Histopathological, Hematobiochemical and Urinalysis Changes in Experimental Consumption of Oak (*Quercus brantii*) in Sheep

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**Abstract:** Acorn contains variable amounts of tannins, so that causes occasional livestock toxicity. Because of its cheapness, accessibility and bad economic condition of many farms, oak ration is used in many parts of Iran. The 20 day period experiment was conducted on 9 female sheep (one-year-old and 40±3 kg weight) of the Karakul breed. Sheep were randomly divided into treatment group (n = 6) and control group (n = 3). In the treatment group, the mean amount of acorn powder added to control ration was 2.2 kg day⁻¹. Venous Blood and urine samples were taken on 0, 10th and 20th days of experiment. At the end of experiment all the animals were slaughtered and histopathological samples were taken after necropsy. Then hematocrit and hemoglobin, serum glucose, total protein, albumin, fibrinogen, blood urea nitrogen, aspartate aminotransferase, Urine glucose and protein were measured. The results indicated that serum fibrinogen of treatment group increased significantly (p<0.05) on 10th day. Other parameters didn’t show significant changes. Only mild hepatic fibrosis, lymphocytic hepatitis and interstitial nephritis were observed in one case of treatment group. It was concluded that the gradual increase of acorn powder in diet cause no overt clinical signs of oak poisoning in sheep.

**Key words:** Hepatitis, karakul breed, nephritis, oak, poisoning, *Quercus brantii*, sheep

**INTRODUCTION**

A large part of our country (Iran) such as Zagros Mountains is covered with oak forests. The area in the Fars Province of Iran is characterized by the presence of *Quercus brantii* (Bokhari and Khan, 1976). Because of its cheapness, accessibility and bad economic condition, occasionally some farmers use oak ration for livestock in many parts of Iran, but it might lead to poisoning.

Oak (*Quercus* species) belongs to the family Fagaceae. Oak poisoning primarily occurs in cattle, however, sheep and horses are also poisoned (Garg, 2000), however, Cattle and sheep are the species most often affected by oak poisoning (Ben Salem *et al*., 2003; Kasari, 1986; Spier *et al*., 1987). Cases of oak poisoning are observed in hilly regions during the spring months due to ingestion of buds and autumn due to ingestion of acorns (fruits) (Garg, 2000). However, acorns have the potential to cause fatal poisoning (Bassden and Dailvi, 1987; Ostrowski *et al*., 1989). Leaves and acorns contain large quantities of tannins and gallotannins in addition to some other principles like simple phenol etc (Garg, 2000). Tannins are polyphenolic substances with various molecular weights and a variable complexity. Their multiple phenolic hydroxyl groups lead to the formation of complexes primarily with proteins and to a lesser extent with metal ions, amino acids and polysaccharides (Makkar, 2003). Tannins particularly hydrolysable tannin and their degradation products such as gallie acid and pyrogallol are the most likely toxic principles (Garg, 2000). Tannic acid is especially toxic to the renal tubules and kidney lesions of oak toxicosis in cattle are nearly pathognomonic (Yenham *et al*., 1998).

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Many scientists reported different aspects of oak poisoning in ruminants such as Rogosie et al. (2006), Yeruham et al. (1998), Garg et al. (1992), Spina et al. (1987), Nesper et al. (1982), Wiseman and Thompson (1984) and Daniels (1976). These studies often carried out on cattle and they are included different strains of oak throughout the world. Because of existence of a large number of sheep (generally Karakul breed) in Kazeroun and presence of dominant oak strain (Quercus brentii), evaluation of changes in oak consumption in Karakul breed, maybe of considerable importance. Hence, the main objective of this study is evaluating of chronic consumption effect of acorn powder in sheep toxicity.

MATERIALS AND METHODS

In order to determine the effect of oak consumption on different parameters, the 20 day period experiment was conducted (summer, 2004; Kazeroun, Iran). Nine female sheep (one-year-old and 40±3 kg weight) of the Karakul breed randomly divided into treatment group (n = 6) and control group (n = 3). In the treatment group, the mean amount of acorn powder added to control ration (dried hay and concentrate) was 2.2 kg day$^{-1}$, approximately 26.25% of ration. This amount was added gradually from 100 g (2.5 % of ration) on zero days to 2 kg (50% of ration) on 20th day of the study. The ration for the control group consisted of dried hay. The two groups had free access to water. Blood and urine samples were taken from both groups on 0, 10th and 20th days of experiment. The sera were separated by centrifugation and were stored at -20°C until used for biochemical measurements.

Hematological and Biochemical Measurements

Hematocrit (%) and Hemoglobin (g dL$^{-1}$) were determined. Serum glucose (Glu), total protein (Tp), albumin (Alb), blood urea nitrogen (BUN) and fibrinogen (Fib) were measured respectively by O-toluidine, Biuret, Bromocresol green, diacetyl monoxime, modified Vandenbreg (Burtis and Ashwood, 1999) and routine method. Enzyme of aspartate aminotransferase (AST), was measured by Reitman-Frankel method (Burtis and Ashwood, 1999). Urine glucose (Glu) and total protein (Tp) also measured.

Histopathological Samples

At the end of experiment all the animals were slaughtered and histopathological samples of kidney and liver were taken on the 20th day of experiment following necropsy. Samples kept separately in packages of formalin 10% and after numbering sent to laboratory for preparation of slides and painting with hematoxilin and eosin. In this stage slides were evaluated macroscopically at different magnifications.

Statistical Tests

The test results were analyzed with student t test.

