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Diagnostic Importance of Cytochemical and Cytoenzymatic Patterns of Peripheral Blood Cells in Native Cattle (Zobawng) of Mizoram, India

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ABSTRACT

Background: The cytochemistry and cytoenzymatic profile of the blood cells is an important tool in the hands of clinical veterinary practitioners. It has also applications in various fields of veterinary sciences. Therefore based on its clinical importance present study was conducted on the blood cells of native cattle (Zobawng) of Mizoram to examine the cytochemical and cytoenzymatic characteristics.

Methods: For this study, a total of 12 numbers of blood samples (5 ml) were taken aseptically from adult local cattle of Mizoram, irrespective of sex. The blood smears were prepared and stained with acid ferrocyanide (ACF) for the detection of non-heame iron, toluidine blue (TBO) stain for acid mucopolysaccharides, Periodic Acid-Schiff (PAS) stain for observation of glycogen and Sudan black B (SBB) stain for demonstration of lipid. Acid phosphatase (ACP), alkaline phosphatase (ALP), peroxidase (POX), arylsulphatase (ARS), cytochrome oxidase (CYO), beta-glucuronidase (GUSB) and succinate dehydrogenase (SDH) enzymes were used for cytoenzymatic features of blood cells. The smears were observed under 1000X magnification in an Olympus Trinocular Research microscope.

Result: In the cytochemical studies, basophils showed a positive reaction for mucopolysaccharides in the toluidine blue stain and neutrophils, eosinophils and basophils recorded positive for glycogen in the PAS stain. Eosinophils and basophils also displayed positive for lipids in the SBB stain. The cytoenzymatic studies of eosinophils and basophils recorded positive reactions for ACP, ALP, POX, ARS and CYO activity.

Key words: Blood cells, Cytochemical, Cytoenzymatic, Diagnosis, Native cattle, Zobawng.

INTRODUCTION

Blood tests are required to determine overall health status and to diagnose various hematological diseases. Blood is a fluid connective tissue in which plasma is suspended together with produced elements such as erythrocytes, granulocytes (neutrophils, eosinophils and basophils), agranulocytes (monocytes and lymphocytes) and platelets (Atkins *et al*., 2017; Choudhary *et al*., 2021). Blood is tested regularly to detect hematological problems and diagnose illnesses (Choudhary *et al*., 2022). In some disorders, blood cell identification and awareness of species differences in terms of morphological distinction are also required. Blood cell cytochemistry can be used to diagnose and classify different kinds of leukemia, as well as to investigate different myeloproliferative illnesses (Cline, 1981). The use of cytochemistry in the diagnosis of many types of hematological malignancies is becoming more common. Blood cells are sensitive to changes in the animal's internal physiological states and stimuli from the external environment, the number, morphology and chemical components of different types of blood cells could reflect the health status of the animals (Khan *et al*., 2011; Peng *et al*., 2018; Sarkar *et al*., 2022) and their abnormal alterations may be related to inflammation, pathogenic microorganism infection or other diseases (Salakij *et al*., 2000; Fang *et al*., 2014).

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Earlier some cytochemical studies of blood leucocytes have been investigated by Menaka and Singh (2002) in goat, Weiss (2005; 2006) in dog, Mrigesh (2011) in donkey and Mehta *et al*. (2012) in pig. To comprehend the body's reaction to any form of stress, proper cell identification and localization of the numerous cytoenzymes in them are critical. Some cytoenzymic characteristics of blood cells were

observed by Feldman *et al*. (2000) in horse, Salakij *et al*. (2000) in Asian wild dog, Singh (2000) in buffalo calves, Santos *et al*. (2003) in roadside hawk, Gupta and Singh (2008) in guinea fowl, Salakij *et al*. (2005a) in Asiatic black bear, Salakij *et al*. (2005b) in Asiatic elephant, Mrigesh (2011) in donkey and Mehta *et al*. (2012) in pig. With these considerations in mind, the present study was designed to assess comprehensive cytochemical and cytoenzymatic characteristics of the blood cells of native cattle of Mizoram.

