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PHARMACOLOGICAL EVALUATION OF TOLTRAZURIL WITH SPECIAL REFERENCE TO ITS ADVERSE EFFECTS

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ABSTRACT

Toltrazuril which is a novel anticoccidial agent elicit an inhibitory effect on the isolated smooth muscles of both intestine and oviduct of chicken. It was proved that its effect is due to an antihistamine-like effect. Toltrazuril exerted a negative inotropic effect on isolated chicken's heart with a decrease in coronary outflow. The drug induced hypotension with increased respiratory rate. Moreover, oral adminstration of toltrazuril produced a significant increase in serum GOT, glucose, creatinine and uric acid. The drug elicited a significant decrease in serum GPT, total protein and cholesterol levels. Histopathological findings were discussed.

INTRODUCTION

Infestation with coccidia is one of the major problems affecting poultry industry. The disease is responsible for high mortalities in broilers and adult chicken leading to severe economical losses.

There has been intensive search during the recent years for a brand-new anticoccidial agent that would provide good advantages over the currently used drugs.

Toltrazuril is a symmetrical triazinone. It is effective against all Eimeria species affecting poultry and against all coccidia of mammals[1]. It has been noticed that Toltrasuril was characterized by its short application period, rapid mode of action, significant reduction of oocyst shedding, good compatibility and acceptance thus proving superior to conventional chemotherapy of coccidiosis[2] .

The present study was conducted to investigate the pharmacological activities and possible adverse effects of toltrazuril.

MATERIAL AND METHODS

A modified method[3] was performed for studying the effect of toltrazuril on the motility of isolated chicken duodenum. The duodenum was mounted in to 50 ml capacity organ bath containing Tyrode's solution at 38°C and areated with air. A strip of about 3 cm long from the oviduct isthmus of hens was severed then mounted into an organ bath containing Dale's ringer solution at 38°C and areated with air. The effect of the drugs was tested also on the isolated perfused chicken's heart[4].

The effect of the drug on arterial blood pressure, respiration and electrocardigram (ECG) was also carried out[5].

Some selected biochemical variables were studied on 96 chicks (45 days old). Blood samples were collected from the wing vein of chicken in dry and clean plastic bottles before oral administration and at 1,7,14 and 21 days post-treatment. Sera were separated and kept in dry freeze at 20°C until used. Serum GOT and GPT[6], total proteins[7] cholesterol[8], glucose[9], uric acid[10] and creatinine[11] were determined using commercial kits supplied by Bio Merieux, France, and DMS-100 u/v visible spectrophotometer.

Specimens from liver and kidney were collected in 10% formal saline histopathological examination. The results were presented as mean ± SEM and statistical significance was determined by Student "t" test for paired observations[12].

RESULTS AND DISCUSSION

Toltrazuril induced a dose-dependent inhibitory effect on both chicken ileum and oviduct (Fig. 1,3).

The probability of atropine like effect and ganglionic blocking activity of the drug was tested by adding acetylcholine (2 µg/ml) and nicotine sulphate (2 µg/ml), respectively in the presence of toltrazuril (200 µg/ml). The appearance of the expected stimulatory effect of acetylcholine and nicotine indicated the absence of atropine like effect or ganglionic blocking activity of the drug (Fig. 2 a & d).

