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ANTIANGIOGENIC PROPERTIES OF SCORPION VENOM: A NOVEL APPROACH IN COLON CANCER THERAPY

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Colorectal cancer (CRC) induced angiogenesis is the most harmful stage in the tumor's growth, invasion, and metastasis. Scorpion venom (SV) is a natural source that has a promising approach for the development of new pharmaceuticals. The study aimed to evaluate the anti-angiogenesis efficacy of the scorpion Leiurus quinquestratus venom (LQV) and Androctonus bicolor venom (ABV). Sprague Dawley rats were divided into four groups: Group 1 (Gp 1) (n=10) as a control group. Gp 2, 3, and 4 (15/each) were subcutaneously (S.C.) injected with 1,2-dimethylhydrazine (DMH) (40 mg/kg/ week) for 4 weeks. Gp 2 served as the DMH-induced cancer group, Gp 3 and 4 were intraperitoneally (i.p.) injected with 1/20 LD50 of LQV or ABV for 19 wks. At the end of the experiment, 24 weeks, the colon was analyzed for histopathology, immunohistochemistry (IHC) CD34, vascular endothelial growth factor (VEGF), and VEGF protein expression via enzyme-linked immunosorbent antibody (ELISA). Microvessel density (MVD) and VEGF expression were quantified. The histological data of the colon demonstrated aberrant blood vessel size and quantity in DMH group. Conversely, LQV and ABV treatments reduced neoplasia, blood vessel numbers, and distribution in the mucosa. The IHC staining of CD34 showed a considerable reduction in the MVD, from 37 ± 11.7 in the DMH group to 10 ± 3.5, and 20 ± 6.5 in LQV and ABV treated groups, respectively. VEGF expression was strongly in the DMH group, becoming only moderately stained after treatments with LQV or ABV. Moreover, the test ELISA revealed a decreased expression in the colonic VEGF protein levels. Collectively, LQV and ABV, have a distinctive anti-angiogenesis capacity against CRC, which could support the development of a novel therapeutic approach for colorectal cancer management.

Keywords: *Scorpion venom; Colorectal cancer; Angiogenesis; Microvessel density; CD34; VEGF*

INTRODUCTION

Colorectal cancer (CRC), which is equally common in men and women, is the third most frequent reason for cancer-related mortality worldwide[1]. CRC caused angiogenesis which is a process of creating new capillaries from either pre-existing vasculature or endothelial progenitor cells. The assessment of tumor microvessel density (MVD), which represents angiogenesis and is closely correlated with the clinical features of the malignant process, is the focus of much anticipation[2]. MVD is a derived metric of angiogenesis that has been linked to unfavorable outcomes in many cancer types[3]. Anti-angiogenic therapy is beneficial for CRC patients, it makes sense to look for novel prognostic indicators that would allow for the assessment of this kind of treatment.

Scorpion venom (SV) is a natural source may offer promising approach for the development of new pharmaceuticals, such as anti-microbial, anti-inflammatory, cytotoxic effects, anti-diabetic and anti-cancer[4-8]. The complex mixture of various compounds found in SV includes proteins, peptides, heterocyclic elements, free amino acids, and inorganic salts. Peptides alter, regulate, or prohibit ion channels[9], this implies that the correlations between ion channels and the development of

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cancer suggest that SV may have novel therapeutic applications. In addition to depolarizing immune cells, these ion channels, which can be overexpressed or downregulated, are essential to the growth and invasion of cancer[10]. SV also possess pleiotropic anticancer properties, including the ability to provoke apoptosis and suppress the proliferation, invasion, and metastasis of cancer cells[11]. The antioxidant activity of SV in vitro were reported[12]. The peptide Smp24, from *Scorpio maurus palmatus*, has an anti-proliferative effects[13].

CD34 is a transmembrane glycoprotein member of the sialomucins family of adhesion molecules for cell proliferation and differentiation control and it considered a marker of hematopoietic stem cells and used in a variety of cancer types[14-16]. It was initially reported that the growth of several tumors *in vivo* was dramatically suppressed by the blocking of VEGF-induced angiogenesis by certain monoclonal antibodies[17]. According to recent research, VEGF expression is linked to an increased number of MVD in colon tumors and has been linked to poor outcomes in terms of tumor progression, metastasis, and patient survival[18]. In CRC, increased VEGF expression leads to higher MVD, as more blood vessels are formed to supply the growing tumor. This increased vascularization, characterized by CD34-positive microvessels, provides the tumor with nutrients and oxygen, while also facilitating the spread of cancer cells to distant sites. Therefore, VEGF, CD34, and MVD are all interconnected indicators of tumor angiogenesis and are often investigated as potential targets for anti-cancer therapies[16].

Therefore, the current study aimed to evaluate the potential effect of the scorpion *Leiurus quinquestratus* venom (LQV) and *Androctonus bicolor* venom (ABV), family Buthidae, as anti-angiogenesis agent against DMH induced CRC in rats.

MATERIALS AND METHODS

Collection of scorpions and venom preparation

One hundred scorpions were collected in August 2022 by professional hunters from Aswan area at Upper Egypt and the Mediterranean Northern coast, Egypt. The collected samples were transferred to Invertebrate lab, Zoology Department, Faculty of Science, Tanta University, Egypt. They were classified according to Salama and Sharshar[19]. *L. quinquestartus* and *A. bicolor* were separated into two plastic containers. The scorpion were milked Using electrical stimulation at 12 volts, centrifuged, lypholyzed, and stored until used at -20 °C[19].

Animals Ethics Approval

According to the National Institute of Health's handbook for the care and use of laboratory animals (NIH Publications No. 8023, amended 1978) and ethical standards outlined in the ARRIVE guidelines, the animals were treated with proper care. IACUC SCI-TU- 0270, the Animal Care and Use Committee of Tanta University's Faculty of Science, accepted the experimental procedure.

