

## Assessment of The Acute Toxic Effects of Ceftriaxone in Chicks

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### ABSTRACT

**Objective:** The current research aims to assess the acute behavioral toxicity and brain histological changes of ceftriaxone in chicks as a biological model.

**Methods:** The acute IM median lethal dose (LD<sub>50</sub>) by Dexon method was obtained during the first day. The scoring of toxicity was estimated in the open-field activity and body-weight was measured. The acute toxic effects of ceftriaxone on brain tissue were recorded.

**Results:** The IM ceftriaxone LD<sub>50</sub> value in chicks was 2131mg/kg. Intramuscular administration of ceftriaxone at doses of 0.532, 1.065 and 1.598 mg/kg caused signs of toxicity such as dropping of wings, eyelids closure, decreased movements and gasping. Ceftriaxone caused a statistically significant decrement in the body weight, decreased activity in the open field experiment represented by delay in movement, and decrease in the crossing lines. The brain showed histopathological changes such as congestion, bleeding and inflammatory cell infiltration. The lesion was more severe when exposed to high concentrations especially during the first 24 hours.

**Conclusion:** The results of the current study demonstrated that ceftriaxone caused behavioral toxic effects in addition to histological changes in the brain especially with acute administration.

**Keywords:** Ceftriaxone, chicks, toxicity effects, LD<sub>50</sub>.

## التأثيرات السمية الحادة للسفترياكسون في نموذج افراخ الدجاج تقبم

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### الخلاصة

**الهدف :** كان الهدف من الدراسة الكشف عن التأثيرات السمية الحادة للسفترياكسون على السلوكية في نموذج افراخ الدجاج بالإضافة الى دراسة التأثيرات النسيجية في الدماغ.

**طريقة العمل :** سجلت الجرعة المميطة الوسطية للسفترياكسون بطريقة دكسون خلال اليوم الاول. كما تم تسجيل مراتب التسمم من خلال دراسة النشاط الحركي داخل الميدان المفتوح كما تم قياس اوزان الافراخ بالإضافة الى دراسة التأثيرات النسيجية في ادمغة افراخ الدجاج.

**النتائج :** كانت الجرعة المميطة الوسطية للسفترياكسون 2131 ملغم /كغم عن طريق الحقن العضلي. ادى اعطاء السفترياكسون عن طريق الحقن العضلي بجرع 0.532، 1.065، 1.598غم /كغم والتي تمثل 0.25 و 0.5 و 0.75 بالمئة من الجرعة المميطة الوسطية الى ظهور علامات التسمم وكانت كالاتي :اغماض العينين وخمول ونفث الريش وتدلي الاجنحة بسبب السفترياكسون نقصانا معنويا في اوزان الافراخ وقلة في الحركة داخل الميدان المفتوح المتمثلة في البطء ببدء الحركة وقلة في عدد الخطوط المقطوعة . اظهرت المقاطع النسيجية للدماغ احتقان ونزف مع زيادة في الخلايا الالتهابية وكانت الافات اكثر شدة بالتراكيز العالية وطول فترة التعرض خلال ال 24 ساعة الأولى.

**الاستنتاج :** نستنتج من دراستنا الحالية ان السفترياكسون لديه سمية حادة في افراخ الدجاج ويمتلك تأثيرات سمية عصبية متمثلة بالتغيرات السلوكية والنسيجية وخاصة خلال التعرض الحاد للدواء.

**الكلمات المفتاحية :** سفترياكسون، افراخ الدجاج، التأثيرات السمية، الجرعة المميطة الوسطية.

## INTRODUCTION

Ceftriaxone is a third generation injectable cephalosporin agent, which has both the gram positive and gram negative activity.<sup>1</sup> As they compared to aminoglycosides, they have less significant toxic potential. Therefore, they have been used increasingly in veterinary medicine.<sup>2</sup>

Ceftriaxone is used to treat serious infections especially when aminoglycosides are not indicated because of their adverse effects.<sup>3</sup> Ceftriaxone is not absorbable when it is given per os and must be given by IM or IV routes. It is widely distributed throughout the body; CSF levels are higher in case of meningitis. Ceftriaxone is excreted by both kidney and liver and in humans, the half-life ( $t_{1/2}$ ) is about 6-11 hours.<sup>4</sup>