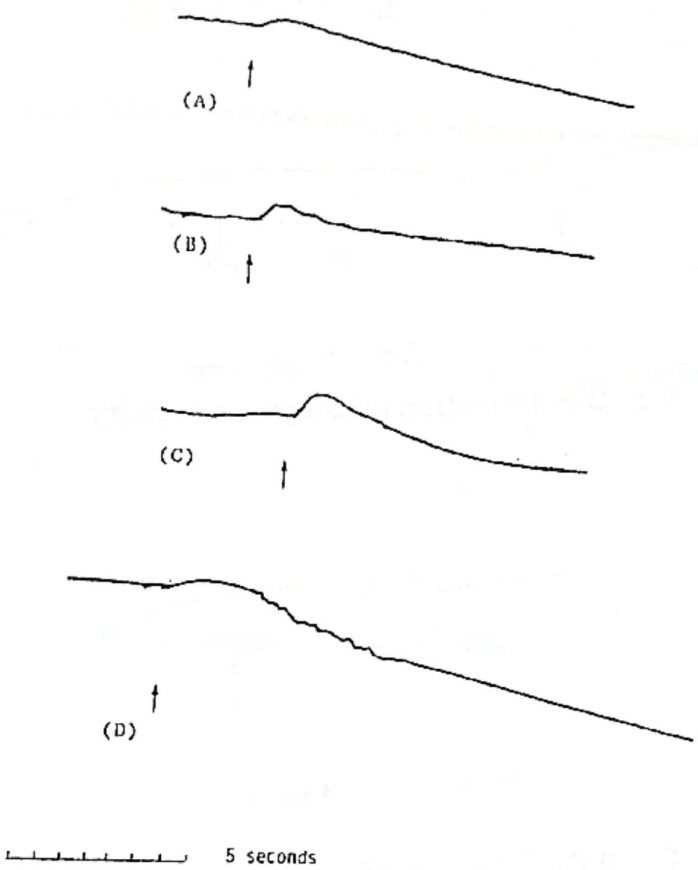

Fig. 1 - The response of isolated chicken ileum to toltrazuril.
(A) 50 ug/ml **(B)** 100 ug/ml, **(C)** 200 ug/ml and **(D)** 400 ug/ml.

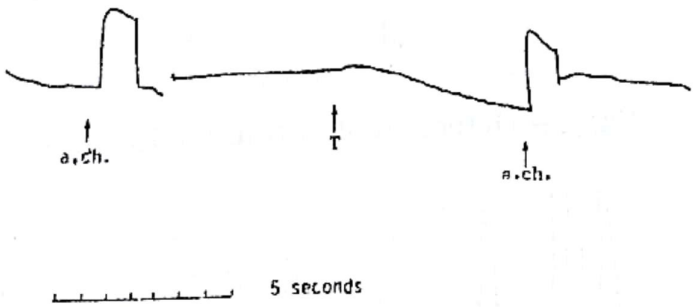

Fig. **2.a-** The site of action of toltrazuril on the chicken ileum.
Detection of atropine like effect.

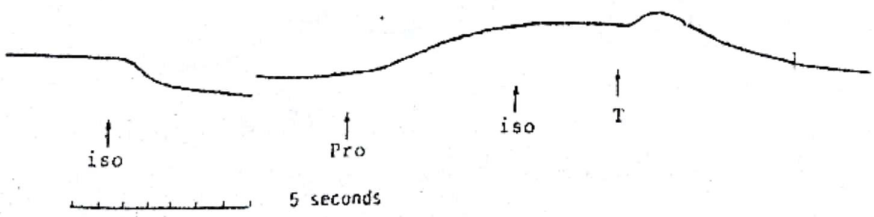

Fig. **2.b-** Detection of ß-agonist activity.

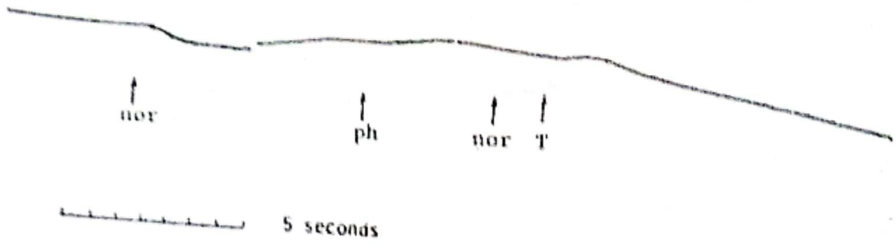

Fig. **2.c-** Detection of α-agonist activity.

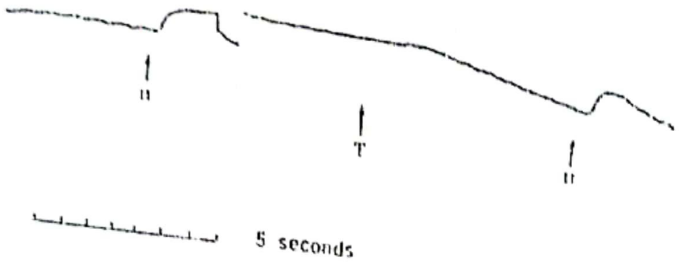

Fig. **2.d-** Detection of ganglionic blocking effect.

