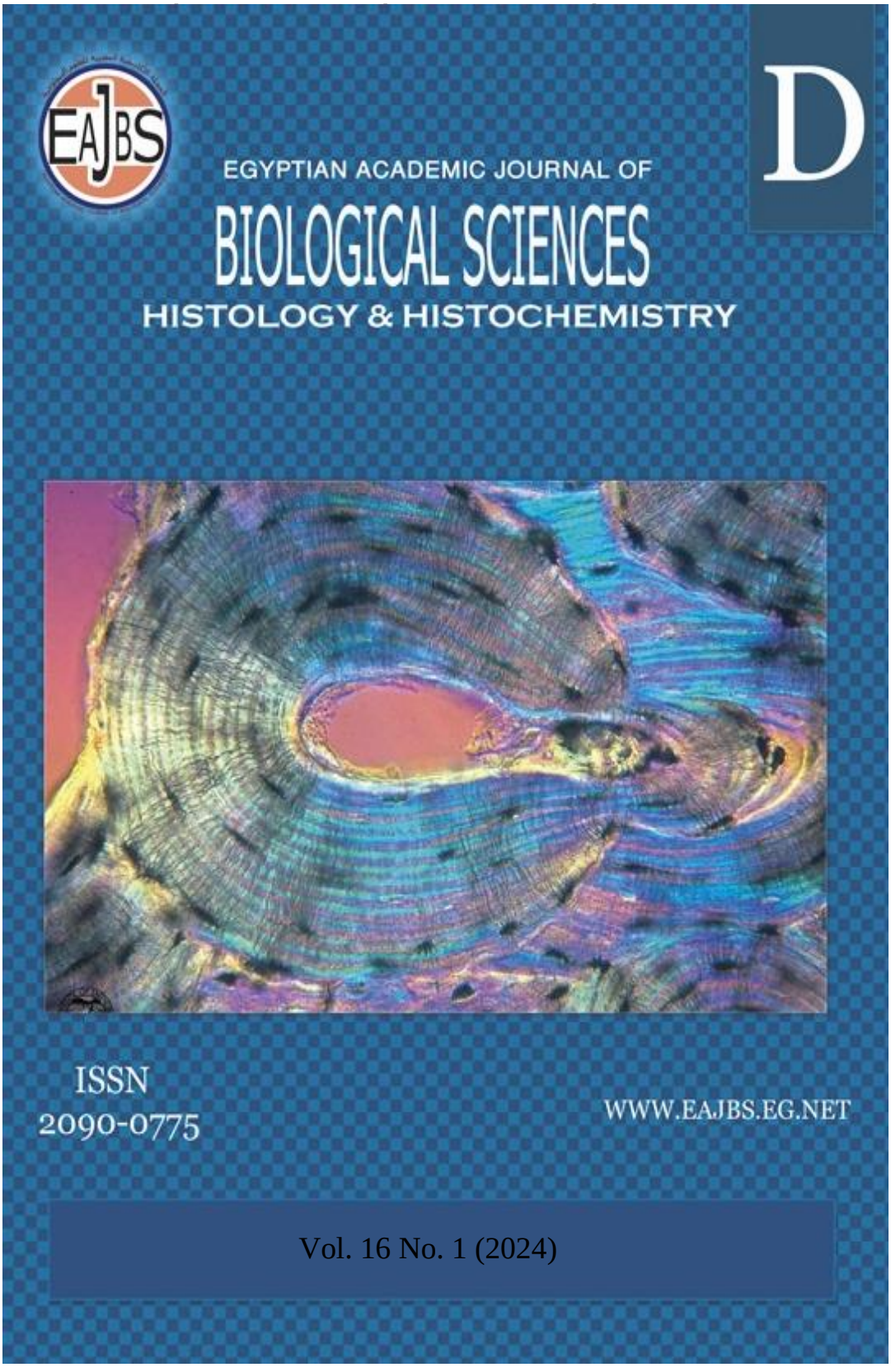

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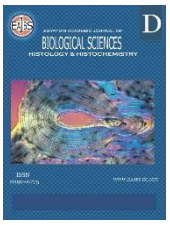

Histological Study on Venom Gland of Hatchling Stage of Egyptian Cobra, *Naja haje* (Squmata: Serpents: Elapidae)

