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PHOTOSENSITIZATION IN SHEEP ASSOCIATED WITH INGESTION OF *TRIBULUS TERRESTRIS* IN AL-NAJAF DESERT, IRAQ

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Abstract

This study was conducted to investigate one of the most issues in Iraqi Awassi sheep by ingesting of *Tribulus terrestris* that disseminated in the Al-Najaf Desert. The Iraqi Awassi sheep is one of the most popular and economically important sheep in Iraq and the Middle East which survive on the low nutrition level. Nutrition also remains one of the major constraints to livestock production in the tropics, particularly the lack of protein during the dry season. *Tribulus terrestris* (Qutub) belongs to family Zygophyllaceae, it is a herbaceous, mat forming plant in nature and grows in warm dry tropics all over the world. A total of 75 blood samples were collected from jugular vein and placed into EDTA and plain tubes. Samples were divided in to two groups depending on the clinical signs, 45 sheep were clinically healthy and 30 sheep possessed clinical signs that are suggestive of photosensitization in Al-Najaf governorate, Iraq. The results showed that the total blood analyses, there were a significant increase ($P > 0.05$) in total leukocyte count and neutrophils, while, there was no significant difference in total red blood cells, PCV, Hb, lymphocytes, and eosinophil of affected sheep compared to the control. Furthermore, affected sheep had significantly higher levels of aspartate aminotransferase (AST), alkaline phosphatase (ALP), sorbitol dehydrogenase (SDH), total protein, total bilirubin, and serum urea nitrogen. In conclusion, the present study recorded that hematological, biochemical and pathological changes related with ingestion of toxic plant *Tribulus terrestris* in Al Najaf desert of Iraq, which causes photosensitization in affected sheep.

Keywords: Sheep, Hematological parameters, Biochemical parameters, *Tribulus terrestris,* photosensitization.

Introduction

Photosensetization is a condition makes the skin strangely sensitive to bright sunshine after the herd eat certain toxic plants, which causing skin damage. Many Photosensitization diseases in livestock cause animal welfare problems, and significant economic losses as a result of weight loss, udder lesions and secondary infections, particularly when it occurs in groups of animals. It is defined as hypersensitivity reaction of non-pigmented and short-haired regions due to photodynamic agents that found in it. It causes great economic losses (Pollock *et al*., 2015a). Furthermore, photosensitization is currently classified depending on the source of the photodynamic agent to type I photosensitivity (primary), type II (hepatogenous) photosensitivity and type III photosensitivity, (aberrant endogenous pigment synthesis) (Hussain *et al*., 2018). Liver is damaged by toxins, infectious agents or neoplasms, which lead to Hepatogenous photosensitization, so that phylloerythrin cannot be sufficiently excreted by the liver. Subsequently, the photodynamic agent phylloerythrin levels increase in the blood and then accumulate in the skin (Quinn *et al*., 2014). *Tribulus terrestris* (commonly known as puncture vine, caltrop, and called "Qutub" in Arabic) is a drought-tolerant, summer growing annual herb with prostrate hairy structure belong to the family of Zygophyllaceae (Al-Bayati, 2008, Shishovska, 2015). It is widely distributed in tropics and subtropics regions all over the world (Tahseen and Mishra, 2013, Chhatre *et al*., 2014), as well as, the *Tribulus* is common distributed in forest zone of Iraq (Sulaimaniya, Mousil, Rutba and Habbania) and the desert of Al-Najaf (Evans, 2009, Qasem, 2014). Under certain circumstances, grazing on *Tribulus terrestris* causes a hepatogenous photosensitization in sheep and goats known as Geeldikkop, yellow big head, or Tribulosis ovis (Aslani *et al*., 2004). Because of steroidal saponin content of *Tribulus terrestris* it lead to hepatotoxicity, causes an outbreak of sporadic hepatogenous photosensitization in small ruminants (Ocal *et al*., 2013b). Therefore, a photosensitization case should be properly diagnosed whether or not the case is by measuring the levels of bilirubin, liver enzymes and bile acids (Ocal, 2013). On the other hand, *Tribulus terrestris* is a medicinal and pharmaceutical interest as it contains a number of steroidal saponins which may account for its use in muscle growth, health status and treatment of certain ailments (Hashim *et al*., 2014; Daniel, 2016). The extract is also used to treat the urinary dysfunction, asthma, antihypertensive, and vaso-dilatory properties (Hashim *et al*., 2014; Abubakar *et al*., 2016). Moreover, *Tribulus terrestris* is extremely rich with substances which have potential biological significances in addition to saponins, which includes: flavonoids, unsaturated fatty, alkaloids, acids, vitamins, and tannins (Heidari *et al*., 2007; Grigorova *et al*., 2017; Khazaei *et al*., 2018). Therefore, the toxicity occurs during the summer when young plants become wilted; in particular, when the rain and / or the humidity are increased and followed by dry hot climate (JA, 2002). According to our knowledge, there was no any study showed to examine the relationship between photosensitization in sheep and ingestion of *Tribulus terrestris* growing in Al-Najaf province desert. The objectives of this study were to evaluate the hematological, pathological and biochemical changes in sheep affected with *Tribulus terrestris* plants toxicity that disseminated in the Al-Najaf desert, and to compare these changes between the normal and affected groups.