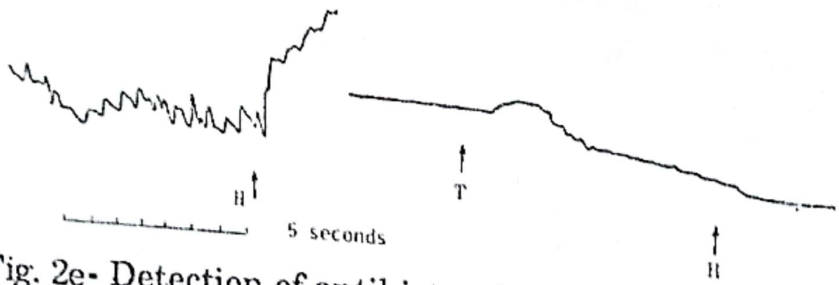

Fig. 2e- Detection of antihistaminic effect.

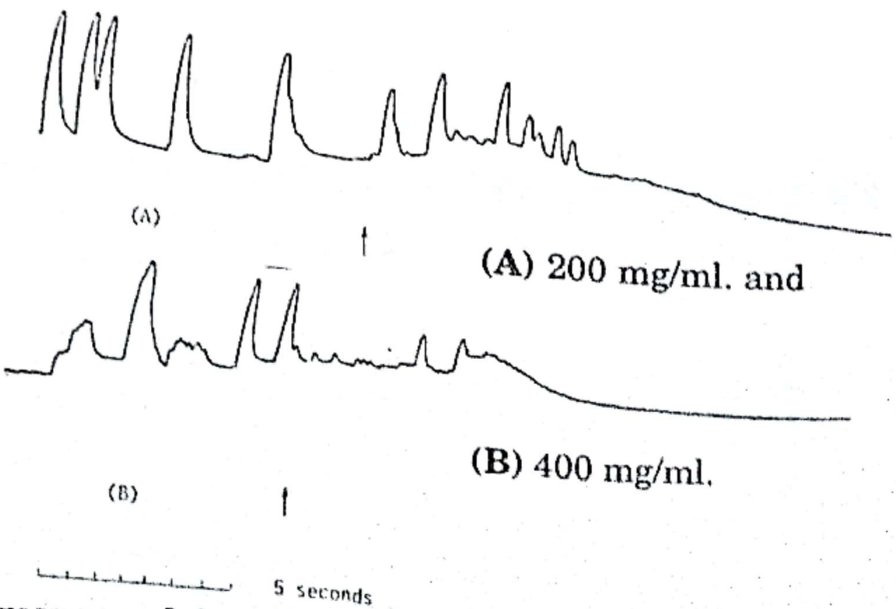

Fig. **3-** The respone of the isolated then oviduct isthmus, to toltrasuril.

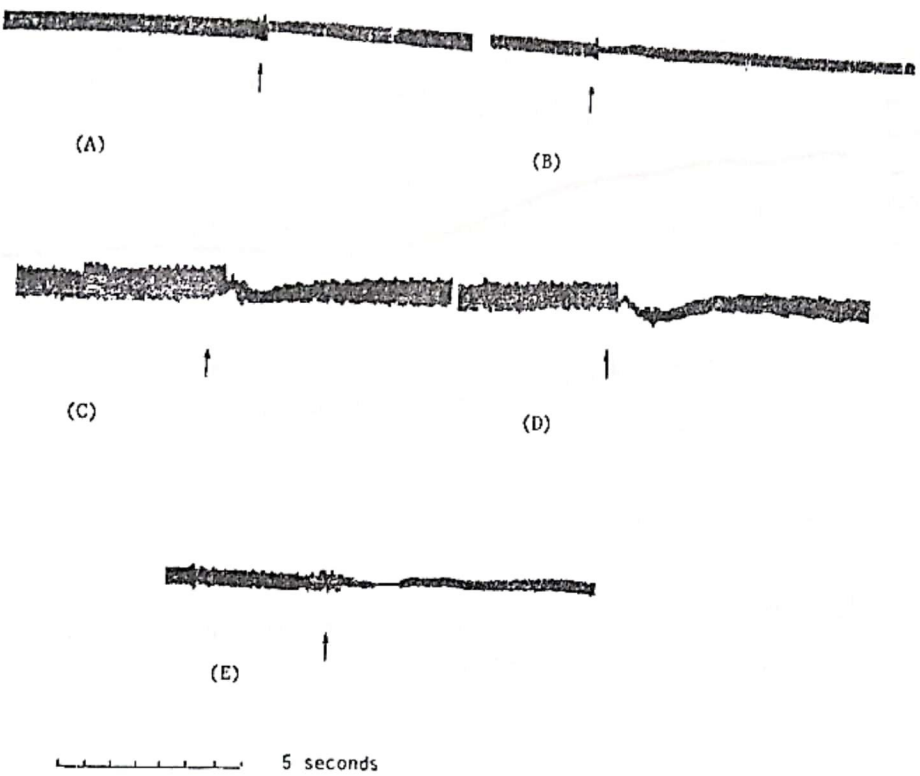

Fig. 4 - Effect of toltrazuril on isolated perfused chicken's heart.