Ceftriaxone penetrates well into the CNS and can cause increased neural excitability even at therapeutic doses. Because of limited uses of ceftriaxone in veterinary medicine, an accurate adverse effect profile has not been observed.<sup>5</sup> Ceftriaxone-induced neurotoxicity is believed to be caused by competitive inhibition of  $\gamma$ -aminobutyric acid (GABA) in brain tissues.<sup>6</sup> As with other beta-lactams, the proposed mechanisms for this neurotoxicity imply; decreased GABA release from nerve terminals, cytokine release, induction of endotoxins and glutaminergic mechanisms.<sup>7,8</sup>

Because ceftriaxone is widely used by clinicians for its antimicrobial broad-spectrum activity and pharmacokinetics profile in the city of Mosul, the present research aims to determine the acute toxicity of ceftriaxone in chicks model.<sup>9</sup>

## MATERIAL AND METHODS

### Animals

The experiment was performed on chicks of 7-14 days and both sex. They received water and food ad libitum and saved in groups of 20 to 30 chicks with lighting day and night at temperature of about 30 -34 °C.

### Chemicals

Ceftriaxone standard powder manufactured by Pioneer Pharmaceutical Company was purchased. The dose was 2 g/kg administered by intramuscular injection (IM) into the sternal muscle of chicks.

### Ceftriaxone Lethal Dose Estimation

The acute median lethal dose ( $LD_{50}$ ) of ceftriaxone was determined by Dexon method.<sup>10</sup> The acute toxicity was observed for two hours and then the number of deaths during the first day was recorded, using 8 chicks for this purpose.

## Toxicity of Ceftriaxone in Chicks

Thirty two chicks were randomly assigned into four groups (8 per group). The doses of ceftriaxone were zero for control group, 0.532, 1.065 and 1.598 mg/kg. The signs of toxicity were observed within the first two hours. Scoring of the severity of toxicity was recorded for all studied groups.<sup>11</sup>

### Acute toxicity of ceftriaxone in chicks:

#### a. Effect of ceftriaxone on open-field experiment:

Twenty four chicks were randomly assigned into four groups (6 chicks per group). The chicks in the control group were injected with distilled water at 5 ml/ kg and ceftriaxone at 0.532, 1.065 and 1.598 mg/kg from the first through the fourteenth day, measurements were recorded on the 7<sup>th</sup> and 14<sup>th</sup> days. The chicks were examined in the open-field for any changes in the activity as described before.<sup>12</sup> After that, each chick was used for the tonic immobility test.<sup>13,14</sup>

#### b. Body weight effects of Ceftriaxone :

Twenty- four chicks were weighed from the first day of dosing of ceftriaxone to the fourteenth day, and the average weights were recorded on the 7<sup>th</sup> and 14<sup>th</sup> day.

### Histological effects of ceftriaxone in the chicks:

The treated chicks with ceftriaxone on the 7<sup>th</sup> and 14<sup>th</sup> day were sacrificed and 1 cm of brain tissue was obtained. 10% neutral buffer formalin was used to fix the brain tissue. The standard histopathological protocol were followed, and the slides (5-6  $\mu$ m thickness) were stained using Hematoxylin and Eosin.<sup>15</sup> Light microscopy is used to examine the sections, and images were captured using digital camera.

### Statistical Analysis

The data were included in SPSS programme and one-way ANOVA test was used for analysis. The frequency data are analyzed using Fisher test. Man Whetiny test was used for non- parametric data, using significance level at  $p \leq 0.05$ .<sup>16</sup>

## RESULTS

### 1) Acute lethality of ceftriaxone

The lethal dose 50 of ceftriaxone was 2131 mg/kg IM by Dexon method. The toxicity recorded were eyelids closure, dropping of wings, feathering, gasping and standstill followed by death as shown in (table 1).

## 2) Signs of acute toxicity

Scoring and signs of toxicity of ceftriaxone are shown in table (2). Further, ceftriaxone at 532, 1065 and 1598 mg/kg caused signs of toxicity in the chicks represented by eyelids closure, wing dropping, feathering, defecation, head twisting, gasping, and immobility.

## 3) Acute effects on body weight :

Ceftriaxone at dose (106.55, 213.1 and 426.2 mg/kg) IM resulted in significant decrease in the body weight of the chicks on the 14<sup>th</sup> days in comparison to the control group as illustrated in (table 3 ).

