

## FIELD EVALUATION OF DIFLOXACIN IN TREATMENT OF COLIBACILLOSIS IN WHITE NEW ZEALAND RABBITS

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### ABSTRACT

*The present study was delineated to evaluate the effect of colibacillosis on hematological parameters, some biochemical constituents of blood and evaluation the effect of difloxacin in treatment of colibacillosis in rabbits. A total of 50 White New Zealand rabbits 4 week old (10 apparently healthy and 40 suffering from diarrhea) from a private rabbits farm in Sharkia Province were used in this investigation. Rectal swabs were collected from all rabbits for identification of rabbits suffering from diarrhea due to E.coli infection. Twenty rabbits (10 apparently healthy and 10 suffering from diarrhea due to E.coli infection) out 50 rabbits were divided into four equal groups (5 in each), 1<sup>st</sup> group healthy rabbits and kept as control group, 2<sup>nd</sup> group healthy rabbits and treated with therapeutic dose of difloxacin, 3<sup>rd</sup> group diarrhoeic rabbits non treated, 4<sup>th</sup> group diarrhoeic rabbits treated with therapeutic dose of difloxacin. At 7<sup>th</sup> and 14<sup>th</sup> days post treatment two blood samples were collected for blood picture and biochemical analysis.*

*Examined rectal swabs collected from rabbits revealed 21 out 50 samples showed positive isolate E. coli in percentage of (42 %).*

*Our study revealed that healthy treated with therapeutic dose of difloxacin and diseased rabbits show significant decrease in total erythrocytic count, haemoglobin content, packed cell volume %, total proteins, albumin, globulin and significant increase in serum liver enzymes (AST, ALT, GGT and alkaline phosphatase) urea and creatinine Diseased rabbits treated with therapeutic dose of difloxacin show improvement in hematological parameters and biochemical constituents of blood at 14<sup>th</sup> days post treatment.*

*In conclusion, Colibacillosis in rabbits resulted in adverse effect in hematological and biochemical parameters. Difloxacin treatment diseased rabbits improved these parameters 14<sup>th</sup> day post treatment.*

### INTRODUCTION

In recent years, the domestic rabbits have

been recommended as a good alternative source of dietary protein for increasing human population in developing countries

(Lukefahr and Cheeke, 1991). Rabbits meet contain a high percentage of protein which increase the value of rabbits. Rabbits have a better productivity of meat and fur (Tylor, 1980). Viral, bacterial and/or parasitic agents were recorded as probable causes of death in rabbits (Campagnolo, et al. 2003). Bacterial infection in rabbits may be accompanied by diarrhea sudden and sudden death Garcia, et al. (2002). Newton, et al. (2004) stated that *E. coli* was the predominant microorganism isolated from diarrheic rabbits. Colibacillosis in rabbits was the most serious problem among the enteric disease Okerman (1994). *E. coli* infection has a great economic influence on rabbit production (Owoad et al. 2004).

Difloxacin is a recently developed fluoroquinolone antimicrobial broad-spectrum antibacterial activity widely used to treatment infections in animals and poultry Gerardo, et al. (2006). The primary target of all fluoroquinolones is DNA-gyrase, (Einstein, et al. 1994). Fluoroquinolones have been seen to interact with bacterial adherence and colonization of epithelial surface, alter the release of proinflammatory bacteria products and modulate phagocytic capacity (Nau and Eiffert, 2002).

The objective of the current study was to elucidate the effects of *E. coli* infection on hematological and some biochemical parameters as well as effect of difloxacin in treatment and control of colibacellosis in rabbit.

## **MATERIALS AND METHODS**

### **Drugs :**

Difloxacin 10% (Dicural)<sup>R</sup> it is clear solu-

tion prepared for use in a dose of 10 mg/kg body weight and is produced by Forte Dodge Veterinaria S.A. Girona-Spain.

### **Animals:**

This study was carried out in a private rabbit farm at Abo-Hamad city (EI-Sharkia Province). A total number of 50 White New Zealand rabbits aged from 3-4 week old and weighting about 650-750gm. (10 healthy and 40 suffering from diarrhea). Rabbits were housed in wire cages under complete hygienic condition. Rabbits were fed on a balanced ration free from any medications and given water ad-libitum.

### **Bacterial examination :**

A total of 50 rectal swab samples from healthy and diarrheic rabbits. Swabs were taken for bacteriological examination for identification of rabbits suffering from diarrhea due to *E. coli* infection. Collected samples were incubated on nutrient broth at 37°C for 24h., then subcultured into nutrient agar according to Woldehiwet, et al. (1990). Isolated bacteria were identified after Holt, et al. (1994).