MATERIALS AND METHODS

Animals and ethical approval

The present study was conducted on blood cells of native cattle of Mizoram state of India from October 2020 to September 2021. The blood samples of Zobawng cattle were collected from the cattle farm, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, India. The animals used for the experiment were ethically approved by the Institutional Animal Ethics Committee (IAEC) vide Approval No.CVSC/CAU/IAEC/20-21/P-3 dated 24.09.2021.

Sample collection

The present study was conducted with twelve numbers of blood samples collected from the adult native cattle of Mizoram, irrespective of sex. From each animal, approximately 10ml of blood was collected from the external jugular vein and was transferred to a sterile siliconized tube filled with heparin or ethylenediaminetetraacetic acid (EDTA).

Cytochemical analysis

For cytochemical studies, the blood smears were prepared immediately after collection on grease-free slides and stained for acid ferrocyanide stain for non-heame iron (Bover, 1964), toluidine stain for acid mucopolysaccharides (Bover, 1964), Periodic Acid Schiff's stain for glycogen (Bover, 1964) and Sudan black- B for lipid (Bover, 1964) (Table 1).

Cytoenzymatic analysis

For cytoenzymatic studies, the blood smears were prepared freshly after collection on grease-free slides and stained samples were treated for acid phosphatase (Bover, 1964), alkaline phosphatase (Bover, 1964), peroxidase (Bover, 1964), arylsulphatase (Bover, 1964), cytochrome oxidase (Bover, 1964), Beta-glucuronidase (Bover, 1964) and succinate dehydrogenase (Bover, 1964), (Table 2).

The stained blood smears and treated slides were examined under oil immersion to record the cytochemical and cytoenzymatic activity of different blood cells with the help of a light microscope (Olympus BX 51, Japan). Critical images were captured with a ProgRes C5 Cool CCD camera (D-07739 Jena, Jenoptik, Germany) for a typical demonstration.

The staining intensity was analyzed as per the activity of different blood cells for the particular chemical or enzyme recorded and graded them using the score from 0-3 (0= no stating/ negative, 1= weak staining, 2= moderate staining, 3= strong staining) (Table 1, 2).

RESULTS AND DISCUSSION

Cytochemical studies

Acid ferrocyanide (ACF) stain for iron

Blood cells did not display any types of reaction (Table 1) when blood smears were stained with ACF stain (Fig 1a).

Toluidine blue (TBO) stain for mucopolysaccharide

The packed granules of basophils displayed a strong positive reaction and stained metachromatically in the form of intense violet colour with the 1% TBO stain (Table 1). Mucopolysaccharides reactivity was absent in the remaining leukocytes (Fig 2a).

Periodic Acid Schiff's (PAS) stain for glycogen

The granules of neutrophils, eosinophils and basophils displayed a positive reaction and pink colour granules were observed in the cytoplasm. The cell membrane of lymphocytes also showed an intensely positive reaction when blood smears were treated with PAS stain (Fig 1b,c,d). The neutrophils and basophils granules were recorded as moderately positive, whereas the granules of eosinophils showed a stronger reaction(Fig 1c);(Table 1).

Sudan black B (SBB) stain for lipid

The eosinophils and basophils displayed a strong positive reaction which revealed darkly stained bluish-black granules (Fig 2c,d). SBB staining was weakly positive in neutrophils (Fig 2b) but lymphocytes and monocytes did not show any reaction to lipids (Table 1).

Table 1: Cytochemical intensity of different blood cells in local cattle of Mizoram.

Name of stain and cells	ACF stain	TBO stain	PAS stain	SBB stain
Erythrocytes	NA	NA	NA	NA
Neutrophils	NA	NA	++	+
Eosinophils	NA	NA	++	+++
Basophils	NA	+++	++	+++
Lymphocytes	NA	NA	+++	NA
Monocytes	NA	NA	NA	NA
Platelets	NA	NA	NA	NA

Gradation for intensity: NA, Negative (0); +, Weak (1); ++, Moderate (2); +++, Strong (3).

