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The Anti-*Echinococcus* Potential of Botanicals and Nano Green Particles: Recent Updating

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INTRODUCTION

Echinococcosis is a zoonotic parasitic disease with global distribution. It is endemic in rural sheep-raising areas. In Iraq, hydatid cysts disease is hyperendemic, with high socio-economic effects due to the infection of humans and their livestock (Benyan et al. 2013; Shnawa et al. 2021). The parasite has an indirect life cycle requiring two hosts. First is the intermediate host, in which the cysts can develop in several organs of humans and domesticated mammals such as sheep, cows, buffalo, goats, camels, and pigs. Second is the final host in which the adult worms develop in the small intestine of carnivorous animals such as dogs, and other wild canids, which pollute the soil with the infective eggs evacuated with their feces (Issa et al. 2022).

Echinococcus granulosus is a parasitic worm (Cestoda) and is the causative agent of hydatidosis or hydatid cysts disease. This is attributable to the development of the larval stage (hydatid cyst) in various human organs (Hizem et al. 2020). The main way that humans become infected is by consuming ova of the parasite that are secreted with the faeces of the definitive host and contaminated food, soil, and water. The ingested eggs hatched and released embryos in the small intestine of the intermediate hosts, which then passed through the intestinal wall and wander through the bloodstream to various tissues, primarily the hepatic and pulmonary tissues, where they developed to give the larval stage or the hydatid cyst (Eckert et al. 2017).

Currently, treating hydatid cysts appears to be difficult and complex; however, there are a number of therapeutic approaches available, including the use of chemical agents like benzimidazole derivatives, PAIR (denoting puncture, Aspiration, Instillation, and PAIR (denoting puncture, Aspiration, Instillation, and Re-aspiration), surgery and even "watch and wait." (Craig et al. 2007). The most significant problem after hydatid cyst surgery is protoscoleces dissemination into the patient's viscera as a result of cyst rupture and hydatid fluid leakage, ultimately resulting in serious complications (Ormeci 2014). Meanwhile, studies revealed that the common scolicidals had some side effects, including cirrhosis, biliary cholangitis, and sclerosing cholangitis (Rajabi 2009; Sharafi et al. 2017). According to the literature, PAIR reportedly uses a variety of scolicidal agents, including albendazole, 95% ethanol, hypertonic saline solution, H2O2, silver nitrate, and cetrimide. However, many of these agents have side effects that limit how often they can be used to treat hydatid cyst disease (Sungur 1979). These factors motivate scientists to develop a novel scolicidal medication to treat Cystic Echinococcosis (CE). For CE to be successfully treated, research into safe and efficient scolicidal agents for surgery is urgently needed.

Due to their ease of use, eco-friendliness, accessibility, and nontoxicity, metal oxide nanoparticle fabrication has received a lot of attention over the past few years. In addition to, their significant and distinctive properties, such as their surface-to-mass ratio that is much higher than that of other particles, their quantum properties, and their capacity to adsorb and carry other materials like drugs, probes, and proteins, nanoparticles have become widely used in medical applications (Barzinjy et al. 2020). The success of numerous disciplines and sciences depends on nanotechnology and nanoscience. The materials' nanoscale size makes them suitable for a variety of potential applications, including drug delivery (Anoop et al. 2020), cancer treatment (Tian et al. 2019), medical treatment (Devi et al. 2019) and other applications. Overall, utilizing medicinal plant extracts as potential reducing and stabilizing agents for the synthesis of nanoparticles, such as ZnO nanoparticles, offers several benefits over traditional physical and extremely toxic chemical techniques (Mittal et al. 2013).

Recently, the treatment of hydatid cysts has been motivated to utilize green biosynthesized nanoparticles as innovative scolicidal materials such as Silver, Nickle oxide, Zinc oxide, Titanium oxide, and others, within in vitro, ex vivo, and in vivo studies (Ibrahim 2020; Shnawa et al. 2021).

In previous work, the application of some nanoparticles, particularly the greener fabrication of nanoparticles, for instance, selenium, zinc oxide, silver, gold, and other nanoparticles, as a novel scolicidal alternative to cure echinococcosis had been reviewed (Shnawa 2018; Shnawa et al. 2021).

