Formulation of Ivermectin 1% Injectable Solution for Veterinary Use

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Summary

New formulation of Ivermectin 1% injectable solution was prepared for Veterinary therapeutic use. Information about all the materials used in preparation of formula were collected from the 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, color, transparency, pH in different storage room temperatures for 20 months. The results of testing the formula both in-vitro against parasitic larvae motility and parasitic infection in diseased cattle and sheep indicating its efficiency as well as its chemical analysis, that indicate nearly no change in its concentrations for almost two years analysis. This formula was approved by I.V.S.C. as a drug for veterinary therapeutic uses after passing all testing schedule. A certificate for such was provided.

Keywords: Formulation, Ivermectin, 1%, injectable, solution, veterinary using.

Introduction

In the mid-1980's, ivermectin was introduced as probably one of the most broad-spectrum anti-parasite medication ever. It is effective against most common intestinal worms (except tapeworms), most mites, and some lice. It is not effective against fleas, ticks, flies, or flukes. It is effective against larval heartworms (the "microfilariae" that circulate in the blood) but not against adult heartworms (that live in the heart and pulmonary arteries), though technically it can shorten their lifespan. Ivermectin is a semisynthetic, anthelmintic agent for oral administration. Ivermectin is derived from the avermectins, a class of highly active broad-spectrum, anti-parasitic agents isolated from the fermentation products of *Streptomyces avermitilis*.

Ivermectin is a mixture containing at least 90% 5-Odemethyl- 22,23-dihydroavermectin A_{1a} and less than 10%5-O-demethyl-25-de(1-methylpropyl)-22, 23- dihydro-25- (1-methylethyl) ivermectin A_{1a}, 22,23-dihydroavermectin B_{1a} B_{1b}, or H_2B_{1a} and H_2B_{1b}, respectively (1 and 2). The respective empirical formulas are $C_{48}H_{74}O_{14}$ and $C_{47}H_{72}O_{14}$, with molecular weights of 875.10 and 861.07, respectively.

Ivermectin is a white to yellowish-white, non-hygroscopic, crystalline powder with a melting point of about 155°C. It is insoluble in water but is freely soluble in methanol and soluble in 95% ethanol. Ivermectin is very effective for treatment and control of gastrointestinal nematodes, lungworms, eye-worms, warbies, mites, lice, mange in cattle, camels, pigs, sheep and goats(3). Ivermectin is an agonist for (GABA) major inhibitory neurotransmitter. The binding of ivermectin to a neuronal membrane increases the release of GABA. Making them less excitatory and decreasing nerve transmission. Hyper-polarization of neuronal membranes (at the Neuro-muscular junction) leading to mediate a flaccid paralysis in arthropods and nematodes (4).

This study aimed to preparation of new therapeutic formulation of Ivermectin solution with assurance of its stability and sterility during different storage environment, Quantative analysis of Ivermectin formulation and in vitro and in vivo evaluations of anthelmintic activity against parasitic

development in culture and in diseased animals in clinic.

Materials and Methods

New formula was prepared under suitable laboratory and productive environment by using sterilized instrument in sterilized place and analar sterilized ingredient. Information about all the materials used in preparation of formula were collected from the well-known pharmacopeia(5), while analar material were provided and used to prepare about three pilot formulae , from which the final one was prepared and selected that include the following material used in formula with following physical , chemical properties and purposes(6) as listed in Table 1 .

Table, 1: Physical and pharmacological properties of drug formulae ingredients.

Ingredients	Character
Ivermectin	Active ingredient: Ivermectin is a white to yellowish-white, nonhygroscopic, crystalline powder with a melting point of about 155°C. It is insoluble in water but is freely soluble in methanol and soluble in 95% ethanol.
sodium sulfite	Stabilizer
Diethelamin alcohol	Stabilizer
Methylparaben	Preservative
Propylparaben	Preservative
Etheylalcohols	Dissolvent
D. water	Vehicle

Preparation of formula (Ivermectin 1% solution): In a clean dry mixing vessel, 20 to 30 ml of D.W was added to ethyl alcohols that mixed at fixed temperature (6o° C) by using magnetic stirrer with incubator. Ivermectin was added to the dilute alcohol gradually and mixed well to avoid turbidity until clear solution was prepared. 9Propylbaraben and Methylparaben were added to the mixture with well mixing at magnetic stirrer and heat to avoid turbidity.1 Adding the Diethelamine alcohol and sodium sulfite in same procedure. Complete the formula volume with distilled water with well mixing and the pH was adjusted to 6 by using NaOH or HCL drops with assurance of transparency. The formula was sterilized by preparation under U.V light and filtration of the end product in 0.2 µ.m Millipore filter paper.

Lastly packing the formula under sterile condition into suitable injection amber bottels that sealed well table 2.

Table, 2: Ingredients of formula for 100 ml vial

Ingredients	Amount
Ivermectin	**1.0 g**
sodium sulfite	**0.02 g**
Diethelamine alcohol	**0.25 ml**
Methylparaben	**0.7 g**
Propylparaben	**0.3 g**
Etheylalcohols	**10 ml**
D. water	**Up to 100 ml**
Total volume	**100 ml**

Stability of the formula was evaluated by storing at different temperature. In an environmental chamber. The samples are removed periodically and visually inspected for physical changes in the appearance of the compositions.

