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Histological Structure of the Cerebellum of the Iraqi Pin-tailed Sandgrouse Bird *Pterocles alchata* (Linnaeus, 1766)

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Received: 6 April 2024 **Accepted: 30 May 2024**

Published: July 2025

DOI: https://dx.doi.org/10.24237/ASJ.03.03.883B

Abstract

A histological study was performed on the cerebellum of the Iraqi pin-tailed sandgrouse bird *Pterocles alchata* (Linaeus, 1766). An abundant sandgrouse species is found in Iraq. The Bird resides in arid plains and partially in rocky deserts near areas where grains are grown. The objective of this study was to thoroughly analyze the histological and morphological features of the Cerebellum in adult *Pterocles alchata* birds. To advance our understanding of the Cerebellum in this specific species, eight healthy bird specimens were obtained from the local market in Baghdad, Iraq. The specimens weighed approximately(223-174g)The study revealed that the cerebellum weighed about (0.33) g and took the parts of the Rhombencephalon, positioned posterior to the cerebrum, comprises folded structures known as cerebellar folia. Specifically, nine folia were identified (I-IX). The folia observed in this study had a histological structure composed of two distinct layers: - the Cerebellar cortex that is called Gray matter and the Medulla region, which is called white matter. The cerebellar cortex is divided into three layers, arranged from the outermost to the innermost layer: - the Molecular layer, the Purkinje cells layer, and the Granular cells layer.

Keywords: Brain, Bird, Cerebellar, Cortex.

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Introduction

The most common type of sandgrouse inhabiting Iraq is the Iraqi pin-tailed sandgrouse bird (*Pterocles alchata*). It inhabits dry plains and semi-desert areas with rocky terrain, often near the regions where grain is cultivated. This bird has a compact body and a small beak. Its feathers are colored to blend in with its natural environment. The Iraqi pin-tailed sandgrouse belongs to the order Pterocliformes and the family Pteroclidae [1]. The brain is a highly complicated organ composed of various regions exhibiting variations across living organisms. In vertebrates, the brain primarily comprises three main regions: The Forebrain (Procencephalon), the Midbrain (Mesencephalon), and the Hindbrain (Rhombencephalon). Additionally, the brain is enveloped by three layers known as meninges, which are further protected by the skull [2-5]. The cerebellum is a component of the Rhombencephalon, specifically its dorsal region. It comprises three lobes: the anterior lobe, the posterior lobe, and the flocculonodular lobe. Each lobe is made up of structures known as folia. The vertebrate cerebellum coordinates muscle movements and receives sensory input from the eyes, ears, joints, and muscles to determine the body's current position [6, 7]. The histological composition of the cerebellum in birds closely resembles that observed in mammals [8]. The Cerebellum comprises two main parts: the outer layer, known as the Grey matter, which contains the cerebellar cortex, and the inner part, the White matter, which represents the Medulla [9]. Several studies have focused on the characterization of specific regions of the brain in various vertebrates [4, 5, 9-23].

Material and Methods

The study employed eight physically fit adult males, whose weights ranged from 174 to 223 g and whose ages ranged from 11 to 12 months. The birds were collected from the nearby market in Baghdad, Iraq. After the birds were cut, they were anesthetized with chloroform and then dissected. The cranial feathers were extracted, and a surgical cut was performed at both the back and front areas of the skull. Afterward, the cranial bones were removed to extract the brain. After acquiring the brain, measurements were conducted utilizing a phone camera (Galaxy A8), a ruler, and a precise balance. Subsequently, the Cerebellum was separated [24].

The specimens were submerged in a 10% formalin solution for a duration of 72 hours, after which they were thoroughly washed with a running water. After that, the samples underwent a series of alcoholic solutions to eliminate water and achieve clarity using xylene. Subsequently, the samples were submerged in a mixture of xylene and paraffin wax with a melting point of 58 °C. Ultimately, the specimens were immersed in paraffin wax to generate molds and subsequently sliced using a rotary microtome with a 5-micrometer thickness. The sections are subjected to staining using the Haematoxylin-Eosin, Methylene blue, and Mallory Stain techniques [25-28].

