The Egyptian Journal of Hospital Medicine (January 2024) Vol. 94, Page 465-472

In Vitro Assessment of Antineoplastic Potential of The Venom Extracted from *Dysdera Sp.* Inhabiting The Egyptian Environment

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ABSTRACT

Background: Cancer is a leading cause of mortality, challenges in conventional nonsurgical cancer treatments, such as chemotherapy and radiation and include poor prognosis, recurrence, and low survival rates.

Objective: The primary objective of this study was to investigate the potential antitumor activity of the venom extracted from an Egyptian spider, preliminarily identified as Dysdera sp. The focus is on exploring the cytotoxicity of the spider venom against various tumor cell lines, including lung (A549), hepatocellular (HEPG-2), colon (HTC-116), and breast (MCF-7) cells, in comparison with non-tumorigenic WI-38 cells.

Material and methods: The Egyptian spider was hunted from Fayoum governorate, and its venom was extracted in PBS for further investigation. MTT assay was employed to assess the potential cytotoxicity of the venom against the specified tumor cell lines. The study aims to determine the IC50 values, representing the concentration of the venom at which 50% inhibition of cell growth occurs.

Results: The results of the MTT assay revealed varying IC50 values, ranging from 163 to more than 1000 µg/ml, against the different tumor cell lines. Notably, the spider venom exhibited promising antitumor activity against HEPG-2 and A549 cells, with IC50 values of 163 and 432 µg/ml, respectively. These findings suggest that the crude venom may contain elements that could be explored for their potential as anticancer medications.

Conclusion: This study demonstrated the potential antitumor activity of the Dysdera spider venom, particularly against HEPG-2 and A549 cells. The varying IC50 values indicated the specificity of the venom's cytotoxic effects on different tumor cell lines.

Keywords: Egyptian spider, *Dysdera*, Antineoplastic, MTT assay, Spider venom.

INTRODUCTION

Spiders, belonging to the order Araneae, are eight-legged arthropods that breathe air. They possess chelicerae, commonly known as fangs, capable of injecting poison, and spinnerets for extruding silk [1]. **Dimitar *et al.*** [2] note that spiders constitute the largest order of arachnids, ranking seventh in total species diversity across all organism orders. Except for Antarctica, spiders are found on all continents, thriving in nearly every land habitat. In 2023, the World Spider Catalogue [3] reported the identification of 51,673 spider species from 136 families by taxonomists. A spider's lifestyle dictates its habitat and influences its adaptive behavior for prey capture and food acquisition.

While spiders are widespread, they are particularly abundant in areas with dense vegetation. Conversely, arid environments such as sand dunes, tidal zones, or mountain tops also serve as habitats for spiders. Spiders have successfully colonized almost every ecological niche on land, displaying adaptability to various physical and biological extremes.

Despite predominantly being terrestrial, spiders include aquatic species, tidal zone residents, and those venturing into the water for hunting purposes [4]. In Egypt, there are 405 spider species distributed among 204 genera and 41 families [5]. Notably, the Salticidae family leads with 33 genera and 74 species, followed by the Gnaphosidae with 20 genera and 47 species [4].

Dysdera spiders are notable among the limited arthropods known for hunting and consuming woodlice, constituting their primary food source. Equipped with large fangs and robust jaws, these spiders adeptly penetrate the tough shells resembling armor that encase woodlice. This ability enables Dysdera spiders to outcompete or even eliminate other spiders and centipedes, establishing them as formidable predators relative to their size. The Dysderidae family of araneomorph spiders, originally described by Carl Ludwig Koch in 1837, predominantly inhabits Eurasia, extending into North Africa, with only a few species found in South America [6].

Five Dysdera species, namely D. crocata, D. ninnii, D. dubrovninnii, D. hungarica, and D. longirostris, persist in Central Europe following the last glacial epoch [7]. These spiders also thrive in Ethiopia, the Iberian Peninsula, Australia, and North African nations such as Morocco and Egypt.