Albalawi et al. (2020) concluded in a review that the most functional nanoparticles for hydatid cyst disease therapy were metal- NPs, metal oxide-NPs, and polymeric NPs.

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Moreover, Chitosan- NPs were proven to be an efficient and safe scolicidal agent at a minimal dose and within short exposure periods (Firouzeh et al. 2021).
Particles with a single dimension between 1 and 100 nm are known as nanoparticles. NPs exhibit a range of traits depending on their size and surface functionalities. Due to their small size and large surface area, NPs are widely used in a variety of industries, including cosmetics, electronics, and diagnostic and medicinal applications. The mode of entry, cellular uptake, and general toxicity of NPs are all influenced by their size. It has been demonstrated that this size-dependent toxicity also occurs in the liver. Silver particles with a size of 10 nm had a higher tissue distribution and more toxic effects on the liver when compared to larger NPs (40 and 100 nm) (Najahi-Missaoui et al. 2020).

Recent Studies of Greenish Fabricated Zinc Oxide Nanoparticles (ZnO NPs)

In a study, Shnawa et al. (2021) reported that the green synthesis of ZnO NPs originating from *Mentha longifolia L.* leaves extracts has an effective scolicidal possibility. At 400 ppm concentration after 150 min of exposure, the ZnO NP showed the highest scolicidal activity with a 100% mortality rate. The treated protoscolices first displayed loss of viability in the parasite's tegument. This was followed by many morphological changes, such as disorganization of the rostellar hooks and loss of calcareous corpuscles as shown in Fig. 1.
Most recently, in a study, *Lavandula angustifolia* extract was used by microwave technique. Several concentrations of the green synthesized ZnNPs (50, 100, and 200 µg/ml) were used alone and in addition to Albendazole (100 µg/ml) in an *in vitro* and *ex vivo* model. By measuring the Caspase-3 activity of protoscoleces treated with a variety of concentrations of ZnNPs, the induction of apoptosis in hydatid cyst protoscoleces was evaluated. At a concentration of 200 g/ml, ZnNPs had the highest level of scolicidal activity, killing 81.6% of protoscolices. The protoscolices were killed by these nanoparticles and ALZ after 10 minutes of exposure, particularly at a concentration of 200 g/ml (Shakibaie et al. 2022). According to these results, ZnNPs and albendazole together demonstrated a powerful protoscolicidal in vitro and ex vivo nanoparticle model against the treatment of hydatid cysts.
Moreover, *Z. spina-christi* leaf extract was used in another investigation (Shnawa et al. 2022a). After 60 minutes of exposure, 400 g/ml of ZnO-NPs exhibited the highest antiparasitic activity with a 100% mortality of the treated protoscoleces. Many morphological alterations and disorganization of rostellar hooks, presence of tegumental and suckers alteration have also been reported as shown in Fig. 2. These authors documented several morphological alterations. The safety and biocompatibility of this nanoparticle against RBCs were also confirmed in which different dosages of ZnO-NPs were considerably less than the positive control. This research proposes that phytochemicals such as polyphenols in the plant extract behave as reducing and stabilizing agents for the creation of ZnO-NPs (Shnawa et al. 2022a).
The antimicrobial mechanisms of ZnNPs were proposed. These nanoparticles can apply their antimicrobial properties through a variety of molecular mechanisms, including preventing cell growth, changing the permeability of cell membranes, promoting induced programmed cell death (apoptosis), inhibiting the production of H2O2 and thereby inducing oxidative stress, and raising the concentration of Zn in the environment prior to entering the body (Sirelkhatim et al. 2015). Additionally, among the fundamental molecular action processes, there is the motivation of programmed cell death, also known as apoptosis. Recent findings demonstrated that ZnNP doses of 50, 100, and 200 g/ml had induced apoptosis at 13.4%, 27.3%, and 34.8%, respectively, dependent on the caspase-3 enzyme (Shakibaie et al. 2022). Similar to this, earlier studies found that ZnNPs significantly increased apoptosis by upregulating the genes for caspase-3, caspase-8, caspase-9, Bax, LC3-II, Atg 5, p53, and Beclin 1 genes, increasing the production of reactive oxygen species, and decreasing the mitochondrial membrane potential in a variety of cells, including human ovarian cancer cells (SKOV3), and mouse Leydig cell line (Wang et al. 2015; Jiang et al. 2018; Jalil et al. 2022).