Afterward, the histological sections were obtained using a MEIJI compound light microscope with a Canon camera at 4X, 10X, 40X, and 100X magnifications.

Results

The sandgrouse bird's brain was discovered to weigh approximately (1.57) g and measure around (1.94) cm in length (Figure 1 and Figure 2). The cerebellum, on the other hand, weighed about (0.33) g, the cerebellum, positioned posterior to the cerebrum. The dorsal surface of the cerebellum exhibited sulci, which divided its surface, and the cerebellum itself had a foliated structure. The histological analysis of the cerebellum in the *Pterocles alchata* bird revealed that it is enveloped by a layer of connective tissue known as the Pia mater. The cerebellum comprises numerous folds and sulci, forming structures called cerebellar folia (Figure 1). There are a total of 9 folia labeled as (I-IX). The anterior lobe of the cerebellum corresponds to folia (I-V), while the posterior lobe corresponds to folia (VI-IX). Additionally, the cerebellum can be histologically divided into two main regions: the Cerebellar cortex and the Medulla. The Cerebellar cortex is the Gray matter region enveloped by a layer of connective tissue known as the Pia mater. The grey matter comprises three layers, namely the molecular layer, Purkinje cells layer, and granular cells layer, as depicted in Figure 3 and Figure 4.

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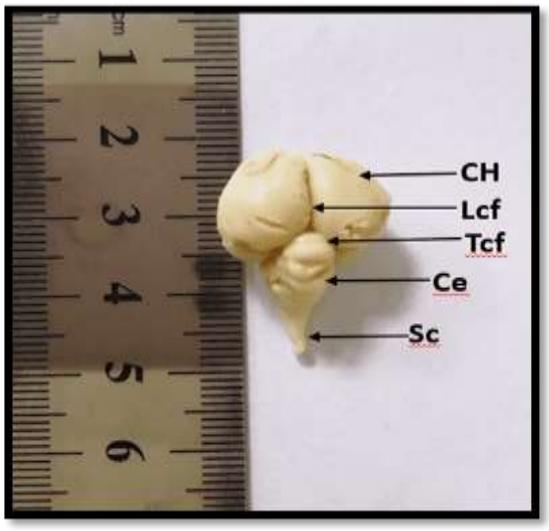

Figure 1: Dorsal view of the brain of the Iraqi pin-tailed sandgrouse *Ptrocles alchata* bird showing the Cerebral hemispherical (CH), Cerebellum(Ce), longitudinal cerebral fissure (LCF), Transverse Cerebral Fissure (TCF), spinal cord (SC).

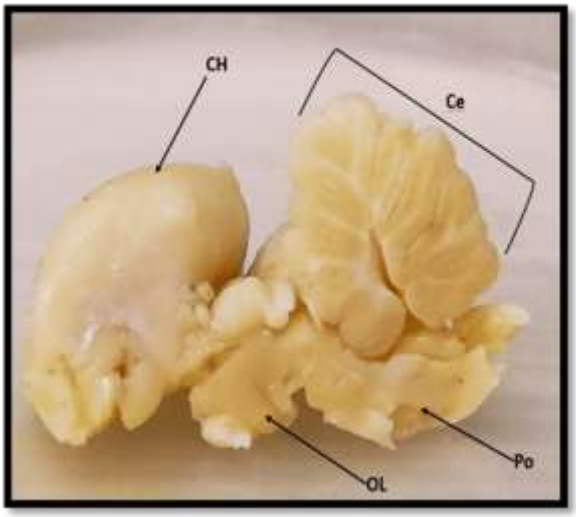

Figure 2: Sagittal segment through the brain of the *Pterocles alchata* showing the cerebral hemisphere (CH), cerebellum (Ce), optic lobe (OL), and Pons (Po).