Among Egyptian spiders, adult Dysdera sp. exhibit a shiny pale beige to yellow-brown abdomen, occasionally dark grey, along with a reddish-brown cephalothorax and legs. With a maximum length of 2 centimeters (0.79 in), females grow slightly larger than males, reaching lengths of 1.1 to 1.5 cm, while males attain lengths of 0.9 to 1 cm. Possessing six closely spaced oval eyes. Dysdera spiders are characterized by their large fangs and wide jaws, causing potentially

Received: 20/08/2023
Accepted: 18/10/2023

painful bites that may result in itching, swelling, or redness. Although, their venom is mildly toxic, causing local reactions in humans according to some reports, the venom does not pose a significant risk to human health [8].

While spiders have long captivated scientists and medical professionals due to potential health risks, only a few species produce venoms fatal to humans. Despite this, spiders present a valuable source of novel insecticidal molecules with potential commercial applications, owing to their selectivity for specific cancer subtypes and membrane ion channels [9].

The use of natural products has played a pivotal role in pharmaceutical development and the exploration of novel molecular entities, contributing to the creation of new medications. Currently, over 50% of pharmaceuticals worldwide are derived from natural sources [10]. Venoms from various venomous creatures such as spiders, sea snails, snakes, and scorpions contain physiologically active compounds, including proteins, peptides, small organic molecules, and salts. These compounds have been isolated and characterized, showcasing pharmacological activities. Notably, some drugs derived from venom components are already in use, demonstrating potential in the development of new anticancer medications [11-13].

Research indicates that biomolecules found in the venoms of scorpions and spiders exhibit chemotherapeutic effects against various cancers, encompassing neuroblastoma, glioma, leukemia, lymphoma, breast cancer, lung cancer, hepatoma, pancreatic cancer, and prostate cancer [14]. The field of venom-derived therapeutics holds significant promise for diverse pharmacological and neurobiological applications, with several venom peptides currently undergoing clinical trials or preclinical development [15]. Spider venom toxins have shown notable cytotoxic activities against a range of cancer cells [16].

Globally, cancer is a major contributor to mortality, accounting for almost 10 million deaths in 2020, or nearly one in six deaths, according to the World Health Organization's 2022 data. Egypt, as reported in the 2020 Global Cancer Observatory (Globocan) fact sheet, faces elevated incidence and mortality rates for specific cancer cases. A challenge in cancer care in Egypt is the late presentation of the disease, leading to less effective treatment and increased morbidity and mortality rates [17].

Despite the significance and abundance of spiders, their biodiversity and importance have been insufficiently explored in Egypt. This study aimed to contribute valuable insights into the existence of spiders in Egypt, encompassing aspects such as habitat, morphology, nutrition, and the potential applications of spider venom in anticancer therapies.

MATERIALS AND METHODS

Spiders were collected using handpicking and beating tray methods, and specimens were then transported alive back to the laboratory. To prevent specimens from consuming each other, traps were set.

Spider Identification: In the field, spiders were easily distinguished by their position, number of eyes, overall shape, length of legs, and form of spinners. Subsequently, they were preserved in a mixture of 70% alcohol and 5% glycerol until identification.

Venom Collection: After anesthetizing the spider, the carapace was removed. The location of the venom gland was then determined, and the venom was extracted. The venom gland was ground up to extract the venom, which was centrifuged at 15,000 rpm and 4°C immediately. The venom was stored at -20°C until needed.

Total soluble protein determination:

Extraction: One gram of air-dried spider venom was extracted at 60°C in a 250 mL conical flask using a mixture of 10 mL distilled water and 5 mL of a 2% phenol solution. The contents of the flasks were shaken well and kept overnight before being filtered, and then they were used for the estimation of soluble proteins [18].

Determination: This assay was conducted using the method described by Lowry [19]. The optical density of the resulting color was then read at a wavelength of 750 nm. The concentration of soluble protein present in the sample was calculated using the constructed standard curve of proteins.

