Case Report

A study on clinical and laboratory features of natural poisoning with Tribulus terrestris in sheep
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Abstract
Tribulus terrestris herb has been used as a drug in traditional medicine and its toxic effects have also been reported. Less attention has been paid to the interpretation of pathological and laboratory findings in the natural form poisoning with this plant in animals. Following a report of mortalities in a sheep farm feeding on a pasture containing Tribulus terrestris herb and involvement of 50 of the animals with similar clinical symptoms, one ewe with these signs i.e. inappetance, serosal nasal discharge, dehydration, severe mucosal and scleral jaundice and rumen impaction was evaluated. Serum biochemical profile and CBC tests revealed an increasing of total protein, blood urea nitrogen, creatinine, glucose, cholesterol, triglycerides, total bilirubin, magnesium, phosphorus values and enzymes activities (ALT, ALP, GGT, CPK) and leukocytosis, neutrophilia, lymphopenia. The presence of bilirubin, protein and blood in the urine was also confirmed by the urine strip. In macroscopic observations, severe pathologic jaundice in different parts of the carcass, rumen impaction with forage materials, petechial hemorrhages in the abomasal mucosa, necrosis and ulcerative lesions in the gallbladder mucosa, presence of pinpoint dark spots on the surface of the kidney, pulmonary edema and focal white deposits on the endocardial surface of the heart were seen. Microscopically, necrosis and degeneration of the hepatocytes and myocardium, acute tubular necrosis, deposition of eosinophilic structures in the renal tubules and hemosiderin pigments in its epithelium were observed. The presence of some toxins in the herb, especially steroidal saponins, is very important in inducing hepatorenal lesions in sheep.

Keywords: Clinical and pathological features, Natural poisoning, Tribulus terrestris, Sheep

Introduction
Tribulus terrestris (family of Zygophyllaceae) is an annual plant mainly distributed in both tropical and mild temperate region such as Khorasan province, Iran. It has prostrate hairy branches with opposite leaves, 5 stellately arranged fruits and yellow petals (Aslani et al., 2004; Adam et al., 2019, Reddy Yanala et al., 2016) (Fig 1). This plant is used in traditional medicine to treat infertility, urolithiasis and cardiovascular diseases in human. It has also some antibacterial, antispasmodic and cytotoxic activity against...
several bacteria, colic pain and cancer cell lines, respectively. The high content of steroidal saponins is a characteristic feature of this plant (Kostova & Dinchey, 2005; Hajhossein Talasaz, 2010). Metabolism of saponins in the forestomach of sheep by ruminal flora, generates epismilagenin, episarsasapogenin and some other metabolites. The crystals which deposit in biliary ducts as lithogenic saponines are the calcium salts of the β-D-glucuronides of epismilagenin and episarsasapogenin. Based on the geographical region of the plant and degree of its maturity, the concentration of lithogenic compounds is different (Miles et al., 1994; Kostova & Dinchey, 2005).

Consumption of *Tribulus terrestris*, particularly by the grazing of young wilted plants or sometimes hay, causes cholangitis and photosensitization in sheep. The plant toxicosis is also known as geeldikkop (yellow bighead), because of icterus and marked edema of ears and face. The disease has also been reproduced experimentally by oral administration of crude extracts of its compounds (Cullen & Stalker, 2015).

**Case description**

In the autumn of 2018, from a flock, consists of 500 sheep in the Sarakhs area (Khorasan Razavi province, Iran) with free grazing, one animal with the following symptoms was referred to the veterinary medicine teaching hospital, Mashhad, Iran. Twenty-five sheep with similar symptoms have already died. According to the livestock owner’s statement, the pasture were filled with *Tribulus terrestris* herbs and fewer *salsola kali*. A pregnant ewe with symptoms of staying away from the herd, loss of appetite, lethargy and recumbency was examined. In physical examination, serosal nasal discharge, severe jaundice of the sclera and mucous membranes of the eyes (Fig 2), as well as ruminal impaction with forage materials (completely firm in the palpation of abdominal cavity) were observed. The heart and respiratory rates were 96 and 22 min/minute; and the rectal temperature and ruminal movements were 38 °C and 0/2 min, respectively; also, dehydration (sunken eye and prolongation of skin tent duration, up to 10 seconds) and dyspnea was observed. The dairy man already used sodium bicarbonate (baking soda), liquid paraffin orally and sodium penicillin G (IM injection) for the treatment of affected animals.

