Rev. Biol. Trop., 44(1): 269-274, 1996

Toxilogical screening of some Nigerian wild legumes

Mukaila Kadiri[1], Babiker A. Bedri[2] and Salami S. Ajao[3]
[1] Department of Biological Sciences, University of Agriculture, Abeokuta, Nigeria.
[2] Department of Biological Sciences, Bayero University, Kano, Nigeria.
[3] University Teaching Hospital, Ilorin, Nigeria.

(Rec. 22-IV-1994. Ac. 28-II-1995)

Abstract: Toxilogical screening was carried out on seeds of five wild leguminous plants: *Delonix regia, Cassia tora, Sesbania sesban, Crotalaria naragutensis* and *Tamarindus indica.* The animals used in the toxicological screening were mice and rats. The leguminoous seeds have high protein contents which range between 21.1% for *S. sesban* and 47.7% for *C. naragutensis,* while the mineral elements detected in appreciable quantities were calcium, magnesium, potassium, iron, cooper, zinc, phosphorus and sodium. Oxalate contents were very low and they range between 0.03% for *S. sesban* and 0.09% for *C. tora.* Acute toxicity test for 12 days using mice and rats given water extracts of the five legumes through the oral and intraperitoneal routes produced no visible negative effects. The treatment instead resulted in increases in fresh weights of the rats and mice when compared with the control animals that were fed or injected distilled water. There were also increases in blood sera protein and sugar contents of rats injected intraperitoneally with concentrated water extracts. Similar results were obtained in the prolonged toxicity screening.

Key words: Toxicological screening, wild legumes, mineral elements, oxalate, fresh weight, rats, mice.

Plant proteins are normally obtained from the seeds of leguminous plants called legumes which are rich in protein because nitrate producing *Rhizobium* lives symbiotically in their roots.

In Nigeria, only three leguminous plants are currently being cultivated and the main sources of plant protein. These are cowpea (*Vigna unguiculata*), groundnut (*Arachis hypogea*) and soya bean (*Glycine max*). Leguminous plants which grow in the wild are not consumed possibly due to absence of experimental data on their nutritional values and the assumption that they are toxic.

Some Nigerian plants have been found to be lethal to sheep, goats and cattle. These are *Erythrophleum africanum, Crotalaria intermedia, Clematis hirsuta, Gloriosa superba, Kalanchoe lanceolata, Morea zambesica, Nerium oleander, Paullinia pinnata* and *Unginea nigritania* (Anon 1936, Mettar 1941, Adaudi 1974). The toxic components of lethal plants have been found to be cardic glycosides for *Adenium hougel, Calatropic procera,* and *Strophanthus hispidus;* fluoroacetates, oxalates, toxalbumins and saponins for *Dichapetalum barteri, Trianthe mapentandra, Portulaca oleracea, Balanites aegyptiaca, Cassia absus,* and *Sesbania punica* (Chen *et al.* 1937, Dalziel 1955, Watt & Breyer-Brandwijk 1962, Patel and Rowson 1964, Everand 1966, Nwude 1976, Powell *et al.* 1990).

Numerous plants have been subjected to toxicological experiments using animals. Using *Indigofera spicata* Christic *et al.* (1975), found the plant to cause death of rats at 48-96% diet content after 2-6 weeks. Similarly, Olsen (1977) working with *Delphinium* sp. found the plant to be toxic to rats at 5 to 6.7 mg/g body wt. while *Kalanchoe integra* given orally was found to cause death in sheep at 200 mg/kg body wt. after 21-28 days of application (Verna *et al.* 1981). Intraperitaneum injection of *Poa bucca* has been observed to be lethal to cattle at 1.5 g/kg body weight (Pomilio *et al.* 1989) while *Albizia stipulata* feeding to rams for 5 to 6 weeks resulted in weight loss, convulsion, degenerated vital internal organs and death (Sharma *et al.* 1969). Nwude *et al.* (1977) showed the leaves of *Dichapetalum barteri* to be toxic to mice, rabbits and goats by causing restlessness, convulsion, congestion of liver, lung, kidney and spleen at 2 g/kg feeding for mice, 0.5 g/kg for rabbit and 2.2 g/kg for goats.

The objectives of the present study were to isolate wild Nigerian legumes that are rich in protein and mineral contents, low in oxalate contents and which are non-toxic to rats and mice.

