COMPARATIVE STUDIES ON MARBOFLOXACIN LEVELS IN PLASMA OF CALVES AFTER ITS INJECTION WITH DIFFERENT ROUTES

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ABSTRACT

Thirty-five friesian calves in one of Gharbia governorate farms, were divided into 3 groups: first group 15 healthy pre-ruminant, second group 15 healthy ruminant and third group 5 diseased pre-ruminant calves . First and second groups were injected with once of marbofloxacin at a dose of 2 mg/kg b.w. either by i.m., s.c. or i.v. route (5 calves for each route of injection), while 3rd group was injected with once by i.m. route at the same dose. Blood samples were collected from each calf just prior to the drug injection, 1/2, 4, 8, 12, 16, 20, and 24h post injection for determination of marbofloxacin concentrations by HPLC with UV detector .

Marbofloxacin concentrations in the plasma of healthy pre-ruminant calves at 4h after i.m., s.c. and i.v. injection were 0.98, 0.85 and 0.95mg/ml respectively, whereas, in healthy ruminant calves it were 0.82, 0.80 and 0.66mg/ml respectively at the same time. After 12h it were 0.55, 0.48 and 0.51mg/ml in healthy pre-ruminant, and 0.18, 0.27 and 0.083mg/ml in healthy ruminant, while after 20h it were 0.34, 0.24 and 0.37mg/ml in healthy pre-ruminant, and 0.060, 0.063 and 0.044mg/ml in healthy ruminant ones after its i.m., s.c. and i.v. injection respectively. The plasma levels of marbofloxacin after its i.m. injection in pre-ruminant calves were 0,55 and 0.66mg/ml after 12h, 0.34 and 0.45mg/ml after 20h, and 0.25 and 0.40mg/ml after 24h in healthy and diseased calves respectively.

INTRODUCTION

Fluoroquinolones are one of the most useful classes of antimicrobial agents used in human and animal medicine today, both because of their spectrum and their physico-chemical properties. As such, their popularity in clinical situations is increasing **(Wolfson and Hooper, 1989, and Sarkozy, 2001)**. These fluoroquinolones were devoloped with addition of a fluorine atom to

the basic quinolone structure, which extend its spectrum of bactericidal activity to include Gram-positive bacteria beside bactericidal activity which directed essentially against Gram-negative bacteria due to presence of quinolone nucleus. Furthermore, presence of the piprazine cycle extends the spectrum further to include pseudomonas and mycoplasmas **(Hooper and Wolfson, 1993, and Appelbaum and Hunter, 2000)**. Marbofloxacin one of a recent member of third generation of this fluoroquinolones has a flat structure that allows its insertion between the chains of the DNA molecule and acts as cocentration-dependent antibiotics for Gram-negative bacteria, whereas their action against certain Gram-positive bacteria is generally considered to be time-dependent **(Bryan and Bedard, 1991; Petracca, 1993 and Bousquet-Melou et al, 2002)**.

Marbofloxacin acts directly on the bacterial DNA by penetrating the bacterium by simple diffusion and the target is a bacterial enzyme DNA gyrase which responsible for the super coiling of bacterial DNA, thus allowing the compression of 1300 mm of DNA in one cell, the size of which is approximately 2m. So through this irreversible and specific inhibition of super coiling the marbofloxacin causes death of the bacteria by two different mechanisms:-

a- Which is common to all quinolones: affects bacteria during the multiplication phase, inhibits cellular replication and blocks respiration, leading to bacterial death.

b- reported for fluoroquinolones: requires neither cellular division nor protein synthesis thus responsible for faster bactericidial action and is not dose-related.

These mechanisms enable marbofloxacin to inhibit both multiplying and inactivte bacteria **(Bryskier, 1993, and Hooper and Wolfson 1993)**.

On the other hand, marbofloxacin unlike many bactericidal antibiotics attacks the internal elements of the bacteria; particularly the genetic material, that causes destruction of each bacterium by filamentation leaving the bacterial wall intact. This process therefore limits the dispersion of any endotoxins in the body and the dramatic deterioration in the animal's general condition **(Hooper and Wolfson, 1993)**. Another criteria of marbofloxacin its difference in particular from other fluoroquinolones on account of its oxadiazine ring which gives the molecule complete bioavailability, excellent tissular diffusion, lower minimum inhibitory concentrations for major pathogens and long elimination half-life **(Bergogne-Berezin, 1997)**. As concerns in cattle, this elimination varies according to the maturity of the animals; (i-e) immature elimination organs lead to prolonged persistence of the molecule in the body **(Petracca, 1993 and Thomas et al, 1994)**.

