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Preparation and Characterisation of Liposomal Formulations of Levamisole and Albendazole Used in Veterinary Medicine

Preparación y caracterización de formulaciones liposomales de levamisol y albendazol utilizadas en medicina veterinaria

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ABSTRACT

The aim of this study is to by converting albendazole and levamisole, which are antiparasitic drugs used in both humans and animals, into liposomal formulations under laboratory conditions. To ascertain the circumstance in practice, characterization studies were additionally conducted. The study was performed by modifying the hydration of the thin lipid film. Experiments were carried out with egg phosphatidylcholine, cholesterol, chloroform and methanol in different amounts. Albendazole and levamisole formulations were made with the substances used in liposomes. Zeta potential, polydispersity index, encapsulation efficiency, particle size measurements and scanning electron microscopy were performed as part of characterization studies. The results show that Lipo LVM has the smallest particle size value at 380.87 ± 19.52 nm, whereas Lipo LVM–PBS has the largest particle size value at 7236.67 ± 443.89 nm. Values for the polydispersity index fall between 0.527 and 0.896. Zeta potential levels, on the other hand, range from -7.6 mV to -46.8 mV. While this value was determined as -8.2 ± 0.4 mV in LD Lipo ABZ and -18.4 ± 0.6 mV in HD Lipo ABZ, respectively. Both HD Lipo ABZ and LD Lipo ABZ have polydispersity indices for ABZ of 0.529 ± 0.066 and 0.896 ± 0.085, respectively. It was found that the particle size rose as the desired amount of liposomal albendazole increased. It was found that the liposomization of albendazole was higher than that of levamisole. Albendazole and levamisole liposomal formulations were successfully developed in the investigation. By carrying out characterization studies, it was discovered that it may be employed in clinical trials. In the upcoming years, it is anticipated that continuous research in the field of nanotechnology will improve human and animal health and aid to more effectively control parasite infestations.

Key words: Albendazole, levamisole, liposome

RESUMEN

El objetivo de este estudio es convertir albendazol y levamisol, fármacos antiparasitarios utilizados tanto en humanos como en animales, en formulaciones liposomales en condiciones de laboratorio. Para comprobar la circunstancia en la práctica, se realizaron además estudios de caracterización. El estudio se realizó modificando la hidratación de la fina película lipídica. Se realizaron experimentos con fosfatidilcolina de huevo, colesterol, cloroformo y metanol en diferentes cantidades. Se realizaron formulaciones de albendazol y levamisol con las sustancias utilizadas en los liposomas. Como parte de los estudios de caracterización se realizaron mediciones del potencial zeta, el índice de polidispersidad, la eficacia de encapsulación, el tamaño de partícula y la microscopía electrónica de barrido. Los resultados muestran que Lipo LVM tiene el valor de tamaño de partícula más pequeño con 380,87 ± 19,52 nm, mientras que Lipo LVM–PBS tiene el valor de tamaño de partícula más grande con 7236,67 ± 443,89 nm. Los valores del índice de polidispersidad se sitúan entre 0,527 y 0,896. Por otra parte, los niveles de potencial zeta oscilan entre -7,6 mV y -46,8 mV. Mientras que este valor se determinó como -8,2 ± 0,4 mV en LD Lipo ABZ y -18,4 ± 0,6 mV en HD Lipo ABZ, respectivamente. Tanto HD Lipo ABZ como LD Lipo ABZ tienen índices de polidispersidad para ABZ de 0,529 ± 0,066 y 0,896 ± 0,085, respectivamente. Se observó que el tamaño de partícula aumentaba a medida que aumentaba la cantidad deseada de albendazol liposomal. Se comprobó que la liposomización del albendazol era mayor que la del levamisol. En la investigación se desarrollaron con éxito formulaciones liposomales de albendazol y levamisol. Al realizar estudios de caracterización, se descubrió que pueden emplearse en ensayos clínicos. Se prevé que en los próximos años la investigación continua en el campo de la nanotecnología mejore la salud humana y animal y ayude a controlar más eficazmente las infestaciones parasitarias.

Palabras clave: Albendazol, levamisol, liposoma

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INTRODUCTION

Tetramisole (levamisole), an ingredient of the imidazothiazole derivatives class of anthelmintic medications, is particularly effective against nematodes in the respiratory tract and gastrointestinal tract. Tetramizole's L – isomer, levamisole, is a broad – spectrum medication. The confidence interval was enlarged by employing the L – isomer alone because it was discovered that the drug's anthelmintic action emanated from the L – isomer. Levamisole is also known to have an immunomodulatory. For example, in a study; It has been stated that the vaccine has a strengthening effect on the immune system by increasing the protective effect of the Brucella vaccine in mice [1, 2].

