Chapter

Effects of Therapeutic and Toxic Agents on Erythrocytes of Different Species of Animals

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Abstract

A preponderance of therapeutic and toxic agents that affect erythrocytes is being used in preclinical and clinical settings. Such agents are responsible for wrong diagnosis of a myriad of diseases and poor prognosis of some therapeutic interventions. In view of this, literature search was carried out with a view to investigate morphometry of erythrocytes in various diseased conditions and species of animals. Findings have shown that erythrocyte size, area, and volume vary in different species of animals under different diseased conditions. Environmental factors, toxicants, toxins, therapeutics, and management system, among others, can cause erythrocyte deformation, leading to anemia. Erythrocyte-related diseases include but not limited to sickle cell anemia, malaria, cancer, psychiatric illness, and chronic inflammation. Hence the principal source of our survival is erythrocyte, because it transports oxygen needed for metabolism of cell nutrients.

Keywords: drug, toxin, toxicant, size, shape, erythrocyte, blood disease, treatment, allometry

1. Introduction

Blood is a connective tissue comprising hematocrit (45%), white blood cells, platelets, red blood cells, and plasma (55%) which is a mixture of protein, water, lipids, amino acids, hormone, carbohydrates, vitamins, and cellular wastes [1]. About 8% of body weight is blood [2, 3], 18% protein and water substances, 15% fat, 7% mineral, and 60% water [4]. Increased surface area of erythrocytes is an adaptation for transportation of oxygen bound to hemoglobin [5]. But destruction of RBCs can lead to anemia based on cause and morphology of erythrocytes. Anemia could be hereditary [6], sickle cell anemia, Mediterranean anemia (thalassemia), glucose-6-phosphate dehydrogenase (6GPD) anemia, aplastic anemia, hemolytic disease of newborn, and acquired hemolytic anemia [7]. Fresh frozen plasma and plasma protein can serve as expander and supply clotting for patients with clotting factor-deficient diseases [8]. Blood can be collected from mice and rat tail, lateral saphenous vein, lateral tail vein, retro-orbital sinus, and heart. Only 1% of blood should be collected at a time [9]. Plasma expanders exert oncotic pressure during infusion and are retained in the vascular compartment [10]. Bed rest of 100–200 days decreased plasma volume by 30% [11]. Blood count consists of erythrocytes, hematocrit, hemoglobin, leucocytes, and differential leucocyte counts. The normal range of

erythrocytes (4.2–6.2 $\times$ 10^{12}/L), hemoglobin (100 g/100 mL), and hematocrit (38–54%) of the total blood volume are the standards for human species [12]. Variation in species, age, environmental factors, management system, and pathological conditions could affect the size, shape, area, and volume of erythrocytes.

2. Methodology

Extensive literature search was carried out to identify differences between normal and abnormal erythrocytes of various species of animals including the ones in the wild. Information on beneficial and toxic effects of drugs, chemical toxicants, toxins, plant extracts, chemicals, and diseased conditions were searched on erythrocyte shape, size, and volume for various species of animals including human. Some developed formulas were modified for determination of anemic, polycythemic, hydrated, and dehydrated erythrocytes. Physiological and pathological features of the erythrocytes were also highlighted. Preclinical and clinical values of the changes in erythrocytes in relation to blood diseases caused by various agents were critically analyzed. Effects of toxic and therapeutic agents on metabolic, cancer, infectious, inheritable, and noninheritable diseases of erythrocytes, such as sickle cell anemia, malaria, and hereditary spherocytosis, among others, were elucidated.

3. Results

The values of erythrocytes, packed cell volume plasma volume, hemoglobin concentration, body weight, and salient features of erythrocytes are presented in **Table 1** [26, 27].

3.1 Morphometry of erythrocytes

Erythrocytes have larger (a) and minor (b) axes, volume (v), and surface area. The measurement is in micrometer (μm):

$$a = \frac{1}{2}; b = \frac{1}{2}.$$

$$\text{Surface area} = 2a^2\left(1 + \frac{c}{ac}\text{sine}^{-1}e\right); \text{where } e^{-1} = 1\frac{-a^2}{c^2} \quad (1)$$

$$\text{Volume} = {}^4/_3\, a^2 \times b \quad (2)$$

Hematological variances can occur between animals of different species and the same species. Erythrocytes of *Piaractus mesopotamicus* (2.57 $\pm$ 0.5 $\times$ $10^6/\mu l$) was higher than that of *Brycon orbignyanus* (2.56 $\pm$ 0.5 $\times$ $10^6/\mu l$), *Oreochromis niloticus* (1.70 $\pm$ 0.4 $\times$ $10^6/\mu l$), and *Rhamdia quelen* (2.11 $\pm$ 0.6 $\times$ $10^6/\mu l$), respectively. Larger axes of *R. quelen* (12.1 $\pm$ 0.3 μm), *O. niloticus* (13.2 $\pm$ 0.6 μm), *B. orbignyanus* (14.4 $\pm$ 0.3 μm), and *P. mesopotamicus* (15.0 $\pm$ 0.4 μm), as compared to their minor axes, 9.3 $\pm$ 0.3, 9.3 $\pm$ 0.4, 8.7 $\pm$ 0.2, and 9.8 $\pm$ 0.2 μm, respectively, as well as the surface area and volume of *R. quelen* (317.0 $\pm$ 36.4 μm^2; 545.2 $\pm$ 95.0 μm^3), *O. niloticus* (343.1 $\pm$ 43.4 μm^2; 612.6 $\pm$ 119.4 μm^3), *B. orbignyanus* (337.3 $\pm$ 30.771 μm^2; 585.4 $\pm$ 84.0 μm^3), and *P. mesopotamicus* (400.6 $\pm$ 36.5 μm^2; 765.8 $\pm$ 108.7 μm^3), respectively, show that erythrocyte shape, area, and volume vary even in the same species of animals [28].

