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RESEARCH ARTICLE

Biochemical characterization and *in vitro* cytotoxic activity of the Egyptian scorpion *Leiurus quinquestriatus* whole body extract

Wesam M. Salama, Sabry A. El-Naggar, Abdelwahab N. El-Newahy and Nabila I. El-Desouki

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ABSTRACT

Invertebrates are considered potential sources for drug development. This study was evaluated the biochemical characterization and the in vitro cytotoxic effect of *Leiurus quinquestriatus* whole-body extract (LQWBE) against HepG-2 and MCF-7 cell lines. For characterization, the total proteins (T.P), lipids (T.L), carbohydrates (T.C), sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), gas chromatography-mass spectroscopy (GC-MS), and minerals contents were evaluated in LQWB. The results showed that the contents of T.P, T.L, and T.C were 712 ± 8.5 mg/g, 68 ± 3.8 mg/g, and 216 ± 5.2, respectively. SDS-PAGE analysis showed that LQWBE has three bands of 12.5, 20, and 30 kDa. In addition, GC-MS analysis revealed that the presence of 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester at the highest peak area. Sodium, potassium, magnesium, and calcium levels were 9.1 ± 0.16, 35 ± 0.28, 0.2± 0.01, and 5.5 ± 0.77 mg/g extract, respectively. LQWBE showed a mild cytotoxic activity in vitro against HepG-2 and MCF-7 human cancer cells. The LQWBE IC50 against HepG-2 and MCF-7 cell lines were 992 µg/ml ± 49.2 and more than 1000µg/ml after 24 hours of treatment.

Keywords: GC-MS, Cytotoxic, *In vitro*, Scorpion, *Leiurus quinquestratus*, HepG-2, MCF-7

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INTRODUCTION

Cancer is characterized by uncontrolled multiplication of modified normal cells. It is the most common cause of death after cardiovascular diseases worldwide (Mubeen and Kini, 2012). Chemotherapy, radiotherapy, surgery, and immunotherapy are the potential agents for cancer treatments, however, search for novel, more efficacious, and better tolerated agents without harming vital organs is ultimate. Several natural compounds have been investigated as candidates for developing new anticancer drugs and ameliorating the adverse effect of the conventional chemotherapies (Narang and Desai, 2009; Al-Bagoury et al., 2023). These compounds derived from animal have been considered as sources of substances with zootherapeutic effects to treat different diseases (Marrotini and Pane, 2010; Djagoun et al., 2013, Salama et al., 2022). These compounds extracted either from the whole animals or its special part or from animal-derived products (Alves and Rosa, 2005). Therefore, invertebrates' natural products were used to treat numerous diseases (Costa-Neto, 2005).

Scorpions are venomous arthropods of class Arachnida, included 16 extant families of scorpions distributed around the world (Soleglad and Fet 2003; Ozkan and Karear, 2007). Family Buthidae scorpion members, in particular have hazardous and danger impact on human due to their large size and fatal sting (Salama and Sharshar, 2013). In Egypt *Leiurus quinquestriatus* scorpion is widely distributed in Aswan. *L. quinquestratus* length ranged between 9 and 9.5 cm, with orange-yellowish body. The prosomal carapace and metasomal segment V with brown color. It has elongated and gracile chela. Vesicle has yellow color with reddish and brown aculeus at the end (Salama and Sharshar, 2013). The external skeleton of scorpion are made of chitin which has an antitumor activity (Salah et al., 2013).

Recent studies showed potent anti-tumor effect of the *L. quinquestratus* venom (LQV) and their whole body extract in vitro and in experimental mouse model (Salama and El-Naggar, 2021,

Salama et al., 2023a; Salama et al., 2023b). Till now, little information is available about pharmacological properties of the whole body of scorpions.

Therefore, evaluation of bio-medical importance of whole body of scorpion is considered as a new avenue to screen its biomedical application. Previous studies investigated the effect of the scorpion whole body extract as anti-diabetic agents (Xie et al., 2011; Abdel-Rahman et al., 2019). This study aimed to evaluate the biochemical characterization and the cytotoxic effect of the whole body ethanolic extract of the Egyptian scorpion *L. quinquestriatus* (LQWBE) against human hepatocellular carcinoma (HepG-2) and breast cancer (MCF-7) cell lines *in vitro*.

