

MORPHO-HISTOLOGICAL AND HISTOCHEMICAL STUDY OF THE GALL BLADDER OF WHITE – EARED BULBUL (*Pycnonotus leucotis*)

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ABSTRACT

The present work aims to study the morpho-histological and histochemical structure of gall bladder in white – eared bulbul (*Pycnonotus leucotis*) in college of veterinary medicine at university of Basrah. Fifteen samples of adult healthy males were used, which divided in to three groups. Each group include (5) male used for this study. Tissue sections stained with H&E, Mallory's, PAS, Verhoeffs and Toluidine- blue. The result indicated that the gall bladder was appear to have blind ovoid- shaped with green color and situated in the middle ventral surface of the right lobe of the liver. The histological examination revealed that it consists of three layers (tunica mucosa, tunica muscularis and tunica serosa or adventitia).

INTRODUCTION

Birds are common dwellers of ecosystems and they have been considered as environmental bio-indicators of inhabited areas, however, they play an important role in the ecological balance by natural selection and biological control ^(1, 2, 3). The *Pycnonotus leucotis* is considered as one of the 137 species of the bulbul family; Pycnonotidae .It is found in the south and middle of Pakistan, Iraq, Iran and all around the Persian gulf, also in far west as Bahrain ⁽⁴⁾. In addition, insects, berries, fruits and seeds are the main foods of bulbul. This bird has a medium gray body, jet-black head with a large cheek patch. It has pale eye-ring whereas the vent is yellowish in color ⁽⁵⁾.

The digestive system is a long hollow tube or tract that has many functions such as digestion and absorption of nutrients, which are necessary for organisms, ⁽⁶⁾. The gall bladder is one of accessory digestive glands, which is a thin-walled muscular green sac present in most vertebrates it plays an important role in stores of the bile, but there are some animal do not contain gall bladder like lampreys and many birds ⁽⁷⁾.

MATERIALS AND METHODS

Fifteen adult healthy bulbul birds weighted (26-30gm), they were collected and take from a local market of Basra city and divided in to three groups, each group include (5) male to be used in this study, and they were anesthesia by using chloroform. After death, the body cavity was opened by making a longitudinal incision through the mid-ventral surface of each bird to expose- internal visceral organs. The topography and the shape of the gall bladder were studied and documented by using a measuring tape, vernier caliper and electric balance. The organ was immediately removed from the abdominal cavity and washed with normal saline to remove blood and any other adhering debris, and then fixed in natural buffered formalin (10%) for 48 hours. After that routine histological techniques preparing paraffin blocks cutting at 5- 6 μm , staining with (H&E) stain for general tissue structure, in addition to special stains (Mallory's ,PAS, Verhoeffs, Toluidine- blue) to histochemical study ^(8 , 9). All data were analyzed by using the statistical program SPSS (2016, version 24). Data expressed as mean \pm standard error, differences among mean were tested by T- test.

RESULTS

The morphological results of gall bladder showed that it is blind ovoid- shaped hollow musculo-membranous organs that forms as an outgrowth of the bile duct. This gland is situated obliquely in the middle ventral surface of the right lobe of the liver and it have green color (Fig.1). The mean weight, length and width with bile material are (0.28 ± 0.03) gm, (0.53 ± 0.09) mm, (0.96 ± 0.08) mm, respectively (Table 1).

The histological examination showed that it is composed of three layers gradually arranged as tunica mucosa, tunica muscularis and tunica serosa or adventitia. The mucosal layer was lined by non-ciliated simple columnar epithelium that have spherical or ovoid nucleus, and it covered by continuous border of microvilli, the goblet cells was present between the epithelial cells (Fig.2).

The thin lamina propria- submucosa is found under the tunica mucosa that contained loose connective tissue with collagen fibers and some lymphatic tissue. (Fig.3). Also, the results showed that the tunica muscularis is composed of thin layer of smooth muscles that arranged circularly. While, the outer most tunica in free surface of gall bladder was serosa that invested by mesothelium, whereas in attached surface was adventitia, this layer is made up of loose connective tissue contains that many of adipose tissue and blood vessels and the glands were absent (Fig.4). The mean of thickness of tunica mucosa, tunica muscularis and the tunica serosa is (33.25± 3.83), (39.9±5.42) and (19.95±3.83) μm, respectively (Table 2).

