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Histological study of mice kidneys that infected with *Echinococcus granulosus* & the effect of treatment with nano mixture

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Abstract---The current study included knowing the effect of Silver-Copper nano mixture Ag-Cu and Silver-Copper/Albendazole hybrid mixture Ag-Cu\Alb., in the kidney tissues of mice infected with hydatid cysts that caused by the parasite *Echinococcus granulosus* experimentally. After 3 months of infection with one dose per day, then the affected kidneys were dissected and extracted and tissue sections were prepared and stained with eosin and hematoxylin dyes, and this was compared to the animals dosing with the traditional drug of Alb. and with the presence of a positive control (infected without treatment) and negative control (uninfected), The results showed that the traditional drug did not cause any change in the tissue, as well as the concentration of 400 mg / kg of nano mixture, while the concentrations of 800 and 1200 mg / kg of the hybrid mixture had a better effect as the tissue appeared free from vascular congestion, as was the concentration of 1200 mg/kg of the hybrid mixture had a good effect on the tissue, as it did not show vascular congestion compared to the lower concentrations, and when comparing the two mixtures with the control treatments, while the infiltration of inflammatory cells remained, which indicates that the tissue is starting to heal with the possibility of the presence of remnants of the

hydatid cyst in the tissue or the symmetry of the total recovery requires a longer period of time for treatment.

Keywords---Nano mixture, *Echinococcus granulosus*, Histological.

Introduction

Echinococcosis is a zoonotic disease caused by one type of tapeworm that genus *Echinococcus*. The parasite harbored the definitive hosts intestine as adult form. while the larva form found In the intermediate hosts as hydatid cysts, the disease in some countries considered the most common health problems , *Echinococcus granulosus* cause Cystic Echinococcosis which the most prevalent (CFSPH , 2020) . In the countries of the Middle East, the disease acts as a major health obstacle. In rural areas, it has high prevalence rates, as sheep are abundant, which are the main host for the parasite. (Thys *et al.*, 2019; Abdulhameed *et al.*, 2019). Iraq is one of the countries that suffers from the spread of the disease significantly, as it is one of the most important health problems that are considered endemic in it, as there is no control system to limit the spread of the disease (Athmar & Ban-Abbas, 2014). Basra, Dhi Qar and Al-Muthanna are among the governorates of Iraq with the most disease recorded for humans (Abdulhameed *et al.*, 2019). Surgical operations are the best treatment method for the disease, although it is difficult to perform for some cases (Mentes *et al.*, 2000). It represents a danger to the life of the patient in the event of cyst formation in vital organs such as the brain, as well as the risk of leakage of cyst fluid and the possibility of the formation of new cysts , so studies have tended to find new alternative means for surgical operations such as injecting anti-protoscolices inside the cyst as substitutional mode as silver nitrate Ag-nitrate , high tensile physiological solutions Hypertonic saline as well as ethanol, but it was later proven that these substances have many side effects as blood poisoning, liver necrosis and colitis (Rahimi *et al.*, 2015) . Therefore, it has become necessary to find new materials that are more effective, have fewer side effects, and can be used through surgical operations. It have now been found that nanoparticles (Nps) nanoparticles have unique properties due to the large surface-to-volume ratio, and these molecules also have the ability to enter cells repeatedly. Most of the others, of these nanoparticles evaluated are copper (Cu) , magnesium (Mg) , gold (Au) , zinc (Zn) , titanium (Ti) and silver (Ag) (Khan *et al.*, 2017; Retchkiman-Schabes *et al.*, 2006) .

Material & Methods

Injecting animals with Protoscolices

Swiss albino mice *Mus musculus* of Balb/C strain were used in the current study. And kept within the ideal conditions for breeding. The livers of sheep infected with virginity cysts were collected naturally from butcher shops, and the Protoscolices were extracted according to the method (Smyth, 1987). And they were collected as a suspension, the animals were injected with an amount of 0.4 ml of the suspension about 2000 Protoscolices intraperitoneal (Wangoo *et al.*, 1989) as 48 animals were divided into 8 groups, including the positive control that was not treated with any treatment, and for the negative control it was group 9, The

number of Protoscolices was calculated as follows : If the number of Protoscolices in 10 μ l = 50 and 2000 Protoscolices are required for each animal $\frac{10}{50} = \frac{?}{2000}$ then the amount of suspension is 0.4 ml/animal (Al-hamiary, 2010) .

