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2019

COMPARATIVE CYTOMORPHOLOGICAL, CYTOCHEMICAL AND CYTOENZYMIC STUDY ON BLOOD CELLS OF DOMESTIC FOWL, GUINEA FOWL AND PIGEON

By

Dr. Naveen Kumar

Reg. no- V/BAU/3894/2009

DEPARTMENT OF
VETERINARY ANATOMY & HISTOLOGY
FACULTY OF VETERINARY SCIENCE & ANIMAL HUSBANDRY

BIRSA AGRICULTURAL UNIVERSITY
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(JHARKHAND)

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Thesis

Submitted to the

BIRSA AGRICULTURAL UNIVERSITY
RANCHI-834006
(JHARKHAND)

By

Dr. Naveen Kumar

Reg. no- V/BAU/3894/2009

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Master of Veterinary Science

IN

VETERINARY ANATOMY AND HISTOLOGY

2019

Dedicated

To

My Parents

And

Teachers

ACKNOWLEDGEMENT

We desire to achieve our dreams, it is our perseverance and determination that pays and then only we achieve what we dream. So, today it is my dream that I am at the meridian of achieving my goal. As I begin to write these lines after completion of my thesis, my heart is filled with deepest sense of gratitude.

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Date: …… ***(Naveen Kumar)***

Place: Kanke, Ranchi *Author*

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DEPARTMENT OF VETERINARY ANATOMY & HISTOLOGY
FACULTY OF VETERINARY SCIENCE & ANIMAL HUSBANDRY,
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CERTIFICATE

This is to certify that the thesis entitled **"COMPARATIVE CYTOMORPHOLOGICAL, CYTOCHEMICAL AND CYTOENZYMIC STUDY ON BLOOD CELLS OF DOMESTIC FOWL, GUINEA FOWL & PIGEON" s**ubmitted in partial fulfillment of the requirements for the **Degree of Master of Veterinary Science (Veterinary Anatomy & Histology)** of the Faculty of Post-Graduate Studies, Birsa Agricultural University, Ranchi (Jharkhand) is the record of bonafide research carried out by **Dr. Naveen Kumar** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that such help or information received during the course of this investigation and preparation of the thesis have been duly acknowledged.

Endorsed:

(Suresh Mehta)
Major Advisor

Certificate of the Advisory Committee Members

CERTIFICATE

We, the undersigned, members of the Advisory Committee of **Dr. Naveen Kumar** a candidate for the Degree of Master of Veterinary Science with major in Veterinary Anatomy and Histology have gone through the manuscript of the thesis and agree that the thesis entitle **"COMPARATIVE CYTOMORPHOLOGICAL, CYTOCHEMICAL AND CYTOENZYMIC STUDY ON BLOOD CELLS OF DOMESTIC FOWL, GUINEA FOWL & PIGEON"** may be submitted by **Dr. Naveen Kumar** in partial fulfillment of the requirements for the degree.

(Suresh Mehta)
Major Advisor

Members of the Advisory Committee

1. **(M.K. Gupta)**

2. **(A.K. Sharma)**

3. **(A. Ahmad)**

Certificate of approval by the Members of the Advisory Committee and External Examiner

CERTIFICATE

This is to certify that the thesis entitled "**COMPARATIVE CYTOMORPHOLOGICAL, CYTOCHEMICAL AND CYTOENZYMIC STUDY ON BLOOD CELLS OF DOMESTIC FOWL, GUINEA FOWL & PIGEON**" submitted by **Dr. Naveen Kumar** in partial fulfillment of the requirements for the Degree of Master of Veterinary Science (Veterinary Anatomy & Histology) of the Faculty of Post-Graduate Studies, Birsa Agricultural University, Ranchi (Jharkhand) was examined and approved on……

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INTRODUCTION

The livestock and poultry sector globally is highly dynamic. In developing countries, it is evolving in response to rapidly increasing demand for livestock and poultry products. India's poultry industry represents a major success story. While agricultural production has been rising at the rate around 2 percent per annum over the past two to three decades, poultry production has been rising at the rate of around 8 percent per annum, with an annual turnover of US$ 7 500 million.

India being an agricultural primed country has a gross share of our nation economy contributed by animal husbandry sector. Livestock production in general and poultry in particular play important socio-economic roles in developing countries (Alder, 2004; Salam, 2005). In recent years, the livestock and poultry sectors have become one of the fastest growing segments in Indian agriculture and contributing a substantial proportion to the national GDP. Poultry production in India has emerged as one of the fast growing sectors among various livestock based vocations as evident from its transformation from traditional backyard rearing to organized commercial farming over the last four decades. This sector comprises of low, medium and high input/ output systems of rearing and is providing employment to over 7 million people, apart from household income and nutritional security to numerous small poultry keepers in rural and tribal areas of the country. It contributes about 0.5 % to the national GDP and about 10% to the livestock GDP. The multifaceted growth in poultry and allied sectors is attributed due to the incessant efforts in advancement and application of new technologies. The development is not only in size but also in productivity and quality. Factors such as nutrition, housing, management and disease control have led to development of poultry sector which plays a pivotal role in the rural economy through a variety of contributions in the form of income generation, draft power, socio-economic upliftment, employment

avenues and better nutrition to human population through livestock products like eggs, meat & oil etc. Technological support, knowledge and research is, therefore, crucial for the sustained growth of the poultry sector.

The chicken ***Gallus domesticus*** is a domesticated fowl, a subspecies of Red jungle fowl. As one of the most common and widespread domestic bird, with a total population of more than 19 billion as of 2011. There are more chicken than any other birds or domesticated fowl. Human keep chickens primarily as a source of food (consuming both their meat and eggs) and, more rarely, as pets.

Genetic studies have pointed to multiple maternal origins in Southeast Asia, East Asia and South Asia, but with the clade found in the Americas, Europe, the Middle East and Africa originating in the Indian subcontinent. From India, the domesticated chicken was imported to Lydia in western Asia Minor and to Greece by the fifth century BC. Fowl had been known in Egypt since the mid-15th century BC, with the "bird that gives birth every day" having come to Egypt from the land between Syria and Shinar, Babylonia, according to the annals of Thutmose. The indigenous fowl is the back-bone of the backyard poultry farming in Jharkhand as well as in India. Most of the fowls are unique in their adaptation to the agroclimatic conditions of their habitat. These local birds are hardy, resistant against common poultry diseases, bio-controller of insects, good foragers and convertors of low quality fodder. There is a meagre information available on the blood cell morphology of Jharsem fowl. So, the motive of this study was to characterize the morphology of blood cells of adult Fowl and to see if any adaptive variation is there in blood cell picture of this fowl.

Guinea fowl **(*Numida meleagris)*** are the interesting gallinaceous bird and is sometimes called pintades or gleanies. It is family of birds originating from South Africa from where it spreads all over the continent, excluding desert regions, up to the

Mediterranean Sea. For a long period of time, the guinea fowl, and its eggs, was one of the main dishes of Africans. It can explain why this bird is more resistant to hot weather than the chicken. It ranks 3rd after duck and chicken. There are three species of guinea fowl the Vulturine, Crested and Helmeted. The Helmeted guinea fowl is the most common species of guinea fowl. It is estimated that few million birds are raised every year. It is referred by different local names in different regions, viz. chittra in west, chinamurgi in south and titari in the northorn plains. Meat of the guinea gowl is rich in essential fatty acid .it has high yield of 80% after processing with excellent meat to bone ratio. It can also be raised for ornamental value .It is a promising resourse for envolving a low input grain saving, poultry alternative for production in developing world.

It is also a source of ready cash for investment in education, healthcare, crop-livestock farming, purchase of grains to bridge gaps in food availability as well as in fixed assets.

There is a dearth of knowledge on haematological and blood biochemical parameters and factors regulating their levels in helmeted guinea fowls raised in Jharkhand. Also, there is increasing interest, in recent times, in raising exotic helmeted guinea fowls in India. Information on their blood profiles is currently not available to aid in assessing their health status and adaptability. Such information will therefore play a key role in the development of proper breeding and management strategies for disease prevention to enhance performance and adaptability of the bird in Jharkhand.

The domestic pigeon **(*Columba livia domestica*)** is a pigeon subspecies that was derived from the rock pigeon. The rock pigeon is the world's oldest domesticated bird. Mesopotamian cuneiform tablets mention the domestication of pigeons more than 5,000 years ago, as do Egyptian hieroglyphics. Research suggests that domestication of pigeons occurred as early as 10,000 years ago. Pigeon keeping/breeding, practiced for thousands

of years in almost every part of the world, has evolved into a hobby or a commercial enterprise for the purpose of aesthetic satisfaction, recreation, entertainment and food. The hobby of breeding or keeping pigeons by pigeon fanciers, for racing (sport), flying, homing and show, is a popular occupation throughout the world.

Pigeons have made contributions of considerable importance to humanity, especially in times of war. In war the homing ability of pigeons has been put to use by making them messengers. So-called war pigeons have carried many vital messages and some have been decorated for their services.

Pigeons are also kept by enthusiasts for the enjoyment of Flying/Sporting competitions. Breeds such as tipplers are flown in endurance contests by their owners.

In culinary terminology, squab is a young domestic pigeon, typically under four weeks old, or its meat. The meat is widely described as tasting like dark chicken. The term is probably of Scandinavian origin; the Swedish word *skvabb* means "loose, fat flesh". It formerly applied to all dove and pigeon species, such as the wood pigeon, the mourning dove, the extinct-in-the-wild socorro dove, and the now-extinct passenger pigeon, and their meat. More recently, squab meat comes almost entirely from domesticated pigeons. The meat of dove and pigeon game birds hunted primarily for sport is rarely called squab.

Pigeons are domestic bird & it’s very easy to handle them. They start laying eggs from six month of age and produce two baby pigeon per month on an average. Pigeon can be raised easily in the home yard and roof of the house. It takes about 18 days to hatch their eggs. Baby pigeon (squab) become suitable for consumption within their 3 to 4 weeks of age.

You can get maximum profit from them, by investing small capital and labor. Diseases are comparatively less in pigeons. Closet of pigeon is a good manure for crop cultivation.

Different types of toys can be made by the feather of pigeons. Pigeons help to keep the environment safe by eating different types of insects. The squab has a great demand in the market as a good patient's diet. Pigeons start laying eggs at their 5 to 6 months of age.

Pigeon farming can be a great income source for the people of some poor country and developing country.

The weather and vast are as of crop field along with housing premises of Jharkhand is suitable for pigeon farming. The contribution of pigeon have not yet been considered in relation to the contribution of livestock sub-sector and whole poultry production though the pigeons provide alternative source of animal protein. Comparatively low investment, care less, less feed and housing cost involved, easy and economic husbandry practices, short reproduction cycle and less disease occurrence are observed for pigeon farming. Pigeons are used in natural beautification and ornamental birds as source of recreation, source of palatable, delicious and easily digestible animal protein, sources of bio-fertilizer especially for family gardening and used as the laboratory animal in case of genetic and hormonal studies. Hence profitable pigeon farming may be an easy and reliable source of employment opportunity, way of family labourutilization and cash income. Sustainable and increasing rate of pigeon farming may enhance the rate of reducing the gap of animal protein consumption/deficiency; increase the rate of poverty reduction and it may improve the socio-economic status of the rural poor community. The ability of pigeon to carry messages has been reliably

exploited in the warfare, trade, friendship maintenance and political administration. But now a day, the pigeons have also been mainly reared for family nutrition and recreation.

Avian veterinarians often rely heavily on the results of various diagnostic tests, including hematology results. As such, cellular identification and evaluation of the cellular response are invaluable tools that help veterinarians understand the health or condition of their patient, as well as to monitor severity and clinical progression of disease and response to treatment. Therefore, it is important to thoroughly understand how to identify and evaluate changes in the avian erythron and leukon, as well as to interpret normal and abnormal results.

Clinical signs in birds are very non-specific, and physical exams provide limited information. Blood exams are an indispensable tool in bird medicine. Comprehensive health assessments on bird populations, as hematology, can be used to assess the effects of many health related problems, such as contaminant intoxication, malnutrition, and exposure to infection. With hematological exams, it is possible to qualitatively and quantitatively measure changes in the red and white blood cell fractions as well as changes in cell morphology that can assist in the diagnosis of several diseases and pathologies. The blood examination which is performed routinely to access general health, diagnosis of haematological diseases, accesses the body ability to respond to a haematological insult and monitor the course of certain diseases. Several protozoan parasites occur in blood of birds. The most important are Plasmodium, Haemoproteus and Leukocytozoon, but Trypanosoma and Lankersterella species and Piroplasma organism may also be found. Leukocytosis, predominantly from heterophilia occurs during acute inflammation in chickens. Infectious diseases such as coccidiosis, *Escherichia coli*, Air sacculitis and Schistosomiasis produce a similar pattern in chicken. Monocytosis is usually a consequence of chronic disease such as granulomatus lesion,

bacterial infection, parasitism, zinc deficiency etc. (Jain,1993). The mRNA of white blood cells were analysed for comparative demonstration of certain genes of poultry birds and other vertebrates.

