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Effects of Ivermectin and Caper Leaves Extract Loaded-Silver Nanoparticles on *Calmodulin* and *Caspase*-3 genes of *Echinococcus granulosus* Protoscoleces *in vitro*

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ABSTRACT

Hydatidosis is an important zoonotic disease due to treatment difficulties. Recent studies have explored alternative treatments, including plant extracts and nanoparticles to enhance drug delivery and efficacy.

This study investigated the lethal effects of different concentrations (1000, 500 and 125 µg/mL) of ivermectin (IVM) and ivermectin-loaded silver nanoparticles (AgNPs), in addition to different concentrations (100, 50 and 12.5 mg/mL) of extract (EXT) and extract-loaded AgNPs against hydatid cyst protoscoleces (PSCs). AgNPs were characterized by UV-visible, X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM), Fourier transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM). Gene expression was assessed by semi-quantitative RT-PCR after 12 and 24 hours of exposure.

We evaluated the therapeutic effects by quantifying calmodulin and caspase-3 gene expression. *In vitro*, AgNPs showed scolicidal effects against PSCs by activating calmodulin and caspase-3 gene expression. The gene expression in PSCs treated with IVM-loaded AgNPs and EXT-loaded AgNPs Was higher ($P \leq 0.05$) than that of PSCs treated with IVM and extract. These data suggest that IVM-loaded AgNPs and EXT-loaded AgNPs may be a promising alternative to current treatments.

Keywords: *Calmodulin*, *Capparis spinosa* extract, *Caspace-3, Echinococcus granulosus*, Ivermectin, Protoscoleces, Silver nanoparticles, Gene expression.

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تأثيرات الايفرمكتين ومستخلص اوراق الشفلح المحملة على دقائق الفضة النانوية على جينات *calmodulin* و*caspase-3* في الرؤيسات الاولية للمشوكة الحبيبية في الزجاج

رائد صباح رضوان، علي محمد عبد

قسم علوم الحياة، كلية العلوم، جامعة تكريت، تكريت، العراق

الملخص

يُعد داء الاكياس المائية من الأمراض الحيوانية المنشأ المهمة، نظرًا لصعوبة علاجه. وقد استكشفت دراسات حديثة علاجات بديلة، بما في ذلك استخدام المستخلصات والدقائق النانوية لتعزيز توصيل الدواء وفعاليته.

أُجريت هذه الدراسة لايجاد التأثيرات المميتة لتركيزات مختلفة (1000 و500 و125 ميكروغرام/مل) من الايفرمكتين المحمل على دقائق الفضة النانوية، بالإضافة إلى تركيزات مختلفة (100 و50 و12.5 ملغم/مل) من المستخلصات المحملة على دقائق الفضة النانوية ضد الرؤيسات الأولية. وُصفت دقائق الفضة النانوية باستخدام حيود الاشعة فوق البنفسجية UV-visible، حيود الاشعة السينية (XRD)، المجهر الالكتروني الماسح (FESEM)، الاشعة تحت الحمراء (FTIR)، والمجهر الالكتروني النافذ (TEM). وتم تقييم التعبير الجيني باستخدام تفاعل semi-qRT-PCR بعد 12 و24 ساعة من التعرض. وتم تقييم تأثيرات العلاجات من خلال تحديد كمية التعبير عن جينات الكالموديولين والكاسبيس-3.

وأظهرت التجارب المخبرية تأثيرات قاتلة للدقائق النانوية الفضية ضد الرؤيسات الأولية من خلال تنشيط التعبير الجيني للكالموديولين والكاسبيس-3. وكان التعبير الجيني في الرؤيسات الأولية المعالجة بالايفرمكتين المحمل على دقائق الفضة النانوية والمستخلص النباتي المحمل على دقائق الفضة النانوية أعلى منه في الرؤيسات الأولية المعالجة بالايفرمكتين والمستخلص النباتي لوحدها ($P \geq 0.05$). وتشير هذه البيانات إلى أن الايفرمكتين والمستخلص النباتي المحملة بالدقائق النانوية قد تكون بديلاً واعداً للعلاجات الحالية.

INTRODUCTION

Cystic Echinococcosis (CE) is one of the most common and important parasitic diseases caused by infection with the larval stage (metacestode) of *E. granulosus* [1]. This disease leads to many medical, veterinary and economic problems, especially affecting Rural and poor communities, where people raise livestock that come into contact with dogs [2]. In humans, cystic echinococcosis is typically the result of accidental ingestion of E. granulosus eggs and initiates detrimental effects on the liver and lungs. In humans, most cases of hydatid cysts occur in childhood and may continue for many years without being detected (asymptomatic). Clinical signs are directly related to the location, number and size of hydatid cysts [3].

Calmodulin is an important genes, it works to bind with the Ca^{2+} and conversion of the signals of the Ca^{2+} though down-stream proteins for the regulation of a variety of the physiological procedures, like muscle contraction, cell motility and metabolism [4, 5]. Many studies have shown that the expression of *caspase*-3 mRNA is significantly higher in protoscoleces (PSCs) treated with certain agents, the activation of *caspase*-3 in *E. granulosus* PSCs is part of a broader mechanism of regulated cell death, which includes necrosis, apoptosis and autophagy [6, 7].

