

## Research article

CLINICAL EVALUATION OF *Ageratum houstonianum* Mill INTOXICATED GOATSR. Bhatta<sup>1</sup>, P. Sharma<sup>2</sup> and P. Pal\*<sup>1</sup><sup>1</sup> Agriculture and Forestry University, Rampur, Chitwan, Nepal<sup>2</sup> Department of Livestock Services, Harihar Bhawan, Lalitpur

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## ABSTRACT

*Ageratum houstonianum* Mill (*A. houstonianum*) is a widespread, highly invasive, and drought-resistant annual semi-shrub easily found in the pasture fields. It is called *Gandhey Jhar* locally. This study was conducted at the livestock farm of Rampur Campus, Rampur, Chitwan, Nepal, to determine the clinical progression and clinical parameters in *A. houstonianum* intoxicated goats to diagnose its toxicosis in small ruminants. Full blossomed *A. houstonianum* was fed *ad libitum* to six goats until the death of the animals. The time to develop the clinical signs and symptoms in goats ranged from 22 to 49 days. All the goats exhibited similar symptoms and signs such as stiffness of the neck, low temperature, respiratory distress, low pulse, anorexia, ruminal atony, general weakness, and finally, recumbency till death. After the onset of clinical symptoms, the entire clinical course persisted for 10-15 hours, followed by death. Two goats died on the 22nd day, and the rest goats died on 27th, 46th, 48th, and 49th days respectively. Hematologic examination revealed a significant decrease in hemoglobin and an increase in total WBC count. The serum biochemical values showed substantial alterations in ALT, AST, ALP, GGT, albumin, direct bilirubin, total bilirubin, glucose, urea nitrogen, and creatinine concentrations. These biochemical changes suggested hepatic and renal dysfunction. Therefore, clinical signs, hematology, and serum biochemistry can be of optimal diagnostic value for the *A. houstonianum* intoxication in goats.

**Keywords:** *Ageratum houstonianum* Mill, hematology, serum biochemistry, goats

## INTRODUCTION

*Ageratum houstonianum* Mill is a widespread, highly invasive, and drought-resistant annual semi-shrub easily found in the pasture fields, water channels, orchards, kitchen gardens, forest margins, highways, and slopes of the the natural springs and crop fields. Known by various names; *Ageratum*, *Gandhi*, *Flossflower*, and *Blue billygoat weed*, this plant tends to become a pest in gardens and pastures (Dhakal, I. P. 1989). It is cosmopolitan in distribution besides being native to Mexico. In Nepal, it is found to be growing extensively in the tropics and sub-tropics. The plant belongs to *Asteraceae* (Edward & Howe, 1999). The plant material consists of pyrrolizidine alkaloids (PA) (Helmut & Adolfo, 2001). In addition to these, the plant also contains triterpenes, coumarin, and some unidentified substances (Noa *et al.*, 2004). However, the phytochemical study of Nepalese *A. houstonianum* contained lycopsamine like Pyrrolizidine alkaloids and several related isomers at 0.56 mg/g dry weight (Pal *et al.*, 2009).

*A. houstonianum* is generally not palatable to the livestock; however, prolonged drought and the availability of only this plant increase the livestock's probability of grazing on it. It has been considered and reported as a toxic plant for livestock in and outside the country (Alfonso, 1989). The toxicity of this plant in livestock has been reported in Cuba, Mexico, and Nepal (Noa *et al.*, 2004 and Pal, 2008). It has also been reported as a poisonous weed by the farmers of the Chitwan district of Nepal (Dhakal, 1989). From mid-August to September, buffaloes and goats grazed on *A. houstonianum* infested pastureland and were found dead after 24 to 48 hours of grazing (Pal, 2008). However, there is no single study that reported the clinical parameters of *A. houstonianum* toxicosis in goats. Therefore, this study is designed to assess the clinical progression and clinical parameters (partial hematology and serum biochemistry) in goats intoxicated with *A. houstonianum* Mill to facilitate the diagnostic procedure in small ruminants.

## MATERIALS AND METHODS

### Site of the study

The study was conducted at the Livestock Farm of IAAS, Rampur Campus, Rampur, Chitwan, Nepal, from 19th April 2009 to 6th June 2009. The laboratory analysis was performed at Veterinary Teaching Hospital, Rampur Campus Rampur, and Bisheswor Prasad Koirala Memorial Cancer Hospital, Bharatpur, Chitwan, Nepal.

