Iran. J. Ichthyol. (Special issue 1, 2021): 317-329

P-ISSN: 2383-1561; E-ISSN: 2383-0964
http://www.ijichthyol.org

Research Article

The effect of piperine and curcumin on the calmodulin gene of *Echinococcus granulosus* protoscolices *in vitro*

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Abstract: Cystic echinococcosis (CE) has been defined as an illness that results from the larval *Echinococcus granulosus* stage. Treating cystic echinococcosis is fundamentally dependent upon using Albendazole that may typically result in negative side effects, which is why, more sufficient options of treatment being necessary. The re-purposing of the drugs has been considered as a beneficial method to advance the drug development. In *vitro* proto-scolicidal effect of the piperine and curcumin have been assessed in *E. granulosus* and expression of calmodulin (CaM) genes, both of them were linked to the cellular signaling activities. Based on the results, curcumin and piperine's have dose-dependent proto-scolicidal effects to achieve the optimal efficacy that the isolated piperine 20mg/k and 2g/k curcumin showed significant anthelminthic activity compared to the standard anthelminthic drug enhancing the bioavailability of curcumin. Calmodulin, as a dynamic Ca^{2+} sensor, mediates various activities of the cellular signaling from *E. granulosus* will be possibly involved in a significant biological function.

Keywords: Piperine, Curcumin, Albendazole, Calmodulin, *Echinococcus granulosus*, *In vitro.*

Citation: Dakllaoe, H.A.; Al-Hilfi, A.A.A. & Almansour, N.A.A. 2021. The effect of piperine and curcumin on the calmodulin gene of *Echinococcus granulosus* protoscolices *in vitro*. Iranian Journal of Ichthyology 8(Special issue 1): 317-329.

Introduction

Human cystic echinococcosis CE has been defined as one of the global zoonosis that result from larval or meta-cestode stage of dog tape-worm, *Echinococcus granulosus*. Treating the cystic echinococcosis is usually costly and complex, requiring wide-ranging surgery or extended drug treatment (Shie et al. 2021). Benzimidazole compounds albendazole and mebendazole were drug-based cystic echinococcosis treatment corner-stone. In past years, the significance of the Herbal drugs in the medical area was increased massively due to their fewer side effects.

The Piperine can be defined as alkaloid that is naturally found in the plants that belong to Piperaceae like *Piper nigrum*. The piperine has a few anti-mycobacterial, and anti-carcinogenic characteristics (Prashant et al. 2017). Curcumin can be obtained from the *Curcuma longa* L. (Zingiberaceae), and current conventional Indian medicine claims using its powder, against many different illnesses. The pharmacokinetic characteristics of the curcumin are an indication of the fact that after the oral administration, it is poorly absorbed and only compound traces appear in blood, whereas the majority of curcumin is excreted in feces (Sarkar et al. 2016). The transformation of curcumin in unidentified complex throughout the absorption and its glucuronidation in liver possibly account for the low concentrations in the blood. The black pepper was found to result in the enhancement of drug bio-availability through inhibiting the glucuronidation in liver and small intestines (Hasimoto et al. 2020).

CaM is a small calcium sensor protein, has been defined as a very evolutionarily ancient protein in the

Table 1. The chemical test of the aqueous plant extracts from the turmeric plant.

extract	Test					
	resins	lead acetate	Saponines	Drakendrov detector	Meyer's detector	ferric chloride
aqueous	+	+	+	+	+	+

Table 2. The chemical test of the aqueous plant extracts from the Black pepper plant.

extract	Test					
	resins	lead acetate	Saponines	Drakendrov detector	Meyer's detector	ferric chloride
aqueous	+	+	+	+	+	+

Table 3. Protoscolices mortality rate by the effect of the aqueous extract of black pepper *in vitro*.

Aqueous extract mg/ml	The period of exposure to the extract in days - number of death		
	First day	second day	third day
0.25	182 ±0.942	190±2.054	225±2.494
0.5	191±2.494	233±3.399	300±3.858
0.75	195±0.942	235±2.054	521±2.943
1	250±2.943	340±2.054	705±2.494
control	10±1.247	14±0.471	18±0.816

eukaryotes. Calmodulin functions include the Ca^{2+} binding and conversion of the signals of the Ca^{2+} though down-stream proteins for the regulation of a variety of the physiological procedures, like the contraction of the muscles, cell motility and metabolism (Tai et al. 2019). Calmodulin gene is expressed as well in tegument tissue as well as the parenchymal region of the protoscolices (PSC). Even though the calmodulin was commonly researched and well-identified in a wide variety of the organisms, it was not characterized or cloned in the *Echinococcus* spp.. Only in the *E. multilocularis* (a species having close genetic relation with the *E. granulosus*) has been predicted as one of the possible drug targets with a high score and with available chemical leads, which have been referred as the approved compounds or drugs (Mosavi et al. 2019; Shabgah et al. 2021). With an objective of evaluating new compounds against *E. granulosus* PSC with the use of the drug re-purposing, the present work has been designed for the evaluation of the effectiveness of 2 available and safe extracts of the curcumin and piperine.

