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Research Article

# Haematological Studies and Micronucleus Assay of Straw-Coloured Fruit Bats (*Eidolon helvum*)

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**Summary:** The straw-coloured fruit bats (*Eidolon helvum*) are the most widely distributed megachiropteran species in Africa. Studies have shown that they migrate, and are likely exposed to environmental pollutants across population. This study was designed to investigate genotoxicity via the bone marrow micronucleus assay and haematological alterations of *Eidolon helvum* in the tropics. Healthy straw-coloured fruit bats (*Eidolon helvum; n=20*) were captured from two geographical regions, Ogun and Gombe States in Nigeria and were grouped based on sex and age. Blood samples were collected for haematology and osmotic fragility, and bone marrow samples for genotoxicity studies. Results showed no significant differences in erythrocytes and leucocytes values across age and sex. The erythrocytes osmotic fragility was higher in juvenile than in adults at 0 and 0.1%NaCl, while it was higher in adult males than in adult females at 0 and 0.3% NaCl. The erythrocytes and leucocytes parameters in straw-colored fruit bats were within the reference values seen in literature except the higher monocyte counts suggesting chronic inflammation. There were increased levels of micronucleated polychromatic erythrocytes and normochromatic erythrocytes in the straw-coloured fruit bats indicating genotoxicity and cytotoxicity, respectively. The present study provided baseline research data on the haematology and micronucleus profile of the straw-coloured fruit bats in Nigeria. This is perhaps the first study on haematology and micronucleus assay of in straw-colored fruit bats in the tropics.

**Keywords***: Straw-coloured fruit bats, Erythrocyte osmotic fragility, Micronucleus, Haematology*



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## INTRODUCTION

Bats are mammals belonging to the order Chiroptera, which is the second most diverse among mammalian orders, exhibiting great physiological and ecological diversity (Hutson *et al* ., 2001). Their forelimbs form webbed wings, making them the only mammals naturally capable of true and sustained flight. The straw-colored fruit bat (*Eidolon helvum*) is a large fruit bat that is the most widely distributed of all the African megabats. Three subspecies have been previously described: *Eidolon helvum* dupreanum (from Madagascar), *Eidolon helvum* helvum (from continental Africa) and *Eidolon helvum* sabaeum (from Arabia) (Skinner and Chimimba, 2005). The straw-coloured fruit bat got its name from the yellow fur on their back. Its belly has a brown color while the skin of its wings is dark brown or black.

Blood is a tissue consisting of red blood corpuscles (erythrocytes), white corpuscles (leukocytes), and platelets. It transports oxygen, carbon dioxide, metabolites, products of digestion, hormones, enzymes and clotting factors. Blood profile is important in evaluating the physiological conditions as well as the nutritional status of animal populations (Herdt, 2000). External factors such as season, time of the day, food availability and quality can affect the blood profile of animals. Other intrinsic factors like age, gender, environment and reproductive state also play important roles (Bezerra *et al* ., 2017; Shawaf *et al* ., 2018). These indices have been employed in effectively monitoring the responses of bats to environmental factors/stressors and thus their health status under such adverse conditions (Bandouchova *et al* ., 2018). They can thus provide substantial information once reference values are established under standardized conditions. In the past, authors have described haematological parameters in different species of bats in some geographical region of the world (Wołk and Ruprecht, 1988; Paksuz *et al* ., 2009; Selig *et al* ., 2016; Ekeolu and Adebiyi, 2018). Results from these studies reflected a great variation in the haematological parameters of bats based on breeds, ages, sexes and location. There is however a paucity of physiological reference values for the straw-colored bat; *E. helvum* in the tropical environments.

