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Chronic toxicity study on neem, (*Azadirachta indica*) leaf aqueous extract in chicken (*Gallus gallus domesticus*)

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ABSTRACT: This study on chronic toxicity of neem (*Azadirachta indica*) leaf aqueous extract in chicken (*Gallus gallus domesticus*) involved daily intraperitoneal administration of graded doses: 500, 1000, and 2000 mg/kg of the extract for a period of 18 days. Clinical symptoms of ruffled feathers, weakness, anorexia, depression, and dyspnoea were observed at 20 minutes post exposure extract-dose dependent, and death intervened on day 6 post exposure. At post mortem gross lesions were pulmonary oedema and congestion, distended gall bladder with enlarged and congested liver which had pale foci on its surface. Microscopic lesions were also extract-dose dependent and included pulmonary congestion and oedema with interstitial mono nuclear cell infiltration, hepatic vacuolar degeneration with kupffer cell proliferation, sinusoidal congestion, haemorrhage and necrosis with foci of bacterial colonies, nephritis, congestion of vasa vasori, spongiosis, matted and stunted intestinal villi with goblet cell hyperplasia.

Keywords: Neem leaf, Aqueous extract, Chronic toxicity, Chicken.

Introduction

Neem leaves have been reported as medicinal in the treatment of many conditions including malaria, gastrointestinal helminthiasis, bacteria and viruses (Sofowora, 1993), with anecdotal accounts from West Africa of neem leaf teas possibly cause liver and kidney damage when taken over a long period (National Research Council, 1992). Neem bitters have been found to be toxic to guinea pigs and rabbits (Sadre et al., 1984), and leaves fed to goats and guinea pigs reduced their growth (Ali, 1987).

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Despite studies on neem, its toxicological potential has not been reported in chicken of Borno State, thus this study was designed to determine the chronic toxicity of the neem leaf aqueous extract in chicken with a view to elucidating these effects as a measure towards establishing safety usage levels in chicken.

Materials and Methods

Plant collection and identification: The neem (*Azadirachta indica*) plant was identified by a botanist in the Department of Biological Sciences, University of Maiduguri, Nigeria. Fresh matured leaves were thereafter collected, part of which is now a reference specimen deposited in the University herbarium.

Extract preparation: The fresh matured neem leaves were air dried in the laboratory for one week, hand crushed to obtain 2.2kg of neem leaf powder which was extracted in 700mls of distilled water at 60°C for 8 hours in a soxhlet extractor (Quick fit, England^R) and the extract concentrated on an aluminum tray which weighed 440grams which was stored at room temperature 27⁰C until used.

Experimental chickens and toxicity studies: Twenty (20) chickens weighing between 400g and 725g were randomly separated into 4 groups (A, B, C, and D) of 5 birds each. The chickens in groups A, B, and C were exposed intraperitoneally, to graded doses of 500, 1000, and 2000 mg/kg body weight, at a concentration of 60g/100ml of the neem leaf aqueous extract for a period of 18 days. Group D served as control and received only drinking water ad libitum.

Results

Following the chronic test using the neem leaf aqueous extract the chickens became weak, anorexic, depressed with ruffled feathers and had dyspnoea. The microscopic lesions were dose dependent showing moderate pulmonary congestion, oedema, and severe interstitial mononuclear cell infiltration. The liver showed multifocal areas of hepatocellular necrosis and foci of bacterial colonies, widespread vacuolar degeneration and kupffer cell proliferation, sinusoidal congestion and haemorrhage, and hepatocellular necrosis. The kidney showed tubular degeneration with mild interstitial mononuclear cell infiltration. The heart had a congested vasa vasori with hyalinization, while the intestine was stunted, and matted with villous fusion. There was a marked congestion of the brain with spongiosis.

Discussion

The chronic toxicity tests using graded doses of neem leaf aqueous extract to chickens for a period of 18 days in this study produced clinical signs of weakness, ruffled feathers, dyspnoea, anorexia, and death. These signs were similar to those observed in rats by Rabo *et al.*, (2003); and in chickens by Uko and Kamalu (2005), with a view that they are indications of the wide distribution of the active toxic principles to various organs, and tissues of the body. The extract caused leukocytosis which could be attributed to the immune- modulatory activities of Nim-76, a fraction of neem extracts that enhance cellular response (Ajagbonna *et al.*, 1999). The microscopic lesions which were dose dependent, and of varying degrees of severity indicates that there is no adaptation to the toxic principle of neem bitters (Gowda *et al.*, 1998).

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