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# **Clinicopathological Studies on Some Vitamins Used In Copper Toxicity In Broilers**

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## **LIST OF CONTENTS**

<b>Title</b>	<b>Page</b>
<b>INTRODUCTION</b>	<b>1</b>
<b>REVIEW OF LITERATURE</b>	<b>3</b>
<b>MATERIAL AND METHODS</b>	<b>27</b>
<b>RESULTS</b>	<b>40</b>
<b>DISCUSSION</b>	<b>104</b>
<b>SUMMARY</b>	<b>123</b>
<b>CONCLUSION</b>	<b>131</b>
<b>REFERENCES</b>	<b>132</b>
<b>Vita</b>	<b>158</b>
<b>ARABIC SUMMARY</b>	

**LIST OF TABLES**

<b>NO.</b>	<b>Title</b>	<b>Page</b>
1.	The experimental design.	33
2.	Body weight (gram/bird/week) in chickens of different groups during the experimental period (mean values $\pm$ S.E).	41
3.	Body weight gain (gram/bird/week) in chickens of different groups during the experimental period (mean values $\pm$ S.E).	42
4.	Feed consumption (gram/bird/week) in chickens of different groups during the experimental period (mean values $\pm$ S.E).	43
5.	Feed conversion ratio in chickens of different groups during the experimental period (mean values $\pm$ S.E).	44
6.	Erythrogram in chickens of different groups at the end of 3 <sup>rd</sup> week (mean values $\pm$ S.E).	51
7.	Erythrogram in chickens of different groups at the end of 6 <sup>th</sup> week (mean values $\pm$ S.E).	52
8.	Leukogram in chickens of different groups at the end of 3 <sup>rd</sup> weeks (mean values $\pm$ S.E).	53
9.	Leukogram in chickens of different groups at the end of 6 <sup>th</sup> weeks (mean values $\pm$ S.E).	54
10.	Some liver enzymes activities in chickens of different groups at the end of 3 <sup>rd</sup> week (mean values $\pm$ S.E).	64
11.	Some liver enzymes activities in chickens of different groups at the end of 6 <sup>th</sup> week (mean values $\pm$ S.E).	65
12.	Proteinogram in chickens of different groups at the end of 3 <sup>rd</sup> weeks (mean values $\pm$ S.E).	66
13.	Proteinogram in chickens of different groups at the end of 6 <sup>th</sup> weeks (mean values $\pm$ S.E).	67

14.	Lipid profile in chickens of different groups at the end of 3 <sup>rd</sup> weeks (mean values $\pm$ S.E).	68
15.	Lipid profile in chickens of different groups at the end of 6 <sup>th</sup> weeks (mean values $\pm$ S.E).	69
16.	Serum glucose and some renal function tests in chickens of different groups at the end of 3 <sup>rd</sup> week (mean values $\pm$ S.E).	70
17.	Serum glucose and some renal function tests in chickens of different groups at the end of 6 <sup>th</sup> week (mean values $\pm$ S.E).	71
18.	Serum markers for oxidative stress and antioxidant enzymes activities in chickens of different groups at the end of 3 <sup>rd</sup> weeks (mean values $\pm$ S.E).	80
19.	Serum markers for oxidative stress and antioxidant enzymes activities (in chickens of different groups at the end of 6 <sup>th</sup> weeks (mean values $\pm$ S.E).	81
20.	DNA degradation indices in chickens of different groups at the end of 3 <sup>rd</sup> weeks (mean values $\pm$ S.E).	85
21.	DNA degradation indices in chickens of different groups at the end of 6 <sup>th</sup> weeks (mean values $\pm$ S.E).	86