In spite of large quantum of literature available on avian species the reports on cytomorphological features of blood cells are meagre. The blood of birds and mammals differs primarily in that birds have nucleated erythrocytes, nucleated thrombocytes and heterophils (the counterpart of mammalian neutrophils). Birds also differ in coagulation, particularly in intrinsic system, because they appear to lack coagulation factor V, VII,X, XI and XII. Avian erythrocytes function similarly to mammalian erythrocytes with some notable biochemical differences. 2, 3 – bisphosphoglycerate is present only in chicken embryonal erythrocytes and disappears shortly after hatch. Many factors influence the composition of drawn blood. These includes capillary venous large vessels or heart blood, time of day, genetic factors like breed, strain, age, sex, nutrition, environmental condition etc. Haematological parameters provide valuable information on the immune status of animals. Such information, apart from being useful for diagnostic and management purposes, could equally be incorporated into breeding programmes for the genetic improvement of indigenous chickens.

It is desirable to know the normal physiological values under local conditions for proper management, feeding, breeding, prevention and treatment of diseases. Major information about the local birds and their characterization are available in the literature and no reliable data describing poultry production are available. However, normal haematological information on Jharkhand local birds is hardly available in the literature as researches on these lines had rarely been carried out under local conditions. The present study was designed to partly rectify this deficiency and to provide baseline data on haematological parameters of three different species in Jharkhand. There may be

certain differences in cytomorphology of blood cells of two different breed of poultry birds. Before going details to access the health of different birds, we must have idea about general cytomorphological, cytochemical and cytoenzymic details of different blood cells.

So keeping the above facts in view present study had been design with following objectives:

OBJECTIVES OF INVESTIGATION:

1. Comparative cytomorphological studies on blood cells of domestic fowl, guinea fowl and pigeon
2. Comparative cytochemical studies of blood cells on domestic fowl, guinea fowl and pigeon
3. Comparative cytoenzymic studies on blood cells of domestic fowl, guinea fowl and pigeon

The cellular component of blood is three types.

(a) Erythrocytes

(b) Thrombocytes

(c) Leucocytes

The leucocytes are again divided into:

(i) Granulocytes (heterophils, eosinophils and basophils).

(ii) Agranulocytes (lymphocytes and monocytes).

The blood cells are suspended in the plasma. Which is fluid part of blood

2.1 CYTOMORPHOLOGICAL STUDIES:

Erythrocytes:

Sturky and Griminger (1986) stated that the avian erythrocytes were vary in size depending on the species, but they generally ranges between 10.7 × 6.1 µm to 15.8 × 10.2 µm.

Deldar *et al.* (1998) reported that the avian erythrocytes were typically elliptical and about 12 µm in length. They had elliptical, central heterochromatic nuclei and orange-pink cytoplasm. The reticulocytes can be identified with New Methylene Blue stain.

Bounous and Stedman (2000) observed that the mature chicken and turkey erythrocytes found in peripheral blood were large elliptical cells of 12 × 6 µm in size. They had a homogeneous eosinophilic cytoplasm and a central round to oval nucleus with a condensed chromatin pattern. The presence of reticulocytes in peripheral blood of normal chicken and turkey was some what higher than in most mammalian species.

Campbell *et al.* (2000) found that the mature psittacine erythrocytes were elliptical in outline with an elliptical, centrally positioned nucleus. Nuclear chromatin was uniformly clumped and became increasingly condensed with cellular age.

Salakij *et al.* (2003) reported that erythrocytes of the painted stork were homogeneous in colour but moderately heterogeneous in size and shape. Nuclei were oval to pleomorphic.

Thrall *et al.* (2004) stated that mature avian erythrocytes generally were larger than mammalian erythrocytes but smaller than reptilian erythrocytes. Mature avian erythrocytes were elliptical and have an elliptical, centrally positioned nucleus. The reticulocytes had a distinct ring of aggregated reticular material that encircles the nucleus.

Bonadiman *et al.* (2009) reported that ostrich erythrocytes were elliptically shaped with a central oval nucleus with condensed chromatin.

Claver and Quaglia (2009) observed that avian erythrocytes were oval in shape and nucleated. The nucleus of the avian erythrocytes were also oval in shape, and it becomes more condensed with age. The cytoplasm generally stained uniformly orange-pink, except for a thin, pale perinuclear band.

Sulaiman *et al.* (2010) stated that the Muscovy ducks erythrocytes were elliptical and nucleated, with orange-blue or greyish ink cytoplasm.

Gupta *et al.*(2012) found that the erythrocytes of guinea fowl were nucleated and elliptical or oval in shape. Their size varied from 15.7±0.20 µ in length and 7.2±0.16 µ in width.

GRANULOCYTES:

Heterophils:

Sturky and Griminger (1986) stated that the heterophils of the chicken were usually round with diameter of approximately 10-15 µm. There was presence of many rod- or spindle shaped acidophilic crystalline bodies in the cytoplasm. In routinely stained smears, these cytoplasmic bodies were frequently distorted. The nucleus was polymorphic with varying degree of lobulation.

Hawkey and Dennett (1989) found that the cytoplasmic matrix of mature avian heterophils stained colorless or faintly pink.

Randall and Reece (1996) reported that the cytoplasmic granules of all avian species were acidophilic in nature.

Deldar *et al.* (1998) stated that the avian heterophils had segmented heterochromatic nuclei and red-orange cytoplasm with distinctive granules. The nuclear segmentation was more prominent in heterophil than eosinophil and the nuclei were often masked by cytoplasmic granules. The cytoplasmic granules were large and reddish in rabbits, guinea pigs and chickens.

Maxwell and Robertson (1998) found that the avian heterophils were round cells and, their primary fusiform granules appear brick-red in color when stained with Romanovsky stains.

Bounous and Stedman (2000) observed in chicken and turkey the heterophil was prominent granulocyte. The mature heterophil was a round cell approximately 13 µm in diameter. The cytoplasm was colourless, with reddish-orange, rod shaped cytoplasmic granules that often partially obscure the nucleus. The nucleus had two to three lobes.

Campbell (2000) found that the heterophils of psittacine birds were most abundant leucocytes. The cytoplasm of normal mature heterophil appears colourless and contains eosinophilic elongated granules.

Salakij *et al.* (2003) stated that heterophils were the most commonly observed leucocytes in the female painted storks but were the second most commonly observed leucocytes in the males. They contained lobed nuclei and oval to pleomorphic granules which had dull and eosinophilic staining with Wright's-Giemsa stain. They were round and 8-12 µm in diameter.

Thrall *et al.* (2004) reported that in most of the birds the cytoplasm of normal, mature heterophil appears colourless and contain eosinophilic granules. The cytoplasmic granules were elongated. The nucleus of mature heterophil in healthy bird was lobed with course, clumped chromatin.

Kaufman and Murray (2008) found that avian heterophils were round cells with a colourless cytoplasm containing eosinophilic or basophilic rod-shaped granules. Heterophil granules usually had a distinct central body that appears refractile. Mature heterophils had a lobed nucleus with a coarse, clumped chromatin that stains purple. The nucleus was often partially hidden by the cytoplasmic granules.

Bonadiman *et al*. (2009) reported that ostrich heterophils had an eccentrically placed mostly bilobulated basophilic nucleus; however, segmented and trilobulated nuclei were also seen. A faint acidophilic cytoplasm with fusiform-shaped and highly acidophilic granules was observed.

Claver and Quaglia (2009) stated that avian heterophils had colourless cytoplasm and typical eosinophilic, rod-shaped granules, together with some other rounded granules. Specific granules were elliptical, although in some species they might be oval or rounded and had a distinct central body that appears refractile.

Sulaiman *et al.* (2010) found that the Muscovy ducks heterophils had an irregular cellular outline and the nucleus was polymorphs. The cytoplasmic granules were rod shaped and acidophilic.

Eosinophils:

Witkowski and Thaxton (1981) observed that in Japanese quail, the eosinophil cytoplasm contains many small, round granules as well as several defined vacuoles.

Sturky and Griminger (1986) reported that in fowl, polymorpho nuclear eosinophilic granulocytes were of about the same size as the heterophils. The granules were spherical and relatively large.

Maxwell (1987) found that the eosinophil cytoplasm was generally pale blue in colour compared with a colourless matrix of heterophil.

Bounous and Stedman (2000) observed that in chicken and turkey the eosinophil were round to irregular in shape and were approximately 12µm in diameter. They had a lobed nucleus and light blue cytoplasm with eosinophilic round to oval granules.

Campbell *et al.* (2000) reported that the psittacine eosinophil were round and had strongly eosinophilic cytoplasmic granules.

Salakij *et al.* (2003) stated that eosinophils were usually the largest WBCs in painted storks, average 9-19 µm in diameter. They contained lobed nuclei and many small round, bright eosinophilic granules

Thrall *et al.* (2004) reported that the avian eosinophils were having strongly eosinophilic cytoplasmic granules. The cytoplasm of eosinophil stained clear blue, in contrast to the colorless cytoplasm of normal, mature heterophil. The nuclei of eosinophils were lobed and usually stained darker than the heterophil nuclei.

Kaufman and Murray (2008) observed that avian eosinophils were typically round. The cytoplasm stained a clear, pale blue and contained round eosinophilic

granules and nuclei are lobed with coarse, clumped chromatin that stained purple, and nucleus often stains bluer.

Bonadiman *et al*. (2009) stated that ostrich eosinophils had an eccentrically placed basophilic, kidney-shaped, rarely lobulated nucleus. Their cytoplasm was semi translucent with a light blue color, containing many eosinophilic, round granules homogeneously distributed.

Claver and Quaglia (2009) reported that avian eosinophils had round, eosinophilic granules and a pale blue cytoplasm.

Sulaiman *et al.* (2010) found that the Muscovy ducks esinophils had a more regular cellular outline than heterophils and oval or round intracytoplasmic granules.

Basophils:

Hawkey *et al.* (1984b) observed the largest basophil from an African grey parrot with a diameter of 10.7 μm.

Sturky and Griminger (1986) had found in chicken polymorphonuclear basophilic granulocytes. The nucleus was weakly basophililc and round or oval in shape.

Maxwell and Robertson (1995) stated that the nucleus of avian basophil was usually non lobulated.

Deldar *et al.* (1998) found that avian basophil had dark blue granules and were readily distinguished from other leucocytes.

Bounous and Stedman (2000) stated that basophils of chicken and turkey were round cells of approximately 12 μm in diameter with a round, central, light blue nucleus which partially obscured by the deeply basophilic cytoplasmic granules.

Campbell *et al.* (2000) found the basophils of psittacine birds and observed the deeply stained metachromatic granules that often obscure the non-lobed nucleus.

Salakij *et al.* (2003) stated that basophils of painted storks were very small, average 6-9 µm in diameter which was smaller than heterophils or eosinophils.

Thrall *et al.* (2004) reported that the avian basophils frequently were found in the peripheral blood, in contrast to mammalian basophils. Avian basophil contains deeply stained metachromatic granules. The nucleus usually was non-lobed.

Kaufman and Murray (2008) observed that avian basophils were round cells with a round, centrally located nucleus. The nucleus stained a light blue and was often hidden by the cytoplasmic granules.

Bonadiman *et al.* (2009) found that ostrich basophils had a highly lobulated basophilic nucleus and their cytoplasm was basophilic, full of large, round basophilic granules.

Claver and Quaglia (2009) stated that avian basophils had a rounded nucleus and characteristic violet to reddish purple granules.

Sulaiman *et al.* (2010) reported that the Muscovy ducks basophils were ovoid in shape, with a slight basophilic to colourless cytoplasm, with strongly basophilic intracytoplasmic granules.

AGRANULOCYTES:

Lymphocytes:

Sturky and Griminger (1986) stated that the lymphocyte constitutes the majority of the leucocytes in the blood of the fowl. The cytoplasm was usually weakly basophilic consisting of a narrow rim bordering the nucleus in small lymphocytes.

Deldar *et al.* (1998) had examined the avian lymphocytes as small (6 to 9µm) and large (9 to 15 µm) lymphocytes. Small lymphocytes had round nuclei and scant pale blue cytoplasm. The nucleus of large lymphocytes contained a few nucleoli and is indented.

The cytoplasm was more abundant and stains homogenously blue, compared with dark-blue staining cytoplasm of small lymphocytes.

Bounous and Stedman (2000) stated that in chicken and turkey lymphocytes were the prominent leucocyte in the peripheral blood. The small lymphocytes were round cells with a round nucleus. Medium lymphocyte had more abundant and sometimes more pale basophilic cytoplasm.

Campbell *et al*. (2000) observed that the lymphocytes of psittacine birds resembled with mammalian lymphocytes. The lymphocyte cytoplasm usually appears homogeneous and weakly basophilic which lacks granules and vacuoles.

Salakij *et al.* (2003) stated that lymphocytes were the most prevalent circulating cells in the male painted storks. They were small, well differentiated and average 6-8 µm in diameter.

Thrall *et al*. (2004) observed that the avian lymphocytes were round in shape with slightly indented nucleus. The lymphocyte cytoplasm usually appears to be homogeneous and weakly basophilic (pale blue), and it lacks both vacuoles and granules.

Kaufman and Murray (2008) found that avian lymphocytes can be classified into three groups according to cell size (small, medium, and large). Most normal mature lymphocytes in the peripheral blood were small or medium. Lymphocytes were typically round cells. The nucleus was usually round and centrally located. The amount of cytoplasm varies from a narrow band surrounding the nucleus in small lymphocytes to a moderately wide band in medium and large lymphocytes.

