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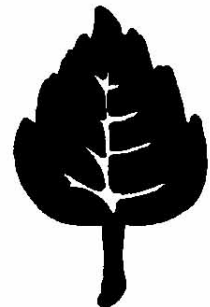
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## ADVERSE EFFECT OF DIFFERENT DOSES OF ANTI-COCCIDIAL DRUG TOLTRAZURIL (BAY-COX) ON CHICKEN

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### Abstract

Toltrazuril (Bay-cox) is a new anti-coccidial drug. It is used in prophylaxis and intermittent treatment. The immunotoxic effect of this drug was studied on 84 Hubbard chickens pre- and post - vaccination with Newcastle disease virus vaccine at 7,18,32 days old.

The present investigation was performed on 84 Hubbard chicks. All chickens were divided into 7 groups (12 chickens per group). All groups were vaccinated against Newcastle disease. The first group was left as control. The second, third and fourth groups were administered toltrazuril at 0.5, 1 and 2ml/L respectively via drinking water for 48 hr pre-vaccination. The fifth, sixth and seventh groups were given toltrazuril at the same doses, respectively as mentioned for 48 hr. post-vaccination. Four chickens from each group were sacrificed at 72 hr. post-vaccination for biochemical analysis of serum and differential leucocytic count lymphocyte blastogenesis, phagocytosis and histopathological lymphoid organs (bursa of fabricius, spleen and thymus), liver and kidneys. The possible immunotoxic effects and pathological alterations due to the treatment with toltrazuril were recorded.

The results showed the alterations in lymphocytes proliferation and phagocytic percent. Titer of the antibody against Newcastle disease virus vaccine was altered. Such alteration was clear with high doses of early age and repeated therapeutic and double therapeutic dose. Moreover, toltrazuril in the liver caused toxic effect cleared by alteration in liver functions. The pathological changes were depletion and necrosis in the lymphoid tissues of the lymphoid organs. Vacuolar and hydropic degenerations were detected in the liver and kidneys with hemorrhages among the tissues. These changes were different in their severity according to the time and dose of the treatment.

### INTRODUCTION

Toltrazuril (Baycox) was a new broad spectrum anticoccidial drug Harder and Haberkorn (1989). Toltrazuril had effect in prevention and treatment of coccidiosis in

chickens Deghid and Hassanain (1988). Toltrazuril used on two consecutive days per week with the feed or in drinking water at various time before and after infection Haberkorn (1984). Voeten (1986) recorded that toltrazuril is a compound from series of a symmetrical triazinone.

Toltrazuril interferes with some reaction steps in DNA synthesis. One target could be dihydroorotate oxidation, a reaction step that was coupled with electron transport system Hammond *et al.* (1985) and was the linkage between the respiratory chain and pyrimidine synthesis. Some of the enzymes in the respiratory chain, such as succinate-cytochrome C reductase, NADH oxidase and succinate oxidase from mouse liver were reduced in the activities with the presence of toltrazuril Harder and Haberkorn (1989). Toltrazuril revealed no evidence of hematopathies (Seuter and perzborn, 1984). The drug has anti-allergic effect on the mast cells of the rat (Hammond and Coomber 1984). Toltrazuril in repeated therapeutic and double therapeutic doses caused increase of serum GOT, GPT and decrease in total protein. Pathologically, it showed fatty change in the liver with periductular fibroblast proliferation, besides, hydropic degeneration in the kidney with subcapsular hemorrhage (Elen, 1993). Moreover, Bomhard and Vogel (1985) recorded that high doses of the drug reduced RBC count, hemoglobin contents, hematocrit, impaired liver function and reduced total protein concentration.

The objective of this study is to assess the immune response of chickens treated with different doses of anticoccidial drug, toltrazuril, and immunized with Newcastle disease virus vaccine. Study on the histopathological changes on lymphoid organs, liver and kidneys in response to drugs was investigated.

## MATERIALS AND METHODS

**Drug:** Toltrauzril (commercial name Baycox)<sup>®</sup> was obtained from Bayer company. Chemical name is 1- [3-methyl - 4 - (4- trifluor methyl thio) phenoxy)- phenyl] - 3 - methyl - 1,3,5- triazin - 2,4,6 (1h, 3h, 5h) - trion.

**Animals:** Eighty-four one day-old Hubbard chicks were divided into seven groups (Table 1). The chickens were reared under good hygenic conditions.

Table 1. showing groups, number of chickens, drug used, dose of administration and time of sacrifice.

Species	Total No. of chicken	Groups	No. of chicken	Time of vaccination according to age			Time of treatment	Doses of treatment	Route of administration	No. of sacrificed			Time of sacrifice
				7 days old	18 days old	32 days old				1 <sup>st</sup> vaccination	2 <sup>nd</sup> vaccination	3 <sup>rd</sup> vaccination	
Chickens	84	1	12	12	8	4	Control	Dist. water	Via drinking water	4	4	4	72 hrs. post-vaccination
		2	12	12	8	4	For 48 h pre-vaccination	1/2 ml /L water		4	4	4	
		2	12	12	8	4		1 ml /L water		4	4	4	
		4	12	12	8	4		2 ml /L water		4	4	4	
		5	12	12	8	4	For 48 hrs post-vaccination	1/2 ml /L water		4	4	4	
		6	12	12	8	4		1 ml /L water		4	4	4	
		7	12	12	8	4		2 ml /L water		4	4	4	

\* Significant at  $P \leq 0.05$ \*\* High significant at  $P \leq 0.01$ 

**Sampling:** Habaritized, citrated blood and serum samples were collected from sacrificed four chickens from each group 72 h post-vaccination (Table 1). Differential leukocytic count were performed on blood films using the battlement technique (Mulley, 1979).