Animals

A total of eighty five (85) male Sprague-Dawley rats, 6-7 weeks of age, weighted 170 ±20, were purchased from Vaccera, Dokki, Egypt. They were housed in the circumstances of the animal facility for a week before being grouped at 55 ± 5% relative humidity and 23 ± 2 ºC. Every day, the animals' body weights, consumption of food and drink, and general health were observed.

Experimental design

Based on our recent findings (Unpublished data), which determined the LD_{50} doses of LQV and ABV in male Sprague Dawley rats, 1/20 LD_{50} were selected (0.5 and 1.09 mg/kg for LQV and ABV, respectively.) Sprague-Dawley male rats were divided randomly into four groups: Group 1 (Gp 1) (10 rats) was used as a normal control group administered 0.09% saline only. Gp 2 (15 rats) was subcutaneously (S.C.) injected with 40 mg/kg/b.wt of DMH (N, N'-dimethylhydrazine, Sigma-Aldrich, Cat. No. D161608, Darmstadt, Germany), once a week starting from week 1 to week 4. Gp 3 and Gp 4 (15 rats/ each) were initiated with DMH similar to G2, and injected intarproteinelly (i.p) with 0.025 mg/kg LQV and 0.05 mg/kg ABV, respectively, starting from week 5 until the end of the experiment, wk 24.

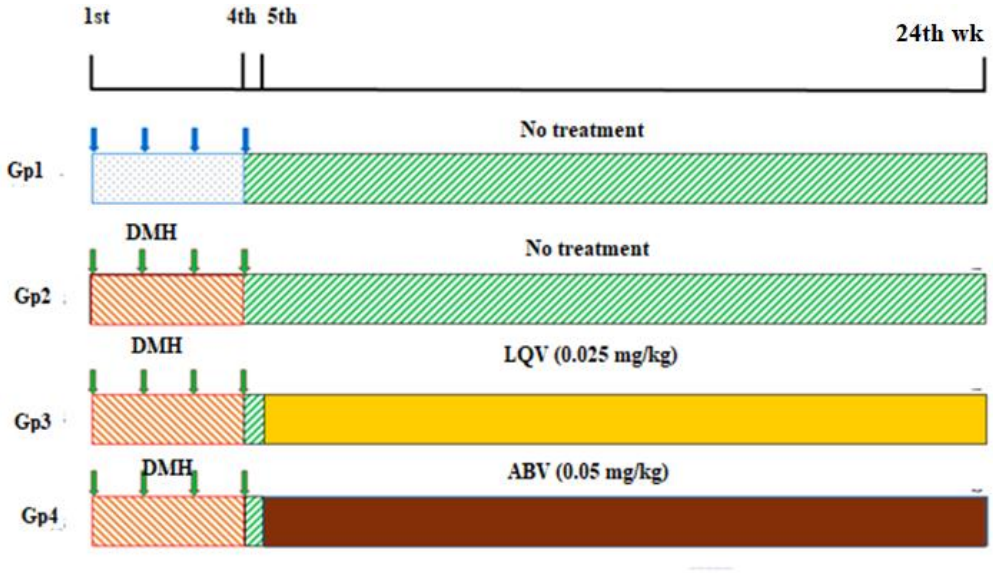

Fig. 1: The experimental design of CRC chemically induced rat groups. Gp1: control group, Gp2: CRC induced group, Gp3; CRC/LQV treated, Gp4: CRC/ABV treated, DMH; carcinogen 1,2 dimethylhydrazine, LQV; *L.quinquestratus* venom; ABV; *A.bicolor* venom.

Preparation of biological samples

At the end of the experiment at wk 24, every rat was sacrificed under excess ethyl ether anesthesia. At necropsy, the livers were perfused with phosphate-buffered saline (PBS) to remove red blood cells and clots, then cut into small pieces to prepare the tissue homogenate. Following removal, the colons were inflated with 0.09% saline, longitudinally cut open, and thoroughly flushed in saline. Colons from 10 rats from each group were retained in 10% phosphate-buffered formalin until it was prepared for routine histopathology and IHC. Next, the colon mucosa of 5 rats from each group was scraped off using clean glass slides and maintained at -86 °C for biochemical and molecular investigations[20]. The colon was found to contain anomalous masses and two animal pathologists made a pathological diagnosis of the lesions, according to[21].

Immunohistochemistry of CD34 and VEGF

Staining for CD34 and VEGF was performed using an anti-CD34 MEC 14.7 monoclonal antibody (Abcam, Cat. No. # ab8158, UK), and anti-VEGF monoclonal antibody (ThermoFisher, Cat #MA1-16629, USA), respectively. Formalin-fixed, paraffin-embedded 4-µm tissue slices underwent xylene deparaffinization, ethanol dehydration, and a 5-minute incubation period with 3% hydrogen peroxidase before being cleaned with phosphate-buffered saline (PBS). Afterward, the tissue sections were incubated in 10% normal horse serum. Next, they were incubated with either an anti-CD34 (1:500) or anti-VEGF (1:200) antibody for an entire night. The slides were dropped with the Super-enhancer TM, allowed to incubate, and then cleaned using PBS. Each slide received a drop of labeled dextran polymer conjugated poly horseradish peroxidase (HRP), which was then incubated for 30 minutes. Subsequently, the sections underwent two 10-minute cycles of cold PBS washing and were meticulously cleaned to eliminate any remaining PBS. Lastly, the pieces were covered with a drop of recently made DAB (32-Diamino benzidine Tetra Hydrochloride, a substrate Chromogen). After removing extra DAB from the slides, they were counter-stained with hematoxylin and rinsed under running distilled water[22].

Calculating the stained vessel quantity (MVD)

According to[23], vessels stained with monoclonal antibodies to CD34 exhibit three basic morphological characteristics: round form, sinusoid-like morphology, and small vessels without visible lumens (endothelial sprouts). The method employed complies with the global consensus approach for evaluating intratumoral motor neuropathy[24] Strong muscle-walled vessels, vessels in sclerotic

regions, and vessels bigger than about eight red blood cells were not included in the count. The immunostained segment was scanned at low magnifications (x40 and x100), and the three regions that had the highest concentration of clearly highlighted microvessels referred to as "hot spots" were chosen. Microvessels were counted at a magnification of x 400. The mean number of vessels per high-power field (x 400) in the three hotspots that were chosen is how MVD is expressed[25].