Bonadiman *et al.* (2009) observed that ostrich lymphocytes were smaller, had more granules, more condensed chromatin, and a higher nucleus : cytoplasm ratio than monocytes.

Claver and Quaglia (2009) stated that avian lymphocytes were small to large, rounded to irregular cells with a round nucleus and scant to abundant basophilic cytoplasm. Small lymphocytes predominantly found in most birds, with irregular projections or blebs frequently observed on this cell type.

Sulaiman *et al.* (2010) studied that the Muscovy ducks lymphocytes showed variability in size and had a thin cytoplasm bordering the nucleus.

Monocytes:

Jain *et al.* (1993) recorded that the monocytes were usually the largest of the leukocytes seen in the blood smears of birds. Their nuclei were usually round or oval in shape however few elongated nuclei with indentation on one side were also seen. Their cytoplasm was usually abundant and frequently vacuolated or foamy.

Deldar *et al.* (1998) found that the monocytes were the largest leucocytes present in avian blood. They had pleomorphic nuclei which appear spherical, ovoid, elongated and indented. Their cytoplasm is relatively abundant, foamy and occasionally vacuolated with no visible granules.

Bounous and Stedman (2000) examined that the chicken and turkey monocytes usually were the largest leukocytes (approximately 14 μm in diameter). Their nuclei were pleomorphic and indented.

Campbell *et al.* (2000) stated that the monocytes in peripheral blood films of psittacine birds as the largest leucocyte.

Thrall *et al*. (2004) found that the avian monocytes were the largest leucocyte. Monocytes had abundant blue-gray cytoplasm containing vacuoles and fine-dust like eosinophilic granules. The monocytes nucleus vary in shape and with less chromatin clumping compared with lymphocyte nuclei.

Kaufman and Murray (2008) found that avian monocytes were large leucocytes that were irregular in shape. The nuclei vary from round to bi-lobed. The cytoplasm of monocytes stained blue-gray with a finely granular appearance and occasionally contained vacuoles.

Bonadiman *et al.* (2009) observed that ostrich monocytes were larger, had rarer granules, less condensed chromatin, and a lower nucleus : cytoplasm ratio than lymphocytes.

Claver and Quaglia (2009) found that avian monocyte were round, with a kidney-shaped nucleus. The cytoplasm were generally deep blue or grayish blue, often presented a pink- or purple-stained granular area near the nucleus.

Sulaiman *et al.* (2010) found that the Muscovy ducks monocytes were observed to be similar to the lymphocytes though larger than the lymphocytes. The nucleus of the monocytes was kidney shaped or oval and the cells had light basophilic cytoplasm.

Thrombocytes:

Campbell *et al.* (2000) observed that the thrombocytes in psittacine birds. The cytoplasm was colorless to pale gray and contained one or more distinct eosinophilic granules. The thrombocytes of duck and goose resemble those of other birds.

Salakij *et al.* (2003) stated that thrombocytes of the painted stork were elongate cells, approximately half the size of mature RBCs. Nuclei were oval, with dense chromatin. When thrombocytes aggregated they turned into round cells. However, they were easily differentiated from lymphocytes by a characteristic perinuclear cytoplasmic vacuolation.

Santos *et al.* (2003) had examined that roadside hawk thrombocytes presented mostly elliptic aspect and a slightly spherical and oval shape as well. The basophilic

nucleus occupies great part of its own volume in relation to the cytoplasm showing chromatin relatively condensed in a rough and grumous way.

Thrall *et al.* (2004) stated that the thrombocytes were nucleated cells. The nucleus was more rounded than the erythrocyte nucleus. Normal, mature thrombocytes have a colorless to pale-gray cytoplasm. Cytoplasmic vacuolation can occur in activated or phagocytic thrombocytes. Thrombocytes frequently contain one or more distinct eosinophilic granules, which usually was located in one area of the cytoplasm. Activated thrombocytes occurring in aggregates had indistinct cellular outlines or cytoplasmic pseudopodia.

Kaufman and Murray (2008) found that avian mature thrombocytes were oval cells that were smaller and more rounded than mature erythrocytes. The thrombocyte nuclei were larger in relation to the amount of cytoplasm and more rounded than erythrocyte nuclei. The cytoplasm was clear but not homogenous and often had a reticulated appearance.

Bonadiman *et al.* (2009) observed that ostrich thrombocytes had heterogeneous shape and were often found in clumps. Their nuclei were highly basophilic, and scarce hyaline cytoplasm without granules and vacuoles was occasionally observed.

Claver and Quaglia (2009) stated that avian thrombocytes were round to oval cells and had an oval to rounded nucleus. The cytoplasm was light blue or colour less, often vacuolated with a few acidophilic granulations.

2.1.2 CYTOCHEMICAL STUDIES:

Erythrocytes:

Salakij *et al.* (2003) stated thatreticulocytes that contained distinct aggregated reticulum were aggregate reticulocytes whereas puntate reticulocytes contained a few dots. The female painted storks had a higher number of aggregate reticulocytes but a

lower number of puntate reticulocytes than the male painted storks. RBCs were positive with SBB staining.

GRANULOCYTES:

Heterophils:

Andreasen and Latimer (1990) observed that the chicken heterophil granules were negative for Sudan Black B and Periodic Acid-Schiff.

Salakij *et al.* (2003) found that heterophils of painted storks were stained weakly or were negative with Sudan black B.

Eosinophils:

Andreasen and Latimer (1990) observed that the chicken eosinophil indicate a positive reactivity for Sudan Black B while heterophils were negative.

Yadav *et al.* (2012) found that the eosinophil showed strong positive reaction in the form of intense black coloured granules when stained with Sudan black B.

Basophils:

Bowers *et al.* (1981) observed that in a comparative study of the histochemical properties of pigeon basophils and mast cells, the pigeon basophils had only a 'trace' or no glycogen, a mucopolysachharide-glycoprotein complex, a small amount of histadin and no histamine

Yadav *et al.* (2012) found that the granules of basophil stained metachromatically in the form of intense violet coloured with 1% toluidine blue for mucopolysaccharides.

Khan *et al.* (2015) reported that in uttra fowl basophil were satained metachromatically in the form of intense violet coloured granules, obscuring the nucleus were stained with 1%toluidine blue for mucopolysaccharide.

AGRANULOCYTES:

Lymphocytes:

Singh *et al.* (1998) stated that the lymphocytes of camel were negative for Sudan black B and PAS.

Singh and Menaka (2004b) found that the cytoplasmic granules of sheep basophil were stained metachromatically with toluidine blue and looked intense violet.

Casal and Oros (2007) observed that juvenile loggerhead sea turtles lymphocytes were negatively stained with Sudan black B, PAS and toluidine blue.

Shigdar *et al.* (2009) found that in Murray cod lymphocytes showed positivity with Sudan black B and PAS staining.

Techangamsuwan *et al.* (2010) reported that Dolphin lymphocytes were negative for sudan black B (SBB).

Monocytes:

Casal and Oros (2007) found that juvenile logger head sea turtles monocytes were negatively stained with Sudan black B, PAS and toluidine blue.

Shigdar *et al.* (2009) observed that in Murray cod monocytes showed negative staining for Sudan black B and PAS staining.

Techangamsuwan *et al.* (2010) stated that Dolphin monocytes were negative for sudan black B (SBB).

Thrombocytes:

Santos *et al.* (2003) examined that roadside hawk thrombocytes showed the positive staining with PAS where as negative staining with Sudan black B.

2.1.3 CYTOENZYMIC STUDIES:

Heterophils:

Andreasen and Latimer (1990) observed that the chicken heterophil granules were negative for alkaline phosphatase, peroxidase, acid phosphatase, naphthol AS-D choloroacetate esterase.

Lam *et al.* (1997) stated that the chicken heterophils contain slight myeloperoxidase activity.

Bounous and Stedman (2000) stated that the chicken and turkey heterophils were lack myeloperoxidase and alkaline phosphate enzymes.

Bonadiman *et al.* (2009) reported that ostrich heterophil granules did not show peroxidase staining.

Claver and Quaglia (2009) stated that avian heterophils were devoid of myeloperoxidase whereas showed positivity with acid phosphatase.

Eosinophils:

Andreasen and Latimer (1990) found that the chicken eosinophil indicate a positive reactivity for peroxidase and acid phosphatase, while heterophils were negative.

Bonadiman *et al.* (2009) reported that ostrich eosinophil granules were highly positive for peroxidase staining.

Claver and Quaglia (2009) found that avian eosinophil granules were peroxidase, acid phosphatase positive.

Basophils:

Maxwell and Robertson (1995) reported that, unlike those of mammals, avian basophil granules contain no peroxidase but do contain acid phosphatase.

Lymphocytes:

Singh *et al.* (1997b) observed that the lymphocytes of camel showed negative reactions for peroxidase, alkaline phosphatase and naphthyl AS-D chloroacetate esterase, where as positive activity for acid alpha naphthyl acetate esterase and acid phosphatase.

Singh *et al.* (1998) found that the lymphocytes of camel showed negative reactions for peroxidase and alkaline phosphatase whereas, positive for acid phosphatase.

Shigdar *et al.* (2009) found that in Murray cod lymphocytes were positive for acid phosphatase, alkaline phosphatase, naphthol AS chloroacetate esterase and naphthyl acetate esterase where as negative for peroxidase and α-naphthyl butyrate esterase activity.

Prasad and Charles (2010) studied that yellow cat fish lymphocytes were negative for alkaline phosphatase, alpha-naphthyl acetate esterase, naphthol ASD chloroacetate esterase and positive for peroxidase.

Techangamsuwan *et al.* (2010) stated that Dolphin lymphocytes stained positive for acid phosphatase, weakly positive for alkaline phosphatase and negative for myeloperoxidase.

Mehta *et al.* (2012) reported that the lymphocyte of pig showed negative reaction when blood smears were stained for acid phosphatase.

Monocytes:

Singh *et al.* (1997b) reported that the monocytes of camel were positive for acid alpha naphthyl acetate esterase and acid phosphatase, whereas, weak or negative for peroxiodase and naphthal SA-D chloroacetate esterase and alkaline phosphatase.

Singh *et al.* (1998) observed that the monocytes of camel were positive for acid phosphatase, whereas weak positive or negative for peroxidase and alkaline phosphatase.

Shigdar *et al.* (2009) found that in Murray cod monocytes were positive for acid phosphatase and naphthyl acetate esterase where as negative for peroxidase, alkaline phosphatase, naphthol AS chloroacetate esterase and α-naphthyl butyrate esterase activity.

Prasad and Charles (2010) stated that yellow cat fish monocytes were negative for alkaline phosphatase, naphthol ASD chloroacetate esterase and peroxidase and weakly positive for alpha-naphthyl acetate esterase.

Techangamsuwan *et al.* (2010) studied that Dolphin monocytes stained positive for myeloperoxidase, weakly positive for alkaline phosphatase and negative for acid phosphatase.

Mehta *et al.* (2012) reported that the monocyte of pig showed negative reaction when blood smears were stained for acid phosphatase.

Thrombocytes:

Santos *et al.* (2003) observed that the roadside hawk thrombocytes showed the positivity with Myeloperoxidase and slight positivity with acid phosphatase.

1ml blood samples were collected using 2ml syringe with 24 gauze needle from wing vein of ten healthy domestic fowl, guinea fowl and pigeon maintained at Department of Veterinary Anatomy & Histology, Ranchi Veterinary College, BAU, Kanke. Immediately after collection blood was transferred to siliconized tube containing EDTA as anticoagulant. Immediately after collection, the blood samples were brought to the laboratory and smears were preparedon grease free slides. Blood films were stained with the following staining procedures.

Cytomorphological studies

1. May GrunwaldGiemsa stain (Bover, 1964)

Cytochemical studies

1. Toluidene blue stain for acid mucopolysaccharide(Bover,1964)
2. Periodic acid Schiff's stain for glycogen (Jain, 1986)
3. Sudan black- B stain for lipid (Jain, 1986)

Cytoenzymic studies

1. Acid phosphatase enzyme (Bover, 1964)
2. Alkaline phosphatase enzyme (Bover, 1964)
3. Peroxidase enzyme (Bover, 1964)
4. Esterase (Non-specific) enzyme (Jain, 1986)

The stained blood smears were examined under oil immersion objective (100X) lens to record the results.

The occulometer was used to record the dimension of different cells.

4. Comparative Cytomorphological, cytochemical and cytoenzymic study on blood cells of domestic fowl, guinea fowl and pigeon

4.1. Comparative Cytomorphological, cytochemical and cytoenzymic study on blood cells ofdomestic fowl

4.1.1 Cytomorphological studies

Erythrocytes

The erythrocytes of domestic fowl were rounded to oval in shape. Their average size was measured 10.21±0.29μm in length and 8.40±0.38 μm in width (Table 1). The nuclei were oval, rounded and elliptical in shape. The cytoplasm was pink in colour and homogenous when stained with May Grunwald Giemsa Stain (Fig. 1). A very few erythrocytes were Crenated and characterized by pointed cell margins.

Granulocytes

Heterophils

The heterophil was the largest cell among leukocytes of domestic fowl, which were almost rounded in shape and measured 10.99±0.18μm in diameter (Table 1).The nuclei were varied from 2-4 lobed and varied in shape, size and number. The arrangement of nuclear materials were spiral, S, and M shaped. The pale cytoplasm was laden with colourless to faint pink coloured fine granules when stained with MGG (Fig.4).

