UNIVERSITI PUTRA MALAYSIA

LIVER OF THE SWIFTLET – A LIGHT AND ULTRASTRUCTURAL STUDY

NADIAH SYUHADA BINTI ROSLAN

LIVER OF THE SWIFTLET – A LIGHT AND ULTRASTRUCTURAL STUDY

NADIAH SYUHADA BINTI ROSLAN

A project paper submitted to the

Faculty of Veterinary Medicine, Universiti Putra Malaysia

In Partial fulfillment of the requirement for the

DEGREE OF DOCTOR OF VETERINARY MEDICINE

Universiti Putra Malaysia

Serdang, Selangor Darul Ehsan.

MARCH 2015

CERTIFICATION

It is hearby certified that I have read this project paper entitle "Liver of the swiftlet – A Light and Ultrastructural Study", by Nadiah Syuhada Binti Roslan and in my opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 – Final Year Project.

PROFESSOR DATO' DR TENGKU AZMI TENGKU IBRAHIM

DVM (BANGLADESH), MVS, PHD (MELBOURNE)

Lecturer,

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Supervisor)

DEDICATION

To my respected lecturers in Faculty of Veterinary Medicine especially the most poise and esteem supervisor Prof Dato' Dr. Tengku Azmi Tengku Ibrahim for all his encouragement, assistance and comfort during the course of this final year project. Not to mention my beloved Prof. Dr. Noordin Mohamed Mustapha for pinning your ears back for our everlasting dilemma and predicament. I owe you so much ~

After about 2 months, I dedicated this thesis to my beloved Rehe for whom I believed my resource of strength generated from. Thank you for your determination in embarking this journey with me.

I also dedicated this thesis to all the characters in my life who are crucial in the making of this paper and the splendid years we had together.

May the voyage be impeccable and triumph will rise

ACKNOWLEDGEMENTS

First of all, I thank Allah S.A.W for giving me the strength and ability to embark on and completed this study. I would like to convey my greatest appreciation to my supervisor Professor Dato' Dr. Tengku Azmi Tengku Ibrahim for his precious time, support and supervision throughout the study. His optimism and encouragement had contributed greatly to the completion of this research.

My special thanks to En. Rafiuz Zaman Haroun and all staff of the Microscopy Unit, Institut Biosains UPM for their kind assistance, time, advice and commitment in this study.

The pillars of my life that keep me on the ground is my family. To my beloved – my lovely and honorable umi Radiah Mahmmod for her affection and endless support, my strong and inspirational abah Roslan Mohamed, my loving and wicked brothers Najib Syahidan, Najiy Shahman and Ahmad Maher, my adoring little sister Atikah Muslihah. Thank you for being born and received me for as I am.

Finally, I would like to thank my best friends Norisal Nasai, Nurul Nadia Rashid and Mohd Sabri Abd Rahman for their lasting support and indulgent throughout this DVM course. Special thanks to my ex-roommates, rota-mates, seniors and juniors.

CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATION

%	percentage
˚C	degree celcius
µm	micrometer
Å	angstrom
BDMA	Benzyl Dimethyl Amine
DDSA	Dodecenyl Succint Anhydride
DVS	Department of Veterinary Services
EBN	Edible bird nest
gm	gram
kV	kilovolt
ml	milliliter
mm	millimeter
MNA	Methyl Nadic Anhydride
TEM	Transmission electron microscope
x	time

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek Tahun Akhir.

KAJIAN ULTRASTRUKTUR HATI BURUNG WALIT

Oleh

Nadiah Syuhada Binti Roslan

2014

Penyelia: Professor Dato' Dr. Tengku Azmi Tengku Ibrahim

Kajian ini bertujuan untuk memberi suatu pemahaman di peringkat ultrastruktur hati burung walit (*Aerodramus fuciphagus*) yang merupakan sebahagian daripada sistem pencernaan yang menyumbang kepada penghasilan sarang burung boleh dimakan (EBN). Dari segi anatomi hati burung walit terletak dikeliling hempedal dan terdiri daripada 3 lobus – lobus kanan yang besar, lobus kiri yang kecil dan lobus kiri dorsal yang lebih kecil. Di bawah mikroskop cahaya, sebahagian besar parenkima hati terdiri daripada hepatosit, vena sentral, dan portal triad. Parenkima hati burung ini bagaimanapun, tidak menunjukkan kewujudan lobul tipikal yang terbentuk daripada vena sentral di tengah-tengah lobul dan portal triad di setiap penjuru lobul. Penglihatan dengan mikroskop elektron, hepatosit mempunyai nukleus yang besar terletak ditengah-tengah sel dan mengandungi nukleolous yang jelas. Sitoplasma hepatosit di penuhi dengan mitokondria dan ruangan antara mitokondria dipenuhi ribosom. Ribosom di ruangan terhad antara mitokondria memberi gambaran mitokondria seolah-olah dikelilingi oleh ribosom. Sitoplasma juga mengandungi granul yang nampak legap kandungannya dan kemungkinan struktur ini adalah granul glikogen. Membran hepatosit menunjukkan struktur yang berbeza apabila bertentangan struktur khusus seperti sel van Kupffer dan kanalikulus. Bertentangan dengan sel Kupffer, membran hepatosit membentuk

lipatan-lipatan dan apabila bertentangan dengan kanalikulus, membran hepatosit membentuk mikrovilus. Cerapan yang menarik yang diperolehi daripada kajian hati burung walit menggunakan mikroskop elektron ialah insidens kanalikulus yang tinggi. Cerapan ini memberi gambaran hati burung walit menghasilkan kuantiti hempedu yang tinggi. Penghasilan hempedu merupakan salah satu tugas utama hati. Penghasilan hempedu yang tinggi ini mungkin diperlukan untuk mencerna lemak, yang diketahui terdapat tinggi kandungannya dalam ulat-ulat halus yang menjadi makanan utama burung walit. Pencernaan lemak menghasilkan tenaga dan tenaga yang tinggi ini diperlukan oleh burung walit yang sentiasa dalam penerbangan daripada pagi hari apabila ia meninggalkan sarangnya hingga ke senja apabila balik semula kesarangnya.

Katakunci: *burung walit, hati, ultrastruktur, kanalikuli, hempedu*

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999 – Final Year Project.