For the treatment of parasitic infestations brought on by helminths, the drug albendazole, a benzimidazole derivative, is used in both veterinary and human medicine. It exhibits great efficacy at low doses and a broad spectrum of anthelmintic effects. Its chemical formulation is $C_{12}H_{15}N_3O_2S$ and its molecular weight is 265.33 g·mol^{-1}. Albendazole is a whitish powder with a melting point of 209°C [3]. It has been stated by researchers that albendazole shows specific and high toxicity in parasites compared to mammals and forms the mechanism of action by inhibiting the polymerization of tubulins into microtubules. Since albendazole is less soluble in water and organic solvents, increasing its solubility greatly increases its absorption and therefore its effectiveness Biotransformation of albendazole is primarily mediated by cytochrome P450 and microsomal flavin monooxygenase enzymes. Following absorption, it undergoes first–pass effects in the liver and intestines and is subsequently metabolized to sulfoxide. The plasma half–life is 4 – 15 hours (h) and its metabolites are excreted through urine, feces, and bile [4, 5].

Liposomes, dendrimers, nanoemulsions, polymeric, and metallic nanoparticles are examples of nanomaterials used in medicine. These materials are primarily used for purposes such as directing drugs to the target tissue, reducing drug side effects, and reducing labor and costs as a result of increasing the bioavailability of drugs. Liposomes were first described as a model for the cell membrane by Alec Bangham in the 1960s. Liposomes, one of the drug delivery systems are biocompatible spherical vesicles with a diameter of about 2 – 3.5 µm, consisting of single or intertwined layers with an aqueous phase between them. They can carry water–soluble active components in the hydrophilic central part and hydrophobic components that are insoluble in water in their membranes. Its advantages are that it is resistant to environmental effects without side effects, and that it is a carrier system that allows the delivery of bioactive components into the cell and even to the compartments within the cell, reducing the side and toxic effects of drugs. It also has properties such as increasing the permeability and bioavailability of drugs with a short half–life, ensuring the stability of drugs, changing the pharmacokinetics of liposomal substances, and bringing the size of drugs to the desired size [6, 7, 8]. The use of nanotechnologically prepared drugs in the field of medicine is limited. The formation of resistance in parasites to commercially available drugs is a major problem in the fight against parasitic infestations. In this sense, new drug delivery systems are needed to increase the effectiveness of drugs.

Some studies, it is aimed to develop a new drug delivery system and to increase drug effectiveness against parasites thanks to this carrier system. For this purpose, the use of liposomal formulations and nanoparticles in drugs used in parasite control has become interesting [9]. The development of drug resistance by parasites appears to be the major obstacle for research in the fight against parasites. This is where the serious benefits of nanoparticles come into play. These are very reasonable systems to reduce the resistance that can develop against traditional drugs used against parasites, to prevent the formation of some of the resistance development mechanisms, and to improve the bioavailability of drugs. In contrast to other uses, the world of medicine now has a new therapeutic option available thanks to nanotechnology. Because the ability to produce and manage nano–sized substances in this field means new opportunities.

The aim of this study was to produce and characterise liposomal formulations of albendazole and levamisole for medical use in the treatment and prevention of parasite infestations. In the subsequent study, the pharmacokinetics of the liposomes produced according to the routes of administration and dosages in animals will be evaluated and their effectiveness will be demonstrated.

MATERIALS AND METHODS

Different physicochemical features of the active ingredient were identified, and pre–formulation trials were conducted to maximize medication distribution. Naeem *et al.* [10] used chloroform as 2.5 ml while preparing lecithin liposomes. Khoshneviszadeh *et al.* [11] used 15 ml chloroform and 5 ml methanol while preparing hydroquinone liposomes. Ahmad *et al.* [12] preferred the ratio of chloroform : methanol as 1 : 1 while preparing isotretinoin liposomes. The solvents and ratios used in this study were decided by considering the chloroform : methanol ratios and amounts used in the studies. During the preparation of liposomal levamisole, chloroform–methanol 2.5 mL–2.5 mL, 5 mL–5 mL used as a solvent were tested. Chloroform–methanol, the solvent used to generate liposomal albendazole, was tested as 2.5 mL–2.5 mL, 5 mL–5 mL, 7.5 mL–7.5 mL, and 12.5 mL–12.5 mL.