Preparation of Drugs

- The Ag-Cu nano mixture prepared as (core-shell) style , by use 10 ml of vitamin C (0.1 N) as a reducing agent, and 2 ml (0.1 N) of copper chloride CuCl₂ and 2 ml (0.01 N) of silver nitrate AgNO₃ were added to it. The mixture was shaken until a gray precipitate was formed . The precipitate was segregation then washed by distilled water then left to dry. It was crushed and kept in glass tubes until used .
- Alb. tablets 200 mg were obtained from pharmacies , The tablets were crushed and a storage solution was prepared from them .
- Alb. was loaded onto the Ag-Cu nano mixture by mixing 250 mg of the mixture with 250 mg of the drug in 25 ml of distilled water with continuous stirring for 12 hours, then left to precipitate the product, separated and washed, left until dry and kept . Glass tubes until use .
- Dose of human of Alb. is 400 mg/kg , typical human weight is 70 kg , so 5.7 mg of Alb. for each kg , the first dose of mixtures was equal to Alb. dose .
- Additional concentrations 800 & 1200 mg/kg where used , give to animals in suitable with weight .
- Stock solution prepared from dissolve Alb. with D.W , but nano mixtures, dissolve with Dimethyl sulfoxide (DMSO) .

Doses of animals

The animals were divided into nine groups for each group eight animals , a group for each concentration of : Alb. , nano mixture & the hybrid , In addition to the presence of the negative and positive control groups .

Histological test

kidney extracted from infected animals were kept in 10% dilute formalin solution for stabilization purpose, and kept until tissue sections were prepared according to Bancroft & Steven (1982) .

Results and Discussion

Figure (1) shows the tissue of the kidney in its normal form with the clarity of the renal tubules, and it represents the negative control in which the animal only dosed distilled water

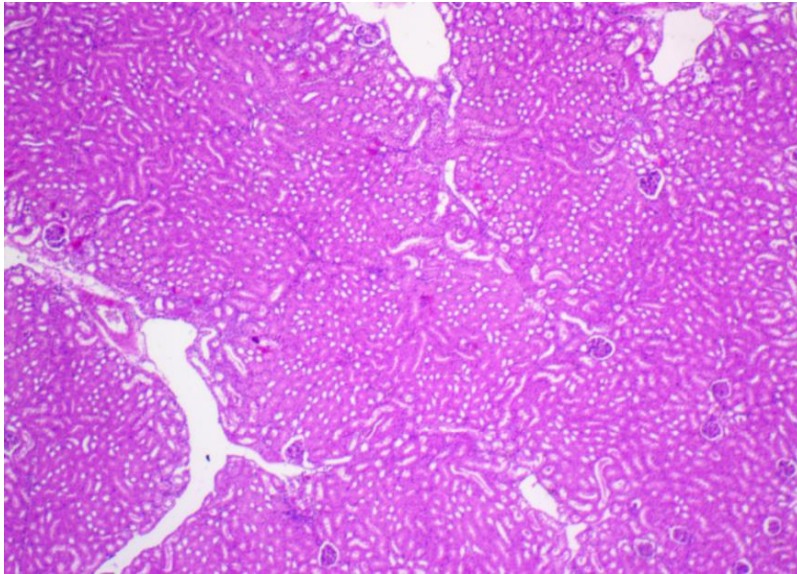


figure (1) : A section in the kidney showing the normal shape of renal tubules (Hematoxylin-eosin stained 20x)

After experimentally infecting the animals with hydatid cysts, Fig. (2) showed a large number of inflammatory cells infiltration of the kidney tissue with congestion in many veins, as this group represented the infected and untreated positive control.

