ABSTRACT
The use of glucocorticoids induces a specific pathology in dogs called steroid hepatopathy. The objective of this study was to determine the possibility of the hepatoprotectants to decrease the corticosteroid-induced alteration in dogs’ liver functional condition. To reach the aim such blood serum enzymes as alaninaminotransferase (ALAT), gammaglutamyltransf erase (GGT), alkaline phosphatase (AP) and corticosteroid-induced thermostable alkaline phosphatase (cAP) were analysed. The study took place in private veterinary clinics in Latvia, during 2013 – 2014, with the permission of dogs’ owners. Twenty animals, which received glucocorticoids due to present diagnosis, were divided into two groups. In the first group long-lasting glucocorticoid methylprednisolone acetate injection was used once, while in the second group, the hepatoprotectants were used after the injection of glucocorticoids. It was discovered that after 45 days of hepatoprotectants use, blood enzymes were significantly lower (p<0.05) than in dogs that did not receive hepatoprotectants. In the group where the hepatoprotectants were used the enzyme values reached the reference limits.

KEY WORDS: dogs, glucocorticoids, liver, hepatoprotectants

INTRODUCTION
Glucocorticoids are widely used in veterinary medicine. Glucocorticoids are anti-inflammatory and immunosuppressive group of drugs, which are used for animals with allergic and anaphylactic reactions, in shock, with autoimmune diseases or in other conditions (Dillon et al., 1980; Lucena et al., 1999; Abraham et al., 2006). Together with desirable effects they can cause undesirable as well: osteoporosis, diabetes mellitus, hypertension, iatrogenic hyperadrenocorticism and others. The telic use because of these factors is extremely important. Local and systemic corticosteroids are used in veterinary medicine, both causing changes in different organ systems of the animal, including the liver morphofunctional condition, which is called steroid hepatopathy (Dillon et al., 1980; Abraham et al., 2006). This is a specific pathology only in dogs (Fittschen and Bellamy, 1984). Some blood serum enzymes indirectly reflect the liver morphofunctional condition, but histological findings of the liver biopsy reflect it directly. Increased values of such blood serum enzymes as alaninaminotransferase (ALAT), aspartataminotransferase (ASAT), gammaglutamyltransferase (GGT), alkaline phosphatase (AP) and corticosteroid-induced thermostable alkaline phosphatase (cAP) are specific for the steroid hepatopathy. The alkaline phosphatase value increases in the serum because of glycogen raised deposit in the liver and the vacuolization of hepatocytes. This process can be caused by corticosteroids. Hepatocytes of dog influenced by corticosteroids produce an isoenzyme of alkaline phosphatase (Dillon et al., 1980). Increased AP value is one of the most used biochemical indicators in the diagnostics of the liver disease (Center et al., 1992). The possibility to distinguish corticosteroid-induced AP from other liver pathologies induced AP has greater differential diagnostic value. The safe differential method is thermic processing of AP isoenzymes – corticosteroid-induced AP isoenzyme is thermostable (Teske, 1999; Feldman and Nelson, 2004).
Different solutions have proved capable of protecting the dog’s body and its liver against the negative glucocorticoid alteration. There exists an evidence of the evaluation of the aminoacid S-adenosylmethionine’s influence on systemic and hepatic effects on prednisolone in dogs (Center et al., 2005), the efficiency of a butafosfan and vitamin B12 on biochemical and hematological blood parameters in dogs treated with dexamethasone (Deniz et al., 2009) and others. Even though there are a lot of different hepatoprotectants, their efficiency towards protecting or reversing corticosteroid-induced changes in dog liver are insufficiently investigated.

Therefore the aim of the study is to investigate the corticosteroid-induced alteration in the liver function in the dogs and the possibility to decrease liver alterations by the use of hepatoprotectants.

MATERIALS AND METHODS
The study took place in private veterinary clinics in Latvia, during 2013 - 2014, with the permission of dogs’ owners. Twenty dogs of various age, weight, breed and gender were used in the present study. All dogs had a confirmed disease and were treated with glucocorticoids. The animals were divided into two groups conditionally, ten animals in each: in the first group dogs received only an injection of glucocorticoid, in the second group dogs received hepatoprotectants after the injection. For this study we selected a long-acting glucocorticoid, methylprednisolone acetate, 40 mg/ml, in intramuscular route once on the first day of the study in a dose of 0.1 mg/kg, but as hepatoprotecctive agent – “GlutaMax” in a recommended dose 1 pill for each 15 kilograms, which contains the extracts of silymarin (Silybum marianum), curcuma (Curcuma longa), artichoke (Cynara scolymus), choline and B group vitamins. These were used once per day per os from the first day of study for 45 days.