THE LIVER OF THE SWIFTLET – AN ULTRASTRUCTURAL STUDY

By

Nadiah Syuhada Binti Roslan

2015

Supervisor: Professor Dato' Dr. Tengku Azmi Tengku Ibrahim

The study was aimed at providing an understanding of the liver of the swiflets (*Aerodramus fuciphagus*) which is a part of digestive system and responsible for the production of edible bird nest (EBN). Grossly the liver of the swiftlet which is located around the gizzard, comprised of three lobes – a large right lobe, a small left lobe and a yet smaller dorsal left lobe. Under the light microscope the liver parenchyma is largely made up of hepatocytes, central veins and portal triad. The parenchyma of this avian species did not however demonstrate the presence of a typical or classical lobule formed by the central vein and portal triads at the corners of each lobule. Under the transmission electron microscope the hepatocyte has a large, centrally located vesicular nucleus with a prominent nucleolus. The cytoplasm of the hepatocyte is largely occupied by mitochondria with a layer of ribosomes surrounding each mitochondria. The ribosomes surrounding the mitochondria could be attributed to the fact that the cytoplasm is packed with mitochondria and there is very little space between the mitochondria which is then, occupied by the ribosomes. Thus the ribosome in the limited space

between the mitochondria gave the impression that the mitochondria is surrounded by ribosomes. In addition to the above mentioned organelles the cytoplasm also contain vacuoles whose content appeared electron opaque which in all probability are glycogen as the liver is known to store glycogen. The cell membrane of hepatocytes demonstrate modifications when facing specific structures namely van Kupffer cells and bile canaliculus. In the case of the former the cell membrane is thrown into numerous folds while in the later the cell membrane form extension microvilli. It is also interesting to note that the incidence of canaliculi is very high in the liver of this avian species. The main function of liver is in the production of bile. The high production of bile could probably be required for the digestion of fats which is high in insects, the main food source of swiftlet. High fats digestion produces high energy required for the swiftlet which is always in constant flight from dawn when it leave the nest to dusk when it return to the nest.

Keywords: *swiftlet, liver, ultrastructure, bile canaliculi, bile.*

1.0 INTRODUCTION

According to the "Malaysia Burung Walit Registration System" 2015, there are approximately 10096 bird premises and 267 processing plants registered under Department of Veterinary Services (DVS) Malaysia. The Malaysian Swiftlet Farming Industry Report stated that the swiftlet farming industry has the potential to grow into a multimillion ringgit industry due to the industry's relatively high return profile as well as continuous growing demand for edible bird nest (EBN). Malaysia exported 120 tonnes of EBN worth RM 114 million in the first 10 months of 2014 and the industry is a high-impact entry point project (EPP) with a targeted export of 870 tonnes worth RM 5.2 billion by 2020 reported Ministry of Agriculture and Agro-based Industry, 2014. Almost 99 % of all swiftlet farms are geared towards the production of white EBN which is produced by *Aerodramus fuciphagus*.

Aerodramus fuciphagus is characterized by dull dark grey brown to black plumage with small beak and limbs. This swiftlet species foraged microscopic insects in the upper strata at the atmosphere during flight. The swiftlet absorbed the nutrient direct from the insect itself (Azreen, 2014) and produced acidic carboxylated mucin which is the main substances used to build the nest (Helen *et al.,* 2011). The swiftlet digestive system is simple and efficient in absorbing the nutrient from the insects (Azreen, 2014) and in the production of EBN from the efficient salivary gland especially in the breeding season (Zainita, 2014).

The liver is part of the digestion system and performs many functions essential for life, the most obvious being the production of bile. Bile functions to emulsify fats and activate pancreatic lipase and amylase, all of which aid in digestion. Apart from aiding in the process of digestion, liver ensures that the products of digestion, which are conveyed in the bloodstream after absorption, are presented to the hepatocytes before entering general circulation (Dyce *et al.,* 2002)

There have been a number of studies on the anatomy of the avian digestive tract. In the past few years a couples of paper had been published to describe the anatomy, histology and ultrastructure on the digestive system of the swiftlet as reported by Azreen *et al.*, (2014) but there is no published study on the histology and ultrastructure of the liver in particular. This study reports on the light and transmission electron microscopic structure of the swiftlet liver.

Specifically the objective of this study is to investigate the light and electron microscopic structure of liver of the swiftlet to provide an understanding of this accessory gland which forms part of digestive system. Hopefully the information generated from this study could provide some information to help in understanding of the physiology of the digestive system and nutrition of the swiftlet.

2.0 LITERATURE REVIEW

2.1 Species of Swiftlet and its Classification

Brooke (1972) classified the swiftlet species (*Aerodramus fuciphagus)* that produce white EBN as below:

Kingdom: Animalia

Phylum: Chordata

Subphylum: Vetebrata

Class: Aves

Order: Apodiformes

Family: Apodidae

Genus: *Aerodramus*

Species: *fuciphagus*

2.2 Swiftlet Morphology and Identification

This swiftlet (*Aerodramus fuciphagus*) species is characterized by a dull, dark black to brown plumage with paler under body. This avian species unable to use limbs for walking, standing and perching due to short metatarsal bone and digits and small muscle of the limb (Zuki *et al.*, 2012). The eggs has of an average size of 20x40 mm and are cylindrical or elliptical in shape. Per clutch there is 2 eggs in the nest and the eggs will hatch on average 25 days (range 19 – 32 days). After incubation the birds have 2 – 3 breeding cycles per year and the natural nesting sites are in cave interiors (Lim and Cranbrook, 2002)

2.3 Anatomy of the Avian Liver

The avian liver comprised of right and left lobes that fuse on the midline in the dorsal middle to cranial third. The right lobe is usually larger than the left lobe in the majority of bird species. However, lobes can be of equal size, and rarely the left lobe being larger than the right lobe in some species (Doneley, 2011).

Microscopically the capsule of liver is comprised of a thin layer of collagen and elastic fibers (Doneley, 2011). The liver is divided into lobules. Each lobule has a central vein which receives drainage of the sinusoids. The portal triads are present at the periphery of each lobules. The lobules of mammals (pig) are well defined by connective tissue. The lobules of birds being devoid of connective tissues makes identification of this functional unit of the liver difficult.

The hepatocytes typically had a nucleus which was round and relatively large, with a large nucleolus. In chicken, the nucleus was usually located toward the perisinusoidal surface (Ghoddusi and Kelly, 2004).

The chicken liver generally consisted of chords of 4 to 6 hepatocytes arranged radially surrounding a central bile canaliculus. Bile is synthesized in the hepatocytes and secreted into bile canaliculi located on the lateral surfaces of adjoining liver cells (Iqbal *et al.*, 2014). The membranes of hepatocytes facing bile canaliculus had numerous short finger-like microvilli. When bordering the perisinusoidal or space of Disse's, the membrane of hepatocyte formed much wider and irregular microvilli (Ghoddusi and Kelly, 2004).

Sinusoidal endothelial cell on the other hand is composed of the cell body that contains a nucleus and the thin membraneous cytoplasmic extension which occupies the partition wall between the perisinusoidal or space of Disse's and the sinusoids. The nucleus of the sinusoidal endothelial cell is oval or spindle-shaped in profile. Other than sinusoidal endothelial cell, van Kupffer cell also occupy sinusoids. Van Kupffer cell can be identified with the fuzzy cell coat

and conspicuous pseudopodia. The pseudopodia, approach the opposite sinusoidal wall to adhere to the endothelial lining. The cell bulge more or less markedly ito the sinusoidal lumen, and the location of an oval or spindle-shaped nucleus is indefinite, probably due to the amoboid movement of cytoplasm (Ohata *et al.*, 1982)

Apart from that, bile canaculi is formed by a gap between parenchymal liver cells (Steiner and Carruthers, 1961) and are well established in livers 6 days old (Karrer and Cox, 1961). The lumen of bile canaliculi is regular and in some cases rounded and there are projections of the cell surface which protrude into the lumen. The lumen do not contain any obvious materials and the observations are the same as a study in rat (Motta and Fumagalli, 1975).