### **Experimental animals :**

Twenty rabbits (10 healthy and 10 suffering from diarrhea due to *E. coli* infection) out 50 rabbits were divided into four equal groups (5 in each), 1<sup>st</sup> group healthy rabbits and kept as control group, 2<sup>nd</sup> group healthy rabbits treated with difloxacin a dose of 10 mg / kgm b.wt in drinking water for 5 successive days, 3<sup>rd</sup> group diarrhoeic rabbits non treated and 4<sup>th</sup> group diarrhoeic rabbits treated with difloxacin (10 mg/kgm b.wt in drinking water for 5 successive days. At 7<sup>th</sup> & 14<sup>th</sup> days post

treatment two blood sample were collected, 1<sup>st</sup> sample was collected in tube contain EDTA for hematological studies according to **Jain (1993)**. 2<sup>nd</sup> sample was collected in centrifuge tubes for obtain clear serum for determination of total proteins (**Doumas, et al. 1981**), albumin (**Young, 1975**), globulin was calculated as difference between total protein and albumin, Activity of transaminases (AST-ALT) according to (**Reitman and Frankel, 1957**) alkaline phosphatase (**John, 1982**), gamma glutamyl transferase (GGT) **Szaz (1969)**, urea (**Tobacco, 1979**) creatinine (**Henry, 1974**).

#### **Statistical analysis:-**

Our data were tabulated and statistically analyzed according to **Spsswin (1995)**.

### **RESULTS AND DISCUSSIONS**

Chemotherapy is one of the most rapidly advancing branches of applied pharmacology. New drugs are continually being introduced with the aim of curing infections with the least possible side effects to host. Therefore, the search for safe antimicrobial agents is a common target (**Rosdy, 2007**).

Clinical signs of diarrhea in rabbits due to E.coli infection were depression, weakness, depraved appetite, body temperature elevated followed by yellowish watery diarrhoea. These observations were coincided with those previously obtained by **Hassan, et al. (2008)** and **Rodriguez, et al. (2008)** in newly born rabbits.

The obtained results in our study revealed that the E. coli was isolated from 21 out 50

samples showed positive isolate E. coli in percentage of (42%). Similar findings were recorded by **Fetah (1985)** who mentioned that E.coli bacteria were the main isolated from diarrhoeic rabbits. Another authors **Eid and Ibraheem (2006)** isolated E.coli in percentage of 59% in diarrhoeic rabbits. Moreover **Hatab and Moustafa (2007)** stated that the main isolated bacteria were E.coli from diarrheic rabbits.

The present work revealed that, both healthy rabbits treated with difloxacin and diarrhoeic rabbits showed significant decrease in total erythrocytic count, hemoglobin content and packed cell volume associated with leukocytosis. Similar finding were reported by **Magdy and Ahmed (1998)** stated another fluoroquinolone enrofloxacin induces significant decrease in erythrocytic count, hemoglobin content and packed cell volume in rabbits. Furthermore, our data clearly reinforced by those obtained previously by **Magdy and Gehad (1997)** stated that danofloxacin induced significant decrease in haemoglobin contents in rats. This increase in total leukocytic count in rabbits treated with difloxacin may be due to peripheral esinophilia with fluoroquinolone treatment suggesting acute hypersensitivity reaction to the drug (**Hootkins, et al. 1989**). The previous results was supported by the finding reported by **Eisa (1998)** who found significant reduction in erythrocytic count, hemoglobin content, packed cell volume and significant increase in total leucocytic count in rabbits infected with E.coli. Change in hematological parameters in infected rabbits with E.coli was attributed to the bacterial endotoxins which cause intravascular destruction of erythrocytic cells and conse-

quently lead to haemolysis and breakdown of hemoglobin (**Karatvanov, 1984**). Another explanation for reduction in erythrogram of rabbit infected with E.coli come from Tserenpungtag, **et al. (2005)** who stated that E.coli lipopolysaccharide has direct effect as it inhibit bone marrow cells and its nephrotoxicity decrease erythropoietin blood level. Also, **Dagmar, et.al. (2002)** stated E.coli infection, produce cell damage toxin (enterohemolysin) that causes changes in cell membrane permeability and formation of surface lesions causes RBC destruction. Leukocytosis in diarrheic rabbits may due to inflammatory response in the gastrointestinal tract due to bacterial infection **Doxey (1983)**.