Breed	English name	Scientific name	Weight (kg)	Erythrocytes ($\times 10^6/\mu l$)	Hematocrit	Hemoglobin concentration (g/dl)	Plasma volume (ml)	Total blood volume (ml)	Comment(s)	References
Canis	Dog	*Canis familiaris*	7.19	6.12 ± 0.25	36.75 ± 1.49	12.25 ± 0.49	363.8	575.2	High blood volume	[13]
Mus	Mouse	*Mus musculus*	0.025	9.60 ± 1.02	36.00 ± 2.58	11.90 ± 0.86	1.28	2.0	High plasma volume	[14]
Meleagris	Turkey	*Meleagris gallopavo*	2.0	1.99–2.26	33.2 ± 3.56	11.07±1.19	106.9	160	High plasma volume	[15]
Mus	Mouse	*Mus musculus*	0.021	1.09 ± 0.04	41.00 ± 2.08	13.67±0.69	1.0	1.7	High hematocrit	[16]
Rattus	Rat	*Rattus norvegicus*	0.16	7.21±0.14	38.17±0.87	13.83±0.06	7.9	12.8	High erythrocytes	[17]
Caprine	Goat	*Capra hircus*	13	11.5±0.4	29.4±0.8	9.8±0.3	734.2	1040	Higher erythrocytes	[18]
Labeo	Fish	*Labeo rohita*	1.48	1.3±0.03	24.30±3.30	8.1±1.10	89.6	118.4	Lower hematocrit	[19]
Struthio	Ostrich	*Struthio camelus*	111	151.58±0.30	36.47±3.78	11.37±0.70	5641.7	8880	Very high erythrocytes	[20]
Streptopelia	Laughing dove	*Streptopelia senegalensis*	0.1	3.76±0.01	42.60±0.86	14.04±0.25	4.6	8.0	Higher hematocrit	[21]
Naja	Indian cobra	*Naja naja*	9.3	0.58±0.04	30.11±1.93	7.6±0.75	520	744	Lower hemoglobin	[22]
Bos	Cow	*Bos indicus*	450	6.7 ± 0.65	28.50±2.05	7.55±3.5	25,740	36,000	Lower hematocrit	[23]
Homo	Child	*Homo sapiens*	23.25	0.36–0.28	24.33–19.09	7.81–4.59	1407.5-1505	1860	Lower parameters	[24]
Homo	Man	*Homo sapiens*	70	4.5–5.9	39–49	13.6–17.2	2856.1–3416.1	5600	Lower plasma volume	[25]
Homo	Woman	*Homo sapiens*	70	3.5–5.0	33–43	12.0–15.0	3192.2–3752.1	5600	Higher plasma volume	[25]

Key: Hemoglobin = 1/3 of hematocrit.

Table 1.
Erythrocytes, packed cell volume, and hemoglobin concentration in various species of animals.

4. Discussion

4.1 Erythrocytes in various species of animals

Erythrocytes in various species of animals vary both in quality and quantity. For example, lactating Holstein breed of cow had hematocrit of 28.50 ± 2.05% and hemoglobin of 7.55 ± 3.5 g/dl on the first lactation and hematocrit (30.02 ± 2.05%) and hemoglobin (12.5 ± 2.1 g/dl) on the sixth lactation, respectively. Hence frequency of lactations changes erythrocytes in dairy cow. Albumin (2.92 ± 0.17 g/dl) on the first lactation increased to 3.69 ± 0.08 g/dl on the sixth lactation, respectively [23]. Hence increased erythrocytes may connote increased albumin. Ostrich (*Struthio camelus*) could weigh between 70 and 150 kg. The erythrocytes (151.58 ± 0.30 × 10^6/mm^3), hemoglobin (11.37 ± 0.70 g/dl), and hematocrit (36.47 ± 3.78%) of ostrich chick were higher than erythrocytes (131.83 ± 0.19 × 10^6/mm^3), hemoglobin (12.01 ± 1.51 g/dl), and hematocrit (40.15 ± 2.44%) of grower ostrich. Total protein was higher (4.32 ± 0.29 g/dl) in ostrich chick than that of young ostrich (3.63 ± 0.54 g/dl), respectively [20], signifying that total protein may be correlated with erythrocytes. Hematocrit of Kano brown buck (55.8 ± 1.12%) is higher than that of Kano brown doe (31.0 ± 0.73%), Borno white buck (34.00 ± 1.2%), Kano brown doe (8.80 ± 0.44%), Sokoto red doe (8.2 ± 0.34%), and Sokoto red buck (8.00 ± 0.29%), respectively. Erythrocytes of Sokoto red doe kid (1.96 ± 0.5 × 10^6/mm^3) are lower than that of buck kid (2.56 ± 6.11 × 10^6/mm^3), Kano brown buck kid (3.4 ± 0.01 × 10^6/ mm^3), and Kano brown doe kid (4.9 ± 6.11 × 10^6/mm^3), respectively. But total protein of Borno white buck (47 ± 1.2 g/dl) is lower than that of Sokoto red buck (69.0 ± 1.33 g/dl), whereas albumin of Borno white doe (26.00 ± 1.1 g/dl) is lower than that of Sokoto red doe (29.0 ± 0.06 g/dl), respectively [29]. Factors affecting erythrocyte management system such as intensive, semi-intensive, and extensive systems of grazing could change erythrocytes. Cattle under intensive care had erythrocytes at the beginning of grazing (6.62 × 10^6/mm^3) as compared to the end of grazing (6.29 × 10^6/mm^3). However, those under extensive care had an erythrocyte increase of 6.69 × 10^6/mm^3 at the beginning of grazing and 7.26 × 10^6/mm^3 after grazing. But cattle that grazed on pasture in group had erythrocytes of 6.93 × 10^6/mm^3 at the beginning in comparison with 7.23 × 10^6/mm^3 after grazing. Total protein was significantly higher in all the groups before grazing as compared to after grazing [30]. Erythrocytes of Nigerian laughing dove (*Streptopelia senegalensis*) after 4 weeks and 8 weeks in captivity are 3.76 ± 0.01 and 3.01 ± 0.11 ×10^6/μl, respectively. The PCV after 4 weeks (42.60 ± 0.86%) was higher than after 8 weeks (34.60 ± 1.47%), respectively. Hemoglobin was significantly higher after 4 weeks (14.04 ± 0.25 g/dl) than 11.26 ± 0.48 g/dl after 8 weeks. But total protein and albumin were slightly lower after 4 weeks than after 8 weeks, suggesting that captivity could lead to decreased erythrocyte count [21]. Erythrocytes of West African dwarf goats are 11.5 ± 0.4 × 10^6/mm^3, hematocrit is 29.4 ±0.8%, and hemoglobin is 9.8 ± 0.3 g/dl, respectively, whereas total protein and albumin are 7.1 ± 01 g/dl and 2.4 ± 0.7 g/dl, respectively [18]. Increase in erythrocytes, hemoglobin, and lactate in *Mugil cephalus* (fish) in comparison with other species of fish such as *Gobius niger*, *Sparus aurata*, and *Dicentrarchus labrax* could be attributed to their feeding behavior, adaptation to the environment, and lifestyle [31]. Erythrocyte count of Balami ewe (9.66 ± 0.12 × 10^6/mm^3) is higher than that of Yankasa ewe (9.31 ± 0.78 × 10^6/mm^3), Ouda ewe (9.25 ± 0.02 × 10^6/mm^3), Yankasa ram (7.80 ± 0.62 × 10^6/mm^3), Balami ram (7.21 ± 0.42 × 10^6/mm^3), and Ouda ram (6.49 ±0.01 × 10^6/mm^3), respectively. Ouda ram has the highest PCV value of 64 ± 2.14%, whereas Yankasa ram has the least PCV value of 28.90 ± 0.02%