The formula was examined by (HPLC) according to method of (7 and 8) in which test solution at different concentrations diluted by using methanol compared with reference solution (a) prepared by dissolving 40.0 mg of ivermectin CRS in methanol R and dilute to 50.0 ml with the same solvent. Reference solution (b) prepared by dilution of 1.0 ml of reference solution (a) to 100.0 ml with methanol R. reference solution (C): dilute 5.0 ml of reference solution (b) to 100.0 ml with methanol R the chromatographic procedure may be carried out by using. Stainless steel columns 0.25 m long and 4-6 mm in internal diameter packed with octadecisity silica gel for chromatography R (5um). As mobile phase at a flow rate of 1.0 ml/min mobile phase A water R. mobile phase B mix 35 volumes of methanol R and 53 volumes of acetonitrile R. As detector a spectroph-otometer set at 254 nm equilibrate the column with a mobile phase ratio AB of 15.86 inject 20ul of reference solution (a).

Pharmacokinetic study: Plasma disposition kinetics of ivermectin was evaluated in cattle local breed. Five clinically healthy cattle weighing 200–250 kg were treated (0.2 mg/ kg) with ivermectin formulation.

Blood samples were collected by jugular puncture at different times between 0.5 h and 20 days post-treatment. After plasma extraction and derivatization, samples were analysed by HPLC with fluorescence detection. Ivermectin was detected in plasma between 30 min and 20 days post-treatment (9, 10 and 11).

In-vitro test: The assay methodology was adapted from (12), in which a 96-well microtiter plate assay used for measuring the effects of ivermectins on the motility of larvae of the ruminant stomach parasite *Haemonchus contortus*. Stock solutions of ivermectin, were prepared at 10 mg/mL, in dimethylsulfoxide and were serially diluted to produce a series of drug dilutions. Aliquots were added at a dilution of 1% to molten agar in a total volume of 200 µL in individual wells of a 96-well microtiter plate. The final drug concentrations in the assay plates consisted of two-fold serial dilutions starting at 1.6 µg/mL for ivermectin. Approximately 30 worms (in 30 µL of water) were placed into each well, and the plate was incubated in the dark at 25°C for 48 hours. The number of separate assay wells used for drug tests varied according to the availability of larvae at different times during the course of the study. All assays consisted of at least two wells at drug concentration (this was increased up to six wells in some cases). Drug sensitivity was generally determined over a series of approximately 10 drug dilutions. However, in some cases, only 4–5 separate drug concentrations were assessed. The number of control wells (no drug added) in each assay varied from 6 up to 21. The effect of the drugs on worm viability was assessed by counting the numbers of motile larvae after the 48-hour incubation period. Prior to counting, the worms were stimulated to move using hot water Satou *et.al.,* (13) for the assessment of motility in *Strongyloides. ratti* and *Strongyloides . venezuelensis*.

The new 1%ivermectin formulation injectable solution formula was tested clinically in the Central Veterinary Hospital by comparing its therapeutic effect with well-known commercial drug at same concentration (Vermectin® 1%, VAPCO-Jordan) given to groups of five cattle and sheep each diagnosed infected with nematodes according to fecal examination. Same doses were used (0.2mg/Kg) S.C. all animal groups were put under observation for two weeks to check the improvement in health status and eradication of its nematode and larva infection by checking their feces microscpically.

Samples of formula were sent by I.V.S.C to the board of drug and biological standardization for testing the new formulated 1% ivervectin injectable solution according the International standard level of quantity, quality, sterility and stability.

The formula was evaluated in laboratory animals to study the possible toxic effect of therapeutics and double doses (therapeutic, double and triple dose) injected s/c to 60 albino mice nearly at same age weighed 25-30 gram and 60 albino rats (30 male and 30 female) with same age and average weight of 200-250 grams that distributed into (10 mice or rats groups) . The animals were observed for a week for any changes in behavioral or developing any clinical signs or mortality (14 and 15).

Statistical analysis was conducted according to SPSS version 13.00. The ANOVA one way used for significances assessment between groups. The $P<0.05$ considered as statistically significant. LSD multiple range tests were carried out for comparing between means.

Results and Discussion

The results of physical stability of the formula at different storage temperature are summarized in table 3. As can be seen from the data in table 3, the composition of the present formulation is stable for at least 18 months at different temperature ranging from zero to fifty indicating long stability of drug for at least two years.

Table, 3*:* Physical Stability of Composition

Month	Temp. C°	Appearance
0	**0** **50**	clear solution
1	**0** **50**	clear solution
2	**0** **50**	clear solution
3	**0** **50**	clear solution
6	**0** **50**	clear solution
12	**0** **50**	clear solution
18	**0** **50**	clear solution

The results of chemical analysis by HPLC for different samples of stored formulae at different storage temperature and period are summarized in Table, 4.

The results showed there are nearly no changes in chemical concentration, pH and possibly activities after different storage temperatures and periods. From the results of stability and chemical analysis listed in table 3and 4, one can conclude that the efficiency of the formula is not changed for at least two years.

