Health effects of residues of ivermectin use in sheep on some organs (liver and kidney) in Karbala areas

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Abstract

This study aims to detect ivermectin (IVM) residues in the liver and kidneys of sheep by using high-performance liquid chromatography (HPLC) technology. 50 samples (each of 25 livers and 25 kidneys) were collected from five different areas in Karbala Governorate. From October 2023 to January 2024. The analysis showed that samples of both liver and kidneys contaminated with ivermectin residues exceed the maximum limits for ivermectin residues, and according to the previously permitted maximum limits for ivermectin residues by the World Health Orgnasation and the Food and Agriculture Orgnasation, they show a significant difference between the regions of the governorate. As shown in the results of sheep liver and according to region (AL Hur, Al Hussaina, center, Tauweraij and Aeen tumer) and compared with the maximum limits of residues in the liver (0.06) The results were higher than the normal limit for residues (3.74, 1.87, 1.13, 0.52 and 0.21) respectively. As for the results of sheep kidneys and according to regions (Al Hussaina, center, Aeen tumer, Tauweraij and AL Hur) and compared with the maximum limits of residues in the kidney (0.02) The results were higher than the normal limit for residues (3.51, 2.44, 1.68, 0.82 and 0.37) respectively. The results showed that contamination of foods of animal origin with pesticides occurs due to incorrect use of the pesticide, failure to take into account the withdrawal period of the pesticide in the tissues of the animal's body, and failure to observe special instructions when using it. The results of this study confirm the need for monitoring programs for eliminating pesticide residues and external parasites in animal products to protect consumer health from risks of exposure to these residues.

Keywords: Ivermectin (IVM), high-performance liquid chromatography (HPLC), residues, contaminated.

Introduction

Ivermectin (IVM) is a macrocyclic lactone, which is a class of antiparasitic Medicines derived from soil microorganisms Streptomyces avermitilis, it is used against internal and external parasites [1], it was discovered in 1976, but its use is related to its control. Internal and external parasites began to appear in animals only in 1981[2]. A class of highly lipophilic substances with low solubility in water. Ivermectin is a mixture of products homologous to B1a and B1b the components differ from each other by one methylene unit [3]. Ivermectin is one of the most widely used medications in the pharmaceutical treatment of parasitic diseases in food production animals due to a wide spectrum of activity, high effectiveness There is a wide margin of safety for the target species.

As in the condition of any therapeutic agent administered for food production [4]. It depends on the drug, but after it is given to animals, the tissue with the highest drug residue are usually the liver and kidneys. The liver is residue control target tissues and unmodified parent drugs have been shown to be the major residue components in animal liver [5]. Ivermectin residues in animal tissues are a major safety concern for animal food consumers. Ivermectin residues are responsible for many health ailments risks it may cause a mild mazotic reaction, including fever, itching, Arthralgia, myalgia, postural hypotension, oedema, lymphadenopathy, hence the gastrointestinal symptoms, sore throat, cough, and headache it becomes necessary for waste to be strictly regulated from a food safety point of view [6].

In that way increasing monitoring of the presence of these compounds has an important role in monitoring food quality and require a method capable of detecting these compounds in smaller levels. For quantitative analysis it is necessary to use it useful techniques such as thin layer chromatography, liquid chromatography, and immunohistochemistry The important approach is based on the high-performance liquid chromatography (HPLC) method [7]. High-performance liquid chromatography (HPLC) methods were used to determine the amount of ivermectin in sheep liver and kidneys. This study aims to determine the residues of ivermectin in sheep meat and fat tail. This study was conducted in Karbala Governorate, Iraq.

Materials and Methods

A study was conducted to examine ivermectin (IVM) residues in sheep liver and kidneys samples from five different areas of Karbala Governorate (Al-Husseiniyah, Al-Hur, Twairyij, City center, and Ain Al-Tumar). 25 sheep liver samples and 25 kidney samples were obtained (5 liver and 5 kidney samples for each area). Meat and tail fat samples (100 g) were collected from the butcher shops. For each region, samples were collected 24 hours before the extraction process, and the samples were kept at moderate temperatures. Samples were extracted from October 2023 to January 2024. All samples were analyzed to determine ivermectin (IVM) residue using a high-performance liquid chromatograph (HPLC) manufactured by Shimadzu Corporation, Japan.

