POISONOUS PLANTS FOR CATTLE IN COLOMBIA: RESEARCH PERSPECTIVES

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ABSTRACT: Even though Colombian cattle-farming makes an important contribution to the national economy it also has great potential for growth; however, this activity is carried out in areas where animals may become naturally exposed to poisonous plants, affecting their health and production. It is recognised that some plants may damage the livestock sector in countries placing an important emphasis on cattle-rearing. Such problem has received scarce attention from academic research in Colombia, and the efforts made to do so date from the 1930s, 1940s, 1970s and 1980s without having had continuity. Many of the studies done in Colombia have been orientated towards establishing nitrate and cyanogenic glycoside content, ignoring other toxic metabolites. Likewise, certain vegetal species which can cause poisoning have not been fully studied, thereby hampering their management and treatment. It is thus important that studies are carried out in greater depth in Colombia concerning poisonous plants affecting livestock. A methodology is thus proposed which includes a search for initial information about suspicious plants, reproducing such poisoning experimentally (selecting the animal species, route, presentation and administration time, and evaluating effects) and searching for the chemical responsible for causing the poisoning. All these aspects are dealt with in the present review.

Key words: cattle, poisonous plant, animal experimentation, phytochemistry

PLANTAS TÓXICAS PARA LOS BOVINOS EN COLOMBIA: PERSPECTIVAS DE INVESTIGACIÓN

RESUMEN: Además de un gran potencial de crecimiento, la ganadería bovina colombiana tiene un importante aporte a la economía nacional. Esta actividad se desarrolla en áreas donde los animales pueden exponerse naturalmente a plantas tóxicas, afectando su salud y producción. En países con una importante vocación ganadera se reconocen las plantas que pueden menoscabar el sector pecuario. En Colombia, escasamente ha sido estudiada esta problemática y los esfuerzos por hacerlo datan de las décadas de 1930, 1940, 1970 y 1980, sin que haya habido continuidad en los mismos. Muchos de los estudios realizados en el país han estado orientados a establecer el contenido de nitratos y glucósidos cianogénicos, ignorando otros metabolitos tóxicos. Así mismo, existen intoxicaciones por ciertas especies de vegetales que no han sido plenamente estudiadas, dificultando su manejo y tratamiento. Es importante que en el país se investigue con mayor profundidad sobre las plantas tóxicas de interés pecuario y para ello se propone una metodología que incluye selección de la planta sospechosa, reproducción experimental de la intoxicación (elección de la especie animal, vía, presentación y tiempo de administración y evaluación de efectos) y búsqueda de principios responsables de causar la intoxicación; estos aspectos se abordan en la presente revisión.

Palabras clave: ganado bovino, plantas tóxicas, experimentación animal, fitoquímica

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INTRODUCTION

Colombia has an important cattle raising tradition, having almost 25 million head of cattle which thus places it amongst the first 9 countries having the greatest bovine production (1). The production systems, however, are mainly large-scale, having extensive areas given over to grazing. It has been calculated that 20 % of the area earmarked for cattle-raising is covered in weeds and stubble as its only vegetation (2), thereby introducing a very high probability that any animals grazing there will consume poisonous plants.

Most plant toxins are secondary metabolites since they are not essential for their growth or reproduction. Such substances represent a survival mechanism for poisonous plants since they are able to produce adverse physiological reactions in their predators and thus reduce the fact that they could become consumed (3).

Many factors predispose these plants to be eaten by cattle; some of them would be the use of fields covered in weeds, animals having nutritional deficiencies, including hunger, the arrival of new individuals which are "ignorant" of the place's particular poisonous plants, overgrazing leading to hardly appetizing forage and also exposing poisonous seeds present in the soil, some poisonous plants' high palatability and/or an alteration in poisonous plants' metabolism leading to the accumulation of some toxins caused by fertilising pastureland, treatment with herbicides or a change in climatic conditions (3,4).

The problem of animals being poisoned by plants has been studied in many countries and it could be stated that, in spite of some plants being able to adapt to different ecosystems, this varies depending on the each region's physiographic characteristics. Several bibliographic sources have thus described plant species which are poisonous for livestock, each being particular to a different country (5, 6), however such studies have been scarce in Colombia.

