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Hematotoxicity in Suckling Pups of Rats Exposed to Veterinary Florfenicol Residues via Lactation

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Background: The veterinary antibacterial florfenicol, an alternative to chloramphenicol that is prohibited for use in food-producing animals due to its hematotoxicity .But some references indicate that florfenicol also induces hematotoxicity**. Objective:** The aim of the study was to use suckling pups of rats as a model to detect the hematotoxicity of florfenicol. **Methods:** Fifteen lactating female rats (8 pups per mother) were divided into three groups C (control), F1 and F2 were treated with florfenicol (0, 50 and 100 mg/kg, intramuscularly (i.m) during the first five days after parturition, respectively. Blood smears were prepared from newborns at 3, 7,14 and 21 postnatal day (PND) and stained with May-Grunwald Giemsa to study changes in the erythrocyte morphology.

Results: Treatment of lactating female rats with a florfenicol(100 mg/kg) in the group (F2) led to severe hematotoxicity represented by appearance of the anulocyte cell as an indication of hypochromia in a state similar to thalassemia and accompanied by the appearance of stomatocytes and target cells at (3 and7 PND) ,but fragmentation of erythrocytes were observed on the 14 PND, which persisted until the 21 PND. Whereas, blood smear from newborns in group (F1) at 3 (PND) showed cells with finger-like projections which are an indicator of hemoglobinopathy, which was followed by the fragmentation of RBC which continued until 7,14 (PND) accompanied by a late appearance of anulocyte and target cells on day 21

Conclusion: We conclude from our current study the success of the newborn model in detecting the hematotoxicity of florfenicol.

Keywords: Florfenicol, Hematotoxicity, Lactation, Residues, Suckling pups.

Introduction

Florfenicol (Flo) is one of the antibacterials widely used in the field of Veterinary Medicine as an alternative to chloramphenicol by replacing the nitro group ($–NO_2$) responsible for the hematotoxic effects of chloramphenicol with a group of sulfomethyl group ($–SO_2CH_3$) group [1]. But with this replacement, Zhang et al. [2] concluded that Flo also induces hematotoxicity .

It was necessary to know the main reason responsible for the occurrence of antibacterial Flo toxicity, which is related to the tendency of Flo for binding and inhibits both the bacterial ribosome (its mechanism of antibacterial action) and the mitochondrial ribosomes in mammals [3] , this is due to the similarity in the structure and chemical properties of the ribosome in bacteria (prokaryotic) and mitochondria (eukaryotic) [4] . Hence the risk of exposure to the residues of this veterinary antibacterial Flo because it does not possess selective toxicity against bacteria only, but its toxic effects extend to the mitochondria of the cells of the body.

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Because of the extensive use of antibacterial Flo in food-producing animals [5], thereby the great importance of detecting the hematotoxic effects of this drug using a sensitive model of laboratory animals in order to ensure the safety and health of consumers exposed to Flo residues found in animal products such as (milk, meat, and eggs) [6].

Florfenicol is excreted through milk [7-9] due to its lipophilic nature [1]. Florfenicol remains excreted in milk for a long time where Power et. al concluded that Flo residues remain for 27 days in the milk of cows treated with the Flo at a dose (20 mg/kg. intramuscularly) twice between them 48 hours, based on the above mentioned about the excretion of Flo through milk, so it is possible to use suckling pups of rats as a model to detect the hematotoxic effects of Flo residues.This model has pharmacokinetic properties that make it highly sensitive to the toxic effects of drugs [10,11]. Where Kaartinen et al. [12] indicated that in newborns the metabolism in the liver and excretion in the kidney is incomplete and the volume of drug distribution is very large because the body contains high levels of water accompanied by a low percentage of binding to plasma protein resulting in a high percentage of the free active drug, because of the pharmacokinetic behavior of the newborns as mentioned above, the drugs to which these newborns are exposed through lactation will accumulate in their various tissues, including the bone marrow, which will result in hematotoxicity .Investigation of the hematotoxicity in suckling pup of rats exposed to Flo through lactation has not yet been reported .Therefore, in our current study, suckling pup rats were chosen as a sensitive model to detect the hematotoxicity of veterinary antibacterial florfenicol .

