Acute oak (*Quercus infectoria*) toxicosis in lambs

Y. EROKSUZ¹, M. DABAK², H. EROKSUZ¹, E. BAYDAR²*, I. TURKOGLU³, I. YILMAZ¹

¹Firat University, Veterinary Faculty, Department of Pathology, 23119 Elazig, Turkey.
²Firat University, Veterinary Faculty, Department of Internal Medicine, 23119 Elazig, Turkey.
³Firat University, Education Faculty, Department of Biology Education, 23119 Elazig, Turkey.
⁴İnonu University, Pharmacy Faculty, Department of Pharmacology, 44315 Malatya, Turkey.

Corresponding author: ebaydar@firat.edu.tr

**SUMMARY**

Acute intoxication caused by consumption of oak (*Quercus infectoria* subsp. *boissieri*) occurred in a flock of 92 lambs in Elazig, a province of the eastern Turkey. Four lambs had died with a history of anorexia and recumbency following introduction approximately 150 g per animal/day oak material for 10-13 days. Clinical, pathological and biochemical findings were documented in 3 lambs. The most striking biochemical changes were detected in blood urea nitrogen levels and gamma-glutamyl transferase activity. At necropsy; subcutaneous edema, mild hydrothorax, moderate ascites, severe perirenal and retroperitoneal edema, severe multifocal renal subcapsular petechiation were the major gross findings detected in all cases. Renal histopathology revealed moderate multifocal tubular cell necrosis, multifocal moderate cell swelling, multifocal tubular dilatation and hyaline cast formations. The results of this study suggest that both plant and animal based factors; namely the young age and high tannin content might contribute to the occurrence of the present toxicosis in the lambs.

**Keywords:** biochemical finding, clinical finding, pathological finding, oak toxicosis, *Quercus infectoria*, lamb

**MATERIALS AND METHODS**

**ANIMALS, CLINICAL EXAMINATION AND SAMPLING**

Three Akkaraman lambs from the same flock presented to the Veterinary Teaching Hospital (VTH) of Firat University in March 2011 for diagnosis were included in this study. The flock was composed of 92 Akkaraman lambs 4 to 8 weeks old and 12 to 18 kg in weight. All the lambs were sucking their mothers. Four lambs had died previously with a history of anorexia and recumbency following the introduction of approximately 150 g per animal/day oak material for 10-13 days. The ewes and lambs were kept in separate units except for the sucking times. Group feeding was applied in both lambs and ewes. Both lambs and ewes consumed 150 and 200 gr the oak plant material voluntarily. Each lamb was fed with daily a mixture of barley (150 gr), ground maize (100 gr) and dreid alfa-alfa (100 gr). The ewes were fed with mixture of the wheat straw (1200 gr) and barley (700 gr) per animal.

After feeding with the plant material for 14 days, 3 lambs were presented to the hospital, they all were down and unable to rise. No therapy was instituted as the animals...
were becoming increasingly moribund. Euthanasia and then necropsy was performed after 24 hours on these animals.

The flock was checked during the 2 months following the first visit and at the end of this period, another randomly selected 10 lambs and 10 sheep were used for the assessment of health status of the flock. Detailed clinical examination of the lambs was performed. Blood samples were taken from a jugular vein of the lambs into the tubes with and without EDTA for hematological and biochemical analyses, respectively. The oak material given to the flock were submitted by the owner for botanical identification and chemical analysis.

HEMATOLOGICAL AND BIOCHEMICAL ANALYSES

Hematological analyses (% PCV, WBC) were conducted manually using conventional techniques. Biochemical analyses including glucose (GLU), blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total protein (TP), total bilirubin (TB), direct bilirubin (DBI) and indirect bilirubin (IBI) were performed using a automated analyzer (Dimension ARX, Dade Behring IL, USA) in serum samples which were obtained by centrifugation of the plain blood tubes at 2000 g at 4°C for 10 minutes of.

