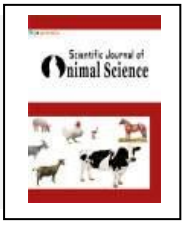
Sjournals
Scientific Journals
Scientific Journal of Animal Science (2013) 2(6) 138-143
ISSN 2322-1704

Contents lists available at Sjournals
Scientific Journal of Animal Science
Journal homepage: www.Sjournals.com



Original article

Screening of some Nigerian plants for pyrrolizidine alkaloids

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ARTICLE INFO

Article history:
Received 06 May 2013
Accepted 20 May 2013
Available online 29 June 2013

Keywords:
Pyrrolizidine alkaloids
Ehrlich's reagent
Plants
Nigeria

ABSTRACT

Pyrrolizidine alkaloids (PAs) are an important class of plant toxins whose hepato- and pneumo-toxicity have been extensively studied. Twenty three field plant materials (leaves, flowers, pulp, seeds) were collected, identified and screened for unsaturated PAs. A field test method with Ehrlich reagent was used. The absorbance of Ehrlich colour of the PAs and N-oxides were obtained at 565nm. Positive reactions were obtained from *Borreria verticillata; Guiera senegalensis; Tamarindus indica*; *Crotolaria doniana; C. gorensis; C. glaucoides; C. mucronata; C. naragutensis; C. lachnosema* and *C. retusa*. The occurrence of PAs are reported for the first time in *C. doniana* and *C. glaucoides. B. verticillata, G. senegalensis* and *T. indica* gave positive Ehrlich reactions which could be due to PAs or other groups giving positive Ehrlich reaction. A number of medicinal and food plants gave negative Ehrlich reaction. Further work is required to confirm and identify the alkaloids in the plants that gave positive reactions.

1. Introduction

Pyrrolizidine alkaloids (PAs) are among the most significant plant toxins in the world. They are an important cause of poisoning in livestock, wildlife and humans resulting in significant economic losses annually (Kellerman et al., 1996; Stegelmeier et al.,1999). Smith and Culvenor (1981) estimated that 3% of the world's flowering plants contain PAs. They occur mainly in the families Boraginaceae (many genera), Compositae (tribes Senecionae and Eupatorieae), Orchidaceae (nine genera) and Leguminosae (mainly the genus Crotolaria) (Dharmananda, 2002). More than 95% of the PA containing plants investigated thus far belonged to these four families (Ober and Hartmann, 1999). The majority of the species within these genera have not yet been investigated but are expected to contain PAs.

Of the 700 PAs and PA- N-oxides identified so far, in over 6000 plants, about half of them exhibit hepato-toxicity (Radominska-pandya, 2010), several have been shown to be carcinogenic in rodents (Fu et al.,2002), and some are non-toxic (Mattocks, 1986). PAs are produced by plants as a defense mechanism against insect herbivores. Most of the species containing these alkaloids are not attractive to grazing animals and some of them are not eaten at all, if other forages are available. This natural restriction on the amount of the plant consumed is very fortunate and has an important bearing on the chronicity of the disease. However, the plant can invade fields or crops and plant parts or seeds can contaminate prepared feed and grains which are then readily eaten by animals and human (Stegelmeier et al., 1999; Rosemann, 2007).

Toxicity is influenced by many factors including species, age, sex, as well as other temporary factors, such as, bio-chemical, physiologic and nutritional status (Stegelmeier et al., 1999). Different species have vastly different susceptibilities to PAs. For instance, the toxic doses of some plants are twenty times higher for sheep, the dose that kill cattle. For this reason, sheep and goat have been used to graze pastures that are dangerous to horses and cattle (Hooper, 1978). Horses appear to be as sensitive to PAs toxicity as cattle, but pigs are reported to be the most sensitive animal specie. The reported toxicity indexes are pigs=1; chicken=5; cattle and horses=14; rat=50; mice=150; sheep and goat=200 (Hooper, 1978). The largest human poisonings occur when plant part or seed are ingested accidentally as a result of food or grain contamination as was the case in Afghanistan in 1974, when over 35,000 people in nearly 100 villages were affected, and many died (Tandon et al., 1978).

PAs contaminated animal products can also enter the human food chain, and although this exposure incidence may not cause immediate toxicity in humans, the effect of long term low level chronic exposure is still uncertain. The most frequently encountered source of residues is the milk of animal that have ingested PA containing plant (Dickinson et al., 1976; Molyneux and James, 1990). Chickens can also transfer PA to their eggs after eating contaminated grains (Edgar and Smith, 2000) and honey has been found to contain high PA level up to 1mg/kg causing a risk to those who consume large amount of honey (Deinzer et al., 1977; Culvenor et al., 1981; Crews et al., 1997). PA residues are, however, unlikely to be present in meat from animals ingesting plants containing PAs since the compounds are rapidly cleared from the tissue, and slaughter would have to occur within few hours after grazing on contaminated pasture (Mattocks et al., 1988). Of concern is the human poisoning risk associated with the use of medicinal herbs that contain PAs. In the past few years, there has been an explosion of interest in the medicinal use of plant and herbal remedies. Exposure to PAs through the use of herbal remedies may be a contributing factor to the high rate of liver cancer and cirrhosis seen in Africa (Steenkamp et al., 2000; ANZFA, 2001).