MATERIAL AND METHODS

Chemicals

Cisplatin (Cis) was obtained from Sigma-Aldrich (St Quentin Fallavier, France). 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO) was purchased from Sigma company (USA). Fetal bovine serum (FBS), Coomassie Blue R-250 Silver, Dulbecco's modified Eagle medium (DMEM), L-glutamine, gentamycin, HEPES buffer solution, and 0.25% trypsin-EDTA were purchased from Lonza (Belgium).

Collection of scorpions and whole-body extract preparation

One hundred of scorpion specimens were collected from Aswan, Egypt by professional hunters in July, 2020 (Figure 1). Scorpions were transferred in plastic containers to Invertebrate Division, Zoology Department, Faculty of Science, Tanta University, Egypt. Specimens were authenticated and identified by a specialist in animal taxonomy. To lower the toxic effect, the telsons containing venom glands were discarded from each scorpion. To prepare the whole-body extract, the scorpions were dried overnight at 60 ˚C and then grinded to obtain scorpion powder. The pooled powder was soaked in ethanol for 3 days then filtered. The filtrates were pooled and centrifuged (3000 rpm, 15 min) to remove impurities and debris. The supernatant of *L. quinquastratus* whole body extract (LQWBE) was lyophilized and kept in −20 ˚C until use (Xie et al., 2011).

Determination of total proteins, lipids, and carbohydrates in LQWB powder

Total proteins content was calculated according to the method of Waterborg (2009). Lipids and carbohydrates of LQWB were determined according to Minnoti and Aust (1987) and Mc Gready (1950), respectively.

Determination SDS–PAGE profile of LQWBE

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) (12% gel) analyses of LQWBE was carried out according to Laemmeli (1970). Proteins were stained with 0.1% Coomassie Blue R-250 Silver. Molecular mass standard (Sigma, S8445) was run in parallel to calculate the molecular weights of proteins. Then, gel was photographed, and the molecular weights were calculated using Molecular Imaging Software (MIS, Kodak).

Gas chromatograph–mass spectrometry (GC-MS) analysis of LQWBE

LQWBE was prepared to determine different chemical profile. The chemical composition was performed using Trace GC 1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m ×0.25 mm ×0.25µm film thickness) according to Medeiros (2018).

Determination of minerals contents of LQWBE

Minerals were determined by using inductive coupling plasma mass spectrometry (ICP) analysis. Briefly, 10 mL of concentrated HNO_3 was added to 0.5 g of LQWBE, and samples were subsequently digested using a microwave digestion machine (Milestone, Ethos Easy model: ACT36). The concentrations of the samples were calculated in mg/dl using the WINLAB 32 programme according to Dolan and Capar (2002).

Human cancer cell lines

HepG-2 and MCF-7 cell lines were purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA) and cultured in DMEM supplemented with 10% FBS (BioWest, Nuaille, France), 100 U/mL penicillin, 100 mg/mL streptomycin, and 100 mg/mL

glutamine at 37 ˚C in a humidified atmosphere containing 5% CO_2. Cells were sub-cultured after every two days.

In vitro cytotoxic effect of LQWBE

To determine the cytotoxic effect of LQWBE *in vitro*, HepG-2 and MCF-7 cells were used. The inhibitory concentration that killed 50% of cells (IC_{50}) was determined by using MTT assay. Different concentrations of LQWBE were applied in triplicate and cancer cells were incubated for 24 hours, then, 10 µL of a 12 mM MTT stock solution (5 mg/mL) in sterile PBS was added to each well. Then, the plates were incubated for 4 h at 37 ˚C. Then, the MTT solution was removed, and the purple formazan crystal formed at the bottom of the wells was dissolved with 100 µL of DMSO for 20 min. Cis was used as a positive standard. The absorbance at 570 nm was read on an enzyme-linked immunosorbent assay reader (StatFax-2100, Awareness Technology, Inc.). IC_{50} was calculated with the sigmoidal curve.

Statistical analysis

The data were expressed as mean ± standard deviation. Comparison between groups was carried out using one-way ANOVA. *P* values < 0.05 were statistically significant. Data and statistical analysis were performed using Excel 2013 (Microsoft Corporation, USA) and Minitab version 18 (Cairo, Egypt).

RESULTS

Total proteins, carbohydrates, and lipids contents in LQWB

The total content of proteins, carbohydrates, and lipids were determined in LQWB powder. The results showed that the total proteins content was the highest content among the different macromolecules and represented as 735 mg/g of LQWB powder. The total carbohydrates and total lipids contents were 230 and 78 mg/g of LQWB powder, respectively (Figure 2).