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ABSTRACT

Several advanced snakes have a distinctive venom delivery system that includes the main venom gland, primary duct, accessory gland, secondary duct, and the fang for defense and to subdue prey. The main part involved in venom secretion is the venom gland, in addition to the accessory gland. *Naja haje* (Elapidae) was used in the current study as an additional model for venomous snakes. One of the snake's distinguishing characteristics is its ability to elevate its anterior body while flattening its neck in response to danger. In the current study, we examine the morphological and histological aspects of the venom gland to demonstrate the venom gland's development according to its histological nature at hatching. Frontally and sagittally serial sections are made to the head of the snake. The study shows the tubular shape of the venom gland. The lateral and post-orbital regions of the upper jaw are home to the venom glands. Around the whole primary duct is the accessory gland, and connected to the main venom gland. These findings contribute to our understanding of how *N. haje's* venom gland is histologically developed at hatching.

INTRODUCTION

The basic state in Iguania is an association of venom with glands in the upper and lower jaws. The venom gland is one of the most important glands among cephalic glands in snakes. It is present in all Caenophidians (advanced snakes) including the front-fanged and non-front-fanged species (Kochva and Gans 1970; Vonk *et al*. 2008). In all Caenophidian snakes, this gland is innervated by the maxillary branch (V2) of the trigeminal nerve and by blood vessels branching from the internal carotid artery (Kochva, 1965; Tauba, 1966). The whole venom apparatus includes specialized components from the main gland, accessory gland, muscles, and specialized teeth (fangs) and has a specialized mechanism in venom repulsion (Kochva, 1978; Kardong, 1979, 1980, 1982; Jackson, 2003). The main venom gland and the accessory gland are the glands that contribute to venom production (Kochva, 1978). The venom gland is one of the main components of the venom delivery system. Its structure is the focus of attention among many descriptive works (Kochva and Gans, 1965; Rosenberg, 1967; Nickerson, 1969; Gabe and Saint Girons, 1971; Halstead *et al*., 1978). We present this study as a part of the descriptive work on the venom gland. We used the cobra (*Naja haje* Linnaeus, 1758) here as an illustration example of Elapids. Elapidae is one of the three families (Viperidae, Elapidae, and Atractaspidinae) of front-fanged Caenophidians snakes (Kochva and Gans 1970; Vonk *et al*. 2008).

The Nile Valley, the western Egyptian desert, and the Mediterranean coastal desert are just a few of the diverse environments that are home to Egyptian *N. haje* (El Din, 2006). We give histological and morphological examinations on the venom gland of hatching *N. haje* (Elaidae) to show how venomous snakes have a particular venom apparatus. We provide the cobra (*Naja haje*) as an example of *Naja* genus for the description of the main gland at the hatching stage.

MATERIALS AND METHODS

For this study, the adult gravid female was obtained after copulation in early summer from the wild in Marsa Mutruh, Egypt and kept in suitable animal cages under field conditions. The fertilized eggs were carefully collected and chosen to incubate within plastic boxes filled with ventilated and moistened perlite. Embryos and newborn specimens were collected by opening eggs in Petri dishes filled with phosphate-buffered saline (PBS). The embryos separated from surrounding extra embryonic membranes.

Embryos were fixed in 4% paraformaldehyde overnight. Specimens were subsequently washed (in PBS) and dehydrated through an ascending series of ethanol. Samples were cleared in xylene (Sigma-Aldrich), infiltrated, and embedded in paraplast (Leica) for sectioning. Samples were cut frontally and sagittally. The sections were 7–9 µm thickness using Slee Cut 5062 microtome. Paraffin sections were deparaffinized through 2x xylol. Sections were hydrated through descending concentrations of ethanol up to H_2O. Some Sections were stained in hematoxylin and eosin (Sigma-Aldrich) and others in Mason's trichrome. After staining, the sections were dehydrated by ascending concentrations of ethanol and mounted in DPX (Sigma-Aldrich) and the coverslipped.

RESULTS

The venom gland has a cylindrical form, situated lateral and post-orbital on both sides of the upper jaw. There is an accessory gland located rostrally to the venom gland with a restriction in between. The whole gland is capsulated with dense connective tissue. The accessory gland surrounds the entire primary duct (Fig. 1). There is a compressor muscle attached to the main venom gland (Fig. 2). The main venom gland consists of acini and loose connective tissue between them. The peripheral cells of acini are columnar peripheral cells with oval and basal nuclei. There is a lumen in the center (Fig. 3). The accessory gland consists of tubules that are connected to each other and enter the primary duct. The entire accessory gland lacks smooth muscle fibers (Fig. 4).