*In vivo* micronucleus (MN) assay has been widely used to evaluate the cytogenetic damage induced by environmental xenobiotics, due to its technical simplicity and sensitivity to xenobiotics capable of inducing genotoxic effects on the DNA (MacGregor *et al* ., 1990; Krishna and Hayashi, 2000). It was recommended as a primary test by regulatory agencies for the safe assessment of carcinogenic and mutagenic chemicals (Morita *et al* ., 1997). Although it was initially developed with mammalian species (Heddle, 1973; Schmid, 1975), it is now extensively applied in the cytogenotoxic assessment of various xenobiotics in lower vertebrates like toads (Malladi *et al* ., 2007), birds (Huang *et al* ., 2007; Alimba and Bakare, 2016) and fish (Cavas, 2008). This is attributed to its reproducibility, cost effectiveness, rapidity and suitability of scoring micronucleated cells at interphase in both laboratory and field studies (Morita *et al* ., 1997).

The purpose of this study is to describe the morphological features of the blood cells as well as haematology baseline values, including erythrocyte osmotic fragility and induction of micronuclei in the bone marrow cells of Eidolon bats Nigeria. This information will assist health monitoring and assessment of this migratory mammal, and as a sentinel animal for environmental pollution.

## MATERIALS AND METHODS

**Experimental Animals:** Twenty straw-colored fruit bats (*Eidolon helvum*; 5 Juveniles and 15 Adults) were used for this study. They were captured with mist nets from Ogun and Gombe State representing the Southwestern and North-eastern zones of Nigeria respectively between May and August 2018. They were immediately removed from the net and put in a metal cage and stabilized for 72 hours; the bats were anesthetized intramuscularly using ketamine at 80-90mg/kg body weight.

**Haematological Studies:** Blood samples were collected from the uropatagial vein into bottles containing EDTA (2mg/mL) as anticoagulant. The full blood count was analyzed using automated hematology analyzer. Fresh smear of adult bats was fixed with 99.9% methanol and stained with Giemsa stain for blood cell morphology.

**Osmotic Fragility Test (OFT):** Erythrocytes osmotic fragility was determined according to the method described by Oyewale (1992). 0.02 mL of blood was added to tubes containing increasing concentrations of phosphate-buffered sodium chloride (NaCl) solution at pH 7.4 (0, 0.1, 0.3, 0.5, 0.7, 0.9% NaCl concentration). The tubes were gently mixed and incubated at room temperature (29°C) for 30 min. The content of each tube was centrifuged at 686g for 10 min, and the supernatant decanted for measurement. Optical density of the supernatant was determined at 540nm using a digital spectrophotometer (model UV-1650; Shimadzu, Tokyo, Japan). Haemolysis in each tube was expressed as a percentage, while taking haemolysis in distilled water (0% NaCl) as 100%.

**Micronucleus Assay:** The bone marrow cells preparation for the MN assay was carried out according to Schmid (1975) with slight modifications. Briefly, the straw-colored fruit bats (*n*=5) were and both femurs of the bats were excised and the bone marrow cells were flushed into Eppendorf tubes with 1 mL of Foetal Bovine Serum (FBS) (Sigma Aldrich, Germany). The micropipette was used to carefully dislodge the bone marrow cells, and centrifuged at 380 g for 5 min. This procedure was repeated three times to properly remove fatty tissues. After discarding the supernatant, fresh FBS (300 µL) was added to the pellets and the cells carefully mixed. Five thin smears were prepared for each bat, stained with May-Grunwald and Giemsa stains. For each bat, 1000 polychromatic erythrocytes with micronucleus were scored at X1000 using the light microscope (Micromaster, Fisher Scientific, China). The percentage PCE: NCE was used as the cytotoxicity index (Krishna and Hiyashi, 2000).

Ethical approval for this work was obtained from University of Ibadan Ethics Committee (UI-ACUREC/App/2016/015)

### Statistical Analysis

Data obtained were analyzed with Student's t-test and expressed as Mean ± SD using GraphPad Prism 7. The results were considered statistically significant for P values $\leq 0.05$.