[32]. Reference value for erythrocytes in cow (5–10 $\times$ 10^6/mm^3) and PCV for sheep (24–45%), cow (24–48%), rabbit (30–50%), guinea pig (37–48%), and swine (32–50%), respectively, show that swine has the highest PCV value in this group of animals. But reference value of hemoglobin concentration for swine (10–16 g/dl), sheep (8–16 g/dl), cow (15–18 g/dl), rabbit (10–15 g/dl), and guinea pig (11–15 g/dl), respectively, indicates that cow has the highest hemoglobin concentration, perhaps resulting from hemolysis. Toxicants, environmental factors, genetics, age, sex, breed, and management system could affect erythrocytes of farm animals [33]. An image-based error (3%) for counting of RBCs has been reported for *Leopardus pardalis*, *Cebus apella*, and *Nasua nasua*. However the RBC values for *Canis familiaris* (5.50–8.50 $\times$ 10^6/mm^3), *Equus caballus* (6.10–11.0 $\times$ 10^6/mm^3), *Leopardus pardalis* (4.07–6.16 $\times$ 10^6/mm^3), *Cebus apella* (3.49–5.48 $\times$ 10^6/mm^3), and *Nasua nasua* (3.88–5.35 $\times$ 10^6/mm^3) with the species showing RBC interval of 3.47–11.0 $\times$ 10^6/μl) [34], respectively, show that erythrocyte volume varies from species to species of animals.

4.2 Diseases of erythrocytes

But changes in erythrocyte shape, area, and volume may be caused by malaria, sickle cell disease, and other related erythrocytes diseases. Malaria parasites of clinical and laboratory importance include *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi* in human and could be treated using orthodox and traditional medicine [35]. The gorilla, chimpanzee, orangutan, gibbon, and monkey also contract various species of malaria. *Aotus trivirgatus* (species of monkey) are experimental models for human *P. vivax* and *P. falciparum*. *P. berghei* discovered in *Grammomys sudaster* is a model of experiment for mammalian malaria. Mice and young rats could also be used as models for *P. berghei* which is very fatal, causing death in 1–3 weeks when less than 10^7 *P. berghei* was inoculated. *P. relictum*, *P. gallinaceum*, *P. cathemerium*, and *P. lophurae* are also used as models of experiment. *P. durae* and *P. juxtanucleare* caused 96% mortality in turkey [36] indicating that malaria could be a source of low erythrocyte count in all the species mentioned above. Consistent acridine orange staining of thin blood film for malaria parasites and rapid staining tests produce superior results in comparison to Giemsa method [37].

Sickle cell disease (SCD) is characterized by dense dehydrated red blood cells (DRBCs) that undergo polymerization and sickling due to sickle cell hemoglobin (Hbs) concentration. DRBCs in sickle cell disease patients caused priapism, renal dysfunction, skin ulcer, deletion of α-thalassemia, hyperbilirubinemia, and increased lactate dehydrogenase [38]. There is comorbidity of SCD and malaria among indigenes of Northwestern Nigeria with highest incidence of SS (51.8%), SC (28.4%), AS (16.2%), and SS + F (3.6%), respectively. Hemophilia, epistaxis, and splenomegaly, among others, are associated with SCD. Weight, packed cell volume, hemoglobin, total blood volume, red blood cell volume, and plasma volume are seriously affected in sickle cell patients that are not therapeutically managed causing the need for blood transfusion. Good prognosis is guaranteed by polypharmacy that involves the use of hematonic, anti-sickling, analgesic, antimalarial, and anti-inflammatory drugs. Patients from Northwestern Nigeria can live up to 49 years [24]. *Raphanus sativus*, *Arbutus unedo*, *Luffa acutangula*, *Lycopersicum esculentum*, *Cucumis melo*, *Brassica oleracea* var. capitata, *Allium porrum*, *Petroselinum sativum*, *Phoenix dactylifera*, and *Ficus carica* can be used for management of blood and blood-related diseases including the diseases of erythrocytes [39, 40]. Efforts made toward antimalarial vaccine may be much near to fruition [35].

