Detection of the Analgesic Effect of Ceftriaxone in Chicks

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ABSTRACT

Objective: To evaluate the analgesic effect of ceftriaxone when administered alone or in combination with paracetamol and diclofenac sodium in boiler chicks.

Methods: This study was conducted in Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine ,University of Mosul , Mosul, Iraq, from November 2021 to February 2022. It was determined using formaldehyde test and the median analgesic effective dose (ED$_{50}$) for ceftriaxone, paracetamol and diclofenac sodium depending on the method of Dixon. An isobolographic ratio of 0.5:0.5 of the ED$_{50}$ for each drug at the analgesic level was calculated.

Results: The administration of ceftriaxone at doses of (523, 1064 and 1330mg/kg) intramuscularly was led to a significant (p value=0.02) and clear analgesic effect through the disappearance of the right foot lift caused by the injection of formaldehyde, in aadition to significant decrease (p value=0.01) in foot thickness at dose of 1330 mg/kg . Analgesic ED$_{50}$ of ceftriaxone , paracetamol and diclofenac sodium by Dixon method were 42.6 ,32.6 and 6.26 mg /kg respectively. The intramuscular administrations of drugs in a ratio of 0.5:0.5 result in a significant decrease (p value<0.001) in ED$_{50}$ values to 6.77, 5.34 and 0.99 mg/kg respectively. The application of the isobolographic analysis revealed the type of interaction between ceftriaxone/paracetamol, ceftriaxone /diclofenac sodium, and the interaction was synergistic when they were given at the ratio 0.5:0.5.

Conclusion: The results showed that ceftriaxone exerts its analgesic effect in chicks by reducing the mechanical hyperalgesia resulting from formaldehyde injection in the planter of the right foot and potentiated the analgesic effects of paracetamol and diclofenac sodium through the synergistic interaction.

Keywords: Ceftriaxone, analgesia, isobolographic, chicks .

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التحري عن التأثير المسكن للسفترياكسون لدى افراخ الدجاج

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الخلاصة

الهدف: لتقييم التأثير المسكن للسفترياكسون عند اعطائه لوحده او مع الباراسيتامول والدايكلوفيناك صودديوم لدى افراخ الدجاج .

طريقة العمل: اجريت هذه الدراسة في فرع الفسلجة والكيمياء الحياتية والادوية/كلية الطب البيطري/جامعة الموصل للفترة من تشرين الثاني ٢٠٢١ ولغاية شباط ٢٠٢٢ولتحقيق هدف الدراسة فقد تم استعمال تجربة الفورمالديهايد وتم حساب الجرعة المسكنة الوسطية للسفترياكسون والباراسيتامول والدايكلوفيناك صوديوم اعتمادا على طريقة ديكسون. تم احتساب نسبة متساوية بمقدار ٠,٥:٠,٥ للجرعة المسكنة الوسطية لكل دواء عند المستوى المسكن.

النتائج: ادى اعطاء السفترياكسون بجرع ٥٢٣و١٠٦٤و١٣٣٠ملغم/كغم من خلال الحقن بالعضلة الى تاثير مسكن معنوي واضح من خلال اختفاء رفع القدم اليمنى والناتج من حقن الفورمالديهايد بالاضافة الى انخفاض معنوي في تثخن القدم وعند جرعة ١٣٣٠ملغم/كغم. كانت الجرعة المسكنة الوسطية للسفترياكسون والباراسيتامول والدايكلوفيناك صوديوم من خلال طريقة ديكسون ٤٢,٦و٣٢,٦و٦,٢٦ملغم/كغم على التوالي. نتج عن الحقن العضلي للادوية اعلاه بنسبة ٠,٥:٠,٥من الجرعة المسكنة الوسطية الى

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حدوث نقصان معنوي في الجرعة المسكنة الوسطية ٦,٧٧ و ٥,٣٤ و ٠,٩٩ملغم/كغم على التوالي. اظهر تطبيق تحليل النسب نوع التداخل بين السفترياكسون /الباراسيتامول والسفترياكسون/الدايكلوفيناك صوديوم. حيث كان التداخل تأزري عند اعطاء الادوية بنسبة ٠,٥:٠,٥من الجرعة المسكنة الوسطية.

