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Evaluation of albendazole and mebendazole-loaded polymeric nanoparticles efficacy against larval stages of the *Echinococcus granulosus* parasite in vitro and in vivo

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A B S T R A C T

This study assessed the effectiveness of albendazole and mebendazole-loaded polymeric nanoparticles against protoscoleces in *vitro* and hydatid cysts *in vivo*. The results showed that the lethal concentration 50% (LC50) of albendazole-loaded nanoparticles (ABZ-NP) and mebendazole-loaded nanoparticles (MBZ-NP) was 27.35 µg/ml and 141.1 µg/ml, respectively, compared to albendazole (ABZ) and mebendazole (MBZ) alone, which exhibited LC50 values 105.5 µg/ml and 265.1 µg/ml, respectively. In contrast, drug-unloaded polymeric nanoparticles (empty nanoparticles) did not exhibit a significant anti-protoscoleces (protoscolicidal) effect *in vitro* depending to the control positive group.

The *in vivo* trials revealed a decrease in the quantity, size, and mass of hydatid cysts in infected mice treated with albendazole- and mebendazole-loaded nanoparticles, in contrast to those treated with unloaded nanoparticles. Albendazole showed superior efficacy compared to mebendazole in both drug-loaded and unloaded forms. Albendazole-loaded nanoparticles at a concentration of 20 mg/kg and albendazole alone at 100 mg/kg achieved the highest reduction rate in the number of hydatid cysts of 73.03% and 78.09%, respectively, over a period of 7 weeks with 3 doses/week compared to the control group. The mice that treated with 10 and 20 mg/kg of albendazole-loaded nanoparticles and 100 mg/kg of albendazole alone exhibited high significant reduction ($P \leq 0.001$) of both diameters and weights of hydatid cysts. It may be concluded that commonly used medications for hydatid cyst treatment, particularly albendazole-loaded polymeric nanoparticles, are effective against hydatid cyst infection in *Mus musculus* mice.

تقييم فعالية البيندازول والميبندازول المحمل على الجسيمات النانوية البوليمرية ضد الاطوار اليرقية لطفيل المشوكة الحبيبية خارج الجسم الحي وداخله

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الخلاصة:

تضمنت الدراسة الحالية تقييم فعالية الجسيمات النانوية البوليمرية المحملة بالبندازول والمبيندازول ضد الرؤيسات الاولية خارج الجسم الحي والاكياس العدرية داخل الجسم الحي. اظهرت النتائج ان التركيز النصفي القاتل LC50% للجسيمات النانوية المحملة بالبندازول (ABZ-NP) وبالمبيندازول (MBZ-NP) كان 27.35 و 141.1 مايكروغرام/مل, على التوالي, مقارنة بالبندازول (ABZ) والمبيندازول (MBZ) وحدهما, والتي اظهرت التركيز النصفي القاتل 105.5 و

265.1 مايكروغرام/مل,على التوالي, في حين لم تظهر الجسيمات النانوية البوليمرية غير المحملة بالادوية (الجسيمات النانوية الفارغة) تأثيرا معنويا مضادا للرؤيسات الاولية خارج الجسم الحي مقارنة بمجموعة السيطرة.

اظهرت التجارب التي اجريت داخل الجسم الحي انخفاضا بمستويات مختلفة في اعداد واقطار واوزان الاكياس العدرية في الفئران المصابة بداء الاكياس العدرية والتي تم معاملتها بالجسيمات النانوية المحملة بالبندازول والمبيندازول مقارنة بتلك المعاملة بالادوية غير المحملة على الجسيمات النانوية وكذلك مجموعة السيطرة, حيث تفوق البندازول في فعاليته المضادة لداء الاكياس العدرية على المبيندازول سواء كان محمل او غير محمل على الجسيمات النانوية. اظهرت الجسيمات النانوية المحملة بالبندازول بتركيز 20 مليغرام/مل والبندازول لوحده بتركيز 100 مليغرام/مل اعلى معدل انخفاض في اعداد الاكياس العدرية بنسبة 73.03% و 78.09% على التوالي, لمدة تعريض 7 اسابيع مع 3 جرعات في الاسبوع مقارنة بمجموعة السيطرة, كما اظهرت الفئران المعاملة بـ 10 و 20 مليغرام/مل من الجسيمات النانوية المحملة بالبندازول و 100 مليغرام/مل من البندازول لوحده انخفاضا معنويا عاليا في اقطار واوزان الاكياس العدرية عند مستوى الاحتمالية $P\leq0.001$. يمكن الاستنتاج ان الادوية الشائعة لعلاج الاكياس العرية وخاصة البندازول المحمل على الجسيمات النانوية البوليمرية يمكن استخدامها كدواء فعال ضد داء الاكياس العدرية في الفئران البيض *Mus musculus*.

الكلمات المفتاحية: *E.granulosus*، جسيمات نانوية بوليمرية، ألبيندازول، ميبيندازول

Introduction

Hydatidosis is a zoonotic illness resulting from infection with the larval stage (metacestode) of *Echinococcus granulosus*. Although the disease is asymptomatic in its early stages, it causes tissue and organ damage due to grow and develop of the hydatid cysts [1]. It is estimated that approximately one million cases of hydatidosis are diagnosed worldwide each year[2]. Previously, surgery was the only treatment for *E. granulosus* cysts. However, with scientific advancements, treatment approaches now include surgery, chemotherapy, and the Aspiration, Injection, and Respiration (PAIR) technique[3]. Recently, advanced therapeutic techniques have been used to treat various diseases, including hydatidosis, by utilizing drugs loaded on different nanoparticles (NP) such as Albendazole (ABZ) and Mebendazole (MBZ).

Nanoparticle-based studies aim to bind or load drugs, especially those with low water solubility, with specialized delivery systems made of organic and inorganic materials at nanoscale sizes (1–100 nanometers). These systems can enhance the water solubility and reduce the side effects of drugs[4]. Most parasitic protozoa are intracellular, which negatively affects the efficacy of chemotherapy due to poor ability of drugs to penetrate cell membranes and move easily inside cells. This limitation can be overcome by loading drugs on various nanoparticles[5], [6].

Medical nanotechnology offers an opportunity to eliminate pathogenic microorganisms by using nanoscale materials as specialized delivery systems for transporting drugs, vaccines, and proteins to their targets within living organisms.

In recent decades, nanoparticles have gained considerable attention as antiparasitic agents because the current drugs often have side effects and low water solubility which reduce the efficacy of drugs, such as albendazole and mebendazole, which are used to treat hydatidosis[7], [8].

The scientific studies have demonstrated the existence of numerous chemotherapeutic agents against *E. granulosus* parasite *in vitro* and *in vivo*. Despite the positive effects of these chemotherapeutic agents in combating hydatid cysts, they are not free from side effects, as they can cause undesirable complications, especially at high concentrations. These complications may affect the internal organs of the host, thereby restricting their usage[9]. The purpose of this work was to determine the 50% lethal concentration (LC50) of polymeric nanoparticles loaded with albendazole and mebendazole in order to evaluate the effectiveness of these nanoparticles against *E. granulosus* protoscoleces *in vitro*. The current study also aimed to evaluate the effectiveness of polymeric nanoparticles loaded with albendazole and mebendazole against *E. granulosus* hydatidosis *in vivo*. This was accomplished by analyzing pathological variables such as the number of hydatid cysts, their diameters, and their weights, as well as the percentage of reduction in cyst numbers in drug-treated mice in comparison to the control group.

Materials and Methods

Collection of hydatid cyst samples

Hydatid cysts were obtained from the livers of sheeps in butcher shops in the city of Mosul. The fertility of the hydatid cysts was confirmed through examination and investigation of the viability of the protoscoleces inside hydatid cysts.

