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Formulation of oxytetracycline 20% injectable solution for veterinary use

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Abstract

Preparation of a new formulation of oxytetracycline 20% injectable solution was done for veterinary therapeutic use. Information about all the materials used in preparation of formula were collected from the well known pharmacopeia while analar material were provided and used to prepare about three pilot formulae, from which the final one was prepared. Stability of new formula was tested in different room environmental conditions by comparing any change in physicochemical properties concerning form, colour, transparency, pH in different storage room temperatures for 18 months as well their MIC value and bacterial inhibition rate formorethan 12 months. The results showed that the new drug proved its stability, antibacterial activity when tested both in vitro against E coli 0.78 was growth in which MIC was 0.2 µg /ml. It was also tested clinically in therapy of group of infected sheep and cows (4 each) with respiratory diseases, at the college of veterinary medicine, University of Baghdad as well as in the central veterinary hospital in comparison with other similarly infected group treated with common commercially used 20% oxytetracycline drug (Alamycin)® while control group treated with normal saline. The results showed improvement of the animals of both treated groups when compared with control one, which its animal health deteriorated and improved when treated with the formula. Both quantitative and qualitative results for oxytetracycline 20% formula as well as its physicochemical properties and steritity was tested by Board of drug and biological standardization, Iraqi Veterinary State Company (I.V, S.C.) and a certificate of approval of quality, quantity, antibacterial activity was issued by I.V.S.C. for new oxytetracycline 20% injectable solution and registered, it as new drug formulated by the College of Veterinary Medicine, university of Baghdad for veterinary use.

Keywords: Formulation; Oxytetracycline; Veterinary.
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تحضير تركيبة محلول اوكسي تتراسايكلين ٢٠ % حقن للاستعمال البيطري

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الخلاصة

حضرت تركيبة جديده للاوكسي تتراسايكلين ٢٠% محلول حقن للاستعمال البيطري. تم جمع المعلومات عن المواد المستعملة مستحضر التركيبة من دساتير الادوية العالمية وتم توفيرها ضمن المواصفات القياسية العالمية لتحضير ثلاث تركيبات اولية تم اختيار التركيبة النهائية منها. فحصت المواصفات الفيزيائية والكيميائية لتشمل القوام و اللون والشفافية والاس الهيدروجيني بظروف خزن في جو الغرفة بدرجات حرارة مختلفة ولمدة ١٨ شهر تم قياس خلالها اقل تركيز مثبط ونسبة التثبيط لنمو البكتريا لفترة ١٢ شهر.اظهرت النتائج ثباتية الدواء وفعاليتة المضادة لنمو E.coli ٠,٧٨ ملم في الزجاج خلال هذه الفتره حيث كان اقل تركيز مثبط هو ٠,٢ ملغم / مل. الفحص السريري تم اجراءه في عيادة كلية الطب البيطري / جامعة بغداد والمستشفى البيطري المركزي حيث جربت التركيبة لعلاج مجاميع مرضية مكونه من اربع اغنام او ابقار مصابة بالتهابات تنفسية وتم مقارنة العلاج بدواء تجاري معروف للاوكسي تتراسايكلين ٢٠% (الامايسين)® بينما عولجت مجموعة السيطرة بالملح الفسلجي.اظهرت النتائج تعافي الحيوانات بنفس الكفاءه تقريبا" للتركيبة المحضرة والدواء التجاري مقارنة بحيوانات السيطرة التي ساءت حالتها الصحية وتحسنت بعد علاجها بالحقن بالتركيبة المحضرة.تم القياس الكمي والنوعي والعلاجي والتحقق من المواصفات الفيزياوية والكيمياوية للتركيبة المحضره (للاوكسي تتراسايكلين) ٢٠% في مركز السيطرة الدوائية والبايلوجية التابع للشركة العراقية العامة للبيطرة I.V.S.C حيث اصدرت الشركة شهادة قبول المنتج ومطابقته للشروط القياسية العالمية في النوعية والكمية وفعاليته المضاده للجراثيم وتم تسجيل تركيبة الاوكسي تتراسايكلين ٢٠ % سائل حقن كمنتج جديد من كلية الطب البيطري – جامعة بغداد لغرض الاستعمال البيطري.

Introduction

Oxytetracycline is first discovered as result of work of researcher in American trade organization from streptomyces fungal species. It has been extracted from fungus streptomyces rimosus for first time by (1). This drug also produced by other streptomyces genus like S. grasoflavus, S. armilatus and S. aureofacius. Oxytetracycline acts on microbial ribosome by binding with 30 s unit of ribosome and preventing tRNA from taking its position in mRNA so it block amino acids addition to the formed serial peptide. For this reason, it prevents bacterial growth and so consider as bacteriostatic antibiotic acting against bacterial protein synthesis (2).

