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THE INFLUENCE OF KETOPROFEN USE ON THE ACTIVITY OF SUPEROXIDE DISMUTASE IN RAT'S LIVER

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ABSTRACT

Ketoprofen (K) is a popular nonsteroidal antiinflammatory drug (NSAID) used for postoperative and acute pain. Its' use can lead to side effects like bleeding from the gastrointestinal tract, hypertension, oedema, photodermatitis. It is metabolized in the liver and eliminated with urine.
The aim of the study was to measure the effect of K on blood morphology, biochemical blood parameters and effect on oxidative stress in rat's liver by measuring the activity of superoxide dismuthase (SOD).
The experiment was accepted by the Local Ethical Committee (decision No 70/2021). A group of 12 female rats were used. They were randomly divided into 2 groups of 6:
1.controls which received saline by gavage for 5 days
2.study group receiving K at the dose of 8 mg/kg b.w. by gavage for 5 consecutive days.
On day 6 all animals were decatpitated. Their blood and livers were obtained. Blood morphology, alanine transaminase (ALT) activity were measured. The neutrophil - lymphocytes ratio (NLR), platelet-lymphocytes ratio (PLR) and monocytes-lymphocytes ratio (MLR) were calculated. SOD activity was measured in the livers with an Elisa kit.
There was no significant differences among the groups in blood morphology (except for eosinophil and neutrophil count, which were significantly elevated in the group exposed to K), ALT nor SOD activity. Significant differences were recorded in NLR, PLR and MLR, which were higher in the group exposed to K. NLR, PLR and MLR are new prognostic parameters used in monitoring inflammatory, autoimmune diseases and some neoplasms.
Conclusions:
1. K does not produce oxidative stress in the liver and athe the therapeutic dose does not affect neither ALT nor SOD activity.
2. NLR, PLR and MLR are new interesting and cheap markers of inflammatory, autoimmune and neoplastic disorders.
Keywords: ketoprofen, oxidative stress, superoxide dismuthase, liver.

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INTRODUCTION

Ketoprofen (K) is a drug belonging to the class of nonsteroidal anti-inflammatory drugs (NSAIDs), which are the most commonly used medications in pharmacological management of pain. K is characterized by strong anti-inflammatory, antipyretic, analgesic, and antirheumatic effects. Due to its pharmacodynamic properties, K is widely used in medical practice for both acute pain and chronic pain therapy. It is a nonselective inhibitor of the cyclooxygenase (COX) enzyme, which is essential for the conversion of arachidonic acid into thromboxanes, prostaglandins, and prostacyclins. K inhibits both the constitutive isoform COX-1 and the inducible isoform COX-2. COX-1 plays an important role in physiological processes such as maintaining proper blood flow through the kidneys and platelet aggregation. It is responsible for the synthesis of prostaglandins that help maintain the integrity of the gastric mucosa. COX-2, on the other hand, is primarily involved in the development of inflammatory processes and is activated by inflammatory mediators. K also exhibits modulatory effects on the opioid system, as well as on serotonergic and noradrenergic pathways. These systems, when influenced by K, enhance the analgesic effect, which is particularly significant in the treatment of neuropathic pain. K stabilizes lysosomal membranes and reduces the release of collagenase and elastase, which are proteolytic enzymes that contribute to tissue destruction during inflammation. Additionally, K inhibits the activity of lipoxygenase (LOX), leading to a decrease in leukotriene synthesis. Leukotrienes have chemotactic properties, increase vascular permeability, and enhance leukocyte migration and activation [8,15,26].

Inflammation is a biological response of the immune system triggered by tissue damage, invasion of pathogens, cancer, autoimmune reactions, or exposure to ionizing radiation [2,6]. During the inflammatory response, symptoms such as fever, redness, swelling, pain, and loss of function of the affected tissue or organ occur. Capillary permeability increases, leading to the formation of edema. Bradykinin and histamine cause the dilation of small blood vessels, allowing increased blood flow to the injured tissue. This increased blood flow facilitates the delivery of more immune cells to the damaged area. Excessive production of prostaglandins, particularly PGE2, leads to various symptoms of inflammation, including fever, pain, and swelling [2,6].