(A) 7 mg (B) 14 mg **(C)** 28 mg **(D)** 56 mg and **(E)** 112 mg.

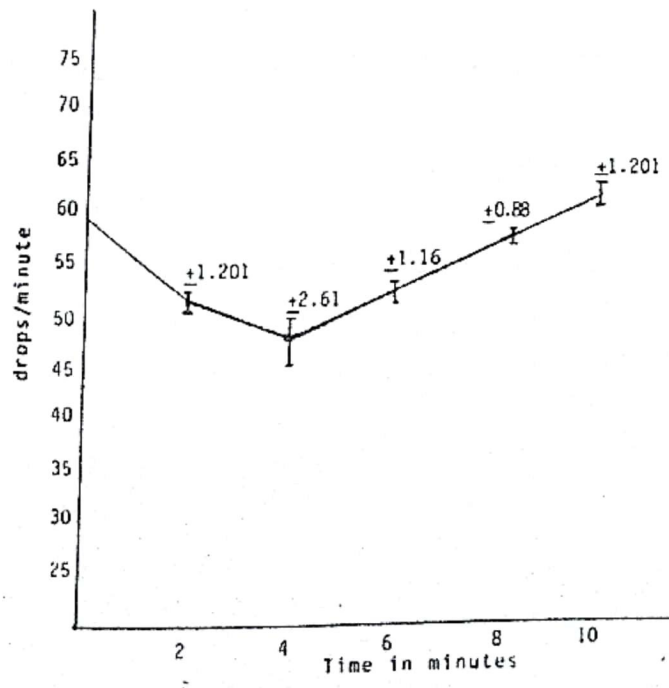

Fig. 5 - Effect of toltrazuril, 56 mg/kg.b.wt. on the coronary outflow of isolated perfused chicken's heart.

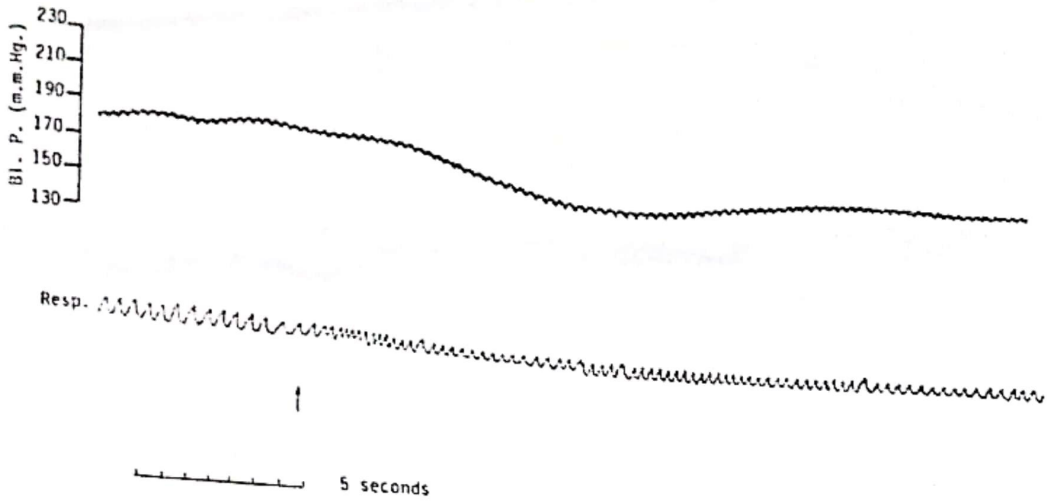

Fig. 6- Response of the arterial blood pressure and respiration of chicken to toltrazuril; 7 mg/kg.b.wt.