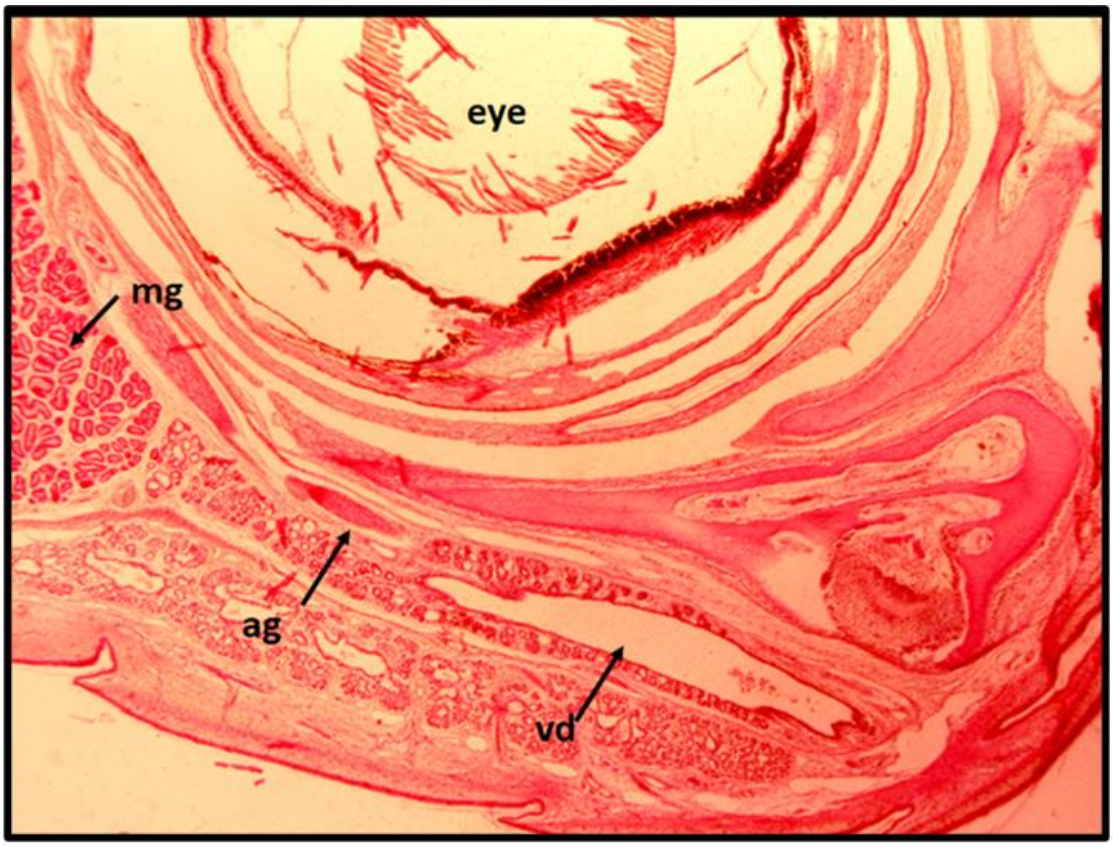

Fig.1: Sagittal section in *N. haje* at hatching stage shows the main venom gland and accessory gland. Abbreviations; mg; main glad, ag; accessory gland & vd, venom duct.