The inflammatory response proceeds in two phases. The first is the proinflammatory phase, with tumor necrosis factor-alpha (TNF-α) acting as the primary mediator. Other proinflammatory mediators include cytokines, such as interleukins and interferons (IL-1β, IL-2, IL-6, IL-8, IL-15, and IFN-gamma). The second phase is the anti-inflammatory phase, in which the increased level of proinflammatory cytokines promotes the synthesis of anti-inflammatory cytokines. The most important of these include soluble TNF receptors, IL-1 antagonists, IL-4, IL-10, IL-13, and TNF-β. Markers of inflammation are considered to be elevated counts of white blood cells (WBC), c- reactive protein (CRP), TNF-α, IL-1, and IL-6 in blood tests. A controlled inflammatory response is considered beneficial in fighting infection, however, when poorly regulated, it can become dangerous, for example leading to septic shock [2,6].

Pain is characterized as an unpleasant sensory and emotional experience. It may be associated with actual or potential tissue damage. Pain can arise from the irritation of pain receptors (nociceptors) or a lowered threshold for their activation. It may also be caused by damage to the peripheral or central nervous system, in which case it is referred to as neuropathic pain. Psychogenic pain occurs when a person experiences pain despite the absence of any identifiable tissue damage [6,32].

Oxidative stress can be defined as an imbalance between the generation of reactive oxygen species (ROS) and the body's antioxidant defense capacity. Free radicals are molecules that contain one or more unpaired electrons on their outer electron shell. Approximately 90% of free radicals originate from the respiratory chain, while the remaining 10% are produced through physiological reactions. Their formation can also be influenced by ionizing radiation, ultraviolet radiation, ultrasound, elevated temperatures, and drug metabolism processes. Free radicals are highly reactive and strive to either capture an additional electron from another molecule or dispose of an excess electron. They play an essential role in numerous vital physiological processes, including gene expression, regulation of intracellular calcium levels, protein phosphorylation, activation of cell division related proteins, and elimination of pathogens from the body. The generation of oxygen in the form of ROS is a natural part of aerobic metabolism. However, an excess of free radicals leads to harmful effects. They can damage both structural and functional components of cells, disrupt homeostasis, and result in cell death through necrosis or apoptosis. ROS are capable of causing significant damage within the body and are associated with many pathologies, such as neurodegenerative diseases, cancer, atherosclerosis, hypertension, and diabetes [the most highly reactive free radicals include the hydroxyl radical (OH•) and the superoxide anion radical (O_2•−). The superoxide radical is primarily generated in the mitochondria during the electron transport in the respiratory chain. The likelihood of free radical formation increases in damaged or aging mitochondria. The O_2•− radical, under the influence of the enzyme superoxide dismutase (SOD), can be converted into hydrogen peroxide (H_2O_2), which itself is not a free radical. However, in the presence of reduced iron or copper cations, through the Fenton reaction, hydrogen peroxide can be transformed into the most reactive free radical- the hydroxyl radical.

The superoxide radical can react with nitric oxide (NO), forming peroxynitrite, which is not a free radical but is highly reactive and interacts with DNA, proteins, and lipids, causing their nitration or oxidation. Peroxynitrite can subsequently be converted into the dangerous hydroxyl radical. Phospholipase A2, under the influence of calcium ions and adenosine triphosphate (ATP), promotes the release of arachidonic acid from phospholipid membranes. Further transformations of arachidonic acid occur with the participation of COX and lipoxygenase, leading to the synthesis of hydroxyl and superoxide free radicals [12,14,23,24].

The antioxidant system serves a protective role in the body by defending it against free radicals. Redox homeostasis is essential for life, as redox processes are involved in nearly all fundamental biological functions, from bioenergetics to metabolism and vital cellular

