Indian Journal of Animal Sciences **80** (7): 647–649, July 2010

Histological changes and protective effect of pipali (*Piper longum*) in arsenic intoxicated cockerels

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Received: 3 November 2007; Accepted: 2 January 2010

ABSTRACT

The toxic effects of arsenic on the visceral organs of cockerel and protective effect of pipali (*Piper longum*) were evaluated in this study. Male White Leghorn chicks were divided randomly and equally into group 1(control), 2(arsenic 50 ppm), 3 (arsenic 100 ppm), 4 (arsenic 50 ppm + pipali 100ppm) and 5 (arsenic 100 ppm + pipali 100ppm). The experimental birds were kept in deep litter system of housing and maintained on grower ration. Birds were sacrificed after completion of 12 weeks feeding trial. At the time of necropsy organs such as liver, spleen, kidney, intestine and bursa were examined for the presence of pathological lesion. Histopathological examination reveals haemorrhages and infiltration of mononuclear cells in intestine, hydropic degeneration of hepatocytes and congestion, degeneration, necrosis, haemorrhages and infiltration of mononuclear cells in hepatic cord. Oedema, congestion, hemorrhage and infiltration of inflammatory cells in lung and degeneration of renal tubular epithelium were observed in dose dependent manner in the cockerels fed on arsenic containing feed for 12 weeks. Histopathological changes were not observed in control group as well as in pipali + arsenic fed cockerels. It is concluded from the present study that the pipali produced protective effect in arsenic induced histopulmonary and renal histopathological changes in poultry.

Key words: Arsenic, Cockerel, Histopathology, Pipali (*Piper Longum*)

In India, underground water is the most prominent source of arsenic poisoning and thus man and animals are likely to be exposed to arsenic at a concentration much higher than the permissible limit (Rahman *et al*. 2001). Arsenic causes several adverse effects on growth and productivity of livestock and poultry causing a substantial economic loss to animal husbandry sector (Sakurai *et al.* 2004, Beane Freeman *et al.* 2004). Pipali (*Piper longum*) contains piperine, the peper alkaloid, possesses several pharmacological actions like anti-infertility, CNS depressant, anti-inflammatory, inhibition of hepatic drug metabolizing enzymes and enhancement of drug bioavailability (Atal *et al.* 1981). Fruit extract of *P. longum* possess hepatoprotective action in rodents in carbon tetrachloride induced hepatotoxicity (Raje *et al*. 1984). The study was carried out to examine the toxic effect of arsenic on visceral organs and protective effect of simultaneous feeding of pipali (*Piper longum*) in cockerels.

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MATERIALS AND METHODS

Analytical grade of arsenic trioxide (As_2O_3) procured from loba chemie was used in this study. The fruits of pipali (*Piper longum*) were collected from Research and Development Farm, Medicinal and Aromatic Plant, Department of Horticulture N.D. University of Agriculture and Technology, Kumarganj, Faizabad, and identified taxonomically, pulverized and used in this investigation.

Arsenic (100ppm) treated feed was prepared by mixing 660.17 mg of arsenic trioxide (containing arsenic equivalent to 500 mg) thoroughly in 5 kg feed. All the birds were fasted over night prior to the start of the experiment. Medicated ration was prepared for about a week period and kept in close container. Pipali fruits were pulverized to prepare 100ppm pipali medicated ration by mixing 600 mg of powder of pipali fruits in about 500 g of ration and then more feed was added to make 6 kg feed.