3.0 MATERIALS AND METHODS

Six swiftlets (*Aerodramus fuciphagus*) were used in this study. The birds were caught in the morning from a bird house in Jalan Bakau, Kluang, Johor and immediately brought back to the laboratory. Following slaughter, the abdominal cavity was opened and the liver was excised and its gross anatomy examined.

3.1 Light Microscopy

Samples of liver were collected and fixed in Bouin's solution (Appendix 1) for light microscopy. The samples were submerged in Bouin's solution for 16 hours and then the tissues washed with 50% alcohol, 3 times each for 30 minutes before fixed in 70% alcohol. Samples of liver then arranged in the labeled cassettes and fixed with 40% formalin for at least 30 minutes before processed in an Automatic Tissue Processor (Histokinett). The tissues were embedded in paraffin wax before sectioning using a microtome. The sections were stained with heamatoxylin and eosin (H&E) stain and examined under light microscope starting from low to higher magnifications.

3.2 Transmission Electron Microscopy

Samples of liver were fixed in 4% Glutaraldehyde (Appendix 2) for two hours to harden the tissue before dicing them into 1mm cubes. The tissues were further fixed overnight in 4% Glutaraldehyde. After thrice washing with 0.1 M Sodium cacodylate buffer (Appendix 3) for 10 minutes each, the tissues were post-fixed in 1% Osmium tetraoxide (Appendix 4) for 2 hours at 4˚C. The tissues were washed again with 0.1 M Sodium cacodylate buffer thrice for 10 minutes each changes. The tissues were then dehydration in a series of acetone of ascending concentrations (35%, 50%, 75%, and 95%) for 10 minutes each and finally in 100% acetone for 15 minutes in 3 changes. Following final dehydration the samples were infiltrated with 50:50 acetone resin mixture (Appendix 5). The samples were embedded in beam capsule filled

with resin mixture and polymerized in oven at 60˚C overnight. Semithin sections (4µm thick) was obtained using an ultramicrotome. The semithin sections were stained with Toulidine Blue stain (Appendix 6) and viewed under light microscope to identify the area of interest prior to the ultrathin sectioning. Ultrathin sections of 1Å thick on the 200-mesh-cooper grids were stained with uranyl acetate (Appendix 7) and lead citrate (Appendix 8) each for 10 minutes and washed with double distilled water. The stained sections were examined under a transmission electron microscope (Hitachi H711, Japan) operating at 80 kV.

4.0 RESULTS

Grossly, the liver of swiftlet being dark red-brown in color was observed to be located around the gizzard and caudal to the heart (Figure 1).

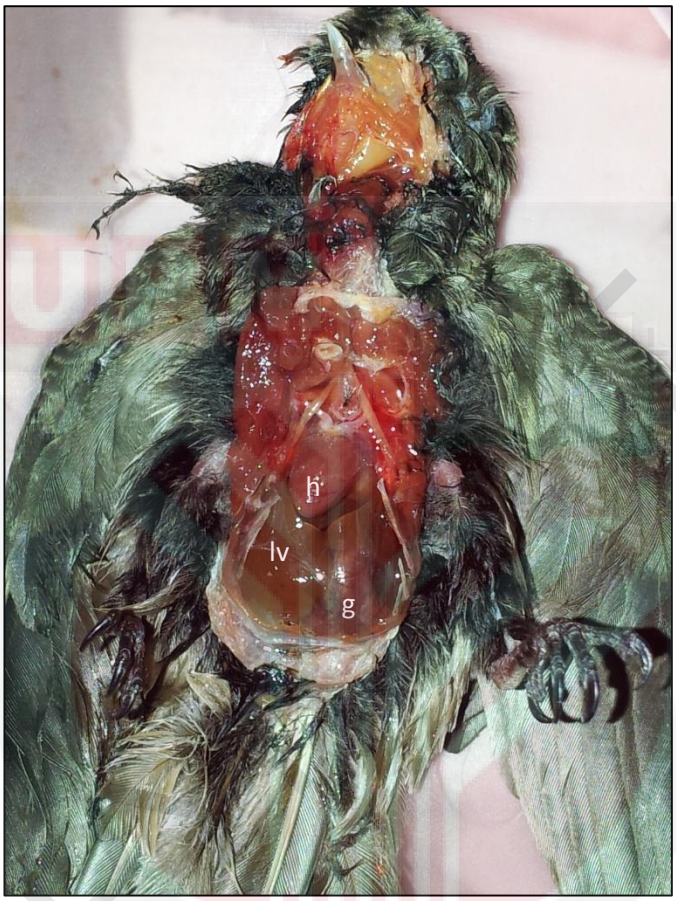

Figure 1: Gross anatomy of the swiftlet liver (lv) identified by its dark brown color located around the gizzard (g) and caudal to the heart (h).

The liver comprised of 3 lobes: a large right lobe, a small left lobe and a yet smaller caudal left lobe located dorsal to the gizzard (Figure 2).

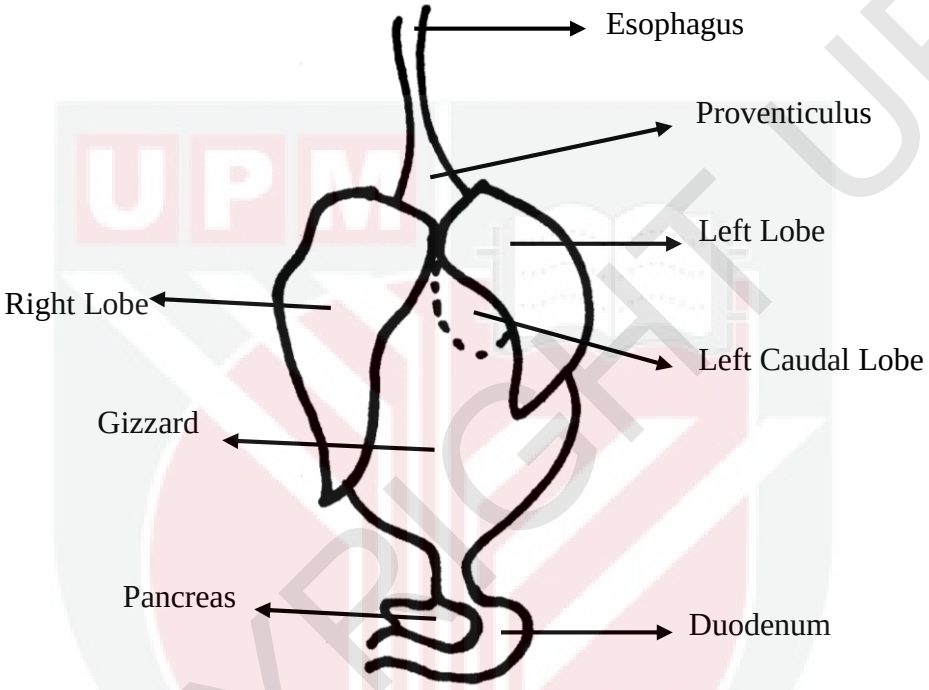

Figure 2: Schematic diagram of swiftlet liver comprised of 3 lobes: a large right lobe, smaller left lobe and yet a smaller caudal left lobe

Examined under the light microscopic the swiftlet liver was surrounded by a thin connective tissue capsule (Figure 3).