Materials and Methods

Animals

In this study, lactating female rats of the type (Albino rats) were used with their pups, obtained from the animal house of the College of Veterinary Medicine / University of Mosul. The weights of the mothers were confined between (195-280) grams and the newborns were between (5-44) grams. In order for suckling pups to be equally exposed to Flo through milk, 8 pups were determined for each mother, with the day of birth being considered as day zero [13,14].The animals were raised in special laboratory conditions characterized by a light cycle of 10 hours of light and 14 hours of darkness, and the temperature of the laboratory was (22^0 2±C) during the breeding took place. Each mother was placed separately with her newborns in special plastic cages prepared for this purpose and provided with water and fodder in abundant quantities and continuously.

Design of the experiment

- The first group: Control group (C): included five lactating female rats who were treated with normal saline (2ml/kg, intramuscularly) once a day for the first five days after parturition.
- The second group: (F1) included five lactating female rats treated with Flo (Introflor-300 - Holland) at a dose of (50 mg/kg, intramuscularly) once a day for the first five days after parturition .
- The third group: (F2) included five lactating female rats treated with Flo at a dose of (100 mg/kg, intramuscularly) once a day for the first five days after parturition

In order to detect hematotoxicity in the model of suckling pups of rats exposed to Flo residues through breastfeeding, blood smears were taken from newborns at (3, 7, 14 and 21 PND) [15] and stained with May-Grünwald-Giemsa staining (MGG) to study changes in the erythrocyte morphology.

Ethical approval

We obtained the official approval for the study protocol (No.1396) from the Committee of Postgraduate Studies at the College of Medicine, University of Mosul, Iraq according to institutional regulations on animal handling and use in research.

Results

The blood smear from suckling pups of rats exposed to florfenicol through breastfeeding in the group (F1) at (3 PND)showed the occurrence of hematotoxicity, represented by changes in the sizes (anisocytosis) and shape (poikilocytosis) of RBC, including cell with finger-like projections (mitten-like cells") that express as an indicator and a marker of the hemoglobinopathies type (HbSC),these changes in the membrane of RBC (finger-like projections) led to a defect in the permeability of this membrane and leakage of potassium and water, resulting in cellular dehydration and the fragmentation and destruction of red blood cells, accompanied by the appearance of polychromatophil erythrocyte in order to compensate for the destruction of red blood cells (Fig. 1)

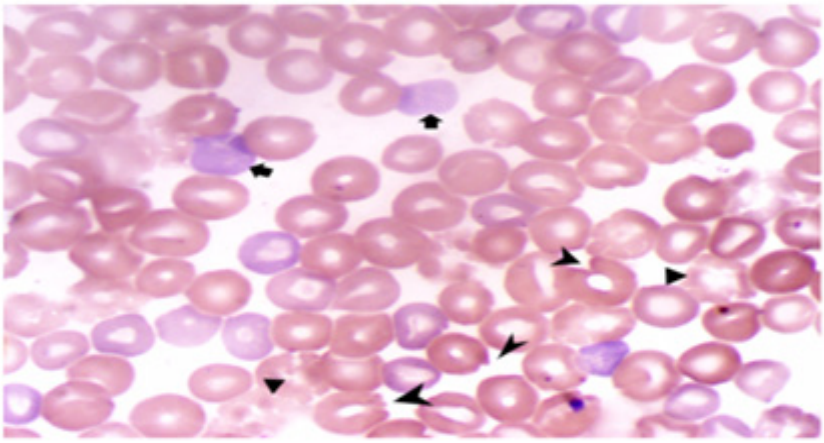


Fig. 1. Peripheral blood smear from suckling pups of rats exposed to florfenicol by breastfeeding in group (F1) at third postnatal day, showing hematotoxicity represented by marked differences in size (anisocytosis) and shape (poikilocytosis) such as cell with finger-like projections (pointed arrow) , polychromatophil erythrocyte (arrow) and fragmented erythrocyte (triangle) . May-Grünwald-Giemsa stain (MGGs), X 1000

