Case-series on Lantana Camera : The Notorious Killer

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ABSTRACT

The present work reports an outbreak of intoxication in cattle by *Lantana camera,* in a village Sanawad, near Indore, M.P. in Two thousand fourteen (2014). Few animals from a herd, entered in areas that was infested by Lantana species. A series of animal deaths, caused due to exposure of lantadenes during grazing by the animals. The gastric lavage, urine and blood sample of affected animals were analyzed in analytical toxicology lab of Forensic Medicine department. The poisoning with *lantana camera* was revealed by using thin layer chromatography in gastric content by extraction and comparison with alcoholic extract of plant material. The lantadene which are pentacyclic triterpene acid are identified.

Keyword

Lantana camera; lantadene; pentacyclic triterpine acid; thin layer chromatography; cattle poisoning.

INTRODUCTION

Poison includes both naturally produced compounds and chemicals manufactured by humans. Natural poisons are produced by species of bacteria, fungi, protists, plants and animals. Poisonous plants are those, which cause serious problems or even death, if a small quantity of its stem, leaves, seeds, fruits and roots are ingested. One of such plant is *Lantana camara*, a member of the Verbenaceae, is an exotic ornamental Shrub that is of particular importance in many countries as a cause of cattle intoxication.

Though it is among the category of medicinal plant, but in India this plant is categorized in poisonous plant since it is among top ten invasive weeds and toxic plant on the earth.[1] Lantana has been reported to make animals ill after ingestion, since its foliage contains the toxic pentacyclic triterpenoids called lantadenes. Livestock foraging on the plant has led to widespread losses in the various countries including India.[2] Present study reports the case series of intoxication of Lantana camera along with extraction and analysis of lantadenes.

CASE-REPORTS

Case 1 (involving 3 fatal cases)

On sixth of November two thousand fourteen, a veterinary doctor in the village *Sanawad*, near Indore, Chhattisgarh reported 3 cattle mortalities. The history given by animal owner revealed that animals were allowed to graze in a nearby field. After grazing the animals developed features characterized by anorexia, diarrhea, swelling (edema) of the affected parts, leading to death of cattle in two to three days. Similar features were present in some other cattle and reported poisoning next day, aving same history. Later on the postmortem examination was done, by the veterinary doctor, but could not conclude the cause of death.

Somehow he came to know about the availability of our analytical toxicology lab and henceforth he enquired the possibility of determination of toxic compound which may be sprayed in the field as an insecticide, with an assumption that high concentration of insecticide in the plants and weeds could have been grazed by the animals, leading to such sign and symptoms. So the autopsy surgeon wanted us to confirm the presence of insecticide in the gastric content and blood. Three biological samples namely the blood sample, gastric content and urine, of all three cattle, were sent to departmental analytical toxicology lab. The samples were analyzed for pesticide, common insecticides and weedicide. However, all the tests were negative for the common poisons prevalent in the area.

Case 2 (involving 4 more fatal cases)

A similar history was again received few days after the above incidence i.e. on fourteen November two thousand fourteen, four cattles had died having similar symptoms. Again, the biological samples of all four new deaths were sent for chemical examination, which were meticulously tested. The entire routine test for analysis of common pesticides, insecticides and weedicides, were negative in samples provided. However, the gastric lavage contained few residues of some plants. Later on after detailed

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discussion we decided to visit the scene. A plant weed was identified having toxic effects on cattle. This was identified to be Lantana camera (Fig 1), which might have been consumed by animals during grazing.

Fig 1: showing the vegetation of Lantana Camara with flowers.

DISCUSSION

Lantana camara L. (Verbanaceae family), universally known as wild or red sage is the most widespread species of this genus and regarded as a popular ornamental garden plant but is notorious in character. This plant has particular importance in India as a cause of cattle intoxication.[3] It was originally introduced from Central and South America and occurs commonly in many tropical and subtropical regions of the world.[4]

The most important toxic principle of Lantana camara is lantadene A, which is a pentacyclic triterpene acid[5]. This substance has been shown to cause injury to the bile canalicular membranes, with subsequent cholestasis and hepatocellular damage. Ingestion of the plant by cattle and sheep results in, secondary photosensitivity, icterus and constipation, or in some cases diarrhea.[5] Significant Lantana toxins are the triterpene acids lantadene A, lantadene B, and their reduced forms, which cause the death of horse, cattle, sheep, goat, and rabbit.[6] Live stocks are being poisoned after ingesting the entire plants and suffered from liver damage, renal tubular disorder and showed secondary Photosensitization.[7] Acute poisoning was clinically characterized by anorexia, dehydration, ruminal stasis and severe icterus within 1–4 days of consuming the plant but sometimes no photosensitization occurred.

The phytochemistry of L. camara is complex as it contains a wide variety of chemical substances, including triterpenes, mono and sesquiterpenes, iridoid and phenyl ethanoid glycosides, nafthoquinones, and flavonoids, among other compounds.[8,9] The leaves contain a mixture of flavonoids.[10] In animal, toxicity is most likely due to lantadene A and the leaves appear to be poisonous.[11] The hepatotoxic action of L. camara has been attributed to two pentacyclic triterpenes known as lantadene A and B. The lantadene content in L. camara plants is variable, and potentially toxic plants contain at least 80 and 200 mg/kg of lantadenes A and B, respectively.[5]

- Toxic effects of lantadenes - Lantadenes are biotransformed by hepatic cytochrome P-450 enzymes into toxic compounds that damage the bile canaliculi, producing intrahepatic cholestasis and impairment of the normal flow of bile.[12] The primary toxic action of the lantadenes may result in secondary photosensitization due to the reduced excretion of phylloerythrin, a natural metabolite product of the anaerobic fermentation of chlorophyll and normally excreted in bile. Disruption in the biliary elimination of phylloerythrin increases its blood level and deposition in subcutaneous tissues. In non-pigmented areas of the skin or in areas without dark hair, phylloerythrin reacts with solar light, forming reactive molecules that damage the local tissue causing erythema, edema, inflammation, and necrosis of the epidermis.[13]

a. Signs shown by poisoned animals:[14,15]