RESULTS

Biochemical results completely listed in Table 1. The results indicated that Glu, Tp, Alb, BUN and aspartate aminotransferase (AST), do not show significant changes between treatment and control groups but fibrinogen (Fib) significantly increased on 10th day in treatment group (p<0.05). Mild histopathological changes in the kidneys and liver (mild hepatic fibrosis, lymphoeyctic hepatitis and interstitial nephritis) were observed in one case of treatment group (Fig. 1-3).

Urinalysis show no changes in total protein and urine cast in treatment and control group during experiment. But glucose was found in low concentration of 0.05±0.01, 0.17±0.03 and 0.16±0.06 mg dL$^{-1}$, respectively in 0, 10th and 20th days of experiment.
Table 1: Blood biochemical factors on zero, 10th and 20th day of experiment, in treatment and control group

<table>
<thead>
<tr>
<th>Biochemical factors in blood</th>
<th>Normal control (mmol)</th>
<th>Treatment (Day)</th>
<th>Control (Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(g dl⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (g dl⁻¹)</td>
<td>2.7-3.7</td>
<td>3.3±0.076</td>
<td>3.0±0.064</td>
</tr>
<tr>
<td>Fibrinogen (mg dl⁻¹)</td>
<td>100-600</td>
<td>236.67±62.62†</td>
<td>325.33±70.4†</td>
</tr>
<tr>
<td>Blood urea nitrogen (BUN) (mg dl⁻¹)</td>
<td>10.3±6.0</td>
<td>10.13±7.9†</td>
<td>11.35±6.3†</td>
</tr>
<tr>
<td>Aspartate ami no transferase (AST) (UL⁻¹)</td>
<td>49.0±12.3</td>
<td>155.58±52.382</td>
<td>152.33±18.15</td>
</tr>
<tr>
<td>Blood Glucose (GLU) (mg dl⁻¹)</td>
<td>44.0±8.1</td>
<td>33.3±6.8†</td>
<td>43.6±6.8</td>
</tr>
<tr>
<td>Hemoglobin (g dl⁻¹)</td>
<td>9.1±1</td>
<td>9.4±0.86</td>
<td>8.7±0.84</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>27.45</td>
<td>28.9±2.30</td>
<td>25.5±6.51</td>
</tr>
</tbody>
</table>

†: Significant differences (p<0.05)

Fig. 1: Mild liver fibrosis, Hematoxilin e Eosin (x100)

Fig. 2: Focal lymphocyte infiltration in liver, Hematoxilin e Eosin (x100)
DISCUSSION

Results showed low severity toxicity in our experimental sheep. Serum protein factors of experimental group such as TP and Alb in comparison with control group, showed slight decrease in 10th and 20th day of experiment (p<0.05), however, Yerulam et al. (1992) revealed decreases in total serum protein and albumin in oak toxicosis of cattle and Garg et al. (1992) announced hypoproteinemia in oak leaf poisoning of cattle.

Obviously increase of Fih, especially its significant rise in 10th day of experiment (p<0.05) is in accordance with Spin et al. (1987), that announced increase of Fih in oak poisoned cattle.

Based on the other findings (Daniels, 1976; Hesser et al., 1982; Wiseman and Thompson, 1984; Yerulam et al., 1998), the Blood Use Nitrogen (BUN), the most important parameter which highly increases in oak poisoning, did not increase in our study. In this study BUN and hemoglobin declined slightly but changes were not significant and blood Glu did not change significantly, however there is a significant reduction in blood hemoglobin (Garg et al., 1992).

Humble et al. (1998), revealed that The oak-fed calves developed the clinical signs and lesions characteristic of renal failure. Proteuria developed up to 72 h in calves. Both calves developed hematuria on day 4 and glucosuria on day 5. On the other hand in our study urine Glu and cast did not determined but Urine protein was distinguishable. Garg et al. (1992) also announced proteuria. Nevertheless AST of both control and treatment groups was over standard range and also it raised in treatment group, there was not any significant changes between them. Garg et al. (1992) announced and greatly increased activities of serum aspartate aminotransferase and Yerulam et al. (1998) revealed increase in aspartate aminotransferase (AST).

Although the most prominent reported macroscopic and microscopic lesion in oak toxicosis animals is interstitial nephritis (Nesser et al., 1982) and severe nephrosis, chronic interstitial nephritis and occasional intestinal ulceration (Yerulam et al., 1998), Garg et al. (1992) also announced extensive nephro and hepatotoxicity in the affected cattle due to hydrolysable tannins and simple phenols in the oak leaves (Garg et al., 1992). Sandikci et al. (1977), in Oak poisoning of cattle observed multifocal necrosis of the proximal convoluted tubules, which is a characteristic feature of this type of poisoning and Dixon et al. (1979), notified the main pathological findings in Acorn poisoning in cattle were severe nephrosis and some intestinal ulceration. But in our study histopathological lesions were limited to mild hepatic fibrosis, lymphocytic hepatitis and interstitial nephritis that only observed in one case of treatment group.
Although acorn poisoning is commonly seen in livestock, several factors such as species of acorns also have a distinct bearing on the degree of acorn toxicity (Basden and Dalvi, 1987). Therefore, on the basis of the results of this study, it is showed that the gradual use of acorn powder (Quercus bruni) as used in our study does not cause a real poisoning in sheep. High amount of oak is needed to be consumed over a long period of time to appear clinical signs. So farmers can apply these amounts gradually in short periods of time in livestock (sheep) rations instead of expensive rations (such as grain concentrates) without significant poisoning risk.

REFERENCES


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