Of the few documented on plant poisoning in livestock in Nigeria (Adaudi, 1975; Otesile and Akpokodje, 1986; Onyeyili et al., 1994; Abatan et al., 1996) there are no reports of PAs poisoning in animal despite the general toxicological interest in plant containing PAs. The ubiquitous nature of PA containing plants (*Crotolaria* and *Senecio* species) in waste places in Nigeria (Nwude,1986) and a livestock population of 13.9 million cattle, 22.1 million sheep and 34.5 million goats (RIMS,1992), suggests that PA intoxication in livestock is under- diagnosed and under-reported. Human poisoning when plant parts or seeds were ingested accidentally as a result of food or grain contamination has been reported in Nigeria (Mattocks, 1986). The present study was carried out to screen some Nigerian plants for PAs or the N-oxide.

2. Materials and methods

2.1. Plant materials

All the plant materials were collected around Ahmadu Bello University dam, Zaria. The plants were confirmed at the Herbarium Section , Department of Biological Sciences, Ahmadu Bello University, Zaria, where voucher specimens have been deposited.

2.2. Reagents

(a) MeoH containing ethanediol (ethylene glycol 5% v/v)
(b) Light petroleum (b.p 80-100^{0}c)
(c) $Feso_4$ (2% w/v) in methanol (life 8hrs).
(d) Ortho- chloranil (0.5%w/v) in acetonitrile (life 8hrs)
(e) Ascorbic acid; a saturated solution in NaoH (life 24hrs)
(f) Ehrlich reagent. 4-dimethyl amino benzaldehyde (5g) dissolved in a mixture of EtoH (75ml); HoAc (25ml) and 60% perchloric acid (1ml) (life 1 week if kept dark).

2.3. Experimental procedure

Fresh plant material (about 0.2 -1g) was ground in a mortar until it was thoroughly disrupted. Dried plant tops, roots, and seeds were first pulverized. Dried material was left in contact with the solvent for 5 minutes before filteration, to enhance extraction of alkaloids. The mixture was filtered through a fluted paper and the filtrate shaken with its own volume of light petroleum in a separating funnel. The green petroleum layer was discarded and the Meoh phase was shaken with further petroleum as many more times (usually 2) as are needed to remove most of the chlorophyll. Three equal portion of the Meoh solution were transferred to test tube (blank designated B, samples designated A and N). Ortho-chloranil reagent (0.1 – 0.2 ml) was mixed with A; $Feso_4$ mixed with N. If the later form a green precipitate, more $Feso_4$ (up to max. of 1ml) was added. The tubes were heated at 70^0 to 80^0 c for 1 minute. A few drops of methanolic ascorbic acid was added to A to dispel the orange colour. Ehrlich reagent (1ml) was then added to each tube and heating continued for a further 1 minute. A magenta colour in A compared with B indicated the presence of an unsaturated basic PAs. A magenta colour in N indicated an unsaturated PA – N- oxide.

3. Results

3.1. Test for pas and pa-n-oxides

The results are presented in table I. *Crotolaria retusa* (seeds). *C.* l*achnosema* (seeds) *C. mucronata* (seeds), *C. doniana* (seeds), *C. gorensis* (seeds). *C. glaucoides* (seeds); *Borreria verticillata* (leaves and flowers); *Guiera senegalensis* (leaves) and *Tamarindus indica* (fruit) gave positive reactions while the other plants gave negative reactions for both alkaloid and N-oxide.

3.2. Absorbance of Ehrlich Colours of positive plant extracts

The absorbance (at 565nm) of the magenta colour formed by the plant extract showing positive reactions are shown in table II. They are in the range of 0.1 to 0.2.

Crotolaria retusa containing monocrotaline and used as standard had an absorbance of 0.21 for PA. The absorabance for N-oxide range from 0.1 to 1.10 (*G*. *senegalensis*), *C. retusa* (0.46). The absorbance of *C. naragutensis*, *C. glaucoides*, *C.mucronata* and *C. lachnosema* were not taken.

Table 1
Result of field test for unsaturated pyrrolizidine alkaloids and n-oxides