Lymphocyte blastogenesis microassay test: lymphocytes proliferation were measured according to Denizot and Long, (1986) with some modification carried out as Maslak and Reynolds (1995). Phagocytosis assay was determined according to Rouse *et al.* (1980). Serum samples were analyzed for antibody titer against Newcastle disease virus vaccine using hemagglutination inhibition test (HI) (Beard and Wilkes, 1973). Serum total protein, albumin and globulin were determined using SGM Italia kits according to Henry (1976). Serum globulins were calculated as the difference between total protein and albumin. Aspartate amino transferase and alanine amino transferase were determined using Diamond kits according to Reitman and Frankel (1957).

## Histopathological Examination

All experimental chickens were examined daily during the period of the experiment. The clinical signs and post-mortem changes were recorded. Specimens were collected from lymphoid organs (bursa of fabricius, spleen and thymus), liver and kidneys, then, fixed in 10 % neutral buffered formalin. Paraffin sections of 5 micron thickness were prepared and stained with hematoxylin and eosin (H&E) then, examined microscopically (Bancroft *et al.*, 1990).

Statistical analysis: the data obtained were calculated as mean  $\pm$  standard error and statistically analysed by T test using stata program.

## RESULTS

### Clinical Signs

No signs were recorded in most chickens received 0.5, 1 and 2 ml/L toltrazuril pre- and post- 2<sup>nd</sup> vaccination against Newcastle diseased virus vaccine. Other chickens received the same doses of the drug post 1<sup>st</sup> and 3<sup>rd</sup> vaccination were depressed and had ruffled feathers.

Results of differential leucocytic count, lymphocyte blastogenesis, phagocytosis assay, total protein, albumin, globulin, hemagglutination titers and liver enzymes are presented in Tables 2-9.

### Pathological Finding

**Macroscopically**, the lymphoid organs (bursa of Fabricius, spleen and thymus), liver and kidneys in the majority of sacrificed chickens in the 7 groups sacrificed at 72 h, pre-and post- 2<sup>nd</sup> vaccination were apparently normal and showed no abnormalities from the normal features of these organs. Bursa and spleen appeared enlarged than normal in chicken in six groups which received drugs pre- and post- 3<sup>rd</sup> vaccination. The liver and kidneys were enlarged with dark red spots from the surface in some chicken particularly in 3 groups post- 1<sup>st</sup> and 3<sup>rd</sup> vaccination.

**Microscopically**, the Bursa of Fabricius: showed interfollicular edema with lymphoid depletion and necrosis in lymphocytes of the lymphoid follicles (Fig. 1). The lym-

Table 2. Effect of toltrazuril on differential leucocytic count (%) of pre- and post- 1<sup>st</sup> vaccination of chicken by ND vaccine (Mean  $\pm$  S.E) . (n = 4)

Groups	Doses of treatment	Time of treatment	Route	Time of vaccination	Lymphocyte	Heterophil	Eosinophil	Monocyte	Basophil
1	Vaccine only	Control	Via drinking water	At 7 days old	77.8 $\pm 3.7$	11.5 $\pm 2.22$	8.5 $\pm 1.19$	3.75 $\pm 0.48$	1.25 $\pm 0.25$
2	1/2 ml /L water	For 48 hrs. Pre-vaccination			73.8 $\pm 1.97$	12 $\pm 1.58$	9.5 $\pm 1.19$	4 $\pm 0.913$	0.75 $\pm 0.25$
3	1 ml /L water				69.25* $\pm 0.48$	14 $\pm 0.913$	14.5** $\pm 0.96$	2 $\pm 0.41$	1.25 $\pm 0.25$
4	2 ml /L water				68.5* $\pm 0.87$	13 $\pm 2.7$	9.5 $\pm 0.65$	4.75 $\pm 0.48$	2.75 $\pm 1.11$
5	1/2 ml /L water	For 48 hrs. post-vaccination			70.5* $\pm 1.32$	15.25 $\pm 1.25$	12.25 $\pm 2.9$	5.25 $\pm 1.97$	1.5 $\pm 0.65$
6	1 ml /L water				68.5* $\pm 2.25$	14.75 $\pm 2.17$	10 $\pm 0.71$	4.25 $\pm 0.78$	0.75 $\pm 0.478$
7	2 ml /L water				69* $\pm 1.22$	13 $\pm 0.408$	12.75 $\pm 2.5$	4.5 $\pm 0.65$	1.25 $\pm 0.48$

\* Significant at  $P \leq 0.05$ \*\* High significant at  $P \leq 0.01$ Table 3. Effect of toltrazuril on differential leucocytic count (%) of pre- and post- 2<sup>nd</sup> vaccination of chicken by ND vaccine (Mean  $\pm$  S.E) . (n = 4).

Groups	Doses of treatment	Time of treatment	Route	Time of vaccination	Lymphocyte	Heterophil	Eosinophil	Monocyte	Basophil
1	Vaccine only	Control	Via drinking water	At 18 days old	76 $\pm 1.35$	10.5 $\pm 0.5$	10 $\pm 1.23$	1.5 $\pm 0.65$	1 $\pm 0.41$
2	1/2 ml /L water	For 48 hrs. Pre-vaccination			71 $\pm 3.9$	12.25 $\pm 0.95$	11.25 $\pm 1.315$	3.75 $\pm 0.95$	2 $\pm 1.08$
3	1 ml /L water				70.7 $\pm 1.32$	10 $\pm 1.22$	11.5 $\pm 1.19$	4.25 $\pm 1.11$	1.25 $\pm 0.49$
4	2 ml /L water				70** $\pm 0.408$	14.5** $\pm 0.29$	11.5 $\pm 0.65$	3.75* $\pm 0.48$	1 $\pm 0.41$
5	1/2 ml /L water	For 48 hrs. Post-vaccination			71.3 $\pm 0.75$	19.5 $\pm 0.5$	9.5 $\pm 1.3$	3 $\pm 1.08$	1.5 $\pm 0.65$
6	1 ml /L water				71 $\pm 2.8$	10.75 $\pm 0.48$	9.75 $\pm 1.79$	4 $\pm 1.22$	1.75 $\pm 0.86$
7	2 ml /L water				59** $\pm 0.71$	17** $\pm 0.71$	14.2** $\pm 0.75$	4.5 $\pm 1.32$	2.25 $\pm 0.85$