It is well established that the formulation and preparation methodologies have a pivotal effect on controlling particle size, shape, polydispersity, and finally capability of liposomes [13, 14]. The Bangham method, also called the thin film hydration method, has been preferred in the preparation of liposomal formulations. This approach has a lot of benefits, including simplicity of use and a low time and labor requirement. This technique works by drying lipids that have been dissolved in an organic solvent, creating and acquiring liposomes in an aqueous medium, and then analyzing the liposomes that have been produced [15].

Levamisole ((LVM), Santa Cruz Biotechnology – sc – 205730, USA), albendazole ((ABZ) Cayman – 23705, USA), egg phosphatidylcholine ((PC) L – α – phosphatidylcholine, USA), and cholesterol ((CL) Acros Organics (Belgium), active substances that are intended to be liposomal, were weighed on a precise scale (Denver Instrument SI–234, Germany), and administered in beakers. Methanol (Sigma – Aldrich, USA) and chloroform (Sigma – Aldrich, USA) were added to them. The beaker's contents were entirely dissolved. To obtain the lipid film, evaporation was carried out for 15 min at a rotational speed (Isolab Laborgerâte GmbH 605.01.001, Germany) of 200 G in a rotary evaporator (Isolab Laborgerâte GmbH 605.01.001, Germany), set to 37°C. In order to take the resulting dry lipid film, 10 mL of distilled water (Lipo LVM–PBS, Oxoid – BR0014G UK) was added to the evaporation bottle and rotated without vacuum. The mixture was then vortexed (Vortex MS 3 basic ika 3617000, Germany), for 3 min. To reduce particle size, it was placed in an ultrasonic bath (MEDISSON, Turkey) for 5 min. The final mixture was placed in tubes for centrifugation and stored in the refrigerator (Arçelik 270530EB, Turkey) at 4°C with the mouths

tightly closed. Centrifugation (ALLEGRA-X64R, USA) was performed at 27000 G for 40 min. The collapsing liposomal part and the aqueous part were placed in separate test tubes and stored in the refrigerator at 4°C for analysis. The amounts and lipid / cholesterol ratios of all the ingredients in the formulations, and the amounts of active drug molecules, solvents, and dispersion liquids are shown in TABLE I.

TABLE I
Liposome-forming substances and their amounts

Formulation Name	Lipid/Cholesterol		Active Drug		Amount of Solvent		Amount of Dispersion
	PC (mg) / CL (mg)		LVM (mg)	ABZ (mg)	Chloroform : Methanol (ml)		Distilled Water (ml)
Lipo LVM	2.5 (50)	1.0 (20)	37	-	1,0 (5)	1.0 (5)	10
HD Lipo LVM	2.0 (50)	1.0 (29)	49	-	1.0 (5)	1.0 (5)	10
LD Lipo LVM-1	3.0 (49)	1.0 (19)	25	-	1.0 (5)	1.0 (5)	10
LD Lipo LVM-2	2.0 (49)	1.0 (21)	25	-	1.0 (5)	1.0 (5)	10
Lipo LVM-PBS	2.0 (49)	1.0 (25)	37	-	1.0 (5)	1.0 (5)	10 (PBS)
HD Lipo ABZ	3.0 (50)	1.0 (19)	-	50	1.0 (12.5)	1.0 (12.5)	10
LD Lipo ABZ	3.0 (50)	1.0 (19)	-	25	1.0 (12.5)	1.0 (12.5)	10

Lipo LVM: Liposomal Levamisole; HD Lipo LVM: High Dose Liposomal Levamisole; LD Lipo LVM-1: Low Dose Liposomal Levamisole-1; LD Lipo LVM-2: Low Dose Liposomal Levamisole-2; Lipo LVM-PBS: Liposomal Levamisole-Phosphate Buffer Solution; HD Lipo ABZ: High Dose Liposomal Albendazole; LD Lipo ABZ: Low Dose Liposomal Albendazole

Particle size, polydispersity index, and zeta potential of the liposomes were measured using Malvern Zetasizer Nano - ZS (ZEN3600, UK) equipped with dynamic light scattering (DLS) and electrophoretic light scattering techniques [16]. For this purpose, 1 ml of distilled water was added to the precipitated liposomes as a result of centrifugation and dispersed. 120 µl of these samples were taken and placed in the measuring cuvette, and 1880 µl of distilled water was added to it. All measurements were performed at 25±0.1°C. Obtained results are presented in TABLE II.