### Experiment model

#### Experimental animals

Six clinically healthy goats aged between 5 to 6 months and weighing between 12-15 kg of both sexes were purposively selected and maintained at the livestock farm of Rampur Campus, Rampur, Chitwan. The goats were brought to the farm before a month of an experiment for the environmental acclimatization and dewormed with Albendazole at 10mg/kg body weight.

#### Feeding plan of the plant

*houstonianum* Mill. was collected near the vicinity of Rampur, Chitwan, Nepal, while in the flowering stage. The whole fresh plant *ad libitum* was given to all the six goats. Animals did not receive any other food except water during the entire experiment period.

**Clinical pathology:** The clinical pathology included clinical signs and symptoms, partial hematology, and serum biochemistry.

#### Clinical signs and symptoms

The animals were examined daily for the presence of any clinical symptoms and signs developed by the goats. The Physiological vitals were also recorded prior to ingestion of *A. houstonianum* at the weekly interval following feeding of the plant and after the onset of symptoms.

#### Collection and analysis of blood

Blood samples were taken before ingestion of *A. houstonianum* and after the onset of symptoms following ingestion of *A. houstonianum*. 10 ml of blood was drawn from the goats by venipuncture via the jugular vein into sterile blood collecting tubes. 3 ml of whole blood was immediately poured into the tubes containing Ethylene Diamine Tetra Acetic Acid (EDTA) for the estimation of total White Blood Cell count (WBC) and Hemoglobin (Hb). EDTA tubes were inverted and rotated gently to mix the blood thoroughly with the anticoagulant. Estimation of hematological indices was carried out according to (Chauhan, 2006).

#### Serum separation and Evaluation

The serum was separated using a centrifuge machine. The tube was labeled corresponding to the goats in the experiment and centrifuged at 1500 rpm for 20 minutes. Separated serum was then collected in serum collecting vials and analyzed for the Total Protein (TP), Albumin, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Gamma-glutamyl Transpeptidase (GGT) Total Bilirubin and Direct Bilirubin, Glucose (GLU), Creatinine and Blood Urea Nitrogen (BUN) using commercial kits of Coral Company in Autoanalysing Spectrophotometer.

#### Data Analysis

The pre and post-treatment mean values of hematological and biochemical values of experimented goats were compared using Matched pair Student's test in statistical software Excel -2003. A value of  $P < 0.05$  was considered statistically significant.

## RESULTS AND DISCUSSION

### Clinical signs and symptoms

The findings demonstrated that the clinical onset, clinical course and death due to *A. houstonianum* intoxication were corresponded to one other. The clinical course, clinical signs and symptoms exhibited by the experimented goats are presented in Table 1. Clinically all the goats were characterized by anorexia, general weakness, depression, difficulty to stand, wasting, vomiting, mild bloat, recumbency and stiffness of neck, hypothermia, low pulse and terminally respiratory distress (abdominal respiration).

**Table 1. Onset of clinical signs and clinical course after feeding of *A. houstonianum***

Goats	Onset of clinical signs (day)	Clinical Course (hour)	Outcome Death in days
1	22	12	22
2	22	10	22
3	27	15	27
4	46	15	46
5	48	12	48
6	49	12	49

### Hematological examination

The hemoglobin values significantly reduced whereas the total white blood cell count was significantly increased in comparison to nontoxic state of the experimental goats. The pre and post feeding hematological findings are shown below in the table 2.

**Table 2. Comparison of the partial hematological findings (Hemoglobin and total WBC counts)**

Goats	Hb(g/dl)		WBC ( $\times 10^3$ / ml)	
	I	C	I	C
1	9.9	7.5	6.7	11.5
2	8.1	6.5	5.7	9.4
3	8.9	6.0	8.7	11.5
4	8.2	5	4.5	12.4
5	9.2	5.5	8.7	10.2
6	9.2	6	9.2	9.5
Mean differences	8.9 $\pm$ 1.06	6.0 $\pm$ 0.82*		7.2 $\pm$ 0.8 10.75 $\pm$ 0.9*

I= Initial Values, C= Clinical Values, \* = Significantly different

### Serum biochemistry

There is a significant ( $P < 0.05$ ) difference in total protein, albumin, ALT, AST, ALP, GGT, direct bilirubin, total bilirubin, BUN, creatinine and glucose values during clinical course compared to the pre intoxicated values (table 3).