- Liver damage - yellow discolouration of the eyes and gums.
- Reddening and inflammation of Skin of the nose and unpigmented (white) skin; moist, ulcerated and very painful (pink nose) which sloughs off.
- Swelling of ears and eyelids.
- Reddening and discharge from the eyes (conjunctivitis).
- Ulceration of the tip and under surface of the tongue.
- Photosensitization

Major mortalities in livestock has been reported by Lantana species due to its toxic effects, still very less work has been done in analysis of toxicity and identity of the toxic compound. Clinical sign of poisoned animals give us a clue regarding the poison, but most of the times they cannot be finally identified, as in this case series no photosensitization occurred in first few animals, but in later cases photosensitization was observed. The earlier analysis of lantana poisoning is essential to keep away the animals from the plant and for the early treatment.

Keeping this in view, we performed a simple method of extraction of lantadens from gastric content of affected animals and their analysis was done by using thin layer chromatography. This method is so simple that no advanced laboratory set up is required to detect such poison.

b. Toxicological analysis - Lantadenes could not be detected in liver, bile, gall bladder, blood and urine samples. Lantadene A (LA) and Lantadene B (LB), their derivatives, reduced lantadene A (RLA), reduced lantadene B (RLB) and two unidentified metabolites could be detected in the gastric content, contents of lower gastro intestinal tract and faeces.[16] The gastric content is commonly used for the identification in such cases, as it is very simple and quick method. The gastric content was extracted with hexane by liquid- liquid extraction method. The alcoholic extract of plant material was subjected to various qualitative tests for the identification of various plants constituents.

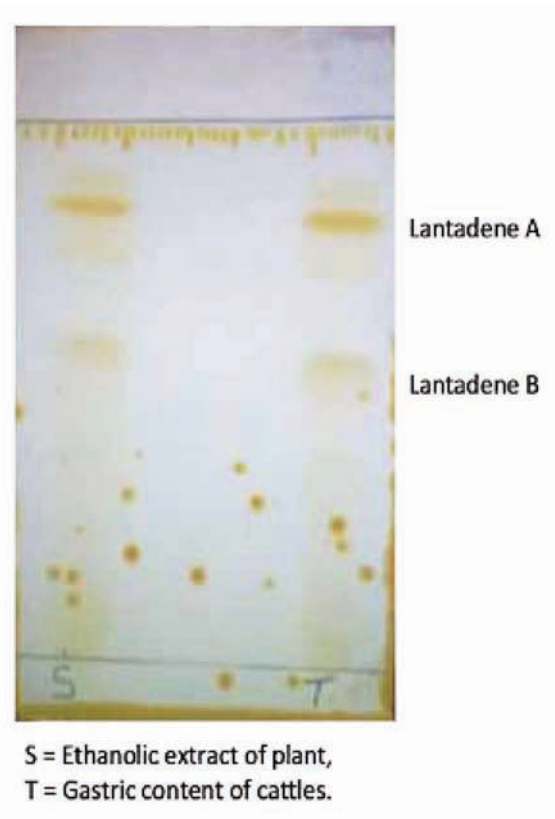
- Test for Triterpenoids: Extract (300 mg) was mixed with 5 ml chloroform and warmed for 30 minutes. Few drops of concentrated sulphuric acid was added and mixed well. The appearance of red color indicates the presence of triterpenes.
- Tests for Alkaloid: Extract was treated with 1ml of Dragendorff's reagent. An orange-red precipitate indicates the presence of alkaloid.
- Tests for Tannin: 1ml of the extract is added with ferric chloride solution. Formation of a dark blue or greenish black color product shows the presence of tannins.

Phyto-chemical analysis was done using the above mentioned chemical tests, for confirming the presence of alkaloids, triterpenoid, and tannins. The test revealed their presence. For the confirmatory test the gastric content is extracted and spotted in TLC plate. The procedure is as follows -

20 ml of hexane mixed with 10 ml of Gastric content and kept in boiling water bath for 30 min. The content of the flask is poured into separating funnel and shaken thoroughly. Two separate layers of water and hexane are visible. Hexane layer is passed through anhydrous sodium sulphate and then poured into petridish until it is dried. Ethanolic extract of plant material is prepared by mixing 20 ml ethanol to it. It is boiled for 30 min and then filtered.

The mobile phase used here is Ethyl acetate: methanol (80:20) for Thin Layer Chromatography (TLC).[17] Solvents is added into TLC chamber and a filter paper is put for saturation of chamber. TLC plate is spotted by the standard and sample, using capillary tube. The plate is then kept into saturated TLC chamber. Observe till the solvent front reaches up to 10 cm. The plate is removed, dried and kept in iodine chamber. Two spots of lantadene are observed. Lantana contains lantadene A and B, as major toxins, which are involved in poisoning (Fig. 2).

Fig 2: Thin-layer-chromatography (TLC) plate depicting the results of analytes containing the extract of plant Lantana camara and the gastric content of poisoned cattles.



CONCLUSION

Thus looking to be an insecticide poisoning the gross examination of gastric content revealed the presence of a lethal weed, which was confirmed by simple chemical tests and TLC method. This weed, named as Lantana Camera is a very commonly seen in the farms and play grounds. Probably it is one of the commonest cause of poisoning among cattle. Although looking to be harmless, this plant has turned out to be a notorious killer in our case series.

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CONFLICT OF INTEREST

Declared None.

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