Ivermectin (IVM) is an antiparasitic agent, a broad-spectrum drug primarily used to fight parasitic worms in human and veterinary medicine.

This unmatched compound has fundamentally been used in humans as an oral remediation for treating filarial aliments but is also effective versus other contagious-related worms. It could be used against nematodes, as well as increased Cl^- permeability in the parasite's muscle cells, resulting in parasite paralysis [8, 9].

Caper (*Capparis spinosa*), an evergreen perennial spiny shrub, is classified among plants that have various medicinal effects; it belongs to the Capparaceae family, genus Capparis and species *spinosa*. This medicinal plant is used widely since ancient times to treat various diseases affect human and animals, because this plants contain effective substances capable of treating many parasitic diseases without side effects [10, 11]. This plant contains many effective components such as rutin glycosides, myronase enzyme, caproic acid, pectic acid, saponins, alkaloids and flavonoids [12].

Nanoparticles properties include large surface area in relation to its volume, its ability to enter and penetrate physiological barriers and play an important role in infection resistance [13]. Nanoparticles produced from plant extract, because of their medicinal properties, could be used in drugs, targeted drug delivery and cosmetic applications [14]. Recent research has focused on using nanoparticles carriers to improve the delivery and efficacy of IVM and EXT against *E. granulosus* PSCs and indicating a potential for drug development against the parasites [15]. Numerous studies have examined the effect of metal nanoparticles such as silver nanoparticles against PSCs of hydatid cysts in vivo and in vitro [16]. The current study aimed to detect effects of IVM and *C. spinosa* EXT-Silver nanoparticles (AgNPs) against *calmodulin* and *caspase*-3 gens of hydatid cyst PSCs.

MATERIALS AND METHODS

Sheep hydatid cysts of *E. granulosus* were collected since February 2024 till March 2025.

Collection and preparation of plant extract (*C. spinos*):

C. spinosa L. leaves were collected from different areas in Kirkuk, brought to the laboratory of the college of science college/ University of Tikrit for botanical identification.

50 grams from air-dried and crushed *C. spinosa* leaves were put into a whipping of cellulose and extracted in a Soxhlet extractor (Germany) with 250 mL alcohol (ethanol 96%). Using a rotary evaporator at 45 Co, the solvent was evaporated until the experiment was finished. The extract was lyophilizd by a Lyophilizer (KARL KOLB, Alpha 1-2 LD plus, Germany) (Freeze drying), the residues were kept at 4 C^o [17].

Diagnosis of active compounds ethanolic plant EXT of *C. spinosa* leaves was performed using GC-MS technology in Samarra University/ College of Applied Sciences/ Central Laboratory.

Biosynthesis of silver nanoparticles (green synthesis) and IVM-loaded AgNPs

Silver nanoparticles of *C. spinosa* plant EXT were prepared according to the method by [18] with some modifications. By stirring 400 mL of the silver nitrate in 1L flask on the hot plate and adding 100 mL of alcoholic plant EXT gradually, and noted the color of the solution was changed during the addition. The solution was centrifuged (4000 rpm) for 30 minutes. The sediment was collected, then washed with deionized distilled water more than once, then dried in the oven under vacuum pressure at 60 C° to obtain a powder form of silver nanoparticles. Mixture of IVM with silver nanoparticles was prepared in the same method according to [18].

Characterization and Diagnosis of AgNPs

Methods used to diagnose hybrid nanoparticles included: UV-Visible Analysis, the color change can be checked using a UV-visible spectrophotometer and the UV-visible spectrum of the green AgNPs displayed a characteristic peak at 423 nm (fig.1).

Fourier transform infrared (FT-IR) spectrum of the green AgNPs (fig.2), FT-IR spectrum of the IVM compound (fig.3). X-ray diffraction (XRD) spectrum: The XRD analysis were used to examine

the crysallisation level of silver nanoparticles and represents the XRD pattern of green AgNPs (fig.4). Field Emission Scanning Election Microscope (FESEM, Quanta 600 Feg, FEI Co., France): The *C. spinosa* EXT from the FESEM analysis revealed a heterogeneous crystalline surface with an average size of (23.67 – 99.03) nm (Fig.5). The green AgNPs FESEM analysis revealed irregularly formed cubical crystals, spherical shape and fused cubical shape with an average size of (21.39-96.50) nm (Fig.6), this result was agreed with [19, 20].

FESEM analysis showed that the IVM and green AgNPs had an irregular, fused spherical shape on the surface of the AgNPs (Fig. 7). TEM imaging of the green AgNPs and IVM with green AgNPs (Fig. 8) further revealed a particle size of approximately 35-90 nm, which aligns with findings from [21, 22].

