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EFFECTS OF *HOGNA CAROLINENSIS* AND *PHIDIPPUS OCTOPUNCTATUS* SPIDER VENOMS ON CULTURED HEART CELLS: MORPHOLOGICAL STUDIES

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ABSTRACT:

The aim of this study is to investigate the effect of the venoms from wolf spider *Hogna carolinensis* and the Jumping spider *Phidippus octopunctatus* on the morphology and viability of cultured 1-2 days old rat embryonic cardiac cells. After treatment with spiders venom, marked morphological changes in cardiac cells were observed, illustrated by rounding-up of the cells, reduction in cell size, loss of cellular projections and clustering. This was followed by cell detachment from the substratum, as revealed by light microscopy. Cells proliferation were also susceptible to the toxic effect of both *Hogna carolinensis* and *Phidippus octopunctatus*, and it caused a significant time- and dose-dependent decrease in cell number when the cells were treated with 0.05, 5, 50 or 200ug/ml of the venom for five days.

INTRODUCTION:

There are over 34,000 species of spider world-wide (except Antarctica). Almost of all are fanged and venomous. Fortunately less than 0.5% are able to penetrate human skin and, of those, only a handful are considered dangerous. Most bites occurring when the spider is provoked or trapped. Spiders are predatory animals, which consume other animals (including other spiders), for food. For the vast majority of spider species, biting is the way by which a spider subdues its prey; the spider will use its venom to paralyze or kill its victim, often consuming it later. Spiders also use biting as a defensive mechanism; though the primary purpose of spider venom is to capture food[1-3].

Spiders (Araneae) make up an integral component of the animal life of the vast and diversified land in terrestrial habitats in the hot dry domains and warm climates all over the world, offers ideal viability conditions for spinning especially in the Kingdom of Saudi Arabia[4-6].

The chief concern with the bite of medically significant spiders is the effect of the spider's

venom. A spider envenomation occurs whenever a spider bites. Not all spider bites involve injection of venom into the wound. The amount of venom injected can vary based on the type of spider. With very few exceptions, such as the so-called camel spider (which is not a true spider), the mechanical injury from a spider bite is not a serious concern for humans. Some spider bites leave a large enough wound that infection may be a concern, and other species are known to consume prey which is already dead, which also may pose a risk for transmission of infectious bacteria from a bite[7, 8].

However, the toxicity of spider venom which poses the most risk to human beings; can be fatal to humans in the amounts that a spider will typically inject when biting[9, 10].

Many spider venoms contain peptide neurotoxins active on ion channels. One major class of such toxins is the gating modifiers which activate Na^+ channels by shifting their voltage dependence or removing inactivation or both[11, 12].

The cellular depolarization and/or repetitive firing of action potentials that results when these toxins act on neurons may explain many excitotoxic activities of such venoms and their components observed in vivo[13] or in vitro[14] as well as the liberation of neurotransmitters that these toxins induce in brain slices or synaptosomes[15]. Similarly, stimulation of sensory neurons by the opening of Na^+ channels may cause, at least in part, the intense local pain reported by victims of some spider bites. The clinical manifestations of spider envenomation also include visual disturbances, priapism, neurogenic shock, tachycardia and arrhythmias. However, in Saudi Arabia there are no reports on severe human accidents by spiders of the suborder Mygalomorph. The symptoms are slight local pain, swelling and redness that disappeared after few hours. In severe cases, renal failure can occur leading to death, especially in children[16, 17].

Sphingomyelinase D is the major component of Loxosceles venom, and has already been demonstrated to be the factor responsible for inducing dermonecrosis in humans, rabbits and guinea pigs. Sphingomyelinase D cleaves the choline moiety of sphingomyelin, resulting in ceramide phosphate, in contrast to endogenous mammalian sphingomyelinases, which cleave the choline-phosphate moiety of sphingomyelin generating ceramide itself[18].

It is well known that ceramides, the breakdown products resulting from the action of sphingomyelinases on sphingomyelin, are very important cellular regulators of various biological processes such as proliferation, migration, differentiation and apoptosis[19]. Sphingomyelin (N-acylsphingosine-1-phospho-choline or ceramide phosphocholine) the primary source of ceramide in signal transduction, is preferentially concentrated in the outer leaflet of the plasma membrane of mammalian cells[20].