**LIST OF FIGURES**

<b>NO.</b>	<b>Title</b>	<b>Page</b>
1.	Weekly body weight (g) of chickens in different groups during the experimental period.	44
2.	Weekly body weight gain (g) of chickens in different groups during the experimental period.	45
3.	Weekly feed consumption (g/ bird) of chickens in different groups during the experimental period.	46
4.	Weekly feed conversion ratio of chickens in different groups during the experimental period.	47
5.	RBCs count in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	54
6.	Hb concentration in chickens of different groups at 3 <sup>rd</sup> and 6 <sup>th</sup> week.	54
7.	PCV% in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	55
8.	MCV in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	55
9.	MCH in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	56
10.	MCHC in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	56
11.	TLC in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	57
12.	Hetrophils count in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	57

13.	Lymphocytes count in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	58
14.	Monocytes count in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	58
15.	Eosinohils count in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	59
16.	Basohils count in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	59
17.	Serum ALT activity in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	71
18.	Serum AST activity in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	71
19.	Serum ALP activity in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	72
20.	Serum TP level in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week	72
21.	Serum albumin level in chickens of differen groups at at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	73
22.	Serum total globulins level in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	73
23.	Serum triglyceride levels in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	74
24.	Serum total cholesterol level in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	74
25.	Serum LDL cholesterol level in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week..	75
26.	Serum VLDL cholesterol level in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	75

27.	Serum HDL cholesterol level in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	76
28.	Serum glucose level in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	76
29.	Serum creatinine level in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	77
30.	Serum uric acid level in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	77
31.	Serum MDA levels in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	81
32.	Serum GSH-P <sub>x</sub> activities in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	81
33.	Serum SOD activities in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	82
34.	Comet % in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	86
35.	%DNA in tail in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	86
36.	Tail length in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	87
37.	Tail moment in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	87
38.	Olive tail moment in chickens of different groups at the 3 <sup>rd</sup> and 6 <sup>th</sup> week.	88

## LIST OF PHOTOS

NO.	Title	Page
1-6	<p>Nucleus of hepatic cells of chicken from different groups, stained with ethidium bromide (400x):</p> <p><b>1:</b> (control group) shows no DNA damage, as demonstrated by the lack of DNA fragment migration away from the nucleus core.</p> <p><b>2,3:</b> (Cu group) shows a high degree of DNA damage, with a greatly reduced nucleus core and a large cloud of DNA fragments migrating away from the core forming the characteristic comet tail.</p> <p><b>4:</b> (Cu+Vit C) shows the moderated degree of DNA damage.</p> <p><b>5:</b> (Cu +Vit E) shows the moderated degree of DNA damage.</p> <p><b>6:</b> (Cu+Vit C+ Vit E) group shows a minimal degree of DNA damage.</p>	90
7	Liver of chicken (gp.2) showing hyperplastic, and necrotic biliary epithelium (arrow) with various degenerative and necrotic changes in the hepatic cells (arrow head), at the end of third week H&E	96
8	Liver of chicken (gp.2) showing cholestasis, necrotic bile duct epithelia (arrow), beside portal lymphocytic aggregation and fibroblast proliferation (arrow head) ), at the end of six week H&E	96
9	Liver of chicken (gp.3) showing moderate swelling of hepatic cells (arrow) and hyperplastic Kupffer cells (arrow head) at the end of third week H&E.	97
10	Liver of chicken (gp.3) showing partially contracted hepatic cells (arrow) proliferative Kupffer cells and dilated sinusoids (arrow head) at the end of six week H&E.	97
11	Liver of chicken (gp.4) showing portal lymphocytic aggregation (arrow) within apparently normal hepatic parenchyma (arrow head) at the end of third week H&E.	98