Isolation and Viability Assessment of Protoscoleces

Sterile circumstances were used to harvest the protoscoleces from the hydatid cysts that were found in the livers of sheep that had been naturally afflicted. Following the aspiration of the hydatid fluid that contained the protoscoleces, the protoscoleces were separated from the hydatid fluid with the use of centrifugation. Phosphate-buffered saline (PBS) used to wash the protoscoleces for a period of five minutes at a speed of three thousand revolutions per minute. In the second wash, the protoscoleces were washed with PBS containing antibiotics (ampicillin at a concentration of 20000IU and streptomycin at a concentration of 1 g/L). A phosphate-buffered saline solution that did not contain any antibiotics was utilized for the third washing. The supernatant was discarded, and the protoscoleces were suspended in a very small volume of phosphate-buffered saline[10]. In order to

determine whether or not the protoscoleces were viable, an aqueous eosin stain at a concentration of 0.1% was utilized[11].

Production of Polymeric Nanoparticles

Albendazole and mebendazole-loaded polymeric nanoparticles were obtained from the University of Strathclyde, UK, Laboratory of Dr. Clare Hoskins. The polymeric nanoparticles (PAA-CH5) were synthesized based on the method of Thompson *et al.*[12].

Determination of lethal concentration (LC50)

In terms of the effectiveness of existing medications against protoscoleces of *E.granulosus* was evaluated *in vitro* using lethal concentration 50% (LC50) assay. A 24-well plate (24-well plate) with RPMI-1640 culture medium was used, with drug exposure for 72 hrs at 37°C and 5% CO_2. Drug dilutions were prepared using a 2-fold dilution pattern, and a series of concentrations of drugs that tested in current study were applied to determine the LC50 for both drug-loaded polymeric nanoparticles and unloaded drugs onto nanoparticles[13].

Drug treatment experiments for 3 weeks

In order to produce an experimental hydatid cyst infection in *Mus musculus* mice, an intraperitoneal injection of two thousand live protoscoleces per mouse was administered. The injected mice were divided into six groups, with 5 mice/group. Oral administration of drug to the experimentally infected mice began two months after infection with hydatid cysts. Albendazole and mebendazole, both loaded and unloaded on polymeric nanoparticles, were administered at various concentrations according to the following: (Group 1: Control group that was injected with protoscoleces and was not treated with any medicines), Group 2: the subject was treated with nanoparticles that were empty at a dose of 20 mg/kg, Group 3: albendazole loaded polymeric nanoparticles were administered at concentrations of 10 and 20 mg/kg to the experimental subjects, Group 4: albendazole was administered on its own at concentrations of 10, 20, and 100 mg/kg on the patients, Group 5: treated with polymeric nanoparticles loaded with mebendazole at a dosage of twenty milligrams per kilogramme, Group 6: treated with mebendazole alone at concentrations of 20 and 100 mg/kg). The treatments were administered at a frequency of 3 doses/week for 3 weeks. The mice were dissected one week after the last dose (i.e., the total infection period with protoscoleces was 3 months).

Drug treatment experiments for 7 weeks

The experimental infection with hydatid cysts was induced in white mice by injecting the mice with 2000 viable protoscoleces/mouse via the intraperitoneal cavity. The injected mice were categorized into six groups, comprising 5 mice each group. The experimentally infected mice were then orally treated with albendazole and mebendazole, both loaded and unloaded onto polymeric nanoparticles, at different concentrations as follows: (Group 1: control group that injected with protoscoleces and untreated with drugs, Group 2: the subject was treated with nanoparticles that were empty at a dose of 20 mg/kg, Group 3: treated with albendazole-loaded polymeric nanoparticles at concentrations of 10 and 20 mg/kg, Group 4: treated with albendazole alone at concentrations of 10, 20, and 100 mg/kg, Group 5: administered with polymeric nanoparticles loaded with mebendazole at a dosage of twenty milligrams per kilogramme, Group 6: treated with mebendazole alone at concentrations of 20 and 100 mg/kg), with an average of 3 doses/week for 7 weeks. The mice were sacrificed one week after the last dose (the total infection duration with protoscoleces was 5 months).

Statistical analysis

All statistical analyses were carried out with the assistance of GraphPad Prism 5.0 software (GraphPad Software, Inc., San Diego, California, United States of America). The One-Way ANOVA with Dunnett's post-test was utilized in order to compare the means of a number of different data groups. The probability value (*P*-value) was calculated to compare the degree of variation between variants and considered to be significant at $P \leq 0.05$=*, $P \leq 0.01$=** and $P \leq 0.001$=***[14].

Results

Effect of loaded and unloaded drugs onto polymeric nanoparticles against protoscoleces viability *in vitro*

The *in vitro* results of drug screening during 72 hrs, showed that albendazole was superior in its protoscolicidal activity, whether loaded or unloaded onto polymeric nanoparticles, compared to mebendazole. The *in vitro* protoscolicidal effect of albendazole-loaded nanoparticles showed lethal concentration 50% (LC50) of 27.35 µg/ml, compared to albendazole alone, which exhibited 105.5 µg/ml. While, mebendazole-loaded nanoparticles exhibited LC50 of 141.1 µg/mL, compared to mebendazole alone, which exhibited LC50 of 265.2 µg/ml. On the other hand, **empty nanoparticles** exhibited a slight decrease in protoscoleces viability without recording LC50 due to their low effectiveness, compared to the control group, after 72 hrs of drug exposure (Figure 1).

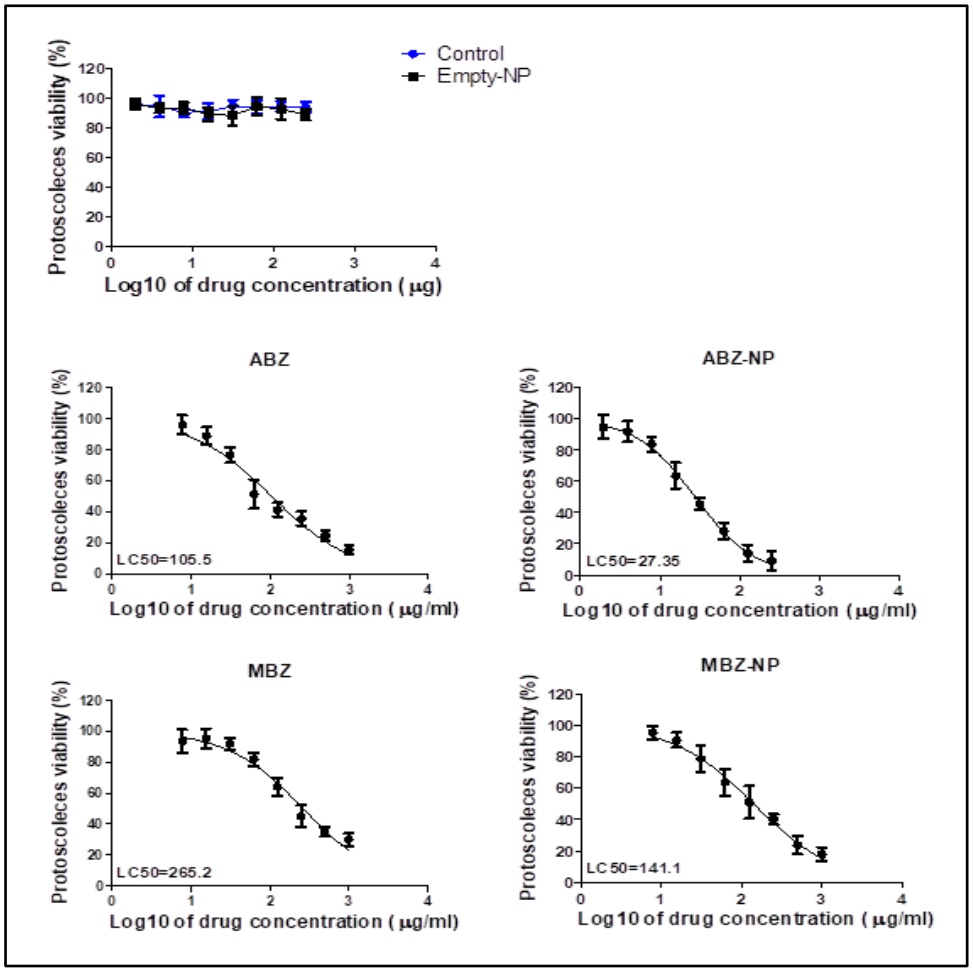

Figure (1): *In vitro* LC50 of drugs against protoscoleces of *E. granulosus* including albendazole alone (ABZ), albendazole-loaded nanoparticles (ABZ-NP), mebendazole alone (MBZ), mebendazole-loaded nanoparticles (MBZ-NP) and empty nanoparticles (Empty-NP), in addition to control group at 72 hrs.