Table 3. Comparison between pre and post biochemical values in *A. houstonianum* intoxicated goats

Goats	ALT IU/L		AST IU/L		ALP IU/L		GGT IU/L		Direct Bilirubin mg/dl		Total Bilirubin mg/dl		Urea mg/dl		Creatinine mg/dl		Total Protein g/dl		Albumin g/dl		Glucose mg/dl	
	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C
1	24	145	50	2241	30	123	60	164	0.1	0.19	0.2	0.51	22	42	0.6	0.8	5.5	6.6	1.2	1.1	64	56
2	20	101	55	791	30	177	73	175	0.01	0.11	0.2	0.42	23	49	0.6	0.8	4.1	6.1	1.1	0.9	63	48
3	25	46	57	255	30	44	34	159	0.02	0.22	0.19	0.7	22	44	0.6	0.7	4.4	5.6	1.3	1.1	75	60
4	33	80	48	1500	37	77	40	170	0.02	0.8	0.23	1.53	24	54	0.6	0.77	5.5	6.1	1.2	0.9	59	35
5	41	121	60	866	40	87	55	166	0.02	0.12	0.3	0.39	25	31	0.8	1.1	6	6.8	1.4	1.2	78	51
6	37	64	65	439	60	84	60	153	0.01	0.2	0.02	0.4	21	51	0.7	0.8	6	6.8	1.2	1.2	64	57
30	92.8*	55.8	1015*	37.8	98.6*	53.6	164.*	0.03	0.24*	0.19	0.65*	22.8	45.*	0.65	0.82*	5.25	6.3*	1.23	1.06	67	51*	

Findings of this study showed that *Ageratum houstonianum* is toxic to goats. The result of the clinical examination showed individual variation in susceptibility to *A. houstonianum* (table 1). The findings of various investigators Cheeke *et al.*, (1985) reported that there is a wide variation among animal species in their susceptibility to PAs toxicity. These differences among animal species are associated to microsomal enzyme levels in the liver, and there are many evidences that the greater the microsomal activity greater the resistance of animal to PA toxicity (Panter and James, 1988).

In this study, the clinical course ran about 18-22 hours with similar and signs in all the goats. We are partially in agreement with Cheeke (1994) who recorded the deaths within about seven days in acute PA poisoning due to severe liver damage and clinical signs include jaundice, wasting, a rough hair coat, diarrhea, prolapsed rectum, dullness and sometime photosensitization. Findings of this study are also similar with the previous investigators who reported that exposed animals commonly develop signs of hepatic failure several weeks or months later (Stegelmeier *et al.*, 1996). Affected animals lose condition, and develop icterus; cattle may also develop photosensitization (Schmitz, 1998).

The partial hematology revealed significant reduction ( $p < 0.05$ ) in white blood cell count. Present finding is in general agreement with the observation of Baker (1991), Increased level of WBC may support the claim that many members of the spurge family *Euphorbiaceae* -an alkaloid and saponin containing are poisonous and establish the inflammation in hematopoietic system. Similar finding was also observed by Dow *et al.*, (1997). Adeoye *et al.*, (2004) in *Jatropha curcas*, (alkaloid and saponin containing plant). This suggests that the plants containing alkaloids and saponin are capable of reducing hematologic indices so the *A. houstonianum*.

The significant reduction of hemoglobin values in this study might be due to hepatic injury. Since coagulopathies and vascular thrombosis are likely to occur when there is alteration in synthesis of blood coagulation factors in the liver. Clinical signs of hepatic failure include coagulopathies and hemorrhage due to decreased production of clotting factors by the liver and possibly increased utilization in inflammatory processes (Stegelmeier, 1996).

Serum biochemical assay for liver function showed significant increase ( $P < 0.05$ ) in the concentration of total protein. The alkaloidal fraction may be accelerating the degradation of liver proteins as well as increase in amino acids metabolism a situation that could lead to the high elevation of plasma proteins (Imazumi *et al.*, 1982). Earlier researchers have observed increased concentration of total protein in animals fed with PA containing plants due to dehydration and fluid deposition in the cavities (Pal, 2008). Stegelmeier (1996) stated that toxic insult to liver by pyrrolizidine alkaloids might cause disturbances in protein metabolism.

Increased value for total protein in this study might be due to ascites and edema in abdominal cavity, which more often results after overwhelming injury to liver. In this condition extracellular fluids (transudates) might have contributed to changes in protein segment.