Autoimmune hemolytic anemia is associated with erythrocytes characterized by hemolysis and autoantibodies of anti-erythrocytes. Neurological sign is common in pernicious anemia. However normal morphology of erythrocytes and leucocytes is necessary for diagnosis of idiopathic thrombocytopenic purpura. But pernicious anemia is characterized by dyserythropoiesis and low vitamin B12 with anti-intrinsic factor and anti-gastric mural cell antibodies being positive. Hence pernicious anemia is treated using vitamin B12 [41]. Complete blood count (CBC) identifies anemia, thrombocytopenia, leukopenia, polycythemia, thrombocytosis, and leukocytosis, but 10–20% of results are abnormal [42].

The life span of erythrocytes in human is 120 days; 20 μl of the erythrocytes are produced daily. But the circulating red blood cells vary among individuals of the same age and gender by over 10%. Mechanisms of anemia in solid tumors are by intrinsic or iatrogenic blood loss; iron or folic acid deficiency; autoimmune, traumatic, or drug-induced hemolysis; bone marrow factor caused by myelofibrosis; marrow necrosis; infection; inflammation; and cancer elsewhere in the body. Erythropoietin is important in the production of erythrocytes. Erythropoietin maybe impaired by tumor inflammatory cytokines [43]. Erythrocytes and hemoglobin promote tumor cell growth by increasing nucleotide-binding oligomerization domain-like receptors' expression and cause induction of IL-1b release, macrophage recruitment, and polarization. Therefore, hemorrhage could be used as a sign of therapeutic failure in cancer patients, because it promotes tumor cell growth and anticancer drug resistance [44]. Facilitation of breast cancer treatment by nano-scaled erythrocytes is via a combination of photodynamic, photothermal, and chemotherapy [45]. Erythrocytes can be lost in the blood lost from cancer surgery and anticancer drugs which affect fast-dividing normal cells and cancerous cells, indicating the drugs cannot differentiate good cells from bad cells [41].

Low calcium concentrations were reported in erythrocytes of patients with depressive disorders [46] indicating that erythrocytes could be used to assess therapeutic success of depressive illness and high calcium level could abate the disease. There was higher concentration of soluble catechol-O-methyltransferase (COMT) in erythrocytes of patients suffering from bulimia nervosa and binge eating disorder than anorexia nervosa [47] indicating that erythrocytes could be used for diagnosis of eating disorders. Favism, neonatal icterus, hereditary non-spherocytic hemolytic anemia, drug-induced hemolytic anemia, and hemolytic anemia due to infection are caused by increased destruction of erythrocytes with enzyme deficiencies. Quinine, chloroquine, sulfadiazine, phenytoin, isoniazid, chloramphenicol, ascorbic acid, and colchicine, among others, could be given in therapeutic doses to G-6-PD-deficient patients without non-spherocytic hemolytic anemia [48]. Hereditary spherocytosis, hereditary elliptocytosis, hereditary pyropoikilocytosis, and hereditary stomatocytosis are hemolytic anemias characterized by heterogeneity and treated by splenectomy which in turn causes complications of cardiovascular diseases, thromboembolic disorders, pulmonary hypertension, and penicillin-resistant pneumococci [49]. Human erythrocytes are subject to degree of genetic diversity. This variability results in anemia, cyanosis, polycythemia, non-hematologic alterations, sickling disorders, unstable hemoglobinopathies, or hemoglobinopathies associated with polycythemia, methemoglobinemia, and α- and β-thalassemias [48]. The cardiovascular effects of SCD and thalassemia are due to iron accumulation and hypoxia, respectively. The risk of thromboembolism should be assessed after splenectomy in the case of congenital chronic hemolytic anemia [50].

Autoimmune hemolytic anemia (AIHA) is potentially severe, characterized by destruction of RBCs and immunoglobulin G (IgG) anti-RBC [51]. Hence, RBC membrane could be used to diagnose autism spectrum disorders using biophotonics [52]. Therefore, relative deformability difference between tumor cells and blood

cells as indicated by the length of time [53] may be used to diagnose and determine prognosis of some erythrocyte-related diseases and therapeutic interventions. Small intestinal resection and anastomosis by 70% decreased RBC from 6.23 to 5.1% pre-administration of glutamine. However, RBC decreased from 5.8 to 4.5% on the 12th day of experimentation. Honey, ascorbic acid, and glutamine caused decreased erythrocytes in intestinal resection and anastomosis in dogs [54]. Canine parvoviral enteritis causes anemia with highest incidence in Nigerian local dog occurring in January of every year and prevalence of 5.7% for the past 7 years [55]. Erythrocyte sedimentation is the speed with which erythrocyte levels fall in the blood of normal animals over a period of time. Hence diseased animals have high ESR, whereas healthy animals have low ESR [56] signifying that erythrocyte diseases can be diagnosed using ESR.

4.3 Effects of toxic agents on erythrocytes

Potassium permanganate (16 mg/kg) decreased hematocrit from 41.00 ± 2.08 to 39.29 ± 2.43% with attendant hypochloremia [16]. Salinity increased hematocrit and decreased hemoglobin of juvenile Nile tilapia (*Oreochromis niloticus*) [57]. Hypertonic saline (20%) could decrease hematocrit, total protein, and albumin [58] invariably decreasing erythrocytes and perhaps increasing hemoglobin. Toxic agents such as plants, drugs, venoms, antivenoms, chemicals, uroliths, and some food additives could damage erythrocytes and consequently cause anemia [59–61]. Halofantrine, sulfadimidine, chloramphenicol, and other many drugs and chemicals are toxic to erythrocytes [10, 15, 62]. But erythrocytes decreased in Baladi goat during the last 3 weeks of parturition and remained low 2 weeks after parturition [63] perhaps due to significant blood loss.

