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Acute toxicity study on neem (*azadirachta indica*, juss) leaf aqueous extract in chicken (*gallus gallus domesticus*)

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ABSTRACT: **Background**: Acute toxicity of *Azadirachta indica* (neem) leaf aqueous extract was tested on chickens; and the median lethal dose (LD_{50}) was calculated. Both clinical signs, gross lesion and microscopic lesions at post mortem were recorded.

Objectives: To evaluate the toxicity of neem leaf extract in chicken, so as to establish safe usage of neem products.

Methods: Five experimental groups of chickens were used in this study. Four of the groups were respectively given intraperitoneal doses of 800mg/kg, 1600mg/kg, 3200mg/kg and 6400mg/kg of the extract, while group five was control. The LD_{50} was calculated. Clinical signs and tissue post mortem and microscopic lesions recorded.

Results: The calculated median lethal dose (LD_{50}) was 4800mg/kg. The severity of the clinical signs, gross and microscopic lesions of tissues observed at post mortem was dose- dependent.

Conclusion: This study has shown that *Azadirachta indica* (neem) leaf aqueous extract is toxic, with toxicity being dose- dependent, and should be used with caution in ethno-veterinary practice.

Keywords: *Azadirachta indica* **(Neem) leaf, aqueous extract, acute toxicity, chicken.**

Introduction

The neem (*Azadirachta indica* A. Juss), is a fast growing drought resistant multipurpose, exotic tree species central to the afforestation programmes in many west African countries. In Nigeria, neem forms about 90% of the trees in the forestry plantations established in the 12 states within the Savanna vegetation zone (Donli and Buahin 1998)

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The use of plants as medicine predates written human history (Saxena, 2000) with herbal medicines cheaper than orthodox medicine both in cost of purchase and advertisements more accessible and more acceptable (Rabo *et. al*., 2000). It has been documented that for over thousands of years, the neem has been used for medicinal purposes (Khalid *et. al.,* 1989). Stimulated by the varied medicinal usage and its presence in the locality (Donli and Buahin 1998; Aladesanmi *et. al.*, 1988) this present study was conducted to determine the toxic effects of the neem leaf aqueous extract in chickens, with the hope that the results will be significant in establishing the safe use of neem products.

Materials and Methods

Plant collection and identification: The neem (*Azadirachta indica*) leaves used in this study were collected from around the University of Maiduguri campus, in 2002 and identified by a botanist in the Department of Biological Sciences, University of Maiduguri, Nigeria. Some of the neem leaves have been deposited in Department for reference.

Extract preparation: The fresh matured neem leaves collected were air-dried in the laboratory (to prevent solar leaching) for 1 week, hand crushed to obtain 2.2 kg powder, which was exhaustively extracted in 700mls distilled water for 8hrs at 60^{o}C using the Soxhlet extractor (Quickfit, England). The neem leaf extract was concentrated on an aluminum tray, placed in an oven and maintained overnight at 60^{o}C to dry, then stored at room temperature (27^{o}C) until used.

Experimental design: A total of twenty five (25) chickens aged 4 weeks old and weighing between 76.4 and 125 grams were randomly separated into 5 groups (A,B,C,D and E) of five (5) each. The chickens were bought from the Evangelical Church of west Africa (ECWA) poultry farm, Maiduguri, and kept in cages in the Department of veterinary pathology and given water and feed (Growers mash ECWA, Nigeria PLC) *ad libitum*. Having acclimatized to the laboratory environment for a period of 2 weeks, the acute toxicity test was conducted to determine the LD_{50} of the neem leaf aqueous extract, using groups A, B, C, D while group E received only drinking water as control. The chickens were intraperitoneally treated with single doses of 800, 1600, 3200 and 6400 mg /kg weight at 60g/100ml concentration of the extract. The chickens were observed over a period of 24hours for clinical signs and death. The LD_{50} of the extract was calculated using the arithmetic method of Karber (Aliu and Nwude, 1982). Post mortem examination of dead animals was performed and samples collected in 10% formal saline, embedded in paraffin wax and cut at 5µm thick before staining with haematoxylin and eosin (Drury and Wallington, 1976). Tissue slides were examined with light microscope at x100 magnification.

Results

Clinical signs and Mortality scores (LD_{50}): Weakness, depression, ruffled feathers, transient anorexia, dyspnoea was noticed immediately with administration of the neem leaf aqueous extract, while dehydration, coma, and death appeared after 1 ½ hours. The dose of the extract that produced 100% mortality was 6400mg/kg. The calculated LD_{50} was 4800mg/kg. At doses of 800, 1600, and 3200 mg/kg there was no mortality (Table.1).

Gross pathology: The livers of the birds were enlarged and had pale focal areas of necrosis while the lungs were congested and edematous. Severity of lesions was dose dependent and observed to be more with the highest dose of 6400mg/kg.