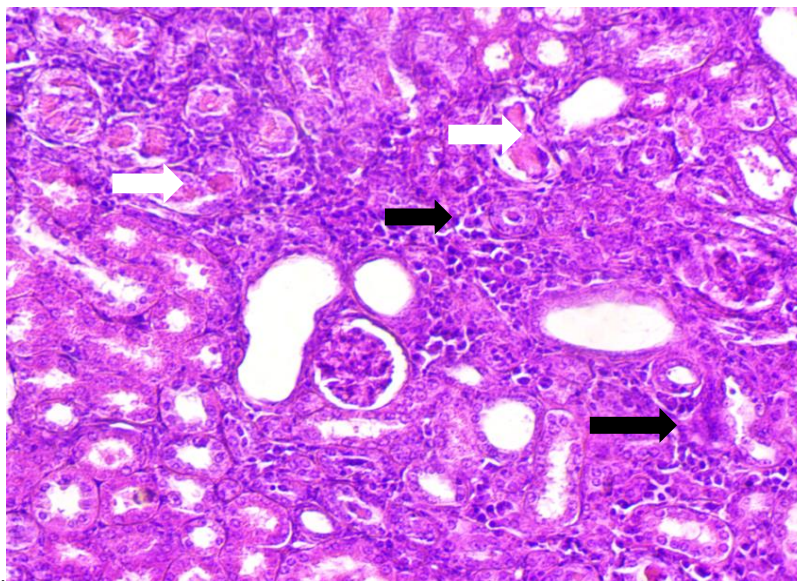


Figure (2) : A section of infected kidney tissue without treatment showing a large infiltration of inflammatory cells (black arrow) and congestion in many vessels (white arrow) (Hematoxylin-eosin stained 40x)

When treating infected animals with Alb. commercial therapy for two days, the results showed that the pathological symptoms of the kidney tissue did not change, as the presence of inflammatory cells infiltration and congestion of the vessels persisted, and this is shown in figure (3) .

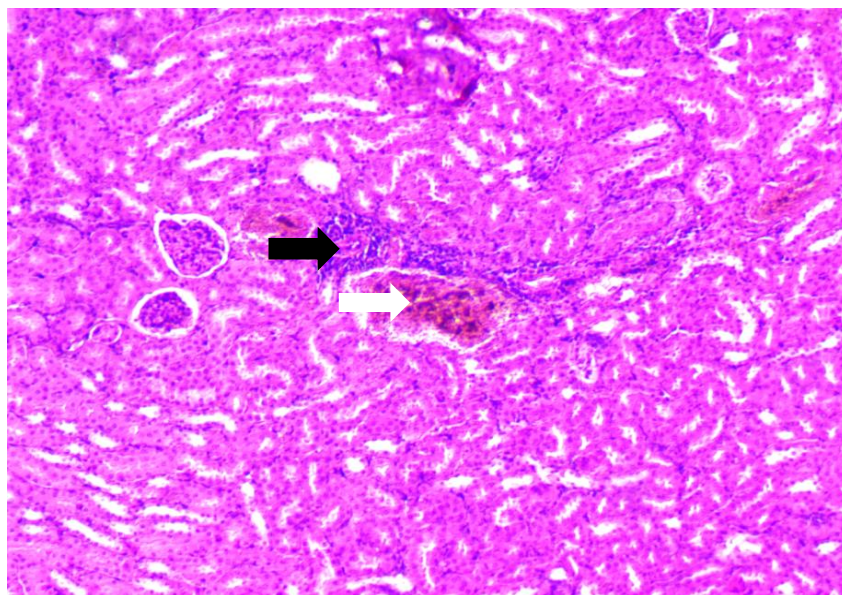


Figure (3) : A section of infected kidney tissue dosage with Alb. showing congestion in blood vessels(white arrow) and infiltration of inflammatory cells(black arrow) (Hematoxylin-eosin stained 40x)

Figure (4) shows the condition of the tissue after treating the animals with the nano mixture 400 mg/kg, as the results did not show any improvement in the condition of the tissue and the persistence of infiltration of inflammatory cells with congestion of the vessels.