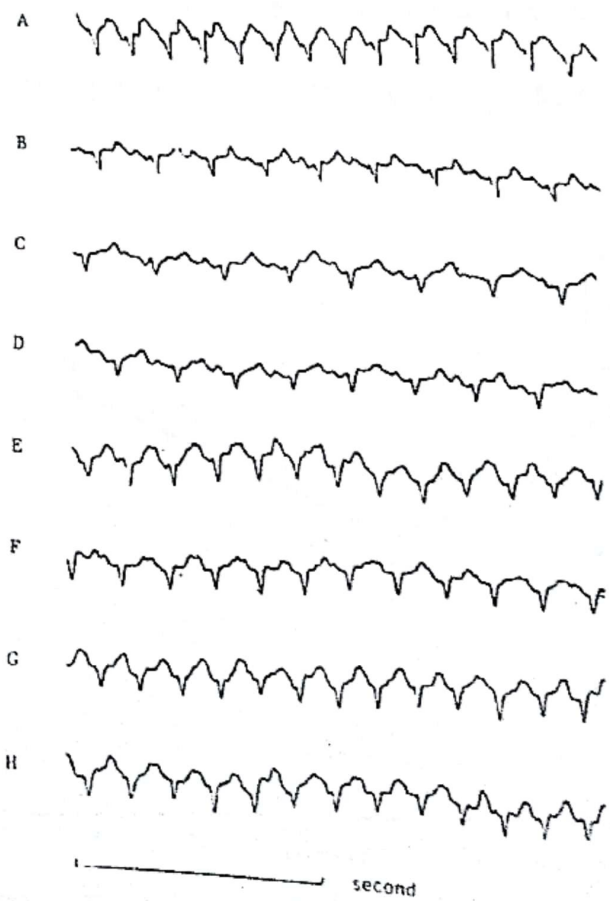

Fig. 7- The effect of intravenous injection of toltrazuril 7 mg/kg.b.wt. on the ECG of unanaesthetised chicken.

The possibility of α and ß adrenoceptor agonist activity were tested by blocking the receptors by phenoxybenzamine (40 µg/ml) and propranolol (100 µg/ml) respectively.

An inhibition was observed after adding toltrazuril (200 µg/ml) indicating the absence of any α or ß adrenergic activity (Fig. 2 c & b).

The antihistaminic action of the drug was investigated by adding histamine (3 ug/ml) in the presence of toltrazuril (200 ug/ml). Histamine failed to produce its stimulatory effect proving the antihistaminic effect of the drug (Fig. 2 e).

The previous results are in full agreement with Hammond and Coomber[13], who observed that toltrazuril has a slight antiallergic effect. Moreover, Gardiner[14] found that toltrazuril reduced the contraction induced by histamine.

Toltrazuril, in different doses, elicited a dose dependent negative inotropic and chronotropic activities on isolated chicken heart. Heart beats were reduced by 33% (Fig. 4). The drug evoked a dose-dependent decrease of the coronary out flow. Maximum reduction of 20% was achieved after the addition of toltrazuril (56 mg/kg.b.wt.) (Fig. 5).

Toltrazuril (7 mg/kg. b. wt.) produced a hypotensive effect of about 35 mmHg. with a concomitant increase in respiratory rate (Fig. 6). The EGG pattern revealed 10% decrease in the heart rate (Fig. 7).

Concerning the effects of toltrazuril on the cardiac contractility, coronary outflow and EGG, it seems conceivable that such effects might be, most probably, due to a quinidine like effect of the drug, since it is well known that some antihistaminics possess a quinidine like effect on the heart[15]. In keeping with these lines, the hypotensive effect of the drug appears to be a secondary effect to inhibition of the cardiac muscle. The respiratory rate was increased probably representing reflex stimulation of the respiratory center reflecting a stimulation of baroreceptors consequent to hypotension and bradycardia.

Table (1): The effect of oral administration of toltrazuril on serum transaminases, total protein, colesterol and glucose in chicken sacrificed one day after treatment.

Groups	Dose	GOT unit/litre	GPT unit/litre	Total protein gm/100 ml	Cholesterol gm/litre	Glucose gm/litre
Control		29.7 ± 3.804	7.28 ± 1.126	3.062 ± 304	111.035 ± 5.710	267.500 ± 10.705
Therapeutic	7 mg/kg (b.wt.) for 2 days.	30.967 ± 4.568	9.565 ± 1.220	2.834 ± 0.242	118.368 ± 6.804	266.667 ± 17.452
Repeated therapeutic	7 mg/kg (b.wt.) for 2 days repeated again after 5 days.	29.358 ± 5.650	10.133* ± 0.359	2.435 ± 0.092	103.59 ± 9.32	250.000 ± 5.628
Double therapeutic	14 mg/kg (b.wt.) for 2 days.	39.167 ± 4.970	7.653 ± 9.930	2.305* ± 0.092	107.313 ± 5.453	254.167 ± 15.082

(mean values ± S.E.) N = 6
* Significant difference at ($P < 0.05$) compared with control.