TABLE II
Characterization results of liposomal LVM and ABZ formulations

Formulation	Particle size (nm) (mean±SD)	Polydispersity index (mean±SD)	Zeta potential (mV) (mean±SD)	Encapsulation efficiency (%) (mean±SD)
Lipo LVM	353.4±19.52	0.527±0.037	-46.8±0.3	25.82±0.03
HD Lipo LVM	1397.67±72.63	0.852±0.158	-15.7±0.7	34.19±0.17
LD Lipo LVM-1	1169.67±66.51	0.630±0.130	-43.9±0.8	35.90±0.19
LD Lipo LVM-2	2000.00±84.67	0.701±0.191	-14.9±0.4	32.31±0.85
Lipo LVM-PBS	7236.67±443.89	0.756±0.081	-7.6±0.2	43.14±0.17
HD Lipo ABZ	4777.33±1150.22	0.529±0.066	-18.4±0.6	99.33±0.00
LD Lipo ABZ	2243.00±288.31	0.896±0.085	-8.2±0.4	99.56±0.00

nm: nanometer, mV: milivolt, SD: standard deviation, Lipo LVM: Liposomal Levamisole, HD Lipo LVM: High Dose Liposomal Levamisole, LD Lipo LVM-1: Low Dose Liposomal Levamisole-1, LD Lipo LVM-2: Low Dose Liposomal Levamisole-2, Lipo LVM-PBS: Liposomal Levamisole-Phosphate Buffer Solution, HD Lipo ABZ: High Dose Liposomal Albendazole, LD Lipo ABZ: Low Dose Liposomal Albendazole

The encapsulation efficiency (EE) of liposomes was calculated using the following formula (Eq.1) [17].

$$EE(\%) = \frac{Total\ drug - Free\ drug\ in\ supernatant}{Total\ drug} \times 100 \quad \textbf{Eq.1}$$

For this purpose, firstly separate calibration samples were prepared for LVM and ABZ. After a known amount of LVM was dissolved in some distilled water, it was completed to a certain volume with distilled water. Based on this stock solution of LVM, a series of dilutions were made with distilled water and the absorbances of the calibration samples prepared at 0.05, 0.5, 2.5, 5, and 10 µg·ml^{-1} concentrations were measured with a UV - Vis spectrophotometer (Shimadzu, Japan) at a wavelength of 213 nm. The calibration equation is obtained as $y = 0.1344x + 0.0098$ and $y = 0.1438x + 0.0126$, the R^2 value of both equations are 0.9997 and 0.9996 for LVM and ABZ, respectively. Calibration graphs were shown in FIG. 1.

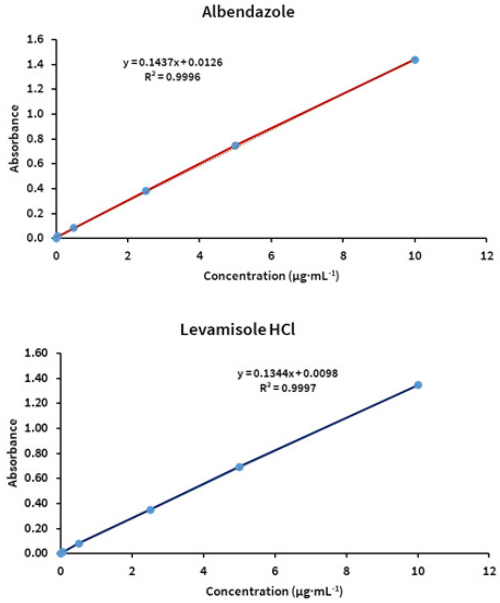

FIGURE 1. Calibration graphs of active substances

By using the calibration equation of the relevant drug (LVM or ABZ), first of all, the amount of free drug in the supernatant was determined. Then, the amount of LVM and ABZ - loaded into liposomes with Eq.1 was calculated. After these procedures, a known amount of ABZ was dissolved in some methanol and then completed to the desired volume with methanol. A stock solution was prepared by taking 5 ml of this solution and adding 5 ml of distilled water. Calibration samples at the same concentrations as LVM were prepared by making a series of dilutions with methanol : distilled water (1 : 1 v/v) mixture using this stock solution of ABZ. The absorbances of the samples were measured using a UV - Vis spectrophotometer (Shimadzu UV - 1900i, Japanese) at a wavelength of 215 nm.