Research into plants which are demonstrably poisonous for cattle represents an urgent need in Colombia, bearing in mind that Colombia is the second country in the world regarding biodiversity (7), that the cattle-raising population is considerable and that it has large-scale production systems. The present paper was thus aimed at proposing a research strategy regarding poisonous plants in this tropical country.

BACKGROUND

Economic losses arising from poisoning by plants in the livestock sector are widely scrutinised in countries having recognised cattleraising production. In Brazil that has the second greatest number of cattle herds; it has been calculated that 800,000 to 1,120,000 cattle die from this cause annually (0.5 % to 0.7 % of bovine mortality) (8, 9). Data from1976 estimated that mortality in cattle caused by this type of flora was around 0.5% annually in Colombia (10). Torres (1983) calculated that economic losses rose to U\$ 160,000 annually, their real value currently remaining unknown (11). Other factors which could lead to losses caused by poisoning with plants (in addition to mortality) would be reduced weight gain, reduced reproductive efficiency, expenses incurred in treating poisoned animals, restricted grazing and loss of usable forage due to direct competition with such plants (12).

Another impact produced by poisonous plants is related to toxins' effect on animal subproducts. The cattle's internal organs/intestines could be confiscated due to changes in their colouring or to them giving off disagreeable odours; such cases could be presented after consuming Buchonsia pseudonitida (13) which produces a pinkish colouring in tissue, or Petiveria alliacea which produces a bad smell in affected animals' meat (11). There is also the possibility that such toxins could be excreted in milk and thus become consumed by humans. Phytotoxins having these characteristics would be pyrrolizidine alkaloids present in plants from the genera Senecio and Crotalaria causing hepatotoxicity, piperidine alkaloids present in Conium spp., Nicotiana spp. and others causing neurotoxicity, selenium stored by accumulator plants (Astragalus spp., *Oonopsis* spp., *Aster* spp., etc.) which could cause multisystemic alterations (13) and ptaquilosides from ferns from the genus Pteridium which are considered to be carcinogenic (15).

The few studies published in Colombia to date, related to plants which are poisonous for cattle, resulted from work done by professors from the Universidad Nacional during the 1930s and 1940s and later on by functionaries from ICA (Instituto Colombiano Agropecuario) during the 1970s and 1980s; it described differ types of toxicosis, such as "cattle fall syndrome", photosensitivity, myopathy, bovine vesical haematuria, bovine chromatosis, selenosis, gastrointestinal disease and abortions (11,16, 17). The botanical species causing much of this poisoning have not been fully identified and, in many cases, poisonous ingredients and the clinical-pathological pictures originating them are not known in detail.

The technology implemented to date in Colombia for characterising the poisonous compounds present in the plants eaten by animals has been limited almost exclusively to spectrophotometrically determining nitrates, nitrites and qualitative analysis of cyanogenic glycosides (18). However, more than one hundred poisonous substances present in plants have been identified up to now. On the other hand, most knowledge about which plants are poisonous for animals has mainly been based on research carried out in other countries which has not born in mind the Colombia's natural regions' physiographical conditions influencing plants' toxin production.

POISONOUS PLANT RESEARCH METH-ODOLOGY

Research into poisonous plants of interest regarding livestock must involve different aspects ranging from suspecting that disease is being caused by vegetal species to recognising the chemical ingredients responsible for the toxicosis. The research methodology adopted in this critical review of plants of interest regarding livestock has involved the following aspects: a search for initial information: toxic suspicion, experimental reproduction of the poisoning (selecting the animal species, route, presentation and vegetal material administration time, evaluating the poisonous effects) and the search for the active ingredients responsible for causing the poisoning. Each aspect is briefly described below.

SEARCHING FOR THE INITIAL INFOR-MATION: SUSPICIOUS PLANTS

When trying to clarify the cause of a disease in which the possible aetiological agent is a plant then the specific region's botanical species must be surveyed (9). One way of doing this is to use ethnobotany which studies human groups' interaction with plants where popular knowledge about vegetal species which could alter human beings and animals' health represents a valuable source of information (19). Another way to initiate the study of suspicious plants is by studying cases of mass poisoning in livestock species of interest where a systematic report of the case has been made and recognising the plants which might be responsible for such event by observation (20, 21, 22).