Evaluation of VEGF Expression

According to[26], the IHC semi-quantitative expression analysis of VEGF was performed in the following way: Extent (Quantity of immunoreactive cells): zero: not present; 1: less than 5%; 2: between 5 and 50%; and 3: greater than 50%. The staining Intensity was semi-qualitatively evaluated as zero: negative; 1: weak; 2: intermediate; and 3: strong. The immunoreaction's final score was determined by multiplying the two factors (Extent X Intensity), and it was categorized as follows: negative (0), weak (0–2), moderate (2-4), and strong (4-6). Strong immunoreaction final scores were regarded as high expression while negative and weak immunoreaction final scores were regarded as low expression for statistical purposes.

Measurement of protein expression of VEGF in tissue samples by ELISA assay

The colon mucosa tissues were carefully minced into minute pieces and then completely rinsed in ice-cold PBS (0.01 M, pH = 7.4) to remove any residual blood. The experiment was conducted using China's Rat Vascular Endothelial Growth Factor ELISA Kit (Cat. No. E-EL-R1058), which enables the performance of ELISA testing. A spectrophotometer (ELx 800; Bio-Tek Instruments Inc., Winooski, VT, USA) equipped with a microplate reader was used to measure the absorbance at 450 nm after the substrate was added and the reaction was cleaned and incubated.

Statistical analyses

Group values expressed as means ± standard deviations were evaluated by the *t*-test or analysis of variance while percentage data were analyzed by the *chi*-squared test using Statistical Package for Social Science ver. 17 (USA). P *<0.05* was considered to be statistically significant.

RESULTS AND DISCUSSION

Treatment with LQV and ABV histologically changed the blood vessels' size and numbers.

Sections stained with hematoxylin and eosin (H&E) were used to rank the characteristics of colon cancer. The mucosa and tumors of the DMH-only administered group indicate prominent blood vessels in size and number compared to normal rats. In contrast, the groups' blood vessel distribution and quantity in mucosa and neoplasia were decreased after receiving LQV and ABV treatments; the LQV group's drop was obvious, as shown in **(Fig. 2)**.

LQV and ABV inhibit CD34 and VEGF, the angiogenesis markers

To evaluate the MVD levels in the colonic tissues and tumors, the vasculature was stained with monoclonal antibodies against CD34. Generally, the microvessels were detected by brown circular lines surrounding angiogenetic vessels **(Fig. 3)**. The CD34-immunoreactive vasculature displayed three morphological features; (a) sinusoid-like, (b) round-shaped, and (c) tiny vessels lacking observable lumina (endothelial sprouts). The only vessels counted here were those without severely thick muscle walls, with visible lumens, and those with a caliber of less than eight red blood cells. When compared with the normal group, the DMH-only treated group indicated the highest average MVD levels in both mucosa and tumors **(Fig. 4)**. Notably, there was a substantial decrease in the MVD counts in the groups treated with LQV or ABV compared with the DMH-only treated group, especially in the LQV group (in both mucosa and neoplasia).