\* Significant at  $P \leq 0.05$ \*\* High significant at  $P \leq 0.01$

Table 4. Effect of toltrazuril on differential leucocytic count (%) of pre- and post- 3<sup>rd</sup> vaccination of chicken by ND vaccine (Mean  $\pm$  S.E) . (n = 4).

Groups	Doses of treatment	Time of treatment	Route	Time of vaccination	Lymphocyte	Heterophil	Eosinophil	Monocyte	Basophil
1	Vaccine only	Control	Via drinking water	At 32 days old	68.5 $\pm 0.96$	15.5 $\pm 0.96$	10.5 $\pm 0.87$	3.5 $\pm 0.96$	1.25 $\pm 0.48$
2	<sup>1/2</sup> ml /L water	For 48 hrs. Pre-vaccination			69 $\pm 1.08$	15.25 $\pm 0.48$	11.25 $\pm 0.48$	2 $\pm 0.00$	1 $\pm 0.41$
3	1 ml /L water				69.75 $\pm 3.04$	17 $\pm 0.41$	11.75 $\pm 0.63$	3.75 $\pm 0.85$	0.75 $\pm 0.41$
4	2 ml /L water				59.5** $\pm 0.65$	16.5 $\pm 1.32$	12.25 $\pm 1.109$	7.75* $\pm 1.031$	2 $\pm 1.08$
5	<sup>1/2</sup> ml /L water	For 48 hrs. Post-vaccination			67.25 $\pm 1.03$	15.25 $\pm 0.48$	10.5 $\pm 0.86$	5.75 $\pm 0.85$	1.75 $\pm 0.854$
6	1 ml /L water				63.75** $\pm 1.25$	17.25 $\pm 0.75$	11.5 $\pm 0.65$	6.25* $\pm 0.25$	1.5 $\pm 0.63$
7	2 ml /L water				61.75** $\pm 2.18$	18.7* $\pm 0.85$	13.25 $\pm 2.85$	5.25 $\pm 1.25$	1.25 $\pm 0.48$

\* Significant at  $P \leq 0.05$ \*\* High significant at  $P \leq 0.01$ Table 5. Effect of toltrazuril on cell mediated immunity of chicken pre- and post- vaccination by ND vaccine monitored by lymphocyte blastogenesis. (Mean  $\pm$  S.E). (n = 4).

Groups	Doses of treatment	Time of treatment	Route	Lymphocyte transformation		
				Vaccine at 7 days	Vaccine at 18 days	Vaccine at 32 days
1	Vaccine only	Control	Via drinking water	1.703 $\pm$ 0.016	1.761 $\pm$ 0.029	1.782 $\pm$ 0.037
2	<sup>1/2</sup> ml /L water	For 48 hrs. Pre-vaccination		1.432** $\pm$ 0.28	1.734 $\pm$ 0.045	1.807 $\pm$ 0.035
3	1 ml /L water			1.227** $\pm$ 0.045	1.655 $\pm$ 0.093	1.262** $\pm$ 0.027
4	2 ml /L water			1.212** $\pm$ 0.023	1.280** $\pm$ 0.026	1.150** $\pm$ 0.023
5	<sup>1/2</sup> ml /L water	For 48 hrs. Post-vaccination		1.506** $\pm$ 0.023	1.716 $\pm$ 0.73	1.697 $\pm$ 0.058
6	1 ml /L water			1.380** $\pm$ 0.021	1.646 $\pm$ 0.098	1.25** $\pm$ 0.025
7	2 ml /L water			1.252** $\pm$ 0.02	1.287** $\pm$ 0.023	1.256** $\pm$ 0.103

\* Significant at  $P \leq 0.05$ \*\* High significant at  $P \leq 0.01$

Table 6. Effect of toltrazuril on cell mediated immunity of chicken pre- and post- vaccination by ND vaccine monitored by phagocytic activity (%). (Mean  $\pm$  S.E). (n = 4).

Groups	Doses of treatment	Time of treatment	Route	Phagocytosis		
				Vaccine at 7 days	Vaccine at 18 days	Vaccine at 32 days
1	Vaccine only	Control	Via drinking water	93 $\pm$ 0.82	89.5 $\pm$ 1.041	90.25 $\pm$ 0.63
2	1/2 ml /L water	For 48 hrs. Pre-vaccination		88** $\pm$ 0.41	88.5 $\pm$ 1.19	87.5** $\pm$ 3.3
3	1 ml /L water			83.25** $\pm$ 1.44	86 $\pm$ 1.47	71.5** $\pm$ 0.5
4	2 ml /L water			77.25* $\pm$ 0.478	86.25* $\pm$ 0.48	68.5** $\pm$ 0.96
5	1/2 ml /L water	For 48 hrs. Post-vaccination		90.0* $\pm$ 0.82	87.25 $\pm$ 1.108	87.5* $\pm$ 0.86
6	1 ml /L water			83** $\pm$ 0.82	85.5 $\pm$ 2.06	77** $\pm$ 0.82
7	2 ml /L water			75.25** $\pm$ 0.63	87.75 $\pm$ 1.79	75** $\pm$ 0.82

\* Significant at  $P \leq 0.05$

\*\* High significant at  $P \leq 0.01$



Table 7. Effect of toltrazuril on total protein, albumin and globulin of chickens pre- and post-vaccination by ND vaccine (Mean  $\pm$  S.E) (n = 4).