Cytoenzymatic studies

Acid phosphatase (ACP)

The eosinophils showed a strongly positive reaction and formed brown-colored granules in the cytoplasm (Fig 3a) but the neutrophils, basophils and lymphocytes did not exhibit any reaction when blood smears were stained for ACP activity (Table 2).

Alkaline phosphatase (ALP)

The eosinophils exhibited a strongly positive reaction and formed brown-coloured granules (Fig 3c), whereas a few cytoplasmic granules of neutrophils and basophils were stained light brown (Fig 3b,d). The lymphocytes and monocytes did not show any reaction to ALP activity (Table 2).

Peroxidase (POX)

The neutrophils (Fig 4a), basophils, lymphocytes and monocytes did not show any reaction to POX activity. The granules of eosinophils exhibited a strong positive reaction for POX (Table 2) in the form of brick-red coloured granules (Fig 4b).

Arylsulphatase (ARS)

The eosinophils and basophils granules showed a positive reaction as dark brown colour granules in the cytoplasm (Fig 4d, 5a). But other leukocytes were non-reactive (Fig 4c) for ARS activity (Table 2).

Cytochrome oxidase (CYO)

The eosinophils and basophils showed a strongly positive reaction in the shades of blue coloured granules of various intensities and some granules were darkly stained (Fig 5c,d). The granules of neutrophils elucidated negative reactivity (Fig 5b) but the monocytes and lymphocytes were non-reactive for CYO (Table 2).

Beta-glucuronidase (GUSB)

The neutrophils, basophils, eosinophils, monocytes and lymphocytes showed no reactivity for GUSB activity (Table 2).

Succinate dehydrogenase (SDH)

The cytoplasm of granulocytes and agranulocytes did not exhibit any reaction when blood smears were treated for SDH activity (Table 2).

Table 2: Cytoenzymic intensity of different blood cells in local cattle of Mizoram.

Name of enzymes and cells	ACP	ALP	POX	ARS	CYO	GUSB	SDH
Erythrocytes	NA	NA	NA	NA	NA	NA	NA
Neutrophils	NA	+	NA	NA	NA	NA	NA
Eosinophils	+++	+++	+++	+++	+++	NA	NA
Basophils	NA	+	NA	+++	+++	NA	NA
Lymphocytes	NA	NA	NA	NA	NA	NA	NA
Monocytes	NA	NA	NA	NA	NA	NA	NA
Platelets	NA	NA	NA	NA	NA	NA	NA

Gradation for intensity: NA, Negative (0); +, Weak (1); ++, Moderate (2); +++, Strong (3).