Preparation of reagents

Special reagents for the extraction process were prepared in the Nutrition and Public Health Laboratory / College of Veterinary Medicine, University of Karbala.

Preparation of reagents A: Acetonitrile 99.9%, 1-methylimidazole, trichloroacetic acid and distilled water. 5 mL of 1-methylimidazole and 5 mL of 99.9% acetonitrile were placed in an amber glass bottle.

Preparation of reagents B: 0.1 g of trichloroacetic acid (TCA) was mixed with 100 ml of distilled water, 10 ml of the resulting solution was taken, placed in an amber glass vial and 5 ml of acetonitrile 99.9% (ACN) was added, thus, the second reagent B was prepared.

Sample preparation

Extraction process

To prepare a simple and easy sample of ivermectin in sheep tissue, solid-liquid extraction (SLE) was adopted on the basis of the initial report on this study, one of the most common methods of extracting antiparasitic from solid matrices is solid-liquid extraction, where the solid tissue is chopped up into small pieces and then homogenized with organic solvent.

The experimental sample was ground using a blender for 5 minutes to obtain an equal and uniform reaction. After completing the grinding, 5 grams of the ground sample were taken and placed in the test tube. Add 25 ml of acetonitrile to each test tube containing the ground sample. After adding acetonitrile and mixing the samples, place the test tubes on the magnetic stirrer for half an hour. This step aims to facilitate the effective extraction of drug substances. After the magnetic stirrer period ends, leave the samples for a quarter of an hour until they settle again. After that, separate the liquid (extracted) part again from the solid part.

Repeat the same steps of the previous process on the same sample, so that the liquid portion becomes approximately 50 ml. After separating the liquid fraction, transfer it to centrifuge tubes. Place the centrifuge tubes in the centrifuge and perform the centrifugation for a quarter of an hour at a cycle of about 4500. This step aims to concentrate the substance and separate other impurities. After extracting the concentrated liquid portion from the centrifuge, stored in a clean conical flask (250 ml) and evaporated to dryness in a water bath.

Adjust dryness conditions appropriately, such as temperature not exceeding 50 Celsius according to approved procedures. After completing the drying process, the dried residue was reconstituted using 1 ml of acetonitrile, after washing the sample add the reagents that were previously prepared for detection, the first reagent A (100 micro pipette) Consists of 5ml from 1-Methylimidazole and 5ml of acetonitrile 99.9% and adding the second reagent B (150 micro pipette) Consists of 10ml of Trichloro acetic acid (TCA) and 5ml of Acetonitrile 99.9% (ACN).

Sample analysis

HPLC analysis

Chromatographic Conditions

Samples were collected and prepared for quantitative analysis using HPLC The first solution is called the mobile phase from which it was prepared from ACN: D.I (70: 30). The stationary phase is the specific column analysis column by type C18, which are column components silica and carbon, and adjust the calibrator to the designated stage at a rate inject 20 µL for 10 minutes. Standard solutions of ivermectin were prepared by dissolving one mg of the compound in ten ml of methanol to obtain a final concentration of 100ppm. Stock standard solutions were packed in amber glass and stored at 20 0C to prevent photodeterioration. Stock solutions were diluted with methanol to produce a series of working standard solutions (2.5, 3.3,,, 5) that were produced weekly [8,9].

Chromatographic separation condition: The gradient elution by using a mixture of ACN (mobile phase) and deionized water was applied (8,9). The chromatographic column (ivermectin): The mobile phase was acetonitrile flow A = deionized water /10 mM ammonium acetate B: acetonitrile flow rate at 1.0mL/min, the column was C18 – ODS (25 cm * 4.6 mm), and the detector (UV- 240 nm). Injection volume: 100µl [10].

Statistical analysis

The data were analyzed using SAS (10) software and the results were compared using the least significant difference (LSD) value at the probability level of $P< 0.05$.