MATERIAL AND METHODS

Collection of wild leguminous seeds: Five leguminous plants were used in the projet. These are *Tamarindus indica, Casia tora, Crotalaria naragutensis, Delonix regia* and *Sebania sesban.* Seeds of the wild legumes were thoroughly washed with tap water, dried in the sun and ground into powder samples using a blender. The powderer samples were stored in a refrigerator at 10 C pending usage.

Determination of legume protein, mineral elements and oxalate contents: The determination of protein and mineral element contents were carried out at International Institute of Tropical Agriculture (I.I.T.A.), Ibadan, Nigeria, by supplying 1 g of each powdered sample to the Institute. The methods adopted employed the microkejdahl digestion for crude protein contents and atomic absortion spectrophotometer, flamephothometer and phosphovanademolybdate reaction for the mineral element contents. These methods are amply described in I.I.T.A. manual of 1979. Oxalate content of the powdered sample was estimated as oxalic acid equivalent using the method described by Oke (1966), Ogundana & Fagade (1982).

Acute toxicity examination of leguminous seeds: Hot water extraction was made for the different leguminous seeds collected at the ratio of 1:20 of seed powder to distilled water at 100 C for 30 min. and the extract filtered (Fasidi & Kadiri 1994). The extracts were kept in a refrigerator at 5 C pending usage. Throughout the study period, the animal's diet was commercial rat cubes, room temperature was 26 ± 4 C and relative humidity of 47 ± 10%. In acute toxicity experiment, two routes were used, oral and intraperitoneal routes. For oral route, 60 swiss white mice aged 6 weeks and weighing 20-22g, from either sex were observed for one week prior to the toxicity test. Five mice were used for application of each dosage level and 10 different dosages were used, namely: 30 g/kg, 40 g/kg, 50 g/kg, 60 g/kg, 70 g/kg, 80 g/kg, 90 g/kg, 100 g/kg, 110 g/kg and 120 g/kg per mouse body weight. The dosages, which were concentrated and diluted to 30 ml each, were given to mice as drinking water. The control animals were given 30 ml of distilled water each, also as drinking fluid. The mice were observed for 12 days, after which they were reweighed. For the intraperitoneal route, Wister rats aged 6 weeks and reweghing 70-75 g were observed for one week prior to toxicity test. The extracts of 60 g/kg, 90 g/kg and 120 g/kg mentioned above were concentrated by evaporation to 1.5 ml. As before, 1 rat was used for application of each dosage level, the treatment replicated 5 times using 5 rats. The dosages were administered into the rats by injection intraperitoneally. The control animals were of two groups, one group injected with sterile distilled water and the other injected with phalloidin, a lethal mushroom toxin. The animals were given 100 ml distilled water each and observed for 12 days, after which they were reweighed. Thereafter, 4 ml of blood were removed through the tail from each treated rat and control and the blood mixed with EDTA (anticoagulant). This was replicated thrice, the blood samples centrifuged to separate the blood sera from the blood cells and the blood sera analysed for sugar and protein contents.

Prolonged toxicity examination of leguminous seeds: This study was conducted for a period of three months. Six weeks old Wister rats weighting 70-75 g were observed for one week prior to the toxicity test. One rat was employed for application of each treatment dose and three different doses of 60 g/kg, 90 g/kg and 120 g/kg per rat body weight were chosen. All the treatments were replicated five times. These dosages were separately diluted to 900 ml and each given as a dose to a rat replicate while the control rats were given 900 ml of distilled water each. The rats were reweighted at 90 days from the onset of the experiment.

Determination of carbohydrate and protein levels in blood sera of treated and control rats: Blood sera collected under acute toxicity examination of leguminous seeds above were analysed for sugar and protein levels. To 1 ml of blood serum, 2 ml of 3,5-dinitrosalicylic acid (DNSA) reagent were added and the ensuing solution boiled for 5 min. The solution was diluted to 10 ml with distilled water and the optical density read at 540 nm against a blank that contained 1 ml distilled water instead of the serum. Serum sugar was calculated using a calibration curve of various concentrations of glucose that were treated as the serum (Denison & Koehen 1977). For the blood serum protein content, 3 ml of solution A were added to 1 ml of blood serum. Ten min later, 0.3 ml of folin-phenol reagent was added and 30 min later, the optical density of the solution was read at 710 nm against a blank than contained 1 ml of 50 ml of 2% Na_2CO_3 in 0.1 ml NaOH mixed with 1 ml of an equal volume of 1% $CuSO_45H_2O$ and 1% sodium tartrate. The protein concentration in the serum was calculated using calibration curve of various concentrations of casein that were treated as the blood serum (Lowry *et al.* 1951).