4.4 Effects of therapeutic agents on erythrocytes

Hexavalent chromium increased erythrocytes from 1.3 ± 0.03 to 1.05 ± 0.05 × 10^6 /*μl* after 24 h in *Labeo rohita* (Indian Major Carp), whereas hemoglobin was increased from 8.1 ± 6.7 to 6.80 ± 0.96 g/dl in the same species of animal. Also total protein of gill was decreased from 192.79 ±4.08 to 171.78 ± 3.64 g/dl [19] suggesting that hexavalent chromium has effect on gill protein. But RBCs of marine teleost carnivorous fish, *Lates calcarifer* (2.96 ± 0.25 × $10^6/\mu l$), are higher than that of omnivorous *Mugil cephalus* (2.52 ± 0.21 × $10^6/\mu l$) and herbivorous *Chanos chanos* (2.0 ± 0.51× $10^6/\mu l$), respectively. But hemoglobin and hematocrit were higher in *L. calcarifer* than in *M. cephalus*, and total protein was also higher in *L. calcarifer* [22]. RBCs of male *Naja naja* (0.58 ± 0.04 × 10^6/μl) are higher than that of the female *Naja naja* (0.50 ± 0.04 × 10^6/μl). PCV of the male (30.11 ± 1.93%) is higher than that of the female (23.41 ± 1.67%), whereas hemoglobin (7.6 ± 0.75 g/dl) is significantly higher in male than in the female *Naja naja* (3.25 ± 0.74 g/dl), respectively [64]. The difference in the hematological parameters may be due to their nutrition, age, sex, and environment. Aqueous extract of *Abrus precatorius* seed decreased erythrocytes from 6.33 ± 0.2 to 5.33 ± 0.24 × $10^6/\mu l$, packed cell volume from 38.00 ± 1.22 to 32.00 ± 1.41%, and hemoglobin from 12.65 ± 0.9 to 10.68 ± 0.47 g/dl, respectively. Distemper-hepatitis-leptospirosis-parvovirus-parainfluenza (DHLPPi) vaccine decreased in dog, hematological parameters at dose level of >2 mg/kg body weight. The same dose of the extract and DHLPPi caused hypoalbuminemia and hypoglobulinemia and decreased total albumin-globulin ratio. Hence the two could be used in prevention of chronic viral infection in dogs [13], signifying that erythrocytes have relationship with plasma proteins and may be used to determine immune status of animals. Methanol and ethanol

seed extract of *Abrus precatorius* decreased erythrocytes from 9.60 ± 1.02 to 7.13 ± 0.34 × $10^6/\mu l$ and 6.37 ± 0.60 × $10^6/\mu l$, respectively [14], indicating that the plant has anti-erythrocytic principle. Erythrocytes of albino rats increased from 7.2 ± 0.14 × 10^{12}/L to 9.35 ± 0.08 × 10^{12}/L. Packed cell volume, hemoglobin, and albumin were also increased significantly by aqueous ethanolic extract of *Psidium guajava* [17]. *Escherichia coli* caused hemolysis in *Rattus norvegicus* that was attenuated by aqueous root back extract of *Byrsocarpus coccineus*. *E. coli* was eliminated from the intestine and other organs by the extract [65]. *Chinchilla chinchilla* breed of the rabbit had increased erythrocytes, PCV, and hemoglobin as compared with other breeds [66]. *Vernonia amygdalina* and *Carica papaya* showed significant decrease in *P. berghei* parasites and increased RBCs and hematocrit in mice [67].

But *Ficus thonningii* aqueous extract has ameliorative activity against osmotic fragility induced by acetaminophen [68]. Various extracts of *A. precatorius* showed activity against parasites of erythrocytes such as *Plasmodium*, *Leishmania*, and *Trypanosoma* species with $1C_{50}$ of 12.1 ± 4.59 μg/ml [69]. Ceftriaxone caused increased packed cell volume, hypobilirubinemia, and increased bicarbonate ions in turkeys [15]. However human equivalent dose (HED) formula could be modified for determination of hematological and biochemical parameters [70]. About 8% of body weight correlates very well with plasma cell volume and hematocrit [2]. But many varieties of body surface area formulas could also yield different values of erythrocytes. Wang et al.'s formula may provide moderate doses of anticancer drugs against blood cell cancers [71]. Aqueous leaf extract of *A. precatorius* cleared significant percent of *Plasmodium berghei* in 14 days. However 10^7, *P. berghei* appeared in erythrocytes of mice within 24 hr after intraperitoneal inoculation [62]. Hence the percent of parasitized erythrocytes is calculated as follows:

$$\text{The percentage parasitized} = \frac{\text{Number of infected erythrocytes x 100}}{\text{Number of total erythrocytes}} \quad (3)$$

$$\text{Number of parasites per microliter (μl) of blood} = \frac{\text{WBC} \times \text{parasites counted against 100 WBC}}{100} \quad (4)$$

Erythrocytes infected with 10^7 *P. berghei* decreased in 7 days by 19%, after inoculation. But aqueous extract of *A. precatorius* leaf and halofantrine caused 9.8 and 12.7% decrease in parasitemia, respectively. However mice-fed grower's marsh had erythrocyte increase of 6.7% in 7 days [62]. The administration of aqueous leaf extract of *A. precatorius* at 10 mg/kg i.p. caused increased hematocrit from 33.0 ± 4.1 to 40.5 ± 3.1% as compared to 50 mg/kg oral dose that caused 40.3 ± 3.6%, respectively [72]. Gender, age, cholesterol, triglycerides, apoliprotein, and albumin affect hematological parameters [73]. Woman's blood has more fluid with 20% fewer erythrocytes than man's blood. Hence there is less supply of oxygen to body tissues in woman, and therefore she gets tired easily and is more prone to fainting [74]. But hot water increases blood supply to the muscle; hence water is contraindicated in acute bleeding [75].