Eosinophils

The eosinophils were rounded in shape and measured 9.11±0.25 μm in diameter (Table 1). The nuclei were varied from bilobed to trilobed. The cytoplasmic granules

were rounded and numerous, homogeneously distributed throughout the cytoplasm and strongly eosinophilic when stained with MGG (Fig. 7).

Basophils

The basophils were irregularly rounded in shape which messured 7.53±0.20 μm in diameter (Table 1).The nuclei were not clearly visible. The cytoplasm was lighter and give a foamy appearance (Fig. 10).

Agranulocytes

Lymphocytes

Depending upon their size lymphocytes were categorized as small, medium and large lymphocytes. Small sized lymphocytes were almost rounded in shape and measured 7.90±0.07 μm diameter (Table 1) which had almost spherical nuclei and surrounded by a thin peripheral cytoplasm (Fig.19).The cytoplasm was comparatively more bluish in colour. The medium sized lymphocytes were elongated to rounded in shape and measured 8.50±0.12μm in diameter (Table 1) and cytoplasm was comparatively more in comparison to small lymphocyte (Fig.16).The large lymphocytes were rounded to oval in shape (Fig.13), which measured10.09 ±0.18 μm in diameter (Table 1).

Monocytes

The monocytes were almost rounded in shape which measured 10.19±0.11 μm in dameter (Table 1). The nuclei were eccentric in position and rounded in shape. The cytoplasm was foamy in appearance due to presence of numerous vacuoles (Fig.22).

Thrombocytes

The thrombocytes were mostly oval in shape and measured 4.20±0.08 μm in diameter (Table 1). They appeared mostly single or in small group (Fig.25). Their nuclei were irregularly rounded to oval in shape. Cytoplasm was non-granular.

4.1.2 Cytochemical studies

Mucopolysaccharides

The granules of basophils showed weak positive reaction and stained in the form of mild violet coloured granules when the blood smears were stained with toluidine blue (Fig.28) while other leukocytes showed negative reaction for mucopolysaccharides.

Glycogen

The heterophils (Fig.31) showed very weak positive reaction in the form of mild red coloured granules in the cytoplasm when the blood smears were stained with the PAS. Whereas, other leukocytes showed negative reaction.

General lipids

The eosinophils showed strong positive reaction in the form of greenish black coloured granules (Fig.34). While other leukocytes were negative reaction for lipid when blood smear were stained with Sudan black-B.

4.1.3 Cytoenzymic studies

Acid phosphatase

The lymphocytes (Fig.37) showed strong positive reaction in the form of brown patches or dot when blood smears were stained for acid phosphatase however, other leukocytes were negative for acid phosphatase.

Alkaline phosphatase

The eosinophils (Fig. 40) showed weak positive reaction in the form of mild brown coloured granules when blood smear were stained for alkaline phosphatase. While other leukocytes were negative for alkaline phosphatase.

Peroxidase

The eosinophils showed moderately positive reaction (Fig.56a) in the form of bluish coloured granules when blood smears were stain for peroxidase however; other leukocytes (Fig. 43) were negative for peroxidase.

Non-specific esterase

All leukocytes(Fig. 46) showed negative reaction for non- specific esterase.

4.1. Comparative Cytomorphological, cytochemical and cytoenzymic study on blood cells of Guinea fowl

4.1.1 Cytomorphological studies

Erythrocytes

The erythrocytes of guinea fowl were elongated in shape. Their average length was measured 12.03±0.20μmand width was 7.01±0.06 μm (Table 2). The nuclei were elongated in shape. The cytoplasm was light pink in colour and homogenous when stained with May Grunwald Giemsa Stain (MGG) (Fig. 2).

Granulocytes

Heterophils

The heterophils of guinea fowl were almost rounded and with slight bulging in shape which measured 11.35±0.13 μm in diameter(Table 2).The nuclei were varied from three to four lobed, and varied in shape, size and number. The arrangement of nuclear

materials were U and eight shaped. The cytoplasm was dark in colour with few lightly stained area when stained with MGG (Fig.5).

Eosinophils

The eosinophils were rounded in shape and measured 11.50±0.12 μm in diameter (Table 2). The nuclei were varied from three to four lobed. The cytoplasmic granules were densly packed and comparatively darker when stained with MGG (Fig. 8).

Basophils

The basophils were oval to elongated in shape which messured8.30±0.16 μm in diameter (Table 2).The nuclei were not clearly visible. The cytoplasm was comparatively more basophilic and filled with abundent, dark, rounded basophilic granules(Fig. 11).

Agranulocytes

Lymphocytes

Small sized lymphocytes weremeasured8.40±0.15 μm diameter (Table 2) which had almost rounded with few blebs in shape and nuclei surrounded by more cytoplasm at peripheri (Fig.20).The cytoplasm was comparatively pale in colour. The medium sized lymphocytes were rounded to oval in shape and measured 9.92±0.16μm in diameter (Table 2) and cytoplasm was comparatively less in comparison to small lymphocyte (Fig.17). The large lymphocytes were irregularly rounded in shape (Fig.14), which measured 10.94 ±0.11 μm in diameter (Table 2).

Monocytes

The monocytes were the largest cell among leukocytes of guinea fowl, which were elliptical to rounded in shape and measured 12.00±0.13μm in diameter. The nuclei

were eccentric and horse shoe shaped. The cytoplasm was comparatively more and foamy in appearance due to presence of numerous of vacuoles (Fig.23).

Thrombocytes

The thrombocytes were oval to rounded in shape and measured 4.80±0.16 μm in diameter (Table 2). They appeared mostly in large group (Fig.26). Their nuclei were irregularly rounded to oval in shape. Cytoplasm was non-granular.

4.1.2 Cytochemical studies

Mucopolysaccharides

The granules of basophils showed strong positive reaction and stained in the form of violet coloured granules when the blood smears were stained with toluidine blue (Fig.29) whereas other leukocytes showed negative reaction for mucopolysaccharides.

Glycogen

The heterophils (Fig. 32) showed strong positive reaction in the form of red coloured granules in the cytoplasm when the blood smears were stained with the PAS. While other leukocytes showed negative reaction.

General lipids

The eosinophils showed weakly positive reaction in the form of black coloured granules (Fig.35). However, other leukocytes were negative reaction for lipid when blood smears were stained with Sudan black-B.

4.1.3 Cytoenzymic studies

Acid phosphatase

The lymphocytes (Fig.38) showed moderately positive reaction in the form of brown patches or dot when blood smears were stained for acid phosphatase whereas other leukocytes were negative for acid phosphatase.

Alkaline phosphatase

The eosinophils (Fig.41) showed moderately positive reaction in the form of brown coloured granules when blood smears were stained for alkaline phosphatase. On the other hand leukocytes were negative for alkaline phosphatase.

Peroxidase

The eosinophils showed strong positive reaction(Fig.44) in the form of bluish coloured granules when blood smear were stain for peroxidase,while other leukocytes were negative for peroxidase.

Non -specific esterase

All leukocytes (Fig.47) showed negative reaction for non- specific esterase.

4.1. Comparative Cytomorphological, cytochemical and cytoenzymic study on blood cells of pigeon

4.1.1 Cytomorphological studies

Erythrocytes

Erythrocytes of pigeon were largest blood cells which were elongated in shape. The average size was measured 11.66±0.22μm in length and 6.19±0.15 μm in width (Table 3). The nuclei were mostly elongated in shape. The cytoplasm was homogenous and pink in colour when stained with May Grunwald Giemsa Stain. (MGG) (Fig. 3)

Granulocytes

Heterophils

The heterophils of pigeon were almost rounded in shape and measured 10.11±0.17μm in diameter (Table 3).The nuclei were varied from two to three lobed and varied in shape, size and number. The arrangement of nuclear materials were S, U and V shaped. The cytoplasm was lightly stained when stained with MGG (Fig.6).

Eosinophils

The eosinophils were rounded to oval in shape and measured 10.44±0.26 μm in diameter (Table 3). The nuclei were varied from two to four lobed. The cytoplasmic granules were rounded and loosely packed, numerous, homogeneously distributed throughout the cytoplasm and strongly eosinophilic when stained with MGG (Fig. 9).

Basophils

The basophils were rounded in shape which mesured7.73±0.15 μm in diameter (Table 3).The nuclei were not clearly visible. The cytoplasm was comparatively lighter and filled with abundent, light, rounded basophilic granules (Fig. 12).

Agranulocytes

Lymphocytes

Small sized lymphocytes were measured of 8.27±0.11 μm diameter (Table 3) which had almost rounded to oval in shape and nuclei were almost rounded and occupied almost all areas (Fig.21).The cytoplasm was comparatively less and bluish in colour. The medium sized lymphocytes were irregularly rounded in shape and measured 9.50±0.16μm in diameter (Table 3) and cytoplasm was comparatively less in comparison

to small lymphocytes (Fig.18). The large lymphocytes were irregularly rounded in shape (Fig.15), which measured 10.30 ±0.10 μm in diameter (Table 3).

Monocytes

The monocytes were almost rounded in shape which measured 10.82±0.10 μm in dameter (Table 3). The nuclei were eccentric in position and irregular in shape. The cytoplasm was foamy in appearance due to presence of numerous vacuoles (Fig.24).

Thrombocytes

The thrombocytes were oval to rounded in shape and measured 5.86±0.17 μm in diameter (Table 3). They appeared mostly in single (Fig.27). Their nuclei were irregularly rounded to oval in shape. Cytoplasm was non-granular.

4.1.2 Cytochemical studies

Mucopolysaccharides

The granules of basophils showed moderately positive reaction and stained in the form of mild violet coloured granules when the blood smears were stained with toluidine blue (Fig.30) while other leukocytes showed negative reaction for mucopolysaccharides.

Glycogen

The heterophils (Fig. 33) showed moderately positive reaction in the form of mild red coloured granules in the cytoplasm when the blood smears were stained with the PAS. Whereas, other leukocytes showed negative reaction.

General lipids

The eosinophils showed moderately positive reaction in the form of black coloured granules (Fig.36). While other leukocytes were negative reaction for lipid when blood smears were stained with Sudan black-B.

4.1.3 Cytoenzymic studies

Acid phosphatase

The lymphocytes (Fig.39) showed weakly positive reaction in the form of brown patches or dot when blood smears were stained for acid phosphatase however, other leukocytes were negative for acid phosphatase.

Alkaline phosphatase

The eosinophils (Fig. 42) showed moderately positive reaction in the form of mild brown coloured granules when blood smear were stained for alkaline phosphatase. However other leukocytes were negative for alkaline phosphatase.

Peroxidase

The eosinophils showed strong positive reaction (Fig.45) in the form of bluish coloured granules when blood smears were stain for peroxidase, while other leukocytes were negative for peroxidase.

Non -specific esterase

All leukocytes (Fig. 48) showed negative reaction for non- specific esterase.

4.4. Comparative micrometric studies on blood cells of domestic fowl, guinea fowl and pigeon

Analysis of variance presented in Table 4 showed significant ($P<0.01$) effect of group on the blood cells. Further critical difference test presented in Table 5 indicated significantly higher length of erythrocytes observed in guinea fowl (12.03±0.20 µm) followed by pigeon (11.66±.22 µm) and fowl (10.21±0.29 µm).Average length of erythrocytes varied significantly in domestic fowl and guinea fowl however length of erythrocytes differ non significantly in guinea fowl and pigeon. On the other hand width of erythrocytes were higher in fowl (8.4±0.38) then guinea fowl (7.01±0.06 µm) and lowest in pigeon (6.19±0.15 µm) however, differences being significant among all the three group of birds.

Although diameter of heterophil was maximum in guinea fowl (11.35±0.13 µm) followed by domestic fowl (10.99±0.18 µm) and pigeon (10.11±0.17 µm). The critical difference test revealed that significant difference was observed between guinea fowl and pigeon however, difference was non significant between guinea fowl and domestic fowl.

With regard to diameter of eosinophil, the highest value was recorded in guinea fowl (11.5±0.36 µm) and pigeon (10.44±0.26 µm) then fowl (9.11±0.25 µm). However, the differences were significant among all three groups of birds.

Average diameter of basophils was higher in guinea fowl (8.3±0.16 µm) followed by pigeon (7.73±0.15 µm) and fowl (7.53±0.20 µm).The critical difference test revealed that guinea fowl differ significantly to domestic fowl and pigeon however, non significant difference was observed between domestic fowl and pigeon. As regards diameter of lymphocytes, all three i.e. small, medium and large were significantly higher value in guinea fowl followed by pigeon and fowl. Further critical difference test revealed that significant difference was observed among domestic fowl and guinea fowl.

Average diameter of monocytes were higher in guinea fowl(12.0±0.13 µm) followed by pigeon(10.82±0.14 µm) and fowl(10.19±0.11 µm), while difference were significant in all three group of birds.

Thrombocytes had highest value in pigeon (5.86±0.10 µm) then guinea fowl (4.8±0.16 µm) and lowest in fowl (4.2±0.08 µm). All the three groups differ significantly among themselves.