Significantly low ( $P < 0.05$ ) concentration of albumin in this study confirmed the earlier reports (Ford *et al.*, 1968, Stegelmeier *et al.*, 1996). One of the clinical laboratory abnormalities commonly seen in PA toxicosis is hypoproteinemia (Schmitz, 1998). The decreased albumin and globulin fraction resulted in an increased A/G ration in rats treated with monocrotaline (pyrrolizidine alkaloid) (Dimande, 2007). Decreased level of albumin supports the hypothesis that most abundant blood plasma protein and is produced in the liver and forms a large proportion of all plasma protein. Liver injury markedly affects the synthesis of albumin resulting low concentration of albumin (hypoalbuminemia).

ALT and AST demonstrated significant increase in their values ( $P < 0.05$ ) in all goats at their clinical stage. Present study confirmed the results obtained by (Baker *et al.*, 1991; Stegelmeier, 1996; Stegelmeier *et al.*, 1999). Significant elevations were observed in serum AST and ALT activities in the buffalo calves intoxicated with *Ageratum houstonianum* (Pal, 2008). Similar findings were recorded by Craig *et al.* (1991) in rats fed MCT-pyrrolizidine alkaloid, supplementation. These hypotheses suggest, there might be significant increase in liver enzymes in all species of animals intoxicated with pyrrolizidine alkaloid containing plants.

Significant increase in the serum direct bilirubin and total bilirubin confirmed the study of earlier workers Stegelmeier *et al.* (1996). It is hypothesized that following pyrrolizidine alkaloid consumption, iron from accelerated erythrocyte destruction is initially concentrated in the liver accounting for the dark color of liver and increased bilirubin. PA containing *jatropha curca* and *phyllanthus amarus* caused drastic effect in the plasma bilirubin indicating a possible damage to the liver (Oluwole, 1997, Dimande A.F, 2007).

Present finding revealed significant hypoglycemia ( $p < 0.05$ ). This is well in accordance with those previously reported in other species (Stegelmeier *et al.*, 1996). This might be due to decreased hepatic function. Hypoglycemia may be present in severe cases PA toxicosis (Stegelmeier *et al.*, 1996).

This study also showed the significant increase in blood urea nitrogen (BUN) concentration ( $p < 0.05$ ) in all the goats. Imazumi *et al.*, (1982) recorded increased BUN in renal toxicosis caused by plants rich in PA. The alkaloidal fraction may be accelerating the degradation of liver proteins as well as increase in amino acid metabolism, a situation that might lead to the high concentration or level of Plasma urea nitrogen (Luke, 1981). We opined that BUN is not directly associated with liver injury, however, it indicates the damage in kidneys because BUN is more specific to kidney function. This study also found significant increase in creatinine concentration ( $P < 0.05$ ) indicating the renal injury which is in general agreement with the findings of Imazumi *et al.*, (1982).

### CONCLUSION

Based on the findings, *A. houstonianum* is toxic to goats. The clinical onset of symptoms and signs developed after 22 to 49 days of the feeding and the clinical episode ranged from 10-15 hours showing the individual variation in toxicity. The clinical pathology revealed the decreased hemoglobin values, increased total WBC count and increased serum biochemical parameters related to hepatic and renal functions were suggestive of hepatic and renal failure due to *A. houstonianum* toxicosis. We recommend for further studies such as necropsies and histopathology to document the exact changes that occur at cellular and tissue level.

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### REFERENCES

- Adeoye, B. A. and O. O. Oyedapo. 2004. Toxicity of *Erythrophleum guineense* stem bark: Role of alkaloidal fraction. *Afr. J. Trad. I*, 45-54.
- Alfonso, H.A., J.M. Aparicio, M. Evangelina, J.M. Cabrera, R. Mirta and J. Ancisar. 1989. Intoxicacion bovina en el Tropico por consumo de Hierba- Celestina Azul. *Avance Veterinario*. 8(1), 27-29.
- Baker, D. C., P. Fister, J. A. Molyneux and P. Kechele. 1991. *Cynoglossum officinale* toxicity in calves. *J. comp. Pathol.* 104, 403-410.
- Chauhan, R. S and D.K. Agrawal. 2006. Text book of veterinary clinical and laboratory diagnosis. Jaypee brothers. Medical publishers. New Delhi.
- Cheeke, P. R., and M. L. Pierson-Goeger. 1983. Toxicity of *Senecio jacobaea* and pyrrolizidine alkaloid in various laboratory animals and avian species. *Toxicol; Lett.* 18, 343-349.
- Cheeke, P. R. 1994. A review of the functional and evolutionary roles of the liver in the detoxification of poisonous plants, with special reference to pyrrolizidine alkaloids. *Vet. Hum. Toxicol.* 36, 240-247.
- Cheeke, P.R. and D. Bhuler. 1985. More threats from an old foe: Tansy ragwort, *Senecio jacobaea* talks explain human hazards poisonous to humans as well as cattle and horses. *Oreg. Agric. Prog.* 25, 4-5.
- Craig, A. M. E.G Pearson, C. Meyer and J. A. Schmitz. 1991. Serum liver enzyme and histopathologic changes in calves with chronic and chronic delayed *Senecio jacobaea* toxicosis. *Am. J. Vet. Res.* 52, 1969-1978.
- Dhakal, I. P. 1989. Major problems of livestock in Chitwan district. *J. Agric. Anim. Sci.* 85,16-19.