Groups	Doses of treatment	Time of treatment	Route	Vaccinated at 7 days			Vaccinated at 18 days			Vaccinated at 32 days		
				Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
1	Vaccine only	Control	Via drinking water	7.8 $\pm$ 0.22	3.88 $\pm$ 0.14	3.95 $\pm$ 0.19	7.9 $\pm$ 0.45	3.5 $\pm$ 0.48	4.42 $\pm$ 0.36	8.4 $\pm$ 0.213	4.18 $\pm$ 0.36	4.25 $\pm$ 0.24
2	1/2 ml /L water	For 48 hrs. Pre-vaccination		6.95 $\pm$ 0.33	4.25 $\pm$ 0.17	2.95 $\pm$ 0.46	7.2 $\pm$ 0.32	4.08 $\pm$ 0.13	3.15 $\pm$ 0.21	6.95 $\pm$ 0.67	4.08 $\pm$ 0.11	2.9 $\pm$ 0.58
3	1 ml /L water			6.4 $\pm$ 0.74	4.13 $\pm$ 0.092	2.32 $\pm$ 0.74	7.28 $\pm$ 0.33	3.8 $\pm$ 0.17	3.4 $\pm$ 0.83	7.025 $\pm$ 0.43	4.05 $\pm$ 0.21	2.48 $\pm$ 0.79
4	2 ml /L water			6.98 $\pm$ 0.2	4.4 $\pm$ 0.13	2.55 $\pm$ 0.19	6.8 $\pm$ 0.69	4.2 $\pm$ 0.12	2.6 $\pm$ 0.58	6.7 $\pm$ 0.49	4.25 $\pm$ 0.28	2.43 $\pm$ 0.52
5	1/2 ml /L water	For 48 hrs. Post-vaccination		7.25 $\pm$ 0.096	4.25 $\pm$ 0.13	3.25 $\pm$ 0.34	8.95 $\pm$ 0.46	4.5 $\pm$ 0.39	4.45 $\pm$ 0.47	7.63 $\pm$ 0.32	4.86 $\pm$ 0.22	2.77 $\pm$ 0.33
6	1 ml /L water			6.4 $\pm$ 0.075	3.7 $\pm$ 0.26	2.72 $\pm$ 0.34	8.1 $\pm$ 0.42	4.2 $\pm$ 0.25	3.9 $\pm$ 0.66	6.8 $\pm$ 0.72	4.8 $\pm$ 0.41	1.94 $\pm$ 0.39
7	2 ml /L water			5.7 $\pm$ 0.25	3.8 $\pm$ 0.23	1.9 $\pm$ 0.22	7.2 $\pm$ 0.24	4 $\pm$ 0.17	3.2 $\pm$ 0.092	6.28 $\pm$ 0.34	4.5 $\pm$ 0.45	2.20 $\pm$ 0.16

\* Significant at  $P \leq 0.05$       \*\* High significant at  $P \leq 0.01$

Table 8. Effect of toltrazuril on Haemagglutination inhibition (HI) titres in chicken pre- and post- vaccination by ND vaccine(Log base-2titer) (Mean  $\pm$  S.E) . (n = 4).

Groups	Doses of treatment	Time of treatment	Route	Haemagglutination inhibition		
				Vaccine at 7 days	Vaccine at 18 days	Vaccine at 32 days
1	Vaccine only	Control	Via drinking water	1.405 $\pm$ 0.14	1.61 $\pm$ 0.071	2.209 $\pm$ 0.07
2	$\frac{1}{2}$ ml /L water	For 48 hrs. Pre-vaccination		1.303 $\pm$ 0.073	1.61 $\pm$ 0.071	2.208 $\pm$ 0.143
3	1 ml /L water			1.403 $\pm$ 0.143	1.405 $\pm$ 0.073	1.510** $\pm$ 0.000
4	2 ml /L water			1.014* $\pm$ 0.126	1.71 $\pm$ 0.073	1.909** $\pm$ 0.07
5	$\frac{1}{2}$ ml /L water	For 48 hrs. Post-vaccination		1.609 $\pm$ 0.071	1.509 $\pm$ 0.000	1.610** $\pm$ 0.07
6	1 ml /L water			1.302 $\pm$ 0.073	1.402 $\pm$ 0.14	1.910* $\pm$ 0.071
7	2 ml /L water			1.015* $\pm$ 0.13	1.101** $\pm$ 0.07	1.710** $\pm$ 0.071

\* Significant at  $P \leq 0.05$       \*\* High significant at  $P \leq 0.01$

Table 9. Effect of toltrazuril on some biochemical parameters of chickens Pre- and post- vaccination by ND vaccine. (Mean  $\pm$  S.E) (n = 4).