## RESULTS

**Complete blood count:** The haematological parameters of straw-colored fruit bats based on age and sex are presented in Tables 1 and 2. The number of red blood cells (RBC) count, packed cell volume (PCV), hemoglobin (Hb), mean cell volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were not statistically (p>0.05) different between different ages and sex in the bats. In the adult male and female, the RBC fell below the reference values documented in the temperate by Selig *et al* . (2016) whereas in the juvenile; both RBC and Hb values fell below these reference values. The neutrophil count was significantly lower in juvenile (1.3 ± 1.07) compared with adults (2.13 ± 1.55) (Table 3). Other leucocytes parameters; WBC, neutrophils, lymphocytes, monocytes and eosinophils of adults were not significantly different (p >0.05) in the adults and juvenile. The neutrophils values reported were lower than the reference value by Selig *et al* . (2016). The monocytes count across ages and sexes (Table 4) observed in the present study were higher than the reference values.

**Table 1**:
Erythrocytes Values of Adult and Juvenile Straw-coloured Fruit Bats in Nigeria

| PARAMETERS | ADULT (n=15) | JUVENILE (n=5) | REFERENCE VALUES (Selig *et al* ., *2016*) |
|---|---|---|---|
| RBC (x$10^6$ mm) | 7.65 ± 1.60 | 7.42 ± 0.81 | 7.74-10.89 (9.47± 0.69) |
| PCV (%) | 39.50 ± 8.34 | 38.76 ± 4.19 | 33.30-51.00 (42.68± 19.3) |
| Hb(g/dl) | 12.33 ± 2.30 | 11.60 ± 1.36 | 12.00-16.00 (14.89± 1.03) |
| MCV (fL) | 51.67 ± 4.88 | 45.10 ± 17.15 | 40.10-53.90 (45.08± 2.97) |
| MCH (pg) | 16.37 ± 1.31 | 18.62 ± 6.59 | 14.20-17.30 (15.73± 0.73) |
| MCHC (g/dl) | 31.51 ± 2.59 | 34.18 ± 9.83 | 30.00-37.20 (34.99± 1.94) |

**Table 2:**
Erythrocytes Values of Adult Straw-Coloured Fruit bats in Nigeria

| PARAMETERS | MALES (n=7) | FEMALES (n=8) | REFERENCE VALUES (Selig *et al ., 2016*) |
|---|---|---|---|
| RBC ($x10^6$ $mm^3$) | 7.70 ± 1.59 | 7.60 ± 1.70 | 7.74-10.89 (9.47± 0.69) |
| PCV (%) | 41.09 ± 8.97 | 38.11 ± 8.08 | 33.30 – 51.00 (42.68± 19.3) |
| Hb (g/dl) | 12.47 ± 2.31 | 12.20 ± 2.44 | 12.00 -16.00 (14.89± 1.03) |
| MCV (fL) | 53.29 ± 5.22 | 50.25 ± 4.40 | 40.10 -53.90 (45.08± 2.97) |
| MCH (pg) | 16.63 ± 1.04 | 16.15 ± 1.55 | 14.20 -17.30 (15.73± 0.73) |
| MCHC (g/dl) | 30.79 ± 3.23 | 32.14 ± 1.85 | 30.00 -37.20 (34.99± 1.94) |

**Table 3:**
Leucocytes Values of Adult and Juvenile Straw-Coloured Fruit Bats in Nigeria

| PARAMETERS | ADULT (n=15) | JUVENILE (n=5) | REFERENCE VALUES (Selig *et al* ., 2016) |
|---|---|---|---|
| WBC $x10^9$ L | 4.78 ± 2.42 | 4.50 ± 2.64 | 1.20 -7.30 (3.19± 1.48) |
| Neutrophils ($x10^9$ L) | 2.13 ± 1.55 (44.56 ± 22.00) | 1.30 ± 1.07 (28.89 ± 25.33) | 0.29-6.00 (1.36± 1.32) |
| Lymphocytes ($x10^9$ L) | 2.01 ± 1.48 (42.05 ± 22.60) | 2.48 ± 2.89 (55.11 ± 26.68) | 0.64-4.00 (1.70± 0.80) |
| Monocytes ($x10^9$ L) | 0.43 ± 0.32 (9.00 ± 3.83) | 0.44 ± 0.17 (10.47 ± 4.55) | 0.00 -0.30 (0.05± 0.07) |
| Eosinophils ($x10^9$ L) | 0.21 ± 0.14 (4.39 ± 1.64) | 0.28 ± 0.11 (6.22± 3.51) | 0.00 -0.89 (0.07± 0.18) |