activities. Excess ROS are eliminated by endogenous antioxidant defense mechanisms that protect tissues from oxidative damage. The balance between ROS production and antioxidant capacity is delicate, and any disruption of this balance contributes to the pathophysiology of numerous diseases. Antioxidant compounds inhibit oxidative processes by reacting with free radicals, other oxidation products, or oxidizing agents. Antioxidants can be classified into small molecule antioxidants (vitamin C, vitamin E, carotenoids, coenzyme Q, glutathione, and trace elements) and macromolecular antioxidants (SOD, catalase, glutathione peroxidase, and glutathione reductase). Small molecule antioxidants participate in nonspecific reactions aimed at inactivating free radicals. Vitamin C is considered the most important antioxidant in extracellular fluids. Macromolecular (enzymatic) antioxidants exhibit higher specificity and efficacy due to their ability to activate reactions that eliminate excess free radicals. Catalase (CAT) is an enzyme that can function both as a catalase and a peroxidase. Its catalase activity predominates in environments with high concentrations of H_2O_2, where it catalyzes the dismutation of hydrogen peroxide into molecular oxygen and water. Although this enzyme does not directly eliminate free radicals, by deactivating hydrogen peroxide it prevents its conversion into the highly reactive hydroxyl radical. Glutathione peroxidase (GPx) is an enzyme that catalyzes the reaction of hydroperoxides and hydrogen peroxide with reduced glutathione. The products of this reaction are oxidized glutathione and water. Through the activity of glutathione peroxidase, hydroperoxides are effectively reduced [10,14].

Superoxide dismutase (SOD) is a key enzyme essential for maintaining the redox potential of cells. SOD eliminates excess superoxide radicals by converting them into hydrogen peroxide and oxygen. SOD requires cofactors such as iron, manganese, or copper and zinc to exert maximal catalytic activity while metabolizing toxic products. SOD exists in both intracellular and extracellular forms. The intracellular form of SOD occurs as mitochondrial SOD, which has manganese as a cofactor (MnSOD), and as cytoplasmic SOD, with copper and zinc as cofactors (Cu/Zn SOD). There is also an iron based dismutase isoform (FeSOD), which is a homolog of MnSOD, isolated from E. coli cells and primarily found in prokaryotic organisms and some lower eukaryotic organisms. The extracellular form, EC-SOD, breaks down superoxide radicals on the external surfaces of cells and in extracellular spaces. EC-SOD is particularly active in select tissues, including blood vessels, the heart, lungs, kidneys, and the placenta. MnSOD is the first and most important line of defense against free radicals, while Cu/Zn SOD supports the defensive function of MnSOD in eliminating excess superoxide ions. SOD decomposes the superoxide anion radical into molecular oxygen and hydrogen peroxide [17,20,25,34].

Ketonal 50 mg/ml Injection Solution in Ampoules. The medicinal product contains 50 mg of K per milliliter. Each ampoule (2 ml) contains 100 mg of K. The use of K in the form of an injectable solution is indicated for the symptomatic treatment of rheumatic conditions of an inflammatory or degenerative nature, as well as for the relief of other types of pain syndromes. K solution for injection can be administered intramuscularly (*i.m.*) or intravenously (*i.v.*). *I.m.* administration is recommended for degenerative diseases, while *i.v.* administration is used for postoperative pain relief. The dosage for adults and children over the age of 15 varies depending on the route of administration. *i.v.* administration is only applicable in a hospital setting [26].

The most commonly reported adverse effects of K involve the gastrointestinal tract, including nausea, vomiting, and loss of appetite. There have also been reports of gastrointestinal discomfort and stomach pain. Elevated activity of liver enzymes aspartate aminotransferase and alanine aminotransferase (AST, ALT) have been observed, along with increased liver function test results reaching borderline values. Frequent side effects include malaise, fatigue, weakness, nervousness, nightmares, and depression. Regarding the circulatory system, frequent edema with a risk of hyperkalemia has been noted. Organic kidney damage has been observed, which may lead to acute renal failure. K overdose may result in symptoms such as headache, dizziness, vomiting, diarrhea, and gastrointestinal pain. The maximum daily dose of K should not exceed 200 mg [26].

AIM OF STUDY

The aim of this experimental study was to investigate the effect of K on peripheral blood morphology parameters, the activity of the liver enzyme alanine aminotransferase (ALT), and the activity of the antioxidant enzyme superoxide dismutase (SOD) in the livers of female rats.

MATERIAL AND METHODS

The study was conducted at the Experimental Medicine Centre at the Medical University of Lublin (OMD Lublin). Approval for the experiment was obtained from the Local Ethics Committee in Lublin, No. 70/2021, issued on November 8, 2021. Twelve healthy, young, nonpregnant female rats were used in the study. The animals were bred at OMD Lublin. At the start of the experiment, the rats weighed between 190 g and 250 g. The rat strain originated from Charles-River Laboratories (Cologne, Germany). The animals were 7 weeks old at the beginning of the experiment. The first 7 days involved handling technique, allowing the rats to become accustomed to the experimenter by being held.

