The Effect of Ascending Doses of Ketoprofen on Biochemical and Coagulation Parameters in Lambs

Key words
drug safety; haemostatic function; hepatotoxicity; ketoprofen; lambs; nephrotoxicity; non-steroidal anti-inflammatory drug

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Abstract: Ketoprofen (KTP) is a non-steroidal anti-inflammatory drug (NSAID) used as an analgesic, antipyretic and anti-inflammatory agent in human and veterinary medicine. Although KTP is used in the treatment of diseases such as musculoskeletal inflammation, endotoxemia, pneumonia, enteritis in sheep and minor surgical procedures such as dehorning and castration there is no information about its safety. The aim of this study is to determine the effect of KTP on biochemical and coagulation parameters following intramuscular (IM) administration of different doses of KTP to lambs. In the study, 18 clinically healthy lambs were randomly divided into three groups of 6 animals each. KTP was administered IM to lambs at doses of 1.5, 3 and 6 mg/kg. Biochemical and coagulation parameters were evaluated by taking blood samples before drug administration (0 hour) and at 24 hours and 48 hours after administration. No local or systemic side effects were observed in lambs after the administration of KTP at different doses. The aspartate aminotransferase (AST), creatine kinase (CK) and lactate dehydrogenase (LDH) values at 24 hours significantly increased compared to 0 hours in all dosage groups (p<0.05). KTP did not cause a significant change in albumin (ALB), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine (CRE), CK and LDH values in different dose groups. The AST value was only significantly higher in the 6 mg/kg dose group compared to the 1.5 mg/kg dose group at 24 hours (p<0.05). Although there was no statistically significant difference in intragroup prothrombin time (PT), fibrinogen and D-dimer levels in all dose groups, a significant increase was observed in the activated partial thromboplastin time (aPTT) value of 6 mg/kg dose group at the 24 hours compared to the 0 hour (p<0.05). As a result, after IM administration of 1.5, 3 and 6 mg/kg, increased CK and LDH values, which may be associated with muscle damage, may limit use of KTP via IM injection in lambs.

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs), which are widely used in painful and inflammatory conditions such as lameness, foot rot, castration, vasectomy, mastitis and laparoscopy in sheep, act by inhibiting the effect of the cyclooxygenase enzyme, which is responsible for the synthesis of prostaglandins (1-3). Ketoprofen (KTP), is a NSAID belonging to the arypropionic acid group and exists as a chiral compound with R(-) and S(-) isomers present in the racemic formulation. In animals, the S(-) form is more active and there is interconversion between the forms (4). In sheep, the suppression of prostaglandin E2 (PGE2) and thromboxane B2 (TXB2) production has been reported for up to 12 hours following a single intravenous injection of 1.5 or 3 mg/kg of KTP (5). In addition, a study in sheep reported that the 4 mg/kg subcutaneous administration of KTP at 90 minutes before the procedure failed to alleviate the postsurgical pain response (6).

It is known that NSAID therapy has serious side effects such as gastrointestinal ulceration or bleeding, liver and
kidney damage, allergic reactions, myocardial infarction and cardiac sudden death. Coagulation, hematological and biochemical parameters are used to evaluate the effects of drugs on physiological and pathological conditions (7,8). Coagulation parameters (aPTT, PT, fibrinogen, D-dimer) reflect coagulation disorders, while hematological parameters (WBC, RBC, hemogram, hematocrit, platelet) reflect bone marrow functions and fluid electrolyte balance, biochemical parameters (albumin, ALP, ALT, AST, BUN, cholesterol, CK, creatinine, GGT, TP and triglyceride) reflect liver, kidney, muscle and lipid metabolism functions (9,10).

In studies conducted in calves, horses, dogs, pig and children, it has been shown that the administration of KTP at recommended doses does not cause any side effects (11-15). On the other hand, some studies in laboratory animals have reported that KTP administered at recommended therapeutic doses causes side effects such as gastrointestinal irritation, impaired kidney function, hepatopathies, prolonged bleeding and clotting times (13,16). In some cases, NSAIDs may need to be used in high doses. However, high doses of this group of drugs may cause undesirable effects. In the literature review, no reference was found regarding the effect of increasing doses of KTP on biochemical and coagulation parameters in lambs. The aim of this study was to determine the effects of KTP on biochemical and coagulation parameters after single doses of 1.5, 3 and 6 mg/kg in lambs.