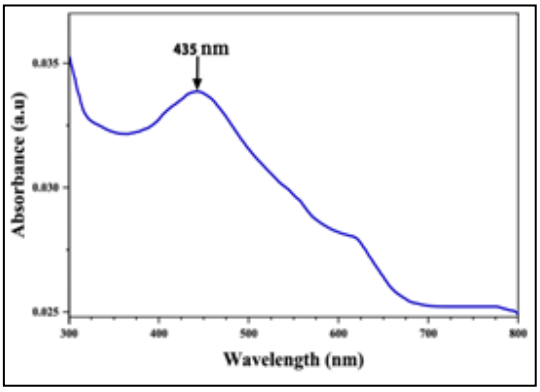

Fig. 1: UV-visible spectrum of the green AgNPs.

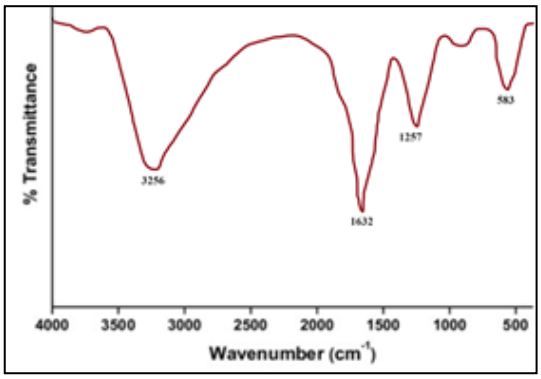

Fig. 2: FT-IR spectrum of the green AgNPs.

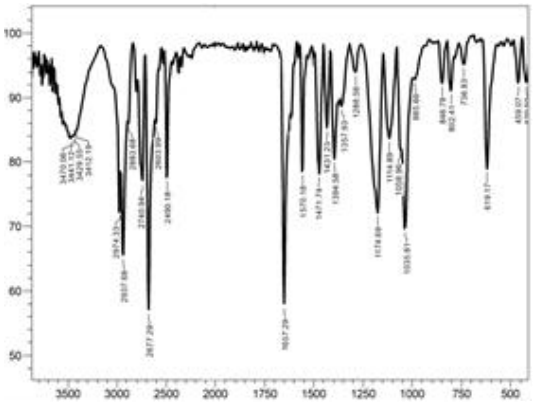

Fig. 3: FT-IR spectrum of the IVM.

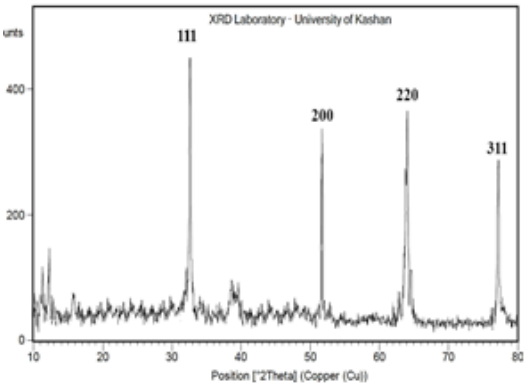

Fig. 4: XRD pattern of green AgNPs.

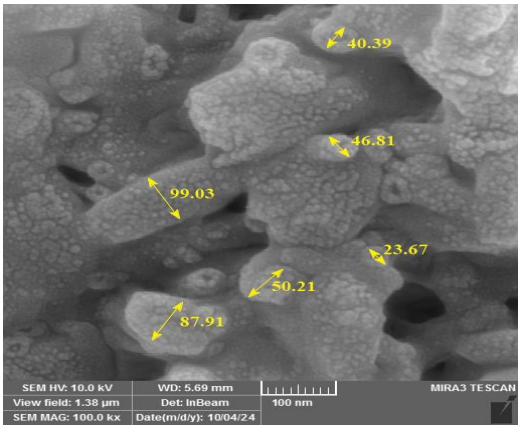

Fig. 5: FESEM of the *C. spinosa* EXT.

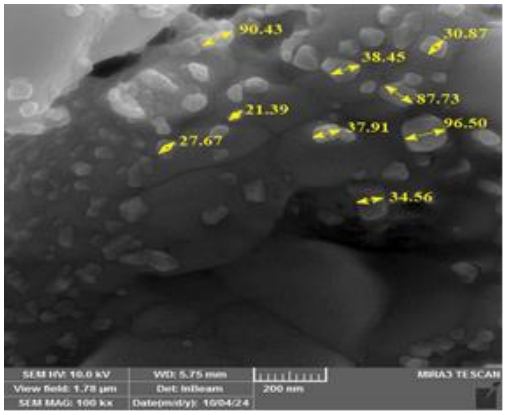

Fig. 6: FESEM of the Green AgNPs.