The experiment was conducted in accordance with European regulations. The temperature during the experiment at OMD Lublin was maintained between 21°C and 22°C, with a constant relative humidity of 55-66%. Both temperature and humidity were continuously monitored in the animals' environment. The animals were exposed to 12 hours of light and 12 hours of darkness daily. Three rats were housed per cage. The animals had continuous access to drinking water and feed. The water was sterilized by UV irradiation, and the feed was provided by Altromin International (Lage, Germany).

K was administered in the form of an injectable solution in ampoules. The Ketonal medication was used at a concentration of 50 mg/ml (Sandoz GmbH, Vienna, Austria). The experimental group received K *via* a gastric tube at a dose of 8 mg/kg body weight for 5 days. The dose was calculated based on the therapeutic dose used in humans, adjusted for the body weight of the animals. The control group received a 0.9% sodium chloride solution via a gastric tube at a dose of 5 ml/kg body weight for 5 days.

After 5 days, the animals were euthanized by decapitation using a guillotine. Blood samples were collected from the rats and analyzed at the veterinary diagnostic laboratory VetDiagnostyka in Lublin. Liver samples were also collected for further studies.

SOD activity in the rats' livers was measured using Cloud-Clone kits (USA). The assay was performed using the ELISA method (Rat SOD ELISA Kit 96T) following the manufacturer's instructions.

A statistical analysis of the obtained results was conducted using the Student's t-test. Basic statistics, including the mean and standard deviation, were calculated. The analysis was performed using the Statistica software, with access provided by Medical University of Lublin. Based on the obtained data, the experimental group was compared to the control group to identify statistically significant differences. The results were considered statistically significant when $p<0.05$. The graphs were generated using GraphPad software, also accessible through UMLUB.

RESULTS

The results are shown in Tab.1.

Tab.1

The results.

	Controls	Study group (ketoprofen 8 mg/kg b.w.)	p<0.05
white blood cells (WBC) [10^3/uL] Mean±SD	9.670±3.566,	9.110±2.020	
eosinophils (EOS) [10^3/uL] Mean±SD	0.090±0.0529	0.280±0.109	*
neutrophils (NEU) [10^3/uL] Mean±SD	0.8900±0.2706	2.920±0.9913	*
lymphocytes (LYM) [10^3/uL]Mean±SD	8.198±3.193	5.070±1.516	
monoctes (MON) [10^3/uL] Mean±SD	0.4617±1.284	0.8150±0.2365	
Erythrocytes [10^6/uL] (ERY) Mean±SD	7.860±0.2532	6.853±1.303	
hemoglobin (HGB) [g/dL]Mean±SD	15.87±0.4761	13.70±2.433	
Platelets (PLT) [10^3/uL]Mean±SD	933.5±180.7	1098±272.2	
alanine aminotransferase (ALT) [U/L]Mean±SD	55.78±10.62	56.03±20.40	
neutrophil to lymphocyte ratio (NLR) Mean±SD	0.1202±0.0420	0.6166±0.2418	*
platelet to lymphocyte ratio (PLR) Mean±SD	138.9±84.03	228.0±66.32	*
monocyte to lymphocyte ratio (MLR) Mean±SD	0.0496±0.0434	0.1629±0.02799	*
superoxide dismutase (SOD) [ng/mL]Mean±SD	2.280±0.2138	2.187±0.3170	

DISCUSSION

Drugs belonging to the NSAIDs class have many well known side effects involving the gastric mucosa, urinary system, cardiovascular system, the liver, and hematopoietic system. It is estimated that 30–50 million people take these drugs daily. K is a derivative of phenylpropionic acid. NSAIDs are classified based on the degree to which they inhibit different COX isoforms. K is a non-selective inhibitor of the COX enzyme [29]. K is rapidly absorbed from the gastrointestinal tract, with peak plasma concentrations reached as early as 15–30 minutes after administration. Its oral bioavailability is