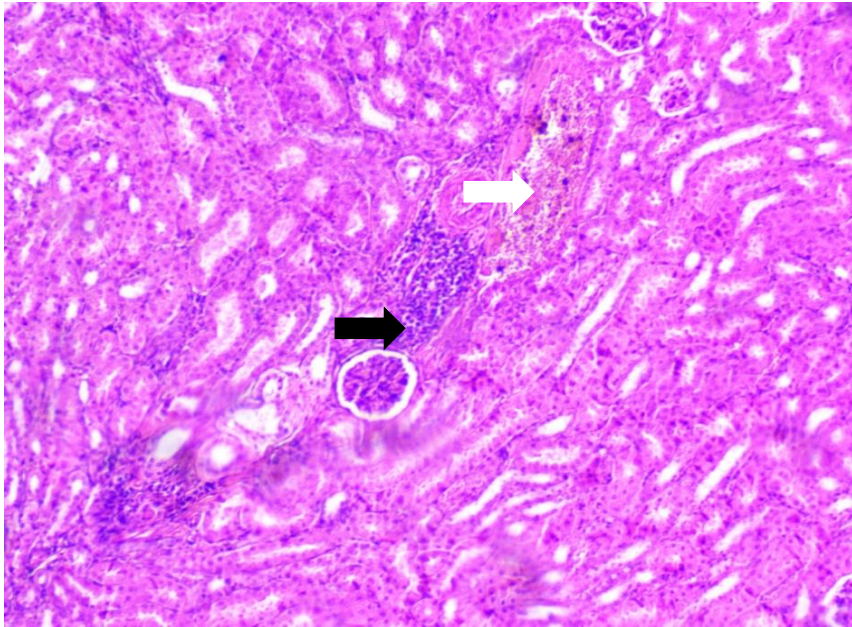


Figure (4) : A section of infected kidney tissue dosage with 400 mg/kg of the nano mixture showing congestion of the vessels (white arrow) and infiltration of inflammatory cells (black arrow) (Hematoxylin-eosin stained 40x)

Figure (5) shows the condition of the tissue after dosing the animals with the 800 mg/kg nano mixture, as vascular congestion did not appear in the section, but the inflammatory cells continued to infiltrate .

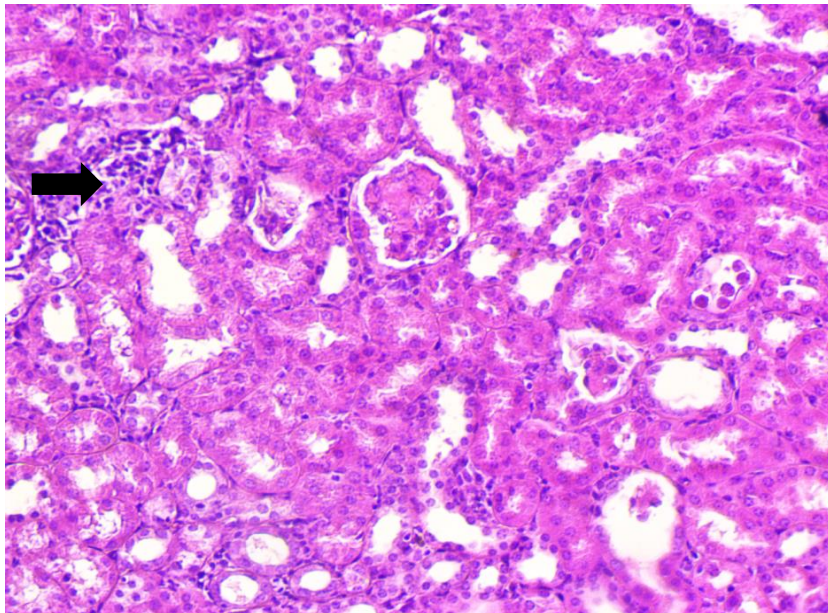


Figure (5) : A section of infected kidney tissue dosage with 800 mg/kg showing infiltration of inflammatory cells (black arrow) (Hematoxylin-eosin stained 40x)

After treating the animals with the concentration of 1200 mg/kg of the nano mixture, the tissue sections showed, as shown in Figure (6), that the pathological condition of the tissue improved after only two days of dosing, while the infiltration of inflammatory cells remained .

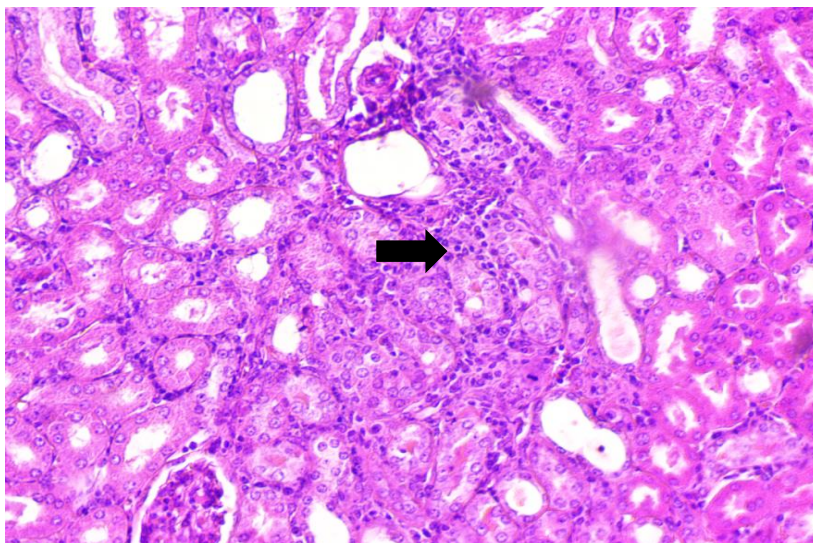


Figure (6) : A section of infected kidney tissue dosage with 1200 mg/kg of the nano mixture showing the infiltration of inflammatory cells (black arrow) (Hematoxylin-eosin stained 40x)

Figure (7) shows the condition of the tissue after treatment with the hybrid mixture 400 mg/kg, as congestion in the vessels and infiltration of inflammatory cells is observed .

