Experimental *Tribulus terrestris* Poisoning in Sheep:
Clinical, Laboratory and Pathological Findings

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**ABSTRACT**

Eleven native sheep, 1–2 years old, of both sexes were randomly divided into two groups, 6 sheep being allocated to the experimental group and 5 serving as controls. The sheep in the experimental group were fed 80% *Tribulus terrestris* and 20% alfalfa hay and wheat straw, while the control sheep were given a mixture of 40% alfalfa hay and 60% wheat straw. Clinical signs of hepatogenous photosensitivity were observed from day 11, including reddening and crust formation on the muzzle, nose, ears and eyelids, depression, weight loss, icterus, conjunctivitis, and yellow discoloration of the urine. Laboratory findings on weekly samples indicated significant differences (*p*<0.05) in white blood cell count, total plasma protein and fibrinogen, total and direct bilirubin, blood urea nitrogen and creatinine concentrations, and aspartate aminotransferase and alkaline phosphatase activities. There were no significant differences in the packed cell volume, in the neutrophil, lymphocyte or eosinophil counts, or in the serum calcium, phosphorus, potassium, sodium or chloride concentrations. At necropsy of the experimental animals, there were various degrees of generalized icterus and the livers were swollen and discolored by bile pigment. Histopathological examination revealed varying amounts of crystallloid material in the bile ducts and renal tubules, hepatocellular degeneration, biliary fibrosis and proliferation, renal tubular necrosis and focal necrosis of cardiac muscle.

**Keywords:** alkaline phosphatase, aspartate aminotransferase, bilirubin, caltrop, clinical signs, diagnosis, jaundice, photosensitivity, plasma protein, sheep

**Abbreviations:** ALP, alkaline phosphatase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; PCV, packed cell volume; WBC, white blood cells

**INTRODUCTION**

*Tribulus terrestris* (caltrop, puncture vine) is a prostrate annual herb belonging to the plant family of Zygophyllaceae and has a worldwide distribution. Under certain conditions, ingestion of this herb induces a hepatogenous photosensitization in sheep and goats, known as geeldikkop, tribulusis ovis or yellow big head (Henning, 1932; Van Tonder et al., 1972; Glastonbury et al., 1984). In Australia, a locomotor disorder of sheep has been reported to be associated with feeding of *T. terrestris* (Bourk, 1984; Bourk et al., 1992).
Tribulosis ovis has great economic importance in South Africa, as more than 0.5 million animals may be affected in a single season (Kellerman et al., 1996) and it has been intensively investigated in that country (Kellerman et al., 1980). It has also been reported from Australia (Bourke, 1983; Glastonbury et al., 1984; Jacob and Peet, 1987), the United States (McDonough et al., 1994), Argentina (Tapia et al., 1994) and Iran (Amjadi et al., 1977).

The disease is one of the photosensitization diseases of small ruminants, characterized by the deposition of optically active, birefringent, crystalloid material in the biliary system (Miles et al., 1994a). The occlusion of the bile ducts by crystalloid material results in failure of the liver to excrete phylloerythrin (a photodynamic porphyrin) in the bile, resulting in its accumulation in the blood (Kellerman et al., 1996). It has been suggested that the crystalloid-inducing factors in T. terrestris are the steroidal saponins dioxigenin and yamogenin, which are converted to epismilagenin and episarsasaponin following ingestion of the plant by ruminants (Miles et al., 1994a,b). It has also been shown that there is a variation in the chemotypes of saponins from T. terrestris collected from different regions (Wilkins et al., 1996).

The plant also contains a mixture of the β-carboline alkaloids: harmene, norharmene, tetrahydroharmene, harmine, harmaline, harmol, harmalol, ruin and dihydrorum (Bourke et al., 1990). It has been suggested that it is these alkaloids that cause a locomotor disorder in sheep associated with prolonged ingestion of large quantities of the plant (Bourke, 1987; Bourke et al., 1992).

Although T. terrestris grows in most regions of Iran, particularly in the central and north-east provinces, there is only one report of photosensitization in sheep associated with its ingestion (Amjadi et al., 1977). The plant is widely distributed in Khorasan and there have recently been a number of undocumented reports of photodermatitis thought to be due to grazing on the plant and observed by farmers and/or local veterinarians. The object of this study was to evaluate the ability of T. terrestris growing in Khorasan province to cause photosensitization in sheep and to describe the clinical, laboratory and pathological findings of the resulting disease.