The aim of this work is concerned to study the marbofloxacin levels in calves (pre-ruminant; healty and diseased, and ruminant) after its injection with different routes (i.m., s.c. or i.v.).

MATERIALS AND METHODS

A-Drug:-

Marbofloxacin :- (Marbocyl ^{R} 10% injectable solution, Vetoquinol). It is a new 3rd generation fluoroquinolone generally have a good therapeutic antibacterial effect for cattle, pigs, dogs, cats and other species but it is developed by Vetoquinol exclusivley for Veterinary use for oral and parenteral administrations to cattle; including lactating dairy cattle, and pigs for treatment of respiratory diseases **(Hooper and Wolfson, 1993 and Thoulon et al, 1999)**. It possesses unique pharmacokinetic properties which distinguish it from other third generation quinolones **(Hooper and Wolfson, 1993)** .

B- Animals:-

Thirty-five Friesian calves in one of Gharbia governorate farms, were divided to 3 groups: first group 15 healthy pre-ruminant, second group of 15 healthy ruminant and third group 5 sick pre-ruminant calves. First and second groups were injected with one dose of marbofloxacin at a dose of 2mg/kg b.w. either by i.m., s.c. or i.v. route (5 calves for each route of injection), while 3rd group was injected with one dose by i.m. route at the same dose rate (as recommended by manufacturer).

C- Sampling:-

Blood samples were collected from each calf just prior to the drug injection, 1/2, 4, 8, 12, 16, 20, and 24h post injection. Blood plasma were stored at -20^{o}C until estimation of drug concentrations. Marbofloxacin molecule was measured by HPLC with UV detector with quantitation limits of 0.01mg/ml according to methods described by **McKellar et al (1999)**.

D- Statistical analysis :-

Were carried out according to **Snedecor and Cochran (1967)**.

RESULTES

In healthy pre-ruminant calves, the plasma concentrations of marbofloxacin at 4h after i.m., s.c. and i.v. injection were 0.98, 0.85 and 0.95mg/ml, while in healthy ruminant ones it were 0.82, 0.80 and 0.66mg/ml respectively. After 12h it were 0.55, 0.48 and 0.51mg/ml in healthy pre-ruminant, and 0.18, 0.27 and 0.083mg/ml in healthy ruminant, while after 20h it were 0.34, 0.24 and 0.37mg/ml in healthy pre-ruminant, and 0.060, 0.063 and 0.044mg/ml in healthy ruminant ones after its i.m., s.c. and i.v. injection respectively as shown in Table (1 and 2) and Fig (1,2 and 3).

After i.m. injection of marbofloxacin in pre-ruminant calves the plasma levels after 8h were 0.72 and 0.78mg/ml. and after 16h were 0.44 and 0.55mg/ml in healthy and diseased calves respectively as shown in table (3) and fig (4).

DISCUSSION

The present study showed that, concentrations of marbofloxacin in the plasma of healthy pre-ruminant calves at 4h after i.m., s.c. and i.v. injection were 0.98, 0.85 and 0.95mg/ml, while in healthy ruminant calves it were 0.82, 0.80 and 0.66mg/ml respectively at the same time. After 12h it were 0.55, 0.48 and 0.51mg/ml in healthy pre-ruminant, and 0.18, 0.27 and 0.083mg/ml in healthy ruminant, while after 20h it were 0.34, 0.24 and 0.37mg/ml in healthy pre-ruminant, and 0.060, 0.063 and 0.044mg/ml in healthy ruminant ones after its i.m., s.c. and i.v. injection respectively. These results reflected a very good absorption of marbofloxacin with different routes of injection used and its plasma levels in pre-ruminant calves were still above its corresponding levels recorded in ruminant ones by considerable values.