Groups	Doses of treatment	Time of treatment	Route	Vaccine at 7 days		Vaccine at 18 days		Vaccine at 32 days	
				AST (U/l)	ALT (U/l)	AST (U/l)	ALT (U/l)	AST (U/l)	ALT (U/l)
1	Vaccine only	Control	Via drinking water	13.45 $\pm$ 2.19	153.4 $\pm$ 3.1	12 $\pm$ 2.039	146.45 $\pm$ 0.298	13.002 $\pm$ 4.18	105.6 $\pm$ 2.9
2	$\frac{1}{2}$ ml /L water	For 48 hrs. Pre-vaccination		13.05 $\pm$ 1.217	136 $\pm$ 2.27	10.05 $\pm$ 0.89	146.9 $\pm$ 4.9	14.002 $\pm$ 1.39	144.18** $\pm$ 2.7
3	1 ml /L water			14.95 $\pm$ 0.44	135.3 $\pm$ 2.4	15.98 $\pm$ 0.42	141.5 $\pm$ 2.69	13 $\pm$ 1.66	140.2** $\pm$ 1.3
4	2 ml /L water			22.2** $\pm$ 2.89	135.8 $\pm$ 2.21	28.3** $\pm$ 3.98	145.2 $\pm$ 1.29	23.95* $\pm$ 3.61	141.85** $\pm$ 0.31
5	$\frac{1}{2}$ ml /L water	For 48 hrs. Post-vaccination		10.53 $\pm$ 0.89	135.3 $\pm$ 2.2	20.16 $\pm$ 4.8	143.48 $\pm$ 2.8	14.45 $\pm$ 1.09	135.01** $\pm$ 5.36
6	1 ml /L water			19.94* $\pm$ 0.543	135.4 $\pm$ 1.85	19.7 $\pm$ 3.49	143.5 $\pm$ 1.45	23.6* $\pm$ 3.39	127.65* $\pm$ 7.99
7	2 ml /L water			19.1* $\pm$ 0.67	135.4 $\pm$ 3.5	35.7** $\pm$ 1.58	144.8 $\pm$ 1.78	22.07* $\pm$ 2.36	141.5** $\pm$ 2.92

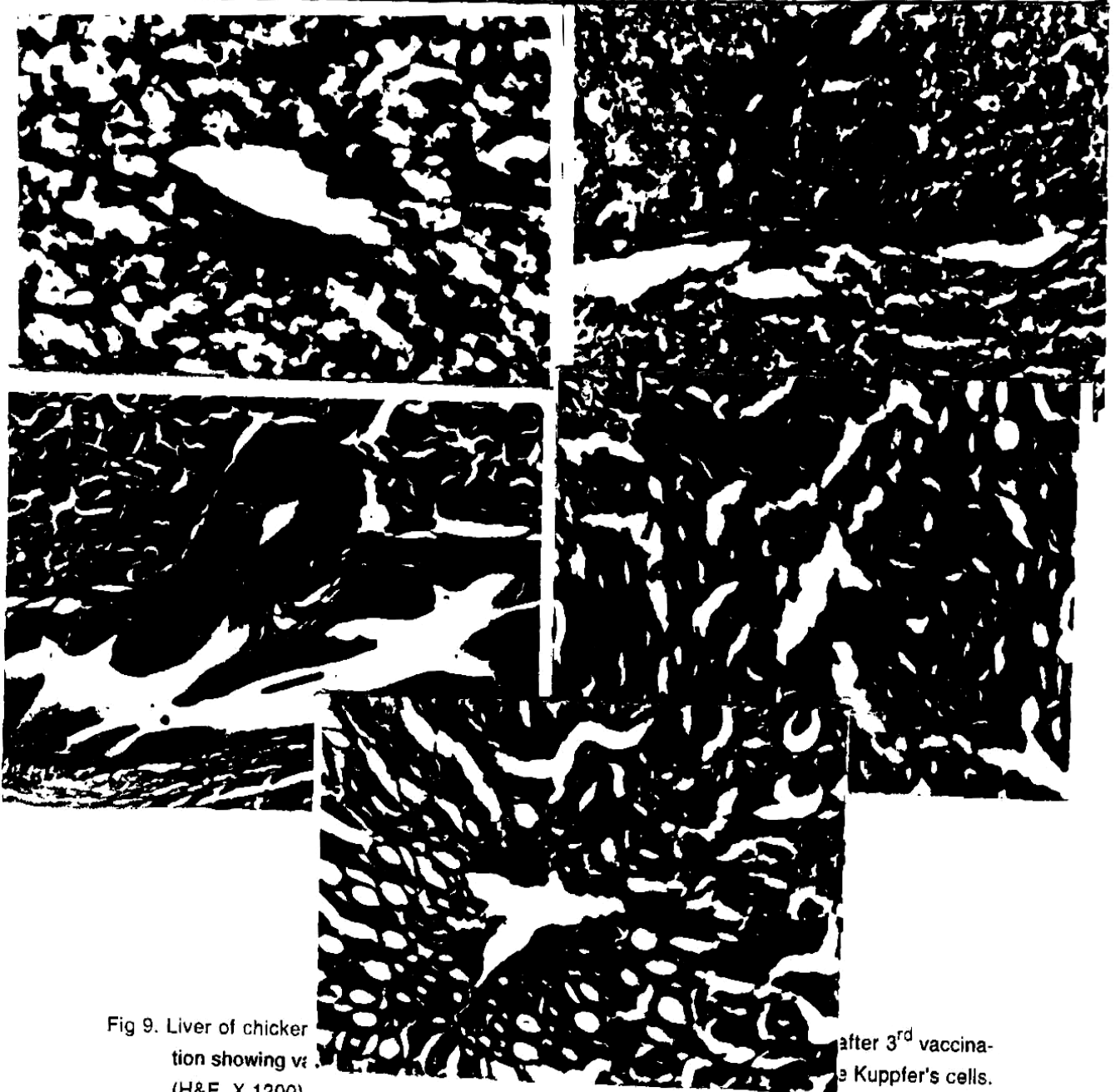
\* Significant at  $P \leq 0.05$       \*\* High significant at  $P \leq 0.01$