Statistical analyses: Treatments and controls were in three or more replicates and the data were analysed statistically using the analysis of variance, significant differences between the treatments and controls were detected by calculating the least significant differences (LSD) at P=0.05.

RESULTS AND DISCUSSION

The most abundant mineral element in the wild legumes was potassium, followed by phosphorus, while the least abundant was manganese (Table 1). *Crotalaria naragutensis* has the highest contest contents of calcium (0.48 mg/g), magnesium (2.0 mg/g), potassium (30.4 mg/g) and sodium (7.0 mg/g) while *Delonix regia* has the highest contents of manganese (0.06 mg/g), iron (2.36 mg/g) and zinc (0.14mg/g) (Table 1). The phosphorous contents which range from 8.5 mg/g in *C. tora* to 12.7 mg/g in *S. sesban* are higher than those of groundnut seed (3.8 mg/g), soya bean (5.8 mg/g) and Cowpea (4.5 mg/g) quoted by Oyenuga (1968). The highest iron content of 2.36 mg/g in *D.· regia* seeds and the lowest value of 0.42 mg/g in *C. naragutensis* are higher than iron contents of groundnut (0.02 mg/g), soya been (0.07 mg/g) and cowpea (0.04 mg/g) reported by Oyenuga (1968). The magnesium contents which range from 1.38 mg/g for *C. tora* to 2.04 mg/g for *C naragutensis* are higher that those of some Nigerian vegetables like *Celosia argentia* and *Telfairia occidentalis* which have magnesium contents of 0.66 mg/g and 0.78 mg/g respectively as reported by Faboya (1983).

The highest protein content of 47.7% was detected in the seeds of *C. naragutensis* (Table1) and this compares favourably well with the highest known protein content of 66% reported for *Macharium acutifolium* by Mayer (1975). Protein contents of *T. indica, C. tora, C. naragutensis* and *D. regia* which range from 36.6% to 47.7% are greater than the protein contents of *Arachis hypogea* (30.4%) and cowpea (24.7%) as reported by Oyenuga (1968). The oxalate contents of the five leguminous seeds range from 0.03% for *S. sesban* to 0.09% for *C. tora* (Table 1). These oxalates values are lower than those detected in some Nigerian vegetables worked upon by Faboya (1983).

The water extracts of the five leguminous seeds did not cause the death of animals in the acute and prolonged toxicity test given through oral route and through intraperitoneal injection of the concentrated water extract (Figs. 1, 2 and 3). On the contrary, most of the treated animals had significant increases in fresh weights (Figs. 1,2 and 3). However, rats injected with mushroom toxin, phalloidin, died on day 2. These results of increases in fresh weights of animals given acute and prolonged toxicity test via the oral route and the death of rats injected with phalloidin indicate that the constituents of the leguminous seeds are non-toxic. Fasidi & Kadiri (1994) similarly found seven wild mushroom species to be non-toxic by feeding their water extracts to rats and observed that the treated rats did not die but instead had

TABLE 1

Protein, oxalate and mineral element contents of leguminous seeds employed in the toxicological screening

Seed type	Ca	Mg	K	Mn	Fe	Cu	Zn	P	Na	Crude Protein	Oxalate %
Tamarindus indica	0.16	1.52	20.4	0.03	1.48	0.18	0.09	8.8	2.7	38.7	0.05
Cassis tora	0.09	1.38	20.4	0.03	1.04	0.10	0.08	8.5	2.6	37.5	0.09
Crotalaria naragutensis	0.48	2.04	30.4	0.04	0.42	0.11	0.13	10.6	7.0	47.7	0.08
Delonix regia	0.08	1.88	28.3	0.06	2.36	0.18	0.14	10.1	5.1	36.6	0.07
Sesbania sesban	0.18	1.92	30.2	0.03	0.88	0.26	0.12	12.7	4.2	21.1	0.03

Data are in mg/g dry weights for mineral elements and percentage dry weigths for crude protein and oxalate contents.