SDS- PAGE analysis of LQWBE

The SDS-PAGE analysis of LQWBE showed that there are 3 bands of proteins with low molecular weights (Figure 3). The protein bands were determined at 12.5, 20 and 30 kDa.

Gas chromatography-mass spectroscopy (GC-MS) analysis of LQWBE

The results showed that the oleic acid has the maximum peak area (14.95%) at the retention time (RT) 22.98 min. 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, ethyl oleate, 9-octadecenoic acid (Z)-, methyl ester, linoelaidic acid, and hexadecanoic acid, methyl ester were detected at different RTs, with peak areas of 14.59, 9.39, 9.98, 7.66, and 6.24%, respectively (Table 1 and Figure 4).

Mineral levels of LQWBE

The results showed that the level of Na was 9.1± 0.16mg/g extract. The levels of K and Mg were 35±0.28 and 0.2±0.01mg/g extract, respectively. The level of Ca in the extract was 5.5 ± 0.77 mg/g extract. While, the Zn were not detected in the extract (Figure 5).

Cytotoxic effect of LQWBE on HepG-2 and MCF-7 cell lines *in vitro.*

The IC_{50} was determined in LQWBE by MTT assay against HepG-2 and MCF-7 cells *in vitro*. The data showed that the LQWBE IC_{50} against HepG-2 and MCF-7 cells were 992 ± 49.2 µg/ml and more than 1000 µg/ml post 24 hours of treatment. The IC_{50} of Cis against HepG-2 and MCF-7 cells were 3.42 ± 0.23 and 5.14 ± 0.31 µg/ml, respectively (Figures 6 and 7).

DISCUSSION

Chemotherapeutic drugs are used for the treatment of different malignancies, but their therapeutic use is limited due to their adverse side effects (Benzer et al., 2018). Invertebrate animals, in particular, are considered as potential sources for drug development (Finke, 2007). In this line, scorpion venom is currently in use in preclinical studies as antitumor, antiepileptic, anti-inflammatory and analgesic agents (Salama and El-Naggar, 2021; Chen et al., 2021; Liu et al., 2021). Recently, the whole body of scorpions was investigated as antidiabetic agent (Abdel-Rahman et al., 2019). Therefore, this study was conducted to evaluate the biochemical characterization and *in vitro* cytotoxic effect of LQWBE against HepG-2 and MCF-7 cell lines.

Table 1. GC-MS analysis of LQWBE

No.	RT (min.)	Name	M.F	M.W	Peak area %
1	11.93	1-Dodecanamine, N,N-dimethyl	$C_{14}H_{31}N$	213	3.23
2	15.75	1-Tetradecanamine, N,N-dimethyl	$C_{16}H_{35}N$	241	1.26
3	16.41	Isobutyl laurate	$C_{16}H_{32}O_2$	256	1.43
4	19.54	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	6.24
5	20.21	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.96
6	20.65	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	1.90
7	22.21	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	14.59
8	22.30	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	9.98
9	22.71	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	1.94
10	22.90	Linoelaidic acid	$C_{18}H_{32}O_2$	280	7.66
11	22.98	Oleic acid	$C_{18}H_{34}O_2$	282	14.95
12	23.22	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308	2.89
13	23.32	Ethyl oleate	$C_{20}H_{38}O_2$	310	9.39
14	23.72	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	1.77
15	26.60	Hexanedioic acid, mono(2-ethylhexyl) ester	$C_{14}H_{26}O_4$	258	1.92
16	26.94	9,12-Octadecadienoyl chloride, (Z,Z)	$C_{18}H_{31}ClO$	298	1.71
17	27.00	9,12-Octadecadienoic acid (Z,Z)-,2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{38}O_4$	831	1.31
18	27.56	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)	$C_{21}H_{36}O_4$	352	2.03
19	30.01	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{38}O_4$	354	2.00
20	30.68	Pregn-5-ene-3,20-diol	$C_{21}H_{34}O_2$	318	1.11
21	35.20	Cholesterol	$C_{27}H_{46}O$	386	4.45

RT: Retention time; MF: Molecular formula; MW: Molecular weight.