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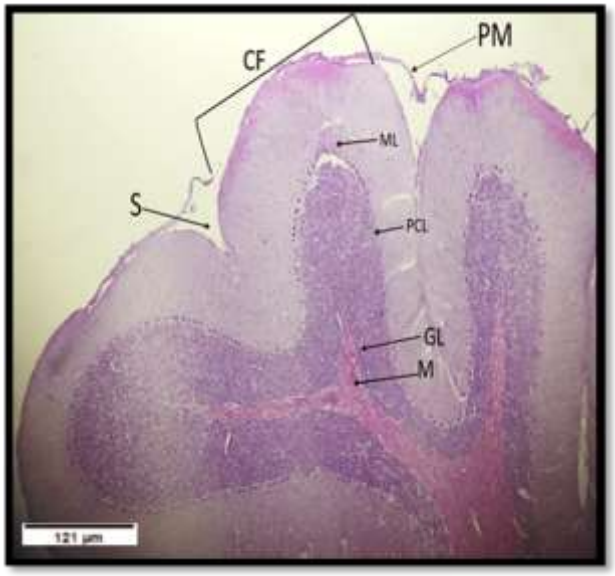

Figure 3: Transverse section through the cerebellar of the *Pterocles alchata* showing cerebellar folia (CF), Granular Layer(GL), pia mater (P), sulci (S), molecular layer (ML), Purkinje cells layer (PCL), medulla region (M), H&E stain (4x).

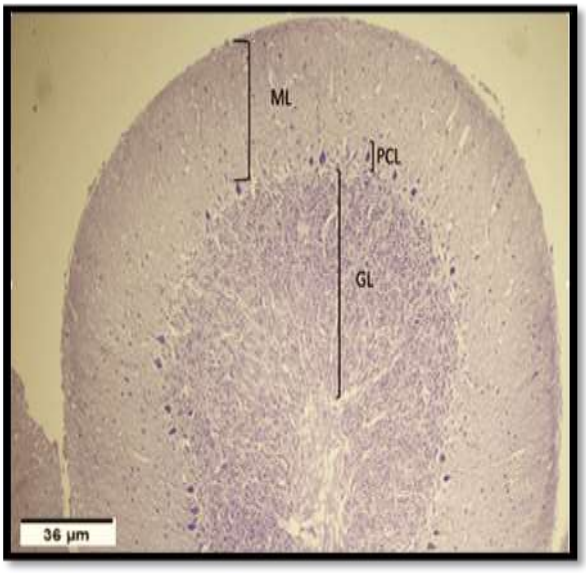

Figure 4: Transverse section through the cerebellar of the *Pterocles alchata* showing the three layers of the cerebellar cortex, Granular layer (GL), molecular layer (ML), Purkinje cells layer (PCL), H&E stain (10x).

The Molecular layer represents the first layer, and the pia mater layer surrounds it. The thickness of this layer was measured to be (59.97) µm at the top of the cerebellar folia. In the sulci area, it was thicker, with an average thickness of (63.03) µm. Fusiform neurons were observed on the superficial side, while pyramidal neurons exhibited dendrites that extended toward Purkinje cells. Additionally, the axons of neurons interacted with the branches of Purkinje cells. The middle region contains stellate cells and small pyramidal cells called basket cells.

Observation revealed the existence of climbing nerve fibers, considered extensions of nerve cells, alongside the dendrites of Purkinje cells (Figure 5).

