Comparative study of fasting blood glucose and haematological parameters of Wistar rat, *Rattus norvegicus* and the common African toad, *Bufo regularis*

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Abstract

Haematological parameters are good indicators of the physiological status of animals and useful in understanding the relationship of blood to the environment. This study was carried out to compare the fasting levels of blood glucose and haematological parameters (Red blood cell count, Packed cell volume, Haemoglobin concentration, White blood cell count, Platelet count, and differential White blood cell counts) in Wistar rats, *Rattus norvegicus* and the common African toad, *Bufo regularis*. Ten rats and ten toads were used in the study. The animals were divided into two groups. Group I consists of 10 rats while Group II consists of 10 toads. The animals were fasted overnight before blood samples were collected for the determination of blood glucose and blood parameters. The blood glucose was determined using modified glucose oxidase method while the blood parameters were determined using standard laboratory techniques. The results of the study showed that the fasting blood glucose level in rats was significantly higher than that of toad. The fasting levels of blood parameters, packed cell volume, red blood cell counts, haemoglobin concentration in the toad were significantly lower than those of rats while the white blood cell counts of the toads were significantly higher compared with the rats. There were no significant differences in neutrophil, monocyte, eosinophil and lymphocyte counts of rats when compared with those of the toad.

Keywords: Blood glucose; red blood cell; haematological parameters; Wistar rat; the common African toad.

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Introduction

Glucose is an important source of energy in most organisms ranging from bacterial to humans. The mechanism of glucose metabolism is complex. The control and regulation of glucose homeostasis have been reported to vary greatly between different species leading to fluctuations in blood glucose levels in different classes of vertebrates (Zhang *et al* 2018). It has been reported that blood glucose levels in vertebrates correlate positively with metabolic rate. In mammals, the blood glucose concentration is about 7mmol/l, while in birds it is double that of mammals. In fish and amphibians, the blood glucose level is lower than that of mammals (Polakof *et al* 2011). Temperature has been reported to affect both the metabolic rate and blood glucose levels in poikilotherms. In amphibians for instance, exposure to low temperature generally causes lowered metabolic rate accompanied by hypoglycemia (Umminger 1970). The amphibians have been reported to have the lowest blood circulating glucose levels among the vertebrates because they do not use glucose as a required energy substrate (Polakof *et al* 2011). Previous studies attributed the low blood glucose levels in amphibians to their peculiar intermediary metabolism, which depends on alternate energy sources other than glucose (Polakof *et al* 2011).

The study of haematological parameters is an important and reliable tool to evaluate the health and physiological status of animals (Al-Samarai and Al-Jbory 2017; Suljevic *et al* 2018). Determination of baseline haematological parameters is important in the clinical diagnosis of some diseases. Amphibians have elliptical nucleated erythrocytes in their blood while mammals have anucleated red blood cells shaped as biconcave discs and vary widely in number and size according to the species (Cabagna *et al* 2005). Variation by sex, age, season and pathological factors in the number of erythrocytes has been reported in amphibians (Arikan and Cicek 2014). Like other vertebrates, erythrocytes in amphibians carry respiratory gases (oxygen and carbon dioxide) by means of haemoglobin (Foxon 1964). The number of red blood cells in amphibians is lower than those of mammals (Arikan and Cicek 2014). The differential white blood cell counts give information concerning the health status of the organism. For example, higher than

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normal proportions of neutrophils in circulation can point to infection (Thrall 2004) while eosinophils are associated with parasitism defense (Kiesecker 2002). The functions of amphibian granulocytes resemble those of other vertebrates (Wright 2001). Lymphocytes are cells that help in immune responses and production of haematopoietic growth factors. Among leukocytes, lymphocytes constitute the dominant cells in amphibians (Campbell 2004). Various factors affect the number of leukocytes in amphibians including sex, age, season, ecological and pathological factors (Arikan and Cicek 2014). Amphibian thrombocytes are nucleated cells and are functionally equal to mammalian platelets (Arikan and Cicek 2014). There is dearth of information on the comparative study of blood glucose and haematological parameters in mammals and amphibians. This study was designed to compare fasting levels of blood glucose and haematological parameters in Wistar rat, *Rattus norvegicus* and the common African toad, *Bufo regularis*.