الاستنتاج: اظهرت النتائج تأثيرا مسكنا للسفترياكسون من خلال تقليل فرط التألم الميكانيكي الناتجة من حقن الفورمالديهايد في قدم افراخ الدجاج وكذلك زيادة التأثير المسكن للباراسيتامول والدايكلوفيناك صوديوم من خلال التداخل التآزري.

الكلمات المفتاحية : سفترياكسون، تسكين، ايزوبولوكرافيك، افراخ الدجاج.

INTRODUCTION

Pain is an important symptom of many harmful stimuli, diseases and bacterial infections; the feeling of pain is a normal reaction of the sensory nerve endings against these stimuli .[1]Therefore, the brain or spinal cord provokes different responses that lead to pain behavior when these peripheral nerve endings are damaged.[2] Glutamate is the major excitatory neurotransmitters in the central nervous system and has an important role in many functions, including pain management and central sensitization.[3] There is no enzyme metabolizes the glutamate in the extracellular space but there is high-affinity and high capacity glutamate transporters, these include glutamate transporter1 (GLT1), which is the predominant type that works to remove glutamate from the extracellular space and spinal cord.[4] The GLT-1 is distributed in astrocytes, Schwann cells, and the peripheral nervous system.[4,5] Down-regulation of GLT-1 is prevalent in various models of neuropathic pain.[6,7]

The FDA has recorded, through tests on thousands of drugs, that drugs of the beta-lactam family, including ceftriaxone, selectively activate and increase the expression of this transporter in the brain and spinal cord.[5] Previous studies conducted by researchers confirmed that the intraperitoneal administration of ceftriaxone have a good penetrate to the CNS in different models of animals suffering from amyotrophic lateral sclerosis and stroke.[8] Ceftriaxone relieves mechanical pain in neuropathic rats.[9] Somatic inflammation and visceral pain are not only associated with glutamate, but also with prostaglandins and other released mediators, so the combination therapy with non-steroidal anti-inflammatory drug (NSAIDs) has an effective value in this context.[10-12] In addition, the combination therapy using a group of drugs with different mechanisms of action leads to the use of sub-maximal doses with less harmful effect and greater effectiveness.[13,14]

Paracetamol is a NSAID used in the management of somatic and visceral pain and its action is related to reducing the synthesis of prostaglandins by inhibiting the cyclooxygenase COX-1,2. Recent studies indicate that the analgesic effect of Paracetamol is due to the inhibition of COX-2 enzyme which is the most common mechanism of action.[15,16] Diclofenac sodium is widely used NSAID and can be obtained without a prescription. It is an effective treatment against inflammation.[17]

The aim of the current study was to detect the analgesic effect of ceftriaxone, as well as the types of interactions with other types of analgesics such as paracetamol and diclofenac sodium in a chicks model.

Materials and Methods
Animals

In this study, Ross chicken broilers of both sexes, one day old chicks weights ranged between75 - 194 g and 6 chicks per group were brought and placed in breeding cages with appropriate temperature conditions (32-35°C), ventilation, lighting, bedding, concentrated feed and water. It was bred until experiments were conducted at the age of 7-14 days.

Preparation of Medicines and Method of Injection

Ceftriaxone pure powder (Pioneer Pharmaceutical Company, Sulymania, Iraq), Diclofenac sodium (Unimed Pharmaceutical Company, Jordan) ,Paracetamol (Medicals Pharmaceutical company,London) and the physiological saline , propylene glycol (Sigma, USA) were purchased and prepared and the injection volume for each one was 5 ml/kg body weight administered intramuscularly in the chest muscle of chicks.

1.Pain response to formaldehyde in chicks treated with ceftriaxone

Formaldehyde in a concentration of 0.1% was injected in a volume of 0.05 ml into the planter right foot of the chicks, and its effect was compared with the same injection volume of physiological saline solution. In this experiment, 30 chicks of 7-14 days of age, their weights ranges from 92-194 g, were divided randomly into five groups each with 6 chicks as follows:-

The first group (control): chicks were injected with physiological saline solution into the chest muscle (5ml/kg) 30 minutes before injecting the physiological saline solution (0.05ml) into the planter of the right foot.