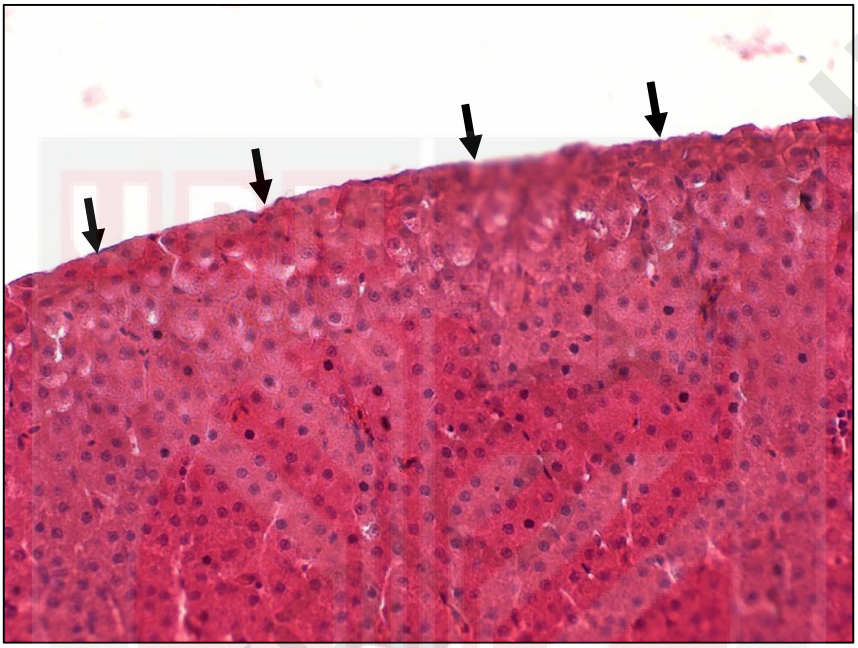

Figure 3: Closely packed parenchyma surrounded by a very thin connective tissue capsule (arrow). Magnification x 40

The liver parenchyma was very compact and made up mostly by hepatocytes. Under higher magnification there was a high incidence of cell undergoing mitosis. Sinusoids could be identified as indicated by the presence of red blood cells and the nuclei of sinusoidal endothelial cells (Figure 4).

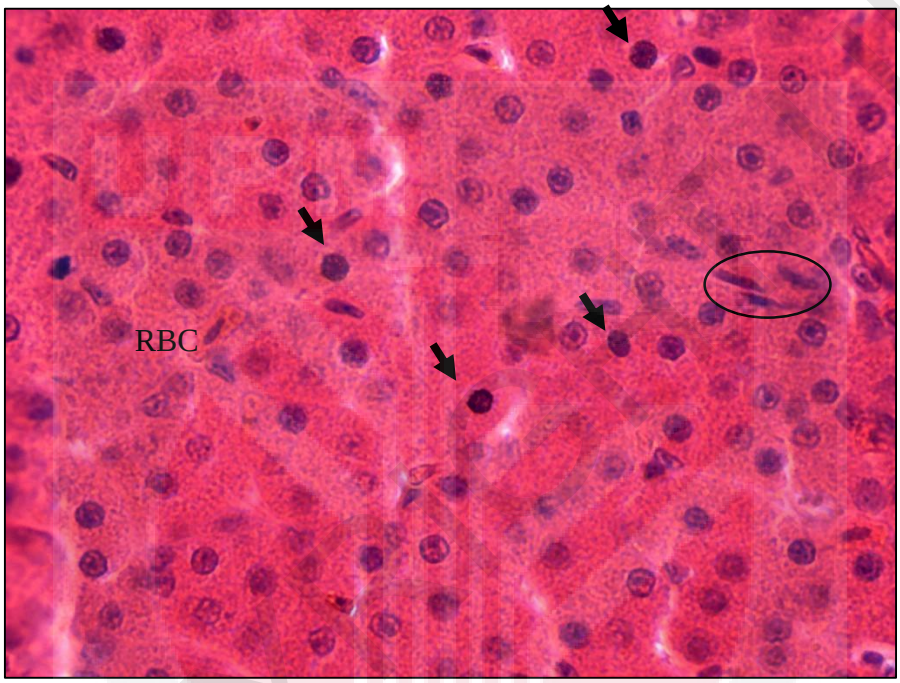

Figure 4: Liver parenchyma showing many hepatocytes undergoing mitosis (arrows). Sinusoids can be identified as indicated by the presence of red blood cell (RBC) and the flattened nuclei of sinusoidal endothelial cell (circled). Magnification x 400

Van Kupffer cells could be identified under oil immersion located at the wall of sinusoids (Figure 5).

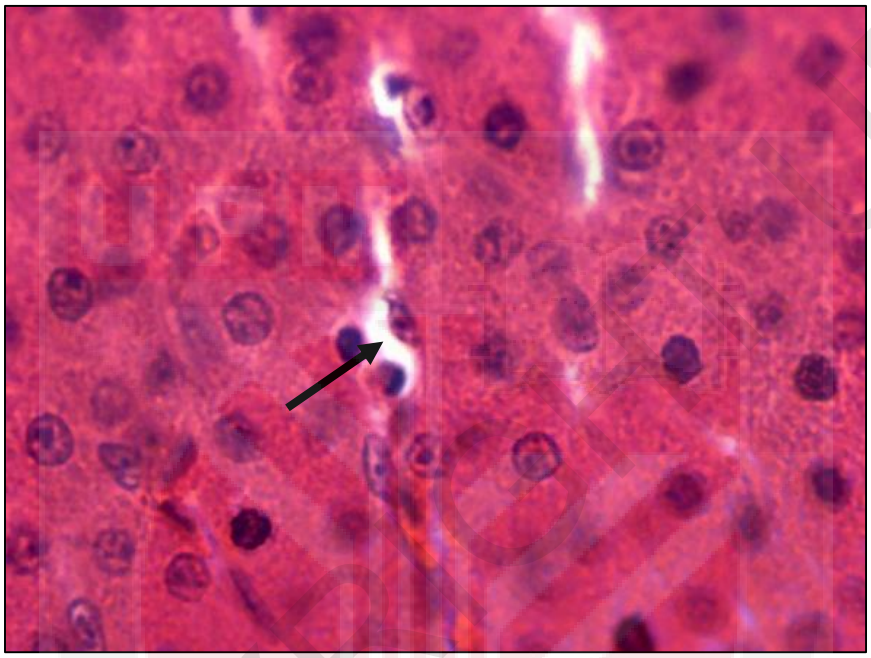

Figure 5: Higher magnification of sinusoid lined van Kupffer cell (arrow).

Magnification x 1000

A typical portal triad could not be identified in the liver parenchyma, only a structure with a vein and an artery without a bile duct could be observed (Figure 6) in the vicinity of a central vein (Figure 7).