4.5 Relationship between body weight, body surface area, erythrocytes, and area under curve

Formulas have been derived from the existing formulas that could be used for calculation of body weight, blood volume, erythrocyte volume, and PCV. The derived formulas are:

$$\text{Total blood volume (TBV)} = 0.08\text{BW} \tag{5}$$

$$\text{TBV} = \text{Plasma volume (PV)} \times \frac{100}{100 - \text{Haematocrit}} \tag{6}$$

$$\text{Haemtocrit (RCV)} = \text{TBV} - \text{PV} \tag{7}$$

Equate Eqs. (5) and (6):

$$\text{TBV} = 0.08\ \text{BW} = \text{PV} \times \frac{100}{100 - \textit{Haematocrit}} \tag{8}$$

$$0.08\text{BW} = \text{PV} \times \frac{100}{100 - \text{Haematocrit}} \tag{9}$$

$$\text{BW} = \text{PV} \times \frac{100}{\frac{100 - \text{Haematocrit}}{0.08}} \tag{10}$$

$$\text{BW} = \text{PV} \times \frac{100}{100 - \textit{Haematocrit}} \times \frac{1}{0.08} \tag{11}$$

But 0.08 = 8% [2].

$$\text{But creatinine clearance } (\textit{CrCl}) = \frac{\text{K} \times (140 - \text{age}) \times \text{BW}}{\text{D} \times \text{Scr} \times 72} \tag{12}$$

Substitute BW of Eq. (11) in Eq. (12):

$$\text{Hence } \textit{CrCl} = \frac{\textit{K} \times (140 - \textit{age}) \times \textit{PV} \times \frac{100}{100 - \textit{Haematocrit}} \times \frac{1}{0.08}}{\text{D x Scr x } 72} \tag{13}$$

$$\text{But serum creatinine (Scr)} = \frac{\textit{Pcr}}{1440} \times 1000\ \text{ml} \tag{14}$$

$$\text{U}_{\text{cr}} = \text{urine creatinine; Pcr} = \text{plasma creatinine}$$

$$\text{D (Depuration)} = \frac{\text{Ucr}}{\text{Pcr}} \tag{15}$$

$$\text{Dose (D)} = \text{AUC} \times [\textit{CrCl} + 25] \tag{16}$$

where D = dose of either therapeutic agent or toxicant that has an effect on erythrocytes, blood volume, and plasma volume and AUC = area under curve.

However, pharmacokinetic data are more useful in relation to disease of pathological findings instead of focusing on the mean data in relation to risk assessment [76]. Baseline variable is important to consider when using AUC for determination of relevant parameters [77]:

$$\text{Creatinine half} - \text{life} \left(\textit{Crt}\ \frac{1}{2}\right) = \frac{14616.8}{P_{CL-25}} \tag{17}$$

where P_{CL} = plasma clearance.

$$\text{Metabolism constant (Km)} = \frac{\text{BW}}{\text{BSA}} \tag{18}$$

$$\text{BW} = \text{Km} \times \text{BSA} \tag{19}$$

where BSA = body surface area [78].

However toxic agent could cause lethality by destroying erythrocytes. The amount of a toxicant that causes death in 50% of test animals is called median lethal dose (LD_{50}). The formula is used for determination of both median lethal and median effective dose of snake venom and antivenom, respectively:

$$\therefore LD_{50} = \frac{ED_{50}}{3} \times BW \times 10^{-4} \tag{20}$$

where LD_{50} = median lethal dose of toxic agent that has an effect on erythrocytes of 50% of test animals, ED_{50} = dose that has therapeutic effect on 50% of test animals, and 10^{-4}= safety factor [79].

Substitute BW of Eq. (19) in Eq. (20):

$$\text{Hence } LD_{50} = \frac{ED_{50}}{3} \times Km \times BSA \times 10^{-4} \tag{21}$$

Equations (18)–(21) are relevant in the study using experimental animals. However, there are various human body surface area formulas that vary from race to race and could be used in calculation of body surface area [80]. But the unique body surface area formula for human and dog may be relevant [81], and it is given below:

$$BSA = BW^{0.528} \times H^{0.528} \times K \text{ (where K = constant = 0.14)} \tag{22}$$

The height of dog must be multiplied by 2, and it is always in meter. More so Treeing Walker Coonhound (65 kg), female Komondor (59 kg), Greater Swiss mountain dog (59 kg), French Mastiff (50 kg), and long-haired St. Bernard (55 kg) have the same body surface area of humans weighing 51.3, 59, 46.7, 44, and 44.8 kg, respectively [81], and the two may have the same erythrocytes and other hematological values. Also, malignant lymphoma (cancer of the white blood cells) and other blood-related cancers can be treated using some other established BSA formulas [80]. But, scorpion sting can cause bleeding, leading to anemia and death. Hence the formula for determination of median lethal dose (LD_{50}) of scorpion venom in experimental animals is given below [82]:

$$LD_{50} = ED_{50}^{1/3} \times BW \times 10^{-4} \tag{23}$$

4.6 Transport of oxygen by erythrocytes

Erythrocytes in contact with alveoli receive oxygen which is combined with hemoglobin (oxyhemoglobin) and transported to various parts of the body. After the delivery of oxygen, the erythrocytes return CO_2 combined with hemoglobin (deoxyhemoglobin) which is bluish in color to the alveoli for expiration. Hence the following reaction occurs in the erythrocytes:

$$H_2O + CO_2 \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^- \rightleftarrows H_2O + CO_2 \tag{24}$$