Recent Studies of Green-based Synthesized Silver Nanoparticles (Ag NPs)

Ag NP biosynthesis is a very efficient process for creating less toxic nanoparticles. A study utilized three different plant extracts from the leaves of *Piper nigrum*, *Ziziphus spina-christi*, and *Eucalyptus globulus* for the biosynthesis of Ag NPs. The scolicidal activity of these nanoparticles against *E. granulosus* has been studied. The outcomes showed that the scolicidal rate of 47.8% after 45 min for the Ag NPs derived from *Eucalyptus globulus* was comparable to that of albendazole treatment (Salih et al. 2020). The proportion of metallic ions in the solution to the capping and stabilizing agents determines the metallic nanoparticle size. The amount of Ag nanoparticles increased with the amount of capping and stabilizing agents present in the higher concentration of plant extract (Kohan Baghkheirati et al. 2016; Moazeni et al. 2019). The released Ag+ gave rise to the Ag nanoparticles' cytotoxicity. Numerous mechanisms explain cell death through interactions with Ag+. Ag ions can interact with the proteins' disulfide bonds and alter the microorganism's three-dimensional structure. The organisms' functionality is prevented by that interaction (Ahmed et al. 2016). Creating complexes with DNA and RNA, breaking mitochondrial membranes, and proton motive force are some additional mechanisms (Medici et al. 2019).
In a recent study, *in vitro* scolicidal effectiveness of the biosynthesized AgNPs from *Zizyphus spina- christi* leaves against *E. granulosus* protoscoleces was pointed out. Numerous morphologic changes were noted on the protoscoleces by both optical as well as scanning electron

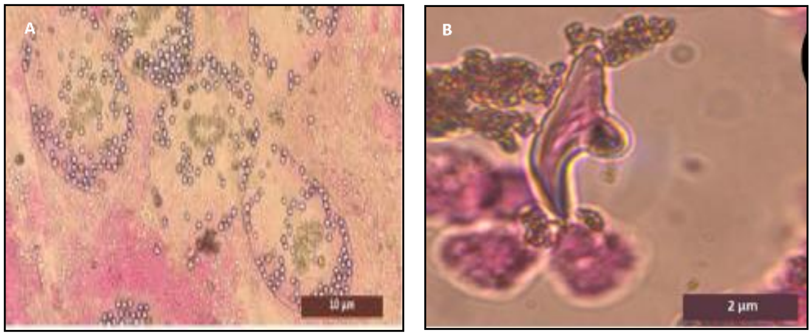

Fig. 1: A&B, dead protoscolices after exposure to ZnO NPs and stained with eosin. The protoscolices are damaged and lose their hooks (Shnawa et al. 2021).