Normal erythrocyte Cell with finger-like projections Fragmented erythrocyte

While the treatment of lactating female rats with a high dose of Flo (100 mg/kg) in the group (F2) led to the passage of high concentrations of the drug through the milk to the suckling pups , which resulted in more severe hematotoxicity effects compared to the group of newborns in the group (F1) represented by changes in the sizes (anisocytosis) and shapes (poikilocytosis) of erythrocytes, such as the appearance of anulocyte cell (wide central pallor- hemigohost cell) and also accompanied by the appearance of anulocyte cells with basophilic cytoplasm as an indication of hypochromia resulting from a defect in the synthesis of hemoglobin in erythrocytes in a state similar to thalassemia and accompanied by the appearance of stomatocytes and target cell at (3 and7 PND) (Fig. 2).

It is important to mention that the fragmentation of red blood cells in the peripheral blood in the group F1 continued until the (21)PND but to a lesser degree than in newborns in the group (F2) , accompanied by the appearance of cells such as anulocyte and target, but later than the group F2 that appeared on the 3rd day after birth .

The peripheral blood picture of the suckling pups in the group (F2) recorded the persistence of red blood cell fragmentation, and to a more severe degree than newborns in the group (F1) at 14,21 PND,with continued appearance of anulocyte cell (wide central pallor- hemigohost cell) . (Fig. 4)

Discussion

In our current study, exposure of suckling pup of rats to florfenicol through breastfeeding in the group (F2) led to hematotoxic effects, represented by the appearance of hypochromia (anulocyte cell) in thalassemia-like condition, the reason for this may be attributed to the fact that with the treatment of lactating female rats with a high dose (100 mg/kg),this led to the passage of high concentrations of Flo through the milk to the newborns, and because the pharmacokinetic behavior of these newborns differs from that of adults in terms of volume of distribution, plasma protein binding of drugs, metabolism and excretion [12] which leads to drug accumulation in their various tissues, including the bone marrow which contains erythroid progenitors (red

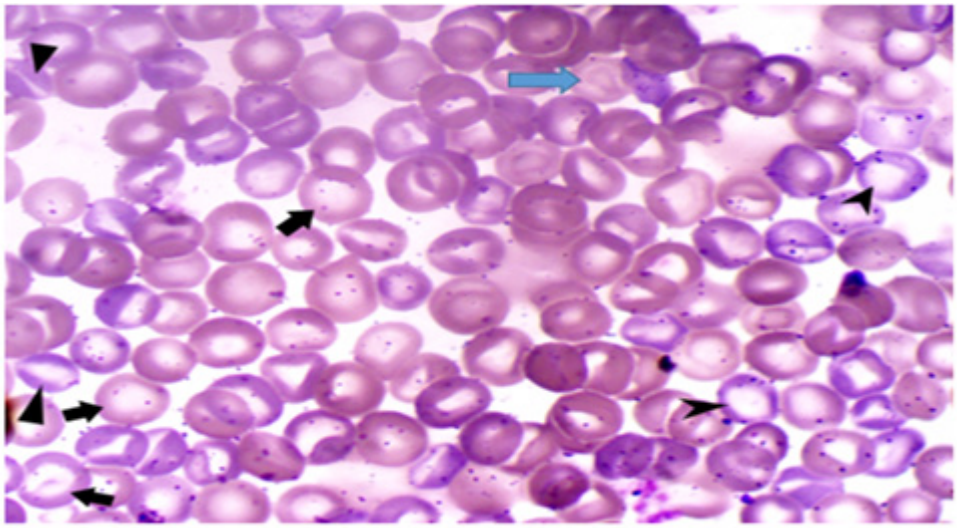
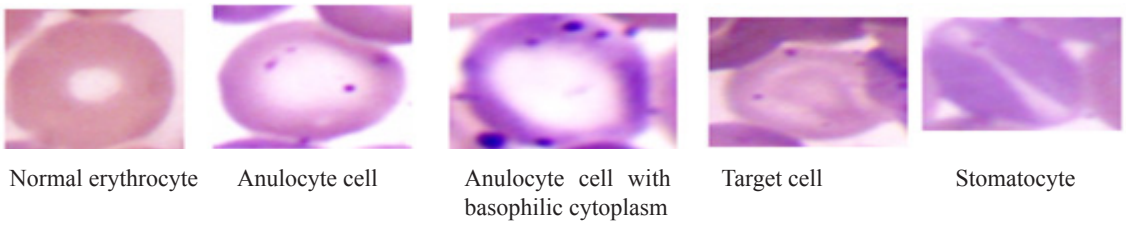