Cytotoxicity assay using MTT method:

To assess the cytotoxicity of crude venom, the antitumor activities of Dysdera sp. venom were determined in vitro against four different cell lines: the breast tumor cell line (MCF-7), the hepatocellular carcinoma cell line (HEPG-2), the lung cancer cell line (A549), and the colon carcinoma cell line (HTC-116), in relation to the normal cell line (Wi-38). A full monolayer sheet was created by adding 1 X 10^5 cells/ml (100 µl/well) to a 96-well tissue culture plate and incubating for 24 hours at 37°C.

Once a confluent sheet of cells had formed, the growth medium was removed, and the cell monolayer was washed twice with wash media. The tested sample was diluted in RPMI medium containing 2% serum (maintenance medium) and added in various wells. Physical indicators of toxicity were examined in the cells, such as shrinkage, rounding, granulation, or partial or total loss of the monolayer. MTT solution (5 mg/ml) was added to each well, and after incubation, the optical density was read at 560 nm. There should be a direct relationship between cell quantity and optical density [20].

Ethical considerations: Ethical considerations were prioritized at various stages. The collection of spiders employed methods that minimized harm and ensured proper handling, adhering to guidelines for animal welfare. Venom extraction involved careful anesthesia to reduce stress, and specimen preservation followed ethical guidelines.

The use of human cell lines in cytotoxicity assays adhered to established ethical standards, including informed consent and approval from relevant ethical review boards. Transparency and accuracy in publication and reporting were maintained to uphold research integrity. Additionally, the exploration of spider venom for potential anticancer medications acknowledged the responsibility associated with therapeutic research, emphasizing patient safety and ethical considerations in potential applications. Throughout the study, compliance with research ethics standards was ensured, reflecting a commitment to ethical conduct in scientific research.

Data Management:

The data were presented as mean ± SE, and each experiment was conducted at least three times. The significance of variations between experimental and control values was assessed using the student's t-test. A variation was considered statistically significant if its p-value ≤ 0.05. Linear interpolation was employed to determine the IC50 values.

RESULTS

Morphological Identification:

Taxonomic knowledge was utilized to guide the identification of specimens, as depicted in figure (1).

The collected specimens were identified down to the genus and even family levels. Identification was facilitated with the help of the following keys, documents, and descriptions: **Jocque and Dippenaar-Schoeman [21]** and **Seymour *et al.* [22].**

An overview of the spider's predator type is provided under the heading "lifestyle." Additionally, as shown in table **(1)**, the distribution of a spider family could be significant, aiding in identification and indicating whether the species has been discovered in a specific area. Descriptive characteristics, such as body size, shape, legs, and eye pattern were summarized in table (2) to define the family and distinguish them from one another.