However, the inner leaflet of the plasma membrane or lysosomal/endosomal compart-ment may be functionally a more important site for sphingomyelin hydrolysis and ceramide generation, given the necessity for interaction between ceramide and intracellular targets.

Ceramides also regulate TNF-α production that causes vascular endothelial cells to express adhesion molecules for leukocytes and stimulates endothelial cells and macrophages to secrete chemokines such as IL-8, the most potent chemotactic factor for neutrophils.

MATERIAL AND METHODS:

Preparation of Spider Venoms:

Wolf spider Hogna carolinensis and Phidippus octopunctatus venoms were purchased from Spider Pharm, Arizona, USA. The venom was kept in a desiccators in the refrigerator until used.

Embryonic Rat Heart Cell Culture:

Wistar rat embryo hearts 1-2 days old were removed under sterile conditions and washed three times with phosphate buffer saline (PBS) and once with minimal essential media (MEM) with out serum. The hearts cells were prepared as described[21, 22]. Briefly, heart were cut into small fragments, minced and then gently agitated in trypsin solution at a concentration of 0.025 mg/ml. The supernatant suspensions containing the dissociated cells was removed and cells were collected by centrifugation at 100 xg. Cells were resuspended in MEM containing 10% heat inactivated (56°C for 30 min) foetal calf serum and 5 mg/ml glucose. Cells were adjusted to $5x10^4$ cells/ml and plated into 35mm culture dishes and incubated in a humidified incubator in an atmosphere of 5% CO_2 at 37°C.

Cells Exposure to Venoms:

Heart confluent mono layers cells were washed and adjust to $5x10^4$ cells/ml. Cells were seeded into 96-well cell culture plates at a concentration of $5x10^4$ cells/ml incubated in humidified 5% CO_2 incubator. Cells were left to adjust for 24 hr before the venoms were added. Venoms used were dissolved in MEM. The venoms concentrations used were selected based on previous reports on LD_{50} in optimization bioassays 0.01-10 µg/ml venom for animals and also on our preliminary toxicity studies on cultured cells. Each venom was used at four different concentrations: 0.05 ug/ml, 5 ug/ml, 50 ug/ml, and 200 ug/ml. Cells were maintained with the venoms for 5 days.

Morphological studies:

Venoms treated cells were harvested by centrifugation at 100x g for 5 min. The pelleted cells were resuspended in the remaining solution and treated with 2 ml of hypotonic solution (0.075 M KCL) drop wise at 37°C for 7 minutes. Cells were collected by centrifugation and the pellets were gently resuspended in the remaining fluid. Two drops of the cell suspension were spread on dry slide angled at 45°, fixed in methanol for 2 seconds and air dried[21]. Slides were stained with Giemsa and examined under light microscope.

Cytotoxicity Assay:

In this study we investigated the effect of Wolf *spider Hogna carolinensis* and *Phidippus octopunctatus* venoms on Wistar rat embryonic heart cells grown in culture. Cells were exposed to venoms at different concentrations. Four concentrations were used for each venom (0.05,

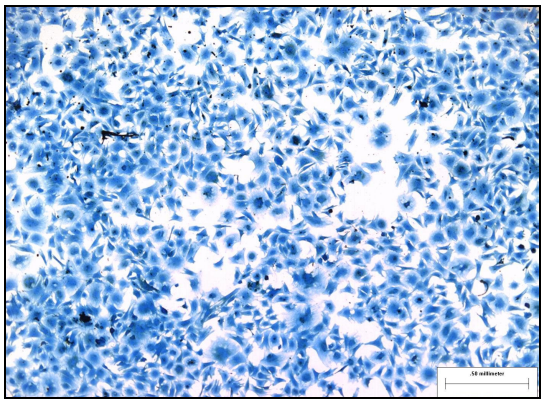
5, 50, and 200 ug/ml) Control was a well containing cell grown in absence of venom. Cells were maintained with the venoms for the period of the experiment, and cell viability was determined every day after venoms treatment for five days. Cells were harvested with trypsin; EDTA and viable cells were counted by trypan blue exclusion test[23] and determined by hemocytometer. All assays were set up in duplicate for each concentration.