Fig. 2. Peripheral blood smear from suckling pups of rats exposed to florfenicol by breastfeeding in group (F2) at third postnatal day,showing hematotoxicity represented by marked differences in size (anisocytosis) and shape (poikilocytosis) such as anulocyte cell (wide central pallor) (arrow), anulocyte cell with basophilic cytoplasm (pointed arrow),stomatocytes (triangle) and target cell (blue arrow). May-Grünwald-Giemsa stain (MGGs), X 1000



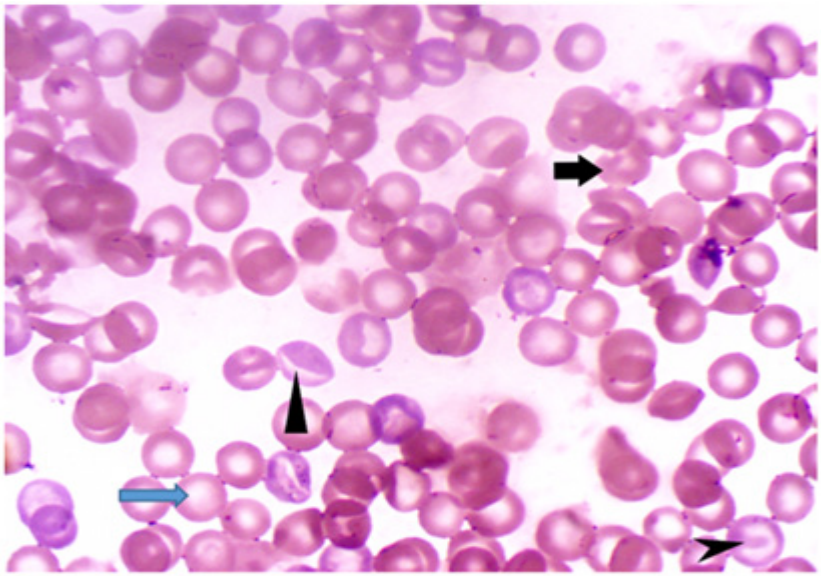


Fig. 3. Peripheral blood smear from suckling pups of rats exposed to florfenicol by breastfeeding in group (F1),shows the persistence of hematotoxicity until the 21 postnatal day, represented by the fragmentation of red blood cell (arrow) accompanied by anulocyte cell (wide central pallor) (blue arrow), stomatocyte (triangle) and target cell (pointed arrow) . May-Grünwald-Giemsa stain (MGGs),X 1000