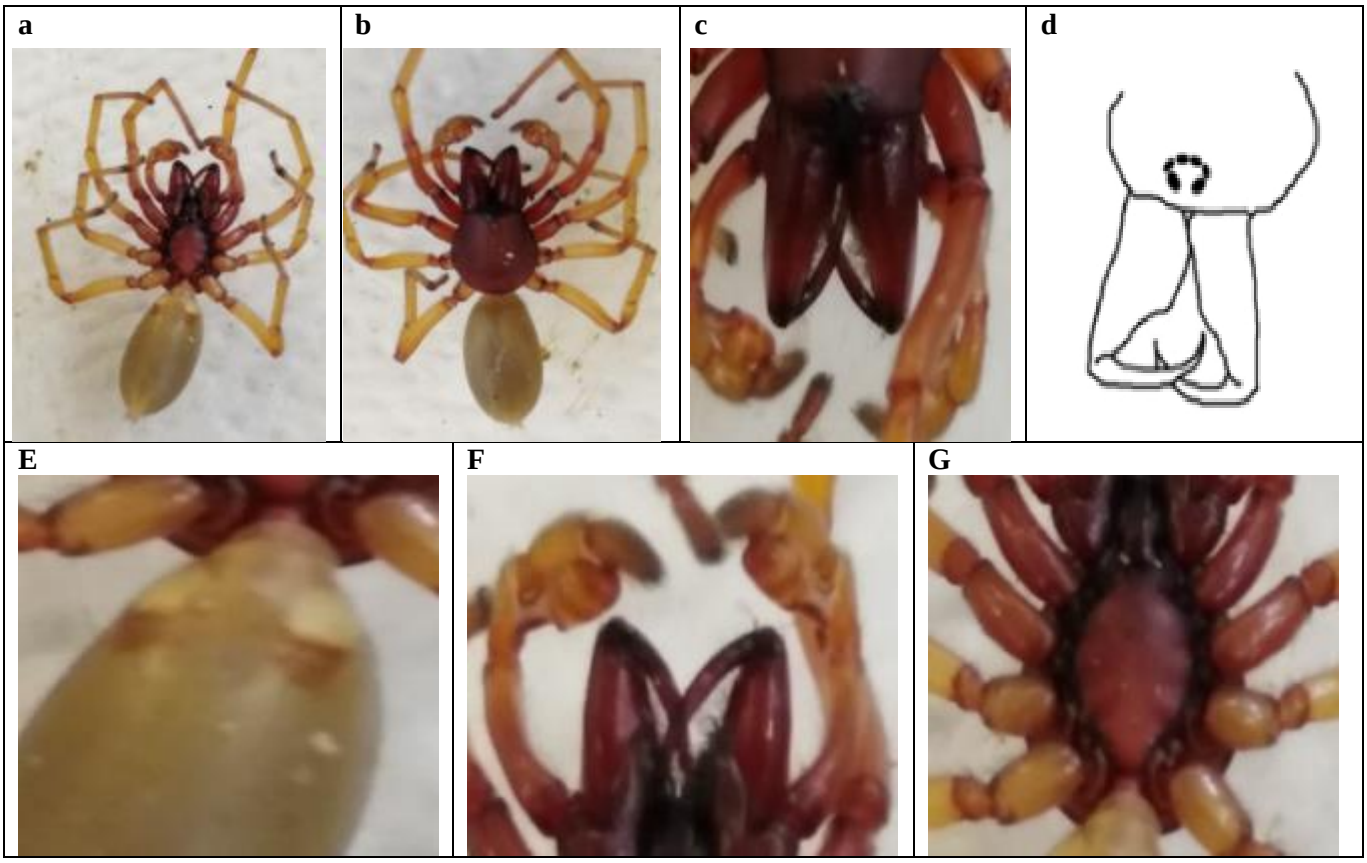

Figure (1): Morphological features of the Egyptian Dysdera sp. (a: ventral view showing spinnerets and chelicera, b: dorsal view showing carapace and legs, c, d: eyes pattern, e: frontal part of abdomen showing tracheal spiracles, f: pedipalp, g: sternum showing intercoxal projections).

Diagnostic characters: Medium-sized araneomorph spiders with three or two tarsal claws, haplogyne, ecribellate, and six eyes. Intercoxal sclerites connect the sternum to the carapace, and posterior spermatheca are present in the female genitalia. The fangs and chelicerae are fully formed.

Descriptive characters

• **Carapace:** Longer than wide, integument sclerotized usually with fine granulation, fovea reduced to a longitudinal black stripe and not nested.

• **Sternum:** Intercoxal sclerites connect it to the carapace.

• Six eyes in a close-knit cluster near the clypeal edge.

• **Chelicerae:** 3-5 teeth in a cheliceral furrow (free, well-developed & subchelate), well-developed fangs and lack of a lamina.

• **Mouthparts:** Endites parallel, labium longer than wide, and frequently with a deep notch at the anterior edge.

• **Legs:** At least tibiae and metatarsi of hind legs with setae and two or three claws.