The presence of COOH and C=O in piroxicam and other chemically related compounds [83] may interfere with chemistry of erythrocytes causing hemolysis and anemia. Also degradation products of some polymers, poly(lactic-co-glycolic acid), polyethylene glycol, polycaprolactone, and poly(propylene glycol) are converted to lactic acid and glycolic acid which are in turn converted to carbon dioxide and water [84] indicating that some polymers could also affect erythrocytes. Polycythemia could be confirmed by hyperpnea. Meat from an animal poisoned with potassium permanganate could react with 0.2% ethanolic benzidine

changing the color of meat to dark green in 1–2 s [85], and potassium permanganate causes hyperpnea. Lowest oxygen saturation could be improved by zolpidem [86]. Erythrocytes of cow, dog, goat, horse, pig, rabbit, rat, and sheep composed greatly of cholesterol and cholesteryl esters, triglycerides, and free fatty acids are present in trace quantity. They may not be true constituents of erythrocytes, but rather contaminants from plasma lipoproteins or leucocytes. Cholesterol is 30% of cell lipid and has molar ratio with phospholipid [87] and may affect oxygen capacity of erythrocytes. Hence cow, sheep, horse, rabbit, and chicken erythrocytes are susceptible to *Vibrio vulnificus* hemolysin with varying degrees of susceptibility [88] and may cause anemia and less oxygen transport. Human coagulation factor 1X (F-1X) activated by human RBCs causes coagulation activated by enzyme in the RBC membrane. However, in dog, cattle, rabbit, and sheep, coagulation did not occur except in pig when procoagulant was used. Hence coagulation activation of enzyme may be present in these species of animals [89].

Lysis of erythrocytes by toxins of cobra could cause anemia and splitting of phospholipids in equal capacity in rabbit, dog, human, and guinea pig, but not in camel and sheep erythrocytes. Phosphatide acylhydrolase is responsible for splitting of the erythrocytes. The action is via lytic factor which is hemolytic. The phospholipase readily hydrolyzes phospholipids of erythrocytes. *Vipera palestinae* could not lyse erythrocytes and hydrolyze phospholipids [90]. Plasma viscosity depends on plasma protein concentration [91], with cattle having 1.72 mpa s and rabbit (1.3 mpa s) with horse, dog, cat, mouse, rat, pig, and sheep having 1.3–1.7 mpa s. Man has a value lower than this range [92]. Deformability of RBCs is dependent on size and shape [93] with pig, hamster, rat, mouse, and rabbit having more deformable RBCs than sheep, horse, elephant, and dog. The deformity is characterized by RBC elongation and aggregation [94]. All these could affect oxygen transport. Non-irreversible sickle cells adhere more at normal oxygen tension, and more than 1% of the cells remained adhered to the monolayer at forces higher than physiologic shear stresses [95].

4.7 Morphologic differences in erythrocytes of animals

Llama, dromedary, and camel have low hematocrit value and low RBC aggregation [96]. Such RBCs may be small and elliptical in shape [97]. Nonmammalian RBCs have nucleus and microtubular bundle connected to a marginal band [98]. The erythrocytes are elliptical, larger, and not bending, with decreased blood cell count. But nonnucleated erythrocytes exhibit nonalignment. Hence erythrocytes of bird are flattened, lenticular, spherical, and folded when deformed [99]. Hemoglobin concentration of nonmammalian erythrocytes is 15–20% higher than that of mammalian species, with increased RBC density, and the nucleus contains 20% of cytoplasmic volume [100, 101] with RBC rigidity [102]. However, RBCs of reptiles are least decreased than that of mammal, the membrane having shear elastic modulus [103]. But fish might have the largest RBC volumes. The cells are elliptical and bulge in the region of their nucleus. Fish hematocrit drops with water temperature and can change due to the environmental temperature [104]. Aggregation capacity of equine erythrocyte is higher than that of dog and sheep, but could not be measured. There is species variation in erythrocyte elongation not linked with the aggregation property. Also deformability of erythrocytes is species-specific [105]. Tannic acid increases or decreases agglutinability of erythrocytes in the presence of immune serum [106]. Attachment of endotoxins, e.g., lipopolysaccharide antigen, to erythrocytes was strongly prevented by mammalian and avian sera followed by that of reptilian (moderate) and amphibian (minimal) [107]. But temperature has no effect on flexibility of horse, cattle, sheep, goat, and some human erythrocytes indicating that blood viscosity varies with temperature [108]. Diameter,

circumference, and surface area are higher in erythrocytes of dog followed by horse, cattle, sheep, and goat in that order [109].

4.8 Metabolic pathway of erythrocytes

Ion transport pathways of the erythrocytes are $Na^+ - K^+ - Cl^-$, $Na^+ - Cl^-$, and $Na^+ - K^+$ through AQPI water and SKI− Gardos channels using ATPase [110]. Major metabolic pathway in erythrocytes is as follows: glucose is converted to glucose-6-phosphate to fructose-6-phosphate to pyruvate to lactate [111]. Reduced or defective erythrocytes result in nonregenerative anemia, and increased cell loss results in regenerative anemia, respectively [112]. Cellular shape and flexibility of erythrocytes are dependent on metabolic process which is via enzymes that are associated with erythrocyte defects [113]. Spherocytes have less diameter and thickness greater than normal resulting from hereditary spherocytosis seen in the peripheral blood smear of neonates with ABO incompatibility [114], releasing lipids, causing adenosine triphosphate depletion, and exposing the cells to shear stress [115]. Methods used for measurements of erythrocytes deformability are filtration microfluidic filtration and laser diffractometry. Deformation of RBCs has to do with the geometry, hemoglobin concentration, rheological properties, osmotic concentration, calcium, nitric oxide, temperature, membrane protein and lipid alteration, erythrocyte ATP, and erythrocyte aging. But measurements of individual cells are by micropipette aspiration, atomic force microscopy, optical tweezers, and quantitative phase imaging. Also, there is correlation between erythrocyte deformability and diabetic microangiopathy [116]. Eosin-5-maleimide (EMA) binding test and osmotic fragility test differentiate hereditary spherocytosis from hereditary stomatocytosis [117]. Morphological changes in the sickle cell hemoglobin caused by deoxygenation of RBCs could lead to high metabolic activity and shortened life span of erythrocytes in sickle cell disease patients [118] invariably leading to anemia. The mechanism extent, levels, and complement involvement differ considerably in autoimmune hemolytic anemia [119]. Erythrocyte membrane disorders including hereditary spherocytosis and elliptocytosis could be diagnosed by red blood cell cytology, ektacytometry, flow cytometry, electrophoresis, and mutational analysis of cell membrane proteins [120].

