Clinical and Pathological Investigations of Accidental *Catharanthus roseus* Toxicity in Sheep

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ABSTRACT

Clinical signs, blood parameters, postmortem and histopathological findings in sheep that were accidentally poisoned with *Catharanthus roseus* are described. Affected animals (40 sheep) showed acute clinical signs such as salivation, dyspnea, anorexia, bloody diarrhea and dehydration. Blood samples were collected from 5 sheep prior to death. Marked increase in Activated Partial Thromboplastin Time (APTT), D-DIMER, hemoglobin, urea and creatinine levels with the decrease in cholinesterase activity and calcium levels were observed in the blood parameters of affected sheep. Necropsy examination was performed in17 sheep and all organs were examined pathologically. According to blood parameters and pathologic findings, Disseminated Intravascular Coagulation (DIC) was observed in different organs supported by the increases in APTT, Prothrombin time (PT) and D-dimer levels in blood. Grossly, linear mucosal hemorrhages in the jejunum and ileum, auricular petechial hemorrhages in the heart were common findings. The livers were pale and friable. Microscopically, the fibrinous exudate consisting of fibrin networks were seen in the lumen of jejunum and ileum. In addition, clumps of fibrin in many small vessels were noted in the brain, lungs, liver, kidneys and intestines. Focal periacinar and mid-zonal necrosis with hemorrhages were observed in the liver. Marked tubular necrosis was seen in the kidneys.

Keywords: *Catharanthus roseus*; Pathology; Blood Parameters; Accidental Toxicity; Sheep

INTRODUCTION

Catharanthus roseus (Madagascar periwinkle) or previously known as *Vinca rosea* is a herb of the *Apocynaceae* family with green leaves and pale pink to white flowers (1-2). The plant contains alkaloids such ascartharathine, lochnenine, vindoline, vindolinenine, vincristine, vinblastine, tetrahydroalstronine, reserpine and serpentine (3). Especially, vincristine and vinblastine are used as chemotherapeutic drugs for some forms of cancer such as lymphomas and testicular carcinoma (4). These drugs can induce axonal degeneration in cancer patients (5). In addition, the organic extract of *C. roseus* is used in some countries for treatment of diabetes, malaria, wasp stings, sore throat, eye irritation, low blood pressure, insomnia and infections (2). Some cases of neurotoxicity have been previously reported with the use of vincristine in patients with acute lymphoblastic leukemia (6). According to another report the use of vincristine at normal dosage resulted in the development of peripheral neurotoxicity in ten out of 20 children. In these patients, weakness of lower limbs, areflexia, neuropathic pain, sensory loss, constipation, urinary retention, seizures, confusion, aphasia, transient blindness and malnutrition were observed (7). In vincristine toxicity,

neuropathy of peripheral nervous system was reported in cats and dogs, however, bone marrow, kidney and gastrointestinal tract are also affected (5). Recently, toxicity studies with the use of *C. roseus* have been reported in experimental animals. In a study, remarkable liver damage was noted in rabbits after taking 0.1 g/kg of aqueous leaf extract of *C. roseus* for 9 days (8). In another study, the oral LD_{50} value of methanol leaf extract of *C. roseus* was reported as 2.1 g/kg in mice (9). Prolonged treatment of repeated doses of 0.5 g/kg and 1.0 g/kg extract of *C. roseus* cause diarrhea and mortality in rats (10).

Up until now, there have been no studies documenting poisoning due to accidental ingestion of *C. roseus* in animals. To the best knowledge of the authors, this is the first study to investigate the clinical signs, blood parameters and pathologic findings resulting from accidental acute poisoning by *C. roseus* in sheep.