Chromatosis represents an example of a case in which a plant has been identified as the causal agent of a disease. This condition is characterised by deposit of pink or violet pigmentation on the teeth, in the mucosa, elastic, subcutaneous and muscular tissues, the urine and sometime in the milk from cattle and sheep. The syndrome was first described in Colombia in 1963 in the Huila and Tolima departments as well as in warm (500-600 masl) and cold regions (2,600 masl). One observational study involved more than 60 plants being collected and identified from cold and hot climates where clinical cases of chromatosis had been presented, the presence of the genus Buchonsia being found in both regions (B. armeniaca in the cold and B. pseudonitida in the hot climate). These plants led to reproducing the disease in experimental animals later on (13). Whether through ethnobotany or clinical reports and observation, many plants indicated as being suspicious could already have been described as poisonous species and, in such case, once taxonomic determination has been made by specialists, the scientific literature is relevant for obtaining more information.

EXPERIMENTALLY REPRODUCING THE POISONING

Biological assays using sensitive species have to be performed to confirm that a suspicious plant is responsible for poisoning (9). Part of the scientific literature related to toxicological tests in animals is orientated towards evaluating the risk that plants used in therapeutic preparations or for nutritional ends could be causing poisoning in human beings (23, 24). In this case, interest in evaluating vegetal specie's toxic potential is framed within safety studies which are carried out on laboratory animals and the results are extrapolated (not always correctly) to human beings. Experimental poisoning should ideally be done in the same animal species or in closelyrelated species when studying poisonous plants of interest regarding livestock (9).

EXPERIMENTAL ANIMAL

Many studies have been carried out using small ruminants (goats and sheep) either because they are the species in which poisoning has initially been described or because an attempt is being made to extrapolate the results to cattle. For example, Medeiros et al., (2009) confirmed that the plant Centratherum brachylepsis was the causal agent of a disease related to anorexia, ruminal indigestion, facial oedema, keratinocyte vacuolisation and vesicle formation in the ruminal epithelium of cattle and goats from the state of Paraíba in northern Brazil when they were fed it; similar signs and lesions were obtained in sheep and goats (22). Another example would be Ipomoea carnea, a plant containing indolizidine alkaloid (swainsonine), causing the cellular accumulation of oligosaccharides by inhibiting the a-mannosidase enzyme, leading to neuronal vacuolisation and the presentation of neurological disorders (25). Cattle and caprines are the most commonly affected species and the disease has been successfully reproduced in the latter species (26).

Some cases have been reported in which the totality of lesions could not be reproduced, in spite of the species involved in natural and experimental presentation of a particular disease being ruminants. The cardiotoxic plant *Nerium oleander* is especially poisonous for sheep; cardiac glycosides from the cardenolides group are its main active ingredient. Barbosa et al., (2008)

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managed to reproduce the clinical picture in goats, characterised by apathy, colic, vocalisation, dyspnoea and ruminal distension; however, they did not report any pathological lesions in the liver and brain (i.e. such events happening in sheep) (27). Even though occurring less frequently, experimental studies have also been carried out on cattle where young animals of around one year old are preferred (28, 30).

Laboratory animals may be of interest when studying poisonous plants, whether for support when isolating active ingredients or as an auxiliary resource in recognising some plants whose poisonous effects could become presented in different species (31). For example, hepatotoxicosis caused by Helianotherpium circinatum (a plant containing pyrrolizidine alkaloids) has been reproduced in rats (32). However, these animals have not been a good model for reproducing the alpha-mannosidosis caused by the swainsonine present in I. carnea (33), whilst the guinea pig has been a suitable model for such toxicosis (34). Likewise, the poisoning caused by Baccharis coridifolia has been successfully reproduced in mice; this plant accumulates macrocyclic trichothecenes (35). Rabbits have been used in biological tests to confirm reports of poisoning by different plants (14, 36).

As plants' toxins can become metabolised and their poisonous effects can become modified in animals' digestive tracts (especially in polygastric animals), then the ideal scenario consists of experimenting with the particular species implicated in cases of poisoning. If the affected species is bovine then cattle must be used for conducting experiments or, failing that, small ruminants. However, using more easily handled, cheaper, laboratory species, allowing more individuals to be used, could provide relevant information about the effect of a poison and, on specific occasions, could represent a useful model for studying poisonous plants of interest regarding livestock. Such studies must be accompanied by preliminary phytochemical studies and selective chemical investigations tending to identify the active compound implicated in cases of poisoning.