Oxytetracycline consider as broad spectrum antibiotics that effect growth of both gram positive and negative bacteria in animal and human being and act against other microbial infection like Rickettsia, Mycoplasma, clamydia. It used against trachauma, urinary and skin infection as well as in treatment of chronic respiratory diseases like pneumonia, Hemophilus influenza while in high doses, it effect growth of protozoal disease in animals like thileria. There is formulation of Oxytetracycline as injectable solution at concentration (10, 20, and 40 %) for veterinary use (3). Oxytetracycline 20% is indicated for use in sheep, cattle and pig in the treatment of atrophic rhinitis caused by Bordetella bomchiseptic Pasturella hemolytica and Pasturella multicida novel joint illness caused by Corynebacterium pyogen, E.coli and Staphylococcus aureus Mastitis caused by Corynebacterium pyogen, E.coli, Staphylococcus aureus, Strepococcus agalactia and Streptococcus uberis Metritis caused by E.coli, Streptococcus pyrogen Pasteurellosis and infection of respiratory tract caused by Pasturella hemolytica and Pasturella multicida Septicemia caused by Samnella duplin and Streptococcus pyrogen Erysipelas caused by Erysipelothrix rhusiopathiae and control of enzootic abortion in sheep (12).

The aims of this study are, preparation of new therapeutic formulation of oxytetracycline 20% injectable solution with assurance of its stability and sterility during different storage environment. - Quantative analysis of oxytetracycline amount, and in vitro and in vivo evaluations of antibacterial activity against E coli growth in culture and in therapy of diseased animals in clinic.

Materials and methods

Oxytetracycline dehydrate

A yellow, odorless, hygroscopic, crystalline powder with a bitter taste, containing not less than 880 units per mg of dried oxytetracycline dehydrate obtained from Sigma. It decomposed above 180 °C. It darkens on exposure to sunlight or moist air above 90° but there is little loss of potency (4).

The reported solubility of Oxytetracycline dihydrate is 1 in 2 for water,1 in 45 of ethyl alcohol soluble, while highly soluble in propylene glycol; insoluble in chloroform and ether, different percent solutions in water has a pH range 2.3 -2.9, while solution in water at neutral pH become turbid on standing owing to hydrolysis and precipitation of oxytetracycline base. Oxytetracycline dihydrate deteriorate in a solution have a pH of less than 2 and is rapidly destroyed by alkalin (5).

Ascorbic acid

White powder provided from (Sigma) -analor standerd have good solubility in distilled water and ethyl alcohol, used as Antioxidant, preservative and pH stabilizer (6).

Propyl and methyl paraben

White powder provided from Sigma-analor standerd, have good solubility in ethyl alcohol, used as preservative (**7).**

Propylene glycol and ethyl alcohol

Vehicle usd to prepare and give final volume of preparation with antiseptic properties.

Preparation of formula (Oxytetracycline dehydrate 20%)

New formula was prepared under suitable laboratory and productive environment by using sterilized instrument in sterilized place and analar sterilized ingredient. Ingredients of formula for 100 ml vial are:-

Item No.	Ingredient	Amount
1	Oxytetracycline dihydrate	20.0 gm
2	Ascorbic acid	1.0 gm
3	Methyl paraben	0.65 gm
4	Propyl paraben	0.35 gm
5	Ethyl alcohol 40%	20.0 ml
6	Propylene glycol aded.	100.0 ml

Preparation steps

1. Dissolving the preservatives propyl and methly parabens, with diluted ethyl alcohol till 15 ml.
2. Dissolving 1 gm ascorbic acide in alittle volume of 40 % ethyl alcohol.
3. Slowly dissolve the active ingredient (oxytetracycline dehydrate by gradual addition to propylene glycol with continuous shaking until it completely dissolved, then step 2 solution was added while step 1 solution later added with continuous shaking for completely mixing and dissolving of all ingredients.
4. Measure the pH for mixture at 5 – 5.3 and complete the volume to 100 ml with propylene glycol.

5. Filtration of mixture with Millipore 0.22 µm filter paper by using Boxner apparatus under U V light.
6. Filling oxytetracycline 20 % formula in sterilized vial of 100 ml that tightly sealed.
7. All the steps were done under sterilized U V light condition.

Assay for oxytetracycline

Spectroscopic method was mentioned by British Veterinary Codex (B.V. C)® (8) Was used by Broad of Veterinary-drug and biological standardization according to the afficial request from the Iraqi Veterinary State Company (I.V.S.C) to analyze and evaluate the final preparation of oxytetracycline 20% injectable solution. This was done by using U.V. spectrophotometer at wave length 383nm for quantitive evaluation of the drug formula as well as other tests for sterility, physical and chemical properties and stability.