12	Liver of chicken (gp.4) showing intense hyperplasia of Kupffer cells (arrow head) and cloudy swelling of hepatic cells with a few lymphocytic aggregation (arrow) at the end of six week H&E.	98
13	Liver of chicken (gp.5) showing little portal (arrow) and interstitial lymphocytic aggregation within apparently normal hepatic parenchyma (arrow head) at the end of third week H&E.	99
14	Liver of chicken (gp.5) showing mild portal lymphocytic aggregation (arrow) and normal hepatic parenchym (arrow head) with dilated blood vessels at the end of six week H&E.	99
15	Kidney of chicken (gp.2) showing extensive necrotic tubular epithelia (arrow) and hypercellularity of glomeruli (arrow head) at the end of third week H&E.	100
16	Kidney of chicken (gp.2) showing pronouced necrosis of renal tubules (arrow) and glomeruli (arrow head) at the end of six week H&E.	100
17	Kidney of chicken (gp.3) showing moderate nephritic changes in renal tubular epithelia (arrow) at the end of third week H&E.	101
18	Kidney of chicken (gp.3) showing degeneration and necrosis of some tubular epithelia (arrow) at the end of six week H&E.	101
19	Kidney of chicken (gp.4) showing various degenerative changes in some tubular epithelia (arrow) and partial intravascular hemolysis (arrow head) at the end of third week H&E.	102
20	Kidney of chicken (gp.4) showing apparently normal renal parenchyma (arrow head) with mild interstitial lymphocytic aggregation (arrow) at the end of six week H&E	102
21	Kidney of chicken (gp.5) showing regenerative attempts from tubular epithelia (arrow head) adjacent to nephritic tubules (arrow) at the end of third week H&E.	103
22	Kidney of chicken (gp.5) showing normal nephron segments and intersitium (arrow) at the end of six week H&E	103

## SUMMARY

The present experiment was performed to investigate the protective effect of vitamin C and E against the toxic impact of copper on growth performance, hematological, some biochemical parameters, DNA damage in broilers as well as, pathological changes

One hundred chicks were divided into 5 groups, each group contained 20 chicks.

Group 1: was given a normal ration from one day old till the end of the experiment (6 weeks)

Group 2: was given a ration contained 350 mg copper sulphate /kg ration from one day old for 6 weeks

Group 3: was given a ration contained 350 mg copper sulphate /kg ration + 250 mg vitamin C /kg ration from one day old for 6 weeks

Group 4: was given a ration contained 350 mg copper sulphate /kg ration + 250 mg vitamin E /kg ration from one day old for 6 weeks

Group 5: was given a ration contained 350 mg copper sulphate /kg ration + 250 mg vitamin C /kg ration + 250 mg vitamin E /kg ration from one day old for 6 weeks.

Chicks of all groups were observed and clinically examined. The body weight, feed consumption, body weight gain and FCR were calculated and recorded weekly.

Five chicks from each group were used for the collecting blood samples. Blood samples were collected from each bird from wing vein at the end of 3<sup>rd</sup> and 6<sup>th</sup> week and divided into 2 parts.

1<sup>st</sup> part: was 1 ml of blood collected on di-potassium salt of EDTA for hematological studies.

2<sup>nd</sup> part: was 3 ml of blood taken without anticoagulant in a clean and dry centrifuge tube, leave to clot at room temperature and then centrifuged at 3000 rpm for 5 minutes. The serum was collected for both biochemical studies.

Necropsy was done and tissue samples (liver, and kidney) were collected at the end of 3<sup>rd</sup> and 6<sup>th</sup> week in formalin 10% for histopathological examination. And samples from the liver (1g) were immediately placed in ice cold phosphate buffer saline (BPS) and were kept on – 20° C until used for the comet assay.

### **Effect on clinical signs:**

No clinical signs or mortality was observed in treating birds except group 2 (copper - treated birds) showed mild diarrhea, decrease weight, decrease appetite and pale comb.

### **Effect on growth performance:**

- Administration of copper sulphate at dose 350 mg/kg diet for 6 weeks affects negatively on feed consumption, body weight, body gain and feed conversion rate of broiler.
- Supplementation with vitamins C or E and combination could partially ameliorate the depressive effect of copper on growth performance.

### **Hematological findings:**

Chickens in gps. 2, 3 only at the end of 3<sup>rd</sup> week showed a significant decrease in Hb conc and PCV% in gps. (2,3) in comparison with the control group, while RBCs count and MCHC% showed a significant decrease only in gp.(2). Meanwhile, MCV value showed a nonsignificant difference.