The histochemical results showed that the apical part of epithelium give positive reaction with PAS stain due to the presence of muco-polysaccharides and carbohydrates material (Fig. 5). With T- blue stain the blue acidophilic muco-substances are present in all surface epithelial cell (Fig. 6) .While the results of Mallory and verhoeff stain appeared that the blue collagenous and brown elastic fibers are found in lamina propria - submucosa and tunica serosa in both birds(Fig. 7, 8).

Table 1: Gross morphometric measurements of weight, length and width of gall bladder (μm), by (Mean ± SE).

Species	Weight	Length	Width
white – eared bulbul	0.28 ± 0.03	0.53±0.09	0.96±0.08

Table 2: Histological measurements of the thickness of gall bladder (μm), by (Mean ± SE).

Gall bladder	(Mean± SE)
Mucosa	33.25 ± 3.83
Muscularis	39.9 ± 5.42
serosa	19.95 ± 3.83

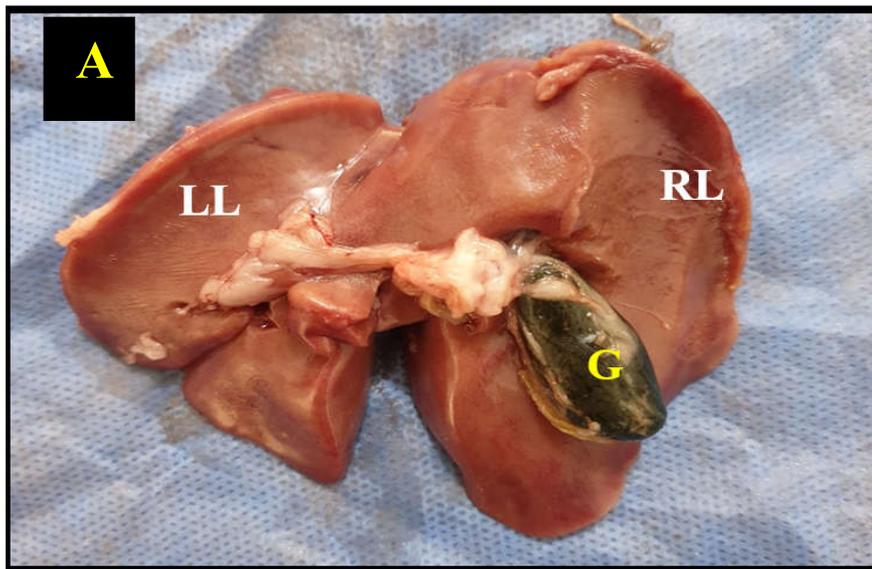


Fig. 1: Cross section of the ventral surface of the liver showing the position of gall bladder.