For encapsulation efficiency analysis, measurement samples were prepared by making various dilutions from the supernatant parts of the prepared and centrifuged liposomes. Distilled water was used to dilute the supernatant of LVM - loaded liposomes. In ABZ - loaded

liposomes, the supernatants were first diluted with methanol at a ratio of 1 : 1 (v/v supernatant : methanol), and then a 1 : 1 methanol:water mixture was used for further dilution when necessary. The absorbance measurements of these samples were performed at 213 and 215 wavelengths for LVM and ABZ, respectively at 25 ± 0.5°C.

Scanning Electron Microscopy (SEM) Analysis

About 0.02 g of liposomized levamisole and albendazole were weighed out to obtain scanning electron microscopic images. The sample was placed on a bidirectional carbon tape. Gold plating was first carried out by applying a vacuum of 8×10^{-1} mbar·Pa^{-1} and a voltage of 10 mA in a quorum coating device (Quorum, Japanese). Liposomal levamisole and albendazole was examined in SEM (JEOL Neoscope JCM – 5000, Japanese) at 2400× magnification, respectively (FIGS. 2a and 2b).

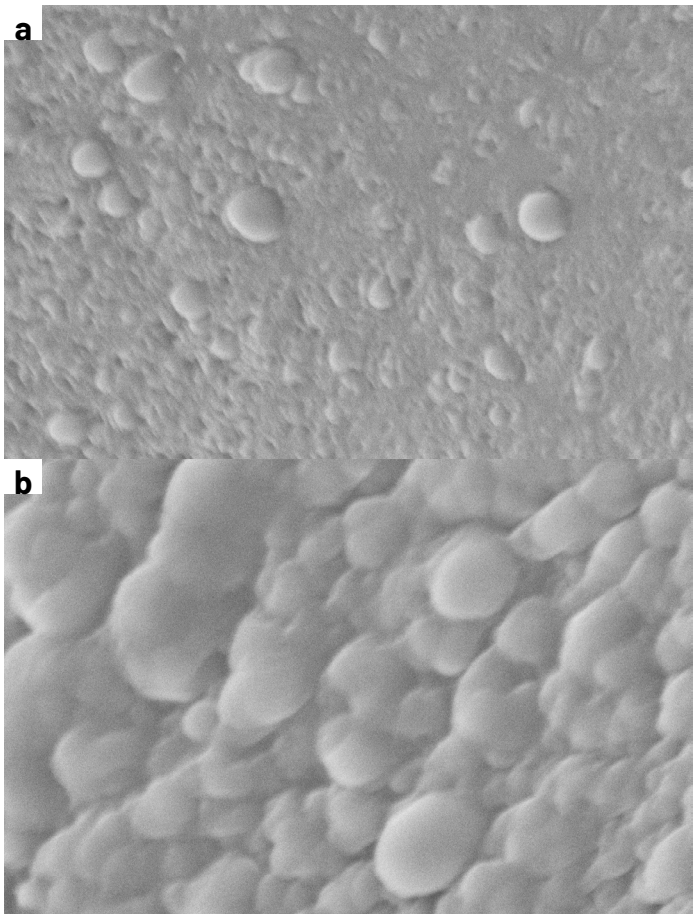

FIGURE 2. a: Liposomal levamizole imaging under SEM, b: Liposomal albendazole imaging under SEM

Statistical Analysis

Statistical analysis of experimental findings was performed using GraphPad Prism 5.0 by student *t*-test and all data were expressed as mean ± standard deviation (SD). *P*-values less than 0.05 were considered statistically significant values.

RESULTS AND DISCUSSIONS

Particle Size, Zeta Potential, Polydispersity Index, Encapsulation Efficiency

In the preparation of liposomal levamisole, initially 2.5–2.5 mL of chloroform–methanol was preferred as the solvent. However, levamisole did not completely dissolve. Therefore, chloroform–methanol in an amount of 5 mL–5 mL was taken. Here, solvent ratios of 1 : 1 were preferred.