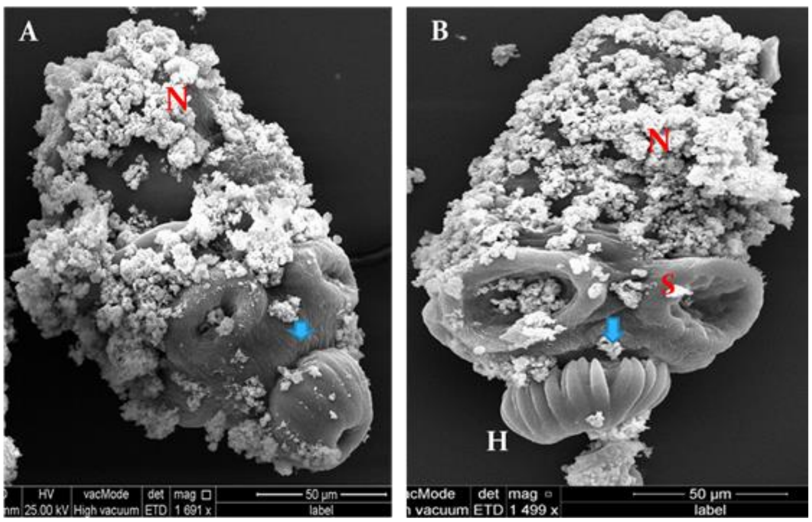

Fig. 2: (A) Evaginated protoscoleces treated with ZnO-NPs. White-colored nanoparticles (N) attached to the tegument of the parasite; the arrow signifies the soma region. (B) Protoscolex incubated with ZnO-NPs, displays contraction of soma region (arrow) And disorganization of rostellar hooks (H), also, the existence of tegumental and suckers alteration; Suckers (S), Hooks (H), (Shnawa et al. 2022 a).

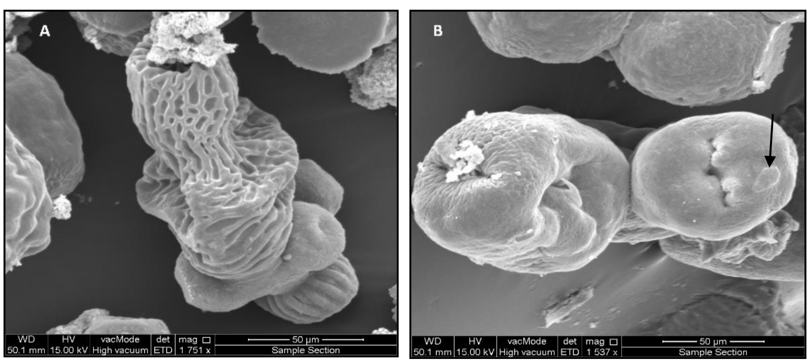

Fig. 3: A &B, protoscoleces incubated with Ag NPs, exhibit the existence of Ag NPs and the development of a bleb with morphological change. The Ag NPs accumulate like white constructions on the protoscoleces (Jalil et al. 2021).

microscopy examination, as illustrated in Fig. 3 (Jalil et al. 2021). Further, this study proposed the biocompatibility of Ag NPs as lysis of erythrocytes at various concentrations of Ag NPs was considerably ($P \leq 0.05$) lower than the value of the positive control.

In a recent paper, Hamad et al. (2022 a) performed the first in vivo evaluation of Ag NPs by *Z. spina-christi* leaves in BALB/c mice. In comparison to the untreated infected mice, the treated-infected mice displayed a variation in the liver hydatid cysts' appearance from hyaline to milky cloudy. This study also showed that Ag NPs administered orally to BALB/c mice (50, 100, 200, and 300 mg/kg) for acute short-term toxicity didn't cause any signs or adverse consequences, and no mortality. Moreover, the histopathological results in the intestine, kidneys and hepatic tissue of the animals treated with Ag NPs exhibited moderate histological impacts in contrast to the control ones, as illustrated in Fig. 4. The histological sections of the hydatid cyst in mice administered with 200 mg/kg showed rupture and destruction of the laminated and germinal layers. The findings also revealed that the cyst wall was destroyed, and vacuoles formed between the laminated and germinal layers.

Recent Studies of Green-based Synthesized Nickle Oxide Nanoparticle (NiO NPS)

In another work, *Z. spina-christi L.* leaf extract was used for synthesizing NiO-NPs. They pointed out that the green fabrication of NiO NPs possesses a powerful antioxidant