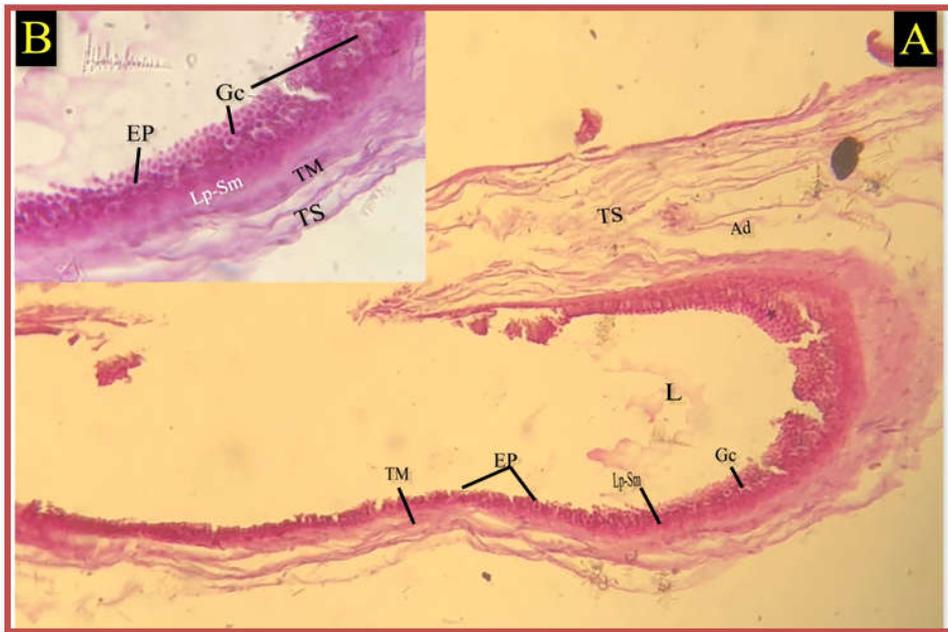


Fig. 2: Cross section from gall bladder in bulbul showing: L. Lumen, Ep. Epithelium, Lp-Sm. Lamina propria-submucosa, Tm. Tunica muscularis, Ts. Tunica serosa, Gc. Goblet cell, Ad. Adipose tissue. H&E stain (A, X100), H&E stain (B, X400).

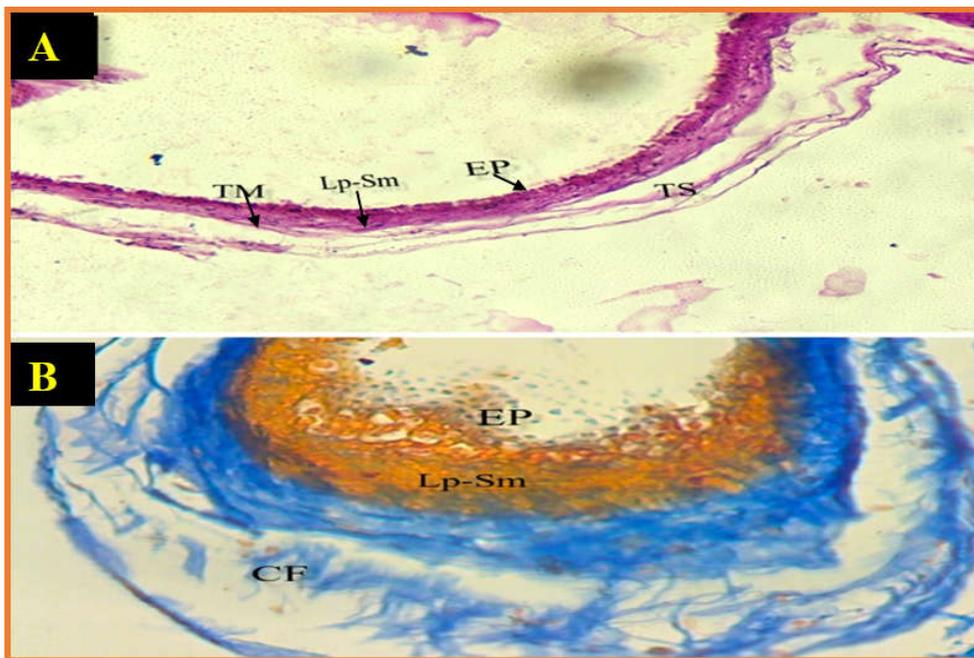


Fig. 3: Cross section from gall bladder showing: EP. Epithelium, Lp-Sm. lamina propria-submucosa, Tm. Tunica muscularis, TS. Tunica serosa, CF. collagen fiber. H&E stain (A, X40), Mallory's stain (B, X400).