90% and is not affected by the presence of food in the stomach. It binds to plasma proteins up to 99%, primarily albumin, which may lead to numerous interactions with other drugs. The biological half life of K is 1.5–2 hours. It crosses both the blood-brain barrier and the placental barrier. Steady state concentrations are achieved within 24 hours of drug administration. When administered *i.v.* K reaches a concentration of 26.4 ± 5.4 μg/ml within 5 minutes of infusion. The bioavailability is 90%. The volume of distribution of K is 0.1–0.2 l/kg. Three hours after administration of a 100 mg dose, the plasma concentration of K is approximately 3 μg/ml, and 1.5 μg/ml in synovial fluid. K penetrates to synovial fluid and is then gradually eliminated from it, while its plasma concentration decreases. A single *i.m.* dose of 100 mgresults in the drug being detectable in both serum and cerebrospinal fluid 15 minutes after administration [26]. K is primarily metabolized by the liver. It undergoes conjugation with glucuronic acid and is then eliminated from the body. The plasma clearance of K after oral administration is 1.16 ml/min/kg. Up to 80% of the K dose is excreted in the urine, and approximately 10% in the feces. In patients with liver failure, K may accumulate in tissues [26]. As K is primarily metabolized by the liver, this organ was selected as the focus for the experimental study.

The effect of K is largely determined by the distribution phase. It binds strongly to plasma proteins, which can displace other drugs from their protein binding sites, thereby enhancing their effects. These include anticoagulants, sulfonamides, or antidiabetic medications. When K is administered concurrently with other drugs, dosage adjustments of those medications may be necessary. Caution is advised when using anticoagulants such as heparin or warfarin, and platelet aggregation inhibitors like clopidogrel, due to the increased risk of bleeding associated with K use [26]. K can cause severe, potentially life threatening skin reactions, including Stevens-Johnson syndrome and toxic epidermal necrolysis (Lyell's syndrome). It also has strong phototoxic properties, increasing the skin's sensitivity to sunlight, which can lead to the development of photodermatoses. The concurrent use of methotrexate and K has resulted in fatal poisoning, likely due to K displacing methotrexate from plasma protein binding sites. K can reduce the effectiveness of diuretics and may increase the risk of renal failure in patients taking diuretics, especially when dehydrated. The simultaneous administration of K with angiotensin converting enzyme inhibitors (ACE inhibitors) or angiotensin II receptor blockers (ARBs, also known as sartans) may also worsen renal function and diminish the antihypertensive effect of these drugs [26]. NSAIDs also contribute to liver damage, hemolytic anemia, granulocytopenia, and impaired platelet function. NSAID therapy should be conducted for the shortest possible duration and at the lowest effective dose. Combining two or more NSAIDs is not recommended, as it increases the risk of adverse effects [16].

Most NSAIDs inhibit both COX isoforms. The adverse effects associated with NSAID use are primarily due to the inhibition of COX-1, while the anti-inflammatory effects result from the inhibition of COX-2. NSAIDs are mostly weak acids and can directly damage the gastric mucosa. By inhibiting prostaglandin production, they compromise the natural protective barrier and impair submucosal blood flow, leading to the formation of erosions and ulcers in the gastric and duodenal mucosa. This process is further intensified by decreased secretion of mucus and bicarbonates. The most dangerous complication, associated with high mortality, is bleeding from the upper gastrointestinal tract. The highest risk of such bleeding is linked to the use of NSAIDs that strongly inhibit COX-1 activity, including K, indomethacin, acemetacin, acetylsalicylic acid, and piroxicam [30].

The present study aimed to investigate the potential effects of K administration on peripheral blood morphology, the activity of the liver enzyme ALT, and the activity of the antioxidant enzyme SOD in the livers of rats. In this study, no significant changes were observed in blood morphology parameters such as WBC, LYM, ALT, PLT, HGB, MON, or ERY. The study was carried out on healthy, young, nonpregnant female rats. K was administered *via* oral gavage for five consecutive days at a dose corresponding to the human therapeutic dose, adjusted for the rats' body weight.

Female rats are more sensitive to the effects of NSAID drugs and their associated adverse reactions compared to males. Sex hormones and the phase of the reproductive cycle influence drug metabolism. Additionally, there are sex related differences in drug metabolism in rats. Enzymes belonging to the cytochrome P450 family, such as CYP2C11, CYP2C13, and CYP3A2, are significantly more active in male rats, whereas CYP2C12 is more prevalent in females [8].