Table (2): The effect of oral administration of toltrazuril on serum transaminases, total protein, cholesterol and glucose in chicken sacrificed 7 days after treatment.

Groups	Dose	GOT unit/litre	GPT unit/litre	Total protein gm/100 ml	Cholesterol gm/litre	Glucose gm/litre
Control		23.883 ± 5.715	6.293 ± 0.977	3.502 ± 0.101	156.993 ± 5.459	270.833 ± 16.707
Therapeutic	7 mg/kg (b.wt.) for 2 days.	40.683 ± 6.068	4.453 ± 1.216	3.331 ± 0.083	151.242 ± 5.918	290.000 ± 16.637
Repeated therapeutic	7 mg/kg (b.wt.) for 2 days repeated again after 5 days.	40.733* ± 4.651	7.12 ± 0.891	3.119** ± 0.052	134.107* ± 5.857	274.167 ± 16.607
Double therapeutic	14 mg/kg (b.wt.) for 2 days.	45.567* ± 4.116	7.147 ± 0.710	3.225 ± 0.076	135.352* ± 8.003	250.00 ± 16.486

(mean values ± S.E.) N = 6
** Significant difference at ($P < 0.01$) compared with control.
* Significant difference at ($P < 0.05$) compared with control.

Table (3): The effect of oral administration of toltrazuril on serum transaminases, total protein, cholesterol and glucose in chicken sacrificed 14 days after treatment.

Groups	Dose	GOT unit/litre	GPT unit/litre	Total protein gm/100 ml	Cholesterol gm/litre	Glucose gm/litre
Control		60.117 ± 4.656	4.640 ± 0.574	3.572 ± 0.148	123.833 ± 9.668	192.233 ± 12.273
Therapeutic	7 mg/kg (b.wt.) for 2 days.	71.233 ± 5.726	4.507 ± 0.761	3.521 ± 0.144	110.167 ± 7.877	249.995* ± 17.606
Repeated therapeutic	7 mg/kg (b.wt.) for 2 days repeated again after 5 days.	77.133* ± 5.428	5.013 ± 0.758	3.518 ± 0.058	112.167 ± 7.792	248.35* ± 17.614
Double therapeutic	14 mg/kg (b.wt.) for 2 days.	77.850* ± 5.484	1.813** ± 0.247	3.420 ± 0.084	109.500 ± 6.836	243.833 ± 18.919

(mean values ± S.E.) N = 6
** Significant difference at ($P < 0.01$) compared with control.
* Significant difference at ($P < 0.05$) compared with control.

Table (4): The effect of oral administration of toltrazuril on serum transaminases, total protein, cholesterol and glucose in chicken sacrificed 21 days after treatment.

Groups	Dose	GOT unit/litre	GPT unit/litre	Total protein gm/100 ml	Cholesterol gm/litre	Glucose gm/litre
Control		46.550 ± 4.557	15.593 ± 0.652	4.424 ± 0.327	114.325 ± 5.602	245.135 ± 6.010
Therapeutic	7 mg/kg (b.wt.) for 2 days.	41.783 ± 2.293	14.120 ± 1.431	3.594* ± 0.090	124.608 ± 3.813	239.580 ± 11.072
Repeated therapeutic	7 mg/kg (b.wt.) for 2 days repeated again after 5 days.	42.167 ± 2.320	11.342 ± 2.203	3.293** ± 0.074	126.942 ± 9.870	230.089 ± 11.135
Double therapeutic	14 mg/kg (b.wt.) for 2 days.	47.850 ± 1.293	10.107** ± 1.496	3.211** ± 0.163	123.138 ± 8.181	218.284 ± 13.254

(mean values ± S.E.) N = 6
** Significant difference at ($P < 0.01$) compared with control.
* Significant difference at ($P < 0.05$) compared with control.

Table (5): The effect of oral administration of tolrazuril on serum creatinine and serum uric acid in chicken sacrificed at different intervals after treatment.