The color of urine was dark yellow and the levels of bilirubin, protein and blood were 2 positive (++) in urine strip test. Microscopic examination of peripheral blood sample (ear vein) showed no infection of blood parasites. Blood samples without and with anticoagulant agents were sent to the lab to determine the serum biochemical profiles and Complete Blood Count (CBC) tests (Table 1). Because of poor prognosis, the sheep was referred for necropsy.

**Results**

The CBC revealed leukocytosis, neutrophilia and lymphopenia and in biochemical tests, an
increase in total protein, blood urea nitrogen (BUN), creatinine, glucose, cholesterol, triglycerides, total bilirubin, magnesium, phosphorus values; enzymes activity (ALT, ALP, GGT, CPK) were also observed (Table 1).

Macroscopic examination of the carcass revealed severe pathologic jaundice in the subcutaneous tissues, trachea, omentum, and large vessels including the aorta and pulmonary trunk. In the gastrointestinal tract and its appendages, there were erosions and petechial hemorrhage, especially in the mucous membrane of abomasum, esophagus and reticular folds. It is noteworthy that the presence of ulcerative lesions was observed in the gallbladder mucosa. Other observations were swelling and paleness of the kidney and the presence of pinpoint dark spots on its surface (Fig 3), focal white deposits on the endocardial surface of the heart and severe hyperemia and pulmonary edema.

Microscopically, necrosis and degeneration of the hepatocytes and myocardium, and also, hyperemia, ulcer, and necrosis of gall bladder mucosa associated with infiltration of inflammatory cells into its wall and a few yellowish colored crystalloid materials in the bile ducts were observed. In addition to acute tubular necrosis, eosinophilic spherical structures (similar to those of hemoglobinuria) were seen in the renal tubules and hemosiderin pigments in its epithelium, which may indicate some degrees of hemolytic crisis (Figures 4 & 5).

Table 1. Complete blood count and biochemical parameters in sheep affected with Tribulus terrestris poisoning

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
<th>Ref value</th>
<th>Parameter</th>
<th>Result</th>
<th>Ref value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV(%)</td>
<td>36.9</td>
<td>27-45</td>
<td>Albumin(g/dL)</td>
<td>2.9</td>
<td>2.4-3</td>
</tr>
<tr>
<td>Hb(g/dL)</td>
<td>12.1</td>
<td>9-15</td>
<td>BUN(mg/dL)</td>
<td>224</td>
<td>8-20</td>
</tr>
<tr>
<td>RBC(×10⁶/µL)</td>
<td>12.6</td>
<td>9-15</td>
<td>Creatinine(mg/dL)</td>
<td>2.7</td>
<td>1.2-1.9</td>
</tr>
<tr>
<td>MCV(fL)</td>
<td>29.3</td>
<td>28-40</td>
<td>Glucose(mg/dL)</td>
<td>120</td>
<td>50-80</td>
</tr>
<tr>
<td>MCH(pg)</td>
<td>9.6</td>
<td>8-12</td>
<td>Cholesterol(mg/dL)</td>
<td>291</td>
<td>52-76</td>
</tr>
<tr>
<td>MCHC(g/dL)</td>
<td>32.8</td>
<td>31-34</td>
<td>Total bilirubin (mg/dL)</td>
<td>20.02</td>
<td>0.1-0.5</td>
</tr>
<tr>
<td>Total WBC(×10⁶/µL)</td>
<td>24500</td>
<td>4000-12000</td>
<td>ALT(U/L)</td>
<td>60</td>
<td>5-20</td>
</tr>
<tr>
<td>Neutrophil (µL)</td>
<td>23765</td>
<td>700-6000</td>
<td>ALP(U/L)</td>
<td>652.4</td>
<td>70-390</td>
</tr>
<tr>
<td>Lymphocyte(µL)</td>
<td>490</td>
<td>2000-9000</td>
<td>GGT(U/L)</td>
<td>488</td>
<td>20-52</td>
</tr>
<tr>
<td>Monocyte(µL)</td>
<td>245</td>
<td>0-750</td>
<td>CPK(U/L)</td>
<td>4134</td>
<td>64-158</td>
</tr>
<tr>
<td>Platelets(×10⁵/µL)</td>
<td>3.1</td>
<td>2.5-7.5</td>
<td>Magnesium(mg/dL)</td>
<td>4.6</td>
<td>2.2-2.8</td>
</tr>
<tr>
<td>Total protein(g/dL)</td>
<td>8.8</td>
<td>6-7.9</td>
<td>Calcium(mg/dL)</td>
<td>9.6</td>
<td>11.5-13</td>
</tr>
<tr>
<td>Fibrinogen(mg/dL)</td>
<td>400</td>
<td>100-500</td>
<td>Phosphorus(mg/dL)</td>
<td>8</td>
<td>5-7.3</td>
</tr>
</tbody>
</table>