In the preparation of liposomal albendazole, initially 2.5–2.5 mL of chloroform – methanol was preferred as the solvent. However, albendazole did not completely dissolve. Therefore, chloroform–methanol in an amount of 5 mL–5 mL was taken. Once more, dissolved was not accomplished. Low–dose albendazole dissolved when chloroform–methanol contained 7.5 mL–7.5 mL. High dose albendazole dissolved in chloroform–methanol 12.5 mL–12.5 mL. Therefore, 12.5 mL–12.5 mL were preferred in both formulations to ensure that the results would not be impacted by the solvent utilized. Here, solvent ratios of 1 : 1 were preferred.

According to the results, the lowest particle size value belongs to Lipo LVM with 380.87 ± 19.52 nm, and the highest particle size value belongs to Lipo LVM – PBS with 7236.67 ± 443.89 nm. When phosphate buffer was used instead of distilled water for liposomal levamisole, it was found that the particle size increased while the encapsulation efficiency increased. Except for the lipo LVM–PBS formulation, it was thought to be in sizes that could be used in animals. The polydispersity index values for LVM are in the range of 0.527–0.756. The polydispersity index of ABZ was found to be 0.529 ± 0.066 for HD Lipo ABZ and 0.896 ± 0.085 for LD Lipo ABZ. When looking at the table, it is seen that the lowest and highest polydispersity indices are in Lipo LVM and LD Lipo ABZ, respectively. Zeta potential values for liposomal levamisole were found to be between -7.6 ± 0.2 and -46.8 mV ± 0.3. Here, the smallest zeta potential value belongs to Lipo LVM–PBS with -7.6 ± 0.2 mV, and the highest zeta potential value belongs to Lipo LVM with -46.8 ± 0.3 mV. On the other hand, this value was measured as -18.4 ± 0.6 mV in HD Lipo ABZ and -8.2 ± 0.4 mV in LD Lipo ABZ. Polydispersity indices for ABZ were found 0.529 ± 0.066 in HD Lipo ABZ and 0.896 ± 0.085 in LD Lipo ABZ. It was determined that the particle size increased as the amount of albendazole desired to be liposomal increased. The liposoming rate of albendazole was found to be higher than levamisol. According to Table II, the encapsulation yields of HD Lipo ABZ and LD Lipo ABZ seem to be quite good. It has been found that the particle size increases as the amount of the drug that is required to be encapsulated in ABZ increases. Although the particle size of albendazole liposomal formulations is high, it is within the usable range in animals. Zeta potential values indicate that it is monodisperse, but the result is better in LD Lipo ABZ where the polydispersity index is close to 1. It was considered to reduce the particle size by prolonging the sonication time. However, it was thought that prolonged sonication may damage the active substance in the liposome. The particle size was considered to be suitable for use in animals. Accordingly, the lowest EE value was found to be 25.82 ± 0.03% and the highest 43.14 ± 0.17% in LVM–loaded liposomes, while this value was approximately 99% in ABZ – loaded liposomes (Table II).

When liposomes are classified according to size and number of layers; Small Unilamellar Vesicles (SUV): 20 – 100 nm, Medium Unilamellar Vesicles (MUV): 40 – 100 nm in size and 1 lipid bilayer, Large Unilamellar Vesicules (LUV): Larger than 100 nm, Giant Unilameller Vesicules (GUV): Larger than 1 µm Multilayer Vesicles (OLV): 100 – 1000

nm Multilayer vesicles (MLV): larger than 500 nm, Multiple Vesicles (MVV): Larger than 5000 nm have been reported [18, 19]. When the prepared liposomes were classified according to their size and layers; Lipo LVM was determined as Large Unilamellar Vesicules (LUV), HD Lipo LVM, LD Lipo LVM – 1, LD Lipo LVM – 2, HD Lipo ABZ, LD Lipo ABZ were determined as Giant Unilameller Vesicules (GUV) and Lipo LVM – PBS was determined as Multiple Vesicles (MVV).

Scanning Electron Microscopy (SEM)

Images of the liposomal formulations are shown in Figure 2. The prepared liposomes were observed to be nano-sized and roughly spherical in shape in suspension by SEM analyses. Liposomal levamisole was visualised at 10 KV × 2400 magnification in 10 µm size and liposomal albendazole was visualised at 10 KV × 2400 magnification in 5 µm size.

Nanotechnology opens up new perspectives for applications in the fields of biology, biotechnology, medicine, and veterinary medicine. New possibilities in animal and human health are made possible by the use of nanotechnology to the development of efficient products and applications for animals. The effectiveness of medications used in human and veterinary medicine will soon change as a result of this research.