Material and Methods

Animals

The study was performed on a commercial farm located in the district of Kadinhani, Konya Province, Türkiye. The study was performed on 18 female Akkaraman lambs (3-6 months old, 23-36 kg) who were determined to be healthy in the general clinical examination and had not received any medication treatment in the one months prior to the study. The animals were divided into different groups 7 days before the start of the study and numbered with oily paint. The lambs were fed with commercial feed (CP-5621, Ankara, Türkiye) twice a day and alfalfa hay, grass hay and water were given ad libitum. All procedures on animals were approved of the Local Ethics Committee for Animal Experiments at Selcuk University on December 27, 2022 with the approval number 2022/140.

Experimental design

A total of 18 lambs were randomly divided into three groups of 6 animals each. KTP (100 mg/ml, Ba-Keto, Injection Solution, Bavet, Turkey) was administered as a single dose via intramuscular injection into the neck region of the lambs in the first, second and third groups at doses of 1.5 mg/kg, 3 mg/kg and 6 mg/kg, respectively, following the dosage references from previous studies (5,6). In the study, blood samples were collected from the jugular vein at 0, 24 and 48 hours using a venipuncture technique. Blood samples were collected into gel tubes (2 ml) for biochemical analysis and into sodium citrate tubes (2 ml) for coagulation tests. After centring the blood samples at 3,500g for 10 minutes, the resulting serum and plasma samples were carefully transferred into 2 ml Eppendorf tubes. The serum samples were stored in a deep freezer at -80°C until the day of analysis, while the plasma samples were analyzed within 3 hours for coagulation tests. Throughout the experimental protocol, the injection site was monitored for swelling, redness and pain and the animals were closely observed for any clinical changes.

Analysis of biochemical parameters

Biochemical parameters including serum albumin (ALB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatine kinase (CK), creatinine (CRE) and lactate dehydrogenase (LDH) levels were determined using an automated analyzer device (Lab-300plus, Instrumentation Laboratory, Milano, Italy) from serum samples stored at -80°C.

Analysis of coagulation parameters

In plasma samples, the measurement of clotting factors including prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen (FIB) and D-Dimer was performed using a coagulation analysis device (Siemens, A-7799, Sysmex CA 1500, Germany).

Statistical analysis

The research data was presented as mean ± standard deviation (SD). Statistical analysis was conducted using the SPSS software (version 26.0, IBM). Biochemical and coagulation parameters were analyzed for homogeneity of variances. Values that showed normal distribution with p-values > 0.05 were considered for parametric statistical analysis. In both intragroup and intergroup comparisons of data, one-way analysis of variance (ANOVA) and post-hoc Tukey test were used (SPSS 26.0). P < 0.05 value was considered statistically significant.

Results

No adverse reactions, general (in their feeding, drinking, defecation and behavior) or local (pain, swelling, redness), were observed during clinical observation of lambs following intramuscular administration of KTP at doses of 1.5, 3 and 6 mg/kg.