The study reported that the total proteins content in the LQWB powder was the highest content among the different macromolecules, followed by carbohydrates, and lipids contents, respectively. This finding was in agreement with a previous study showed that the protein content in the whole-body extract of scorpion was about 78 % (Wali et al., 2019). The whole-body part of a scorpion is used as medicinal material in traditional Chinese medicine. However, few reports are available on the extraction and functional evaluation of scorpion total protein. (Ren et al., 2014; Kamau et al., 2023).

The SDS-PAGE analysis of LQWBE showed that there are 3 bands of proteins at low molecular weights, these bands of proteins were reported at 12.5, 20 and 30 kDa. In a previous study, SDS-PAGE of whole-body extract of scorpion *Buthus maratensii* showed different bands of proteins at 9.5, 20, 27, 45, and 66 kDa (Wali et al., 2019).

GC-MS analysis of the LQWBE showed that there were several chemical compounds at different RTs with varying peak areas ranges between 0.1 to 14.95%. The most abundant chemicals in the LQWBE were octadecanoic acid (PA 14.59%), oleic acid (PA 14.95%), and cholesterol (PA 4.45%).

A previous study showed that octadecanoic acid has anticancer, anti-inflammatory, and hypocholesterolemia activities (Zhang et al., 2015). Furthermore, oleic acid has been found to be antioxidant and antibacterial agents (Dilika et al., 2000). It has been reported that the fatty acid composition of scorpion is mainly saturated fatty acid. The main fatty acids detected in scorpions are oleic acid and cis-9, cis-12-linoleic acid, followed by palmitic acid and stearic acid (Qin et al., 2016). The results showed that the level of K was 9.1±0.16 mg/g extract. The levels of Na and Ca were 9.1±0.16 and 5.5±0.77mg/g extract, respectively. While, the levels of Mg and Zn were 0.35 ± 0.28 and 0.02 ± 0.02 mg/g extract, respectively. A previous study was carried out on *Androctonus australis* scorpion to investigate its proximate composition and minerals content. It has been reported that the predominant metal was K (695.21 mg kg^{-1}) and cobalt found only in traces (2.50 mg kg^{-1}) (Abulude et al., 2006). The results showed that compared to the cytotoxic effect of Cis against HepG-2 and MCF-7 cells, the LQWBE showed a mild effect. Since the scorpion exoskeleton consists from chitin, the mild effect of LQWBE against cancer cells may be due to chitin effect as anti-tumor (Salah et al., 2013).

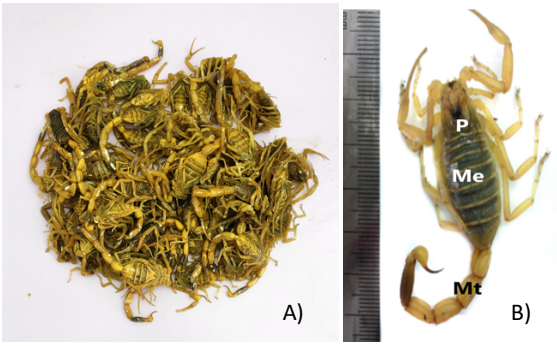

Figure 1. *L. quinquestratus* scorpion whole body (A), showing body parts prosoma (P), mesasoma (Me), and metasoma (Mt) with dark last segment V, the telson end with vesicle and dark aculeus (B).

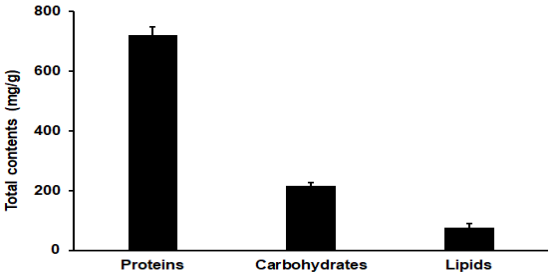

Figure 2. Total proteins, carbohydrates, and lipids contents in LQWB powder.

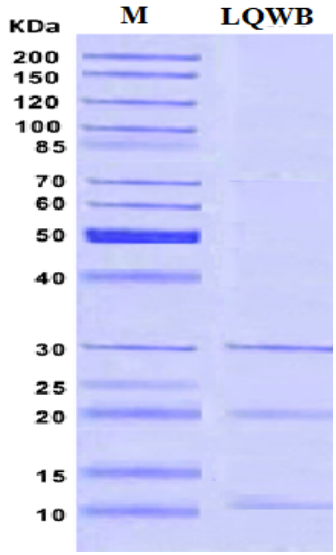

Figure 3. SDS-PAGE of *L. quinquestratus* scorpion whole body.