Nearly similar results were recorded for healthy pre-ruminant calves by **(Petracca, 1993 and Thomas et al, 1994)** and for healthy ruminant ones by **(Petracca, 1993; Drugeon et al, 1994 and Banting et al, 1997)**. They mentioned that, of 4h after i.m., s.c. and i.v. administrations of marbofloxacin to healthy calves its levels in plasma were 1.02, 0.92 and 0.99mg/ml in pre-ruminant whereas it were 0.89, 0.93 and 0.71mg/ml in ruminant while after 12h it were 0.60, 0.55 and 0.58mg/ml in pre-ruminant, and 0.25, 0.35 and 0.098mg/ml in ruminant calves respectively . The authors concluded that, these differences between plasma levels in pre-ruminant and ruminant calves may be due to immaturity of elimination organs in pre-ruminant calves that lead to prolonged persistence of the molecule in the body **(Petracca, 1993 and Thomas et al, 1994)**. Finally they found that, in the pre-ruminant calf, 10% is eliminated in the bile and 75% in the urine and these proportions are different in ruminants; in dairy cows 1% in milk, 54% in faeces and 45% in urine, that can be explained by the immaturity of the hepatic system in pre-ruminant calves. In this respect **(Committee for Veterinary Medicinal Products, 2000)** mentioned that marbofloxacin was well absorbed after oral and parenteral administrations for calves and in comparing its pharmacokinetics in pre-ruminant and ruminant, they found that both absorption and elimination were slower in pre-ruminant and the bioavailability approached 100% after i.m. and s.c. administration. Otherwise **(Petracea, 1993 and Thomas et al, 1998)** stated that, marbofloxacin bioavailability by parenteral route in the calf is 90 to 100% and its clearance in pre-ruminant calves are half its rates recorded in dairy cows. Furthermore, **Drugeon (1994) and Scott (1997)** mentioned that marbofloxacin absorption in cattle from the ad-

ministration site is very good regardless of the administration route used and this reflected in the very high absolute bioavallability, but the presence of divalent cations (calcium, magnesium) can affect this absorption. In general, this long persistence of fluoroquinolones in the plasma may be attributed to substitution of hydrogen atoms by fluorines at position 8 of the ring and on the methyl of the alkyl chain that diminishes its rate of degradation and elimination **(Dudley, 1991 and Sarkozy, 2001)** .

On the other hand, this study revealed that, when marbofloxacin injected i.m. in pre-ruminant calves the plasma levels were still slightly high in sick than healthy ones, it were 0.55 and 0.66mg/ml after 12h, 0.34 and 0.45mg/ml after 20h, and 0.25 and 0.40mg/ml after 24h in healthy and sick calves respectively.

Nearly similar resultes were recorded by **(Petracca, 1993 and Thomas et al, 1994)** They found that, after 12h marbofloxacin level in plasma was 0.60 and 0.69mg/ml, and after 24h was 0.30 and 0.43mg/ml in healthy and sick calves respectively after its i.m. injection . The authors added that, this non-significant difference is probably linked to some reduction in renal clearance in sick calves and does not require an adjustment of the dose.

Finally it could be concluded from this study that, after injection of marbofloxacin in calves (pre-ruminant; healthy or sick, and ruminant) with different routes it rapidly achieves high concentrations that are maintained over a long period of time and its concentrations in pre-ruminants were still above its corresponding levels in ruminants with considerable values which must be put in mind with drug using . On the other hand, although the difference in plasma levels between healthy and sick pre-ruminant calves was non-significant but it is considered one of a good drug criteria .

Table (1) : Marbofloxacin plasma levels in healthy pre-ruminant calves following its injection at a dose of 2mg/kg b.w with different routes (μg/ml).

Time of sampling	i.m. (n =5) mean ± SD	s.c. (n =5) mean ± SD	i.v. (n =5) mean ± SD
Pre-injection	0.00	0.00	0.00
1/2h	1.62 ± 0.062	1.41 ± 0.063	3.98 ± 0.083
4h	0.98 ± 0.029	0.85 ± 0.042	0.95 ± 0.038
8h	0.72 ± 0.034	0.64 ± 0.037	0.75 ± 0.029
12h	0.55 ± 0.038	0.48 ± 0.029	0.51 ± 0.037
16h	0.44 ± 0.062	0.30 ± 0.063	0.46 ± 0.081
20h	0.34 ± 0.042	0.24 ± 0.037	0.37 ± 0.029
24h	0.25 ± 0.081	0.20 ± 0.063	0.28 ± 0.062

Table (2): Marbofloxacin plasma levels in healthy ruminant calves following its injection at a dose of 2mg/kg b.w with different routes (μg/ml).