Materials and methods

Ten (10) Wistar rats and ten (10) common African toads (*Bufo regularis*) were used in the study. The rats were obtained from Central Animal house, College of Medicine, University of Ibadan, Ibadan, Nigeria. The rats were acclimatized to laboratory conditions (12 hours light and dark, standard feed and standard room temperature) for two weeks. They were aerated and fed on standard rat chow. They were allowed free access to water *ad libitum*. The rats were fasted overnight before the start of the experiment. Blood was collected from the rat tail for blood glucose determination while blood was collected from ocular vein into heparinized EDTA bottles and used for determination of blood parameters. The toads were collected at night from slow-moving waters within the University of Ibadan. Collected toads were transported to the laboratory and acclimated in a wire-gauged cage containing water and free from insects.

The toads were fasted overnight before the start of the experiment and then anaesthetized with 3mg/100g sodium thiopental intraperitoneally. Each toad was secured on its back on a dissecting board. The thorax was opened and the truncus arteriosus was dissected free from the surrounding connective tissue. Blood sample was taken from truncus arteriosus and used for blood glucose and blood parameters (packed cell volume (PCV), red blood cell Count (RBC), haemoglobin concentration (Hb), white blood cell count (WBC), platelet count, and differential white blood cell count (WDC) determinations. Due to the small size of the toads, 1ml of blood was obtained from each toad once and for all. Blood glucose was determined immediately using modified glucose oxidase method (Trinder 1969). While blood collected for haematological analyses were stored in EDTA bottles prior to determination of blood parameters. The values of blood parameters were determined immediately after collection using standard laboratory techniques. From the values obtained, the haematological indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated (Jain 1986).

Statistical analysis

The mean±S.E of all measurements were computed. Significance was assessed by student's t-test for two means of independent variables. P-values of 0.05 or less was taken as statistically significant.

Results

The results of the study are presented in Tables 1-3. The results showed that the mean values of fasting blood glucose level, PCV, RBC, Hb and platelet counts in the common African toad were significantly lower than those of the rats (Table 1) while WBC was significantly higher in the toad than in the rat (Tables 3). There were no significant differences between the neutrophil (NEU), monocyte (MONO), eosinophil (EOS), and lymphocyte (LYM) counts of rats and toads (Table 3). However, MCV, MCH and MCHC varied significantly between the rat and toad (Table 3).

Discussion

The results of this study showed that there were significant differences in the mean values of fasting blood glucose and blood parameters levels of rats compared with the common African toads. The differences in fasting levels of blood glucose and blood parameters of rats compared with the toads showed species variability between rats and toads. Toads are amphibians, their body temperature and circulating blood glucose vary with the environment while rats are mammals whose body temperature and blood glucose are regulated within a narrow range, independent of the environment (Polakof *et al* 2011). The fasting blood glucose level observed in the present study is consistent with previous studies in toads (Oyebola *et al* 1998; Isehunwa and Alada 2016).

The mean values of fasting blood glucose in the present study are higher for rats than toads. It has been reported that the normal resting level of blood glucose in amphibians is lower than other vertebrates. The lower blood glucose levels observed in toads in the present study and their ability to survive without signs of hypoglycemia suggests probably that their metabolism does not require glucose for energy source. In mammals, glucose is the required metabolic substrate for energy while amphibians do not use glucose as a required energy substrate (Polakof *et al* 2011). The lower blood glucose levels observed in the toads compared with the rats could also be due to exposure to lower temperature. It has been reported that temperature affects both metabolic rate and blood glucose levels in poikilotherms (Umminger 1970).

Table 1: Comparison of fasting levels of blood glucose and blood parameters (PCV, RBC and Hb) of the common African toads and rats.

Animals	Blood Glucose Level (mg/dl)	PCV (%)	RBC (x1012/l of blood counts)	HB(g/dl)
Rats	***76 ± 2.3**	***44.8 ±1.4**	***7.5 ± 0.2**	***15.2 ± 0.5**
Toads	**52.9 ± 6.5**	**31.5 ±1.5**	**3.3 ± 0.2**	**10.2 ± 0.6**

Values are Mean ± S.E, significant * ($p < 0.05$) n=10

Table 2: Comparison of mean values of mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) of common African toads and rats.