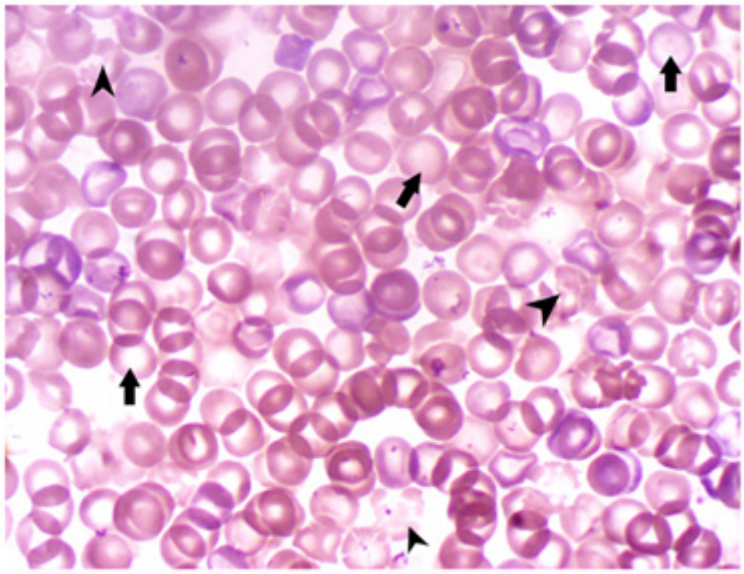


Fig. 4. Peripheral blood smear from suckling pups of rats exposed to florfenicol by breastfeeding in group (F2),shows the persistence of hematotoxicity until the 21 postnatal day, represented by the fragmentation of red blood cell (pointed arrow) accompanied by anulocyte cell (wide central pallor) (arrow) . May-Grünwald-Giemsa stain (MGGs),X 1000

cell precursors) rich in mitochondria (mito) and because the (mito) are responsible for synthesis of hemoglobin , in which the process of binding iron with protoporphyrin IX is stimulated by the enzyme ferrochelatase to form heme, which leaves the (mito) to combine with the globin chain in the cytoplasm to form hemoglobin [16]. Since the antibacterial Flo has a great tendency to bind and inhibit the mitochondrial eukaryotic (mammals) (3)and because (mito)`111 as mentioned above, is responsible for making hemoglobin [16] which led to a defect in the manufacture of hemoglobin and the appearance of hypochromia (anulocyte cell) in thalassemia-like condition, in the blood smear of the newborns exposed to Flo residues through breastfeeding in the F2 group ,what confirms this explanation about the occurrence of a condition similar to thalassemia is the appearance of target cells in the blood smear of the newborns of this group (F2), which is considered as an important marker hemoglobinopathy [17] .

The blood smear from suckling pups of rats exposed to Flo through milk in the (F1) group showed cells with finger-like projections (may look like a mitten) which are an indicator of hemoglobinopathy , these projections represent crystalline aggregates of hemoglobin SC may protrude from the erythrocyte membrane thus, this case is called (Hb SC) **[**18], Where, Rodak and Carr [19] indicated that the cause of the appearance of (Hb SC) is a mutation in the Beta Globin Gene (HBB) leads to the formation of hemoglobin S (HbS) and hemoglobin C (HbC),the tendency to crystallization, which, along with cell dehydration caused by the HbC induced loss of K+ and water, these changes, such as cellular dehydration, are followed by the fragmentation and destruction of erythrocytes [20] based on what was mentioned above, it is possible that in our current study, the Flo residues induced the mutation in the hemoglobin of the red blood cells of the newborns exposed to it through milk, which led to the appearance of cells that have finger-like projections (like look mitten).

It is worth noting that stomatocytes cells were observed in the blood smear of newborns in both groups F1 and F2, and because their presence is associated with toxic effects on hepatocytes [18] ,this means that exposure of suckling pups of rats to florfenicol residues through milk led to harmful effects on the hepatocytes of these newborns.

We conclude from the results of our current study success suckling pup of rats model in detecting the hematotoxicity of veterinary antibacterial florfenicol that is used clinically for the treatment of food-producing animals. Therefore, there is a risk of these toxic effects occurring in consumers exposed to Flo residues in animal products (milk, meat and eggs).

Conflicts of Interest

The authors declare there is no conflict of interest .