The metabolism of NSAIDs involves isoenzymes such as CYP2C9, CYP2C8, CYP3A4, and CYP1A2, which can also be involved in the biotransformation of other endogenous and exogenous compounds. Inhibition of CYP2C9 activity may slow down NSAID metabolism, leading to an increase in drug concentration in the body, which could result in more severe adverse effects. Conversely, accelerated metabolism may cause a quicker elimination of the drug, preventing it from reaching therapeutic concentrations. The primary metabolic pathway for K is conjugation with glucuronic acid, which eliminates almost 80% of the dose. For this reason, CYP2C9's role in K biotransformation is relatively limited, as it primarily affects oxidative metabolism [3].

The isoenzymes COX-1 and COX-2 catalyze the conversion of arachidonic acid into thromboxanes and prostaglandins. Prostacyclin, the primary product of COX-2 activity in the endothelium, acts as a strong inhibitor of platelet aggregation and a vasodilator, while thromboxanes, the main product of COX-1 in platelets, lead to irreversible platelet aggregation. The use of these substances increases the risk of bleeding and prolongs bleeding time [30].

All NSAIDs have the potential to cause hematological issues. However, in this study there were no significant changes in red blood cell count or hemoglobin concentration between groups. Anemia, which is often seen as a side effect of NSAIDs, was not observed.

K is a potent NSAID with a short half life. This class of drugs is known for its high efficacy but also for a greater risk of side effects, particularly related to the upper gastrointestinal tract and kidneys. The relative risk of gastrointestinal bleeding associated with K is considerably considerably higher compared to drugs like ibuprofen, naproxen, diclofenac, or indomethacin [30].

Mitochondrial oxidative stress (MOS) induced by NSAIDs represents a mechanism independent of

prostaglandin inhibition that contributes to gastric mucosal damage. Although the exact mechanism remains unclear, oxidative stress is often observed in inflammatory responses. NSAID induced gastric damage can present as a loss of mucosal integrity, reduced innate antioxidant defense, apoptosis of mucosal cells, bleeding and impaired cellular regeneration. In the study by Bindu et al. it was shown that NSAIDs, using indomethacin as an example in rats, stimulate proinflammatory genes in the gastric mucosa [1].

In our experiment, a statistically significant increase in the markers NLR, MLR, and PLR was observed, which may indicate the presence of gastric inflammation, a common adverse effect associated with the use of NSAIDs.

The liver enzyme AST has two isoforms: cytoplasmic and mitochondrial. ALT is found in its cytoplasmic form. Their activity increases in response to damage caused by hypoxia or toxins. AST is present in liver cells, skeletal muscle, heart muscle, kidneys, brain, and erythrocytes. ALT is mainly found in liver cells. A physiological increase in the activity of aminotransferases occurs under the influence of analgesic drugs. NSAIDs are also among the drugs that affect potassium levels in the blood, causing hyperkalemia [17]. In our experiment, no significant increase in ALT enzyme activity was observed in the group exposed to K. The rats received a therapeutic dose of K appropriate for their species over a short period of 5 days. In a study conducted by Ruszel et al. on 36 female Wistar rats, the animals were divided into six groups: the first group received 50% ethanol, the second received 0.9% NaCl, the third received 0.9% NaCl for one day followed by K, the fourth received 50% ethanol for one day followed by K, the fifth received 0.9% NaCl for one day followed by K lysine salt, and the sixth received 50% ethanol for one day followed by K lysine salt. The experiment lasted 6 days. No changes in liver mass were observed in the rats after administration of K [19]. Ruszel et al. did not find significant differences in ALT activity between the groups. No significant differences were found in the number of white blood cells, red blood cells, platelets, or hemoglobin concentration [19]. Our experiment is consistent with the results obtained by Ruszel et al. We did not observe significant changes in the levels of WBC, RBC, PLT, HGB, nor did I find significant differences in ALT activity between the experimental and control groups.

Damage to the gastrointestinal tract is one of the most common adverse effects associated with the use of NSAIDs. It can manifest as gastrointestinal bleeding, gastric mucosal inflammation, ulcers, or erosions in the stomach or duodenum [30]. In the study by Ruszel et al. a high degree of gastric mucosal inflammation was observed, accompanied by significant thinning of the gastric mucosa and lymphocytic-plasmacytic inflammatory infiltrates. Ruszel et al. found that, except for the control group, the rats experienced similar levels of gastric mucosal damage in all exposed groups. They noted no significant difference in the degree of gastric mucosal damage between K and K lysine salt [22]. In our study, a statistically significant increase in the concentration of neutrophils and eosinophils was observed in the group exposed to K, which may indicate the presence of an inflammatory response.