RESULTS AND DISCUSSION:

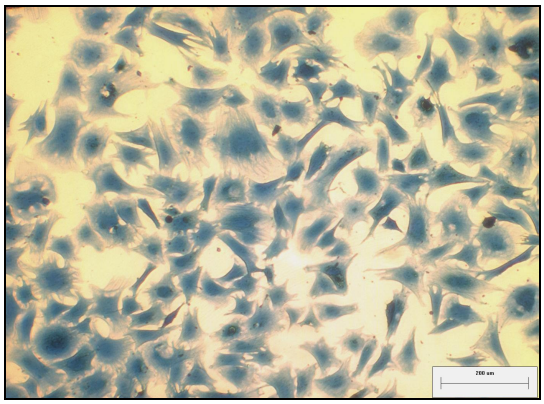
Morphological Changes Induced by Venoms:

Marked morphological changes of cultured rat embryonic cardiac cells were observed when exposed to *H. carolinensis* and *P. octopunctatus* spider venoms at concentrations of 0.05-200 ug/ml.

The gross microscopic response to the two spider venoms was initial rounding-up of the cells, reduction in cell size and loss of cellular projections followed by detachment from the cell culture surface and clustering (Figs.1-3). The morphological changes showed dose-related responses by the cardiac cells in both. The *H. carolinensis* and *P. octopunctatus* venoms.

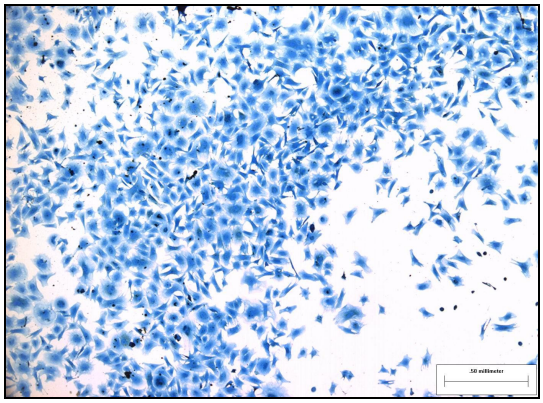


A

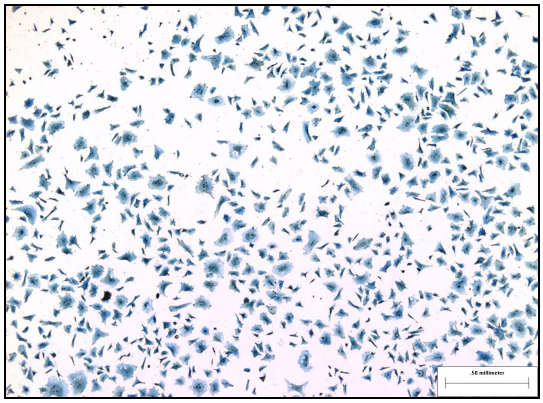


B

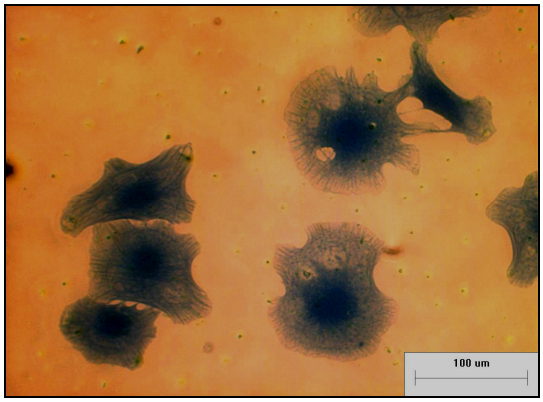
Fig. 1 A&B: Rat embryonic cardiac cells untreated with venom (control), showing nice confluent monolayer attached cells, spindle shape with projections