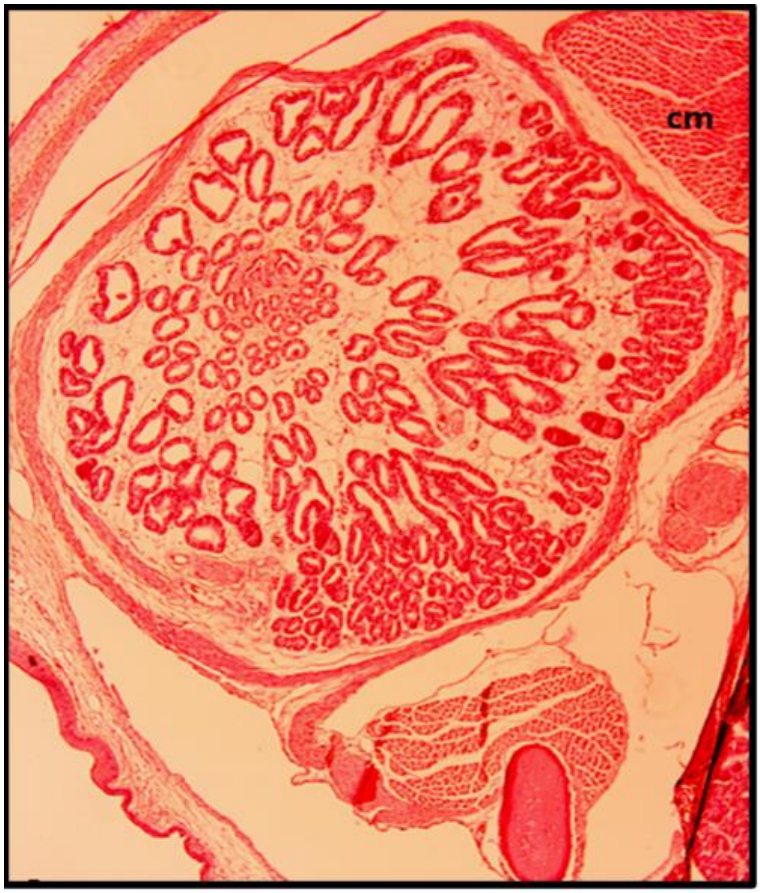

Fig. 2: Frontal section in *N. haje* at hatching stage shows the tubules of the main venom gland and the attached compressor muscle (cm).