VEGETAL MATERIAL'S CONSUMPTION ROUTE, PRESENTATION AND ADMIN-ISTRATION TIME

As the ideal course in experimentation is reproducing the natural conditions of poisoning, then recently collected fresh vegetal material must be orally administered (31), either by voluntary consumption, mixing the plant in the food or via orogastric probe. However, some authors have used dried, finely ground up plants which they have dissolved in water (37) or mixed with the animals' food (38). Vegetal material extracts obtained by using different solvents have also been used (39). Brazilian researchers have reported that *I. carnea* is very palatable for goats, meaning that the vegetal material collected for experimentation must be voluntarily consumed by the caprines (26, 40). Another way to ensure that animals voluntarily consume a plant of interest is by leading the animals to pastures already infested with it (28).

Some authors have reported success in reproducing poisoning by placing small amounts of the fresh plant in the mouths of sheep and goats (20, 22). Some studies in ruminants have proved successful by mixing the vegetal material (fresh or dried) with the animals' food ration (29, 41). However, a more precise dose could be achieved by administering dried and ground up plant, suspended in water, via an orogastric probe (42, 43).

When the poisonous ingredient has become known and the necessary methodology for determining it is available, then the plant administered to the animals could be analysed for determining toxin content and thus calculate the dose to be used in experiments. Such is the case of studies carried out with hypericin, the main poisonous compound in *Hypericum perforatum* (43), monocrotaline present in *Crotalaria retusa* (44) and with cyanogenic glycosides present in *Prunus virginiana* (45).

The dried plant could be supplied mixed in food (32, 34), in watery or oily suspension via probe (35, 36) or in alcoholic extract in studies involving laboratory animals (33, 46). Likewise, extracts and fractions from the plant of interest are habitually administered to small rodents in the search for active compounds (47).

Drying the plant for biological assays (in some cases) could make poisonous compounds lose their toxic activity. It has been shown that the toxicity of *Baccharis coridifolia*, containing macrocyclic trichothecenes, becomes reduced by half if the plant has been dried (35). Other poisonous molecules such as cyanogenic glycosides are very volatile, thus making it necessary to supply the fresh plant or, failing that, keeping it refrigerated from when it has been collected to when it is administered (37).

An investigator's objectives and a plant's characteristics determine the dose to be administered and the duration of the poisoning, respectively. For example, *I. carnea* could cause sudden death by being consumed in large amounts (9) but it has been used in chronic poisoning studies, even being administered for four months in low doses (26). Cyanogenic glycoside-accumulating plants, recognised as causing sudden death, have been administered for 30 days consecutively in experiments designed to evaluate the effects of prolonged administration (37, 45). Toxicosis can become rapidly reproduced, such as the case of the cardiotoxic cardenolides present in *Nerium oleander*, where a single dose of vegetal material has led to the signs appearing even during the first hour after being administered (27, 42). The poisoning could become rapidly reproduced with plants like *Plumbago scandens*, *Mascagnia rigida* and *Palicourea corymbifera*, given that they are responsible for causing sudden death (20, 27, 46); of these three plants, the metabolite responsible for the poisonous effect is only known for the last one (monofluoroacetic acid) (31).

The factors influencing toxicity must be born in mind for biological tests to be successful, such as the administration route, the presentation and the time spent exposed to the plant. It is important that maximum experimental conditions are ensured, as similar to naturally occurring ones as possible.

EVALUATING POISONOUS EFFECTS

Animals must be clinically evaluated during the time spent during an experimental process. Clinical exams are performed more frequently once the symptoms have appeared. Paraclinical tests regarding haematology and blood chemistry provide important information about toxicosis. Likewise, some diagnostic aids could contribute towards a disease being characterised with greater precision, for example, electrocardiograms when experimenting with cardiac glycoside-rich plants (27). It is worth mentioning that experimenting with small ruminants or rabbits could provide better information than doing so with laboratory rodents because of the animal's size.

An animal's weight must be monitored before, during and on finishing an experimental process as large variations in weight could indicate physiological alterations. The weight of the organs, regarding body weight (relative weight), particularly in laboratory animals, also provides relevant information about poisoning (48).