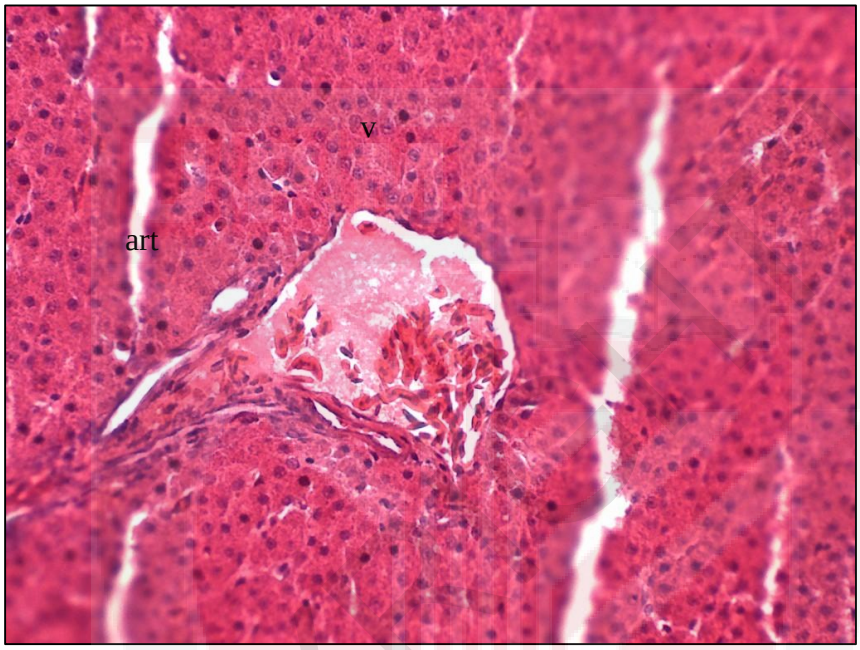

Figure 6: A portal triad identified by a large vein (v) and an arteriol (art) without a bile duct. Magnification x 100

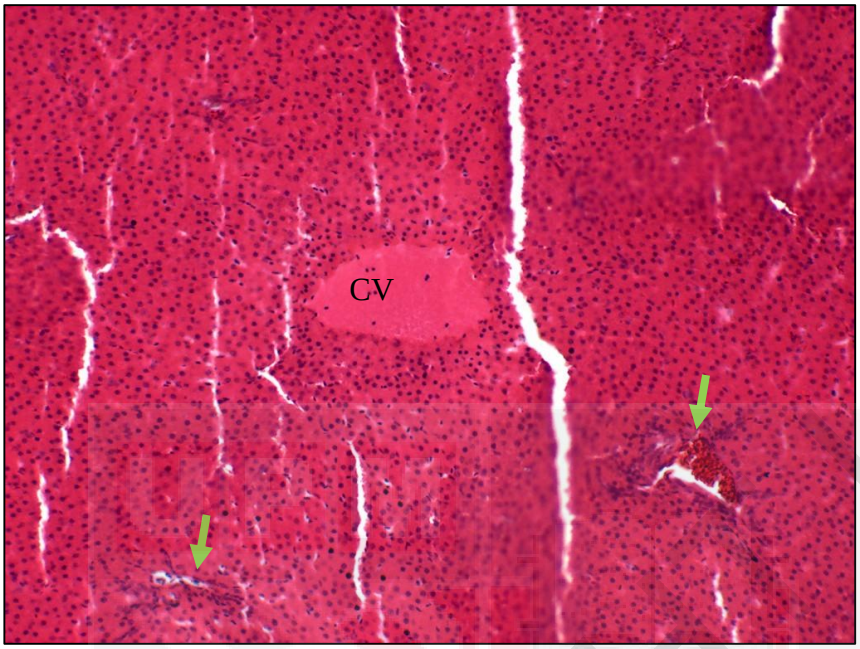

Figure 7: A central vein (CV) in the parenchyma of the liver. Only two possible portal triad (arrows). Magnification x 40

Under the transmission electron microscope hepatocytes were hexagonal shaped with a centrally located nucleus and a prominent nucleolus. The cytoplasm was filled with mitochondria and ribosomes. There were also granules with electron opaque contents (Figure 8).

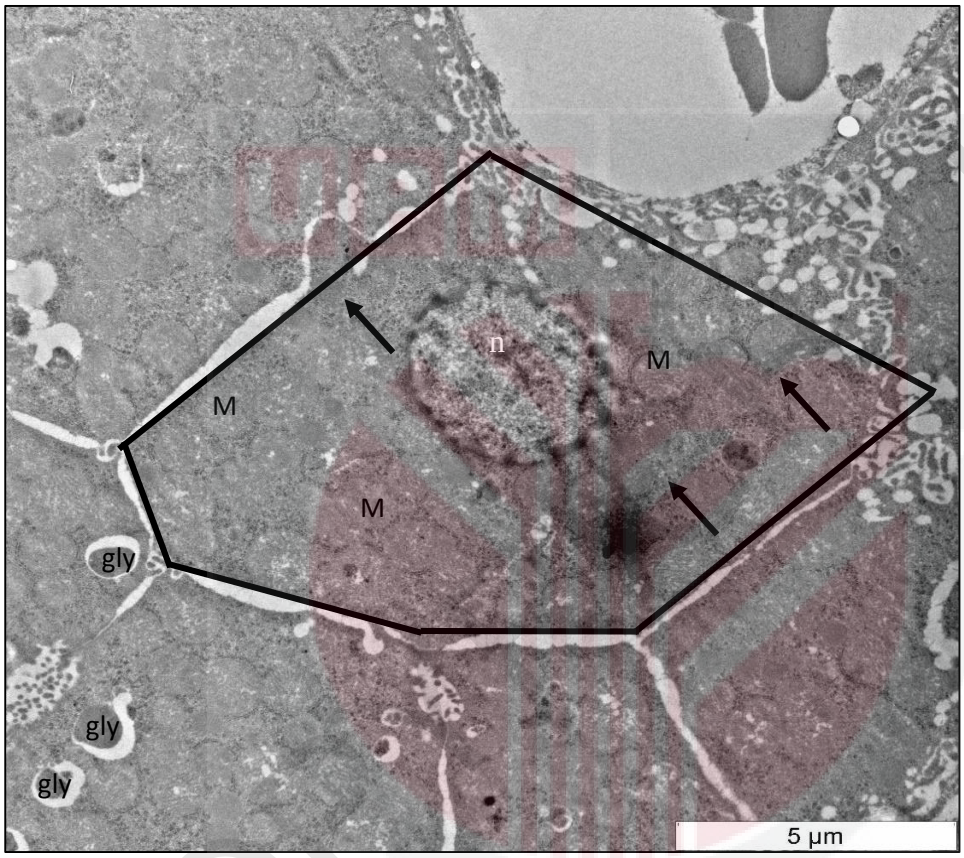

Figure 8: Hepatocytes showing hexagonal shape with centrally located nucleus (n). The cytoplasm is filled mostly by mitochondria (M), ribosomes (arrows) and glycogen granules (gly) in the cytoplasm.

There was high incidence of bile canaliculi observed between the hepatocyte (Figure 9).