**Table 4:**
Leucocytes Values of Adult Straw-Coloured Fruit bats in Nigeria

| PARAMETERS | MALES (n=7) | FEMALES (n=8) | REFERENCE VALUES (Selig *et al* ., *2016*) |
|---|---|---|---|
| WBC $x10^9$ L | 3.90 ± 1.42 | 5.6 ± 2.90 | 1.20 -7.30 (3.19± 1.48) |
| Neutrophils ($x10^9$ L) | 1.51 ± 0.76 (38.72 ± 25.50) | 2.71 ± 1.87 (48.40 ± 19.99) | 2.90 -6.00 (1.36± 1.32) |
| Lymphocytes ($x10^9$ L) | 1.79 ± 1.42 (45.89 ± 27.40) | 2.22 ± 1.60 (39.64 ± 19.46) | 0.64-4.00 (1.70± 0.80) |
| Monocytes ($x10^9$ L) | 0.40 ± 0.37 (10.26 ± 5.18) | 0.46 ± 0.29 (8.21 ± 2.49) | 0.00 -0.30 (0.05± 0.07) |
| Eosinophils ($x10^9$ L) | 0.2 ± 0.16 (5.13 ± 2.19) | 0.21 ± 0.14 (3.75 ± 0.99) | 0.00 -0.89 (0.07± 0.18) |

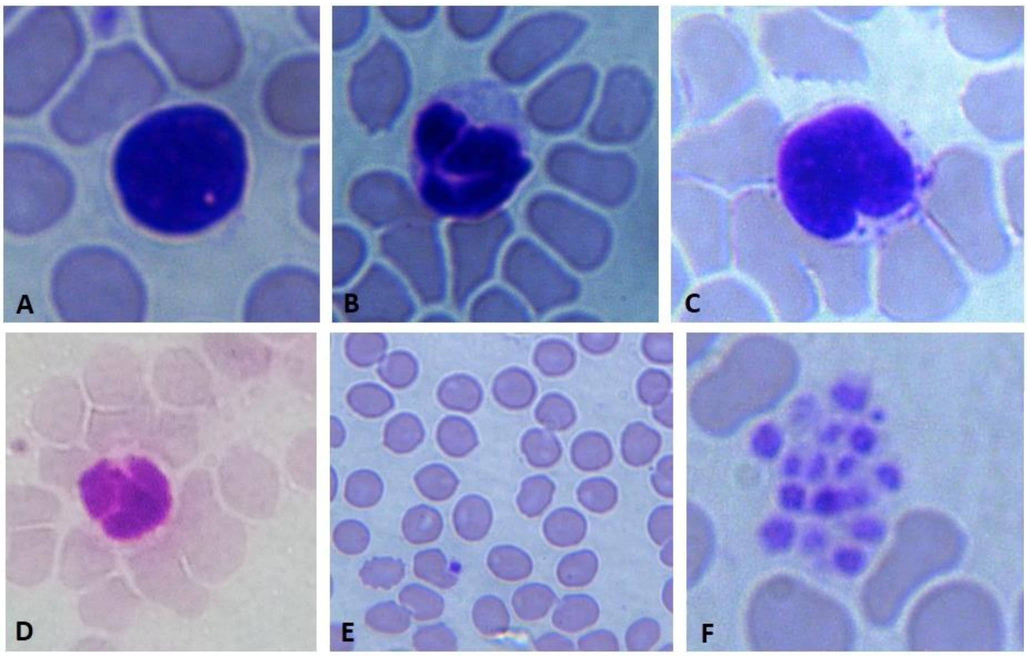


**Plate 1**: Photomicrograph of blood smear showing blood cells of the Adult Straw-coloured fruit bat A-F. Lymphocyte (A); Neutrophil (B); Monocyte (C); Eosinophil (D); Erythrocytes (E); Platelets (F).