Table-1: Micrometric Parameters of Different Blood Cells of Fowl

(Mean Diameter in µm)

Name of cells / No. of cells		1	2	3	4	5	6	7	8	9	10	Mean±S.E
Erythrocytes	Length	8.9	10.5	9.8	11.8	11	9	10.1	10	9.7	11.3	10.21 ±0.29
	Width	8.5	9	8	9.4	9.7	6	8.4	8.1	7	9.9	8.4±0.38
Heterophils		9	10.2	10	10.5	9.1	11.2	10.6	11	9.8	10.9	10.23±0.24
Eosinophils		7.8	8	7.5	8.2	8	9	7	9.4	9	7.2	8.11 ±0.25
Basophils		8	7	7.5	7.2	8	6.2	7.2	7.8	8.4	8	7.53±0.20
Lymphocytes	Small	5.9	6.2	6.2	6	6.5	6	6	6.5	6.5	6	6.18±0.07
	Medium	8	7	7.5	8	7.8	7	8	8	7.5	7.6	7.64 ±0.12
	Large	9	9	8.5	9	8.5	9.4	9	8.5	9.5	9	8.94±0.11
Monocytes		10.2	10	9.6	10	9.5	10	9.8	9.5	10	9.5	9.81±0.083
Thrombocytes		4.5	4	4.5	4.5	3.8	4	4.5	4	4	4.2	4.20±0.08

Table-2: Micrometric Parameters of Different Blood Cells of Guinea fowl

(Mean Diameter in µm)

Name of cells / No. of cells		1	2	3	4	5	6	7	8	9	10	Mean ±S.E
Erythrocytes	Length	13	12.5	12.7	10.9	12	12	12	12	11.5	11.7	12.03±0.19
	Width	7.1	7	7	6.7	6.9	7	7.2	7.4	7	6.8	7.01±0.06
Heterophils		11	11.2	12	12	11.5	11.4	10.8	10.9	11.7	11	11.35±0.14
Eosinophils		12.5	11.5	11	10	10	11.3	12.5	11	12.7	12.5	11.5±0.32
Basophils		9	9.5	8	7.5	7.5	7.9	8.5	8.3	8.3	8.5	8.3±0.19
Lymphocytes	Small	8.3	8.5	9	9.1	8.2	7.8	8	8.1	8.6	8.4	8.4±0.13
	Medium	9	10.2	10	10	11	9.5	9.8	10.1	9.9	9.7	9.92±0.16
	Large	11.4	12	12	11	11	10	10	10	12	10	10.94±0.28
Monocytes		12	12.5	12	11.9	11.6	11.2	12.8	12	13	11	12±0.20
Thrombocytes		4	4.5	5	5.2	3.9	5	5	5.6	4.8	5	4.8±0.16

Table-3: Micrometric Parameters of Different Blood Cells of Pigeon

(Mean Diameter in µm)

Name of cells / No. of cells		1	2	3	4	5	6	7	8	9	10	Mean±S.E
Erythrocytes	Length	11.5	12	11	12.5	12	10.5	10.7	12	12.6	11.8	11.66±0.22
	Width	6.8	6	6.5	5.5	6.5	6.6	5.9	5.4	6.2	6.5	6.19±0.15
Heterophils		10	11	10	10.5	11	10	9	9.9	10	9.7	10.11 ±0.18
Eosinophils		11.7	10	10	10.5	9	9.8	10.7	11.2	10	11.5	10.44±0.26
Basophils		8.5	8	8	7.5	7.9	6.9	7	7.9	8	7.6	7.73±0.15
Lymphocytes	Small	8.5	8	8.7	7.9	7.8	8.6	8	8.5	8	8.7	8.27±0.11
	Medium	9.6	10	10	9.5	9.2	9.5	8.9	9	9.3	10	9.50 ±0.12
	Large	10.5	10	9.9	10.7	9.8	10.5	10.5	10.5	10	10.6	10.30±0.13
Monocytes		11	11	11	10.5	10.4	10.9	10.6	10.5	10.8	11.5	10.82±0.13
Thrombocytes		6.8	5.9	6	6	5.5	5	5	6.2	6	6.2	5.86±0.17

Table-4: Analysis of variance showing the effect of group of birds on morphometry of blood cells

TYPES OF BLOOD CELLS	SOURCE OF VARIATION	df	SS	MS	F
Erythrocytes (Length)	BETWEEN GROUP	2	18.50	9.25	15.01**
	WITHIN GROUP	27	16.6	0.61	
Erythrocytes (Width)	BETWEEN GROUP	2	24.96	12.48	21.49**
	WITHIN GROUP	27	15.67	0.58	
Heterophils	BETWEEN GROUP	2	8.13	4.06	13.83 **
	WITHIN GROUP	27	7.94	0.29	
Eosinophils	BETWEEN GROUP	2	28.68	14.34	18.44**
	WITHIN GROUP	27	20.99	0.77	
Basophils	BETWEEN GROUP	2	3.19	1.59	4.55*
	WITHIN GROUP	27	9.46	0.35	

TYPES OF BLOOD CELLS	SOURCE OF VARIATION	df	SS	MS	F
Lymphocytes (Small)	BETWEEN GROUP	2	1.34	0.67	4.07*
	WITHIN GROUP	27	4.46	0.16	
Lymphocytes (Medium)	BETWEEN GROUP	2	10.64	5.39	22.11**
	WITHIN GROUP	27	6.49	0.24	
Lymphocytes (Large)	BETWEEN GROUP	2	3.92	1.96	4.72**
	WITHIN GROUP	27	11.21	0.41	
Monocytes	BETWEEN GROUP	2	16.88	8.44	39.26**
	WITHIN GROUP	27	5.80	0.21	
Thrombocytes	BETWEEN GROUP	2	14.13	7.06	31.98**
	WITHIN GROUP	27	5.96	0.22	

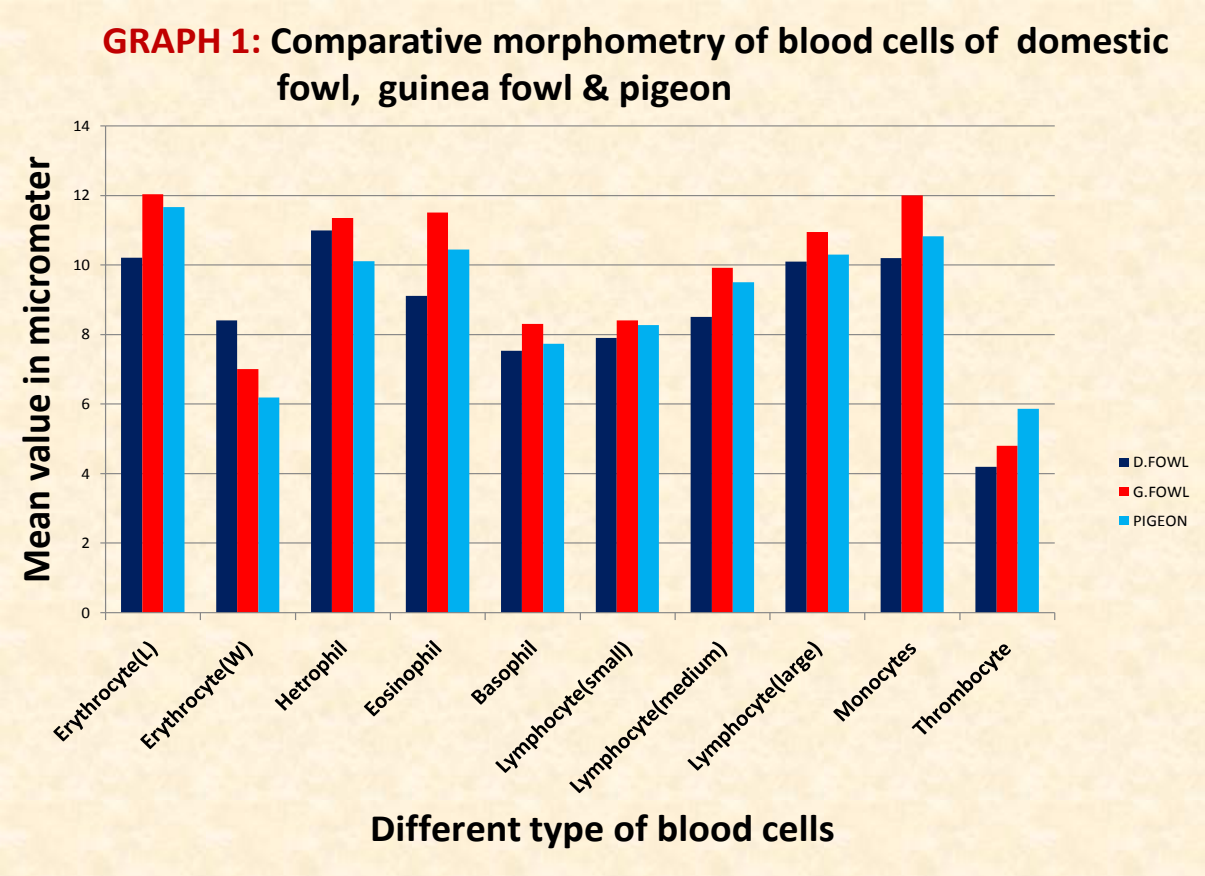
** - P < 0.01,NS - Non significant

Table-5: Comparative morphometry of blood cells ofdomestic fowl, guinea fowl and pigeon.

SPECIES / TYPE OF CELL		FOWL	DUCK	QUAIL	C.D. VALUE
ERYTHROCYTES	LENGTH (µm)	10.21 ± 0.29 [a]	12.03 ± 0.20 [b]	11.66 ± 0.22 [b]	0.72
	WIDTH (µm)	8.4 ± 0.38 [a]	7.01 ± 0.06[b]	6.19 ± 0.15 [c]	0.69
HETEROPHILS(µm)		10.99 ± 0.18 [b]	11.08 ± 0.35 [b]	10.11 ± 0.17 [a]	0.49
EOSINOPHILS (µm)		9.11 ± 0.25 [a]	11.5 ± 0.12[b]	10.44 ± 0.26[c]	0.80
BASOPHILS (µm)		7.53 ±0.20 [a]	8.3 ± 0.16[b]	7.73 ± 0.15 [a]	0.54
LYMPHOCYTES	SMALL (µm)	7.9 ±0.07 [a]	8.4 ± 0.15 [b]	8.27 ± 0.11[ab]	0.37
	MEDIUM (µm)	8.5 ± 0.12[a]	9.92 ± 0.16[b]	9.5 ±0.16 [b]	0.45
	LARGE (µm)	10.09 ± 0.18 [a]	10.94± 0.11[b]	10.30 ± 0.10 [a]	0.59
MONOCYTES (µm)		10.19 ± 0.11[a]	12.0 ± 0.13[b]	10.82 ± 0.14 [c]	0.42
THROMBOCYTES (µm)		4.2 ± 0.08 [a]	4.8 ± 0.16 [b]	5.86 ± 0.10[c]	0.43

Mean under same superscript did not differ significantly.

Bar diagram showing mean values of blood cells size in domestic fowl, guinea fowl and pigeon



5.1 Comparative cytomorphological, cytochemical and cytoenzymic studies on blood cells of domestic fowl, guinea fowl and pigeon

5.1.1 Cytomorphological studies

Erythrocytes

Erythrocytes were round to oval in shape with length of 10.21 ± 0.29 µm and width of 8.4 ± 0.38 µm in fowl. They were elongated in shape with length of 12.03 ± 0.20 µm and width of 7.01 ± 0.06µm in guinea fowl whereas elongated with length of11.66 ±0.22 µm and width of 6.19 ± 0.15 µm in pigeon. Sturky and Griminger (1986) stated that the avian erythrocytes varied in size depending on the species, but they generally ranged between 10.7 × 6.1 µm to 15.8 × 10.2 µm. Deldar *et al.* (1998) find that the avian erythrocytes were typically elliptical and about 12 µm in length.Gupta *et al.* (2012) observed that in guinea fowl the erythrocytes of guinea fowl were elliptical or oval in shape and their size varied from 15.7±0.20 µ in length and 7.2±0.16 µ in width.

The cytoplasm of erythrocytes of domestic fowl and pigeon were homogenous pink colour whereas homogenous with light pink colour in guinea fowl. In fowl the nuclei were elliptical, oval,or round while in guinea fowl and pigeon were elongated. The nuclei stained violet-blue in fowl and pigeon whereas in guinea fowl it was light pink. Bounous and Stedman (2000) stated that in mature chicken and turkey erythrocytes found in peripheral blood had a homogeneous eosinophilic cytoplasm and a central, round to oval nucleus with a condensed chromatin pattern. The present observation was in agreement with the statement of Campbell *et al.* (2000) in psittacine bird. He find that the mature psittacine erythrocytes had an elliptical and centrally positioned nucleus. Nuclear chromatin was uniformly clumped and became increasingly condensed with cellular age. Thrall *et al*. (2004) find that mature avian erythrocytes had an elliptical,

centrally positioned nucleus. Alberio *et al.* (2005) stated that lizard erythrocytes were elliptically shaped with a centrally located oval nucleus. Cytoplasmic inclusions were rarely seen. Contrary to this finding, the present finding tallies with the finding of Bonadiman *et al.* (2009), Claver and Quaglia (2009) in avian species. Bonadiman *et al.* (2009) reported that ostrich erythrocytes had a central oval nucleus with condensed chromatin. Claver and Quaglia (2009) find that avian erythrocytes were nucleated. The nucleus of the avian erythrocytes were also oval in shape, and it becomes more condensed with age. The cytoplasm generally stained uniformly orange-pink, except for a thin, pale perinuclear band. Contrary to this finding Sulaiman *et al.* (2010) reported that the Muscovy ducks erythrocytes were nucleated, with orange-blue or greyish ink cytoplasm.