phoid depletion was manifested by reduction in size and lighter density of the cortex in most chickens particularly in groups (4, 5 and 6) after 1<sup>st</sup> vaccination. The epithelial covering of the bursa had vacuolated cytoplasm, and others showed vesicles formation with few erythrocytes in subepithelial cells, besides, destruction and lysis of the lymphocytes of most lymphoid follicles (Fig. 2) mostly in some chickens in group (1, 2 and 3) which received drugs before the 3<sup>rd</sup> vaccination. Other epithelial cells showed metaplasia to goblet cells with edema and heterophils infiltration in the subepithelial tissues. The bursa showed cyst in the center of the lymphoid follicles, which were filled with eosinophilic fluid besides, focal destroyed areas replaced by fibrous tissues proliferation among the follicles (Fig. 3). This lesion showed in some chickens in groups (2 and 4) pre- and post- 3<sup>rd</sup> vaccination. Depletion and necrosis of lymphocytes in the white pulp of spleen were common in most chickens in all vaccinated groups with hemorrhage among the splenic tissues and subcapsule (Fig. 4). Old hyalinized thrombus was detected in the trabecular blood vessels (Fig. 5). with inflammatory cells in the vascular wall, mainly, lymphocytes and fibrous tissue proliferation. This lesions appeared mostly in chicken in group 3 post- 3<sup>rd</sup> vaccination. Polyarteritis with vacuolation and hyalinization in the wall of splenic blood sinusoids was seen particularly in group 6 post- 3<sup>rd</sup> vaccination. Thickening in the trabecular capsule with fibrous tissues proliferation was noticed in some chickens in group 6 post- 2<sup>nd</sup> vaccination. Endotheliosis, thickening in the wall of blood sinusoids with vacuolation and perivascular edema could be noticed (Fig. 6). In addition, depletion and necrosis in lymphocytes in splenic tissues mostly in 3 groups pre- and post- 1<sup>st</sup> and 3<sup>rd</sup> vaccinations appeared. Thymus showed depletion and necrosis in lymphocytes in the germinal center (Fig. 7). The depletion was severe particularly, in most chickens in all groups received the drugs post- 1<sup>st</sup> vaccination, but, appeared mild in all groups given the drugs post- 3<sup>rd</sup> vaccination. Necrosis with hemorrhage was seen in the center of the lymphoid follicles of the thymus (Fig. 8). Thymus showed destruction in lymphoid elements and macrophages engulfed necrotic lymphocytes formed (star-sky appearance). This lesions were detected in most groups particularly in the 3<sup>rd</sup> group which received drugs post- 1<sup>st</sup> and 3<sup>rd</sup> vaccination. The hepatic lesions were severe and involved the majority of hepatic parenchyma. The hepatic cells revealed various degenerative changes mainly, vascular and hydropic degenerations with hypertrophy of the Kuppfer's cells (Fig. 9) besides, dilated and engorged hepatic sinusoids and veins with blood. Focal hemorrhages were noticed among the de-

generated hepatic cells. These lesions could be noticed in most chickens in all vaccinated groups, but, the lesions were severe in chickens given double doses of the drugs post- 3 vaccination. The liver showed focal aggregation of the inflammatory cells replaced by destroyed hepatic cells near to the portal zones of the hepatic lobules (Fig. 11). Focal fibroblasts proliferation could be seen in the portal areas and extended to the interlobular tissues, particularly, in chickens that received the drugs with double doses after 2<sup>nd</sup> and 3<sup>rd</sup> vaccination. Thrombus of the portal vein with numerous inflammatory cells infiltration, mainly, lymphocytes in the vascular wall was seen in few chicken in group that received drugs pre- 3<sup>rd</sup> vaccination. The kidneys showed thickening with hyperplasia in the epithelial lining of the renal pelvis forming finger-like projections inside the luman surrounded by areas of hemorrhage (Fig. 15). Large focal aggregation of the inflammatory cells replaced the destroyed renal tubules with cystic dilatation (Fig. 12). Degenerative changes, mainly, vacuolar degeneration were noticed in the renal tubules, and others revealed regenerative process, characterized by presence of rows of large basophilic vesicular nuclei from the tubular epithelial. This lesions could be seen in most chickens in all vaccinated groups, but, severe in chickens treated with double doses of drugs pre- and post 1<sup>st</sup> vaccination. Hypercellularity of the glomeruli with mild fibroblasts proliferation in the interstitial tissues was seen in most chickens in group 2 before 1<sup>st</sup> and 3<sup>rd</sup> vaccinations. The kidneys showed necrosis of the renal tubules with cystic dilatation and focal area of inflammatory cells among the renal tubules with hemorrhage, besides, edema of the Bowman's capsules leading to shrinkage of the glomerular tuft (Fig. 13) in most chickens in group 4 post- 1<sup>st</sup> vaccination and group 1 pre- 3<sup>rd</sup> vaccination. Endothelosis in the endothelial lining of the renal blood vessels with thickening and hyalinization in the smooth muscles and perivascular edema were evident in some chicken that received small doses of the drug pre- and post- 1<sup>st</sup> vaccination.