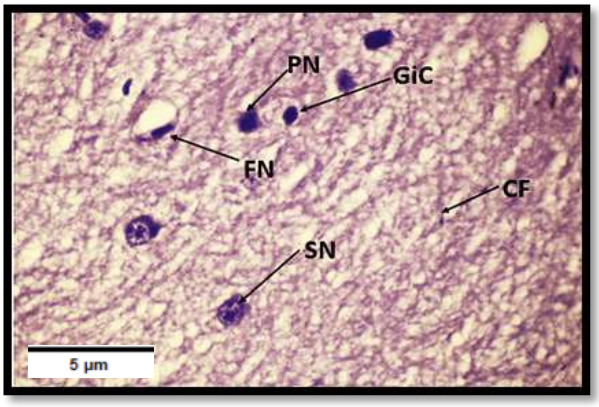

Figure 5: Transverse section in the molecular layer of the *Pterocles alchata* cerebellar cortex showing Climbing nerve Fibers (CF), Fusiform Neurons (FN), Gilal Cells (GiC), pyramidal neurons (PN), stellate neurons (SN), H&E stain. Molecular layer (100x).

The Purkinje cells layer is a narrow layer between the molecular and granular layers and is primarily composed of Purkinje neurons (Figure 6). The study revealed that the mean thickness at the highest point of the folia was 7.04 µm, while at the sulci, the mean thickness was 4.65 µm. Purkinje cells exhibit a distinctive morphology, with a prominent spherical shape and a central nucleus. They are further distinguished by the presence of Nissl granules (Figure 7B). The axon extends to the molecular layer, surrounded by satellite cells known as Golgi cells II, located on the side of the Purkinje cells (Figure 7).

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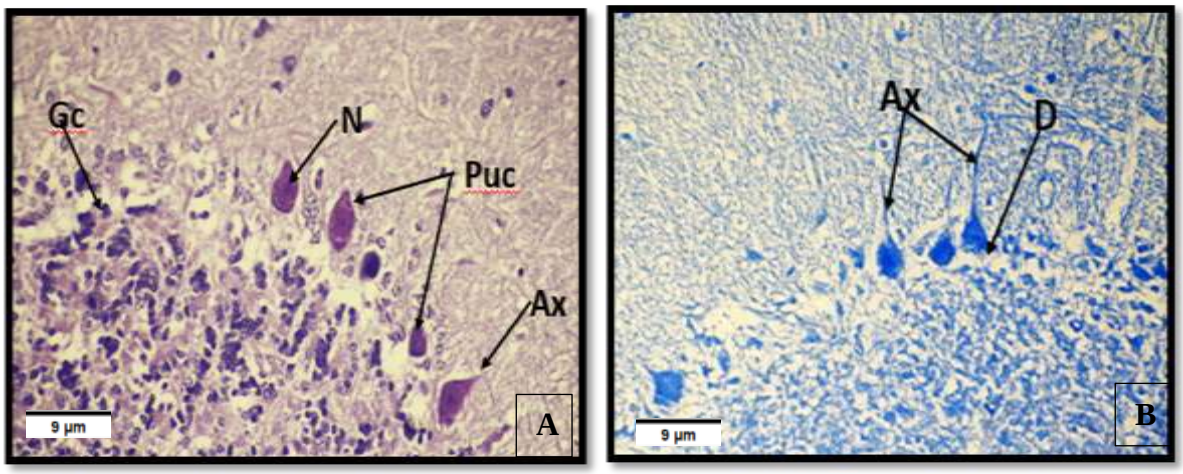


Figure 6: Transverse section passing through the cerebellum of the *Pterocles alchata* showing the Purkinje cells layer (PuC), Axon (A), dendrite (D), Granular Cells (Gc), Nuclei (N), H&E stain, A (40X), Methyelen blue stain B (40x).