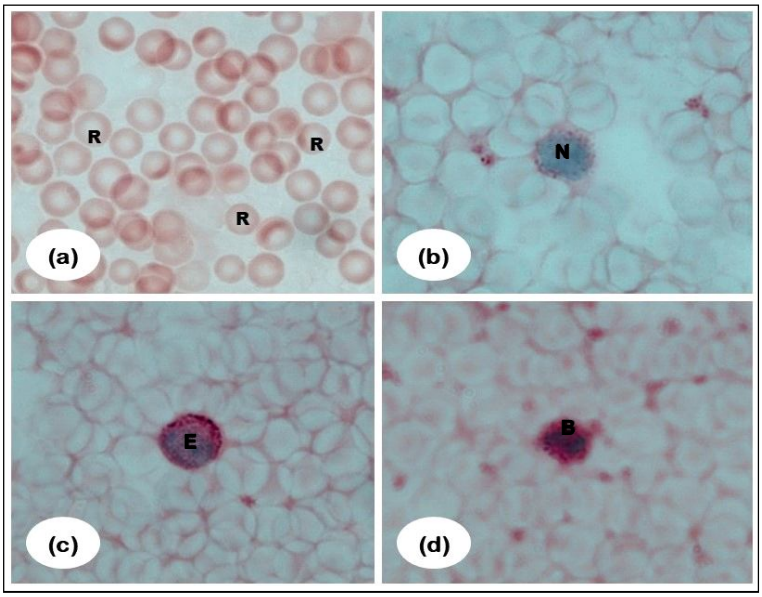

Fig 1: Photomicrograph (a) showing erythrocytes (R) in ACF and (b) neutrophil (N). (c) eosinophils (E). (d) basophil (B) in PAS stain, blood smears of native cattle of Mizoram. (X1000).

Cytochemical studies

There was no cytochemical reactivity in the erythrocytes when blood smears were stained with ACF stain. It could be because of the absence of non-heame iron in the blood. Similar findings were also reported by Mehta *et al*. (2012) in the blood samples of pigs. However, Singh (2000) in buffalo calves, Weiss (2005; 2006) in dog, Mrigesh (2011) in donkey and Yadav *et al*. (2015) in fowl recorded some siderocytes with their small blue colour granular structure inside the erythrocytes.

The packed granules of basophils demonstrated a strong positive reaction and stained a metachromatically intense violet colour with 1% TBO stain. It could be due to the presence of heparin in basophils because heparin is a good example of a mucopolysaccharide. The present findings were similar to that of Singh (2000) in buffalo calves, Yokohama *et al*.(2002) in chronic myeloproliferative disorders, Mrigesh (2011) in donkey, Mehta *et al*. (2012) in pig and Yadav *et al*. (2015) in fowl. Yokohama (2002) reported that the intensity of TBO increased in chronic myeloproliferative disorders, whereas decreased in myeloid leukemia.

The granules of neutrophils, eosinophils and basophils displayed a positive reaction because of glycogen in the

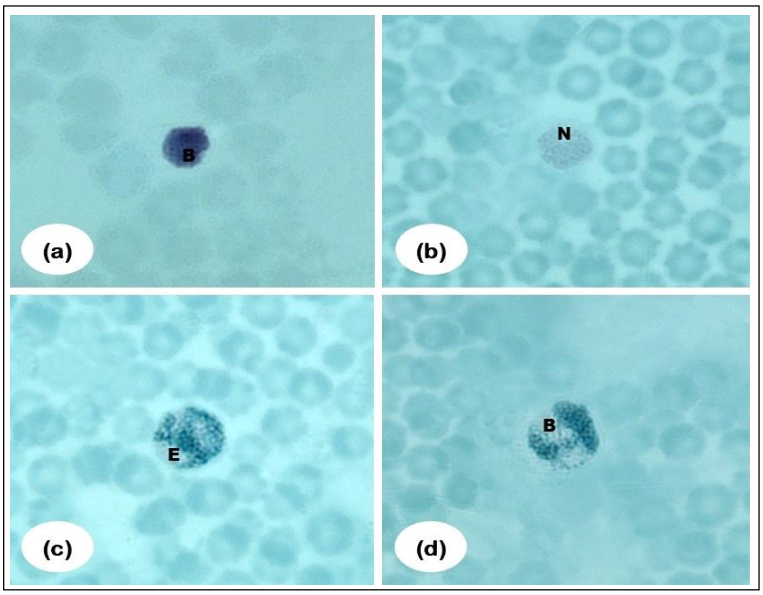

Fig 2: Photomicrograph (a) showing basophil (B) in TBO and (b) neutrophil (N).
(c) eosinophils (E). (d) basophil (B) in SBB stain, blood smears of native cattle of Mizoram. (X1000).