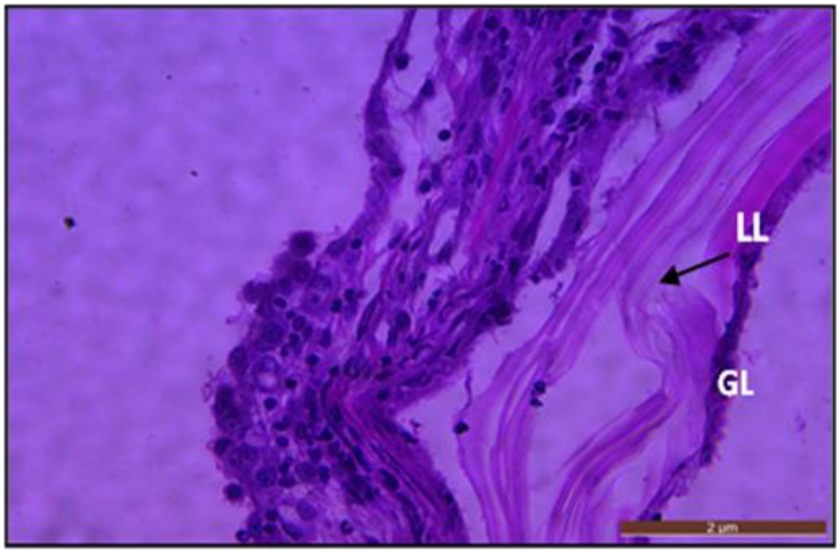

Fig. 4: Hydatid cysts from infected mice treated with AgNPs. The hydatid cysts layer is destroyed. The (LL) laminated layer converted to a loose layer with a vascular appearance. The LL seemed thin and separated from the (GL) germinal layer, stained with hematoxylin and eosin (Hamad et al. 2022 a).

action and minimal toxicity on the RBCs and seem hemocompatible. A high scolicidal action against *E. granulosus* with an efficient antibacterial action on *Escherichia coli* and *Staphylococcus aureus* was also reported (Shnawa et al. 2022b).

Recent Studies of Green-based Synthesized Copper Nanoparticles (Cu NPs)

In a study, the effects of copper nanoparticles (CuNPs) on hydatid cyst protoscoleces in vitro and ex vivo were investigated using *C. spinosa* extract and when mixed with albendazole (ALZ). Protoscoleces were exposed to different concentrations of CuNPs (250, 500, and 750 mg/mL) separately, as well in combination with Albendazole (200 mg/mL). At a concentration of 750 mg/mL in vitro, CuNPs displayed their maximum protoscolicidal activity, killing 73.3% of protoscoleces after 60 minutes of exposure. However, after being exposed for 10 min to 750 mg/mL of CuNPs together with 200 mg/mL of ALZ, protoscoleces died at a rate of 100% (Ezzatkhah et al. 2021). The results of this investigation demonstrated that CuNPs, particularly when combined with Albendazole, have strong protoscolicidal effects.

Furthermore, CuNPs at concentrations of 250, 500, and 750 mg/mL increased the activation of the caspase enzyme by 20, 32, and 36.1%, respectively, after 48 hours of treating protoscoleces. The outcomes also demonstrated that, even though the potential protoscolicidal mechanistic action of CuNPs is not fully clarified, one of the primary protoscolicidal mechanisms of CuNPs is inducing apoptosis through caspases (Ezzatkhah et al. 2021). Further, the mechanism of nanoparticles has been elucidated by previous studies as Mikami et al. (2013) explained the biological action of gold NPs by the creation of ROS, oxidative stress, DNA harm induction, and cell cycle effects. Moreover, this biological metal affects the tegument of the parasite and inhibits the synthesis of proteins (Kar et al. 2014). So the ultrastructural variations in the tegument are suggested to be a probable act of the NPs as an inhibitor of the synthesis of proteins (Napooni et al. 2019) Table 1 illustrated the updates on the effectiveness of biosynthesized nanoparticles against *E. granulosus* protoscoleces.

Proposed Mechanisms of Action

According to previous studies, the treated protoscoleces with plant material or bile acid-like chenodeoxycholic acid displayed decreased viability as well as morphological alterations, including the soma region contracting, bleb development, rostellar disorganization, loss of the parasite hooks, the obliteration of microtriches, and the formation of vesicles and lipid droplets (Shi et al. 2016; Shnawa et al. 2017). Additionally, since microtriches are directly linked to nutrition absorption, the structural changes of the tegument affect the microtriches, which may interfere with protoscoleces nutrition (Xing et al. 2016). The tegumental alteration, rostellar disorganization, loss of rostellum hooks, and the formation of numerous blebs on the tegument were among the changes in the protoscoleces. Previously, the same finding was seen in protoscoleces incubated in the existence of a variety of chemical substances, involving praziquantel and albendazole combined, flubendazole, thymol, and nitazoxanide. The nanoparticles exert their effects in various ways by disrupting DNA , preventing protein synthesis, and creating free radicals (Bajwa et al. 2022).