Biological activity

Microbiological assay by disc infusion method as mentioned by United State Pharmacopeia, 2005 (9) was done by using different concentrations of oxytetracycline 20% at concentration of (0.1, 0.2, 0.3, 0.5, 0.7, 0.9 and 1 µg/ml) that compared with the same concentration of oxytetracycline strandard prepared by dissolving pure oxytetracycline powder in propylene glycole in order to estimate and compare the inhibition zone of both the prepared oxytetracycline and reference standard on the strandard test bacterial growth (E.Coli 0.78).

Pyrogen test was performed as described by (9) in which rabbits restricted in special chamber and injected I.V in ear vein by diluted solution of oxytetracycline 20% formula at one ml dose rate of 1 mg/ml per kg of rabbit weight. Oxytetracycline 20% solution was diluted with sterile normal saline. Rectal temperature recorded hourly after injection to record any change in body temperature.

Clinical examination

Clinical examination for antibacterial activity of oxytetracycline 20% formula was done at the central veterinary hospital on two groups of four diseased sheep and cows suffering from respiratory disease diagnosed as pneumonia in which a recommended dose (20 mg/kg) was given intramuscularly, the antibacterial activity was compared with other commonly used pharmaceutical preparation (oxytetracycline 20% Alamycin® - Norbrook - Ireland) which was given to other equal group of sheep and cows that suffered the same disease condition. Another clinical experiment was performed in december 2008 at the Hospital Clinic of Veterinary Medicine College, University of Baghdad to evaluate the antibacterial activity of the drug formula.In this experiment twelve sheep suffered from upper respiratory disease were divided equally into three groups, two groups of which were treated with either the formula or a commercial drug (Alamycin 20%) given I.M at a dose of 20 mg/kg to compare their antibacterial activity with third control group dosed I.M. with normal saline. Dosing in all groups continue for three days.

Results

Oxytetracycline assay

The results showed that formula comply with pharmacological standardization of preparation for oxytetracycline 20% injectable solution.

Biological activity

The results of antibacterial analysis in-vitro were listed in table (1) and figure (1). The antibacterial activity of drug as well as its minimum inhibition concentration (M.I.C) were estimated at different storage period (0, 3, 6, 12 months). The results were given as percent as listed in table (2).

Table 1: Comparative inhibition effect of different concentration of Oxytetracycline strandard concentration against test bacterial growth (E.Coli 0.78) by disc infusion method (9).

Drug	Concentration µg/ml	Inhibition zone Diameter (mm)
Ox	0.1	+
St	0.1	-
Ox	0.2	8
St	0.2	8
Ox	0.3	10
St	0.3	10.5
Ox	0.5	14
St	0.5	14.5
Ox	0.7	18
St	0.7	18
Ox	0.9	22
St	0.9	22.5
Ox	1.0	23.5
St	1.0	23.3

Ox=Oxytetracycline 20% formula St=Oxytetracycline standard.

These results are nearly compatible with that recorded by (10) in which combination of antibiotics and the change in their antimicrobial activity were tested.

The result of pyrogen test showed no change in rectal temperature was noticed hourly after 24 hour observation, and the product was free from pyrogen.

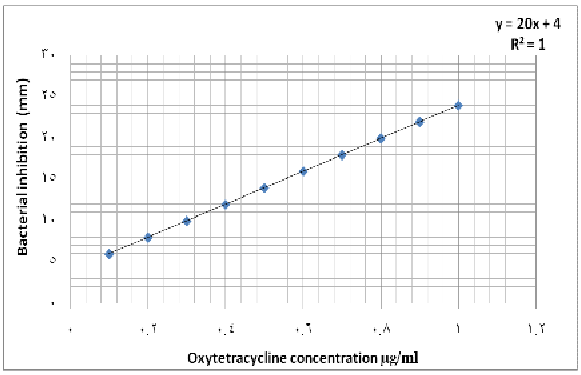

Figure 1: Standard inhibitory effect of different oxytetracycline standerd concentration against test bacterial growth (E.coli 0.78).

Physical examination

Stability of preparation was measured as a change in colour, from and pH of the formula in different storage environment at room temperature as listed in table (3). The acidity (pH) of solution containing 20% W /V oxytetracycline dehydrate is 5.3- 5.4 and doesn't changed during the test of stability as listed in table (3). Solution containing 20% W / V of oxytetracycline dihydrate appear clear and yellow and doesn't change during the test of stability for one and half year, Table (3).

Clinical examination

All formula treated sheep group recovered within few days similarly to that of Alamycin treatment. A good antibacterial activity was approved in the certificate that refers to the report of the Central Veterinary Hospital, Iraq Veterinary State Company (I.V.S.C) in which this clinical examination was performed. In the second clinical examination trial that performed in the Vet college clinic, the two groups of both oxytetracycline treatment (formula and commercial) were recovered completely from all their respiratory symptoms (sneezing, cough, difficult respiration, weakness) at the end of treatment periods, while these symptoms were deteriorated to that of pneumonia (gasping for air, recumbency, emaciation) in animal of control group in which one sheep was died at the end of saline treatment but the other three sheep were saved and recovered by giving them a course of Oxytetracycline 20% formula treatment.