MATERIALS AND METHODS

In November 2012, the Burdur Municipality located in the southwest of Turkey cultivated flowers of *C. roseus* in the city gardens. After the flowers had faded, they were collected from gardens by municipal gardeners. Due to the lack of fresh grass in the winter season in Burdur, animal owners usually feed their animals with these collected green plants. These collected plants were fed ad *libitum* to sheep without knowing that the plant was poisonous. Feeding of the leaves and flowers of *C. roseus* to a flock of 40 sheep (31 female and 9 male) caused an acute toxicosis within 24 h of ingestion of the plant with all animals manifesting salivation, incoordination, staggering, recumbency, dyspnea, anorexia, bloody diarrhea and dehydration. All the sheep died within two days after the start of the signs.

For diagnostic purposes, 17 of the 40 sheep carcasses were presented to Department of Pathology of the University of Mehmet Akif Ersoy, Burdur-Turkey. In addition, specimens of the plants were collected for identification by the director of Municipal gardens. Necropsy was performed on all presented sheep and all organs were examined grossly. Tissue samples were taken from the organs and fixed in 10% buffered formalin. Using standard methods, tissues were blocked in paraffin and cut to 5μm thickness. Sections were stained with hematoxylin and eosin (H&E) and examined microscopically.

After the death of 10 animals, the farm was visited and blood samples were collected from 10 live affected animals. MS9 blood counting equipment (Melet Schloesing Laboratories, France) was used for hematological analysis of the blood drawn in EDTA tubes. Serum biochemical analyses were made using by Dade Behring (Dade Behring/ Dimension RXL MAX, Germany) equipment and reagents. In order to measure PT (Prothrombin time) and APTT (Activated Partial Thromboplastin Time) levels, 9 ml blood from each animal was collected in 1ml 3.8% sodium citrate containing tubes and centrifuged at 1500 x g for 15 minutes. The plasma samples were separated from blood within 30 minutes, followed by blood collection. APTT and PT were measured by Diagnostica Stago STA analyzer (USA).

Gesan Chem 200 autoanalyser (Campobello-Italy) was used for detecting serum cholinesterase levels by Bireagent-cholorimetric method.

RESULTS

C. roseus was identified by its characteristic flowers and leaves (Figure 1). The blood parameters such as APTT, D-DIMER, hemoglobin, urea and creatinine levels showed a marked increase. In addition we found a severe decline in cholinesterase activity and calcium levels in the blood of affected sheep (Table 1.)

At the clinical examination, salivation, incoordination, staggering, recumbency, dyspnea, anorexia, bloody diarrhea and dehydration were the common clinical symptoms.

At necropsy the affected sheep were found to be in good body condition. Poorly masticated leftovers of leaves of

Figure 1: The plant *C. roseus*.

Table 1: Blood parameters of sheep poisoned by *C.roseus*

Parameters	Sheep Number					References Values (11)
	1	2	3	4	5	
APTT (s)	58.7*	64.5*	70.6*	44.4*	61.1*	25.92
PT (s)	51.7*	41.2*	51.9*	42.8*	45.9*	23.29
Fibrinogen (mg/dL)	378	220	454	248	350	200-500
D-DIMER(ng/mL)	953*	381*	781*	681*	316*	162-222
Cholinesterase (nmol/min/ml plasma)	350*	361*	377*	287*	475*	514-1465
ALT (IU/L)	39*	12	22	14	62*	22-38
ALP (IU/L)	350	96	99	191	231	70-390
AST (IU/L)	220	161	203	143	139	60-280
GGT (IU/L)	55*	54*	39	42	56*	20-52
Hemoglobin (g/dL)	17.2*	16.8*	15.5*	17.8*	15.8*	9.0-15.0
PCV (%)	29.6	38.36	37.63	35.12	38.17	27-45
RBC ($x10^{12}$/L)	9.31	13.15	12.43	10.22	12.96	9.0-15.0
WBC ($x10^{9}$/μL)	6.9	6.8	5.5	4.0	4.2	4.0-12.0
PLT ($x10^{9}$/μL)	7.5*	66*	77*	112*	99*	250-750
Urea (mg/dL)	666*	828*	720*	576*	540*	72-122.4
Creatinine (mg/dL)	3.51*	6.76*	4.69*	2.04*	2.25*	1.2-1.9
T Bil (mg/dL)	0.10	0.11	0.13	0.12	0.15	0.1-0.5
D. Bil (mg/dL)	0.25	0.12	0.14	0.12	0.21	0-0.27
Ca (mg/dL)	4.15*	8.72*	6.34*	7.47*	3.25*	11.5-13.0
Mg (mg/dL)	2.25	2.98	3.53	2.63	2.12	2.2-2.8
P (mg/dL)	2.20	4.41*	5.86	2.58*	3.11	5.0-7.3
Sodium (mEq/L)	110*	142*	126*	111*	141*	145-152