4.9 Anemia as a major sign of erythrocyte deformation

Causes of anemia in cats are acute blood loss, chronic inflammatory disease, renal disease, feline leukemia, immune-mediated hemolytic anemia, pure red cell aplasia, myeloproliferative syndrome, mycoplasma infection, cytauxzoonosis, iron deficiency, and nutritional deficiency. The prognosis of feline nonregenerative anemia is variable, reversible, chronic, or fatal [121]. The spleen contributes to anemia by removing the damaged erythrocytes. Hereditary spherocytosis is spectrin-deficient and ankyrin-deficient erythrocytes dependent and could cause hemolysis [122]. Glycogen storage disease could affect erythrocytes. The disease is classified as follows: Type 1 (von Gierke's disease) is caused by deficiency of glucose-6-phosphate, whereas type 2 (Pompe's disease) is generalized glycogenosis. But type 3 (limit dextrinosis) is characterized by deficiency of the amylo-1, 6-glucosidase or debrancher enzyme, and type 4 is characterized by hepatic cirrhosis, abnormal glycogen resembling amylopectin, and deficiency of amylo-1, 4-1, 6-transglucosidase. Type 5 is characterized by weakness of muscle and phosphorylase deficiency in adults, and type 6 is clinically similar to type 1, characterized by higher phosphorylase. But type 3 has the highest concentration of glycogen, in the erythrocytes, but the concentration of glycogen is normal in type 1 and 2 [123].

Plasmodium species, *Babesia* species, and Bartonella species can target erythrocytes directly, whereas immunogens, microbial toxins, crypt antigens, and suppression of erythropoiesis can target erythrocytes indirectly. Duffy blood group antigens, ABO blood group antigens, Knops blood group antigen, Gerbich blood group antigen, babesiosis, bartonellosis, and toxoplasmosis target RBCs primarily. Erythrocytes are targeted for immunogenic clearance of *Mycoplasma pneumoniae*, *Haemophilus influenzae* type B, *Salmonella* species, polyagglutination T activation, *Clostridium perfringens*, parvovirus B19, Epstein-Barr virus, and acquired B antigen [124]. Disorders of erythrocytes hydration are overhydration, hereditary hydrocytosis, cryohydrocytosis, dehydration, and hereditary xerocytosis which are genetic [125]. Chronic liver disease could cause anemia but requires a complex diagnostic approach [126]. Hereditary erythrocyte volume homeostasis is heterogeneous with phenotypes ranging from overhydrated to dehydrated erythrocytes usually characterized by laboratory, physiological, clinical, and genetic findings [127].

Examination of urine sediment could serve as a guide for diagnosis and management of kidney disease [128] in relation to erythrocyte disorders. Erythrocytes have linked type 2 diabetes and Alzheimer disease in human. Superimposed alterations have been observed in Alzheimer disease patients caused by oxidative stress of erythrocytes [129], suggesting that therapeutic target on RBCs could alleviate Alzheimer disease. Hence erythrocytes' mechanical properties toward microfluidics could provide a clinical correlate in diseases of erythrocytes [130]. End-stage renal disease causes alteration of erythrocytes. Therefore, erythrocytes from peritoneal dialysis patients are more prone to aggregation that may be caused by uremia, hypoproteinemia, and high oxidative stress on erythrocytes, impairing blood flow dynamics and causing inadequate microcirculatory perfusion [131]. Erythrocyte complement receptor type 1 (E-CR 1) level of expression could be used as a diagnostic marker for systemic lupus erythematosus (SLE) [132]. The level of concentration of methotrexate polyglutamate in erythrocytes is associated with alleviation of rheumatoid arthritis [133].

Blood transfusion and febrile condition could also affect morphology of erythrocytes and erythrocyte count [134]. However, 15% of cancer patients with anemia are given blood transfusion and with hemoglobin level of <9 g/dl used as index of anemia. After transfusion hemoglobin rises by 1 g/dl, and the transfused erythrocytes last for 100–110 days with complications including but not limited to iron overload, viral and bacterial infections, immune injury, non-Hodgkin lymphoma, and chronic lymphocytic leukemia which are some worst outcome in selected cancers [135]. Trypanosomosis, pediculosis, helminthosis, lousiness, colibacillosis, babesiosis, coccidiosis, and amoebiasis characterized by anemia in advanced condition could be treated using various species of medicinal plants. The therapeutic principles are alkaloids, tannins, saponins, glycosides, flavonoids, phenols, minerals, and vitamins [136]. Health education could lead to disappearing of blood-related diseases such as malaria [137]. Ebola affects the blood leading to hemorrhage, septic shock, and multiple organ failure [138]. This point to the need for transfusion which could not be instituted until blood and erythrocytes are assessed. Because less than 1% dense hematocrit could cause aneurysm in aged dogs, canine hematocrit is an accurate model for human hematocrit [139].

5. Conclusion

Erythrocytes are red blood cells that transport oxygen from the alveoli to other parts of the body. Hence they are very vital connective tissues that play a metabolic

role on the functional organ system. Its pathological features could be used for diagnosis of a myriad of metabolic, non-metabolic, infectious, noninfectious, hereditary, and non-hereditary diseases. Erythrocyte shape, size, area, and volume could be used to determine a prognosis of a disease. Erythrocytes also store some drugs invariably prolonging their half-life. Hemolysis can lead to anemia that is treated using hematonics. But severe blood loss is corrected by blood transfusion.

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