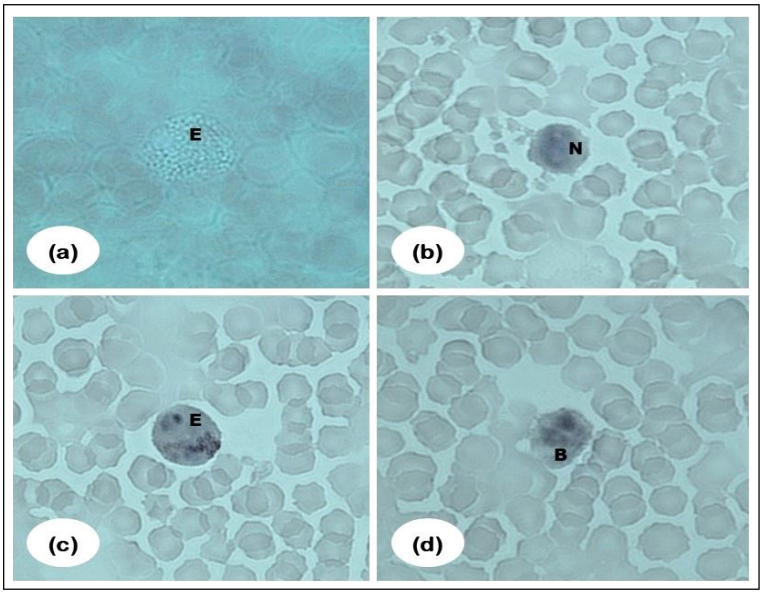

Fig 3: Photomicrograph (a) showing eosinophils (E) in ACP, and(b) neutrophil (N).
(c) eosinophils (E). (d) basophil (B) in ALP, blood smears of native cattle of Mizoram. (X1000).

cells and observed pink colour granules in the cytoplasm when blood smears were treated with PAS stain. Similar findings were reported by Santos *et al*. (2003) in roadside hawk, Mrigesh (2011) in donkey, Mehta *et al*. (2012) in pig and Yadav *et al*. (2015) in fowl. In the present study, some granules of lymphocytes were recorded as positive reactivity. The same result was also observed by Feldman *et al*. (2000) in horse for lymphocytes. The pattern of cytoplasmic PAS reactivity can be used to characterize lymphocytic leukemia (Schwarze,1980).

The eosinophils and basophils demonstrated a strong positive reaction in the form of darkly stained bluish-black granules with an SBB stain. It might be because of the higher amount of lipid materials inside the cell granules. The present investigations also closely agreed with the findings of Santos *et al*. (2003) in roadside hawk, Salakij *et al*. (2007) in Palm civet cat, Mehta *et al*. (2012) in pig for eosinophils and Yadav *et al*. (2015) in fowl. SBB staining was weakly positive for neutrophils but lymphocytes and monocytes did not show any reaction for lipids as reported

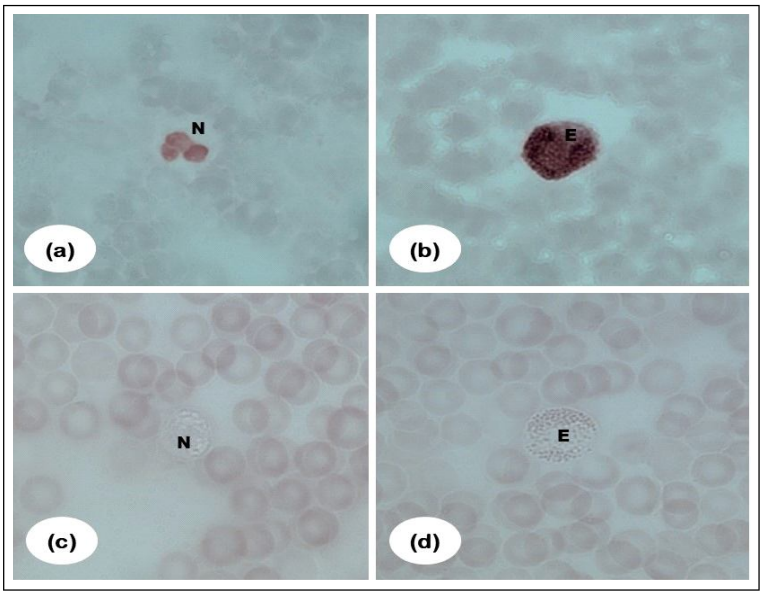

Fig 4: Photomicrograph (a) showing neutrophil (N), (b) eosinophils (E) in POX and(c) neutrophil (N), (d) eosinophils (E) in ARS, blood smears of native cattle of Mizoram. (X1000).