**Blood Smear:** There were no morphological differences in the blood smear across ages and sexes of the straw-colored fruit bats (Plate 1A-F). Lymphocytes were the most abundant leukocytes in the blood smears (Plate 1A). They were round or slightly elliptical, contained small amounts of cytoplasm, and were of two different sizes large lymphocytes and of small lymphocytes. Neutrophils, the second most abundant leukocytes, were similar in size to large lymphocytes but easy to distinguish by the size and shape of the nucleus (Plate 1B). The nucleus of monocytes was U - or bean-shaped, and more than two-thirds of the cell size (Plate 1C). Eosinophils, the fourth most abundant leukocytes (Figure 1D), were also spherical in shape, had some visible large, granules in their cytoplasm. Erythrocytes were biconcave with no nucleus (Plate 1E). Platelets were seen as fragments (Plate 1F) in the blood smear.

**Erythrocyte Osmotic Fragility:** The erythrocyte osmotic fragility was higher in juvenile than in adults at 0 and 0.1% NaCl (Figure 1). On the other hand, erythrocyte osmotic fragility was higher in adult males than in adult females at 0 and 0.3% NaCl (Figure 2). However, there were no statistically significant (p> 0.05) differences in the above results.

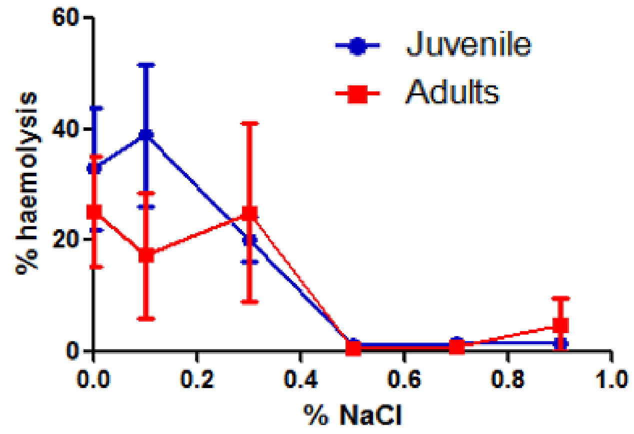


**Figure 1**:
Showing erythrocyte osmotic fragility test in juvenile and adults Straw-coloured fruit bat

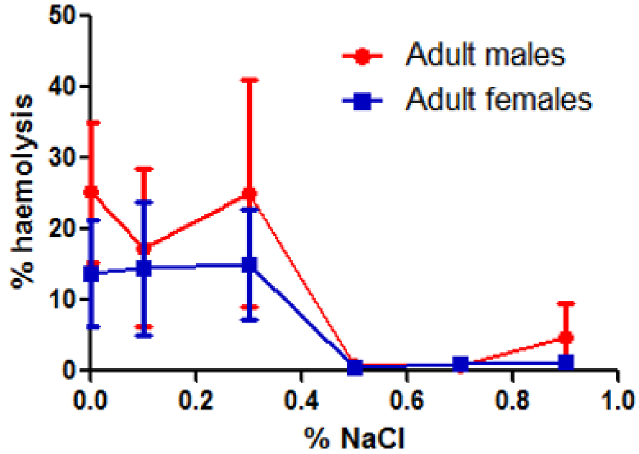


**Figure 2:**
Showing erythrocyte osmotic fragility test in adult males and females Straw-coloured fruit bat

**Micronucleus Assay:** This assay as a biomarker for genotoxicity indicated an elevated level of micronucleated polychromatic erythrocytes (MNPCE) in all the straw-coloured fruit bats (Plate 2). The PCE/NCE an index for assessing cytotoxicity indicates an abnormal proliferation from polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) (Table 5).

## DISCUSSION

The erythrocytes and leucocytes parameters in straw colored fruit bats were within the reference range as reported by Selig *et al* . (2016) except for the monocytes which were higher. High monocytes count is an indication of chronic inflammation, but this may be a normal phenomenon. It has been reported that the average percentage of monocytes is high in all species of bats (Riedesel, 1977).