Materials and methods

Hydatid cyst samples collection and diagnosis: Hydatid cysts have been obtained from lungs and livers of the infected sheep in the Basra slaughterhouse. The samples were brought to the laboratory in plastic bags and examined on the same day. They were distinguished by their spherical shape with a single vacuole. Protoscolices were isolated and their vitality was tested, as the live were green, while the dead ones appeared in red and their numbers were calculated (Varcasia et al. 2007).

Plant sample collection: The roots of the turmeric plant, *Curcuma longa*, and the dry fruits of the black pepper plant, *Piper nigrum*, were obtained from the local markets in the district of Qurna. The plants were classified in the botanical herbarium in the Department of Life Sciences, College of Sciences, University of Basra. The plant parts of turmeric and black pepper were ground separately with an electric mill and kept in sealed glass containers in the refrigerator at a temperature of 4°C until use.

Preparation of aqueous and organic extracts (ethyl alcohol 95% - ethyl acetate – hexane):

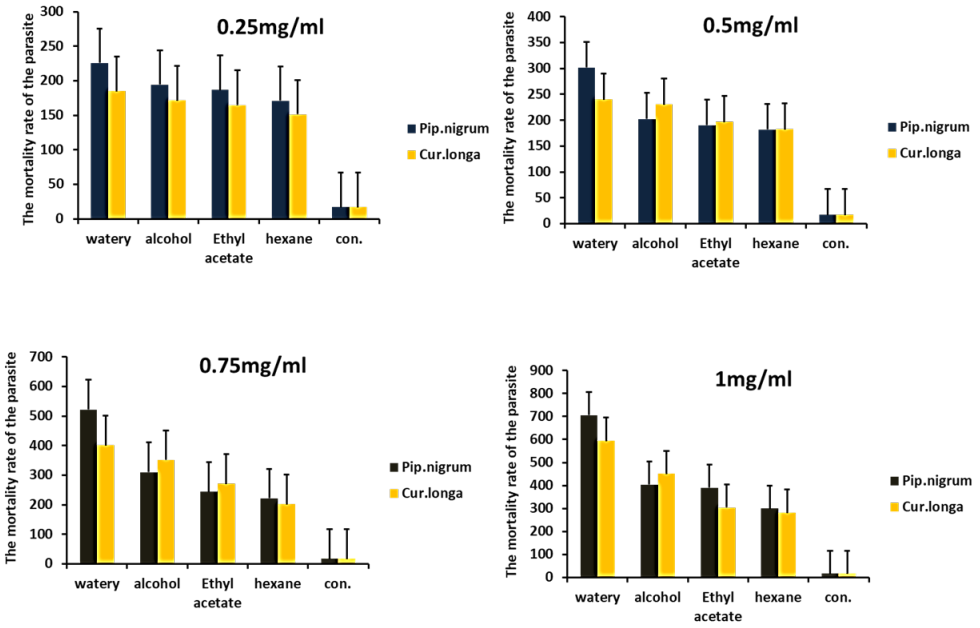

Fig.1. Effect of aqueous and organic extracts of turmeric and black pepper plants on protoscolices *in vitro*.

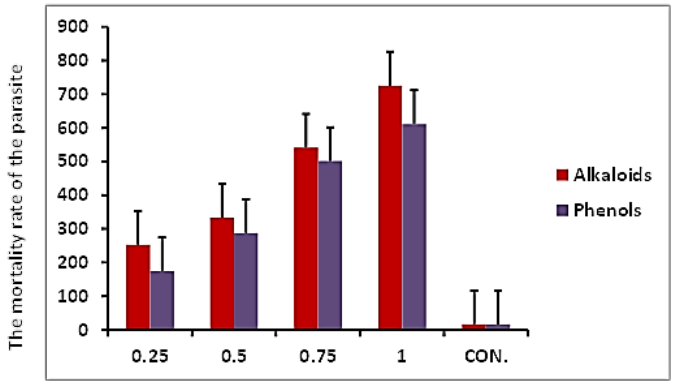

Fig.2. Effect of extracts of alkaloids and phenols on protoscolices *in vitro*.

Table 4. Protoscolices mortality rate by the effect of the alcoholic extract of black pepper *in vitro*.

alcoholic extract mg/ml	The period of exposure to the extract in days - number of death		
	First day	second day	third day
0.25	161±2.494	182±4.320	3.399±193
0.5	180±2.943	195±2.494	201±3.399
0.75	190±2.943	200±1.632	310± 2.054
1	204±2.494	300±4.320	404±1.632
control	10±1.247	14±0.471	18±0.816

Aqueous and organic extracts were extracted according to the method of Harborne (1984).

Chemical tests: A number of the chemical tests for the identification of the quality of the chemical compound in aqueous extract of *P. nigrum* and *C. longa* according to Iqbal et al. 2015.

Preparation of phenolic extract for tumeric plant: The phenolic extract for tumeric plant were extracted according to the method of Harborne (1984).