To estimate the hepatoprotectant influence on dog’s condition and the possibility to protect the liver against glucocorticoid impact, the following blood serum enzymes were determined – alaninaminotransferase (ALAT), gammaglutamyltransferase (GGT), alkaline phosphatase (AP) and corticosteroid-induced thermostable alkaline phosphatase (cAP) values.

The hepatoprotectant „GlutaMax” was used because of its unique composition. The extract of silymarin has antioxidant, hepatoprotective, antifibrotic, and anti-inflammatory effects (Flatland, 2003; Johnson, 2008). It was used in the treatment of experimentally induced mushroom hepatotoxicity in dogs (Vogel, 1984). The extracts of curcuma and artichoke have antioxidant and anti-inflammatory effects and are also used for liver detoxication. Choline regulates the metabolism of fats, works against fatty degeneration of liver (Johnson, 2008).

A day before glucocorticoid usage, blood samples were collected from each animal v. cephalica. Next samples were taken on the days 15, 30 and 45. To separate the serum we centrifuged the blood on 1300 rounds per minute for 10 minutes (Gulbis, 2011). We analyzed the serum not more than 15 minutes after the separation. GGT, ALAT and AP were determined in serum by biochemical analyzer ‘MINDRAY BS-120’. Corticosteroid-induced enzyme thermostable alkaline phosphatase was determined by Teske method (Teske, 1999), the blood serum was handled for 15 minutes in temperature 60 °C, therefore only thermostable isoform will be presented into blood serum and then it was determined by the same biochemical analyzer.

To analyze the data the programs MS Excel and ‘RStudio’ were applied. P-values less than 0.05 were considered to be statistically significant. For the comparison of blood serum enzymes values T-test was used.
RESULTS AND DISCUSSION
Before the study (day 0) blood serum enzyme values were defined for each dog. It was found that serum enzymes as ALAT, GGT, AP and cAP values were within reference limits (Table 1).

Table 1
The values of some blood serum enzymes in dogs during the study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Reference limits, U/L*</th>
<th>Groups</th>
<th>Day 0</th>
<th>Day 14</th>
<th>Day 30</th>
<th>Day 45</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALAT</td>
<td>10 – 94</td>
<td>1.</td>
<td>69.2±8.0</td>
<td>206.4±28.8</td>
<td>177.6±19.5</td>
<td>113.5±48.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.</td>
<td>66.3±6.7</td>
<td>157.5±8.6</td>
<td>141.3±8.6</td>
<td>59.9±25.5</td>
</tr>
<tr>
<td>GGT</td>
<td>0 – 6</td>
<td>1.</td>
<td>5.0±0.7</td>
<td>19.6±4.4</td>
<td>17.8±3.5</td>
<td>9.8±6.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.</td>
<td>4.6±0.8</td>
<td>12.3±1.7</td>
<td>11.0±1.3</td>
<td>4.9±2.0</td>
</tr>
<tr>
<td>AP</td>
<td>0 – 90</td>
<td>1.</td>
<td>59.2±5.5</td>
<td>412.8±75.3</td>
<td>329.2±46.7</td>
<td>157.2±86.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.</td>
<td>63.2±6.1</td>
<td>274.0±20.5</td>
<td>221.2±18.1</td>
<td>89.3±33.9</td>
</tr>
<tr>
<td>cAP</td>
<td>-**</td>
<td>1.</td>
<td>12.5±2.2</td>
<td>265.1±41.4</td>
<td>210.6±32.7</td>
<td>91.0±78.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.</td>
<td>13.4±2.1</td>
<td>205.2±22.5</td>
<td>158.4±17.1</td>
<td>39.8±18.1</td>
</tr>
</tbody>
</table>

* by Willard, Tvedten, 2012
** Corticosteroid-induced alkaline phosphatase (cAP) does not have reference limits.