MATERIALS AND METHODS

Animals

Eleven, 1–2 year old, native and apparently healthy sheep of both sexes were dewormed 14 days before commencement of the experiment by subcutaneous injection of ivermectin (Ivomec, Razak Co., Tehran, Iran) and oral administration of rafoxanide (Ranide, Damloran Co., Tehran, Iran) at dose rates of 0.22 and 7.5 mg/kg body weight, respectively. The animals were sheared and randomly divided into two groups, 6 sheep being allocated to the experimental group and 5 sheep serving as controls. The animals were placed in two adjoining pens without protection against the prevailing climatic conditions.
**Plant material**

*T. terrestris*, in the flowering to seeding stage, was collected in August 1998 in Sabzevar district of Khorasan province, air-dried, transported to the farm of the School of Veterinary Medicine of Mashhad and stored away from sunlight for use during the experiment.

**Haematology and serum biochemistry**

In both groups of sheep, weekly blood samples for haematological studies were taken from the jugular vein into vacutainers containing EDTA as an anticoagulant from day 0 to day 42. For the serum biochemical analyses, blood samples were collected at the same times into vacutainers and the serum was separated by centrifugation. Haematological measurements were made by standard manual methods (Dacie and Lewis, 1984) within 12 h of collection. Differential leukocyte counts were performed on routinely prepared Giemsa-stained blood films using the cross-sectional technique (Jain, 1993); 100 leukocytes were identified. Serum total protein was determined by refractometry (Erma Clinical Refractometer, Erma, Tokyo, Japan). Fibrinogen concentrations were measured by a heat precipitation method (Jain, 1986). The levels of blood urea nitrogen (BUN) were measured by the diacetyl monoxime method; creatinine by the Jaffé method; total and direct bilirubin by the Jendrassik method; calcium by an o-cresolphthalein method; inorganic phosphorus by the ammonium molybdate method; aspartate aminotransferase (AST) by the Reitman and Frankel method; alkaline phosphatase (ALP) by the modified Bowers and Mecomb method; and chloride by a colorimetric (mercuric nitrate) method using a spectrophotometer (Jenway 6105, Dunmow, UK). Sodium and potassium concentrations in serum were determined by a flame photometric method (Flame Photometer, PFP 7 Clinical, Jenway). All the enzymatic activities were measured at 37°C and the results are presented in U/L. All the biochemical procedures were conducted as described by Burtis and Ashwood (1994).

**Feeding regimes, clinical examination and sampling**

The sheep in the experimental group were fed *ad libitum* on an 80% *T. terrestris* and 20% alfalfa hay mixture with chopped wheat straw (40% and 60%, respectively), while the control sheep received only the same mixture of alfalfa hay and chopped wheat straw. These feeding regimes were continued for 6 weeks. The animals were closely observed twice daily for any signs of illness or behavioural changes. They were also examined clinically at weekly intervals for their vital signs and/or abnormalities.
Pathology

The sheep in the experimental group were killed by exsanguination and necropsied on day 42. Tissue samples were collected from the liver, gallbladder, kidney, urinary bladder, heart, lung, lymph nodes, spleen, rumen, abomasum, cerebrum, cerebellum, spinal cord, skin and small and large intestines. The collected tissues were immediately fixed in phosphate-buffered 10% formalin, embedded in paraffin wax, sectioned at 5 μm and stained with haematoxylin and eosin.

Statistical analyses

To evaluate the significance of the differences between the mean values observed during the experiment, statistical analyses were performed using Student’s t-test and analysis of variance (ANOVA) with the multiple range Duncan test; p < 0.05 was considered significant.

RESULTS

Clinical findings

The sheep in the experimental group showed different degrees of clinical manifestations. The first signs were pruritis on the face, mild reddening of the eyelids and a tendency to seek shade in one sheep on day 11 and in two other animals on day 13. During the third week, three other sheep in this group gradually developed clinical signs associated with photosensitivity, including a serous ocular discharge, conjunctivitis, icterus, erythema, crust formation on the muzzle, ears and eyelids, hyperpnoea, reduced appetite, depression, weakness, discoloration of the urine and weight loss. The severity of these signs differed, being most evident in three animals. No significant differences were observed between the two groups of sheep in body temperature or in their heart or respiratory rates during the experiment.