Histopathology: There was severe congestion of the pulmonary blood vessels, interstitial oedema, and leukocytic infiltration which were dose dependent. The liver showed widespread sinusoidal congestion, with periportal necrosis and infiltration of mononuclear cells. There was marked goblet cell hyperplasia and

mononuclear cell infiltration in the lamina propria of the small intestine of some birds. The heart showed fibrinous exudates in the lumen of the endocardium with mild leukocytic infiltration into the endocardium.

Table 1: LD_{50} of neem leaf aqueous extract for chicken

Group (N=5)	Plant Extract (mg/kg body weight)	Dose difference (mg/kg extract)	Dead birds	Mean dead	Dose difference x Mean dead
A	800		0		
		B – A = 800		0	0
B	1600		0		
		C – B = 1600		0	0
C	3200		0		
		D – C = 3200		2.5	8000
D	6400		5		
Total					8000

$LD_{50} = LD_{100} - \frac{\text{(dose difference(Dd) X mean dead(Md)}}{N} = 6400 - \frac{8000}{5} = 4800$ LD_{50} = 4800mg/kg body weight.

Discussion

The results of the acute toxicity study of neem leaf aqueous extract revealed an intraperitoneal LD_{50} of 4800mg/kg, and clinical signs that were dose dependent. Substances with LD_{50} of 500-5000mg/kg are reported (Agaie *et. al.*, 2000) as moderately toxic, and could be administered with some degree of safety especially through the oral route where the absorption might not be complete due to inherent factors limiting absorption in the gastro intestinal tract. The toxicity and degenerative changes observed in this study agrees with the observation that birds fed on diets containing water extract of neem fruit showed toxicity, and degenerative changes in the liver and kidney (Gowda *et. al.*, 1998) and that visceral organ hypertrophy is common when monogastrics are fed insufficiently processed plant products, usually associated with increased enzyme secretions by the organs, in response to presence of enzyme from the plants (Uko and Kamalu, 2005).

In conclusion, this study has indicated that neem leaf aqueous extract causes liver, kidney, lung, hearts, brain and intestinal damage with dose-dependent clinical signs of weakness, depression, anorexia, dyspnoea, dehydration and death, which requires that it should be used with caution in ethno-veterinary practice.

References

Agaie, B. M., Nwatsok, P. and Sonfada, M.I. Toxicological effects of *Ageratum conyzoides* in rats. Sokoto Journal of Veterinary Sciences 2000; **2** (2): 27 -31.

Aladesanmi, A. J., Awe, S.O., Adesanya, S.A. and Bray, D.H. Antimalarial activity of some Nigerian Medicinal Plants. In: Drug Production from Natural Products. Proceedings of the Seventh International Symposium (ed. Adesina, S.K.). Medex Publications Ltd., Lagos, Nigeria. 1988; 32-36.

Aliu, A. Y. and Nwude, N. Veterinary Pharmacology and Toxicology Experiments. Baraka Press Nigeria Ltd., Zaria. 1st ed. 1982; 104 -109.

Donli, P. O. and Buahin, G. K. A. Management of oriental yellow scale insects (*Aonidiella Orientalis*, Newstead) (Homoptera: Diaspididae) on neem (*Azadirachta indica* A. Juss) (Meliaceae) in West Africa. ESN Occasional Publications 1998;. **31**:1-10.

Drury, R. B. and Wallington, E.A. Carleton's Histological Techniques (4th Edition) Oxford University Press, London. 1976; 392-398.

Gowda, S. K., Verma, S. V. S., Elangovan, A. V. and Singh, A. D. Neem (*Azadirachta indica*). Kernel meal in the diet of white leghorn layers. British Poultry Science. 1998; **39**:648-652.

Khalid, S.A., Duddeck, H. and Gonzalez-Sierra, M. Isolation and characterization of an antimalarial agent of the neem tree *Azadirachta indica*. Journal of Natural Products. 1989; **52** (2): 922 – 926.

Rabo, J. S., Onyeyili, P. A., Salako, M. A. and Khalil, M. I. Acute toxicity studies on aqueous extract of stem bark *Butyrosperum paradoxum* in rats. Bull. Anim. Hlth. Prod. Afr. 2000; **48:** 39-43.

Saxena, M.J. Relevance of herbs in improving health index of livestock animals. Proceedings of the Nigerian Veterinary Medical Association (NVMA) 38th Annual Congress.9th 13th Oct. Badagry- Lagos 2000; 14-16.

Uko, O. J. and Kamalu, T. N. Haematological parameters and weight changes of cockerels fed raw or autoclaved neem seed kernels in diets. Nigerian Veterinary Journal. 2005; **26** (1): 10-17.