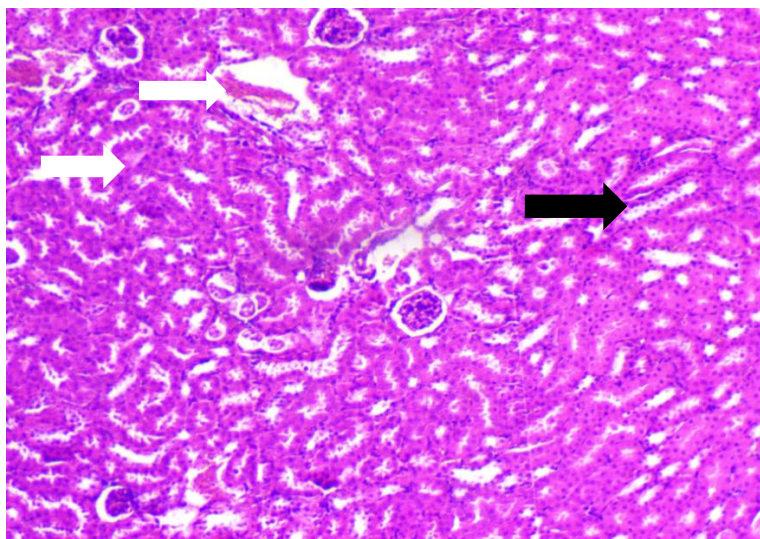


Figure (7) A section of infected kidney tissues dosage with 400 mg/kg hybrid mixture showing congestion in the vessels (white arrow) and infiltration of inflammatory cells (black arrow) (Hematoxylin-eosin stained 40x)

The results showed through figure (8) of the kidney tissue of animals treated with the hybrid mixture 800 mg/kg that the tissue still suffers from vascular congestion and inflammatory cell infiltration .

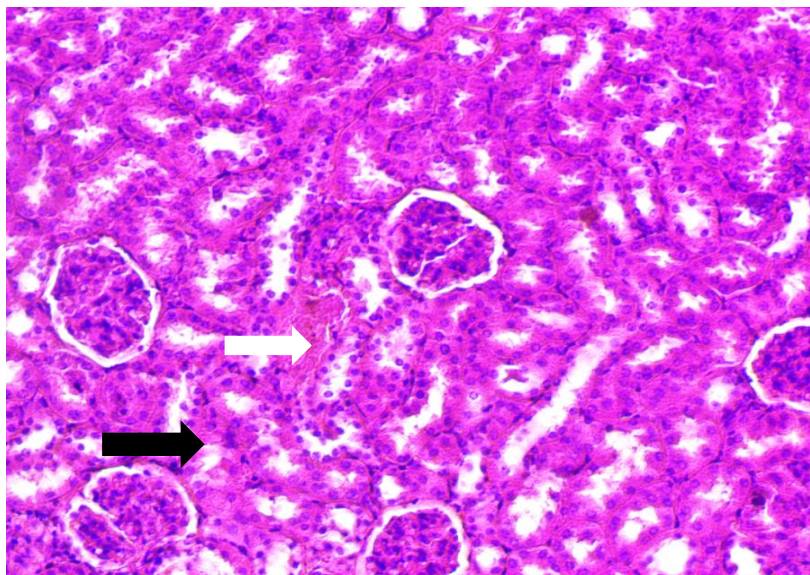


Figure (8): A section of infected kidney tissues dosage with 800 mg/kg hybrid mixture showing congestion in the vessels (white arrow), infiltration of inflammatory cells (black arrow) (Hematoxylin-eosin stained 40x)

When the infected animals were treated with the hybrid mixture 1200 mg/kg, or the results revealed that the congestion of the vessels did not appear in the tissue section and only the infiltration of inflammatory cells remained, and this shows the direction of the tissue towards healing , as showing in figure (9) .