The sealed container should be protected from light (by using amber vial be kept in shade) the formula was stable at room temperature storage environment for one and half year (11). The labeling of the container and drug leaflet was (Oxytetracycline dehydrate 20% injectable solution). A sterile solution containing oxytetracycline dehydrate (Ph. Eur.) equivalent to 200 mg/ml oxytetracycline dehydrate base. The recommended dosage rate is 20 mg/kg body weight (1 ml per 10 kg body weight) by deep intramuscular injection once or twice doses per 3 days for different domestic animal as follow 5, 20, 10 ml for sheep, cattle and pig respectively (12).

Table 2: Estimation of Oxytetracycline 20% antibacterial activity against standard test bacterial growth (E. Coli 0.78) by measuring MIC, and bacterial inhibition % in comparison with standard concentration of Oxytetracycline at different period and storage environments.

Drug	MIC (µg/ml)	Bacterial inhibition %	Period	Date
Ox	0.205	101.0	Zero time	21/1/2008
St	0.21			
Ox	0.22	100	After 3 months	21/4/2008
St	0.22			
Ox	0.21	99.9	After 6 months	21/7/2008
St	0.205			
Ox	0.22	99.9	After 1 year	22/1/2009
St	0.215			

MIC=Minimum inhibitory concentration Ox = Oxytetracycline 20% formula St=Standard concentration

Table 3: The stability of oxytetraxcycline 20% formula by comparing its colour, form, pH at different storage room environment.

Date of estimation Oxytetracycline 20%	Room environment~	Acidity pH	Formula Form *
Zero time 17/1/2008	15°C Sun light present	5.3	Clear, yellow, Without turbidity
After 3 months 17/4/2008	30°C Sun light present	5.4	Clear, yellow, Without turbidity
After 6 month 17/7/2008	45°C Tense sun light present	5.4	Clear, yellow, Without turbidity
After one year 17/1/2009	20°C Sun light present	5.3	Clear, yellow, Without turbidity
After 18 month 1/6/2009	35°C Sun light present	5.3	Clear, yellow, Without turbidity

*Preparation kept in brown vial in the room shade.

Discussion

The new formula contain in addition to the active ingredients oxytetracycline dihydrate 20 %, it contain methyl and propylparabans as preservative and ascorbic acid as antioxident for pH stability at 5 – 5.3. While 4% ethyl alcohol and propylene glycol were used as vehicle, antiseptic and to give the final form, a yellow trasperant appearance. All the preparation steps were done by using standard analar ingredients under sterilized condition, using UV light cabinet for sterilization of the instruments that used for preparation of formula as well as using filtration with 0.2 mm Millipore filter paper in Boxner funnel to sterilize the final formula. The results of microbiological assay showed that antibacterial activity against E coli 0.78 growth for all used formula concentrations matched that of standard oxytetracycline concentrations, while its MIC was not changed at room storage condition for one year. Clinically the drug formula was tested by using it for treatment of sheep and cows suffered from respiratory disease in the clinic of College of Vet Medicine and Central Veterinary Hospital. At both place sheep and cows were divided into two groups of 4 animals one group treated with the formula and the other with known commercial drug of oxytetracycline 20 % (Alamycine)® injected at same therapeutic doses. The same recovery pattern was noticed in both groups that give indication that the formula has same antibacterial activity as that of commercial one. This good antibacterial activity of formula that match the universal standard one was included in the certificate issued from (I.V.S.C) referring to the drug formula. The results of stability of drug formula, showed that the drug was stable since its colour, transparence and pH were not changed under room storage conditions for one year and half at different temperatures and sun shade light. These results were approve by the results of analysis tests on formula done after one and half year of production by the Board of drug and biological standardization / that sent to I.V.S.C. referred that the drug formula within the universal standard of quantity, quality, sterility and stability,and the formula was registered by I.V.S.C.

A certificate of registration was issued approved its therapeutic use in veterinary practice.

The new oxytetracycline 20 % injectable solution is suitable for treatment of respiratory disease (Pneumonia) caused by mycoplasma or Hemophilus influenza as well as skin disease (acne), urinary tract infection and diseases caused by Rickettsiea and clamydia. The drug could be injected once or twice for different animal, so it could be classified as long term therapeutic drug since this was important because of the low following up therapy by the animal owner. We are assure that a good storage condition for two years will not change the formula activity, quantity or stability since it was tested for one and half year under unrecommended room storage condition and not changed.

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