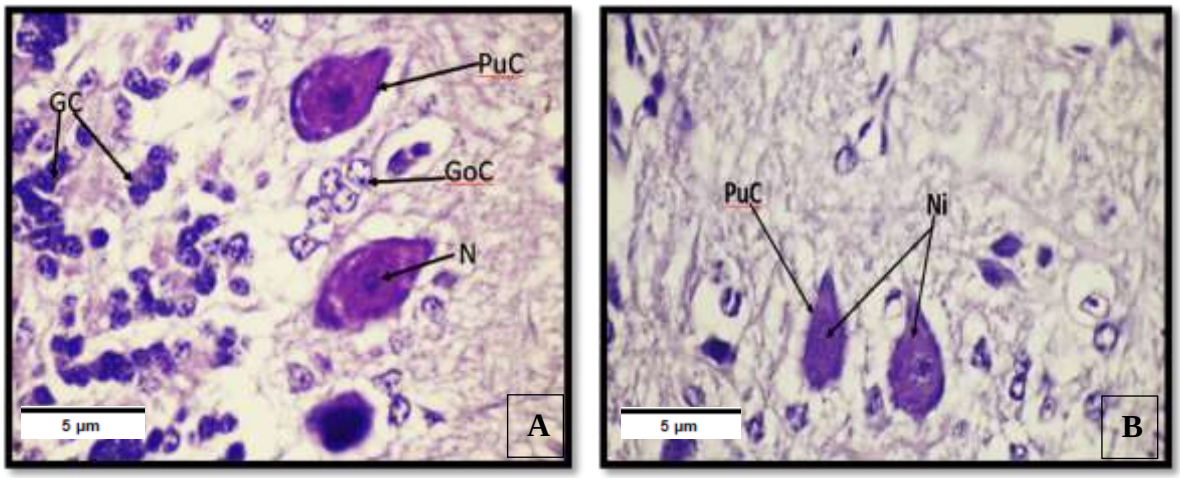


Figure 7: Transverse section through the cerebellum of the *Pterocles alchata* showing: Granular Cell (GC), Golgi Cell II (GoC), Nissls granules (Ni), Nucleus (N), Purkinje cells (PuC), H&E stain, A and B (100x).

The Granule layer: The third layer of the cerebellum, known as the inner layer, is situated between the layer of Purkinje cells and the white matter or medulla. The thickness at the top of the folia is (101.85) µm, while the average thickness at the sulci is (70.58) µm. This condition is distinguished by the abundant presence of granular cells, as shown in Figures 8 and 9. These

cells are found in the mossy fibers, which extend from the medulla region and communicate with granular cells through structures called glomeruli.