Animals	MCV (fl)	MCHC(g/dl)	MCH (pg)
Rats	**59.5 ± 0.5**	***34.0 ± 0.2**	**20.2 ± 0.2**
Toads	***97.9 ± 6.0**	**32.5 ± 0.3**	***31.9 ± 2.1**

Values are Mean ±S.E, significant * ($p< 0.05$), n=10

Table 3: Comparison of mean values of fasting white blood cell counts (WBC), differential white blood cell counts and platelets in rats and the common African toads.

Animals	WBC (x10^9 /l)	PLATELET (x109 /l)	LYM (%)	NEUT (%)	MONO (%)	EOS (%)	BASO (%)
Rats	7945 ±1049.0	142300±493.8	65.2±1.9	29.9±1.99	2.2±0.4	2.7±0.3	0.0
Toads	*14545±872.4	132600±20.4	66.3±1.2	26.9±1.4	2.5±0.4	3±0.4	0.3±0.2

Values are Mean ± S.E, significant *($p < 0.05$), n=10

Observation of RBC, Hb, PCV, WBC, platelets counts, DLC, neutrophils, eosinophils, lymphocytes and monocytes in both the blood of rats and toads showed similarities in the blood parameters of mammals and amphibians. In vertebrates, most blood cells including erythrocytes, thrombocytes and leukocytes are formed in the bone marrow in adults (Arikan and Cicek 2014). The mean values of fasting blood parameters of RBC, PCV and Hb concentrations in the toads were lower than those of the rats. This is consistent with the study of Arikan and Cicek (2014). The differences in the values of blood parameters in toads and rats may be genetically based, developmental or physiological in nature (Sealander 1964). Seasonal changes have been reported to affect the rate of haemopoietic activity and blood parameters in amphibians (Glomski *et al* 1997; Isehunwa and Alada 2013). The observed higher red blood cell counts, haemoglobin concentration and packed cell volume values in rats compared with toads may be associated with blood oxygen capacities. Previous studies have shown that increased oxygen capacity provides means for increasing oxygen delivery to tissues with high metabolic rates (Sealander 1964). The higher red blood cell counts and hemoglobin concentration observed in the rats may assist the animals with increased oxygen supply to tissues. The hematological indices MCV, MCH and MCHC of rats were significantly different compared with those of the toads. Mammals have been reported to have higher metabolic rate than amphibians, therefore, require efficient delivery mechanism for increased oxygen supply to the tissues. Since erythrocyte is the most important carrier of oxygen and carbon dioxide (Sinha 1983), oxygen carrying capacity of the animals is proportional to the amount of RBC counts, or Hb concentration (Prosser 1973). Amphibians because of exposure to lowered environmental temperature have low metabolic rate.

The differences in resting blood parameters of the toad and rat could also be due to differences in habitat and species variation. This agrees with the study of Isehunwa and Alada (2013), which reported that among poikilotherms, the blood parameters correlate with habitat and activity. The higher significant white blood cell counts observed in the toads compared with the rats enables them to withstand hazards in their environment. This is consistent with the observations of Etim *et al* (2014) that animals with high white blood cell counts have high degree of resistance to diseases. Amphibians are found around shallow waters and ponds near

agricultural lands that may be contaminated with chemicals, therefore, are exposed to greater risk. This agrees with the observation of Rowe *et al* (2003). The observation of the present study in which the most abundant cell type of differential white blood cells is lymphocytes followed by neutrophils agrees with previous studies in rats and amphibians (Campbell 2004; Arikan and Cicek 2014). There were basophils in the blood of the common African toad but absent in the blood of the rats. This difference could be due to species variability. The presence of basophils in the blood of the common African toad agrees with the observation of Arikan and Cicek (2011). The results of this study also showed that the MCHC of rats was significantly higher than that of the toads while the MCV and MCH of toads were significantly higher compared with the rats.

Conclusion

This study showed that the fasting level of blood glucose in Wistar rat was significantly higher compared with the common African toad, *Bufo regularis*. The fasting levels of red blood cell counts (RBC), packed cell volume (PCV), hemoglobin concentration (Hb) in the common African toad were significantly lower than those of the rats while the mean value of white blood cell counts in the toads was higher, significantly than that of the rats. There were no significant differences in the neutrophil, monocyte, eosinophil, and lymphocyte counts of Wistar rat compared with the common African toad.

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