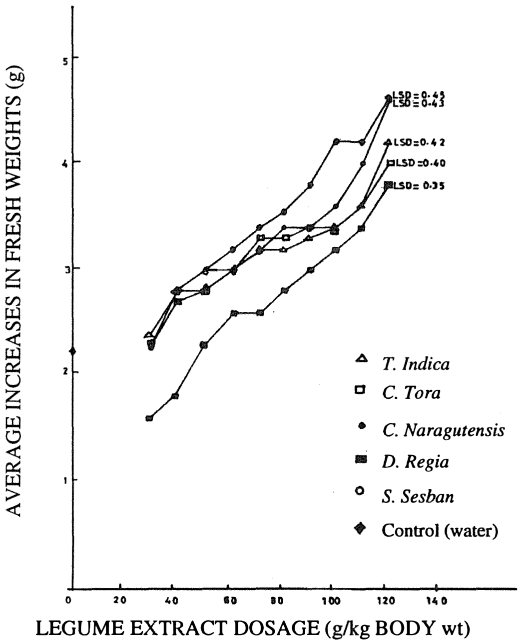

Fig. 1. Changes in fresh weights of mice fed orally with water extracts of *I. indica*, *C. tora*, *C. naragutensis*, *D. regia* and *S. sesban* seeds during the acute toxicity screening.

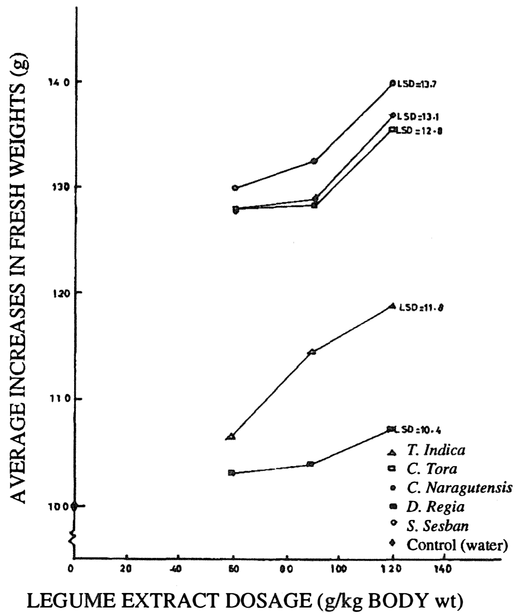

Fig. 2. Changes in fresh weights of rats fed orall with water extracts of *I. indica*, *C. tora*, *C. naragutensis*, *D. regia* and *S. sesban* seeds during the prolonged toxicity screening.

increases in fresh an dry weights. Results contrary to the present finding were seen in the literature. For example, *Cassia occidentalis* was found to cause death of laboratory rats and sheeps (Kamau 1975). Consumption of *Dihapetalum barteri* was found to be lethal to rabbits and goats al 0.5 g/kg and 2.2 g/kg body weight respectively within 4 hr of eating and clinical symptoms of toxicity observed were depression, restlessness, convulsion and death (Nwude *et al* 1977). *Kalanchoe integra* has been found to cause death of sheep fed with its leaf extract at 200 mg/kg body weight for 21-28 days with clinical symptoms of toxicity detected being loss in weight, emaciation and death (Verna *et al* 1981).

The haematological examination of protein and sugar contents of treated and control rats

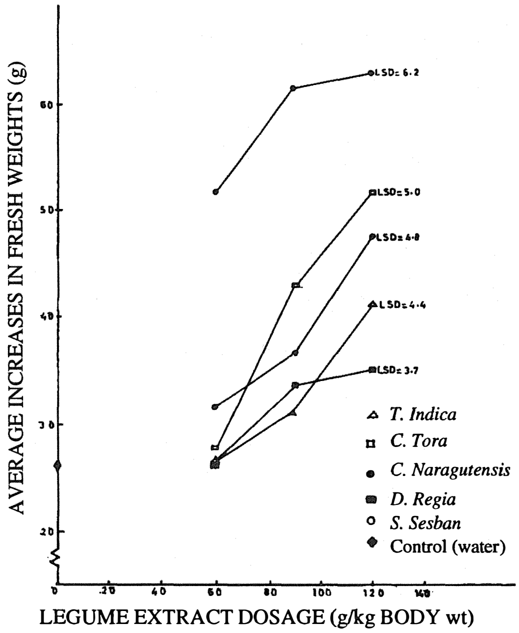

Fig. 3. Changes in fresh weights induced in rats due to interperitoneal injection of rats with concentrated water extracts of *T. indica*, *C. tora*, *C. naragutensis*, *D. regia*, and *S. sesban* seeds during the acute toxicity screening.