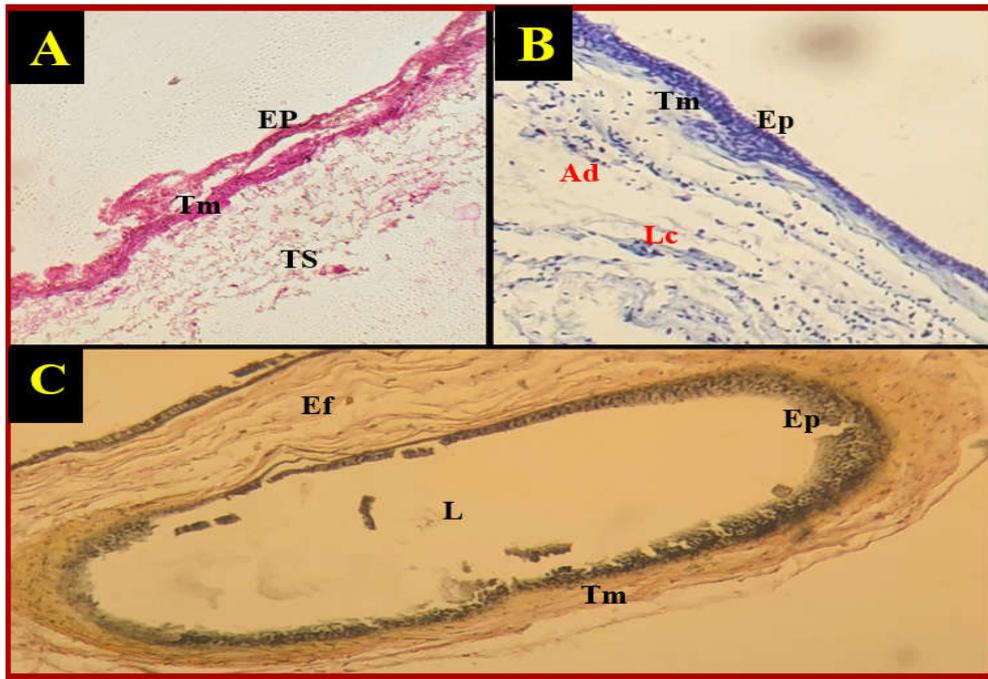


Fig. 4: Cross section from gall bladder in bulbul showing: L. Lumen, EP. Epithelium, Tm. Tunica muscularis, TS. Tunica serosa, Ad. Adipose tissue, Ef. Elastic fibers. H&E stain (A, X100), T-Blue stain (B, X100), Verhoeff's stain (X100).

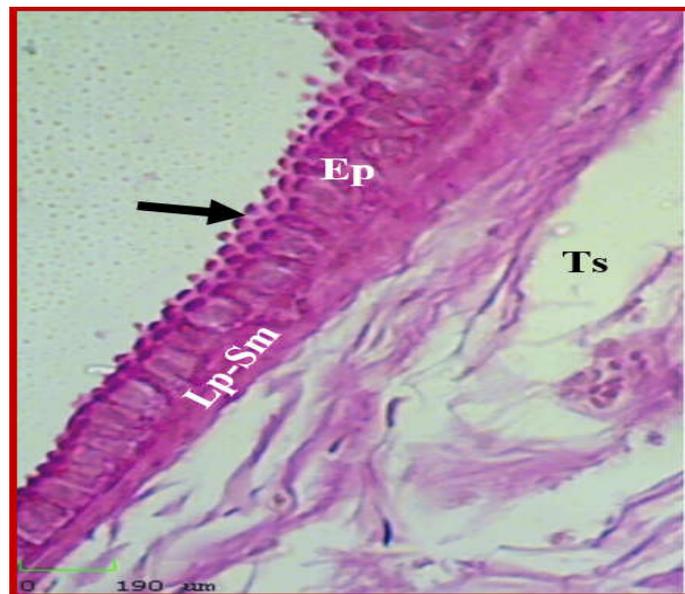


Fig. 5: Cross section from gall bladder showing: PAS-positive materials found in the apical part of the cytoplasm of the epithelial cells (Ep) (arrows), EP. Epithelium, TS. Tunica serosa, Lp-Sm. lamina propria-submucosa. PAS stain (X400).

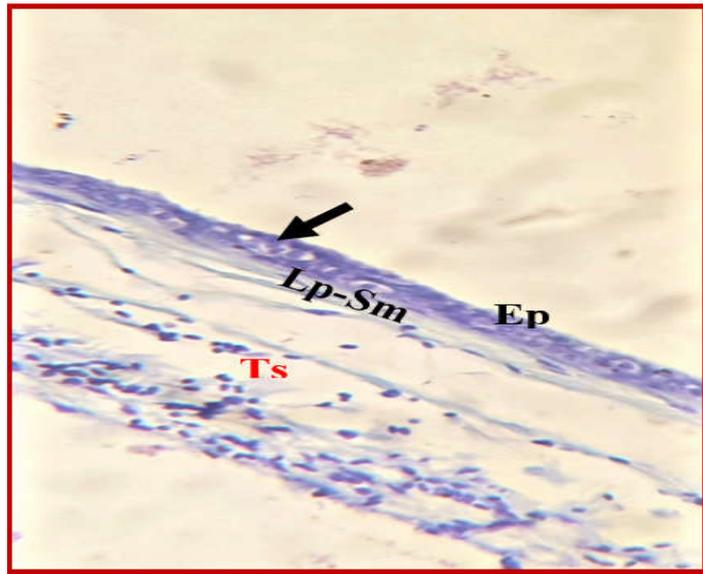


Fig. 6: Cross section from gall bladder in (A) bulbul, (B) starling bird showing: Acidophilic muco-substances found in all epithelial cells surface (Ep) (arrows), EP. Epithelium, TS. Tunica serosa, Lp-Sm. lamina propria-submucosa. T-Blue stain (X400).