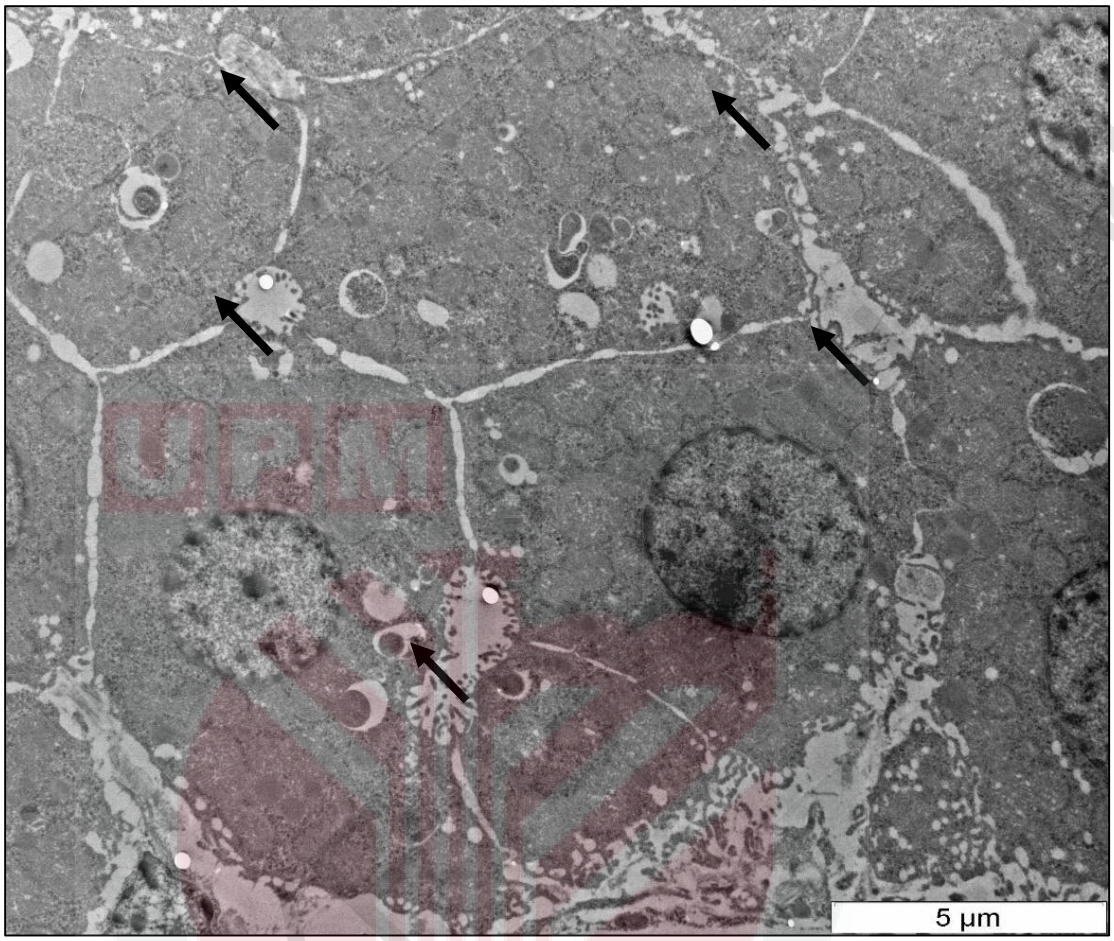

Figure 9: Liver parenchyma showing high incidence of bile canaliculi (arrows).

Cell membrane of hepatocytes facing bile canaliculi showed the presence of extensive microvilli projecting into the lumen of bile canaliculi (Figure 10).

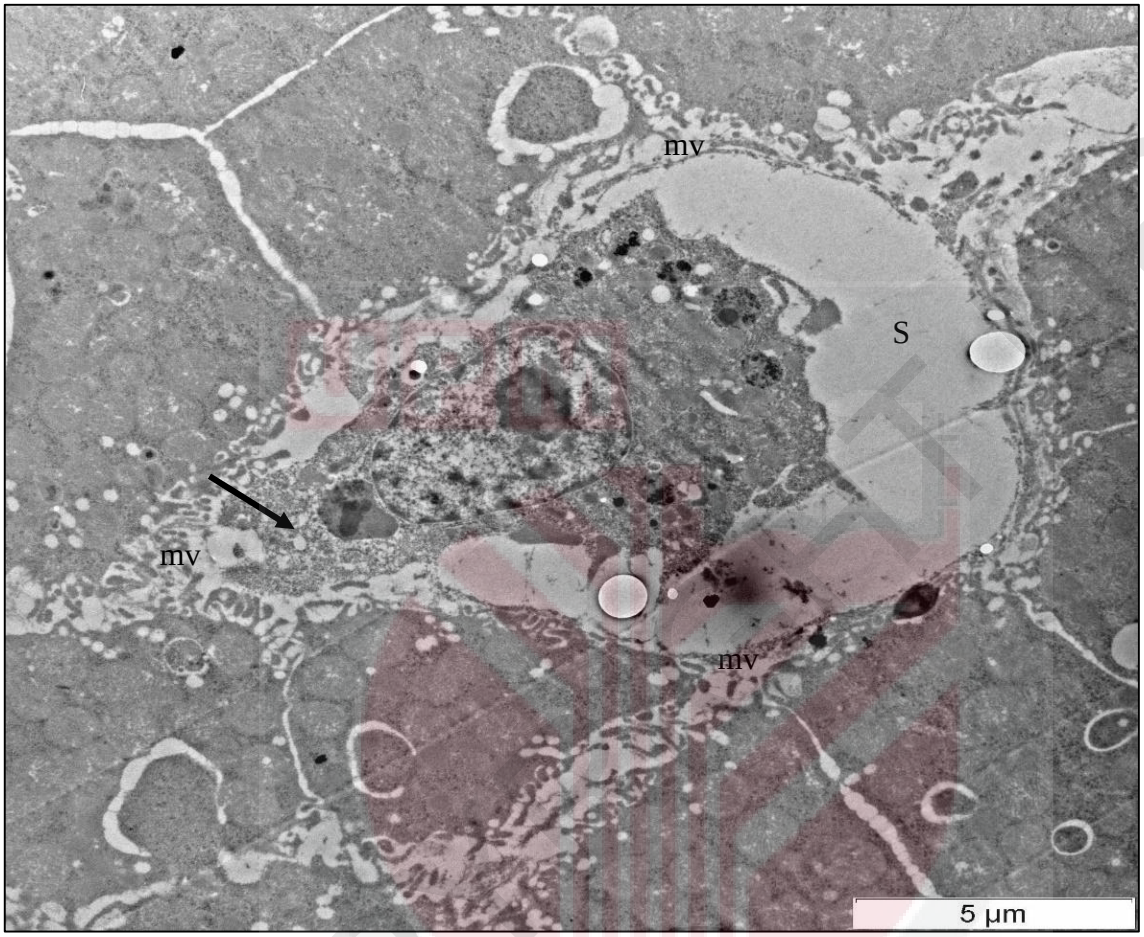

Figure 10: Van Kupffer cell in a sinusoid (s) identified by the eccentrically located nucleus and the cytoplasmic appendages (arrow). Cell membrane of hepatocytes facing sinusoid have cytoplasmic projection or microvilli (mv).

Van Kupffer cells in the liver sinusoids could be identified by the eccentrically located nucleus and the cytoplasmic projections. Cell membrane of hepatocytes facing sinusoids also formed cytoplasmic projection or microvilli (Figure 11). Sinusoidal endothelial cells were identified by their elongated, flattened nucleus.