• **Female palp**: No teeth, but a claw is present.

• **Abdomen:** Ovoid often with a thin layer of short setae covering it. The anterior spinnerets have three segments with the longest segment being the apical one.

• **Respiratory system:** Two booklungs, two different pairs of spiracles, the posterior pair is behind the epigastric groove and leads to the tracheae, and the anterior pair leads to the booklungs.

• **Genitalia:** Tarsus short or long, bulbus originating medioventrally on cymbium, male palp variable and female genitalia with anterior and posterior spermathecae and internal sclerite (endogyne).

• **Size of body:** 2.5–20 mm.

• **Color:** Abdomen is usually grey, but the legs and carapace can be orange, or red.

Lifestyle: Theye are nocturnal, free-living, wandering spiders that inhabit the ground or the trunks of trees. They stay in a silken retreat during the day. *Dysdera* is specialized on woodlice as a prey.

Table (1): Description of the distribution and environmental behavior of the Egyptian *Dysdera sp*

Family	Species	Prey	capture method	Collection area	Habitat	Behavior
Dysderidae (Koch, 1837)	*Dysdera* sp.	prey on woodlice	Active hunters	Fayoum governorate	Found in damp areas under leafy debris on the ground, logs and rocks	Nocturnal hunters

Table (2): Description of the morphological features of the Egyptian *Dysdera sp*

Family	Species	Body	Legs	Eyes
Dysderidae (Koch, 1837)	*Dysdera* sp.	- Medium size (0.3-0.6 inch) - Cream to gray abdomen - Orange-brown carapace - Guite and long chelicera with sharp fangs at the tip	- Orange brown in color - Long and agile	Six eyes arranged in nearly complete and transverse oval pattern

Total soluble protein determination: A total soluble protein in the crude venom of *Dysdera sp.* was 0.3 mg/ml. This result allowed to continue investigating the activity of the spider venom as an antitumor agent.

Antitumor activity of *Dysdera* venom:

The cytotoxic effect of venom of Egyptian *Dysdera* species was investigated on tumor-derived cell lines including hepatocellular carcinoma (HEPG-2), colon carcinoma (HTC-116), breast adenocarcinoma (MCF-7) and lung carcinoma (A549) using MTT assay.

The venom treatment was performed at different concentrations of 31.25, 62.5, 125, 250, 500 and 1000 µg/ml as shown in table (2). After MTT application, the absorbance data obtained spectrophotometrically were evaluated statistically.

The cell viability of five cell lines after *Dysdera* sp. crude venom treatment was illustrated in table (1). The venom induced a reduction of viability against HEPG-2 cells in a concentration-dependent manner, with IC_{50} of 163.6 µg/ml. Figure (2) easily showed that HEPG-2 liver carcinoma cell line was highly affected with the action of spider venom in particular when the results compared to those belongs to the normal cell line WI-38 with IC_{50} value > 1000 µg/ml as revealed in table (3).

Additionally, spider venom showed a significant activity against A549 lung carcinoma cell line with IC_{50} value 432.2 µg/ml as exposed in table (3).

Likewise, figure (2) theoretically confirms success of the spider venom as antineoplastic chemotherapeutic agent against both HEPG-2 and A549 carcinoma cell lines when compared to the resulted curve of the normal cell line. On the other side, two cell lines; HCT-116 and MCF-7 gave close IC50 values with this of the normal cell line, which means that the action of the spider venom could be neglected.

Table (3): Viability and IC_{50} values (µg/ml) of *Dysdera* venom against all tested cell lines

SV Conc.	**Viability**				
µg/ml	**WI-38**	**A549**	**HEPG-2**	**HCT-116**	**MCF-7**
1000	72.9	31.8	23.4	52.8	67.0
500	79.5	42.7	28.8	59.1	74.7
250	82.4	70.8	35.1	83.2	78.6
125	87.2	89.4	53.3	88.7	89.5
62.5	93.8	98.9	97.4	96.8	98.0
31.25	99.8	99.1	98.2	99.6	99.6
IC_{50}	>1000	432.2	163.6	>1000	>1000

Where SV is spider venom.