Results and Discussion

Through statistical analysis, these results appeared compared to the normal residual recommended by the World Health Organization. It was found that there was a significant difference between the data obtained and the natural residual for sheep liver samples, as shown in (Table 1) and (figure 1). Analysis was conducted on 25 samples of sheep liver to determine ivermectin residues in the samples, and the results showed the presence of ivermectin residues exceeding the maximum percentage of residues. In comparison with the percentage of pollution in the regions, it was found that the Al-Hur area had a concentration of (3.74), followed by Al Hussaina area with a concentration of (1.87), the city center area with a concentration of (1.13), the Tauweraij area with a concentration of (0.52), and the Aeen al-Tumer area with a concentration of (0.21), respectively.

Table (1): Ivermectin residues in sheep liver with maximum residue limits

Area	WHO	Al Hussaina	AL Hur	center	Tauweraij	Aeen tumer	LSD
Result	0.06	1.87 ±0.009 B	3.74 ±0.04 A	1.13 ±0.003 C	0.52 ±0.003 D	0.21 ±0.003 E	0.0567

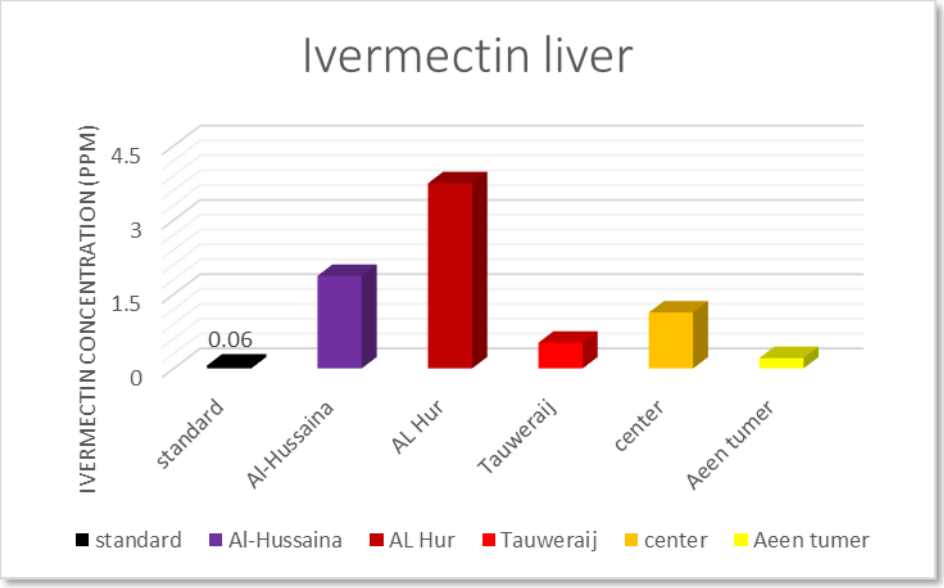

Figure (1): ivermectin concentrations (PPM) in sheep liver

Samples of sheep kidneys to determine ivermectin residues in the samples, and the results showed the presence of ivermectin residues exceeding the maximum percentage of residues. In comparison with the percentage of pollution in the regions, it was found that the Al Hussaina area had a concentration of (3.51), followed by the city centre area with a concentration of (2.44), and the Aeen al-Tumer area with a concentration of (1.68), respectively, as shown in Table (2) and Figure (2).

Table (2): Ivermectin residues in sheep kidney with maximum residue limits

Area	WHO	Al Hussaina	AL Hur	center	Tauweraij	Aeen tu-mer	LSD
Result	0.02	3.51 ±0.01 A	0.37 ±0.002 E	2.44 ±0.01 B	0.82 ±0.003 D	1.68 ±0.008 C	0.0333