Effect of loaded and unloaded drugs onto polymeric nanoparticles against secondary hydatid cyst *in vivo*

Polymeric nanoparticles have been shown to have the ability to improve the effectiveness of albendazole and mebendazole in treating hydatid cyst disease, as indicated by the findings of *in vivo* trials. This was reflected by reduction, to varying degrees, in the numbers, diameters, and weights of hydatid cysts, along with an increase in percentage reduction of cyst numbers in mice treated with drug-loaded nanoparticles, surpassing the performance of unloaded drugs onto nanoparticles, compared to the positive control group at significance levels ($P \leq 0.05$–$P \leq 0.001$). The results also showed the superiority of albendazole in its

anti-hydatid cyst efficacy, whether loaded or unloaded albendazole onto nanoparticles, comparing to mebendazole.

Changes in the rates of numbers, diameters, and weights of hydatid cysts with percentage reduction in their cyst numbers in mice treated with drugs for three weeks

The lowest mean of hydatid cyst numbers were obtained as 4.0, 5.25, and 7.00 cysts (Figure 2-A), with percentage reductions of cyst numbers which was 58.97%, 46.15%, and 28.2%, respectively (Figure 2-B), in mice treated with 100 mg/kg of albendazole alone and 20 mg/kg and 10 mg/kg of albendazole-loaded polymeric nanoparticles for three weeks at a rate of three doses per week, and at significance levels ($P \leq 0.001$, $P \leq 0.01$, and $P \leq 0.05$), respectively, in comparison with control group, which showed 9.75 cysts. In contrast, the reduction in cyst numbers was not significant at other concentrations. It was shown that mice who were given either 100 mg/kg of mebendazole by itself or 20 mg/kg of mebendazole-loaded nanoparticles experienced a significant decrease in the average number of hydatid cysts over the course of their treatment. These mice recorded 6.0 and 7.5 cysts, respectively, representing the lowest significant reduction (Figure 2-A) at significance levels ($P \leq 0.01$ and $P \leq 0.05$), with percentage reductions in cyst numbers of 38.46% and 23.07%, respectively (Figure 2-B).

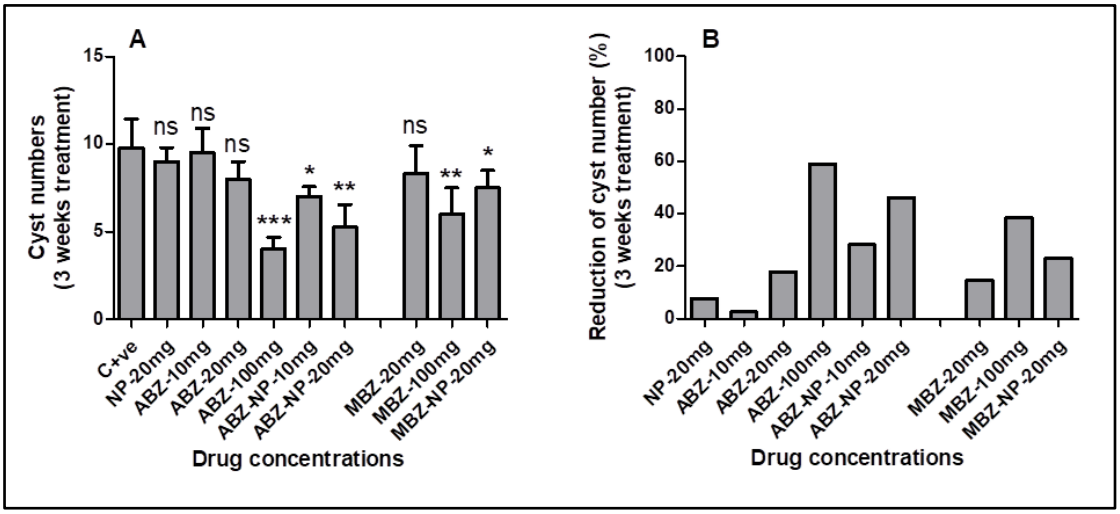

Figure (2): (A) Cyst numbers and (B) reduction percentage of hydatid cyst numbers in mice treated with different concentrations of albendazole (ABZ), mebendazole (MBZ), albendazole- and mebendazole-loaded nanoparticles (ABZ-NP and MBZ-NP, respectively) and empty nanoparticles (NP) for 3 weeks at a rate of 3 doses/week. (Probability; ns=non-significant, $P \leq 0.05$=*, $P \leq 0.01$=, $P \leq 0.001$=***).**

When it came to the reduction in the mean diameters and weights of hydatid cysts, the mice who were given albendazole at a dose of 100 mg/kg exhibited the least significant drop. We observed this phenomenon at a level of significance of ($P \leq 0.001$). A significant reduction in cyst diameters and weights was observed in mice that were treated with albendazole-loaded polymeric nanoparticles at doses of 10 and 20 mg/kg. This reduction was observed at a significance level of ($P \leq 0.01$), On the other hand, mice that were treated with 20 mg/kg of albendazole alone showed a minor but substantial reduction in the diameters and weights of hydatid cysts. This was found at a significance level of ($P \leq 0.05$). The diameters and weights of hydatid cysts in mice that were treated with 10 mg/kg of albendazole alone and 20 mg/kg of empty nanoparticles revealed a reduction that was not statistically significant when compared to the mice that served as the control (Figure 3-A, B).

In terms of the alterations in the diameters and weights of hydatid cysts in mice that were administered mebendazole for a period of three weeks at a rate of three doses per week, it was observed that the mice that were administered 20 mg/kg of mebendazole-loaded polymeric nanoparticles and 100 mg/kg of albendazole alone exhibited a noteworthy reduction at significance levels ($P \leq 0.05$ and $P \leq 0.01$), respectively. Mebendazole at a dose of 20 mg/kg alone demonstrated a decline that was not statistically significant (Figure 3-A, B).

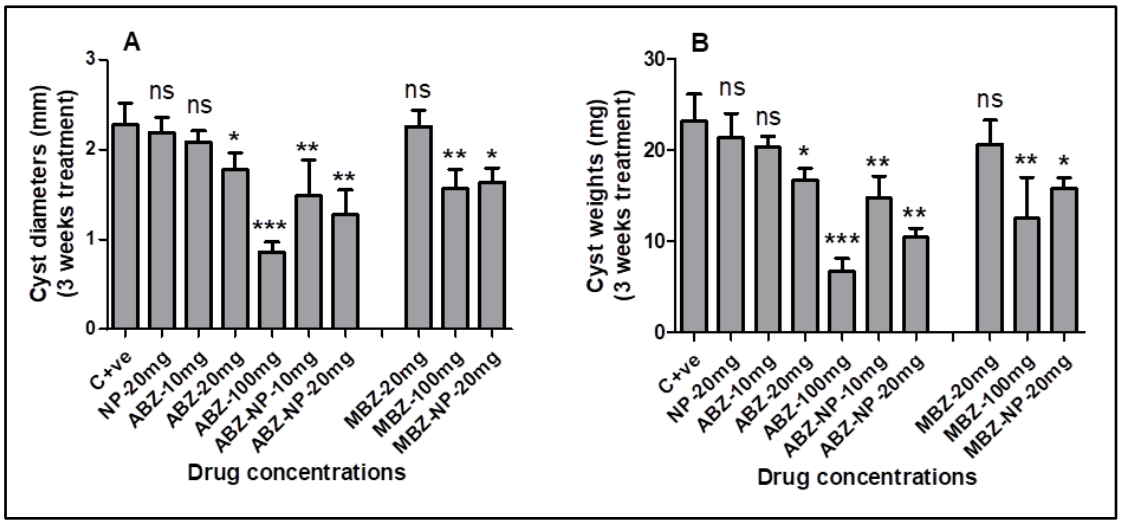

Figure (3): (A) mean of cyst diameters (mm) and (B) weights (mg) in mice treated with different concentrations of albendazole (ABZ), mebendazole (MBZ), albendazole- and mebendazole-loaded nanoparticles (ABZ-NP and MBZ-NP, respectively) and empty nanoparticles (NP) for 3 weeks at a rate of 3 doses/week. (Probability; ns=non-significant, P≤0.05=*, P≤0.01=, P≤0.001=***).**