Laboratory findings

There were no significant differences in the PCV, neutrophil, lymphocyte, monocyte or eosinophil counts between the experimental and control groups. The total numbers of WBC were significantly increased (p < 0.05) in the experimental group in weeks 5 and 6. The total serum protein concentration increased gradually in the animals of experimental group from 64±4.6 g/L on day 0 to 78±6.4 g/L on day 42. The difference was significant (p < 0.001) by weeks 5 and 6. The plasma fibrinogen concentration also increased in the experimental group during the feeding of T. terrestris and there was a significant difference (p < 0.05) between the groups in week 6.
The serum AST and ALP activities increased in the animals in the experimental group during the experiment ($p<0.01$). Total and direct bilirubin concentrations also increased significantly ($p<0.05$) in the sheep in the experimental group. The serum bilirubin concentration was sufficiently elevated in some sheep to cause yellow discoloration of the serum samples. The serum creatinine concentration in the animals in the experimental group increased gradually during the experiment and there was a significant difference ($p<0.05$) in week 6. The BUN in the serum showed a more marked and earlier elevation and differences were significant ($p<0.05$) from week 3 to week 7 (Table I).

The concentrations of Ca, P, Cl, Na and K in the serum showed no significant differences between the experimental and control groups.

**Pathological findings**

At necropsy, all the animals in the experimental group showed various degrees of generalized icterus. Their livers were swollen and discolored by bile pigments. The gallbladders were enlarged and filled with concentrated bile. The kidneys were somewhat swollen and yellow to green in colour. Some animals showed dermatitis with facial scaling and crusts.

Histological examination of the livers revealed varying amounts of crystalloid materials in the bile ducts (Figure 1) and some hepatocellular degeneration. In several of the affected animals, there was also biliary fibrosis, bile duct proliferation and infiltration of mononuclear inflammatory cells. Other lesions included tubular necrosis in the kidneys, with the presence of crystalloid materials in the tubules (Figure 2), focal necrosis of cardiac muscle and epithelial necrosis of the gallbladder, accompanied by infiltration of mononuclear inflammatory cells. There was pustule formation, fibrino-purulent exudate, cell debris and infiltration of inflammatory cells in the dermis of the affected animals. No histological changes were found in other tissues.

**DISCUSSION**

It is clear that *T. terrestris* growing in Sabsevar district of Khorasan province of Iran is capable of causing hepatogenous photosensitization in sheep. Although there have been several reports of successful experimental induction of this disease by feeding *T. terrestris* to sheep, most experimental trials have failed to produce the disease (Henning, 1932; Van Tonder et al., 1972; Glastonbury et al., 1984; Jacob and Peet, 1987).

Biliary deposition of crystalloid materials is thought to be involved in the pathogenesis of poisoning by *T. terrestris* and it has been suggested that the factors responsible for the formation of such occlusive materials are the steroidal saponins present in the plant (Kellerman et al., 1991). A variety of saponins have been isolated from *T. terrestris*, including diosgenin, yamogenin, epimilagenin, tigogenin, neotigogenin, gitogenin and neogitogenin in the ratio 10:7:1:11:7:35:25 (Miles et al., 1994a). Evidence
<table>
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<th>BUN (mmol/L)</th>
<th>Creatinine (µmol/L)</th>
<th>Total bilirubin (µmol/L)</th>
<th>Direct bilirubin (µmol/L)</th>
<th>AST (IU/L)</th>
<th>ALP (IU/L)</th>
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<td>Day 0</td>
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<tr>
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<td>NS</td>
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<td>61.5 ± 15.4</td>
<td>80.7 ± 15.6</td>
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<td>Week 1</td>
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<td>4.82 ± 0.99</td>
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<td>99.01 ± 19.45</td>
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<td>84.86 ± 12.38</td>
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<td>3.93 ± 3.50</td>
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<td>Week 2</td>
<td>6.32 ± 1.5</td>
<td>4.46 ± 0.61</td>
<td>101.66 ± 18.56</td>
<td>110.5 ± 32.71</td>
<td>5.13 ± 4.10</td>
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<td>Week 3</td>
<td>8.96 ± 1.61</td>
<td>6.64 ± 1.21</td>
<td>97.24 ± 8.84</td>
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<td>Week 4</td>
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<td>4.78 ± 0.96</td>
<td>114.04 ± 21.22</td>
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<td>Week 5</td>
<td>10.75 ± 2.82</td>
<td>7.28 ± 1.04</td>
<td>115.90 ± 60.11</td>
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<tr>
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<td>NS</td>
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<td>7.28 ± 1.04</td>
<td>115.90 ± 60.11</td>
<td>80.44 ± 14.14</td>
<td>10.43 ± 6.67</td>
<td>2.74 ± 0.90</td>
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<td>Week 6</td>
<td>11.07 ± 3.99</td>
<td>7.32 ± 1.46</td>
<td>155.58 ± 51.27</td>
<td>84.86 ± 14.14</td>
<td>10.43 ± 6.67</td>
<td>3.42 ± 2.05</td>
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<tr>
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<td>NS</td>
<td>NS</td>
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<td>3.42 ± 2.05</td>
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NS, not significant; S, significant difference (p < 0.05, between groups)

