



## Haematobiochemical and Pathological Alterations of Chronic Copper Toxicity in Ducks

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### ABSTRACT

The present study was designed to investigate the effect of excessive intake of copper sulphate on the haematological and biochemical parameters of domestic ducks. Three months old domestic ducks were given copper sulphate as a daily dose of 5 mg/kg body weight for 12 weeks. Haematological changes revealed decreased haemoglobin concentration in the treated group with increase in total erythrocytic count. The changes were progressive in low dosed group compared to control. Biochemically, there were significant increased levels of serum alkaline phosphatase, acid phosphatase and transaminase enzymes. However, biochemical alterations were progressive in the treated group compared to the control group. Gross lesions were primarily confined to liver with congestion, haemorrhage, moderate congestion and haemorrhages in the kidney, brain, proventriculus, spleen and intestine along with sloughing of mucosa of gizzard. Histopathologically, kidney showed degeneration, necrosis, karyomegaly and infiltration of mononuclear cells. There was sloughing of the koilin layer from the epithelial layer of gizzard.

**Keywords:** Biochemical, chronic, copper sulphate, ducks, histopathology

Assam ranks second in duck population in the country as ducks are excellent converter of low quality waste products to high quality animal protein in the form of egg and meat. Ducks are reared for both eggs and meat by all sections of the society irrespective of their caste or religion. In fact, due to religious sentiments, duck eggs and meat get a preference over those of chickens. Most of the rural people in Assam rear ducks as backyard poultry that help them to earn a steady income. However, this industry frequently suffers severe setback due to various infectious diseases (Konch, 2005) as well as feed and water toxicity. Apart from Aflatoxicosis, a highly potent mycotoxin present in stored grain, feed and water toxicity in ducks also occurs due to presence of heavy metals like copper, lead, mercury, arsenic etc. (Fulton, 2013).

Copper salts are widely employed in agricultural and in veterinary practices as weedicide, growth promoter, anthelmintic, fungicide, haematinic and as molluscicide.

As a molluscicide, copper is used to repel and kill slugs and snails, the intermediate hosts of different parasites (Gupta, 2007). Because of its wide-spread use, copper toxicity in animals and birds is possible as the toxic nature of copper salt has been known for centuries (Garg, 2000). Among the birds, probability of copper toxicity in ducks is more because they prefer to take snail as their food. There exists a voluminous literature on biological effects of copper on farm animals particularly sheep and broiler chickens, but there is paucity of literature on the cytotoxic effects of copper sulphate in ducks. Therefore, the present study has been undertaken to study the haemato-biochemical and pathological alterations of chronic copper toxicity in domestic ducks.

### MATERIALS AND METHODS

#### Experimental animal

Total 20 domestic ducklings of 3-months of age irrespective



of sex were procured from organized duck farms. The ducks were maintained with a balanced nutritional diet and *ad libitum* water. All ducks were observed daily during the entire period of study and handled as per the Institutional Animal Ethics Guidelines.

### Chemicals

Commercial products of Copper Sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) used in this study was procured from Merck Specialities Private Limited.

### Experimental design

Twenty domestic ducklings were divided into two groups comprising of 10 ducks in Group I (control) and Group II (treated) and fasted for 6 hours prior to dosing. Following the period of fasting, the ducks were weighed and the doses were calculated according to the body weight. Copper sulphate was diluted in distilled water to obtain the desired concentration. Group I ducks were given distilled water orally which served as control. Group II ducks were administered orally with a daily dose of 5mg/kg body weight suspended with water daily for 12 weeks. The ducks were minutely observed for clinical signs till the end of the treatment. Ducks of the control group were sacrificed at the end of the experiment.

### Sampling

About 2ml of blood from the ducks were aseptically collected from the jugular vein / wing vein with a sterile 2ml disposable syringe. In Group II, blood was collected at 0-day and after treatment with copper sulphate, collection was done at weekly interval considering 6 days a week. In the control group blood was collected at the same time and same day of collection as in the case of the treated group. About 1ml of blood was taken in a vial containing EDTA as anticoagulant @ 1mg/ml for estimation of Hb and TEC and the remaining 1ml of blood was kept in a dry wide-mouthed tube in slanting position at room temperature for separation of serum. The serum was separated after 6-8 hrs following collection and stored at  $-20^\circ\text{C}$  for biochemical analysis.