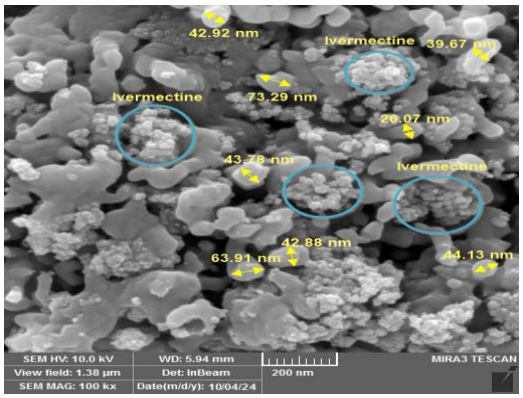

Fig. 7: FESEM of the IVM and green AgNPs.

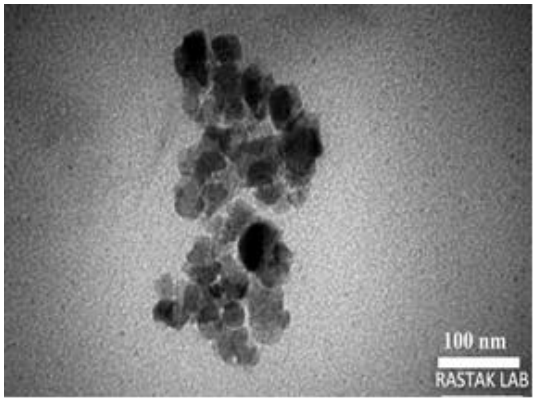

Fig. 8: TEM of the of the IVM and green AgNPs.

Preparation of protoscoleces with viability determination

PSC samples were collected and prepared using the method described by [23]. These were obtained from naturally infected sheep livers sourced from the Kirkuk slaughterhouse in Iraq. The infected livers were then transported to the parasitological laboratory at the College of Science, University of Tikrit, for further analysis.

The eosin dye assay was used to assess the viability of PSCs [24] (Figure 9b). A 10 µL solution of pooled PSCs was transferred onto a slide, mixed with 10 µL of aqueous eosin dye, and examined under a light microscope. Live PSCs did not absorb the dye, appearing green, while dead PSCs absorbed eosin dye, appearing red [25].

Treatment of PSCs *in vitro*

A total of 42 tubes were selected and divided into 4 groups (IVM, IVM-loaded AgNPs, the alcoholic EXT, the alcoholic EXT-loaded AgNPs). Each group used 9 tubes for different concentration: IVM and IVM-loaded AgNPs groups with concentrations of 1000, 500 and 125 µg/mL, while the alcoholic EXT, the alcoholic EXT-loaded AgNPs groups with concentrations 100, 50 and 12.5 mg/mL. In addition, it is used to control medium (3 tubes) and control DMSO (3 tubes). After adding EXT or the IVM at each stage, the tubes are placed in the incubator at 37°C.

In this study, to evaluate the protoscolicidal activity of IVM, initially, 0.5 mL of IVM or IVM-AgNPs with concentrations of 1000, 500 and 125 µg/mL was added to 0.5 mL from the hydatid fluid containing PSCs into the tubes. Similarly, added 0.5 mL the EXT or the EXT-AgNPs of *C. spinosa* at concentrations of 100, 50 and 12.5 mg/mL was added to 0.5 mLof the hydatid fluid containing PSCs for 1, 3, 6, 12, 24 and 48 hours of incubation, and some samples of PSCs treated and untreated (control) were isolated after 12 and 24 hours of exposure to RT-PCR.

Separation of total RNA and synthesis of complementary DNA (cDNA)

The total cellular RNA was separated from the processed and not processed PSCs after 12 and 24 hours of exposure time. RT-PCR was applied to estimate the level of gene expression by using *Transzol* Up plus RNA extraction kit. posteriorly, RNA was inverse-transcribed into cDNA and utilized as the template for PCR amplification using an adverse transcriptase kit (*Easy Script* First-Strand cDNA Synthesis).

Primers designing and qRT-PCR

The total RNA of treated PSCs after 12 and 24 hours of exposure time was extracted using the *Transzol* Up plus RNA Kit in order to effects of IVM, AgNPs-loaded IVM, EXT and AgNPs-loaded EXT on the cultured PSCs. The specific primers of *E. granulosus* calmodulin (caM) and caspase-3 genes sequences and cycling conditions have been described [26]. Real-time PCR was performed to determine the level of caM messenger RNA expression. The cDNA played the role of the templates for the qRT-PCR that has been carried out with employ of SYBR-Green PCR essence reagents. The *calmodulin* and *caspase-3*

genes, as well as β-actin (housekeeping gene) used the primers in Table1 [27]. The *caspase*-3 primer was designed using Primer3 Program (www.neb.com) in the molecular laboratory at college of science-Anbar University.