Macroscopic and microscope pathology is a tool allowing affected systems to be defined with greater precision and also ascertain characteristic lesions contributing towards a diagnosis of spontaneous poisoning. Using histochemical techniques is useful in cases of toxicosis related to some plants causing structural cellular alterations as happens with those accumulating swainsonine (i.e. *I. carnea* and *Sida carpinifolia*) thereby leading to the formation of cellular carbohydrate deposits made visible by reacting with specific lectins (34). Some intracellular lesions may also be characterised by using an electron microscope (29, 40).

THE SEARCH FOR ACTIVE INGREDI-ENTS RESPONSIBLE FOR CAUSING POISONING

Research groups focusing on poisonous plants of interest regarding livestock have not traditionally elucidated the actual substances responsible for poisoning. In many cases, the researchers' final goal has been concerned with trying to reproduce particular types of poisoning in experimental animals and characterise the main pathological lesions, possibly due to the complexity represented by isolating, purifying and identifying as yet unknown chemical compounds and/or the absence of interdisciplinary work with experts in the field of chemistry who could thus make a great contribution to such work.

Characterising the active ingredients present in plants could be useful for relating toxins' mechanisms of action to suggesting possible treatment. Likewise, this could provide relevant information leading to synthesising useful molecules. In fact, many drugs have been prepared from toxins isolated from plants; such is the case of taxol, an alkaloid derived from the plant Taxus baccata causing sudden death in cattle; it is currently being used as an antitumor drug (25). Chemotaxonomy (the discipline classifying plants regarding the structure of their chemical constituents) represents a means by which the poisonous metabolites present in a botanical species from a particular family may be deduced (49). A series of processes, including extraction, purification and structural analysis, must be performed when chemically characterising a plant.

Adding solvents to dry pulverised vegetal material leads to different components being extracted from a plant, according to solvent polarity. The initial preparation of ethanol extracts means that they can be used for evaluating activity in laboratory animals. Using simple techniques, including colorimetry and thin-layer chromatography with developers, extracts may be evaluated for determining the presence of certain groups of compounds (terpenes, phenols, alkaloids, cardenolides and flavonoids) (50).

The following step is to apply separation techniques aimed at purifying active substances from amongst the rest of the components from the vegetal extract's matrix. One way of doing this is to use fractioning, using chromatographic techniques (open column chromatography being most widely used). The fractions so obtained are then biologically tested for determining activity (49). The pure compounds of interest could be submitted to structural elucidation techniques such as UV spectrometry, infra-red, nuclear magnetic resonance or mass spectroscopy. If a

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molecule cannot be purified by the above, then hybrid techniques combining separation and detection could be used for isolating a compound of interest which could then become structurally elucidated. Liquid or gas chromatography coupled to mass spectrometry is the technique which provides the best information from amongst the aforementioned techniques (49).

Investigating plants of interest for livestock is an integral process which must include studying these species' active components. Identifying the responsible molecules and also submitting them to activity studies could contribute towards the search for useful compounds for humanity.

STRATEGIES FOR APPROACHING KNOWLEDGE REGARDING POISON-OUS PLANTS

Given the large amount of plants which are poisonous for animals, it is necessary to group them to facilitate their study. The most usual ways of classifying them are according to geographical region (e.g. poisonous plants from the state of Texas), the infected animals' pathological clinical picture (plants causing acute mortality, plants causing photosensitivity), the affected systems (cardiotoxic plants, hepatotoxic plants), the botanical families (Poaceaes, Apocynaceaes) and the predominant poisonous compound (nitrateaccumulating plants, cyanogenic glycoside-accumulating ones, etc.). Table 1 lists some of the poisonous plants present in Colombia grouped according to their chemical classification as well as indicating the main effects produced by each of them (3). Table 2 lists the main poisonous plants present in Colombia, classified according to the affected system (3).