*Significant difference with first sampling time (p < 0.05, within groups)
has also been presented that indicates that only saponins capable of being metabolized to epismilagenin and episarsasapogenin are lithogenic, and the biliary crystals in the sheep with experimentally induced geelddkop have been shown to be composed of a 6:1 mixture of the calcium salts of the β-D-glucuronides of epismilagenin and episarsasapogenin (Miles et al., 1994a,b). Accumulation of birefringent crystalloid material (microliths) is the most conspicuous histopathological feature of *T. terrestris* poisoning, and the principal occluding mechanism responsible for retention of phyloerythrin. An increase in the concentration of circulating phyloerythrin can result in photosensitization (Row, 1989).

On the other hand, both lithogenic and non-lithogenic populations of *T. terrestris* have been identified in South Africa, because of differences in the types of saponins they contain, and it is thought that this difference may be determined by genetic or environmental factors (Wilkens et al., 1996). Seasonal variations in the concentration of the saponins have also been reported in the leaf and stem of Chinese *T. terrestris* (Wang and Lu, 1991).

*T. terrestris* collected in August in Khorasan province of Iran could produce the disease and was lithogenic. Differing results in previous experiments (Henning, 1932; Van Tonder et al., 1972; Jacob and Peet, 1987) may have been due to seasonal variations in the saponins in the plants or to different chemotypic populations of the plant.
Although the findings, which included icterus, elevation of serum AST and ALP and of total and direct bilirubin, as well as the histopathological lesions in the sheep that had received T. terrestris, indicated liver lesions and particularly a biliary disorder, the clinical manifestations of photosensitization were generally mild in comparison to other reports (Van Tonder et al., 1972; Glastonbury et al., 1984; McDonough et al., 1994; Tapia et al., 1994). This may be related to the time of the experiment, which was performed in October and November, when there are shorter days and a lower intensity of sunlight. Such conditions may not have provided enough irradiation to cause severe photodermatitis. Natural cases of tribulosis ovis have usually been reported to occur in hot and sunny seasons (Van Tonder et al., 1972; Tapia et al., 1994; Kellerman et al., 1996). Moreover, the individual susceptibility of the animals and the varying levels of circulating photodynamic agents and bile pigments may have influenced the occurrence and severity of the disease. Various forms of the disease, from mild to severe, have been observed in both natural and experimental cases (Van Tonder et al., 1972).

The elevations in the concentrations of BUN and creatinine following feeding of T. terrestris are evidence of renal lesions, which is in agreement with previous reports (McDonough et al., 1994). From the histopathological findings, in addition to the hepatic lesions, there was renal tubular necrosis, with deposition of crystalloid
materials in the tubules. These findings are in agreement with those of other reports (Van Tonder et al., 1972; Amjadi et al., 1977; Bourke, 1983; Glastonbury et al., 1984).

Cardiac lesions have occasionally been reported in cases of disease induced by *T. terrestris* (Van Tonder et al., 1972) and in diseases caused by ingestion of other plants that contain saponins. It has been suggested that the saponins are toxic to the myocardium (Dollahite et al., 1977; Bridges et al., 1987).

Further studies are necessary to determine whether there are natural cases of *T. terrestris* poisoning in Iran and its economic importance.

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