The changes in the rates of numbers, diameters, and weights of hydatid cysts with percentage reduction in their cyst numbers in mice treated with drugs for seven weeks

A decrease in the rates of growth and development of hydatid cysts was noticed in mice that were treated with drugs for a period of seven weeks at a rate of three doses per week. This particular treatment was administered to the mice. The results of the current investigation demonstrated that the degree of reduction varied widely. A highly significant decrease of hydatid cyst numbers was observed in group of mice that treated with 10 and 20 mg/kg of albendazole-loaded nanoparticles and 100 mg/kg concentration of albendazole alone, where the average number of cysts was 14.75, 8.0, and 6.5 cysts at a probability level ($P \leq 0.001$), respectively, compared to the control group of mice, which showed an average 29.67 cysts. The percentage reduction in the number of hydatid cysts reached 50.28%, 73.03%, and 78.09%, respectively. As for the mice treated with 10 and 20 mg/kg of albendazole alone, they showed a lower significant decrease, at a probability level of $P \leq 0.05$ and $P \leq 0.01$, respectively. In contrast, the mice who were given empty nanoparticles at a dose of 20 mg/kg demonstrated a decline in the number of hydatid cysts that was not statistically significant. The mice that were treated with a concentration of 100 mg/kg of mebendazole alone and 20 mg/kg of albendazole-loaded polymeric nanoparticles showed a significant reduction in the number of hydatid cysts. The average number of cysts seen in these mice was 14.25 and 17.75, respectively, at a probability level of $P \leq 0.001$ and $P \leq 0.01$, with percentage reductions of hydatid cyst numbers of 51.97% and 40.12%, respectively, as shown in (Figure 4-A,B).

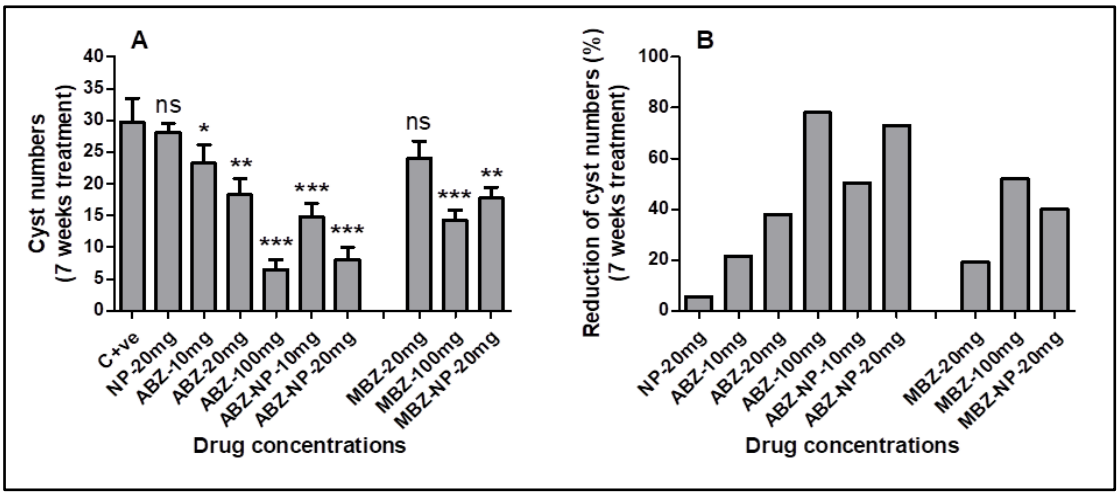

Figure (4): (A) Cyst numbers and (B) reduction percentage of hydatid cysts in mice treated with different concentrations of albendazole (ABZ), mebendazole (MBZ), albendazole- and mebendazole-loaded nanoparticles (ABZ-NP and MBZ-NP, respectively) and empty nanoparticles (NP) for 7 weeks. (Probability; ns=non-significant, $P \leq 0.05$=*, $P \leq 0.01$=, $P \leq 0.001$=***).**

It is evident from (Figure 5) that there were decreases, with varying levels of significance, in the diameters and weights of hydatid cysts in the groups of mice treated with albendazole and mebendazole, both loaded and unloaded onto nanoparticles, for 7 weeks at a rate of 3 doses /week. The mice treated with 10 and 20 mg/kg of albendazole-loaded nanoparticles and 100 mg/kg of albendazole alone showed the lowest reductions in cyst diameters, with averages of 2.92, 1.63, and 2.06 mm at a probability level ($P \leq 0.001$), respectively, compared to the control group, which showed 5.89 mm. On the other hand, the mice that were administered 10 and 20 mg/kg of albendazole on their own shown a minor but statistically significant reduction at a probability threshold of ($P \leq 0.05$). On the other hand, the mice who were given empty nanoparticles at a dose of 20 mg/kg demonstrated a decrease in cyst sizes that was not statistically significant (Figure 5-A).

Meanwhile, the lowest significant reduction in hydatid cyst diameters was observed at a probability level of $P \leq 0.05$ and $P \leq 0.01$ in mice treated with 20 mg/kg of mebendazole-loaded polymeric nanoparticles and 100 mg/kg of mebendazole alone, respectively. Additionally, the groups of mice treated with 20 mg/kg of mebendazole alone showed a non-significant reduction in cyst diameter rates (Figure 5-A).

When compared to the control group, it is obvious from the data presented in Figure 5-B that there was a highly significant drop in the mean weights of hydatid cysts in mice that were treated with 10 and 20 mg/kg of albendazole-loaded nanoparticles and 100 mg/kg of albendazole alone. This decrease was observed at the probability level ($P \leq 0.001$). At the same time, the mice that were administered with 10 and 20 mg/kg of albendazole on their own demonstrated a reduction in cyst weights that was statistically significant at the probability level of ($P \leq 0.01$). This is in contrast to the 20 mg/kg of empty nanoparticles, which demonstrated a reduction in the weight mean of hydatid cysts that was not statistically significant (Figure 5-B). When compared to the control group, it is obvious from the data presented in Figure 5-B that there was a noteworthy reduction in the weight means of hydatid cysts in mice that were treated with 100 mg/kg of mebendazole alone and 20 mg/kg of mebendazole-loaded nanoparticles. This reduction was observed at the probability level of ($P \leq 0.01$). Regarding the group that was administered 20 mg/kg of mebendazole on its own, it demonstrated a marginally significant reduction in cyst weights at the probability threshold of ($P \leq 0.05$).

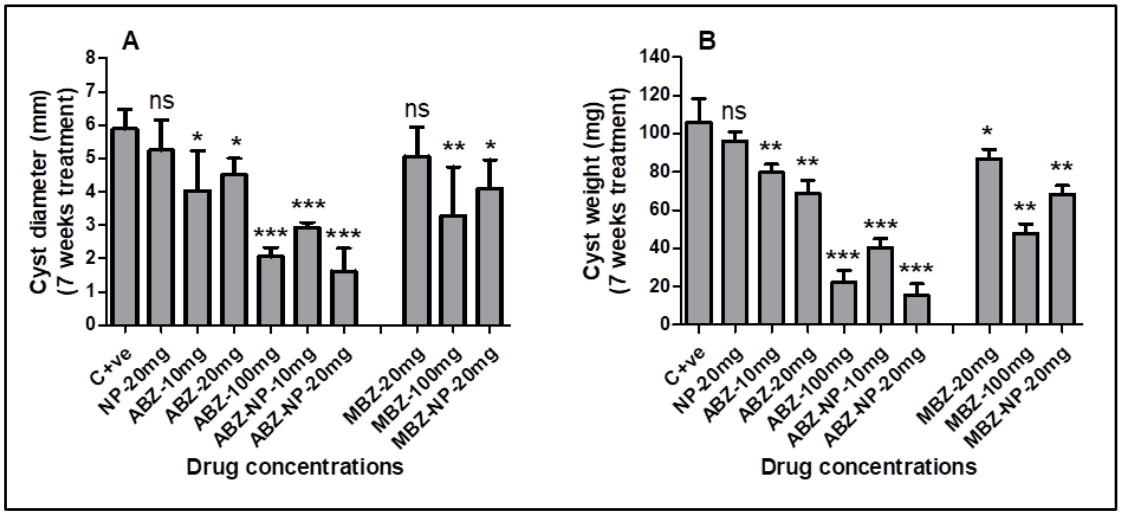

Figure (5): (A) Cyst diameter (mm) and (B) weight (mg) means of hydatid cysts in mice treated with different concentrations of albendazole (ABZ), mebendazole (MBZ), albendazole- and mebendazole-loaded nanoparticles (ABZ-NP and MBZ-NP, respectively) and empty nanoparticles (NP) for 7 weeks at a rate of 3 doses/week. (Probability; ns=non-significant, P≤0.05=*, P≤0.01=, P≤0.001=***).**