* Abnormal results

C. roseus were found in their mouths. Some carcasses showed early and rapid autolytic and putrefactive changes especially of the liver, brain, spleen and kidneys. Lesions were most severe in the gastrointestinal tract. The main macroscopic finding of gastrointestinal tract was linear mucosal hemorrhages in the jejunum and ileum together with watery to mucoid diarrhea with or without blood. Masses of fibrin were present in the form of cylindrical casts in the intestinal lumen which tended to cause complete obstruction (Figure 2). The gut mucosa was hyperemic and edematous. Externally, the serosa of the affected portion of ileum and jejunum was red in color and blood vessels were congested. Severe hyperemia was noted in the brain. Marked auricular petechial hemorrhages were seen in the hearts of five of the sheep examined. The lungs were dark purple-red in color, swollen and oozed a large amount of sero-sanguineous fluid from the cut surface in six of the sheep. The livers were enlarged, pale and friable in all affected sheep. Pale and swollen kidneys were commonly observed.

At the microscopic examination, the fibrinous exudate in the lumen of jejunum and ileum was found to consist of a fibrin network with varying quantities of mucus, neutrophils and desquamated epithelial cells. Clumps of fibrin

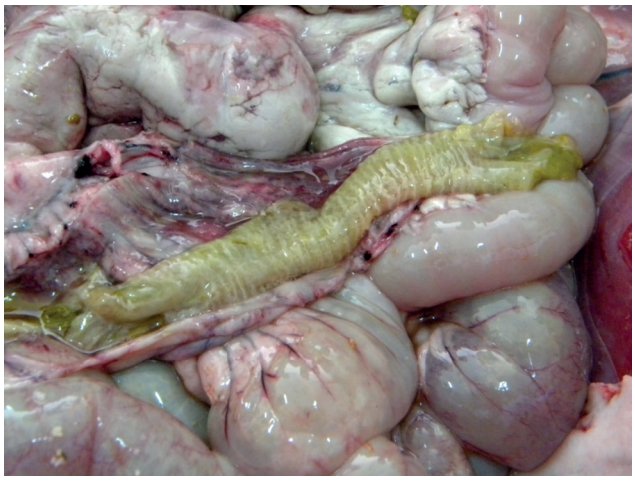

Figure 2: Fibrin masses in the intestinal lumen which blocked the lumen.

were present in the brain, lungs, liver, kidneys and intestines in numerous small vessels. Many arterioles and capillaries contained clumps of fibrin without evidence of organization or invasion of fibroblasts.

Focal periacinar and midzonal necrosis and fatty degeneration with hemorrhages were observed in the liver (Figure 3). The capillaries in the alveolar walls were congested with the alveoli filled with eosinophilic material characteristic for pulmonary edema (Figure 4). Hyperemia, edema and slight mononuclear cell infiltrations consisting of lymphocytes, plasma cells and macrophages with extensive hemorrhages of the villi, propria mucosa and submucosa were observed in the other parts of intestines.

Acute tubular necrosis was commonly seen in the kidneys. The presences of pyknosis and karyolysis with intense eosinophilic homogenous cytoplasm in the tubular epithelium are regarded as a characteristic feature for acute tubular necrosis. Lumens of tubules contained necrotic cellular debris and proteinous materials (Figure 5).