The effect of KTP on the biochemical parameters of lambs after intramuscular administration at doses of 1.5, 3 and 6 mg/kg is presented in Table 1. In the evaluation within the group, there was no statistically significant difference in
Table 1: The effects on biochemical parameters of intramuscular administration of ketoprofen at doses of 1.5, 3 and 6 mg/kg to lambs (n=6, mean ± SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sampling Time (Hour)</th>
<th>1.5 mg/kg</th>
<th>3 mg/kg</th>
<th>6 mg/kg</th>
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<tr>
<td>ALB (g/L)</td>
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<td>29.83±5.91</td>
<td>33.25±3.03</td>
<td>32.58±4.34</td>
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<tr>
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<td>24</td>
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<td>35.33±3.33</td>
<td>34.42±2.73</td>
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<td></td>
<td>48</td>
<td>33.66±1.63</td>
<td>35.08±6.74</td>
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</tr>
<tr>
<td>ALT (U/L)</td>
<td>0</td>
<td>16.08±3.64</td>
<td>19.17±3.60</td>
<td>17.92±4.76</td>
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<tr>
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<td>19.67±6.22</td>
<td>22.75±7.44</td>
<td>24.08±6.59</td>
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<td>15.50±5.00</td>
<td>19.33±1.08</td>
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<td>AST (U/L)</td>
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<td>97.25±16.67</td>
<td>104.00±12.15</td>
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<tr>
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<td>24</td>
<td>147.17±10.44</td>
<td>164.08±12.71</td>
<td>180.33±13.85</td>
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<td></td>
<td>48</td>
<td>115.42±13.25</td>
<td>121.33±29.44</td>
<td>124.58±20.31</td>
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<tr>
<td>BUN (mg/dL)</td>
<td>0</td>
<td>72.08±6.71</td>
<td>73.33±9.01</td>
<td>73.75±6.05</td>
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<td>75.58±7.79</td>
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<td>70.58±6.33</td>
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<td>70.25±7.24</td>
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<tr>
<td>CRE (mg/dL)</td>
<td>0</td>
<td>0.65±0.04</td>
<td>0.64±0.06</td>
<td>0.68±0.04</td>
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<td>0.79±0.18</td>
<td>0.76±0.16</td>
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<td>0.72±0.14</td>
<td>0.61±0.14</td>
<td>0.72±0.15</td>
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<tr>
<td>CK (U/L)</td>
<td>0</td>
<td>223.75±22.94</td>
<td>243.58±11.00</td>
<td>231.33±41.68</td>
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<td>399.25±73.72</td>
<td>384.42±38.51</td>
<td>426.33±145.25</td>
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<td>48</td>
<td>252.67±35.07</td>
<td>268.25±16.58</td>
<td>285.92±55.26</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>0</td>
<td>620.00±47.18</td>
<td>640.92±35.72</td>
<td>629.92±47.10</td>
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<td></td>
<td>24</td>
<td>891.50±128.45</td>
<td>880.25±152.23</td>
<td>877.58±153.09</td>
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<td>48</td>
<td>827.00±81.93</td>
<td>830.50±113.06</td>
<td>818.09±140.62</td>
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</table>

Superscripts with different letter in the same row show statistically significant differences (P < 0.05). Superscripts with different letter in the same column show statistically significant differences (P < 0.05).

ALB, Albumin, AST, aspartate aminotransferase, ALT, alanine aminotransferase, BUN, blood urea nitrogen, CRE, Creatinine, CK, creatine kinase and LDH, lactate dehydrogenase.

The effect of KTP on coagulation parameters in lambs at doses of 1.5, 3 and 6 mg/kg after intramuscular administration is presented in Table 2. In the within-group evaluation, there was no statistically significant difference in PT and D-Dimer values in all dose groups (P>0.05). However, the aPTT value increased significantly at 6 mg/kg at 48 hours compared to 0 hours (P<0.05). In the evaluation between groups, there was no statistically significant difference in PT and D-Dimer values (P>0.05). However, the aPTT value in the 3 mg/kg dose group was significantly lower at 48 hours compared to the value in the 1.5 mg/kg dose group.
and the fibrinogen value in the 3 mg/kg dose group was significantly higher at 24 hours compared to the value in the 1.5 mg/kg dose group (P<0.05).

Discussion

KTP, a widely used NSAID in humans and animals, is a potent cyclooxygenase inhibitor. Unlike other NSAIDs, it also inhibits lipoxygenase and creates a double blockade in arachidonic acid metabolism. The recommended dose of KTP is 2 mg/kg for cats and dogs, 2.2 mg/kg for horses and 3 mg/kg for cattle and small ruminants (4,16). Since the effect of KTP varies depending on the dose, it can be applied in increasing doses (17,18), but undesirable effects may occur when used in increasing doses. The doses (1.5, 3 and 6 mg/kg) selected in this study and sample collection times were determined by considering previous studies in pigs, sheep and cattle (5,19,20). Common side effects of KTP are observed when it is used at higher doses than recommended or for a prolonged period of time. However, side effects are rarely reported after a single administration at the recommended dose (21). KTP administered intravenously at a dose of 3 mg/kg for 5 days was well tolerated in calves (11).

It has been found that gastrointestinal side effects tend to occur when dogs are given KTP for 30 days, but the lesions healed after administration have been discontinued (13). Horses did not show any clinical adverse effects during administration of 2.2 mg/kg three times daily KTP (12). Oral administration of KTP in children has been reported to be well tolerated for up to three weeks (15). In pigs, there was no change in serum biochemical values at the single intramuscular dose of 3, 6 and 9 mg/kg and 3 mg/kg for 3 days (14). In this study, no side effects were observed after IM administration of 1.5, 3 and 6 mg/kg KTP to lambs, but statistically significant intra and intergroup differences were observed in some biochemical and coagulation parameters. Most of the reports on the side effects of KTP administered at recommended treatment doses are obtained from humans and laboratory animals. These effects include gastrointestinal irritation, impaired kidney function, hepatopathies, prolonged bleeding and clotting times and photosensitivity (10,14). Gastrointestinal ulceration occurs due to inhibition of PGE2 synthesis and decreased production of mucosal protective agents (22). Nephrotoxicity is related to the inhibition of prostaglandins in the kidneys that are essential for salt and water balance, vascular tone, blood flow and renin secretion (23). Coagulation disorders are the result of thromboxane A2 deficiency in platelets after administration of COX1 inhibitors (24). Phototoxicity results from damage to cell membranes by radical intermediates (25).