Materials and Methods

Plant Collection

The plant is well-known in pastoralism, spreading in Al-Najaf desert and locally called "Qutub" (Figure 1). It was collected and authenticated from the Herbal Center for

scientific identification in Department of Physiology and Pharmacology, Faculty of pharmacy, University of Kufa, Iraq. Also has been classified scientifically (*Tribulus terrestris*) and certified by the Iraqi State Board for seed testing through isolation of saponin from the plant by a method of HPLC analysis which was performed on a Waters HPLC system (Angstrom advanced Inc.).

Fig. 1 : *Tribulus terrestris* plant.

Blood Collection

A total of 75 blood samples were collected from two groups of animals, including 45 normal and 30 affected sheep. Allocation of groups was based on the presence or absence of the characteristic clinical signs (The location and appearance of skin damage and shade-seeking behavior), during the summer season of sheep with different ages. All samples were obtained from AL-Najaf desert of sheep with the same managemental and environmental conditions.

The blood samples were taken by syringe with a vacutainer tube with EDTA from jugular vein of the sheep, and each sample was divided into two parts, whole blood sample (for hematologic tests) and plasma sample (for biochemical tests).

Blood samples in plain tubes were centrifuged at 3000 rpm for 15 minutes for serum biochemical analyses, and then the serum was harvested according to standard methods of (Kaneko *et al*., 2008). All the samples were stored at -20 ºC for analysis.

Evaluation of Blood Film

The blood was used directly for detecting the complete blood count (CBC). Red blood cells and white blood cells counts were evaluated using hemocytometer method according to (Thrall *et al*., 2012). Packed cell volume (PCV) was measured using micro-hematocrit centrifuge according to (Thrall *et al*., 2012). The Hb was converted into cyanmethaemoglobin using drabkins reagent and measured by spectrophotometer (Weiss and Wardrop, 2011). Blood films were prepared and stained using Giemsa stain for differential leukocyte count DLC according to (Weiss and Wardrop, 2011) in which 100 leukocytes were used for DLC.

Biochemical Analyses

Serum samples were tested for biochemical analysis of aspartate aminotransferase (AST), Sorbitol dehydrogenase (SDG), alkaline phosphatase (ALP), blood urea nitrogen (BUN), total bilirubin and total protein concentrations, by using spectrophotometer with commercial chemical kits supplied by Randox lab. LTD.

Statistical Analysis

Data are expressed directly for calculation of group means and standard errors of the means. The least significant differences test (LSD) was used to determine differences among groups, which performed by one-way analysis of variance (Bulashev *et al*., 2017) and Duncan range test.

Results of statistical analyses were considered significant if they produced values of $p < 0.05$). The computerized SPSS version 21 was the software that used for calculations and statistical analyses of data.

Results

Clinical Findings

The gradually developed clinical signs restricted to areas of skin unprotected from sunlight represented by erythematous and edematous with exudation and crust formation of the affected area as ear, eye lids, lips, and extremities. Photophobia, fever. The progressed lesions include sloughing and ulceration of the skin over the ear pinna, face, muzzle, and extremities (*photodermatitis*). Loss of lips and ears. Serous nasal discharge, discoloration of the urine and black colored diarrhea. No significant differences were observed between the two groups in relation to heart rate and respiratory rates.

Clinical Pathology

At necropsy, the liver of sheep was moderately enlarged, greyish-brown slightly sunken areas of variable size were scattered throughout the parenchyma. The lobulation in these areas were more distinct than elsewhere. In addition to mild edema of the gall bladder wall, contained a small amount of dark-green bile in which fine chalky-white sediment was suspended. Apart from slight swelling and yellowish-brown discoloration of the kidneys, no other macroscopic lesions were seen in other organs and tissues.

The results of hematological parameters were presented in (Table 1) which revealed a significant increased ($P<0.05$) in white blood cell count and neutrophil. While there were no significant differences ($p\cdot 0.05$) in red blood cells, packed cell volume, hemoglobin, lymphocyte, monocyte and eosinophil in affected sheep compared with normal sheep.

The results of biochemical parameters are presented in which revealed that a significant increased ($P<0.05$) in total plasma protein, total bilirubin, blood urea nitrogen, aspartate aminotransferase, alkaline phosphatase (ALP) and Sorbitol dehydrogenase (SDH) in affected sheep compared with normal sheep.

Table 1 : The hematological parameters for normal and affected sheep; ranges and means+ SE.