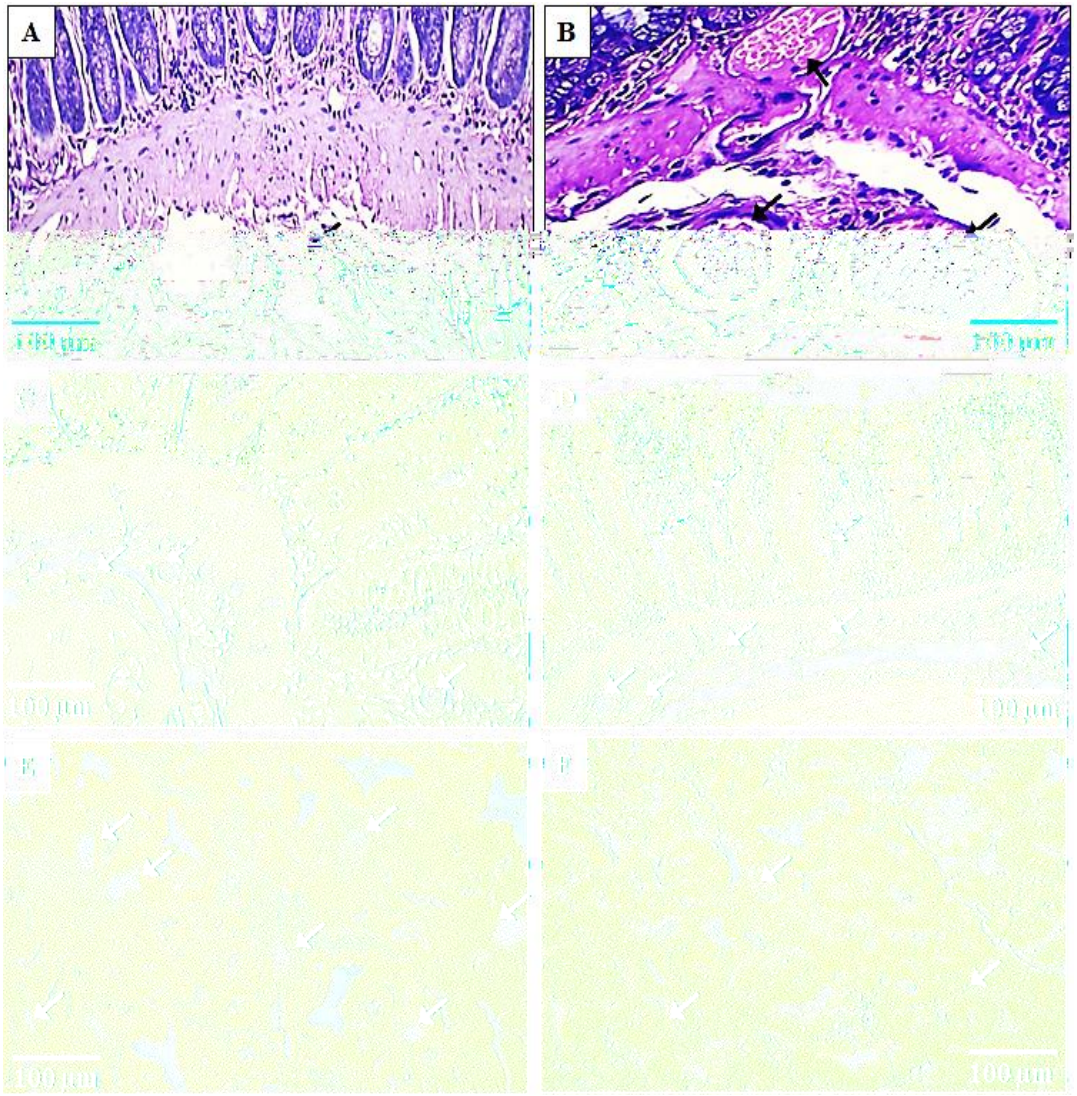

Fig. 2: Photomicrographs showing general blood vessel distribution in rat colons of different groups. **A)** the distal area of a normal rat's colon with typical blood vessels (arrows); **B)** the middle area of the colon of a DMH-only administered rat with large and numerous vascular infiltration (arrows); **C**) middle colonic area from an LQV-treated rat indicating significantly shrunk and fewer blood vessels (arrows); **D**) middle colonic area of a rat from the ABV-treated group showing small and few blood vessels (arrows). **E**) invasive adenocarcinoma from the colon of a rat treated with DMH only showing many blood vessels (arrows); **F**) invasive adenocarcinoma from the colon of a rat treated with ABV showing a marked fewer number of blood vessels (arrows).H&E

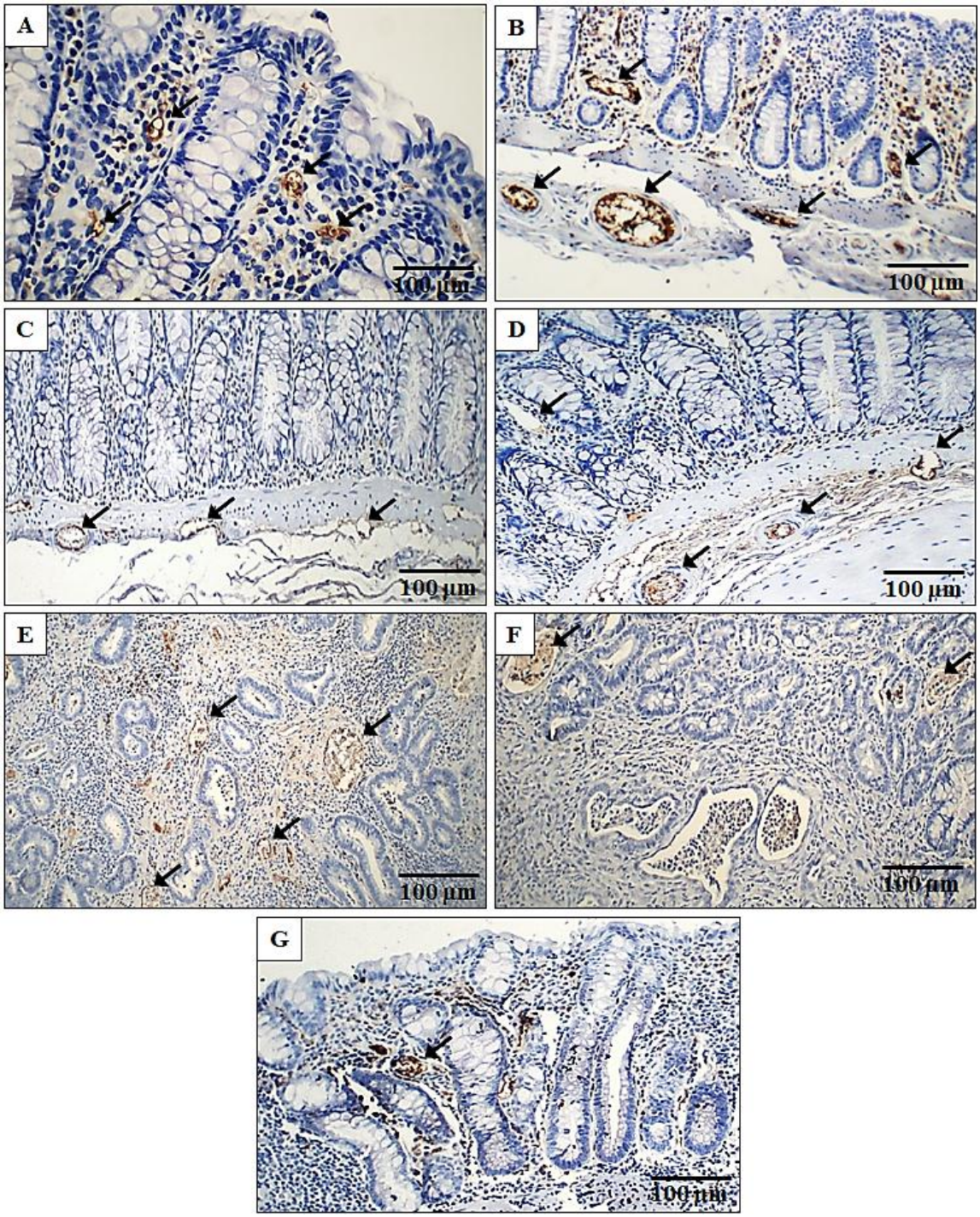

Fig. 3: IHC of CD34 staining for quantifying MVD in normal and cancerous areas. (**A**) Normal colonic area stained with CD34, showing strongly stained MVD (arrows) (X400), **B**) a cancerous area treated with DMH-only, showing increased numbers and size of blood vessels labeled with CD34, (**C**) a significant decrease in CD34-labeled cells treated with LQV. (**D**) a colon treated with ABV with decreased CD34-labeled cells, (**E**) invasive adenocarcinoma from DMH only group with marked vascular infiltration (arrows), (**F**) invasive adenocarcinoma from a rat treated with ABV showing less vascular infiltration (arrows). (**G**) Dysplastic area from the colon of a rat treated with LQV showing vascular infiltration (arrows) (no large tumor masses were detected in LQV group).