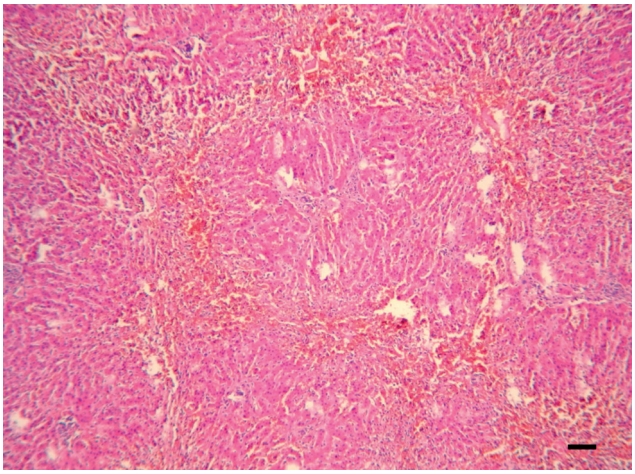

Figure 3: Periacinar and midzonal necrosis with hemorrhages in the liver. HE, bar = 30 µm.

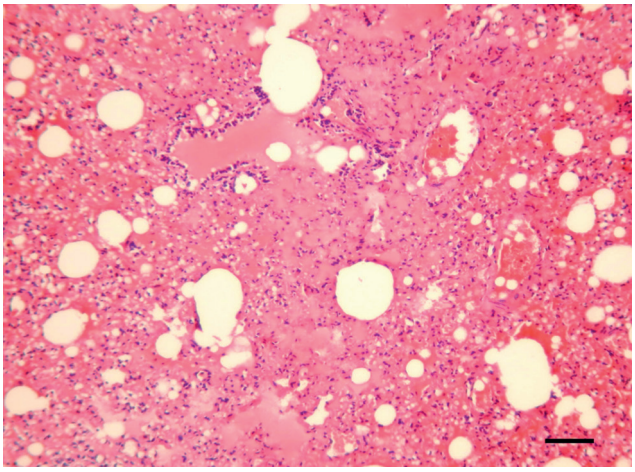

Figure 4: Eosinophilic material in lungs that characteristic for pulmonary edema. H&E, bar = 50 µm.

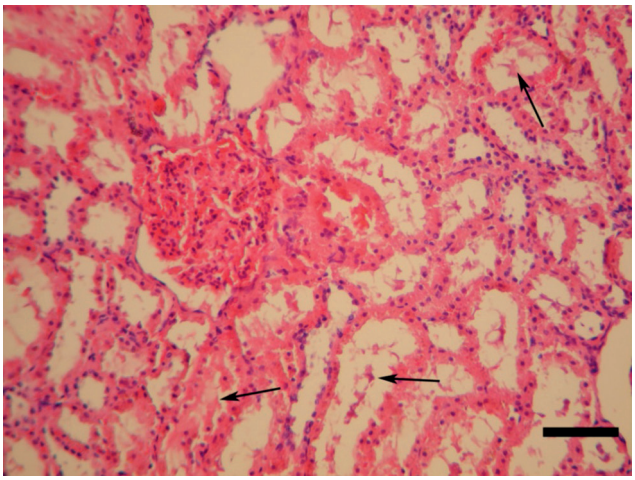

Figure 5: Histopathologic appearance of acute tubular necrosis in kidney, proteinous materials in tubules (arrows). H&E, bar = 50 µm.

DISCUSSION

C. roseus is a medicinal plant from which secondary metabolites are used for treatment of certain diseases. One hundred and thirty alkaloids can be found in this plant and these may cause toxicities in humans and animals in the course of treatment (2, 12).The alkaloid of *C. roseus*, vincristine cause vincristine neuropathy and also affect other organs and systems among them bone marrow, kidneys and the gastrointestinal tract (5). A report documents a 67-year-old woman with hepatitis C-related liver cirrhosis and hepatoma treated with *C. roseus* as an alternative anticancer treatment which caused severe bone marrow suppression. Vomiting, diarrhea, oral ulceration, severe gastrointestinal disturbances, bacteremia, urinary tract infection and fever were noted about 1 week after a 5-days' course of *C. roseus*. In addition,bone marrow biopsy material showed rapid autolysis (13). In the present study, early and rapid autolytic and putrefactive changes were observed in some carcasses. This condition was thought to be mainly due to bacteremia. In addition, some gastrointestinal tract disturbances such as linear mucosal hemorrhages, bloody diarrhea, dehydration, masses of fibrin in the lumen of intestines were observed.