Acknowledgment

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السمية الدموية في صغار الجرذان الرضع المعرضه لبقايا الفلورفينكول البيطري عن طريق الرضاعة

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الخلفية العلمية :المضاد البكتيري البيطري الفلورفينكول الذي حل بديلا عن الكلورمفينكول المحرم استخدامه في الحيوانات المنتجة للغذاء بسب امتلاكه تأثيرات سمية دموية ولكن بعض المراجع العلمية اشارت الى ان الفلورفينكول أيضا يحدث سمية دموية

الهدف : كان الهدف من دراستنا الحالية هو استخدام صغار الجرذان الرضع كنموذج حساس لكشف السمية الدموية لبقايا الفلورفينكول .

طرائق العمل :لاجراء هذه الدراسة فقد تم تقسيم اناث الجرذان المرضعات الى ثلاثة اقسام (سيطرة واف١ واف٢) بواقع خمسة أمهات لكل مجموعة (٨ صغار لكل ام) عوملت الأمهات المرضعات في مجموعة السيطرة بمحلول الملحي العادي (٢ مل /كغم) بينما عوملت الأمهات المرضعات في المجاميع (اف١ واف٢) بالفلورفينكول (٥٠ و١٠٠ ملغم/كغم بالعضل) مرة واحدة يوميا وخلال الخمسة أيام الأولى بعد الولادة . تم تحضير المسحة الدموية من الصغار الرضع في الأيام ٣ و٧ و١٤ و٢١ بعد الولادة وتم صبغها بصبغة الكمزا- ماي كرونلد وذلك لدراسة التغيرات في اشكال خلايا الدم .

النتائج:أدى معاملة اناث الجرذان المرضعات بالفلورفينكول بالجرعة العالية (١٠٠ ملغم/كغم) في المجموعة (اف٢) الى عبور تراكيز عالية من الدواء خلال الحليب الى الصغار الرضع مما نتج عنها سمية دموية شديدة تمثلت بظهور خلايا الانيولسايت (شحوب مركزي واسع) كمؤشر على نقص الصبغيات في حالة مشابهة لمرض الثلاسيميا ومصحوبة بظهور خلايا الستوماتوسايت وخلايا الهدف في اليوم الثالث والسابع بعد الولادة اما التجزء وتبعه التحطيم في خلايا الدم الحمر فقد تم ملاحظته في اليوم ١٤ بعد الولادة والذي استمر الى اليوم ٢١ بعد الولادة . في حين أظهرت المسحة الدموية من الصغار المعرضين للفلورفينكول عن طريق الرضاعة في المجموعة (اف١) في اليوم الثالث بعد الولادة خلايا ذات نتوءات شبيهة بالأصابع والتي تعد مؤشرًا على اعتلال الهيموكلوبين ،أدى هذا التشوه الخلوي (البروزات) إلى الجفاف الخلوي ، الذي أعقبه تجزء وتحطيم في خلايا الدم الحمراء ، مصحوبًا بظهور خلايا الدم الحمراء متعددة الألوان لتعويض عن خلايا الدم الحمر المحطمة ،ومن المهم ذكره ان تجزئة كريات الدم الحمراء استمر في اليومين ٧ و١٤ بعد الولادة ، مصحوبًا بظهور متأخر للخلايا الانيولسايت وخلايا الهدف في اليوم ٢١ بعد الولادة بالمقارنة مع الصغار في المجموعة (اف٢)

الاستنتاج :نستنتج من نتائج دراستنا الحالية نجاح نموذج صغار الجرذان الرضع في الكشف عن السمية الدموية للمضاد البكتيري البيطري الفلورفينكول المستخدم سريريا لعلاج الحيوانات المنتجة للغذاء لهذا السبب ، هناك خطر حدوث هذه التأثيرات السامة لدى المستهلكين المعرضين لبقايا الفلورفينكول في المنتجات الحيوانية (الحليب واللحوم والبيض).

الكلمات الرئيسية: الفلورفينكول ، السمية الدموية ، الرضاعة ، بقايا ، الصغار الرضع.