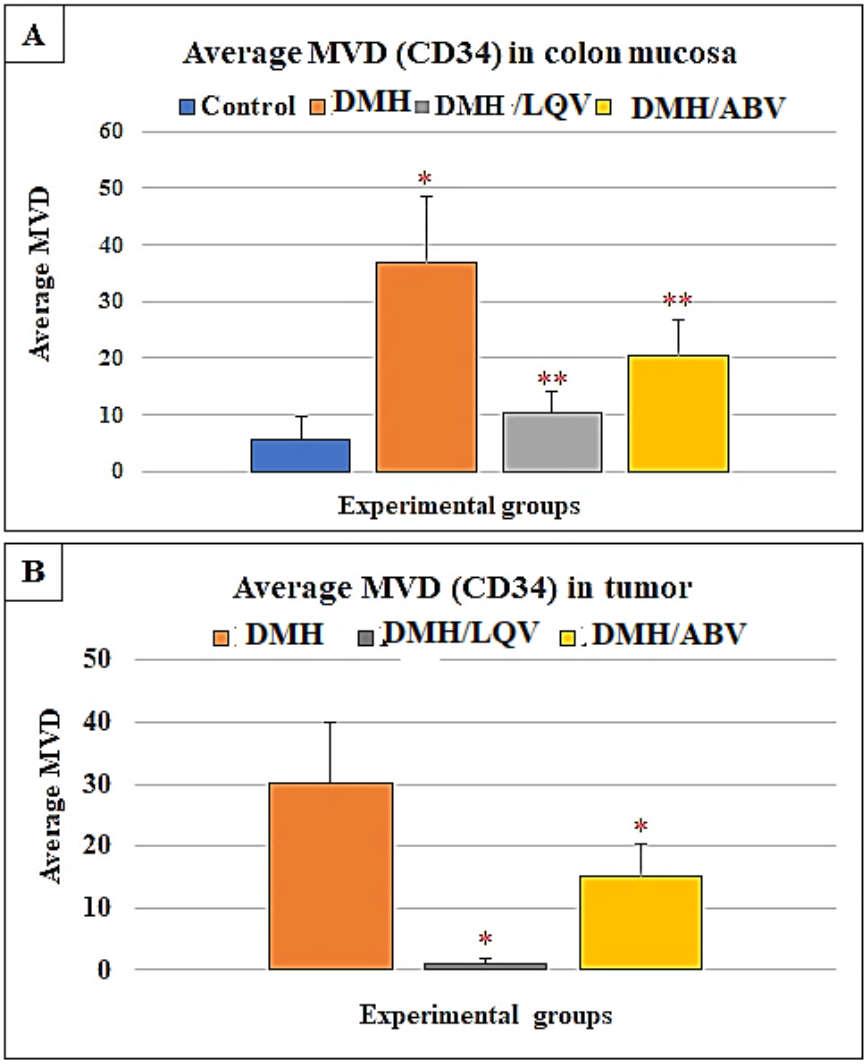

Fig. 4: (A) Average MVD of CD34 in normally appearing mucosa for control and treated groups. **(B)** Average MVD of CD34 appearing from tumors in DMH, LQV, and ABV treated groups *: Significant vs. MVD of G1 at *P< 0.05*. **: Significant vs. G2 at *P<0.05*. MVD: micro-vessel density.

The cytoplasm of the tumor cells showed brownish cytoplasmic staining that was reactive with the VEGF antigen as shown in **(Fig. 5)**. A semi-quantitative scale of 0–3 was used to express the results, and the mean of the multiplications of the various values was used. The staining intensity score was divided into four categories: 0, no staining at all, 1, weakly stained cells, 2, moderately stained cells, and 3, highly stained cells. The degree of staining was rated as follows: 0 denoted no staining, 1 mildly stained, 2 highly stained, and 3 strongly stained. The VEGF immunoreactivity in normal epithelium was observed with a weak intensity of 1+ and a score of 1, according to our findings. Yet, the mean of the quantitative scale multiplied by staining intensity yielded an IHC score of 7+ for group DMH alone. As the average values for the IHC score, the treated groups (LQ and AB) received a score of 4+ and 5+ respectively, as seen in **(Fig. 6)**.

LQV and ABV treatments down-regulated VEGF expression

VEGF protein expressed in tissues was analyzed using ELISA. **(Fig. 7)** demonstrates the expression of the VEGF protein in various groups. In G2, which received DMH only, a significantly higher level of VEGF expression (264.7%) was detected as compared to the normal control group. Meanwhile, the treatment with LQV and ABV demonstrated a statistically significant reduction in VEGF protein expression (78.05% and 167.57%, respectively).