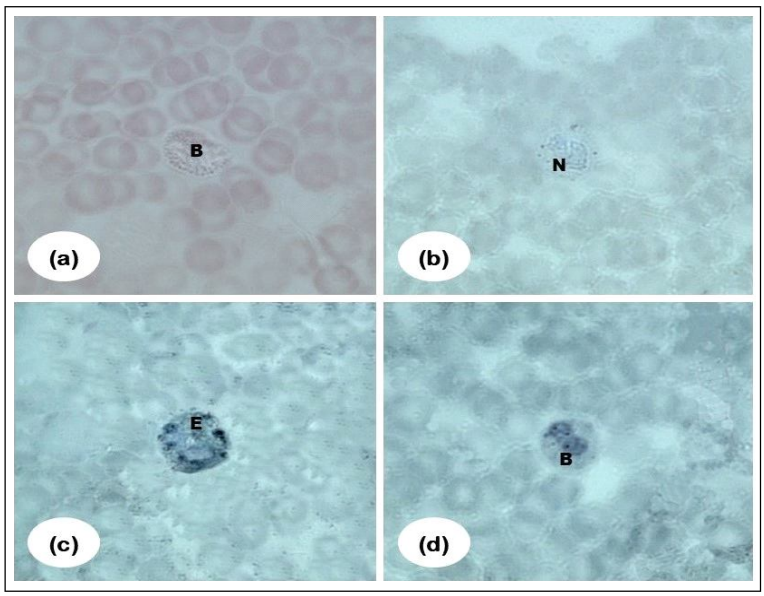

Fig 5: Photomicrograph (a) showing basophil (B) in ARS and (b) neutrophil (N), (c) eosinophils (E), (d) basophil (B) in CYO, blood smears of native cattle of Mizoram. (X1000).

by Mrigesh (2011) in donkey and Mehta *et al*. (2012) in pig. The affinity of SBB stain in neutrophils and eosinophils increased in myeloblastic leukemia without maturation, myeloblastic leukemia with maturation and monocytic leukemia whereas lymphoid malignancies lack positive reaction (Jain, 1993).

Cytoenzymaticstudies

The eosinophils showed a strong positive reaction in the form of brown-coloured granules in the cytoplasm. It could be because of the ACP enzymatic activity of the granules. In the eosinophils, Feldman *et al*. (2000) and Shigdar *et al*. (2009) recorded positive reactions in the granules of eosinophils for ACP activity in horse and Murray cod, respectively. In the current study, the neutrophils, basophils and lymphocytes did not show any reaction when the blood smear was stained for ACP activity, as also reported by Mehta *et al*. (2012) in pig. ACP activity within the lymphocytes was correlated with various types of lymphoproliferative disorders (Schwarze, 1980; Savage *et al*., 1981). The highest levels of ACP were found in sickle cell disease or multiple myeloma or lysosomal disorders, such as Gaucher's disease, which showed moderately increased levels (Moul, 1998).