Table 1: Update on the effectiveness of biosynthesized nanoparticles against *E. granulosus* protoscoleces.

Nanoparticles	Plant	Concentrations	Model	Against	Activity	Reference
zinc oxide nanoparticles (ZnO NPs)	*Mentha longifolia* leaf extracts	100 ppm, 200 ppm & 400 ppm	*in vitro*	Protoscolices	The highest protoscolicidal effect was at 400 ppm., followed by 200, and 100 ppm. The scolicidal effect at 400 ppm after 150 min kills all the protoscolices.	(Shnawa et al. 2021)
zinc nanoparticles (ZnNPs)	*Lavandula angustif olia* extract	50, 100, and 200 µg/ml lonely and mixed with Albendazole (100 µg/ml)	*in vitro* and *ex vivo*	Hydatid cyst protoscolices	At 200 µg/ml, ZnNPs had the greatest scolicidal effect, killing 81.6% of protoscolices. After 10 minutes of mixing with ALZ at 200 g/ml, it damaged the protoscolices. At 200 µg/ml100% of protoscoleces were killed after 20 min in an *Ex vivo* trial. The dosage promoted the caspase-3 enzyme by 13.4%, 27.3%, and 34.8% at 50, 100, and 200 g/ml, respectively.	(Shakibaie et al. 2022)
zinc oxide nanoparticles (ZnO NPs)	*Ziziphus spina-christi* leaves	50,100, 200, 300, and 400 µg /ml	*in vitro*	protoscolices	At 400 µg / ml after 60 min of exposure,all protoscoleces were killed. Numerous morphological changes were detected in the protoscoleces. The lysis of erythrocytes at diverse concentrations of ZnO-NPs was significantly lower than the positive control.	(Shnawa et al. 2022 a)
Silver nanoparticles (Ag NPs)	*Ziziphus spina-christi* leaves	0.05, 0.1, 0.2, 0.3 & 0.4 mg/mL	*in vitro*	protoscolices	After 2 hours of treatment with Ag NPs , the entire killing of protoscoleces at 0.4 mg/ml was proved.	(Jalil et al. 2021)
Silver nanoparticles (Ag NPs)	*Ziziphus spina-christi* leaves	Mice were administered with 200 mg Ag NPs/kg. For toxicity experiments 50, 100, 200, and 300 mg/kg were tested.	*in vivo*	Hydatid cyst	The toxicity outcomes revealed that Ag NPs did not cause any adverse impacts or indications and no mortality in mice. The mice's liver, kidneys, and intestine revealed mild histological effects. The liver hydatid cysts in the treated-infected mice with Ag NPs changed from hyaline to milky cloudy in appearance.	(Hamad et al. 2022 a)
Silver nanoparticles (Ag NPs)	Leaves of *Ziziphus Spina-Christi* , *Piper nigrum*, and *Eucalyptus globulus*	0.025, 0.05, 0.1 and 0.15 mg/mL	*in vitro*	protoscolices	Scolicidal action against *E. granulosus* was detected at 47.8 % after 45 min.	(Salih et al. 2020)
Nickle oxide nanoparticles (NiO NPS)	*Ziziphus spina-christi* leaves	0.1, 0.2, 0.3, and 0.4 mg/mL	*in vitro*	protoscolices	Levels of 400, 300, and 200 µg/mL of NiO NPs presented more quick results with non-significant differences, killing almost all protoscolices after one hour.	(Shnawa et al. 2022 b)
Copper nanoparticles (CuNPs) only and mixed with Albendazole	*Capparis spinosa* Fruits	250, 500, and 750 mg/mL separately and mixed with Albendazole of (200 mg/mL)	*in vitro* and *ex vivo*	protoscolices	At 750 mg/mL in vitro, CuNPs had the highest protoscolicidal action and caused 73.3% mortality after 60 minutes of exposure. After 10 minutes, CuNPs mixed with 200 mg/mL of ALZ have 100% mortality. Caspase activity was also induced by 250, 500, and 750 mg/mL of CuNPs.	(Ezzatkhah et al. 2021)