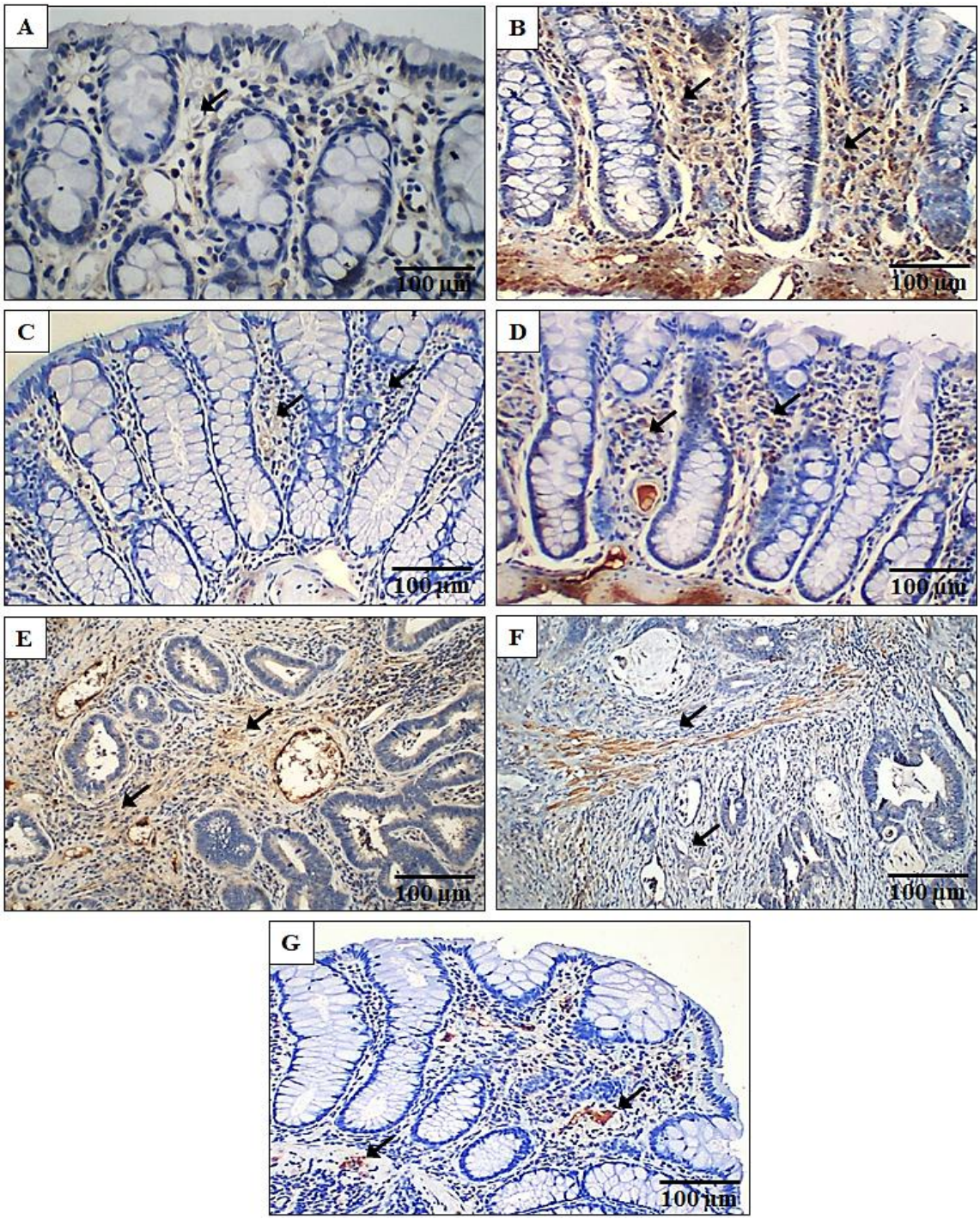

Fig. 5: IHC of VEGF staining: (**A**) weakly stained normal colonic epithelium. (**B**) strongly stained DMH-only colonic epithelium. (**C**) Moderately stained colonic areas treated with LQV. (**D**) Moderately stained, treated with ABV. (**E**) Invasive adenocarcinoma from the colon of a rat treated with DMH only showed strong staining. (**F**) Invasive adenocarcinoma from the colon of a rat treated with ABV, showing moderate staining. (**G**) Dysplastic area from the colon of a rat treated with LQV showing moderate staining.

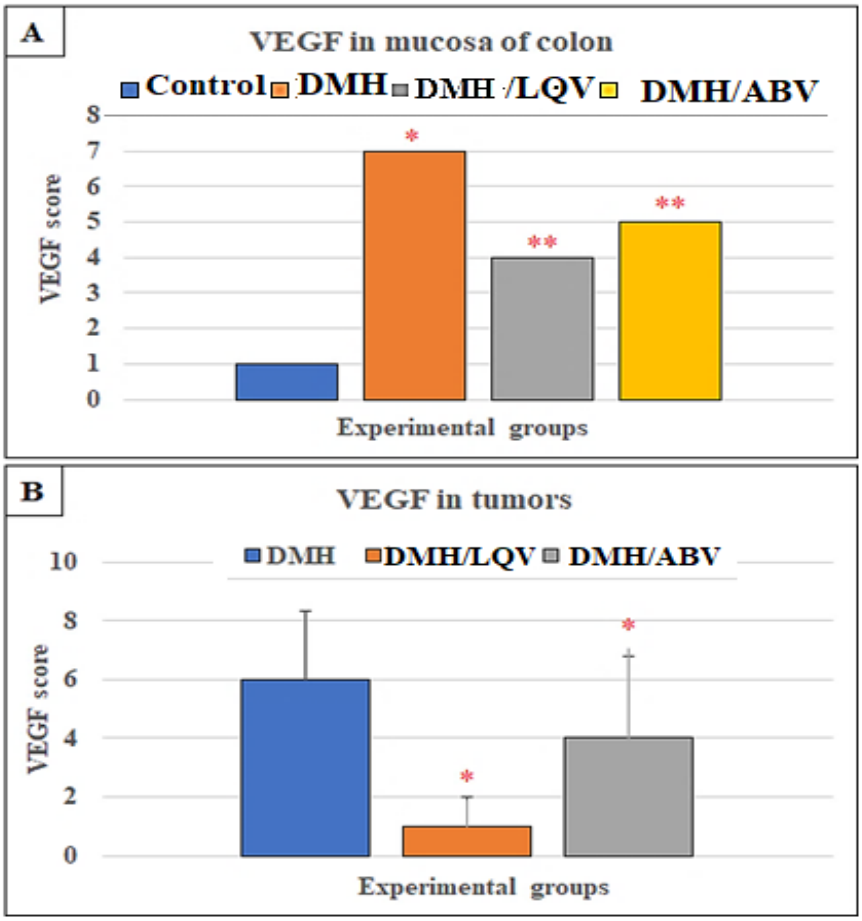

Fig. 6: (A) IHC analysis of VEGF expression in mucosa for normal, and treated groups. **(B)** VEGF expression in tumors in colon of treated groups. *: Significant vs. VEGF of G1 at $P< 0.05$. **: Significant vs. VEGF of G2 at $P<0.05$.