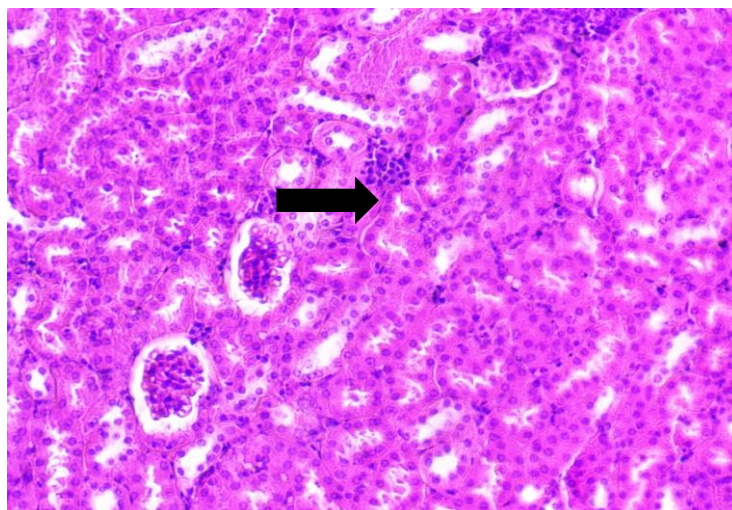


Figure (9): A section of infected kidney tissue dosed with 1200 mg/kg hybrid mixture, showing the infiltration of inflammatory cells (black arrow) (Hematoxylin-eosin stained 40x)

Hydatid cysts can develop and grow in all parts of the body, so the tissues of the affected organs are subject to destruction due to the factors of pressure and displacement of cells resulting from the growth of the cyst (Ali & Salih, 2000) . Vuitton (2003) explained that the reason for the parasite to cause histological changes may be due to its ability to avoid the immune system and secretion of immune antigens and its effect on the process of antigen presentation and the formation of granular foci in an immune attempt of the host to defend itself .

Figure (3) shows that treatment with Alb. did not affect the pathological condition of the tissue after two days of dosing , and this agreed with Aljanabi (2021) who used Alb. to treat the affected liver tissue, as the treated tissue did not show any change compare to the affected tissue , Dumitru (2019) showed that , the reason for this is that Alb. needs long periods of time to show its therapeutic efficacy .

The pathogens show high resistance to the drugs common for treatment , so they are given in larger quantities, and this seemed to cause high toxicity and the tendency to produce renewable drugs at a higher cost, so it became necessary to find alternatives to these drugs, including nanoparticles , Which is characterized by many characteristics such as low manufacturing cost, the possibility of storing for long periods and not losing its properties with high temperatures compared to different drugs (Huh & Kwon, 2011). Shown by Zheng *et al.* (2008) that nanoparticles have many mechanisms in the elimination of pathogens, as they contribute to the strong connection between the components of the cell membrane such as phosphorous and sulfur. Thus, it causes damage to the cell membrane by obstructing the formation of proteins, and thanks to this property, it can penetrate the pathogen and destroy the DNA enzymes containing phosphorus and sulfur, and may collect in large quantities within mitochondria , causing a weakening of its function due to oxidative stress (Asharani *et al.*,2009) . The nanoparticles are also one of the important systems in delivering the active chemical drug substance to the area of injury in the body (Ahmadpour *et al.*, 2019). The nano mixture can penetrate through the germ layer of the hydatid cyst through biological barriers such as degrading enzymes that hinder the arrival of the chemical drug to the target site of infection with high stability (San, 2019)

Liang *et al.* (2014) Show that when loading Alb. on a specific nanoparticles will lead to a reduction in the size and weight of the hydatid cyst, as well as it increases the concentration of the drug in the plasma and liver tissue by up to 93%, while its use in its free form makes it less effective therapeutically due to its irregular distribution Regular as well as out of the body quickly. Abulaihaiti *et al.* (2015) say that the Alb-Chitosan hybrid mixture is effective in reducing the size and weight of the hydatid cyst caused by *E. multicoloris* with better results than Alb. alone. This may explain the reason why the nano mixture and the hybrid mixture was better than Alb. in improving tissue condition and not showing congestion of the vessels, especially with concentrations of 800 and 1200 mg/mg.

Conclusion

According to the foregoing, the study proved that the nano mixture and hybrid mixture was better than Alb. if it was used freely in the treatment of animals

infected with hydatid cysts, as the kidney tissue showed a significant improvement when comparing them.

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