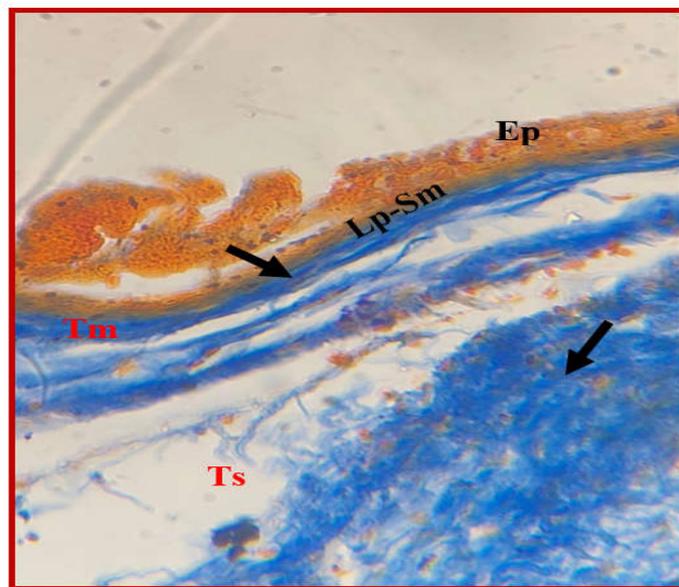


Fig. 7: Cross section from gall bladder showing: Collagenous fibers (arrows) in lamina propria-submucosa and tunica serosa, EP. Epithelium, TS. Tunica serosa, Lp-Sm. lamina propria-submucosa, TM. Tunica muscularis. Mallory's stain (X400).

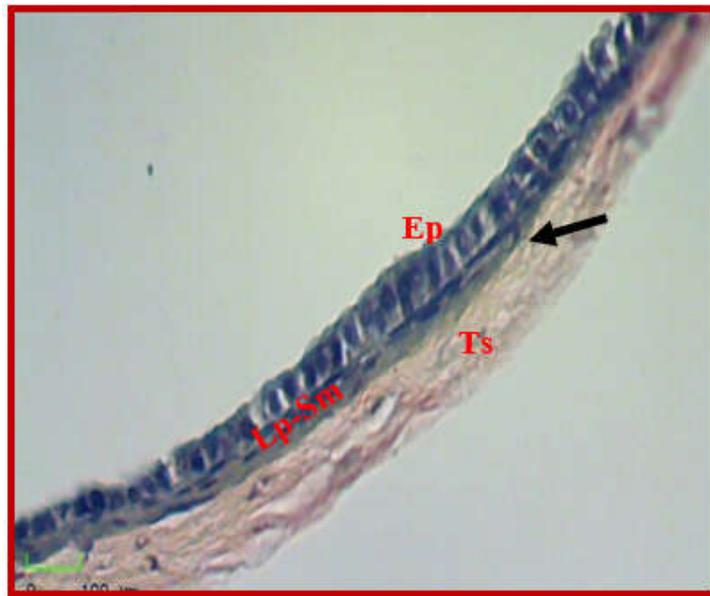


Fig. 8: Cross section from gall bladder showing: Elastic fibers (arrows) in lamina propria-submucosa and tunica serosa, EP. Epithelium, TS. Tunica serosa, Lp-Sm. lamina propria-submucosa. Verhoeff's stain (X400).

DISCUSSION

The morphological results revealed that the gallbladder is blind ovoid- shaped musculo-membranous organs and it is situated in the middle ventral surface of the liver, this result is consistent with ⁽¹⁰⁾ who explained that the avian gall bladder is sac like a structure located on the visceral surface of the right lobe, and with ⁽¹¹⁾ that showed the gall bladder in geese (*Anser anser*) appear as hollow musculo- membranous sac for bile collection and concentration and it does not extend down to the ventral hepatic border , whereas it is disagreed with ^(12, 13) that said the gland was elongated and pear in shape in bustard and *Passer domesticus*. Also, it disagreed with ⁽¹⁴⁾ who mentioned that the gall bladder in *Larus canus* and *Numida meleagris* looks like a grain of pumpkins, whereas it lack in *Agaporins fischeri*.