Time of sampling	i.m. (n =5) mean ± SD	s.c. (n =5) mean ± SD	i.v. (n =5) mean ± SD
Pre-injection	0.00	0.00	0.00
1/2h	1.32 ± 0.062	1.00 ± 0.063	3.90 ± 0.081
4h	0.82 ± 0.042	0.80 ± 0.037	0.66 ± 0.062
8h	0.46 ± 0.038	0.61 ± 0.029	0.27 ± 0.037
12h	0.18 ± 0.041	0.27 ± 0.062	0.083 ± 0.006
16h	0.085 ± 0.006	0.093 ± 0.006	0.070 ± 0.008
20h	0.060 ± 0.004	0.063 ± 0.004	0.044 ± 0.008
24h	0.038 ± 0.006	0.040 ± 0.004	0.020 ± 0.003

Table (3): Marbofloxacin plasma levels in pre-ruminant calves (healthy and sick) following its i.m. injection at a dose of 2mg/kg b.w (μg/ml).

Time of sampling	Healthy (n =5) mean ± SD	Sick (n =5) mean ± SD
Pre-injection	0.00	0.00
1/2h	1.62 ± 0.062	1.68 ± 0.08
4h	0.98 ± 0.029	0.98 ± 0.07
8h	0.72 ± 0.034	0.78 ± 0.042
12h	0.55 ± 0.038	0.66 ± 0.025
16h	0.44 ± 0.062	0.55 ± 0.081
20h	0.34 ± 0.042	0.45 ± 0.063
24h	0.25 ± 0.081	0.40 ± 0.062

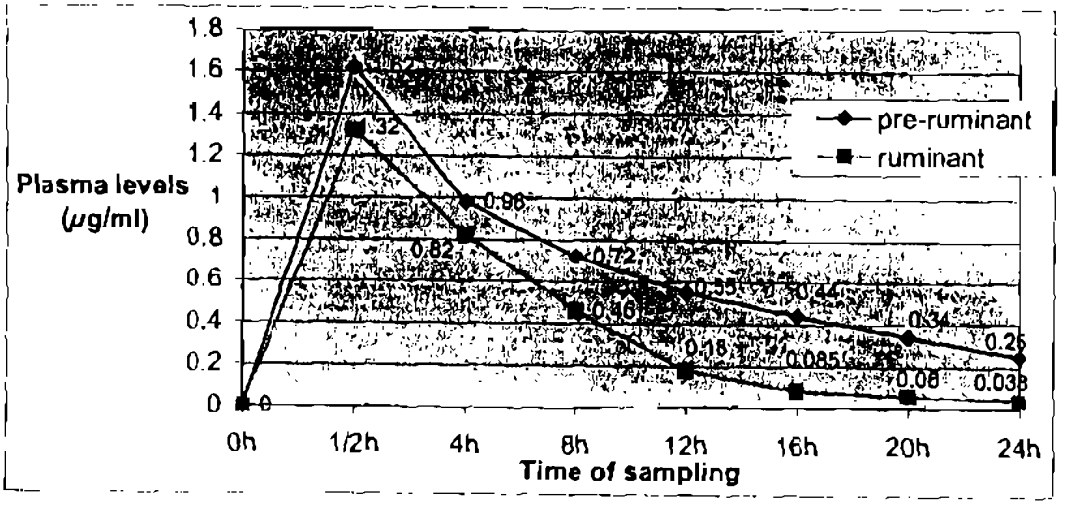

Fig. (1): Marbofloxacin plasma levels in healthy calves (pre-ruminant and ruminant) following its i.m. injection at a dose of 2mg/kg b.w (μg/ml).μ