### Hematology and biochemical assays

Haemoglobin (Hb) and Total Erythrocyte Count (TEC)

were estimated by the standard haematological protocols. The various serum biochemical parameters such as Alkaline Phosphatase (ALP), Aspartate Amino Transferase (AST), Acid phosphatase (ACP) and Alanine Amino Transferase (ALT), were estimated spectrophotometrically as per the method of Reitman and Frankel (1957) as mentioned on the diagnostic reagent kit literature (Span Diagnostic Ltd. and Coral Diagnostic Limited).

### Histopathological study

Representative samples irrespective of lesions from liver, kidney, lungs and gizzard were collected at necropsy and preserved in 10% neutral formalin. After washing in running water and dehydration in alcohol, tissues were embedded in paraffin and  $5\mu$  paraffin sections cut and stained with haematoxylin and eosin as per standard method (Luna, 1968).

### Statistical analysis

The statistical analysis of data and calculation of means and standard errors were performed following the methods described by Snedecor and Cochran (2004).

## RESULTS AND DISCUSSION

The symptoms of chronic toxicity were moderate with increased thirst as compared to control. Gradually, the birds returned to their normal state with reduced feed intake throughout the experimental period. The clinical signs observed in the present study agreed with the findings of Tokarnia *et al.* (2000) in different animals and birds. Immediately after dosing, ducks developed vigorous shaking of the head might be due to severe irritation caused by corrosive nature of copper sulphate as well as neuronal degeneration recorded in the histopathological investigation. The hepatotoxic effect of copper sulphate might be the reason for manifestation of inappetance and resultant weakness (Peterson and Talcott, 2006). However, in the chronic group, neuronal changes due to intoxication occurred in the brain of ducks from 8<sup>th</sup> week of the experiment, which resulted in slow movement.

### Haematological changes

The Hb and TEC concentration of the chronic group

on weekly basis after administration of copper sulphate are presented in Table 1. Among the haematological parameters studied, no significant changes were observed in the concentration of haemoglobin in Group II ducks which showed gradual decrease in the haemoglobin level throughout the experiment. The result was in agreement with the findings of Hengmin *et al.* (2005) and Minervino *et al.* (2009) in animals and birds due to copper sulphate toxicity. It was also stated that intravascular hemolysis is a typical sign of copper toxicity resulting in reduced haemoglobin level.

However, in chronic group, elevation of TEC was gradual and no significant difference was noticed with the control ducks throughout the study. Similar observation was also observed by Ozcelik *et al.* (2002). The increased total erythrocyte count recorded in the study might be due to haemo-concentration resulted from dehydration (Benjamin, 1979; Sastry, 1983). Further transient polycythemia occurs due to release of RBC from the stored organs due to action of epinephrine (Benjamin, 1979).

**Table 1:** Effect of copper sulphate on hematology in domestic ducks

Weeks	Control		Treated	
	Hemoglobin	TEC	Hemoglobin	TEC
0	13.7 ± 0.021	4.89 ± 0.08	13.8 ± 0.04	4.91 ± 0.08
1	13.7 ± 0.420	4.92 ± 0.06	13.7 ± 0.20	5.02 ± 0.04
2	13.6 ± 0.04	4.98 ± 0.08	13.2 ± 0.44	5.06 ± 0.04
3	13.8 ± 0.18	4.95 ± 0.04	12.6 ± 0.08	5.03 ± 0.02
4	13.6 ± 0.14	4.99 ± 0.04	12.3 ± 0.08	5.07 ± 0.01
5	13.7 ± 0.04	4.95 ± 0.08	12.1 ± 0.14	5.08 ± 0.12
6	14.2 ± 0.130	4.93 ± 0.20	11.9 ± 0.12	5.11 ± 0.30
7	13.7 ± 0.502	4.96 ± 0.01	11.7 ± 0.40	5.16 ± 0.33
8	13.6 ± 0.401	4.98 ± 0.01	11.7 ± 0.05	5.21 ± 0.45
9	13.8 ± 0.044	4.99 ± 0.02	11.7 ± 0.40	5.23 ± 0.44
10	14 ± 0.260	4.93 ± 0.02	11.7 ± 0.33	5.20 ± 0.04
11	13.8 ± 0.04	4.99 ± 0.04	11.7 ± 0.25	5.20 ± 0.08
12	13.6 ± 0.201	4.98 ± 0.42	11.8 ± 0.23	5.21 ± 0.08