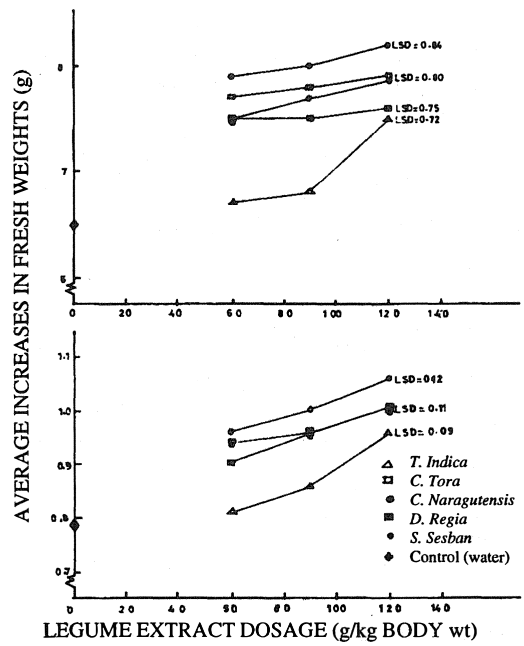

Fig. 4. Sugar and protein levels in blood sera of rats injected intraperitoneally with concentrated water extracts of *T. indica*, *C. tora*, *C. naragutensis*, *D. regia* and *S. sesban* seeds.

sera showed significant increases in the sera sugar and protein contents of some treated rats (Fig. 4). This suggest that the wild legumes contain nutritional factors. Sera protein contents of the treated rats vary from 6.7 mg/ml to 8.2 mg/ml (Fig. 4). This value compares well to the 8.3 mg/ml serum protein content obtained for sheep that were fed *Tribulus terrestris* (Bourke 1975).

In conclusion, the present finding clearly show the richness of the five wild legumes in protein and minerals. Their low oxalate contents and their induction of increases in fresh weights of rats and mice after acute and prolonged toxicity tests. The significance of these finding is that the five leguminous species seeds are non-toxic to mice and rats and by inference, they may not be toxic to livestock and man. They are therefore recommended as animal feeds. They could also be incorporated into animal feeds in order to enhance the feeds' protein and mineral contents and thereby reduce the pressure on use of groundnut, cowpea, soya bean, fish, blood meal etc. as protein sources in animal feeds.

RESUMEN

Se hizo una evaluación toxicológica de semillas en cuatro leguminosas silvestres: *Delonix regia, Cassia tora, Sesbania sesban, Crotalaria naragutensis* y *Tamarindus indica,* usando ratones y ratas. Las semillas tienen un alto contenido proteico que va de 21.1% en *S. sesban* a 47.7% en *C. naragutensis.* Los minerales detactados en cantidades significativas fueron calcio, magnesio, potasio, hierro, cobre, zinc, fósforo y sodio. Hubo muy bajos contenidos de oxalatos: de 0.03% en *S. sesban* hasta 0.09% en *C. tora.* Las pruebas de toxicidad aguda por 12 días con animales a los que se dio extractos acuosos por vías oral e intraperitoneal no produjeron efectos negativos notables, por el contrario, hubo un aumento de peso en comparación con los testigos que recibieron agua destilada. También hubo aumentos en las proteinas del suero sanguíneo y en los contenidos de azúcar de las ratas que recibieron extractos vegetales acuosos intraperitonealmente. Los resultados fueron similares en el análisis toxicológico prolongado.

REFERENCES

Adaudi, A. O. 1974. Poisoning in goats caused by *Dichapetalum barteri.* Trop. Animal Health Prod. 7: 56-57.

Anon. V. A. 1936. Annual Report of Veterinary Dept. Vom, Nigeria. p.22.

Bourke, C. A. 1975. Staggers in sheep associated with ingestion of *Tribulus terrestris.* Austral. Vet. J. 61: 360-363.