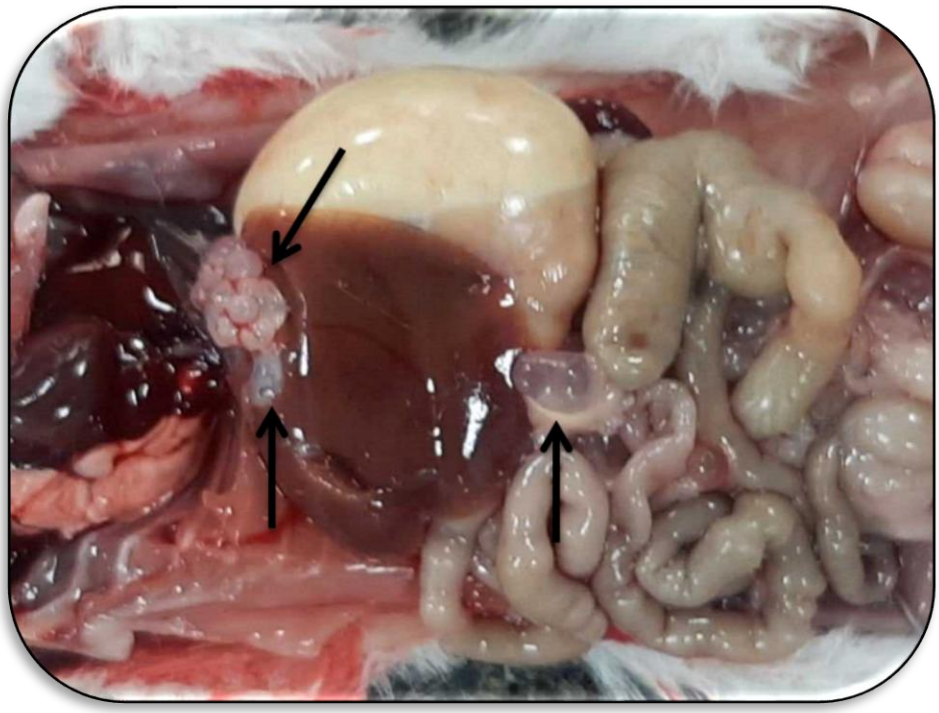

Figure (6): Hydatid cysts developed for three months in the positive control group

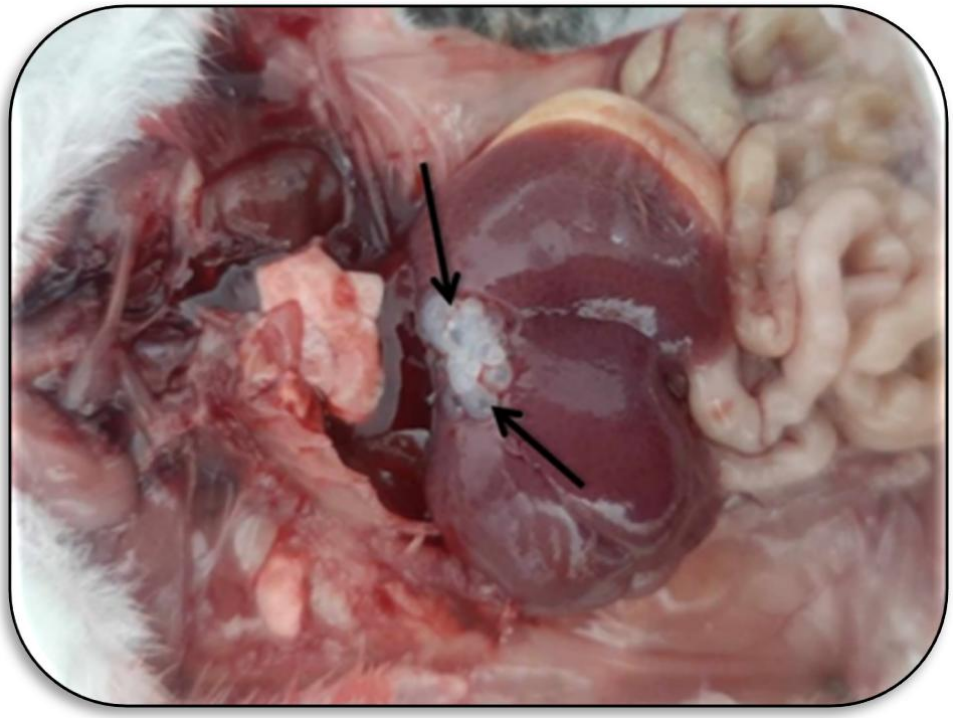

Figure (7): Hydatid cysts developed for three months in the group treated empty nanoparticles at a concentration of 20 mg/kg of body weight for three weeks.

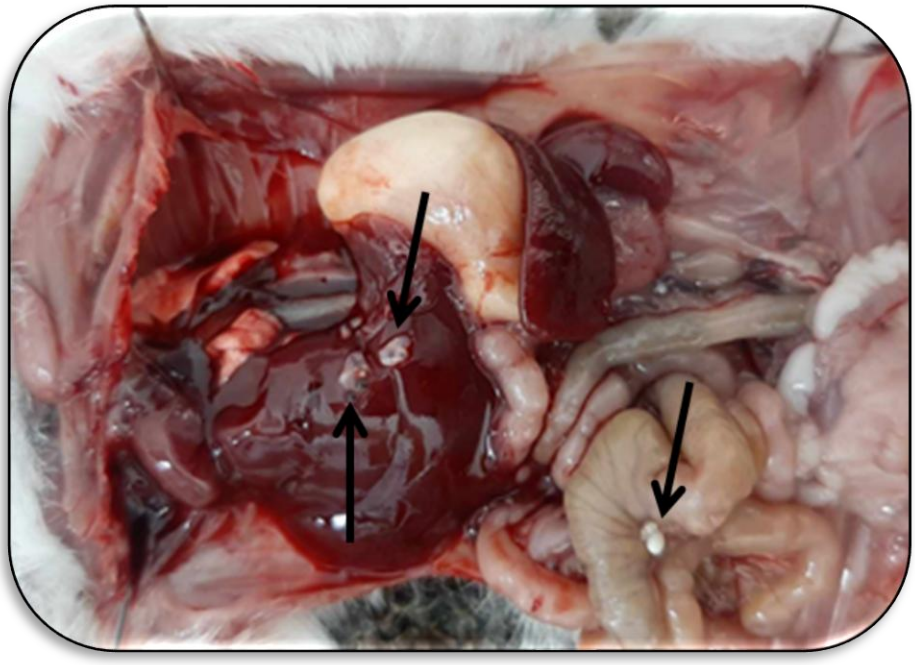

Figure (8): Hydatid cysts developed for three months in the group treated with albendazole alone at a concentration of 100 mg/kg of body weight for three weeks.