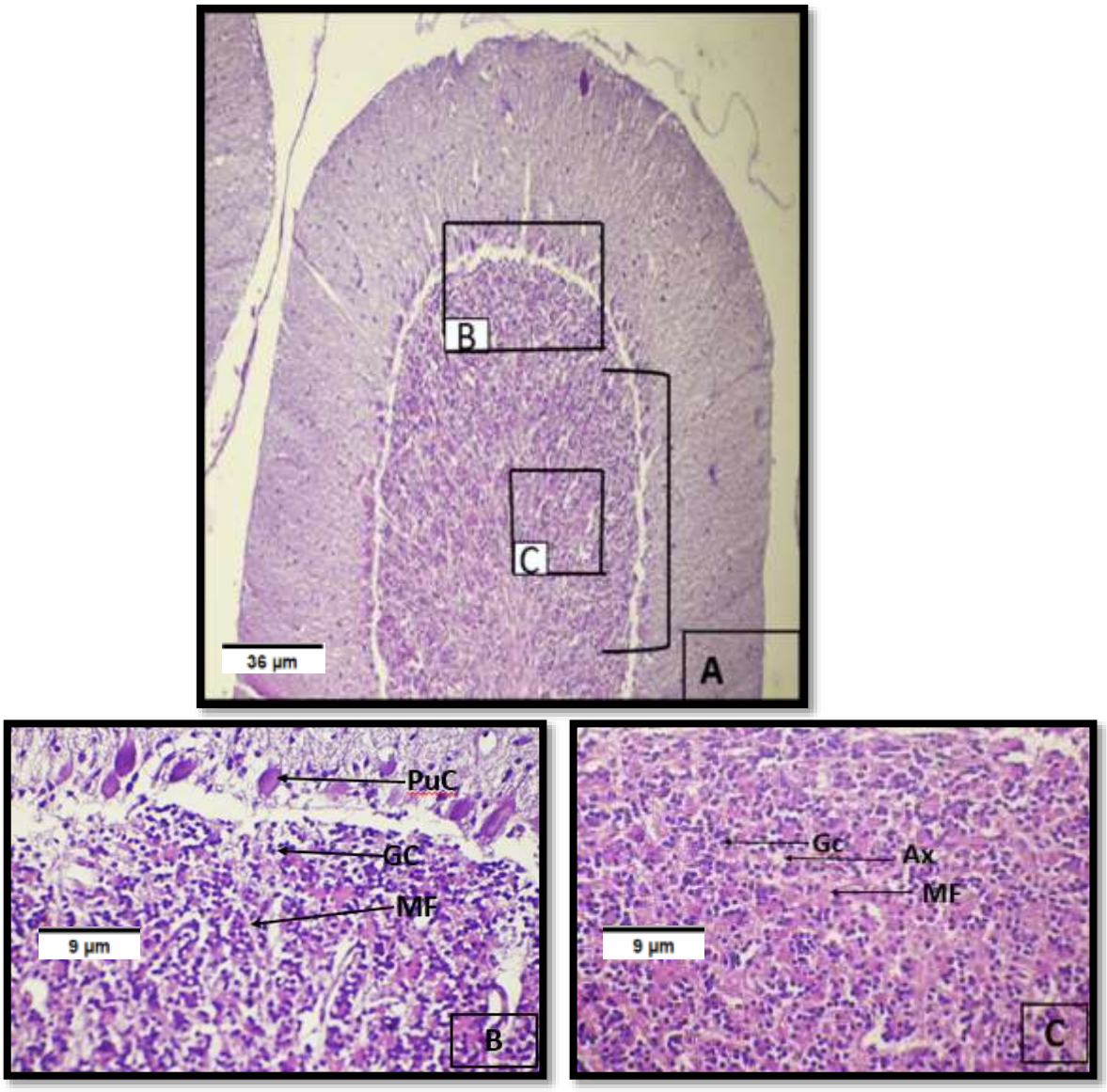


Figure 8: Transverse section through the cerebellum of the *Pterocles alchata* showing the Axon (Ax), Granular layer (GL), Granular Cells (GC), Mossy Fibers (MF), Purkinje cells (PuC), H&E stain A. Cerebellum (10x), B. and C. Granular layer (40x).

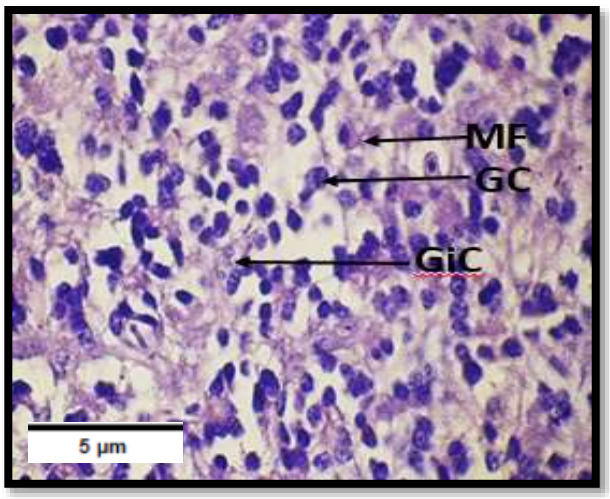
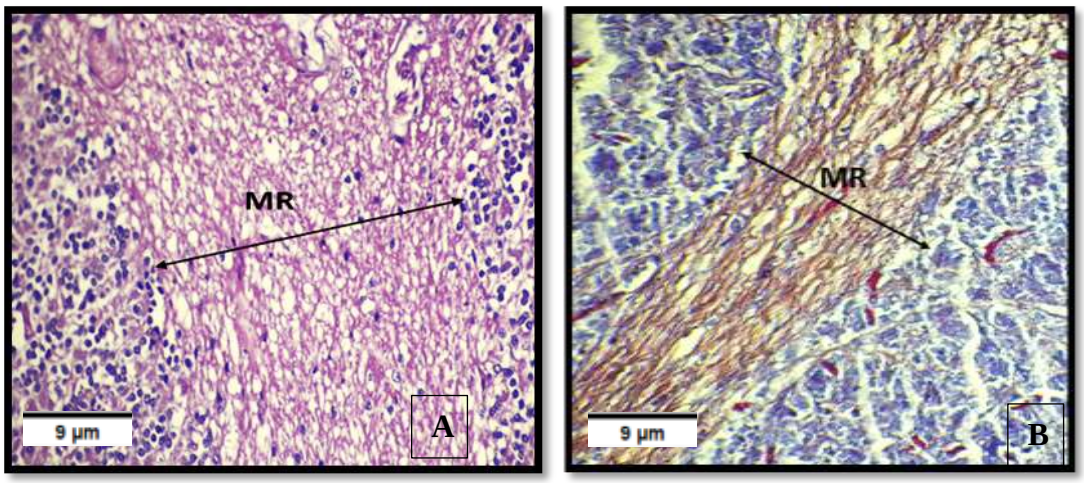