Preparation of alkaloids extract for black pepper plant: The alkaloids extract for black pepper plant were extracted according to the method of Harborne

Table 5. Protoscolices mortality rate by the effect of the ethyl acetate extract of black pepper in vitro.

Ethyl acetate extract mg/ml	The period of exposure to the extract in days - number of death		
	First day	second day	third day
0.25	151±2.054	162±2.494	185±3.858
0.5	169±2.494	182±3.399	189±2.943
0.75	180±2.494	188±3.858	243±2.943
1	191±3.399	269±2.943	389±3.399
control	10±1.247	14±0.471	18±0.816

Table 6. Protoscolices mortality rate by the effect of the hexane extract of black pepper in vitro.

hexane extract mg/ml	The period of exposure to the extract in days - number of death		
	First day	second day	third day
0.25	141±3.399	153±2.943	169±4.320
0.5	149±2.943	179±1.632	181±2.494
0.75	173±2.943	179±3.399	220±2.624
1	186±2.943	251±3.399	299±2.943
control	10±1.247	14±0.471	18±0.816

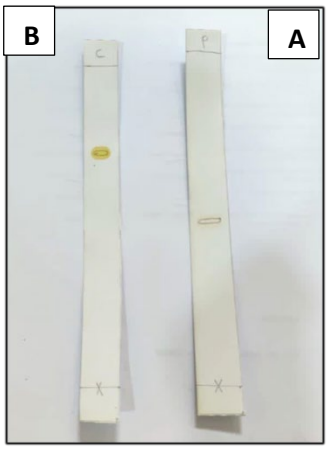

Fig.3. Thin layer chromatography (A) piperine (B) curcumin.

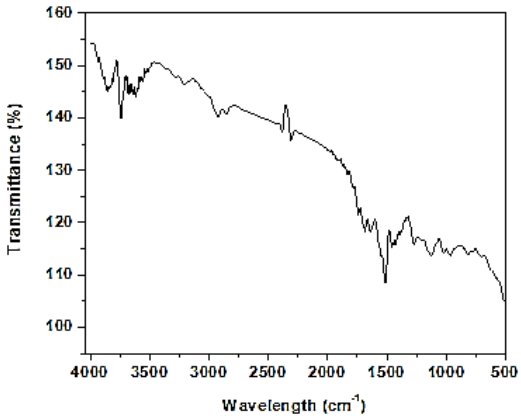

Fig.4. Infrared spectrum of curcumin.

(1984).

The effect of aqueous, organic extracts and phenolic, alkaloid compounds on the protoscolices *in vitro*: For the studying the effect of plant extracts on the protoscolices, an appropriate medium must be provided for culture. A medium RPMI 1640 (Roswell Park Memorial Institute) was used. Four groups containing equal volumes of 0.1ml hydatid cyst fluid containing the 700 protoscolex were treated with 0.25, 0.5, 0.75 and 1mg/ml of the extract in culture bottles with cover of 19.7, 19.4, 19.2 and 18.9ml of medium, respectively, so that the final volume of each culture vial was 20ml, and the control group was left untreated with three replicates for each group and calculated dead protoscolices daily for 3 days after treatment (Hamady Obeid et al. 2019).

Isolation of curcumin and piperine: Curcumin separated from phenols extract for turmeric roots with use of a thin layer chromatography and eleuent system (Chloroform: Ethanol: glacial acetic acid) by a 94:5:1 ratio and piperine was separated from alkaloids extract for black pepper using eleuent system (Toluene: Ethyl acetate) by ratio of 7:3 (Bolat et al. 2020).

Diagnosis of curcumin and piperine

a. Infra-red spectra*:* IR spectra have recorded discs technology of potassium bromide (KBr discs) in a range from 4,000 to 500cm^{-1}.

b. Electronic spectra*:* Those spectra have been

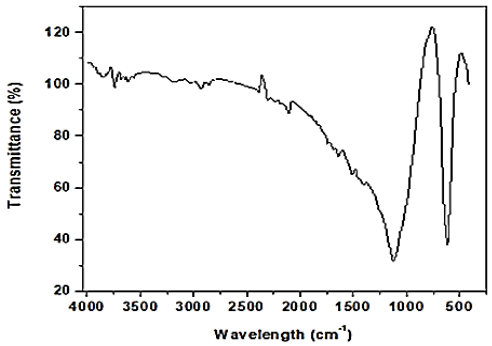

Fig.5. Infrared spectrum of piperine.

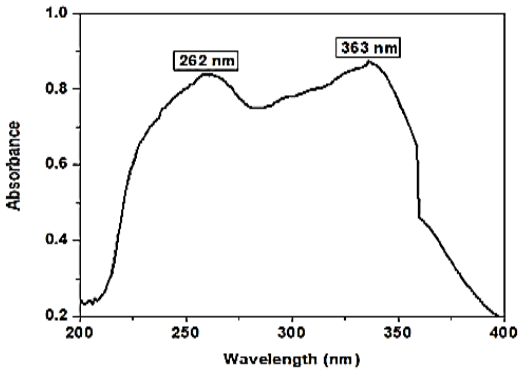

Fig.6. Spectrum of the ultraviolet region of curcumin.