In this study, AST, CK and LDH values increased at 24 hours compared to 0 hours in all dose groups (P<0.05). It was observed that AST and CK values decreased (P<0.05) in 48 hours compared to 24 hours, but LDH value did not change. The increase in serum CK and LDH levels may occur due to muscle damage caused by IM drug administration (26,27).

Table 2: The effects on coagulation parameters of intramuscular administration of ketoprofen at doses of 1.5, 3 and 6 mg/kg to lambs (mean ± SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sampling Time (Hour)</th>
<th>1.5 mg/kg</th>
<th>3 mg/kg</th>
<th>6 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>PT (sn)</td>
<td>12.80±0.47</td>
<td>14.65±2.32</td>
<td>12.75±0.66</td>
<td>13.42±0.13</td>
</tr>
<tr>
<td></td>
<td>14.65±2.32</td>
<td>13.55±1.15</td>
<td>12.70±0.74</td>
<td>13.42±0.13</td>
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<tr>
<td></td>
<td>13.42±0.13</td>
<td>12.87±1.03</td>
<td>13.42±0.13</td>
<td>12.87±1.03</td>
</tr>
<tr>
<td>aPTT (sn)</td>
<td>38.17±0.83</td>
<td>39.30±1.87</td>
<td>40.75±3.03a</td>
<td>40.53±7.21a</td>
</tr>
<tr>
<td></td>
<td>36.47±2.46</td>
<td>35.85±1.20</td>
<td>35.62±3.15b</td>
<td>44.0±1.75ab</td>
</tr>
<tr>
<td></td>
<td>37.28±2.04</td>
<td>40.53±7.21a</td>
<td>44.0±1.75ab</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>2.21±0.17</td>
<td>1.53±0.56a</td>
<td>1.98±1.05</td>
<td>2.41±0.75</td>
</tr>
<tr>
<td></td>
<td>1.88±0.25</td>
<td>2.35±0.50a</td>
<td>2.41±0.56</td>
<td>2.41±0.75</td>
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<td></td>
<td>1.96±0.28</td>
<td>1.60±0.55ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-DIMER (ng/L)</td>
<td>457.67±64.97</td>
<td>551.00±57.84</td>
<td>524.67±82.46</td>
<td>518.50±62.91</td>
</tr>
<tr>
<td></td>
<td>501.83±85.35</td>
<td>487.33±104.93</td>
<td>518.50±62.91</td>
<td>512.00±63.22</td>
</tr>
</tbody>
</table>