Values are Mean ± SE. Mean values bearing different superscripts differed significantly

### Serum biochemical changes

The results of biochemical parameters studied are given in

Table 2. Group II ducks showed dose dependent increase of AST and ALT activities. There were significant rise in the enzyme activity after 8<sup>th</sup> week of treatment. Increased levels of these enzymes have also been reported by various workers in the serum of animals and birds in copper toxicosis (Fuentealba *et al.*, 2000; Hengmin *et al.*, 2005). Exposure to excess copper was reported to cause accumulation of copper in the liver that led to extensive damage and necrosis of the liver. The damaged and necrotic liver would subsequently produce increased level of AST and ALT (Theil and Calvert, 1978) which is reflected in the serum of the treated birds in the present experiment. Increase of ALT in the serum has a high specificity for liver damage. The increase of these enzymes in our experiment clearly demonstrated that the treatment with copper sulphate disrupts the liver function (Kaneko, 1997; Almansour, 2006).

Group II ducks showed significant ( $P < 0.05$ ) increase in the ALP level from 7<sup>th</sup> week till the termination of the experiment. The increase in ALP is linked with liver damage in the form of hepatitis, bile duct obstruction and biliary cirrhosis (Sacher and McPherson, 1991). The histopathological changes in the present study also revealed massive damage to the hepatic parenchyma characterized by fatty changes with necrosis as well as pathological alterations of the duodenum.

### Pathological changes

Gross lesions were mainly visible in the liver, kidneys, lungs and gizzard comprising of congestion and petechial haemorrhages which confirm the observations of Jensen *et al.* (1991) and Anjum *et al.* (1992) in copper sulphate toxicity in different animals and birds. However, the lesions were found to be increased gradually in Group II ducks reflecting the dose and time dependent effect of copper sulphate, which relates to the findings of Henderson *et al.* (1974). Henderson *et al.* (1974) and Jensen *et al.* (1991) also found similar lesions during copper toxicosis in Canada goose and broiler chicks. The koilin layer of the gizzard revealed edema and sloughing of the mucosa towards the end of the experiment reflecting the dose and time dependent effect of copper sulphate. Fisher *et al.* (1973) and Jensen *et al.* (1991) also found similar lesions during copper toxicosis in Canada goose and broiler chicks.