S/NO	Plants	Part	Alkaloid	N-oxide
1.	*Crotolaria retusa*	Seeds	+ve	+ve
2a.	*C. lachnosema*	Seeds	+ve	-ve
2b.	*C. lachnosema*	Leaves	-ve	-ve
3a.	*C. mucronata*	Seeds	+ve	-ve
3b.	*C. mucronata*	Leaves	-ve	-ve
4a.	*C. naragutensis*	Seeds	+ve	-ve
4b.	*C. naragutensis*	Leaves	-ve	-ve
5a.	*C. doniana*	Seeds	-ve	+ve
5b.	*C. doniana*	Leaves	-ve	-ve
6a.	*C. gorensis*	Seeds	-ve	+ve
6b.	*C. gorensis*	Leaves	-ve	-ve
7a.	*C. glaucoides*	Seeds	-ve	+ve
7b.	*C. glaucoides*	Leaves	-ve	-ve
8	*Borreria verticillata*	Leaves & flowers	+ve	+ve
9	*Tamarindus indica*	Pulp & seed	+ve	+ve
10	*Guiera senegalensis*	Leaves	+ve	-ve
11a.	*Indigofera spicata*	Seeds	-ve	-ve
11b	*I. spicata*	Leaves	-ve	-ve
12a.	*Carica papaya*	Seeds	-ve	-ve
12b.	*C. papaya*	Leaves	-ve	-ve
13a	*Cassia occidentalis*	Seeds	-ve	-ve
13b.	*C. occidentalis*	Leaves	-ve	-ve
14.	*Cassia tora*	Seeds	-ve	-ve
15.	*Lantana camara*	Leaves	-ve	-ve
16.	*Azadirachta indica*	Leaves	-ve	-ve
17.	*Oryza sativa (Rice)*	Seeds	-ve	-ve
18.	*Glycine max (Soy bean)*	Seeds	-ve	-ve
19.	*Vernonia amygdalina*	Leaves	-ve	-ve
20	*Irvingia gabonensis*	Seeds	-ve	-ve
21.	*Tecona Stans*	Leaves &pods	-ve	-ve
22.	*Calotropis procera*	Leaves &pods	-ve	-ve
23.	*Macrua anglolensis*	Leaves &pods	-ve	-ve

Table 2
Absorbance at 565 nm of Ehrlich colour of plants that gave positive reactions

S/NO	Plants	Alkaloid	N-Oxide	Remarks
1.	*Guiera senegalensis*	0.19	1.10	N-oxide was –ve
2.	*Borreria verticillata*	0.10	0.25	
3.	*Tomarindus indica*	0.12	0.22	
4.	*Crotolaria doniana*	0.11	0.10	Alkaloid was –ve
5.	*C.gorensis*	0.06	0.11	Alkaloid was –ve
6.	*C. retusa*	0.21	0.46	Pilot test
7.	*C. naragutensis*	-	-	Not taken
8.	*C.glaucoides*	-	-	Not taken
9.	*C. mucronata*	-	-	Not taken
10.	*C. lachnosema*	-	-	Not taken

4. Discussion

Ten of the twenty three plants screened gave positive reactions for pyrrolizidine alkoloids or PA-N-oxide. Of these, seven are *Crotolaria* species. This is not surprising as PAs and the N-oxides are known to occur in *Crotolaria* species. Of the *Crotolaria* species that showed positive reactions PAs have been reported in *C. retusa, C. lachnosena, C. naragutensis and C. mucronata* (Mattocks 1986; Mattocks and Nwude, 1988). However, no report of the occurrence of the alkaloid or its N-oxide has been found in respect of *Crotolaria doniana* and *C. glaucoides*. This would appear to be the first report of occurrence of PAs in these *Crotolaria* species. The other three plants that showed positive reactions are *Borreria Verticillata, Guiera senegalensis* and *Tamarindus Indica*. These need further investigations as the magenta colour could have been given by groups other than pyrrolizidine alkaloids. Such groups that give positive Ehrlich reactions include indoles and pyrroles (Mattocks, 1986). In these cases the blank will also show Ehrlich positive reactions. If these plants are confirmed to have PAs then, this would appear to be the first report of their occurrence in the plants. It would also be a significant finding as the plants are important medicinal plants. *T. Indica* is a popular food plant in Northern Nigerian. The pulp is used for making pap *commonly* known as "kunnu tsamiya" among Hausas. The plant is also known for its purgative effect which is often attributable to its tannin content. Dalziel (1937) reported the medicinal use of *Gueira senegalensis* and *Borreria verticillata* in Northern Nigeria and Gambia respectively. There is a possibility that these plants may constitute health hazard to those who use them. There are several reports of infants developing Veno-occlusive disease when they were treated with herbal teas or when their mothers used such an herbal preparation during pregnancy (Stillman et al., 1977; Roulet et al., 1988; Sperl et al., 1995).

On the other hand, majority of the plants screened were negative for PAs. This is fortunate as many of them like *Carica papaya*, *Cassia occidentalis, Azadiracta indica* are important medicinal plants, while others such as, *Oryza sativa*, *Glycine max, Vernonia amygdalina, and Irvingia gabonensis* are important food plants. Further work is required to confirm and identify the alkaloids in the plants that showed positive reactions.

5. Conclusion

In this study twenty three plants including medicinal, food and hedge plants were screened for unsaturated PAs. Ten gave positive reactions. These were: *Crotolaria retusa, C. naragutensis, C. lachnosema, C. mucronata, C. doniana, C. gorensis, C. glaucoides, Borreria verticillata, Guiera senegalensis,* and *Tamarindus indica.* A number of important food and medicinal plants gave negative reactions. The study also revealed some specific hazardous situations where plants that gave positive Ehrlich reactions are associated with home remedies in human population. This merits further investigation. Additional information is also needed on the effect of low dose exposure over extended period of time in human.

Acknowledgement

The contribution and supervision of late professor Nwako Nwude to this work is highly appreciated.

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