Bruere, P. 1942. A coffee subsititute *Cassia occidentalis* , that is toxic before roasting. Pharm. Chem. J. 2: 321-324.

Chen, K. K., R. C. Anderson & E. B. Kabbins. 1937. Potency of five additional cardiacglycosides. J. Amer. Pharm. Assoc. 26: 214-218.

Christic, G. S., M. Nilson & M. P. Hegarty. 1975. Effects on the liver in the rat ingestion of *Indigofera spicata,* a legume containing an inhibitor of arginine metabolism. J. Pathol. 117: 195-204.

Dalziel, J. M. 1955. Useful plants of west tropical Africa. Grown, London. p. 23-26, 44, 47,60, 63.

Denison, A. D. & D. R. Koehen. 1977. Cellulase activity of *Poronia oedipus.* Mycologia 69: 592-603.

Everand, M. M. 1966. The pathology of *Crotalaria spectabilis* seed poisoning in domestic fowl. J. Amer. Vet. Med. Assoc. 43: 627-634.

Faboya, O. O. P. 1983. The oxalate contents of some Nigerian Vegetables. Nigerian J. Appl. Sci. 1: 55-58.

Fasidi, I. O. & M. Kadiri. 1994. Toxicological screening of seven Mushroom. Food Chem. 42: in press .

I.I.T.A. 1979. International Institute of Tropical Agriculture: Manual. Ibadan, Nigeria.

Kamau, J. A. 1975. Kiliambiti plant (*Adenia valkensii*) toxicity in animals. Bull anim. Health Prod. Africa 23: 189-205.

Lowry, O. H., H. N. Rosebrough, L. A. Farvi & J. R. Randal. 1951. Protein measurement with folinphenol reagent. J. Biol. Chem. 193: 265-275.

Mayer, A. M. & A. P. Mayber. 1975. The germination of seeds. Pergamon, Oxford. p. 10-13, 15-17.

Mettam, R. W. W. 1941. Plant poisoning in the domestic animals. Farm Forest 1: 58-60.

Nwude, M. 1976. Toxicological investigation of *Lasiosiphon kraussianus.* Ph.D. thesis, Ahmadu Bello University, Nigeria.

Nwude, M., L. E. Person & A. O. Adaudi. 1977. Acute toxicity of the leaves and extracts of *Dichapetalum barberi* in mice, rabbits and goats. Toxicology 7: 23-29.

Ogundana, S. K. & O. E. Fagade. 1982. Nutritive value of some Nigerian Edible mushroom. Food Chem. 8: 263-268.

Oke, L. O. 1966. Chemical studies of the more commonly used leaf vegetables in Nigeria. J. West Afric. Sci. Assoc. 11: 42-48.

Olsen, J. O. 1977. Rat bioassay for estimating toxicity of plant material from larkspur (*Delphinium sp.*). Amer. J. Ver. Res. 38: 277-279.

Oyenuga, V. A. 1968. Nigeria foods and feeding stuffs. Ibadan University, Ibadan, Nigeria. p. 9-15.

Patel, M. B. & J. M. Rowson. 1964. Investigation of certain Nigerian Medicinal plants. Planta Medica 12: 33-42.

Pomilio, A. B., R. D. Rofi, M. B. Gambino, C. A. Mazzini & R. T. Oebinedetti. 1989. The lethal principle of *Poa bucca* (Corín blanco): a plant indigenous to Argentina. Toxicon 27: 1251-1262.

Powell, R. G., D. Olaltnu & M. Suffress. 1990. Occurence of sesbanimide in seeds of toxic sesbania species. Weed Sci. 38: 148-152.

Sharma, D. D., S. Chander & S. S. Negi. 1969. Nutritive value and toxicity of Abizzia stipulata bowin tree leaves. J. Res. Punjab Agric. Univ. 6: 388-393.

Verma R. K., B. D. Garg, M. U. Kharole & A. Ahmad. 1981. Chronic toxicity studies on *Kalanchoe integra* in sheep. Indian J. Anim. Sci. 51: 511-526.

Watt, J. M. & M. G. Breyer- Brandivijk. 1962. The medicinal and poisonous plants of southern and eastern Africa. Evans and Evans, Livingstone, Edinburg. p. 36-40.