Granulocytes

Heterophils

Heterophils were almost round in shape with average diameter10.99 ± 0.18 µm in fowl. While they were almost round with slight bulging in shaped with size of 11.35 ± 0.13 µm in guinea fowl and rounded shaped and measured 10.11 ± 0.17 µm in pigeon. Bounous and Stedman (2000) find that in chicken and turkey the heterophil was prominent granulocyte.The mature heterophil was a round cell approximately 13 µm in diameter. Salakij *et al.* (2003) examined that heterophils were the most commonly observed leucocytes in the female painted storks but were the second most commonly observed leucocytes in the males. They were round and 8-12 µm in diameter.

The nuclei of heterophils were 2-4 lobed in fowl, 3-4 lobed in guinea fowl and 2-3 lobed in pigeon. In fowl, the arrangement of nuclear segments were spiral, S, and M .In guinea fowl, the arrangement of nuclear segments were U and 8. In pigeon the nuclei were 2 to 3 lobed. The arrangement of nuclear segments were S, U and V shaped. This

observation was inconsonance with the statement of Sturky and Griminger (1986) in avian species. Cytoplam stained pale in fowl, comparatively dark in colour in duck and light bluish in quail. This finding tallies with finding of Hawkey and Dennett (1989). Randall and Reece (1996) reported that the cytoplasmic granules of all avian species were acidophilic in nature. Hawkey and Dennett (1989) found that the cytoplasmic matrix of mature avian heterophils stained colorless or faintly pink. Randall and Reece (1996) observed that the cytoplasmic granules of all avian species were acidophilic in nature.Deldar *et al.* (1998) examined that the avian heterophils had segmented heterochromatic nuclei and red-orange cytoplasm with distinctive granules. The nuclear segmentation was more prominent in heterophil than eosinophil and the nuclei were often masked by cytoplasmic granules. The cytoplasmic granules were large and reddish in rabbits, guinea pigs and chickens. Maxwell and Robertson (1998) reported that the avian heterophils granules appear brick-red in color when stained with Romanovsky stains. Campbell (2000) observed that the heterophils of psittacine birds were most abundant leucocytes. The cytoplasm of normal mature heterophil appears colourless and contains eosinophilic elongated granules. This finding was inagreement with the finding of Thrall *et al.* (2004) in bird. Thrall *et al.* (2004) reported that the cytoplasm of normal, mature heterophil appears colourless and contain eosinophilic granules in most of the birds. The cytoplasmic granules were elongated. The nucleus of mature heterophil in healthy bird was lobed with course, clumped chromatin. Murray (2008) found that avian heterophils had colourless cytoplasm containing eosinophilic or basophilic rod-shaped granules. Heterophil granules usually had a distinct central body that appears refractile. Mature heterophils had a lobed nucleus with a coarse, clumped chromatin that stains purple. The nucleus was often partially hidden by the cytoplasmic granules. Bonadiman *et al.* (2009) reported that ostrich heterophils had an eccentrically placed mostly

bilobulated basophilic nucleus; however, segmented and trilobulated nuclei were also seen. A faint acidophilic cytoplasm with fusiform-shaped and highly acidophilic granules were observed.The present examination was in agreement with the finding of Claver and Quaglia (2009) in avian species. Claver and Quaglia (2009) observed that avian heterophils had colourless cytoplasm and typical eosinophilic, rod-shaped granules, together with some other rounded granules. Specific granules were elliptical, although in some species they might be oval or rounded and had a distinct central body that appears refractile. Sulaiman *et al.* (2010) found that the Muscovy ducks heterophils had an polymorphic nucleus. The cytoplasmic granules were rod shaped and acidophilic.

Eosinophils

Eosinophils were rounded in shape in fowl as well as guinea fowl and round to oval in pigeon. The average dimeter was 9.11 ± 0.25μmin fowl, 11.5± 0.12 μm in guinea fowl and 10.44 ± 0.26 μm in pigeon

Griminger (1986) found that eosinophilic granulocytes were of about the same size as the heterophils. Bounous and Stedman (2000) found that in chicken and turkey theeosinophils were round to irregular in shape and were approximately 12μm in diameter. Campbell *et al.* (2000) reported that the psittacine eosinophil were round. Salakij *et al.* (2003) observed that eosinophils were usually the largest WBCs in painted storks, average 9-19 μm in diameter. Sulaiman *et al.* (2010) found that the Muscovy ducks esinophils had a more regular cellular outline than heterophils.

In fowl, the nuclei were mostly two to three lobed .The darkly stained, chromatin materials were distributed in patches. The cytoplasmic granules were round, coarse, numerous, and homogeneously distributed throughout the cytoplasm and eosinophilic when stained with MGG. In guinea fowl, the nuclei were generally three to four lobed.

The size and arrangement of lobes varied greatly. The cytoplasm was relatively darker and cytoplasmic granules were densly packed .The cytoplasm was moderately eosinophilic when stained with MGG.In pigeon, the nuclei were 2-4 lobed. The cytoplasm was comparatively darkly stained. The cytoplasmic granules were round, and, loosely packed. Cytoplasm was strongly eosinophilic when stained with MGG. Witkowski and Thaxton (1981) observed that in Japanese quail, the eosinophil cytoplasm had many small, round granules as well as several defined vacuoles. Maxwell *et al.* (1987) observed that the eosinophil cytoplasm was generally pale blue in colour compared with a colourless matrix of heteroph. Thrall *et al.* (2004) reported that the avian eosinophils were had strongly eosinophilic cytoplasmic granules.The cytoplasm of eosinophil stained clear blue, in contrast to the colourless cytoplasm of normal, mature heterophil. The nuclei of eosinophils were lobed and usually stained darker than the heterophil nuclei. Bonadiman *et al.* (2009) found that ostrich eosinophils had an eccentrically placed basophilic, kidney-shaped, rarely lobulated nucleus. Their cytoplasm was semi translucent with a light blue colour, containing many eosinophilic, round granules homogeneously distributed. Claver and Quaglia *et al.* (2009) examined that avian eosinophils had round, eosinophilic granules and a pale blue cytoplasm.

Basophils

Basophils were irregularly rounded in fowl, oval to elongated in guinea fowl and rounded in pigeon. The average size was 7.53 ± 0.20μm in fowl, 8.30 ± 0.16 μm in guinea fowl and 7.73 ± 0.15 μm in pigeon. Hawkey *et al.* (1984b) noted the largest basophil in African grey parrot with a diameter of 10.7 μm. Bounous and Stedman (2000) observed that basophils of chicken and turkey were round cells of approximately 12 μm in diameter. Salakij *et al.* (2003) stated that basophils of painted storks were very

small, with average diameter of 6-9 µm which was smaller than heterophils or eosinophils.

In all the three species nuclei were not clearly visible. The cytoplasm was comparatively lighter in fowl as well as pigeon and generally darkly stained in guinea fowl. The cytoplasmic granules were comparatively lighter in fowl and pigeon whereas comparatively darker in guinea fowl. It gives foamy appearance in fowl. When stained with MGG.

Sturky and Griminger (1986) stated that in chicken polymorpho nuclear basophilic granulocytes were seen in cytoplasm .The nucleus was weakly basophililc and round or oval in shape. Maxwell and Robertson (1995) observed that the nucleus of avian basophil was usually non lobulated. The present finding in fowl was in consonance with the finding of Deldar *et al.* (1998). Deld *et al.* (1998) observed that avian basophil had dark blue granules and were readily distinguished from other leucocytes. Campbell *et al.* (2000) evaluated that the basophils of psittacine birds and reported the deeply stained metachromatic granules that often obscure the non-lobed nucleus. Thrall *et al.* (2004) stated that the avian basophils frequently were found in the peripheral blood, in contrast to mammalian basophils. Avian basophil contains deeply stained metachromatic granules. The nucleus usually was non-lobed. Kaufman and Murray (2008) found that avian basophils contained a round, centrally located nucleus. The nucleus stained a light blue and was often hidden by the cytoplasmic granules. Contrary to this finding, Bonadiman *et al.* (2009) observed that ostrich basophils had a highly lobulated basophilic nucleus and their cytoplasm was basophilic, full of large, round basophilic granules. Claver and Quaglia (2009) reported that avian basophils had a rounded nucleus and characteristic violet to reddish purple granules. Harvey and Sulaiman (2010)

examined that the in Muscovy ducks cytoplasm of basophil was slight basophilic to colourless, with strongly basophilic intracytoplasmic granules

Agranulocytes

Lymphocytes

Small lymphocytes were almost round in fowl, almost rounded with few blebs in guinea fowl where as almost rounded to oval in pigeon .Their size was 7.90 ±0.07 μmin fowl, 8.40 ± 0.09μm in guinea fowl and 8.27 ±0.11 μm in pigeon. Medium sized lymphocytes were elongated to rounded in fowl, rounded to oval in guinea fowl whereas irregularly rounded in pigeon. Their size was 8.5 ± 0.12μm in fowl, 9.92 ± 0.16μm in guinea fowl and 9.5 ±0.16 μm in quail. Large lymphocytes were rounded to oval in fowl while irregularly rounded in guinea fowl as well as in pigeon. Their size was 10.09 ± 0.18μm in fowl, 10.94 ± 0.11μm in guinea fowl and 10.30 ±0.10 μm in pigeon.

Deldar *et al.* (1998) examined that the avian lymphocytes as small (6 to 9μm) and large (9 to 15 μm) lymphocytes. Salakij *et al.* (2003) reported that lymphocytes were the most prevalent circulating cells in the male painted storks. They were small, well differentiated and average 6-8 μm in diameter.

In fowl, small lymphocytes had almost spherical nuclei surrounded by a peripheral cytoplasm. The cytoplasm stained comparatively darker in colour. In medium sized lymphocytes cytoplasm was comparatively more in compare to small lymphocytes. In large sized lymphocytes, nuclei were irregularly oval in shaped. Cytoplasm was comparatively more than small and medium sized lymphocytes.

In guinea fowl, small lymphocytes almost irregular shaped nuclei surrounded by a thik cytoplasm. The medium sized lymphocytes had comparatively less amount of cytoplasm than compared to small lymphocytes. In large sized lymphocytes, nuclei were

irregular shaped which had small amount of cytoplasm. The cytoplasm was comparatively darkly stained.

In pigeon, small lymphocytes had almost rounded in shape and occupied almost all area. The medium sized lymphocytes had irregularly rounded shaped nuclei with comparatively less amount of cytoplasm than small lymphocytes. The large sized lymphocytes had almost round nuclei with large amount of cytoplasm than small and medium sized lymphocytes. The cytoplasm stained comparatively darker in colour.

Sturky and Griminger (1986) found that the lymphocyte constitutes the majority of the leucocytes in the blood of the fowl. The cytoplasm was usually weakly basophilic consisting of a narrow rim bordering the nucleus in small lymphocytes. Bounous and Stedman (2000) reported that in chicken and turkey lymphocytes were the prominent leucocyte in the peripheral blood. The small lymphocytes had a round nucleus. Medium lymphocyte had more abundant and sometimes more pale basophilic cytoplasm. Campbell *et al.* (2000) observed that the lymphocytes of psittacine birds resembled with mammalian lymphocytes. The lymphocyte cytoplasm usually appears homogeneous and weakly basophilic which lacks granules and vacuoles. Thrall *et al*. (2004) studied that the avian lymphocytes had slightly indented nucleus. The lymphocyte cytoplasm usually appears to be homogeneous and weakly basophilic (pale blue), and it lacks both vacuoles and granules. Kaufman and Murray (2008) observed that avian lymphocytes can be classified into three groups according to cell size (small, medium, and large). Most normal mature lymphocytes in the peripheral blood were small or medium. The nucleus was usually round and centrally located. The amount of cytoplasm varies from a narrow band surrounding the nucleus in small lymphocytes to a moderately wide band in medium and large lymphocytes. Bonadiman *et al*. (2009) studied that ostrich lymphocytes were smaller, had more granules, more condensed chromatin, and a higher

nucleus : cytoplasm ratio than monocytes. Claver and Quaglia (2009) reported that avian lymphocytes had a round nucleus and scant to abundant basophilic cytoplasm. Small lymphocytes predominantly found in most birds, with irregular projections or blebs frequently observed on this cell type. Sulaiman *et al.* (2010) observed that the Muscovy ducks lymphocytes had a thin cytoplasm bordering the nucleus.

Monocytes

Monocytes were rounded in fowl, elliptical to rounded in guinea fowl and almost rounded in pigeon. Their size was 10.19 ± 0.11μm in fowl, 12.0 ±0.13μm in guinea fowl and 10.82 ± 0.14μm in pigeon.

Bounous and Stedman (2000) reported that the chicken and turkey monocytes usually were the largest leukocytes (approximately 14 μm in diameter). Campbell (2000) observed the monocytes in peripheral blood films of psittacine birds as the largest leucocyte.

In fowl, the nucleus was eccentric and rounded in shape. The cytoplasm was foamy in appearance due to presence of large number of vacuoles. In guinea fowl, the nuclei were eccentric, irregular and horse shoe shaped. The cytoplasm was lightly stained with MGG.