The results of the pharmacokinetic of ivermectin 1% in plasma samples of treated cattle are summarized as following:

Observed peak plasma concentration (C_{max}) was 76.3 ± 13.8 ng ml^{-1}

Time to reach C_{max} (t_{max}) was 0.9 ± 0.2 day.

The absorption half-life (t1/2ab) 0.3 ± 0.2 days. Elimination half-life (t1/2el) 2.8 ± 0.7 days. Apparent area under the concentration–time curve (AUC) was 164.2 ± 12.1 ng day ml−1.

Mean residence time (MRT) was 4.2 ± 1.3 days.

No clinical symptoms, mortality or change in behaviors were recorded in all dosed mice and rats of both sexes groups indicating the formula at its therapeutic doses. The results of clinical examination revealed that both treated drug groups (formula and commercial) showed same recovery pattern from nematodal infection in both sheep and cattle. The follow up therapy indicated that complete eradication of parasites, larvae or eggs from collected fecal samples examined microscopically indicated good therapeutic effect that listed in the report sent to I.V.S.C.

The results of testing the quantity and quality of 1% ivermectin injectable solution formula by the Board Of Drug And Biological Standardization that listed in the report sent to I.V.S.C. indicating that the formula comply with the international standards of quantity, quality, sterility and stability for such concentration product.

As the drug concentration increased, a marked and rapid change from motile to non-motile was observed for both ivermectin treated *Strogyloides. ratti* and *Strogyloides Venzeualli* larvae. The transition in pattern of motion from motile to non-motile involved a change from rapid and sustained sinusoidal motion to an only transient sinusoidal movement that was sustained for no more than 3–4 strokes, and which was often combined with a readily apparent stiffness in a section of the body (the location of this stiffness along the body varied). The movement of these non-motile worms was clearly identifiable as a twitching motion in contrast to the smooth sinusoidal motion of control worms(16), after 30 seconds, the extent and velocity of twitching decreased $P<0.05$ significantly due to Ivermectin is an agonist for (GABA) major inhibitory neurotransmitter. The binding of ivermectin to a neuronal membrane increases the release of GABA. Making them less excitatory and decreasing nerve transmission.

Hyperpolarization of neuronal membranes (at the Neuro-muscular junctiom) leading to mediate a flaccid paralysis in arthropods and nematodes (17).

The same recovery pattern were noticed, when the drug formula tested clinically by the Central Veterinary Hospital in comparison with commercial one for treatment of nematodes infection in both sheep and cattle groups indicating its good therapeutic effect. A report for such a result was sent to I.V.S.C. Also the drug formula when tested by the board of drug and biological standardization approved its matching the universal standard of quantity, quality, sterility and stability. The formula also approved its stability at different

storage temperature, as well as its efficacy concluded from the no change in its chemical analysis results for nearly two years, indicating that that the expiry dates for the formula will be at least two years. These results in addition to the report from Central Veterinary Hospital about its good therapeutic effect, make (I.V.S.C) issued a certificate indicating matching of the new 1% ivermectin injectable solution formulation that of the universal standard for such drug concentration and approved the drug for veterinary therapeutic uses.

Table, 4: Results of chemical analysis

Time of analysis	Temp ◦C	pH	activity	Date
zero time	30	5 – 5.5	101%	2008/4/14
After 2 months	45	5 – 5.3	99.8%	2008/6/18
After 8 months	15	5.4	99.8%	2008/12/18
After 14 months	45	5.3	% 99.8	2009/6/8
After 20 months	15	5.4	% 99.5	2009/12/5

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تركيبة الافرمكتين 1% كمحلول حقن للإستعمال البيطري

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الخلاصة

تم تحضير تركيبة جديدة لعقار الافرمكتين كمحلول حقن 1% للإستعمال البيطري طبقا للمعلومات المسقاة من دساتير الأدوية العالمية وبمواد قياسية استعملت لتحضير ثلاثة تراكيب تجريبية والتي منها حضرت التركيبة النهائية. تم فحص ثباتية التركيبة الجديدة في مختلف درجات الحرارة والفترات الزمنية وذلك بالإعتماد على التغيرات في الصفات الفيزيائية والكيميائية (اللون، الشفافية،درجة الحامضية) ولمدة عشرون شهرا. أظهرت نتائج فحص التركيبة في الزجاج وفي الابقار والأغنام المصابة بالطفيليات فعاليتها كمضاد لحركة اليرقات والطفيليات ومعالجة الإصابات الطفيلية عند تجريبها سريريا. أظهرت نتائج التحليل الكيمياوي عدم وجود أي تغير في تركيز الأفرمكتين في التركيبة ولمدة سنتين. وهذه التركيبة ممكن ان تحقن تحت الجلد او بالوريد و تمت المصادقة على التركيبة الجديدة من قبل الشركة العامة للبيطرة/ العراق كعقار للإستخدام البيطري واعطيت شهادة بذلك.

الكلمات المفتاحية:, تركيبة , افرمكتين , محلول , حقن 1% ,استعمال بيطري.