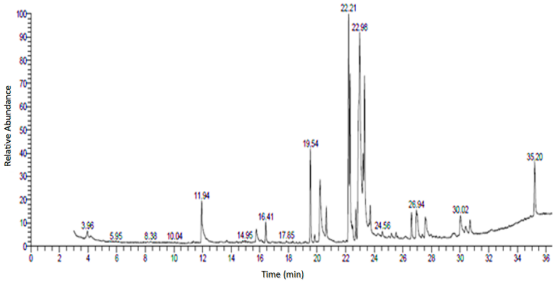

Figure 4: GC-MS chromatogram of *L. quinquestratus whole* body extract

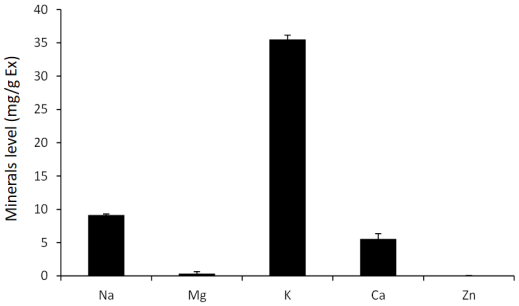

Figure 5. Sodium, magnesium, potassium, calcium and zinc concentrations of LQWBE.

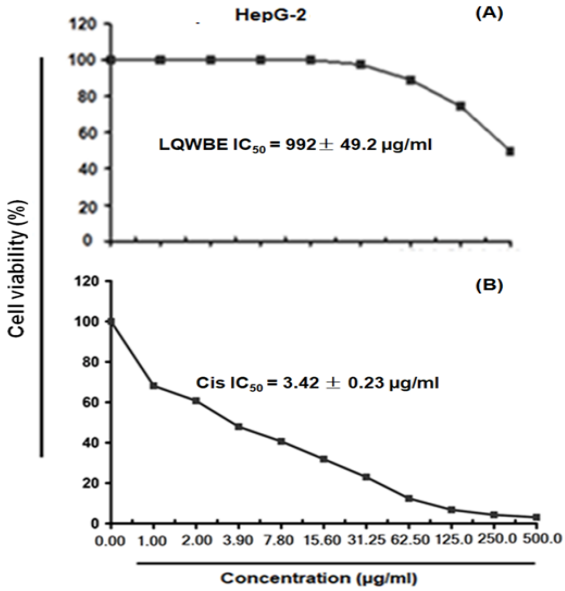

Figure 6. Percentages of HepG-2 cells viability (A) Post-24 hrs. of LQWBE treatment (B) Post-24 hrs. of Cis treatment

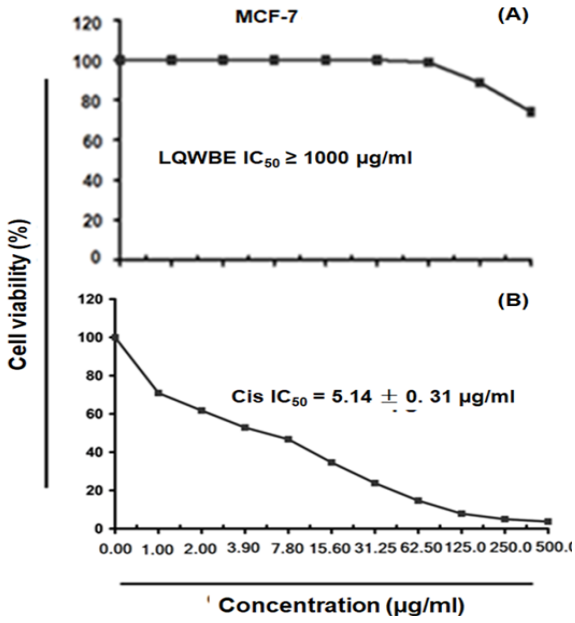

Figure 7. Percentages of MCF-7 cells viability (A) Post-24 hrs. of LQWBE treatment (B) Post-24 hrs. of Cis treatment

These results were in accordance with the study of Bouhenna et al. (2015), who evaluated the cytotoxic effect of chitin extracted from shrimp shells against human larynx and embryo rhabdomyosarcoma cancer cells *in vitro*. This finding indicated that the LQWBE is not a potent cytotoxic against HepG-2 and MCF-7 cancer cell lines *in vitro*.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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