On the other side, our results of gall bladder showed that it has green color ,this is on the line with ^(15, 16) that mentioned the gall bladder in local broiler fowl, common moorhen and indigenous gazelle appear with dark green color, while it is different from the results that performed by ⁽¹⁷⁾ in *Capra hircus* in that the gland was observed as yellowish-white color,

which stayed filled with green colored bile juice. In addition, the results of the mean of weight, length and width of gall bladder was consistent with ^(18, 19, 20).

Our histological study showed that the wall of this gland is composed of tunica mucosa, tunica muscularis and tunica serosa or adventitia, which similar to those of guinea fowl ⁽²⁰⁾. The mucosal layer was lined by non-ciliated simple columnar epithelium, this result agreed with ⁽¹³⁾ who report a similar result in house sparrow, and this finding agreed with many studies such as ⁽²¹⁾ in chukar partridge, ⁽²²⁾ in Iranian chukar partridge ⁽²³⁾ in Japanese quail, and with ⁽²⁴⁾ in common quail.

The results appeared that the epithelial layer is covered by border of microvilli, however this was deal with ⁽²³⁾ when resulted that the epithelium layer in Japanese quails that varied from stratified columnar to tall columnar and enclosed by a continuous striated border of microvilli. Our results explained that the lining epithelial is not formed as folded and contain goblet cells , this is similar to the previous findings of ^(15, 24, 25), but un like with ⁽¹⁶⁾ who found that the epithelium of the tunica mucosa does not contain goblet cells in local broiler fowl and common moorhen.

The lamina propria-submucosa consists of loose connective tissue with collagen fibers and some lymphatic tissue, which is in agreement with the finding of ⁽²⁵⁾ in chicken. On other hand, the result of the tunica muscularis showed that it is compose of thin layer of smooth muscles, this result disagreed with ⁽²⁰⁾ who reported that the tunica muscularis in guinea fowl is composed of an outer longitudinal layer and an inner layer that consists of outer circularly arranged muscle fibers, but it is similar with ⁽¹¹⁾ and ⁽¹⁶⁾. The tunica serosa showed that it consists of loose connective tissue contains adipose tissue; these results are similar to that confirmed by ⁽²⁶⁾, the results of the mean of the thickness of layer this finding agreed with ^(10, 16).

By using histochemical stains, the results appeared that PAS-positive material is in the apical part of the cytoplasm of the epithelial cells, collagenous and elastic fibers are found in the lamina propria-submucosa and tunica serosa, these results are similar to ^(16, 21, 27) that explained the apical surface of the columnar cells became positive with PAS staining in chukar partridge , local broiler fowl and common moorhen. However, the acidophilic muco-substances are present in all surface epithelial cells can be showed with T- blue stain , this was similar to that of other poultry ^(28, 29, 30) which indicated that the γ muco-substances that is

composed of acid and neutral glycols- aminoglycan complexes, but some investigators have reported that the presence of mucous secretion might important in relation to the water absorbing function of the gall bladder epithelium (the mucus might form a water-absorbing surface gel) for the concentration of the bile ⁽³¹⁾.

دراسة نسجية-شكلية وكيمونسجية للمرارة في البلب ابيض الاذنين (*Pycnonotus leucotis*)

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الخلاصة

الهدف من هذه الدراسة هو لمعرفة التركيب النسجي - الشكلي والكيمونسجي للمرارة في البلب ابيض الاذنين (*Pycnonotus leucotis*) في كلية الطب البيطري في جامعة البصرة. بمعدل (١٥) عينة من الذكور الصحية البالغة تم استخدامها، والتي قسمت الى ثلاث مجموعات كل مجموعة تشمل (٥) ذكور. المقاطع النسجية تم تصيغها بواسطة صبغات الهيماتوكسلين والايوسين والملوري والبيروديك والتولادين -بلو. اشارت النتيجة الى ان المرارة تبدو ذات شكل بيضوي مغلق بلون اخضر وتقع في منتصف السطح البطني للفص الايمن للكبد. بين الفحص النسيجي انها تتكون من ثلاث طبقات من الغلالة المخاطية والغلالة العضلية والغلالة المصلية او البرانية.

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