX-1 by Flunixin meglumine may result in decreased prostaglandin synthesis, leading to a decline in glomerular filtration rate and renal hemodynamics. This, in turn, can cause an increase in both urea and creatinine levels. Inhibition of COX due to nonsteroidal anti-inflammatory drug (NSAID) use can induce acute kidney injury and induce structural changes in the kidneys, including damage to the brush border, dilation of tubules, and the development of tip lesions, which are indicative of kidney damage (Mohammed *et al*., 2022). Flunixin, in particular, has been associated with the development of renal papillary necrosis in horses, renal tissue necrosis in dogs, and similar findings have been reported in quails and budgerigars (Ramzan *et al*., 2012). The occurrence of active metabolites is associated with the nephrotoxic effects of nonsteroidal anti-inflammatory drugs (NSAIDs). Through the participation of cytochrome P-450, these drugs generate electrophilic intermediate metabolites that form covalent bonds with cellular macromolecules, disrupting the normal functioning of mitochondria and the nucleus. This disruption subsequently triggers the production of reactive oxygen species (ROS) and leads to protein oxidation (Lee *et al*., 2020). In situations where the body's antioxidant mechanisms are unable to promptly eliminate ROS, oxidative stress arises (Salah, 2020). The destruction of mitochondrial membranes and the release of cytochrome C are attributed to ROS (Hassan & Asim, 2020). Imbalances between the cell's antioxidant capacity and the production of ROS can result in irreversible damage to cellular macromolecules, leading to cell death through processes such as apoptosis and necrosis (Mustafa & Al-Baggou, 2020). Our results were consistent with those obtained by (Mohammed *et al*., 2022), as they also observed significantly higher levels of creatinine and urea in the male rats treated with flunixin (2.5 mg/kg b.wt, i.p.) compared to the control and silymarin groups. However, Our results were inconsistent with those obtained by Luna *et al.* (2007), who found no significant changes in urea and creatinine values among dogs treated with flunixin meglumine (1 mg/kg for 3 days, with 4-day intervals) or between the treatment groups at each time. In our study, male rats treated with alpha-lipoic acid alone showed insignificant changes in serum urea and creatinine levels compared to the control group on the 4th and 8th week of the experiment. Interestingly, the administration of

alpha-lipoic acid orally in combination with subcutaneous administration of flunixin meglumine at both dose levels (2.5 and 5 mg/kg b.wt.) resulted in a significant decrease in serum urea and creatinine levels on the 4th and 8th week of the experiment, compared to the groups treated with flunixin meglumine alone. Our findings are in line with the results reported by Osfor *et al*. (2010), who investigated the effects of alpha-lipoic acid (ALA) on male albino rats intoxicated with copper and lead. They administered ALA orally at a dose of 40 mg/kg body weight and observed a reduction in serum urea and creatinine levels in rats exposed to lead and copper intoxication.

Antioxidants are molecules that interact with free radicals in a safe manner, effectively interrupting the chain reaction before essential molecules are harmed. Within the body, various enzymatic systems scavenge free radicals, as noted by Bhattacharjee and Sil (2006); Anbuselvam *et al*. (2007). Malondialdehyde (MDA), a toxic byproduct of lipid peroxidation caused by ROS, is highly indicative of oxidative stress. It not only contributes to degradation of unsaturated fatty acids in cell membranes but also signifies tissue damage (Pınar *et al*., 2017). It was found that there were significant increases ($p<0.05$) in renal malondialdehyde (MDA) levels and significant decreases in total antioxidant capacity on the 4th and 8th week of the experiment in rats treated with Flunixin meglumine (FM) at both dose levels (2.5 and 5 mg./ kg. b.wt.) compared to the control group. Notably, male rats treated with FM at a dose of 5 mg/kg b.wt. exhibited the most pronounced elevation in MDA levels as compared to the control group. Consistent with our findings, Yakan *et al*. (2018) reported a significant decrease in glutathione (GSH) and superoxide dismutase (SOD) levels in calves treated with Flunixin meglumine (administered intravenously at a dose of 2.2 mg/kg).

Similarly, AbdAl-Salam *et al*. (2021) found significant decreases in catalase, glutathione, and superoxide dismutase levels in pregnant sheep treated with flunixin meglumine (at a dose of 2.5 mg/kg subcutaneously for 6 successive days) compared to the control group at the 6th day of pregnancy.