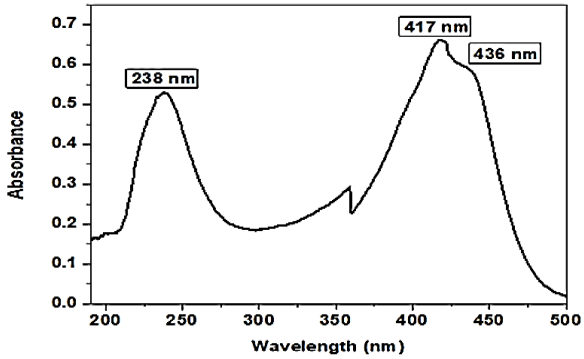

Fig.7. Spectrum of the ultraviolet region of piperine.

recorded in UV range. The distilled water has been utilized for measuring in this range and concentration values that have been utilized were 0.004gm/5mL of the solvent (Silverstein 1991).

Effect of curcumin, piperine and albendazole on protoscolices *in vitro*: Effect of curcumin, piperine and albendazole on protoscolices were examined using 2g/k curcumin and 20mg/k piperine according to Shoba et al. (1998).

Molecular analysis of Calmodulin gene: Protoscolices treated with curcumin, piperine, curcumin+piperin and albendazole *in vitro* were used to study gene expression for Calmodulin protiens compared with control by qRT- PCR method using suspension 0.25-1mg/ml after 48-72h (Shiee et al. 2021)**.**

Total RNA extraction: Total RNAs have been extracted from protoscolices based on a procedure of GENEzolTM TriRNA Pure Kit (Promega, USA)**.**

Reverse RNA transcription: Totally 300ng of the RNA from every one of the samples has been utilized for the generation of the c-DNA with the use of the GoTaq®2-Step RT-qPCR System (Promega, U.S.). the condition of the thermal cycler for the synthesis of the cDNA has been represented in a 25^{o} annealing temperature for 5min, incubation at 42^{o}C for 1h, inactivating reverse transcriptase at 70^{o}C for 15min with one cycle**.**

Quantitative real -time PCR (qRT-PCR): cDNA played the role of the templates for the qRT-PCR that has been carried out with the use of SYBR-Green PCR core reagents. Totally 120ng of the cDNA from every one of the samples has been utilized for measuring the (caM and β-actin (ACTB) gene expression as a housekeeping gene (HK) The used primers for the caM were forward primer of (FP) 5′-GAAGGATACCGATAGTGAGGAAGA-3′ and reverse primer of (RP) 5′-ATCATTTC GTCAACCTCCTCGTC-3'; ACTB: FP 5′-ATGG TTGGTATGGGACAAAAGG-3′and RP 5′-TTCGT CACAATACCGTGCTC-3′ (Mousavi et al. 2019). The volumes of every of the reactions were used in GoTaq® qPCR as Master 10ml, FP 1ml, RP 1ml, the cDNA template 6ml, Nuclease-Free Water 2ml. The DNA amplifications have been carried out under the following conditions of the reaction: an initial 95^{o}C heating cycle for 2min; 40 cycles that alternate between the denaturation at 95^{o}C for 15sec, primer annealing and extension at 60^{o}C for 1min. The data have been analyzed through the calculation of the level of expression of the genes of interest using cycle threshold number (CT) value. Relative quantification of the expression of caM genes have been obtained with the use of the β-actin as an HK

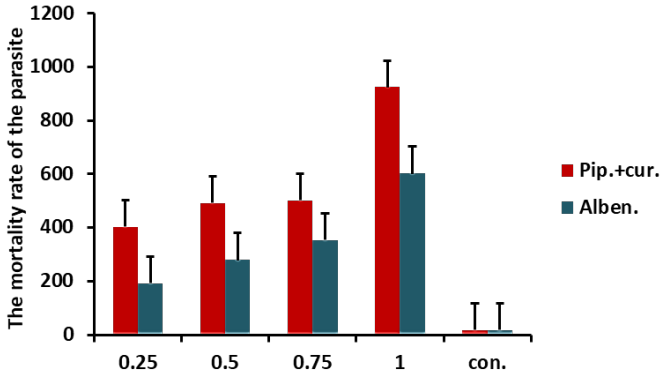

Fig.7. The effect of the curcumin - piperine and albendazole on protoscolices *in vitro*.

Table 7. Protoscolices mortality rate by the effect of the aqueous extract of turmeric plant *in vitro*.

Aqueous extract mg/ml	The period of exposure to the extract in days - number of death		
	First day	second day	third day
0.25	153±1.247	160±1.632	185±0.816
0.5	181±2.054	201±0.471	241±0.816
0.75	188±0.471	220±0.816	401±2.160
1	243±0.816	305±1.247	593±1.247
control	10±1.247	14±0.471	18±0.816

Table 8. Protoscolices mortality rate by the effect of the alcoholic extract of turmeric plant *in vitro*.