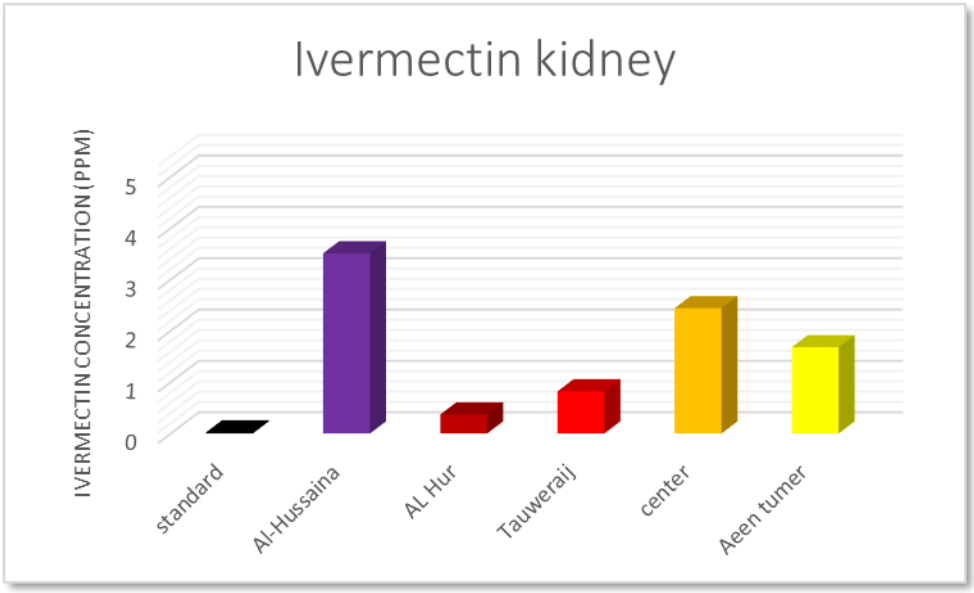

Figure (2): ivermectin concentrations (PPM) in sheep kidneys

Table (3): Concentrations of ivermectin residues in both sheep liver and kidney

Area	Means ± S.E	
	ivermectin	
	Liver	Kidney
Al-Hussaina	1.87± 0.009 B	3.51± 0.01 A
AL Hur	3.74± 0.04 A	0.37± 0.002 E
Tauweraij	0.52± 0.003 D	0.82± 0.003 D
center	1.13± 0.003 C	2.44± 0.01 B
Aeen tumer	0.21± 0.003 E	1.68± 0.008 C
LSD	0.0567	0.0333

Means with common or similar letters do not differ significantly

Table (3) shows that both sheep liver and kidney samples have similar concentrations to the percentages of ivermectin residues. [11] demonstrated not only higher residuals levels in the liver but the drug also has the longest duration half-life in the liver. The liver is the last tissue to remove drug residue, but not all tissues contain detectable residue by 42 days after the dose.

The results of ivermectin residues in sheep liver samples at the level of Karbala governorate show that all governorate regions, in order: Al-Hur > Al-Hussaina > City Center > Tauweraij >Aeen al-Tumer. For the kidneys, the percentages of residuals were in order: Al-Hussaina > City Center > Aeen al-Tumer> Tauweraij >Al-Hur. Compared to the maximum residue limits, it was found that both the liver and kidneys have high rates of contamination with ivermectin, and this is due to incorrect use, as well as failure to take into

account the depletion time of the substance in the animal's body before slaughtering the animal. Thus, it leads to the appearance of high levels of waste in the animal's body tissues, which leads to harm to consumers.

The liver is residue control target tissue and unmodified parent drugs have been shown to be the major residue components in animal liver [5]. It was revealed by the researcher [11] using an HPLC device the highest residue Levels in the liver but the drug also has the longest duration half-life in the liver. The liver is the last tissue that removes drug residues. As for [12] A study was also conducted on proving the presence or absence of ivermectin residue in the liver of food-producing farm animals in sheep. the study also demonstrated that there were no positive results in the samples analyzed. [13], it contains the second highest levels of ivermectin after lipids. Kidney and muscle tissue had lower levels of residue, and this study is similar to the findings [14]. [15] As shown through the study, Ivermectin residue the maximum residue level (MRL) was found to be exceeded in one sample. As in [16] The study aimed to identify ivermectin residues in the liver of cattle. Only 22% (20/90) of the samples analyzed showed ivermectin residues.

To ensure the safety of the consumer from the side effects of eating products of animal origin contaminated with residues of veterinary medicines. Automated techniques and accurate detection of chemical residues at levels used by the HPLC device in this study show, using the HPLC device, high levels of ivermectin residues compared to the maximum residue limits.

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