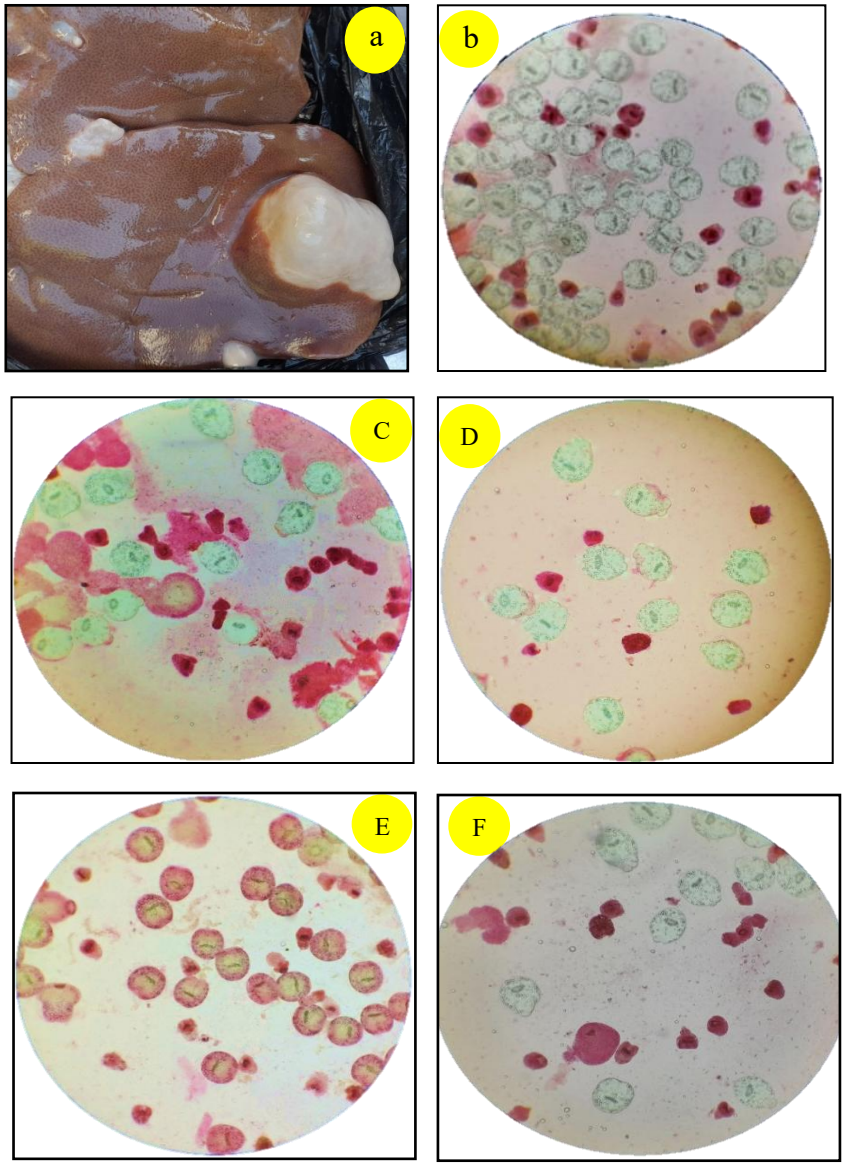

Fig. 9: a- Liver of sheep infected naturally with fertile hydatid cysts. b- Live (green) and death (red) protoscoleces (PSCs) (10X). c- PSCs treated with IVM (10X). d- PSCs treated with IVM loaded-AgNPs (10X). e- PSCs treated with EXT (10X). f- PSCs treated with EXT loaded-AgNPs (10X).

Table1: Genes and correspondent primers for the gene expression analyses of *E. granulosus*.

Gene Name	Symb.	Sequence (5′-3′)	References
calmodulin	F	GAAGGATACCGATAGTGAGGAAGA	[28]
	R	ATCATTTCGTCAACCTCCTCGTC	
caspase-3	F	GAAATGGTTGGTTGGTGGTT	In this study [29]
	R	CAATTGCAACTACCTGACTGGA	(www.neb.com)
β-actin (ACTB) (Housekeeping)	F	ATGGTTGGTATGGGACAAAAGG	[28]
	R	TTCGTCACAATACCGTGCTC	

Level Calculation of Gene Expression

The level of gene expression for all genes has been calculated using the Livak approach [30].

ΔCT infection = CT target gene – CTHK gene

ΔCT control = CT target gene – CTHK gene

ΔΔCT = ΔCT infection – ΔCT control

Gene expression € = 2^- ΔΔCT

ΔCT infection =ΔCT of infected group

ΔCT control = ΔCT of control group

CT (HK gene) = values of CT of housekeeping (ACTB)

CT (target gene)= values of CT of target genes (caM)

Fold change = Exp. Of infection / Exp. Of control

Statistical analysis of data

Statistical analysis was performed by GraphPad PRISM (GraphPad Software, La Jolla, CA, USA; http://www.graph pad.com) P-value (P≤ 0.05) was utilized as statistically meaningful and data were determined as the mean ± standard error.

RESULTS

Gene expression of *E. granulosus* PSCs in vitro

The *caspase-3* and *calmodulin* gene expression levels in the PSCs processed with compounds (IVM, IVM-loaded AgNPs at concentrations 1000, 500 and 125 µg/mL, and EXT and EXT-loaded AgNPs at concentrations 100, 50 and 12.5 mg/mL) at were assessed by the qRT-PCR after 12 and 24 hours of exposure.