Aqueous extract mg/ml	The period of exposure to the extract in days - number of death		
	First day	second day	third day
0.25	153±1.247	160±1.632	185±0.816
0.5	181±2.054	201±0.471	241±0.816
0.75	188±0.471	220±0.816	401±2.160
1	243±0.816	305±1.247	593±1.247
control	10±1.247	14±0.471	18±0.816

Table 9. Protoscolices mortality rate by the effect of the ethyl acetate extract of turmeric plant *in vitro*.

Ethyl acetate extract mg/ml	The period of exposure to the extract in days - number of death		
	First day	second day	third day
0.25	130±1.247	143±0.816	166±2.943
0.5	142±1.247	172±0.942	198±0.816
0.75	155±2.054	188±1.247	270±0.816
1	182±0.942	201±1.247	302±1.247
control	10±1.247	14±0.471	18±0.816

gene, the level of expression of every one of the genes has been computed based upon a Livak approach (Livak & Schmittgen 2001) as follow:

$\Delta CT_{infection} = CT_{target\ gene} - CT_{HK\ gene}$

$\Delta CT_{control} = CT_{target\ gene} - CT_{HK\ gene}$

$\Delta\Delta CT = \Delta CT_{infection} - \Delta CT_{control}$

Gene expression (E) = 2^- ΔΔCT

- $\Delta CT_{infection}$ =ΔCT of infected group
- $\Delta CT_{control}$ = 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

Table 10. Protoscolices mortality rate by the effect of the hexane extract of turmeric plant in vitro.

Hexane extract mg/ml	The period of exposure to the extract in days - number of death		
	First day	second day	third day
0.25	121 ± 1.247	133± 0.816	151±2.054
0.5	130± 1.699	162±2.054	182±2.494
0.75	144±0.942	180±1.699	202±2.054
1	173±2.494	191±0.942	280±1.247
control	10±1.247	14±0.471	18±0.816

Table 11. Protoscolices mortality rate by the effect of the alkaloid extract of black pepper in vitro.

Alkaloid extract mg/ml	The period of exposure to the extract in days - number of death		
	First day	second day	third day
0.25	2.943±192	3.399±203	3.299±255
0.5	2.494±204	0.942±225	2.494±333
0.75	2.943±234	2.494±255	3.399±540
1	2.624±290	2.624±366	2.494±725
control	10±1.247	14±0.471	18±0.816

Table 12. Protoscolices mortality rate by the effect of the phenolic extract of turmeric plant in vitro.

Phenolic extract mg/ml	The period of exposure to the extract in days - number of death		
	First day	second day	third day
0.25	2.054±163	2.943±170	1.632±191
0.5	2.494±191	2.943±230	1.247±288
0.75	2.054±203	2.494±271	2.494±501
1	1.247±255	1.632±356	3.858±600
control	10±1.247	14±0.471	18±0.816

Table 13. IR data of curcumin (with potassium bromide disk technique).

Wave number (cm^{-1})	Assignment
3741.74	stretching vibration of phenolic OH
3282.85-3205.93	stretching vibration of OH in enol form
3004.96	stretching vibration of aromatic CH
2925.24, 2856.28	stretching vibration of aliphatic CH
1733.77	stretching vibration of aliphatic C=O
1687.93	stretching vibration of aliphatic C=C
1514.83	vibrations of υC=O, δCCC, δCC=O and aromatic υC=C, υCCH
1461.19, 1447.09, 1428.26	deformation vibrations of two CH_3 groups
1272.44	in plane CH vibrations of aromatic rings
1130.60, 1024.13	in plane deformation of phenyl rings and skeletal in-plane deformations
966.27	CH out-of-plane vibration of aromatic rings

Statistical analyses: Statistical analysis has been carried out with the use of ANOVA test (Kao & Green 2008).

Results

Chemical test of aqueous plant extracts: The results of these test indicated that they contained the alkaloid and phenolic compounds (Tables 1, 2)

Effect of aqueous and organic extracts of turmeric and of black pepper on protoscolices *in vitro*: The results showed the direct effect of the aqueous and organic extracts of the turmeric and black pepper plants on protoscolices and their high effectiveness with gradual rates of killing compared with the

Table 14. Infrared spectrum data for piperine (potassium bromide disc technology).

Wave number (cm^{-1})	Assignment
3024.25	stretching vibration of aromatic CH
2930.50, 2856.77	stretching vibration of aliphatic CH
1643.19, 1512.44	stretching vibrations of C=C, -CO-N
1448.59	bending vibration of CH_2
1125.60	stretching vibrations of =C-O-C and in-plane bending vibrations of phenyl C-H
614.96	C-H bending vibration of –CH=CH- and stretching vibration of C-O

Table 15. Infrared spectrum data for piperine (potassium bromide disc technology).