The eosinophils recorded a strongly positive reaction in the form of brown-coloured granules. It may be because of the ALP enzymatic activity of the granules of eosinophils. A similar result was also noticed by Singh (2000) in buffalo calves and Feldman *et al*. (2000) in horse. In the current investigation, a few cytoplasmic granules of neutrophils and basophils were stained light brown colour as also recorded by Mehta *et al*. (2012) in pig. The lymphocytes and monocytes did not show any reaction which was also recorded by Singh (2000) in buffalo calves and Mrigesh (2011) in donkey for ALP activity. Higher levels of leukocyte ALP were seen in polycythemia vera, essential thrombocytosis, primary myelofibrosis and the leukemoid reaction and lower levels were found in chronic myelogenous leukemia and paroxysmal nocturnal hemoglobinuria. The ALPactivity of circulating neutrophils can facilitate the differentiation of chronic myelogenous leukemia from leukemoid reactions or neutrophilic leucocytosis associated with non-malignant causes (Okum and Tanaka, 1978).

The neutrophils, basophils, lymphocytes and monocytes did not show any types of reaction for POX enzyme activity as also reported by Singh (2000) in buffalo calves, Santos *et al*. (2003) in roadside hawk, Mrigesh (2011) in donkey and Mehta *et al*. (2012) in pig. It was observed that the granules of eosinophils exhibited a strong positive reaction for POX in the form of brick-red coloured granules. It could be because of the POX enzymic activity of the granules. Feldman *et al*. (2000) in horse and Salakij *et al*. (2007) in Palm civet cat eosinophils also found similar types of results for POX activity. The negative POX activities of lymphocytes help in the differentiation of lymphocytic leukemia from granulocytic leukemia (Mehta *et al*., 2012).

The granules of basophils and eosinophils showed a positive reaction and recorded dark brown colour granules in the cytoplasm. It could be because of the ARSenzymatic activity of the granules of basophils and eosinophils. The current findings were also closely similar to the findings of Singh (2000) in buffalo calves, Mehta *et al*. (2012) in pig. In the present study, the remaining leukocytes showed no reaction when stained for ARS activity, as also noticed by Mrigesh (2011) in donkey.

The basophils and eosinophils showed a strong positive reaction as shades of blue coloured granules. It may be due to the presence of the CYO enzymatic activity of the granules of basophils and eosinophils. The present investigations were also similar to the findings of Singh (2000) in buffalo calves, Gupta and Singh (2008) in Guinea fowl and Mrigesh (2011) in donkey. In the current findings, the granules of neutrophils recorded moderate reactivity, but the reaction was absent in monocytes and lymphocytes. Mehta *et al*. (2012) in the neutrophils of pig also noticed a positive reaction while the reaction was absent in monocytes and lymphocytes for CYO.

Neutrophils, basophils, eosinophils, monocytes and lymphocytes did not show any reaction to the GUSB enzyme. It could be the absence of beta-glucuronidase enzymatic activity in the cells. However, Singh (2000) in buffalo calves, Salakij *et al*. (2000) in Asian wild dog, Salakij *et al*. (2005a) in Asiatic black bear and Salakij *et al*. (2005b) in Asiatic elephant recorded positive reactions for GUSB activity. The absence of GUSB activity in the present study might be due to variations in species and age. Deficiencies in GUSB result in the non-recessive inherited metabolic disease known as Sly syndrome or mucopolysaccharidosis.GUSB is used in veterinary medicine primarily to detect focal staining in T lymphocytes and acute undifferentiated leukemia (Jain, 1993).

The cytoplasm of granulocytes and agranulocytes remained non-reactive for SDH enzyme activity. It may be due to the absence of SDH enzymatic activity of the granulocytes and agranulocytes. However, Singh (2000) in buffalo calves, Mehta *et al*. (2012) in pig, Mrigesh (2011) in donkey observed positive reactions to SDH activity. The absence of SDH activity in the present study might be due to variations in species and age.

CONCLUSION

This present study exposed the cytochemical profile and showed a positive reaction for mucopolysaccharides of basophils in TBO stain; glycogen and lipids recorded positive for PAS stain of neutrophils, eosinophils and basophils; eosinophils and basophils also displayed positive for SBB stain. Thecytoenzymatic studies of eosinophils and basophils recorded positive reactions for ACP, ALP, POX and ARS enzyme activity.

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Conflict of interest: None.

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