Zoubair et al. conducted a study on 66 male Swiss albino mice to evaluate the effects of K on animals subjected to oxidative stress induced by hydrogen peroxide. The researchers observed a significant decrease in the activity of oxidative stress markers (CAT, SOD, GPx) in the group treated with K alongside hydrogen peroxide, as well as in the group receiving vitamin C with hydrogen peroxide. A reduction in the generation of reactive oxygen species (ROS) in the liver was noted, indicating a protective effect by restoring redox homeostasis. Vitamin C, known for its antioxidant properties, was recognized for its ROS scavenging ability. Interestingly, the authors found that K also exhibited ROS scavenging potential. They noted the pro oxidative action of hydrogen peroxide and the protective effect of both K and vitamin C, which lowered antioxidant enzyme activity compared to the control group. Their findings demonstrated that K possessed antioxidant capabilities, with effectiveness comparable to that of ascorbic acid [35].

In our study, the rats were exposed to K without additional oxidative stress being induced. The results showed no statistically significant difference in hepatic SOD activity between the treatment group and the control group. Furthermore, there was no observed increase in SOD activity following K administration, suggesting that K did not promote ROS production in the liver.

In a study by A. de la Lastra et al. the effects of K on gastrointestinal damage and bleeding in rats were investigated. The authors observed that K administration led to the development of longitudinal ulcers in the intestinal lumen, an increased neutrophil infiltration, and a significant decrease in superoxide dismutase (SOD) activity. These findings suggest that K might significantly influence the formation of intestinal lesions, neutrophil migration, and the activity of the antioxidant enzyme SOD [4].

In another study by A. de la Lastra et al. the impact of K on gastric toxicity, free radical production, and neutrophil influx in Wistar rats was examined. The researchers found that K exhibited ulcerogenic properties and led to a decrease in SOD activity [5].

In our experiment the results showed a significant increase in neutrophil levels, as well as elevated levels of eosinophils and inflammatory markers such as NLR, MLR, and PLR, indicating the presence of an inflammatory state. However, no statistically significant difference in SOD activity was observed between the experimental and control groups.

One of the adverse effects that may occur during the use of NSAIDs is hepatotoxicity. High concentrations of NSAIDs in the liver and bile ducts may result in the penetration of the substance through the membranes of hepatocytes and damage to mitochondria. Before initiating NSAID therapy, attention should be paid to the risk of hepatotoxicity, and concurrent use of hepatotoxic drugs should be avoided whenever possible [30].

In the study by Rangasamy B. et al. conducted on zebrafish (*Danio rerio*), the effect of different doses of K on embryos and adult zebrafish was analyzed. The activity of ALT and AST in adult zebrafish was significantly increased at each of the tested concentrations of K. A statistically significant decrease in the activity of antioxidant enzymes in liver tissues, such as SOD, GPx, CAT was also observed [21].In our experiment, no significant changes in the activity of the ALT enzyme, nor SOD enzyme in rats' livers were found.

Our study showed a statistically significant increase in the level of neutrophils, which are the first line of defense during inflammation or infection. During inflammation, an increased number of immature granulocytes may be observed in a peripheral blood test, a phenomenon known as a left shift [29]. In this

experimental study, additional markers were determined, including the neutrophil to lymphocyte ratio (NLR), monocyte to lymphocyte ratio (MLR), and platelet to lymphocyte ratio (PLR). Granulocytes, which include neutrophils, eosinophils and basophils, are key cells of the nonspecific immune response, constituting the body's first line of defense against infectious agents such as bacteria and fungi. They arise in the bone marrow from stem cells. Neutrophils are the most numerous group of granulocytes, constituting up to 70% of all leukocytes. They are the first to reach sites of microbial invasion, and have the ability to kill bacterial cells, fungi and other pathogens through phagocytosis or the release of cytolytic substances from cytoplasmic granules [29]. Eosinophils are responsible for fighting parasitic infections. Mature eosinophils produce mediators of inflammatory reactions, which sustain inflammation and activate other cells of the immune system to fight infections. They participate in antiviral and antibacterial responses and in eliminating cancer cells. Eosinophils also participate in allergic reactions. The causes of eosinophilia are most often associated with the occurrence of allergic diseases, such as bronchial asthma, hay fever and parasitic diseases [29]. These indicators play a role in predicting the risk of postoperative infections at the stage of preclinical studies, and are treated as markers of inflammation. The platelet to lymphocyte ratio (PLR) describes the proportion of platelets to lymphocytes. In a study conducted by Wójcik E. et al. 159 individuals with small cell lung cancer (SCLC) were examined. The authors demonstrated a significant impact of PLR, IL-6, and other factors on the 5 year survival rate of patients with SCLC. Although IL-6 was identified as the most important disease marker, the PLR level was also recognized as a significant prognostic factor [31].