Chickens in gps.(2, 3 and 4) at the end of 6<sup>th</sup> week showed a significant decrease in RBCs count, Hb concentration, PCV% and MCHC% values with significant increase in MCV value. The most pronounced significant change was seen in chickens of gp.(2). However, gps. (3 and 4) showed an improvement as there was a significant increase in RBCs count, Hb conc and PCV% compared to copper group. MCH values in all groups showed a non significant difference all over the experimental period when compared with the control group.

Chickens in gp.(5) revealed no significant change in all erythrocytic parameters which returned towards the normal values of control group all over experimental period.

Total and differential leukocytic count of gps. (2,3, and 4) showed a significant decrease in total leukocytic, lymphocytic, and monocyte count, but eosinophils and basophils count did not show any significant difference all over the experimental period compared with the control group. Heterophils count shows a non significant decrease at end third week and become significant decrease at end sixth week. The most pronounced decrease was in gp.(2), while gps.(3 and 4) results were improved showing a significant increase in total leukocytic, lymphocytic and heterophils count, but not return to the value of control compared with copper- treated group. Chickens in gp.(5) recorded a non - significant

decrease in leukocytes parameter at the end of third and sixth week compared with copper treated groups.

### **Change in some liver enzyme activities:**

The serum ALT, AST and ALP activities compares to control revealed a significant increase in chickens in gps. (2, 3 and 4) all over the experimental period. However, ALT, AST and ALP activities compared with the copper group showed a significant decrease in the gps. (3 and 4) subside toward control values in chicken of gp.(5) all over the experimental period.

### **Change in some biochemical parameters:**

The results revealed that copper group (2) showed a higher serum uric acid and creatinine levels, in addition to a significant decrease in serum glucose, total proteins, albumin, globulins, triglycerides, cholesterol, HDL-C, LDL-C and VLDL-C values particularly at the end of the sixth week in comparison to the control group. Our results showed an improvement in these previous parameters in groups supplied with either VitC or Vit E and combination when compared with copper group.

### **Changes of serum markers for oxidative stress and antioxidant enzymes activities:**

Copper group showed a significant increase in serum MDA all over the experimental period with a significant decrease in GSH-P<sub>x</sub> and SOD activities at the end of the 6<sup>th</sup> week. Groups supplied with either VitC or Vit E and their combination, partial improves the deleterious effect of copper on serum MDA, GSH-P<sub>x</sub> and SOD activity values.

### **Changes of DNA:**

Concerning degradation of DNA, which measure by comet assay. Comet test assay in liver Cu treated group revealed a significant rise of all indices, including tail length, % DNA in tail, tail moment, and olive tail moment as compared to control. Groups supplied with either Vit C or Vit E and combination (gps. 3, 4 and 5) showed partial improvement manifested by a significant decrease in these previous parameters compared with copper group but not return to control value.

### **Pathological findings:**

#### **A-Macroscopic examination (PM):**

- Chicken in the 2<sup>nd</sup> group (copper group): showed marked congestion and enlargement in size of liver, and kidneys at end of 3<sup>rd</sup> and 6<sup>th</sup> week in addition to a firm consistency notice in the liver at the end of 6<sup>th</sup> weeks.
- Chicken in the 3<sup>rd</sup> group (copper +VitC group) and 4<sup>th</sup> group (copper +VitE group): showed slightly enlarged liver and pale to yellowish patches of hemorrhage beside, moderate congestion of the kidney at the end of 3<sup>rd</sup> and 6<sup>th</sup> week.
- Chicken in the 5<sup>th</sup> groups (copper +Vit C+ Vit E group): did not show any changes except liver was yellowish in color with small patches of hemorrhage and kidney showed paleness and slight enlargement at the end of 3<sup>rd</sup> week but, at the end 6<sup>th</sup> week showed no gross pathological lesions.

#### **B-Microscopic examination:**

Examined hepatic tissues of chicken in the copper treated group at the end of 3<sup>rd</sup> week revealed hepatic toxicity target bile duct (cholangiopathy) and hepatic cells, represented in hyperplastic, and

necrotic biliary epithelium with various degenerative and necrotic changes in the hepatic cells while, at the end of 6<sup>th</sup> week pronounced cholangiopathy and toxic hepatitis was the most common lesion, cholestasis, necrotic bile duct epithelia, beside portal lymphocytic aggregation and fibroblast proliferation was noticed in the majority of portal areas.