Vincristine neuropathy of the peripheral nervous system

has been reported in vincristine toxicity (*C. roseus*) especially in cats and dogs (5). In this study, poorly masticated remains and leaves of *C. roseus* were found in the mouth of most sheep the digestion of which may indicate the possibility of neuropathic effects of this plants alkaloids in the sheep (14).

A histological study to ascertain the toxic effect of *C. roseus* on liver and kidneys in New Zealand rabbits revealed centrilobular hemorrhagic necrosis in the liver and glomerulonephritis, without cardiac pathology (8). Generally, the toxic effects of alkaloids are due to their metabolites produced by biotransformation in the liver, and not by the alkaloids themselves (15). In this study, periacinar or centrilobular hemorrhagic necrosis were seen in the liver which may have been due to the toxic effect of *C. roseus* alkaloids. In addition, acute tubular necrosis in the kidney, auricular petechial hemorrhages in the heart and pulmonary edema were also noted.

The APTT measures the integrity of the intrinsic and common pathways of coagulation. D-dimer is a fibrin degradation product, a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis. It is so named because it contains two cross-linked D fragments of the fibrinogen protein. D-dimers are not normally present in blood plasma, except when the coagulation system has been activated, for instance due to the presence of thrombosis or disseminated intravascular coagulation (DIC) (16). APTT and D-dimer levels were elevated in all five examined animals compared to reference levels. Lower cholinesterase activity levels were attributed to central nervous system damage (17). Marked increase of the urea and creatinine levels supported kidney failure.

According to pathological findings a process in which DIC may have been present in the different organs was apparent. These included sinusoidal platelet or fibrin thrombi, hemorrhages and focal necrosis in the liver; acute focal tubular necrosis in the kidneys; capillary platelet and fibrin thrombi with focal infarcts in the brain; hemorrhages, focal ulcers and gastroenteritis in the gastrointestinal tract; capillary fibrin thrombi and alveolar hemorrhages in the lungs (18). We also observed capillary fibrin thrombi in the liver, brain and lungs and focal periacinar and midzonal necrosis and hemorrhages in the liver. At necropsy, hemorrhages and enteritis were observed in the gastrointestinal tract along with fibrin aggregates in jejunal and ileal lumen. Pulmonary edema was observed in the affected lung while changes observed in the kidney included acute tubular necrosis. It is considered that these findings may have occurred due to DIC effects of the alkaloids of *C. roseus*. Clinical and pathological findings showed that coagulopathy was a marked finding in this poisoning.

DIC diagnosis can be made based on the various tests results (19). Traditionally diagnosis of overt DIC in animals include a combination of two or more test abnormalities, specifically thrombocytopenia, prolonged coagulation times (PT and APTT), hypofibrinogenemia, low antithrombin activity and high fibrin degradation product or D-dimer (20). DIC can be diagnosed when at least three of five tests included in a coagulation profile are abnormal (21). The most common cause of prolonged APTT and PT is increased consumption of clotting factors during DIC (22). In this study, higher APTT, PT and D-dimer levels supported DIC formation.

To the best knowledge of the authors no study of *C. roseus* poisoning in sheep in the veterinary medicine literature has been documented. In the present study, accidental *C. roseus* poisoning of blood parameters and pathological effects were investigated in sheep. This study demonstrates that *C. roseus* can cause toxicosis in sheep resulting in mortality probably due to DIC and circulatory disturbances related to diarrhea and dehydration. In conclusion, results of this study suggested that the diagnosis of this poisoning depends upon a combination of anamnesis, clinical signs, laboratory parameters and pathological examination.

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