## 4) Acute effects of ceftriaxone on the open-field test:

Table (4) showed the effects of ceftriaxone on chicks treated with 106.55, 213.1 and 426.2 mg/kg IM on the 14<sup>th</sup> day of treatment, which resulted in significant decrease in the number of lines crossing, number of jumping and defecation as compared to control group. The dose of ceftriaxone 426.2 mg/kg (20% of LD<sub>50</sub>) caused significant increase in the time to walk in comparison to control group.

## 5) Histopathological changes of ceftriaxone:

The brain of chicks treated with ceftriaxone at dose of 2000 mg/kg after 30 minutes of IM injection of 7-14 days, aged showed congestion as demonstrated in figure (1). In figure (2), the light microscopy of brain in chicks given ceftriaxone at dose of 2000 mg/kg of the same age but after 1 hour of IM injection showed bleeding and inflammatory cell infiltration. The histological examination of the brain of chicks given the same dose but after 24 hours of IM injection, there were demyelination of axon, perivascular edema and vasogenic edema as shown in figure (3). The histological examination of chick's brain again showed congestion, inflammatory cell infiltration, increase oligodendroglial cells and shrunken in purkinji as demonstrated in figure (4).

## DISCUSSION

The current study was designed to investigate the possible acute toxicity effects of ceftriaxone in the chick's model which is the first one up to our knowledge. The LD<sub>50</sub> of ceftriaxone obtained in this study was 2131mg/kg IM, while the available data of LD<sub>50</sub> were obtained from different routes of administration in animal model other than IM. For example, the LD<sub>50</sub> of ceftriaxone in mice and rats

(both sexes) was > 10000 mg/kg body weight orally while it is > 5000 mg/kg body weight after SC administration where in rabbit the LD<sub>50</sub> was 240 mg/kg body weight when it is given intravenously which means that ceftriaxone is rather safe in rats, mice and rabbit and toxic in chicks under study.<sup>17,18</sup>

Ceftriaxone caused signs of acute toxicity such as eyelids closure, wing dropping, feathering, gasping, and immobility followed by death. While the clinical neurotoxic signs following acute subcutaneous injection in rat is drowsiness, loss of appetite, ataxia, analgesia, abnormal respiration, convulsions and cecum enlargement.<sup>19</sup> Ceftriaxone-induced neurotoxicity is believed to be caused by competitive inhibition of  $\gamma$ -aminobutyric acid (GABA) in brain tissues.<sup>6</sup> As with other beta-lactams, the proposed mechanisms for this neurotoxicity imply; decreased GABA release from nerve terminals, cytokine release, induction of endotoxins and glutaminergic mechanisms.<sup>7,8</sup>

Acute doses of ceftriaxone caused significant increase in body weight in comparison to the control group in the 14<sup>th</sup> day. This result was in agreement with previous findings of Roura *et al.*, and Angelakis who found that antibiotics resulted in weight gain in the animal and that antibiotic addition to the food resulted in improvement of weight gain, feed consumption and efficient feed utilization. These results indicated that feeding antibiotics may permit growth by preventing immunological stress and associated metabolic changes brought about by monokines including interleukin-1.<sup>20,21</sup> However, it was disagreed with previous findings that antibiotics result in animal weight gain. These findings implied that the growth of animals may be affected by the intervention time, dose, types and properties of antibiotics.<sup>22</sup>

The adverse effects of antibiotics on health are not well known except for drug resistance. Recently, the use of antibiotic have been reported to increase the risk of mental diseases.<sup>23</sup> The importance of this study in chicks treated with ceftriaxone at low toxic doses are decreased in the activity in open-field test represented by delay to move especially at different concentrations of the LD<sub>50</sub> and increased in the tonic immobility test represented by decreased number of line crossed, especially at the dose of 5% of LD<sub>50</sub>. These behavioral changes can be a result of the direct toxic effect of ceftriaxone on the brain. Alteration of the nerve-endocrine-immunological network may be a possible mechanism that explain the abnormal behaviors induced by impairment of the gut microbiota.<sup>24,25</sup>

The hippocampus provides the brain with a spatiotemporal framework which regulate various sensory, emotional, and cognitive components.<sup>26</sup> Previous studies has reported that hippocampus

degeneration leads to a decline in cognition and that gut dysbiosis after ceftriaxone administration can lead to cognitive function impairment.<sup>27,28</sup> The histopathological changes seen in this study could explained the behavioral effect of ceftriaxone given in toxic dose and during short period . In addition , the increased inflammatory cell infiltration in the brain seen in this study could explained the changes in behavioral profile which are in agreement with findings of Reardone *et al* who found that inflammation-induced anxiety could result in behavioral changes seen in mice after ceftriaxone administration .<sup>29</sup>

**CONCLUSION**

The current study concluded that ceftriaxone produced acute toxic effect in chicks, and responsible for the observed behavioral effects , especially with large dose and produced tissue changes in the brain especially when administered in high dose and during short period.