The RBC count 7.54 $x10^{12}$/L is lower than 9.15 $x10^{12}$/L reported in *Epomops franqueti* (Ekeolu and Adebiyi, 2018), 12.39 $x10^{12}$/L in the serotine bat (Wolk and Ruprecht, 1988) and 11.35 $x10^{12}$/L in *Myotis myotis* (Albayrak *et al* ., 2016). There was no significant difference in the WBC counts of adults when compared with Juvenile likewise in adult males when compared with adult females in this study. The total WBC counts 4.69 $x10^9$/L obtained in this study is similar to that reported in the insectivorous microchiropterans of the temperate breeds, *Myotis myotis* with 4.87 $x10^9$ /L (Albayrak *et al* ., 2016) but higher than some other species such as vampire bats, 3.68$x10^9$/L (Arevalo *et al* ., 1992), *Myotis veliper*, 2.20 $x10^9$/L (Krutzsch and Hughes, 1959) and *Myotis elegans*, 2.05 $x10^9$/L. However, they differ from 13.46 $x10^9$/L obtained for franquet's fruits bat as reported by Ekeolu and Adebiyi (2018). Blood cells morphology is similar to that reported in the little brown bat by Cooper *et al* . (2014) and in some species of insectivorous bats by Paksuz *et al* . (2009). This study is the first to our knowledge to provide baseline information on the haematology and morphology in the straw-colored fruit bats in the tropics.

Osmotic fragility is a measure of the resistance of RBC to lysis as a function of increasing NaCl concentration. Females of the *Epomops franqueti* have higher erythrocytes osmotic fragility than the males of the same species (Ekeolu and Adebiyi, 2018). Although the erythrocyte osmotic fragility was higher in the juvenile than in the adults, there was no significant difference. It shows that the adult of *Eidolon* bats are more resistant to intravascular hemolysis. Similarly, erythrocytes osmotic fragility is higher in the adult males than in adult females. It indicates that the adult males are more fragile and less resistant to lysis.

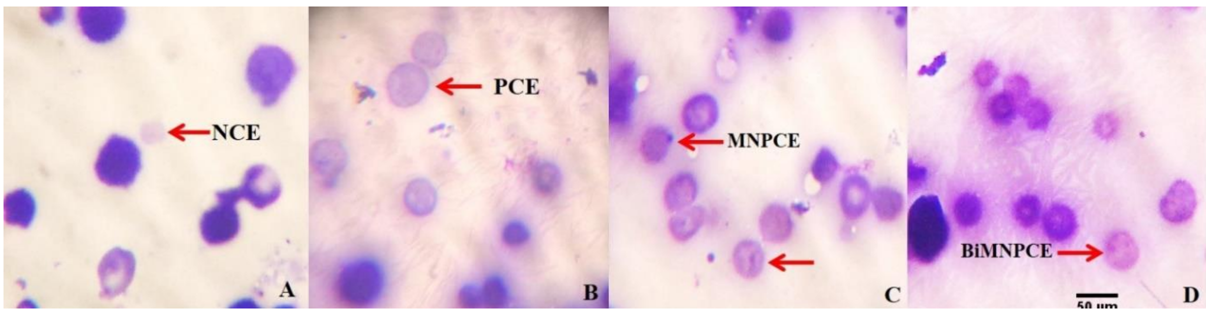


**Plate 2:**
Showing bone marrow smear of straw-colored fruit bat for micronucleus assay
A) NCE: Normochromatic erythrocyte; B) PCE: Polychromatic erythrocyte; C) MNPCE: Micronucleated erythrocyte; D) BiMNPCE: Bi-nucleated polychromatic erythrocyte. x1000 magnification.