## DISCUSSION

Our work was performed on the effect of toltrazuril on the immune response of chickens vaccinated with Newcastle disease virus vaccine. Chickens that received different doses of toltrazuril post- 1<sup>st</sup> and 3<sup>rd</sup> vaccination were depressed and had ruffled feathers. This result was in agreement with Haberkorn and Scheer (1984). They record-



- Fig 9. Liver of chicker after 3<sup>rd</sup> vaccination showing vacuolation and aggregation of the Kupffer's cells. (H&E.,X 1200)
- Fig 10. Liver of chicken received toltrazuril with double therapeutic dose (2ml/L) after 3<sup>rd</sup> vaccination showing focal aggregation of the inflammatory cells replaced the destroyed hepatic cells near to the portal zones of the hepatic lobules. (H&E.,X 150)
- Fig 11. Kidneys of chicken given toltrazuril with double therapeutic dose (2ml/L) before 1<sup>st</sup> vaccination showing thickening with hyperplasia in the epithelial lining of the renal pelvis forming finger-like projections inside the lumen, which surrounded by areas of hemorrhage. (H&E.,X 150)
- Fig 12. Kidneys of chicken received toltrazuril with double therapeutic dose (2 ml / L) after 1<sup>st</sup> vaccination showing large focal aggregation of the inflammatory cells replaced the destroyed renal tubules with cystic dilatation. (H&E.,X 1200)
- Fig 13. Kidneys of chicken administrated toltrazuril with half therapeutic dose (0.5 ml / L) after 1<sup>st</sup> vaccination showing necrosis of the renal tubules with cystic dilatation and focal area of inflammatory cells among the renal tubules with hemorrhage. Edema of the Bowman's capsules lead to shrinkage of the glomerular tuft. (H&E.,X 300)

ed that high doses of toltrazuril caused impairment of general condition and ruffled fur in rats. Our results may be attributed to stress of drug and vaccine on the immune system of the birds. So, decrease in feed consumption and depression occurred.

Toltrazuril treated chickens pre- and post- vaccination with ND vaccine revealed that toltrazuril caused reduction in lymphocyte count, especially, in chickens at early age pre- and post- 1<sup>st</sup> vaccination. Also, double therapeutic doses resulted in reduction in lymphocyte count pre- or post- 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> vaccination. This result partially, agreed with Bomhard and Vogel (1985). They recorded decrease in leukocytic count due to large dose of toltrazuril. Our investigation may be attributed to drug toxicity and immune suppressive effect. This result was confirmed with depletion in the lymphocyte of bursa of Fabricus, spleen and thymus.

Toltrazuril caused reduction in lymphocyte transformation in all groups of chickens of the early age pre- and post- 1<sup>st</sup> vaccination by ND vaccine. These results may be attributed to the toxic effect of toltrazuril on the immune system which is not well developed in the early age. The chickens in groups 4 and 7 given double therapeutic doses pre- and post- 2<sup>nd</sup> vaccination suffered from reduction of lymphocyte transformation. The same result was recorded with therapeutic and double therapeutic doses pre- and post- 3<sup>rd</sup> vaccination. Our result agreed with Bomhard and Vogel (1985). They mentioned that toltrazuril decreased leukocytic count. These results can be explained by the fact that repeated therapeutic and high doses of toltrazuril have immune suppressive effect. This effect may be due to the anti allergic and anti histaminic effect of the drug on mast cells (Hammond and Coomber, 1984) and Eten (1993). Glucocorticoids released in excess and resulted in decrease of the circulating lymphocyte count, size of thymus and increasing destruction of lymphocyte (Ganony, 1983).

In the current study, phagocytic activity was decreased in all groups pre- and post- 1<sup>st</sup> and 3<sup>rd</sup> vaccination. This may be due to the inhibiting effect of glucocorticoide on the activity of leukocytes and macrophages, which, became inactive and unable to phagocyte as stated by Eistein *et al.* (1994) and Laurent *et al.* (2001). This result was confirmed by the destroyed areas in the follicles of bursa of fabricus replaced by fibrous tissues proliferation in chickens which received therapeutic and double therapeutic doses post- 3<sup>rd</sup> vaccination.

Administration of toltrazuril caused reduction in total protein, especially, in the group that administered high doses pre- 1<sup>st</sup> and 3<sup>rd</sup> vaccination and in group given therapeutic and double therapeutic doses post- 1<sup>st</sup> and 3<sup>rd</sup> vaccination. This result agreed with Bomhard and Vogel (1985) and Elen (1993). Hypoproteinaemia might be due to degenerative changes in the liver and kidneys evidenced in this work. These results agreed with Dein (1982), who reported that hypoproteinaemia occurs in cases of chronic hepatic and renal diseases and stress. This record is in full agreement with our investigation that showed vacuolar and hydropic degenerations in the hepatic cells besides, degeneration and necrosis in the renal tubules of kidneys.

Globulin levels were decreased in chickens of early age due to therapeutic and double therapeutic doses. Also, the same results were detected in chickens at 3<sup>rd</sup> vaccination (group 4, 5, 6 and 7). From the obtained results, it could be noticed that the results of serum globulin comes parallel with the cellular immune response. This could be attributed to the impaired protein synthesis due to hepatic dysfunction, where the liver is the primary site of protein synthesis (Giralt *et al.* 1997).

Concerning the effect of Baycox<sup>®</sup> on specific antibody titre against Newcastle disease, the results of Hemagglutination inhibition test revealed that double therapeutic doses of toltrazuril resulted in reduction of antibody titer against ND vaccine pre- and post- vaccination in early age. Also, decrease in the antibody titer was detected due to repeated therapeutic and double therapeutic doses pre- and post- 3<sup>rd</sup> vaccination. The decreased antibody titer has been associated with reduction in lymphocyte transformation. This is in agreement with Katzung and Trevor (1993) and Lachman (1971). They mentioned that T-cells act as helper cells to allow the B-cells to produce antibody to certain antigen, where, B-cells are responsible for serologic immunity via their differentiation into specific antibody forming cells. This result confirmed by lysis of lymphocyte in all lymphoid organ. Our results were in correlation with Warner *et al.* (1962) who claimed that bursa of Fabricius controlled antibody mediated immunity including production of antibody.