**Conflict of Interest**

The authors have no conflict of interest.

Table (1): The acute median lethal dose of ceftriaxone (LD<sub>50</sub>) in chicks

Value	Results
LD50 ( mg/ kg)	2131mg/kg
Range of doses ( mg /kg)	3000-1000=2000 mg/kg
First dose ( mg/kg)	3000 mg/kg
Last dose ( mg/kg)	2500 mg/kg
Increase or decrease in the dose ( mg/kg)	500 mg/kg
Number of chicks	6(xoxox)
Toxicity signs	Eyelids closure, wing dropping, feathering, gasping, decreased motion, standstill and death.
Range of latency to the onset of toxicity (min)	-0,737

\*X = died; O = survived

Table (2): Signs and scoring of toxicity of ceftriaxone in chicks treated by 25%, 50% and 75% of LD<sub>50</sub>.

Toxicity signs Doses	Onset of toxicity signs (mint )	Depression and feathered	Closed eyelids	Wing drooping	Defecation	Paralysis	Recumbency	Gasping	Toxicity scores
<b>Control ( DW)</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	
<b>Ceftriaxon ( 532.75) mg/kg)</b>	<b>11,16±2,13*</b>	<b>83*</b>	<b>83*</b>	<b>83*</b>	<b>83*</b>	<b>0,00</b>	<b>0.00</b>	<b>83*</b>	<b>24</b>
<b>Ceftriaxon (1065.5) mg/kg)</b>	<b>12,34±0,84*</b>	<b>83*</b>	<b>100*</b>	<b>100*</b>	<b>67*</b>	<b>0,00</b>	<b>0,00</b>	<b>100*</b>	<b>21</b>
<b>Ceftriaxon ( 1598.3) mg/kg)</b>	<b>3,12±0,57</b>	<b>100*</b>	<b>67*</b>	<b>100*</b>	<b>33</b>	<b>100*</b>	<b>100*</b>	<b>83*</b>	<b>29</b>

Value= Mean ± Standard Error

\*Significantly different from the control group at p ≤ 0.05

N= 8 chicks

Table (3): Body weight effects using ceftriaxone at dose 5%, 10% and 20% of LD50 at 14<sup>th</sup> day.

Dose ( mg/kg )	Body weight ( g )
Control group (DW)	126,50±20,15
Dose 106.55 mg/kg (5%)	186,00±7,05*
Dose 213.1 mg/kg (10%)	196,34±6,62*
Dose 426.2 mg/kg (20%)	194,67±6,42*

Value= Mean ± Standard Error

\* Significantly different from the control group at p ≤ 0.05

N= 8 chicks

Table (4): Effects of ceftriaxone in open field-test in the 14<sup>th</sup> day from administration.

Variable s	Control (DW )	5 % of LD50	10% of LD50	20% of LD50
Latency to move / s	14,33±0,98	39,83±4,03*	29,16±4,34*	37,16±5,83*
Lines crossed	12,53±0,65	2,40±1,12*	5,40±1,28*	14,67±1,56
Jump escaping	0,34±0,21	0,17±0,15	0,50±0,34	0,17±0,16
Distress calls	2,83±0,16	2,50±0,34	2,67±0,21	2,16±0,40
Pecking	0,00±0,00	0,00±0,00	0,00±0,00	0,00±0,00
Defecations	0,83±0,53	0,67±0,34	1,16±0,47	0,83±0,30
Tonic immobility / s	42,67±6,08	39,34±4,34	27,00±7,31	37,34±7,49

Values are Mean ± Standard Error

\* Significantly different from the control value, P ≤ 0.05.