Analysis of blood serum constituents of rabbits show significant decrease in serum total proteins, albumin and globulin in healthy rabbits treated with difloxacin and diarrheic one. These results are reinforced those obtained by **Mohamed (2004)** who found another fluoro-quinolone danofloxacin produced a significant decrease in serum total proteins level in healthy hens. These results were supported by previous studies **Eisa (1998)** who recorded that infection of rabbits with E. coli resulted in a significant decrease in total protein, albumin and globulin. Similar results were recorded by **Hitaler and Blum (2002)** mentioned that E. coli induce significant decrease in total protein and albumin. Decreased albumine in diarrheic rabbits might be attributed to its small size and osmotic sensitivity to fluid movement. Further explanation of hypoalbuminemia may be referred to the fact that the liver in the sole of albumin synthesis and hypoalbuminemia is important feature of liver disease (**Kaneko,**

**1980**). Moreover could be attributed to the destructive effect on the intestinal villi which lead to malabsorption. Hypoproteinaemia induced by sepsis was reported by **Vlahos, et al. (2005)** and **Schuerholz, et al. (2005)**. **Radostitis, et al. (2002)** mentioned that decrease protein level in diarrheic animal may be due to a state of anorexia and inability of the synthesis proteins.

In the current work obtained data indicated significant increase in the liver enzymes (AST, ALT, GGT and alkaline phosphatase) urea and creatinine in healthy rabbits treated with difloxacin and diarrheic rabbits. These results may be due to alteration of membrane permeability or damage of the hepatic cells by direct effect of the drug resulting in escape of these enzymes to the plasma (**Hanafy 1993**). Similar results were reported in diarrheic rabbits by (**Eisa 1998**). On the same ground **Joan and Pannel (1981)** recorded that bacterial infection produced alteration in cellular permeability due to changes in cell membrane which allows the escape of these enzymes (AST, ALT, GGT and ALP) into serum in abnormal high level. The investigated enzymes are mostly of hepatic origin and so their increase levels in the serum was indicative to hepatocellular damage (**Campbell and Coles, 1986**). Our results agree with those reported by **El-Boushy, et al. (2005)** who recorded that E.coli infected rabbits evoked a significant increase in AST, ALT, GGT and ALP. These recorded results were supported by (**Kaneko 1980**) who mentioned that the increase uric acid, creatinine in the infected rabbits could be attributed to the degenerative changes in the kidney tubules preventing excretion of uric acid and creatinine increas-

ing their levels in serum. Elevation of serum creatinine due to endotoxins of *E.coli* in rat was reported by **Collin, et al. (2005)**.

For trials of treatment, our study revealed that, the use therapeutic dose of difloxacin in treatment colibacillosis among rabbits orally resulted in a good rapid recovery of diarrhetic rabbits, reduced the clinical symptoms, improved the health status and improved hematological and biochemical parameters towards the normal level at 14<sup>th</sup> days post treatment. These results agreed with that of **Mohamed (2004)** who found that the treatment chick infected with *E.coli* by another fluoroquinolone (danofloxacin) induce improved the health status and hematological and biochemical parameters. In keeping with this line, **Bryan, et al (1998)** stated that danofloxacin improved performance of infected chick

with *E.coli*. Our results were supported by **Rosdy (2007)** who found that pefloxacin appeared to be an effective treatment for controlling colibacillosis diarrhoea. Improved haematological and biochemical parameters post treatment may be due to antibacterial effect of the drug Abd **El-Aziz (2002)**. Same was recorded by **Alexandra, et al. (2010)** who mentioned that use difloxacin in treatment *E.coli* infection in chickens induce reduced the clinical symptoms.

Summing up our observations, It could be concluded from the present study that colibacillosis in rabbits induce many adverse effect on both haematological and biochemical parameters but administration of difloxacin in therapeutic dose considered value in medication of *E.coli* infection in rabbits.

Table 1: Isolation of E.coli from rectal swab samples collected from diarrhoeic rabbits. (n=50).

Total	No. of samples(n)		Cultural examination		Biochemical examination		Total +ve E.coli	
	healthy	diseased	+ve	-ve	+ve	-ve	No.	%
50	10	40	21	29	21	29	21	42

Table 2: Effect E.coli and difloxacin on erythrogram and total leukocytic count in rabbits (n=5).