Parameters	Groups	
	Normal Sheep No.45	**Affected Sheep No.30**
RBC ($10\times^{6}/\mu$ L)	8.88-14.20 10.98±0.20 A	8.89-13.32 10.36±0.21 A
PCV (%)	28 – 42 33.84±0.51 A	28 – 38 31.36±0.41 A
Hb (g/dL)	8.9-13.9 11.0±0.21 A	8.6-13.2 10.12±0.20 A
WBC (/ μ L)	3950-13000 10882±294.48 B	9050.- 23550 13783±598.40 A
N%	21.5-58.5 40.8±1.41 B	35.5-73.6 50.78±1.57 A
L%	37.5-72.5 56.45±1.43 A	32.0-71.0 52.10±1.82 A
E%	0-9.0 2.35±0.32 A	0-9.0 2.11±0.34 A
M %	0.5-5.0 1.54±0.17 A	0.5-6.0 2.21±0.24 A

The differences in capital letters horizontally refer to the presence of significant value at (<0.05).

Table 2 : The biochemical values in normal and affected sheep; range and mean +SE.

Parameters	Groups	
	Normal Sheep No.45	**Affected Sheep No.30**
TP (g/dl)	5.37-9.31 7.06±0.17B	8.18-12.85 10.12±0.24 A
BUN (mmol/L)	4.31-11.18 7.59±0.25 B	9.90-13.64 12.06±0.18 A
TB (mmol/L)	1.55-8.59 5.06±0.27B	8.80-13.91 11.26±0.25 A
SDH U/L	9.50-29.40 20.19±0.94 B	28.80-35.20 31.68±0.34 A
AST U/L	90.19-240.29 142.33±5.79 B	201.33-306.45 288.77±3.33 A
ALP U/L	100.69-299.66 178.63±7.35 B	299.78-402.97 391.68±3.27 A

The differences in capital letters horizontally refer to the presence of significant value at (<0.05).

Discussion

In general, when green plant material is eaten (which contain chlorophyll substance materials), it will break down into phylloerythrin by the action of microorganisms of the rumen (Pollock *et al*., 2015b). when it absorbed into the hepatic circulatory system, it will excreted by the bile duct and gall bladder (Bischoff *et al*., 2018). It is believed that toxins from further possible causes destroy the liver cells and impede the secretion of phylloerythrin, leading to collection in the general circulatory system, and in exposed areas produces free radicals that can damage the membranes of cells (Baird, 2000).

Although, the plant *Tribulus terrestris* is a nutritious semi-annual prostrate herb which sporadically becomes toxic to sheep under certain conditions, in etiolate young plants through warm dry period (Rivero *et al*., 2011). Also, all hepatotoxins may cause secondary photosensitization in ruminants due to an alteration in the metabolism of chlorophyll leading to skin damage when ruminants are bared to the sun (Ocal, 2013).

However, ovine photosensitivity due to ingestion of *Tribulus terrestris* is a great economic importance in desert Al- Najaf of Iraq due to steroidal sapogenin toxins that found in the plant which cause liver damage and increase levels of phylloerythrin in circulation and eventually in skin and subsequent excitation of it under the effect of sunshine result in clinical case of the syndrome (Talasaz, 2010; Pollock *et al*., 2015b*).* Moreover, it can cause neurotoxicity and renal toxicity in ruminant animals which known as Geeldikkop and Staggers.

No significant differences ($p < 0.05$) were found in the red blood cell, packed cell volume, hemoglobin, lymphocyte or eosinophil counts. In addition, there were a significant increased ($p < 0.05$) in a total white blood cell count and neutrophil in affected animals compared with healthy animals, these results agree with Kellerman *et al*., 1980 and Aslani *et al*., 2004 (Kellerman, 1991; Aslani *et al*., 2004) (Table1).

The significant increase ($p < 0.05$) in body temperature, total leukocyte counts and neutrophils were most possibly associated with the inflammatory process in photosensitization. Leukocytosis is frequently occurred in infections or during the inflammatory processes (Ocal, 2013).

Biochemical values indicated that the significant increase ($p < 0.05$) of Sorbitol dehydrogenase (SDH) and aspartate aminotransferase (AST) actions were harmonic with hepatocellular disease. Furthermore, increased alkaline phosphatase (ALP), total bilirubin, point out cholestasis. Moreover, a significant rise in total protein ($p < 0.05$) was most likely due to dehydration, as well as mildly raised serum urea nitrogen was considered a relative increase representative prerenal azotemia associated with dehydration and these results agree with Ocal *et al*., 2013 and Aslani *et al*., 2004 (Aslani *et al*., 2004; Ocal *et al*., 2013a).

Finally, according to our knowledge, this was the first study on photosensitization in sheep association with ingestion of *Tribulus terrestris* in Al Najaf desert in Iraq. Veterinarians should be aware not only with those plants commonly associated with outbreaks of the disease in the desert Al-Najaf but also with less well-known plants that may also be in the environment.

Conclusion

The present study recorded a leukocytosis, neutrophilia, increased liver enzymes, and some pathological changes such as enlargement and discoloration of liver and kidney, and edema of the gallbladder wall, with chalky-white sediment.

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