The second group: chicks were injected with physiological saline solution into the chest muscle (5 ml/kg) 30 minutes before formaldehyde injection (0, 05 ml) in the planter of the right foot. The third group: Ceftriaxone was injected at a dose of (523 mg/kg) of body weight into the chest muscle 30 minutes before formaldehyde was injected (0, 05 ml) into the planter of the right foot. Fourth group: Ceftriaxone was injected with a dose of (1064 mg/kg) of body weight into the chest muscle 30 minutes before formaldehyde injection (0, 05 ml) in the sole of the right foot. Fifth group: Ceftriaxone was injected at a dose of (1330 mg/kg) of body weight into the chest muscle 30 minutes before formaldehyde injection (0,05 ml) in the planter of the right foot. After 30 minutes of formaldehyde or physiological saline injection, each chick was subjected to the following measurements for 3 minutes : [18]

1-The time (in seconds) it takes for the chick to lift the right foot injected with formaldehyde
2-The number of times the right foot injected with formaldehyde is raised

The thickness of the foot formed as a result of the injection of formaldehyde into the planter of the right foot was measured by an electronic digital caliber before the formaldehyde was injected and an hour after the injection.[19] The percentage of the thickness of the foot was calculated according to the following equation:

Reduction percentage =

$$\frac{\text{the control group - the ceftriaxone treated group}}{\text{Control group}} \times 100$$

2-Determination of the median effective analgesic dose (ED$_{50}$):
A. Determination of the median effective analgesic dose (ED$_{50}$) in ceftriaxone-injected chicks using an electro-stimulator by Dixon method:

The median analgesic dose of ceftriaxone was determined using a locally manufactured electrical stimulator which has the same specifications as the original electrical stimulator , the device's frequency is (50 Hz) and the electrical pulse width is (5 ms), and the pain threshold has been determined at a voltage of (10 volts), noting that the device's voltage is (30 volts) and one of the characteristics of this device is that the stimulating voltage can be measured in millivolts and the device's poles were placed on the skin under the wing of the chicks while wetting the skin area with distilled water before placing the electrodes for the purpose of ensuring the arrival of the electric current and the voltage was gradually increased until we hear the chick screaming as an expression of pain, then we adopt that this voltage is a preliminary reading. In this experiment, (8 chicks) age of (7-8 days) with weights ranging from (75-95 g) were used. They were injected with ceftriaxone intramuscularly at different concentrations (0.45 mg/kg, 0.4 mg/kg, 0.35 mg/kg, 0.3 mg/kg and 0. 25 mg/kg) based on preliminary experiments. The occurrence or non-occurrence of analgesia was monitored using the electrical stimulator after a quarter of an hour from the time of the intramuscular injection. The rate of increase and decrease in the dose of ceftriaxone for the later chicks was a constant of 0. 05 mg/kg, and the median analgesic dose of ceftriaxone was calculated as it was mentioned.

B- Determination of the median analgesic effective dose ED$_{50}$ in paracetamol-injected chicks using an electro-stimulator by Dixon procedure:

The median analgesic dose of paracetamol was determined as mentioned in the above experience. In this experiment, (8 chicks) aged (7-8 days) with weights ranging from (80-105 g) were used. They were injected with paracetamol intramuscularly at different doses (60 mg/kg, 50 mg/kg, 40 mg/kg and 30 mg/kg) based on preliminary experiments. The occurrence or non-occurrence of analgesia was monitored using the electrical stimulator after a quarter of an hour from the time of the intramuscular injection. The rate of increase and decrease in the dose of paracetamol for the subsequent chicks was a 10 mg/kg, and the median analgesic dose of paracetamol was calculated.

C- Determination of the median effective analgesic dose ED$_{50}$ in chickens injected with diclofenac sodium using an electro-stimulator by Dixon method:

In this experiment, (8 chicks) aged (7-8 days) with weights ranging from (70-98 g) were used. They were injected with diclofenac sodium into the muscle at different doses (10,9,8,7,and 6 mg/kg) based on preliminary experiments. The occurrence or non-occurrence of analgesia was monitored using the electrical stimulator after a quarter of an hour from the time of intramuscular injection. The rate of increase and decrease in the dose of diclofenac sodium for the subsequent chicks was a constant of 1 mg/kg, and the median analgesic dose of diclofenac sodium was calculated by Dixon method as follows:

ED$_{50}$ = Xf+ K d
Xf = the last dose used in the experiment
k = tabular value is taken from the Dixon table.[20]
d = last dose used in the experiment

Analgesic interaction between ceftriaxone and paracetamol at ratio (0.5:0.5) of ED$_{50}$:

In this experiment, 6 chicks (7-8 days) with weights ranging from (82-98 g) were used. They were injected with ceftriaxone intramuscularly with different doses, depending on the ED$_{50}$ of ceftriaxone (42.6 mg/kg) in an up-and-down method and after 15 minutes ceftriaxone was injected with paracetamol intramuscularly at different doses, depending on the ED50 of paracetamol (32.5 mg/kg) and also in an up-and-down manner as mentioned. After 15 minutes from the time of injection of paracetamol, the pain threshold was also determined using the electrical stimulator device at a voltage of (10 volts). Gradually until we hear the chick screaming then we adopt that this voltage is a second reading.

Analgesic interaction between ceftriaxone and diclofenac sodium at ratio (0.5:0.5) of ED$_{50}$:

In this experiment, 6 chicks aged (7-8 days) and weights ranging between (72-97 g) were used. They were injected with ceftriaxone intramuscularly with different doses, depending on the ED$_{50}$ of ceftriaxone (42.6 mg/kg) in an up-and-down manner and 15 minutes after the time of ceftriaxone injection They were injected with diclofenac into the muscle with different doses, depending on the ED$_{50}$ of diclofenac sodium (6.26 mg/kg) and also by the up and down method as mentioned. After 15 minutes from the time of injection with diclofenac, the pain threshold was also determined using the electrical stimulator device at a voltage of (10 volts). The voltages are gradually increased until we hear the chick screaming as an expression of pain, then we adopt that this voltage is a second reading.[21,22]

Statistical Analysis

Standard statistical methods were used to determine the mean and standard deviation..The statistical analysis program (SPSS) was used to analyze the data. One way ANOVA test followed by an LSD test were used . P-value ≤0.05 was considered statistically significant .

RESULTS
1-Pain response to formaldehyde in chicks treated with ceftriaxone.

The injection of formaldehyde with a volume of (0.05 ml) and a concentration of 0.1% in the planter of the right foot caused a painful effect through the increase in the time it took for the chick to raise the right leg until it was lowered compared to the control group injected with physiological saline solution, the IM administration of ceftriaxone in doses 532,1064 and 1330 mg/kg led to a clear relief of pain by not raising the right foot in all groups compared to the control group treated with formaldehyde (table 1). Injecting formaldehyde (0,05) into the planter of the right foot led to a significant increase in the thickness of the foot compared to the control group (normal saline) with a percentage 64.6% (table 2). Also, the injection of ceftriaxone at doses 1330 mg / kg of body weight caused significant decrease in the thickness of the foot compared with the control group with percentages (51.4%).

2- Determination of the ED$_{50}$ in chicks injected with ceftriaxone ,paracetamol and diclofenac sodium using an electro stimulator by Dixon method:

Our results recorded the dose that produce 50% of analgesic effect in chicks for ceftriaxone ,paracetamol and diclofenac sodium were 42.6,32.6 and 6.26 mg/kg IM respectively by using Dixon procedure table (3).

By applying the isobolographic analysis, the value of the median analgesic dose for each drug was calculated separately .Then the value of the ED$_{50}$ for drug combination (ceftriaxone/paracetamol and ceftriaxone/diclofenac sodium) , which show the synergistic effect in the chicks were recorded (Figure 1,2). In addition, the value of Y was calculated from the equation where it was found that the value of Y is less than 1 (0.322 and 0.317) table (4,5).

DISCUSSION

Peripheral inflammation induced by formalin causes glutamate release from primary sensory afferents and excitatory interneurons in the dorsal horn of the spinal cord, producing inflammatory hyperalgesia via the activation of peripheral and spinal glutamate receptors. Ceftriaxone can selectively upregulate the expression of glutamate transporter GLT-1 and reduce intrasynaptic glutamate.[23] In addition, Evidence suggests that the β-lactam antibiotic, ceftriaxone, is a potent stimulator of GLT-1 expression10 and that it can attenuate neuropathic and visceral pain.[24]

Formalin test is considered as an activating model for inflammatory pain, and that pain is one of the most important components of inflammation. The transmission of nerve impulses to the spinal cord via the peripheral nerves via the peripheral and cranial nerves to the cranial ganglia.[25]