- Dimande, A. F. P. 2007. The toxicity of *Senecio inaequidens* D.C. Ji S. Afr. Vet.Ass.78(3), 121-129.
- Dow, J. G. Lindsay and J. Morrison. 1997. Metabolic of Nitrogen containing compounds. In Biochemistry: Molecules, cells and the body. Addison-Wesly Publishers Limited England.pp.293-328.
- Edward, S.H and R.R. Howe. 1999. Classification of *A. houstonianum*. Retrieved on Jan.5, 2009 from [http://www.floridata.com/ref/A/ager\\_hou.cfm](http://www.floridata.com/ref/A/ager_hou.cfm)
- Ford, E.J., H.E. Ritchie and E. Thorpe.1968. Serum changes following the feeding of ragwort to calves. J. Comp. Pathol.78, 207-218.
- Helmut, W. and A. Andrade-Cetto.2001.Phytochemistry. 57, 1269-1271.
- Imaizumi, Y., M. Katoh and Sugimota.1982. Effects of malotilate on synthesis and movement of RNA in rat liver. Fool. Pharmacol. Japan. 9, 285-291.
- Luke, R.G. 1981. Uremia and the BUN. Engl. J. Med. 305(20),1213-5.
- McLeannan, M.W., M.E. Dodson and R. Rac. 1972. Heliotropium poisoning in cattle. Aust. Vet. J.48:480.
- Noa, M., L.M. Sanchez and R. Durand. 2004. *Ageratum houstonianum* toxicosis in Zebu cattle. Vet. Hum. Toxicol. 46(4),193-4.
- Oluwole, F.S. and A. F. Bolarinwa. 1997. *Jatropha curcas* causes anaemia in rats. Phyto Res 11, 538-539.
- Pal, P. 2008. Clinico-pathological evaluation of buffalo calves intoxicated with *Ageratum houstonianum* Mill. Thesis, M. V. Sc. Institute of Agriculture and Animal Science, Rampur 97p.
- Panter, K.E, R.F. Keeler and D. Baker. 1988. Toxicoses in livestock from the hemlocks (*Conium* and *Cicuta* spp.). J. Anim. Sci. 66, 2407-2413.
- Schmitz, D. G. 1998. Toxicological problem. In: Reed S.M. Bayly (eds). Equine Internal Medicine. pp.981-1042.WB Saunders, Philadelphia, PA.
- Schoch, T.K., D.R. Gardner and B. L. Stegelmeier. 1996. GC/MS/MS detection of pyrrolic metabolites in veno-occlusive disease due to *Crotalaria nan* Burm. Indian J. Med. Res. 68, 790-797.
- Stegelmeier, B. L., J. A. Edgar, S. M. Colegate, D. R. Gardner, T. K. Scoch, R. A. Coulombe and R. j. Molyneux.1999. Pyrrolizidine alkaloid plants, metabolism and toxicity. Journal of Natural Toxins. 8, 96-110.
- Stegelmeier, B. L., R. D. Gardner, F. James, R. J. Molyneux. 1996. Pyrrole detection and the pathologic progression of *Cynoglossum officinale* (houndstongue) poisoning in horses. J. Vet. Diagn. Invest. 8, 81-90.
- Theophile, D., T. S. D. Emery and D. D. Paul.2006. Effects of *Alafia multiflora* stap on lipid peroxidation and antioxidant enzyme status in carbon tetrachloride-treated rats. Pharmacology online. 2, 76-89.