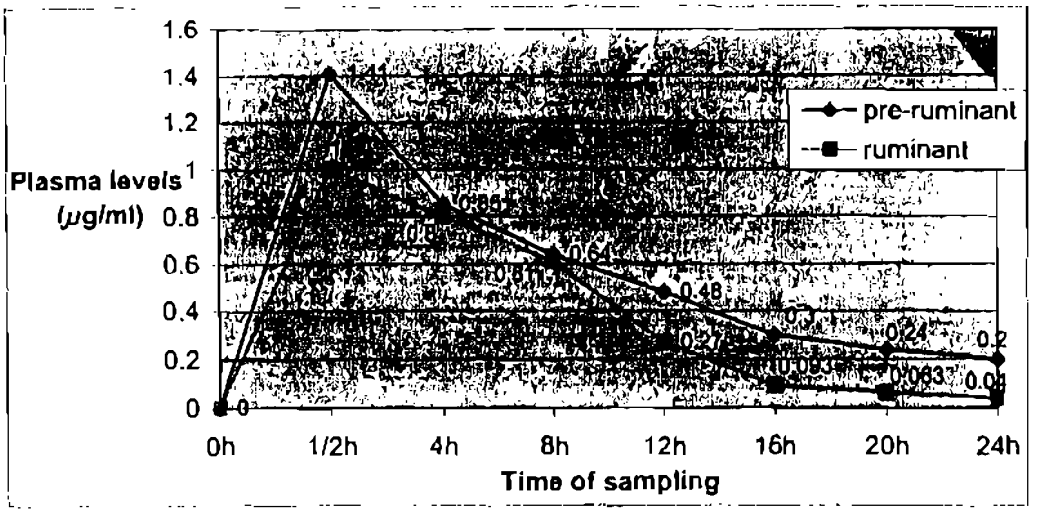

Fig (2) : Marbofloxacin plasma levels in healthy calves (pre-ruminant and ruminant) following its s.c. injection at a dose of 2mg/kg b.w (µg/ml).

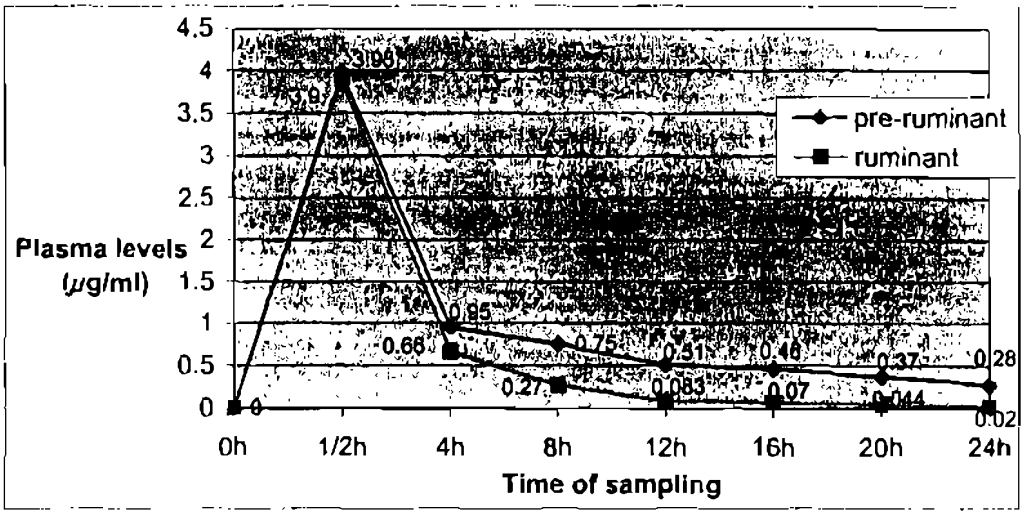

Fig (3) : Marbofloxacin plasma levels in healthy calves (pre-ruminant and ruminant) following its i.v. injection at a dose of 2mg/kg b.w (µg/ml).