Superscripts with different letter in the same row show statistically significant differences (P < 0.05). Superscripts with different letter in the same column show statistically significant differences (P < 0.05). PT; prothrombin time, aPTT; activated partial thromboplastin time.
The half-life of CK is approximately 2 hours (28), thus indicating that the transient increase in serum CK level seen in this study is due to IM drug administration. In our study, it was concluded that mild and transient increases in enzyme levels, particularly within the same dosage groups, are related to the metabolism and elimination processes of KPT. In the current study, it was determined that KTP did not cause a significant change in ALB, ALT, BUN, CRE, CK and LDH values in different dose groups. Only the AST value in the 6 mg/kg dose group was significantly higher at 24 hours compared to the 1.5 mg/kg dose group (p<0.05). It was determined that the elevated AST level returned to its normal levels in the 48 hours. AST also known as serum glutamate-oxaloacetate transaminase (SGOT), is found mainly in the heart, liver, kidneys and muscle tissue and high blood concentrations indicate liver damage or disease (29). Serum AST and ALT levels can be used as an indicator of liver damage. ALT is more liver specific than AST. ALT instead of ALT can only be used when ALT is not present and there is no known muscle pathology causing an increase in AST (30). In previous studies, it was reported that KTP administration increased the AST enzyme in mice (31), dogs (32) and donkeys (33). These results are consistent with the results of our study. Low elevations in serum enzyme values have been reported during KTP therapy and this has rarely been associated with significant acute liver injury (31). KTP blocks the formation of thromboxane A2 by inhibiting thromboxane cyclooxygenase. It creates a systemic bleeding tendency by disrupting platelet aggregation and thus prolonging bleeding time (34, 35). In this study, although no statistically significant difference was found in intragroup PT, fibrinogen and D-dimer levels in all dose groups, a significant increase was observed in aPTT value at 6 mg/kg in 24 hours compared to 0 hour (p<0.05). PT and aPTT are commonly used coagulation measurement parameters in the evaluation of secondary hemostasis in humans and animals (36). aPTT level in healthy sheep has been reported to be between 29.2 ± 3.2 and 41.1 ± 8.7 seconds (37). It was reported that the administration of flunixin meglumine and meloxicam in sheep prolonged aPTT, but these changes were not statistically significant (38). It has been determined that carprofen increased the aPTT value in dogs on the 5, 7 and 12 days after administration (36). It has been reported that the administration of meloxicam and KTP in ponies did not cause any difference in fibrinogen, PLT, PT and aPTT values and did not affect the coagulation parameters (39). In this study, it was concluded that the fact that the fibrinogen value in the 3 mg/kg dose group was higher at 24 hours compared to the value in the 1.5 mg/kg dose group was not significant.

Conclusion

In conclusion, after IM administration of 1.5, 3 and 6 mg/kg of KTP, it was observed that CK and LDH values, which may be associated with muscle damage, were increased. This may limit use of KTP via IM route in lambs. In all dose groups, AST values, which may be associated with metabolism processes of KTP, were increased. The aPTT value increased at the dose of 6 mg/kg. For the effective and safe use of KTP in lambs, its hematological, biochemical, molecular and pathological safety must be demonstrated after repeated administrations and other administration routes.

Acknowledgements

The authors would like to express their gratitude to the people working on the farm where the experimental procedure on the lambs took place.

Conflict of interest: The authors declare that they have no potential conflict of interest with respect to the authorship and/or publication of this article.

References


Vpliv naraščajočih odmerkov ketoprofena na biokemične in koagulacijske parametre pri jagnjetih

M. N. Ural, K. Üney

Izvleček: Ketoprofen (KTP) je nesteroidno protivnetno zdravilo (NSAID), ki se v humani in veterinarski medicini uporablja kot sredstvo proti bolečinam, povišani temperaturi in vnetju. Čeprav se KTP pri ovcah uporablja za zdravljenje mišično-skeletnih vnetij, endotoksemije, pljučnice, enteritisa in pri manjših kirurških posegih, kot sta odstranjevanje rogov in kasstracija, ni podatkov o varnosti zdravila. Namen te študije je bil ugotoviti vpliv biokemijskih in koagulacijskih parametrov po intramuskularni (IM) aplikaciji različnih odmerkov KTP pri jagnjetih. Vrednosti aspartataminotransferaze (AST), kreatin kinaze (CK) in laktat dehidrogenaze (LDH) so se po 24 urah v primerjavi z 0 urami v vseh skupinah znatno povečale (p < 0,05). KTP ni povzročil značilnih sprememb vrednosti albumina (ALB), alanin aminotransferaze (ALT), dušika sečnine v krvi (BUN), kreatinina (CRE), CK in LDH v različnih skupinah odmerkov. Vrednost AST je bila po 24 urah pomembno višja le v skupini z odmerkom 6 mg/kg v primerjavi s skupino z odmerkom 1,5 mg/kg (p < 0,05). Čeprav znotraj posameznih skupin ni bilo statistično pomembnih razlik v vrednostih protrombinskega časa (PT), fibrinogena in D-dimerov, smo v skupini z odmerkom 6 mg/kg v 24 urah v primerjavi z uro 0 opazili znatno povečanje vrednosti aktiviranega delnega tromboplastinskega časa (aPTT) (p < 0,05). Posledično bi lahko povečane vrednosti CK in LDH (ki so lahko povezane s poškodbami mišic) omejile uporabo IM aplikacije KTP pri jagnjetih.

Ključne besede: varnost zdravil; hemostatska funkcija; hepatotoksičnost; ketoprofen; jagnjeta; nefrotoksičnost; nesteroidno protivnetno zdravilo