Our current results showed that ceftriaxone exerts its analgesic effect in chicks by reducing the mechanical hyperalgesia resulting from formaldehyde injection in the planter of the right foot. This effect was in agreement with the findings of Stepanovic´-Petrovic et al., in their studies on rodents who found that ceftriaxone exerts antihyperalgesia/antinociception in both somatic and visceral inflammatory pain in both mice and rats.[22] This may be attributed to the ceftriaxone that potentiates the activation of the glutamate transporter GLT-1, and this may be the reason why ceftriaxone works as analgesic.[5,9]

The calculation of the median analgesic dose of the drug is an important indicator of the action of this drug in laboratory animals used in experiments in addition to determining the doses used in other experiments. Our results revealed that the analgesic effect of the ceftriaxone alone or when mixed with paracetamol once and diclofenac sodium again (by electrical stimulation test) and their effect was verified together by applying the isopolographic analysis at the ratio of 0.5:0.5 as well as finding the value of the Y which was less than 1 (0.322 and 0.317) and the relationship was synergistic between them. The results of the current study are consistent with Stepanovic´-Petrovic et al .,conducted on mice and rats.[22] The analgesic effect of paracetamol is attributed to its inhibitory effect on the peripheral and central COX-2 [24], as well as its role in the activation of the serotonin and opiate pathway.[25,26]

Previous studies confirmed that the opioid-related effect of diclofenac sodium could be responsible for the analgesia induced by diclofenac, knowing that birds have opiate receptors that can be compared with opiate receptors in humans.[27] Among the mechanisms that contribute to the analgesic behavior of diclofenac sodium are the blockage of the voltage-dependent sodium channels in addition to the pharmacological effect of non-steroidal anti-inflammatory drugs that inhibit COX1, 2 enzymes, which stimulate the conversion of arachidonic acid to prostaglandins, which in turn cause pain, heat and inflammation.[28-30]

The drug interactions between ceftriaxone and the analgesics examined in this work can be used clinically as drug combinations, because they show a prolongation of the drug's action and a decrease in the dose used, in addition to enhancing the effect of the drug's action.

In conclusion, our results indicate that ceftriaxone has an analgesic effect on formalin-induced pain in chicks. Ceftriaxone has a current interaction when mixed with analgesics such as paracetamol and diclofenac sodium, thus providing a useful approach in the clinical treatment of inflammatory pain.

Table (1): Measurement of the analgesic effect of ceftriaxone against the pain caused by injecting formaldehyde into the right foot.

Groups	Time taken to raise the right foot (in seconds)	The number of times to raise the right foot	The difference in the thickness of the foot	% anti-inflammatory potency
Normal saline +formaldehyde	0.72±0.48	6.83 ± 1.01	0.72± 0.48	
Ceftriaxone523mg/kg + formaldehyde(0.05ml)	0.66±0.12	*0.00 ± 0.00	0.66±0.12	8.3
Ceftriaxone1064mg/kg + formaldehyde(0.05ml)	0.59±0.12	*0.00 ± 0.00	0.59±0.12	18
Ceftriaxone1330mg/kg + formaldehyde(0.05ml)	*0.35±0.09	*0.00 ± 0.00	*0.35±0.09	51.4

The values expressed as means ± SE
*The value differs significantly from the control group at a significant level (p=0.02).

Table (2): The percentage of increase in the thickness of the right foot in chicks treated with formaldehyde (0.05ml)

Groups	The difference in the thickness of the foot	The percentage of increase in the thickness of the right foot%
Normal saline + Normal saline	0.17 ±0.06	
Normal saline + Formaldehyde (0.05ml)	0.72 ± 0.48*	64.6

The values expressed as means ± SE
* The value differs significantly from the control group at a significant level (p=0.01).

Table (3): Determination of median effective doses of ceftriaxone, paracetamol and diclofenac sodium by Dixon procedure

Variable	Ceftriaxone	Paracetamol	Diclofenac sodium
Analgesic ED$_{50}$	42.6 mg/kg	32.6 mg/kg	6.26 mg/kg
Dosage range	100-40=60	60-30=30	10-6=4
First dose	100	60	10
Last dose	50	40	7
Number of chicks used	8(xxxxoxox)	7(xxxoxox)	8(xxxxoxox)
Up and down in doses	10mg/kg	10mg/kg	1mg/kg

 x - Analgesia; o - no analgesia. The ED50 were determined by the up and down method.