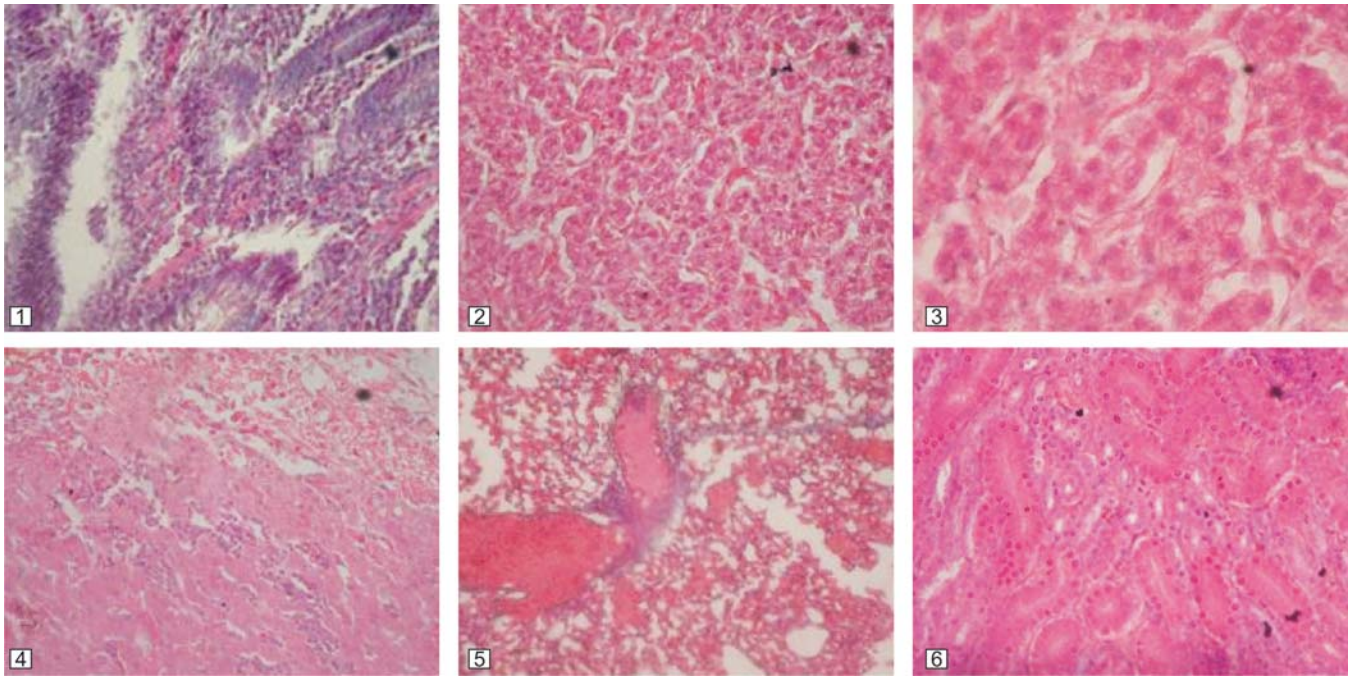
Male White Leghorn chicks (35), 4-week old, were

randomly divided into group 1 (control), 2 (arsenic 50 ppm), 3 (arsenic 100 ppm), 4 (arsenic 50 ppm + pipali 100ppm) and 5 (arsenic 100 ppm + pipali 100ppm) of 7 birds each and were kept in deep litter system of housing and maintained on grower ration. Uniform feeding and management practices were maintained throughout the experiment. Feed and water were provided *ad lib.* throughout the study. Birds were sacrificed after completion of 12-week feeding trial. At the time of histological examination, organs such as intestine, liver, lung and kidney were examined for the presence of any gross lesions.

Visceral organs such as intestine, liver, lung, and kidneys were collected in formal saline solution. Tissues were prepared for histological examination by employing the method of Parsons *et al*. (1984). Paraffin embedded section of tissue were cut at 5–6 µm thickness and stained with haematoxylin and eosin stain (Parsons *et al*. 1984) and examined microscopically for different pathological changes.