Examined liver sections of chicken in Cu+ Vit C treated group revealed slightly ameliorated severity of previous mentioned lesions in Cu treated group represented by moderate acute cell swelling of the hepatic cells with hyperplastic kupffer cells, The majority of portal areas had lymphocytic aggregation, hyalinized wall of hepatic arteriole, endotheliosis with mild proliferation of bile duct epithelium were seen at of 3<sup>rd</sup> week. Meanwhile, at the end of 6<sup>th</sup> week there was a slight reduction in the hepatic lesions represented in hepatic cell become partially contracted, proliferative Kupffers cells and dilated sinusoids, beside the majority of the hepatic area still suffered from partial necrosis or degeneration.

Examined liver sections of chicken in Cu+Vit E treated group revealed improvement the lesions of hepatic toxicity was detected in copper group represented by the hepatic parenchyma showed mild vascular degeneration with portal lymphocytic aggregation at the end of 3<sup>rd</sup> week while, mild hepatic changes mainly cloudy swelling and intense proliferation of Kupffer cells with a few portal lymphocytic aggregation were detected at end of 6<sup>th</sup> week.

Examined liver sections of chicken in Cu+ Vit C+Vit E treated group revealed intense ameliorative effect for the hepatic lesion in copper group represented by apparently normal hepatic parenchyma with little portal and interstitial lymphocytic aggregations and little portal and

interstitial lymphocytic aggregations and hyperplastic Kupffer cells were notice at the end of 3<sup>rd</sup> week, meanwhile, At the end of 6<sup>th</sup> week, the hepatic parenchyma apparently normal, beside the Kupffer cells were proliferative.

Examined kidney tissues of chicken in the copper treated group at the end of 3<sup>rd</sup> week revealed nephrotoxicity represented by extensive necrosis of the renal tubular epithelial with or without hyper cellularity of the glomeruli, moreover, extensive hemorrhages focally replaced renal parenchyma with congested blood vessels and capillaries in addition, cellular or hyaline cast with or without urates deposits inside some tubular lumina are observed mean while, at the end of 6<sup>th</sup> week renal damaged become extensive and characterized by pronounced necrosis of the renal tubules and glomeruli and extensive hemorrhages and hemolysis were seen.

Examined kidney tissues of chicken in the Cu+Vit C treated group at the end of 3<sup>rd</sup> week revealed moderate nephritic changes involved the majority of renal tubular epithelia with presence of a few extra wasted erythrocytes were common , the majority of glomeruli restore its normal picture and a few had necrosed glomerular tuft, in addition , at the end of 6<sup>th</sup> week there was a moderate renal lesion represented by degeneration or necrosis of some tubular epithelia with hypercellulaity of the glomeruli.

Examined kidney tissues of chicken in the Cu+ Vit E treated group at the end of 3<sup>rd</sup> week revealed various mild degenerative changes mainly cloudy swelling or hydropic degeneration of tubular epithelia with partial intra vascular hemolysis still seen also, a few regenerative attamps from the tubular could be seen meanwhile, at the end of 6<sup>th</sup> week the majority of renal parenchyma showed improvement of lesions with restore their



histomorphologic picture of all nephron segments and intersitium with Mild interstitial lymphocytic aggregation could be seen.

Examined kidney tissues of chicken in the Cu<sup>+</sup> Vit C<sup>+</sup> Vit E treated group revealed reduced in renal toxicity represented great regenerative attempts from the adjacent tubular epithelia were encounter at the end of 3<sup>rd</sup> week, meanwhile, at the end of 6<sup>th</sup> week the renal parenchyma had intense reduction in lesion of copper toxicity beside, all segments of nephrons restore its normal histomorphologic picture with intense regenerative attempts in the tubules.