Max wavelength (λmax) nanometers	Compound
238, 417, 436	curcumin
262, 363	piperine

control groups (Tables 3-10) as they varied according to the type of plant extract. The effect of aqueous extracts was higher compared to the organic extracts for the third day for concentrations of 0.25-0.5 - 0.75-1mg/ml, as the highest value was recorded for black pepper (225±2.494, 300±3.858, 521±2.943, 705±2.494, respectively). Followed by the aqueous extract of turmeric (185±0.816, 241±0.816, 401±2,160, 593±1.247, respectively) (Fig. 1).

Effect of extracts of alkaloid and phenolic on proscolices *in vitro*: The secondary compounds (alkaloid-phenol) showed a significant differences from the first day of the experiment in the number of parasite deaths and for all treatments (Tables 11, 12). The highest value of fatalities was recorded by the alkaloid compound on the third day at concentrations of 0.25-0.50-0.75-1mg/ml and 255±3.299, 333±2.494, 540±3.399, 725±2.494, respectively, and the fatalities decreased using the compound Phenol (191±1.632, 288±1.247, 501±2.494, and 600±3.858, respectively) (Fig. 2)

Isolation of curcumin and piperine: Curcumin and piperine were separated by thin layer and column chromatography. Two spots appeared separately, and the flow coefficient for the separated piperine was 0.5, while for curcumin was 0.7, compared with the pure standard compounds (Fig. 3).

Curcumin - Piperine diagnosis

a. Infrared spectra: Infrared spectra of piperine and curcumin were recorded through the appearance of the resulting spectra absorption bands and their respective structural aggregates (Tables 13-14; Figs. 4, 5).

b. Electronic spectra: The electronic spectra of the active compounds were recorded by the appearance of the π-π* electron transitions (Table 15; Fig. 6, 7).

Effect of curcumin-piperin and albendazole on protoscolies in vitro: The results showed the effect of the active compounds (piperin, curcumin, curcumin+piperine) and albendazole treatment on protoscolices in varying proportions and with a significant difference for all treatments compared to control groups (Tables 16-19). And the highest value of parasite mortality was recorded using a mixture of curcumin and piperine for the third day by concentrations of 0.25-0.50-.0.75-1mg/ml and 401±2.943, 490±4.784, 501±3.399, 952±0.942, respectively. Compared with Standard albendazole (191±3.858, 279±1.632, 350±2.054, 601±3.399, respectively) (Fig. 8).

Molecular study

caM gene expression *in vitro*: The gene expression levels of caM proteins showed remarkably high progressive levels in the treated protoscolices at a concentration of 0.25mg/ml 48h and 72h after the start of exposure to all compounds compared to the control groups. Melting curves with one peak were obtained for the caM gene for the purpose of illustrating the specific association of primers with target gene (Fig. 9). The highest gene expression of

Table 16. Protoscolices mortality rate by the effect of the piperine *in vitro*.

Piperine mg/ml	The period of exposure to the extract in days - number of death		
	First day	second day	third day
0.25	2.054±255	2.449±280	2.494±303
0.5	2.828±298	2.160±395	1.885±397
0.75	2.943±301	3.091±402	3.299±401
1	3.681±600	2.054±694	1.699±785
control	10±1.247	14±0.471	18±0.816

Table 17. Protoscolices mortality rate by the effect of the piperine *in vitro*.

Curcumin mg/ml	The period of exposure to the extract in days - number of death		
	First day	second day	third day
0.25	2.943±172	2.160±181	1.699±225
0.5	2.867±200	2.494±243	2.943±332
0.75	2.494±225	2.054±282	2.867±525
1	2.449±276	2.357±360	2.867±630
control	10±1.247	14±0.471	18±0.816

Table 18. Protoscolices mortality rate by the effect of the curcumin + piperine mix *in vitro*.

Curcumin + piperine mix mg/ml	The period of exposure to the extract in days - number of death		
	First day	second day	third day
0.25	2.943±299	2.494±325	2.943±401
0.5	2.943±330	2.494±443	4.784±490
0.75	2.943±399	2.494±480	3.399±501
1	2.494±675	2.943±780	0.942±925
control	10±1.247	14±0.471	18±0.816

Table 19. Protoscolices mortality rate by the effect of the Albendazole *in vitro*.

Albendazole mg/ml	The period of exposure to the extract in days - number of death		
	First day	second day	third day
0.25	2.494±162	2.943±170	3.858±191
0.5	2.494±185	2.867±220	1.632±279
0.75	2.943±190	2.494±230	2.054±350
1	2.494±245	4.320±310	3.399±601
control	10±1.247	14±0.471	18±0.816

caM gene has been discovered in the protoscolices treated with compounds (curcumin, albendazole, piperine, curcumin+piperine) at 0.25mg/ml after 48 hours (26.46±1.28, 22.44±1.24, 8.58±0.93 and 5.27±0.82, respectively). The same expression pattern has been observed as well after 72 hours, but at lower levels (8.68±3.48, 7.28±1.20, 4.09±1.29 and 2.94±1.55, respectively) and all samples were compared with the control groups (Fig. 10). While the gene expression levels decreased in the treated protoscolices at a concentration of 1mg/ml for the compounds used in the study (curcumin, albendazole, piperine, curcumin+piperine) after 48 hours (14.33±1.24, 12.86±2.59, 7.81±1.65, 6.33±0.99, respectively), it declined to a lower level after 72 hours (5.29±1.66, 4.34±1.62, 2.93±0.07 and 1.68±0.43, respectively) compared with the control samples (Fig. 11). The fold changes of this gene in protoscolices observed in compared to healthy control (Table 20)