POSTMORTEM AND HISTOPATHOLOGICAL EXAMINATION

Necropsy was performed on the 3 lambs. Tissue samples from the liver, kidneys, lungs, spleen, mesenteric lymph nodes, large and small intestines, cerebrum and cerebellum were collected and fixed in 10% buffered formalin, routinely processed in paraffin, cut in 5 µm in thickness and stained routinely with haematoxylin and eosin.

TOTAL PHENOL AND TOTAL TANNIN DETERMINATION

The oak leaf samples were analyzed for total phenolics and total tannins according to Makkar (11). Briefly, dried plant material (200 g) was extracted using acetone/water (10 ml; 70:30, v/v) in an ultrasonic bath for 20 min. The contents were then centrifugated (4°C, 10 min., 3000 g) and the supernatant was kept on ice. Total phenols, by using Folin-Ciocalteau reagent, were detected at 725 nm. A calibration curve was prepared by using tannic acid. Total phenol was calculated as tannic acid equivalents and expressed as equi. g/kg dry matter. Total tannins were calculated as the difference between total phenols and non-tannin phenols.

Results

Anorexia, severe depression, and recumbency were determined in the clinical examination of three lambs brought to the VTH. The history revealed that another 4 lambs had died within 10 to 13 days after the initiation of feeding with oak materials. All the lambs were on feeding with oak material approximately 150 g per animal/day for two weeks. The sampled oak material given to the flock was identified as Quercus infectoria subsp. boissieri in the morphological examination (Fig. 1). The results of hematological and biochemical examinations are shown in the Table I. The phenolic content and tannen levels were detected as 231.79 g/kg and 165.16 g/kg, respectively.

As seen in the Table 1, serum BUN and creatinine levels were markedly increased over the reference ranges in the three lambs. Hematological (PCV%, WBC) and biochemical analyses (GLU, BUN, CREA, AST, ALT, ALP, GGT, TP, TBI, DBI, IBI) of 10 lambs examined at the end of the second month after the oak feeding stopped were in the reference

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Cases</th>
<th>Reference ranges (*)</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>GLU (mg/dl)</td>
<td>52</td>
<td>29</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
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<td>251</td>
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<td>CREA (mg/dl)</td>
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<td>AST (U/L)</td>
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<td>ALP (U/L)</td>
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<td>GGT (U/L)</td>
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<td>TP (g/dl)</td>
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<td>TBI (mg/dl)</td>
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<td>DBI (mg/dl)</td>
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<tr>
<td>IBI (mg/dl)</td>
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<td>0.9</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>28</td>
<td>27</td>
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<tr>
<td>WBC (x10⁶)</td>
<td>14.6</td>
<td>10.7</td>
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Table I: The results of biochemical (serum glucose (GLU), blood urea nitrogen (BUN), creatinine (CREA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), total protein (TP), total bilirubin (TBI), direct bilirubin (DBI) and indirect bilirubin (IBI) and hematological (%PCV, WBC) examinations.
ranges. Clinical findings of all ten lambs were within the physiological limits. Neither the clinical signs nor mortality occurred in the ewes and rams which had been fed about 200 g oak material.

Necropsy findings were the same in all the lambs with some varying minor degrees in severity of the lesions. The most prominent gross lesion was moderate to severe gelatinous-hemorrhagic perirenal and retroperitoneal edema (Fig. 2) extending into the intermuscular and subcutaneous tissues to lumbosacral region. Edema fluid had jelly-like appearance and tinged with blood. Diffuse subserosal pulmonary and epicardial petechial hemorrhages were also detected.

The kidneys were swollen and had a moderately pale appearance in the cut surfaces. There was numerous areas of petechiation on the subcapsular surface of the kidneys (Fig. 3). Pleural and abdominal cavities contained 75 to 100 and 300 to 550 ml of amber colored transudate, respectively (Fig. 4).

The rumen had 500 to 800 ml of green-tinged watery content and contained pieces of oak leaves. Large and small intestinal walls had an edematous and congestive appearance. There was no erosion or ulceration on the mucosal surfaces of the gastro-intestinal system.