In the first group the hepatoprotectant was not used, in the second group the hepatoprotectant was used for 45 days. Fourteen, thirty and forty five days after the injection of long-lasting glucocorticoid methylprednisolone acetate, we collected blood samples from all dogs.

The mean values of serum enzymes in the both groups on the fourteenth and thirtieth day were significantly (p<0.05) higher than reference limits. The tendency to decline in the mean values was noticeable in both groups, but it should be noted that the results from the second group where significantly (p<0.05) lower then in the first group. Only in forty five days the mean values of serum enzymes from the second group dogs achieved the reference limits (see Table 1).

In forty five days the dogs from the first group showed following results: the mean value of ALAT (113.5±48.8 U/L) was approximately 1.6 times higher than on day 0 (69.2±8.0 U/L); the mean value of GGT increased 2 times (9.8±6.2 U/L versus 5.0±0.7 U/L); the mean value of AP increased approximately 2.7 times from the day 0 (157.2±86.4 versus 59.2±5.5 U/L), but cAP mean value in serum was approximately 7 times higher than on the day 0 (91.0±78.6 U/L and 12.5±2.2 U/L, respectively) (see Table 1).

The purpose of this study was also to find out how effectively the hepatoprotectant “GlutaMax” can decrease the alteration in the biochemical parameters on the serum, which was caused by methylprednisolone acetate. It became apparent that all the investigated enzyme values in blood serum obtained from the dogs from group two, which were using hepatoprotective agents after the long-lasting glucocorticoid injection were significantly lower (p<0.05) than these in the dogs from group one (see Table 1).

The mean value of ALAT in dogs from the group two on day 45 was 59.9±25.5 U/L, which is within reference limits and significantly lower than the value from the animals of the group one – 113.5±48.8 U/L (p<0.05) (see Table 1). The mean value of GGT in the group two was 4.9±2.0 U/L (within reference limits), compared with 9.8±6.2 U/L, as seen in group one (see Table 1). The increase of the mean value of AP was 89.3±33.9 U/L, and this was only 1.4 times higher than in the same group on day 0 in comparison with group one where the increase was 3 times higher (see Table 1). Corticosteroid-induced AP (cAP) mean value in group two
on the forty fifth day was high – 39.8±18.1 U/L, that was 3 times higher than on day 0, but in group one the same value was approximately 3 times higher than on day 0 (see Table 1).

It should be noted that corticosteroid-induced thermostable AP in serum was found in small amounts (12.5±2.2 U/L and 13.4±2.1 U/L) even on the day 0. This can be explained by the stress of the dogs because of the veterinarian presence and blood collecting. It is acknowledged that it is higher glucocorticoid, e.g. cortisol level, in blood when animal is in stress (Feldmann et al., 1994). It is experimentally proven that there are small amounts of thermostable alkaline phosphatase even in healthy dogs’ blood as the authors describe it with being in stress condition (Fukui et al., 2006).

It should be noted that the mean values of ALAT, GGT, AP and cAP in the serum of the dogs from the second group on day 45 influenced by hepatoprotectant “GlutaMax” were significantly lower. That indicates the negative effect of long-acting methylprednisolone acetate is decreased. Every enzyme mean value was lower compared to the same mean values from the animals of the first group, and these achieved the reference limits (see Table 1).

The results of our study prove the fact of glucocorticoid-induced negative effects on liver functional condition (Badylak and van Vleet., 1981; Lucena et al., 1999; Abraham et al., 2006). These negative effects have been reflected by enzymes ALAT, GGT, AP and especially cAP significant increase in blood serum. The hepatoprotectant “GlutaMax” could completely prevent these functional failures of liver during this study.

CONCLUSIONS
1. The corticosteroid methylprednisolone acetate statistically significantly increases the values of alaninaminotransferase (ALAT), gammaglutamyltransferase (GGT), alkaline phosphatase (AP) and corticosteroid-induced thermostable alkaline phosphatase (cAP) in dogs’ blood serum.
2. The hepatoprotectant ‘GlutaMax’ in dosage of 1 pill for 15 kg of bodyweight, used 45 days after the one injection of long-lasting methylprednisolone acetate for dogs, significantly decreases the values of alaninaminotransferase (ALAT), gammaglutamyltransferase (GGT), alkaline phosphatase (AP) and corticosteroid-induced thermostable alkaline phosphatase (cAP) in dogs’ blood serum.

REFERENCES