*Reference (Ref) value for triglyceride is taken from Kaisar Rahman et al.(2018) and others are obtained from the textbook of veterinary medicine (Constable et al., 2017).


**Discussion**

In intoxication with *tribulus terrestris*, a number of essential and most important metabolic pathways are simultaneously disturbed (Brown, 1963). The presence of some clinical and necropsy symptoms, including weakness, ruminal stasis, nasal discharge, tachycardia, dehydration, jaundice, urine discoloration, gallbladder necrosis, hepatorenal and cardiac lesions is consistent with the findings of experimental studies in sheep and goats (Aslani, 2004; Aslani, 2003). Recumbency may be due to the toxic effects of the plant as chronic, progressive, irreversible, locomotor disorder which commences as a bilaterally symmetrical, mild, flaccid paresis of the pelvic limbs which eventually becomes sever and asymmetrical and extends to the thoracic limbs (Bourke, 2006).

Sever neutrophilia, leukocytosis and lymphopenia maybe due to some pathologic phenomena such as necrosis, hemolysis, infarction and stress (corticosteroid effects) (Stockham & Scott, 2002; Smith, 2015).

Also, some biochemical parameters can be interpreted as follows: hyperglycemia may be due to the excitement or stress and is mediated by increases in catecholamine and glucocorticoid hormones. The high blood circulation in the kidney, makes it more exposed to toxins, as well as increased vulnerability to ischemia. In ruminants, creatinine is a more reliable indicator of renal failure than BUN. Nearly two thirds to three fourths of the nephrons must be nonfunctional before the serum creatinine level clearly exceeds the normal range. Dehydration, not only concentrates the toxin in the tubular filtrate and exacerbates nephrotoxicity, but also, increases BUN by decreasing glomerular filtration rate. Although, tissue necrosis can markedly upregulate C-reactive protein levels and cause hyperproteinemia, the ratio of total protein to fibrinogen (21) indicates the domination of dehydration rather than inflammation (Stockham & Scott, 2002; Smith, 2015).

Observation of proteinuria, hematuria, and casts are related to tubular necrosis (TN). The presence of blood in urine may be due to blood vessel damage by inflammation, trauma, or necrosis (Stockham & Scott, 2002; Smith, 2015) and haemosidrosis may be related to the haemolytic effects of the plant (Aslani, 2004).

In ruminants, kidney is a main organ in controlling magnesium excretion, so, hypermagnesemia may be related to TN. Reduction in phosphorus excretion by urine or saliva (during anorexia) and tissue hypoxia (due to pulmonary edema) may result in hyperphosphatemia. Hypocalcemia is also common in TN because of reduced calcium intake, GI stasis, urinary losses, and the competitive effect of hyperphosphatemia (Smith, 2015; Stockham & Scott, 2002). Other plasma electrolytes have also been studied and a tendency towards sodium retention during the first week of the intoxication has been recorded (Brown, 1963).
Icterus, elevation of serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), Gamma-glutamyl transferase (GGT) and total bilirubin as well as the histopathological lesions in the studied sheep indicated involvement and dysfunction in liver and biliary tract (Smith, 2015; Stockham & Scott, 2002).