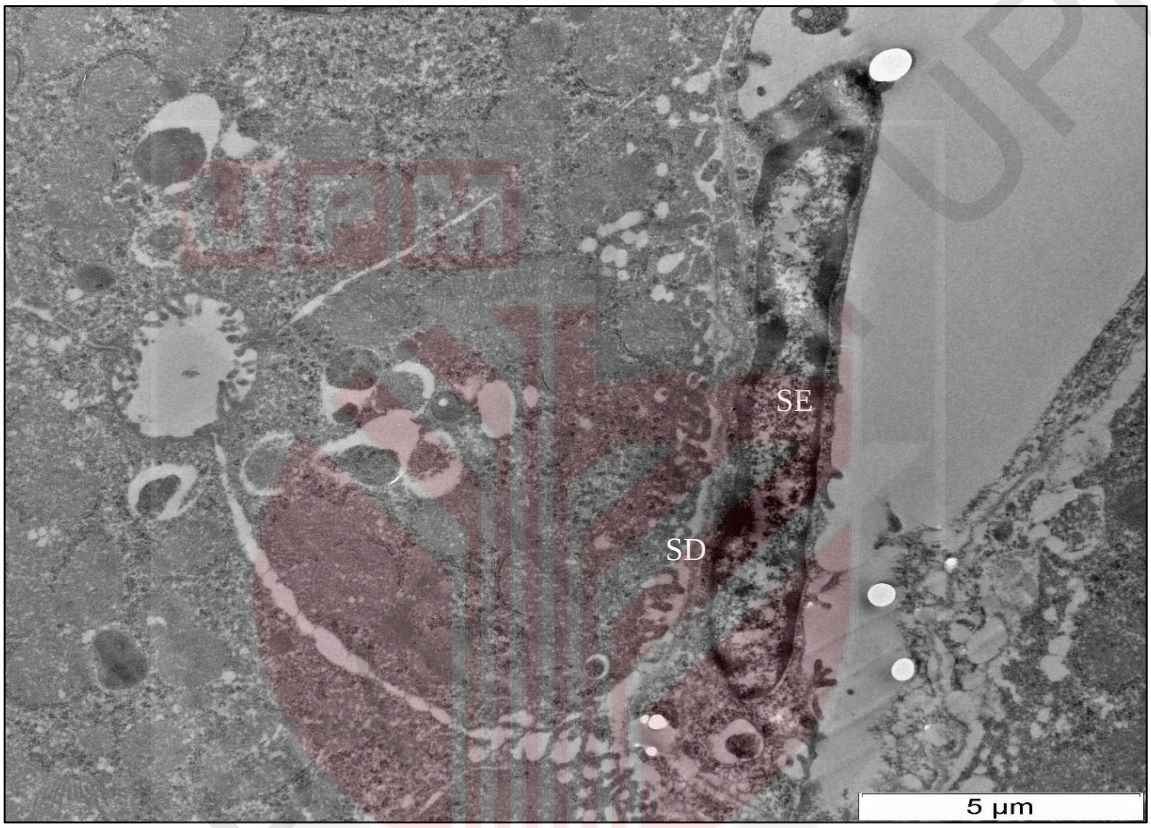

Figure 11: A sinusoidal endothelial cell (SE) identified by the elongated nucleus that filled most of cytoplasm. The cell membrane of hepatocyte facing the sinusoidal endothelial cell form numerous folds projecting into the space of Disse.(SD)

5.0 DISCUSSION

Examined under light microscope the liver parenchyma is very compact and there is no arrangement of the hepatocytes in chords or plates as observed in other mammalian species or avian species. Sinusoids could be identified by the presence of sinusoidal endothelial cell or red blood cells. Identification of a typical lobule is also not possible in the liver parenchyma in this avian species as no definite portal triads around the central vein could be identified. A maximum of two portal triads could be identified in the vicinity at a central vein which did not allow the reconstruction of a typical liver lobule as characterized by a central vein and a number of portal triads at the corners of each lobule. A portal triad could not be identified either as there was only a vein and an artery while the bile duct which is normally a component of a portal triad is absent.

Examined under the transmission electron microscope the more prominent structure observed in the parenchyma of the liver is the high incidence of bile canaliculi and also the large canaliculi between contiguous hepatocytes. As there is no bile duct in the portal triad as observed under the light microscope bile in all probability flow from the bile canaliculi direct into the gall bladder.

It is also tempted to speculate that the high incidence of bile canaliculi could be associated with the high production of bile for digestion of the insects foraged by the swiftlet in the upper strata of the atmosphere. Insects are known to contain a high proportion of fat in comparison to their body mass. It has been reported that the body of edible grasshopper and termites are known to contain 67% and 49% fats respectively (Huis *et al.,* 2013). Thus, in the case of the swiftlet the insects foraged by this avian species in all probably also contain a high fat content. Thus considerable bile is required to digest the fats to produce the high energy

required by the swiftlet for it continuous flight from dawn when its leaves its nest until dusk where it return to the nest.

It is concluded from this study that the liver of the swiftlet is complex as the hepatocytes are not arranged in plates or chords and in the absence of the bile duct in the portal triad thus the blood and bile flow which is opposite to each other as in mammalian cannot be determine.

6.0 CONCLUSION

The following conclusion can be drawn from the present study. The liver of swiftlet comprised of 3 lobes: a large right lobe, a small left lobe and a yet smaller caudal left lobe. The hepatocytes are hexagonal in shape with a centrally located nucleus. Unlike other the mammalian or avian species the hepatocytes of swiftlet are not arranged in chords or plates. A typical portal triad cannot be identified and typical lobule cannot be identified either in the liver parenchyma.There are high incidence of bile canaliculi which could indicate that liver of swiftlet is actively synthesizing bile. The large production of bile is related to the digestion of insects which has a high fat content. The liver of the swiftlet is functionally efficient but complex from the point of view. The blood flow from the portal triads and the flow of bile from bile canaliculi to bile duct cannot be distinguished.

7.0 RECOMMENDATION

In the present study, we cannot identify the liver lobule and portal triad of the liver in this avian species. Hence, future studies should be focus to identify liver lobule and portal triad to determine blood circulation and the flow of bile in the liver of the swiftlet.

REFERENCES

Abu-Absi, S. F., Friend, J. R., Hansen, L. K., and Hu, W. S. (2002). Structural Polarity and Functional Bile Canaliculi in Rat Hepatocyte Spheroids. Experimental Cell Research 274. Pp. 56 – 67.

Attia, H. F., and Soliman, S. M. (2005). Histology and Histochemical Studies on the Ostrich's Liver. Benha Veterinary Medicine Journal. Vol: 16. No: 2.

DVS Online Registration Portal. (2015, February 23). Retrieved from http:ebndvs.efoodpremise.gov.my.

Ghoddusi, M., and Kelly, W. R. (2004). Ultrastructure of In Situ Perfusion-Fixed Avian Liver, with Special Reference to Structure of the Sinusoids. Microscopy Research and Technique. 65: 101 – 111.

Hanan, A. S. (2013). Morphological and Histological Study for Liver in Local Coot Birds *Fulica atra*. Basrah Journal of Veterinary Research. Vol: 12. No: 1, Pp. 152 – 156.