Caspase-3* gene expression *in vitro

The expression of *the caspase-3* gene (Fig. 10) was low at both the 12- and 24-hour, with a slight increase observed at 24 hours ‹Significant differences (P<0.001) were noted following treatment with IVM (1000 µg/mL). The gene expression of *caspase-3* with 100 mg/mL of EXT was lower after 12 hours compared to 24 hours, with no significant difference observed (P=0.450). In contrast, PSCs treated with IVM-AgNPs at a concentration of 1000 µg/mL showed a significant increase in gene expression (P<0.001). Meanwhile, EXT-AgNPs at 100 mg/mL did not significantly increase expression compared to EXT alone, with no statistically significant difference observed (P=0.955) between the 12- and 24-hour marks.

The gene expression levels of *caspase*-3 were increased in the PSCs treated with IVM at 500 µg/mL (P=0.055), and in plant EXT at 50 mg/mL (P=0.075) after 24 hours compared with 12 hours exposure time. While the gene expression levels of PSCs treated with IVM-AgNPs at 500 µg/mL, and EXT-AgNPs at 50 mg/mL of were decreased after 24 hours compared with 12 hours, indicating significant difference (P=0.005, P=0.002), respectively (Fig.11).

The gene expression levels of *caspase-3* were increased in PSCs treated with EXT-AgNPs with 12.5 mg/mL at 24 hours, indicating a statically significant (P<0.001), and the *caspase*-3 gene expression of PSCs treated with EXT were recorded statistically significant (P=0.002). In other groups (IVM, IVM-AgNPs) no significant difference, P-vales (0.802 and 0.784, respectively) (Fig. 12).

Calmodulin* gene expression *in vitro

The gene expression levels of *calmodulin* were increased in the PSCs treated with IVM at 1000 µg/mL, and EXT-AgNPs at 100 mg/mL after 24 hours compared with 12 hours. While the gene expression was decreased in the PSCs treated with IVM- AgNPs at 1000 µg/mL after 24 hours compared with 12 hours. The gene expression levels didn't change in PSCs treated with EXT 100 mg/mL, there is no significant difference (P>0.999) between 12- and 24-hours exposure times. While statistical values in: IVM (P=0.833), IVM-AgNPs (P=0.602), and EXT-AgNPs (P=0.178) indicating no statistically significant differences (Fig. 13).

The gene expression levels of *calmodulin* has been increased in the PSCs treated with (IVM-AgNPs at 500 µg/mL, and the EXT-AgNPs at 50 mg/mL) after 24 hours compared with 12 hours, reported significant difference (P=0.026 and P=0.002) respectively. While the gene expression levels of PSCs treated with IVM at concentrations 500 µg/mL and EXT at 50 mg/mL exhibited a little

change after 24 hours of exposure compared with 12 hours (Fig. 14).

The gene expression levels of *calmodulin* showed remarkably decrease in the PSCs treated with IVM at 125 µg/mL and EXT at 12.5 mg/mL after 24 hours compared with 12 hours of exposure. While the gene expression of PSCs treated with IVM-AgNPs at 125 µg/mL and EXT- AgNPs at 12.5 mg/mL were increased after 24 hours for all compounds compared with 12 hours (Fig. 15).

DISCUSSION

At present, the application of nanoparticle goods in biomedical sciences highlights the need for efficient approaches in addressing parasites. Nanoparticle's functional important role in drug delivery, diagnosis, and gene therapy. The biosynthesis of nanoparticles by the medical plants are cost effective and environmentally pal, that did not require using energy, temperature, and toxic chemicals. These features are considered useful compared with physical and chemical methods [31]. The *C. spinosa* has many medicinal properties, one of the remarkable reasons of this exchanging is fewer side effects of grassy drugs than chemical medicines [32].

A variety of natural extracts can effectively eliminate protoscolices (PSCs) at high concentrations within a short period, making them useful as protoscolicidal agents to minimize the risk of PSC spillage during hydatid cyst surgery [33, 34]. If a cyst ruptures, PSCs may be released and lead to secondary hydatid cyst formation in the intermediate host [35]. This study demonstrates that the extract of *Capparis spinosa* (EXT of *C. spinosa*) induces PSC death in a concentration- and time-dependent manner, which aligns with findings from [34].

The prepared AgNPs using biotechnological methods can be considered as an effective scolicidal agent that could be applied for pharmaceutical applications, because of the mentioned eco-friendly process is likely to be cost effective, safe which requires non-toxic materials compared to existing chemical methods [16]. The main mechanisms identified of nanoparticles in the biological response are the production of ROS and oxidative stress, DNA damage, cell cycle effects, and potential interference [36].

Ultra structural changes in the tegument are attributed to the effectiveness of the nanoparticles affecting the tegument of parasite and inhibit the synthesis of proteins [37].