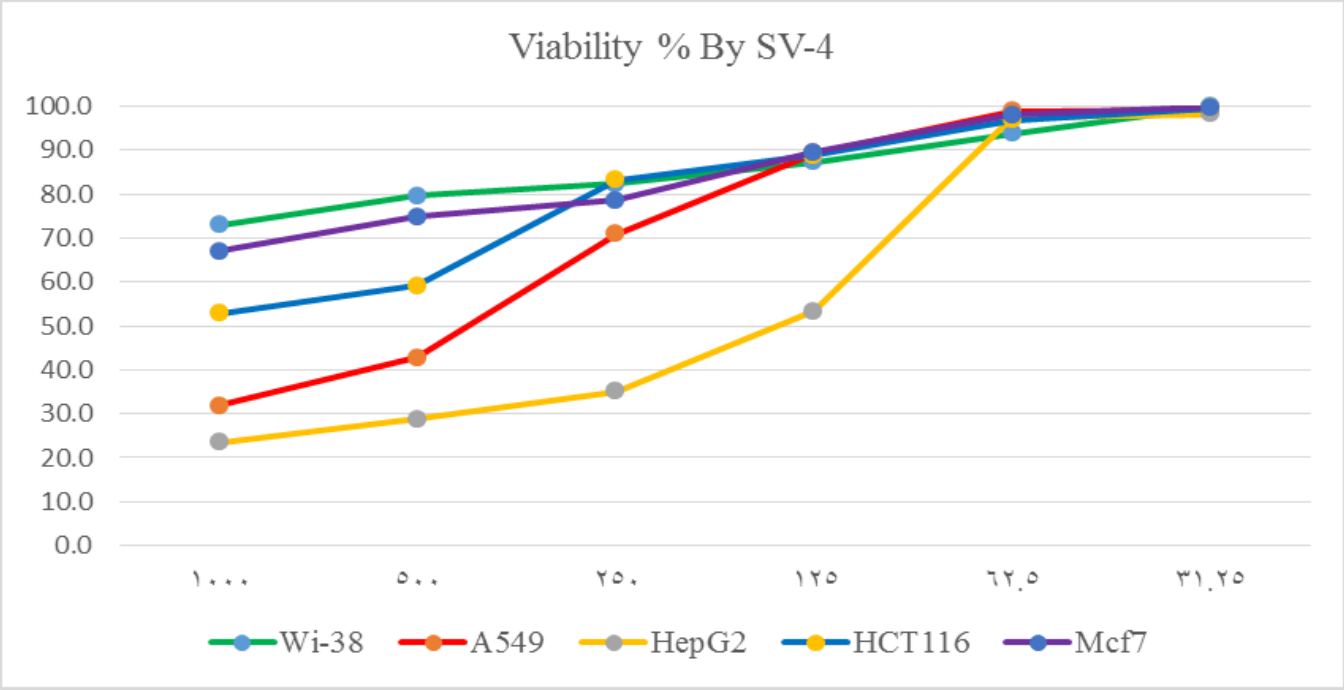

Figure 2. Cytotoxic effect of Dysdera venom against all tested cell lines.

Morphological study of cell Line:

After treatment with descending concentrations of venom, and prior to the addition of MTT salt, monolayers of tumor cell lines per experimental group were photographed. The morphological changes of cells were analyzed using a Nikon bright field inverted light microscope (Japan) at 100X magnification and compared to the control group.

Figure (3) illustrated the cell viability of the five cell lines after Dysdera sp. crude venom treatment. The activity of spider venom was indicated by the decrease in cell density.

The figure demonstrated a significant reduction in cell count when compared to the corresponding controls. Reduction of cellular adhesion, cell condensation, and membrane blebbing were observed in A549 and HEPG2 cells treated with high concentrations (250 µg/mL and above) of Dysdera spider venom indicating the cytotoxic effects of Dysdera sp. crude venom.

