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

[1]A. Derakhshanfar, [2]M. Pourjafar, [3]K. Badiei, [1]H. Talebanfard and [2]M. Shakhse-Niaie
[1]Faculty of Veterinary Medicine, Kazeroun Azad University, Kazeroun, Iran
[2]Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
[3]Faculty of Veterinary Medicine, Shiraz University, Shiraz, Iran

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 toxicosis (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 (Basden and Dalvi, 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 gallic 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 pathognomic (Yeruham *et al*., 1998).

Corresponding Author: Amin Derakhshanfar, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran Tel: +98 341 3222047 Fax: +98 341 3222047

Many scientists reported different aspects of oak poisoning in ruminants such as Rogosic *et al*. (2006), Yeruham *et al*. (1998), Garg *et al*. (1992), Spire *et al*. (1987), Neser *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 brantii*), 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 Vandenberg (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 hematoxiline 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, lymphocytic 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 days of experiment, in treatment and control group

Biochemical factors in blood	Normal content (Aiello, 1998)	Treatment (Day)			Control (Day)		
		0	10	20	0	10	20
Total protein (TP) ($g\,dL^{-1}$)	5.9-7.8	7.3±0.82	6.97±1.05	7.43±0.98	7.67±0.58	7.75±0.35	8.05±0.21
Albumin (Alb ($g\,dL^{-1}$)	2.7-3.7	3.33±0.76	3.03±0.64	3.13±0.64	3.4±0.14	3.28±0.29	3.35±0.14
Fibrinogen ($mg\,dL^{-1}$)	100-600	236.67±82.62↑	323.33±78.4↑	348.33±87.05	180	200±34.64	270.0±15588
Blood urea nitrogen (BUN) ($mg\,dL^{-1}$)	10.3-26.0	19.13±7.78	13.35±5.33	17.78±4.05	18.33±9.87	20.67±5.86	24±8.54
Aspartate amino transferase (AST) ($U\,L^{-1}$)	49.0-123.3	153.33±32.58	132.33±18.15	180±31.19	143.83±25.19	120.5±46.07	148.17±23.06
Blood Glucose (Glu) ($mg\,dL^{-1}$)	44.0-81.2	34.33±9.87	40.67±5.86	44±8.54	44.67±9.83	42±6.39	41.83±9.5
Hemoglobin ($g\,dL^{-1}$)	9-15	9.4±0.86	8.7±0.84	8.78±0.74	9.57±0.97	8.7±1.04	9.83±1.56
Hematocrit (%)	27-45	28±2.56	23.67±3.51	28±5.29	27.3±3.39	23±3.03	24±3.22

↑: Significant differences ($p<0.05$)

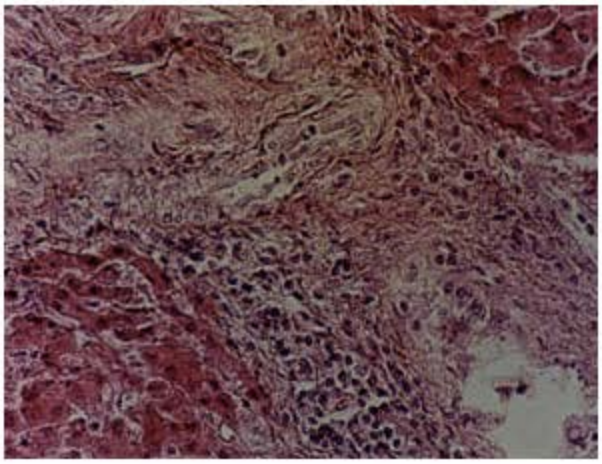

Fig. 1: Mild liver fibrosis, Hematoxiline and Eosin (x100)

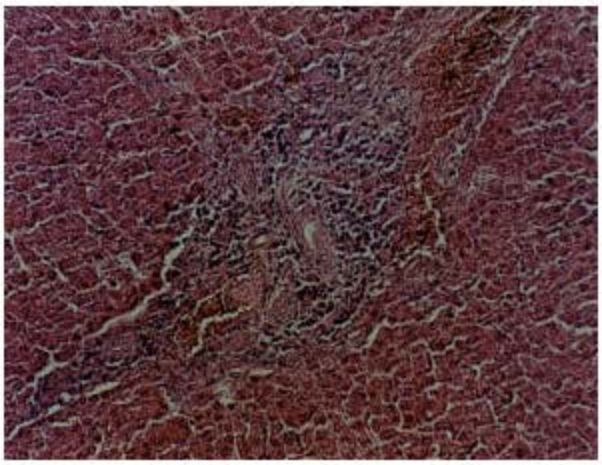

Fig. 2: Focal lymphocyte infiltration in liver, Hematoxiline and Eosin (x100)

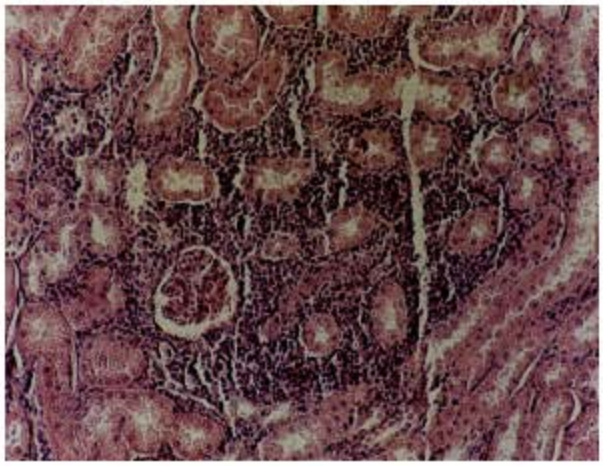

Fig. 3: Interstitial nephritis, Hematoxiline and Eosin (x100)

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, Yeruham *et al*. (1998) 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 Fib, especially its significant rise in 10th day of experiment ($p<0.05$) is in accordance with Spire *et al*. (1987), that announced increase of Fib in oak poisonous cattle.

Based on the other findings (Daniels, 1976; Neser *et al*., 1982; Wiseman and Thompson, 1984; Yeruham *et al*., 1998), the Blood Urea 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 reductions report in blood hemoglobin (Garg *et al*., 1992).

Plumlee *et al*. (1998), revealed that The oak-fed calves developed the clinical signs and lesions characteristic of renal failure. Proteinuria 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 proteinuria.

Nevertherless AST of both control and treatment groups was over standard range and also it rised 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 Yeruham *et al*. (1998) revealed increases in aspartate aminotransferase (AST).

Although the most prominent reported macroscopic and microscopic lesion in oak toxicosis animals is interstitial nephritis (Neser *et al*., 1982) and severe nephrosis, chronic interstitial nephritis and occasional intestinal ulceration (Yeruham *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). Sandusky *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 (*Q. brantii*) 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.

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