In this study various concentrations (1000, 500 and 125 mg/mL) of IVM of biosynthesized Ag-NPs with different exposure times (1, 3, 6, 12, 24, 48 hours) were employed for scolicidal assessment. The results of current study clarified the fast tegument change of the PSCs by IVM and EXT loaded with silver nanoparticles are important to support and estimation of the protoscolicidal efficacy of these compounds, this agreement with[38].

The plants, especially medicinal plants are an important source of many organic and inorganic compounds of pharmaceutical and medicinal importance, that can treat many diseases, bacterial and parasitic infections[39]. *In vitro* scolicidal effects of gold nanoparticles against PSCs of hydatid cyst was reported by provoking the apoptosis process of *caspase*-3 was changing and activation the PSCs ultrastructure with no significant cytotoxicity against the normal cells of human [40].

In this study various concentrations of IVM and IVM-loaded AgNPs, the alcoholic EXT and EXT-loaded AgNPs groups with concentrations at different exposure times were employed for scolicidal assessment and caused many effects on PSCs gene expression of hydatid cysts. The *caspase*-3 expression of PSCs in control, and PSCs treated with IVM, IVM-AgNPs, EXT, and EXT-AgNPs after 12 and 24 hours exposure. The *caspase*-3 expression was more in each PSCs processed with IVM-loaded AgNPs and PSCs processed with EXT-loaded AgNPs than IVM, plant EXT and the groups of control after 12 hours of exposure time. A convenient protoscolicidal factor must have certain characters, like ability to

little doses, the great effect at the short time. The ability to maintain the effect of the cyst dilute liquid, increased availability, decreased toxicity and the possibility of rapid preparation as mentioned in previous studies [33, 41, 42].

In this study noted that the gene expression levels of *calmodulin* were increased after exposed treatments. Calmodulin protein link with calcium, which is important for cellular signs. This linkage was changes the protein form and associated with other proteins, such as (kinase, Protein phosphatases and phosphodiesterases). That is necessary to organize physiological operations, like contractions of muscles and metabolism [43, 44].

Previous studies have confirmed that silver nanoparticles exhibit cytotoxic effects on hydatid cysts by inducing oxidative stress and activating apoptosis [45], the researchers reported that AgNPs enhanced the expression of apoptosis-related genes, such as *caspase*-3, supporting the current findings [40], it has demonstrated that combining AgNPs with other chemical agents enhances the therapeutic efficacy against hydatid cysts.

Some studies were disagree with the current study [46], who observed the effect of AgNPs alone may be not significant at lower concentrations, which aligns with the reduced *caspase*-3 expression at lower concentrations.

Previous studies have confirmed that AgNPs affected parasites by inducing oxidative stress, which affects vital processes, such as the gene expression of calcium-related proteins, including *calmodiulin*, this study agree with D'Elia and Weinrauch [47] who reported that EXT-AgNPs decreased *calmodulin* expression at low concentrations. Many studies confirm the extracts with nanoparticles have a stronger common effect, this treatments enhance the anti-parasitic effects [48], and other studies have highlighted to AgNPs can enhance apoptosis responses by increasing oxidative stress and activating *caspase*-3 [49]. Several studies have confirmed the role of IVM in inducing apoptosis by multiple pathways [50].

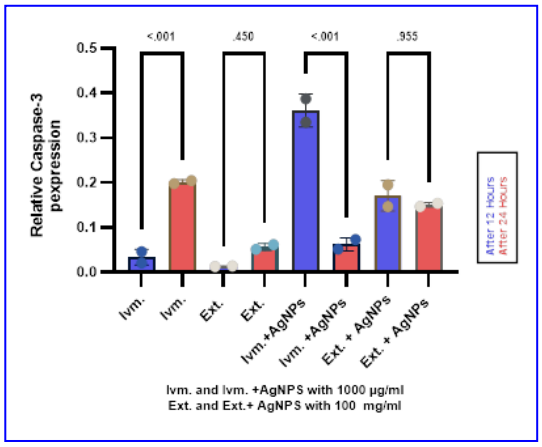

Fig. 10: The *caspase*-3 gene expression in PSCs processed with (1000 µg/mL of IVM and IVM+AgNPs and 100 mg/mL of EXT and EXT+AgNPs) after 12, 24 hours.

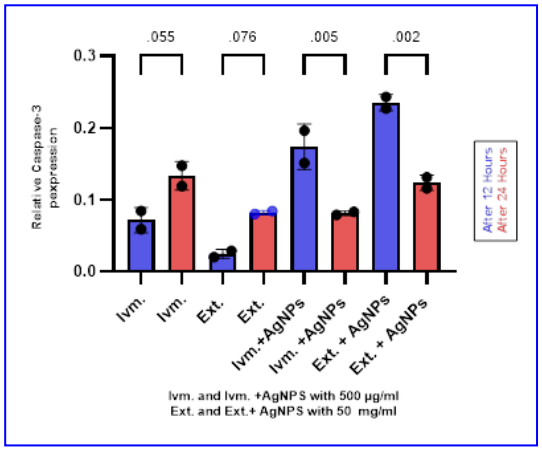

Fig. 11: The *caspase*-3 gene expression in PSCs processed with (500 µg/mL of IVM and IVM+AgNPs and 50 mg/mL of EXT and EXT+AgNPs) after 12, 24 hours.