Micronucleus used as a biomarker for genotoxicity in this study indicated that there may be environmental toxins (heavy metals, organic and inorganic chemicals), which accumulated over time in the bats. Since they are migratory birds, there is a great likelihood that the environment is highly polluted with genotoxins that are capable of inducing DNA damage, hence, increasing the micronucleated polychromatic erythrocytes. Naidoo *et al* . (2014) reported that pollutant-exposed bats have significantly higher DNA damage and diminished antioxidant capacity. In the study, there was an elevated level of MNPCE which is indicative that the environmental toxicants are likely clastogens and or aneugens inducing chromosome/chromatid breakage and inhibiting spindle fibres, respectively. In addition, it was observed that there was an abnormal rapid rate of proliferation from PCE to NCE, which is indicative of bone marrow toxicity and aging (Gonzalez-Borroto *et al* ., 2003; Heuser *et al* ., 2008) and likely reduction of the life span of the bats.

In conclusion, with the knowledge that bats are known reservoirs of many infectious disease that are transmissible to man and other mammals, the result of this study may provide a good basis to further explain the role of WBC and the immune system of this bat species following inflammation or infections with a means to early detection and control strategies for disease outbreak. The mechanism of the DNA damage induced by these environmental toxicants is unknown; however, further studies require identification of these toxicants as well as their mechanistic pathway in the induction of DNA damage. This is of importance to the general public as these toxicants may affect human health and the environment over time.

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## REFERENCES

Albayrak, I., Özcan, H.B and Baydemir, M. (2016). Some hematological parameters in Myotis myotis and Myotis blythii (Mammalia: Chiroptera) in Turkey. Turk. J. Zool., 40,388–91.

Alimba, C.G. and Bakare, A.A.(2016). In vivo micronucleus test in the assessment of cytogenotoxicity of landfill leachates in three animal models from various ecological habitats. Ecotoxicology,25, 310–319.

Arevalo, F., Parez, S.G and Lopez, L.P. (1992). Seasonal changes in blood parameters in the bat species Rhinolophus ferrumequinum and Miniopterus schreibersii. Arch. Physiol.Biochem.,100, 385–7.

Bandouchova, H., Bartonička, T., Berkova, H., Brichta, J., Kokurewicz, T., Kovacova, V., Linhart, P., Piacek, V., Pikula, J., Zahradníková, A. and Zukal, J.(2018). Alterations in the health of hibernating bats under pathogen pressure. Sci. Rep., 8,461-465.

Bezerra, L.R., Oliveira, W.D., Silva, T.P., Torreão, J.N., Marques, C.A., Araújo, M.J. and Oliveira, R.L. (2017). Comparative hematological analysis of Morada Nova and Santa Inês ewes in all reproductive stages. Pesquisa Veterinária Brasileira, 37, 408 – 414.

Cavas. T. (2008). In vivo genotoxicity of mercury chloride and lead acetate: micronucleus test on acridine orange stained fish cells. Food Chem. Toxicol., 46, 352–358.

Cooper, M., Hooper, S., Amelon, S. and Wiedeneyer C. (2014). Haematological and Electrolyte changes in little brown bats treated for white nose syndrome. In: merial-NIH Veterinary scholars' symposium.

Ekeolu, O.K. andAdebiyi, O.E. (2018). Hematology and erythrocyte osmotic fragility of the Franquet's fruit bat (Epomops franqueti). J Basic Clin Physiol Pharmacol. doi.org/10.1515/jbcpp-2017-0169.

Gonzalez-Borroto, J.I., Creus, A., Marcos, R., Molla, R., Zapatero, J. (2003). The mutagenic potential of the furylethylene derivative 2-furyl-1-nitroethene in the mouse bone marrow micronucleus test. Toxicol. Sci.,72, 359-362. 10.1093/toxsci/kfg038.

Heddle, J.A. (1973). A rapid in vivo test for chromosome damage. Mutat. Res., 18, 187–192.

Herdt, T.H. (2000). Variability Characteristics and Test Selection in Herdlevel Nutritional and Metabolic Profile Testing. Vet. Clin. N. Am.: Food AnimPract., 16, 387–403. doi:10.1016/s0749-0720(15)30111-0.