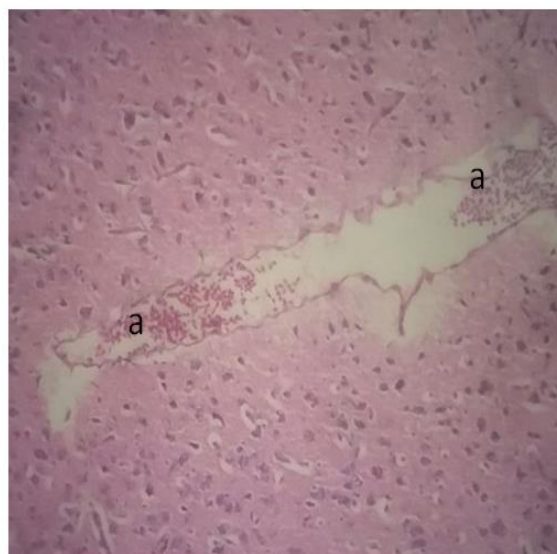


Figure (1): Brain section of chicks (7-14 days old) treated by ceftriaxone (2000 mg/kg) after 30 minutes shows congestion (a) ( H α E 6 × 2.8 \* 10)

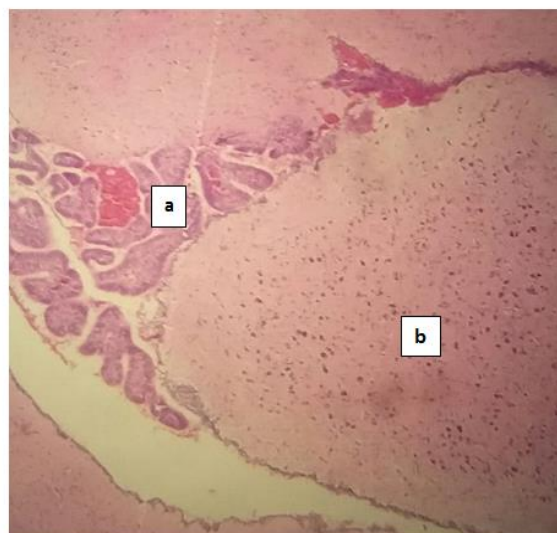


Figure (2): Brain section of chicks (7-14 days old) treated by ceftriaxone (2000 mg/kg) after 1 hour shows bleeding (a) and inflammatory cell infiltration (b) ( H α E 6 × 2.7 \* 10)

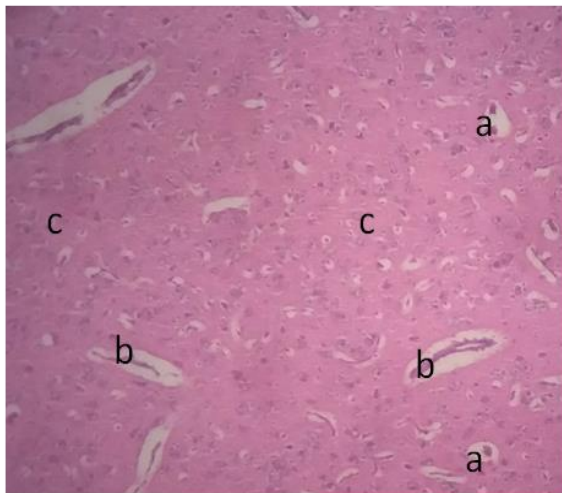


Figure (3): Brain section of chicks (7-14 days old) treated by ceftriaxone (2000 mg/kg) after 24 hour shows demyelination of axon (a) , perivascular edema (b) and vasogenic edema (c) ( *H α E 6 × 2.3 \* 10* )

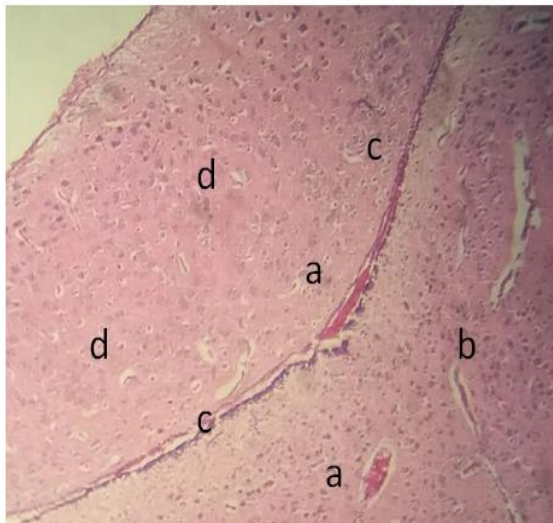


Figure (4): Brain section of chicks (7-14 days old) treated by ceftriaxone (2000 mg/kg) after 24 hour shows congestion (a), inflammatory cell infiltration (b) ,increase oligodendroglial cells (c) and shrunken in purkinji (d) ( *H α E 6 × 2.1 \* 10* )

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