Helen, M., Intan-Shameha, A. R., Kamaruddin, M. I, and Zuki, A. B. Z. (2013). Morphological Evaluation of Submandibular Salivary Glands of White (*Aerodramus fuciphagus*) and Black (*Aerodramus maximus*) Edible Bird's Nest Swiftlet. Proceeding of WPSA (Malaysia Branch) and WVPA (Malaysia Branch) Science Conference. Pp. 47-48

Huis, A. V., Itterbeeck, J. V., Klunder, H., Mertens, E., Halloran, A., Muir, G., and Vantomme, P. (2013). Chapter 6: Nutritional value of insects for human consumption. Edible Insects. FAO Forestry Paper. 72 – 88.

Ibrahim, M. M., Zuki, A. B. Z., and Omar, A. R. (2013). Developed and Undeveloped Submandibular Glands of EBN Swiftlet (*Aerodramus fuciphagus*). . Proceeding of WPSA (Malaysia Branch) and WVPA (Malaysia Branch) Science Conference. Pp. 65 – 66.

Iqbal, J., Bhutto, A. L., Shah, M. G., Lochi, G. M., Hayat, S., Ali, N., Khan, T., Khan, A. M., and Khan, S. A. (2014). Gross Anatomical and Histological Studies on the Liver of Broiler. Journal of Applied Environmental and Biological Sciences. 4(12). 284 – 295.

Karrer, H. E. and Cox, J. (1961). Electron Microscope Observations on Chick Embryo Liver: Glycogen, Bile Canaliculi, Inclusion Bodies and Hematopoiesis. Journal Ultrastructural Research. 5: 116-141.

Lourie, S. A., and Tompkins, D. M. (2000). The Diets of Malaysian Swiftlets. Ibis (International Journal of Avian Science). Vol: 142. Pp. 596 – 602.

Merican, H. S. (2007). *2007 Malaysian Swiftlet Farming Industry Report*. Retrieved from SMI Association of Penang website: enquiries@smipenang.com

Motta, P., and Fumagalli, G. (1975). Structure of Rat Bile Canaliculi as Revealed by Scanning Electron Microscopy. The Anatomical Record. Vol: 182. Issue: 4. Pp. 499 – 513.

Ohata, M., Tanuma, Y., and Ito, T. (1982). Electron Microscopic Study on Avian Livers with Special Remarks on the Fine Strcuture of Sinusoidal Cells. Okajimas Folia Anat. Jpn., 58 (4-6) : 325 – 368.

Steiner, J. W. and Carruthers, J. S. (1961). Studies on the Fine Structure of the Terminal Branches of the Biliary Tree: The Morphology of Normal Bile Canaliculi, Bile Pre-Ductules (Ducts of Herring) and Bile Ductules. The American Journal of Pathology. Vol: 38 (6), 639 – 660.

Syed, W. S. and Norazlin, A. A. (2014). Morphology of the Lingual Apparatus of the Swiftlet, *Aerodramus fuciphagus* (Aves, Apodiformes, Apodidae). Journal of Microscopy and Ultrastructure. 2: 100 – 103.

Zuki, A. B. Z., Abdul Ghani, M. M. Khadin, K. K., Intan-Shameha, A. R. and Kamaruddin, M. I. (2012). Anatomical Structures of the Limb of White-nest Swiftelet (*Aerodramus fuciphagus*) and White-headed Munia (*Lonchura maja*). Pertanika Journal Tropical Agricultural Science. 35 (3): 613 – 622.

APPENDICES

Transmission Electron Microscope and Histological Slides Preparation

CHEMICAL PREPARATION FOR TRANSMISSION ELECTRON MICROSCOPE

The entire procedure and chemical used during this process was handled in a fume chamber and hand gloves were worn during the chemical preparations to prevent exposure to the hazardous and carcinogenic material.

Appendix 1

Bouin's Solution

Powdered Picric acid in saturated aqueous solution was prepared.	750 ml
37 – 40% formalin	250 ml
Acetic Acid	50 ml

Appendix 2

Buffered Gluteraldehyde

1. Sodium Cacodylate buffer

   Sodium Cacodylate 42.8Double distilled water 1000 ml

2. 25% Gluteraldehyde

4% Buffered Gluteraldehyde

Sodium Cacodylate buffer	50 mg
25% Gluteraldehyde	10 mg
Double distilled water	40 ml

Appendix 3

Sodium Cacodylate Buffer (100ml)

Sodium Cacodylate	42.8 gm

Methods:

Sodium Cacodylate was dissolved in distilled water and adjusted to pH 7.3 by using 1N HCl. To make 01.M buffer solution, equal amount of the above buffer and distilled water was added. The buffer was stored in a clear glass bottle in 2-4˚C.

Appendix 4

Osmiun Tetraoxide

To prepare the 2% stock solution, an ampoule of 0.1g Osmium (O_8O_4) was broken and mixed with 50ml distilled water in a clear bottle which was subsequently shaken well. The solution should be made one day ahead in small quantities and stored in tapered flask fitted with glass stopper as O_8O_4 is extremely volatile and rapidly decreased in concentration. The bottle were then wrapped with aluminium foil and kept inside a glass jar to prevent the internal portion of refrigerator becoming black.

To prepare 1% stock solution, equal volume of fixative and distilled water were mixed.

Appendix 5

100% Resin

Agar 100 Resin	10.0 ml
DDSA (Dodecenyl Succint Anhydride)	6.0 ml
MNA (Methyl Nadic Anydride)	5.5 ml
BDMA (Benzyl Dimethyl Amine)	0.5 ml

Freshly prepared resin were prepared by measured and mixed or stirred the above chemicals well in a plastic measured cup for 5 minutes to ensure the proper mixing. For initial infiltrations, 100% resin was mixed with Acetone in 1:1 ratio for 1 hour. Then in 1:3 ratio for 2 hours before infiltration with 100% resin overnight. The tissue then infiltrated again with a new batch 100% resin for another 2 hours.

Appendix 6

Touline Blue

1% Touline Blue	1.0 gm

1 g of Touline Blue was dissolved in 100 ml of distilled water.

Appendix 7

Uranyl Acetate

Uranyl Acetate was dissolved in 50% alcohol until it becomes saturated. It was prepared a day ahead, filtered and kept away from strong light.

Additional stain before dehydration was prepared using 0.5% Uranyl Acetate for 30 minutes called En Bloc Staining.

Appendix 8

Lead Citrate

Reynold Lead Stain

Lead Citrate	0.65 gm
Sodium Citrate	0.88 gm
Distilled water	15 ml

The mixture was shaken vigorously at 30 minutes interval in a container. The milky suspension appearance was obtained after a complete conversion of lead nitrate to lead citrate. This was followed by adding 4ml NaOH (4%) into the suspension and it was diluted with distilled water to 25 ml. the suspension was mixed by inversion until lead citrate dissolves and cleared up completely with pH around 12.

The distilled water used in this preparation was boiled for 8 minutes to remove any Carbon Dioxide (CO_2). The container was covered and allowed to cool before it was added into the suspension. The NaOH also should be free from CO_2 and freshly prepared. This stain can be stored for up to 6 months in a tight container and discarded if precipitation appears. It should be centrifuged before used