Heuser, V., Deandrade, V., Peres, A., Gomesdemacedobraga, L., and Bogochies, J. (2008). Influence of age and sex on the spontaneous DNA damage detected by Micronucleus test and Comet assay in mice peripheral blood cells. Cell Biol. Int., 32, 1223–1229. doi:10.1016/j.cellbi.2008.07.005.

Huang, D., Zhang, Y., Wang, Y., Xie, Z., Ji, W. (2007). Assessment of the genotoxicity in toad Bufo raddei exposed to petrochemical contaminants in Lanzhou Region, China. Mutat. Res., 629, 81–88.

Hutson, A.M., Mickleburgh, S.P. and Racey, P.A. (2001). Microchiropteran Bats: Global Status Survey and Conservation Action Plan, IUCN/SSC chiroptera specialist group, IUCN, Gland, Switzerland.

Krishna, G. and Hayashi, M. (2000). In vivo rodent micronucleus assay; protocol, conduct and data interpretation. Mutat. Res., 20, 155-66.

Krutzsch, P.H. and Hughes, A.H. (1959). Hematological changes with torpor in the bat. J. Mammal, 40,547–54.

MacGregor, J.T., Wehr, C., Henika, P.R., Shelby, M.D. (1990). The in vivo erythrocyte micronucleus test: measurement at steady state increases assay efficiency and permits integration with toxicity studies. Fund. Appl. Toxicol., 14, 513–522.

Malladi, S.M., Bhilwade, H.N., Khan, M.Z, Chaubey, R.C. (2007). Gamma ray induced genetic changes in different organs of chick embryo using peripheral blood micronucleus test and comet assay. Mutat. Res., 630, 20–27.

Morita, T., Asano, N., Awogi, T., Sasaki, Y.F., Sato, S., Shimada, H., Sutou, S., Suzuli, T., Wakata, A., Sofuni, T., Hayashi, M. (1997). Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (Group 1. 2A and 2B). The summary report of the 6th collaborative study by CSGMT/JEMS MMS. Mutat. Res.,389, 3–122.

Naidoo, S., Vosloo, D. and Corrie-Schoeman, M. (2014). Haematological and genotoxic responses in an urban adapter, banana bat, foraging at waste water treatment works. Eco. Env. Saf., 114, 304-311. doi.org/10.1016/j.ecoenv.2014.04.043.

Oyewale, J.O. (1992). Effects of temperature and pH on osmotic fragility of erythrocytes of the domestic fowl (Gallus domesticus) and guinea fowl (Numida maleagridis). Res. Vet. Sci.,52, 1-4.

Paksuz, S., Paksuz, E.P. and Ozkan, B. (2009). White blood cell (WBC) count of different bat (Chiroptera) species. Trakya. Univ. J. Sci., 10,55-59.

Riedesel, M.L. (1977). Blood physiology. In: "Biology of Bats". Ed. Wimsatt WA. Vol. III. Academic Press: pp485—517, New York.

Schmid, W. (1975). The micronucleus test. Mutat. Res.,31, 9-15.

Selig, M., Lewandowski, A. and Kent, M.S. (2016). Establishment of reference intervals for hematology and biochemistry analytes in a captive colony of straw-colored fruit bats (*Eidolon helvum*). J. Zoo. Wild. Med., 47, 106–112.

Shawaf, T., Hussen, J., Al-Zoubi, M., Hamaash, H., and Al-Busadah, K. (2018). Impact of season, age and gender on some clinical, haematological and serum parameters in Shetland ponies in east province, Saudi Arabia. Int. J. Vet. Sci. Med., 6, 61–64. https://doi.org/10.1016/j.ijvsm.2018.03.007

Skinner, J.D. and Chimimba, C.T. (2005). The Mammals of the Southern African Sub-region. 3rd edition. Cambridge University Press, Cambridge, UK.

Wołk, E. and Ruprecht, A.L. (1988). Haematological values in the serotine Bat, Eptesicus serotinus (Schreber, 1774). Acta Theriol., 33, 545–53.