A-day 1 following venom treatment

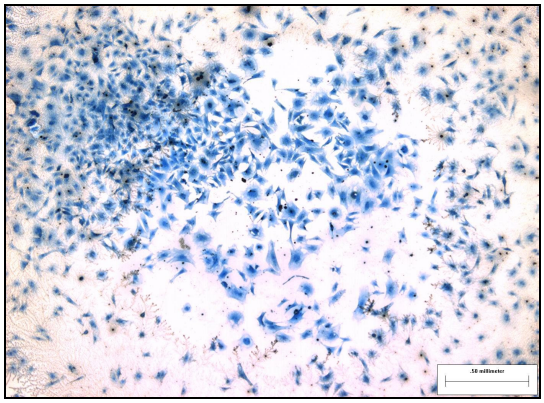


B-Day 3 following venom treatment showing reduction in cell number

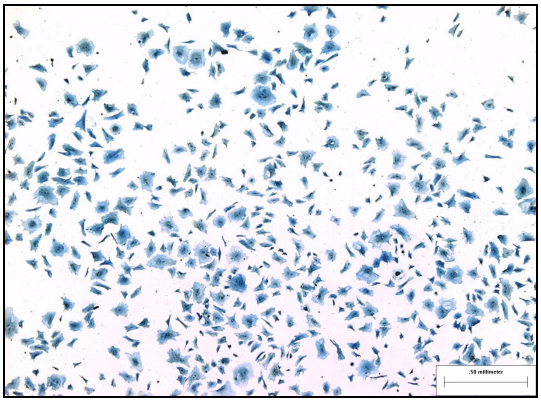


C-Magnified cells 3 days following venom treatment showing rounding of cells, loss of spindle shape and projections. Also, cells had less contact with each other

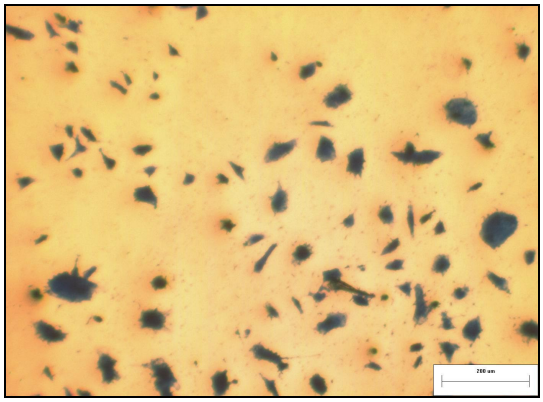
Fig. 2: Rat embryonic cardiac cells treated with *Hogna carolinensis* venom



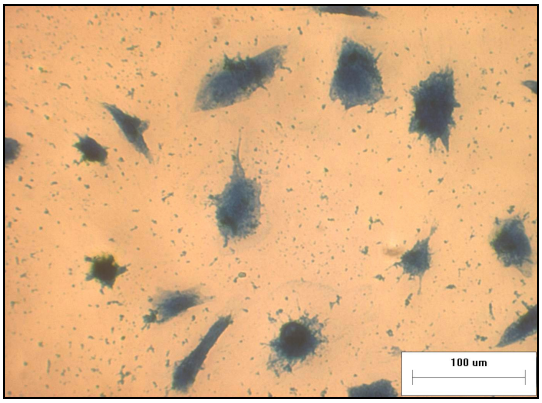
A-Day 1 following venom treatment



B-Day 3 following venom treatment showing reduction in cell number



C-Magnified cells 4 days following venom treatment



D-cardiac cells showing rounding of cells, loss of spindle shape and projections following treatment with the venom. Also, cells had less contact with each other

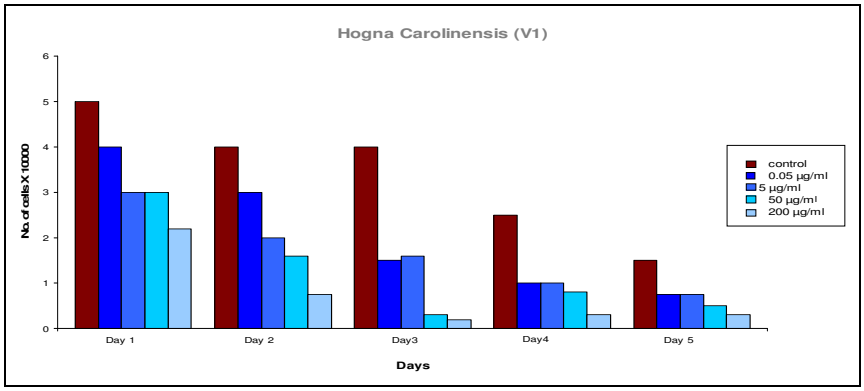
Fig. 3: Rat embryonic cardiac cells treated with *Phidippus octopunctatus* venom

Effect of Venoms on Growth Rate and Cell Viability:

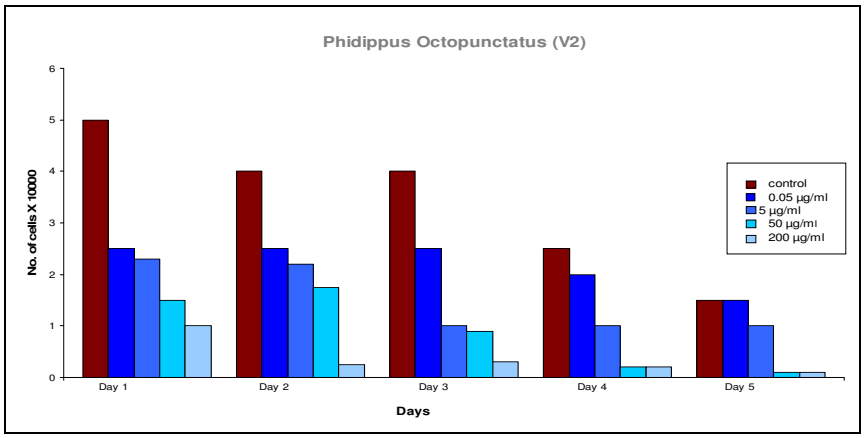
Spider venoms at concentrations of 0.05-200 µg/ml caused dose- and time-dependent inhibition of cultured cardiac cells proliferation. The effect of 5 days exposure to Hogna carolinensis venom on the growth of embryonic rat cardiac cells is shown in Figure 4. The extent of growth inhibition was dose dependent and cells treated with venom grew at a slower rate than the untreated control cells. The venom acts very rapidly and by the third day of incubation at concentrations of 50ug/ml and 200 ug/ml more than 90% of cell growth was inhibited (Fig. 4A).

Similarly the Cytotoxicity of Phidippus octopunctatus venom was at its maximum at 50 ug/ml and 200 ug/ml causing more than 93%

cell growth inhibition after 5 days of exposure to the venom (Fig. 4B). Phidippus octopunctatus venom act more rapidly than Hogna carolinensis venom, after 24 hr of exposure, *P. octopunctatus* 200 ug/ml caused 80% cell growth inhibition, meanwhile *H. carolinensis* caused only 56% cell growth inhibition. Also, the *P.octopunctatus* venom is more cytotoxic to the cultured cardiac cells and it showed more toxicity at a lower venome concentrations compare to *H. carolinensis* venom (Fig. 4 a&B). The inhibition of cardiac cells by the venoms of the spider could be due to apoptosis, toxicity damage or direct lyses. Additional biochemical studies are needed to delineate that.



A



B

Fig. 4: Effect of (A) *Hogna carolinensis* venom and (B) *Phidippus octopunctatus* on viability of rat embryonic cardiac cells. Cardiac cells were seeded in a 96-well cell culture plate at a density of 5×10^4 cells/ml and left to settle at 37 °C in a 5% CO_2 incubator overnight. Cells were exposed to 0.05, 5, 50, and 200 ug/ml of *venoms* in tissue culture medium. Parallel wells were incubated with cells without venom as a control

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التأثير المورفولوجي لسموم العنكبوت الذئبي و العنكبوت الوثاب على خلايا القلب الجنينية للجرذان

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من أهداف هذه الدراسة معرفة مدى تأثير سم العنكبوت الذئبي والعنكبوت الوثاب على قدرة نمو خلايا القلب الجنينية للجرذان، وذلك من خلال تعرض هذه الخلايا التي أخذت من أجنة الجرذان من عمر 1–2 يوم لسموم كل من هذين العنكبوتين بتركيزات تتراوح من 0.05–200 ميكروجرام/مل لمدة خمسة أيام. وقد أظهرت الدراسة أن هذه السموم كان لها تاثيراً كبيراً على الشكل الظاهري للخلايا القلبية المنزرعه من حيث الشكل والاستدارة وفقدان النتوءات وضمور الحجم ثم انفصالها عن سطح الأطباق التي تمت عملية الزرع فيها، وذلك باستخدام المجهر الضوئي. كما أن هذه السموم (سموم العناكب) كان لها تاثيراً على عملية تكاثر هذه الخلايا. وقد تبين ان نقص عدد الخلايا القلبية الجنينية المنزرعه في الأطباق مرتبطاً ارتباطاً طردياً مع جرعة المواد السامة، وكذلك مع وقت التعرض لهذه السموم.