RESULTS AND DISCUSSION

On histological examination haemorrhage and infiltration of mononuclear cells in small intestine (Fig. 1) were found similar to findings of Hatch (1988) and Paul and Robert (1975). Hydropic degeneration of hepatocytes (Fig. 2), congestion, degenerative changes, necrosis (Fig. 3) and haemorrhage and infiltration of mononuclear cell in hepatic cord (Fig. 4) were observed in arsenic treated group in this study. This finding is in agreement with findings as described by Klaasen (2001). As shown in Fig. 5, the oedema, congestion, hemorrhage and infiltration of inflammatory cells in lung in arsenic (100 ppm) treated group are in agreement with the finding of Biswas *et al*. (2000) and Hatch (1988). The degenerative changes in renal tubular epithelium in 100 ppm arsenic group observed in this study (Fig. 6) were similar to findings of Gernhardt *et al.* (1978) and Kleinfeld (1980). Noticeable gross and microscopic changes were, however, not observed on histological examination in liver, lungs and kidneys of control group as well as pipali + arsenic fed cockerels. The extract of *Thespesia populnea* flowers, *Electtaria cardamom*, *Zingiber officinalis*, *Glyeyrrhiza glabra*, *Piper longum* and honey reversed the liver damage induced by carbon tetra chloride in rats @ 0.4 ml/100 g b. wt. daily for 10 days (Shriwaiker *et al.* 1991). *Emblica officinalis* was reported to prevent lead and aluminium induced cytotoxic damages in mice (Roy *et al.* 1992, Dhir *et al.* 1993). Kombucha tea supplementation also relieved from the lead-induced immunosuppression to an appreciable level (Dipti 2003). On gross examination mild to moderate haemorrhages and



Figs 1–6. **1.** Photomicrograph of intestine from cockereles fed on arsenic (100 ppm) in diet for 12 weeks showing infliltration of mononuclear cells (MNC) (H&E×40). **2.** Photomicrograph of liver from cockerels fed on arsenic (50 ppm) in diet for 12 weeks showing hydropicdegeneratoin (H&E×40). **3.** Photomicrograph of liver from cockerels fed on arsenic (100 ppm) in diet for 12 weeks showing congestion, degenerative changes and necrosis (H&E×100). **4.** Photomicrograph of liver from cockerels fed on arsenic (100 ppm) in diet for 12 weeks showing haemorrhage and infiltration of mononuclear cells (H&E×40). **5.** Photomicrograph of lung from cockerels fed on arsenic (100 ppm) in diet for 12 weeks showing oedema, congestion, haemorrhage and infiltration of inflammatory cells (H&E×40). **6.** Photomicrograph of kidney from cockerels fed on arsenic (100 ppm) in diet for 12 weeks showing degeneration of renal tubular epithelium (H&E×40).

inflammation were found on lung, heart, peritoneum, kidney, proventriculus and gizzard in a few chicks of arsenic (50 and 100 ppm) groups. Reddening of the mucosa of small intestine and necrotic foci on liver were observed in few birds fed on high arsenic dietary levels. The chicks fed on pipali alone or in combination of arsenic did not reveal any significant gross lesion. No apperent changes were observed except atrophy in few birds of high arsenic dietary levels. Thus, it is concluded that feeding of arsenic at dietary levels of 50 and 100 ppm for 12 weeks produced mild to moderate histological changes in liver, lungs and kidney indicating gastrointestinal, hepatotoxic and nephrotoxic effect in cockerels. The simultaneous feeding of pipali (100 ppm) produce mild to moderate protective action against arsenic induced gastrointestinal, hepatotoxic and nephrotoxic effects in cockerels after 12 – week feeding trial. This is due to piperine (1-piperolypiperidine) an alkaloid *of Piper longum* that possesses several pharmacological actions like anti-infertility, CNS depressant, hepatoprotective, anti-inflammatory, inhibition of hepatic drug metabolizing enzymes and enhancement of drug bioavailability. It is a nonspecific inhibitor of drug metabolism, and shows little discrimination among different forms of cytochrome p-450 enzymes. Piperine interacts with hepatic drug metabolizing enzymes and was found to inhibit aryl hydrocarbon hydroxylationethyl-N-demethylation,T-ethoxycoumarin-o-de-ethylation, 3–hydroxy benzopyrine glucuronidation and activity of hepatic arylhydrocarbon hydroxylase and UDP -glucuronyl transferase in mice and rats (*Atal et al.* 1984, Raje *et al*. 1984, Koul and Kapil 1993).

ACKNOWLEDGEMENT

Authors would like to thank to Director of Research, ND University of Agriculture and Technology, Faizabad (UP) for providing facility to carried out the research in the College of Veterinary Sciences and Animal Husbandry.

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