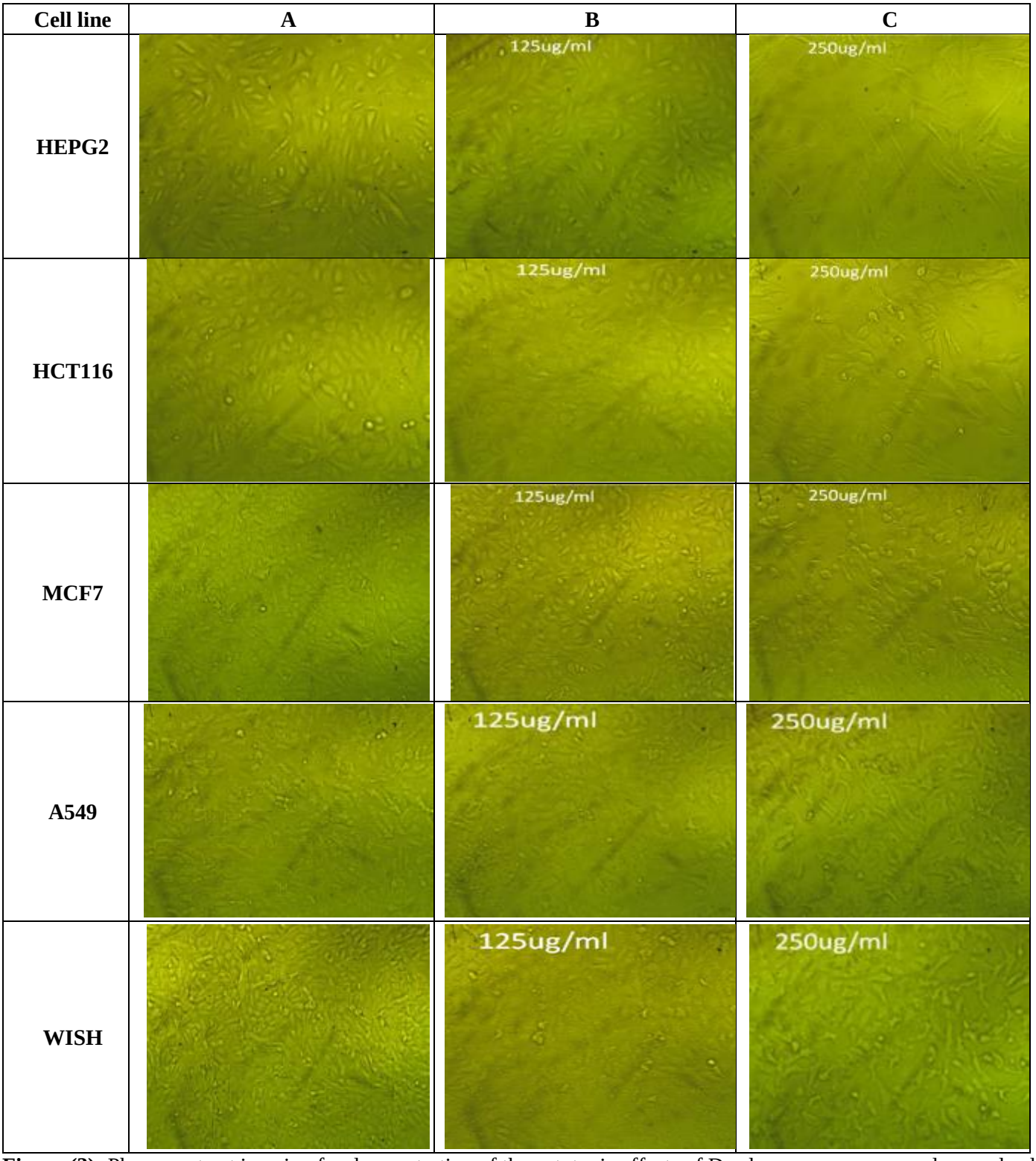

Figure (3): Phase-contrast imaging for demonstration of the cytotoxic effects of Dysdera sp. on cancer and normal cell lines. A: control, B: dose of 125µg/ml, C: dose of 250µg/ml.