Group		Parameters	erythrogram			TLC 103/cmm
			RBCs(10 <sup>6</sup> /c.mm)	Hb (Gm%)	PCV (%)	
Healthy rabbits			6.36 ±0.32	11.09±0.75	32.48±0.90	12.41±0.26
Healthy rabbits difloxacin treated	7 <sup>th</sup> day		5.32±0.15*	8.19±0.61*	29.16±0.76*	14.02±0.32**
	14 <sup>th</sup> day		6.16±0.21	9.97±0.82	31.79±0.88	12.87±0.22
Diseased rabbits non treated	7 <sup>th</sup> day		5.07±0.34**	8.12±0.70*	28.34±0.92	13.89±0.24**
	14 <sup>th</sup> day		5.15±0.25**	8.40±0.52*	28.17±0.90*	14.06±0.37**
Diseased rabbits difloxacin treated	7 <sup>th</sup> day		5.50±0.20*	8.90±0.32*	30.05±0.49*	13.15±0.19*
	14 <sup>th</sup> day		6.08±0.39	10.04±0.39	31.39±0.89	12.65±0.18

\* P &lt; 0.05

\*\* P &lt; 0.01

Table 3 : Effect E.coli and difloxacin on protein profile in rabbits (n=5).

Group		Parameters	T.protein (gm/dl)	Albumin (gm/d)	Globulin (gm/dl)	A/G Ratio
Healthy rabbits			5.96±0.62	3.10±0.44	2.86 ±0.29	1.08 ±0.19
Healthy rabbits difloxacin treated	7 <sup>th</sup> day		3.78±0.54*	1.98±0.22*	1.80 ± 0.23*	1.10±0.17
	14 <sup>th</sup> day		5.48±0.76	2.90±0.20	2.58±0.19	1.12±0.23
Diseased rabbits non treated	7 <sup>th</sup> day		3.50±0.66*	1.87±0.10*	1.63 ±0.31*	1.15±0.16
	14 <sup>th</sup> day		3.64±0.59*	1.90±0.15*	1.74 ±0.23*	1.09±0.13
Diseased rabbits difloxacin treated	7 <sup>th</sup> day		3.80±0.40*	2.05±0.12*	1.75±0.25*	1.17±0.19
	14 <sup>th</sup> day		5.72±0.54	3.13±0.15	2.59±0.24	1.21±0.19

\* P &lt; 0.05

\*\* P &lt; 0.01

**Table 4:** Effect E.coli and difloxacin on liver enzymes enzymes in rabbits (n=5).

Group	Parameters	AST (U/L)	ALT (U/L)	ALph (U/L)	GGT (U/L)
Healthy rabbits		39.65±1.78	25.05±1.13	30.16±1.50	17.12±0.30
Healthy rabbits difloxacin treated	7 <sup>th</sup> day	46.97±1.93*	30.23±1.79*	36.30±1.72*	18.24±0.28*
	14 <sup>th</sup> day	43.95±1.76	27.39±1.65	32.12±1.85	17.53±0.22
Diseased rabbits non treated	7 <sup>th</sup> day	47.62±1.98*	30.64±1.90*	37.17±1.88*	18.46±0.39*
	14 <sup>th</sup> day	47.89±1.89*	31.25±1.77*	36.89±1.76*	18.51±0.37*
Diseased rabbits difloxacin treated	7 <sup>th</sup> day	45.78±1.99*	29.97±1.65*	34.97±1.42*	18.30±0.29*
	14 <sup>th</sup> day	41.85±1.78	26.20±1.70	33.29±1.67	17.39±0.35

\* P &lt; 0.05

**Table 5:** Effect E.coli and difloxacin on kidney function in rabbits (n=5).

Group	Parameters	Urea (mg/d)	Creatinine (mg/dl)
Healthy rabbits		22.28±0.19	1.21±0.22
Healthy rabbits difloxacin treated	7 <sup>th</sup> day	24.30±0.78*	2.32±0.34*
	14 <sup>th</sup> day	23.71±0.56*	1.92±0.20*
Diseased rabbits non treated	7 <sup>th</sup> day	24.01±0.61*	1.26±0.29
	14 <sup>th</sup> day	23.30±0.19*	1.30±0.17
Diseased rabbits difloxacin treated	7 <sup>th</sup> day	24.12±0.48*	1.91±0.18*
	14 <sup>th</sup> day	22.60±0.28	1.32±0.15