Table (4): Parameters of Isobolographic Analysis for Ceftriaxone–Analgesics Combinations in chicks

Variable	Ceftriaxone	paracetamol		ceftriaxone	diclofenac sodium
ED$_{50}$	6.77	5.34		6.77	0.99
Rang of doses used	21.3-5.4=15.9	16.3-4.3=12		21.3-5.4=15.9	3.15-0.78=2.37
Initial dose	21.3	16.3		21.3	3.15
Last dose	10.7	8.3		10.7	1.57
Increase and decrease in dose	5.3	4		5.3	0.79
Number of chicks used	7(xxxoxox)	7(xxxoxox)		7(xxxoxox)	7(xxxoxox)

x - analgesia; o - no analgesia. The ED50 were determined by the up and down method.

Table (5): Analgesic interaction between ceftriaxone-paracetamol and ceftriaxone- diclofenac sodium at ratio (0.5:0.5) of ED$_{50:}$

Drug /drug combination	Y value	Percentage of decrease in ED$_{50}$
Ceftriaxone + paracetamol	0.322	84%
Ceftriaxone + diclofenac sodium	0.317	84%
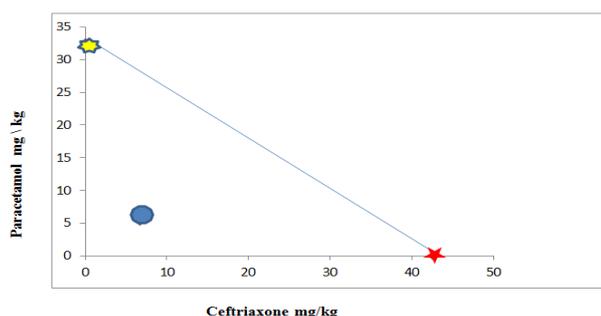
Figure(1) : Isobolographic analysis of interaction between ceftriaxone and paracetamol at ratio 0.5:0.5 in chicks .

🔵 0.5:0.5 of ED_{50} combinations of ceftriaxone and paracetamol. The diagonal line between both drugs ED_{50}s is synergistic.

⭐ ED_{50} of ceftriaxone 42.6 mg/kg IM

✳️ ED_{50} of paracetamol 32.6 mg/kg IM

$$Y\ value = \frac{ED_{50}\ ceftriaxone + ED_{50}\ paracetamol}{ED_{50}\ ceftriaxone} +$$

$$\frac{ED_{50}\ ceftriaxone + ED_{50}\ paracetamol}{ED_{50}\ paracetamol}$$
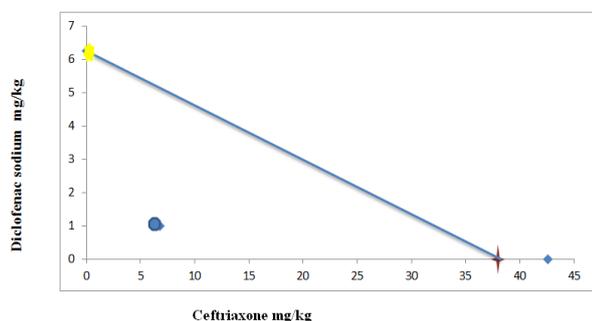

Figure (2): Isobolographic analysis of interaction between ceftriaxone and diclofenac sodium at ratio 0.5:0.5 in chicks .

🔵 0.5:0.5 of ED_{50} combinations of ceftriaxone and Diclofenac sodium. The diagonal line between both drugs ED50s is synergistic

✴️ ED_{50} of ceftriaxone 42.6 mg/kg IM

✴️ ED_{50} of diclofenac sodium mg/kg IM

$$Y\ value = \frac{ED_{50}\ ceftriaxone + ED_{50}\ diclofenac}{ED_{50}\ ceftriaxone} +$$

$$\frac{ED_{50}\ ceftriaxone + ED_{50}\ diclofenac}{ED_{50}\ diclofenac}$$

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Conflict of Interest

The authors decline no conflict of interest

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