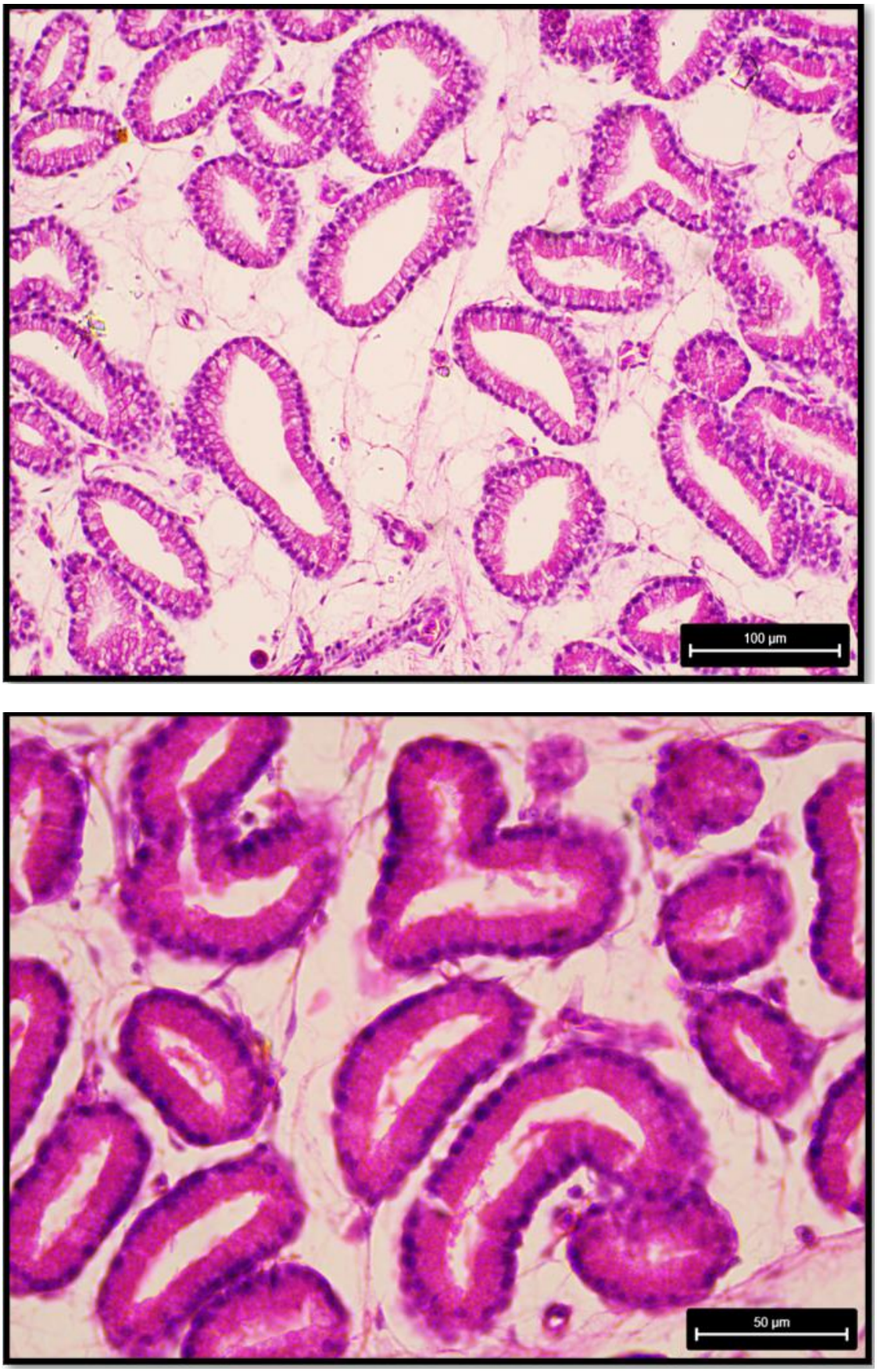

Fig. 3: Sagittal section in *N. haje* at hatching stage shows the structure of acini in the gland at power 20X (A) and 40X (B). note: the columnar cells at the peripheral and lumen in the center.

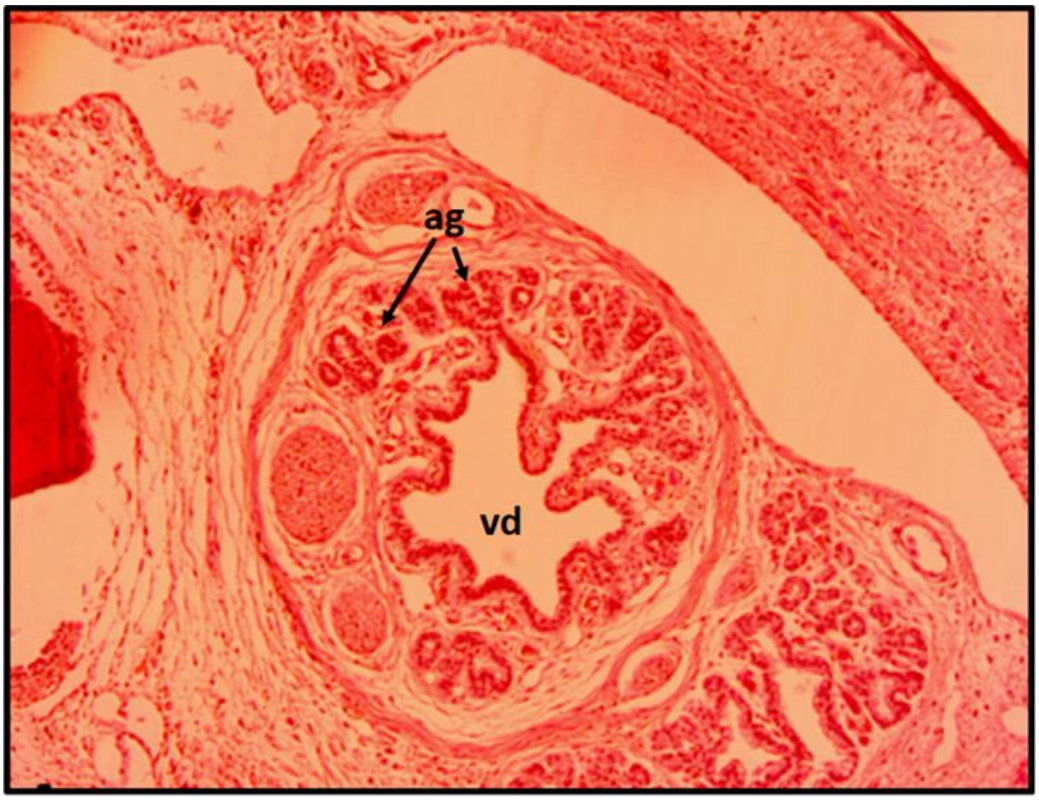

Fig. 4: Frontal section in *N. haje* at hatching stage shows the tubules of the accessory gland around venom duct. Abbreviations; ag; accessory gland & vd, venom duct.