RESEARCH PERSPECTIVES REGARD-ING POISONOUS PLANTS IN COLOM-BIA

Colombia is the second country in the world having the greatest diversity of flora as well as great cattle-raising potential this being mainly

Table 1. Examples of plants which are po	isonous for animals	present in C	Colombia,	classified a	ccor-
ding to their main chemical compounds					

Chemical Group	Toxic Substance	Plant	Effect	
Alcohols and acids	Monofluoroacetic acid	Palicourea spp.	Neurotoxic	
	Cicutoxin	Cicuta maculata.	Neurotoxic.	
	Calcium insoluble oxalates	Philodendron spp., Monstera spp., Caladium spp.	Irritation of the oral cavity.	
	Soluble oxalates	Rumex sp., Chenopodium sp., Rheum sp.	Hypocalcemia, nephrotoxic.	
	Diterpenes	Aconitum spp.	Neuromuscular.	
Alkaloids	Indolizidine	Ipomoea carnea, Sida rhom- bifolia.	Neurotoxic, infertility.	
	Piperidine	Conium maculatum	Neurotoxic	
	Piridine	Nicotiana tabacum.	Neurotoxic.	
	Prrolizidine	Senecio spp, Crotalaria spp.	Hepatotoxic, photosensitivity	
	Quinolizidine	<i>Lupinus</i> spp.	Teratogenic, myotoxic, neuro- toxic	
	Tropane	Datura spp.	Anticholinergic	
Glycosides	Cardiac	Nerium oleander, Digitalis purpurea.	Cardiotoxic	
	Cyianogenic	Prunus sp., Sorghum sp., Trifolium repens.	Respiratory, nervous.	
	Phytoestrogen	Medicago sativa	Infertility	
	Ptaquiloside	Pteridium aquilinum,	Enzootic hematuria, Aplastic anemia.	
Proteins and	Lectins	Momordica charantia, Rici- nus communis.	Gastrointestinal.	
aminoacides	Thiaminase	Pteridium aquilinum, Equise- tum spp.	Neurotoxic.	

Affected system	Plant	Toxic substance
	Thevetia peruviana	Cardenolides
Cardiovascular	Nerium oleander	Cardenolides
	Sorghum vulgare	Nitrates
	Ricinus communis	Lectins
Gastrointestinal	Panicum coloratum	Steroidal saponines
	Senecio spp.	Pyrrolizidine alkaloids
	Rumex spp.	Oxalates
Urinary	Amaranthus spp.	Oxalates
	Pteridium aquilinum	Ptaquiloside
	Equisetum spp.	Tiaminase
Nervous	Conium maculatum	Piperidine alkaloids
	Prunus spp.	Cyanogenic glycosides
Skeletal muscular	Senna occidentalis	Anthraquinones
	Fagopyrum esculentum.	Fagopyrin
Skin and appendages	Lantana spp.	Triterpenes
	Brachiaria decumbens.	Sporidesmin (produced by <i>Pyhtomyces chartarum</i>)
	Trifolium subterraneum.	Isoflavones
Reproductive	Medicago sativa	Steroidal saponines
	Lolium perenne	Nitrates

Table 2. Examples of plants which are poisonous for animals present in Colombia, classified according to the affected system

carried out on natural pastures which man has scarcely interfered with; large-scale production systems are used thereby representing a high risk of poisoning by the plants eaten by the cattle. Colombia does not have a system for recording possible cases of animals becoming poisoned by plants, making this phenomenon practically unknown to such an extent that nitrates and cyanogenic glycosides are usually considered as being exclusively responsible for poisoning by plants. There are very few publications about poisonous plants in Colombia, most existing ones having been published in non-scientific journals and practically none in internationally indexed journals, thereby creating a large conceptual vacuum concerning material related to this problem.

Research into poisonous plants of interest regarding livestock in Colombia must consider different aspects involving compiling an inventory by natural regions regarding plants which have been empirically recognised as being poisonous in each area, identifying poisonous plants by performing suitably designed toxicological studies, carrying out phytochemical studies leading to recognising the main metabolites responsible for toxicity and estimating the economic losses generated by poisonous plants for cattle-raising, bearing in mind the morbidity, mortality and costs incurred in prevention and treatment.

CONCLUSIONS

Cattle-raising potential, exploitation system characteristics and the great biodiversity of flora in Colombia are factors which, added to the emergent investigation being developed to date regarding plants which are poisonous for animals, justify suitable exploration of this problem. A methodology must be designed for correctly researching this field which has been based on identifying suspicious plants until the responsible active ingredients have been elucidated, characterising the clinical, paraclinical and pathologic picture in species which habitually become affected. Studying plants which are poisonous for animals could make exploitation systems more productive, thereby endeavouring to ensure a lower economic impact or contributing towards knowledge about this subject concerning public health.

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