In a study by Liu *et al.* [20], engineered stem cell biomimetic liposomes carrying levamisole were prepared. They reported that the particle size of liposomal levamisole was 108.4 ± 1.2 nm, 138.3 ± 2.9 nm, 116.1 ± 1.9 nm, respectively. The small particle size facilitates the entry of the drug into the cells, but shortens the circulation time. Because particle size affects the distribution of liposomes in the body and those with very small size are eliminated from the body faster. This may cause a decrease in the bioavailability of the drug. They found that the polydispersity index was 0.108 ± 0.042, 0.280 ± 0.062, and 0.102 ± 0.026, and the zeta potential was -16.85 ± 0.91 mV, -6.72 ± 0.50 mV, -7.23 ± 0.91 mV. Zeta potential values are similar with HD Lipo LVM, LD Lipo LVM–2, Lipo LVM–PBS in this study. Zeta potential values are one of the stability indicators of liposomal formulation. Therefore, monodisperse or polydisperse values are important parameters. When the stability of the liposomal formulation is good, bioresistance in the body, mean residence time, area under the curve values are higher than the free formulation. Although the active substance is different, it is similar to HD Lipo ABZ and LD Lipo ABZ. Particle size and polydispersity index gave better results than this study. The researchers did not calculate the encapsulation rate. It was thought that the different results may be due to the materials used in liposome preparation and the preferred method. In addition, the charge of the liposomal formulation obtained, surface conditions, number of layers and electrostatic interactions between the substances added to the formulation may have caused different results. Liposomal albendazole was prepared by Fülöp *et al.* [21]. Here, although zeta potential and polydispersity index values were better than this study, encapsulation efficiency values were found to be low. Zhang *et al.* [22] conducted a study on the pharmacokinetics and tissue distribution of liposomal albendazole. Particle size, polydispersity index, zeta potential, encapsulation efficiency and SEM analyses of liposomal albendazole characterisation studies were not performed here.

According to TABLE II, although the encapsulation efficiency is low, the best results in terms of particle size and zeta potential were obtained with Lipo – LVM for LVM – loaded liposomes. The low encapsulation efficiency of the liposomal formulation limits its usability. Since other results are better, the usefulness of the drug can be increased by processes that can increase the encapsulation efficiency (such as increasing the amount of lipid). When the use of PBS instead of distilled water as the dispersion medium (Lipo LVM – PBS), the highest particle size and lowest zeta potential were obtained with Lipo LVM – PBS. EPC is a neutral phospholipid, on the other hand in some literature it is said that is a zwitterionic lipid [23, 24, 25, 26]. The zeta potential of liposomes prepared with these lipids is usually lower [27]. Increased negative values of the zeta potential can lead to the system becoming more monodisperse. However, this would be more favourable if the value is greater than -30 mV. However, in TABLE II, see that the zeta potentials of liposomes are different from zero. This may be due to the adsorption of Cl^- ions formed in the aqueous environment as a result of the ionization of the active substance (LVM HCl) to the liposome surface.

The system is considered monodisperse if the positive or negative zeta potential is greater than 30 mV positive or -30 mV negative, respectively. However, values that are around the neutral value that is, less than 5 mV and greater than -5 mV can also lead to aggregation [24]. In the study of the zeta potential values are consistent with the literature. Lipo LVM and LD Lipo LVM–1 formulations are polydisperse, others are monodisperse.

Since the Na^+ and K^+ ions in the buffer will also be adsorbed on the surface of the liposome when PBS is used as the dispersion medium, the decrease in the zeta potential can be explained by this situation [26, 28]. Because the dispersion medium affects the electrostatic interactions of the substances added to the liposomal formulation. The zeta potential value varies according to electrostatic interactions. In addition, by increasing the ionic strength of the medium, PBS may have reduced the electrical double layer barrier around the liposomes and caused the particles to aggregate, and then because of aggregation particle size reduction may have occurred [29, 30]. When PBS was used as a dispersion medium, the encapsulation efficiency of the liposomes significantly increased (P<0.0001).