DISCUSSION

N. haje's venom gland is situated in the upper jaw's lateral and post-orbital regions. As mentioned earlier, venom is present only in the upper jaw of snakes and is lost from the lower jaw, whereas in poisonous lizards, the venom gland disappears from the upper jaw (Fry *et al*., 2008). The venom gland is located along the upper jaw of front-fanged snakes (Kochva, 1978). The venom gland is situated laterally on the superficial aspect of the head (Taub, 1966). Among snakes, these glands are diverse in their structures and arrangements in upper and lower jaws (Kochva 1978; Wollberg *et al*., 1998; Fry *et al*., 2008).

Typically, the primary duct is absent in Elapids (Mackessy and Baxter 2006). As shown above, the accessory gland surrounds the entire primary duct. The venom gland is surrounded by a fibrous sheath for the attachment of compressor muscles. The connective tissues are capsulated the whole venom gland and accessory gland. The tubules of the main venom gland form the acinar structures (acini). Simple columnar epithelial cells with basal rounded nuclei at the periphery of the acini and a large lumen in the center were observed. This can be viewed also in other Elapids; *Naja siamensis* and *Oxyuranus microlepidotus* (Logan *et al*., 2021). The lumen of acini connected to each other to form the primary duct. The lined epithelial cells of acini extend inside the primary duct and become flat (Logan *et al*., 2021). There is homoplasy in the structure of the venom gland among the three groups; Viperidae, Elapidae, and Atractaspidinae (Jackson, 2003).The venom gland in venomous snakes is homologs to the Duvernoy's gland in most Colubrid, and the post-orbital position of both the two glands is evident (Kardong, 1982), and no snake has both glands. Taub (1967) suggested that Duvernoy's gland appeared early in colubroid and then subsequently specialized independently into venom glands in the Elapids, Viperids, and Atractaspidids. Although there are morphological and functional differences between the venom glands and Duvernoy's glands (Kochva *et al*., 1967; Kardong 2002), the venom gland of both Viperid and Elapid Snakes differ quite

anatomically from Duvernoy's gland. This gland contains secretory parenchyma defined by a capsule of connective tissue and there is a large space within the gland that acts as a reservoir to store the ready venom extracellular (Kardong 2002). The different Caenophidian snakes have homologous venom glands. They arise from a single origin at the base of Colubrid radiation 80 million years ago (Fry 2005).

Declarations:

Ethics Approval: All of the animal work was approved by the Committee on the Ethics of Animal Experiments of the Zoology Department, Faculty of Science, Fayoum University

Conflict of Interest: The authors declare no conflicts of interest.

Author contribution: The author, Hagar, has carried out all of the work under the supervision of Dr. Ahmad A. Kandeel and Prof. Dr. Naglaa R. Ismael.

Data availability statement: Data related to this research can be obtained from the author based on appropriate request.

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ARABIC SUMMARY

دراسة نسيجية على غدة السم للكوبرا المصرية فى مرحلة الفقس

هاجر ابراهيم بيومى , أحمد على قنديل , نجلاء رفعت اسماعيل
قسم علم الحيوان كلية العلوم جامعة الفيوم

فى العصور الحالية تمتلك العديد من الثعابين الحديثة جهازا متخصصا لإنتاج وإيصال السم من أجل الدفاع عن نفسها وإخضاع فرائسها. يتكون هذا الجهاز المتخصص من غدة السم الرئيسية، والقناة الأولية والغدة الملحقة والقناة الثانوية والناب. الجزء الرئيسي الذي يشارك في إفراز السم هو غدة السم بالإضافة إلى الغدة الملحقة. في هذه الدراسة الوصفية تم إستخدام *Naja haje* (Elapidae) كنموذج للثعابين السامة. إحدى الخصائص المميزة لهذا الثعبان هي قدرته على رفع جسمه الأمامي مع تسطيح رقبته إستجابة للخطر. وقد عنيت هذه الدراسة بالجوانب المورفولوجية والنسيجية لغدة السم لتوضيح تركيبها النسيجي وتطورها عند الفقس. وقد تم ذلك من خلال عمل قطاعات (سهمية وعرضية) متسلسلة لرأس الثعبان. وقد أظهرت الدراسة أن غدة السم الرئيسية تقع على جانبي الفك العلوى من الناحية البطنية لحجاج العين وهى أنبوبية الشكل. توجد الغدة الملحقة حول القناة الأولية عند الجهه الأمامية لغدة السم الرئيسية متصلة بها. وقد أسهمت هذه النتائج في فهم كيفية تطور غدة السم لدى *N. haje* تشريحيًا ونسيجيًا عند الفقس.