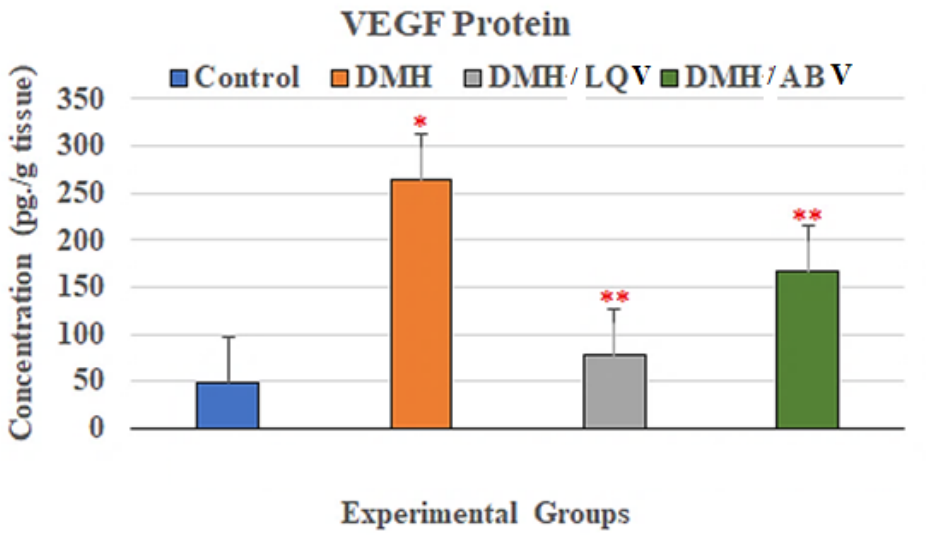

Fig. 7: VEGF protein expression in the control and treated groups. *****: Significant vs. the protein expression of VEGF in G1 at *P<0.05.* **: Significant vs. the protein expression of VEGF in G2 at $P<0.05$.

Discussion

Colorectal cancer (CRC) is one type of cancer that affects the colon. CRC is the third leading cause of cancer-related deaths worldwide, affecting both genders[1]. The development of new blood vessels sometimes referred to as neo-vascularization or angiogenesis, is crucial for the growth and spread of tumors in both human and animal models of colorectal cancer and is one of the characteristics that predict the likelihood of relapse[26]. MVD has been a widely used prognostic factor for a variety of malignancies. The concept of MVD for evaluating tumor angiogenesis was first proposed by[27]. The number of blood vessels inside a predetermined number of microscopic field "hotspots" is called MVD[28].

The hotspot method is mostly used to measure intertumoral blood vessels that are identified by von Willebrand factor (vWF), and CD34 which are pan-endothelial cell markers. Hotspots are identified using light microscopy, and in the identified locations, each microvessel is counted at a high magnification[29]. High MVD was significantly correlated with poor survival[2]. Since MVD counts reflect the total result of all angiogenic routes, they are a valuable gauge of how well anti-angiogenic medication is working. According to[30], VEGF is the most potent angiogenic factor among other angiogenesis-stimulating proteins. It is frequently linked to elevated MVD and unfavorable clinic-pathological parameters and outcomes. Therefore, creating new cancer treatments is a major priority for research worldwide, as colorectal cancer is one of the most dangerous global public health issues. Several investigations have demonstrated the presence of medicinal compounds in scorpion venom that may find application as anticancer medications[31].

Our current histological findings demonstrate the strong anti-tumor effects of the ABV and LQV on rats. The tumors and mucosa of the DMH-only treated group had observable blood vessels in terms of both size and abundance when compared to the normal rats. In contrast, after receiving LQV and ABV, mucosa and neoplasia blood vessel counts decreased, with a discernible decrease in the LQV group. Therefore, monoclonal antibodies against CD34 were used to stain the vasculature to evaluate the levels of MVD in the colonic tissues and tumors[32]. Our study's results showed that in comparison to the normal group, the DMH-only treated group's average MVD levels were highest in both tumors and mucosa. The MVD counts in the groups treated with LQV or ABV showed a noteworthy and significant decrease as compared to the DMH-only treated group, especially in the LQV group (in both mucosa and neoplasia). Likewise,[33] suggests that melittin, a constituent of bee venom, could potentially mitigate the impact of osteosarcoma on EPC-mediated angiogenesis. The tumors were injected with melittin, and its effects were assessed by immunohistochemistry using CD34. According to the findings, melittin reduced the MVD levels in the tumors. Prior research on the effects of various scorpion venoms, including those of *A. bicolor*, *A. crassicauda*, *L. quinquestriatus*, *Hemiscorpius lepturus*, *A. australis*, and *Scorpio maurus palmatus*, on various human malignant cell lines, were studied[13,31,34,35]. These studies revealed specific and differential mechanisms of anti-tumorigenesis.

Furthermore, as reported by[36] the treatment of VGB4 resulted in the regression of 4T1 murine MCT development by significantly reducing cancer cell proliferation and angiogenesis, as demonstrated by CD34 expression in immunohistochemistry. In addition, the results of our study showed that LQV and ABV inhibited the growth of tumors and decreased angiogenesis in rats with colon cancer. Our results showed that immunohistochemical VEGF in normal epithelium was seen by IHC with a weak intensity and a score of 1+ However, a score of 7+ for group DMH-only was obtained by multiplying the mean of the quantitative scale by strongly stained. The treated groups (LQV and ABV) scored moderately stained, with an average of 4+ and 5+ for the IHC score, respectively.