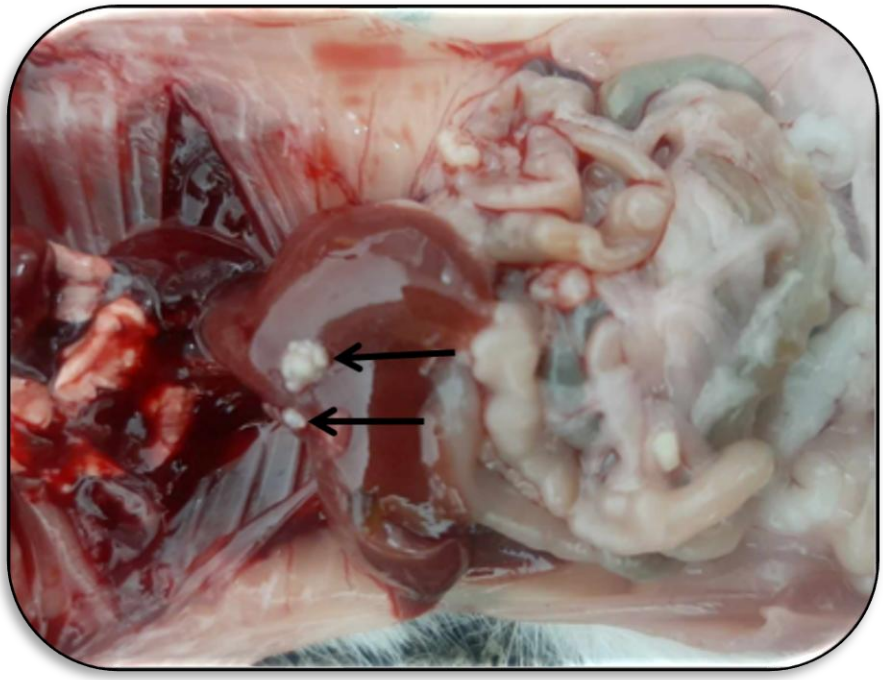

Figure (9): Hydatid cysts developed for three months in the group treated with polymeric nanoparticles loaded with albendazole at a concentration of 20 mg/kg for three weeks.

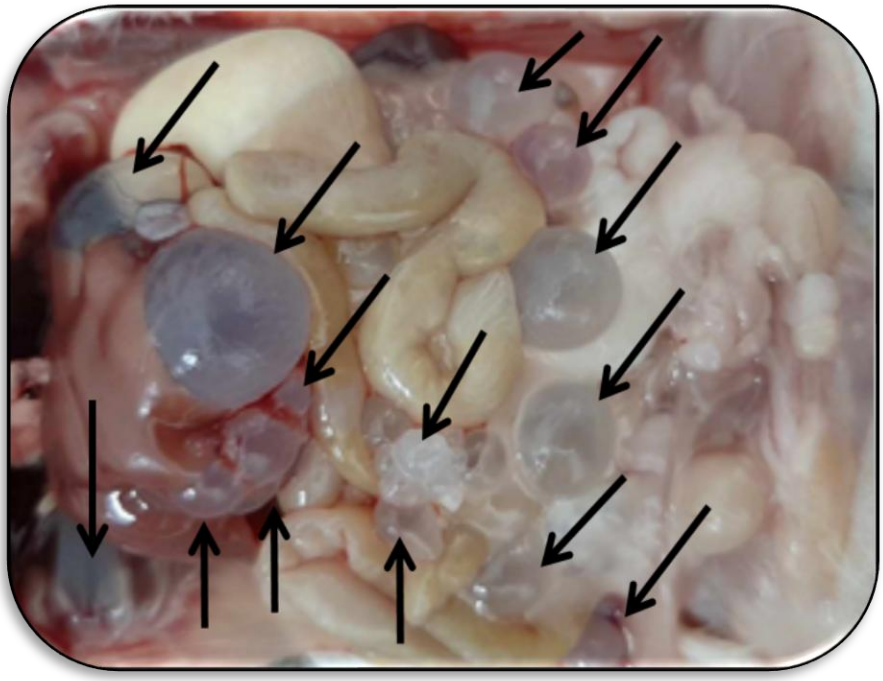

Figure (10): Hydatid cysts developed for five months in the positive control group (untreated with drugs).

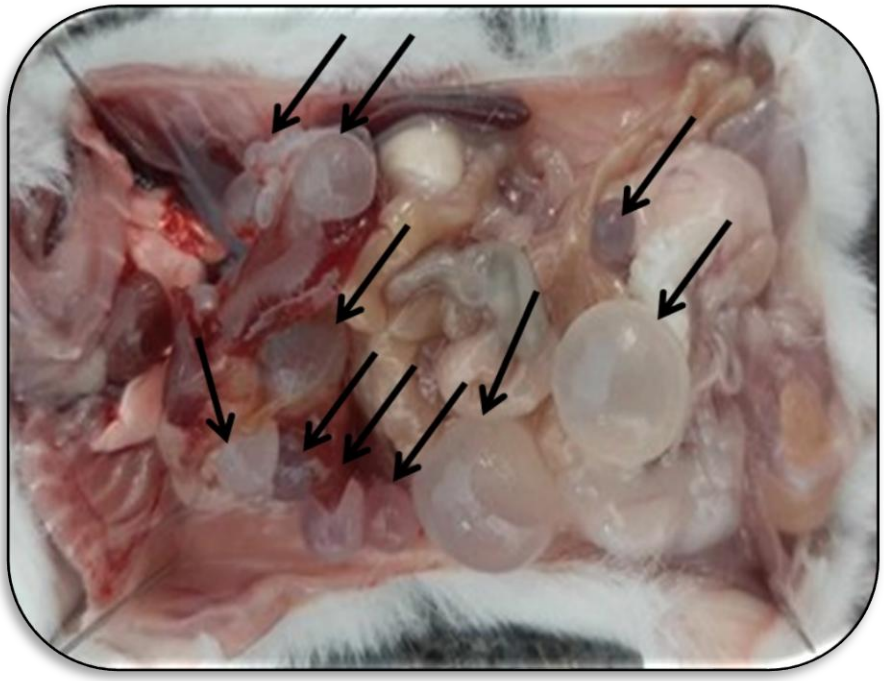

Figure (11): Hydatid cysts developed for five months in the group treated with empty polymeric nanoparticles at a concentration of 20 mg/kg for seven weeks.

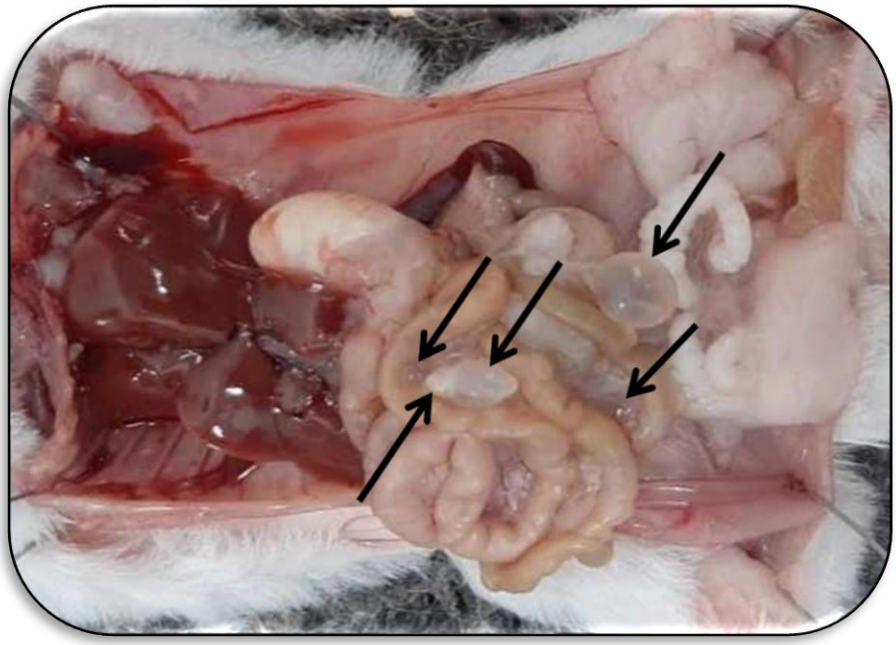

Figure (12): Hydatid cysts developed for five months in the group treated with albendazole alone at a concentration of 100 mg/kg of body weight for seven weeks.