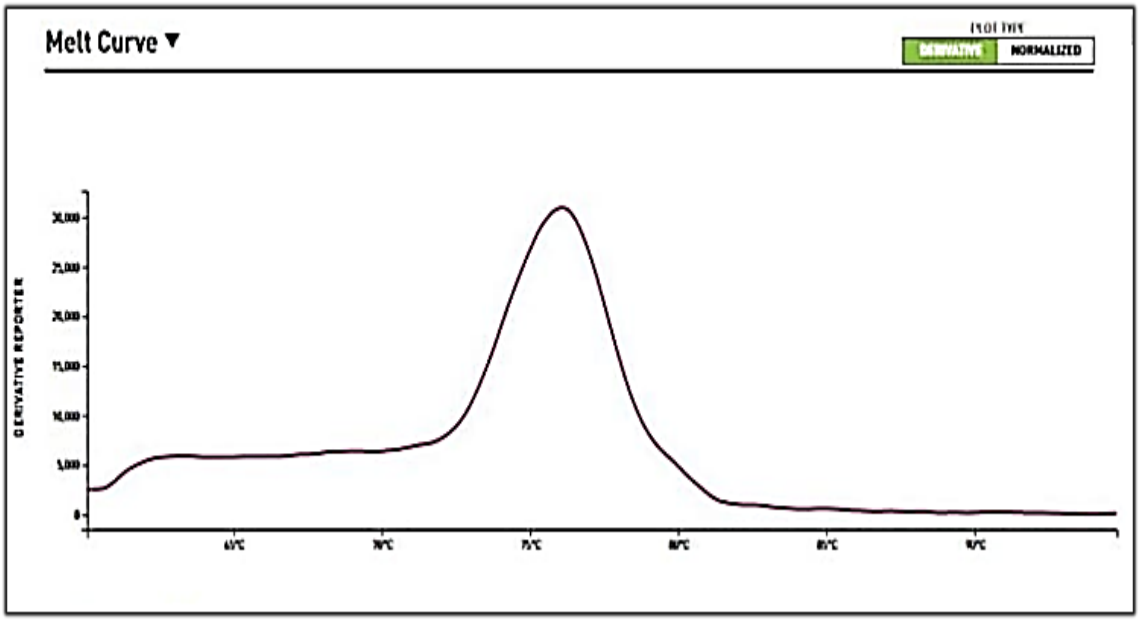

Fig.9. Melting curves of the real time - PCR products. One peak that represents specific SYBER green dye binding for the genes of interest. `caM gene in protoscolices treated with curcumin

Table 20. Protoscolices mortality rate by the effect of the Albendazole *in vitro*.

Mg/ml	Gene caM							
	Curcumin+piperine /h.		Piperine/h.		Albendazole/h.		Curcumin/h.	
	72	48	72	48	72	48	72	48
0.25	0.008	0.06	0.02	0.09	0.04	0.26	1.86	0.35
1	0.5	1.92	0.65	0.42	1.15	2.30	1.92	2.46

Discussion

Presently, numerous in vitro studies were carried out for of finding new natural compounds to treat the hydatid cysts infections. Natural products have been utilized, which prospected their anti-parasitic potentials (Mohammed 2019). For treating adult stage cestoda in gastrointestinal of dogs and larval stage in human several natural products were used. In this study, we showed that both curcumin and piperine have dose-dependent protoscolicidal effects, the current results showed significant difference between protoscolicidal effects of the piperine and curcumin is most potentially a result of dual molecular signaling path-way (Snyder et al. 2021). The association of 2g of curcumin+ 5mg piperine has shown a three-fold increases in relation to the pure curcumin. Piperine is a natural black pepper alkaloid (i.e. *Piper nigrum*) that can be described as a potent bio-transformation inhibitor and particularly glucuronidation, considerd natural product capable of modifying curcumin disposition. In this study, it showed significant anthelminthic activity as compare to standard reference and control (Dei Cas & Ghidoni 2019). Also, the piperine has enhanced curcumin's bio-availability and that the rapidity in the parasite death, possibly due to the higher curcumin levels with the piperine in comparison to the curcumin alone. Albendazole can be defined as a medication whose proto-scolicidal effects are well known, and it is presently utilized for the cystic echinococcosis chemo-therapy.