The present study showed that double therapeutic doses resulted in increased level of AST pre- vaccination, while, therapeutic and double therapeutic doses caused increased level post-vaccination in all treated groups. ALT levels were increased in all

treated groups pre- and post- 3<sup>rd</sup> vaccination. These results agreed with Bomhard and Vogel (1985) and Elen (1993). This elevation may be due to hepatocellular damage in chickens (Roskopf *et al.*, 1982, Rayau *et al.*, 1982). Toltrazuril affected on some liver enzymes as mentioned by Harder and Haberkorn (1989). This result is agumented by the pathological lesion of the liver in the form of vacuolar and hydropic degeneration with hypertrophy of the Kupffer's cells besides, focal hemorrhage among degenerated cells. Also, the liver showed focal fibroblast proliferation around the portal area.

It can be concluded that, toltrazuril (Baycox)<sup>®</sup> should not be used in chickens at early ages, where, the immune system is not well developed. The drug should be used in indicated concentration because the higher doses resulted in immune suppression with other disorders especially, liver and kidneys dysfunction. It is advised to use the drug pre-vaccination and limit the use of toltrazuril especially, in repeated therapeutic doses post-vaccination programs.



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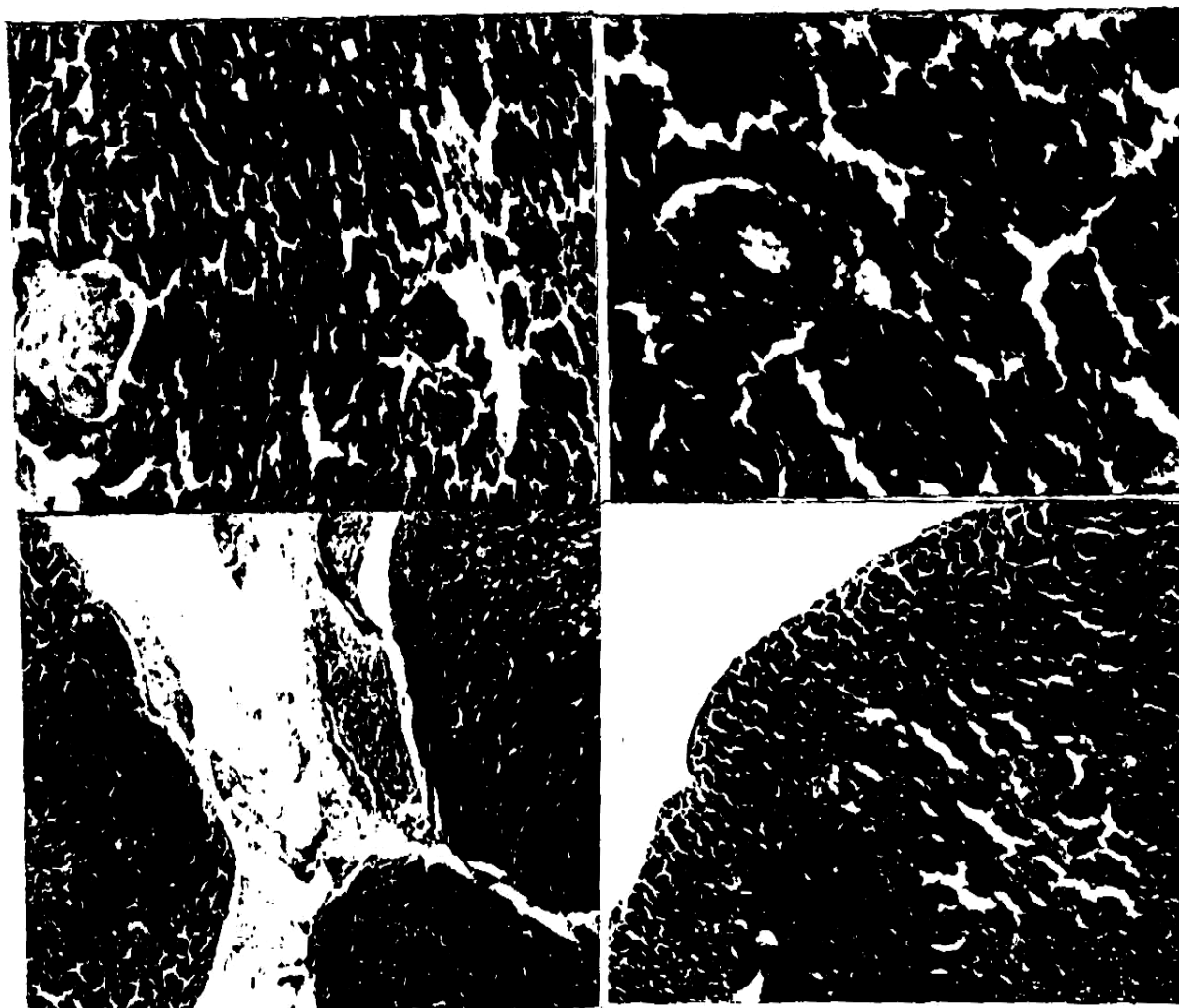


Fig 5. spleen of chicken received toltrazuril with double therapeutic dose (2ml/L) post 3<sup>rd</sup> vaccination showing old hyalinized thrombus in the trabecular blood vessels. ( H&E.,X 300 )

Fig 6. spleen of chicken received toltrazuril with therapeutic dose (1ml/L) before 1<sup>st</sup> vaccination showing endotheliosis, thickening in the wall of blood sinusoids with vacuolation and perivascular odema. ( H&E.,X 1200)

Fig 7. Thymus of chicken received toltrazuril with double therapeutic dose ( 2 ml / L ) after 1<sup>st</sup> vaccination showing depletion and necrosis in lymphocytes in the germinal center. ( H&E.,X 150)

Fig 8. Thymus of chicken administrated toltrazuril with double therapeutic dose (2 ml / L) after 3<sup>rd</sup> vaccination showing necrosis with hemorrhage in the center of the lymphoid follicles. ( H&E.,X 300)