\* P &lt; 0.05

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## الملخص العربي

### التقييم الحقلّي للدائفلوكساسين في علاج الاصابه بالميكروب القولوني العصوي في الارانب النيوزلاندي الابيض

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معهد بحوث صحة الحيوان- فرع الزقازيق

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الإصابة بمرض القولون العصوي تمثل خطرا علي صناعة الارانب وهذا يرجع إلي أضرارها المتعددة منها الاسهالات نقص في معدل التحويل الغذائي وزيادة في نسبة النفق لذلك استهدفت هذه الدراسة استبيان تائر الاصابه بالميكروب القولوني العصوي على صورة الدم وبعض الوظائف البيوكيميائية ومعرفة المزيد من المعلومات عن كفاءة عقار الدائفلوكساسين لعلاج الارانب المصابة بالميكروب العصوي . تم إجراء هذا البحث في احدى مزارع الارانب النيوزلاندي الابيض بمحافظة الشرقية يتم فحص الولادات في عمر ٤ اسبوع والتي تعاني من وجود اسهال .و يتم أخذ مسحات شرجيه من فتحة المجمع لعدد ٥٠ ارناب (١٠ سليمة ظاهريا واكلينيكية و٤٠ تعاني من وجود اسهال) للفحص البكتريولوجي وذلك لتحديد الارانب التي تعاني من وجود اسهال نتيجة للاصابه بالميكروب القولوني العصوي.تم تقسيم (١٠ ارناب سليمة ظاهريا واكلينيكية و١٠ ارناب تعاني من وجود اسهال نتيجة للاصابه بالميكروب القولوني العصوي ) الى اربع مجموعات كلا منها يحتوى على ٥ ارناب .المجموعة الأولى أرناب سليمة ظاهريا واكلينيكية ولم تعالج باى أدوية (مجموعة ضابطة).المجموعة الثانية أرناب سليمة ظاهريا واكلينيكية وتعالج باستخدام الدائفلوكساسين بجرعة ١٠ مجم/كجم من وزن الجسم عن طريق مياه الشرب لمدة خمس أيام متتالية. المجموعة الثالثة أرناب مصابة إصابة طبيعيه بالميكروب القولوني العصوي ولم يتم علاجها بينما المجموعة الرابعة ارناب مصابة إصابة طبيعيه بالميكروب القولوني العصوي ويتم علاجها باستخدام الدا يفلوكساسين بجرعة ١٠ مجم/كجم من وزن الجسم عن طريق مياه الشرب لمدة خمس أيام متتالية. عند اليوم السابع والرابع عشر من نهاية العلاج يتم جمع عينتين دم من كل ارناب.العينه الاولى تم تجميعها على EDTA وذلك لدراسة تأثير الاصابه بالميكروب العصوي والدائفلوكساسين على صورة الدم والأخرى لفصل المصل وذلك لقياس بعض المؤشرات البيوكيميائية (صورة البروتين وظائف الكبد والكلى).

وبالفحص البكتريولوجي للمسحات الشرجيه الماخوذه من الارانب التي تعاني من وجود اسهال اسفر عن وجود الميكروب القولوني العصوي في ٢١ عينه من ٥٠ عينه بنسبه ٤٢٪ .

تشيرالنتائج أن الارانب المصابه بالميكروب العصوي والسليمه المعالجه بالجرعه العلاجه من الدائفلوكساسين احدثت نقص معنوي في العدد

الكلى لكرات الدم الحمراء والبيضاء تركيز الهيموجلوبين، حجم خلايا الدم المرصوة، البروتين الكلى، الزلال والجلوبولين وزيادة معنوية فى الترانس أمينيزيس GGT (AST ALT) الفوسفاتيز القاعدى اليوريا والكرياتينين. ولكن علاج الارانب المصابه باستخدام الداى فلوكساسين ادى الى اختفاء الاعراض الظاهره وتحسن فى صورة الدم والوظائف البيوكيميائه حيث عادت لوضعها الطبيعى تقريبا بعد ١٤ يوم من نهايه العلاج.

نستخلص من هذه الدراسه ان الاصابه بالميكروب القولونى العصى احدثت تاثيرات عكسه على صورة الدم وبعض القياسات البيوكيميائيه فى الارانب ولكن استخدام الدايفلوكساسين ادى الى تلاقى تلك التاثيرات. لذلك ينصح باستخدام الدايفلوكساسين لعلاج الالتهابات المعويه الناتجه عن الاصابه بالميكروب القولونى العصى فى الارانب.