In contrast, Konyalioglu *et al*. (2007) reported findings contradictory to our study. They observed that mice treated with lipopolysaccharide (LPS), which induces endotoxic shock, exhibited a significant increase in malondialdehyde (MDA) levels (a recognized marker of oxidative stress) in various tissues, including the heart, kidney, and spleen. The levels of SOD, GPX, CAT, and GSH were also altered. However, when Flunixin meglumine (administered subcutaneously at a dose of 2.5 mg/kg body weight) was administered simultaneously with LPS, it inhibited the increase in MDA levels induced by LPS in all tissues. Therefore, while LPS led to increased oxidative stress and MDA levels, Flunixin meglumine mitigated this effect.

The present study revealed that male rats treated with Alpha lipoic acid alone showed significant decrease in renal malondialdehyde (MDA) levels and significant increase in renal total antioxidant capacity as compared with the control group on the 4th and 8th of the experiment. The obtained results showed that oral administration of Alpha lipoic acid with subcutaneous administration of flunixin meglumine at both dose levels (2.5 and 5 mg./ kg. b.wt.) induced a significant decrease in renal malondialdehyde (MDA) levels and total antioxidant capacity on the 4th and 8th days of the experiment as compared with Flunixin meglumine alone treated groups. Alpha-lipoic acid (ALA) is recognized as a natural antioxidant that can reduce the risk of oxidative stress-related diseases. Its antioxidant properties allow it to scavenge reactive oxygen species (ROS) (Bast & Haenen, 2003). Additionally, ALA has the ability to regenerate endogenous antioxidants such as vitamin C, vitamin E, and intracellular glutathione (GSH) (Maczurek *et al*., 2008). ALA has been investigated as a potential therapeutic agent for mitigating heavy metal toxicity (Biewenga *et al*., 1997) and has been used as a therapeutic or protective agent in various liver-related conditions (Tanaka *et al*., 2015). Morsy *et al*. (2012) observed significant reductions in hepatic malondialdehyde (MDA) levels along with increased hepatic glutathione (GSH) levels in male Wistar rats treated with Alpha-lipoic acid (ALA) (30 mg/kg body weight) orally for one week before and concurrently with carbon tetrachloride (CCl4) injections, compared to the group receiving CCl4 alone. Our findings compatible with those of Pınar *et al*. (2018), who reported that the combination of methotrexate and ALA (at a dose of 100 mg/kg intraperitoneally) in rats resulted in lower levels of MDA, total oxidant status (TOS), and total antioxidant status (TAS), as well as increased activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), myeloperoxidase (MPO), and serum testosterone levels compared to the methotrexate-only group. Additionally, our results are consistent with those of Ceylanlı *et al*. (2022), who found that ALA treatment (at a dose of 100 mg/kg intraperitoneally once a day for one week) significantly reduced tumor necrosis factor-alpha (TNF-α) and MDA levels in both tissues and serum, while increasing reduced SOD and GPx activities in a murine model of 5-Fluorouracil-induced gastrointestinal mucositis. Furthermore, our findings compatible with the study conducted by El-Maddawy and Abd El Naby (2018), which revealed that ivermectin administration induced an increase in MDA content and a reduction in GSH and CAT values in the testicular tissue of male rats. However, concurrent administration of ALA (at a dose of 50 mg/kg body weight once daily/five times weekly for 8 weeks) with ivermectin provided complete protection against oxidative stress in the testicular tissue.

Conclusion

Administration of Flunixin Meglumine (FM) induces hematological and renal impairments in male rats. The elevation of white blood cells (WBCs), serum urea, creatinine levels, and malondialdehyde (MDA), along with the reduction in total antioxidant capacity (TAC), histopathological changes in the kidneys, indicates the detrimental effects of FM on hematological parameters and renal function. However, the co-administration of Alpha Lipoic Acid (ALA) effectively mitigates these impairments, suggesting its potential protective role against FM-induced hematological and renal disturbances. These findings highlight the therapeutic potential of ALA in ameliorating the hematological and renal toxicity associated with FM administration.

Conflict of interest

The authors declare that they have no conflict of interest.

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