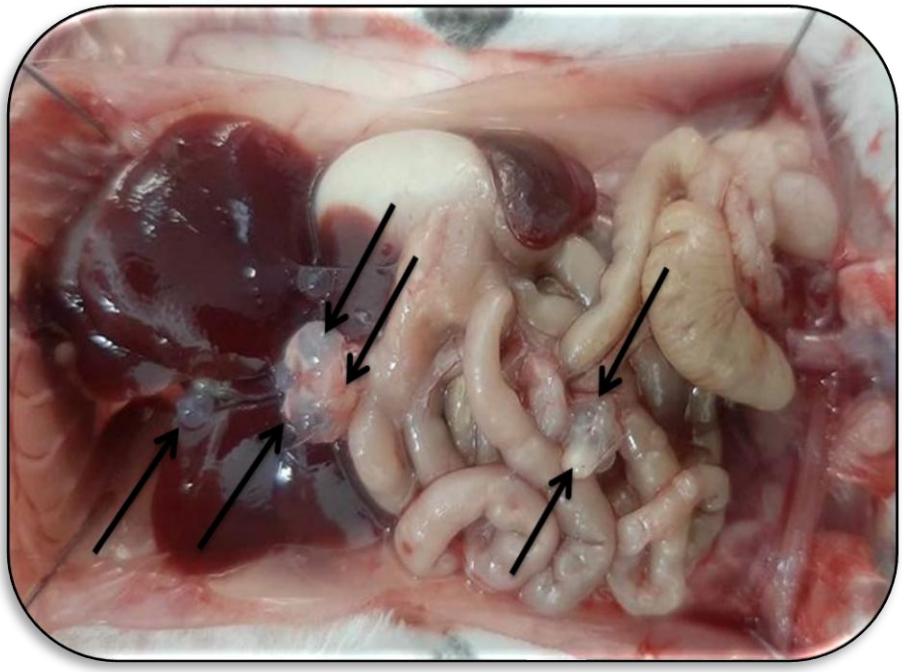

Figure (13): Hydatid cysts developed for five months in the group treated with polymeric nanoparticles loaded with albendazole at a concentration of 20 mg/kg for 7 weeks.

Discussion

For *in vitro* experiments, the current study demonstrated the superiority of albendazole over mebendazole, whether the drugs were loaded or unloaded on polymeric nanoparticles. The polymeric nanoparticles also showed the ability to enhance the efficacy of the selected drugs in the current study against the protoscoleces of *Echinococcus granulosus*. Albendazole and mebendazole loaded on polymeric nanoparticles exhibited higher efficacy against protoscoleces compared to albendazole and mebendazole unloaded on nanoparticles. Meanwhile, the empty polymeric nanoparticles demonstrated very low efficacy against protoscoleces *in vitro* compared to the control group (Figure 1). This may indicate that the empty polymeric nanoparticles are biocompatible, the results of which are in agreement with the conclusions of a study that was carried out by Hoskins and *et al*., (2010)[15], additionally, their study demonstrated that empty polymeric nanoparticles exhibit low cytotoxicity.

Based on the current data, polymeric nanoparticles can enhance the efficacy of albendazole and mebendazole against protoscoleces, which may be attributed to the increased water solubility of the tested drugs, thereby improving their bioavailability in plasma as well as increasing drug accumulation at the drug action sites. Additionally, albendazole and mebendazole enhance the activity of

Caspase-3, an enzyme known as an apoptotic marker, in protoscoleces exposed to these drugs[16], [17].

The *in vitro* efficacy of solid lipid nanoparticles (SLNs) against protoscoleces at concentrations of 250 and 500 µg/ml of unloaded albendazole on nanoparticles for 1–7 days, with daily examination of protoscoleces viability, showed a killing rate of 52% and 60%, respectively, after three days. But at seventh day of treatment, a 100% killing rate was observed. Whilst, albendazole-loaded solid lipid nanoparticles at the same concentrations showed a killing rate of 66% and 78%, respectively, against protoscoleces on the third day of treatment [18].

The time required to achieve a 100% killing rate of *E. granulosus* protoscoleces of G1 genotype (sheep strain) was reduced from 28 days to 17 days when treated with 1 µg/ml of albendazole powder compared to albendazole nanocrystals, respectively [19]. In another experiment, albendazole demonstrated superiority over mebendazole loaded into nanocapsules. Albendazole and mebendazole-loaded nanocapsules showed killing rates of 46.67% and 36.33%, respectively, at a concentration 250 µg/ml after 2hrs of exposure. While, at 500 µg/ml and the same exposure duration, killing rates of 52.33% and 46.67% were observed, respectively [20].

For *in vivo* investigations, the findings of the current study demonstrated a highly significant reduction in the number of hydatid cysts, as well as their weights and diameters, in mice that were treated with drug-laden nanoparticles as opposed to animals that were given nanoparticles that were not loaded with pharmaceuticals. In addition to, albendazole efficacy outperformed mebendazole against hydatid cysts during both study periods—three and seven weeks of *in vivo* treatment. This may be attributed to the ability of nanoparticles to enhance drug efficacy by increasing their water solubility, leading to improve drug absorption in the intestines and enhance drug bioavailability in bloodstream [21]. This, in turn, leads to an increase in concentration of the albendazole metabolite (albendazole sulfoxide) in plasma and within the hydatid cyst. The concentration of albendazole sulfoxide inside the cyst [22]. The two metabolites of albendazole (albendazole sulfoxide and albendazole sulphone) are highly effective against protoscoleces and hydatid cysts *in vivo* [23]. The greater inhibition of hydatid cyst growth and development in mice treated with albendazole compared to mebendazole may be attributed to the ability of albendazole sulfoxide to penetrate more easily through the cyst membrane [24], [25]. The results of *in vivo* experiments in the current study are consistent with the findings of a study conducted at the University of Kufa/College of Education for Women, the purpose of which was to determine whether or not silver nanoparticles loaded with

albendazole and mebendazole achieved the desired results. Albendazole-loaded nanoparticles were shown to be superior to mebendazole-loaded nanoparticles [26].

Another study conducted by Torabi and his colleagues (2018) [27] to investigated effect of drug-loaded nanoparticles against hydatid cysts in BALB/c mice. The study demonstrated that praziquantel and albendazole-loaded chitosan nanoparticles exhibited anti-hydatid effects compared to praziquantel and albendazole alone. When compared to animals who were treated with unloaded pharmaceuticals or the control group. A different study found that the quantity of hydatid cysts, as well as their diameters and weights, were dramatically reduced in mice that were treated with albendazole loaded on silver nanoparticles [28].

The capacity of albendazole to trigger programmed cell death mechanisms, may be responsible for the efficacy of albendazole in treating hydatid illness. This efficacy is demonstrated by the reduction in the number of hydatid cysts, as well as their diameters and weights [29]. Additionally, albendazole can stimulate autophagy in treated cells by activating LC3 (Atg8) and Ag7, as well as Beclin-1, which is considered a key factor in the autophagy pathway. [30]. Albendazole can also cause the destruction of the components of the hydatid cyst wall, thereby reducing the viability of the cysts. Transmission electron microscope (TEM) graph in mice treated with albendazole revealed cellular changes in the germinal layer of the hydatid cyst wall, including the breakdown of microtriches, glycogen, the loss of several cellular organelles[31].

Conclusion

Enhancing the effectiveness of drugs by loading them onto nanoparticles helps reduce the effective concentrations of drugs, which may reduce their side effects. Significant reduction in the numbers, diameters and sizes of hydatid cysts in the groups of mice that treated with drugs-loaded polymeric nanoparticles compared to drugs unloaded on nanoparticles, with albendazole outperforming mebendazole in the type of drugs loaded and unloaded on polymeric nanoparticles during the two treatment periods of three and seven weeks were obtained.

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References:

[1] A. Casulli, M. Siles-Lucas, and F. Tamarozzi, "Echinococcus granulosus sensu lato," *Trends Parasitol*, vol. 35, no. 8, pp. 663–664, 2019, doi: 10.1016/J.PT.2019.05.006.

[2] World Health Organisation, "Echinococcus," 2021. [Online]. Available: https://www.who.int/news-room/fact-sheets/detail/echinococcosis

[3] H. Pham, D. Kupshik, C. King, and P. Romano, "Cystic echinococcosis," *Appl Radiol*, vol. 48, no. 5, pp. 42–43, 2019, [Online]. Available: https://appliedradiology.com/Communities/CT-Imaging/cystic-echinococcosis

[4] D. Natelson, *Nanostructures and Nanotechnology*. Cambridge University Press, 2015. doi: 10.1017/CBO9781139025485.