In pigeon, the nuclei were irregular shaped and eccentrically placed. The cytoplasm was foamy in appearance with few vacuoles and granular dots. The cytoplasm stained comparatively darker in colour.

Jain *et al.* (1993) pointed that in avian species the monocytes the nuclei were usually round or oval in shape however, few elongated nuclei with indentation on one side were also seen. Their cytoplasm was usually abundant and frequently vacuolated or foamy. Deldar *et al.* (1998) observed that the avian monocytes had pleomorphic nuclei

which appear spherical, ovoid, elongated and indented. Their cytoplasm is relatively abundant, foamy and occasionally vacuolated with no visible granules. Thrall *et al.* (2004) reported that the avian monocytes had abundant blue-gray cytoplasm containing vacuoles and fine-dust like eosinophilic granules. The monocytes nucleus varied in shape and with less chromatin clumping compared with lymphocyte nuclei. Kaufman and Murray *et al.* (2008) pointed that avian monocytes had round to bi-lobed nuclei. The cytoplasm of monocytes stained blue-gray with a finely granular appearance and occasionally contained vacuoles. Bonadiman *et al.* (2009) observed that ostrich monocytes, had rarer granules, less condensed chromatin, and a lower nucleus: cytoplasm ratio than lymphocytes. Claver and Quaglia (2009) reported that avian monocyte had a kidney-shaped nucleus. The cytoplasm was generally deep blue or grayish blue, often presented a pink- or purple-stained granular area near the nucleus. Sulaiman *et al.* (2010) founded that the in Muscovy ducks, the nucleus of the monocytes was kidney shaped or oval and the cells had light basophilic cytoplasm.

Thrombocytes

Thrombocytes were mostly oval in fowl while oval to rounded in guinea fowl as well as in pigeon. Their size was 4.20 ± 0.08 μm in fowl, 4.80 ±0.16 μm in guinea fowl and 5.86 ± 0.10 μm in pigeon. Thrombocytes were the smallest blood cell in all three species.

Santos *et al.* (2003) found that roadside hawk thrombocytes presented mostly elliptic aspect and a slightly spherical and oval shape as well.

In fowl, the thrombocytes stained reddish violet with MGG. They occurred mostly singly or in small group .The nuclei were moderately basophilic and irregularly rounded in shape. Cytoplasm was non-granular.

In guinea fowl, the thrombocytes stained moderately violet in colour when blood smears were stained with MGG. They mostly occur red in cluster of large group. Their nuclei were slightly basophilic and rounded in shape. In pigeon, the thrombocytes stained light pink is with MGG. They always occurred in single. The nuclei were elongated in shape. The cytoplasm was less in amount and purple in colour.

Campbell *et al.* (2000) observed that in psittacine birds cytoplasm of thrombocytes was colour less to pale gray and contained one or more distinct eosinophilic granules. The thrombocytes of duck and goose resemble those of other birds. Salakij *et al*. (2003) reported that thrombocytes of the painted stork had oval nuclei with dense chromatin. When thrombocytes aggregated they turned into round cells. However, they were easily differentiated from lymphocytes by a characteristic perinuclear cytoplasmic vacuolation. Thrall *et al.* (2004) pointed that the thrombocytes were nucleated in birds. The nucleus was more rounded than the erythrocyte nucleus. Normal, mature thrombocytes had a colorless to pale-gray cytoplasm. Cytoplasmic vacuolation can occur in activated or phagocytic thrombocytes. Thrombocytes frequently contain one or more distinct eosinophilic granules, which usually was located in one area of the cytoplasm. Activated thrombocytes occurring in aggregates had indistinct cellular outlines or cytoplasmic pseudopodia. Kaufman and Murray (2008) observed that avian mature thrombocytes nuclei were larger in relation to the amount of cytoplasm and more rounded than erythrocyte nuclei. The cytoplasm was clear but not homogenous and often had a reticulated appearance. Bonadiman *et al.* (2009) found that ostrich thrombocytes had highly basophilic nuclei and scarce hyaline cytoplasm without granules and vacuoles was occasionally observed. Claver and Quaglia (2009) found that avian thrombocytes had an oval to rounded nucleus. The cytoplasm was light blue or colourless, often vacuolated with a few acidophilic granulations.

5.1.2 Cytochemical studies

Mucopolysachharides

The basophilic granules showed weak positive reaction in fowl while, strong in guinea fowl and moderately positive in pigeon when the blood smears were stained with toluidine blue stain. Bowers *et al.* (1981) reported that in a comparative study of the histochemical properties of pigeon basophils and mast cells, the pigeon basophils had only a 'trace' or no glycogen, a mucopolysachharide-glycoprotein complex, a small amount of histadin and no histamine. Yadav *et al.* (2012) observed that the granules of basophil stained metachromatically in the form of intense violet coloured with 1% toluidine blue for mucopolysaccharides in fowl.

Glycogen

Heterophils showed strong positive reaction in guinea fowl, while moderate in pigeon and weak in fowl in their cytoplasm when the blood smears were stained with the periodic acid-Schiff stain. Andreasen and Latimer (1990) observed that the chicken heterophil granules were negative Periodic Acid-Schiff. Menaka and Singh (2002) reported that the cytoplasmic granules of goat neutrophils showed diffuse positive reaction with Periodic Acids Schiff's stain. The present finding was also in agreement with the statement of Santos *et al.* (2003) in roadside hawk. Singh and Menaka (2004a) stated that the neutrophil granules of horse showed strongly positive reaction with Periodic Acid Schiff's stain. Singh and Menaka (2004b) found that the cytoplasmic granules of sheep neutrophil showed diffuse type of positive reaction when stained with Periodic Acid Schiff's stain. Casal and Oros (2007) reported that juvenile logger head sea turtles heterophil, eosinophil and thrombocytes were stained with PAS whereas basophil, lymphocyte and monocyte did not stained. Shigdar *et al.* (2009) found that Periodic acid Schiff's positivity was detected in Murray cod heterophil, basophil and

lymphocyte whereas monocyte and thrombocytes showed negative staining for PAS. Mehta *et al.* (2012) observed that the neutrophil and eosinophil of pig showed positive reaction in the form of pink granules when the blood smear were stained with Periodic acid Schiff's stain.

General lipids

Eosinophil showed strong positive reaction in form of black colour granule in fowl, while moderate in pigeon and weak in guinea fowl when the blood smears were stained with Sudan Black B. Singh *et al.* (1998) found that the neutrophil and eosinophil of camel were positive for Sudan black B and the lymphocytes of camel were negative for Sudan black B. Singh *et al.* (2000) stated that in buffalo calves the neutrophil and eosinophil granules showed positive reaction when stained with Sudan black B. Menaka and Singh *et al.* (2002) reported that the cytoplasmic granules of goat neutrophil and eosinophil showed positive reactivity with Sudan Black- B. The above finding in fowl was in agreement with the finding of Santos *et al.* (2003) in roadside hawk. Salakij *et al.* (2003) reported that reticulocytes that contained distinct aggregated reticulum were aggregate reticulocytes whereas puntate reticulocytes contained a few dots. The female painted storks had a higher number of aggregate reticulocytes but a lower number of puntate reticulocytes than the male painted storks. RBCs were positive with SBB staining and heterophils of painted storks were stained weakly or were negative with Sudan black B. Singh and Menaka (2004b) observed that the cytoplasmic granules of sheep neutrophil and eosinophil and showed positive reaction with Sudan black B with taking light black colour. Techangamsuwan *et al.* (2010) reported that Dolphin neutrophil and eosinophil were positive for sudan Black B (SBB) showing brown-black staining whereas lymphocyte and monocyte were negative for Sudan Black B (SBB). Mehta *et al.* (2012) observed that the neutrophil of pig showed very weak to negative

reaction in the form of brownish-black granules with Sudan black B whereas eosinophils of pig blood showed positive reaction in the form of brownish-black granules with Sudan black B. The above observation was inconsonance with the statement of Yadav *et al.* (2012) pointed that the eosinophil showed strong positive reaction in the form of intense black coloured granules when stained with Sudan black B.

5.1.3 Cytoenzymic studies

Acid phosphatase

Lymphocyte showed strongly positive reaction in fowl while moderate in guinea fowl and weak in pigeon in the form of black-brown patches when blood smears were stained for acid phosphatase. Andreasen and Latimer (1990) reported that the chicken eosinophil indicate positive reactivity for acid phosphatase, while heterophils were negative Maxwell and Robertson (1995) found that, unlike those of mammals, avian basophil granules contain acid phosphatase. Singh *et al.* (1998*)* observed that the neutrophils of camel blood were devoid of acid phosphatase, eosinophils of camel blood had weakly positive for acid phosphatase and lymphocytes and monocyte of camel showed positive reaction for acid phosphatase. Santos *et al.* (2003) pointed that roadside hawk thrombocytes showed with acid phosphatase. Claver and Quaglia (2009) observed that avian heterophil and eosinophil showed positivity with acid phosphatase.

Alkaline phosphatase

Eosinophil of guinea fowl as well as pigeon showed moderate positive reaction however, reaction was weak in fowl for alkaline phosphatase in the form of brown coloured granules. Andreasen and Latimer (1990) observed that the chicken heterophil granules were negative for alkaline phosphatase. Singh *et al.* (1997b) pointed that the neutrophil and lymphocyte and monocyte of camel blood were devoid of alkaline

phosphatase activity and eosinophil of camel blood had strongly positive for alkaline phosphatase. Singh *et al.* (1998) found that the neutrophil and lymphocyte of camel blood were devoid of alkaline phosphatase activity and eosinophils of camel blood had strongly positive for alkaline phosphatase and monocytes of camel were weak positive or negative for alkaline phosphatase. Bounous and Stedman (2000) reported that the chicken and turkey heterophils lacked alkaline phosphate enzymes. Singh *et al.* (2000) observed that in buffalo calves the granules of neutrophil and eosinophil showed positive reactions with alkaline phosphatase. Techangamsuwan *et al.* (2010) found that Dolphin neutrophil, eosinophil, lymphocyte and monocyte stained weakly positive for alkaline phosphatase. Mehta *et al.* (2012) pointed that the neutrophil of pig showed moderately positive reaction in the form of brown granules when blood smears were stained for alkaline phosphatase.

Peroxidase

Eosinophil of guinea fowl as well as pigeon showed strongly positive reaction while reaction was moderately positive in fowl for peroxidase. The current finding was in agreement with the statement of Andreasen and Latimer (1990) in chicken. Maxwell and Robertson (1995) reported that, unlike those of mammals, avian basophil granules did not contain peroxidase. Lam *et al.* (1997) found that chicken heterophils contain slight myeloperoxidase activity. Singh *et al.* (1997b) observed that the neutrophil, eosinophil of camel blood had positive for peroxidase whereas basophil, lymphocyte and monocyte were negative for peroxidase. Singh *et al.* (1998) pointed that the neutrophil, eosinophil of camel blood had positive reactions for peroxidase whereas basophil, lymphocyte and monocyte were negative for peroxidase. Bounous and Stedman (2000) observed that the chicken and turkey heterophils were lack myeloperoxidase. Singh *et al.* (2000) found that in buffalo calves the granules of neutrophil and eosinophil showed

positive reactions with peroxidase. Santos *et al.* (2003) found that roadside hawk thrombocytes showed the positivity with Myeloperoxidase. Alberio *et al.* (2005) examined that four different granulocyte types were observed in lizards. Type I & Type III were considered as heterophils, Type II as eosinophils and Type IV as basophils. Neutral peroxidase activity was localized in granules and endoplasmic reticulum found in type I granulocytes and type III was not observed. Bonadiman *et al.* (2009) pointed that ostrich heterophil granules did not show peroxidase staining while eosinophil granules were highly positive for peroxidase staining. The above observation was in consonance with the statement of Claver and Quaglia (2009) in avian. Techangamsuwan *et al.* (2010) found that Dolphin neutrophil, eosinophil and monocyte showed positive reaction with myeloperoxidase and lymphocytes were negative for myeloperoxidase. Mehta *et al.* (2012) observed that the granules of eosinophil and basophil showed strong positive reaction for peroxidase in pig.

Non- specific esterase

All cells were negative in all the three species for Non-specific esterase. Singh *et al.* (1997b) observed that the eosinophil, basophil, lymphocyte of camel blood had negative for naphthol AS-D chloroacetate esterase enzyme whereas monocytes of camel were positive for acid alpha naphthyl acetate esterase. Singh (2000) found that in buffalo calves the granules of neutrophils showed positive reactions with esterase. Shigdar *et al.*(2009) reported that Murray cod heterophil, eosinophil, basophil, lymphocyte and thrombocyte showed positive activity for Naphthol AS chloroacetate esterase (NCE) whereas negative for α-naphthyl butyrate esterase activity. Monocytes were positive for naphthyl acetate esterase whereas negative for naphthol AS chloroacetate esterase and α-naphthyl butyrate esterase activity. Prasad and Charles (2010) observed that yellow cat fish neutrophil, eosinophil, basophil, lymphocyte were negative for alpha-

naphthylacetateesterase and naphthol ASD chloroacetate esterase. Monocytes were negative for naphthol ASD chloroacetate esterase and weakly positive for alpha-naphthyl acetate esterase. Thrombocytes showed a weakly positive stain for naphthol ASD chloroacetate esterase whereas negative for α-naphthyl acetate esterase. Mehta *et al.* (2012) observed that in pig, the granules of eosinophil, basophil were positive for non-specific esterase.