Groups	Dose	Time of sacrification							
		After 1 day		After 7 days		After 14 days		After 21 days	
		Creatinine mg/litre	Uric acid mg/litre	Creatinine mg/litre	Uric acid mg/litre	Creatinine mg/litre	Uric acid mg/litre	Creatinine mg/litre	Uric acid mg/litre
Control		0.542 ± 0.028	5.573 ± 0.257	0.790 ± 0.138	4.607 ± 0.372	0.661 ± 0.092	2.879 ± 0.245	0.481 ± 0.026	2.645 ± 0.175
Therapeutic	7 mg/kg body weight for 2 days.	0.630 ± 0.031	5.560 ± 0.466	1.600 ± 0.478	5.380 ± 0.284	0.599 ± 0.108	3.038 ± 0.280	0.456 ± 0.040	2.884 ± 0.158
Repeated therapeutic	7 mg/kg body weight for 2 days repeated again after 5 days.	0.569 ± 0.025	6.107 ± 0.424	1.600 ± 0.346	5.093 ± 0.268	0.666 ± 0.089	2.767 ± 0.223	0.454 ± 0.013	2.589 ± 0.098
Double therapeutic	14 mg/kg body weight for 2 days.	0.597 ± 0.046	5.987 ± 0.172	1.817* ± 0.398	5.720* ± 0.185	0.444 ± 0.091	3.342 ± 0.223	0.452 ± 0.042	2.393 ± 0.119

(mean values ± S.E.) N = 6

* Significant difference at ($P < 0.05$) compared with control.

The forementioned results are in accordance with Bomard and Vogel[16], who found that the intravenous administration of toltrazuril in mice resulted in accelerated respiration.

Oral administartion of toltrazuril in repeated therapeutic (7 mg/kg.b.wt.) and double therapeutic doses (14 mg/kg. b. wt.) produced a significant increase in serum GOT, creatinine and uric acid 7 and 14 days post treatment. However, there was a significant decrease in serum GPT, glucose, total proteins and cholesterol (Tables, 1,2,3,4,5). The doses were repeated every five days.

The above result was in correlation with Rosskopf[18] who reported that serum GOT is not liver specific in birds. However, increased activity has been associated with hepatocellular damage in chicken. The obtained results revealed hydropic degenerative changes in kidneys, which agree with the previous reported observations[19,20], that hypoproteinaemia occured in cases of chronic hepatic and renal diseases.

Moreover, the results agreed with the reported[12] low serum cholesterol level which has been assoiated with liver diseases. On these bases it has been shown [16] that creatinine and uric acid are increased in renal disease and massive tissue destruction.

Thus, it could be concluded that toltrazuril could be used safely in therapeutic doses and care must be taken with the double therapeutic dose which has harmuful effects on the liver and kideny function.

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تقييم فارماكولوجى لدواء التولترازوريل مع إشارة خاصة لتأثيراته الضارة

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قسم الفارماكولوجى والطب الشرعى والسموم
كلية الطب البيطرى - جامعة الزقازيق

بدراسة تأثير التولترازوريل على العضلات اللإإردية فى الدجاج وجد أن له تأثير مثبط لحركة الأمعاء الدقيقة وقناة البيض فى الدجاج وذلك نتيجة لتأثيره المصاد للهستامين .

وأثبتت الدراسة أن التولترازوريل يسبب تثبيطا لعضلة القلب كما أنه يحدث هبوط فى ضغط الدم وزيادة فى معدل التنفس وتغيير فى رسم القلب فى الدجاج .

ولقد وجد أن الدواء يسبب إرتفاعا معنويا فى نشاط خميرة الجلوتاميك أوكسال أسيتيك ترانس أمينيز وسكر الدم والكرياتينين وحمض البوليك وإنخفاضا معنويا فى نشاط خميرة الجلوناميك بيروفيك ترانس أمينيز ونسبة البروتين الكلس وأيضا نسبة الكوليسترول .

ولقد أحدث تغييرات تحطيمية فى خلايا الكبد والكلى فى الدجاج المعامل بضعف الجرعة العلاجية (١٤ مجم/كجم وزن) لمدة يومين .

ولقد إتضح من هذه الدراسة أن التأثيرات الفارماكولوجية للتولترازوريل نتيجة لغلق مستقبلات الهستامين .

ثم من هذه الدراسة يمكن إستنتاج أنه يمكن إستخدام التولترازوريل بأمان فى الجرعات العلاجية ومراعاة عدم زيادة الجرعة العلاجية حيث أن الجرعات المضاعفة لها تأثير ضار على وظائف الكبد والكلى .