[5] L. D. Silva *et al.*, "Elucidating the influence of praziquantel nanosuspensions on the in vivo metabolism of Taenia crassiceps cysticerci," *Acta Trop*, vol. 161, pp. 100–105, 2016, doi: 10.1016/j.actatropica.2016.06.002.

[6] F. Tomiotto-Pellissier *et al.*, "Nanotechnology as a potential therapeutic alternative for schistosomiasis," *Acta Trop*, vol. 174, pp. 64–71, 2017, doi: 10.1016/j.actatropica.2017.06.025.

[7] S. Khan and M. K. Hossain, "Classification and properties of nanoparticles," in *Nanoparticle-based Polymer Composites*, Woodhead Publishing, 2022, pp. 15–54. doi: 10.1016/B978-0-12-824272-8.00009-9.

[8] M. J. Mitchell, M. M. Billingsley, R. M. Haley, M. E. Wechsler, N. A. Peppas, and R. Langer, "Engineering precision nanoparticles for drug delivery," *Nat Rev Drug Discov*, vol. 20, no. 2, pp. 101–124, 2021, [Online]. Available: https://www.nature.com/articles/s41573-020-0090-8

[9] M. H. Kohansal, A. Nourian, M. T. Rahimi, A. Daryani, A. Spotin, and E. Ahmadpour, "Natural products applied against hydatid cyst protoscolices: a review of past to present," *Acta Trop*, vol. 176, pp. 385–394, 2017, doi: 10.1016/j.actatropica.2017.09.013.

[10] J. D. Smyth, "In vitro culture of Echinococcus spp.," in *Proc. 13th Int. Cong. Hydat.*, 1985, pp. 84–95.

[11] J. D. Smyth and N. J. Barrett, "Procedures for testing the viability of human hydatid cysts following surgical removal, especially after chemotherapy," *Trans R Soc Trop Med Hyg*, vol. 74, no. 5, pp. 649–652, 1980, doi: 10.1016/0035-9203(80)90157-1.

[12] C. J. Thompson *et al.*, "The effect of polymer architecture on the nano self-assemblies based on novel comb-shaped amphiphilic poly (allylamine)," *Colloid Polym Sci*, vol. 286, pp. 1511–1526, 2008, doi: 10.1007/s00396-008-1925-8.

[13] M. Walker, J. F. Rossignol, P. Torgerson, and A. Hemphill, "In vitro effects of nitazoxanide on Echinococcus granulosus protoscoleces and

metacestodes," *Journal of Antimicrobial Chemotherapy*, vol. 54, no. 3, pp. 609–616, 2004, doi: 10.1093/jac/dkh386.

[14] H. J. Motulsky, *Prism 5 statistics guide, 2007*, vol. 31, no. 1. GraphPad Software, 2007.

[15] C. Hoskins *et al.*, "In vitro and in vivo anticancer activity of a novel nano-sized formulation based on self-assembling polymers against pancreatic cancer," *Pharm Res*, vol. 27, pp. 2694–2703, 2010, doi: 10.1007/s11095-010-0268-6.

[16] N. Doudican, A. Rodriguez, I. Osman, and S. J. Orlow, "Mebendazole induces apoptosis via Bcl-2 inactivation in chemoresistant melanoma cells," *Molecular Cancer Research*, vol. 6, no. 8, pp. 1308–1315, 2008.

[17] G. Xing *et al.*, "Sodium arsenite augments sensitivity of Echinococcus granulosus protoscoleces to albendazole," *Exp Parasitol*, vol. 200, pp. 55–60, 2019, doi: 10.1016/j.exppara.2019.02.008.

[18] S. Aminpour, A. Rafiei, A. Jelowdar, and M. Kouchak, "Evaluation of the protoscolicidal effects of albendazole and albendazole loaded solid lipid nanoparticles," *Iran J Parasitol*, vol. 14, no. 1, p. 127, 2019, doi: 10.18502/ijpa.v14i1.726.

[19] R. Fateh *et al.*, "In vitro evaluation of albendazole nanocrystals against Echinococcus granulosus protoscolices," *Ann Parasitol*, vol. 67, no. 2, 2021, doi: 10.17420/ap6702.3308.

[20] N. Soleymani, S. Sadr, C. Santucciu, A. Rahdar, G. Masala, and H. Borji, "Evaluation of the In-Vitro Effects of Albendazole, Mebendazole, and Praziquantel Nanocapsules against Protoscolices of Hydatid Cyst," *Pathogens*, vol. 13, no. 9, p. 790, 2024, doi: 10.3390/pathogens13090790.

[21] X. Xiao *et al.*, "Polymeric nanoparticles—Promising carriers for cancer therapy," *Front Bioeng Biotechnol*, vol. 10, p. 1024143, 2022, doi: 10.3389/fbioe.2022.1024143.

[22] P. E. Pensel *et al.*, "Cystic echinococcosis therapy: albendazole-loaded lipid nanocapsules enhance the oral bioavailability and efficacy in experimentally infected mice," *Acta Trop*, vol. 152, pp. 185–194, 2015, doi: 10.1016/j.actatropica.2015.09.016.

[23] G. Adas, S. Arikan, O. Kemik, A. Oner, N. Sahip, and O. Karatepe, "Use of albendazole sulfoxide, albendazole sulfone, and combined solutions as scolicidal agents on hydatid cysts (in vitro study)," *World journal of gastroenterology: WJG*, vol. 15, no. 1, p. 112, 2009, doi: 10.3748/wjg.15.112.

[24] A. B. Dehkordi *et al.*, "Albendazole and treatment of hydatid cyst: review of the literature," *Infectious Disorders-Drug Targets*, vol. 19, no. 2, pp. 101–104, 2019, [Online]. Available: https://www.ingentaconnect.com/content/ben/iddt/2019/00000019/00000002/art00002

[25] A. G. Saimot *et al.*, "Albendazole as a potential treatment for human hydatidosis," *The Lancet*, vol. 322, no. 8351, pp. 652–656, 1983.
[26] S. D. Jari and J. J. Yousif, "Therapeutic effects of silver nanoparticles loaded with albendazole, mebendazole drugs in male albino mice infected with hydatid cysts," *International Research Journal of Advanced Science*, vol. 1, no. 1, pp. 13–18, 2020.
[27] N. Torabi, F. Dobakhti, S. Faghihzadeh, and A. Haniloo, "In vitro and in vivo effects of chitosan-praziquantel and chitosan-albendazole nanoparticles on Echinococcus granulosus Metacestodes," *Parasitol Res*, vol. 117, pp. 2015–2023, 2018, doi: 10.1007/s00436-018-5849-z.
[28] N. E. Nassef *et al.*, "Evaluation of the therapeutic efficacy of albendazole-loaded silver nanoparticles against Echinococcus granulosus infection in experimental mice," *Journal of parasitic diseases*, vol. 43, pp. 658–671, 2019, doi: 10.1007/s12639-019-01145-z.
[29] Y. Y. Jung, S. H. Baek, I. J. Ha, and K. S. Ahn, "Regulation of apoptosis and autophagy by albendazole in human colon adenocarcinoma cells," *Biochimie*, vol. 198, pp. 155–166, 2022, doi:10.1016/j.biochi.2022.04.014.
[30] J. A. Loos, P. A. Caparros, M. C. Nicolao, G. M. Denegri, and A. C. Cumino, "Identification and pharmacological induction of autophagy in the larval stages of Echinococcus granulosus: an active catabolic process in calcareous corpuscles," *Int J Parasitol*, vol. 44, no. 7, pp. 415–427, 2014.
[31] X. M. Ma *et al.*, "Therapeutic effects of Sophora moorcroftiana alkaloids in combination with albendazole in mice experimentally infected with protoscolices of Echinococcus granulosus," *Brazilian Journal of Medical and Biological Research*, vol. 40, pp. 1403–1408, 2007, doi: 10.1590/S0100-879X2006005000167.