Erythrocytes were round to oval in shape with length of 10.21 ± 0.29 µm and width of 8.4 ± 0.38 µm in fowl. They were elongated in shape with length of 12.03 ± 0.20 µm and width of 7.01 ± 0.06µm in guinea fowl whereas elongated with length of11.66 ±0.22 µm and width of 6.19 ± 0.15 µm in pigeon. The cytoplasm of erythrocytes of domestic fowl and pigeon were homogenous pink colour whereas homogenous with light pink colour in guinea fowl. In fowl the nuclei were elliptical, oval,or round while in guinea fowl and pigeon they were elongated. The nuclei stained violet-blue in fowl and pigeon whereas in guinea fowl it was light pink. Heterophils were almost round in shape with average diameter10.99 ± 0.18 µm in fowl. While they were almost round with slight bulging in shaped with size of 11.35 ± 0.13 µm in guinea fowl and rounded shaped and measured 10.11 ± 0.17 µm in pigeon. The nuclei of heterophils were 2-4 lobed in fowl, 3-4 lobed in guinea fowl and 2-3 lobed in pigeon. In fowl, the arrangement of nuclear segments were spiral, S, and M .In guinea fowl, the arrangement of nuclear segments were U and 8. In pigeonthe nuclei were 2 to 3 lobed. The arrangement of nuclear segments were S, U and V shaped .Eosinophils were rounded in shape in fowl as well as guinea fowl and round to oval in pigeon. The average dimeter was9.11 ± 0.25µmin fowl, 11.5±0.12 µm in guinea fowl and 10.44 ± 0.26 µm in pigeon. In fowl, the nuclei were mostly two to three lobed. The darkly stained, chromatin materials were distributed in patches. The cytoplasmic granules were round, coarse, numerous, and homogeneously distributed throughout the cytoplasm and eosinophilic. In guinea fowl, the nuclei were generally three to four lobed .The size and arrangement of lobes varied greatly. The cytoplasm was relatively darker and cytoplasmic granules were densly packed. The cytoplasm was moderately eosinophilic. In pigeon, the nuclei were 2-4 lobed. The cytoplasm was comparatively darkly stained. The cytoplasmic granules were round, and,

loosely packed. Basophils were irregularly rounded in fowl, oval to elongated in guinea fowl and rounded in pigeon. The average size was 7.53 ± 0.20μm in fowl, 8.30 ± 0.16 μm in guinea fowl and 7.73 ± 0.15 μm in pigeon. In all the three species nuclei were not clearly visible. The cytoplasm was comparatively lighter in fowl as well as pigeon and generally darkly stained in guinea fowl. The cytoplasmic granules were comparatively lighter in fowl and pigeon whereas comparatively darker in guinea fowl. It gives foamy appearance in fowl. Small lymphocytes were almost round in fowl, almost rounded with few blebs in guinea fowl where as almost rounded to oval in pigeon. Their size was 7.90 ±0.07 μmin fowl, 8.40 ± 0.09μm in guinea fowl and 8.27 ±0.11 μm in pigeon. Medium sized lymphocytes were elongated to rounded in fowl, rounded to oval in guinea fowl whereas irregularly rounded in pigeon. Their size was 8.5 ± 0.12μm in fowl, 9.92 ± 0.16μm in guinea fowl and 9.5 ±0.16 μm in quail. Large lymphocytes were rounded to oval in fowl while irregularly rounded in guinea fowl as well as in pigeon. Their size was 10.09 ± 0.18μm in fowl, 10.94 ± 0.11μm in guinea fowl and 10.30 ±0.10 μm in pigeon. In fowl, small lymphocytes had almost spherical nuclei surrounded by a peripheral cytoplasm. The cytoplasm stained comparatively darker in colour. In medium sized lymphocytes cytoplasm was comparatively more in compare to small lymphocyte. In large sized lymphocytes, nuclei were irregularly oval in shaped .Cytoplasm was comparatively more than small and medium sized lymphocytes.In guinea fowl, small lymphocytes almost irregular shaped nuclei surrounded by a thik cytoplasm. The medium sized lymphocytes had comparatively less amount of cytoplasm than compared to small lymphocytes. In large sized lymphocytes, nuclei were irregular shaped which had small amount of cytoplasm. The cytoplasm was comparatively darkly stained. In pigeon, small lymphocytes had almost rounded in shape and occupied almost all area. The medium sized lymphocytes had irregularly rounded shaped nuclei with

comparatively less amount of cytoplasm than small lymphocytes. The large sized lymphocytes had almost round nuclei with large amount of cytoplasm than small and medium sized lymphocytes. The cytoplasm stained comparatively darker in colour. Monocytes were rounded in fowl, elliptical to rounded in guinea fowl and almost rounded in pigeon. Their size was 10.19 ± 0.11μm in fowl, 12.0 ±0.13μm in guinea fowl and 10.82 ± 0.14μm in pigeon. In fowl, the nucleus was eccentric and rounded in shape. The cytoplasm was foamy in appearance due to presence of large number of vacuoles. In guinea fowl, the nuclei were eccentric, irregular and horse shoe shaped. In pigeon, the nuclei were irregular shaped and eccentrically placed. The cytoplasm was foamy in appearance with few vacuoles and granular dots. The cytoplasm stained comparatively darker in colour. Thrombocytes were mostly oval in fowl while oval to rounded in guinea fowl as well as in pigeon. Their size was 4.20 ± 0.08 μm in fowl, 4.80 ±0.16 μm in guinea fowl and 5.86 ± 0.10 μm in pigeon. Thrombocytes were the smallest blood cell in all three species. In fowl, the thrombocytes stained reddish violet. They occurred mostly singly or in small group. The nuclei were moderately basophilic and irregularly rounded in shape. Cytoplasm was non-granular. In guinea fowl, the thrombocytes stained moderately violet in colour. They mostely occurred in cluster of large group. Their nuclei were slightly basophilic and rounded in shape. In pigeon, the thrombocytes stained light pinkish. They always occurred in single. The nuclei were elongated in shape, The cytoplasm was less in amount and purple in colour. The basophilic granules showed weak positive reaction in fowl while, strong in guinea fowl and moderately positive in pigeon when the blood smears were stained with toluidine blue stain. Heterophils showed strong positive reaction in guinea fowl, while moderate in pigeon and weak in fowl in their cytoplasm when the blood smears were stained with the periodic acid-Schiff stain. Eosinophil showed strong positive reaction in form of black colour granule in fowl,

while moderate in pigeon and weak in guinea fowl when the blood smears were stained with Sudan Black B. Lymphocyte showed strongly positive reaction in fowl while moderate in guinea fowl and weak in pigeon in the form of black-brown patches when blood smears were stained for acid phosphatase. Eosinophil of guinea fowl as well as pigeon showed moderate positive reaction however, reaction was weak in fowl for alkaline phosphatase in the form of brown coloured granules. Eosinophil of guinea fowl as well as pigeon showed strongly positive reaction while reaction was moderately positive in fowl for peroxidase. All cells were negative in all the three species for Non-specific esterase.

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Fig. 1 Photomicrograph of blood smear showing erythrocytes of domestic fowl.May Grunwald Giemsa stain X 1000

Fig. 2 Photomicrograph of blood smear showing erythrocytes of guinea fowl. May Grunwald Giemsa stain X 1000

Fig. 3 Photomicrograph of blood smear showing erythrocytes of pigeon.May Grunwald Giemsa stain X 1000

Fig. 4 Photomicrograph of blood smear showing heterophil of domestic fowl. May Grunwald Giemsa stain X 1000

Fig. 5 Photomicrograph of blood smear showing heterophil of guinea fowl.May Grunwald Giemsa stain X 1000

Fig. 6 Photomicrograph of blood smear showing heterophil of pigeon.May Grunwald Giemsa stain X 1000

Fig. 7 Photomicrograph of blood smear showing eosinophil of domestic fowl.May Grunwald Giemsa stain X 1000

Fig. 8 Photomicrograph of blood smear showing eosinophil of guinea fowl.May Grunwald Giemsa stain X 1000.

Fig. 9 Photomicrograph of blood smear showing a eosinophil of pigeon.May Grunwald Giemsa stain X 1000.

Fig. 10 Photomicrograph of blood smear showing a basophil of domestic fowl.May Grunwald Giemsa stain X 1000.

Fig. 11 Photomicrograph of blood smear showing a basophil of guinea fowl.May Grunwald Giemsa stain X 1000.

Fig. 12 Photomicrograph of blood smear showing a basophil of pigeon.May Grunwald Giemsa stain X 1000.

Fig. 13 Photomicrograph of blood smear showing a large sized lymphocyte of domestic fowl.May Grunwald Giemsa stain X 1000.

Fig. 14 Photomicrograph of blood smear showing a large sized lymphocyte of guinea fowl.May Grunwald Giemsa stain X 1000.

Fig. 15 Photomicrograph of blood smear showing a large sized lymphocyte of pigeon.May Grunwald Giemsa stain X 1000.

Fig. 16 Photomicrograph of blood smear showing a medium sized lymphocyte of domestic fowl.May Grunwald Giemsa stain X 1000.

Fig. 17 Photomicrograph of blood smear showing a medium sized lymphocyte of guinea fowl.May Grunwald Giemsa stain X 1000.

Fig. 18 Photomicrograph of blood smear showing a medium sized lymphocyte of pigeon.May Grunwald Giemsa stain X 1000.

Fig. 19 Photomicrograph of blood smear showing a small sized lymphocyte of domestic fowl.May Grunwald Giemsa stain X 1000.

Fig. 20 Photomicrograph of blood smear showing a small sized lymphocyte of guinea fowl.May Grunwald Giemsa stain X 1000.

Fig. 21 Photomicrograph of blood smear showing a small sized lymphocyte of pigeon.May Grunwald Giemsa stain X 1000.

Fig. 22 Photomicrograph of blood smear showing a monocyte of domestic fowl.May Grunwald Giemsa stain X 1000.

Fig. 23 Photomicrograph of blood smear showing a monocyte of guinea fowl.May Grunwald Giemsa stain X 1000.

Fig. 24 Photomicrograph of blood smear showing a monocyte of pigeon.May Grunwald Giemsa stain X 1000.

Fig. 25 Photomicrograph of blood smear showing a thrombocyte of domesticfowl May Grunwald Giemsa stain X 1000

Fig. 26 Photomicrograph of blood smear showing a thrombocyte of guinea fowlMay Grunwald Giemsa stain X 1000.

Fig. 27 Photomicrograph of blood smear showing a thrombocyte of pigeon.May Grunwald Giemsa stain X 1000.

Fig. 28 Photomicrograph of blood smear showing basophil with metachromatically stained granules of domestic fowl.Toluidine blue stain X 1000

Fig. 29 Photomicrograph of blood smear showing basophil with metachromatically stained granules of guinea fowl. Toluidine blue stain X 1000

Fig. 30 Photomicrograph of blood smear showing basophil with metachromatically stained granules of pigeon.Toluidine blue stain X 1000

Fig. 31 Photomicrograph of blood smear showing weak positive reacting heterophil of domestic fowl.Periodic acid Schiff's stain X 1000

Fig. 32 Photomicrograph ofblood smear showing strongpositive reacting heterophil of guinea fowl.Periodic acid Schiff's stain X 1000

Fig. 33 Photomicrograph of blood smear showing moderate positive reactingheterophil of pigeon.Periodic acid Schiff's stain X 1000

Fig. 34 Photomicrograph of blood smear showing positive reacting eosinophil of domestic fowl.Sudan black B X 1000

Fig.35 Photomicrograph of blood smear showing weak positive reacting eosinophil of guinea fowl.Sudan black B X 1000

Fig.36 Photomicrograph of blood smear showing moderate positive reacting eosinophil of pigeon.Sudan black B X 1000

Fig. 37 Photomicrograph of blood smear showing strong positive reacting lymphocyte of domestic fowl. Acid phosphatase X 1000

Fig. 38 Photomicrograph of blood smear showing moderate positive reacting lymphocyte of guinea fowl.Acid phosphatase X 1000

Fig. 39 Photomicrograph of blood smear showing weak positive reacting lymphocyteof pigeon.Acid phosphatase X 1000

Fig. 40 Photomicrograph of blood smear showing weak positive reacting eosinophilof domestic fowl. Alkaline phosphatase X 1000

Fig. 41 Photomicrograph of blood smear showingmoderate positive reacting eosinophilof guinea fowl. Alkaline phosphatase X 1000

Fig. 42 Photomicrograph of blood smear showingmoderate positive reacting eosinophil of pigeon. Alkaline phosphatase X 1000

Fig. 43 Photomicrograph of blood smear showing moderate reacting eosinophil of domestic fowl. Peroxidase X 1000

Fig. 44 Photomicrograph of blood smear showingstrongpositive reacting eosinophilof guinea fowl. Peroxidase X 1000

Fig. 45 Photomicrograph of blood smear showingstrongpositive reacting eosinophil of pigeon. Peroxidase X 1000

Fig. 46 Photomicrograph of blood smear showing negative reacting eosinophil of domestic fowl.Non-specific esterase X 1000

Fig. 47 Photomicrograph of blood smear showing negative reacting eosinophil of guinea fowl.Non-specific esterase X 1000

Fig. 48 Photomicrograph of blood smear showing negative reacting eosinophilof pigeon.Non-specific esterase X 1000