Although in this intoxication, fragility of erythrocytes and low-grade intravascular hemolysis has been raised, but it seems that hepatic injury or biliary obstruction is a major cause of pronounced jaundice (Aslani, 2004; Brown, 1963; Smith, 2015). In large animal species, the enzymes of ALP and especially GGT are more indicative of biliary tract disease or proliferation and damage to the liver, so, increasing their levels are often associated with chronic liver disease, cholangitis and cholelithiasis. Impaired bile flow and accumulation of bile acids in hepatocytes due to intra or posthepatic cholestasis stimulate ALP production (Stockham & Scott, 2002). Hepatocytes of sheep have so little ALT, so damage to them due to a variety of insults e.g. toxicant and hypoxia may partially increase the serum ALT activity. CPK is highly sensitive and at the same time a specific indicator of muscle damage; therefore, an increase in CPK in serum of recumbent animals and sheep with myocardial lesions (as the patient here) is justified (Smith, 2015; Stockham & Scott, 2002). It is assumed that the saponins of this and other plants are toxic to the myocardium (Aslani, 2004; Aslani, 2003).

Plant saponins are metabolized in the rumen and liver to episapogenin glucuronides, which in the presence of calcium may precipitate, forming the characteristic biliary crystals. Cholestasis is likely related to reduced bile acid secretion into the lumen of the canaliculi, rather than biliary occlusion by these plant sapogenins. This explains the evidence of cholestasis before crystals can be seen histologically. Other unidentified plant components may also play a role in hepatocellular and biliary injury (Cullen & Stalker, 2015).

Although in this intoxication, the concentration of circulating phylloerythrin usually increases, no photosensitization was observed in the sheep. This may be related to the time of the animal referred (29 October) with low intensity of sunlight and without enough skin irradiation to cause photodermatitis as well as the brown skin color of the sheep (Aslani, 2004; Aslani, 2003).

Negative energy balance (NEB) caused by anorexia and lack of grain intake may be related to the increasing of blood cholesterol and triglycerides, because in order to offset the NEB, the animal must mobilize body fat and protein stores in the form of triglycerides and amino acids for gluconeogenesis (Smith, 2015). Hypercholesterolemia and hypertriglyceridemia are also recorded in horses with chronic renal failure, prolonged anorexia, hepatic lipidosis and severe physiologic stress (Smith, 2015).

In another study, a man who consumed the extract of the plant to prevent urolithiasis was
described and hepatotoxicity, nephrotoxicity (acute tubular necrosis), neurotoxicity as well as, elevation of serum ALT, creatinine and BUN concentration, mild proteinuria, sterile pyuria, haematuria, episodes of seizure, severe weakness in the lower limbs and thus poor appetite was recorded (Hajhossein Talasaz et al., 2010).

The first report of poisoning with T.terrestris was from sheep grazed in the suburbs of Saveh with symptoms such as photosensitization dermatitis, keratitis, jaundice increasing of body temperature (up to 39.5°C), pulmonary and nostril edema, photophobia, thickness of renal capsule and pinpointed brownish yellow bile pigments in hepatic cells. Location and season of grazing (summer) are possible causes of differences in the findings of the present study. Subtoxic amount of selenium in the diet has been considered to have a predisposing role in photosensitization by T.terrestris (Amjadi et al., 1977).

Figure 1. *Tribulus terrestris* herb (Photo is adapted from Reddy Yanala et al., 2016)

Figure 2. Pathological jaundice in the carcass of involved sheep
Figure 3. Large pale kidneys in the sheep involved with poisoning

Figure 4. Acute tubular necrosis and dilatation of the cortical tubules of the kidney (hematoxylin and eosin staining, scale bar 300 µm).
**Figure 5.** Acute tubular necrosis and the presence of eosinophilic structures in the epithelial cells and ducts of the cortical tubules of the kidney (hematoxylin and eosin staining, scale bar 50 µm).

**References**