In another study, the opposite results were observed in cinnamaldehyde–loaded liposomes, and the decrease in encapsulation efficiency was attributed to the increase in ionic strength by the researchers [31]. However, this difference may be because cinnamaldehyde is water–insoluble [32]. This may be because water – soluble LVM creates a common ion effect (Cl^-) with salts from PBS, decreasing the affinity of the active substance to the dispersion medium and consequently increasing both its adsorption to the liposome bilayer and retention in the internal phase of the liposome [33]. Moreover, sodium counter ions in the dispersion medium can reduce the charge of phospholipid and cause a decrease in the interlamellar space of multilayer vesicles formed during the hydration phase, thereby reducing the volume of aqueous inner phase and encapsulation efficiency [34]. LD Lipo LVM – 2 has a lipid / cholesterol ratio of 2 / 1 and contains more cholesterol for the same amount of lipid than LD Lipo LVM – 1. It was shown that the zeta potential of liposomes decreased as the amount of cholesterol in them increased [35]. In this investigation, LD Lipo LVM – 1 and LD Lipo LVM – 2, this was proven. On the other hand, as the amount of cholesterol increased there was a significant decrease in encapsulation efficiency despite the increase in particle size. (P<0.0042).

Active substances with high water solubility, such as levamisole, are located in the internal aqueous phase of the liposome [36]. According to TABLE II, the EE value of levamisole was in the range of 25 – 43%. These values are similar to Egg PC liposomes Katragadda *et al.* [36] containing the hydrophilic active ingredient stavudine. In addition, as shown in this study, it is seen in this results that the lipid : cholesterol ratio does not affect the EE value.

The encapsulation efficiency of ABZ – loaded liposomes was higher than LVM – loaded liposomes. It has been thought that the reason for this is due to the high content of ABZ in the bilayer structure of the liposome instead of the aqueous dispersion medium due to its lipophilic natüre. It was observed that the zeta potential of ABZ – loaded liposomes was lower than that of LVM – loaded liposomes. This may be because ABZ is not ionized in an aqueous medium such as LVM. Although many factors can affect the zeta potential value, this results for the ABZ–loaded liposome are considered acceptable. Because liposomes prepared with egg phosphatidylcholine loaded with cyclosporine Chen *et al.* [37], another lipophilic drug, have also been observed to have low zeta potentials. However, since ABZ is located in the bilayer of the liposome, the particle size and zeta potential increased as the amount of ABZ in the formulation increased.

Pensel *et al.* [38] studied liposomal albendazole in experimentally infected mice. However, characterisation studies are also lacking here. Even if liposomes are used in animal experiments, characterisation studies should be carried out first. Ergin *et al.* [39] prepared and evaluated cholesterol – free liposomes. Although the particle sizes were smaller than this study, the results of polydispersity index, zeta potential and encapsulation efficiency were similar. Zhang *et al.* [40] prepared liposomal ciprofloxacin. They found the particle size smaller than this study. Although encapsulation rates were similar to levamisole, they were much lower than albendazole. Reigada *et al.* [41] prepared liposomal isotretinoin and loratadine formulations. They found the particle size smaller than this study. Although encapsulation rates were similar to levamisole, they were much lower than albendazole. This may be due to the preparation method.

CONCLUSIONS

With the help of this research tried to prepare liposomal formulations of levamisole and albendazole from antiparasitic drugs that are often used in medicine. Different formulation ratios were tried. The tests applied in the characterization studies of liposomes were performed within the laboratory facilities. In this respect, even if the highest encapsulation efficiency in LVM – loaded liposomes is obtained with Lipo LVM – PBS, it will not be the best formulation because the zeta potential is too low – it can negatively affect the colloidal stability – and also the particle size is too high. In the remaining LVM – loaded liposomes, it concluded that HD Lipo LVM is the optimal formulation, which has particle size compatible with the literature data, the highest encapsulation efficiency, and high zeta potential. When viewed with a similar approach, it can be said that HD Lipo ABZ with higher zeta potential is the optimal formulation, since the particle size of both liposomes is compatible with the literature data and the encapsulation efficiency is over 99% in ABZ–loaded liposomes. When the possible mechanisms of action of liposomes on parasites are evaluated, it is possible to benefit from the fact that they provide more drug permeability and increase the bioavailability of the drug, or even directly inactivate the parasites by targeting and delay the re–occurrence of resistance. In order to better understand these effects of liposomal levamisole and albendazole, it was thought that they should be studied in natural or experimentally induced parasitic infestations.

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Since the research carried out is a laboratory study and does not include animal experiments, it does not contain any ethical issues.

Conflict of Interest

The authors declared that there is no conflict of interest.

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