The neutrophil to lymphocyte ratio (NLR) is a commonly used inflammatory marker, calculated by dividing the number of neutrophils by the number of lymphocytes. In a study conducted by Fuss et al. on 234 surgical patients with colorectal cancer, a higher NLR was found to correlate with an increased risk of infectious complications in the postoperative period. Additionally, NLR values were used as a criterion for determining the possibility of early hospital discharge. Surgical procedures are known to trigger a systemic inflammatory response, characterized by elevated neutrophil levels and reduced lymphocyte counts in peripheral blood [7]. In our study, a statistically significant increase in NLR and neutrophil count was observed in the group exposed to K compared to the control group, suggesting the occurrence of an inflammatory response. Kuźniar-Placek et al. emphasized the relevance of NLR as a predictive marker in cardiovascular diseases. Elevated NLR levels have been linked to a higher risk of hypertension and arrhythmias, such as nonvalvular atrial fibrillation. Furthermore, patients with a history of coronary thrombosis showed increased NLR levels alongside a marked reduction in lymphocyte count. NLR was additionally identified as a potential prognostic indicator of mortality or the need for heart transplantation in patients with heart failure [16].

The monocyte to lymphocyte ratio (MLR) represents the proportion of monocytes to lymphocytes. In a study conducted by Xu Z. et al. involving 100 patients diagnosed with prostate cancer, it was demonstrated that the values of PLR, MLR, and NLR were significantly higher in patients with prostate cancer compared to healthy individuals in the control group. Monocytes have the ability to suppress lymphocyte activation, which can accelerate tumor progression. The authors concluded that combining the assessment of MLR, NLR, and prostate-specific antigen (PSA) levels in blood offers the highest specificity for diagnosing prostate cancer [33]. In a study by Wiąk-Walerowicz et al., conducted on 93 patients with inflammatory spondyloarthropathies such as psoriatic arthritis (PsA) and ankylosing spondylitis (AS) who were treated with TNF-α inhibitors, researchers examined the relationship between NLR, MLR, PLR levels and the duration of disease remission. These markers were measured before treatment, and then at 6 and 12 months in AS patients, with an additional evaluation at 18 months for PsA patients. The study did not reveal a statistically significant association between hematological marker values (NLR, MLR, PLR) and remission duration in AS patients. However, in PsA patients, higher levels of these indices were observed in those with shorter remission periods [27]. In our experiment a statistically significant increase in the levels of the NLR, MLR, and PLR after exposure to K were recorded. These indicators serve as markers of inflammation. Conducting a blood morphology test and calculating these indicators is an easily accessible, noninvasive procedure that incurs minimal costs. Our study, along with the research mentioned above, indicates the potential of these markers in diagnosing various diseases and monitoring their progression. Additionally, this study noted an elevated level of eosinophils. Eosinophilia is commonly associated with allergic reactions or infections, however it can also result from the use of NSAIDs.

The next step in this area of research would involve studies on rats exposed to oxidative stress, such as through the induction of sepsis. Exposing rats to oxidative stress could help determine the extent to which K influences the production of free radicals and the activity of antioxidant enzymes in a diseased organism.

CONCLUSION

1. K does not significantly affect the generation of oxidative stress, thus does not influence the synthesis of free radicals in the rats under study. The activity of the SOD did not differ significantly between the experimental and control groups.
2. The NLR, MLR, and PLR indices, which serve as markers of inflammation, are promising indicators for assessing the inflammatory state, diagnosing inflammatory diseases, and monitoring disease progression, including cancers.

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