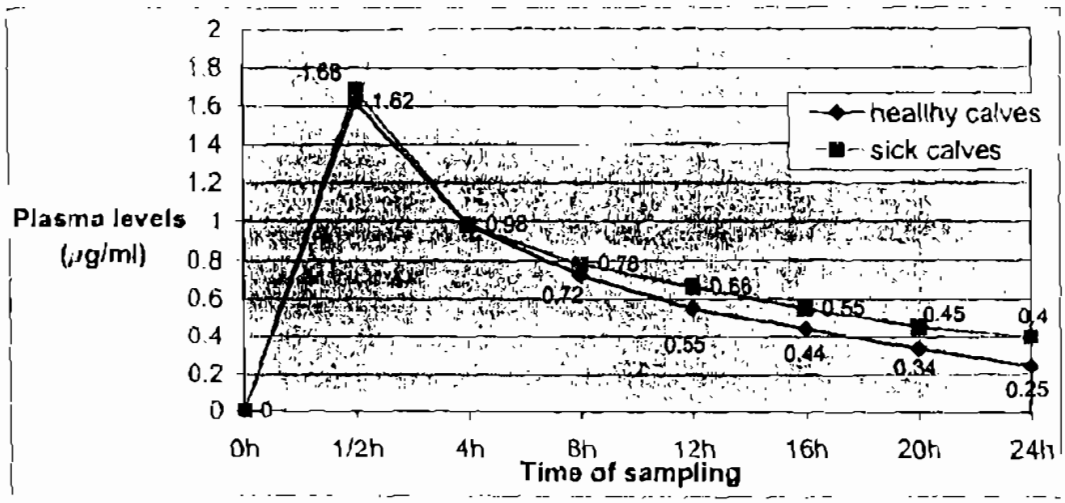

Fig (4) : Marbofloxacin plasma levels in pre-ruminant calves (healthy and sick) following its i.m. injection at a dose of 2mg/kg b.w (µg/ml).

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الملخص العربى

دراسات مقارنة على مستويات الماربوفلوكساسين فى بلازما العجول بعد حقنه بالطرق المختلفة

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تم تقسيم الحيوانات تحت الدراسة إلى ثلاث مجموعات : المجموعة الأولى عدد ١٥ عجل (سليمة - رضيعة) والثانية عدد ١٥ عجل (سليمة - مجترة) والثالثة عدد ٥ عجول (مريضة - رضيعة) وتم حقن المجموعتين الأولى والثانية بعقار الماربوفلوكساسين جرعة واحدة بمعدل ٢ملجم / كجم من وزن الحيوان إما بالعضل أو تحت الجلد أو بالوريد (عدد ٥ عجل لكل مجموعة علاجية) وحقنت المجموعة الثالثة جرعة واحدة بالعضل بنفس المعدل وأخذت عينات من الدم قبل حقن العقار وبعد ١/٢ و ٤ و ٨ و١٢ و١٦ و٢٠ و٢٤ ساعة من حقنة لقياس مستوى الماربوفلوكساسين.

وجد أن تركيز الماربوفلوكساسين فى بلازما العجول السليمة الرضيعة بعد ٤ ساعات كان ٩٨ر٠ و٨٥ر٠ و ٩٥ر٠ ميكروجرام/مل فى حين كان تركيزه فى العجول السليمة المجترة ٨٢ر٠ و ٨٠ر٠ و ٦٦ر٠ ميكروجرام/مل بعد حقنه بالعضل وتحت الجلد وبالوريد على الترتيب فى حين كان تركيزه بعد ١٢ ساعة ٥٥ر٠ و ٤٨ر٠ و٥١ر٠ ميكروجرام/مل فى العجول السليمة الرضيعة و١٨ر٠ و٢٧ر٠ و٨٣ر٠ ميكروجرام/ مل فى العجول السليمة المجترة، وبعد ٢٠ ساعة كان ٣٤ر٠ و ٢٤ر٠، ٣٧ر٠ ميكروجرام/مل فى العجول السليمة الرضيعة و٦٠ر٠ و ٦٣ر٠ و٤٤ر٠ ميكروجرام/مل فى العجول السليمة المجترة بعد حقنه بالعضل وتحت الجلد وبالوريد على التوالى. أما بالنسبة للعجول الرضيعة فكان تركيز العقار بعد ١٢ ساعة من حقنه بالعضل ٥٥ر٠ و ٦٦ر٠ ميكروجرام/مل وبعد ٢٤ ساعة ٢٥ر٠ و ٤٠ر٠ ميكروجرام/مل فى العجول السليمة الرضيعة والمريضة الرضيعة على الترتيب.

وبدراسة مستوى العقار فى المجموعتين الأولى والثانية نجد أن العقار يمتص بسرعة كبيرة بغض النظر عن طريقة حقنه ويظل مستواه فى العجول الرضيعة أعلى منه فى المجترة بقيم ملحوظة، وبالمقارنة بالمجموعة الثالثة نجد أن مستوى العقار يظل فى العجول المريضة الرضيعة أعلى منه فى العجول السليمة الرضيعة ولكن بقيم أقل.