Histopathological examination of the kidneys showed tubular cell necrosis, degeneration, tubular dilatation and hyaline cast formations. Necrotic changes were more prominent in the renal cortex and especially in proximal convoluted tubular epithelium which was mostly replaced by amorphous eosinophilic material (Fig. 5). The medullar tubules also contained variable degree of necrotic cells and hyaline casts. In one case, thyroidisation was detected in medullar tubules. There was severe, multifocal, perivascular, perilglomerular and subcapsular hemorrhages in the renal cortex (Fig. 6).

Hepatocytes throughout the liver contained clear cytoplasmic lipid droplets consistent with the moderate to severe, diffuse fatty change.
Discussion

Most animal species are susceptible to oak poisoning, and losses in sheep and cattle being reported most commonly with occasional cases occurring in horses [5, 6, 9, 10, 13]. Goats are more resistant than other ruminants because of greater tannase enzymes concentration in their ruminal mucosa [13]. There are no specific clinical findings of oak toxicosis in ruminants [13, 16]. The history of oak feeding, anorexia, severe depression and recumbency determined in the clinical examination of the lambs suggested a tentative oak toxicosis. Increased serum BUN and creatinine levels confirmed by the renal damage determined in the histopathologic examinations were consistent with studies indicating that the kidney is the primary target organ in oak toxicosis in cattle [1, 5, 6, 12, 15, 16]. The occurrence of more pronounced necrotic changes in proximal convoluted tubular epithelium in the kidneys could be explained by their higher enzymatic activity of these cells and, their first exposure to the toxic metabolites.

Degenerative liver lesions in the histopathological examination together with the increased serum GGT in all 3 lambs and serum ALP in one lamb are also consistent with the results of an earlier report in cattle [12]. The diagnosis of oak toxicosis was supported by the visual detection of oak plant materials found in the ruminal content in all the cases. It also was supported the diagnosis the immediate ceasing of the deaths when the oak consumption was prevented.

The reason why only 7 out of 92 lambs were affected and died in the flock might be explained by various factors including the differences in the age, amount of oak parts consumed, and individual differences in susceptibility. Another possible explanation is that it might be due to the developing an adaptation after daily ingestion of non-lethal doses of oak. There are several pathways for the adaptation. It has been reported that saliva proteins bind tannins in a highly specific manner in sheep (14). Another possible mechanism can be the rapid adaptation of ruminal microbes for the synthesis of tannase enzyme (10). Repeated consumption of small amounts of oak parts by the nursing lamb might have induced an adaptation to the oak toxication. Such feeding practice with oak might protect the lambs before the larger amount of oak ingestion. Alternatively, single or twice high dose of oak consumption might also induce adaptation in nursing lamb. Further studies on controlled feeding trials are needed to prove these hypothesis.

The total phenolic and tannin contents in Quercus infectoria subsp. boissieri (231.79 g/kg, 165.16 g/kg) were higher than some oak species [3] including Quercus persica (78 g/kg, 73 g/kg), Quercus libani (104 g/kg, 100 g/kg) and even Quercus infectoria ( 115 g/kg, 109 g/kg ).

Yildiz et al. [17] reported no clinical signs in lambs aged 8 to 9 months old fed on Quercus hartwiissiana leaves containing 63.70 g/kg total tannin at a dose of 370 g/day for 60 days. Conceivably, this discrepancy depends upon newly introduced to oak feeding, being young animal [10] and considerably high tannin content of the present plant material in this report. Considering that calves are more likely to be poisoned by oak than mature animals [5, 6, 9, 12, 15], the present toxicosis fit rather well with two different explanations that degradations of hydrolysable tannins by rumen microbes [10] and the animals consuming tannin rich feeds might have developed a defense mechanism against dietary tannins [8].

Conclusion

This study suggests that both plant and animal based factors; young age and high tannin content of plant material might contribute to toxicosis in lambs. Further phytochemical studies and experimental feeding experiments in livestock are needed to further elucidate the toxic and nutritive properties of the plant material and to ensure the adaptation.
References