Consistent with the earlier research (Negi 2017), this study showed that the proto-scolicidal Albendazole effects has been less effective compared to the piperine at all of the 4 administrated concentration levels. It was found that the piperine has stronger *in vitro* protoscolicidal effects compared

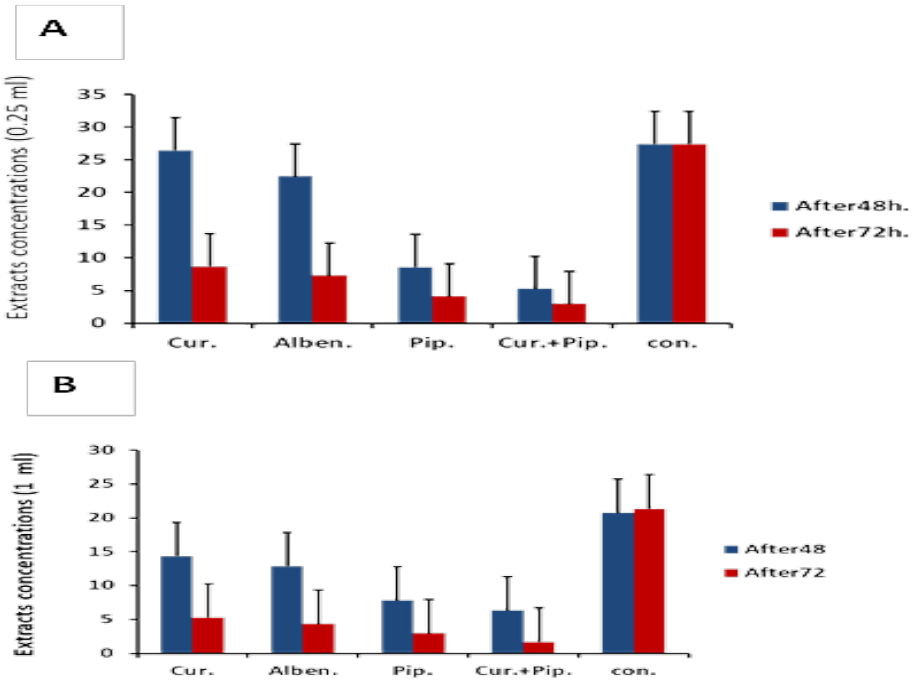

Fig.10. The levels of gene expression of *caM* genes in protoscolices treated after 48, 72 h. (A): *caM* gene expression in protoscolices treated 0.25mg/ml (curcumin, Albendazole, piperine, curcumin+piperine mix) (B): *caM* gene expression in protoscolices treated 1mg/ml (curcumin, Albendazole, piperine and curcumin+piperine mix).

to the Albendazole. Calmodulin, as dynamic sensor of Ca^{2+}, exists in all of the eukaryotic cells and mediates various cellular signaling activities, like regulating enzymatic activities, gene expression, modulation of the activities of the ion channel, mitochondrial events, and specific synaptic transmission mechanisms, even though the calmodulin was commonly researched and well-characterized in a wide range of the organisms, there have been little data on molecular and bio-chemical characterizations of the calmodulin in the *E. granulosus*. (Mehta et al. 2014). Several Ca-binding "CaM-like" proteins were characterized in the *E. granulosus*, however, none of them have a "classical" calmodulin structure and sequence. Calmodulin is usually a 16kDa protein, which comprises 2 globular domains that are connected with a flexible alpha helix hinge.

In this work, the successful expression of "classical" calmodulin of *E. granulosus* has been accomplished, in addition to characterizing its locations in *E. granulosus* and assessing its m-RNA expressions of the PSCs in different states. Those data are an indication of the fact that the CaM had stayed highly conserved across the protoscolices, which are treated by curcumin and albendazole while were low conserved when used piperine and mix piperine+curcumin. Calmodulin was previously (Wang et al. 2017) identified as a highly conserved protein from *Schistosoma mansoni* parasite; the alignments of the SmCaM-1 as well as the SmCaM-2 shared an identity of 97-98% with other calmodulins from the mammals, insects and flatworms. In addition, the mutations and composition of CaM intergenic spacer in the species of the Leishmania were researched as one of the molecular targets, which could be having taxonomic values. In *E. granulosus*, calmodulin-like proteins have shown low identity with the Eg-CaM; which is why, it would be significant exploring the correlations between calmodulin and calmodulin-like genes in the case where more cestode homologs are included in future. (Thomas & Timson 2018; Qasim & Al-Mayali 2019; Tahmasebi et al. 2021)

In conclusion, curcumin and piperine's health potential, which is assessed undoubtedly, must be

always assessed by correlation with well-defined administration way, and used both curcumin and piperine have dose-dependent proto-scolicidal effects to achieve the optimal efficacy, that the isolated piperine 20mg/k and 2g/k curcumin showed significant anthelminthic activity when compared with the standard anthelminthic drug also enhances the bioavailability of curcumin. Calmodulin, as a dynamic Ca^{2+} sensor, mediates various activities of the cellular signaling from *E. granulosus* will be possibly involved in a significant biological function.

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