**Table 2:** Effect of copper sulphate on biochemical parameters in domestic ducks

Weeks	Control				Treated			
	AKP	ACP	AST	ALT	AKP	ACP	AST	ALT
0	25.6 ± 0.08	0.58 ± 0.08	46.5 ± 0.30	84.6 ± 0.04	25.9 ± 0.08	0.6 ± 0.02	47.4 ± 0.02	83.8 ± 0.50
1	25.8 ± 0.04	0.43 ± 0.04	46.8 ± 0.42	82.8 ± 0.40	26.2 ± 0.06	0.64 ± 0.04	48.8 ± 0.04	90.2 ± 0.26
2	28.2 ± 0.04	0.62 ± 0.06	48.9 ± 0.22	88.3 ± 0.44	27.6 ± 0.04	0.68 ± 0.02	50.4 ± 0.06	90.5 ± 0.31
3	26.8 ± 0.08	0.68 ± 0.02	47.2 ± 0.28	84.8 ± 0.04	28.9 ± 0.08	0.69 ± 0.02	54.6 ± 0.08	91.4 ± 0.33
4	27.6 ± 0.02	0.55 ± 0.04	45.9 ± 0.02	84.2 ± 0.52	30.9 ± 0.01	0.76 ± 0.04	57.5 ± 0.30	94.05 ± 0.29
5	29.2 ± 0.04	0.48 ± 0.02	47.5 ± 0.04	82.2 ± 0.04	33.3 ± 0.18	0.80 ± 0.06	61.7 ± 0.31	96.2 ± 0.42
6	30.8 ± 0.18	0.67 ± 0.30	46.8 ± 0.08	85.3 ± 0.02	35.8 ± 0.14	0.93 ± 0.02	65.7 ± 0.09	99.6 ± 0.44
7	27.6 ± 0.08 <sup>a</sup>	0.63 ± 0.04	49.2 ± 0.04	84.6 ± 0.44	41.4 ± 0.20 <sup>b</sup>	0.96 ± 0.08	68.4 ± 0.40	108.3 ± 0.05
8	28.2 ± 0.02 <sup>a</sup>	0.53 ± 0.02 <sup>a</sup>	48.8 ± 0.20 <sup>a</sup>	84.3 ± 0.30 <sup>a</sup>	44.6 ± 0.16 <sup>b</sup>	1.06 ± 0.02 <sup>b</sup>	74.5 ± 0.05 <sup>b</sup>	119.3 ± 0.09 <sup>b</sup>
9	28.4 ± 0.04 <sup>a</sup>	0.54 ± 0.04 <sup>a</sup>	47.3 ± 0.21 <sup>a</sup>	82.6 ± 0.41 <sup>a</sup>	47.8 ± 0.08 <sup>b</sup>	1.22 ± 0.04 <sup>b</sup>	78.2 ± 0.44 <sup>b</sup>	125.1 ± 0.01 <sup>b</sup>
10	29.8 ± 0.31 <sup>a</sup>	0.64 ± 0.08 <sup>a</sup>	49.8 ± 0.50 <sup>a</sup>	86.4 ± 0.04 <sup>a</sup>	49.4 ± 0.18 <sup>b</sup>	1.28 ± 0.08 <sup>b</sup>	79.2 ± 0.38 <sup>b</sup>	126.7 ± 0.25 <sup>b</sup>
11	30.2 ± 0.04 <sup>a</sup>	0.67 ± 0.05 <sup>a</sup>	50.6 ± 0.45 <sup>a</sup>	88.0 ± 0.05 <sup>a</sup>	53.1 ± 0.04 <sup>b</sup>	1.31 ± 0.09 <sup>b</sup>	81.8 ± 0.27 <sup>b</sup>	131.3 ± 0.03 <sup>b</sup>
12	26.5 ± 0.04 <sup>a</sup>	0.68 ± 0.04 <sup>a</sup>	52.8 ± 0.04 <sup>a</sup>	88.8 ± 0.06 <sup>a</sup>	57.1 ± 0.07 <sup>b</sup>	1.32 ± 0.03 <sup>b</sup>	84.9 ± 0.22 <sup>b</sup>	135.1 ± 0.08 <sup>b</sup>

Mean values bearing different superscripts differed significantly

Histologically, the kidneys revealed focal to diffuse haemorrhages, cytoplasmolysis in the renal tubular epithelium, which were in progressing order along with dose and period of exposure to copper sulphate treated groups. This resembles with earlier reports of copper toxicosis in chicks by Kamel *et al.* (1971) and Kaur Bala (1998). These changes in kidney might be due to effect of metabolites of copper sulphate as kidneys are the major route for elimination of copper. In lungs, there was mild vascular congestion, focal haemorrhages and mild to moderate infiltration of heterophils mixed with mononuclear cells. By 8<sup>th</sup> week of the experimental period, changes revealed marked vascular congestion, diffuse haemorrhages, presence of serous exudates mainly in the parabronchiolar lumen and peribronchiolar spaces. Marked hyperplasia with hypertrophy was seen in the bronchiolar epithelium. In spleen, there were progressive focal haemorrhages and thickened vascular wall with hypertrophy and hyperchromasia of the endothelial cells in the copper sulphate treated group. There was formation of secondary follicles by 10<sup>th</sup> week in Group II ducks. These follicles were surrounded by a well developed connective tissue. Thorbecke *et al.* (1957) described that secondary follicles in the spleen appeared in response to infection.

## CONCLUSION

From the present investigation, it can be inferred that

intoxication with copper sulphate produces deleterious hematological and biochemical alterations in domestic ducks.

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