Additionally, the production of the VEGF protein expression by ELISA (enzyme-linked immunosorbent assay) was markedly decreased, from 264.7% in the DMH group to 78.05% in the LQV group and 167.57% in the ABV group, to strengthen our confirmation of these declines in IHC by VEGF. Similarly,[13]

investigated the peptide Smp24, which is found in the venom of *Scorpio maurus palmatus*, in mice that have Ehrlich carcinoma (SEC). The findings showed that the Smp24 peptide showed a decrease in VEGF level. The data shown here also aligns with the findings of[37] in mice with murine hepatoma tumors which had antiproliferative and antiangiogenic effects after using the polypeptide PESV extracted from *Buthus martensii Karsch's* (BmK) venom.

Many different peptides, proteins, mucoproteins, phospholipase A2 enzyme (PLA2), enzymes like metalloproteinase, hyaluronidase, and serine proteases, as well as nucleotides, salt, and biogenic amines, are found in scorpion venom[38]. PLA2 exhibits a broad spectrum of pharmacological characteristics with anti-tumor and anti-cancer activities. Three major proangiogenic factors are reduced in expression levels by PLA2: vascular endothelial growth factor receptor-1 and -2 (VEGFR-1 and VEGFR-2), vascular endothelial growth factor-A, -C, and -D (VEGF-A, VEGF-C, VEGF-D) as well as endoglin expression (CD105). Moreover, it has an anti-angiogenesis effect on human pulmonary artery endothelial cells (HPAECs), human umbilical vein endothelial cells (HUVECs), and the chorioallantoic membrane (CAM) of chick embryos both in vivo and in vitro[38,39]. Our recent findings by GC-MS (unpublished data; supplementary files **Fig. 1, Fig. 2, Table 1, and Table 2**) show the presence of 1,4-benzenedicarboxylic acid and bis(2-ethylhexyl) ester, a PLA2 inhibitor in the composition of the scorpion venoms of *L. quinquestratus* and *A. bicolor*. Besides being PLA2 inhibitors, benzenedicarboxylic acid and bis(2-ethylhexyl) ester, compromise several biological activities in the regulation of angiogenesis as well as promoting protein phosphorylation, antioxidant rendering, and anticancer properties[40,41,42]. The present immunohistochemistry results for CD34 and VEGF as well as in the ELISA data of VEGF, could be key regulatory factors in reducing MVD, pointing to a prospective role in colon cancer therapy.

Conclusion

In conclusion, *L. quinquestratus* and *A. bicolor* venom produced a notable and focused cytotoxic and anti antiangiogenic effect against colon cancer *in vivo*. This fact was ensured by decrease the MVD average, CD34, and VEGF expressions compared to DMH treated group. This could be a prospective for a promising anti-colorectal cancer therapy with no side effects. The comparison with potential other pharmaceuticals and chemotherapy drugs deserves attention.

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الخصائص المضادة لتكوين الأوعية الدموية لسم العقرب: نهج جديد في علاج سرطان القولون

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يعد تكوين الأوعية الدموية الناجم عن سرطان القولون والمستقيم (CRC) هو المرحلة الأكثر ضررًا في نمو الورم وغزوه وانتشاره. ويعتبر سم العقرب (SV) هو مصدر طبيعي له العديد من التطبيقات العلاجية ضد الأمراض. هدفت هذة الدراسة إلى تقييم فعالية سم عقربي *ليوريوس كوينواسكتراتس* *Leiurus quinquestratus* (LQV) و *اندروكتوناس بيكولر* *Androctonus bicolor* (ABV) ضد تكوين الأوعية الدموية في سرطان القولون. تم تقسيم جرذان سبراغ داولي إلى أربع مجموعات: المجموعة ١ (Gp 1) (ن = ١٠) هي المجموعة الضابطة غير المعالجة. وتم حقن المجموعات الثانية و الثالثة و الرابعة (١٥ / لكل منهما) تحت الجلد (SC) بـ ١،٢ ثنائي ميثيل هيدرازين (DMH) (٤٠ مجم / كجم / أسبوع) لمدة ٤ أسابيع. المجموعة الثانية (Gp 2) هي المجموعة المتسرطنة غير المعالجة . وتم حقن المجموعة الثالثة و الرابعة داخل الصفاق (IP) بـ ١/٢٠ من نصف الجرعة المميتة LD_{50} من LQV أو ABV لمدة ١٩ أسبوعًا. في نهاية التجربة، ٢٤ أسبوعًا، تم فصل القولون وتحليله هستولوجيا والكيمياء المناعية للنسيج لكل من CD34 وعامل نمو بطانة الأوعية الدموية (VEGF) وتعبير بروتين VEGF بإستخدام ال (ELISA). تم قياس كثافة الأوعية الدقيقة (MVD) وتعبير VEGF. أظهرت التراكيب النسيجية للقولون حجم وكمية الأوعية الدموية الشاذة في مجموعة DMH. على العكس من ذلك، أدت علاجات LQV وABV إلى تقليل الأورام وأعداد الأوعية الدموية وتوزيعها في الغشاء المخاطي. أظهرت الكيمياء المناعية لأنسجة القولون لـ CD34 انخفاضًا كبيرًا في MVD، من ٣٧ ± ١١.٧ في مجموعة DMH إلى ١٠ ± ٣.٥ و٢٠ ± ٦.٥ في المجموعات المعالجة بـ LQV وABV، على التوالي. وكان تعبير VEGF قويًا في مجموعة DMH، وأصبح معتدل فقط بعد العلاج باستخدام LQV أو ABV. علاوة على ذلك، كشف اختبار ELISA عن انخفاض التعبير في مستويات بروتين VEGF في القولون. نستنتج من ذلك ان سم العقربين LQV و ABV لة قدرة مميزة على مقاومة تكوين الأوعية ضد سرطان القولون، والتي يمكن أن تدعم تطوير نهج علاجي جديد ضد سرطان القولون.