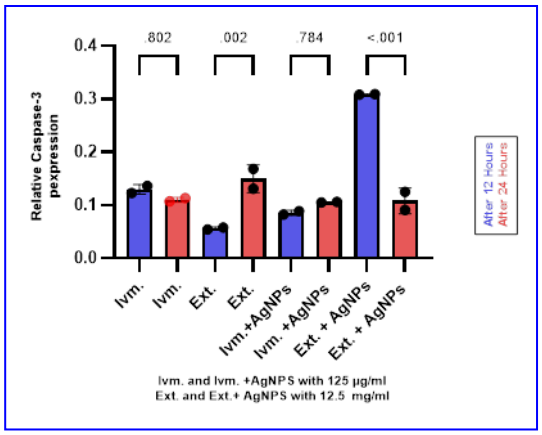

Fig. 12: The *caspase*-3 gene expression in PSCs processed with (125 µg/mL of IVM and IVM+AgNPs and 12.5 mg/mL of EXT and EXT+AgNPs) after 12, 24 hours.

DOI:https://doi.org/10.25130/tjps.v31i4.2026

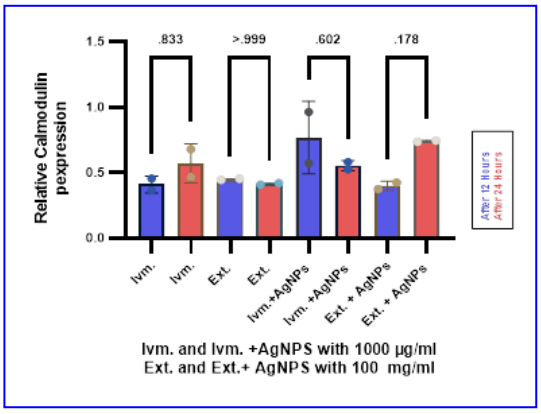

Fig. 13: The *calmodulin* gene expression in PSCs processed with (1000 µg/mL of IVM and IVM+AgNPs and 100 mg/mL of EXT and EXT+AgNPs) after 12, 24 hours.

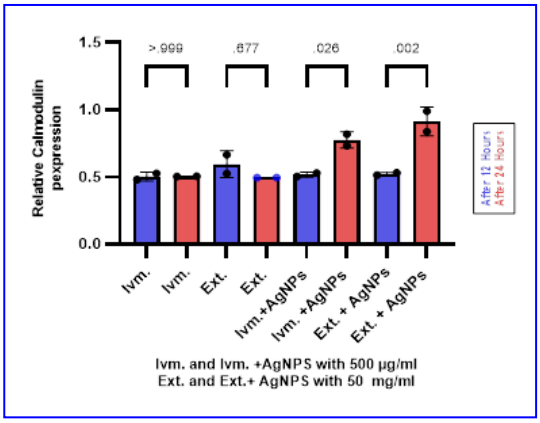

Fig. 14: The *calmodulin* gene expression in PSCs processed with (500 µg/mL of IVM and IVM+AgNPs and 50 mg/mL of EXT and EXT+AgNPs) after 12, 24 hours.

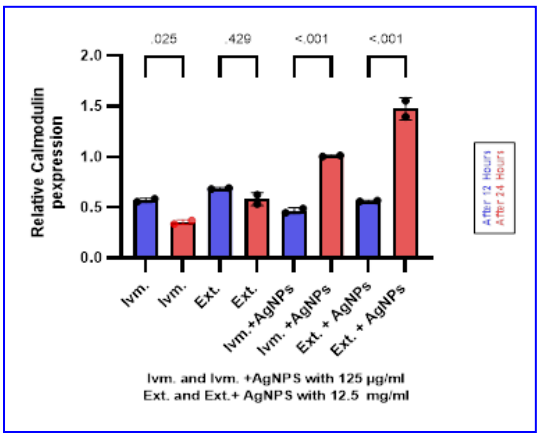

Fig. 15: The *calmodulin* gene expression in PSCs processed with (125 µg/mL of IVM and IVM+AgNPs and 12.5 mg/mL of EXT. and EXT.+AgNPs) after 12, 24 hours.

CONCLUSION

This study demonstrates that IVM, IVM-AgNPs, EXT, and EXT-AgNPs modulate the expression of *caspase*-3 and *calmodulin* genes in *E. granulosus* PSCs in a concentration and a time-dependent manner, may be a promising alternative to current treatments. The *caspase*-3 expression exhibited significant difference between 12 and 24 hour exposure across different treatments. Higher concentrations of loaded-AgNPs increased *caspase*-3 expression. Calmodulin expression was generally stable across treatments, with fewer statistically significant changes than *caspase*-3. These therapeutic nanoparticles can be utilized as a substitute in hydatid cyst treatment to overcome the disadvantages of chemical treatments, particularly their side effects.

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