Figure 9: Transverse section of the cerebellum of the *Pterocles alchata* through the Granular layer. Showing Granular cells (Gc), Gilal Cells (GiC), Mossy Fibers (MF), H&E stain (100x).

The Medulla region: Also known as white matter, it reached at the folia about 59.97µm while it was larger at the sulci at 63.03µm .The medulla of the cerebellar folia is composed of axons of neurons. This layer also contains two types of glial cells, including astrocytes and oligodendrocytes, with branches. (Figure 10 A, B ,C) Furthermore, it has been noted that clusters of neurons known as deep cerebellar nuclei exist (Figure 11).



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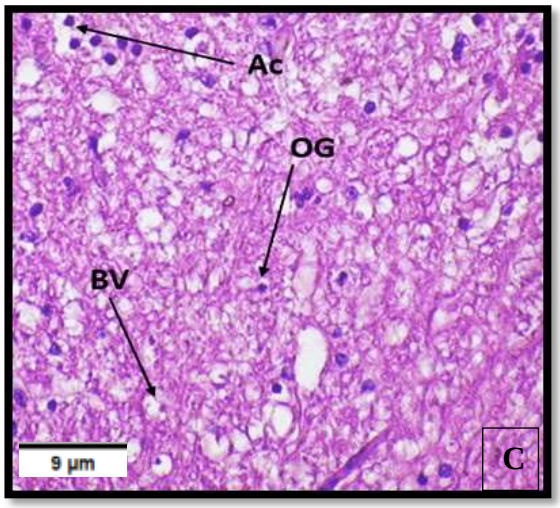

Figure 10: Transverse through the medulla region of the cerebellum of the *Pterocles alchata* showing the Astrocytes (Ac), Blood Vessels (BV), Medulla Region (MR), Oligodendrocytes (OG), H&E stain, and Mallory stain, Aand B Cerebellum (40x), C Medulla Region (40x).

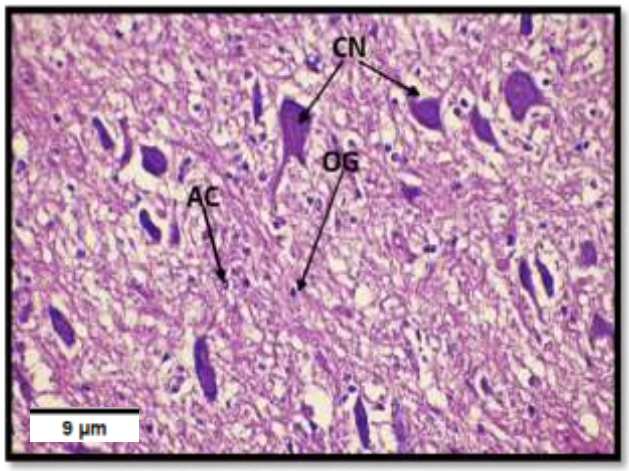

Figure 11: Transverse section in the cerebellum of the *Pterocles alchata* showing the deep cerebellar nuclei in the medulla region Cerebellar Nuclei (CN), Astrocyte (AC), Oligodendroglia (OG), H&E stain (40x).