The ability of hydatid fluid antigens to cause apoptosis in surrounding tissue was demonstrated by a recent study that revealed a high percentage of caspase-3 in mouse liver samples (Hamad et al. 2022b). It is a parasite strategy for surviving and managing the immune response, according to Hussein et al. (2020). and found that the parasite could stimulate apoptosis in hepatic and spleen tissues after the induction of a cystic echinococcosis mouse model. Immunohistochemistry analysis also demonstrated that cytokeratin and caspase3 levels were higher in echinococcus tissues than in healthy tissues. According to Yang et al. (2022), Hepatic echinococcosis causes the liver to express high levels of cytokeratin and other molecules related to apoptosis, which indicate liver damage, as shown in Fig 5.

Conclusion

According to previous research, the use of nanoparticles along with albendazole eradicated the parasite within in vitro, ex vivo, and in vivo; nevertheless, additional research is needed to evaluate the efficacy and safety of these NPs as a promising scolicidal agent in the preclinical setting (in animals) and clinical setting, not only when administered intraperitoneally but also via other routes like oral administration. The induction of apoptosis through caspases is one of the main mechanisms, even though the potential protoscolicidal mechanisms of NPs are not fully understood. But it's important to understand how these biogenic NPs work against CE at the cellular and molecular levels.

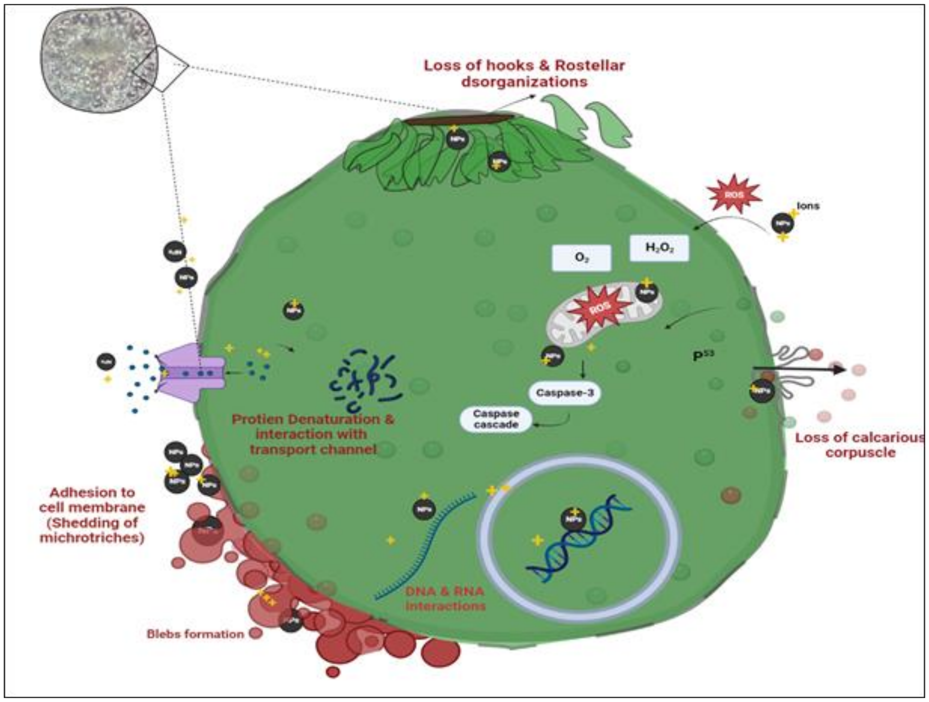

Fig. 5: The potential mechanism of green synthesized nanoparticles against hydatid cyst protoscolices.

These may offer a fresh perspective on NP targets and possibly the opportunity to develop a new, more potent drug for human CE on an immediate basis.

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