DISCUSSION

The findings of our study align with **Elmeligy *et al.*** [23], who investigated the impact of Cerastes vipera crude venom on HepG-2 cells. Exposure to various doses (0.78, 1.56, 3.125, 6.25, 12, 25, 50, and 100 µg venom/ml culture medium) resulted in a significant reduction in cell count compared to the corresponding controls, indicating the cytotoxic effects of Cerastes vipera crude venom. The half-maximal inhibitory concentration (IC50) value for the crude venom was determined to be 16.3 µg/ml. In a similar vein, **Zhang *et al.*** [24] explored the effects of Lycosa vittata spider venom on various human cancer cell lines, including K562, U937, PC3, and MDA-MB-231, along with the control cell line HEK-293. Their results demonstrated a noticeable reduction in cell viability induced by the venom. Moreover, **Siedlakowski *et al.*** [25] highlighted the apoptotic-inducing ability of Pancratistatin (PST), a natural compound derived from the Hawaiian spider lily, specifically targeting human breast cancer cell lines MCF-7 and Hs-578-T in comparison with noncancerous counterparts.

In a more recent study in 2020, **Mayor *et al.*** [9] observed cellular changes, including reduced adhesion, cell condensation, and membrane blebbing, in A549 cells treated with high concentrations (200 µg/mL) of fraction AT5-3 from Phlogiellus bundokalbo spider venom. Notably, variations in activity were observed among collected fractions, with AT5-3 exhibiting the highest inhibitory activity and an IC50 value of 13.18 µg/ml. **Lian *et al.*** [26] reported morphological changes induced by spider venom, including decreased cell numbers, shorter cell length, and reduced cell adhesion. Their study on HepG2 cells revealed potent suppression of cell proliferation in a dose- and time-dependent manner, with an IC50 of 126.00 µg/ml. **Santiago-Bautista *et al.*** [5] presented the protein profile of Phlogiellus bundokalbo venom, an endemic Philippine tarantula, and observed a significant cytotoxic effect against MCF-7 cells at concentrations of 25, 50, 100, and 200 µg/ml, with IC50 values ranging from 52.25 µg/ml to 110.20 µg/ml.

Finally, **Guo *et al.*** [27] investigated the in vitro antitumor activity of Agkistrodon blomhoffii ussurensis snake venom on HepG2 cells. The novel L-amino acid oxidase Akbu-LAAO purified from the snake inhibited HepG2 growth in a time- and dose-dependent manner, with an IC50 of approximately 38.82 µg/ml. Additionally, **Gupta *et al.*** [28] noted the growth-inhibitory effects of M. raveni venom on various cancer cell lines, including HepG2, BEL-7402, A549, and MCF-7.

CONCLUSIONS AND FUTURE RECOMMENDATIONS

In this in vitro study, we investigated the concentration-dependent response of Dysdera spider venom on various human cell lines, including the breast tumor cell line (MCF-7), hepatocellular carcinoma cell line (HEPG-2), lung cancer cell line (A549), and colon carcinoma cell line (HTC-116), in comparison with the normal cell line (Wi-38). The study revealed a lower cytotoxic effect against normal breast cells than the cancer cell lines, suggesting cancer-specific effects. Based on the preliminary results, even though spider venom is primarily used as a veterinary drug, its observed cytotoxic effect on human cancer cells implies the need for a re-evaluation as a potential cytotoxic agent. We recommend further analyses to identify the functional compounds within spider venom and advocate for future studies that delve into its mechanism of action on cancer cells.

Financial support and sponsorship: Nil
Conflict of Interest: Nil.

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