Discussion

The present study revealed that the cerebellum's surface is composed of sulci. The cerebellum has a foliated structure resembling a series of leaves. It comprises nine folia (I-IX), with the

anterior lobe containing folia I-V and the posterior lobe containing folia IV-IX. The primary fissure separates these two lobes, and the secondary fissure separates the eighth folia from the ninth folia. These results align with [21, 29].

The histological structure of the cerebellum in birds is comparable to that of other vertebrates. The cerebellum comprises the cerebellar cortex, organized into folded structures known as cerebellar folia [30]. Furthermore, it has been discovered in the *Pterocles alchata* cerebellum that the cerebellar cortex is divided into three layers: The Molecular layer, The Purkinje cells layer, and The Granular layer. This finding aligns with the research conducted by [21, 22, and 23]. The thickness of the Molecular layer in *Petrocles alchata*, which corresponds to white matter, measured at the highest point of the cerebellar folia was (59.97) µm. In contrast, the average thickness of the sulci region, which is a larger area, was (63.03) µm. Furthermore, basket cells are neurons with astrocyte-like shapes that extend their nerve fibers to the Purkinje cell layer. These results do not match with Abid and Abid. [19], Abid and Al-Bakri [21], and Abid [23]. The present study demonstrated that the intermediate layer of the cerebellar cortex is comprised of Purkinje cells, with an average thickness of (7.04) µm at the Folia while it reached (4.65) µm at the Sulci. These cells are distinguished by their substantial size and contain both the nucleus and Nissl granules. Paulsen [31] states that the axons of Purkinje cells serve as efferent nerve fibers that extend to the granular cell layer, where they form synapses in the medulla region. The granular layer of the cerebellar cortex is named as such due to the high concentration of granular cells prominently stained with hematoxylin-eosin, and this aligns with Abid and Abid [19]. In this study, The granular layer has reached at the Foli (101.85) µm while it reached at the sulci (70.58) µm and this doesn't match with Abid and Al-Bakri [21] in their study on the bird Quail *Coturnix coturnix*, The granular cells possess axons that project into the molecular layer and dendrites that establish connections with mossy fibres to form synapses. Additionally, the presence of glomeruli, which serve as sites for synapses, has been observed. These findings align with the research conducted by Abid and Abid [19] on *Columba livia gaddi*. Below the grey matter lies the Medulla, which constitutes the white matter and serves as the inner portion of the cerebellum. The Medula region contains both efferent and afferent nerve

fibres originating from the cerebellar cortex. The medulla region comprises astrocytes and oligodendroglia glial cells, along with deep cerebellar nuclei, as demonstrated by the research conducted by Abid and Al-Bakri [21]. According to Smith et al [33], these oligodendroglia cells are responsible for myelination, while astrocytes play a role in repairing damage to the central nervous system when affected by diseases. Deep cerebellar nuclei were observed in the cerebellum of Pterocles alchata and this is aligned with the findings of Abid and Al-Bakri [21], and Chen et al [34].

Conclusion

This study concluded that the cerebellum of *Pterocles alchata* consists of Gray matter and white matter, the Gray matter is divided into three layers (Molecular layer, Purkinje cell layer, and Granular layer), the results of this study also showed that the size of the cerebellum is less than other birds and that's due to the environment that *pterocles alchata* live in.

Source of funding: This research received no funding.

Interest Conflict: There are no conflicts of interest.

Ethical Clearance: The samples were gained according to Local Research Ethics Committee approval in the College of Education for Pure Sciences (Ibn Al-Haitham), University of Baghdad, No.EC-5 in 4/3/2024.

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